CHARACTERIZATION AND INTEGRATED MANAGEMENT OF *Fusarium oxysporum* f. sp. *cubense* (E. F. SMITH) SNYDER AND HANSEN CAUSING FUSARIUM WILT DISEASE OF BANANA

By

LISHMA N. P.

(2017-21-018)

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

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THESIS

Submitted in the partial fulfilment of the requirements for the degree of

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Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA
2020

DECLARATION

I, **Lishma N. P. (2017-21-018)** hereby declare that this thesis entitled **"Characterization and integrated management of *Fusarium oxysporum* f. sp. *cubense* (E. F. Smith) Snyder and Hansen causing Fusarium wilt disease of banana"** is a bonafide record of research work done by me during the course of research and that the thesis has not been previously formed for the award of any degree, diploma, fellowship or other similar titles, of any other University or Society.

Place: Vellanikkara

Date: 24.11.2020

Lishma N. P.

(2017-21-018)

CERTIFICATE

Certified that this thesis entitled **"Characterization and integrated management of *Fusarium oxysporum* f. sp. *cubense* (E. F. Smith) Snyder and Hansen causing Fusarium wilt disease of banana"** is a record of research work done independently by **Mrs. Lishma N. P. (2017-21-018)** under my guidance and supervision and that it has not been previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

Place: Vellanikkara

Date: 24.11.2020

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

CERTIFICATE

We, the undersigned members of the advisory committee of **Mrs. Lishma N. P. (2017-21-018)**, a candidate for the degree of **Doctor of Philosophy in Agriculture**, with major field in **Plant Pathology**, agree that this thesis entitled **"Characterization and integrated management of *Fusarium oxysporum* f. sp. *cubense* (E. F. Smith) Snyder and Hansen causing Fusarium wilt disease of banana"** may be submitted by **Mrs. Lishma N. P. (2017-21-018)** in partial fulfillment of the requirement for the degree.

24/11/20

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

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

24/11/20

Dr. Sainamole Kurian P.
(Member, Advisory Committee)
Professor (Plant Pathology)
College of Horticulture, Vellanikkara

Dr. D. Girija
(Member, Advisory Committee)
Professor (Retd.)
Agricultural Microbiology
College of Horticulture, Vellanikkara

24/11/20

Dr. Deepa Mathew
(Member, Advisory Committee)
Assistant Professor (Plant Biotechnology)
College of Horticulture, Vellanikkara

EXTERNAL EXAMINER

Dr. (Mrs) M. L. JEEVA
Principal Scientist (Plant Pathology)
Central Tuber Crops Research Institute
Thiruvananthapuram - 695 017
Kerala, India

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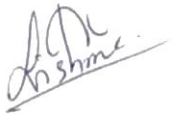

Lishma N. P.

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ABBREVIATIONS

mg	: Milligram
g	: Gram
µg	: Microgram
%	: Per cent
DNA	: Deoxyribo nucleic acid
min.	: Minute
h	: Hour
sec.	: Second
kb	: Kilo bases
bp	: Base pairs
PCR	: Polymerase chain reaction
NaCl	: Sodium chloride
CTAB	: Cetyltrimethyl ammonium bromide
EDTA	: Ethylene diamine tetraacetic acid
TAE	: Tris-acetate-EDTA
TE	: Tris-EDTA
PVP	: Polyvinylpyrrolidone
ml	: Millilitre
l	: Litre
ha	: Hectare
kg	: Kilogram

PDI	: Per cent disease incidence
PDS	: Per cent disease severity
rpm	: Rotations per minute
cm	: Centimetre
DAI	: Days after inoculation
BLAST	: Basic local alignment sequence tool
ng	: Nanogram
µl	: Microlitre
OD	: Optical denisty
PGPR	: Plant growth promoting rizhobacteria
m	: Meter
PDA	: Potato dextrose agar
°C	: Degree celcius
V	: Volt
CFU	: Colony forming units

INTRODUCTION

1. INTRODUCTION

Banana (*Musa* spp.) is one of the most important fruit crops in the tropical as well as subtropical countries of the world. It belongs to the family Musaceae. Based on genome constitution, botanical names of cultivated banana are *Musa acuminata*, *M. balbisiana*, *M. paradisiaca* or a hybrid of *M. acuminata* x *M.balbisiana*.Year-round availability, affordability, varietal diversity, nutritive and medicinal value makes banana a favourite fruit across the globe. Also, banana is the fourth vital food crop in production after rice, wheat and maize in developing countries. It is a major source of energy, dietary fibre, proteins and minerals, especially potassium and vitamins like A, C and B_6. India is the leading producer of banana with an average output of 28.6 million tonnes per annum and it is the second most important fruit crop next to mango (FAO, 2018).

Cultivation of banana is often constrained by the incidence of many pests and diseases worldwide. The major diseases of banana are Fusarium wilt, leaf spot diseases, bacterial wilt, anthracnose, bunchy top, bract mosaic, streak disease, *etc*. Among these, Fusarium wilt disease of banana, commonly known as Panama wilt which is caused by the soil-borne fungus, *Fusarium oxysporum* f. sp. *cubense* (Foc) is one of the most devastating diseases affecting the crop. It is a typical wilt and a lethal vascular disease of banana. It was first reported from Panama of Tropical America and then named as Panama wilt (Stover, 1962). Since then, the disease was widely spread throughout the world. In India, it has become one of the most important diseases in all banana growing states with a report of disease incidence of about 30 per cent and 85 per cent in the first crop and ratoon crop respectively (Mustaffa and Thangavelu, 2011). In general, the yield loss ranged from 30 to 90 per cent in the country. In the state of Kerala, it is reported to be one of the major limiting factors for the cultivation of banana varieties like Rasthali/Poovan (AAB), Njalipoovan (AB) and Kadali (AA) and causes huge economic loss to the farmers.

The occurence of Fusarium wilt disease is very severe in ill drained soils. The pathogen survives in the soil for several years through its thick-walled spores called chlamydospores. Initial obvious symptoms are expressed externally, which include

wilting and slight yellowing of the older leaves starting from margin to midrib with green younger leaves and get dried completely. Longitudinal splitting of the base of the pseudostem and buckling of the leaf petiole are also some of the characteristic symptoms noticed. Internal symptoms of the disease such as the discolouration of vascular bands become visible when the infected banana pseudostem or the rhizome is cut open transversely or longitudinally. Generally, fruits do not express any symptoms. Suckers of infected plants show symptoms after four to six months of planting or during flowering.

The pathogen attacks primarily banana and other banana relatives and it is a very diverse pathogen. The pathogenic isolates of Foc have been traditionally grouped into four physiological races based on pathogenicity to host cultivars under field condition. Race 1 infects Gros Michel (AAA), caused Gros Michel epidemics in America, it also infects AAB group varieties such as Rasthali, Lady Finger and Silk. Whereas race 2 affects ABB group cooking bananas like Bluggoe and Monthan and Race 3 infects *Heliconia* sp., a close relative of cultivar banana. Race 4 has been divided into 2 groups *viz.*, subtropical R4 (SR4) and tropical R4 (TR4). Subtropical race 4 affects the Cavendish group and the varieties susceptible to race 1 and 2 in subtropics. Tropical race 4 pathogens attack banana both in tropical as well as subtropical conditions. In India, race 1 of Foc is distributed in almost all banana growing states and Foc tropical race 4 also has been reported from Bihar (Katihar district) in 2015 (Damodaran *et al*., 2019; Thangavelu *et al*., 2020). In Kerala, so far race 1 of Fusarium wilt pathogen is reported from banana varieties like Rasthali/Poovan (AAB), Njalipoovan (AB), Kadali (AA), Monthan (ABB) and Karpooravalli (ABB). It causes huge damage to the economy of banana farmers of the state.

Though internationally significant progress has been made to understand the biology of this pathogen, no attempts have been made to study the variability and diversity of the pathogen isolates from Kerala. Studies on pathogenic variability are also very important to develop integrated management practices. Hence, an attempt to characterize various isolates and diversity analysis using molecular tools was undertaken in the present study. The recent reports of symptoms resembling Fusarium

wilt in banana varieties like Cavendish, Nendran and Grand Naine are of concern to scientists and banana producers. Among the four races of the pathogen reported so far, race 4 has not been reported from Kerala. However, growing incidence of Fusarium wilt disease on banana in the state necessitate further probing on the occurrence and identification of various races of the pathogen using advanced quick detection technologies.

The conventional methods for identifying Foc is difficult and time-consuming, particularly when symptoms are confusing with that of bacterial wilt. Nowadays, a variety of advanced techniques such as polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP) were developed elsewhere for the identification and early detection of fungal pathogens. These advanced techniques could provide more rapid, accurate and reliable detection of the pathogen in the early stages of infection. It helps to take early and timely management measures which in turn, makes it possible to reduce the impact of disease outbreaks. Hence, an attempt was undertaken to develop the LAMP, a technique for the quick detection of Foc race 1. It is an isothermal approach for the amplification of nucleic acid and does not require a thermal cycler.

Various management practices such as biological, cultural and chemical control have been tried to mitigate the pathogen causing Fusarium wilt in banana since its discovery and no single method is effective for the control of the disease. Hence, a sustainable approach utilizing genetic resistance, disease-free plantlets, cultural practices, new generation fungicides and eco-friendly bioagents needs to be formulated to limit further spread of the pathogen and to contain the disease.

In view of such concerns, the project entitled "Characterization and integrated management of *Fusarium oxysporum* f. sp. *cubense* (E. F. Smith) Snyder and Hansen causing Fusarium wilt disease of banana" was undertaken with the following objectives.

- Purposive sampling surveys in selected banana growing areas of Kerala
- Cultural, morphological and molecular characterisation of the pathogen

- Study the genetic diversity of the pathogen
- Development of a technique for quick detection of the pathogen
- Evaluation of host plant disease resistance
- Development of an integrated management strategy against the disease

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Banana is an important fruit crop in tropical and subtropical countries and is the major diet for around 400 million people in several regions of the world. It is a perennial monocotyledonous plant which belongs to the order Zingiberales and the family Musaceae. Commonly cultivated bananas are triploids (AAA, AAB or ABB) and formed as a result of intraspecific or interspecific crosses between two diploids *viz*., *Musa acuminata* (AA) and *Musa balbisiana* (BB) (Simmonds and Shepherd, 1955; Heslop-Harrison and Schwarzacher, 2007). It produces parthenocarpic fruits whereas, the wild diploid banana produces seeds (Li *et al*., 2013a). Its production is seriously threatened by several pests and diseases. Among the diseases, the most destructive one is Fusarium wilt that affect the yield and quality of the fruits. The disease is caused by a soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc).

OCCURRENCE AND IMPACT OF FUSARIUM WILT DISEASE

"*Panama disease can transform a living plantation to a dead loss in a few months*" (Carefoot and Sprott, 1969).

Fusarium wilt of banana and plantains was first explained by Bancroft (1876) in Australia, who unknown that this disease would be most dangerous in the history of banana cultivation worldwide. He isolated the fungus associated with wilting from banana for the first time.

Higgins (1904) reported that a fungus found in banana plants causes lethal yellowing and wilting of the entire plants. Thereafter in 1908, the fungus associated with this wilting was realised by Smith (Smith, 1910) in sick plants collected from Cuba and he termed it as *Fusarium cubense*. The detailed explanation about the pathogen was given by Ashby (1913). The Koch postulates of the causal agent was carried out in Gros Michel (AAA), Manzano (AAB) and Bluggoe (ABB) varieties (Brandes, 1919). *F. oxysporum* causing wilt diseases in banana plants was renamed as *F. oxysporum f. sp. cubense* after the formation of *formae specialis* system (Snyder and Hansen, 1940).

In India, Fusarium wilt of banana was first reported from West Bengal in 1911 in the cultivar Kanthali (ProMusa, 2020). Then it became the most destructive disease

in banana cultivating areas (Thangavelu *et al*., 1999). In Kerala, Fusarium wilt is reported from banana varieties like Rasthali/Poovan, Njalipoovan, Kadali and Karpooravalli. It causes huge damage to the economy of Kerala farmers (AICRP, 2017).

At present, the Fusarium wilt of banana has been distributed to almost all banana growing areas in the world. It includes Asia, Australia, Africa, South pacific provinces and Latin America excluding the Papua New Guinea and some of the Mediterranean nations (Bakry *et al*., 2009).

A purposive sampling survey was conducted by Sivamani and Gnanamanickam (1987) in various banana growing areas of Tamil Nadu which is affected by Fusarium wilt disease and reported the disease incidence of 2-90 per cent.

Sivamani (1987) reported that Fusarium wilt was a major threat to banana cultivation with a per cent disease severity of 80 to 90 per cent. Also, in Bihar, it caused a yield reduction of 50 to 70 per cent where more than 55 per cent of the banana cultivation was highly susceptible varieties. Narendrappa and Gowda (1995) reported that the Fusarium wilt affected cultivation of variety Nanjagod Rasabale severely which caused a reduction in the area of cultivation from 500 ha to 50 ha.

In the first half of the 20th century, Fusarium wilt devastated Panama and caused a huge yield loss. Since then, the disease was commonly known as Panama wilt (Jeger *et al.*, 1996).

In the northern states of India, the disease occurrence of up to 80 per cent and yield loss of up to 40 per cent have been reported. Whereas, in southern states, yield loss up to 90 per cent has been reported (Thangavelu *et al*., 1999). Thangavelu *et al*. (2001) reported that the incidence of Fusarium wilt in banana was 30 per cent in the plant crop and 85 per cent in the ratoon crop in India. The varieties like Rasthali (AAB), Karpuravalli (ABB), Monthan (ABB) and Virupakshi (AAB) were severely affected by wilt disease.

Survey carried out in coastal districts of Andhra Pradesh revealed that the Fusarium wilt disease incidence was more severe in Silk cultivars such as Rasthali/Poovan and Amritapani. Apart from these cultivars, Karpuravalli, a Pisang Awak cultivar and Bluggoe group were also affected with the wilt disease considerably (Prasadji, 2006).

Molina *et al*. (2007) reported that in Asia, the pathogen caused a huge loss to the banana production in countries like Taiwan (1967), Indonesia and Malaysia (1990), China (2004), etc.

In a survey, Leong *et al*. (2009) collected thirteen Foc isolates from various parts of Malaysia and reported that all the isolates were closely related with respect to cultural as well as molecular characteristics regardless of the location and cultivars. This is one of the most serious and destructive diseases of banana and it is the major limiting factor in banana production (Lin, *et al*., 2009; Visser *et al*., 2010).

Incidence of Fusarium wilt of banana caused severe economic loss in almost all banana growing countries in the world especially in Asia, Africa, Australia and the tropical America. Over 50 years, it destroyed around 40,000 ha of banana production in Central and South parts of America (Thangavelu *et al*., 2012). The disease is now prevalent in all banana growing regions (Butler 2013).

Somu *et al.* (2013) carried out a roving survey and reported the wilt disease incidence ranging from zero to hundred per cent in banana growing regions of Karnataka in the year 2011-12. They observed that the maximum incidence of disease was in Kadali variety in Bangalore followed by Devarasanahalli in Mysore and Rasthali in Nanjanagudu.

A survey was conducted by Kumar and Saxena (2015) to study thoroughly about the symptoms and severity of Fusarium wilt of banana at Panchgaon, Hariyana during 2014-2015. The population of the pathogen Fusarium was maximum in the rhizosphere soil (83.4) and minimum in rhizoplane (46.3) while, it was 71.6 per cent in infected stem and 51.6 per cent in collar region of the plant.

A purposive survey was conducted by Mostert *et al*. (2017) to investigate the distribution, host range and diversity of the Fusarium wilt fungus, Foc in ten Asian countries. They collected 594 isolates and classified based on vegetative compatibility groups.

According to Ghag (2019), in India, Fusarium wilt caused a severe loss in the production of banana cultivars like Silk, Rasthali/Poovan, Karpooravalli, Monthan, Ney poovan, Virupakshi, Amrithapani, etc and the yield loss recorded due to race 1 strain was up to 50 to 70 per cent.

SYMPTOMATOLOGY

Symptoms produced by Fusarium wilt of banana are of two types *viz*., external and internal symptoms. External symptoms are again classified into two, namely yellow leaf syndrome and green leaf syndrome (Stover, 1962). Yellow leaf syndrome is the most visible and classic symptom of the disease. It starts with yellowing of older leaves and progress to the younger leaves. Later, the leaves collapse gradually, bends near to the midrib and forms a 'skirt' like appearance with dead leaves. Whereas in some varieties, the leaves remain green after the infection but leaves bend and collapse. This is termed as green leaf syndrome.

External symptoms start with pale green streaks on the base of petiole two weeks before the appearance of typical symptoms and brownish-red discolouration of vessels under the epidermis of the petiole. Infected plants often show splitting of pseudostem, wilting and then die (Stover, 1972).

The internal symptoms start with discolouration of the vascular system. Initially, yellowing of the root and rhizome vascular tissue occurs which gradually forms reddish or brownish vascular strands in the pseudostem (Perez-Vicente, 2004). The pathogen enters plants and colonises inside the vascular system of pseudostem and rhizome. The fungus grows very rapidly inside the vascular bundles of the host plant and sporulates abundantly especially producing microconidia which block the water-conducting system (Li *et al*., 2011). The soil-borne fungus reaches plant through fine root hairs and inhabits in the xylem vessels thereby blocking the passage of water and

nutrients (Ploetz and Churchill, 2011). Thus, it leads to water deficiency in plants and induces characteristic wilting symptoms accompanied by necrosis and rotting of roots, rhizome and pseudostem vessels and plant dies finally.

The races of Foc can not be differentiated based on symptoms produced. All races produce same symptoms irrespective of the varieties (Dita, *et al*., 2018).

In the study conducted by Dong *et al*. (2014) revealed that yellowing of the Fusarium wilt infected plants are due to the production of fusaric acid, a phytotoxin. It damages chloroplast and reduces the photosynthetic efficiency of photosystem II. Structural and biochemical changes were also seen associated with the disease.

The symptoms of banana Fusarium wilt disease are similar to the Fusarium wilt of other crops. The main symptoms are characteristic yellowing and then subsequent wilting of the entire plant (Ghag *et al*., 2015).

Foc moves upwards through the xylem vessels of host plants and also produces microconidia during the process. The microconidia produced block the conducting vessels and germinate there. The hyphae penetrate the host barriers and travel along the xylem tissue. It results in discolouration of the vascular strands from initial pale yellow to dark brown or black in the advanced stage (Kumar and Saxena, 2015).

Symptoms of Fusarium wilt affected banana start with reddish discolouration of the xylem vessels in root hairs or feeder roots and yellowing of lower leaves (Thangavelu *et al*., 2020).

PATHOGEN OF FUSARIUM WILT

Fusarium wilt of banana is caused by the fungal pathogen *Fusarium oxysporum* f. sp. *cubense* (E. F. Smith) Snyder and Hansen (Foc) (Parham, 1934; Snyder and Hansen, 1940). *Fusarium* sp. is one of the most important and diversified genera among the microorganisms. The pathogen is classified under Domain: Eukaryota, Kingdom: Fungi, Phylum: Ascomycota, Class: Sordariomycetes, Subclass: Sordariomycetidae, order: Hypocreales and Family: Nectriaceae (Snyder and Hansen, 1940).

Booth (1971) classified the fungi *Fusarium* spp. based on their morphological and cultural characters by morphological species concept by growing it on a specific medium using the single spore isolation technique. He also stated that it is a septate fungus which shows abundant growth on potato dextrose agar medium when incubated at a temperature of 25°C.

According to Agrios (2005), the genus *Fusarium* comes under the class Fungi Imperfectii or Deuteromycetes. It belongs to the Kingdom Mycota, Division Eumycota, Subdivision Deuteromycotina, Class Hyphomycetes and the order Moniliales. Leslie and Summerell (2006) stated that the Foc comes under the class ascomycetes and according to Groenewald (2006), it belongs to the section Elegans.

Foc is a hemibiotrophic organism which primarily forms a biotrophic association with banana plant and then turns to a necrotroph after killing the plant tissue (Dita *et al*., 2018; Jones, 2018).

The pathogen is heterogeneous and polyphyletic and it is characterized based on vegetative compatibility. So far, more than twenty vegetative compatibility groups have been reported (Jones, 2018).

Sexual reproduction of Foc has not been reported (Koenig *et al*., 1997) and it is infrequent if occurs (Kerenyi *et al*., 2004). Yun *et al.* (2000) reported that genes such as MAT1-1 and MAT1-2 are present in sexual species of *F. oxysporum*. Further studies have to be conducted to understand the sexual cycle of Foc.

HOST RANGE AND VARIABILITY OF FOC

Fusarium wilt of banana is caused by the forma speciales cubense of *F. oxysporum* complex of pathogens. Waite and Dunlap (1953) reported that the pathogen can infect hosts other than banana such as *Chloris inflata*, *Commelina diffusa*, *Ensete ventricosum*, *Euphorbia heterophylla*, *Tridax procumbens* and *Panicum purpurescens*. The pathogen does not produce any symptoms on these weeds on infection. This group of *Fusarium* infects only a limited number of host species such as *Musa acuminata*, *M. balbisiana*, etc (Waite and Stover, 1960).

Race 1 infects Gros Michel caused Gros Michel epidemics in America, it also infects AAB group varieties such as Rasthali, Maqueno, Silk, Pome and Pisang Awak whereas, Race 2 affects ABB group cooking bananas like Bluggoe (Moore *et al*., 1995) and Race 3 infects *Heliconia* spp., a close relative of banana. Race 4 has been divided into 2 groups *viz.*, subtropical R4 (SR4) and tropical R4 (TR4) (Groenewald *et al*., 2006). Subtropical Race 4 affects the Cavendish group and the varieties susceptible to Race 1 and 2 in subtropics. TR4 pathogens attack both in tropical as well as subtropical conditions (Buddenhagen, 2007).

In an investigation carried out by Waman *et al.* (2013) to screen the ornamental plants against Foc, stated that detached leaf assays are desirable to assess the susceptibility of the ornamentals to infection by Foc. They also reported that the fungus can survive and spread to new areas through plants belonging to the orders Zingiberales and Alismatales.

Race designations of Foc help in comparison of variability in its populations. This is required for in-depth study of the fungal cultivars and host specificity. Four types of races which are specific to distinct varieties have been reported so far from various parts of the world (Ploetz, 2015).

In India, race 1 of Foc is distributed in almost all banana growing states. Foc tropical race 4 also has been reported from Bihar (Katihar district) in 2015 (Damodaran *et al*., 2019; Thangavelu *et al*., 2020). The pathogen has transmitted from Bihar to Utter Pradesh and the disease incidence exceeds 50 per cent in affected areas.

BIOLOGY AND ECOLOGY

Stover (1962) stated that the spread of the Fusarium wilt pathogen occurs in several manners and infected corms are the most common and effective mode of dissemination.

During unfavourable conditions, Foc produces chlamydospores for the survival. It can survive in the soil for more than twenty years (Stover, 1972). In sick soils, the pathogen exists by inhabiting on non-susceptible host plants and produces

chlamydospores that perform as the source of inoculum (Schippers and Van Eck, 1981). Planting materials collected from infected field would be a source of pathogen even though it is symptomless. Infected leaves, fruit bunch stalks, fibres and other infected banana pieces left at the field or transported to other places can carry over the disease (Deacon, 1984). Foc can spread through water by flooding or by irrigating the field (Su *et al*., 1986).

Foc infects both resistant and susceptible plants but causes plugging of vascular bundles only in the susceptible plants as the resistant plants produce defensive materials like tyloses, gums and gels in xylem vessels at the earlier stage of infection (Beckman, 1987;1990). Foc limits to the xylem cells and some neighbouring cells when the host plant is alive (Ploetz and Pegg, 2000). However when, the host dies, the pathogen penetrates the parenchyma cells and sporulates abundantly.

Perez-Vicente *et al.* (2003) reported that, under *in vitro* conditions, Foc can survive at a temperature between 9 °C to 38 °C. The favourable temperature for the growth is between 23 °C and 27 °C. Under field conditions, the infection occurs in wet and warmer months of the year. Sometimes, the infection is dependent on the factors such as resistance or susceptibility of the cultivar, pathotype of Foc, etc.

Saravanan *et al.* (2003) stated that the Fusarium wilt of banana is a typical vascular disease and pathogen enters the plant through water-conducting xylem vessels thus, causes the blockage of water and results in wilted appearance.

Till date, no reports are showing the role of fruits from infected plants in the dispersal of the disease even though the fruit stalk is infected (DAFF, 2004).

According to Li *et al*. (2011), chlamydospores germinates when it reaches proximity to banana roots and the infection occurs as a result of primary and secondary root exudates. Root hairs are affected directly, then it spreads towards major roots and rhizome of banana. After germination, hyphae stick on to epidermis and penetrates it. Then reaches the xylem through cortex and intracellular spaces. After reaching the xylem, it produces microconidia and toxins and colonizes the nearby cells. After colonization inside the epidermal cells, the fungus occupies intercellular spaces and produces plenty of spores.

The pathogen inhabits intercellular and intracellular spaces and succeeds to the cortex and subsequently to the endodermis to reach the vascular system (Yadeta and Thomma, 2013).

The chance for the spread of spores through insects is also significant (Meldrum *et al*., 2013). It is also reported the presence of the pathogen in the exoskeleton of *Cosmopolites sordidus*.

Fusarium wilt is a polycyclic disease, the pathogen is capable of causing more than one disease cycle per season (Ploetz, 2015).

The pathogen overcomes the primary and secondary defence system of host plants and get entry into the root system of the plant through root hairs. Chlamydospores adhere to the root caps and colonize on the surface of roots. Then it enters the epidermal cells of root hairs *via* penetration or through injuries (Ghag *et al*., 2015).

The pathogen spreads from plant to plant actively through root proximity or passively by carrying out the inoculum by other means (Pegg *et al*., 2019). The movement can be occurred by both animals and human activities.

ISOLATION OF THE PATHOGEN

Ainsworth (1971) used half strength potato dextrose agar consisting of 100 g peeled and diced potatoes, 10 g dextrose and 20 g agar per litre of distilled water for the better growth of *F. oxysporum* was (Ainsworth, 1971).

In a study conducted by Bragulat *et al*. (2004), six different media *viz*., Nash and Snyder medium, dichloran-chloramphenicol peptone agar medium, modified Czapek Dox agar, Czapek Dox iprodione dichloran agar, potato dextrose iprodione dichloran agar and malachite green agar were evaluated to isolate the colonies of various *Fusarium* spp. such as *F. anthophilum, F. culmorum, F. dlamini, F. graminearum, F. napiforme, F. nygamai, F. oxysporum, F. proliferatum, F. semitectum, F. solani, F. subglutinans* and *F. verticillioides*. Results revealed that no

significant difference was recorded regarding the colony count, whereas colony diameter was comparatively less in the case of malachite green agar medium.

Nel *et al.* (2006) isolated 100 isolates of non-pathogenic *F. oxysporum* from rhizosphere region of banana plants by soil serial dilution method and grown on Komada's medium. The isolates were purified by single spore isolation using the PDA medium.

In an experiment conducted by Dita *et al*. (2010) for the characterization of Foc TR4, the pathogen was isolated from sections of infected pseudostem strands of size 2 cm long by placing on Komada's medium. They reported that fungal colonies appeared after 3-5 days of incubation as white and pink coloured mycelia.

Das *et al.* (2012) isolated Foc from strands of infected banana plants collected from southern parts of India and maintained on PDA. Pure cultures were sub-cultured on to Czapek Dox agar medium for further studies.

PATHOGENICITY

In an experiment conducted by inoculating the injured and uninjured roots of banana plantlets Foc, the symptoms were produced 15 days after inoculation in plants with injured roots (Sivamani and Gnanamanickam, 1988; Sowmya, 1993). Inoculated plants with healthy roots remained disease-free.

Garibaldi *et al.* (2004) proved pathogenicity of *F. oxysporum* causing wilt in gerbera plants by inoculating the pathogen suspension by root dipping method. Inoculated plants showed vascular discolouration and wilting within a month of inoculation and the re-isolated pathogen appeared the same as original culture. Uninoculated plants remained healthy throughout the experiment.

Purwati *et al*. (2008) proved pathogenicity of Foc in banana with injured and uninjured roots by root dipping in conidial suspension. The results of the experiment revealed that the plants with injured roots expressed symptoms earlier than plants with uninjured roots.

Pathogenicity of Foc was proved by root dip inoculation of the suckers of banana cultivar Neypoovan in spore suspension (Somu, 2012). The results revealed that inoculated suckers produced symptoms after 90 days of inoculation whereas, the control plants were healthy.

Maymon *et al*. (2020) confirmed the pathogenicity of Foc isolates collected from various regions of Israel by artificial inoculation and verified by assessing Koch postulates.

CHARACTERIZATION OF ISOLATES

Cultural characterization of isolates

In PDA medium, Foc produces pale violet to dark coloured pigmentation and some produce pionnotal to flat humid or white to peach coloured mycelium (Stover, 1962).

Three different types of spores are produced by the pathogen Foc. They are macroconidia, microconidia and chlamydospores. Macroconidia consist of 4-8 cells bounded in a saclike structure whereas, microconidia are kidney-shaped consisting of only 1-2 cells. The globular shaped chlamydospores are resistant structures of the fungus which helps to overcome the unfavourable conditions (Smith, 2006) and can be viable for more than three decades.

According to Honnareddy and Dubey (2007) pigmentation of *F. oxysporum* f. sp. *ciceris* isolates causing wilt in chickpea collected from various locations of India ranged from white to violet, pink, brown and dark green. The isolates produced either aerial and fluffy mycelium or flat and suppressed mycelium. Most of the isolates produced terminal or intercalary chlamydospores singly or in pairs, but rarely in a chain.

In modified Komada media (K2), some isolates of TR4 develop laciniate radial colonies, which are not found in isolates of races 1 and 2 (Qi *et al*., 2008). However, this characteristic is not a determinant of a *Foc* TR4 diagnosis.

In an investigation conducted by Dubey *et al.* (2010), they collected 112 isolates of *F. oxysporum* f. sp. *ciceris* causing wilt in chickpea and studied the cultural

characters such as radial growth as well as colony characters. Radial growth ranged from 8 to 13 mm/day. Most of the isolates were white to pink coloured with floccose colonies.

Sharma and Pandey (2010) studied the mycelial growth, colony characters, sporulation of ten fungal isolates isolated from decaying vegetable waste on various media *viz*., PDA, Czapek's Dox + Yeast Extract Agar (CYA) and Lignocellulose Agar (LCA) and reported that sporulation was depended on the type of media used. LCA showed maximum mycelial growth whereas sporulation was found to be highest in PDA and CYA media.

The study conducted by Patel *et al.* (2011) indicated that isolates of pigeon pea wilt pathogen *F. udum* produced whitish to light pink or orange to pink coloured mycelium with a dry mycelial weight of 221 mg to 494 mg. Sporulation of the isolates varied from 3.2 to $32.5x10^6$ spores per ml.

Cultural variability in 15 isolates of *F. udum* causing pigeon pea wilt collected from the Bihar, Jharkhand, Orissa and West Bengal was studied, the results revealed that the colony diameter of isolates varied from 2.96 cm to 5.73 cm in potato dextrose agar medium after eight days of incubation (Kumar and Upadhyay, 2014). Among the 15 isolates, 10 produced white, fluffy mycelium with a serrated margin and yellow pigmentation whereas, five isolates were white having appressed mycelium with a serrated margin and yellow to brown pigmentation.

An investigation was conducted by Chopada *et al.* (2014) to study the variability among 10 isolates of *F. oxysporum* f. sp. *lycopersici* causing wilt in tomato collected from various locations of Gujarat. Results revealed that the isolates varied in mycelial colour, mycelial growth and pigmentation. These produced moderate, profuse fluffy, thin flat to slight fluffy mycelium and submerged growth with white, yellow and pink colouration with orange and purple to orange pigmentation.

Rana *et al*. (2017) collected 14 isolates of Foc from lower Gangetic plain and studied the cultural characters such as mycelial growth, biomass production and

sporulation in different media. The results showed that PDA performed superiorly compared to oat meal agar and banana pseudostem extract agar medium.

Morphological characterization of isolates

Morphological distinction of Foc from other formae speciales of *F. oxysporum* causing wilt disease in plants is not practically possible (Booth, 1971)

As per Kontoyiannis *et al*. (2000), the pigmentation of *F. oxysporum* varies greatly and may be yellowish, purple or brown. The colony colour may be observed as white, purple, pink, violet, tan, *etc* in the culture medium.

Studies on biological, physiological and pathogenic diversity of 26 isolates of Foc subtropical race 4, the causal agent of Fusarium wilt of banana resulted in grouping the isolates into sporodochial, cottony and slimy pionnotal types (Groenewald *et al*., 2006). They reported that macroconidia were produced in plenty by all isolates, but it varied in quantity.

Foc is an anamorphic fungus lacking a known teleomorph stage. It produces three types of spores *viz.*, macroconidia, microconidia and chlamydospores for reproduction. Macroconidia and microconidia are produced in sporodochium, an orange coloured fruiting body of the fungus. Chlamydospres are produced in hyphae terminally or intercalary in single or in chains. Size of macroconidia and microconidia ranges from 27-55 x 3.3-5.5 µm and 5-16 x 2.4-3.5 µm respectively. Macroconidia are produced abundantly, falcate to erect with 3-5 septations and foot-shaped basal cell whereas, microconidia are single-celled, oval to kidney-shaped and abundantly produced on short phialides. Chlamydospores are 7-11 µm in diameter and are the resistant structures of the fungus which help to overcome the adverse environmental conditions (Fourie *et al*., 2011).

Hafizi *et al*. (2013) studied the morphological characteristics of 51 isolates of *F. solani* and 40 isolates of *F. oxysporum* causing crown disease in oil palm collected from Indonesia and Malaysia and assessed the genetic diversity. They cultured the isolates on carnation leaf agar media and observed septation, length as well as width of

50 macroconidia randomly. Based on these characters, isolates were classified into morphotype I and morphotype II. In the case of *F. solani*, macroconidia of morphotype I produced septation ranging from 3 to 5, whereas it is 3 to 7 in morphotype II. Length of macroconidia of morphotype I was 27.0 to 37.3 µm and width was 3.1 to 4.3 µm. The length and width of morphotype I was 36.6 to 46.2 µm and 3.7 to 5.3 µm respectively. While septation of *F. oxysporum* was 3 to 7 and 3 to 5 in morphotype I and morphotype II respectively.

Kumar and Upadhyay (2013) collected 15 isolates of wilt pathogen *F. udum* from various pigeon pea growing areas of Bihar and studied the morphological characteristics. The weight of dry mycelium varied from 98.3 to 201.3 mg whereas, the sporulation varied from 0.8 to 3.6 million ml^{-1} in potato dextrose broth medium after 15 days of incubation at 27±2 °C. The length and breadth of macro and microconidia ranged from 15.4-35.0×2.0-8.2 µm and 4.1-16.5×2.0-6.1 µm respectively.

The results of an experiment on tomato wilt pathogen *F. oxysporum* f. sp. *lycopersici* conducted by Sonakar *et al.* (2013) indicated that the size of the macroconidia ranged between 15-37.5 µm x 2.5-4 µm and that of the microconidia was 2.5-15 µm x 2-3 µm among the isolates. The macroconidia were 3 to 5 septate and the microconidia were aseptate or single septate.

In an experiment conducted to study the morphological characters of *F. oxysporum* f. sp. *lycopersici* causing wilt in tomato, it was found that the sporulation of various isolates ranged from 2.77×10^6 to 21.68×10^6 spores/ml. The maximum and minimum dry mycelial weight observed was 193.33 mg 120.67 mg respectively. The size of macroconidia and microconidia varied from 15.46-21.8 × 4.91-5.45 µm to 21.42-44.28 × 7.35-9.14 µm with 1-6 septa and 3.57-14.28 × 2.68-4.46 µm to 7.14-14.28 × 3.57-5.35 µm respectively with 0-1 septum in different isolates (Chopada *et al.*, 2014).

Mulekar *et al*. (2017) studied variability among 24 isolates of *F. oxysporum* f. sp. *ricini*, the causal agent of castor wilt collected from several topographical places of India. The average length and breadth of microconidia varied from 7.23 µm to 9.47 µm

and 3.77 μm to 5.02 μm correspondingly. Likewise, average length and breadth of macroconidia varied from 12.32 μm to 34.56 μm and 3.45 μm to 4.38 μm respectively.

Morphological identification methods for microorganisms are still quite difficult and time consuming. It needs expert techniques in spotting and identifying the diagnostic features of the organisms.

Molecular characterization of isolates

Detection of the disease by morphological identification of the pathogen is difficult and time consuming (Alves-Santos *et al*., 2002). Morphological identification needs deep knowledge about the taxonomy of pathogen (Jurado *et al*., 2006).

Cultural and morphological characterization is not sufficient for the exact identification of Foc as these methods do not give the genetic information. To avoid the difficulty in identification of Foc, more reliable methods such as molecular characterization can be adopted.

Isolation of genomic DNA

Bentley *et al.* (1994) used liquid nitrogen and extraction buffer consisting of 2 per cent SDS, 40 mM EDTA, 100 mM Tris-HCl and 25 mM sodium diethyldithiocarbamic acid for the isolation of genomic DNA from Foc. Proteins present were removed by the addition of 24:1 chloroform: isoamyl alcohol and the nucleic acid was precipitated by treating with ice-cold isopropanol.

Leong *et al.* (2009) extracted total DNA from fifteen isolates of Foc collected from Malaysia and Indonesia using DNeasy Mini Plant kit provided by QIAGEN.

A CTAB method was developed by Ingle and Ingle (2013) for the extraction of genomic DNA from Foc for the identification and characterization of isolates collected from Maharashtra region.

Mishra *et al.* (2014) developed a simple and rapid method for the extraction of genomic DNA from filamentous fungi using the CTAB extraction buffer by grinding

with or without liquid nitrogen. Isolation of total genomic DNA from Foc race 4 infected plants and pure fungal culture was carried out by Muhammad *et al.* (2017) using CTAB method.

Genomic DNA of Foc was extracted by Aguilar-Hawod *et al.* (2020) after growing in potato dextrose broth medium for 5 to 7 days of incubation and grinding the mycelial mat using liquid nitrogen. Then CTAB buffer was used for extraction followed by treatment with isoamyl alcohol and 70 per cent ethanol.

Polymerase chain reaction

The conventional methods for identifying Foc is difficult and time consuming, particularly when symptoms are confusing with that of bacterial wilt. Nowadays, a variety of polymerase chain reaction (PCR) based methods have been developed for identification and early detection of fungal pathogens. These advanced techniques could provide more rapid, accurate and reliable detection of the pathogen in the early stage of infection.

Bruns *et al.* (1991) stated that the internal transcribed spacer region of rDNA is highly variable among organisms and so that it can be used for analysing the relationship between organisms at the species level.

Intra-specific variation within internal transcribed spacer regions (ITS1 and ITS2) of the fungus *F. sambucinum* (*Gibberella pulicaris*) was detected by PCR and rDNA sequencing of 86 strains collected from various geographical locations was carried out (O'Donnell, 1992).

Identification of *F. oxysporum* can be done using ITS primers, even though the difference between species is less at the ITS region of rDNA (Edel *et al*., 1995).

Prashant *et al*. (2003) reported the identification of *F. oxysporum* at the molecular level using PCR based methods.

Abd-Elsalam *et al.* (2003) designed two specific ITS primers *viz.*, ITS-Fu-f and ITS-Fu-r for the identification of *Fusarium* genus. The primers performed in a better

way for the genus Fusarium and the amplification was done at a product size of approximately 389 bp. The quantity of PCR product varied from 100 fg to 10 ng for DNA isolated from *F. oxysporum.*

Molecular characterization of *F. oxysporum* using species-specific primers was done by Mishra *et al.* (2003). It gives the better validation at the species level of organisms even by PCR techniques. They also developed a PCR based technique for the rapid confirmation of *F. oxysporum* by amplification of rDNA region using ITS primers

Leong *et al*. (2009) collected thirteen Foc isolates from Malaysia and two isolates from Indonesia and carried out the molecular characterization. They extracted fungal DNA using DNeasy Mini Plant Kit and the amplification was done with ITS F1 and ITS R4 primer pair. Reaction mixture (25 µl) consisted of 1X PCR buffer, 2.5 mM MgCl2, 0.6 mM of each dNTPs, 0.25 µM of each primer, 1.25 U Taq polymerase and 4 ng genomic DNA. For all FOC isolates, DNA fragments were sized approximately 550 bp and were closely related regardless of cultivars.

Visentin *et al*. (2009) studied the variation between morphologically similar pathogens *viz*., *Fusarium verticillioides* (*Fv*) and *Fusarium proliferatum* (*Fp*) in maize at the species level. The ITS region sequencing method was employed to distinguish between the *Fv* and *Fp* and to get taxonomic information.

Molecular characterization and identification of *Fusarium* spp. isolates *viz., F. proliferatum* and *F. solani* associated with yellowing disease of black pepper was carried out by Shahnazi *et al*. (2012) by sequencing of 5.8S ribosomal DNA using ITS1 and ITS 2 primers. Sequence analysis of nucleotides revealed the identification of isolates at the species level.

Molecular characterization of nine Foc isolates isolated from infected root samples of banana collected from Maharashtra region was performed by Ingle and Ingle (2013). Extraction buffer consisting of 1 per cent CTAB (Cetyl trimethylammonium bromide), 0.7 M NaCl (sodium chloride), 25 mM EDTA (Ethylenediaminetetraacetic

acid), 50 mM Tris Hcl and 1 per cent ß-mercaptoethanol was used for the extraction of fungal DNA. Amplification of DNA templates was carried out using ITS primers which yielded amplicons of size 600 bp for all of the isolates except for one sample.

Molecular characterization of 12 isolates of *Fusarium* was carried out to identify the isolates at species level through ITS - rDNA sequence analysis using universal primers ITS 1 and ITS 4 (Sreeja, 2014). Size of the PCR amplification product obtained was of 500-530 bp. BLAST analysis of aligned nucleotide sequences showed an identity of 99-100 per cent between the isolates.

Singha *et al.* (2016) conducted ITS sequencing of the conserved rDNA using ITS 1 and ITS 4 primers for the identification and characterization of *Fusarium* spp. the causal agent of wilt in tomato collected from various locations of Assam. Further amplification was done by random amplified polymorphic DNA (RAPD) using 40 primers.

Molecular characterization of fusarium wilt infected sampls collected from different parts of Kerala was carried out using ITS 1F and IS 4R primers and produced single band of size 580 bp (Louis *et al*., 2018). A phylogenetic tree was construced using Maximum likelihood method and all the isolates showed sequence homology to *F. oxysporum* f. sp. *cubense*.

Molecular characterization of 48 isolates of *F. oxysporum* f. sp. *ciceris*, the causal agent of wilt in chickpea collected from various parts of Maharashtra was done using ITS primers by Jadhav *et al.* (2019) and obtained an amplicon size of 302 bp.

Advanced detection and identification methods could provide a more rapid, accurate and reliable diagnosis of plant diseases in their early stages, which can, in turn, make it possible to reduce the impact of disease outbreaks.

Sequencing and phylogenetic tree analysis of isolates

Sequencing and UPGMA cluster phylogenetic analysis of Foc isolates based on RAPD-PCR banding pattern indicated the presence of genetic variability among the

various isolates collected from different cultivars of infected banana (Bentely *et al.,* 1995). The cluster analysis separated all isolates into two major groups based on the variability.

Hirano and Arie (2009) isolated DNA of various *F. oxyspourm* isolates causing wilt in vegetable crops and sequencing of two endopolygalacturonase genes (*viz., pg1 and pg5*) and exopolygalacturonase genes (*viz., pgx1 and pgx4*) responsible for cell wall degradation was carried out to study the range of nucleotide dissimilarity. Phylogenetic tree analysis was done by neighbour-joining (NJ) method with 1000 bootstraps showed the evolutionary relationship among these four genes.

A study was conducted by Fourie *et al.* (2009) to observe the relationship pattern of 70 Foc isolates from banana through sequencing and phylogenetic tree analysis of the mitochondrial genome. The isolates were categorized into two clades and then to eight lineages based on their coevolution.

Genetic variability of 98 Foc isolates from banana through phylogenetic tree analysis based on inter simple sequence repeats (ISSR) analysis revealed the polyphyletic nature of Foc isolates and its diversity among them (Thangavelu *et al*., 2012).

Karangwa *et al.* (2018) studied the genetic diversity of Foc in banana collected from various parts of East and Central Africa by phylogenetic tree analysis using maximum likelihood method with a bootstrap value of 1000.

Phylogenetic analysis of Foc collected from various banana growing regions of Indonesia was done employing the maximum likelihood method by Maryani *et al*. (2019).

IDENTIFICATION OF RACE

Stover and Waite (1960) described that race 1 of Foc causes infection in AAA triploids of *M. acuminata* especially in Gros Michel whereas, race 2 has been reported

from ABB triploids of *M. acuminata* x *M. balbisiana viz*., Bluggoe, Chato, Rulo and Chamaluco.

Waite (1963) reported that numerous types of *Heliconia* plants were infected with wilt pathogen *F. oxysporum* in Central and South America in the mid-1900s. He termed the causal agents as race 3 of Foc. He also reported that race 1, 2 and 4 affects Gros Michael, Bluggoe and Cavendish respectively.

According to Ploetz and Pegg (2000), banana cultivars affected by race 1 are Maqueno, Silk, Pome and Pisang Awak; by race 2 are some tetraploids and enset; by race 4 are plantains, cavendish, cooking cultivars and all the cultivars infected by race 1 and 2.

Based on host specificity to banana cultivars, Foc strains have been classified into race 1, 2 and 4. Race 1 and 2 are reported from banana genotypes other than Cavendish types (Thangavelu and Mustaffa, 2010a). Race 4 is specific to Cavendish cultivars. In India, Fusarium wilt is reported from race 1 and 2 susceptible varieties of banana. Tropical race 4 is considered more aggressive than other known races of the pathogen (Jones, 2018).

Loop mediated isothermal amplification assay (LAMP)

Assessing morphological and cultural characters of *Fusarium* are often difficult and time consuming. Loop mediated isothermal amplification assay (LAMP) is a different amplification method which is very sensitive, requires less time and labour compared to conventional PCR based approaches (Kaneko *et al*., 2007; Tomita *et al*., 2008). LAMP technology comprises of four to six primers *viz*., forward internal, backward internal, forward external, backward external, forward and backward loop primers and brings out DNA synthesis using a DNA polymerase enzyme through high strand displacement activity.

A novel technique namely loop mediated isothermal amplification (LAMP) of DNA has been developed by Notomi *et al*. (2000). This technique uses four to six

primers that recognize six to eight regions of the target DNA and provides high specificity with *Bst* DNA polymerase enzyme.

Nowadays, several appreciated kinds of research for the detection of *Fusarium* sp. carried out by the LAMP assay are available (Niessen and Vogel, 2010; Abd-Elsalam *et al.*, 2011; Denschlag *et al*., 2012). Many investigations on recognition of bacterial, fungal and viral plant pathogens have been going on (Dai *et al*., 2012; Ravindran *et al.*, 2012).

Peng *et al.* (2012) developed a one-step reverse transcription LAMP assay for the easy and rapid recognition of *Cucumber mosaic virus* in banana and plantain (*Musa* spp.) at 63 °C for 90 min with a fluorescent dye for visual detection.

LAMP method has been extensively used for the recognition of several significant infections in humans, animals as well as plants (Mori *et al*., 2013). Therefore, it can be used as an effective method for the recognition of fungal pathogens infecting plants.

Zhang *et al.* (2013) used a real time fluorescence LAMP technology for the rapid and quantitative detection of Foc tropical race 4 isolates from the soil causing Fusarium wilt in banana. Primers were designed based on the IGS region of nuclear ribosomal operon using the software PrimerExplorer V4.

Benjin *et al*. (2013) performed a LAMP reaction for the sensitive, quick and specific field detection Foc race 4 strain from infected banana plant tissue using primers designed based on the sequence of specific SCAR markers with PrimerExplorer V4 software. Primers consisted of F3, B3, FIP, BIP, LF and LB and the reaction was carried out employing a Loopamp DNA amplification kit provided by Eiken Chemicals Co. Ltd, Japan.

Almasi *et al*. (2013) developed a reliable and rapid pathogen detection protocol for detection of *F*. *oxysporum* f. sp. *lycopersici* and revealed that even though both PCR and LAMP assays could successfully detect positive infected samples, considering the time, safety, cost and simplicity, the latter was superior in performance.

Quick detection of Foc race 4 of banana by LAMP assay was developed by Li *et al.* (2013b) based on the SCAR marker sequence of accession number EF155535. The primers were designed by PrimerExplorer V4 software and reaction was set up at 60-65 °C on a water bath.

Peng *et al.* (2014) designed LAMP primers based on RAPD marker sequences and used real time fluorescence LAMP for the rapid detection of Foc race 4 from the soil causing wilt in banana.

Ghosh *et al*. (2015) developed LAMP assay targeting the *gene elongation factor 1 alpha* for the visual detection of *F. oxysporum* f. sp. *ciceris* causing Fusarium wilt in chickpea. They conducted amplification at 63 °C for 60 min.

LAMP assay for the rapid detection of root wilt disease of coconut and yellowing of arecanut caused by phytoplasma was developed by Nair *et al.* (2016). Primers were designed using the PrimerExplorer V4 software based on the 16S rDNA sequence of accession number JX 273772. The reaction was run with template DNA, regular and loop primers, betaine, $MgSO_4$, *Bst* DNA polymerase and thermopol buffer at 65 °C for 30 min. The reaction was detected visually using hydroxynaphthol blue (HNB) dye and by gel electrophoresis. Positive samples turned from violet to blue colour in HNB dye detection and showed a ladder-like pattern in gel electrophoresis.

Colourimetric detection of *F. oxysporum* f. sp. *melonis* by LAMP assay employing primers designed based on translation elongation factor 1-alpha gene was developed by Almasi (2019). Hydroxynaphthol blue (HNB) dye was used for the visual detection of positive and negative samples. Negative samples remained violet/purple colour whereas positive samples turned to blue colour in the reaction. Positive samples showed ladder-like bands in gel electrophoresis whereas negative samples didn't show any bands.

LAMP assay for the detection of Foc tropical race 4 was developed by Ordonez *et al*. (2019). The primers were designed using PrimerExplorer V4 software. The reaction was set at a temperature of 65 °C for 30 min and terminated at 80 °C.

EVALUATION OF HOST PLANT DISEASE RESISTANCE

Screening for host disease resistance

Rekha *et al*. (1996) conducted screening of 189 banana germplasm against the Fusarium wilt and revealed that the disease incidence varied irrespective of the genomic groups.

Resistance screening of 102 germplasms of *Cicer bijugum*, *C. echinospermum, C. judaicum, C. pinnatifidum* and *C. reticulatum* were carried out in greenhouse by Infantino *et al.* (1996) against *F. oxysporum* f. sp. *ciceri* causing Fusarium wilt. All accessions of *C. bijugum* and some of the other species showed high resistance towards wilt disease.

Singh *et al.* (1998) evaluated 30 chilli germplasm lines against *F. oxysporum* in Himachal Pradesh. Out of 30 lines tested, none of the lines were found to be highly resistant. Nine accessions were moderately resistant and 21 were susceptible or highly susceptible.

Madhukar (2001) screened 30 chilli accessions for resistance to chilli Fusarium wilt disease caused by *F. solani* in pot culture. Among the lines evaluated, 17 were susceptible and 11 were highly susceptible. None of the accessions were found to be resistant except 2 which exhibited moderately resistance reaction.

A study was conducted by Chaudhry *et al.* (2006) for the resistance screening of 414 germplasm lines to Fusarium wilt in chickpea. Among these, 35 accessions were found to be resistant, 208 were intermediate resistant, 77 were susceptible and 94 were highly susceptible.

Germplasm screening of 146 pigeon pea accessions from 53 countries were tested to identify the resistance sources towards Fusarium wilt (root dip technique) and sterility mosaic disease (leaf stapling technique) (Sharma *et al*., 2012). Six accessions were resistant to wilt and 24 accessions were highly resistant to mosaic disease with <10 per cent incidence.

Benzohra-Belaidi (2016) screened 13 chickpea germplasm accessions for resistance against *F. oxysporum* f. sp. *ciceris* (race1 and race 2) the causal agent of Fusarium wilt on chickpea. Results revealed that 3 accessions (Flip4107, Kadri and Flip97-555) showed resistance and other 10 were susceptible to the pathogen.

Zuo *et al*. (2018) screened 109 banana accessions in greenhouse and 100 accessions in the field level for resistance against Fusarium wilt and classified the banana germplasm into various categoaries *viz*., immune, highly resistant, resistant, moderately resistant, susceptible and highly susceptible based on the degrees of resistance.

Biochemical basis of disease resistance

Horsfall and Cowling (1980) stated that the disease resistance and pathogen virulence are the collective actions of various biochemical mechanisms. Host plant resistance is not only based on anatomical and cytological features but also biochemical components of the plants (Bell, 1981).

Kumar *et al*. (2009) analysed the biochemical characters of Foc isolates from India and stated that the biochemical studies were efficient and effective in determining various Foc isolates.

Role of phenolic compounds in disease resistance

Several studies have been reported that the production of phenolic compounds in plants during the infection of pathogens. Most of them are harmful to certain pathogens such as fungi, bacteria, virus, etc (Kosuge, 1969).

Resistant, moderately resistant and susceptible varieties of *Brassica* sp. with downy mildew pathogen *Peronospora parasitica* at 3 growth stages (30 days, 60 days and 90 days after sowing) was examined by Singh (2000). As per the results obtained, the level of phenol content was higher in resistant and moderately resistant varieties compared to susceptible varieties at all growth stages. A similar study was conducted

by Ramanathan *et al*. (2000), which revealed that the accumulation of phenolics showed resistance to Fusarium wilt in tomato.

Phenolic content in resistant and susceptible wheat varieties inoculated with Karnal bunt pathogen *Neovassia indica* was observed at regular intervals. The results obtained revealed that the phenol content was found to be highest at 2 days after inoculation in susceptible bread wheats. Whereas in resistant durum varieties, the phenol content was increased up to 10 days after inoculation and then decreased (Gogoi *et al.,* 2001).

Singh *et al*. (2003) analysed the biochemical basis of disease resistance in chickpea against Fusarium wilt. They examined the activity of total phenols in callus of infected and resistant plants. The results obtained revealed that the phenolic content was higher in resistant lines and lower in susceptible genotypes.

A challenge inoculation of *F. oxysporum* f. sp. *ciceri* in chickpea was conducted by Arfaoui *et al*. (2005) to analyse the pattern of change in phenol content induced by *Rhizobium* isolates at 24 h interval. Results of the study revealed that the highest level of phenolic content was noticed in the resistant varieties, 72 h after the inoculation with chickpea wilt pathogen.

Analysis of biochemical response in rapeseed-mustard genotypes infected with *Alternaria brassicae* revealed that the quantity of total phenols and flavanols was higher in resistant genotypes compared to susceptible plants (Mathpal *et al*., 2011).

Mohd *et al*. (2012) studied the biochemical basis of host plant resistance in tomato plants inoculated with *F. oxysporum* f. sp. *lycopersici* causing wilt disease. In this study, *Trichoderma harzianum*, *T. viride*, *Aspergillus niger*, *Chaetosphaeridium globosum* and *Pseudomonas flourescens* were used to induce plant defense. Total phenol content was recorded maximum in plants treated with *T. harzianum*. Also, phenol content showed an increasing trend in resistant and tolerant plants than disease affected plants.

An investigation was carried out by Li *et al.* (2017) to study the role of phenolic compounds in banana against Fusarium wilt disease. They extracted total phenol content using the Folin-Ciocalteu method. The expression level of phenols was found to be more in resistant varieties than in susceptible ones.

Changes in phenol content of susceptible and resistant varieties of black pepper inoculated with *Phytophthora capsici* was observed by Vandana *et al.* (2014) at 24 h interval for ten days. The amount of total phenol content was found to be more in resistant variety 04-P24 than susceptible variety Sreekara and has a significant role in disease resistance.

Role of sugars in disease resistance

Sugars act as the primary source of energy in plants. It affords the components for defense related activities in plants and controls the plant immune system by activating the reactive oxygen species (Morkunas and Ratajczak, 2014).

Sugars are needed for all regulatory functions and play an important role in plant defense against the diseases caused by fungi and oomycetes (Stokes *et al*., 2013).

Jayapal and Mahadevan (1968) reported that artificial inoculation of leafspot pathogens such as *Cercospora musae* in banana plants caused a reduction in the level of reducing sugars.

A study conducted by Easwaran (1972) in sorghum infected with bacterial disease revealed that the level of reducing sugar was maximum in susceptible and moderately susceptible cultivars compared to the resistant ones and also inoculation caused a significant reduction in existing sugar content.

An experiment was performed by Veeramohan *et al.* (1994) on inoculation of chilli leaves with *Alternaria solani* to study the variation in the amount of sugar content due to disease infection. According to them, the quantity of reducing, non-reducing and total sugars reduced after infection with the pathogen.

Tauzin and Giardina (2014) stated that pathogen infection causes the change of available sugars in plants for colonization by pathogen. This change in sugars activates the defense response in many plants. They also reported that the sucrose hydrolysing enzymes undergo modification especially invertases.

Role of defense enzymes in disease resistance

Arfaoui *et al*. (2005) studied the role of *Rhizobium* isolates in the induction of defense related enzymes inoculated with wilt pathogen *F. oxysporum* f. sp. *ciceri* in chickpea. The results revealed that the level of peroxidases and polyphenol oxidases were found to be extreme at 24 h and 72 h after inoculation respectively.

As per Vanitha *et al.* (2009), the activity of defense related enzymes such as phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) in imparting resistance against bacterial wilt pathogen *Ralstonia solanacearum* in tomato plants was found to be maximum at 12 h and 15 h of induction respectively. Total phenol content was also compared in susceptible and resistant varieties and it was significantly higher in resistant ones.

According to the study conducted by Anand *et al*. (2009), the activity of peroxidase (PO), phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO) and catalase was more in chilli fruits inoculated with *Colletotrichum capsici* and *Alternaria alternata* rather than in healthy chillies. Activity of these phenols and enzymes was found to be maximum at 2-3 days after inoculation and then gradually declined.

According to Ardebili *et al.* (2011), inoculation of *P. fluorescens* induced the accumulation of defense enzymes, mainly peroxidase (POX), polyphenol oxidase (PPO), superoxide dismutase (SOD) and phenylalanine ammonia lyase (PAL) in tomato plants infected with *F. oxysporum* f. sp. *lycopersici* causing Fusarium wilt disease.

Kumar *et al.* (2017) conducted a study on defense related enzymes associated with infection of *Dickeya zeae* causing bacterial stalk rot in maize. They analysed the activities of three antioxidant enzymes, *viz.*, phenylalanine ammonia lyase (PAL),

peroxidase (PO) and polyphenol oxidase (PPO) in eighty maize lines belonging to three categories *viz.*, moderately resistant, moderately susceptible and highly susceptible. According to them, the activity of these enzymes was significantly more in moderately resistant lines than highly susceptible lines. Maximum activity of PAL and PPO was at 48 h and PO was at 72 h of challenge inoculation.

Biochemical basis of host resistance concerning defense related enzymes such as PO, PAL and PPO in banana against Fusarium wilt was studied by Nagar *et al*. (2016). The activity of these enzymes was higher in resistant hybrids compared to tolerant hybrids.

Activity of defense enzymes such as peroxidase (PO), superoxide dismutase (SOD), catalase, ascorbate peroxidase (APX), β-1,3 glucanase etc in castor inoculated with *F. oxysporum* f. sp. *ricini* was studied by Bharathi *et al*. (2019) and the results revealed that all the enzymes except APX were higher in resistant varieties.

HISTOPATHOLOGY

Carnation roots infected with the fungus Fusarium wilt pathogen *F. dianthi* showed distortion in cortical cells as well as endodermis which led to the exposure of vascular bundles (Bickerton, 1942).

Chambers and Corden (1963) reported that vascular vessels of tomato roots infected with Fusarium wilt exhibited micro and macroconidia abundantly. Fungal mycelium was also present in the roots of infected plants.

The anatomy of carnation plants infected with *F. oxysporum* f. sp. *dianthi* was studied by Pennypacker and Nelson (1972) and found that the fungus resides only in vessel cells and spreads through vessel cavities to adjacent cells. Vascular cells of infected plants were distorted and reduced in size compared to the healthy cells. Hypertrophy and hyperplasia were noticed in xylem and parenchyma cells of infected plants.

A study on the histopathology of banana infected with Foc revealed that hypertrophy and hyperplasia were present in xylem and phloem of roots and rhizome (Vishwanath *et al*., 2011). Entry of pathogen into vascular vessels caused fructification in roots and severe necrosis of cortex which caused the gap formation between cortical and stellar region.

Histopathological characters of passion fruit infected with *F. oxysporum* and *F. solani* were studied by Ortiz *et al.* (2014) and the results revealed that infected xylem vessels were colonized by hyphae and microconidia of the pathogen which led to the degradation of xylem vessels compared to healthy plants. Whereas, the infected phloem cells were found to be disrupted and was associated with hypertrophy and hyperplasia.

Microscopic studies of grapevine infected with *Phakopsora euvitis* showed that the pathogen formed appressoria on epidermal cells and stomata. In infected plants, chloroplasts with plastoglobules and mesophyll cells with callose were noticed (Rasera *et al*., 2019).

Histopathological parameters of castor infected with *F. oxysporum* f. sp. *ricini* was studied employing an electron microscope and the results showed brown discolouration in xylem vessels of vascular bundles (Bharathi *et al*., 2019).

MANAGEMENT OF FUSARIUM WILT OF BANANA

In vitro studies for the evaluation of fungicides and bioagents/botanicals

Evaluation of chemical fungicides

An experiment was carried out by Nel *et al.* (2007) to evaluate the efficacy of few fungicides against Foc and the results revealed that prochloraz and propiconazole inhibited the mycelial growth at 1 mg/ml as well as 5 mg/ ml concentrations.

According to Nikam *et al.* (2007), thiram + carbendazim showed 90 per cent inhibition in colony diameter of *F. oxysporum* f. sp. *ciceri* when evaluated *in vitro* along with other chemical treatments like thiram, captan, carbendazim and thiram + captan for the management of wilt in chickpea.

Carboxin and carbendazim are highly effective fungicides against *F. oxysporum* and *Rhizoctonia solani* under *in vitro* conditions and showed 100 per cent inhibition over control at 100 ppm and 200 ppm (Soma *et al*., 2008).

Vinit *et al.* (2010) revealed that carboxin and carbendazim controlled growth of *F. oxysporum* f. sp. *lentis* causing wilt in lentil in the *in vitro* conditions.

Amini and Sidovich (2010) evaluated chemical fungicides such as benomyl, carbendazim, prochloraz, fludioxonil, bromuconazole and azoxystrobin for their efficacy against *F. oxysporum* f. sp. *lycopersici* causing wilt in tomato *in vitro* and found that prochloraz and bromuconazole were the most operative fungicides against the pathogen followed by benomyl and carbendazim. Similarly, Sharma *et al.* (2010) reported that the fungicides such as tebuconazole and carbendazim were found to be highly effective against the *F. oxysporum* f. sp. *lycopersici* in tomato.

As per the results revealed by Ram and Pandey (2011), carbendazim (500 µg ml^{-1}), difenoconazole (100 µl ml^{-1}), hexaconazole (200 µl ml^{-1}) and combination product of captan+hexaconazole (250 pg ml^{-1}) and carbendazim+mancozeb (500 µg ml^{-1}) completely controlled the mycelial growth of *F. oxysporum* f. sp. *udum* causing wilt in pigeon pea.

Fungicides such as carbendazim, hexaconazole, bitertanol, myclobutanil, captan, mancozeb and zineb were tested against *F. solani* causing rot in potato *in vitro*. Among these, carbendazim showed the highest potential against the pathogen (Wani and Mir, 2011).

Efficacy of carbendazim, hexaconazole, bitertanol, myclobutanil, mancozeb, captan and zineb was evaluated against the mycelial growth and spore germination of *F. oxysporum*. Hexaconazole showed maximum inhibition of mycelial growth and spore germination at 1000 ppm followed by other chemicals (Nisa *et al*., 2011).

Chennakesavulu *et al*. (2013) conducted an experiment to manage the Fusarium wilt in red gram caused by *Fusarium udum* using different fungicides in combination

with *P. fluorescens* and reported that the fungicide tebconazole gave 100 per cent inhibition of the pathogen.

Efficacy evaluation of chemical fungicides for the management of *F. oxysporum* causing Fusarium wilt in cowpea revealed that tebuconazole (0.1 %), carboxin+thiram (0.4 %) and mancozeb (0.25 %) suppressed the mycelial growth *in vitro* (Sreeja, 2014).

An *in vitro* experiment was conducted by Somu *et al*. (2014) for the evaluation of six fungicides *viz*., carbendazim, carboxin, propiconazole, difenoconazole, azoxystrobin and benomyl at various concentrations against Foc. They reported that all fungicides except azoxystrobin showed total inhibition of the fungus *in vitro*.

Evaluation of bioagents/botanicals

Biological management of diseases trusts mainly on the disruption of the relationship between host and pathogens using biological methods. It suggests the management of diseases through active microbes under their natural or artificial conditions (Garrett, 1965).

Jager *et al.* (1991) reported that *T. harzianum* was used for the management of wilt diseases in several plants very effectively. It helped in decreasing the severity of wilt disease up to 8.9 per cent when applied in the soil.

Biocontrol agents are potential, environment-friendly tools against several plant pathogens. It includes various parasites, non-pathogenic microorganisms and other natural enemies (Larkin and Fravel, 1998; Pal and Gardener, 2006).

Etebarian (2006) stated that *Trichoderma* sp. are widely used as the biocontrol agent against several diseases in plants spread through the soil as well as seeds. These biocontrol agents belong to the group of filamentous fungi that grow saprophytically (Mohamed and Haggag, 2006). It produces numerous conidia for the survival of long years. Biological control is an effective eco-friendly method for the control of soil-borne pathogens, such as *Fusarium* spp. (Gohel *et al.*, 2007).

Thangavelu and Mustaffa (2010b) studied the effect of 110 isolates of *T. viridae* isolated from the rhizosphere of banana and found that thirty-one isolates were effective in the initial screening tests conducted *in vitro* against banana wilt pathogen Foc.

Wani *et al* (2014) conducted an extensive survey in Kashmir valley and collected 10 *Trichoderma* isolates (Tr1, Tr2, Tr3, Tr4, Tr6, Tr8, Tr10, Tr11, Tr12, Tr16) from the rhizosphere of healthy chilli plants. All the isolates inhibited the growth of *F*. solani, but Tr16 showed the maximum inhibition of 77 per cent in dual culture technique whereas Tr8 showed maximum inhibition (89 %) in volatile tests.

Several studies have been dealt with biological control of Fusarium wilt in banana worldwide. Some of the tested biocontrol agents are used for the management of this disease and proved their efficiency (Bubici *et al.*, 2019).

Different strains of *T. harzianum* were used to evaluate the effect on Foc under *in vitro* conditions using dual culture method and volatile organic compounds production test (Napitupulu *et al*., 2019). They reported that all the strains inhibited Foc growth at various level and produced toxic metabolites.

Kinge *et al*. (2019) used four types of botanicals from *Azadiracta indica* leaves, *Moringa oleifera* oil, *Withania somnifera* roots and *Tithonia diversifolia* leaves to control the fungal isolates of *F. oxysporum f. sp. elaeidis* causing vascular wilt in oil palm collected from various regions of Cameroon and found that leaf extracts spray of *A. indica and T. diversifolia* could manage the fungal growth compared to the others.

Management of Fusarium wilt in field condition

The pathogen, Foc is soil born and survives in the soil as chlamydospores for more than thirty years. Hence sustainable approach utilizing genetic resistance, new generation fungicides and eco-friendly bioagents needs to be formulated.

Chemical control

Soil drenching of carbendazim fungicide at 0.2 per cent or corm injection of the same at 2 per cent performed better in managing the Fusarium wilt of banana caused by Foc (Thangavelu *et al*., 2001).

Cherian and Menon (2001) suggested an integrated management package including carbendazim and biocontrol agents for the control of Fusarium wilt disease in banana.

Nel *et al.* (2007) reported that the fungicides coming under the group benzimidazole *viz.,* benomyl, carbendazim and thiabendazole successfully managed the Fusarium wilt of banana under greenhouse constructions.

Field as well as pot culture experiments were conducted by Sreeja (2014) to evaluate the efficacy of new generation fungicides for the management of Fusarium wilt in vegetable cowpea. The results indicated that soil drenching of tebuconazole (0.1 %), flusilazole (0.1 %) and carbendazim (0.1 %) recorded the lowest index of Fusarium wilt caused by *F. oxysporum.*

AICRP (2017) technology for the management of Fusarium wilt of banana includes the use of disease free suckers, application of neem cake at 250 g per plant, sucker dipping in carbendazim, followed by carbendazim drenching and injection.

In an experiment, Nayak (2017) used rhizome dipping in carbendazim, rhizome dipping along with corm injection and rhizome dipping in carboxin followed by corm injection. Among these, the treatment corm dipping and injection with carbendazim along with the application of neem cake and calcium carbonate was the best with zero per cent vascular wilt index followed by carboxin application in combination with neem cake and calcium carbonate.

Biological control

As per Liu *et al*. (1995), plant growth-promoting rhizobacteria (PGPR) like *P. fluorescens* and *Serratia marcescens* induces systemic resistance in cucumber against fusarium wilt caused by *F. oxysporum* f. sp. *cucumerianum*.

Nowadays, biological control of Fusarium wilt diseases using certain microorganisms has been getting more popular as it is an eco-friendly management strategy compared to the indiscriminate use of chemical fungicides (Weller *et al*., 2002; Fravel *et al*., 2003).

Combined application of seed treatment and soil drenching of *T. viride* along with soil application of neem cake and mancozeb effectively controlled the incidence of wilt in cowpea caused by *F. oxysporum* with better yield in Kerala (Senthil, 2003).

According to Saravanan *et al.* (2004), soil application of *P. fluorescens* was the most effective among the various methods *viz.*, corm injection, sucker dipping and capsule application tried for the management of Fusarium wilt of banana in the field experiment.

Dubey *et al.* (2007) isolated *T. harzianum*, *T. viride* and *T. virens* from soil to evaluate the efficacy of *Trichoderma* species against Fusarium wilt pathogen *F. oxysporum* f. sp. *ciceris* in chickpea through dual culture method as well as the production of volatile and non-volatile inhibitors. They revealed that the application of *Trichoderma* inhibited the growth of mycelium to a great extent, boosted seed germination, increased root and shoot length and reduced the wilt incidence in greenhouse cultivation.

Chawla and Gangopadhyay (2009) tested bioagents such as *T. harzianum*, *T. viride*, *P. fluorescens* and *Bacillus subtilis* along with organic amendments against *F. oxysporum* f. sp. *cumini* causing wilt disease in cumin. *T. harzianum* and *P. fluorescens* were found to be significantly better in the presence of farmyard manure or mustard cake.

Ardebili *et al.* (2011) reported that application of *P. fluorescens* considerably stimulated the plant vigour of tomato plants infected with *F. oxysporum* f. sp. *lycopersici*. It inhibited the growth of the pathogen by 50 per cent when carried out the *in vitro* evaluation.

Belgrove *et al*. (2011) evaluated the non-pathogenic *F. oxysporum* endophytes isolated from healthy banana plants for the management of Fusarium wilt of banana and stated that ten isolates could reduce the disease significantly.

Leaf extracts and bio-control agents were tested against Foc by Akila *et al.* (2011). They reported that the leaf extract of Datura (10 %) inhibited the mycelial growth completely *in vitro*. Under field conditions, the combined application of leaf extract along with biocontrol agents like PGPR controlled the wilt incidence significantly.

Biocontrol efficacy of twelve *Trichoderma* isolates was tested against the *F. oxysporum* f. sp. *lycopersici*, the pathogen of Fusarium wilt in tomato by seed and soil application. Some of the isolates were effective in enhancing seed germination up to 92 per cent and controlling the wilt disease up to 80 per cent (Bhagat *et al*., 2013).

Zhang *et al.* (2014) used a bio preparation of *T. harzianum*, *Bacillus* spp. and *Paenibacillus* sp. for the management of Fusarium wilt of banana and concluded that disease incidence was suppressed up to 80 per cent under pot culture experiment.

Ubaud and Requina (2016) reported the effect of Arbuscular Mycorrhizal Fungi (AMF) and *T. harzianum* against Foc on Cavendish banana plantlets. The plantlets treated with 70 g of VAM + 50 g of *T. harzianum* reduced Foc development in terms of disease symptom and disease incidence.

Application of endophytes and rhizobacteria in two banana field conditions controlled Fusarium wilt up to 78 per cent and gave a significantly higher yield (Kavino and Manoranjitham, 2018).

Hesse *et al*. (2018) stated that Pseudomonads consists of more than 100 species and some of them have antagonistic property against Foc. Pseudomonads with these biocontrol properties can reduce the impact of various phytopathogens and enhance the plant growth (Schreiter *et al*., 2018).

Under field conditions, application of *Pseudomonas* spp. and *Trichoderma* spp. managed Fusarium wilt of banana up to 79 per cent and 70 per cent respectively. Efficiency of AMF, *Bacillus* spp., and non-pathogenic Fusarium strains in controlling the disease was found to be up to 42 to 55 per cent (Bubici *et al*., 2019).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Investigation on "Characterization and integrated management of *Fusarium oxysporum* f. sp. *cubense* (E. F. Smith) Snyder and Hansen (Foc) causing Fusarium wilt disease of banana" was conducted in the Department of Plant Pathology, College of Horticulture, Vellanikkara, Thrissur and Banana Research Station, Kannara during 2017-2020. The details of the materials used and the methodologies followed are given in this chapter.

3.1 SURVEY AND COLLECTION OF SAMPLES

3.1.1. Purposive sampling survey

Purposive sampling surveys were conducted in selected banana growing districts of Kerala *viz.,* Thiruvananthapuram, Ernakulam, Thrissur, Palakkad, Kozhikode and Wayanad to assess the incidence of disease and to collect infected samples for further studies. During the survey, symptoms, incidence of the disease, wilt index and meteorological data such as rainfall and temperature were recorded from each field. The per cent disease incidence (PDI) was analysed using the formula given below.

$$\text{Per cent disease incidence (PDI)} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

Per cent disease severity (PDS) of the Fusarium wilt infected banana plants were assessed with the standard score chart using a 0-4 scale given in Table 3.1 (Mak *et al.,* 2004).

Table 3.1. Standard score chart for assessing per cent wilt index of Fusarium wilt (external symptoms) under natural conditions

Score	External symptoms
0	No streaking or yellowing of the plant, the plant appears healthy.
1	Slight streaking and/or yellowing of lower leaves
2	Streaking and/or yellowing of most of the lower leaves
3	Extensive streaking and/or yellowing on most or all of the leaves
4	Dead plant

Disease severity was calculated using the formula described below.

$$\text{Per cent disease severity (PDS)} = \frac{\sum (\text{Class} \times \text{number of plants in that class}) \times 100}{4 \times \text{Total number of assessed plants}}$$

3.1.2. Collection of the samples

Samples were collected from pseudostem and rhizome of the infected banana plants. Vascular strands were separated from the infected pseudostem and placed in between tissue papers (Plate 3.1). These samples were placed in paper bags and labelled properly with the number of the isolate, name of the variety, location of survey and date of collection. These were brought to the laboratory for further studies. Since the plastic bags are known to cause sweating and bacterial infection, the paper bags were used for collection of samples.

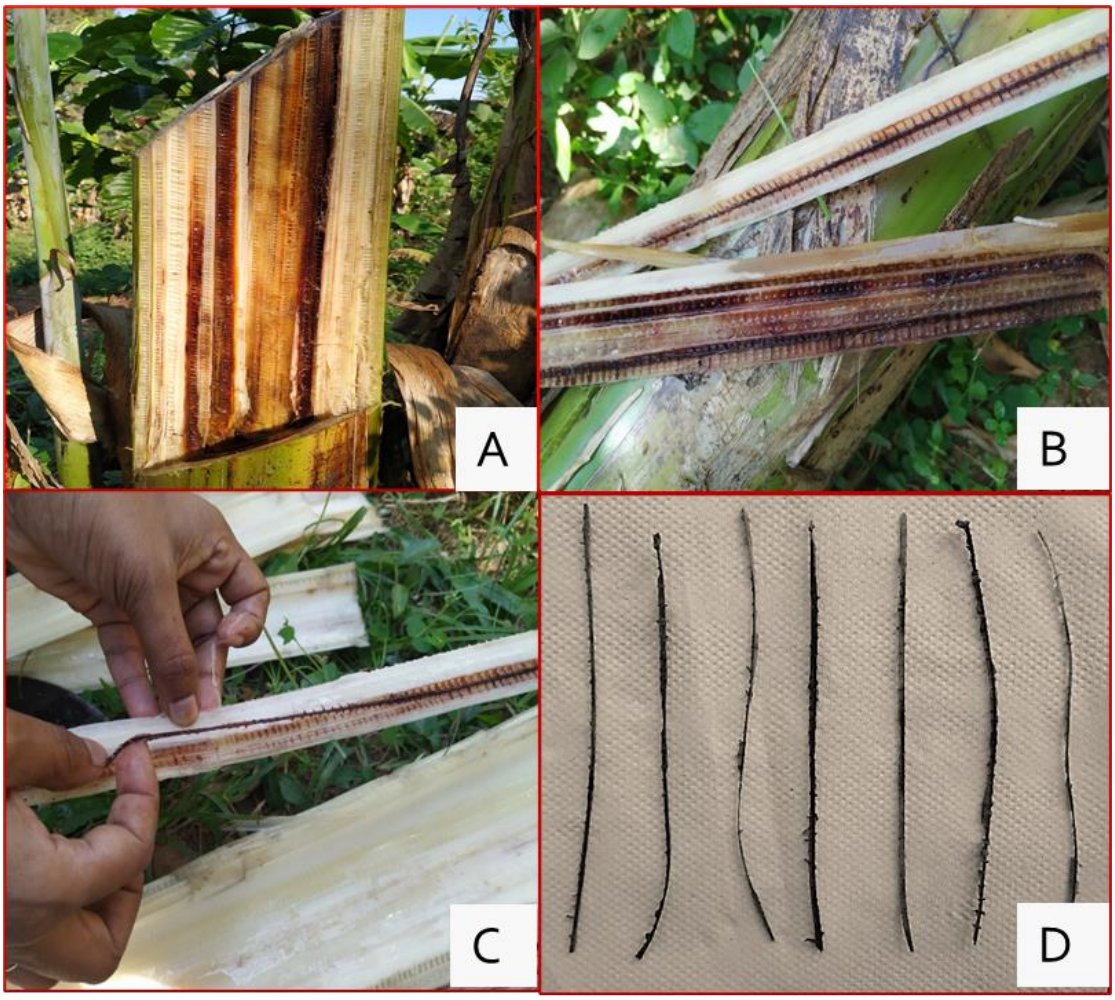


Plate 3.1. Procedure for sample collection from Fusarium wilt suspected banana pseudostem. A and B: Pieces of an infected pseudostem, C: Separating discoloured vascular strands from pseudostem and D: Fragments of necrotic vascular strands taken in tissue paper.

3.2. SYMPTOMATOLOGY

Symptoms exhibited by Fusarium wilt infected banana plants under natural field condition were recorded during the survey and also under artificial conditions by challenge inoculation.

3.2.1. Natural condition

The external and internal symptoms developed in banana plants due to Fusarium wilt under natural condition were recorded during the purposive sampling surveys conducted in farmers' fields.

3.2.2. Artificial condition

The Fusarium wilt symptoms produced under artificial condition was studied by inoculating the pathogen into two months old tissue culture plants of various varieties *viz.*, Rasthali/Poovan, Njalipoovan, Kadali and Chenkadali. The development of symptoms and the number of days taken for shifting external symptom from one score category to another were recorded.

3.3. ISOLATION OF THE PATHOGEN

The pathogen was isolated from discoloured pseudostem strands of infected plants collected from all the locations surveyed. Culture media used for isolation and maintenance of the fungal isolates was half strength potato dextrose agar (PDA) (Appendix I).

3.3.1. Isolation from pseudostem

Isolation of the pathogen was done after the strands kept in between tissue papers become dry. Strands of size 0.5-1.0 cm were sterilized in 70 per cent alcohol and placed in sterile Petri plates with half strength PDA and incubated at 25 °C. Fungus grown from these strands were purified by single hyphal tip method and subcultured. The pure cultures were maintained on PDA slants at 4 °C.

3.4. PATHOGENICITY TEST

The pathogenicity of all fungal isolates collected from infected banana plants during the survey was proved by following the Koch's postulates. Each isolate was inoculated into the same variety from which it was isolated.

3.4.1. Planting materials used

Two months old, hardened disease free tissue culture plantlets were bought from the Department of Plant Biotechnology, College of Horticulture, Kerala Agricultural University. The pathogenicity was tried on different varieties *viz*., Rasthali/Poovan (AAB), Kadali (AA), Chenkadali (AAA) and Njalipoovan (AB).

3.4.2. Preparation of inoculum

Inoculum was prepared separately for the isolates in sterilised half strength potato dextrose broth in 250 ml conical flasks by transferring mycelial disc of 5 mm of five to seven days old culture grown on PDA. This was incubated at 25 °C until the surface of the medium is completely covered by the mycelial mat (approximately five to seven days). The culture was then shaken in Orbitek shaker for 3 days at 150 rpm (Zuo *et al.*, 2018). The concentration of conidial suspension was finally diluted up to $5x10^6$ CFU/ml and used for inoculation.

3.4.3. Preparation of potting mixture

Potting mixture prepared by mixing sand, soil and cow dung in the ratio 1:1:1 was sterilised by fumigating with 40 per cent formaldehyde solution and kept covered with polythene cover for seven days. Then uncovered, turned and kept open for seven days to remove the excess formalin.

3.4.4. Inoculation

For proving the pathogenicity, root dipping along with soil drenching was done for each isolate. Two months old plantlets were uprooted from hardening grow bag and roots were dipped in ground conidial suspension ($5x10^6$ /ml concentration) for 30 min.

100 ml of conidial suspension was used for soil drenching after planting in grow bags containing sterile medium.

The inoculated plants were labelled properly and kept in polyhouse and watered regularly. Regular monitoring was done by observing for any symptom development. Observations on external symptoms were taken for the calculation of per cent wilt index (PWI) using standard score chart (Table 3.2 and Plate 3.2) given by Perez-Vicente *et al*. (2014). After two months of planting, the plants were uprooted and stellar region scoring was done for calculating per cent vascular wilt index (PVWI) as per the standard score chart (Table 3.3. and Plate 3.3) given by Perez-Vicente *et al*. (2014). The time taken for shifting from one score category to other was also recorded.

3.5. CHARACTERISATION AND IDENTIFICATION OF THE PATHOGEN

All isolates of the pathogen isolated from collected samples from various locations were identified by its cultural, morphological and molecular characterisation.

3.5.1. Cultural characterization of isolates

Cultural characters such as colour, texture and rate of mycelial growth, shape of margin, pigmentation and development of spores for all isolates were studied as a part of preliminary identification of the pathogen. This was done by inoculating the fungal disc of size 5 mm on half strength PDA medium plated in sterile Petri plates. Mycelial fragments were taken on a microscopic slide and stained with cotton blue stain and observed under the light microscope (Olympus) with 40X magnification.

3.5.2. Morphological characterization of isolates

The morphological characters *viz*., Septation and size (length and breadth) of macroconidia and microconidia, as well as the diameter of chlamydospores and fruiting body development. Mycelial fragments were taken on a microscopic slide containing cotton blue stain and observed under a Leica Image analyser. The dimensions of hyphae and spores were measured.

Table 3.2. Standard score chart for assessing per cent wilt index (external symptoms) under polyhouse condition

Score	External symptoms
1	No symptoms
2	Initial yellowing mainly in the lower leaves
3	Yellowing of all the lower leaves with some discolouration of younger leaves
4	All leaves with intense yellowing
5	Plant dead

Table 3.3. Standard score chart for assessing per cent vascular wilt index (internal symptoms) under polyhouse condition

Score	Internal symptoms
1	No symptoms
2	Initial rhizome discolouration
3	Slight rhizome discolouration along the whole vascular system
4	Rhizome with most of the internal tissues showing necrosis
5	Rhizome fully necrotic

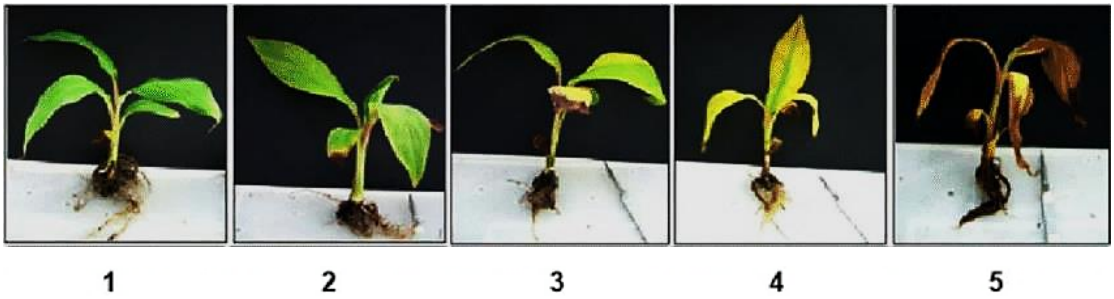


Plate 3.2. Scale for rating external symptoms of Fusarium wilt of tissue culture banana under polyhouse condition. 1: No symptoms; 2: Initial yellowing mainly in the lower leaves; 3: Yellowing of all the lower leaves with some discolouration of younger leaves; 4: All leaves with intense yellowing; 5: Plant dead.

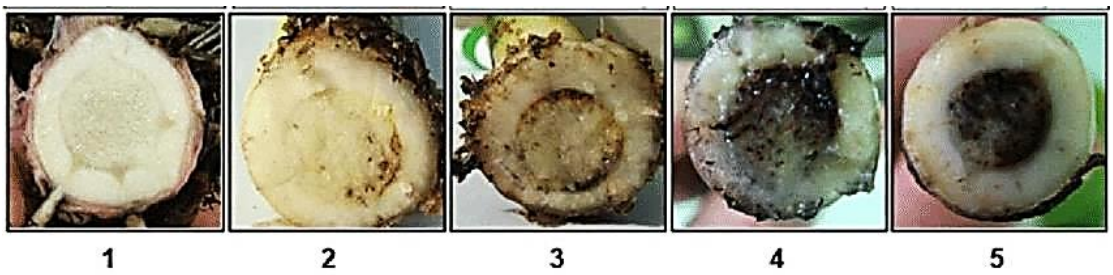


Plate 3.3. Scale for rating internal symptoms of Fusarium wilt of tissue culture banana under polyhouse condition. 1: No symptoms; 2: Initial rhizome discolouration; 3: Slight rhizome discolouration along the whole vascular system; 4: Rhizome with most of the internal tissues showing necrosis; 5: Rhizome fully necrotic.

3.5.3. Cluster analysis of isolates based on cultural and morphological characters

Cluster analysis of all 30 isolates was done using cultural and morphological characters as similarity coefficient by Minitab 19 software. The data was classified into quantitative and qualitative data and the dendrogram was constructed based on these variables. Quantitative parameters included colony diameter, length of macroconidia and microconidia, breadth of macroconidia and microconidia and the diameter of chlamydospores. Qualitative parameters used for cluster analysis were colour of mycelium, the texture of mycelium and pigmentation.

3.5.4. Molecular characterization of isolates

Molecular detection and characterization of all isolates were carried out for further confirmation of the pathogen and diversity analysis. Sequence analysis of the internal transcribed spacer (ITS) region amplified through PCR and the *in silico* analysis were carried out to characterize the pathogen and to study the diversity of the isolates.

3.5.4.1. Isolation of fungal DNA from culture

Genomic DNA of the collected samples was extracted by modifying the CTAB method described by Ingle and Ingle (2013). Isolates identified by cultural and morphological characters were inoculated in 150 ml of half strength potato dextrose broth and incubated for 7 days at 25 °C. 0.1 g of the mycelial mat was air dried and ground in liquid nitrogen using ice-cold pestle and mortar. This homogenised mixture was resuspended in 1 ml extraction buffer consisting of 2 per cent CTAB (Cetyl trimethylammonium bromide), 0.7 M NaCl (sodium chloride), 20 mM EDTA (Ethylene diamine tetraacetic acid), 50 mM Tris HCl and 50 µl of ß-mercaptoethanol. This mixture was incubated in a water bath at 65 °C for one h. After incubation, it was treated with an equal volume of isoamyl alcohol and centrifuged at 10000 rpm for 10 minutes at 4 °C. The supernatant obtained was mixed with an equal volume of ice-cold isopropanol and kept for two h incubation at -20 °C. Again, centrifuged at 13000 rpm for 10 min. at 4 °C. After decanting the supernatant, the DNA pellet was rinsed with 200 µl of 70 per cent ethanol by centrifuging at 8000 rpm for two minutes at 4 °C and

the pellet obtained was air dried. The pellet was then dissolved in 200 µl 1X TE buffer (Tris HCl and EDTA) and added 5 µl of RNase (20 mg/ml) to avoid RNA contamination. The solution was incubated at 37 °C for one h and then centrifuged at 13000 rpm for 15 minutes at 4 °C. The supernatant obtained decanted and the pellet was washed with 200 µl of each 70 per cent and 100 per cent ethanol subsequently with centrifugation at 8000 rpm for two min. at 4 °C. The pellet was air dried, dissolved in 50 µl sterile distilled water and stored at -20 °C in deep freezer for further use.

3.5.4.2. Isolation of fungal DNA from infected plant parts

A simple and rapid method for the isolation of genomic DNA from the rhizome of Foc infected banana was carried out. This method avoids the isolation of the pathogen from plant parts as well as grinding step with liquid nitrogen. 0.1 g of plant tissue was weighed and ground with the extraction buffer containing 2 per cent CTAB (cetyltrimethylammonium bromide), 0.7 M NaCl, 20 mM EDTA (ethylene diamine tetraacetic acid), 50 mM Tris HCl, 50 µl of ß-mercaptoethanol and polyvinylpyrrolidone. This mixture was incubated in a water bath at 65 °C for 30-60 min. After incubation, it was treated with an equal volume of isoamyl alcohol and centrifuged at 10000 rpm for 10 min. at 4 °C. The supernatant obtained was mixed with an equal volume of ice-cold isopropanol and kept for two h incubation at -20 °C. Again, centrifuged at 13000 rpm for 10 min. at 4 °C. After decanting the supernatant, the DNA pellet was rinsed with 200 µl of 70 per cent and 100 per cent ethanol subsequently. The pellet was air dried and dissolved in 50 µl of TE buffer. The DNA isolated was stored as above.

3.5.4.3. Gel electrophoresis for visualising the DNA

The isolated DNA was visualized using agarose gel electrophoresis. Agarose gel (100 ml) of 0.8 per cent was prepared in 1X TAE buffer which provides a source of ions for the electric field during electrophoresis. The gel was allowed to cool to 60 °C and then added 3 µl of ethidium bromide. The solution was poured into a gel casting tray with a comb. Once the solution has solidified, the comb was removed and placed in the electrophoresis chamber filled with 1X TAE buffer. Loading sample was prepared by mixing 6 µl of DNA and 2 µl of 6X DNA loading dye. The samples and

reference ladder were loaded into wells near the negative electrode of gel and electrophoresis was run at 75 V until the dye has reached near the positive end of the gel. The gel was taken out and visualized under BIORAD Molecular Imager (Gel Doc^{TM} XR+) and the image was documented. The composition of reagents used for DNA isolation of all the collected isolates and its agarose gel electrophoresis are given in Appendix II.

3.5.4.4. Assessment of quantity and quality of DNA

Genomic DNA extracted from all fungal isolates collected from various locations was quantified using the instrument NanoDrop 1000 spectrophotometer (ThermoScientific, USA). Calibration of the spectrophotometer was done by loading 2 µl of nuclease-free water as the blank. Afterwards, 2 µl of DNA samples were loaded and the absorbance at 260 nm and 280 nm wavelength was logged. This revealed the concentration of DNA in the sample and λ260/280 ratio indicated the purity of DNA isolated.

3.5.4.5. Polymerase chain reaction using ITS primers

PCR amplification reactions were carried out in a 20 µl reaction mixture using ITS primers. The components and the quantity of reaction mixture used for the PCR amplification is given in Table 3.4.

3.5.4.5.a. Standardization of annealing temperature for PCR using ITS primers

The standardization of annealing temperature for PCR reaction using ITS primers was conducted using a gradient PCR in master cycler (Eppendorf). The range of temperature used for the standardization was from 54.6 to 56.0 ºC is. Temperatures used are presented in Table 3.5. Among these, the temperature at which good amplicons obtained was used for the further amplification of the isolates.

Table 3.4. Composition of reaction mixture used for PCR amplification using ITS primers

Sl. No.	Component	ITS primers (µl)
1	10 X PCR buffer	2.00
2	25 mM $MgCl_2$	1.50
3	0.2 mM dNTP	2.00
4	Forward primer	1.00
5	Reverse primer	1.00
6	Template DNA	2.00
7	Taq DNA polymerase	0.50
8	Distilled water	10.00
Total		20.00

Table 3.5. Range of annealing temperature tested for PCR using ITS primers

Sl. No.	Temperature (ºC)
1.	54.6
2.	54.9
3.	55.1
4.	55.4
5.	55.6
6.	55.8
7.	55.9
8.	56.0

The internal transcribed spacer (ITS) region of rDNA was amplified using the universal primers, ITS 1F and ITS 4R. Reactions were completed in a PCR Master Cycler (Eppendorf).

Details of primer used for amplification using ITS primers (White *et al*., 1990);

Target	**Primer Name**	**Direction**	**Sequence**
Internal transcribed spacer region	ITS-1F	Forward	5'TCCGTAGGTGAACCTGCGG3'
	ITS-4R	Reverse	5'TCCTCCGCTTATTGATATGC3'

PCR amplification profile is given below;

Sl. No.	**Programme**	**Temperature (°C)**	**Time (Min.)**
1	Initial denaturation	94	10:00
2	Denaturation	94	01:00
3	Annealing	*	01:00
4	Extension	72	01:00
No. of cycles: 35			
5	Final extension	72	10:00

*Standardization of annealing temperature was done as described under section 3.5.4.5.a. using gradient PCR and the optimum temperature was taken.

3.5.4.5.b. Analysis of PCR amplicons by agarose gel electrophoresis

Amplicons obtained through PCR were resolved in 1.5 per cent agarose gel prepared in 1X TAE buffer with ethidium bromide. PCR product (10 µl) and 6X DNA loading dye (4 µl) were mixed and loaded into wells along with reference ladder. Electrophoresis was carried out in 1X TAE buffer at 75 V for 1 h and viewed under BIORAD Molecular Imager (Gel Doc^{TM} XR+) and the image was recorded.

3.5.4.6. Sequencing of amplicons

The sequencing of the amplified products of all isolates obtained through PCR using universal primers ITS 1F and ITS 4R was done. The PCR products were sent to AgriGenome Labs Pvt. Ltd., Kochi, Kerala, India and the sequencing was carried out by Sanger dideoxy sequencing method.

3.5.4.7. In silico analysis

Analysis of the nucleotide sequences obtained from AgriGenome Labs Pvt. Ltd. was done by comparing the sequences available in the NCBI (National Centre of Biotechnology Information) database through BLASTn (http://ncbi.nlm.nhm.gov./blast.cgi) tool. Accessions of top hit sequences during analysis were used for the confirmation of isolates and further phylogenetic studies.

3.5.4.8. Deposition of sequences in NCBI

Nucleotide sequences of all Foc isolates confirmed through *In silico* analysis were deposited in NCBI database, USA using NCBI BankIt software and provided the accession number for each isolate.

3.5.4.9. Phylogenetic analysis

The nucleotide sequences obtained by Sanger dideoxy method based on ITS – rDNA region of the Foc isolates collected from various locations were aligned with the top hit accessions recovered from NCBI database using the BLAST algorithm. All the sequences were aligned with ClustalW tool accessible in MEGAX software and the phylogenetic tree was constructed employing Neighbor-joining method with a bootstrap value of 1000 (Saitou and Nei, 1987).

3.6. IDENTIFICATION OF RACES

The isolates of Foc pathogen have been classified into four races based on host specificity. Race identification was done by artificial inoculation to race-specific banana varieties as well as through a molecular technique, loop mediated isothermal amplification (LAMP) assay. The novel technique of LAMP assay was standardized

for the occurrence of race 1 and the primers reported by Li *et al*. (2013b) was used for the identification race 4 if any among the collected samples.

3.6.1. Artificial inoculation

Foc isolates collected from various locations during the survey were inoculated to banana varieties such as Cavendish, Plantain, Rasthali/Poovan, Monthan and *Heliconia* sp. raised in polybags for the identification of races. Race 1 infects Rasthali whereas, race 2 affects Monthan and race 3 infects *Heliconia* sp., a close relative of the banana. Race 4 affects the Cavendish group and the varieties susceptible to race 1 and 2 in subtropics. The inoculum was prepared from each isolate in half strength potato dextrose broth and soil drenching was done. The plantlets were inoculated for the development of any symptoms to identify the race of the pathogen.

3.6.2. Loop mediated isothermal amplification assay (LAMP) for the quick detection of Foc

DNA samples from the collected isolates (DNA isolation method as given in 3.5.4.1) of Foc were subjected to LAMP assay using the designed primers for race 1 as well as using reported primers for Race 4. This method was standardized as it requires only 1 h for amplification with a simple water bath and it can be used for the identification of pathogenic races with the specific primers.

3.6.2.1. Designing of primers

Primers for detection of Foc Race 1 by LAMP assay were designed based on *endoglucanase 4* gene sequence (Accession number: KB730431.1) of Foc race 1 obtained from NCBI database using PrimerExplorer V5 software (Eiken Chemical Co. Ltd., Tokyo, Japan) (http://primerexplorer.jp/e/). Whereas, the primers for detection of Foc race 4 were selected as per Li *et al*. (2013b). The primers include forward outer primer (F3), backward outer primer (B3), forward inner primer (FIP), backward inner primer (BIP), loop forward primer (LF) and loop reverse primer (LB). The primers synthesized from M/s Sigma-Aldrich Chemicals Pvt. Ltd. Bangalore were obtained in the lyophilized form. The primers were subjected to spinning and a stock solution of 100 p*M*/µl was made as per the instructions given by the manufacturer and was kept at

-20 ºC. A working solution of 10 p*M*/µl was then prepared from the stock solution by adding nuclease-free water.

3.6.2.2. Standardization of LAMP assay

Standardization of different components such as *Bst* DNA polymerase, betaine, DNA sample, dNTPs and $MgSO_4$ required for LAMP assay was done as the first step. The total reaction volume (25 µl) included 50 ng template DNA, 10 pM / µl of each primers F3, B3, FIP, BIP, LF and LB, 2.5 mM dNTPs, 25 mM MgSO4, 5M betaine (Sigma-Aldrich), 8000 U/ml *Bst* DNA polymerase large fragment (New England BioLabs), 10X thermopol reaction buffer and nuclease-free water (Sisco Research Laboratories Pvt. Ltd.) (Table 3.6). The entire isothermal reaction was set up in a water bath and the reaction mixture was incubated at 60.0, 61.0, 62.0, 63.0, 64.0 and 65.0 °C for 30, 45 and 60 min. to standardise the temperature and time. The reaction was terminated by inactivating the enzyme *via* incubating the mixture at 80 °C for 10 min. A control tube containing only nuclease-free water without the template DNA (no template control - NTC) was also maintained.

3.6.2.3. Colorimetric detection of amplification

The colorimetric detection of amplified products was done by the addition of 1 µl 150 µM of Hydroxynaphthol blue (HNB) dye (Sisco Research Laboratories Pvt. Ltd.) to the reaction mixture prior to amplification. The positive reaction was determined by a colour change from purple to blue whereas, the control remained purple.

3.6.2.4. Agarose gel electrophoresis

Electrophoretic detection of the LAMP products was observed on 2 per cent agarose gel stained with ethidium bromide in 1 per cent TAE buffer. Agarose gel (100 ml) was prepared in 1X TAE buffer by heating. The gel was allowed to cool to 60 °C and then added 3 µl of ethidium bromide. The solution was poured into a gel casting tray with a comb. The comb was removed after the solidification of the mixture and the gel was placed in the electrophoresis chamber filled with 1X TAE buffer. Loading sample was prepared by mixing 10 µl of LAMP product and 2 µl of 6X DNA loading

Table 3.6. Composition of reaction mixture for LAMP assay

Sl. No.	Components	Volume (µl)
1	Forward outer primer (F3) (10 pM / µl)	1.00
2	Backward outer primer (B3) (10 pM / µl)	1.00
3	Forward inner primer (FIP) (10 pM / µl)	1.00
4	Backward inner primer (BIP) (10 pM / µl)	1.00
5	Loop forward primer (LF) (10 pM / µl)	1.00
6	Loop reverse primer (LB) (10 pM / µl)	1.00
7	Thermopol reaction buffer (10X)	2.50
8	dNTPs (2.5 mM)	3.00
9	$MgSO_4$ (25 mM)	2.00
10	Betaine (5 M)	2.50
11	*Bst* DNA Polymerase (8000 U/mL)	1.00
12	Template DNA (50 ng)	2.00
13	Nuclease-free water	6.00
	Total volume	**25.00**

dye. The samples and controls were loaded into the respective wells near the negative electrode of gel and electrophoresis was run at 80 V for 30 min. The gel was taken out and visualized under BIORAD Molecular Imager (Gel Doc^{TM} XR+) and the image was documented.

3.6.3. Polymerase chain reaction using specific primers

PCR amplification reactions were carried out in a 25 µl reaction mixture using specific primers (F3 and B3 primers designed for Race 1 in LAMP assay) The composition of the reaction mixture used for the amplification is given in Table 3.7.

3.6.3.1. Standardization of annealing temperature for PCR using specific primers

The standardization of annealing temperature for PCR reaction was conducted using a gradient PCR in master cycler (Eppendorf). The range of temperature used for the standardization is given in Table 3.8. The temperature at which good amplicons obtained was used for the further amplification of the isolates.

PCR amplification profile is given below;

Sl. No.	**Programme**	**Temperature (°C)**	**Time (Min.)**
1	Initial denaturation	94	05:00
2	Denaturation	94	01:00
3	Annealing	*	01:00
4	Extension	72	01:00
No. of cycles: 35			
5	Final extension	72	10:00

3.6.3.2. Analysis of PCR amplicons by agarose gel electrophoresis

The analysis of PCR amplicons by agarose gel electrophoresis was done as given in 3.5.4.5.b and the image was recorded.

Table 3.7. Composition of reaction mixture used for PCR amplification using specific primers

Sl. No.	Component	Specific primers (µl)
1	10 X PCR buffer	2.50
2	25 mM $MgCl_2$	1.20
3	0.2 mM dNTP	2.00
4	Forward primer	1.00
5	Reverse primer	1.00
6	Template DNA	2.00
7	Taq DNA polymerase	0.50
8	Distilled water	14.8
Total		25.00

Table 3.8. Range of annealing temperature tested for PCR using specific primers

Sl. No.	Temperature (ºC)
1.	52.2
2.	53.4
3.	54.6
4.	55.2
5.	56.4
6.	57.3
7.	58.1

3.7. EVALUATION OF HOST PLANT DISEASE RESISTANCE

Host plant disease resistance was evaluated by screening the germplasm maintained at Banana Research Starion (BRS), Kannara and by estimating the biochemical parametes.

3.7.1. Evaluation of germplasm under natural conditions

Periodic monitoring of germplasm at Banana Research Starion, Kannara was done for recording the expression of any wilting symptoms in banana varieties.

3.7.2. Screening of germplasm accessions

Screening of selected accessions of the gene bank of Banana Research Station Kannara, KAU for assessing the level of disease resistance was done by artificial inoculation under pot culture (Plate 3.4).

3.7.2.1. Planting materials

Banana germplasms were provided by the Banana Research Station Kannara. Twenty-six germplasm collection were tested including diploids, triploids and tetraploids. The accessions were selected from the field gene bank maintained at BRS Kannara based on the genome of varieties to study whether there is any relationship between plant genotype and the disease resistance. The accessions used for the study are given below (Table 3.9).

3.7.2.2. Preparation of inoculum

A fungal culture isolated from banana cultivar Rasthali was grown in PDA for 5 days and inoculum was prepared as described in 3.4.2.

3.7.2.3. Pot culture experiment

Four suckers from each accession were selected and planted in grow bags of size 20" x 18.5" filled with potting mixture prepared as given in 3.4.3. The experiment was carried out in completely randomized design (CRD) with four replications for each accession. The plants were inoculated by drenching with the spore suspension (100 ml)

Plate 3.4. Pot culture experiment for screening of banana germplasm for Foc resistance

Table 3.9. Banana genotypes evaluated for disease resistance against Foc race 1 under artificial inoculation

Sl. No.	Variety	Genome	Ploidy level
1	Nendran Hybrid	AAAB	Tetraploid
2	Attunendran	AAB	Triploid
3	Zanzibar	AAB	
4	Grand Naine	AAA	
5	Big Ebanga	AAB	
6	Yangambi Km5	AAA	
7	Nedunendran	AAB	
8	Nendran (Kumbavazha)	AAB	
9	BRS II – (Vannan x Pisang lilin)	AAB	
10	Thiruvananthapuram	AAB	
11	Chinese Cavendish	AAA	
12	Palayankodan (Alpan)	AAB	
13	Pachanadan I	AAB	
14	Padathy	AAB	
15	Velipadathy	AAB	
16	Cheriya Poovan	AAB	
17	Valiya Poovan	AAB	
18	TMP 2 x 2829	AA	Diploid
19	Kadali	AA	
20	Cultivar Rose	AA	
21	Pisang Lilin	AA	
22	*Musa acuminate* sub. sp. *burmanica*	AA	
23	Njalipoovan	AB	
24	Sanna Chenkadali	AA	
25	Chenkadali	AAA	
26	Pisang Jari Buaya	AA	

thrice at one-month interval. The plants were watered regularly to ensure the sufficient moisture content in the soil. External symptoms appeared were recorded after seven months of planting and per cent wilt index (PWI) was calculated using a standard score chart (Mak *et al.*, 2004) as given in Table 3.1. The rhizome of all accessions was uprooted after seven months of planting and rhizome scoring was recorded using the standard score chart (Table 3.10) given by Zuo *et al.* (2018) to find out per cent vascular wilt index (PVWI) (Plate 3.5).

Table 3.10. Standard score chart for assessing per cent vascular wilt index (internal symptoms) under natural conditions

Score	Internal symptoms
0	Corm completely clean/no symptoms
1	Discolouration up to 1-5 per cent of corm
2	Discolouration between 6-25 per cent of corm
3	Discolouration between 26-50 per cent of corm
4	Discolouration between 51-75 per cent of corm
5	Discolouration over 75 per cent of corm

The per cent disease incidence (PDI) and per cent disease severity (PDS) using PWI and PVWI were assessed.

3.7.3. Biochemical basis of disease resistance

Disease resistance and pathogen virulence are the collective action of various biochemical mechanisms. Host plant resistance is not only based on anatomical and cytological features but also biochemical components of the plants. Hence, the study was undertaken to assess the role and activity of phenols, sugars and various defense-related enzymes in host plant disease resistance. Tissue culture banana plants of two months old were collected from the Department of Plant Biotechnology and used for the biochemical studies. The susceptible cultivars used for the study were Rasthali/Poovan (AAB) and Kadali (AA) and resistant cultivars were Nendran (AAB) and Robusta (AAA). The plants were dipped in Foc inoculum suspension of 5 x10^6

0. Corm completely clean/no symptoms

1. Discolouration up to 1-5 per cent of corm

2. Discolouration up to 6-25 per cent of corm

3. Discolouration up to 26-50 per cent of corm

4. Discolouration up to 51-75 per cent of corm

5. Discolouration over 76 per cent of corm

Plate 3.5. Scale for rating internal symptoms of Fusarium wilt of banana under field conditions

CFU/ml concentration for 30 min. and transplanted into grow bags prepared as in 3.3.3. Biochemical factors estimated are total phenols, defense related enzymes such as peroxidase, polyphenol oxidase and Phenylalanine ammonia lyase and reducing and non-reducing sugars. Observations were taken for healthy and inoculated plants at an interval of 48 h and change in values were recorded.

3.7.3.1. Estimation of total phenols

Estimation of total phenols in inoculated and healthy plants in 48 h interval was done according to the method explained by Sadasivam and Manickam (1996). In this method, 1 g of leaf sample was used for the analysis. Samples were balanced out and ground in a sterile pestle and mortar with 10 ml of 80 per cent ethyl alcohol. The blend was subjected to centrifugation for 20 min. at 10,000 rpm. The supernatant gained was collected and the process was repeated with residue using 5 ml of 80 per cent ethyl alcohol. The supernatant was pooled and kept in a water bath until the content became dried. Then, 5 ml of distilled water was added to the dehydrated product and dissolved thoroughly. Afterwards, from this 5 ml, different volumes of aliquots ranging from 0.2 ml to 2 ml were transferred to various test tubes. The volume of aliquots was made up to 5 ml by adding distilled water and 0.5 ml of Folin-Ciocalteau reagent was added to each tube. This was mixed thoroughly and kept for exactly 3 min. Later, 2 ml of 20 per cent Na_2CO_3 was poured. After proper mixing, the contents were kept in a boiling water bath for exact one min. The tubes were subjected to cooling and the optical density was recorded at 650 nm in a spectrophotometer against a reagent blank. Different concentration of catechol was used for the preparation of a standard curve. The quanity of phenol was estimated by plotting the standard graph with absorbance on y-axis and concentration on x-axis.

3.7.3.2. Estimation of reducing sugars

Reducing sugars at different intervals after inoculation were estimated using the DNS method explained by as explained by Miller (1959). For this, 0.2 g of leaf samples were weighed and ground with 2 ml of methanol in a pestle and mortar. The contents were transferred to a test tube and kept in a boiling water bath for 5 min. for evaporation. DNS reagent in 3 ml quantity was poured into each test tube. Into this, 40 per cent

Rochelle salt was added and placed on a boiling water both 10 min. and then cooled. The absorbance was measured at 575 nm in a spectrophotometer against the blank. Different concentration of glucose was used for plotting the standard graph with absorbance on y-axis and concentration on x-axis.

3.7.3.3. Estimation of non-reducing sugars

Non-reducing sugar was also estimated using the DNS method as explained by Miller (1959). 0.2 g of leaf sample was ground with 1 ml of alcohol in a pestle and mortar. The contents were taken in a test tube and kept in a water bath until dryness of the extract. Then, added 1 ml of distilled water and 1 ml of 1N H_2SO_4 and again placed on a water bath at 49 °C for 30 min. After cooling, 2 drops of methyl red indicator were added followed by neutralizing with 1N NaOH. The volume of content was brought up to 10 ml by adding distilled water. Then, 3 ml of DNS reagent and 1 ml of 40 per cent Rochelle salt was added to 5 ml of the above extract. This was then kept on a boiling water bath for 10 min. After cooling, the intensity of the colour developed was measured using a spectrophotometer at 575 nm. Graph plotted with various concentration of glucose was used as the standard for the calculation of non-reducing sugars with absorbance on y-axis and concentration on x-axis..

3.7.3.4. Estimation of peroxidase (PO)

The activity of the peroxidase enzyme in leaves was analysed using the method explained by Sadasivam and Manickam (1996). One gram of leaf sample was ground in a pestle and mortar with 3 ml of 0.1 M phosphate buffer (pH 7). The supernatant obtained after centrifugation of extract at 18,000 g for 15 min. at 5 °C was used for the estimation. In a cuvette, 3 ml of phosphate buffer and 0.05 ml of guaiacol (prepared by mixing 240 mg guaiacol in 100 ml of distilled water) were mixed. Into this, 0.02 ml enzyme extract and 0.03 ml H_2O_2 (0.14 ml of 30 per cent H_2O_2 in 100 ml distilled water) were added and dissolved thoroughly. The UV spectrophotometer was standardized with 3.1 ml of phosphate buffer at 436 nm wavelength. The cuvette was kept in the instrument and time (delta t) taken for the increase in OD by 0.1 was recorded. The enzyme activity (units/g) was calculated using the following formula;

$$\text{Enzyme activity units/ gram} = \frac{3.18 \times 0.1}{6.39 \times \text{delta t} \times 0.1}$$

The results were expressed as Enzyme activity unit/g of fresh tissue.

3.7.3.5. Estimation of polyphenol oxidase (PPO)

Estimation of PPO was done by the protocol described by Malik and Singh (1980). Leaf samples were collected from all treatments and 0.5 g was weighed out. The samples were ground in a pestle and mortar with 10 mM sodium phosphate buffer. The homogenate was centrifuged at 12,000 rpm for 20 min. at 4 °C. the resultant supernatant was used as the enzyme extract for the estimation. From this, 0.2 ml of extract was taken and mixed with 0.7 ml of sodium phosphate buffer. Then, 0.1 ml f 0.2 M catechol was added. The intensity of colour was measured at 30 Sec. intervals at 420 nm in a spectrophotometer. The results were expressed as Enzyme activity unit/g of fresh tissue.

3.7.3.6. Estimation of phenylalanine ammonia lyase (PAL)

The activity of the PAL enzyme in leaves was analysed using the method explained by Sadasivam and Manickam (1996). The homogenate for enzyme analysis was prepared using 0.5 g of leaf samples from inoculated as well as control plants collected at 48 h intervals after inoculation. The samples were weighed out and ground in 5 ml buffer containing 25 mM borate-HCl of pH 8.8 and 5 mM mercaptoethanol. This enzyme extract was subjected to centrifugation at 12,000 g for 20 min. and the supernatant obtained was used for further analysis. The reaction mixture was made by blending 0.5 ml borate buffer (0.2 M), 0.2 ml supernatant of enzyme extract and 1.3 ml distilled water in a test tube. Into this, 1 ml of L-Phenylalanine solution (0.1 M) was added and incubated for 20-60 min. at 32 °C. Afterwards, 0.5 ml trichloroacetic acid (1 M) poured to end the reaction. A blank was prepared by adding phenylalanine to the reaction mixture. The OD value was documented using a UV spectrometer at 290 nm. A standard graph was plotted with different concentrations of *trans*-cinnamic acid with absorbance on y-axis and concentration on x-axis. The results were expressed as Ezyme activity unit/g of fresh tissue.

3.8. HISTOPATHOLOGY

An attempt was undertaken to study the histopathological changes in Fusarium wilt infected tissues. Thin sections of healthy and infected leaves and roots from infected plants were taken using a sharp razor and made microscopic slides using safranin stain. The slides were observed under Leica Image analyser at 10 X magnification to study the internal symptoms and anatomical changes in infected and diseased tissues. The changes in different tissues *viz.*, cortex, palisade, spongy mesophyll cells and vascular tissues due to the pathogen infection was examined and compared with healthy tissue.

3.9. MANAGEMENT OF FUSARIUM WILT OF BANANA

3.9.1. *In vitro* evaluation of fungicides, bioagents and botanicals against the pathogen causing Fusarium wilt of banana

In vitro suppression of Foc using chemical fungicides, biocontrol agents (KAU) and botanicals (plant extracts) were done by poisoned food technique described by Nene and Thapliyal (1993). Plant extracts collected from Aromatic and medicinal plants research station, Odakkali, KAU were dissolved in equal volume of dimethyl sulfoxide (DMSO) and employed for the evaluation. The desired concentration of the formulation *ie*., lower, recommended and the higher dose was amended with melted and cooled half-strength PDA medium. The media incorporated with fungicides were poured into Petri plates and inoculated with 7 mm mycelial discs of 4 days old Foc culture under aseptic condition. The biocontrol agents such as *Trichoderma viride* and *Pseudomonas fluorescens* against Foc were evaluated using dual culture method also. The inoculated plates were incubated at a temperature of 25 °C along with control. Details of treatments used are given the Table 3.11. The percentage inhibition was calculated using the formula given below (Vincent, 1947).

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

Where, I – Per cent inhibition

C - Growth of fungus in unamended medium

T – Growth of fungus in amended medium

Table 3.11. Details of treatments used for *in vitro* studies

Type	Name	Concentration
Contact fungicides	1. Bordeaux mixture	0.5, 1.0, 1.5 %
	2. Mancozeb	0.15, 0.2, 0.25 %
	3. Copper oxychloride	0.15, 0.2, 0.25 %
	4. Copper hydroxide	0.15, 0.2, 0.25 %
Systemic fungicides	1. Tebuconazole	0.05, 0.1, 0.15 %
	2. Propiconazole	0.05, 0.1, 0.15 %
	3. Carbendazim	0.15, 0.2, 0.25 %
	4. Azoxystrobin	0.15, 0.2, 0.25 %
Combination fungicides	1. Iprodione + Carbendazim	0.05, 0.1, 0.15 %
	2. Captan + Hexaconazole	0.05, 0.1, 0.15 %
	3. Carbendazim + Mancozeb	0.15, 0.2, 0.25 %
Biocontrol agents	1. PGPR Mix II (*Pseudomonas fluorescens, Bacillus subtilis and Lactobacillus*)	1.5, 2.0, 2.5 %
	2. PGPM (*P. fluorescens* and *Bacillus megatherium*)	1.5, 2.0, 2.5 %
	3. Trichoderma viride	1.5, 2.0, 2.5 %
	4. *Pseudomonas fluorescens*	1.5, 2.0, 2.5 %
Botanicals	*1. Azadirachta indica* (leaves)	0.05, 0.1, 0.15 %
	2. Myristica fragrans (mace)	0.05, 0.1, 0.15 %
	3. Lawsonia inermis (leaves)	0.05, 0.1, 0.15 %
	4. Areca catechu (seed kernel)	0.05, 0.1, 0.15 %
	5. Kaempferia galanga (rhizome)	0.05, 0.1, 0.15 %
	6. Curcuma caesia (rhizome)	0.05, 0.1, 0.15 %
	7. Calotropis gigantea (flower)	0.05, 0.1, 0.15 %
	8. Bacopa monnieri (aerial part)	0.05, 0.1, 0.15 %
	9. Curcuma angustifolia (rhizome)	0.05, 0.1, 0.15 %
	10. Justicia gendarussa (leaves)	0.05, 0.1, 0.15 %

The best treatments obtained after *in vitro* evaluation was used for pot culture and field experiments.

3.9.2. Pot culture experiment

A pot culture experiment was conducted for the management of Fusarium wilt using the best treatments selected after *in vitro* evaluation (Plate 3.6). The experiment was laid out inside the net house at the Department of Plant Pathology in CRD with three replications. Healthy suckers of Rasthali/Poovan cultivar brought from BRS Kannara were used as the planting materials. Potting mixture was prepared by mixing sand: soil: cow dung in the ratio 1:1:1. Potting mixture was sterilized using 40 per cent formaldehyde before filling the pots. The plants were inoculated by drenching with 100 ml of spore suspension thrice at one-month interval. Treatment details are given in Table 3.12. Per cent wilt index and per cent vascular wilt index were calculated using the standard score chart given by Mak *et al*. (2004) (Table 3.1) and Zuo *et al*. (2018) (Table 3.7) respectively.

3.9.3. Field experiment

A field experiment to develop a management strategy for Fusarium wilt of banana was conducted in the sick plot at Banana Research Station Kannara from July 2018 to November 2019 (Plate 3.7). The treatments were selected based on the results obtained from *in vitro* evaluation of chemical fungicides, biocontrol agents and botanicals. The planting material used was 3-4 months old disease-free sword suckers of susceptible variety Rasthali/Poovan and experiment was carried out in Randomized block design. Treatment details are given in Table 3.13. The experiment details are as follows;

Location	: Sick plot, BRS Kannara
Design	: RBD
No. of treatments	: Ten
No. of replications	: Three
No. of plants per replication	: Five
Variety	: Rasthali/Poovan

Plate 3.6. Pot culture experiment for the management of Fusarium wilt of banana

Plate 3.7. Field experiment for the management of Fusarium wilt of banana

Table 3.12. Schedule of treatments for pot culture experiment

Sl. No	**Treatment**	**Schedule of application**
T1	0.2 per cent Copper hydroxide (Kocide 2000)	Sucker treatment Soil drenching at 1, 2 and 3 MAP
T2	0.1 per cent Tebuconazole (Folicur 25.9 EC)	Sucker treatment Soil drenching at 1, 2 and 3 MAP
T3	0.2 per cent Carbendazim + mancozeb (Saaf)	Sucker treatment Soil drenching at 1, 2 and 3 MAP
T4	2 per cent *Trichoderma viride*	Sucker treatment Soil drenching at 1, 2 and 3 MAP
T5	AMF (100g/plant) + *Trichoderma* enriched cow dung - neem cake mixture (5 Kg/plant) + Soil drenching of 2 per cent *Pseudomonas fluorescens*	At the time of planting Soil drenching at 1, 2 and 3 MAP
T6	IDM: *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + Soil drenching of selected best fungicide	Sucker treatment at the time of planting Soil drenching at 1 and 2 MAP
T7	2 per cent PGPR Mix II (*P. fluorescens, Bacillus megatherium and Lactobacillus*)	Sucker treatment and soil drenching at 1, 2 and 3 MAP
T8	2 per cent *P. fluorescens*	Sucker treatment and soil drenching at 1, 2 and 3 MAP
T9	0.2 per cent Carbendazim	Sucker treatment and soil drenching at 1, 3 and 5 MAP
T10	Untreated control	

Table 3.13. Schedule of treatments for field experiment

Sl. No	Treatment	Schedule of application
T1	0.2 per cent Copper hydroxide (Kocide 2000)	Sucker treatment Soil drenching at 1, 3 and 5 MAP
T2	0.1 per cent Tebuconazole (Folicur 25.9 EC)	Sucker treatment Soil drenching at 1, 3 and 5 MAP
T3	0.2 per cent Carbendazim + mancozeb (Saaf)	Sucker treatment Soil drenching at 1, 3 and 5 MAP
T4	2 per cent *Trichoderma viride*	Sucker treatment Soil drenching at 1, 2 and 3 MAP
T5	AMF (100g/plant) + *Trichoderma* enriched cow dung - neem cake mixture (5 Kg/plant) + Soil drenching of 2 per cent *P. fluorescens*	At the time of planting Soil drenching at 1, 2 and 3 MAP
T6	IDM: *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + Soil drenching of selected best fungicide	Sucker treatment at the time of planting Soil drenching at 2 and 4 MAP
T7	2 per cent PGPR Mix II (*P. fluorescens, Bacillus subtilis and Lactobacillus*)	Sucker treatment and soil drenching at 1, 2 and 3 MAP
T8	2 per cent *P. fluorescens*	Sucker treatment and soil drenching at 1, 2 and 3 MAP
T9	0.2 per cent Carbendazim	Sucker treatment and soil drenching at 1, 3 and 5 MAP
T10	Untreated control	

The land preparation was carried out by weeding, pulverizing and digging the soil. Pits of size 50 cm x 50 cm x 50 cm were used for planting the suckers. The suckers were planted at a spacing of 2.1 m x 2.1 m and manuring was done as per the KAU package of practices recommendation (KAU, 2016).

The plants were regularly monitored for the development of any external wilting symptoms such as yellowing of leaves and splitting of pseudostem. The per cent disease incidence (PDI) was calculated at 8 MAP and 10 MAP. As given in 3.1.1, per cent wilt index (PWI) was recorded at 10 MAP and per cent vascular wilt (PVWI) was recorded during the uprooting of plants. Biometric observations were documented at monthly interval from 5^{th} month onwards. Biometric characters observed were plant height, girth, number of functional leaves, yield characters such as bunch weight, number of hands per bunch and fingers per hand, pulp: peel ratio of ripe fruits and total soluble solids (TSS) of ripe fruits using brix meter.

RESULTS

4. RESULTS

The present study on "Characterization and integrated management of *Fusarium oxysporum* f. sp. *cubense* (E. F. Smith) Snyder and Hansen causing Fusarium wilt disease of banana" was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2017-2020. The field experiment was conducted at Banana Research Station, Kannara. The objectives of the study were to characterize the pathogen through cultural, morphological and molecular basis, to identify the races, to develop quick detection methods, to study the basis of host plant resistance and to develop integrated management strategies using biological/botanicals and chemicals for the control of Fusarium wilt pathogen. The results revealed in this study are presented in this chapter.

4.1 SURVEY AND COLLECTION OF SAMPLES

Purposive sampling surveys were conducted during the period from June 2018 to November 2018 in farmers' fields at various locations (Fig. 4.1-4.6) in six districts of Kerala *viz*., Thiruvananthapuram (Southern zone), Ernakulam (Central zone), Thrissur (Central zone), Palakkad (Northern zone), Kozhikode (Northern zone) and Wayanad (High range zone) covering various agroclimatic zones. The surveyed locations are given in Table 4.1. A total of thirty isolates were collected from infected fields for further studies.

4.1.1 Occurrence of the disease

The Fusarium wilt disease was observed in Kazhakuttom, Nedumangad, Mudapuram, Attingal, Karyavattom and Azhoor in Thiruvananthapuram district and Karukutty, Angamaly, Aluva, Kadungallur and Muppathadam in Ernakulam district. In Thrissur district, the disease was observed in Kannara, Nadathara, Chalakudy, Thrikkur, Ollukkara, Nandikkara and Anandapuram. Kottayi, Kuzhalmannam, Chithali and Kannadi were the places in which disease was observed in Palakkad district. Whereas in Kozhikode district, the infected fields were observed at Mukkom, Koduvally and Omassery. In Wayanad district, the disease was noticed in Ambalavayal, Kenichira, Sulthan Bathery and Kakkavayal.

Table 4.1. Details of locations surveyed for the incidence of Fusarium wilt disease in Kerala

Sl. No.	Locations	Latitude and Longitude
1	Kazhakuttom	8.569 °N, 76.873 °E
2	Mudapuram	8.662 °N, 76.813 °E
3	Nedumangad	8.608 °N, 77.005 °E
4	Attingal	8.698 °N, 76.814 °E
5	Neyyattinkara	8.403 °N, 77.086 °E
6	Karyavattom	8.568 °N, 76.891 °E
7	Azhoor	8.644 °N, 76.799 °E
8	Karukutty	10.227 °N, 76.375 °E
9	Angamaly	10.193 °N, 76.387 °E
10	Aluva	10.100 °N, 76.357 °E
11	Kadungallur	10.109 °N, 76.326 °E
12	Muppathadam	10.083 °N, 76.315 °E
13	Kannara	10.536 °N, 76.336 °E
14	Nadathara	10.505 °N, 76.270 °E
15	Chalakudy	10.307 °N, 76.334 °E
16	Thrikkur	10.463 °N, 76.273 °E
17	Ollukkara	10.532 °N, 76.252 °E
18	Nandikkara	10.405 °N, 76.277 °E
19	Anandapuram	10.380 °N, 76.262 °E
20	Kottayi	10.747 °N, 76.543 °E
21	Kuzhalmannam	10.719 °N, 76.601 °E
22	Chithali	10.690 °N, 76.583 °E
23	Kannadi	10.730 °N, 76.640 °E
24	Mukkom	11.321 °N, 75.996 °E
25	Koduvally	11.360 °N, 75.913 °E
26	Omassery	11.365 °N, 75.962 °E
27	Ambalavayal	11.620 °N, 76.210 °E
28	Kenichira	11.725 °N, 76.149 °E
29	Sulthan Bathery	11.663 °N, 76.257 °E
30	Kakkavayal	11.644 °N, 76.141 °E

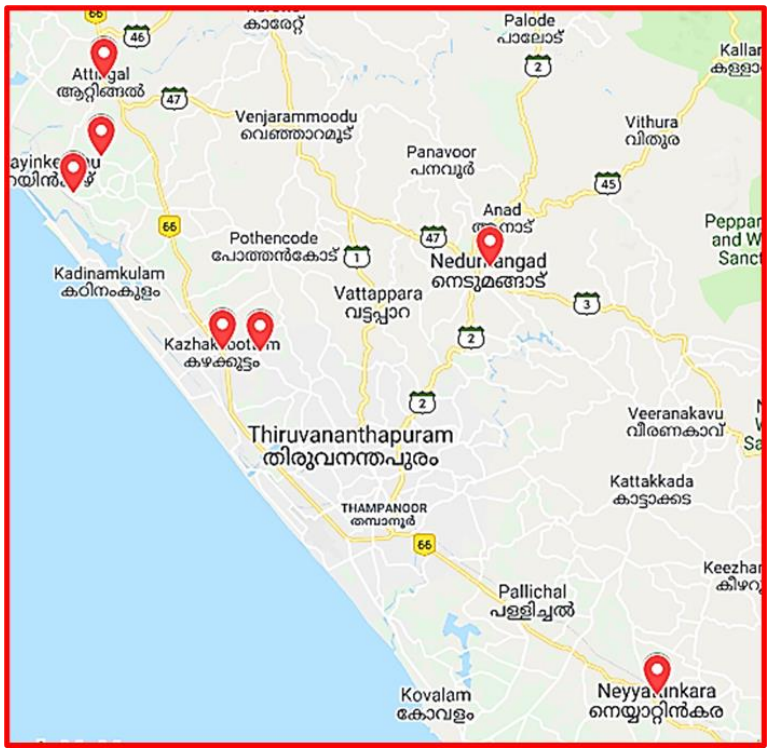


Fig. 4.1: Locations of survey in Thiruvananthapuram district

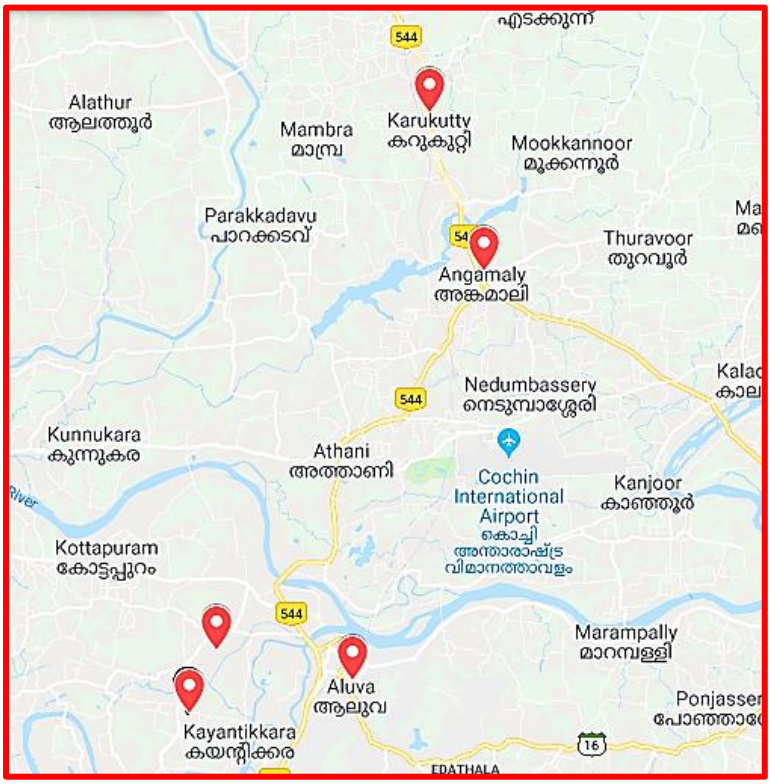


Fig. 4.2: Locations of survey in Ernakulam district

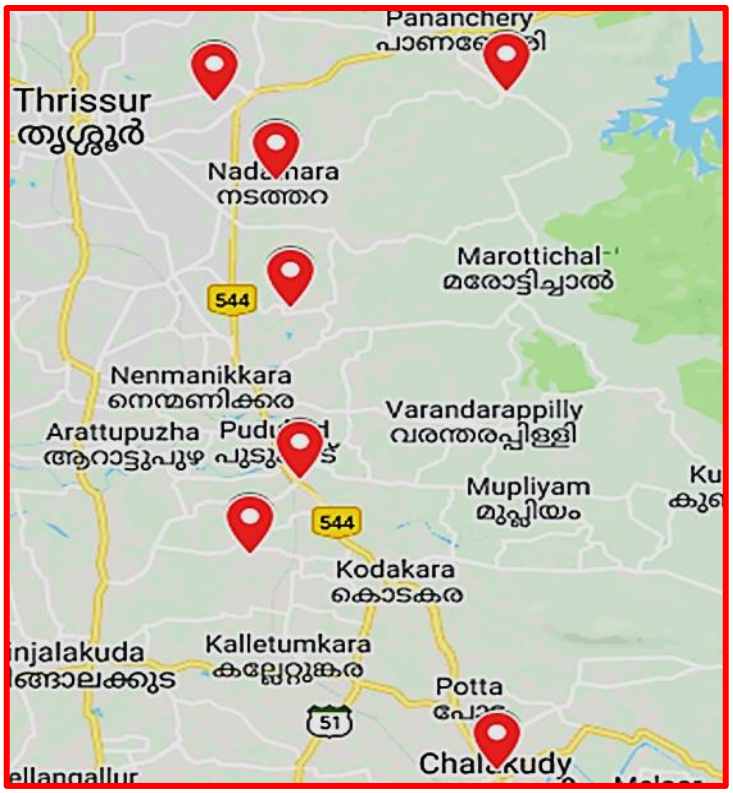


Fig. 4.3: Locations of survey in Thrissur district

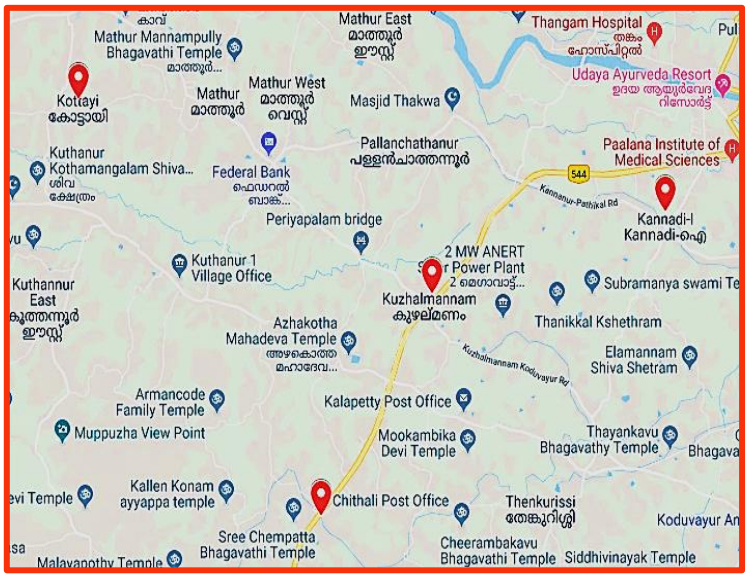


Fig. 4.4: Locations of survey in Palakkad district

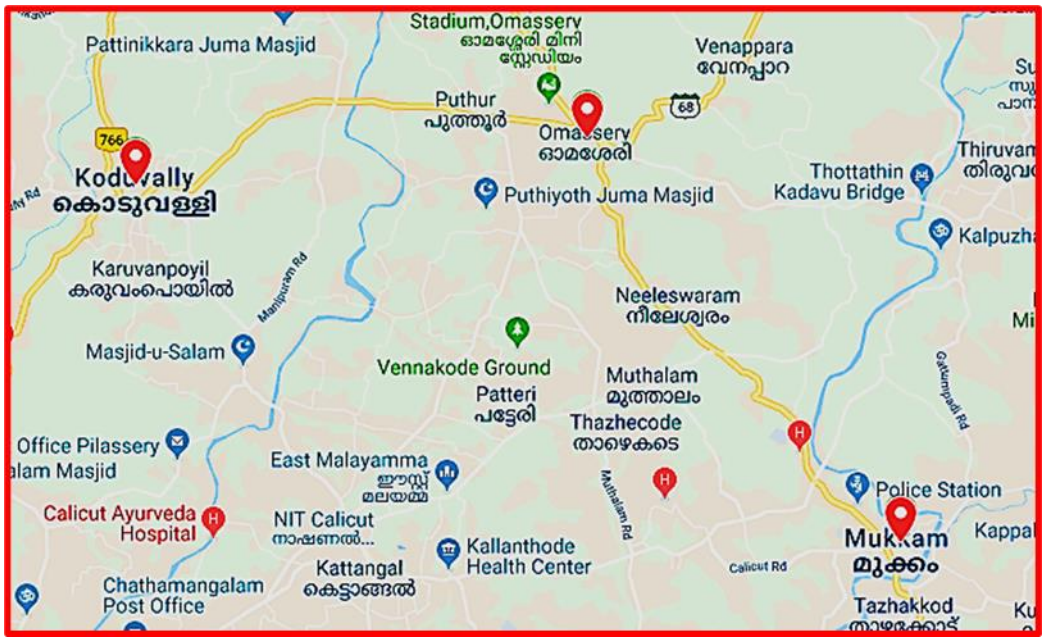


Fig. 4.5: Locations of survey in Kozhikode district

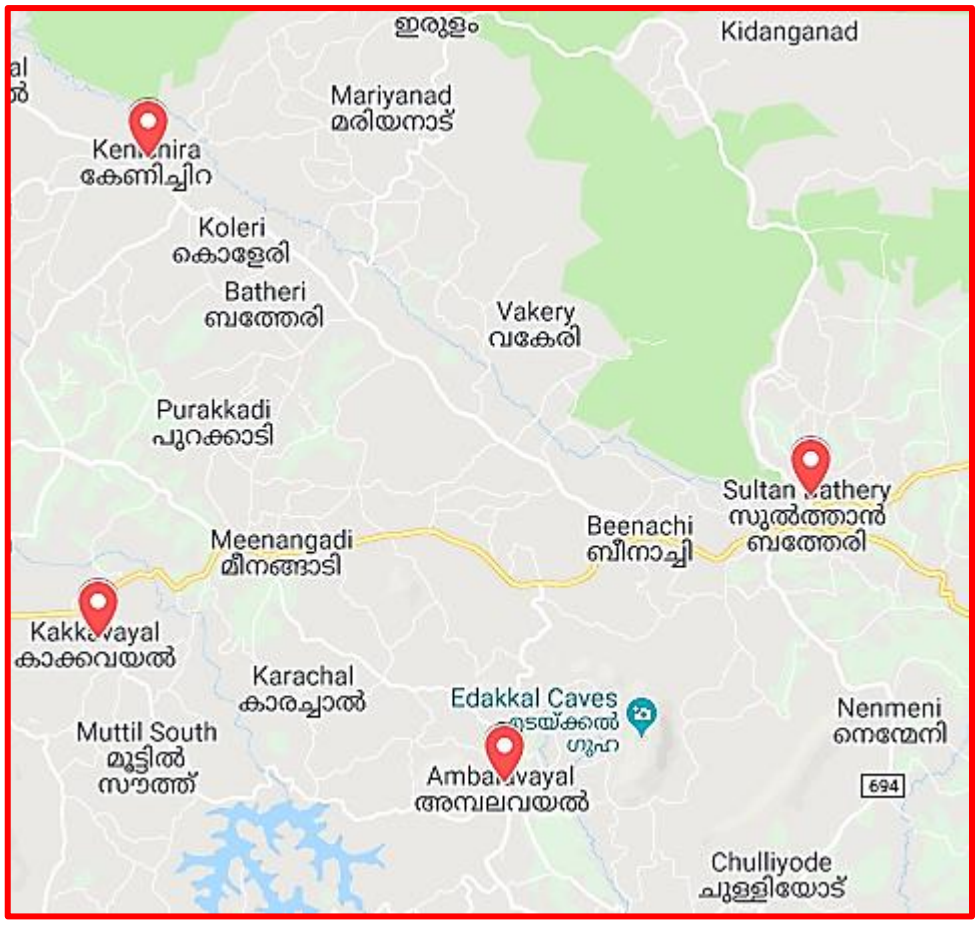


Fig. 4.6: Locations of survey in Wayanad district

The per cent disease incidence (PDI) and per cent disease severity (PDS) were recorded from surveyed locations and are presented in Table 4.2. During the survey, it was observed that the disease incidence ranged from 1.52 to 4.64 per cent in Thiruvananthapuram, 19.44 to 27.40 per cent in Ernakulam, 6.40 to 23.78 per cent in Thrissur, 5.33 to 7.58 per cent in Palakkad, 12.30 to 17.22 per cent in Kozhikode and 17.66 to 43.65 per cent in Wayanad district. The PDI was lowest (1.52 %) in Thiruvananthapuram and highest (43.65 %) in Wayanad district.

The results of the survey revealed that the lowest (20.41 %) PDS was recorded in Ernakulam while the highest PDS of 49.57 per cent was recorded in Wayanad district. The PDS varied from 21.30 to 38.18 per cent, 20.34 to 42.22 per cent, 20.67 to 32.63 per cent, 28.70 to 34.11 per cent, 26.81 to 29.68 per cent and 34.74 to 49.57 per cent in Thiruvananthapuram, Ernakulam, Thrissur, Palakkad, Kozhikode and Wayanad districts respectively.

In Thiruvananthapuram district, PDI was highest in Attingal (4.64 %) in Rasthali/Poovan variety followed by Kazhakuttom (4.10 %) in Kadali variety. The lowest PDI was noted in Neyyattinkara (1.52 %) on Kadali variety. The highest PDS was observed in Nedumangad (38.18 %) in Rasthali/Poovan preceded by Kazhakkuttom (36.77 %) in Kadali variety with the least PDI in Mudapuram (21.30 %) in Rasthali/Poovan variety. In this district, the disease incidence was reported in cultivars such as Rasthali/Poovan, Kadali, Chenkadali and Njalipoovan.

In the case of Ernakulam district, the highest PDI of 27.40 per cent was recorded in Kadungallur followed by Angamaly (24.60 %) in Rasthali/Poovan variety. The least PDI of 19.44 per cent was observed in Karukutty in Rasthali/Poovan variety. The PDS was observed lowest in Aluva (20.34 %) and highest in Karukutty (42.22 %) followed by Muppathadam (26.36 %). In the Ernakulam district, all the surveyed fields were planted with Rasthali/Poovan variety.

The results of the survey in Thrissur district revealed that the PDI was highest in Nadathara with 23.78 per cent which was followed by Ollukkara (21.70 %) and minimum at Kannara (6.40 %). The highest PDS of 32.63 per cent was recorded in the

field at Kannara followed by Chalakudy (31.11 %) and the least PDS of 20.67 per cent was recorded in field of Ananadapuram. In this district, all the surveyed fields were planted with Rasthali/Poovan except Ollukkara which was planted with Kadali.

In the Palakkad district, the field at Kottayi was recorded with the highest (7.58 %) PDI followed by Chithali (6.66 %) and Kuzhalmannam (5.50 %). The least (5.33 %) PDI was recorded in the field at Kannadi. The highest (34.11 %) PDS was observed in the field at Chithali followed by the fields at Kannadi (31.43 %) and Kuzhalmannam (28.70 %). The lowest PDS (25.71 %) was noticed in the field at Kottayi. All the fields were planted with Rasthali/Poovan variety except Kottayi which was planted with the variety Njalipoovan.

During the survey in Kozhikode district, the highest PDI (17.22 %) was noticed in the field at Koduvally planted with Kadali variety followed by the field at Omassery (15.90 %) planted with Rasthali/Poovan variety. The lowest PDI (12.30 %) was recorded in the field at Mukkom planted with Rasthali/Poovan variety. The highest PDS was recorded in the field at Mukkom (29.68 %) followed by Koduvally (29.31 %). The lowest PDS was observed in the field at Omassery (26.81 %).

The results of the survey in Wayanad district revealed that the highest PDI (43.65 %) was recorded in Ambalavayal field which was followed by Kakkavayal and Kenichira with a PDI of 31.81 per cent and 26.80 per cent respectively. The least PDI (17.66 %) was noticed in Sulthan Bathery field. Whereas, the highest (49.57 %) PDS was observed in Kenichira field followed by Sulthan Bathery and Kakkavayal fields with a PDS of 42.67 per cent and 35.56 per cent respectively. The least (34.74 %) PDS was noticed in the field Ambalavayal. All the infected fields in this district were planted with the Rasthali/Poovan variety.

During the survey, it was observed that the varieties such as Rasthali/Poovan, Kadali, Njalipoovan and Chenkadali were infected by the disease.

Table 4.2. Details of disease incidence and severity in the surveyed locations

Sl. No.	Location of survey	Variety	PDI (%)	PDS (%)
S1.	Kazhakuttom	Kadali	4.10	36.77
S2.	Mudapuram	Rasthali/Poovan	3.40	21.30
S3.	Nedumangad	Rasthali/Poovan	1.77	38.18
S4.	Attingal	Rasthali/Poovan	4.64	33.33
S5.	Neyyattinkara	Kadali	1.52	25.24
S6.	Karyavattom	Njalipoovan	3.10	26.66
S7.	Azhoor	Chenkadali	3.60	26.40
S8.	Karukutty	Rasthali/Poovan	19.44	42.22
S7.	Angamaly	Rasthali/Poovan	21.67	23.41
S10.	Aluva	Rasthali/Poovan	24.60	20.34
S11.	Kadungallur	Rasthali/Poovan	27.40	24.44
S12.	Muppathadam	Rasthali/Poovan	21.42	26.36
S13.	Kannara	Rasthali/Poovan	6.40	32.63
S14.	Nadathara	Rasthali/Poovan	23.78	24.28
S15.	Chalakudy	Rasthali/Poovan	9.57	31.11
S16.	Thrikkur	Rasthali/Poovan	15.33	28.14
S17.	Ollukkara	Kadali	21.70	22.22
S18.	Nandikkara	Rasthali/Poovan	18.60	30.56
S19.	Anandapuram	Rasthali/Poovan	16.33	20.67
S20.	Kottayi	Njalipoovan	7.58	25.71
S21.	Kuzhalmannam	Rasthali/Poovan	5.50	28.70
S22.	Chithali	Rasthali/Poovan	6.66	34.11
S23.	Kannadi	Rasthali/Poovan	5.33	31.43
S24.	Mukkom	Rasthali/Poovan	12.30	29.68
S25.	Koduvally	Kadali	17.22	29.31
S26.	Omassery	Rasthali/Poovan	15.90	26.81
S27.	Ambalavayal	Rasthali/Poovan	43.65	34.74
S28.	Kenichira	Rasthali/Poovan	26.80	49.57
S29.	Sulthan Bathery	Rasthali/Poovan	17.66	42.67
S30.	Kakkavayal	Rasthali/Poovan	31.81	35.56

4.1.2. Correlation analysis of disease incidence with weather parameters

Weather parameters such as rainfall, minimum temperature and maximum temperature of the surveyed locations were collected (Table 4.3). The correlation analysis of disease incidence with weather parameters such as rainfall and average temperature ((maximum temperature + minimum temperature)/2) was carried out (Table 4.4 and Fig. 4.7 – 4.8). The results revealed that a significant positive correlation with the rainfall and per cent disease incidence (PDI). The correlation coefficient obtained was 0.839 which indicates a strong positive relationship between these two parameters. Whereas, the correlation coefficient obtained between average temperature and PDI was -0.610 which indicates a moderate negative relationship between these parameters. Hence the correlation analysis revealed that the per cent disease incidence increased with increasing rainfall and decreased with increasing temperature.

4.2. SYMPTOMATOLOGY

4.2.1. Symptoms under natural condition

Under natural conditions, two types of symptoms were noticed *viz.*, external (Plates 4.1) and internal symptoms (Plates 4.2). External symptoms observed were yellowing of leaves starting from older to younger leaves. Then the leaves collapsed and formed a skirt-like appearance around the pseudostem. Afterwards, the whole leaves became dried. Another characteristic external symptom noticed was the splitting of pseudostem. The splitting was more intensive in the Rasthali/Poovan varieties. Severely infected plants showed wilting and finally collapsed. The internal symptoms observed were vascular and rhizome discolouration. It started with the discolouration of root and rhizome vascular tissue. The discolouration of vascular strands in the pseudostem were reddish or brownish.

4.2.2. Symptoms under artificial condition

Under artificial condition also external and internal symptoms were noticed. The external symptoms started with yellowing of the leaf tip and expanded gradually. The

Table 4.3. Weather parameters of surveyed locations during 2018 June-November

Sl. No.	Locations of survey	June – November 2018		
		Maximum temperature (°C)	Minimum temperature (°C)	Rainfall (cm)
1	Kazhakuttom	30.77	24.78	247.33
2	Mudapuram	30.75	24.45	266.83
3	Nedumangad	30.87	24.98	223.00
4	Attingal	30.88	24.55	271.17
5	Neyyattinkara	30.08	24.92	192.83
6	Karyavattom	30.60	24.43	243.67
7	Azhoor	30.75	24.93	266.83
8	Karukutty	30.38	24.10	443.33
9	Angamaly	30.57	24.57	445.00
10	Aluva	30.38	24.52	456.50
11	Kadungallur	30.38	24.52	457.33
12	Muppathadam	30.35	24.48	458.17
13	Kannara	30.50	24.58	409.50
14	Nadathara	30.25	24.33	427.50
15	Chalakudy	30.48	24.53	433.50

Plate 4.1.a. Yellowing of leaves

Plate 4.1.b. Skirt like appearance of leaves

Plate 4.1.c. Drying of infected plant

Plate 4.1.d. Splitting of pseudostem

Plate 4.1. External symptoms of Fusarium wilt of banana under natural conditions

Plate 4.2.a. Pseudostem discolouration

Plate 4.2.b Rhizome discolouration

Plate 4.2. Internal symptoms of Fusarium wilt of banana under natural conditions

Table 4.3. Weather parameters of surveyed locations during 2018 June-November (Contd.)

Sl. No.	Locations of survey	June – November 2018		
		Maximum temperature (°C)	Minimum temperature (°C)	Rainfall (cm)
16	Thrikkur	30.42	20.50	428.83
17	Ollukkara	30.43	24.47	424.83
18	Nandikkara	31.42	24.52	431.83
19	Anandapuram	30.42	24.53	434.00
20	Kottayi	30.67	24.27	344.67
21	Kuzhalmannam	30.68	24.23	321.00
22	Chithali	30.58	24.52	324.67
23	Kannadi	30.13	24.12	304.67
24	Mukkom	30.45	24.45	373.48
25	Koduvally	30.25	24.35	385.68
26	Omassery	30.43	24.47	380.08
27	Ambalavayal	25.72	19.43	468.17
28	Kenichira	26.89	20.20	461.20
29	Sulthan Bathery	26.20	19.73	456.20
30	Kakkavayal	26.97	20.28	467.67

Table 4.4. Correlation analysis of disease incidence with weather parameters

Sl. No.	Weather parameter	Correlation coefficient
		PDI *
1	Minimum temperature	-0.593
2	Maximum temperature	-0.630
3	Rainfall	0.839

*Per cent disease incidence

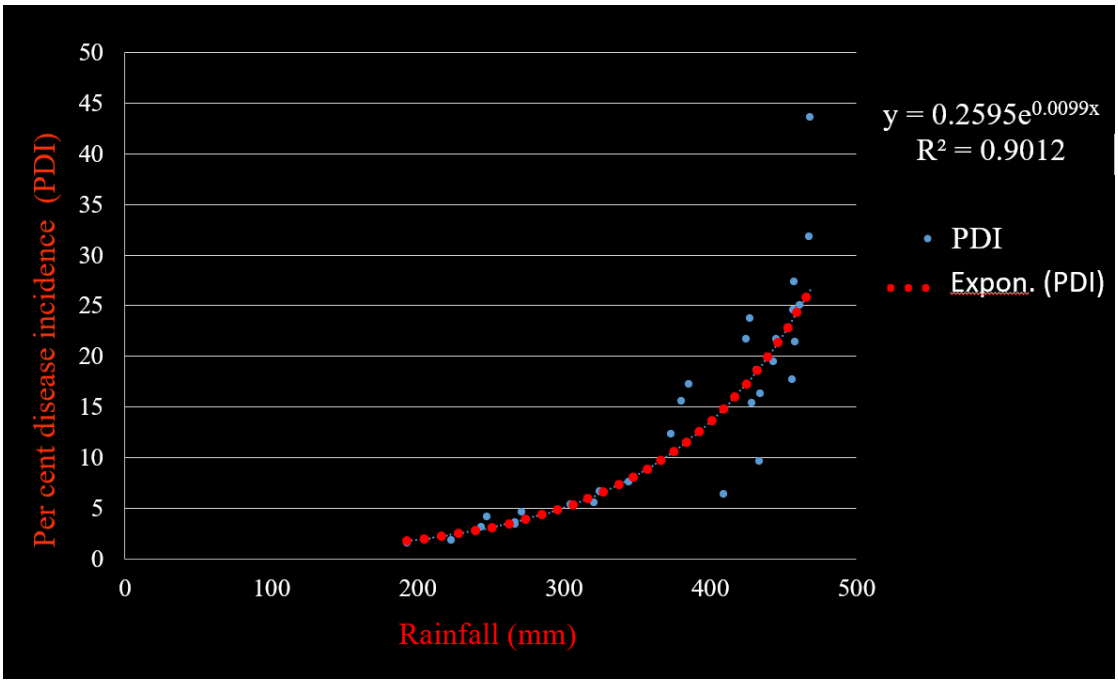


Fig. 4.7. Influence of rainfall on per cent disease incidence

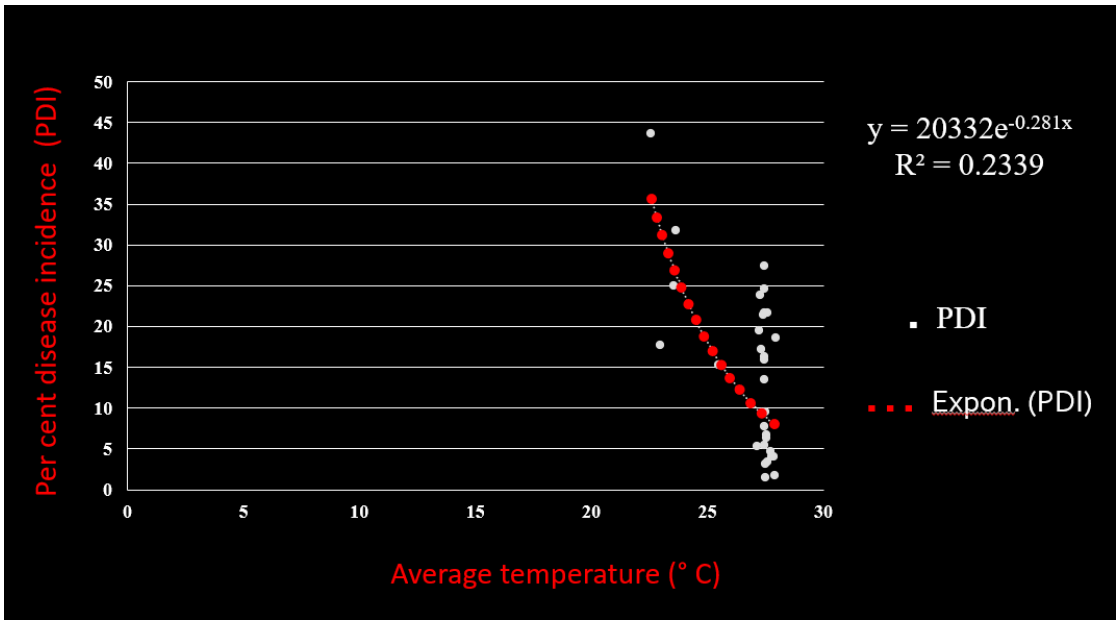


Fig. 4.8. Influence of average temperature on per cent disease incidence

plants succumbed to death in the final stage. While the internal symptom noticed was the discolouration of the stellar region (Plates 4.3).

The external symptoms under artificial condition were grouped into 4 categories based on the external scoring (Plates 4.4). The number of days taken for shifting from one category of scoring to another was taken in the varieties such as Rasthali/Poovan, Njalipoovan, Kadali and Chenkadali (Table 4.5). The number of days taken for shifting from category 0 to 1 was recorded highest (15.33) in Njalipoovan followed by Chenkadali (11. 67), Kadali (11.33) and Rasthali/Poovan (10.67). The number of days taken for shifting from category 1 to 2 was maximum in Njalipoovan (12.33) followed by Chenkadali (9.67) and Kadali (8.67) and was minimum in Rasthali/Poovan (7.67). Days taken for shifting from the category 2 to 3 was also highest (10.33) in Njalippovan followed by Chenkadali (9.33), Kadali (7.33) and Rasthali/Poovan (6.33). The highest number of days (10.00) required for shifting from the category 3 to 4 was also observed in Njalipoovan followed by Chenkadali (7.00) and Rasthli/Poovan (5.00) whereas, the lowest number of days taken for shifting was noticed in Kadali (3.67).

Total number of days taken for symptom expression and shifting from first category to forth category was recorded maximum in Njalipoovan (47.99) followed by Chenkadali (37.67) and Kadali (31) whereas, the minimum number of days (29.67) was taken in the variety Rasthali/Poovan.

4.2.3. Fusarium wilt like symptoms in Nendran variety

Symptoms similar to that of Fusarium wilt of banana such as yellowing of leaves was observed in Nendran variety in a field at Wadakkanchery, Thrissur (Plate 4.5). The sample was collected and subjected to isolation of the microorganism, pathogenicity study and molecular characterization.

4.3. ISOLATION OF THE PATHOGEN

The pathogen associated with the diseased samples of banana collected during the survey was isolated on half strength potato dextrose agar (PDA) medium (Plate 4.6). A total of 30 isolates of *Fusarium oxysporum* f. sp. *cubense* (Foc) were collected from

Plate 4.3. Internal symptoms of Fusarium wilt of banana under artificial conditions

Category 1

Category 2

Category 3

Category 4

Plate 4.4. Different categories of external symptoms of Fusarium wilt of banana under artificial conditions

Plate 4.5. Fusarium wilt like symptoms noticed in the Nendran variety of banana

Plate 4.6.a. Isolation of the pathogen in half strength PDA medium

Plate 4.6.b. Growth of the pathogen in half strength PDA medium

4.5.c. Pigmentation of the pathogen

Plate 4.6. Isolation and growth of the pathogen Foc in half strength PDA medium

Table 4.5. Number of days taken for the development of symptoms on different cultivars

Cultivar	Days taken to shift to the next category of index			
	Category 0-1	**Category 1-2**	**Category 2-3**	**Category 3-4**
Rasthali/Poovan	10.67	7.67	6.33	5.00
Njalipoovan	15.33	12.33	10.33	10.00
Kadali	11.33	8.67	7.33	3.67
Chenkadali	11.67	9.67	9.33	7.00

various locations in six districts *viz*., Thiruvananthapuram, Ernakulam, Thrissur, Palakkad, Kozhikode and Wayanad of Kerala. Among these, four isolates were obtained from the variety Kadali, two from Njalipoovan, one from Chenkadali and twenty three from Poovan/Rasthali. All the isolates were obtained from pseudostem strands of infected plants. All the isolates were purified and maintained on PDA by frequent subculturing.

4.4. PATHOGENICITY STUDIES

Pathogenicity studies of all 30 isolates of Foc were completed by proving Koch's postulates on their respective host varieties. Artificial inoculation was done on healthy banana tissue culture plants by root dipping and soil drenching with conidial suspension of Foc. Inoculated plants produced yellow coloured symptoms on the leaf tip 10 to 15 days after inoculation in various varieties. The yellowing was then gradually spread to whole leaf and caused drying of the leaves within 30 days after inoculation. The rhizome of the plant was cut open to see the discolouration of the stellar region just after the whole leaves have dried. The stellar region was brown coloured in infected plants whereas, no symptoms were produced on control plants (Plate 4.7). Re-isolation of the pathogen from inoculated plants produced fungal colony the same as the mother culture. The per cent wilt index (PWI) and per cent vascular wilt index (PVWI) of all the isolates were recorded after 30 days of inoculation and given in Table 4.6. The PWI was highest (93.33 %) in the isolates S1(Kadali), S2 (Rasthali/Poovan), S5 (Kadali), S12 (Rasthali/Poovan), S20 (Njalipoovan) and S27 (Rasthali/Poovan). The lowest PWI (40 %) was observed in the isolate S14 (Rasthali/Poovan). Whereas, the PVWI was observed maximum (100 %) in the isolate S2 (Rasthali/Poovan) and the least (33.33 %) PVWI was recorded in the isolate S14 (Rasthali/Poovan).

The microorganism isolated from Nendran variety showing Fusarium wilt like symptoms also showed characters of the *Fusarium* sp. in the culture medium but showed negative results in the pathogenicity studies.

Plate 4.7.a. Chenkadali

Plate 4.7.b. Kadali

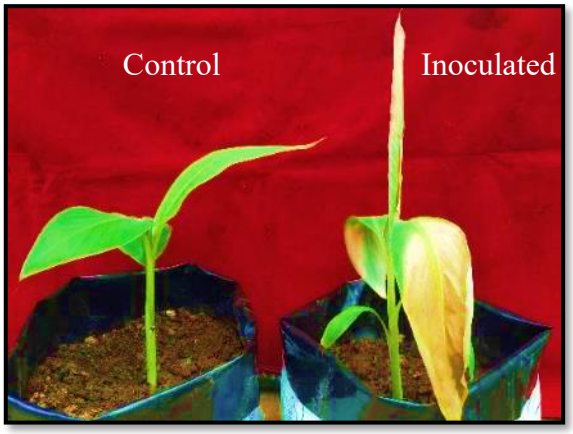


Plate 4.7.c. Rasthali/Poovan

Plate 4.7. Pathogenicity studies of the collected isolates in different varieties collected during the survey

Table 4.6. Disease severity shown by the isolates during pathogenicity test

Isolate No.	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
PWI*	93.33	93.33	86.67	60.00	93.33	46.67	33.33	86.67	66.67	80.00	46.67	93.33	53.33	40.00	80.00
PVWI**	88.89	100.0	77.78	55.56	94.44	44.44	38.89	88.89	61.11	83.33	44.44	83.33	50.00	33.33	77.78

Table 4.6. Disease severity shown by the isolates during pathogenicity test (Contd.)

Isolate No.	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30
PWI*	46.67	73.33	80.00	53.33	93.33	60.00	53.33	66.67	80.00	66.67	86.67	93.33	40.00	53.33	53.33
PVWI**	38.89	72.22	72.22	44.44	83.33	55.56	50.00	66.67	72.22	61.11	83.33	88.89	38.89	66.67	50.00

*Per cent wilt index

**Per cent vascular wilt index

4.5. CHARACTERIZATION OF ISOLATES

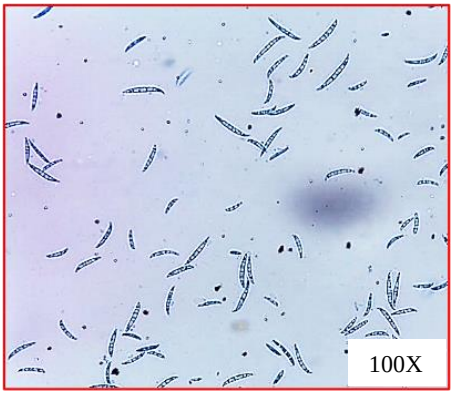
The pathogen associated with the disease was identified based on cultural, morphological and molecular characters of the collected isolates.

4.5.1. Cultural characterization of isolates

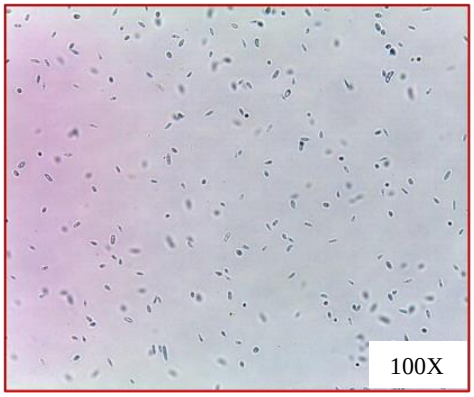
Among the 30 isolates, twenty five isolates produced white coloured aerial mycelium, four of them were greyish white and one was grey. White or pinkish white (S1, S2, S6, S8, S11, S19, S21, S22, S23 and S26) pigmentation was common in most of the isolates while dull-white (S5, S16 and S27), pink (S12, S13, S15, S17, S18, S24 and S25) and violet (S4 and S14) pigmentations were also noticed. Isolates were also variable with respect to colony texture. Cottony and fluffy mycelial mat was the commonly found texture but thin and sparse growth were also seen. Colony diameter ranged from 59.6 to 90.0 mm at seven days after incubation at 25 ºC. Based on the colony diameter after seven days of inoculation, isolates were grouped into 3 categories. Out of the thirty isolates, twenty-three included in the first category which produced more than 80.00 mm colony diameter. Among the remaining isolates, three were classified under the second group coming in between 70.00 to 80.00 mm. Whereas, four isolates belonged to the third category *ie*., the mycelial growth was less than 70.00 mm. The rate of mycelial growth ranged from 0.83 cm/day to 2.40 cm/day. The highest mycelial growth rate (2.40 cm/day) was recorded in the isolate S26 followed by the isolate S10 (2.30 cm/day) and S3 (2.23 cm/day). The lowest mycelial growth rate was recorded in the isolate S25 (0.83 cm/day) The results are presented in Table 4.7.

4.5.2. Morphological characterization of isolates

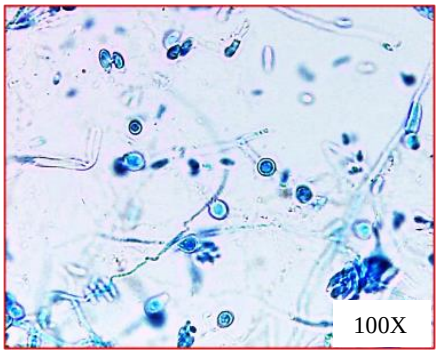
Significant changes were noticed with the size of macroconidia and microconidia between the isolates (Table 4.8). The length and breadth macroconidia ranged from 15.01 to 20.20 µm and 2.14 to 5.07 µm respectively whereas, it ranged from 4.29 to 7.42 µm and 1.35 to 3.13 µm respectively in the case of microconidia. The diameter of chlamydospores varied from 5.68 to 11.07 µm. The macroconidia were 3 to 5 septate and the microconidia were aseptate or single septate . All the isolates were found to be sporodochial in nature (Plate 4.8) and the hyphae were septate. The length and breadth of hyphae ranged from 16.14 µm to 22.94 µm and 4.22 µm to 6.57 µm.



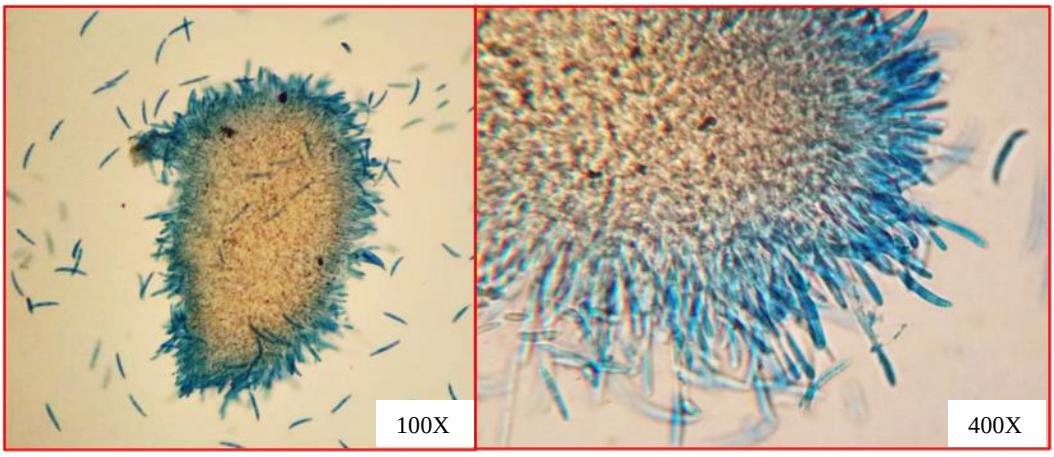
4.8.a. Macroconidia



4.8.b. Microconidia



4.8.c. Chlamydospores



4.8.d. Sporodochium of the pathogen

4.8. Asexual spores and fruiting body of Foc

Sl. No.	Colour and Texture	Pigmentation	Radial growth at 7 DAI (cm)	Rate of mycelial growth (cm/day)
S1	Greyish white, cottony, thin	Whitish pink	9.00	2.20
S2	White, thick, fluffy	Whitish pink	9.00	1.98
S3	Greyish white, cottony	Purplish pink	8.93	2.23
S4	White, cottony	Violet	7.03	1.4
S5	White, cottony	Dull white	9.00	2.10
S6	White, cottony, sparse	Pinkish white	6.53	0.93
S7	White, cottony	White	8.47	1.21
S8	Greyish white, thin	Pinkish white	8.90	1.28
S9	White, thin with zonation	White	8.23	1.18
S10	Grey, cottony	White	9.00	2.30
S11	White, fluffy	Whitish pink	9.00	2.26
S12	White, moderate fluffy	Bright pink	8.73	1.25
S13	White, net like and sparse	White	6.23	0.88
S14	White, very thin	Violet	7.57	1.09
S15	White, thick fluffy	Pink	9.00	2.09

Table 4.7. Cultural characters of the isolates

Table 4.7. Cultural characters of the isolates (Contd.)

Sl. No.	Colour and Texture	Pigmentation	Radial growth at 7 DAI (cm)	Growth rate (cm/day)
S16	White, moderate fluffy	Dull white	8.10	1.16
S17	White, thin and sparse	Pink	8.33	1.20
S18	White, cottony	Pink	9.00	1.99
S19	Greyish white, profuse fluffy	Pinkish white	9.00	2.14
S20	White, sparse	White	6.70	0.97
S21	White, thin	Pinkish white	7.37	1.07
S22	White, cottony	Pinkish white	9.00	2.20
S23	White, cottony	Pinkish white	8.46	1.22
S24	White, thick fluffy	Pink	9.00	2.05
S25	White, very thin	Pink	5.96	0.83
S26	White, thick	Pinkish white	9.00	2.40
S27	White, cottony	Dull white	9.00	2.10
S28	White, moderate fluffy	White	8.07	1.13
S29	White, cottony	White	9.00	2.24
S30	White, cottony	White	8.83	1.26

Table 4.8. Morphological characters of the isolates

Sl. No.	Macroconidia (µm)		Microconidia (µm)		Chlamydospores (µm)	Hyphae (µm)	
	Length	Breadth	Length	Breadth		Inter septal length	Breadth
S1	19.15	5.01	7.31	2.24	9.58	22.94	6.57
S2	16.79	3.49	5.01	1.35	6.66	18.64	6.09
S3	16.24	3.01	5.75	2.01	5.81	17.89	4.91
S4	15.01	2.76	5.69	2.19	6.43	17.63	4.88
S5	18.31	3.18	7.24	1.95	8.49	21.63	5.11
S6	18.29	3.45	6.98	2.40	6.93	18.11	5.80
S7	18.22	3.45	6.03	2.15	8.25	19.25	5.02
S8	16.44	2.92	7.19	2.42	7.32	18.45	4.46
S9	19.13	4.37	5.06	2.84	6.58	17.22	5.01
S10	17.25	3.24	4.89	2.93	6.18	16.86	4.64
S11	17.29	2.91	5.19	2.17	6.07	18.78	4.92
S12	16.65	2.63	4.72	1.84	8.92	19.13	5.28
S13	18.45	3.24	5.66	2.04	6.19	19.63	4.89
S14	16.16	2.72	6.16	1.84	6.62	19.66	6.24
S15	18.28	3.55	5.97	3.13	5.99	17.10	5.23

Table 4.8. Morphological characters of the isolates

Sl. No.	Macroconidia (µm)		Microconidia (µm)		Chlamydospores (µm)	Hyphae (µm)	
	Length	Breadth	Length	Breadth		Inter septal length	Breadth
S16	17.46	2.69	4.29	2.18	7.26	18.57	5.58
S17	19.20	5.07	7.42	2.13	9.34	19.88	6.93
S18	15.16	2.41	5.64	2.40	5.68	16.29	4.35
S19	14.91	2.15	5.03	1.87	7.55	16.14	4.57
S20	18.40	3.40	7.35	2.62	6.36	17.96	5.86
S21	18.09	3.67	4.91	1.65	6.38	18.25	5.34
S22	16.58	2.37	6.11	1.87	8.43	17.32	4.22
S23	15.91	2.24	4.97	2.34	6.82	18.43	4.76
S24	18.36	4.21	5.42	2.12	7.36	19.01	5.53
S25	19.04	4.49	5.88	1.41	9.98	19.21	6.28
S26	17.69	3.99	5.72	2.16	6.54	17.56	4.96
S27	16.33	2.22	5.36	2.19	5.87	17.13	5.08
S28	16.70	2.33	6.28	1.47	8.04	16.47	4.33
S29	17.49	3.19	4.49	2.21	6.46	18.03	4.59
S30	16.40	2.14	6.18	1.75	9.29	17.16	5.42
Average	**17.31**	**3.24**	**5.76**	**2.13**	**7.25**	**18.34**	**5.23**

Based on the cultural and morphological characters, the pathogen associated with the collected isolates were identified as *Fusarium oxysporum* f. sp. *cubense*.

4.5.3. Cluster analysis of Foc isolates based on cultural and morphological characters

Cluster analysis of all 30 isolates was done using cultural and morphological characters as similarity coefficient by Minitab 19 software. The data was classified into quantitative and qualitative data and the dendrogram was constructed based on these variables.

Quantitative parameters included colony diameter, length of macroconidia and microconidia, breadth of macroconidia and microconidia and the diameter of chlamydospores. The dendrogram constructed with quantitative data of cultural and morphological characters is given in Fig. 4.9. The maximum variation among the Foc isolates was observed at zero per cent similarity where two clusters were formed namely A1 and A2. Only five isolates were included in the A2 cluster and the remaining 25 isolates were included in the cluster A1. All the Foc isolates collected from Rasthali/Poovan (AAB) and Njalipoovan (AB) varieties of banana belonged to the cluster A1. Whereas, all the Foc isolates collected from Kadali (AA) and Chenkadali (AA) varieties of banana belonged to the cluster A2. Three subclusters were formed at 33.33 per cent similarity namely B1, B2 and B3. The cluster A1 branched into two sub-clusters (B1 and B2) whereas, the cluster A2 has only one sub-cluster (B3). All the isolates included in the sub-cluster B1 namely 3, 27, 18, 4, 14, 19, 23, 8, 12, 22, 30 and 28 were isolated from Rasthali/Poovan and was again branched into three at 66.67 per cent similarity. Whereas, in the sub-cluster B2, the isolates 6 and 20 were isolated from the variety Njalipoovan having a similarity of nearly 90 per cent and the remaining isolates 2, 10, 11, 29, 16, 9, 15, 24, 26, 13 and 21 were isolated from the Rasthali/Poovan variety. The isolates in the sub-cluster B3 *viz*., 1, 17, 5 and 25 were isolated from Kadali variety and the isolate number 7 was from Chenkadali variety.

Qualitative parameters used for cluster analysis were colour of mycelium, the texture of mycelium and pigmentation. The dendrogram constructed with qualitative data of cultural and morphological characters is given in the Fig. 4.10. The maximum variability with respect to qualitative data was observed at 100 per cent dissimilarity. All the isolates were classified into two clusters namely A1 and A2. Nine isolates were included in the cluster A1 which consists of only one sub-cluster (B1). Whereas the remaining 22 isolates included in the A2 cluster which is again divided into two sub-clusters (B2 and B3). Among the isolates belonged to B1 sub-cluster, the isolates 2, 9, 8, 21, 15, 24 and 14 were isolated from Rasthali/Poovan and17 and 25 were isolated from Kadali variety. In the B2 sub-cluster, the isolates 3, 22, 23, 4, 18, 7, 27, 29, 30 and10 were isolated from Rasthali/Poovan, isolate 6 from Njalipoovan and isolate 5 from Kadali variety whereas, in B1 sub-cluster, the isolates 1, 11, 13, 20, 28, 12, 16, 19 and 26 were isolated from Rasthali/Poovan, isolate 1 from Kadali and the isolate 20 from Njalipoovan.

4.5.4. Molecular detection and characterization of the isolates

Molecular detection and characterization of all thirty isolates collected from six districts of Kerala were carried out for the further confirmation of pathogen.

4.5.4.1. Isolation of fungal genomic DNA

Genomic DNA of the collected samples was extracted from fungal cultures by modifying the method described by Ingle and Ingle (2013) as mentioned in 3.5.4.1.

4.5.4.2. Assessment of quality and quantity of isolated DNA

Genomic DNA extracted from all the isolates collected from various locations was quantified using NanoDrop 1000 spectrophotometer (ThermoScientific, USA). The concentration and absorbance ratio at 260 nm and 280 nm of isolated DNA are given in the Table 4.9. The concentration of extracted DNA varied from 125.70 to 2627.32 ng/µl. OD ratio at $\lambda_{260}/\lambda_{280}$ was in between 1.8 to 2.0 indicated the good quality of DNA. The absorbance ratio of isolated DNA ranged from 1.82 to 2.19, indicated thar the DNA is of good quality and free from impurities such as RNA.

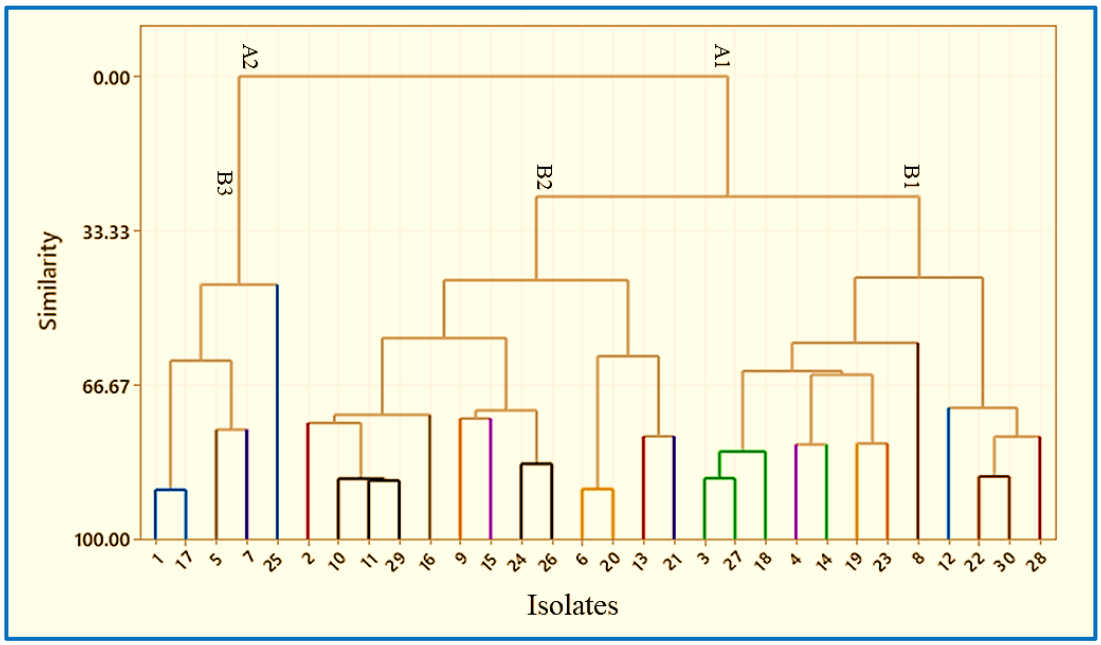


Fig. 4.9. The dendrogram of quantitative data of cultural and morphological characters of the isolates

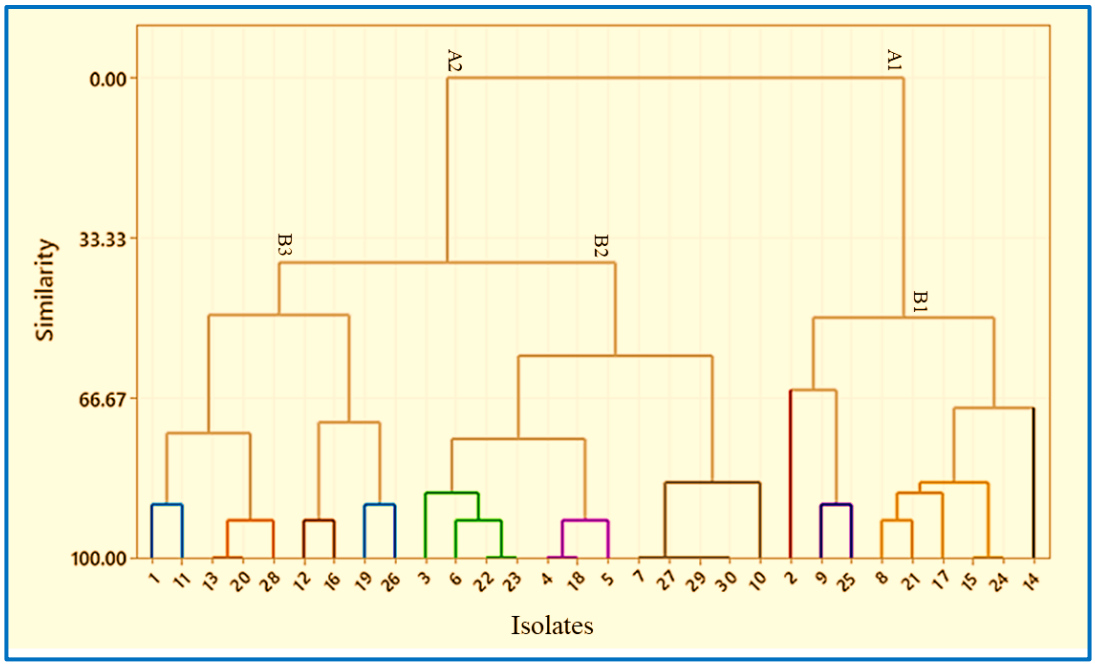


Fig. 4.10. The dendrogram of qualitative data of cultural and morphological characters of the isolates

The isolated DNA was visualized using gel documentation system BIORAD Molecular Imager (Gel Doc^{TM} XR+). It produced distinct bands for all the thirty isolates (Plate 4.9).

4.5.4.3. Molecular characterization of the pathogen by thermal cycling using ITS primers

Amplification of internal transcribed region of rDNA isolated from all thirty isolates was done using ITS primers *viz*., ITS 1F (5'TCCGTAGGT GAACCTGCGG3') and ITS 4R (5'TCCTCCGCTTATTGATATGC3') in a PCR master cycler.

4.5.4.3.a. Standardization of annealing temperature

Among the different temperatures used for standardization of annealing temperature employing gradient PCR, 54.9 °C produced good quality bands. So, this temperature was selected as optimum and used for the further amplification of isolates using ITS 1F and ITS 4R primers (Plate 4.10).

4.5.4.4. Analysis of amplicons

Amplicons produced by amplification of rDNA region using universal ITS primers also were documented using the gel documentation system, which produced single band of 580 bp (Plate 4.11). No band was produced in non-template control reactions.

4.5.4.5. Sequencing of the amplicons

The rDNA amplicons were Sanger sequenced employing automated sequencing at AgriGenome Pvt. Ltd., Kochi. The nucleotide sequences obtained for thirty isolates are given in the Appendix III.

4.5.4.6. In silico analysis of the sequences

The nucleotide sequences of all the isolates were compared with other sequences of Foc available in NCBI database employing BLASTn program. Isolates had 96 to 100 per cent sequence homology to the sequences of Foc (Table 4.10. and Fig. 4.11 - 4.12).

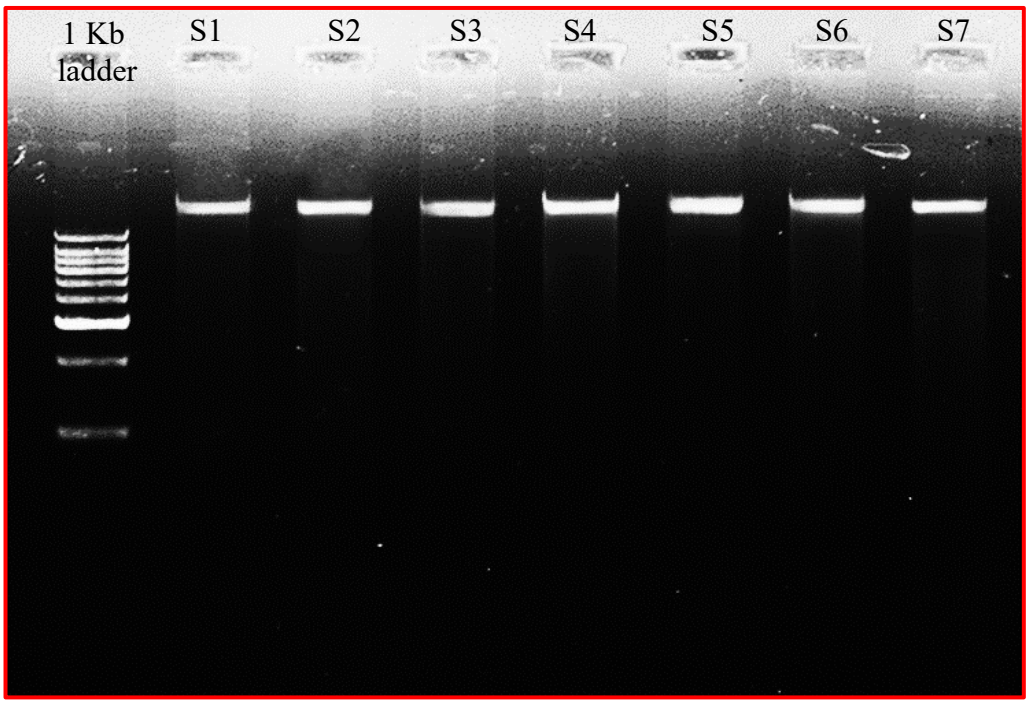


Plate 4.9. Gel documentation of isolated DNA from different isolates

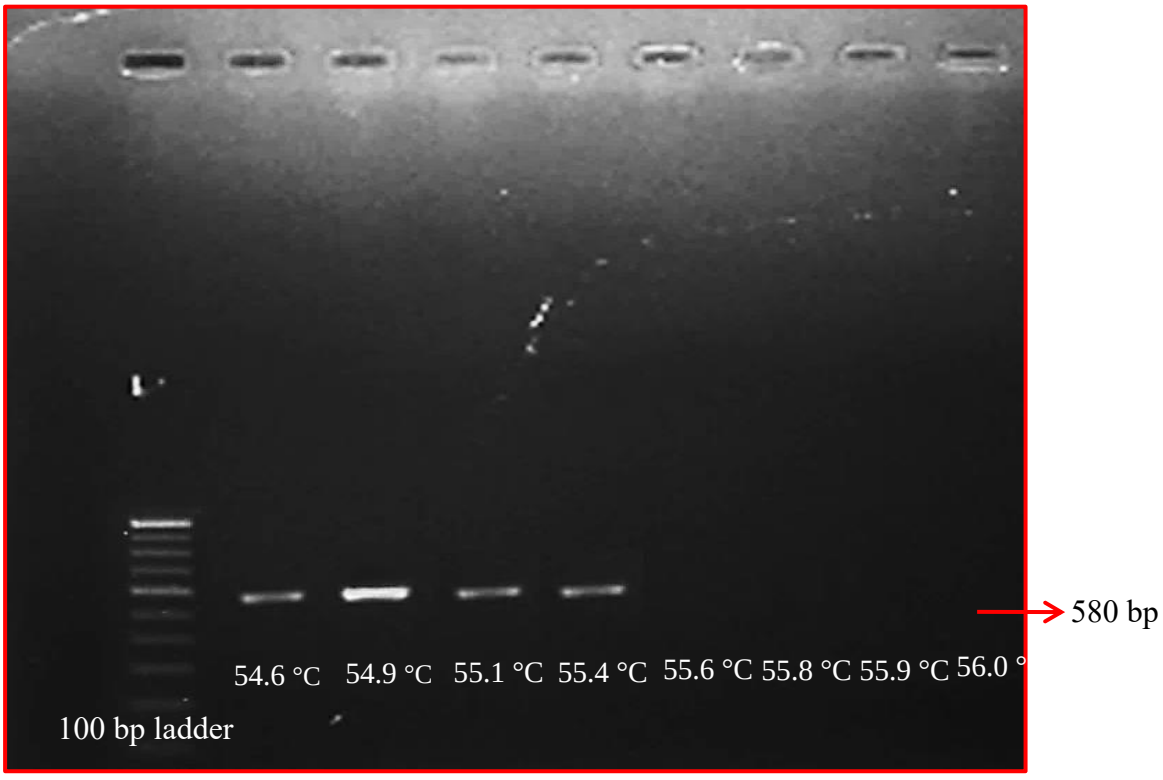


Plate 4.10. Standardization of annealing temperature for PCR using ITS primers

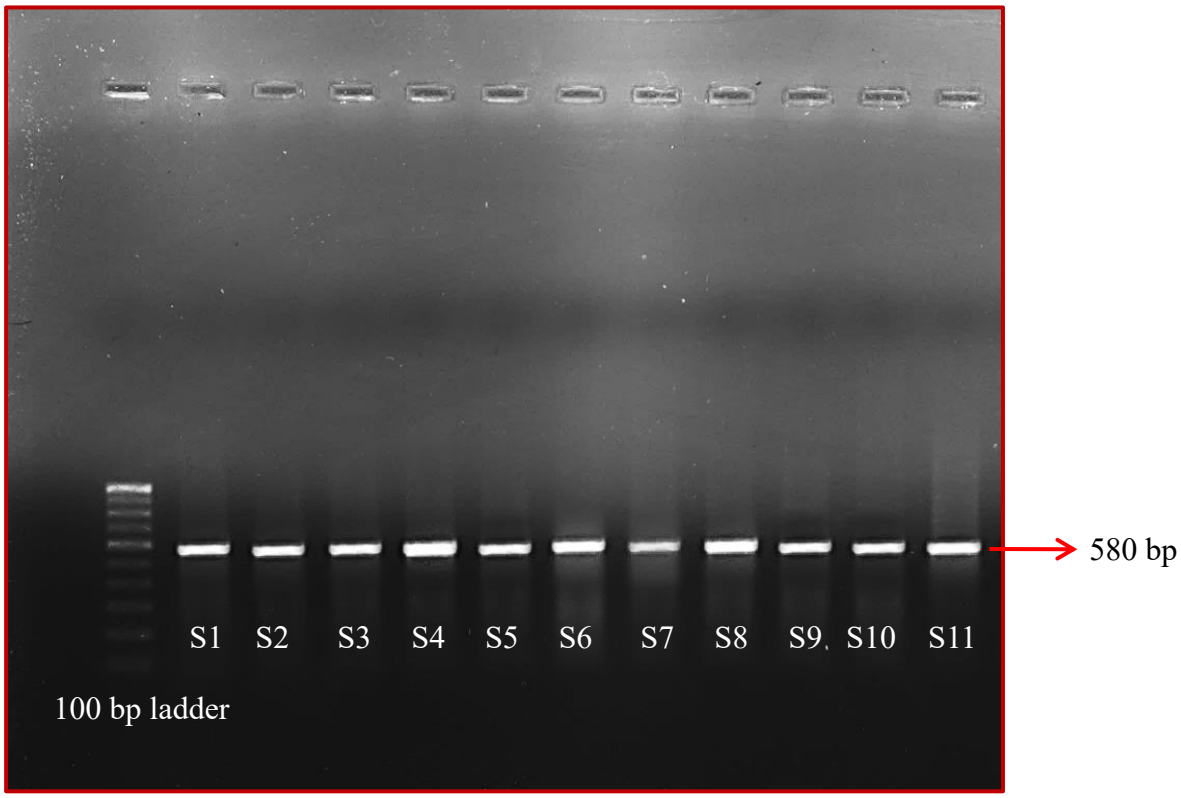


Plate 4.11. Gel documentation of PCR amplicons using ITS primers

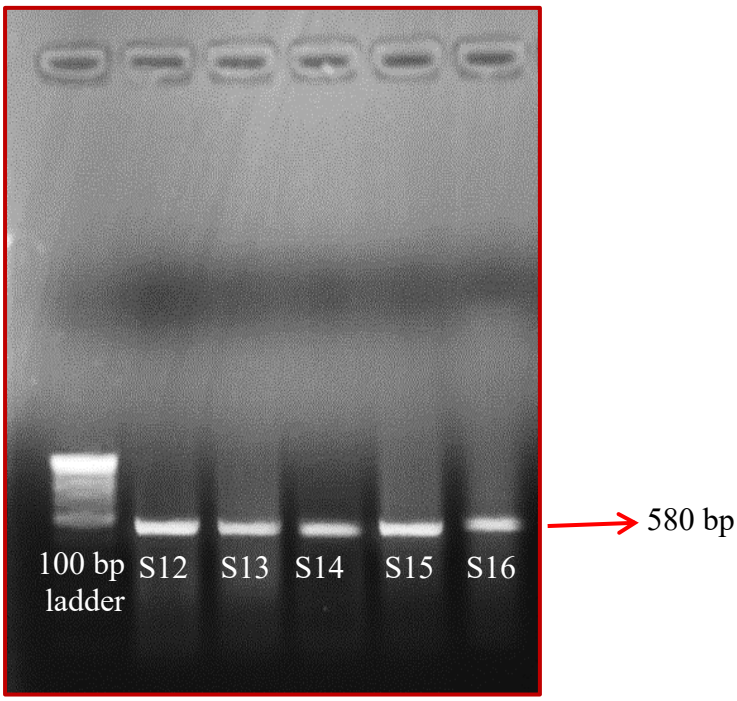


Plate 4.11. Gel documentation of PCR amplicons using ITS primers (Contd.)

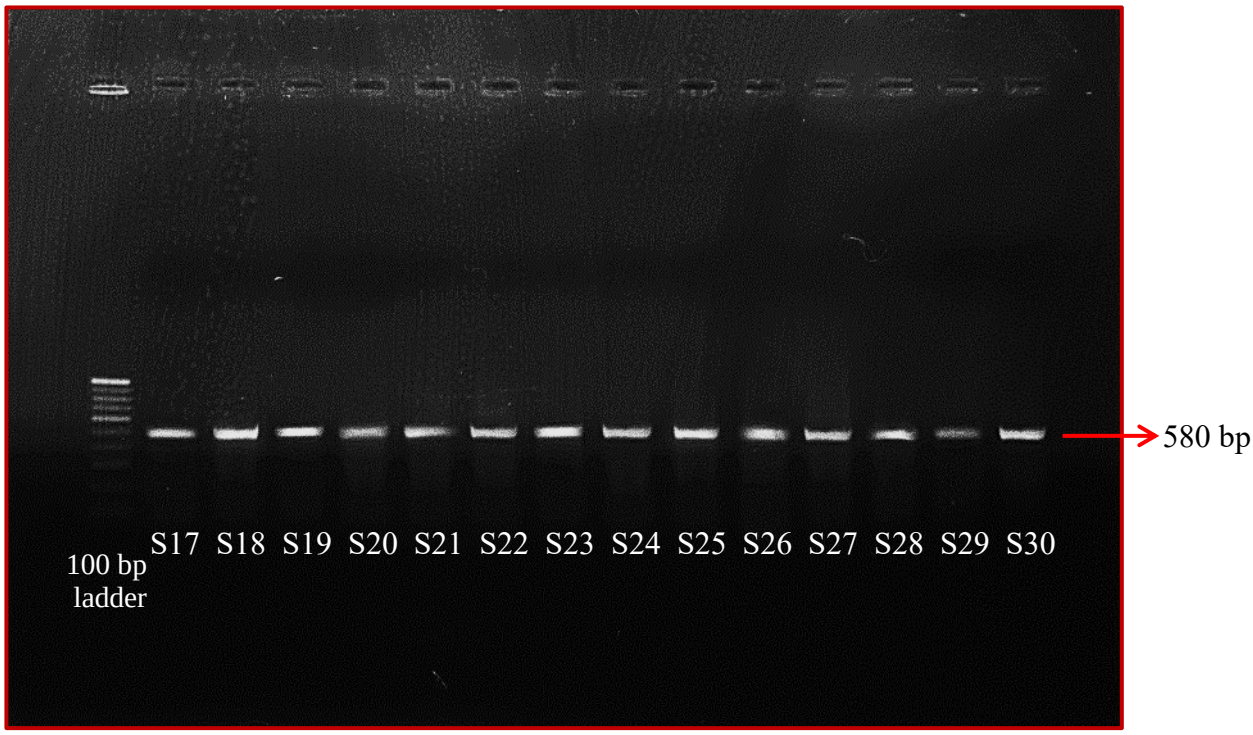


Plate 4.11. Gel documentation of PCR amplicons using ITS primers (Contd.)

Table 4.9. Quality and quantity of DNA isolated from collected isolates during survey

Sl. No.	Quality (260/280 OD)	Quantity (ng/µl)
S1	2.05	1589.7
S2	2.08	852.0
S3	1.93	2080.9
S4	1.83	1647.8
S5	1.97	2398.7
S6	1.97	1935.0
S7	1.89	463.7
S8	1.90	2627.32
S9	1.86	147.7
S10	1.94	207.51
S11	1.82	411.8
S12	1.88	125.70
S13	1.82	136.22
S14	2.14	471.43
S15	2.11	2061.8

Table 4.9. Quality and quantity of DNA isolated from collected isolates during survey (Contd.)

Sl. No.	Quality (260/280 OD)	Quantity (ng/µl)
S16	1.92	386.88
S17	2.01	1545.8
S18	1.91	360.6
S19	1.96	1449.5
S20	2.13	1331.1
S21	2.03	172.93
S22	2.22	887.95
S23	2.14	1733.1
S24	1.85	424.22
S25	2.19	522.84
S26	2.10	736.1
S27	1.95	1253.2
S28	1.84	562.7
S29	2.12	2314.4
S30	1.99	417.93

Table 4.10. Nucleotide BLASTn analysis of the collected isolates during survey

Isolate No.	Query coverage (%)	E value	Per cent identity (%)	Most similar isolate	Accession number from NCBI
S1	100	0.0	100.00	MH681689 (*Fusarium oxysporum* f. sp. *cubense*)	MN519712.1
S2	100	0.0	99.25	MF540565 (*F. oxysporum* f. sp. *cubense*)	MN520842.1
S3	99.00	0.0	100.00	EF590328.1 (*F. oxysporum* f. sp. *cubense*)	MN752172.1
S4	97	0.0	99.78	MF540565 (*F. oxysporum* f. sp. *cubense*)	MN520598.1
S5	99	0.0	100.00	MH854824 (*F. oxysporum* f. sp. *cubense*)	MN520604.1
S6	100	0.0	100.00	MH681689 (*F. oxysporum* f. sp. *cubense*)	MN520606.1
S7	99	0.0	100.00	MF540565 (*F. oxysporum* f. sp. *cubense*)	MN520635.1
S8	100	0.0	99.54	MH681689 (*F. oxysporum* f. sp. *cubense*)	MN527251.1
S9	100	0.0	99.77	MH681689 (*F. oxysporum* f. sp. *cubense*)	MN527254.1
S10	100	0.0	100.00	EF590328 (*F. oxysporum* f. sp. *cubense*)	MN527256.1

Table 4.12. Nucleotide BLASTn analysis of the collected isolates during survey (Contd.)

Isolate No.	Query coverage (%)	E value	Per cent identity (%)	Most similar isolate	Accession number from NCBI
S11	99	0.0	99.35	MF540565.1 (*F. oxysporum* f. sp. *cubense*)	MN527257.1
S12	100	0.0	99.76	MF540565.1 (*F. oxysporum* f. sp. *cubense*)	MN527356.1
S13	94	0.0	99.30	MH681689.1 (*F. oxysporum* f. sp. *cubense*)	MN527468.1
S14	100	0.0	99.76	MF540565.1 (*F. oxysporum* f. sp. *cubense*)	MN527520.1
S15	99	0.0	99.82	EF590328.1 (*F. oxysporum* f. sp. *cubense*)	MN527522.1
S16	99	0.0	99.80	EF590328.1 (*F. oxysporum* f. sp. *cubense*)	MN528143.1
S17	99	0.0	99.82	EF590328.1 (*F. oxysporum* f. sp. *cubense*)	MN528565.1
S18	86	0.0	99.43	MH454073.1 (*F. oxysporum* f. sp. *cubense*)	MN749627.1
S19	100	0.0	100.00	EF590328.1 (*F. oxysporum* f. sp. *cubense*)	MN663127.1
S20	100	0.0	100.00	MF540565.1 (*F. oxysporum* f. sp. *cubense*)	MN663130.1

Table 4.12. Nucleotide BLASTn analysis of the collected isolates during survey (Contd.)

Isolate	Query coverage (%)	E value	Percent identity (%)	Most similar isolate	Accession number from NCBI
S21	99.00	0.0	100.00	EF590328.1 (*F. oxysporum* f. sp. *cubense*)	MN663148.1
S22	99.00	0.0	99.62	EF590328.1 (*F. oxysporum* f. sp. *cubense*)	MN663157.1
S23	100.00	0.0	99.78	MH454072.1 (*F. oxysporum* f. sp. *cubense*)	MN749615.1
S24	100.00	0.0	100.00	MF540565.1 (*F. oxysporum* f. sp. *cubense*)	MN749616.1
S25	99.00	0.0	100.00	MH454072.1 (*F. oxysporum* f. sp. *cubense*)	MN749617.1
S26	100.00	0.0	100.00	MH454072.1 (*F. oxysporum* f. sp. *cubense*)	MN752175.1
S27	100.00	0.0	100.00	EF590328.1 (*F. oxysporum* f. sp. *cubense*)	MN752174.1
S28	100.00	0.0	100.00	MH454072.1 (*F. oxysporum* f. sp. *cubense*)	MN749620.1
S29	81.00	0.0	96.03	EF590328.1 (*F. oxysporum* f. sp. *cubense)*	MN749621.1
S30	99.00	0.0	100.00	MH454072.1 (*F. oxysporum* f. sp. *cubense)*	MN749622.1

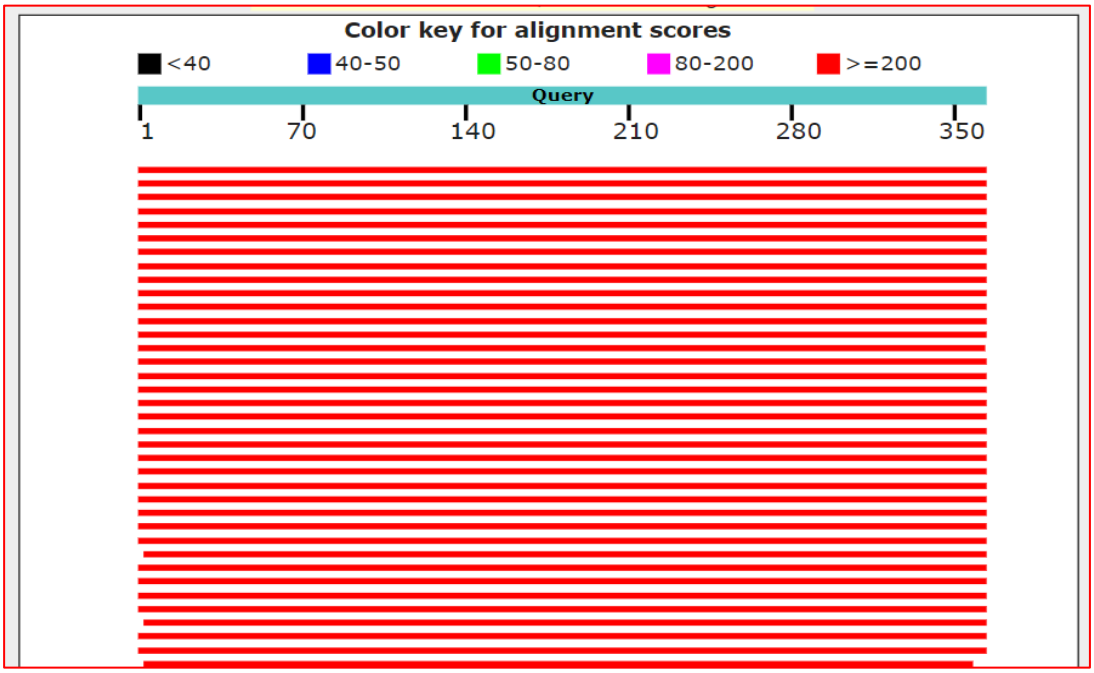


Fig. 4.11. Graphical representation of BLASTn analysis of ITS region in Foc

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	Per. Ident	Accession
Fusarium oxysporum f. cubense isolate A53 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosoma	669	669	100%	0.0	100.00%	MF540565.1
Fusarium oxysporum f. cubense isolate KARA10 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribos	669	669	100%	0.0	100.00%	MH681689.1
Fusarium oxysporum f. cubense strain tropical race 4 isolate WBKBCKV3 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal trar	669	669	100%	0.0	100.00%	MH454073.1
Fusarium oxysporum f. cubense strain tropical race 4 isolate WBKBCKV2 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal trar	669	669	100%	0.0	100.00%	MH454072.1
Fusarium oxysporum f. cubense strain tropical race 4 isolate WBKBCKV1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal trar	669	669	100%	0.0	100.00%	MH454071.1
Fusarium oxysporum f. cubense isolate PAR44 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosc	669	669	100%	0.0	100.00%	MH681690.1
Fusarium oxysporum f. cubense isolate KABA9 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosc	669	669	100%	0.0	100.00%	MH681688.1
Fusarium oxysporum f. cubense strain BW1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA ge	669	669	100%	0.0	100.00%	KC869369.1
Fusarium oxysporum f. cubense strain FJ-23-2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA	669	669	100%	0.0	100.00%	KC869368.1
Fusarium oxysporum f. cubense strain BobaiFJ internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA	669	669	100%	0.0	100.00%	KC869361.1
Fusarium oxysporum f. cubense 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S rib	669	669	100%	0.0	100.00%	HQ694500.1
Fusarium oxysporum f. cubense isolate EPPI04 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequer	669	669	100%	0.0	100.00%	EU022521.1
Fusarium oxysporum f. cubense strain ATCC 96285 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete se	669	669	100%	0.0	100.00%	EF590328.1
Fusarium oxysporum f. cubense strain Race1-VCG 0124 large subunit ribosomal RNA gene, partial sequence	667	787	99%	0.0	100.00%	KJ131497.1
Fusarium oxysporum f. cubense strain CBS 149.25 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, com	664	664	100%	0.0	99.72%	MH854824.1
Fusarium oxysporum f. cubense strain Race-3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA	664	664	100%	0.0	99.72%	KC869371.1
Fusarium oxysporum f. cubense isolate A63 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosoma	658	658	100%	0.0	99.45%	MF540564.1

Fig. 4.12. Text output of BLASTn analysis of ITS region of Foc

The sequences consisted of the partial sequence of 18S rDNA and 28S rDNA and the full sequence of ITS 1 region, ITS 2 region and 5.8S rDNA.

The isolate collected from Nendran variety showing Fusarium wilt like symproms were also subjected to PCR and nucleotide sequencing (Appendix IV). The *in silico* analysis of the ITS rDNA sequence revealed that the isolate had a homology of 94.83 per cent to the sequence of *Fusarium redolens*.

4.5.4.7. Deposition of sequences

Nucleotide sequences of 30 Foc isolates after *in silico* analysis were deposited in the NCBI database through NCBI BankIt software and the accession number for each isolate was received. The details of deposited sequences are shown in the Appendix V.

4.5.4.8. Phylogenetic analysis

The phylogenetic tree was constructed with 30 local isolates and 17 *F . oxysporum* sequences including that of *F. oxysporum* f. sp. *cubense*, *F. oxysporum* f. sp. *pisi*, *F. oxysporum* f. sp. *cepae*, *F. oxysporum* f. sp. *vasinfetum* and *F. oxysporum* f. sp. *melonis*, *F. oxysporum* f. sp. *conglutinans and F. oxysporum* f. sp. *lycopersici*, and two sequences of *F. solani*, one sequence of *F. tricinctum* and *F. graminium* taken from NCBI database (Fig. 4.13). It showed that all the isolates have a greater similarity to the formae speciales of *F. oxysporum. F. oxysporum* formed a cluster including all the formae speciales of *F. oxysporum* and *F. tricintum.* Whereas, the other *Fusarium* spp. formed separate cluster in the phylogenetic tree. The former cluster was again divided into sub-clusters in which, one sub-cluster consists of all isolates including all formae speciales of *F. oxysporum*. Whereas, the other sub-cluster involves the *F. tricinctum*. All the fungi formed a monophyletic group and it indicates that all the *Fusarium* spp. have a common ancestor. All the isolates were diverged into different groups of the sub-cluster irrespective of the location and the grouping may be due to some mutations in the organisms.

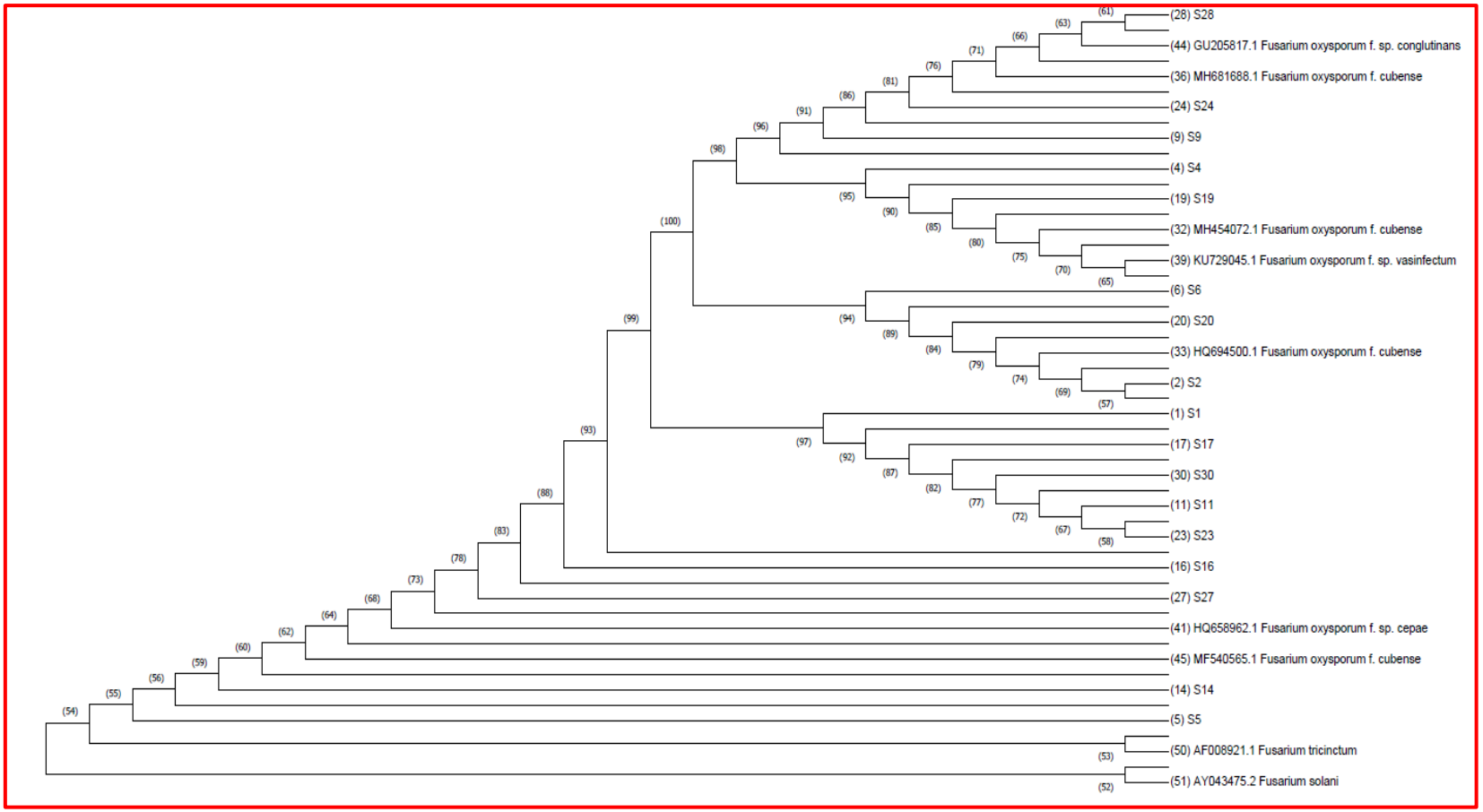


Fig. 4.13. Phylogenetic tree generated from ITS-rDNA sequences of *Fusarium* spp.

4.5.4.9. Barcoding of the sequences

The sequence alignment of Foc isolates with other formae speciales of *F. oxysporum* revealed that there was no changes in the sequences at ITS rDNA region (Fig. 4.14). But variations were noticed in the sequences of Foc isolates with other *Fusarium* spp. at various positions. In this alignment, a gap of 1 bp was observed at 576th position in *F. oxysporum* whereas it was present in all other *Fusarium* spp. (Fig. 4.15).

4.5.4.10. Isolation of fungal DNA directly from infected tissue and the characterization of the pathogen

Extraction buffer used in this protocol was the modifications of an earlier method for extraction of DNA from Foc culture (Ingle and Ingle, 2013). In this protocol, 2 per cent CTAB and 20 mM EDTA were added instead of 1 per cent CTAB and 25 mM EDTA used in the former method. In addition to this, 50 µl of ß-mercaptoethanol and 1 per cent polyvinylpyrrolidone were also used during the grinding of 1 mg infected tissue which was collected from the field experiment at Banana Research Station, Kannara. This method of DNA isolation avoids the time consuming and difficult process explained in former methods. In this protocol, isolation of DNA directly from infected tissue and avoidance of liquid nitrogen help to reduce the time and labour consuming step during DNA isolation. EDTA inactivates the endonucleases and ß-mercaptoethanol prevents the phenolic oxidation. CTAB avoids the polysaccharides contamination whereas, the isopropanol and ethanol allow actual precipitation of DNA and removes the salts and detergents, making it more stable. The quantity of DNA extracted was measured using a NanoDrop spectrophotometer. The concentration of DNA yield was 751.6 ng/µl. OD ratio λ260/ λ280 obtained was 1.89. Gel electrophoresis of the isolated DNA was carried out and documented (Plate 4.12).

The isolate was amplified at 580 bp with ITS 1F and ITS 4R primers and the PCR product obtained was compared with GeNeiTM StepUpTM 100 bp DNA ladder. Plate 4.13 shows the amplification of the PCR product in 1.5 per cent agarose gel.

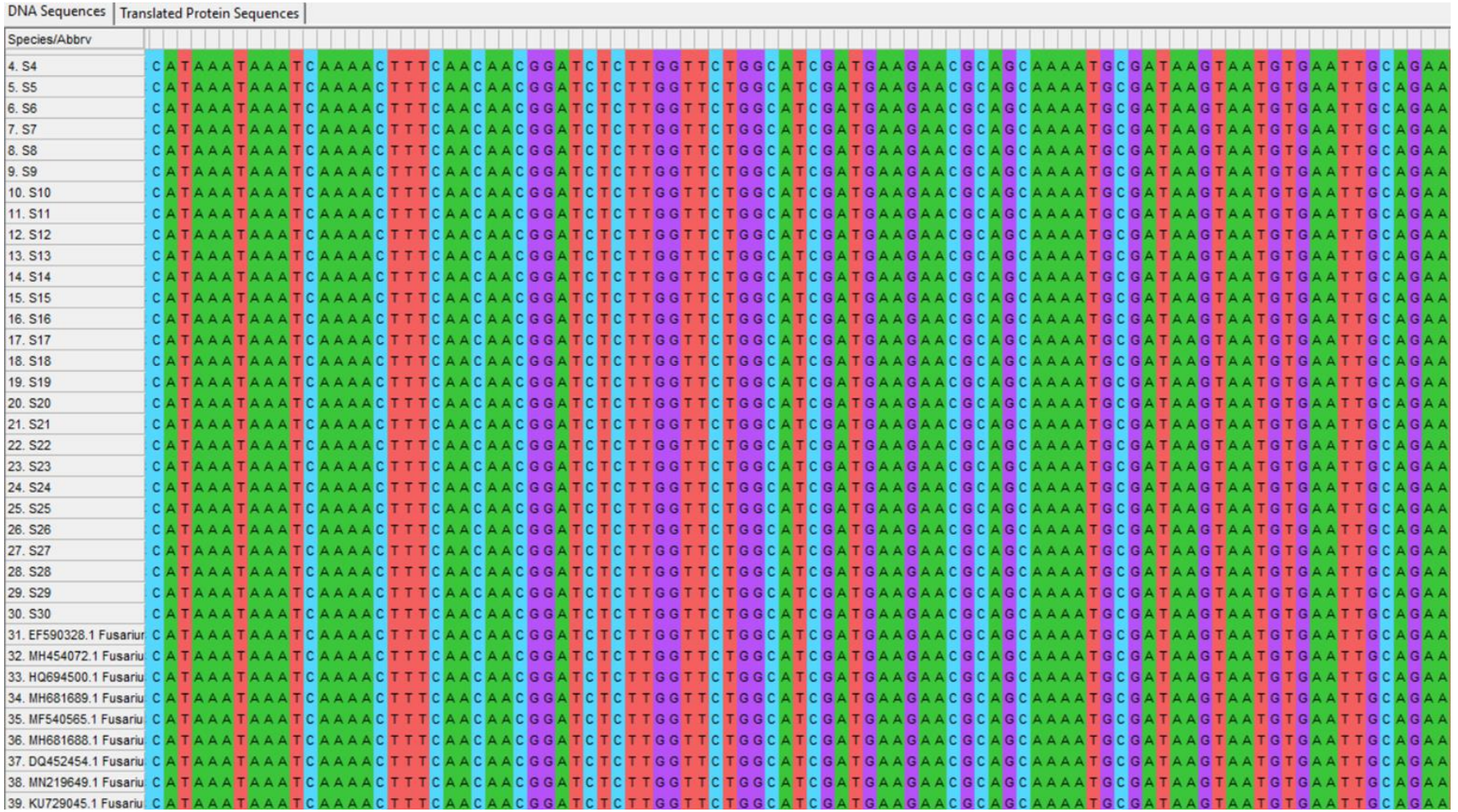


Fig. 4.14. Sequence alignment of formae speciales of *Fusarium oxysporum*

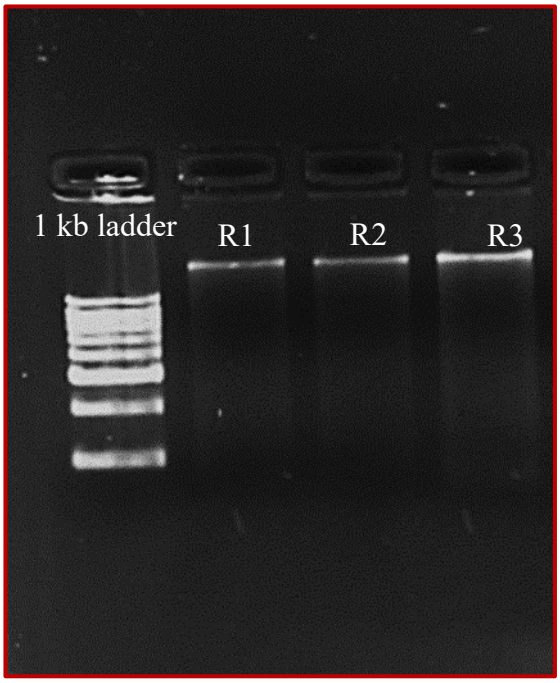


Plate 4.12. Gel documentation of DNA isolated directly from Foc infected plant tissue

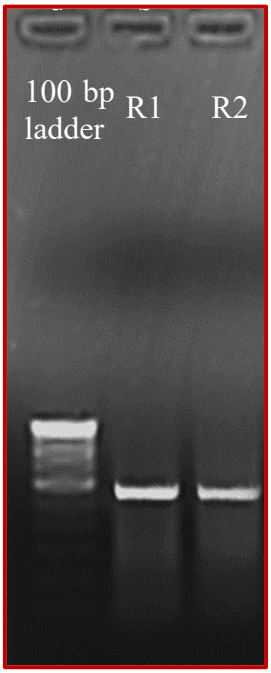


Plate 4.13. Gel documentation of amplicons of DNA isolated directly from Foc infected tissue

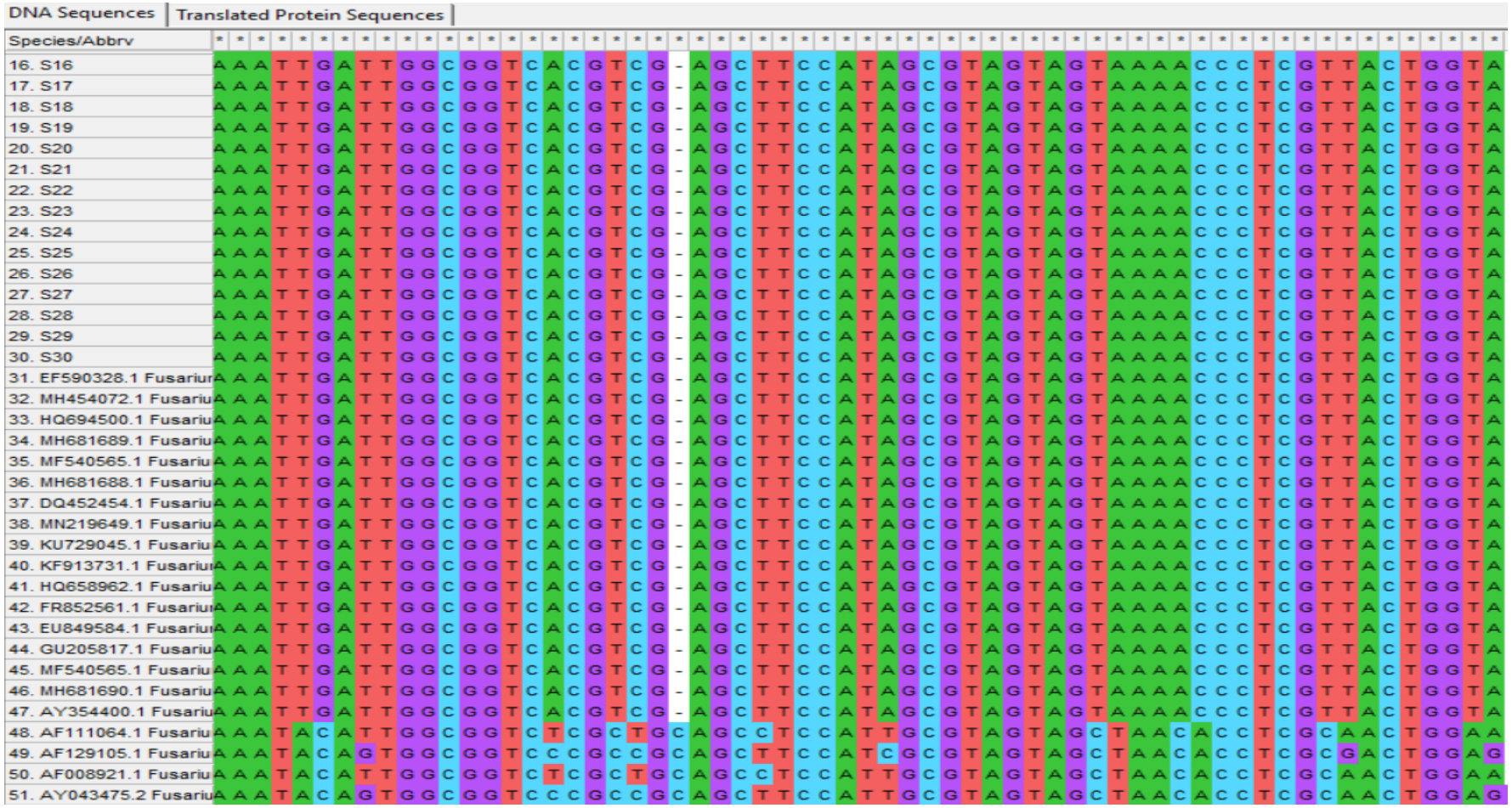


Fig. 4.15. Sequence alignment of formae speciales of *F. oxysporum* compared to other *Fusarium* spp.

The sequencing of amplicon obtained through PCR was done (Appendix VI) and compared with top hit accessions available in NCBI database. *In silico* analysis showed that the isolate exhibited 100 per cent similarity with *F. oxysporum* f. sp. *cubense* accessions infecting banana. The sequence was submitted in the NCBI database through BankIt program and obtained the accession number MN953004.

The phylogenetic analysis of the isolate MN953004 was done by aligning the sequence with the top hit accessions of *F. oxysporum* f. sp. *cubense* available in NCBI. The phylogenetic tree constructed by the Neighbor-Joining algorithm with the bootstrap method was shown in the Fig. 4.16. The sequences were classified into two major clades. The isolate MN953004 was clustered in the first clade along with seven other Foc isolates taken from NCBI and showed maximum similarity with the accession MH454072 from West Bengal of Foc.

4.6. IDENTIFICATION OF RACE

Identification of Foc races was done by artificial inoculation into race-specific banana cultivars as well as through a molecular technique, loop mediated isothermal amplification (LAMP) assay.

4.6.1. Artificial inoculation

Isolates collected from various locations during the survey were inoculated to banana cultivars such as Cavendish, Plantain, Rasthali/Poovan, Monthan and *Heliconia* sp. raised in polybags to identify the race of the pathogen (Table 4.11 and Plate 4.14). All the 30 isolates caused infection in Rasthali/Poovan varieties but none in Cavendish, Plantain, Monthan or *Heliconia* sp. The results revealed that all the isolates belonged to the race 1 of the pathogen Foc.

4.6.2. Loop mediated isothermal amplification assay (LAMP)

All the DNA samples from collected isolates of Foc were subjected to LAMP assay for the quick detection of pathogen races.

Plate 4.14.a. Cavendish

Plate 4.14.b. Monthan

Plate 4.14.c. *Heliconia* sp.

Plate 4.14.d. Nendran Rasthali/Poovan

Plate 4.14. Identification of different races of Foc by artificial inoculation in assay

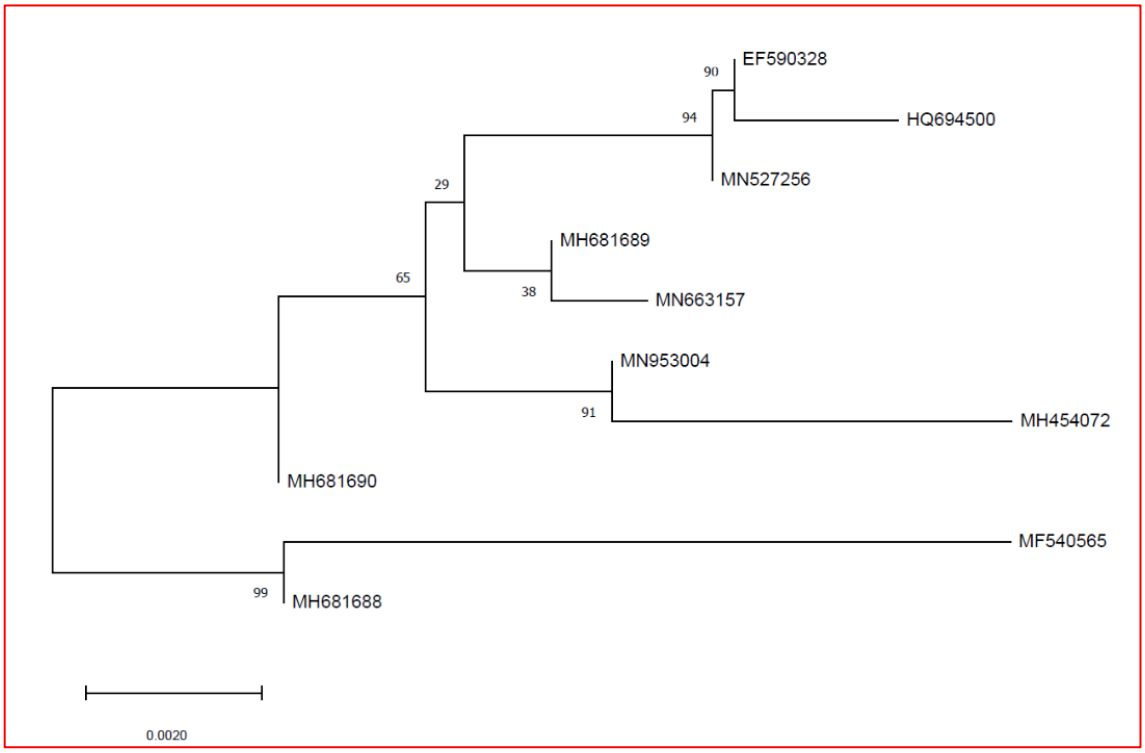


Fig. 4.16. Phylogenetic tree generated from ITS-rDNA sequence of the isolate MN953004 with other *Fusarium* spp.

Table 4.11. Differential host assay for the identification of Foc races

Isolate No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Nendran, Cavendish, Monthan and *Heliconia* sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Poovan/ Rasthali	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 4.11. Differential host assay for the identification of Foc races (Contd.)

Isolate No.	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Nendran, Cavendish, Monthan and *Heliconia* sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Poovan/ Rasthali	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

4.6.2.1. Designing of primers

The standardization of LAMP for the easy detection of Foc race 1 was carried out using the primers designed based on the *endoglucanase 4* gene sequence (Accession number: KB730431.1) of Foc Race 1 obtained from the NCBI database using PrimerExplorer V5 software (Eiken Chemical Co. Ltd., Tokyo, Japan) (http://primerexplorer.jp/e/). The sequences of primers developed are given in the Table 4.12. The positions of designed primers are shown in Fig. 4.17. The primers for the identification of race 4 as suggested by Li *et al*. (2013) were also tested for all the isolates.

4.6.2.2. Standardization of temperature and time

Among the different temperatures (60.0, 61.0, 62.0, 63.0, 64.0 and 65.0 °C) and time (30, 45 and 60 min.) used for the standardization, a temperature of 63 °C for 60 minutes was found to be optimum for the reaction.

4.6.2.3. Standardization of LAMP assay

The entire reaction was set up in a simple water bath at 63 °C for 60 minutes . All the thirty isolates were tested with the race 1 and race 4 primers. The amplification was terminated by incubating the reaction mixture at 80 °C for 10 min. A no template control tube containing only nuclease-free was also maintained.

4.6.2.4. Detection of amplified products

The colorimetric detection of amplified products was done by the addition of 1 µl 150 µM of Hydroxynaphthol blue (HNB) dye (Sisco Research Laboratories Pvt. Ltd.) to the reaction mixture prior to amplification. The reaction mixture turned to blue colour in positive reaction whereas, the control and negative reactions remained purple/violet. The results are shown in Plate 4.15.

Table 4.12. Details of LAMP primers used for the assay

Sl. No.	Primer name	Sequence 5'-3'	No. of base pairs
1	RACE 1 - F3	5′TCCTAAGCGTATATCCCGCC3′	20
2	RACE 1 - B3	5′AGTCAGGCCAGAGAGTCC3′	18
3	RACE 1 - FIP	5′AGTGTAGCCGTTGCACTGCATA-CAATTCCAGGCAACGGACC3′	41
4	RACE 1 - BIP	5′GCTGGTGGTATCAAGGGAAGCT-TCAGGTTGACAGTTGACCCA3′	42
5	RACE 1 - LF	5′CGATGGATGTGACATCCTCAACA3′	23
6	RACE 1 - LB	5′CTGCTGCTCTTCACGCCGAT3′	20
7	RACE 4 - F3	5′AGGACCTCTTCGAATGGCA3′	19
8	RACE 4 - B3	5′GACGCTGCAGCTATGACAA3′	19
9	RACE 4 - FIP	5′GGTGGCTCAATAGCCCAGTGAA-CCGATACCTGTGAAGTCGC3′	41
10	RACE 4 - BIP	5′CGACATCATCAGCATCTCCGCT-AGCTTTGGCTCTTGTGACAG3′	42
11	RACE 4 - LF	5′GCCTAATTGAACATTCAGTATAAAC3′	25
12	RACE 4 - LB	5′ACTCCAAGGAACTAGACGACG3′	21

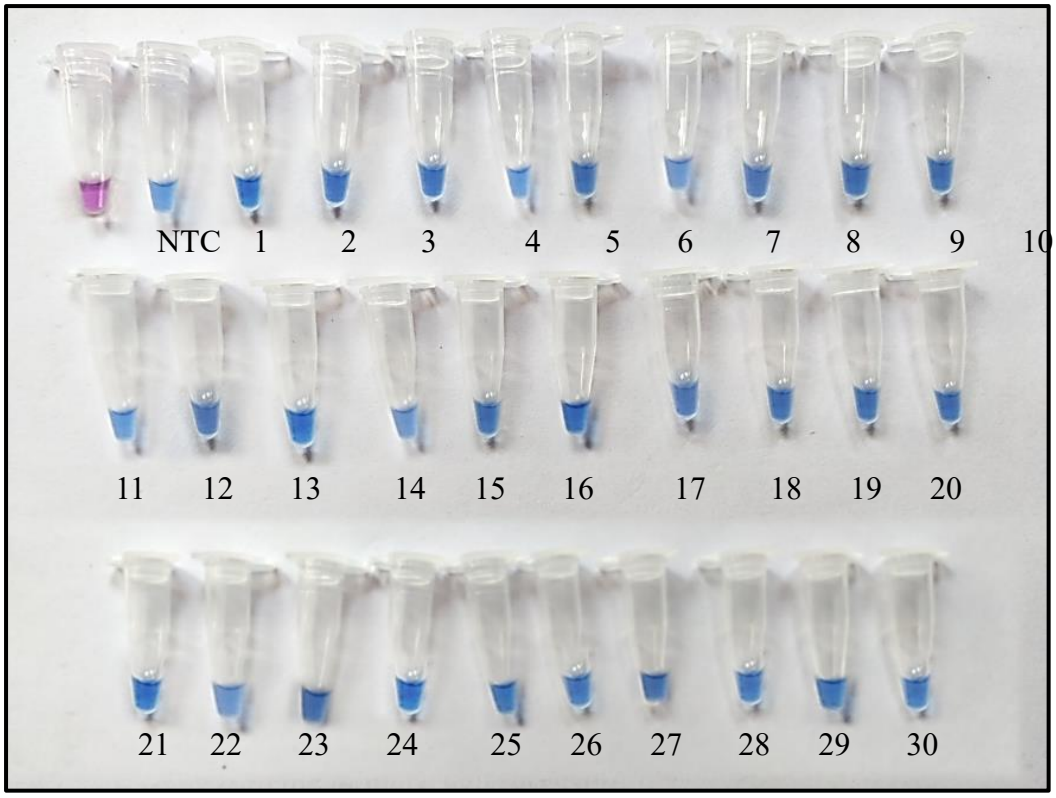


Plate 4.15.a. Amplification using race 1 primers

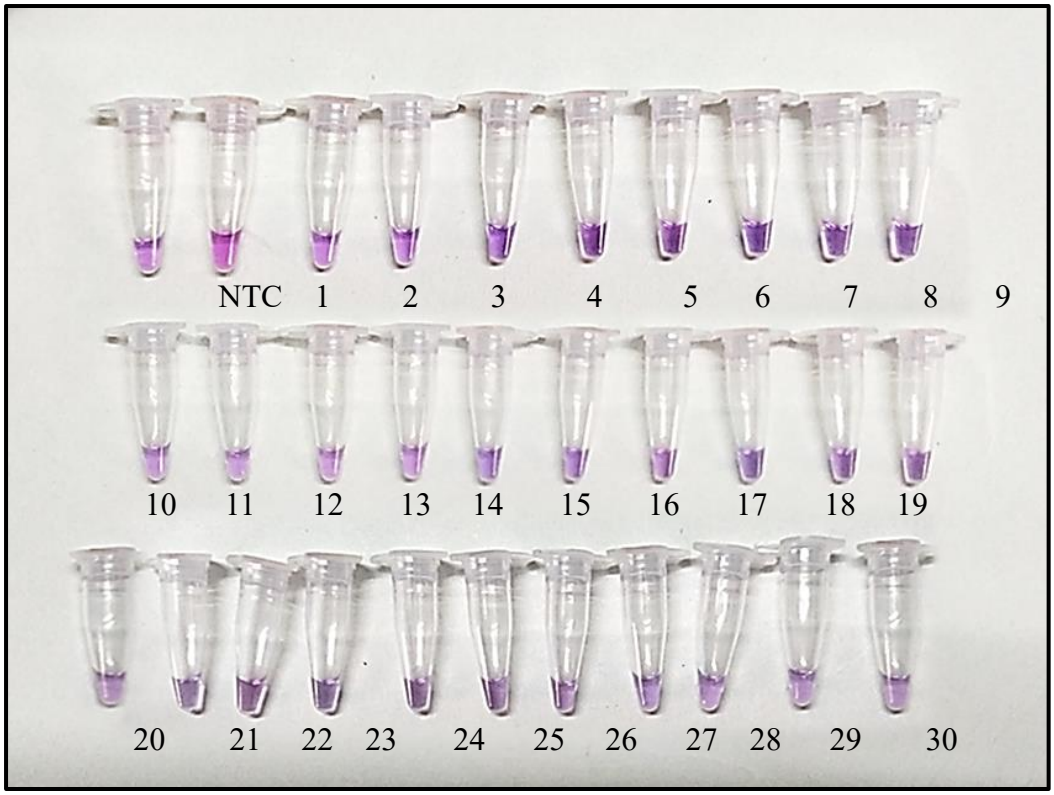


Plate 4.15.b. Amplification using race 4 primers

Plate 4.15. Colorimetric detection of amplicons obtained in the LAMP assay of collected isolates

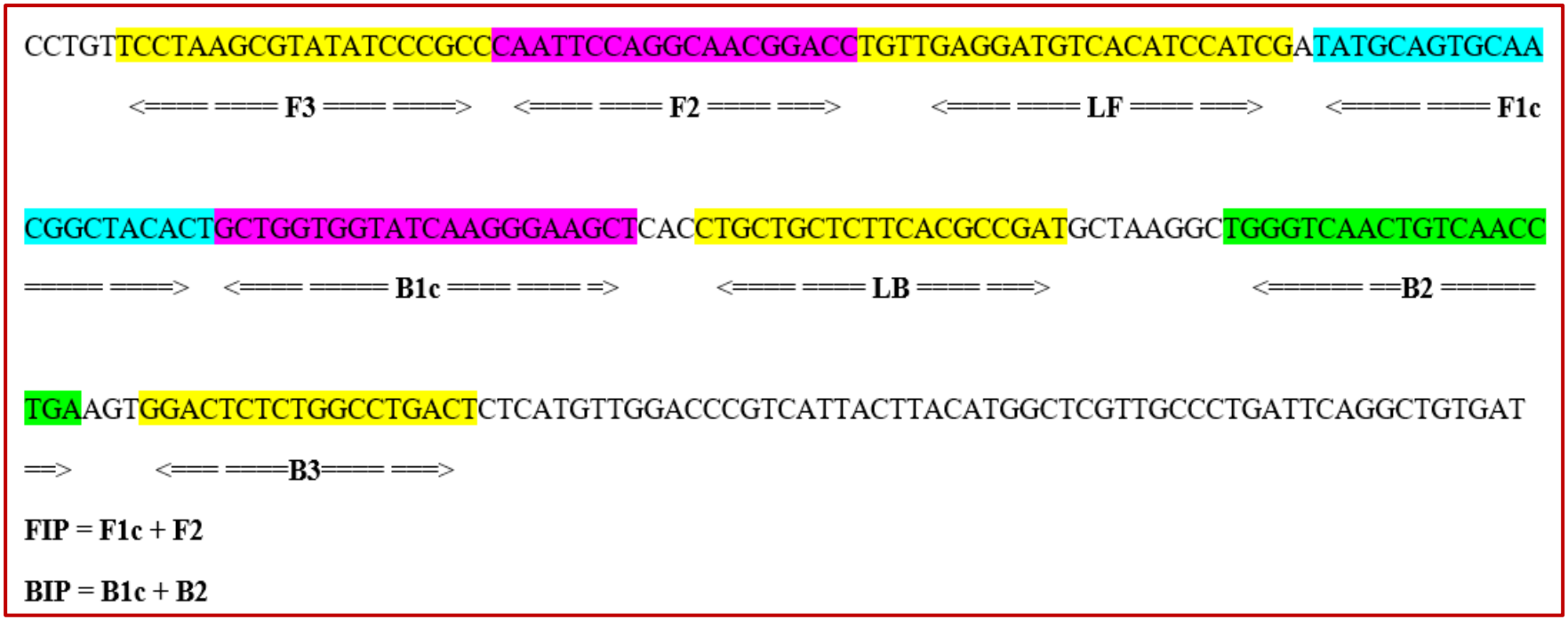


Fig. 4.17. Graphical representation of positions of LAMP primers designed for detecting Foc race 1

All the LAMP products were subjected to 2 per cent gel electrophoresis and visualised under BIORAD Molecular Imager (Gel Doc™ XR+). The positive reactions showed a ladder-like bands under UV light whereas negative/control reactions did not produce any bands (Plate 4.16).

All the isolates showed a positive reaction to the race 1 primers but negative to the race 4 primers. Therefore, the collected isolates belong to the race 1 of Foc.

4.6.3. Polymerase chain reaction using specific primers

In order to confirm the results, a polymerase chain reaction using specific primers was carried out. Amplification of *endoglucanase 4* gene of genomic DNA isolated from all thirty isolates was done using specific primers *viz*., F3 (5′TCCTAAGCGTATATCCCGCC3′) and B3 (5′AGTCAGGCCAGAGAGTCC3′) in a thermal cycler.

4.6.3.1. Standardization of annealing temperature

Among the different temperatures used for standardization of annealing temperature employing gradient PCR, 53.4 °C produced good quality bands of size 240 bp. So, this temperature was selected as optimum and used for the further amplification of isolates using F3 and B3 primers (Plate 4.17).

4.6.3.2. Analysis of amplicons

The analysis of amplicons produced by thermal cycling using Foc race 1 specific F3 and B3 primers was analysed using the gel documentation system BIORAD Molecular Imager (Gel Doc™ XR+). It produced distinct bands for all the thirty isolates of size about 240 bp. The images are shown in Plate 4.18. No bands were produced in no-template control reactions.

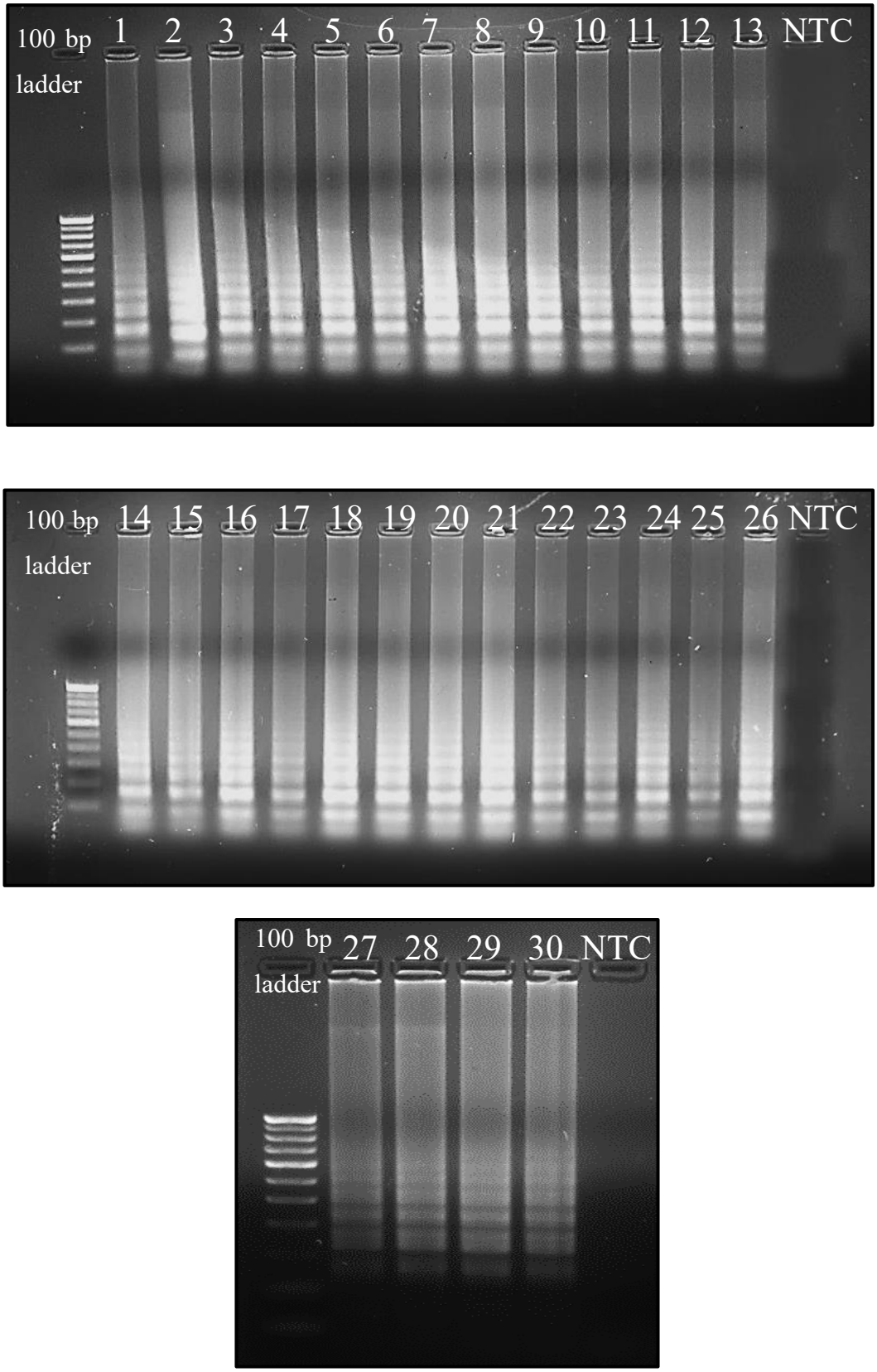


Plate 4.16. Gel documentation of amplicons obtained in the LAMP assay of isolates using race 1 primers

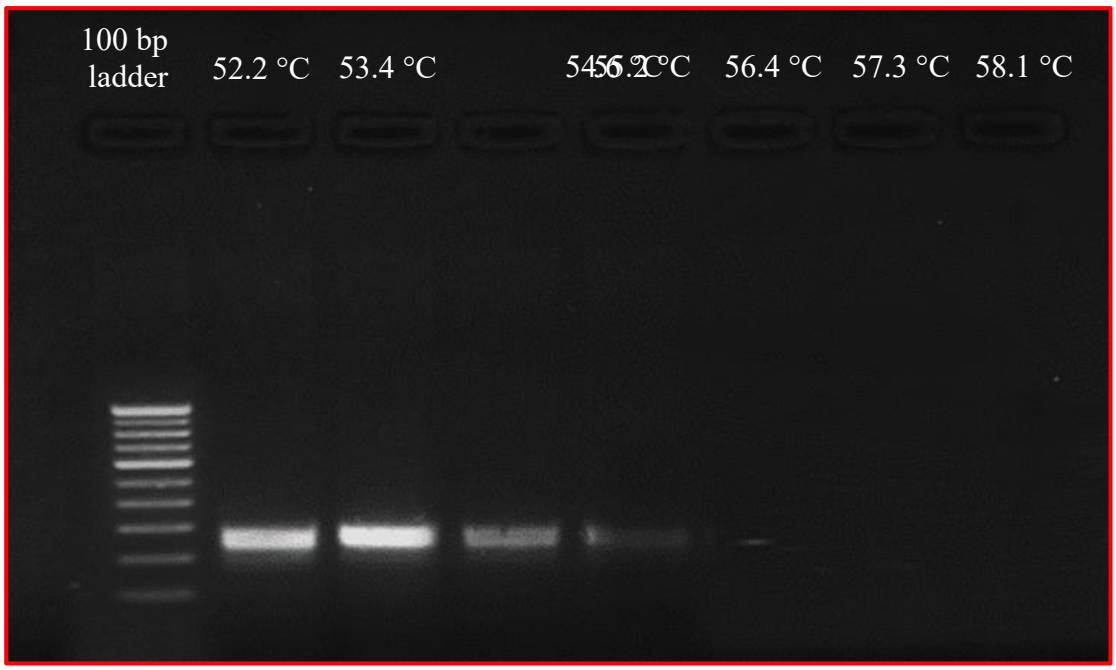


Plate 4.17. Standardization of annealing temperature for PCR of collected isolates using specific primers

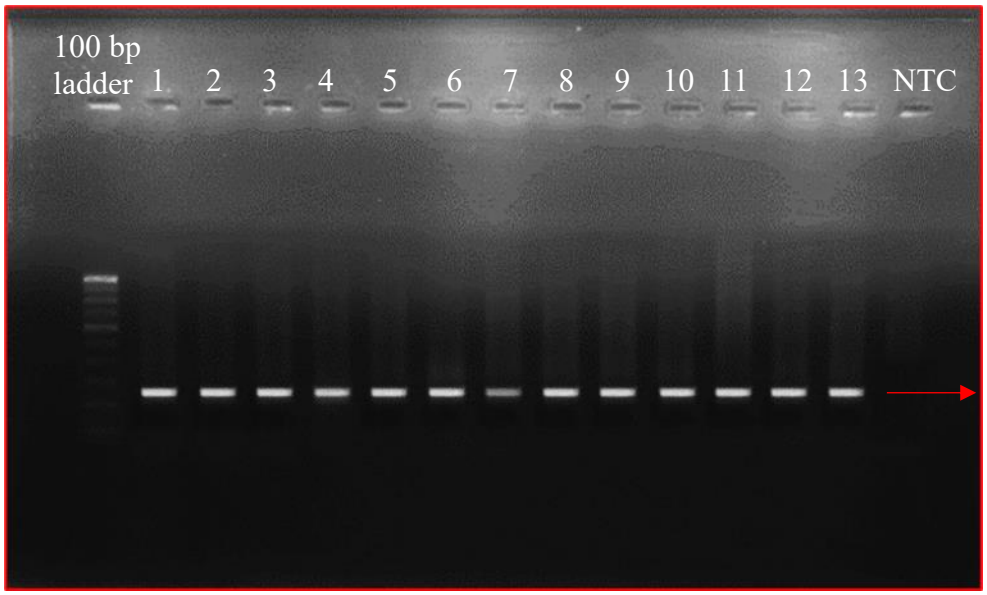


Plate 4.18. Gel documentation of PCR amplicons of collected isolates using specific primers

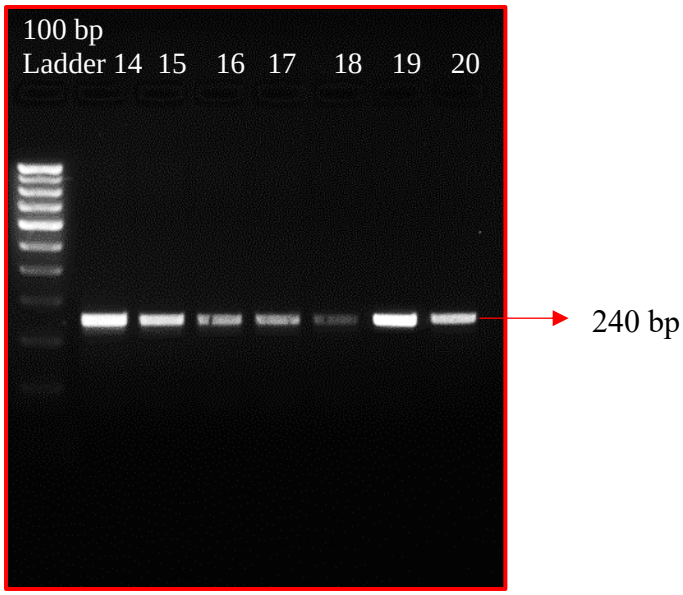


Plate 4.18. Gel electrophoresis of PCR amplicons of collected isolates using specific primers (Contd.)

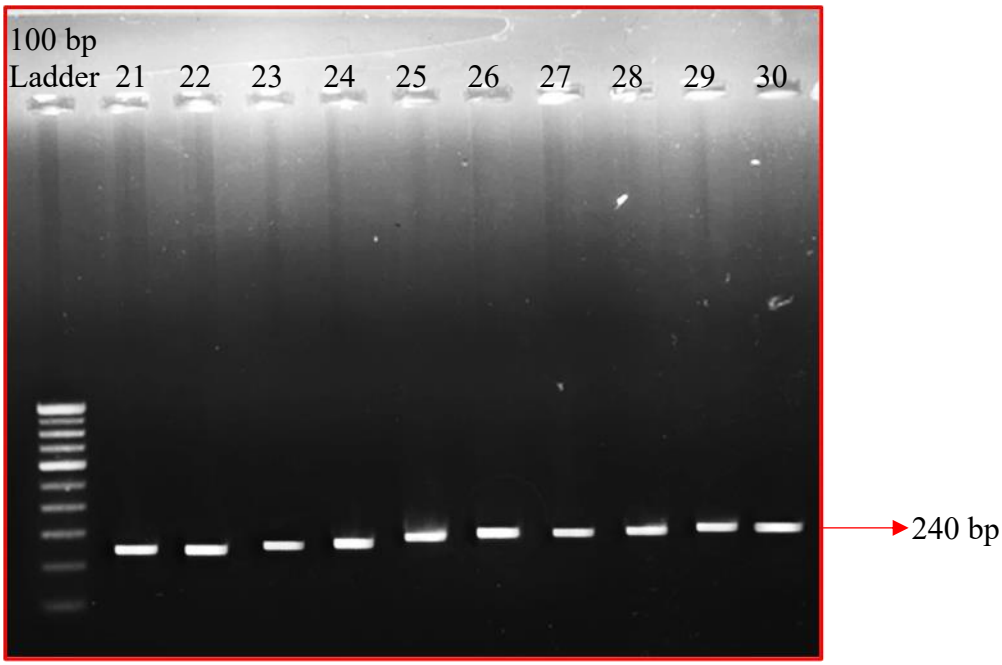


Plate 4.18. Gel electrophoresis of PCR amplicons of collected isolates using specific primers (Contd.)

4.7. EVALUATION OF HOST PLANT DISEASE RESISTANCE

4.7.1. Screening for host plant resistance

The host plant resistance was studied by screening the twenty six germplasm lines selected from the gene bank of Banana Research Station, Kannara by artificial inoculation. Per cent disease incidence (PDI) and per cent disease severity (PDS) based on per cent vascular wilt index (PVWI) and per cent wilt index (PWI) were calculated.

4.7.1.1. Per cent disease incidence (PDI)

Per cent disease incidence was calculated for all twenty six germplasm tested (Table 4.13). Maximum PDI (100 %) was recorded in varieties such as Kadali, Njalipoovan, Cheriya Poovan and Valiya Poovan. Whereas, no disease incidence was recorded in the varieties such as Attunendran, Zanzibar, Big Ebanga, Nedunendran, Nendran, BRS II, Thiruvananthapuram, Pachanadan I, Cultivar Rose, Pisang Lilin, Pisang Jari Buaya, Grand Naine, Yangambi Km5, Chinese Cavendish and Nendran hybrid.

Of the 13 accessions belonging to AAB genome group, eight were immune (Attunendran, Zanzibar, Big Ebanga, Nedunendran, Nendran, BRS II (Vannan x Pisang Lilin), Thiruvananthapuram and Pachanadan I) and free of disease and five were found to be infected. Among the infected, two varities were highly susceptible (Cheriya Poovan and Valiya Poovan) with 100 per cent infection, two were susceptible (Palayankodan and Velipadathy) with 75 per cent infection and one was resistant (Padathy) with 25 per cent infection. The minimum PDI (0 %) was recorded in Attunendran, Zanzibar, Big Ebanga, Nedunendran, Nendran, BRS II (Vannan x Pisang Lilin), Thiruvananthapuram and Pachanadan I.

Among the seven genotypes belonging to AA group, three were immune (Cultivar Rose, Pisang Lilin and Pisang Jari Buaya) to the disease, one was resistant (*Musa acuminata* sub sp. *burmanica*), one was moderately resistant (TMP 2x 2829), one was susceptible (Sanna Chenkadali) and one was highly susceptible (Kadali). The

Table 4.13. Per cent disease incidence of various germplasm inoculated with Foc

Sl. No.	Variety	PDI* (%)
1	Attunendran	0
2	Zanzibar	0
3	Grand Naine	0
4	Big Ebanga	0
5	Yangambi Km5	0
6	*Musa acuminata* sub. sp. *burmanica*	25
7	Nedunendran	0
8	Kadali	100
9	Sanna Chenkadali	75
10	Nendran (Kumbavazha)	0
11	Cultivar Rose	0
12	Pisang lilin	0
13	BRS II – (Vannan x Pisang lilin)	0
14	Thiruvananthapuram	0
15	Pisang Jari Buaya	0
16	TMP 2 x 2829	50
17	Chinese Cavendish	0
18	Palayankodan (Alpan)	75
19	Pachanadan I	0
20	Padathy	25
21	Njalipoovan	100
22	Velipadathy	75
23	Chenkadali	66.67
24	Nendran Hybrid	0
25	Cheriya Poovan	100
26	Valiya Poovan	100

*Per cent disease incidence

highest PDI was recorded in Kadali (100 %) which was followed by Sanna chenkadali (75 %), TMP 2x 2829 (50 %) and *Musa acuminata* sub sp. *burmanica* (25 %). The minimum PDI (0 %) was observed in Cultivar Rose, Pisang Lilin and Pisang Jari Buaya.

All the varieties belonging to AAA group namely Yangambi Km5, Grand Naine and Chinese cavendish were immune (0 %) except Chenkadali (66.67 %). The Chenkadali variety belonged to the susceptible category. The Nendran hybrid belonging to AAAB group was also immune (0 %) to the disease whereas Njalipoovan (100 %) belonging to the AB group was included in the highly susceptible category. Categorization of banana germplasm based on disease resistance to Foc Race 1 is given in Table 4.14.

4.7.1.2. Per cent wilt index (PWI)

PWI was calculated for assessing the per cent disease severity based on external scoring using a 0-4 scale descibed by Mak *et al*. (2004). Among the twenty-six germplasm, the highest disease severity was noticed in Valiya Poovan (50.00 %) followed by Cheriya Poovan (41.67 %), Kadali (37.50 %) and Njalipoovan (31.25). Whereas, no disease incidence was recorded in the varieties such as Attunendran, Zanzibar, Big Ebanga, Nedunendran, Nendran, BRS II, Thiruvananthapuram, Pachanadan I, Cultivar Rose, Pisang Lilin, Pisang Jari Buaya, Grand Naine, Yangambi Km5, Chinese Cavendish and Nendran hybrid. The results are presented in Table. 4.15.

Of the 13 accessions belonging to AAB genome group, eight were immune (Attunendran, Zanzibar, Big Ebanga, Nedunendran, Nendran, BRS II (Vannan x Pisang Lilin), Thiruvananthapuram and Pachanadan I) to the disease, three were highly resistant (Palayankodan, Velipadathy and Padathy) and two were moderately resistant (Valiya Poovan and Cheriya Poovan). The highest PDS was recorded in Valiya Poovan (50.00 %) followed by Cheriya Poovan (41.67 %), Palayankodan (18.75 %), Velipadathy (18.75 %) and Padathy (6.25 %). The minimum PDS (0 %) was observed

Table 4.14. Categorization of banana germplasm based on disease resistance to Foc race 1

Genome	Immune	Resistant	Moderately resistant	Susceptible	Moderately susceptible	Highly susceptible
AAB	Attunendran, Zanzibar, Big Ebanga Nedunendran, Nendran, BRS II, Thiruvananthapuram, Pachanadan I	Padathy	–	Palayankodan, Velipadathy	–	Cheriya Poovan, Valiya Poovan
AA	Cultivar Rose, Pisang Lilin, Pisang Jari Buaya	*Musa acuminata* sub sp. *burmanica*	TMP 2x 2829	Sanna Chenkadali	–	Kadali
AAA	Yangambi Km5, Grand Naine, Chinese Cavendish	–	–	Chenkadali	–	–
AAAB	Nendran hybrid	–	–	–	–	–
AB	–	–	–	–	–	Njalipoovan

Table 4.15. Per cent wilt index of various germplasm inoculated with Foc

Sl. No.	Variety	PWI* (%)
1	Attunendran	0
2	Zanzibar	0
3	Grand Naine	0
4	Big Ebanga	0
5	Yangambi Km5	0
6	*Musa acuminata* sub. sp. *burmanica*	6.25
7	Nedunendran	0
8	Kadali	37.50
9	Sanna Chenkadali	18.75
10	Nendran (Kumbavazha)	0
11	Cultivar Rose	0
12	Pisang lilin	0
13	BRS II – (Vannan x Pisang Lilin)	0
14	Thiruvananthapuram	0
15	Pisang Jari Buaya	0
16	TMP 2 x 2829	12.50
17	Chinese Cavendish	0
18	Palayankodan (Alpan)	18.75
19	Pachanadan I	0
20	Padathy	6.25
21	Njalipoovan	31.25
22	Velipadathy	18.75
23	Chenkadali	16.67
24	Nendran Hybrid	0
25	Cheriya Poovan	41.67
26	Valiya Poovan	50.00

*Per cent wilt index

in Attunendran, Zanzibar, Big Ebanga, Nedunendran, Nendran, BRS II (Vannan x Pisang Lilin), Thiruvananthapuram and Pachanadan I**.**

Among the eight genotypes belonging to AA group, three were immune (Cultivar Rose, Pisang Lilin and Pisang Jari Buaya), four were highly resistant (Sanna Chenkadali, Chenkadali, TMP2x2829 and *Musa acuminata* sub sp. *burmanica*) and one was resistant (Kadali). The highest PDS was recorded in Kadali (37.50 %), followed by Sanna Chenkadali (18.75 %), Chenkadali (16.67 %), TMP2x2829 (12.50 %) and *Musa acuminata* sub sp. *burmanica* (6.25 %) The minimum PDS (0 %) was observed in Cultivar Rose, Pisang Lilin and Pisang Jari Buaya.

All the three varieties belonging to the AAA group *viz*., Grand Naine, Yangambi Km5 and Chinese Cavendish were to the disease immune (0 %). The Nendran hybrid belonging to AAAB group was also found to be immune (0 %) to the disease while Njalipoovan (31.25 %) belonging to the AB group was included in the resistant category.

4.7.1.3. Per cent vascular wilt index (PVWI)

Disease severity based on PVWI was calculated as per the 0-5 scale given by Zuo *et al.* (2018). Among the twenty-six germplasm tested, the highest disease severity was recorded in the variety Valiya Poovan (86.67 %) followed by Cheriya poovan (85 %), Kadali (75 %) and Njalipoovan (70 %). Whereas, no disease incidence was recorded in the varieties such as Attunendran, Zanzibar, Big Ebanga, Nedunendran, Nendran, BRS II, Thiruvananthapuram, Pachanadan I, Cultivar Rose, Pisang Lilin, Pisang Jari Buaya, Grand Naine, Yangambi Km5, Chinese Cavendish and Nendran hybrid. The results are presented in (Table 4.16 and Plate 4.19).

Among the 13 accessions belonging to AAB genome group, eight were immune, two were highly resistant, one was moderately resistant and two were highly susceptible. The highest PDS was recorded in Valiya Poovan (86.67 %) followed by Cheriya Poovan (85 %), Velipadathy (45 %), Padathy (15 %) and Palayankodan (15%).

Zanzibar

Big Ebanga

Palayankodan (Alpan)

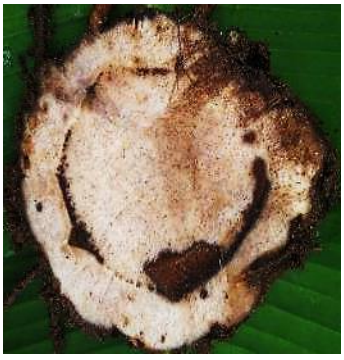
Musa acuminata sub. sp. *burmanica*

Kadali

Padathy

Nendran

Pisang Lilin



Velipadathy

Plate 4.19. Rhizome discolouration in various banana cultivars used for Foc resistance screening

Nendran Hybrid

Nedunendran

Yangambi Km5

Sanna Chenkadali

Cultivar Rose

BRS II

Chinese Cavendish

Pachanadan I

Njalipoovan

Plate 4.19. Rhizome discolouration in various banana cultivars used for Foc resistance screening (Contd.)

Grand Naine

Cheriya Poovan

Attunendran

Chenkadali

Pisang Jari Buaya

Valiya Poovan

Thiruvananthapuram

TMP 2 x 2829

Plate 4.19. Rhizome discolouration in various banana cultivars used for Foc resistance screening (Contd.)

Table 4.16. Per cent vascular wilt index of various germplasm inoculated with Foc

Sl. No.	Variety	PVWI* (%)
1	Attunendran	0
2	Zanzibar	0
3	Grand Naine	0
4	Big Ebanga	0
5	Yangambi Km5	0
6	*Musa acuminata* sub. sp. *burmanica*	25
7	Nedunendran	0
8	Kadali	75
9	Sanna Chenkadali	20
10	Nendran (Kumbavazha)	0
11	Cultivar Rose	0
12	Pisang lilin	0
13	BRS II – (Vannan x Pisang lilin)	0
14	Thiruvananthapuram	0
15	Pisang Jari Buaya	0
16	TMP 2 x 2829	15
17	Chinese Cavendish	0
18	Palayankodan (Alpan)	15
19	Pachanadan I	0
20	Padathy	15
21	Njalipoovan	70
22	Velipadathy	45
23	Chenkadali	20
24	Nendran Hybrid	0
25	Cheriya Poovan	85
26	Valiya Poovan	86.67

*Per cent vascular wilt index

Whereas, the lowest PDS (0 %) was observed in Attunendran, Zanzibar, Big Ebanga, Nedunendran, Nendran, BRS II (Vannan x Pisang Lilin), Thiruvananthapuram and Pachanadan I.

Among the eight genotypes belonging to the AA group,three were immune (Cultivar Rose, Pisang Lilin and Pisang Jari Buaya), one was highly resistant (TMP2 x 2829), three were resistant (*Musa acuminata* sub sp. *burmanica,* Sanna Chenkadali and Chenkadali) and one was susceptible (Kadali). The highest PDS was recorded in Kadali (75 %) which was followed by *Musa acuminata* sub sp. *burmanica* (25 %), Sanna chenkadali (20 %), Chenkadali (20 %) and TMP2x 2829 (15 %). The minimum PDS (0 %) was observed in Cultivar Rose, Pisang Lilin and Pisang Jari Buaya.

All the three varieties belonging to the AAA group *viz*., Grand Naine, Yangambi Km5 and Chinese Cavendish and the Nendran hybrid belonging to AAAB group were immune (0 %) to the disease, whereas Njalipoovan (70 %) belonging to the AB group was included in the susceptible category.

4.7.2. Biochemical basis of disease resistance

Biochemical basis of disease resistance was studied by quantifying the biochemical factors. The activities of total phenol content, reducing and non-reducing sugars, peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) enzymes were assessed at 48 h interval for 14 days in pathogen inoculated and uninoculated control plants of resistant and susceptible cultivars of banana. The susceptible cultivars used for the study were Rasthali/Poovan (AAB) and Kadali (AA) and resistant cultivars were Nendran (AAB) and Robusta (AAA). The quantity of phenol, reducing and non reducing sugars and defense related enzymes in healthy leaves of resistant and susceptible cultivars is presented in Table 4.17.

4.7.2.1. Total phenol

The quantity of total phenol in healthy leaves was noticed more in resistant cultivars (Robusta and Nendran) compared to susceptible cultivars (Rasthali/Poovan and Kadali). Statistically significant differences were observed in the quantity of total

Table 4.17. Total phenol content, sugars and defense related enzymes in the healthy leaves of various banana varieties

Sl. No.	Variety	Phenols (µg/g)	Reducing sugar (mg/g)	Non reducing sugar (mg/g)	Peroxidase (EU/g)	Polyphenol oxidase (EU/g)	Phenylalanine ammonia lyase (EU/g)
1	Rasthali/Poovan	14.39^{d}	10.36^{b}	3.37^{b}	1.96^{c}	0.75^{d}	1.25^{d}
2	Kadali	16.67^{c}	11.64^{a}	4.58^{a}	2.11^{bc}	0.77^{c}	1.50^{c}
3	Nendran	21.55^{a}	8.93^{d}	3.11^{c}	2.39^{a}	0.86^{b}	1.71^{b}
4	Robusta	19.31^{b}	9.55^{c}	2.31^{d}	2.26^{ab}	1.79^{a}	1.96^{a}
CD (0.05)		0.697	0.255	0.195	0.170	0.077	0.069

phenol content among resistant and susceptible cultivars. Among the resistant cultivars, the total phenol content was highest in Nendran (21.55 mg/g) preceded by Robusta (19.31 mg/g). While in susceptible cultivars, the quantity of total phenol was recorded highest in Kadali (16.67 mg/g) and lowest in Rasthali/Poovan (14.39 mg/g).

After artificial inoculation with Foc, the activity of total phenol was maximum on 4th day in Nendran, Robusta and Kadali, whereas in Rasthali/Poovan the highest phenol content was recorded on 6th day and then decreased near to the basal level. In resistant cultivars *viz*., Nendran and Robusta, the maximum phenol activity recorded was 26.90 mg/g and 24.74 mg/g and the increase in total phenol content activity was 1.24 and 1.28 fold respectively. Whereas in susceptible cultivars *viz*., Rasthali/Poovan and Kadali, the highest phenol activity observed was 17.67 mg/g and 19.74 mg/g respectively. The activity of total phenol content was increased by only1.23 fold in Rasthali/Poovan and 1.18 fold in Kadali. In control plants, the activity of total phenol remained constant throughout the 14 days of study. The changes in total phenol content in Foc race 1 resistant and susceptible cultivars of banana for 14 days after artificial inoculation is given in Table 4.18.

4.7.2.2. Reducing sugar

In healthy leaves, the quantity of reducing sugar was recorded highest in susceptible cultivars (Rasthali/Poovan and Kadali) when compared to resistant cultivars (Nendran and Robusta). While comparing statistically, significant differences were noticed in the quantity of reducing sugar in resistant and susceptible cultivars. The leaves of Kadali (11.64 mg/g) cultivar exhibited the highest quantity of reducing sugar followed by Rasthali/Poovan (10.36 mg/g). This was preceded by the resistant cultivar Robusta (9.55 mg/g) and the lowest quantity was observed in Nendran (8.93 mg/g).

After challenge inoculation, the quantity of reducing sugar showed a decreasing trend in both resistant (Nendran and Robusta) and susceptible (Rasthali/Poovan and Kadali) cultivars. Among resistant cultivars, the quantity of reducing sugar decreased from 8.91 mg/g to 7.12 mg/g and 9.56 mg/g to 8.50 mg/g in Nendran and Robusta respectively. Whereas in susceptible cultivars, the quantity of reducing sugar declined

Table 4.18. Change in total phenol content of banana leaves after challenge inoculation with Foc

DAI*	Rasthali/ Poovan (inoculated) µg/g	Rasthali/ Poovan (control) µg/g	Kadali (inoculated) µg/g	Kadali (control) µg/g	Nendran (inoculated) µg/g	Nendran (control) µg/g	Robusta (inoculated) µg/g	Robusta (control) µg/g
0	14.39	14.42	16.67	16.85	21.63	21.33	19.35	19.51
2	15.82	14.30	17.25	16.59	23.91	21.76	22.47	19.24
4	16.06	14.41	19.74	16.63	26.90	21.62	24.74	19.81
6	17.67	14.29	19.21	16.77	26.46	21.46	24.27	19.74
8	16.83	14.48	18.94	16.82	25.79	21.13	23.91	19.45
10	14.76	14.33	18.41	16.44	24.75	21.89	23.31	19.79
12	13.24	14.65	18.09	16.38	23.04	21.83	21.82	19.54
14	11.43	14.37	16.22	16.71	22.07	21.48	20.65	19.65

from 10.36 mg/g to 5.57 mg/g and 11.64 mg/g to 6.24 mg/g in Rasthali/Poovan and Kadali respectively. The activity of reducing sugar decreased by 1.25 fold in Nendran and 1.12 in fold in Robusta. Whereas in susceptible cultivars, its activity was decreased by 1.86 fold in Rasthali/Poovan and 1.87 fold in Kadali cultivars. In control plants, the quantity of non reducing sugar remained constant throughout the study. The changes in reducing sugar content in Foc race 1 resistant and susceptible cultivars for 14 days after artificial inoculation is given in Table 4.19.

4.7.2.3. Non reducing sugar

The quantity of non reducing sugar was noticed to be more in susceptible cultivars (Rasthali/Poovan and Kadali) compared to resistant cultivars (Nendran and Robusta). Statistically significant differences were observed in the activity of non reducing sugar in healthy leaves of resistant and susceptible cultivars. It was found to be highest in Kadali (4.58 mg/g) variety followed by the Rasthali/Poovan (3.37 mg/g). Among the resistant cultivars, the quantity of non reducing sugar was recorded highest in Nendran (3.11 mg/g) which was preceded by Robusta (2.31 mg/g).

After challenge inoculation, the quantity of non reducing sugar showed a decreasing trend in both resistant (Nendran and Robusta) and susceptible (Rasthali/Poovan and Kadali) cultivars. In the resistant cultivars, the quantity of non reducing sugar declined from 3.10 mg/g to 2.22 mg/g and 2.35 mg/g to 1.67 mg/g in Nendran and Robusta respectively. Among the susceptible cultivars, its quantity was reduced from 3.37 mg/g to 0.41 mg/g and 4.58 mg/g to 0.86 mg/g in Rasthali/Poovan and Kadali respectively. The activity of non reducing sugar decreased by 1.40 fold in Nendran and 1.41 fold in Robusta. Whereas in susceptible cultivars, its activity was decreased by 8.22 fold in Rasthali/Poovan and 5.33 fold in Kadali cultivars. The changes in non reducing sugar content in Foc race 1 resistant and susceptible cultivars for 14 days after artificial inoculation is given in Table 4.20.

4.7.2.4. Peroxidase (PO)

In healthy leaves (control), the activity of peroxidase enzyme was recorded more in the resistant cultivars *viz*., Nendran and Robusta than the susceptible cultivars namely

Table 4.19. Change in reducing sugar of banana leaves after challenge inoculation with Foc

DAI*	Rasthali/ Poovan (inoculated) mg/g	Rasthali/Poovan (control) mg/g	Kadali (inoculated) mg/g	Kadali (control) mg/g	Nendran (inoculated) mg/g	Nendran (control) mg/g	Robusta (inoculated) mg/g	Robusta (control) mg/g
0	10.36	10.32	11.64	11.65	8.91	8.93	9.56	9.59
2	8.68	10.29	11.06	11.62	8.73	9.04	9.37	9.58
4	8.10	10.33	9.71	11.66	8.64	8.95	9.26	9.60
6	7.84	10.37	8.32	11.60	8.36	8.92	9.04	9.54
8	7.52	10.34	7.41	11.58	7.84	9.05	8.91	9.56
10	7.05	10.31	7.11	11.61	7.59	9.00	8.79	9.63
12	6.51	10.35	6.71	11.64	7.39	8.90	8.65	9.61
14	5.57	10.32	6.24	11.59	7.12	8.87	8.50	9.58

*Days after inoculation

Table 4. 20. Change in non-reducing sugar of banana leaves after challenge inoculation with Foc

DAI*	Rasthali/Poovan (inoculated) mg/g	Rasthali/ Poovan (control) mg/g	Kadali (inoculated) mg/g	Kadali (control) mg/g	Nendran (inoculated) mg/g	Nendran (control) mg/g	Robusta (inoculated) mg/g	Robusta (control) mg/g
0	3.37	3.48	4.58	4.52	3.10	3.08	2.35	2.32
2	2.58	3.37	3.84	4.49	3.02	3.07	2.32	2.36
4	2.42	3.39	3.26	4.50	2.94	3.14	2.25	2.29
6	2.04	3.44	2.77	4.55	2.79	3.07	2.14	2.31
8	1.78	3.42	2.32	4.53	2.64	3.10	2.01	2.32
10	1.30	3.45	1.66	4.47	2.43	3.09	1.95	2.27
12	0.92	3.40	1.48	4.51	2.35	3.11	1.78	2.28
14	0.41	3.39	0.86	4.59	2.22	3.08	1.67	2.32

*Days after inoculation

Rasthali/Poovan and Kadali. Significant differences were recorded with the activity of peroxidase in resistant and susceptible cultivars. The quantity of peroxidase in healthy leaves of Nendran (2.39 EU/g) was found to be superior but was on par with peroxidase content in healthy Robusta leaves (2.26 EU/g) followed by Kadali (2.11 EU/g). Likely, the quantity of the peroxidase enzyme in healthy leaves of Robusta was on par with that of Kadali. The lowest peroxidase content was recorded in Rasthali/Poovan variety (1.96 EU/g).

After artificial inoculation with Foc, the activity of PO enzyme was found to be maximum on 8th day and then decreased to the base level. In Nendran and Robusta, the maximum PO activity recorded was 7.54 EU/g and 7.09 EU/g and the increase in PO activity was 3.23 and 3.12 fold respectively. Whereas in Rasthali/Poovan and Kadali, the highest PO activity observed was 3.98 EU/g and 5.42 EU/g respectively. The PO activity was increased only 2.03 fold in Rasthali/Poovan and 2.57 fold in Kadali. In control plants, the activity of PO enzyme remained constant throughout the 14 days of study. The changes in peroxidase content in Foc race 1 resistant and susceptible cultivars for 14 days after artificial inoculation is given in Table 4.21.

4.7.2.5. Polyphenol oxidase (PPO)

The activity of polyphenol oxidase in healthy leaves of resistant cultivars (Nendran and Robusta) was observed to be highest than in susceptible cultivars (Rasthali/Poovan and Kadali). Statistically significant differences were noticed in the activity of PPO in healthy leaves of resistant and susceptible cultivars. The quantity of PPO was observed to be significantly superior in resistant cultivar Robusta (1.79 EU/g) which was followed by the resistant cultivar Nendran (0.86 EU/g). Among the susceptible cultivars, Kadali (0.77 EU/g) showed the highest activity of the PPO enzyme which was preceded by Rasthali/Poovan (0.75 EU/g).

The activity of PPO was recorded maximum on 6th day after inoculation with Foc and then decreased near to the base level. The highest PPO activity was observed in resistant cultivars *viz.*, Nendran and Robusta was 4.27 EU/g and 5.69 EU/g respectively whereas in the susceptible cultivars *viz*., Rasthali/Poovan and Kadali it was

Table 4.21. Change in peroxidase of banana leaves after challenge inoculation with Foc

DAI*	Rasthali/ *Poovan (inoculated) Eu/g	Rasthali/ Poovan (control) Eu/g	Kadali (inoculated) Eu/g	Kadali (control) Eu/g	Nendran (inoculated) Eu/g	Nendran (control) Eu/g	Robusta (inoculated) Eu/g	Robusta (control) Eu/g
0	1.96	2.05	2.11	2.14	2.32	2.34	2.24	2.26
2	2.37	2.01	2.77	2.19	4.22	2.38	3.93	2.22
4	3.14	1.99	4.01	2.23	5.46	2.31	5.23	2.22
6	3.80	1.97	5.11	2.17	5.96	2.39	6.40	2.25
8	3.98	2.02	5.42	2.22	7.54	2.30	7.09	2.29
10	3.46	2.11	4.58	2.19	6.44	2.30	5.81	2.26
12	2.90	2.07	3.51	2.15	4.89	2.33	4.76	2.30
14	2.06	2.04	2.34	2.25	3.19	2.36	3.03	2.27

*Days after inoculation

1.95 EU/g and 2.38 EU/g respectively. The increase in activity of PPO activity on 6th day after inoculation was 4.85, 3.31, 2.6 and 3.10 fold in Nendran, Robusta, Rasthali/Poovan and Kadali respectively. While in control plants, the activity of PPO enzyme remained constant during the study. The changes in PPO content in Foc race 1 resistant and susceptible cultivars for 14 days after artificial inoculation is given in Table 4.22.

4.7.2.6. Phenylalanine ammonia lyase (PAL)

In healthy leaves, the activity of phenylalanine ammonia lyase was noticed to be highest in resistant cultivars (Nendran and Robusta) compared to susceptible (Rasthali/Poovan and Kadali) cultivars (Rasthali/Poovan and Kadali). Statistically significant differences were observed in the quantity of PAL in tested varieties. The activity of PAL was higher in resistant variety Robusta (1.96 EU/g) followed by the resistant variety Nendran (1.71 EU/g). Among the susceptible varieties, the activity of PAL in Kadali (1.50 EU/g) leaves was significantly superior to that of Rasthali/Poovan (1.25 EU/g) variety.

The activity of PAL enzyme was found to be maximum on 8th day after challenge inoculation both in resistant and susceptible cultivars and then declined near to the base level. In Nendran and Robusta, the maximum PAL activity recorded was 4.80 EU/g and 5.62 EU/g and the increase in PAL activity was 2.79 and 2.82 fold respectively. Whereas in Rasthali/Poovan and Kadali, the highest PAL activity observed was 2.69 EU/g and 2.98 EU/g respectively. The PAL activity was increased only 2.15 fold in Rasthali/Poovan and 1.99 fold in Kadali. In control plants, the activity of PAL enzyme remained constant throughout the 14 days of study. The changes in PAL content in Foc race 1 resistant and susceptible cultivars for 14 days after artificial inoculation is given in Table 4.23.

4.8. HISTOPATHOLOGY

Histopathological changes due to the infection of Foc in Rasthali/Poovan variety was studied by preparing sections using sharp razor and staining with safranin followed by observing under the microscope. The histopathological changes due to infection in

Table 4.22. Change in poly phenol oxidase of banana leaves after challenge inoculation with Foc

DAI*	Rasthali/ Poovan (inoculated) Eu/g	Rasthali/ Poovan (control) Eu/g	Kadali (inoculated) Eu/g	Kadali (control) Eu/g	Nendran (inoculated) Eu/g	Nendran (control) Eu/g	Robusta (inoculated) Eu/g	Robusta (control) Eu/g
0	0.75	0.62	0.77	0.71	0.88	0.85	1.72	1.80
2	1.46	0.61	0.93	0.68	1.03	0.86	3.52	1.75
4	1.70	0.58	1.69	0.73	3.08	0.85	4.81	1.76
6	1.95	0.60	2.38	0.70	4.27	0.87	5.69	1.76
8	1.81	0.56	2.03	0.74	3.81	0.85	5.17	1.75
10	1.22	0.57	1.33	0.65	2.54	0.83	4.30	1.75
12	0.98	0.59	0.86	0.73	1.63	0.84	3.93	1.79
14	0.57	0.61	0.70	0.73	0.94	0.87	2.86	1.81

*Days after inoculation

Table 4.23. Change in phenylalanine ammonia lyase of banana leaves after challenge inoculation with Foc

*Days after inoculation

DAI*	Rasthali/ Poovan (inoculated) EU/g	Rasthali/ Poovan (control) EU/g	Kadali (inoculated) EU/g	Kadali (control) EU/g	Nendran (inoculated) EUg	Nendran (control) EU/g	Robusta (inoculated) EU/g	Robusta (control) EU/g
0	1.25	1.24	1.50	1.52	1.72	1.69	1.99	1.97
2	1.56	1.25	1.96	1.52	2.38	1.71	3.32	1.96
4	2.03	1.30	2.57	1.47	2.94	1.74	4.06	2.00
6	2.44	1.25	2.88	1.53	3.85	1.72	4.93	1.98
8	2.69	1.26	2.98	1.54	4.80	1.73	5.62	2.10
10	2.14	1.28	2.73	1.51	4.08	1.74	4.44	1.97
12	1.83	1.26	2.29	1.53	3.12	1.70	3.80	2.01
14	1.40	1.23	1.73	1.50	2.06	1.72	2.58	1.99

roots (Plate 4.20), leaves (Plate 4.21) and rhizome (Plate 4.22). The sections of healthy plant parts were also taken and compared with diseased ones. It was observed that the amount of chloroplast was less in the cytoplasm of infected leaves compared to the healthy tissues. The cells in the palisade layer, as well as the spongy layer, were flaccid. In the cortex of infected root and rhizome, the deposition of secondary metabolites was more compared to the healthy roots. The distorted cells were noticed in the root cortex of the infected tissue. In the xylem vessels of infected roots, some irregular growth was present. Crushed cells were seen distributed in the rhizome cortex.

4.9. MANAGEMENT OF FUSARIUM WILT OF BANANA

4.9.1. *In vitro* studies for the evaluation of chemical fungicides, bioagents and botanicals

In vitro evaluation of total twenty two chemical fungicides, bioagents and botanicals at lower, recommended and higher dose was done by poisoned food technique to assess its efficacy for the management of Foc (Plate 4.23). The results are presented in Table 4.24.

The results of the experiment revealed that, among the contact fungicides used, Bordeaux mixture gave 100 per cent inhibition on the growth of the pathogen at lower, recommended and the higher dose and it was significantly different from other contact fungicides. It was followed by mancozeb (51.33 %), copper hydroxide (39.78 %) and copper oxychloride (32.33 %) at lower, recommended and higher dose. The mean mycelial growth of the pathogen was recorded lowest when treated with Bordeaux mixture (0.00 cm) followed by mancozeb (4.38 cm), copper hydroxide (5.42 cm) and copper oxychloride (6.09 cm).

Among the systemic fungicides, all the fungicides namely tebuconazole, propiconazole and carbendazim gave 100 per cent inhibition of the pathogen except the azoxystrobin (70.78 %). No mycelial growth of the pathogen was recorded in tebuconazole, propiconazole and carbendazim whereas it was 2.63 cm when treated with azoxystrobin.

All the combination fungicides used in the study *viz.*, captan + hexaconazole, carbendazim + mancozeb and propiconazole + difenoconazole showed 100 per cent

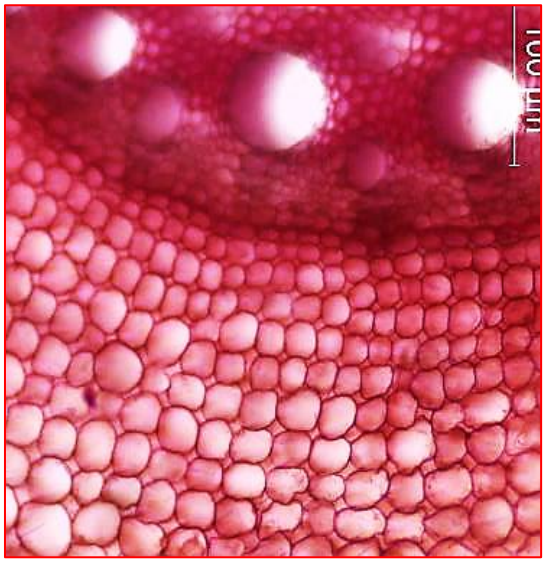


Plate 4.20.a. Healthy root

Plate 4.20.b. Foc infected root

Plate 4.20.c. Healthy rootlet

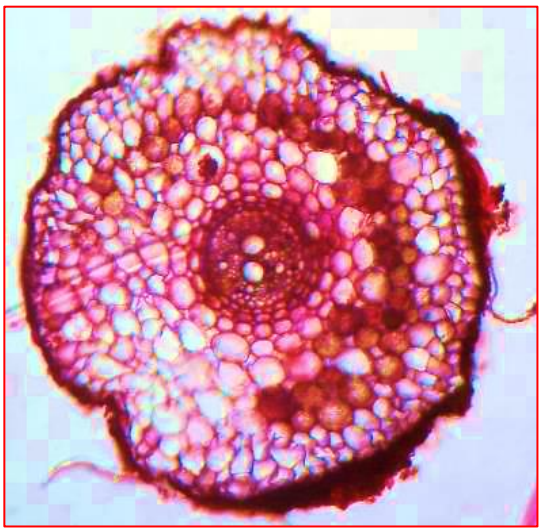


Plate 4.20.d. infected rootlet

Plate 4.20. Histopathological changes in the root of healthy and Foc infected banana

Plate 4.21.a. Healthy leaf

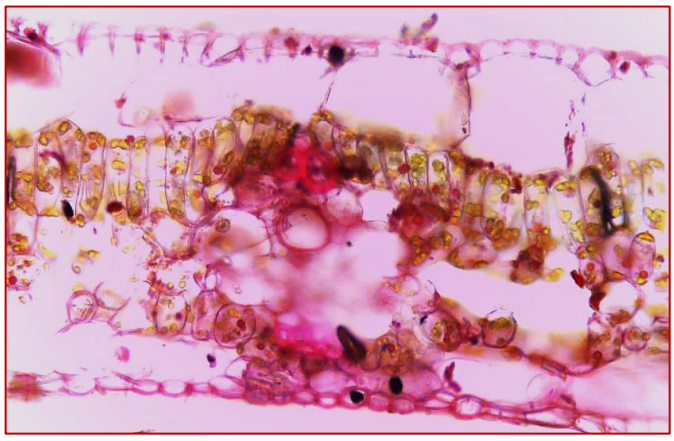


Plate 4.21.b. Foc infected leaf

Plate 4.21. Histopathological changes in the leaf of healthy and Foc infected banana

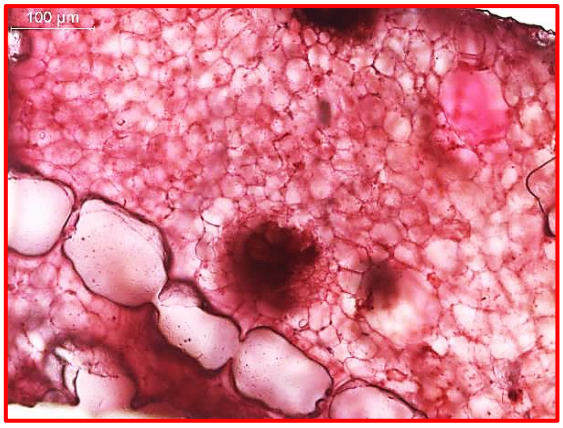


Plate 4.22.a. Healthy rhizome

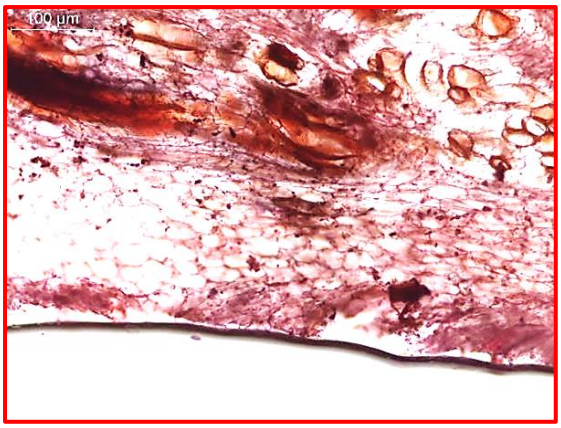


Plate 4.22.b. Foc infected rhizome

Plate 4.22. Histopathological changes in rhizome of healthy and Foc infected banana

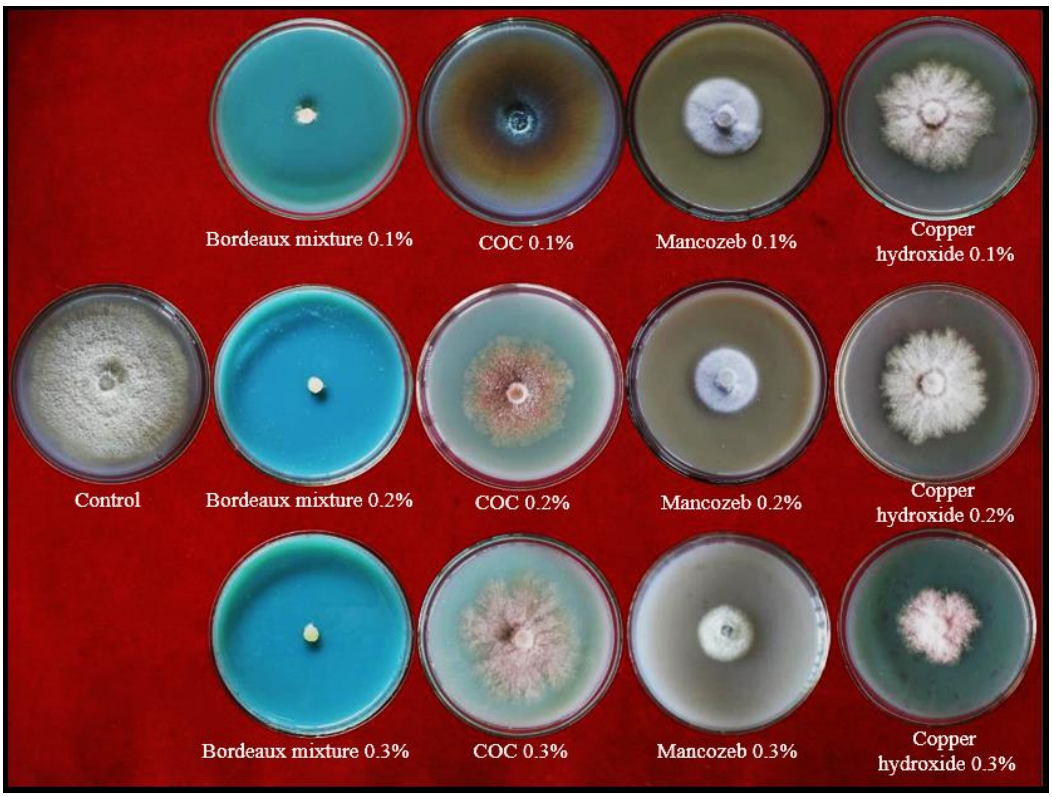


Plate 4.23. Effect of chemical fungicides, biocontrol agents and botanicals on the growth of Foc under *in vitro*

Plate 4.23. Effect of chemical fungicides, biocontrol agents and botanicals on the growth of Foc under *in vitro* (Contd.)

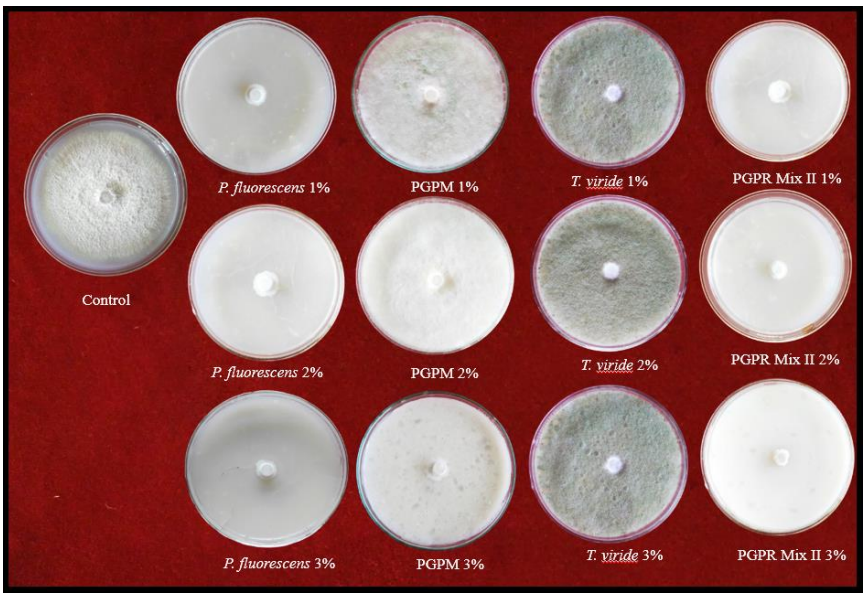


Plate 4.23. Effect of chemical fungicides, biocontrol agents and botanicals on the growth of Foc under *in vitro* (Contd.)

Plate 4.23. Effect of chemical fungicides, biocontrol agents and botanicals on the growth of Foc under *in vitro* (Contd.)

Plate 4.23. Effect of chemical fungicides, biocontrol agents and botanicals on the growth of Foc under *in vitro* (Contd.)

Table 4.24. Effect of fungicides, bicontrol agents and botanicals on the growth of the pathogen Foc

Sl. No.	Treatments	Mycelial growth (Diameter in cm)				Percentage inhibition (%)
		Lower dose	Recommended dose	Higher dose	Mean	
1	Bordeaux mixture	0.00 (0.00)^{f}	0.00 (0.00)^{j}	0.00 (0.00)^{k}	0.00	100
2	Copper oxy chloride	6.60 (0.881)^{bc}	5.93 (0.841)^{e}	5.73 (0.828)^{f}	6.09	32.33
3	Mancozeb	6.06 (0.849)^{c}	3.96 (0.696)^{h}	3.13 (0.616)^{I}	4.38	51.33
4	Copper hydroxide	6.16 (0.777)^{d}	5.66 (0.824)^{f}	4.43 (0.735)^{h}	5.42	39.78
5	Tebuconazole	0.00 (0.00)^{f}	0.00 (0.00)^{j}	0.00 (0.00)^{k}	0.00	100
6	Propiconazole	0.00 (0.00)^{f}	0.00 (0.00)^{j}	0.00 (0.00)^{k}	0.00	100
7	Carbendazim	0.00 (0.00)^{f}	0.00 (0.00)^{j}	0.00 (0.00)^{k}	0.00	100
8	Azoxystrobin	3.23 (0.626)^{e}	2.96 (0.598)^{i}	1.70 (0.431)^{j}	2.63	70.78
9	Captan + Hexaconazole	0.00 (0.00)^{f}	0.00 (0.00)^{j}	0.00 (0.00)^{k}	0.00	100
10	Carbendazim + Mancozeb	0.00 (0.00)^{f}	0.00 (0.00)^{j}	0.00 (0.00)^{k}	0.00	100
11	Propiconazole + Difenoconazole	0.00 (0.00)^{f}	0.00 (0.00)^{j}	0.00 (0.00)^{k}	0.00	100

Values in the parenthesis are log transformed

Table 4.24. Effect of fungicides, bicontrol agents and botanicals on the growth of the pathogen Foc (Contd.)

Sl. No.	Treatments	Mycelial growth (Diameter in cm)				Percentage inhibition (%)
		Lower dose	Recommended dose	Higher dose	Mean	
12	PGPR Mix II	0.00 (0.00)^{f}	0.00 (0.00)^{j}	0.00 (0.00)^{k}	0.00	100
13	PGPM	0.00 (0.00)^{f}	0.00 (0.00)^{j}	0.00 (0.00)^{k}	0.00	100
14	*Trichoderma viride*	0.00 (0.00)^{f}	0.00 (0.00)^{j}	0.00 (0.00)^{k}	0.00	100
15	*Pseudomonas fluorescens*	0.00 (0.00)^{f}	0.00 (0.00)^{j}	0.00 (0.00)^{k}	0.00	100
16	*Azadirachta indica* (oil)	7.20 (0.914)^{b}	6.56 (0.879)^{d}	6.16 (0.853)^{e}	6.64	26.22
17	*Myristica fragrance* (mace)	6.50 (0.869)^{bc}	5.12 (0.790)^{g}	4.73 (0.758)^{g}	5.45	39.44
18	*Lawsonia inermis* (leaves)	7.20 (0.914)^{b}	6.77 (0.890)^{c}	6.23 (0.859)^{e}	6.72	25.33
19	*Areca catechu* (seed kernel)	7.27 (0.917)^{b}	7.10 (0.908)^{b}	6.87 (0.896)^{c}	7.08	21.33
20	*Kaempferia galanga* (rhizome)	8.70 (0.987)^{a}	8.03 (0.956)^{a}	6.60 (0.881)^{d}	7.68	14.66
21	*Curcuma caesia* (rhizome)	8.67 (0.985)^{a}	8.13 (0.961)^{a}	7.67 (0.938)^{a}	8.16	9.33
22	*Curcuma angustifolia* (rhizome)	8.53 (0.979)^{a}	8.07 (0.957)^{a}	7.33 (0.921)^{b}	7.98	11.33
	CD (0.05)	0.049	0.009	0.012		

Values in the parenthesis are log transformed

inhibition of the pathogen. The mean mycelial growth was recorded zero in these chemicals.

The bioagents used namely PGPR Mix II, PGPM, *Trichoderma viride* and *Pseudomonas fluorescens* gave 100 per cent inhibition of the pathogen with no mycelial growth under *in vitro* condition. Also, the effect of *T. viride* and *P. fluorescens* on the growth of Foc was tested by the dual culture method (Plate 4.24). The results revealed that both bioagents gave 100 per cent inhibition of Foc in the dual culture method.

Among the botanicals used for the experiment, the highest (39.44 %) per cent inhibition of the pathogen was observed in treatments applied with extract of *Myristica fragrans* mace followed by an extract of *Azadirachta indica* oil (26.22 %) and *Lawsonia inermis* leaves (25.33 %). The lowest per cent of inhibition of the pathogen was noticed in the treatment *Curcuma caesia* (9.33 cm). The highest mean mycelial growth was recorded in the treatment *Curcuma caesia* (8.16 cm) followed by *Curcuma angustifolia* (7.98 cm) and *Kaempferia galanga* (7.68 cm). Whereas the lowest mean mycelial growth was observed in the treatment with *Myristica fragrans* (5.45 cm) followed by *Azadirachta indica* (oil) (6.64 cm) and *Lawsonia inermis* leaves (6.72 cm).

4.9.2. Pot culture experiment for the management of Fusarium wilt

A pot culture experiment on integrated management of Fusarium wilt of banana was conducted at the Department of Plant Pathology, COH Vellanikkara. The experiment was laid out with ten treatments and three replications. The inference of the experiment was drawn by statistical analysis on per cent disease incidence (PDI) and per cent disease severity (PDS) by calculating per cent wilt index (PWI) and per cent vascular wilt index (PVWI).

4.9.2.1. Per cent disease incidence (PDI)

Statistically significant differences were noticed in the PDI with various treatments and the treatments were significantly superior to the control plants. The results are shown in Table 4.25.

Table 4.25. Effect of treatments on per cent disease incidence under pot culture experiment

Sl. No.	PDI* (%)	Per cent reduction over control
T1 (Copper hydroxide)	88.89 (77.14)^{ab}	11.11
T2 (Tebuconazole)	55.56 (48.25)^{bc}	44.44
T3 (Carbendazim + mancozeb)	77.78 (65.94)^{ab}	22.22
T4 (*Trichoderma viride*)	88.89 (77.14)^{ab}	11.11
T5 (*Trichoderma* enriched cow dung - neem cake mixture + *Pseudomonas fluorescens*)	77.78 (65.94)^{ab}	22.22
T6 (*P. fluorescens* + AMF and *Trichoderma* enriched cow dung + selected best fungicide)	22.22 (24.06)^{c}	77.78
T7 (PGPR Mix II)	88.89 (77.14)^{ab}	11.11
T8 (*P. fluorescens*)	88.89 (77.14)^{ab}	11.11
T9 (Carbendazim)	66.67 (54.74)^{b}	33.33
T10 (control)	100.00 (88.35)^{a}	
CD (0.05)	30.020	

Values in the parenthesis are Arc sin transformed

Plate 4.24.a. Effect of *T. viride* on the growth of Foc

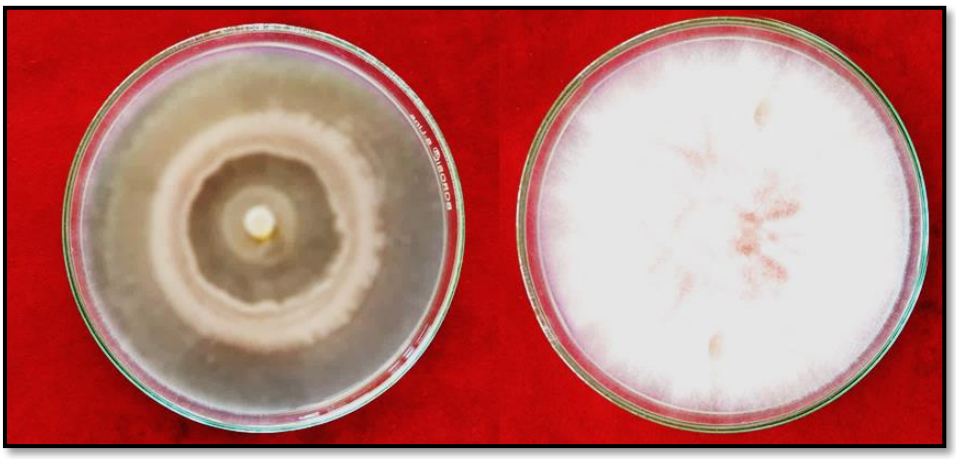


Plate 4.24.b. Effect of *P. fluorescens* on the growth of Foc

Plate 4.24. Effect of biocontrol agents on the growth of Foc under dual culture method

The disease was observed in all the treatments. The PDI ranged from 22.22 per cent to 100.00 per cent. The highest PDI (100.00 %) was noticed in control plants (T10) followed by T1 (copper hydroxide), T4 (*Trichoderma viride*), T7 (PGPR Mix II) and T8 (*P. fluorescens*) having a PDI of 88.89 %. The lowest PDI (22.22 %) was recorded in plants applied with T6 (IDM – *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) followed by T2 (tebuconazole) and T9 (carbendazim) having a PDI of 55.56 per cent and 66.67 per cent respectively.

The per cent reduction over the control of the disease in all treatments were calculated. The maximum per cent reduction over control (77.78 %) was observed in T6 (IDM – *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) followed by T2 (tebuconazole) and T9 (carbendazim) having a reduction of 44.44 per cent and 33.33 per cent respectively. The minimum reduction (11.11 %) in disease over control was recorded in T1 (copper hydroxide), T4 (*Trichoderma viride*), T7 (PGPR Mix II) and T8 (*P. fluorescens*).

4.9.2.2. Per cent disease severity (PDS)

Effect of various treatments on per cent disease severity was calculated by assessing the per cent wilt index (PWI) and per cent vascular wilt index (PVWI). Statistical analysis of data on disease severity showed significant differences among the treatments. The treatments were significantly superior when compared to the control plants. The results are shown in Table 4.26.

Disease severity based on the PWI was recorded at 3 MAP by scoring the external symptoms. The highest disease severity (66.64 %) was noticed in plants kept as control (T10) followed by T1 (copper hydroxide), T7 (PGPR Mix II) and T4 (*Trichoderma viride*) having the disease severity of 44.43 per cent, 41.60 per cent and 36.10 per cent respectively. The lowest disease severity (8.33 %) was recorded in plants treated with T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) preceded by T2 (tebuconazole) and T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) having the severity of 13.89 per cent and 22.22 per cent respectively.

Table 4.26. Effect of treatments on per cent disease severity based on PWI and PVWI under pot culture experiment

Sl. No.	PWI*	Per cent reduction over control (PWI)	PVWI**	Per cent reduction over control (PVWI)
T1	44.43^{b}	33.33	48.91^{ab}	11.39
T2	13.89^{ef}	79.16	20.01^{de}	64.00
T3	33.33^{bcd}	49.98	31.13^{cd}	43.99
T4	36.10^{bcd}	45.83	28.90^{cd}	48.00
T5	22.22^{def}	66.66	33.35^{c}	40.00
T6	8.33^{f}	87.50	13.34^{e}	76.00
T7	41.60^{bc}	37.58	35.57^{c}	36.00
T8	36.05^{bcd}	45.90	37.80^{bc}	31.99
T9	27.77^{cde}	58.33	26.68^{cd}	52.00
T10	66.64^{a}	-	55.58^{a}	-
CD (0.05)	14.871		11.915	

*Per cent wilt index

**Per cent vascular wilt index

The per cent disease reduction over the control based on PWI was assessed. The highest disease reduction (87.50 %) was noticed in T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) followed by T2 (tebuconazole), T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) and T9 (carbendazim having the per cent disease control of 79.16 per cent, 66.66 per cent and 58.33 per cent respectively. The lowest disease reduction over control (33.33 %) was recorded in T1 (copper hydroxide) which was followed by T7 (PGPR Mix II) and T4 (*Trichoderma viride*) having the per cent disease control of 37.58 per cent and 45.83 per cent respectively.

Assessment of disease severity based on PVWI was carried out by scoring the vascular symptoms in the rhizome after uprooting the plants (Plate 4.25). The highest disease severity (55.58 %) was recorded in untreated plants (T10) followed by T1 (copper hydroxide), T8 (*P. fluorescens*) and T7 (*Trichoderma viride*) having the severity of 48.91 per cent, 37.80 per cent and 35.57 per cent respectively. The least disease severity (13.34 %) was found in plants treated with T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) followed by T2 (tebuconazole), T9 (carbendazim) and T4 (*Trichoderma viride*) 64.00 per cent, 26.68 per cent and 23.33 per cent respectively.

Per cent disease reduction over control based on PVWI was assessed. The highest disease reduction (76.00 %) over control treatment was noticed in T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) followed by T2 (tebuconazole) and T9 (carbendazim) with a disease reduction of 64.00 per cent and 52.00 per cent respectively. Whereas the lowest disease reduction over control (11.39 %) was found in T1 (copper hydroxide) followed by T8 (*P. fluorescens*) and T7 (PGPR Mix II) having a disease reduction of 31.99 per cent and 36.00 per cent respectively.

4.9.3. Field experiment for the management of Fusarium wilt

The field experiment for the integrated management of Fusarium wilt of banana was conducted at a sick plot in Banana Research Station Kannara. The inference of the experiment was drawn by statistical analysis of the data on per cent disease incidence

(PDI), per cent disease severity (PDS), vegetative characters such as the height of plants, the girth of plants, number of functional leaves and number of suckers, yield characters such as bunch weight, number of hands per bunch, fingers per hand, length of fingers, the circumference of fingers, weight of green fruits, weight of ripe fruits, peel to pulp ratio and total soluble solids (TSS).

4.9.3.1. Per cent disease incidence (PDI)

Statistically significant differences were observed in the PDI at 8 MAP and 10 MAP with various treatments and were significantly superior when compared to the control plants. The results are shown in Table 4.27 and Fig. 4.18 - 4.19

Observations at 8 MAP revealed that there was disease incidence in all the treatments with variations. The PDI was ranged from 6.67 per cent to 53.33 per cent. The highest PDI (53.33 %) was noticed in control plants (T10) followed by T1 (copper hydroxide) and T7 (PGPR Mix II) having a PDI of 46.67 % and 40.00 % respectively. The lowest PDI (6.67 %) was recorded in plants applied with T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) succeeded by T2 (tebuconazole) and T9 (carbendazim) having a PDI of 13.33 per cent for both.

At 10 MAP, the disease incidence has increased in all treatments ranging from 13.33 per cent to 73.33 per cent. However, the highest PDI (73.33 %) was recorded in control plants (T10) which was on par with T1 (copper hydroxide) and T7 (PGPR Mix II) having a PDI of 66.67 per cent and 60.00 per cent respectively. While, the lowest PDI (13.33 %) was recorded in plants treated with T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) followed by T2 (tebuconazole) and T9 (carbendazim) with 20.00 per cent and 26.67 per cent correspondingly.

While comparing the mean values of PDI at 8 MAP and at 10 MAP, the results revealed that the highest PDI was observed in untreated plants (63.33 %) followed by T1 (copper hydroxide) and T7 (PGPR Mix II) having a mean PDI of 56.67 per cent and 50.00 per cent correspondingly. The lowest PDI (10.00 %) was recorded with the plants treated with T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) which precedes T2 (tebuconazole) and T9 (carbendazim) with a PDI of 16.67 per cent and 20.00 per cent respectively.

Plate 4.25. Rhizome discolouration in banana under pot culture experiment for the management of Foc

Table 4.27. Effect of treatments on per cent disease incidence under field experiment

Sl. No.	PDI* at 8 MAP	PDI at flowering	Mean	Per cent reduction over control
T1	46.67^{ab}	66.67^{ab}	56.67	10.52
T2	13.33^{cd}	20.00^{ef}	16.67	73.68
T3	20.00^{bcd}	33.33^{cdef}	26.67	57.89
T4	33.33^{abcd}	53.33^{abcd}	43.33	31.58
T5	20.00^{bcd}	40.00^{bcdef}	30.00	52.63
T6	6.67^{d}	13.33^{f}	10.00	78.95
T7	40.00^{abc}	60.00^{abc}	50.00	21.05
T8	26.67^{abcd}	46.67^{abcde}	36.67	43.00
T9	13.33^{cd}	26.67^{def}	20.00	68.42
T10	53.33^{a}	73.33^{a}	63.33	-
CD (0.05)	28.705			

*Per cent disease incidence

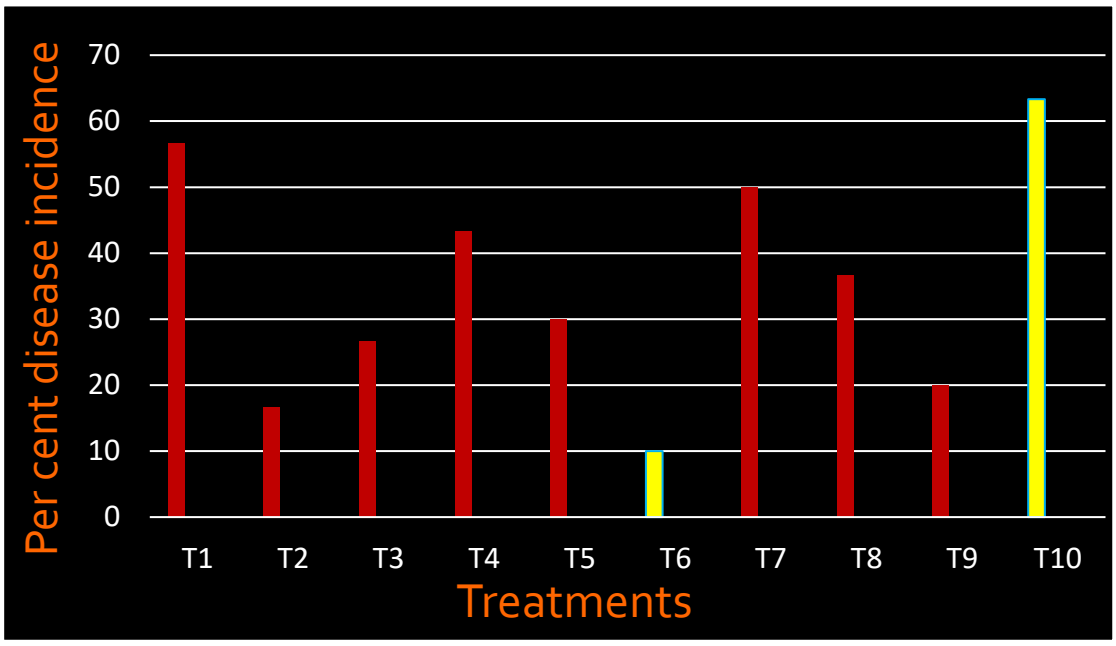


Fig. 4.18. Effect of treatments on per cent disease incidence in the field experiment

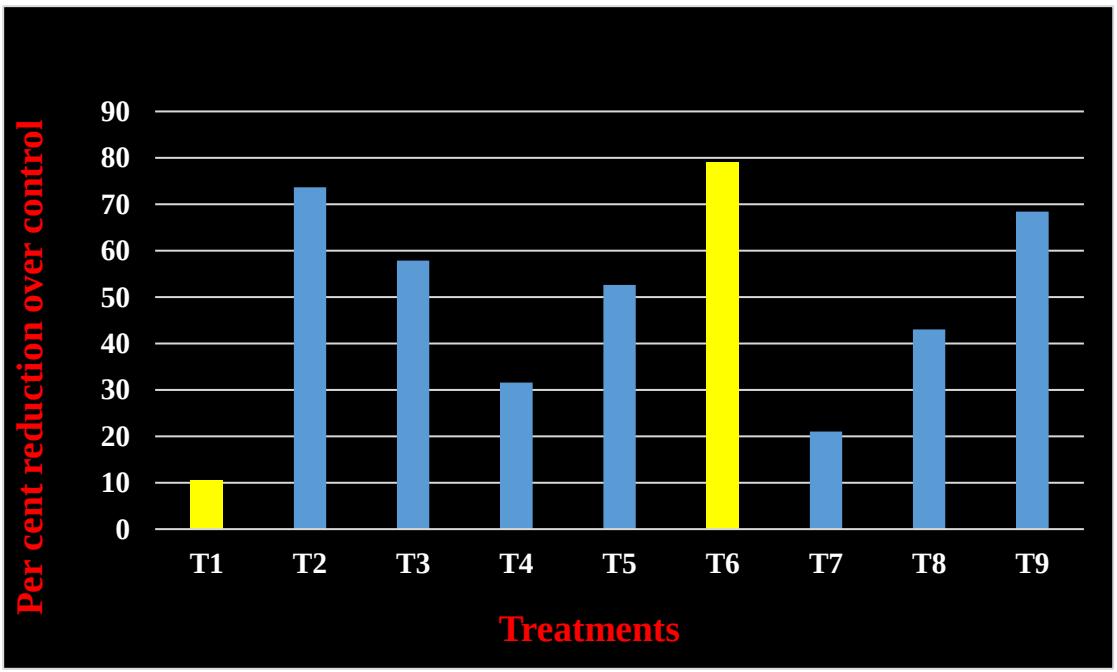


Fig. 4.19. Per cent reduction of disease incidence over control plants in the field experiment

The assessment of per cent reduction of the disease in all treatments over control revealed that the maximum per cent reduction (78.95 %) was observed in T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) followed by T2 (tebuconazole), T9 (carbendazim), T3 (carbendazim + mancozeb) and T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) having a reduction of 73.68 per cent, 68.42 per cent, 57.89 per cent and 52.63 per cent respectively. The minimum reduction (10.52 %) of the disease over control was recorded in T1 (copper hydroxide) followed by T7 (PGPR Mix II) and T4 (*Trichoderma viride*) with 21.05 per cent and 31.58 per cent respectively.

4.9.3.2. Per cent disease severity (PDS)

Effect of various treatments on disease severity was studied by assessing the per cent wilt index (PWI) and per cent vascular wilt index (PVWI). Statistical analysis of data on disease severity showed significant differences among the treatments. The treatments were significantly superior when compared to the control plants. The results are shown in Table 4.28.

Disease severity based on the PWI was recorded at the 10 MAP by scoring the external symptoms. The highest disease severity (56.67 %) was noticed in plants kept as control (T10) followed by T1 (copper hydroxide), T4 (*Trichoderma viride*) and T7 (PGPR Mix II) having the disease severity of 43.33 per cent, 33.33 per cent and 30.00 per cent respectively. The lowest disease severity (11.67 %) was recorded in plants treated with T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) preceded by T2 (tebuconazole) and T3 (carbendazim + mancozeb) having the severity of 16.67 per cent and 21.67 per cent respectively.

Per cent disease reduction over the control based on PWI was assessed. The highest disease reduction (79.41 %) was noticed in T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) followed by T2 (tebuconazole), T3 (carbendazim + mancozeb) and T9 (Carbendazim) having the per cent disease control of 70.58 per cent, 61.76 per cent and 58.83 per cent respectively. The lowest disease reduction over control (23.53 %) was recorded in T1 (copper hydroxide) which

Table 4.28. Effect of treatments on per cent disease severity under field experiment

Sl. No.	PWI*	Per cent reduction over control (PWI)	PVWI**	Per cent reduction over control (PVWI)
T1	43.33^{b}	23.53	45.00^{ab}	22.85
T2	16.67^{de}	70.58	18.33^{cd}	68.58
T3	21.67^{cde}	61.76	23.33bcd	60.00
T4	33.33^{bc}	41.19	36.67^{abcd}	37.13
T5	28.33^{cd}	50.01	25.00^{bcd}	57.14
T6	11.67^{e}	79.41	13.33^{d}	77.15
T7	30.00^{c}	47.06	41.67^{abc}	28.56
T8	25.00^{cd}	55.88	33.33^{bcd}	42.86
T9	23.33^{cde}	58.83	20.00^{cd}	65.71
T10	56.67^{a}	-	58.33^{a}	-
CD (0.05)	12.119		24.947	

*Per cent wilt index

**Per cent vascular wilt index

was followed by T4 (*Trichoderma viride*) and T7 (PGPR Mix II) having the per cent disease reduction over control of 41.19 per cent and 47.06 per cent respectively.

Assessment of disease severity based on PVWI was carried out by scoring the vascular symptoms in the rhizome after uprooting the plants (Plate 4.26). The highest disease severity (58.33 %) was recorded in untreated plants (T10) followed by T1 (copper hydroxide), T7 (PGPR Mix II) and T4 (*T. viride*) having the severity of 45.00 per cent, 41.67 per cent and 36.67 per cent respectively. The least disease severity (13.33 %) was found in plants treated with T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) followed by T2 (tebuconazole), T9 (carbendazim) and T3 (carbendazim + mancozeb) 18.33 per cent, 20.00 per cent and 23.33 per cent respectively.

Per cent disease reduction over the control based on PVWI was assessed. The highest disease reduction (77.15 %) over control treatment was noticed in T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) followed by T2 (tebuconazole) and T9 (carbendazim) with a disease reduction of 68.58 per cent and 65.71 per cent respectively. Whereas the lowest disease reduction over control (22.85 %) was found in T1 (copper hydroxide) followed by T7 (PGPR Mix II) and T4 (*Trichoderma viride*) having a disease reduction of 28.56 per cent and 37.13 per cent respectively.

4.9.3.3. Height of plants

The height of the plants was recorded six times from 5 MAP to 10 MAP for all treatments at the one month interval. No statistical differences were noticed among the treatments applied. The results are presented in Table 29.

During the fifth MAP, the highest plant height (141.83 cm) was recorded in T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) followed by 134.67 cm in T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and 133.83 cm in T7 (PGPR Mix II) applied plants. Whereas the lowest plant height observed in this period was 121.33 cm in T8 (*P. fluorescens*) applied plants.

Table 4.29. Effect of treatments on height of plants under field experiment

Sl. No.	5th MAP*	6th MAP*	7th MAP*	8th MAP*	9th MAP*	10th MAP*
T1	128.50	140.67	153.33	162.67	192.67	231.67
T2	128.33	140.33	157.33	162.83	193.17	237.83
T3	130.00	136.67	153.33	160.44	191.17	228.33
T4	127.33	138.33	149.17	158.67	185.00	226.67
T5	141.83	148.00	168.33	173.33	200.00	240.00
T6	134.67	145.00	160.83	168.33	202.67	242.33
T7	133.83	141.33	157.66	165.67	197.33	234.50
T8	121.33	137.03	144.00	152.83	190.33	229.50
T9	125.53	138.77	148.66	156.83	186.00	228.17
T10	126.00	141.50	146.00	153.33	182.33	221.00
CD (0.05)	NS	NS	NS	NS	NS	NS

*Months after planting

NS – Non significant

Plate 4.26. Rhizome discolouration in banana under field experiment for the management of Foc

The maximum plant height (148.00 cm) noticed in the sixth MAP was in T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) preceded by T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and T10 (untreated control) having the plant height of 145.00 cm and 141.50 cm respectively. The lowest plant height (136.67 cm) was recorded in T3 (carbendazim + mancozeb).

During the seventh MAP, the highest plant height (168.33 cm) was recorded in T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) followed by T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and T7 (PGPR Mix II) having the plant height of 160.83 cm and 157.66 cm respectively. The least plant height (144.00 cm) was observed in T8 (*P. fluorescens*).

The highest plant height (173.33 cm) in the eighth MAP also was observed in T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) followed by T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and T7 (PGPR Mix II) with a plant height of 168.33 cm and 165.67 cm respectively. The lowest plant height (152.83 cm) in the eight MAP was recorded in T8 (*P. fluorescens*).

The highest plant height (202.67 cm) in the ninth MAP was recorded in T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) followed by T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) and T7 (PGPR Mix II) with a plant height of 200.00 cm and 197.33 cm respectively. Whereas the lowest plant height (182.33 cm) was observed in T10 (untreated control) applied plants.

During the tenth MAP also the plant hight was highest (242.33 cm) in T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) followed by T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) and T2 (tebuconazole) with a plant height of 240.00 cm and 237.83 cm respectively. The lowest plant height (221.00 cm) was noticed in T10 (untreated control) applied plants.

From the observations, it was clear that the treatments T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) and T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) were the best for enhancing the height of the plants under field condition.

4.9.3.4. Girth of plants

The measurement on plant girth was recorded for all treatments for six times from 5 MAP to 10 MAP at the one-month interval. Statistically significant differences were noticed among the different treatments applied. The results are shown in Table. 4.30.

In all months, the highest plant girth was observed in plants applied with the treatment T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*).

During the fifth MAP, the highest plant girth (17.07 cm) was followed by T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and T3 (carbendazim + mancozeb) having a plant girth of 15.93 cm and 14.78 cm. The lowest plant girth (13.53 cm) recorded in this month was in T1 (copper hydroxide).

The highest plant girth (17.79 cm) during the sixth MAP was preceded by T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and T7 (PGPR Mix II) having the plant girth of 17.52 cm and 17.19 cm respectively. While the lowest plant girth (15.14 cm) was recorded in this period was in plants applied with T8 (*P. fluorescens*).

The highest plant girth noticed in the seventh MAP was 19.83 cm followed by 19.15 cm (T6 - IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and 17.73 cm (T2 - tebuconazole). The lowest plant girth (15.89 cm) was observed in plants applied with T8 (*P. fluorescens*).

The highest plant girth in the eighth MAP observed was 22.10 cm preceded by T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole)

Table 4.30. Effect of treatments on girth of plants under field experiment

Sl. No.	5th MAP* (cm)	6th MAP* (cm)	7th MAP* (cm)	8th MAP* (cm)	9th MAP* (cm)	10th MAP* (cm)
T1	13.53^{c}	15.78^{e}	17.30^{bc}	17.83^{bc}	19.96^{cde}	22.00^{cd}
T2	14.00^{bc}	16.24^{cd}	17.73^{bc}	18.20^{bc}	20.23^{c}	22.64^{bc}
T3	14.78^{bc}	15.28^{f}	16.82^{c}	17.23^{c}	19.64^{e}	21.70^{cde}
T4	14.27^{bc}	16.42^{c}	17.13^{c}	18.67^{bc}	19.83^{de}	21.83^{cd}
T5	17.07^{a}	17.79^{a}	19.83^{a}	22.10^{a}	23.12^{a}	24.44^{a}
T6	15.93^{ab}	17.52^{a}	19.15^{ab}	20.70^{ab}	22.67^{b}	24.16^{ab}
T7	13.97^{bc}	17.19^{b}	17.62^{bc}	19.43^{abc}	20.35^{c}	22.74b^{c}
T8	14.49^{bc}	15.14^{f}	15.89^{c}	16.76^{c}	18.15^{g}	20.12^{e}
T9	13.80^{c}	15.19^{f}	16.60^{c}	18.15^{bc}	20.07^{cd}	22.00^{cde}
T10	13.57^{c}	16.08^{d}	17.07^{c}	17.00^{c}	18.68^{f}	20.94^{de}
CD (0.05)	**1.968**	**0.275**	**1.985**	**3.103**	**0.390**	**1.625**

*Months after planting

and T7 (PGPR Mix II) having the plant girth of 20.70 cm and 19.43 cm respectively. The lowest plant girth (17.00 cm) was recorded in T10 (control).

During the ninth MAP also, plant girth of T5 (23.12 cm) applied plants were followed by T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and T7 (PGPR Mix II) with a plant girth of 22.67 cm and 20.35 cm respectively. The least plant girth (18.15 cm) was observed in T8 (*P. fluorescens*).

The highest plant girth (24.44 cm) in 10 MAP was preceded by T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and T7 (PGPR Mix II) with a plant girth of 24.16 cm and 22.74 cm respectively. The lowest plant girth (20.12 cm) observed in 10 MAP was in T8 (*P. fluorescens*).

Among the all treatments, T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) was found to be the best treatment which promoting the girth of plants.

4.9.3.5. Number of functional leaves

The number of functional leaves was recorded from 5 MAP to 10 MAP at the one-month interval for all treatments. No statistically significant differences were noticed in the number of functional leaves among the treatments in any of the months from 5 to 10 MAP. The results are shown in Table 4.31.

The highest number of functional leaves (8.38) in the fifth MAP was observed in plants applied with T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) followed by T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and T7 (PGPR Mix II) with 8.23 and 8.10 number of functional leaves respectively. The lowest number of functional leaves (7.20) in the fifth MAP was recorded in T10 (untreated control).

During the sixth MAP, the highest number of functional leaves (8.72) was recorded in T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) which was followed by T6 (IDM - *P. fluorescens* + AMF and *Trichoderma*

Table 4.31. Effect of treatments on number of functional leaves under field experiment

Sl. No.	5th MAP*	6th MAP*	7th MAP*	8th MAP*	9th MAP*	10th MAP*
T1	7.87	8.38	9.00	9.33	8.42	7.40
T2	7.96	8.55	9.15	9.58	8.96	8.10
T3	7.75	8.25	9.13	9.53	8.90	8.10
T4	8.00	7.88	8.33	9.47	8.43	6.77
T5	8.38	8.72	9.47	9.87	8.36	7.58
T6	8.23	8.67	9.22	8.72	8.25	8.70
T7	8.10	8.00	8.47	9.22	7.80	7.17
T8	7.47	8.13	8.47	8.90	8.25	8.30
T9	7.40	8.29	8.77	8.58	7.93	8.37
T10	7.20	7.81	8.28	9.04	7.84	7.16
CD (0.05)	NS	NS	NS	NS	NS	NS

*Months after planting

NS – Non significant

enriched cow dung + tebuconazole) and T2 (tebuconazole) having the number of functional leaves of 8.67 and 8.55 respectively. Whereas, the lowest number of functional leaves (7.81) was noticed in T10 (untreated control).

The highest number of functional leaves (9.47) in the seventh MAP was noticed in plants treated with T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) followed by T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and T2 (tebuconazole) with 9.22 and 9.15 functional leaves respectively. The lowest number of functional leaves recorded in this period was 8.28 which was in the T10 (untreated control) applied plants.

In the eighth MAP, the number of functional leaves was found to be maximum (9.87) in T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) preceded by T2 (tebuconazole) and T3 (carbendazim + mancozeb) with 9.58 and 9.53 functional leaves. The lowest number of functional leaves (8.58) was recorded in T9 (carbendazim).

During the ninth month, the number of functional leaves (8.96) was noticed highest in T2 (tebuconazole) followed by T3 (carbendazim + mancozeb) and T4 (*Trichoderma viride*) with 8.90 and 8.43 functional leaves correspondingly. While the lowest number of functional leaves (7.80) was observed in T7 (PGPR Mix II).

The maximum number of functional leaves (8.70) in the tenth MAP was recorded in T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) preceded by T9 (carbendazim) and T8 (*P. fluorescens*) with 8.37 and 8.30 functional leaves respectively. The least number of functional leaves (6.77) in this month was found to be in T4 (*Trichoderma viride*).

The total number of functional leaves was increased slowly up to 8 MAP whereas, after 8 MAP the number of functional leaves decreased due to the incidence of disease. It was highest in the treatment T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) up to 8 MAP whereas, in the 9 MAP and 10 MAP

the number functional leaves was highest in T2 (tebuconazole) and T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) respectively.

4.9.3.6. Number of suckers

The number of suckers was recorded from 5 MAP to 10 MAP at the one-month interval for all treatments. No statistically significant differences were noticed in the number of suckers among the treatments in any of the months from 5 to 10 MAP. The results are shown in Table. 4.32.

The highest number of suckers (0.67) observed in the fifth MAP was in plants applied with T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) followed by T7 (PGPR Mix II) and T4 (*Trichoderma viride*) with 0.60 and 0.53 number of suckers correspondingly. The least number of suckers was noticed in T9 (carbendazim) and T10 (untreated control) with 0.07 number of suckers.

During the sixth MAP also, the maximum number of suckers (0.81) was found to be in T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) preceded by T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and T7 (PGPR Mix II) with 0.73 and 0.63 suckers respectively. Whereas the least number (0.28) of suckers in this month was recorded in T10 (untreated control).

During the seventh MAP also the highest number of suckers (0.98) was observed in T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) which was followed by T3 (carbendazim + mancozeb) and T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) with 0.93 suckers for both. While the lowest number (0.33) of suckers was recorded in T8 (*P. fluorescens*).

The highest number of suckers (1.40) in the eighth MAP was noticed in T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) followed by T1(copper hydroxide) and T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) having the number of suckers 1.07

Table 4.32. Effect of treatments on number of suckers under field experiment

Sl. No.	5th MAP*	6th MAP*	7th MAP*	8th MAP*	9th MAP*	10th MAP*
T1	0.47	0.52	0.73	1.06	2.40	4.07
T2	0.33	0.52	0.83	0.92	2.30	3.90
T3	0.20	0.53	0.93	0.87	2.42	3.87
T4	0.53	0.47	0.80	0.73	2.28	3.90
T5	0.67	0.81	0.98	1.40	2.77	4.62
T6	0.33	0.73	0.93	1.07	2.63	4.58
T7	0.60	0.63	0.73	0.82	1.92	3.17
T8	0.27	0.50	0.33	0.42	1.87	3.53
T9	0.07	0.37	0.63	0.97	1.83	3.16
T10	0.07	0.28	0.42	0.48	1.93	3.33
CD (0.05)	NS	NS	NS	NS	NS	NS

*Months after planting

NS – Non significant

and 1.06 respectively. While the lowest number of suckers (16.78) in this month was observed in T8 (*P. fluorescens*).

The highest number of suckers in the ninth (2.77) and tenth (4.62) MAP was observed in T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens)*. In the ninth MAP, the maximum number of suckers was followed by T6- (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and T3 (carbendazim + mancozeb) with 2.61 and 2.42 suckers respectively. Whereas in the tenth MAP, the maximum number of suckers was preceded by T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and T1 (copper hydroxide) with 4.58 and 4.07 suckers respectively. The lowest number of suckers in the ninth (18.15) and tenth (20.12) MAP was observed in T8 (*P. fluorescens*).

In all the months, the number of suckers was found to be highest in the plants applied with the treatment T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens)*.

4.9.3.7. Effect of treatments on yield characters

The data on the effect of various treatments on yield characters are given in Table 4.33.

4.9.3.7. a. Bunch weight

Statistically significant differences were observed in all treatments with respect to bunch weight of banana and all treatments were significantly superior to the untreated control. The highest bunch weight (6.10 kg) was recorded in plants applied with T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) followed by T2 (tebuconazole) and T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) with bunch weight of 5.97 kg and 5.83 kg respectively. The lowest bunch weight (4.28 kg) was noticed in control plants deprived of any treatment. The effect of various treatments on the yield is given in Fig. 4.20 and Plate 4.27.

Table 4.33. Effect of treatments on yield characters under field experiment

Treatments	Weight of bunch (kg)	No. of hands	No. of fingers/hand	Per cent increase in yield over control	B:C ratio
T1: Copper hydroxide	5.13^{de}	4.33	11.90^{b}	19.16	1.25
T2: Tebuconazole	5.97^{b}	4.93	13.47^{a}	39.49	1.48
T3: Carbendazim + Mancozeb	5.09^{e}	4.47	11.47^{bc}	18.93	1.34
T4: *Trichoderma viride*	5.11^{e}	4.53	11.27^{bc}	19.39	1.31
T5: AMF +*Trichoderma* enriched cow dung - neem cake mixture + *Pseudomonas fluorescens*	5.83^{c}	4.73	13.07^{a}	36.21	1.40
T6: IDM - *Pseudomonas fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole	6.10^{a}	4.93	13.00^{a}	42.52	1.44
T7: PGPR Mix II	4.90^{f}	4.47	10.87^{cd}	14.49	1.31
T8: -*Pseudomonas fluorescens*	5.18^{d}	4.93	10.73^{cd}	21.03	1.35
T9: Carbendazim (Chemical check)	4.84^{g}	4.73	10.07^{de}	13.08	1.27
T10: Untreated control	4.28^{h}	4.34	9.27^{e}	-	1.18
CD (0.05)	0.059	NS	0.880	NS	

*NS – Non significant


T1
T2
T3
T4
T5
T6
T7
T8
T9
T10

Table 4.33. Effect of treatments on yield characters under field experiment (Contd.)

Treatments	Length of fingers (cm)	Circumference of fingers (cm)	Fresh weight of fingers (g)	Ripe weight of fingers (g)	Peel to pulp ratio	TSS (^{o} brix)
T1: Copper hydroxide	16.13^{d}	13.00^{de}	82.54^{c}	67.66^{c}	0.31	25.80
T2: Tebuconazole	16.30^{d}	13.17^{d}	74.47^{d}	59.70^{de}	0.32	26.13
T3: Carbendazim + Mancozeb	17.20^{c}	14.50^{bc}	81.82^{c}	65.10^{cd}	0.27	25.37
T4: *Trichoderma viride*	17.50^{bc}	14.73^{ab}	82.81^{c}	70.69^{c}	0.33	25.93
T5: AMF +*Trichoderma* enriched cow dung - neem cake mixture + *Pseudomonas fluorescens*	17.80^{b}	15.33^{a}	94.79^{b}	81.67^{b}	0.26	25.73
T6: IDM - *Pseudomonas fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole	18.50^{a}	14.77^{ab}	114.66^{a}	99.55^{a}	0.28	25.53
T7: PGPR Mix II	17.83^{b}	14.83^{ab}	71.92^{d}	55.33^{ef}	0.30	26.17
T8: -*Pseudomonas fluorescens*	16.00^{d}	12.70^{de}	82.18^{c}	67.30^{c}	0.26	25.27
T9: Carbendazim (Chemical check)	17.90^{b}	14.00^{c}	82.83^{c}	64.56^{cd}	0.35	26.07
T10: Untreated control	15.83^{d}	12.40^{e}	67.99^{e}	50.28^{f}	0.29	25.33
CD (0.05)	0.562	0.653	3.234	6.147	NS	NS

NS – Non significant

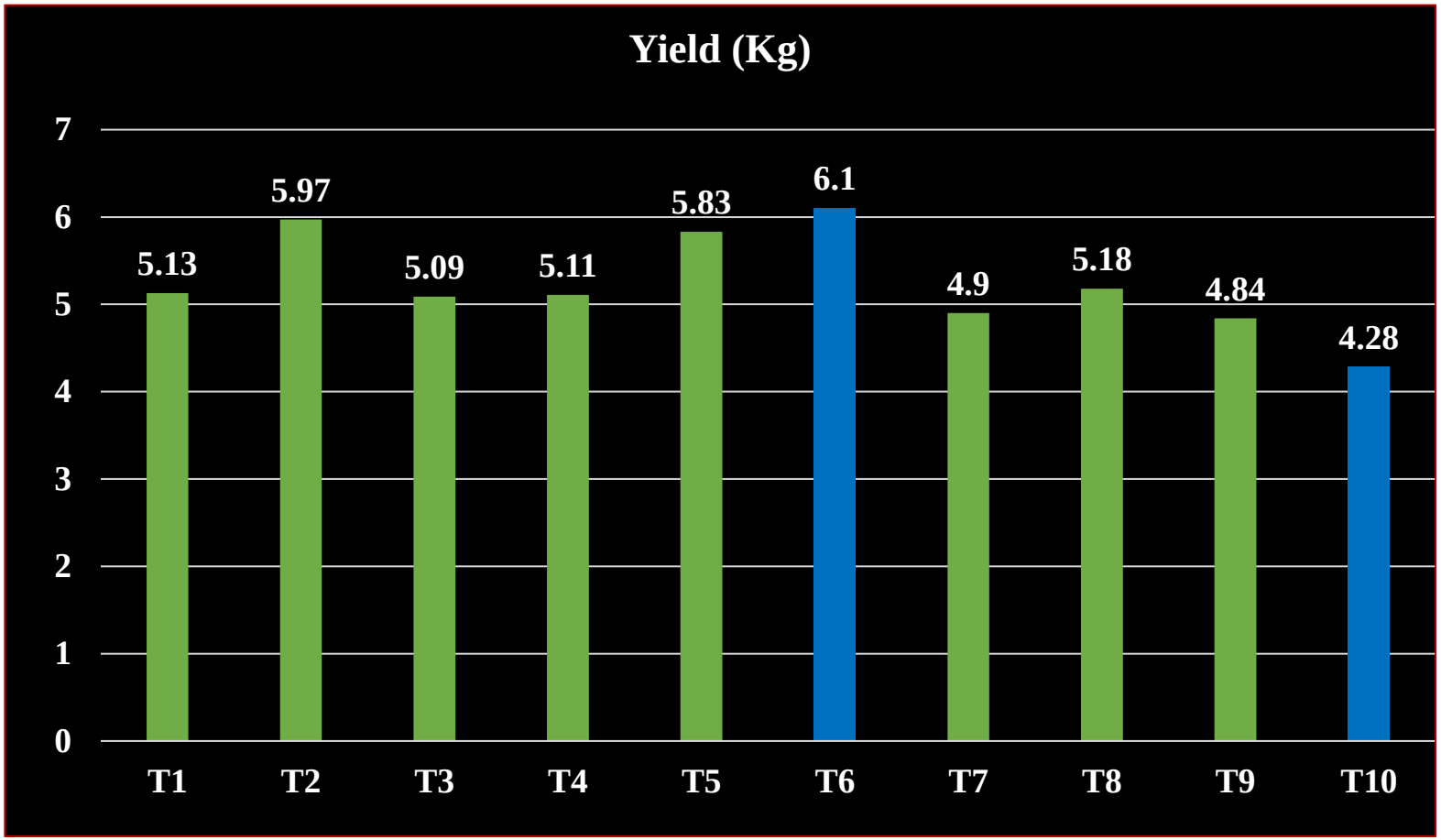


Fig. 4.20. Effect of various treatments on the yield of plants under field experiment

The per cent increase in yield over control plants was tabulated for all treatments. The results showed that the maximum increase (42.52 %) in yield over control was observed in plants applied with T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) which was preceded by T2 (tebuconazole), T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) and T8 (*P. fluorescens*) with a yield increase of 39.49 per cent, 36.21 per cent and 21.03 per cent respectively.

4.9.3.7. b. Number of hands

No statistically significant differences were noticed in the number of hands per bunch among the treatments applied. The highest number of hands was recorded in plants treated with T2 ((tebuconazole), T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and T8 (*P. fluorescens*) with 4.93 number of hands. These treatments were followed by T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) and T9 (Carbendazim) having 4.73 number of hands. The lowest number of hands (4.32) was observed in plants applied with T1 (copper hydroxide).

4.9.3.7. c. Number of fingers per hand

Statistically significant differences were observed in the number of fingers per hand among the treatments applied. The highest number of fingers (13.47) was noticed in plants treated with T2 (tebuconazole). This treatment was statistically on par with T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) and T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) having 13.07 and 13.00 number of fingers. The lowest number of fingers (9.27) was noticed in plants without any treatment (control).

4.9.3.7. d. Length of fingers

Statistically significant differences were noticed in the length of fingers among the treatments. Highest length of fingers (18.50 cm) was recorded in plants applied with T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole)

which was significantly superior to the remaining treatments. This treatment was followed by T9 (carbendazim) and T7 (PGPR Mix II) having a finger length of 7.90 cm and 7.83 cm respectively. The lowest length of fingers (15.83 cm) was observed in T10 (untreated control).

4.9.3.7. e. Circumference of fingers

Statistical analysis of circumference of fingers revealed that the plants applied with T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) showed the highest circumference (15.33 cm) and was significantly superior to the other treatments. This treatment was followed by T7 (PGPR Mix II) and T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) having a circumference of 14.83 cm and 14.77 cm respectively. The lowest circumference of fingers (12.40 cm) was noticed in plants without any treatment (untreated control).

4.9.3.7. f. Weight of green fruits

All the treatments were statistically significant concerning to fresh weight of fingers. The highest weight of green fruits (114.66 g) was observed in plants treated with T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) followed by T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) and T9 (carbendazim) having a fresh weight of 94.79 g and 82.83 g respectively. The lowest weight of green fruits (67.99 g) was recorded in plants without any treatment.

4.9.3.7. g. Weight of ripe fruits

Statistically significant differences were noticed in the weight of ripe fruits among the various treatments. The highest weight of ripe fruits (99.55 g) was recorded in plants applied with T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) followed by T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) and T4 (*Trichoderma viride*) having a ripe weight of 81.67 g and 70.69 g respectively. The lowest weight (50.28 g) of ripe fruits was noticed in control plants deprived of any treatment.

4.9.3.7. h. Peel to pulp ratio

The maximum peel to pulp ratio (0.35) was obtained in plants applied with T9 (carbendazim) followed by T4 (*Trichoderma viride*) and T2 (tebuconazole) having a ratio of 0.33 and 0.32 respectively. The lowest peel to pulp ratio (0.26) was recorded in T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) and T8 (*P. fluorescens*) applied plants. No statistically significant differences were observed among the treatments.

4.9.3.7. i. Total soluble solids (TSS)

Total soluble solids of ripened fruits obtained from all treatments varied from 25.27 °brix to 26.17 °brix which comes in the normal range of TSS of variety Rasthali/Poovan. No statistically significant differences were noticed in the TSS among various treatments. The highest TSS (26.17 °brix) was observed in fruits of plants applied with T7 (PGPR Mix II) followed by T2 (tebuconazole) with 26.13 °brix whereas, the lowest TSS (25.27 °brix) was noticed in fruits of plants applied with T8 (*P. fluorescens*).

4.9.3.8. Economic analysis

The benefit to cost ratio (B: C ratio) was calculated for all the treatments and is given in Table 4.33. At the market price of Rs. 50/kg of fruit, the highest (1.48) benefit to cost ratio was obtained in the treatment T2 (tebuconazole) followed by T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) with a B: C ratio of 1.44 and 1.40 respectively. Whereas, the lowest (1.18) B: C ratio was observed in untreated control plants.

4.9.3.9. Residue analysis

The residue analysis was carried out to check the persistence and degradation of the fungicides on fruits harvested from plants applied with tebuconazole. The study revealed that no residues of this fungicide or metabolites left in fruits. So these chemical can be effectively used for the management of the disease.

DISCUSSION

5. DISCUSSION

Banana (*Musa acuminata*) is one of the most important fruit crops in India as well in different parts of the world. Most of the cultivars of banana are triploids (AAA, AAB or ABB) of *Musa acuminata* (AA) and *Musa balbisiana* (BB) formed by intraspecific or interspecific cross-breeding (Simmonds and Shepherd, 1955; Heslop-Harrison and Schwarzacher, 2007). Cultivated bananas are seedless but produce parthenocarpic seeds, whereas, wild banana produces seeds (Li *et al*., 2013a). India is the largest producer of banana in the world with an average output of 28.6 million tonnes per annum and it is the second most important fruit crop next to mango (FAO, 2018). It occupies the fourth most vital food after rice, wheat and maize. It is a good source of essential vitamins and minerals such as potassium, calcium, manganese, magnesium, iron, folate, niacin, riboflavin and B6. These all contribute to the good functioning of the human body.

The banana production is seriously constrained by several pests and diseases. Among the diseases, the Fusarium wilt is the most destructive one which is incited by the pathogen, *Fusarium oxysporum* f. sp. *cubense.* It is a classical example of vascular wilt disease in plants. The pathogen is soil-borne and produces three types of spores namely microconidia, macroconidia and chlamydospore. It can survive for several years through chlamydospores. The pathogen causes up to 20 to 80 per cent yield loss. The pathogen has four recognised races from 1 to 4 based on the host specificity. Foc race 1 attacks cultivars Gros Michel (AAA), Silk (AAB), Rasthali (AAB) and Pome (AAB); Foc race 2 causes disease in the varieties such as Bluggoe and other cultivars (ABB genome). Whereas, race 3 was previously described as Foc (Waite and Stover, 1960; Stover, 1962) which is pathogenic to *Heliconia* spp. an ornamental flowering plant, but is no longer considered to belong to race structure of Foc (Ploetz, 2005). Race 4 attacks Cavendish and all banana cultivars susceptible to races 1 and 2.

Fusarium wilt of banana causes significant economic loss in agriculture scenario of tropical and subtropical countries in the world. It is a major threat to banana cultivation with 80 to 90 per cent disease severity (Sivamani, 1987). In India, the pathogen causes up to 80 per cent disease incidence and yield loss of up to 40 per cent

in northern states. Whereas, in southern states yield loss up to 90 per cent have been reported (Thangavelu *et al*., 1999). However, growing incidences of Fusarium wilt disease on banana in the country needs further studies on the occurrence and identification of various races using advanced technologies. A sustainable approach utilizing genetic resistance, new generation fungicides and eco-friendly bioagents needs to be formulated for the integrated management of the disease. Studies on pathogenic variability are also very important to develop integrated management practices. In the present study, an attempt was undertaken to characterize various isolates and diversity studies using molecular tools, to develop quick detection techniques and management strategy against the disease.

The project started with purposive sampling survey in farmers' fields of various locations in six districts of Kerala *viz.*, Thiruvananthapuram (Southern zone), Ernakulam (Central zone), Thrissur (Central zone), Palakkad (Northern zone), Kozhikode (Northern zone) and Wayanad (High range zone) during the period from June 2018 to November 2018. The observations such as the number of plants, number of infected plants and disease severity were recorded. The cultivars such as Rasthali/Poovan, Njalipoovan, Kadali and Chenkadali were widely cultivated and found highly infected by Fusarium wilt in Kerala. Though the Nendran variety was also highly cultivated, no disease incidence was noticed. A total of thirty isolates were collected from various locations for further studies.

The per cent disease incidence ranged from 1.52 per cent (Thiruvananthapuram) to 43.65 (Wayanad) per cent and thus varied from place to place. In Thiruvananthapuram district, the PDI ranged from 1.52 per cent (Neyyattinkara) to the 4.64 per cent (Attingal). In this district, the disease incidence was reported from cultivars such as Rasthali/Poovan, Kadali, Chenkadali and Njalipoovan. Whereas in Ernakulam district, the PDI varied from 19.44 per cent (Karukutty) to 27.40 per cent (Kadungallur). In this district, all the affected fields were planted with the Poovan variety. In the Thrissur district, the results of the survey revealed that the PDI ranged from 6.40 per cent (Kannara) to 23.78 per cent (Nadathara). All the infected fields were planted with the Poovan variety except Ollukkara where the Kadali variety was planted. While in the Palakkad district, the PDI varied from 5.33 per cent (Kannadi) to 7.58 per

cent (Kottayi). The Kottayi field was planted with Njalipoovan variety whereas all other fields were with Poovan variety. The PDI ranged from 12.30 per cent (Mukkom) to 17.22 per cent (Koduvally) in Kozhikode district where all the fields except Koduvally (Kadali) were planted with the Poovan variety. In the Wayanad district, all the infected fields were planted with the Poovan variety and the PDI ranged from 17.66 per cent (Sulthan Bathery) to 43.65 per cent (Ambalavayal). An attempt was taken to analyse the relationship of PDI with rainfall and temperature. The PDI increased with an increase in rainfall and decreased with increase in temperature. Rishbeth (1955) observed that the incidence of Fusarium wilt of banana increased soon after the heavy rainfall for two months. Similar results were also reported by other Foc researchers such as Stover (1962) and Simmonds (1966). Epp (1987) reported a positive correlation between heavy rainfall and the incidence of Fusarium wilt disease in Cavendish type of banana. Regarding temperature, the present study was in accordance with the study of Peng *et al.* (1999) who reported that the higher temperature above 33 °C inhibited the growth of the pathogen.

The per cent disease severity in the surveyed districts varied from location to location. The maximum PDS (20.34 %) was noticed in the field at Aluva (Ernakulam) and the minimum PDS (49.57 %) was observed at Kenichira field (Wayanad). In the Thiruvananthapuram district, PDS varied from 21.30 (Mudapuram) per cent to 38.18 per cent (Nedumangad). Whereas in Ernakulam district, the PDS varied from 20.34 per cent (Aluva) to 42.22 per cent (Karukutty). It ranged from 20.67 per cent (Anandapuram) to 32.63 per cent (Kannara) and 25.71 per cent (Kottayi) to 34.11 per cent (Chithali) in Thrissur and Palakkad districts respectively. While in the Kozhikode district, the PDS ranged from 26.81 per cent (Omassery) to 29.68 per cent (Mukkom) and in Wayanad district it varied from 34.74 per cent (Ambalavayal) to 49.57 per cent (Kenichira).

In the present study, types of symptoms produced by the infected plants were recorded. External and internal symptoms were noticed. During the natural infection, yellowing of leaves starting from older to younger, collapsing of leaves resulted in 'skirt' appearance and splitting of pseudostem were the external symptoms observed. The severely infected plants showed wilting and finally died. The splitting of

pseudostem were severe in the Rasthali/Poovan varieties. Whereas, the discolouration of rhizome and vascular tissues were the internal symptoms noticed. Severe wilting caused the death of plants. Under artificial condition, yellowing of the leaves and wilting were the external symptoms produced. Similar observations were recorded by several other workers (Stover, 1962; Perez-Vicente, 2004; Kumar and Saxena, 2015; Thangavelu *et al*., 2020). The external symptoms were grouped into 4 categories based on scoring using a standard score chart. The number of days taken for shifting from one stage to another was recorded. The highest (15.33) number of days taken for shifting from category 0 to 1 was observed in Njalipoovan followed by Chenkadali (11. 67), Kadali (11.33) and Rasthali/Poovan (10.67). The number of days taken for shifting from category 1 to 2 was maximum in Njalipoovan (12.33) followed by Chenkadali (9.67) and Kadali (8.67) and was found to be minimum in Rasthali/Poovan (7.67). The number of days taken for shifting from the category 2 to 3 was also found to be highest (10.33) in Njalippovan followed by Chenkadali (9.33), Kadali (7.33) and Rasthali/Poovan (6.33). The highest number of days (10.00) required for shifting from the category 3 to 4 was also observed in Njalipoovan followed by Chenkadali (7.00) and Rasthli/Poovan (5.00) while, the lowest number of days taken for shifting was noticed in Kadali (3.67).

The isolation of the pathogen from collected samples was carried out on half-strength Potato Dextrose Agar (PDA) medium. Ainsworth (1971) also used the half-strength PDA for the isolation of Foc from banana samples. A total of 30 Foc isolates were collected from various locations surveyed. Among these, four isolates were from the variety Kadali, two from Njalipoovan, one from Chenkadali and twenty-three from Poovan/Rasthali. The pathogen was isolated from pseudostem strands of infected plants. Das *et al.* (2012) isolated Foc from pseudostem strands of infected banana and maintained on PDA.

The pathogenicity test was carried out to test the potential capacity of all collected isolates on respective host plants by proving Koch postulates. All the isolates started producing symptoms from 10 to 15 days after the inoculation of the plants with injured roots by dipping in conidial suspension. The number of days taken for symptom expression depended on the variety of the cultivar used. Among the thirty isolates, nine isolates were most virulent which produced a disease severity of more than 80 per cent.

Earlier workers also .proved the pathogenicity of Foc in banana varieties. Similar results were obtained when an experiment was conducted by Sivamani and Gnanamanickam (1988) and Sowmya (1993) to prove the pathogenicity of Foc isolates in root injured and uninjured plants. Purwati *et al*. (2008) experimented proving pathogenicity of Foc in banana with injured and uninjured roots by root dipping and obtained similar results. Garibaldi *et al*. (2004) and Somu (2012) also proved the pathogenicity by root dipping in conidial suspension. Some other workers also demonstrated the pathogenicity of Foc in banana (Thangavelu *et al*., 2019; Damodaran *et al*., 2019).

The characterization of isolates was an important objective of the present study. The cultural characters of 30 isolates were studied on half strength PDA medium. Variation in the colour of the aerial mycelium and pigmentation was observed. Out of 30 isolates, twenty-five isolates produced white coloured aerial mycelium, four of them were greyish white and one was grey. White or pinkish white colour was the mostly found pigmentation however, dull-white, bright pink and violet pigmentations were also noticed in few isolates. Similar results were reported by Stover (1962) and Ploetz (1990) while working in Foc infecting banana. Honnareddy and Dubey (2007) also reported the similar results of pigmentation of *F. oxysporum* f. sp. *ciceris* isolates causing wilt in chickpea Difference in the colony texture was also noticed among the isolates. Most of the isolates were with cottony and fluffy mycelium whereas thin and sparse growth were also recorded. The maximum diameter of mycelium recorded on the seventh day of incubation was 9.00 cm while the minimum diameter observed was 5.96 cm. All the isolates were grouped into three categories based on the colony diameter. However, twenty-three isolates belonged to the first category which produced a colony diameter of more than 8.00 cm, three isolates in between 7.00 to 8.00 cm, belonged the second group and four isolates belonged to the third category *ie*., the colony diameter is less than 7.00 cm. The rate of mycelial growth varied from 0.83 cm/day to 2.40 cm/day. Similar results were reported by Joshi *et al*. (2013) in *F. oxysporum* causing wilt disease in tomato.

The morphological characterization is also important for studying the variability among fungal isolates. For this, characters such as length and breadth of microconidia and macroconidia and diameter of chlamydospores were recorded. Length and breadth

macroconidia ranged from 15.01 to 20.20 µm and 2.14 to 5.07 µm whereas, it varied from 4.29 to 7.42 µm and 1.35 to 3.13 µm in the case of microconidia. Length and breadth of hyphae varied from 16.14 µm to 22.94 µm and 4.22 µm to 6.57 µm. The results were in accordance with the earlier findings by Fourie *et al*. (2011). The macroconidia were 3 to 5 septate and the microconidia were aseptate or single septate. The diameter of chlamydospores ranged from 5.68 to 11.07 µm. Similar results were reported by Smith (2006) in Foc of banana.

In the present study, cluster analysis of the all isolates based on cultural and morphological characters by similarity coefficient method using Minitab 19 software was conducted. For this, the characters were classified into qualitative and quantitative data. Separate dendrogram was constructed for qualitative and quantitative parameters. The quantitative data included rate of colony diameter, length of macroconidia and microconidia, breadth of macroconidia and microconidia and the diameter of chlamydospores. Two clusters were formed *viz*., A1 and A2 at zero per cent similarity. Only five isolates belonged to the A2 cluster and the remaining 25 isolates belonged to the cluster A1. Cluster A1 includes all the Foc isolates collected from Rasthali/Poovan (AAB) and Njalipoovan (AB) varieties. Whereas, the cluster A2 includes all the Foc isolates collected from Kadali (AA) and Chenkadali (AA) varieties. The cluster A1 has two sub-clusters (B1 and B2) whereas, the cluster A2 has only one sub-cluster (B3). While, the qualitative parameters used for cluster analysis were the colour of mycelium, the texture of mycelium and pigmentation. Two main clusters namely A1 and A2 were formed with qualitative data at 100 per cent dissimilarity. Among the 30 isolates, nine were grouped into the cluster A1 which consists of only one sub-cluster (B1) and the remaining 22 isolates belonged to the A2 cluster which is again divided into two sub-clusters (B2 and B3).

Molecular characterization of the all 30 isolates was carried out in this study. Genomic DNA of all the isolates was extracted using the CTAB method described by Ingle and Ingle (2013) with modifications. During the standardization of the protocol, the quantity of sample was reduced from 1.0 to 0.1 g and extraction buffer was reduced from 10 to 1 ml, thus the extraction can be done in 2 ml centrifuge tube. Hence, the contamination and the loss of DNA can be reduced (Mishra *et al*., 2014). The mycelial

mat was ground with liquid nitrogen instead of sterilized sand helps in the proper grinding of the cell wall. Moreover, the per cent of CTAB used was increased from 1 to 2 per cent and the concentration of EDTA was decreased from 25 mM to 20 mM. The quantity of isopropanol used for the precipitation of DNA was increased from 0.6 volume to 1 so that the incubation period could be reduced from overnight to only 2 h. The temperatures used for the centrifugations also modified for the extraction of good quality DNA. The quality and quantity of isolated DNA were assessed. The amount of the isolated DNA ranged from 125.70 to 2627.32 ng/µl which indicates the good quantity of DNA can be extracted through the method used. The optical density ratio at $\lambda_{260}/\lambda_{280}$ of the isolated DNA was ranged from 1.82 to 2.19 indicates the obtained DNA is of good quality and is free from impurities such as RNA. Also, high-quality bands were produced when the isolated DNA was documented in agarose gel electrophoresis system. CTAB method for the extraction of good quality DNA from filamentous fungi was also used by several other workers (Moller *et al*., 1992; Muhammad *et al.*, 2017; Aguilar-Hawod *et al*., 2020)

The polymerase chain reaction provides rapid, reliable and easy detection of the pathogen at the early stages of infection instead of the time consuming conventional methods. The standardization of annealing temperature clearly showed that 54.9 °C was the optimum annealing temperature for the polymerase chain reaction of the isolates using ITS 1F and ITS 4R primers. The rDNA internal transcribed spacer region is highly variable among organisms and so that it can be used for analysing the relationship between organisms (Bruns *et al.*, 1991). Agarose gel documentation of the amplicons formed by PCR produced single bands of about 580 bp for all isolates. The amplicons obtained through PCR were sequenced and *in silico* analysis of the 30 isolates was carried out to study the similarity with other sequences available in NCBI. The isolates had more than 96 per cent sequence homology to Foc sequences available in NCBI. Each nucleotide sequence involves the partial sequence of 18S rDNA and 28S rDNA and the full sequence of ITS 1 region, ITS 2 region and 5.8S rDNA. Several other workers also targeted the ITS region for the characterization and identification of *F. oxysporum* (Ingle and Ingle, 2013; Jadhav *et al.*, 2019).

The phylogenetic tree analysis using the neighbour-joining (NJ) method with 1000 bootstraps of 30 isolates with other formae speciales of *F. oxysporum* and other *Fusarium* spp. formed two main clusters. All isolates and other *F. oxysporum* formae speciales along with *F. tricinctum* formed one cluster whereas remaining *Fusarium* spp. formed another cluster. The former cluster was again subdivided into two sub-clusters. In this, one sub-cluster included all the isolates along with other formae speciales of *F. oxysporum* and the other sub-cluster involves the *F. ticinctum*. The analysis revealed that all the *Fusarium* spp. originated from a common ancestor. The Foc isolates showed more similarity with the formae speciales of *F. oxysporum*. All the isolates were diverged into different clades irrespective of the geographic region. Variations were observed among the isolates and this may be due to some mutational changes. Similar studies were also conducted by Hirano and Arie (2009) and Fourie *et al.* (2009) for the analysis of the evolutionary relationship among the isolates of *F. oxysporum*.

No differences in sequences were observed between Foc isolates with other formae speciales of *F. oxysporum* at the ITS rDNA region. Whereas, variations in sequences were observed between formae speciales of *F. oxysporum* and other *Fusarium* spp. at various positions. A gap of 1 bp was observed at 576th position in *F. oxysporum* while it was present in all other *Fusarium* spp. (Fig. 5.).

In the present study, an attempt was undertaken to isolate the fungal DNA directly from infected tissues of the plant. The method employed here is rapid and inexpensive which avoids the time consuming and difficult process explained in former methods as the isolation of pathogen, its artificial cultivation and grinding with liquid nitrogen (Zhang *et al*., 2013). In this protocol, 2 per cent CTAB and 20 mM EDTA were added instead of 1 per cent CTAB and 25 mM EDTA used in the former method (Ingle and Ingle, 2013). In addition to this, 50 µl of ß-mercaptoethanol and 1 per cent polyvinylpyrrolidone were also used during the grinding of 1 mg tissue. EDTA inactivates the endonucleases (Rogers and Benedich, 1994) and ß-mercaptoethanol prevents the phenolic oxidation. CTAB avoids the polysaccharides contamination whereas, the isopropanol and ethanol allow the actual precipitation of DNA and remove the salts and detergents, making it more stable. The quantity of DNA extracted was measured using a NanoDrop spectrophotometer. The concentration of DNA yield was

751.6 ng/µl. OD ratio λ260/ λ280 obtained was 1.89 which relies between 1.8 to 2 indicates the DNA is free from protein and RNA impurities (Mishra *et al*., 2003). The isolate used for the study was sequenced and *in silico* analysis displayed 100 per cent similarity with Foc accessions. The sequence was deposited in NCBI database and the accession number was obtained as MN953004. The phylogenetic tree clearly showed that the isolate MN953004 was closely related with Foc isolates with a good bootstrap value. Hence, this method can be used for isolation of high quality DNA required for molecular research directly from the infected tissues.

Race identification of the isolates collected from various locations during the survey was done by artificial inoculation of the pathogen in banana cultivars such as Cavendish, Plantain, Rasthali/Poovan, Monthan and *Heliconia* spp. All the isolates produced symptoms in Rasthali/Poovan varieties but no symptoms were produced by the isolates in Cavendish, Plantain, Monthan or Heliconia. Hence, it can be concluded that all the isolates belong to Foc Race 1 group and Foc Race 2, 3 or 4 were not present among the isolates. Foc race 1 infects Gros Michel, group Rasthali, Maqueno, Silk, Pome, Pisang Awak, whereas race 2 affects cooking bananas like Bluggoe and Monthan (Moore *et al*., 1995) and race 3 infects *Heliconia* spp. a close relative of cultivar banana. Among the race 4, subtropical R4 (SR4) and tropical R4 (TR4) (Groenewald *et al*., 2006). Subtropical race 4 affects the Cavendish group and the varieties susceptible to race 1 and 2 in subtropics. TR 4 pathogens attack both in tropical as well as subtropical conditions (Buddenhagen, 2007). The Cavendish type of banana is susceptible to the race 4 of Foc (Thangavelu and Mustaffa, 2010a).

In the present study, loop mediated isothermal amplification assay (LAMP) was conducted for the easy detection of Foc races. The standardization of temperature needed for the amplification revealed that a temperature of 63 °C for 60 min. was optimum for the reaction. The primers were designed for Foc race 1 using PrimerExplorer V5 software based on the endoglucanase 4 gene sequence. All the 30 isolates were subjected to amplification using the primers of Foc race 1 and Foc race 4 (Li *et al*., 2013b). All the isolates were positive in amplification using Foc race 1 primers whereas, the negative reaction was shown in the case of Foc race 4 primers. In the colourimetric detection using hydroxynaphthol blue (HNB) dye, all the isolates turned

from purple to blue colour in positive reaction while remained purple/violet in negative reaction and non-template control. LAMP products of all isolates were undergone 2 per cent gel electrophoresis and the positive reactions showed a ladder-like bands under UV light whereas negative/control reactions were without any bands. It indicates that all the isolates were Foc race 1 and no race 4 isolates were obtained. Similar results were obtained in the colourimetric detection and gel electrophoresis of *F. oxysporum* f. sp. *melonis* by LAMP assay using Hydroxynaphthol blue (HNB) dye (Almasi, 2019). In order to confirm th results the isolates were subjected to polymerase chain reaction (PCR) using the specific primers (F3 and B3 primers of LAMP assay for Race 1). All the isolates produced single bands of size about 240 bp at an annealing temperature of 53.4 °C.

Host plant resistance is an effective method for escaping plants from certain diseases. It is used when a certain variety of plants are resistant to certain races of the pathogen which is pathogenic to other varieties of the host plants. An attempt was done to evaluate the host plant disease resistance of banana to Fusarium wilt by screening the twenty six varieties available at gene bank of Banana Research Station, Kannara. Per cent disease incidence (PDI) and per cent disease severity (PDS) were calculated.

Among the AAB group, the lowest PDI (0 %) was recorded in Attunendran, Zanzibar, Big Ebanga, Nedunendran, Nendran, BRS II (Vannan x Pisang Lilin), Thiruvananthapuram and Pachanadan I and were immune to the disease. Whereas, two varieties (Cheriya Poovan and Valiya Poovan) were highly susceptible (100 %). In AA group, the maximum PDI (100 %) was observed in Kadali and the least PDI (0 % disease incidence) was noticed in Cultivar Rose, Pisang Lilin and Pisang Jari Buaya. All the three varieties belonging to the AAA group namely Grand Naine, Yangambi Km5 and Chinese Cavendish were immune (0 % disease incidence) to the disease. The Nendran hybrid belonging to AAAB group was immune (0 % disease incidence) to the disease whereas Njalipoovan belonging to the AB group was highly susceptible (100 % disease incidence) to the disease.

The disease severity was calculated using per cent wilt index (PWI) by scoring of external symptoms employing 0-4 scale (Mak *et al*., 2004). Out of 13 varieties

belonging to AAB genome group, eight (Attunendran, Zanzibar, Big Ebanga, Nedunendran, Nendran, BRS II (Vannan x Pisang Lilin), Thiruvananthapuram and Pachanadan I) were immune to the disease with zero per cent severity, three varieties were highly resistant (Palayankodan, Velipadathy and Pdathy) and two were moderately resistant (Valiya Poovan and Cheriya Poovan). Of the eight genotypes belonging to the AA group, three were immune (Cultivar Rose, Pisang Lilin and Pisang Jari Buaya), four were highly resistant (Sanna Chenkadali, Chenkadali, TMP2x2829 and *Musa acuminata* sub sp. *burmanica*) and one was resistant (Kadali) to the disease. All the three varieties belonging to the AAA group *viz*., Grand Naine, Yangambi Km5 and Chinese Cavendish were immune to the disease. The Nendran hybrid (AAAB) was also immune to the disease while Njalipoovan (AB) was resistant to the disease.

An attempt was made to calculate the disease severity based on per cent vascular wilt index also by scoring internal symptoms of rhizome using the 0-5 scale (Zuo *et al.*, 2018). Of the 13 accessions belonging to AAB genome group, eight were immune (Attunendran, Zanzibar, Big Ebanga, Nedunendran, Nendran, BRS II (Vannan x Pisang Lilin), Thiruvananthapuram and Pachanadan I), two were highly resistant (Padathy and Palayankodan), one was moderately resistant (Velipadathy) and two were highly susceptible (Valiya Poovan and Cheriya Poovan). Among the seven varieties belonging to the AA group, three were immune (Cultivar Rose, Pisang Lilin and Pisang Jari Buaya), one was highly resistant (TMP 2x 2829), two were resistant (*Musa acuminata* sub sp. *burmanica* and Sanna chenkadali) and one was susceptible (Kadali) to the disease. Among the four varieties belonging to the AAA group, three were (Grand Naine, Yangambi Km5 and Chinese Cavendish) immune and one (Chenkadali) was resistant. The Nendran hybrid belong to AAAB group was found to be immune to the disease. The Njalipoovan (70 %) belonging to the AB group was found to be susceptible to the disease.

In general, the varieties such as Attunendran (AAB), Zanzibar (AAB), Big Ebanga (AAB), Nedunendran (AAB), Nendran (AAB), BRS II (Vannan x Pisang Lilin) (AAB), Thiruvananthapuram (AAB), Pachanadan I (AAB), Cultivar Rose (AA), Pisang Lilin (AA), Pisang Jari Buaya (AA), Grand Naine (AAA), Yangambi Km5 (AAA), Chinese Cavendish (AAA) and Nendran hybrid (AAAB) were observed to be

immune to the disease. Whereas, the varieties such as Valiya Poovan (AAB), Cheriya Poovan (AAB), Kadali (AA) and Njalipoovan (AB) were susceptible to the disease. Hence, from the results, it can be concluded that the resistance/susceptibility towards the Fusarium wilt is not based on the genome of the cultivar. Similar studies were conducted by Mondal *et al*. (2001) and revealed that Njalipoovan (AB) was highly susceptible to the Fusarium wilt. Huang *et al*. (2005) also conducted a screening experiment and reported that Pisang Jari Buaya (AA) was resistant to the disease.

Plants have various defense strategies to counteract with pathogens. It includes the activity of secondary metabolites/biochemicals produced by plants. Biochemical basis of disease resistance is a key strategy found in plants. Being a soil-borne pathogen, Foc can enter the host plant at any time after planting. If the plants are resistant or immune with phenols and defence-related enzymes, the invasion of the pathogen can be easily warded off. The phenolic compounds are a key group of secondary metabolites as these are involved in the defense mechanisms of plants (Sharma *et al*., 2018). The present study was undertaken to determine the activity of secondary metabolites such as phenols, reducing sugars, non reducing sugars, peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase in defense mechanisms after artificial inoculation. The activity of these secondary metabolites in healthy leaves were also studied (Fig. 5.1).

The quantity of total phenol was more in resistant cultivars *viz*., Nendran and Robusta than susceptible cultivars namely Rasthali/Poovan and Kadali. The activity of total phenol was increased up to 6^{th} day after challenge inoculation in Nendran, Robusta and Kadali however in Rasthali/Poovan the maximum phenol content was recorded on 4^{th} day after inoculation and then decreased near to the basal level (Fig. 5.2). A similar study was conducted by Ramanathan *et al*. (2000), which revealed that the accumulation of phenolics showed resistance to Fusarium wilt in tomato. The phenol content was increased by 1.24 and 1.28 fold in Nendran and Robusta respectively while 1.23 and 1.18 in Rasthali/Poovan and Kadali respectively. This was in tune with the study conducted by Arfaoui *et al*. (2005) to analyse the pattern of change in phenol content induced by *Rhizobium* isolates at 24 h interval in response to challenge inoculation of *F. oxysporum* f. sp. *ciceri* in chickpea. Similar observations were reported by Thakker *et al*. (2007) while working on the banana with challenge inoculation by Foc.

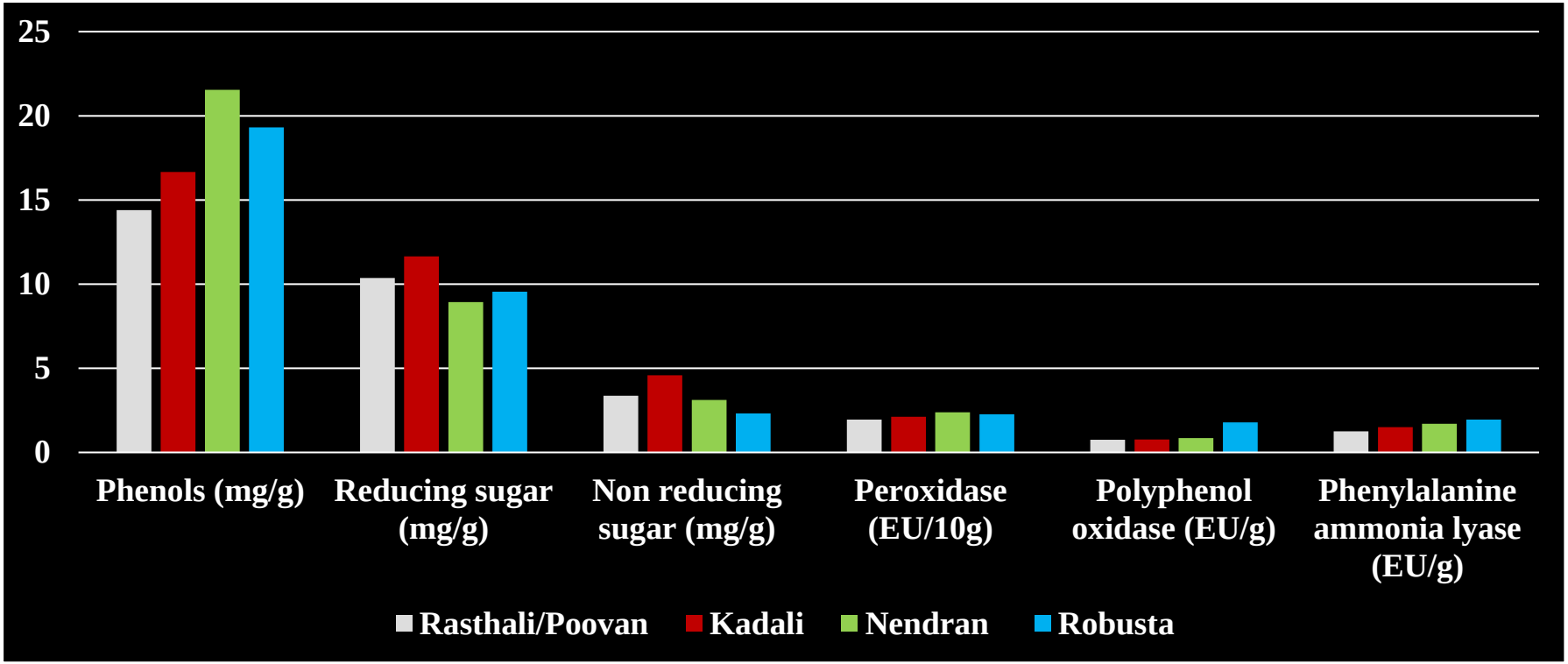


Fig. 5.1. Activity of total phenols, sugars and defense related enzymes in healthy leaves of banana

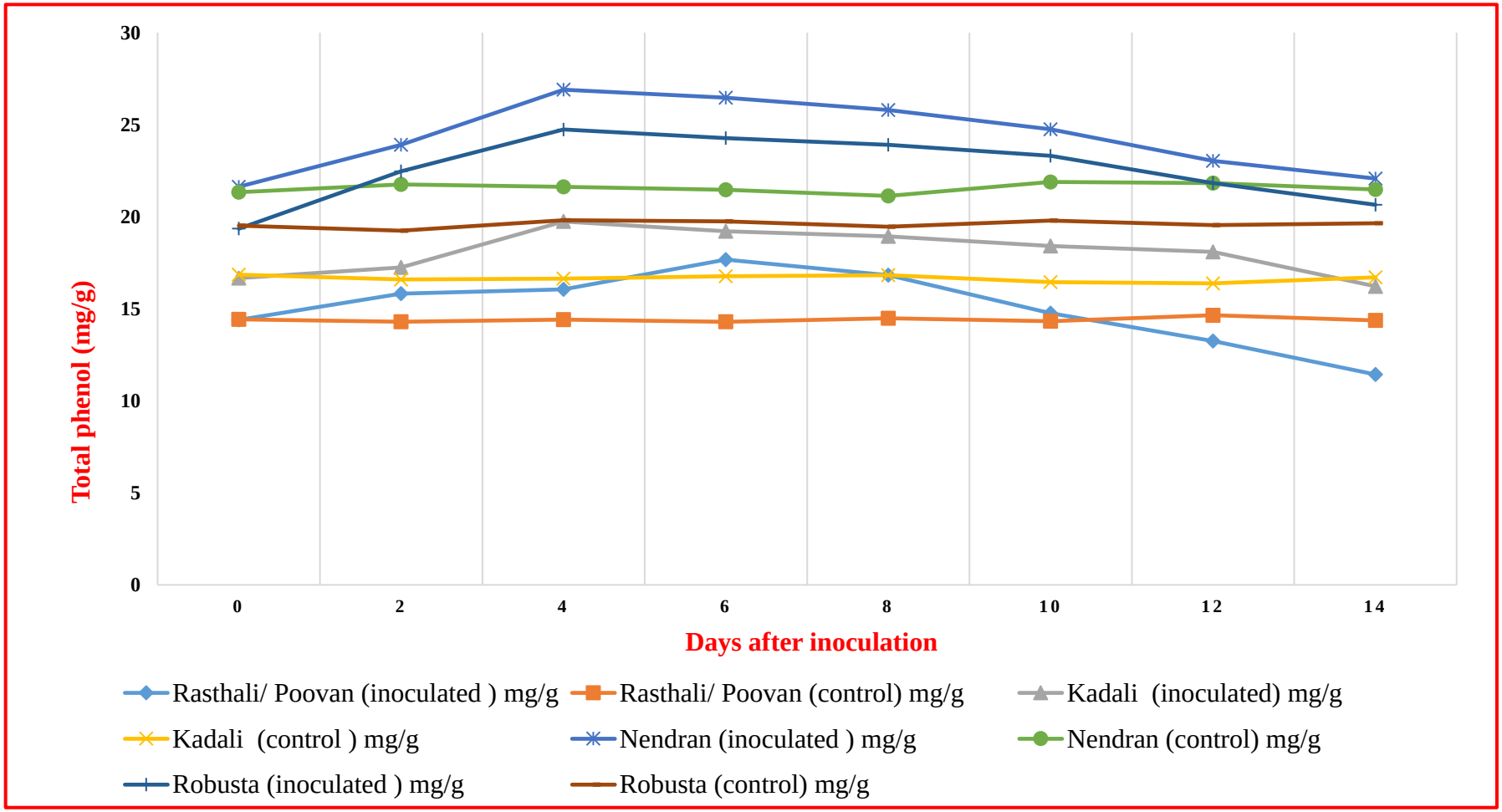


Fig. 5.2. Activity of total phenols in different varieties of banana after inoculation with Foc

The activity of reducing and non reducing sugar was more in susceptible cultivars *viz*., Rasthali/Poovan and Kadali compared to the resistant varieties (Nendran and Robusta). This result was in line with the observations reported by Easwaran (1972) in sorghum infected with the bacterial disease. After challenge inoculation, the activity of these sugars decreased continuously (Fig. 5.3 - 5.4). This might be due to the pathogen utilizes the sugars present in host plants for their own needs. Moreover, the host plant converts the sugars into antimicrobial agents for imparting disease resistance (Tauzin and Giardin, 2014). Similar reports were given by Jayapal and Mahadevan (1968) when artificial inoculation of leaf spot pathogens such as *Cercospora musae* was done in banana plants and Veeramohan *et al.* (1994) on inoculation of chilli leaves with *Alternaria solani.*

The quantity of peroxidase enzyme was more in resistant cultivars than susceptible cultivars. After artificial inoculation with Foc, the activity of peroxidase showed an increasing trend in resistant (Nendran and Robusta) and susceptible (Rasthali/Poovan and Kadali) varieties up to 8th day and then decreased near to the base level when assessed for 14 days at 48 h interval (Fig. 5.5). Anand *et al*. (2009) also got the similar results when they were working in chilli fruits inoculated with *Colletotrichum capsici* and *Alternaria alternata.* In Nendran and Robusta the quantity of PO enzyme increased 3.23 and 3.12 fold respectively whereas, in Rasthali/Poovan and Kadali, the increase was only 2.03 and 2.57 fold respectively. This study was in line with the experiment done by Thakker *et al.* (2013) on the induction of defence-related enzymes in banana by inoculation with Foc.

The quantity of polyphenol oxidase enzyme was more in resistant cultivars than susceptible cultivars. A similar report was given by Nagar *et al*. (2016) in banana hybrids against the Fusarium wilt. On artificial inoculation with Foc, the activity of polyphenol oxidase also showed an increasing trend initially in both resistant and susceptible cultivars and then decreased after 6th day of inoculation (Fig. 5.6). In resistant varieties *viz*., Nendran and Robusta, increase in activity of PPO activity on 6th day after inoculation was 4.85 and 3.31 fold respectively while it was 2.6 and 3.10 fold in Rasthali/Poovan and Kadali respectively. Similar observations were reported by

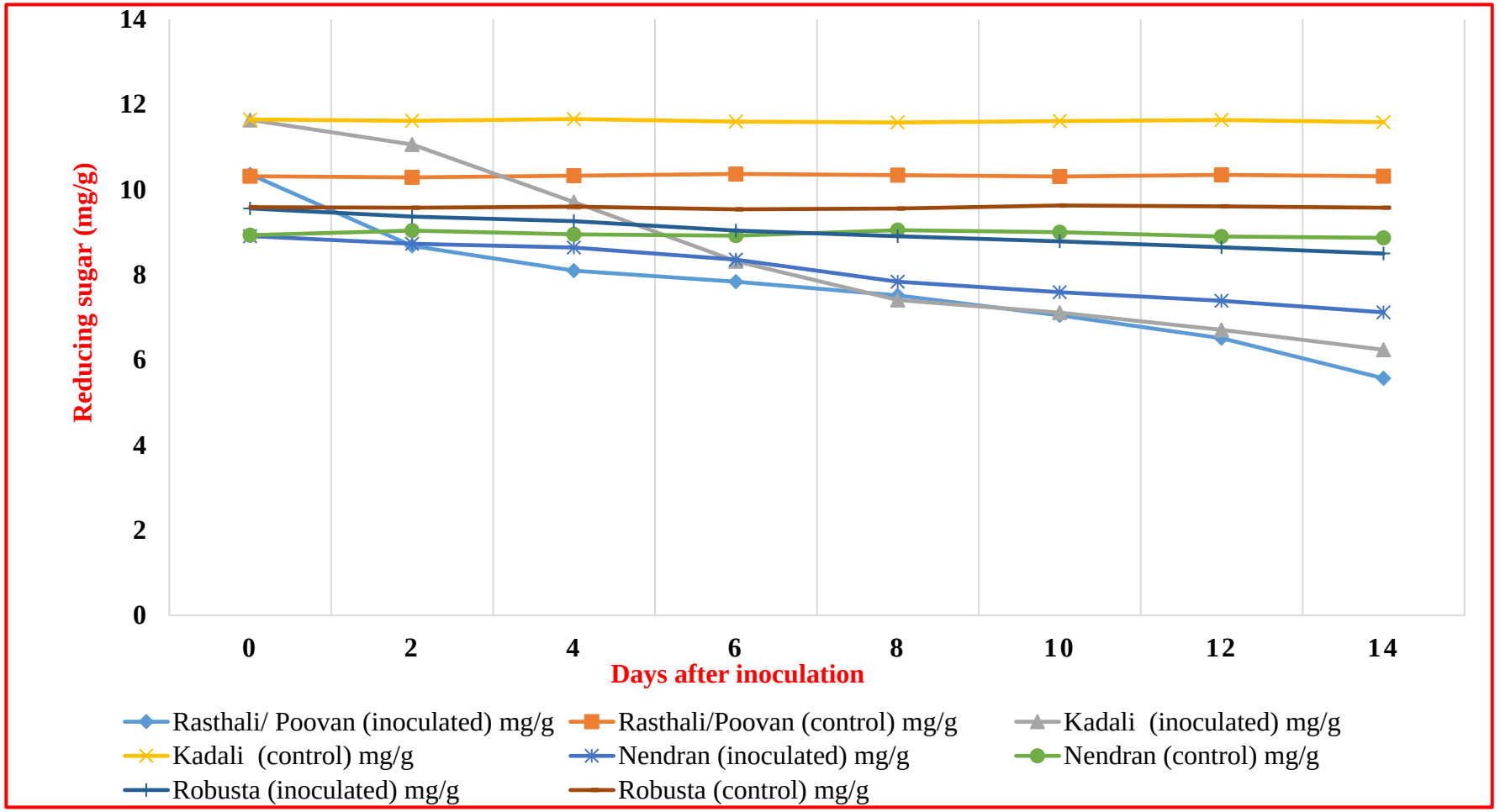


Fig. 5.3. Activity of reducing sugars in different varieties of banana after inoculation with Foc

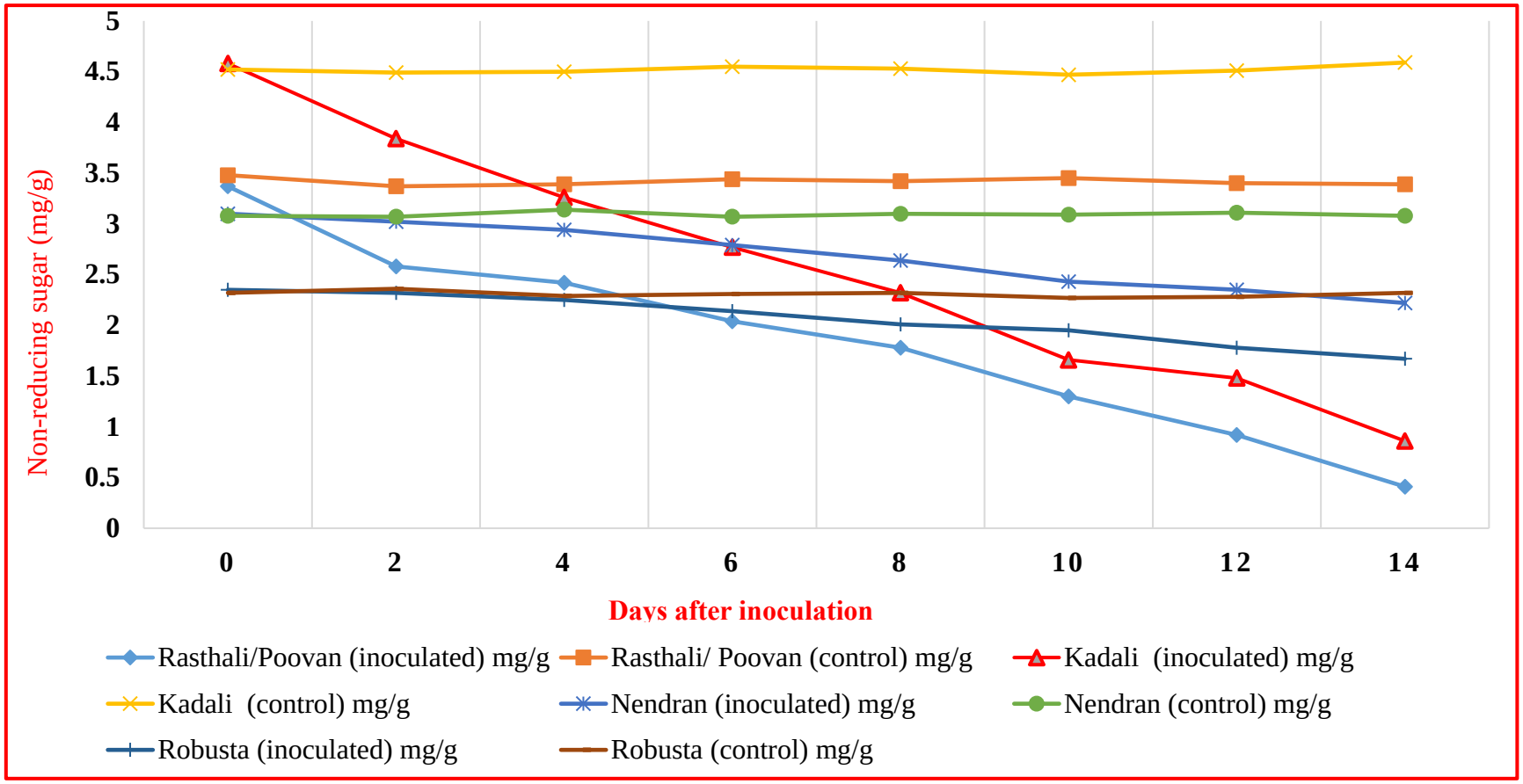


Fig. 5.4. Activity of non reducing sugars in different varieties of banana after inoculation with Foc

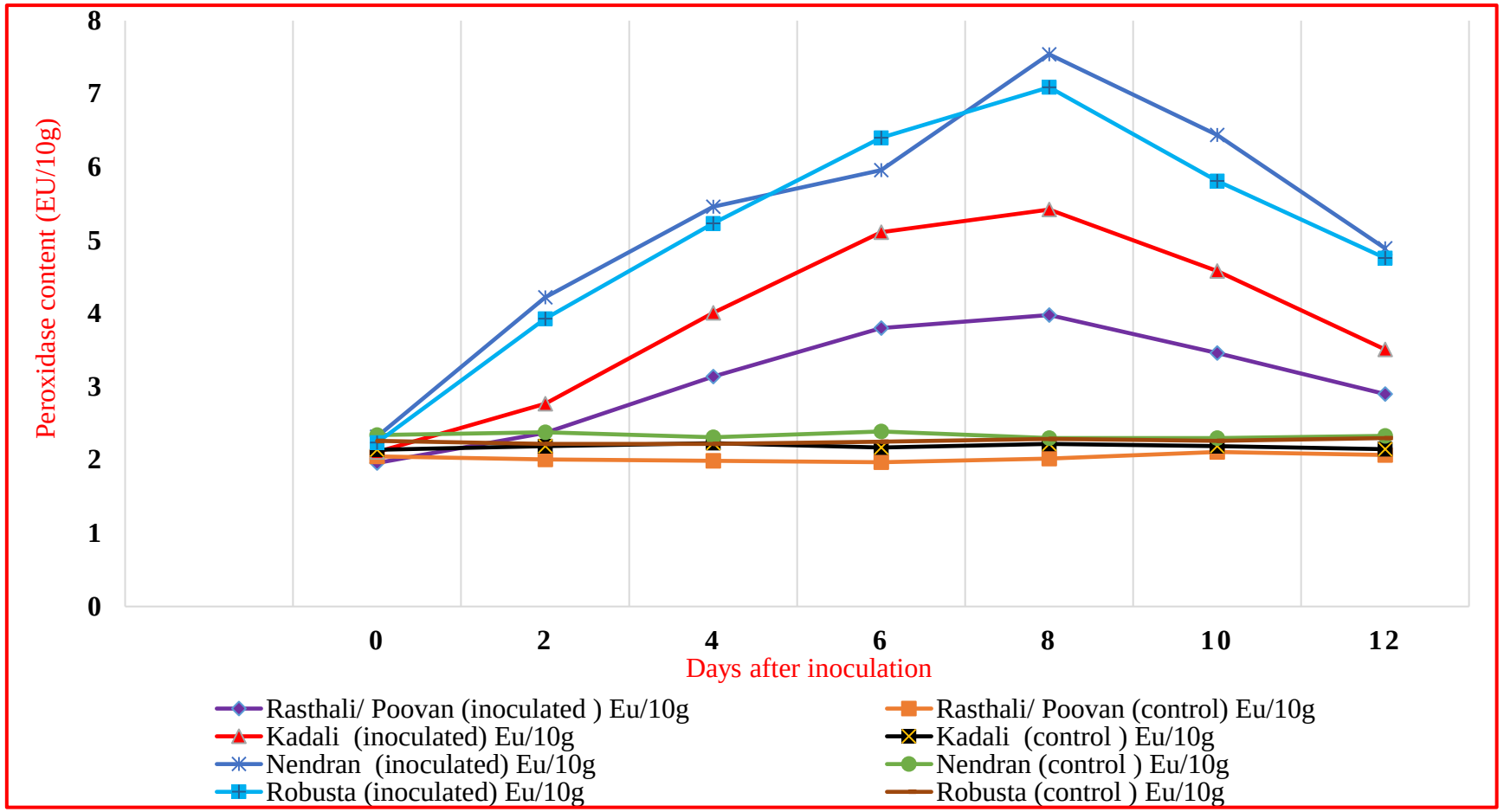


Fig. 5. 5. Activity of peroxidase enzyme in different varieties of banana after inoculation with Foc

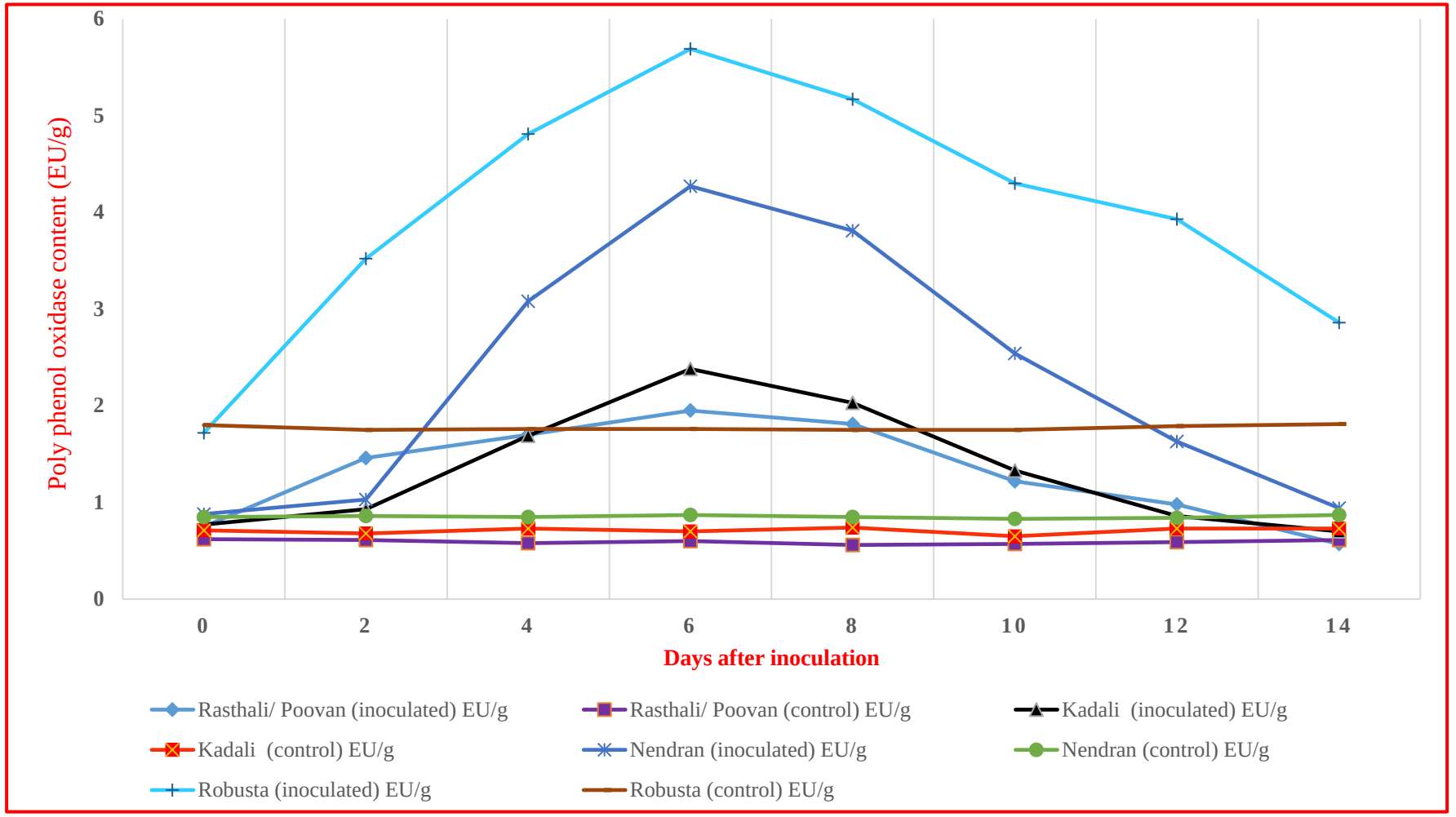


Fig. 5. 6. Activity of poly phenol oxidase enzyme in different varieties of banana after inoculation with Foc

Vanitha *et al.* (2009) in tomato plants against bacterial wilt pathogen *Ralstonia solanacearum.*

The activity of PAL was found to be more in resistant cultivars compared to the susceptible cultivars. This was in sync with the results given by Bharathi *et al*. (2019) in castor inoculated with *F. oxysporum* f. sp. *ricini*. The plants showed a rapidly increasing trend up to 8th day in resistant and susceptible cultivars after artificial inoculation with Foc (Fig. 5.7). The activity of PAL was increased by only 2.15 fold in Rasthali/Poovan and 1.99 fold in Kadali and 2.79 and 2.82 fold in Nendran and Robusta respectively. Similarly, Kumar *et al.* (2017) conducted a study on defense-related enzymes associated with infection of *Dickeya zeae* causing bacterial stalk rot in maize and got the maximum PPO and PAL after 48 h of inoculation.

The histopathological changes associated with the infection of Foc in different parts of banana were examined and documented. In the infected leaves, the amount of chloroplast was less and the cells in palisade and spongy layer were found to be flaccid. The accumulation of secondary metabolites were noticed cortex of root and rhizome and crushed cells was observed in the rhizome. Some irregular growth was noticed in the xylem vessels of the roots and distorted cells were also found in the root cortex. Similar results were also reported by Vishwanath *et al*. (2011) in Foc infected banana tissues.

An evaluation of eleven chemical fungicides, four biocontrol agents and seven botanicals at lower, recommended and higher doses was conducted by poisoned food technique for the mitigation Foc *in vitro*. The chemical fungicides included four contact fungicides, four systemic fungicides and four combination fungicides. The results of the experiment revealed that among the contact fungicides used, 100 per cent inhibition was showed by Bordeaux mixture followed by mancozeb (51.33 %), copper hydroxide (39.78 %) and copper oxychloride (32.33 %). The mean mycelial growth was zero in the case of Bordeaux mixture while it was highest in copper oxychloride (6.09 cm). All the systemic fungicides (tebuconazole, propiconazole and carbendazim) gave 100 per cent inhibition of the pathogen except azoxystrobin (70.78 %). The mean mycelial growth was 2.63 cm when azoxystrobin was used. The 100 per cent inhibition of Foc

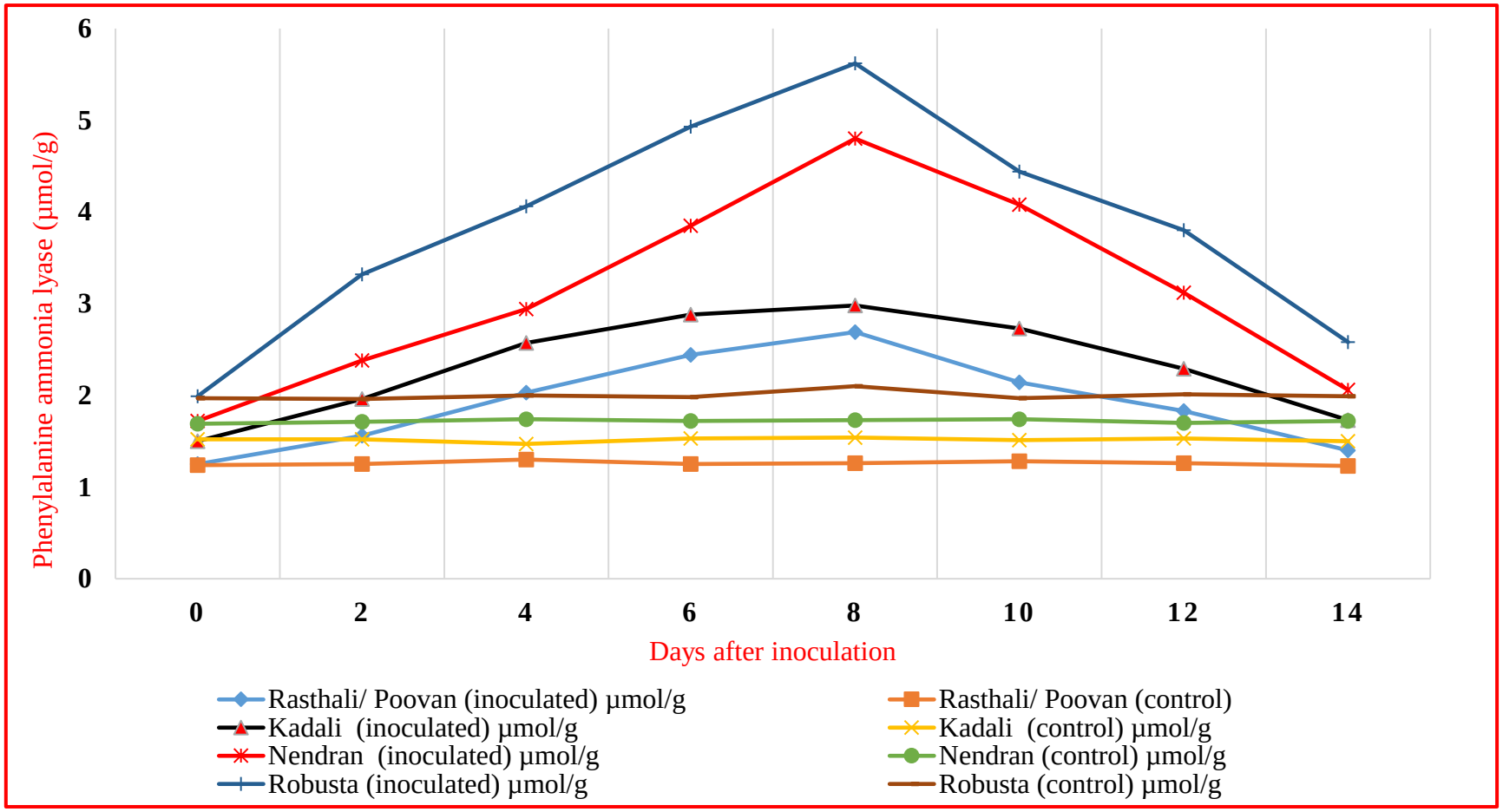


Fig. 5. 7. Activity of phenylalanine ammonia lyase enzyme in different varieties of banana after inoculation with Foc

by triazoles was reported by Nel *et al.* (2007) and Shukla (2019) in the evaluation of new fungicides against Foc. Soma *et al*. (2008) reported the complete inhibition of *F. oxysporum* using the carbendazim *in vitro*. Several other workers also reported similar results (Amini and Sidovich, 2010; Vinit *et al.*, 2010; Ram and Pandey, 2011; Wani and Mir, 2011). All combination fungicides (captan + hexaconazole, carbendazim + mancozeb and propiconazole + difenoconazole) and biocontrol agents (PGPR Mix II, PGPM, *T. viride* and *P. fluorescens)* gave 100 per cent inhibition and the mean mycelial growth was zero cm. *T. viride* and *P. fluorescens* gave 100 per cent in the dual culture method also. Ram and Pandey (2011) revealed that the combination products of captan + hexaconazole (250 pg ml^{-1}) and carbendazim + mancozeb (500 µg ml^{-1}) completely controlled the mycelial growth of *F. oxysporum* f. sp. *udum* causing wilt in pigeon pea. Thangavelu and Mustaffa (2010b) reported that *T. viride* can be effectively used for the management of Foc. Among the botanicals used, none of the treatments gave 100 per cent inhibition of the pathogen. The maximum (39.44 %) per cent inhibition was observed when treated with extract of *Myristica fragrans* mace followed by the *Azadirachta indica* oil (26.22 %) and *Lawsonia inermis* leaves (25.33 %). The minimum per cent of inhibition of the pathogen was recorded when treated with extract of *Curcuma caesia* rhizome (9.33 cm). The mean mycelial growth was maximum when treated with *Curcuma caesia* (8.16 cm) followed by *Curcuma angustifolia* (7.98 cm) and *Kaempferia galanga* (7.68 cm). Whereas it was lowest in the treatment with *Myristica fragrans* (5.45 cm) followed by *Azadirachta indica* (oil) (6.64 cm) and *Lawsonia inermis* leaves (6.72 cm). Similar results were reported by Kinge *et al*. (2019) in an experiment against *F. oxysporum f. sp. elaeidis* causing vascular wilt in oil palm.

A pot culture experiment was carried out to develop an effective method for the management of Fusarium wilt of banana. Sucker treatment and soil drenching with four chemical fungicides and three biocontrol agents, two integrated management strategies and the no treatment control. The per cent disease incidence (PDI) and per cent disease severity (PDS) were recorded. The highest PDI (100.00 %) was recorded in the plants treated with T10 (control) whereas the lowest PDI (22.22 %) was noticed in T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole). Similar results were reported by Sharma *et al*. (2010) while evaluation of the fungicides for the

management of Fusarium wilt in tomato. The highest per cent reduction over the control plants (77.78 %) was recorded in T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole whereas, the lowest reduction (11.11 %) of the disease over control plants was recorded in T1 (copper hydroxide), T4 (*T. viride*), T7 (PGPR Mix II) and T8 (*P. fluorescens*). The present study was in line with the experiment conducted by Chennakesavulu *et al.* (2013) who reported that the tebuconazole along with *P. fluorescens* gave 100 per cent inhibition of Fusarium wilt in red gram incited by *Fusarium udum*.

The per cent disease severity based on the PWI was recorded at 3 MAP by scoring the external symptoms. The highest disease severity (66.64 %) was observed in control plants (T10). While the lowest disease severity (8.33 %) was recorded in T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole). The highest disease reduction (87.50 %) was noticed in T6 (IDM – *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) whereas, the least disease reduction over control (33.33 %) was recorded in T1 (copper hydroxide).

The per cent disease severity based on PVWI was carried out by scoring the vascular symptoms in the rhizome at 3 MAP. The maximum disease severity (55.58 %) was observed in control plants (T10) whereas the lowest disease severity (13.34 %) was found in plants treated with T6 (IDM – *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole). The per cent disease reduction over control based on PVWI was maximum (76.00 %) in T6 (IDM – *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole). The lowest disease reduction over control (11.39 %) was recorded in T1 (copper hydroxide).

A field experiment was laid in the sick plot at Banana Research Station, Kannara to develop an effective, environment friendly and affordable management strategy against the Fusarium wilt of banana. The treatments used were the same as in the pot culture experiment. The factors such as per cent disease incidence (PDI), per cent disease severity (PDS), vegetative characters such as the height of plants, the girth of plants, number of green leaves and number of suckers, yield characters such as bunch weight, number of hands per bunch, fingers per hand, length of fingers, the

circumference of fingers, fresh weight of fingers, ripe weight of fingers, peel to pulp ratio and total soluble solids (TSS) were recorded for each treatment.

The per cent disease incidence was recorded at 8 MAP as well as 10 MAP. The disease incidence was observed in all treatments. The highest PDI (53.33 %) was noticed in control plants whereas the lowest PDI (6.67 %) was recorded in plants applied with T6 (IDM – *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole). At 10 MAP, the disease incidence has increased in all treatments ranging from 13.33 per cent to 73.33 per cent. During this period also the highest PDI (73.33 %) was recorded in control plants (T10) and the lowest PDI (13.33 %) was recorded in plants treated with T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole). The mean PDI was 63.33 per cent in untreated control plants whereas, 10.00 per cent in plants treated with T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole). The per cent reduction of the disease over control was maximum (78.95 %) in T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) while, the minimum reduction (10.52 %) of the disease over control was recorded in T1 (copper hydroxide). The present study was in accordance with the results reported by Sreeja (2014) for the *F. oxysporum* causing wilt in cowpea by soil drenching of tebuconazole. Similar observations were reported by Chawla and Gangopadhyay (2009), they revealed that the bioagents such as *T. harzianum, T. viride* and *P. fluorescens* in the presence of farmyard manure can be effectively used against *F. oxysporum* f. sp. *cumini.*

The severity of the disease in the field experiment was assessed by calculating the per cent wilt index (PWI) and per cent vascular wilt index (PVWI) at 10 MAP. The treatments were statistically superior over the control plants. The PWI was recorded by scoring the external symptoms using a 0-4 scale (Mak *et al*., 2004). The maximum disease severity (56.67 %) based on PWI was noticed in plants kept as control the lowest disease severity (11.67 %) was recorded in plants treated with T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole. Per cent disease reduction over the control based on PWI was also assessed in the present experiment. The highest disease reduction (79.41 %) was noticed in T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole), while the lowest disease reduction

over control (23.53 %) was recorded in T1 (copper hydroxide). The assessment of disease severity based on PVWI was carried out by scoring the vascular symptoms in the rhizome using a 0-5 scale (Zuo *et al.*, 2018). The disease severity was maximum (58.33 %) in untreated plants (T10) whereas minimum (13.33 %) in plants treated with T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) followed by T2 (tebuconazole). Per cent disease reduction over the control based on PVWI was calculated and the highest disease reduction (77.15 %) over control treatment was noticed in T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole). Whereas the disease reduction over control (22.85 %) was minimum in plants applied with T1 (copper hydroxide). The Arbuscular Mycorrhizal Fungi (AMF) and *T. harzianum* were effective against *F. oxysporum f. sp. cubense* on banana plantlets under field conditions (Ubaud and Requina, 2016). The present study was in tune with the experiment conducted by (Bubici *et al*., 2019) who reported that application of *Pseudomonas* spp., *Trichoderma* spp. and arbuscular mycorrhizal fungi could manage the disease. Shukla (2019) reported that the maximum inhibition of PWI and PVWI in banana could be achieved by the treatment with tebuconazole containing fungicides. Hence, an effective integrated management strategy including *Trichoderma* spp. *P. fluorescens*, AMF and tebuconazole can be effectively used the management of Fusarium wilt of banana under field conditions.

The height of plants increased from 5th MAP to 10th MAP in all the treatments. The best treatment enhancing the plant height was observed to be T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) followed by T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*)) with a plant height of 242.33 cm and 240.00 cm respectively. The plant height was minimum (221.00 cm) in T10 (untreated control) applied plants. The treatments were found to be not significantly different from each other. The plant girth was increased from 5th MAP to 10th MAP and statistically significant differences were observed among the treatments applied. The highest plant girth (24.44 cm) was observed in plants applied with the treatment T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) followed by T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) with 24.16 cm. Whereas the lowest

(20.12 cm) plant girth was obtained in T8 (*P. fluorescens*). The number of functional leaves increased up to 8th MAP and then decreased due to the incidence of disease. No significant differences were noticed among the treatments. The highest number of functional leaves was (9.87) observed in T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) preceded by T2 (tebuconazole) with 9.58 functional leaves. It was lowest (8.58) when the plants were applied with T9 (carbendazim). The number of suckers increased from 5th MAP to 10th MAP and the treatments were on par. The highest (4.62) number of suckers was noticed in T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens)* followed by T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and T1 (copper hydroxide) with 4.58 suckers. The lowest (20.12) number of suckers was observed in the T8 (*P. fluorescens*). The application of cow dung along with other fertilizers increases the vegetative growth of the plants (Solaiman and Rabbani, 2006). The application of AMF enhances the growth and nutrient uptake of the plants. The present study was in accordance with the study conducted by Emara *et al*. (2018) which revealed that the application of AMF in banana enhanced the plant height, pseudostem girth, number of leaves and root dimensions. Similar observations were also reported by Yano-Melo *et al.* (1999) and Rodriguez-Romero *et al.* (2005) in banana plants.

Yield attributes such as bunch weight, number of hands, number of fingers per hand, length of fingers, circumference of fingers, fresh weight of fingers, ripe weight of fingers, peel to pulp ratio and total soluble solids (TSS) were recorded. Statistically significant differences were noticed in all treatments with respect to bunch weight, number of fingers per hand, length of fingers, the circumference of fingers, fresh weight and ripe weight of fingers, whereas no significant differences were observed in the number of hands, peel to pulp ratio and TSS.

The bunch weight was maximum (6.10 kg) in the treatment T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and the lowest (4.28 kg) bunch weight was noticed in control plants. Per cent increase in yield over control plants was found to be maximum (42.52 %) in plants applied with T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) followed by T2 (tebuconazole) and T5 (AMF +*Trichoderma* enriched cow dung - neem cake

mixture + *P. fluorescens*) with a yield increase of 39.49 per cent and 36.21 per cent respectively. The maximum (4.93) number of hands were same in the treatments T2 ((tebuconazole), T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and T8 (*P. fluorescens*). The number of hands was minimum (4.33) in the treatment T1 (copper hydroxide). The highest number of fingers (13.47) was noticed in plants treated with T2 (tebuconazole). Whereas, the lowest number of fingers (9.27) was noticed in plants without any treatment (control). The length of fingers was highest (18.50 cm) in plants applied with T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole) and lowest (15.83 cm) in T10 (untreated control) applied plants. The plants applied with T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*) showed the highest circumference (15.33 cm) while, the lowest circumference of fingers (12.40 cm) was noticed in plants without any treatment (untreated control). The highest fresh (114.66 g) and ripe (99.55 g) weight were observed in T6 (IDM - *P. fluorescens* + AMF and *Trichoderma* enriched cow dung + tebuconazole). Whereas, the lowest fresh (67.99 g) and ripe (50.28 g) weight were observed in control plants. The peel to pulp ratio was highest (0.35) in the treatment T9 (carbendazim) and lowest (0.26) in the treatment T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*). The quantity of TSS ranged from 25.27 °brix to 26.17 °brix which comes in the normal range of TSS of variety Rasthali/Poovan. It was maximum in fruits of plants applied with T7 (PGPR Mix II) and minimum in the fruits of plants applied with T8 (*P. fluorescens*). The application of biocontrol agents decreased the incidence of Fusarium wilt in banana and increased the yield (Bubici *et al*., 2019). The application of AMF in banana improves the root growth and hence improves the water and nutrient uptake. Thus, the plant growth and yield attributes would be increased (Jefwa *et al*., 2008). Soil drenching with tebuconazole is also found to be very effective against Fusarium wilt (Sreeja, 2014). Hence, an integrated management approach including biocontrol agents and chemical fungicides at recommended rate could be effectively used for the management of Fusarium wilt of banana.

SUMMARY

6. SUMMARY

Banana is considered as an important fruit crop in the livelihood of many people in tropical and subtropical countries. However, the banana crop is threatened by Fusarium wilt incited by the pathogen *Fusarium oxysporum* f. sp. *cubense*, a soil borne fungus. The disease has a destructive effect on the crop and causes up to 80 per cent of yield loss.

The study entitled "Characterization and integrated management of *Fusarium oxysporum* f. sp. *cubense* (E. F. Smith) Snyder and Hansen (Foc) causing Fusarium wilt disease of banana" was conducted in the Department of Plant Pathology, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur and Banana Research Station, Kannara during 2017-2020 with the view to study the symptomatology, pathogen diversity and characterization of the pathogen. The present study was also undertaken to develop a quick detection technique for the pathogen and an integrated management package for the control of disease.

Purposive sampling surveys were conducted in farmers' fields of various locations in six districts of Kerala *viz.*, Thiruvananthapuram (Southern zone), Ernakulam (Central zone), Thrissur (Central zone), Palakkad (Northern zone), Kozhikode (Northern zone) and Wayanad (High range zone) during the period from June 2018 to November 2018. The per cent disease incidence (PDI) varied from 1.52 per cent (Neyyattinkara, Thiruvananthapuram) to 43.65 per cent (Ambalavayal, Wayanad). The highest (20.34 %) per cent disease severity (PDS) was noticed in the field at Aluva in the Ernakulam district. and the lowest (49.57 %) PDS was observed at Kenichira field in the Wayanad district. Significant positive correlation was observed between the rainfall and per cent disease incidence (PDI) while, a negative correlation was observed between temperature and the PDI. The PDI increased with increasing rainfall and decreased with increasing temperature.

Two types of symptoms viz., external and internal symptoms were noticed both under natural and artificial condition. Yellowing and collapsing of leaves, splitting of pseudostem and plant wilting were the external symptoms recorded, whereas the discolouration of rhizome and vascular tissues were the internal symptoms noticed

under natural condition. Under artificial condition, yellowing of the leaves and wilting were the external symptoms produced whereas, the discolouration of stellar region was the internal symptom recorded. The number of days taken for shifting from one stage to another was highest in Njalipoovan variety and minimum in Rasthali/Poovan followed by Kadali.

The pathogen associated with the diseased samples of banana collected during the survey was isolated on half strength potato dextrose agar (PDA) medium. A total of 30 isolates were collected from pseudostem of different cultivars namely Rasthali/Poovan, Kadali, Chenkadali and Njalipoovan various locations in six districts. All the isolates were purified and maintained on PDA by frequent subculturing.

The pathogenicity studies of all 30 isolates of Foc were done by proving Koch's postulates on their respective host varieties. The inoculated plants started producing yellow coloured symptoms on the leaf tip from 10 to 15 days after inoculation in various varieties. The yellowing was spread gradually to whole leaf and drying occurred within 50 days after inoculation. The stellar region showed internal discolouration in infected plants. The per cent wilt index (PWI) was observed to be highest (93.33 %) in the isolates S1, S2, S5, S12, S20 and S27 and lowest (40 %) in the isolate S14. Whereas, the per cent vascular wilt index (PVWI) was observed maximum in the isolate S2 (100 %) and the least PVWI was recorded in the isolate S14 (33.33 %).

The characterization of the pathogen was carried out using cultural, morphological and molecular characters. Twenty five isolates produced white coloured aerial mycelium, four of them were greyish white and one was grey. The most common pigmentation found was pink. Cottony and fluffy mycelial mat was the commonly found texture but thin and sparse growth were also seen. The diameter of colony varied from 59.6 to 90.0 mm at seven days after incubation at 25 ºC for various isolates. Twenty-three included in the first category which produced more than 80.00 mm colony diameter, three were in between 70.00 to 80.00 mm and for four isolates, the mycelial growth was less than 70.00 mm. The length and breadth macroconidia ranged from 15.01 to 20.20 µm and 2.14 to 5.07 µm respectively whereas in the case of microconidia, it ranged from 4.29 to 7.42 µm and 1.35 to 3.13 µm respectively. The

chlamydospores varied from 5.68 to 11.07 µm in diameter. The macroconidia were 3 to 5 septate and the microconidia were aseptate or single septate. All the produced sporodochium and the hyphae were septate in nature.

The cluster analysis of the all isolates based on qualitative and quantitative data of cultural and morphological characters was done. The dendrogram of quantitative data formed two main clusters *viz*., A1 and A2 at zero per cent similarity. Among the 30 isolates, five isolates belonged to the A2 cluster and the 25 isolates belonged to the cluster A2. All the Foc isolates collected from Rasthali/Poovan (AAB) and Njalipoovan (AB) varieties were under cluster A1, whereas the cluster A2 includes all the Foc isolates collected from Kadali (AA) and Chenkadali (AAA) varieties. The dendrogram based on the qualitative parameters also produced two main clusters namely A1 and A2 at 100 per cent dissimilarity. The cluster A1 consisted of nine isolates and the remaining 22 isolates belonged to the A2 irrespective of the host variety from which it was collected.

Molecular detection and characterization of the isolates were carried out using universal primers ITS 1F and ITS 4F. The DNA was isolated using CTAB method with modifications. The quantity of isolated DNA varied from 125.70 to 2627.32 ng/µl and the quality ranged from 1.82 to 2.19 which indicate the DNA is of good quality and free from impurities such as RNA. The annealing temperature selected for polymerase chain reaction was 54.9 °C using ITS 1F and ITS 4R primers. Agarose gel electrophoresis of the rDNA amplicons produced single bands of 580 bp for all isolates. The amplicons were sequenced and *in silico* analysis of the 30 isolates showed a sequence homology of more than 96 per cent to Foc sequences available in NCBI. The phylogenetic tree analysis revealed that the isolates showed more similarity with the formae speciales of *F. oxysporum*. All the isolates were diverged into different clades irrespective of the geographic region. Variations were observed among the isolates and this may be due to some mutational changes. The barcoding of isolates revealed that the sequences of Foc isolates were similar to the sequences of other formae speciales of *F. oxysporum* at the ITS rDNA region. Whereas, variations in sequences were observed between formae speciales of *F. oxysporum* and other *Fusarium* spp.

Race identification of the pathogen was done by artificial inoculation and by developing loop mediated isothermal amplification assay (LAMP). During artificial inoculation, all the isolates produced infection in Rasthali/Poovan varieties but none in Cavendish, Plantain, Monthan or Heliconia. The LAMP assay was developed for the easy detection of Foc Race 1 using the primers designed based on the endoglucanase 4 gene sequence of Foc Race 1. A temperature of 63 °C for 60 min. was found to be the optimum condition for the reaction. The isolates were tested with primers of Foc Race 1 and Foc Race 4. The amplification products were subjected to colorimetric detection using Hydroxynaphthol blue and 2 % gel electrophoresis. All the isolates showed a positive reaction to the Race 1 primers but negative to the Race 4 primers. A conventional polymerase chain reaction was also conducted using the specific primers (F3 and B3 primers for the detection of Race 1) for further confirmation of the results. All the isolates produced bands of size around 240 bp. Therefore, it can be concluded that the collected isolates belong to the Race 1 of Foc.

The evaluation of host plant disease resistance of banana to Fusarium wilt pathogen Foc race 1was conducted by screening the twenty six varieties available at gene bank of Banana Research Station, Kannara and were grouped into six categories. Fifteen immune varieties *viz*., Attunendran, Zanzibar, Big Ebanga, Nedunendran, Nendran, BRS II, Thiruvananthapuram, Pachanadan I, Cultivar Rose, Pisang Lilin, Pisang Jari Buaya, Yangambi Km5, Grand Naine, Chinese Cavendish and Nendran Hybrid and four highly susceptible varieties *viz*., Cheriya Poovan, Valiya Poovan, Kadali and Rasakadali were identified.

The estimation of biochemical parameters for the assessment of host plant disease resistance against Foc Race 1 revealed that the activity of total phenols and defense related enzymes was more in resistant varieties (Nendran and Robusta) compared to susceptible varieties (Rasthali/Poovan and Kadali) and the activity of reducing and non reducing sugars was more in susceptible varieties. The activity of total phenol was increased up to 6^{th} day after challenge inoculation in Nendran, Robusta and Kadali however in Rasthali/Poovan the maximum phenol content was recorded on 4^{th} day after inoculation and then decreased near to the basal level. The activity of these sugars decreased continuously after challenge inoculation in all varieties. Whereas, the activity of peroxidase showed an increasing trend in resistant and susceptible varieties up to 8^{th} day and then decreased near to the base level. Similarly, the activity of

polyphenol oxidase also showed an increasing trend initially in both resistant and susceptible cultivars and then decreased after 6th day of inoculation. The plants showed a rapidly increasing trend in the quantity of phenylalanine ammonia lyase in resistant and susceptible varieties up to 8th day after inoculation with Foc.

Characteristic histopathological changes were noticed in the tissues of infected and healthy plants. In the infected leaves, the amount of chloroplast was less and the cells in palisade and spongy layer were found to be flaccid. The accumulation of secondary metabolites was noticed cortex of root and rhizome and crushed cells were observed in the rhizome. Some irregular growth was noticed in the xylem vessels of the roots and distorted cells were also found in the root cortex.

An *in vitro* experiment was conducted for the evaluation of chemical fungicides, biocontrol agents and botanicals at lower, recommended and higher doses was conducted by poisoned food for control of the pathogen. The effective treatments from *in vitro* evaluation were carried over to pot culture and field experiments for the disease management. Among the various treatments, the highest biometric characters of the plants were recorded in the treatment T5 (AMF +*Trichoderma* enriched cow dung - neem cake mixture + *P. fluorescens*). Whereas, an integrated package comprising of *Pseudomonas fluorescens* + arbuscular mycorrhizal fungi and *Trichoderma* enriched cow dung + tebuconazole (T6) was recorded the best for yield and disease management under pot culture and field experiment.

It is concluded that an integrated management approach comprising of biocontrol agents and chemical fungicides at recommended rate could be effectively used for the management of Fusarium wilt of banana. The present study was helpful to characterize the pathogen causing fusarium wilt disease of banana, to study the pathogenic diversity and to develop quick detection techniques and management strategy against the disease. Identification and exploitation of resistant genes for the development of resistant varieties, regular monitoring and keeping vigilance on the appearance of Race 4 in future in Kerala and conduct of the on farm trials on the integrated management package and its popularisation among farmers can be done in future to eradicate the Fusarium wilt pathogen in banana.

REFERENCES

REFERENCES

Abd-Elsalam, K. A., Aly, I. N., Abdel-Satar, M. A., Khalil, M. S., and Verreet, J.A. 2003. PCR identification of *Fusarium* genus based on nuclear ribosomal-DNA sequence data. *Afr. J. Biotechnol.* 2(4): 82-85.

Abd-Elsalam, K., Bahkali, A., Moslem, M., Amin, O. E., and Niessen, L. 2011. An optimized protocol for DNA extraction from wheat seeds and Loop-Mediated Isothermal Amplification (LAMP) to detect *Fusarium graminearum* contamination of wheat grain. *Int. J. Mol. Sci.* 12: 3459-3472.

Agrios, G. N. 2005. *Plant Pathology* (5th Ed.). Academic Press, New York, 922 p.

Aguilar-Hawod, K. G. I., de la Cueva, F. M., and Cumagun, C. J .R. 2020. Genetic diversity of *Fusarium oxysporum* f. sp. *cubense* causing panama wilt of banana in the Philippines. *Pathogens*, *9*(1): 32.

AICRP (All India Ccordinated Research Project on Fruits). 2017. Proceedings of fourth group discussion, 4-7 January 2017, IIHR, Bangaore. 65p.

Ainsworth, G. C. 1971. *Ainsworth and Bisby's Dictionary of the Fungi*. (6th Ed.). Commonwealth Mycological Institute, Kew, Surrey, England. 663p.

Akila, R., Rajendran, L., Harish, S., Saveetha, K., Raguchander, T., and Samiyappan, R. 2011. Combined application of botanical formulations and biocontrol agents for the management of *Fusarium oxysporum* f. sp. *cubense* (Foc) causing Fusarium wilt in banana. *Biol. Control* 57(3): 175-183.

Almasi, M. A. 2019. Development of a colorimetric loop-mediated isothermal amplification assay for the visual detection of *Fusarium oxysporum* f. sp. *melonis*. *Hort. Plant J. 5*(3):129-136.

Almasi, M. A., Dehabadi, S. M. H., Moradi, A., Eftekhari, Z., Ojaghkandi, M. A., and Aghaei, S. 2013. Development and application of loop-mediated isothermal

amplification assay for rapid detection of *Fusarium oxysporum* f. sp. *lycopersici. Plant Path. Microbiol.* 4(5): 71-78.

Alves-Santos F. M., Ramos, B., García-Sánchez, M. A., Eslava, A .P., and Díaz-Mínguez, J. M. 2002. A DNA-based procedure for in plant detection of *Fusarium oxysporum* f. sp. *phaseoli*. *Phytopathol.* 92: 237-244.

Amini, J. and Sidovich, D. 2010. The effects of fungicides on *Fusarium oxysporum* f. sp. *lycopersici* associated with Fusarium wilt of tomato. *J. Plant Prot. Res.* 50(2): 172-178.

Anand, T., Bhaskaran, R., Raguchander, T., Samiyappan, R., Prakasam, V., and Gopalakrishnan, C. 2009. Defence responses of chilli fruits to *Colletotrichum capsici* and *Alternaria alternata. Biol. Plant.* 53(3): 553-559.

Ardebili, Z. O., Ardebili, N. O., and Mahdi Hamdi, S. M. 2011. Physiological effects of' *Pseudomonas fluorescens* CHA0 on tomato (*Lycopersicon esculentum* Mill.) plants and its possible impact on *Fusarium oxysporum* f. sp. *lycopersici*. *Aust. J. Crop Sci*. 5(12): 1631-1638.

Arfaoui, A., Sifi, B. E. l., Hassni, M. E. l., Hadrami, I., Boudabbous, A., and Cherif, M. 2005. Biochemical analysis of chickpea protection against Fusarium wilt afforded by two Rhizobium isolates. *Plant Pathol. J*. 4(1): 35-42.

Ashby, S. F. 1913. *Banana diseases in Jamaica*. Bulletin of the Department of Agriculture Jumaica volume 2, Department of Agriculture, Jumaica, 95p.

Bakry, F., Carreel, F., Jenny, C., and Horry, J. P. 2009. Genetic improvement of banana. In: Jain, S. M., Priyadarshan, P. M. (eds), *Breeding Plantation Tree Crops: Tropical Species*. Springer, New York, pp. 3-51.

Bancroft, J. 1876. Report of the board appointed to inquire into the cause of disease affecting livestock and plants. In: *Votes and Proceedings,* Queensland, pp.1011-1038.

Beckman, C. H. 1990. Host responses to the pathogen. In: Ploetz (ed.), *Fusarium Wilt of Banana*. The American Phytopathological Society, St. Paul, MN, pp.93-105.

Beckman, C.H., 1987. *The Nature of wilt Diseases of Plants*. APS press, St. Paul, MN.

Belgrove, A., Steinberg, C., and Viljoen, A., 2011. Evaluation of non-pathogenic *Fusarium oxysporum* and *Pseudomonas fluorescens* for Panama disease control. *Plant dis.* 95(8): 951-959.

Bell, A. A. 1981. Biochemical mechanisms of disease resistance. *Annu. Rev. Plant Physiol.* 32(1): 21-81.

Benjin, L., Du, J., Lan., C., Liu, P., Weng, Q., and Chen, Q. 2013. Development of a loop-mediated isothermal amplification assay for rapid and sensitive detection of *Fusarium oxysporum* f. sp. *cubense* race 4. *Eur. J. plant Path.*135: 903-911.

Bentley, S., Pegg, K. G., and Dale, J. L. 1994. Optimization of RAPD-PCR fingerprinting to analyse genetic variation within populations of *Fusarium oxysporum* f. sp. *cubense*. *J. Phytopathol.* 142(1): 64-78.

Bentley, S., Pegg, K. G., and Dale, J. L. 1995. Genetic variation among a world-wide collection of isolates of *Fusarium oxysporum* f. sp. *cubense* analysed by RAPD-PCR fingerprinting. *Mycol. Res.* 99(11): 1378-1384.

Benzohra-Belaidi, H. 2016. Screening of some chickpea germplasm accessions for resistance to two races of *Fusarium oxysporum* f. sp. *ciceris*, the causal of chickpea wilt disease. *Am. Eur. J. Agric. Environ. Sci.* 16 (11): 1758-1763.

Bhagat, S., Bambawale, O.M., Tripathi, A.K., Ahmad, I. and Srivastava, R.C., 2013. Biological management of fusarial wilt of tomato by Trichoderma spp. in Andamans. *Indian J. Hortic. 70*(3), pp.397-403.

Bharathi, E., Prasad, M. S. L., Yadav, P., and Bee, H. 2019. Defense responses to *Fusarium oxysporum* f. sp. *ricini* infection in castor (*Ricinus communis* L.) cultivars. *Indian Phytopathol.* 72: 647-656.

Bickerton, J. E. 1942. *Fusarium wilt of carnations caused by Fusarium dianthi* Prill et Del. Cornell University Agricultural Experiment Station Bulletin No.788, Cornell University Agricultural Experiment Station, New York, 31p.

Booth, C. 1971. *The Genus Fusarium.* Common wealth Mycological Institute, Kew, England, 137p.

Bragulat, M. R., Martinez, E., Castella, G., and Cabanes, F. J. 2004. Selective efficacy of culture media recommended for isolation and enumeration of *Fusarium* spp. *J. Food Prot.* 67(1): 207-211.

Brandes, E. W. 1919. Banana wilt. *Phytopathol.* 9: 339-83.

Bruns, T. D., White, T. J., and Taylor, J. W. 1991. Fungal molecular systematics. *Ann. Rev. Ecol. Syst*. 22: 525-564.

Bubici, G., Kaushal, M., Prigigallo, M. I., Gómez-Lama Cabanás, C., and Mercado-Blanco, J. 2019. Biological control agents against Fusarium wilt of banana. *Frontiers Microbiol.* 10(616): 1-33.

Buddenhagen, I. W. 2007. Understanding strain diversity *in Fusarium oxysporum* f. sp. *cubense* and history of introduction of 'tropical race 49 to better manage banana production. In: Jones D, and Bergh, V. D. I., (eds), *Proceedings of the International Symposium on Recent Advances in Banana Crop Protection for Sustainable Production and Improved Livelihoods*, 10-14 September 2007, White River, South Africa. ISHS Acta Horticulturae, pp.193–204.

Butler, D. 2013. Fungus threatens top banana [online]. Available: https://www.natureasia.com/en/nmiddleeast/article/10.1038/nmiddleeast.2013.241 [29 November 2019].

Carefoot, G. L. and Sprott, E. R. 1969. *Famine on the Wind – Plant Diseases in Human History.* Angus & Robertson, London, 222 p.

Chambers, H. L. and Corden, M. E. 1963. Semeiography of Fusarium wilt of tomato. *Phytopathol.* 53: 1006-1010.

Chaudhry, M. A., Muhammad, F., and Afzal, M. 2006. Screening of chickpea germplasm against Fusarium wilt. *J. Agric. Res.* 44(4): 307-312.

Chawla, N. and Gangopadhyay, S.,2009. Integration of organic amendments and bioagents in suppressing cumin wilt caused by *Fusarium oxysporum* f. sp. *cumini*. *Indian Phytopathol.* 62(2): 209-216.

Chennakesavulu, M., Reddikumar, M., and Reddy, N.P.E., 2013. Evaluation of different fungicides and their compatibility with *Pseudomonas fluorescens* in the control of redgram wilt incited by *Fusarium udum. J. Biol. Control 27*(4):354-361.

Cherian, A. K. and Menon, R. 2001. Integrated management of panama wilt disease of banana caused by *Fusarium oxysporum* f. sp. *Cubense. In: Proceedings of National Symposium on Ecofriendly Appoaches for Plant Disease Managenment,* 22-24 January 2001, Chennai. University of Madras, Chennai, 31p.

Chopada, G. B., sing, P., and Chandulal, K. 2014. Cultural and morphological variability among *Fusarium oxysporum* f. sp. *lycopersici* wilt of tomato in south Gujarat region. *Archives Phytopathol. Plant Prot*. 48(2): 104-110.

DAFF [Department of Agriculture, Fisheries and Forestry] 2004. Importation of fresh bananas from the Philippines [online]. Available : http://www.agriculture.gov.au/biosecurity/risk-analysis/plant/banana-philippines [0 7 August 2019].

Dai, T. T., Lu, C. C., Lu, J., Dong, S., Ye, W., Wang, Y., and Zheng, X. 2012. Development of a loop-mediated isothermal amplification assay for detection of *Phytophthora sojae*. *FEMS Microbiol. Letters* 334(1): 27-34.

Damodaran, T., Mishra, V. K., Jha, S. K., Gopal, R., Rajan, S., and Ahmed, I. 2019. First report of Fusarium wilt in banana caused by *Fusarium oxysporum* f. sp. *cubense* tropical race 4 in India. *Plant Dis.* 103(5): 1022-1022.

Das, A., Venkataramana, M., Chandranayaka, S., Murali, H. S., and Batra, H.V. 2012. Molecular characterization of *Fusarium oxysporum* f. sp. *cubense* isolates from banana. *Pest Manag. Hortic. Ecosyst.* 18(2): 171-178.

Deacon, J. W. 1984. Panama disease of bananas in South Africa. *Hortic. Sci.* 1: 29-31.

Denschlag, C., Vogel, R. F., and Niessen, L. 2012. Hyd5 gene-based detection of the major gushing-inducing *Fusarium* spp. in a loop-mediated isothermal amplification (LAMP) assay. *Int. J. Food Microbiol.* 156: 189-196.

Dita, M. A., Waalwijk, C., Buddenhagen, I. W., Souza Jr, M. T., and Kema, G. H. J. 2010. A molecular diagnostic for tropical race 4 of the banana fusarium wilt pathogen. *Plant Pathol.* 59(2): 348-357.

Dita, M., Barquero, M., Heck, D., Mizubuti, E. S., and Staver, C. P. 2018. Fusarium wilt of banana: current knowledge on epidemiology and research needs toward sustainable disease management. *Frontiers Plant Sci. 9*: 1468.

Dong, X., Xiong, Y., Ling, N., Shen, Q., and Guo, S. 2014. Fusaric acid accelerates the senescence of leaf in banana when infected by *Fusarium*. *World J. Microbiol.* 30: 1399-1408.

Dubey, S. C., Singh, S. R., and Singh, B. 2010. Morphological and pathogenic variability of Indian isolates of *Fusarium oxysporum* f. sp. *ciceris* causing chickpea wilt. *Archives Phytopathol. Plant Prot.* 43(2): 174-190.

Dubey, S. C., Suresh, M., and Singh, B. 2007. Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. *ciceris* for integrated management of chickpea wilt. *Biol. control* 40(1): 118-127.

Easwaran, K. S. S. 1972. Physiology of resistance to a bacterial disease in sorghum II. carbohydrate metabolism in normal and inoculated tissues. *J. Phytopathol.* 73(2): 163-170.

Edel, V., Steinberg. C., Avelange, I., Laguerre, G., and Alabouvette, C. 1995. Comparison of three molecular methods for the characterization of *Fusarium oxysporum* strains. *Phytopathol*. 85: 579-85.

Emara, H.A., Nower, A., Hmza, E., Saad, M., and El Shaib, F., 2018. Role of mycorrhiza as biofertilization of banana grand naine on nursery stage. *Int. J. Curr. Microbiol. App. Sci*. *7*(10):805-814.

Epp, M. D. 1987. Somaclonal variation in bananas: a case study with Fusarium wilt. Banana and Plantain Strategies. In: *Transport of Fresh Fruit and Vegetables.* Proceedings of a workshop held at CSIRO Food Research Laboratory, 5-6 February 198, North Ryde, Sydney. Australian Centre for International Agricultural Research, Australia**.** pp.140–150.

Etebarian, H. 2006. Evaluation of *Trichoderma* isolates for biological control of charcoal coal stem rot in melon caused by *Macrophomina phaseolina*. *J. Agric. Sci. Technol.* 8: 243-250.

FAO [Food and Agriculture Organization]. 2018. FAOSTAT home page [online]. Available: http://www.fao.org/faostat/en/#home [14 November 2019].

Fourie, G., Steenkamp, E. T., Gordon, T. R., and Viljoen, A. 2009. Evolutionary relationships among the *Fusarium oxysporum* f. sp. *cubense* vegetative compatibility groups. *Appl. Environ. Microbiol*. 75(14): 4770-4781.

Fourie, G., Steenkamp, E. T., Ploetz, R. C., Gordon, T. R., and Viljoen, A. 2011. Current status of the taxonomic position of *Fusarium oxysporum* formae specialis *cubense* within the *Fusarium oxysporum* complex. *Infection Genet. Evol. 11*(3):533-542.

Fravel, D. R., Olivain, C., and Alabouvette, C. 2003. *Fusarium oxysporum* and its biocontrol. *New Phytologist* 157: 493-502.

Garibaldi, A., Minuto, A., Bertetti, D., and Gullino, M. L. 2004. Fusarium wilt of gerbera in soil and soilless crops in Italy. *Plant Dis.* 88(3): 311-311.

Garrett, S. D. 1965. Towards biological control of soil-borne plant pathogens. In: Baker, K.F. and Snyder, W.C. (eds), *Ecology of soilborne plant pathogens. Prelude to biological control.* University California Press, Berkeley, p.571.

Ghag, S. B. 2019. Fusarium Wilt Disease of Banana in India: Current Scenario. *Agrotechnol*. 8(1): 121.

Ghag, S. B., Shekhawat, U. K., and Ganapathi, T. R., 2015. Fusarium wilt of banana: biology, epidemiology and management. *Int. J. Pest Manag.* 61(3): 250-263.

Ghosh, R., Nagavardhini, A., Sengupta, A., and Sharma, M. 2015. Development of Loop-Mediated Isothermal Amplification (LAMP) assay for rapid detection of *Fusarium oxysporum* f. sp. *ciceris*-wilt pathogen of chickpea. *BMC research notes 8*(1): 40.

Gogoi, R., Singh, D. V., and Srivastava, K. D. 2001. Phenols as a biochemical basis of resistance in wheat against Karnal bunt. *Plant Pathol.* 50(4): 470-476.

Gohel, V., Maisuria, V., and Chatpar, H. 2007. Utilization of various chitinous sources for production of mycolytic enzymes by *Pantoea dispersa* in bench-top fermenter. *Enzyme Microbial Technol*. 40: 1608-1614.

Groenewald, S. 2006. Biology pathogenicity and diversity of *Fusarium oxysporum* f. sp. *Cubense.* Ph.D. thesis, University of Pretoria, Pretoria, 176p.

Groenewald, S., Van den Berg, N., Marasas, W. F. O., and Viljoen, A. 2006. Biological, physiological and pathogenic variation in a genetically homogenous population of *Fusarium oxysporum* f. sp. *cubense*. *Aust. Plant Pathol.* 35(4): 401-409.

Hafizi, R., Salleh, B., and Latiffah, Z. 2013. Morphological and molecular characterization of *Fusarium solani* and *F. oxysporum* associated with crown disease of oil palm. *Brazilian J. Microbiol.* 44(3): 959-968.

Heslop-Harrison, J. S. and Schwarzacher, T., 2007. Domestication, genomics and the future for banana. *An. bot.* 100(5): 1073-1084.

Hesse, C. , Schulzb, f., Carolee, T., Bullc. , Brenda, T., Shaffera , Yand , Q., Shapirob , N., Karl, A., Hassane , Vargheseb , N., Liam, D. H., Elbournef , Ian, T., Paulsenf , Kyrpidesb , N., Woykeb , T., and Lopera, J. E. 2018. Genome-based evolutionary history of *Pseudomonas* spp. *Environ. Microbiol.* 20(6):2142-2159.

Higgins, J. E. 1904. The banana in Hawaii. Hawaii Agricultural Experiment Station Bulletin No.7, Hawaiian Gazette Company Ltd, Honolulu, 51p.

Hirano, Y. and Arie, T. 2009. Variation and phylogeny of *Fusarium oxysporum* isolates based on nucleotide sequences of polygalacturonase genes. *Microbes Environ.* 24(2): 113-120.

Honnareddy, N. and Dubey, S. C. 2007. Morphological characterization of Indian isolates of *Fusarium oxysporum* f. sp. *ciceris* causing chickpea wilt. *Indian Phytopathol*. 60(3): 373-376.

Horsfall, J. G. and Cowling, E. B. 1980. *Plant Disease: An Advanced Treatise. Vol. V. How Plants Defend Themselves.* Academic Pres, New York, 534 p.

Huang, B., Xu, L., and Molina, A .B. 2005. Preliminary evaluation of IMTP-III varieties and local cultivars against fusarium wilt disease in South China. In: *Advancing banana and plantain R&D in Asia and the Pacific*. Proceedings of the 3rd BAPNET Steering Committee meeting held in Guangzhou, 23-26 November 2004, China. International Plant Genetic Resources Institute,Rome, pp. 187-192.

Infantino, A., Portapugua, A., and Singh, K. B. 1996. Screening wild *Cicer* species for resistance of Fusarium wilt. *Plant Dis*. 80: 42-44.

Ingle, A. and Ingle, R. 2013. Isolation and Identification of *Fusarium oxysporum* infecting *Musa* plants in Maharashtra region and their molecular characterization. *Asiat. J. Biotech. Res*. *4*: 28-34.

Jadhav, B., Sarika. R., Bhalerao., and George.2019. Molecular characterization of F*usarium oxysporum* causing chickpea wilt by internal transcribed spacer (ITS) marker. *Legume Res*. 42.

Jager, G., Velvis, H., Lamers, J. G., Mulder, A., and Roosjen, J. 1991. Control of *Rhizoctonia solani* in potato by biological, chemical and integrated measures. *Potato Res*. 34: 269-284.

Jayapal, R. and Mahadevan, A. 1968. Biochemical changes in banana leaves in response to leaf spot pathogens. *Indian Phytopathol*. 21: 43-48.

Jefwa, J., Vanlauwe, B., Coyne, D., Van Asten, P., Gaidashova, S., Rurangwa, E., Mwashasha, M., and Elsen, A. 2008. Benefits and potential use of arbuscular mycorrhizal fungi (AMF) in banana and plantain (*Musa* spp.) systems in Africa. In: *IV International Symposium on Banana: International Conference on Banana and Plantain in Africa: Harnessing International partnership to increase research impact,* 5-9 October 2008, Kenya. International society for horticultural science, Belgium, pp. 479-486.

Jeger, M. J., Waller, J. M., Johnson, A., and Gowen, S. R. 1996. Monitoring in banana pest management. *Crop Prot.* 14: 339-340.

Jones, D. 2018. *Handbook of Diseases of Banana, Abacá and Ense.* CABI Publishing, Wallingford, 632p.

Joshi, M., Srivastava, R., Sharma, A. K., and Prakash, A. 2013. Isolation and characterization of *Fusarium oxysporum,* a wilt causing fungus, for its

pathogenic and non-pathogenic nature in tomato (*Solanum lycopersicum*). *J. Appl. Nat. Sci.* 5(1): 108-117.

Jurado, M., Vázquez, C., Marín, S., Sanchis, V., and González-Jaéna, M. T. 2006. PCR-based strategy to detect contamination with mycotoxigenic *Fusarium* species in maize. *Syst. Appl. Microbiol.* 29: 681–689.

Kaneko, H., Kawana, T., Fukushima, E., and Suzutani, T. 2007. Tolerance of loop-mediated isothermal amplification to a culture medium and biological substances. *J. Biochem. Biophys. Methods*, 70(3): 499-501.

Karangwa, P., Mostert, D., Ndayihanzamaso, P., Dubois, T., Niere, B., zum Felde, A., Schouten, A., Blomme, G., Beed, F., and Viljoen, A. 2018. Genetic diversity of *Fusarium oxysporum* f. sp. *cubense* in East and Central Africa. *Plant Dis. 102*(3):552-560.

KAU (Kerala Agricultural University) 2016. *Package of Practices Recommendations: Crops* (15th Ed.). Kerala Agricultural University, Thrissur, 393p.

Kavino, M. and Manoranjitham, S. K. 2018. *In vitro* bacterization of banana (*Musa* spp.) with native endophytic and rhizospheric bacterial isolates: Novel ways to combat Fusarium wilt. *Eur. J. Plant Pathol.* 151(2): 371-387.

Kerenyi, Z., Moretti, A., Waalwijk, C., Olah, B., and Hornok, L. 2004. Mating type sequences in asexually reproducing *Fusarium* species. *Appl. Environ. Microbiol.*, 70(8): 4419-4423.

Kinge T. R., Moforcha, Z. L., and Mih, A. M., 2019. Cultural variability of *Fusarium oxysporum* f. sp. *elaeidis* isolates from oil palm in South Western Cameroon and sensitivity to four plant extracts. *Plant Pathol. Quarantine* 9(1): 116-127.

Koenig, R. L., Ploetz, R. C., and Kistler, H. C. 1997. *Fusarium oxysporum* f. sp. *cubense* consists of a small number of divergent and globally distributed clonal lineages. *Phytopathol*. 87(9): 915-923.

Kontoyiannis, B., Lee, S., and Taylor, J. W. 2000. Genetic Diversity in the plant-pathogenic fungus *Fusarium oxysporum. Am. Phytopathol. Soc.* 10: 475-874.

Kosuge, T. 1969. The role of phenolics in host response to infection. *Annu. Rev. Phytopathol*. 7: 195-222.

Kumar, A., Hunjan, M. S., Kaur, H., Dhillon, H. K., and Singh, P. P. 2017. Biochemical responses associated with resistance to bacterial stalk rot caused by *Dickeya zeae* in maize. *J. Phytopathol.* 165(11-12): 822-832.

Kumar, B. H., Shankar, U. A. C., Nayaka, C. S., Kini, R. K., Shetty, H. S., and Prakash, H. S. 2009. Biochemical characterization of *Fusarium oxysporum f. sp. cubense* isolates from India. *Afr. J. Biotech*. 9(4): 523-530.

Kumar, N. and Saxena, N. 2015. Fusarium wilt of banana at Panchgaon. *Int. J. Curr. Microbiol. App. Sci*. 4(11): 724-730.

Kumar, S. and Upadhyay, J. P. 2013. Cultural, morphological and pathogenic variability in isolates of *Fusarium udum* causing wilt of pigeon pea. *J. Mycol. Plant Pathol.* 43(1): 76-79.

Kumar, S. and Upadhyay, J. P. 2014. Studies on cultural, morphological and pathogenic variability in isolates of *Fusarium udum* causing wilt of pigeon pea. Indian *Phytopathol*. 43(1): 55-58.

Larkin, R. P. and Fravel, D. R. 1998. Efficacy of various fungal and bacterial biocontrol organisms for control of Fusarium wilt of tomato. *Plant Dis*. 82: 1022-1029.

Leong, S. K., Latiffah, Z., and Baharuddin, S. 2009. Molecular characterization of *Fusarium oxysporum* f. sp. *cubense* of banana. *Am. J. Appl. Sci.* 6(7): 1301-1307.

Leslie, J. F. and Summerell, B. A. 2006. *The Fusarium Laboratory Manual (*1st Ed.). Blackwell Publishing , Ames, Iowa, USA, 30p.

Li, B., Du, J., Lan, C., Liu, P., Weng, Q., and Chen, Q. 2013b. Development of a loop-mediated isothermal amplification assay for rapid and sensitive detection of *Fusarium oxysporum* f. sp. *cubense* race 4. *Eur. J. Plant Pathol.* 135(4): 903-911.

Li, C., Chen, S., Zuo, C., Sun, Q., Ye, Q., Yi, G., and Huang, B. 2011. The use of GFP-transformed isolates to study infection of banana with *Fusarium oxysporum* f. sp. *cubense* race 4. *Eur. J. Plant Pathol*. 131:327-340.

Li, C.Y., Mostert, G., Zuo, C. W., Beukes, I., Yang, Q. S., Sheng, O., Kuang, R. B., Wei, Y. R., Hu, C. H., Rose, L., and Karangwa, P. 2013a. Diversity and distribution of the banana wilt pathogen *Fusarium oxysporum f. sp. cubense* in China. *Fungal Genomics & Biol. 3*(2): 1-6.

Li, W., Li, C., Sun, J., and Peng, M. 2017. Metabolomic, biochemical, and gene expression analyses reveal the underlying responses of resistant and susceptible banana species during early infection with *Fusarium oxysporum* f. sp. *cubense*. *Plant Dis.* 101(4): 534-543.

Lin, Y. H., Chang, J. Y., Liu, E. T., Chao, C. P., Huang, J. W., and Chang, P. F. L. 2009. Development of a molecular marker for specific detection of *Fusarium oxysporum* f. sp. *cubense* race 4. *Eur. J. Plant Pathol.* 123(3): 353-365.

Liu, L., Kloepper, J. W., and Tuzun, S. 1995. Induction of systemic resistance in cucumber against Fusarium wilt by plant growth-promoting rhizobacteria. *Phytopathol.* 85(6): 695-698.

Louis, V., Paul, L., Namitha, P. M., Vinny, K.V., Dilip, D., and Pushpalatha, P. B. 2018. Molecular characterization of Fusarium oxysporum f sp. cubense from Kerala (Ab). In: *National Symposium on Cutting Edge Approaches for Sustainable Plant Disease Management and Ensuring Farmers' Profit,* 21-23 December 2018, Trichy. Indian Phytopathological Society (Southzone Chapter) and NRCB, Trichy.

Madhukar, H. M. 2001. Ecology and management of Fusarium wilt of chilli. M.Sc. (Ag) thesis, University of Agricultural Sciences, Dharwad, 134p.

Mak, C., Mohamed, A. A., Liew, K. W., and Ho, Y. W. 2004. Early screening technique for Fusarium wilt resistance in banana micropropagated plants. In: Jain, S. M. and Swennen, R. (eds), *Banana Improvement: Cellular, Molecular Biology, and Induced Mutations.* Science Publishers, Enfield, USA, pp. 219–227.

Malik, C. P. and Singh, M. B. 1980. *Plant Enzymology and Histo- Enzymology.* Kalyani Publishers, New Delhi, 215p.

Maryani, N., Lombard, L., Poerba, Y. S., Subandiyah, S., Crous, P. W., and Kema, G. H. J. 2019. Phylogeny and genetic diversity of the banana Fusarium wilt pathogen *Fusarium oxysporum* f. sp. *cubense* in the Indonesian centre of origin. *Stud.Mycol. 92*: 155-194.

Mathpal, P., Punetha, H., Tewari, A. K., and Agrawal, S. 2011. Biochemical defense mechanism in rapeseed-mustard genotypes against Alternaria blight disease. *J. Oilseed Brassica* 1(2): 87-94.

Maymon, M., Sela, N., Shpatz, U., Galpaz, N., and Freeman, S. 2020. The origin and current situation of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 in Israel and the Middle East. *Sci.Rep. 10*(1):1-11.

Meldrum, R. A., Daly, A. M., Tran- Nguyen, L. T. T., and Aitken, E. A. B. 2013. Are banana weevil borers a vector in spreading *Fusarium oxysporum* f. sp. *cubense*. *Aust.. Plant Pathol.* 42: 543-549.

Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 31: 426-428.

Mishra, I. G., Tripathi, N., and Tiwari, S. 2014. A simple and rapid DNA extraction protocol for filamentous fungi efficient for molecular studies. *Indian J. Biotechnol.* 13: 536-539.

Mishra, P. K., Fox, R. T., and Culham, A. 2003. Development of a PCR-based assay for rapid and reliable identification of pathogenic *Fusaria. FEMS Microbiol. Letters* 218(2): 329-332.

Mohamed, H. A. and Haggag, W. M. 2006. Biocontrol potential of salinity tolerant mutants of *Trichoderma harzianum* against *Fusarium oxysporum*. *Braz. J. Microbiol.* 37: 181-191.

Mohd, R., Biswas, S .K., and Shiv, S. 2012. Biochemical basis of defense response in plant against Fusarium wilt through bio-agents as an inducers. *Afr. J. Agric. Res. 7*(43):5849-5857.

Molina, A. B., Fabregar, E., Sinohin, V. G., Yi, G., and Viljoen, A. 2007. Recent occurrence of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 in Asia. In: Jones D, and Bergh, V. D. I., (eds), *Proceedings of the International Symposium on Recent Advances in Banana Crop Protection for Sustainable Production and Improved Livelihoods*, 10-14 September 2007, White River, South Africa. ISHS Acta Horticulturae, pp.109-11.

Moller, E. M., Bahnweg, G., Sandermann, H., and Geiger, H. H. 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Res.* 20(22): 6115.

Mondal, S. K., Ray, B., Thakur, S., and Ghosal, P. 2001. Isolation, purification and some structural features of the mucilaginous exudate from Musa paradisiaca. *Fitoterapia* 72(3):263-271.

Moore, N. Y., Bentley, S., Pegg, K. G., and Jones, D. R. 1995. Musa disease fact sheet Nº. 5 - Fusarium wilt of banana [on-line]. Available*:*

https://www.frontiersin.org/articles/10.3389/fpls.2018.01468/full [17 October 2019].

Mori, Y., Kanda, H., and Notomi, T. 2013. Loop-mediated isothermal amplification (LAMP): recent progress in research and development. *J. Infection Chemother*. 19(3): 404-411.

Morkunas, I. and Ratajczak, L. 2014. The role of sugar signaling in plant defense responses against fungal pathogens. *Acta Physiologiae Plantarum* 36(7): 1607-1619.

Mostert, D., Molina, A. B., Daniells, J., Fourie, G., Hermanto, C., Chao, C. P., Fabregar, E., Sinohin, V. G., Masdek, N., Thangavelu, R., and Li, C. 2017. The distribution and host range of the banana Fusarium wilt fungus, *Fusarium oxysporum* f. sp. *cubense,* in Asia. *PloS one* 12(7): 1-24.

Muhammad, A., Hussain, I., Khanzada, K. A., Kumar, L., Ali, M., Yasmin, T., and Hyder, M. Z. 2017. Molecular characterization of *Fusarium oxysporum* f. sp. *cubense* (Foc) tropical race 4 causing Panama disease in Cavendish banana in Pakistan. *Pak. J. Agric. Sci.* 54(1): 1-8.

Mulekar, V. G., Desai, A. G., and Chavan, A. A. 2017. Studies on morphological variability in *Fusarium oxysporum* f. sp. *ricini* causing wilt of castor. *Trends Biosci*. 10(4): 1232-1236.

Mustaffa, M. M. and Thangavelu, R. 2011. Status of fusarium wilt in india. In: Bergh, I. V. D., Smith, M., Swennen, R., and Hermanto, C. (eds), *Proceedings of International ISHS-ProMusa Symposium on Global Perspectives on Asian Challenges*, 14-18 September 2009, Guangzhou. ISHS Acta Horticulturae, pp.323-329.

Nagar, D., Kumar, N., Debbarama, R., and Reshma, V.S. 2016. Investigation on the biochemical basis of resistance to Fusarium wilt in newly synthesized banana hybrids. *Int. QJ Environ. Sci. 9:*593-596.

Nair, S., Manimekalai, R., Raj, P. G., and Hegde, V. 2016. Loop mediated isothermal amplification (LAMP) assay for detection of coconut root wilt disease and arecanut yellow leaf disease phytoplasma. *World J. Microbiol. Biotechnol.* 32(7): 108-115.

Napitupulu, T. P., Ilyas, M., Kanti, A., and Sudiana, I. M. 2019. In vitro evaluation of *Trichoderma harzianum* strains for the control of *Fusarium oxysporum* f. sp. *cubense*. *Plant Pathol. Quarantine* 9(1): 152-159.

Narendrappa, T. and Gowda, B. J. 1995. Integrated management of Panama wilt on banana cv. Nanjangud Rasabale. *Curr. Res.* 24:53-55.

Nayak, D. D. 2017. Study of Panama wilt of banana caused by *Fusarium oxysporum* f. sp. *cubense* and its management. M.Sc. (Ag) thesis, Orissa University of Agriculture and Technology, Bhubaneswar, 102p.

Nel, B., Steinberg, C., Labuschagne, N., and Viljoen, A. 2006. Isolation and characterization of non-pathogenic *Fusarium oxysporum* isolates from the rhizosphere of healthy banana plants. *Plant Pathol.* 55(2): 207-216.

Nel, B., Steinberg, C., Labuschagne, N., and Viljoena, A. 2007. Evaluation of fungicides and sterilants for potential application in the management of Fusarium wilt of banana. *Crop. Prot*. 26(4): 697-705.

Nene, Y. L. and Thapliyal, P. N. 1993. *Fungicides in Plant Disease Control* (3rd Ed.). International Science Publisher, 691p.

Niessen, L. and Vogel, R. F. 2010. Detection of *Fusarium graminearum* DNA using a loop-mediated isothermal amplification (LAMP) assay. *Int. J. Food Microbiol*. 140: 183-191.

Nikam, P. S., Jagtap, G. P., and Sontakke, P. L. 2007. Management of chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri*. *Afr. J. Agric. Res.* 2(12):692–697.

Nisa, T. U., Wani, A. H., Bhat, M. Y., Pala, S.A., and Mir, R. A. 2011. In vitro inhibitory effect of fungicides and botanicals on mycelial growth and spore germination of *Fusarium oxysporum*. *J. Biopesticides*, 4(1): 53-56.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., and Amino, N. 2000. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res*. [e-journal] 28(12). Available: https://www.ncbi.nlm.nih.gov/pubmed/ 10871 386. ISSN 1362-4962. [15 December 2017].

O'Donnell, K. 1992. Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum* (*Gibberella pulicaris*). *Curr. Genet.* 22(3): 213-220.

Ordóñez, N., Salacinas, M., Mendes, O., Seidl, M. F., Meijer, H. J .G., Schoen, C.D., and Kema, G. H .J. 2019. A loop-mediated isothermal amplification (LAMP) assay based on unique markers derived from genotyping by sequencing data for rapid in planta diagnosis of Panama disease caused by Tropical Race 4 in banana. *Plant Pathol. 68*(9): 1682-1693.

Ortiz, E., Cruz, M., Melgarejo, L. M., Marquínez, X., and Hoyos-Carvajal, L. 2014. Histopathological features of infections caused by *Fusarium oxysporum* and *F. solani* in purple passionfruit plants (*Passiflora edulis* Sims). *Summa Phytopathol.* 40(2): 134-140.

Pal, K. K. and Gardener, B. S. 2006. Biological control of plant pathogens. P*lant Health Instructor [e-journal]*. Available: http://dx. doi.org/10.1094/PHI-A-2006-1117-02. ISSN 1935-9411 [21 October 2019].

Parham, B. E. V. 1934. Report on banana disease. *Agric. J.* 7 (1) :43-57.

Patel, S. I., Patel, R. L., Desai, A. G., and Patel, D. S. 2011. Morphological, cultural and pathogenic variability among *Fusarium udum* and root dip inoculation technique for screening pigeon pea germplasm. *J. Mycol. Plant Pathol.* 41(1): 57-62.

Pegg, K. G., Coates, L. M., O'Neill, W. T., and Turner, D.W. 2019. The epidemiology of Fusarium wilt of banana. *Frontiers Plant Sci.* 10:1395.

Peng, H. X., Sivasithamparam, K., and Turner, D. W. 1999. Chlamydospore germination and Fusarium wilt of banana plantlets in suppressive and conducive soils are affected by physical and chemical factors. *Soil Biol. Biochem.* 31: 1363–1374.

Peng, J., Shi, M., Xia, Z., Huang, J., and Fan, Z. 2012. Detection of *Cucumber mosaic virus* isolates from banana by one-step reverse transcription loop-mediated isothermal amplification. *Arch. Virol.* 157(11): 2213-2217.

Peng, J., Zhang, H., Chen, F., Zhang, X., Xie, Y., Hou, X., Li, G., and Pu, J. 2014. Rapid and quantitative detection of *Fusarium oxysporum* f. sp. *cubense* race 4 in soil by real-time fluorescence loop-mediated isothermal amplification. *J. Appl. Microbiol.* 117(6): 1740-1749.

Pennypacker, B. W. and Nelson, P. E. 1972. Histopathology of carnation infected with *Fusarium oxysporum* f. sp. *dianthi. Phytopathol*. 62: 1318-1326.

Perez-Vicente, L. 2004. Fusarium wilt (Panama disease) in bananas: an updated review of the present knowledge about its causal agent. *Phytosanity* 8 (4):27-38.

Perez-Vicente, L. F., Dita, M. A., and Parte, E. M. 2014. Prevention and diagnostic of Fusarium Wilt(Panama Disease) of banana caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4(TR4). In: *Proceedings of the Regional Workshop on the Diagnosis of Fusarium Wilt (Panama Disease) Caused by Fusarium oxysporum f. sp. cubense Tropical Race 4: Mitigating the Threat and Preventing its Spread in the Caribbean,* 5-9 May 2014, Trinidad. University of the West Indies, Port Spain.

Perez-Vicente, L., Batlle-Viera, A., and Chacón-Benazet, J. 2003. *Fusarium oxysporum* f. sp. *cubense* en Cuba: biología de las poblaciones, reacción de los clones híbridos de la FHIA y biocontrol. In: Rivas, G. and Rosales, F. (eds). *Memorias del Taller*

Manejo Convencional y Alternativo de la Sigatoka negra, Nemátodos y Otras Plagas asociadas al Cultivo de Musáceas en los Trópicos. Guayaquil, EC. pp. 141-155.

Ploetz, R. C. 1990. Population biology of *Fusarium oxysporum* f.sp. *cubense.* In: Ploetz, R. C.(ed.), *Fusarium wilt of banana.* Proceedings of First International Conference on fusarial wilt of banana, 27-30 August 1989, Florida. Tropical Research and Education Center, Florida, pp.63-76.

Ploetz, R. C. 2005. Panama disease: an old nemesis rears its ugly head - the cavendish era and beyond [on-line]. Available: https://www.researchgate.net/publication /228979987 Panama Disease An Old NemesisRears its Ugly Head -Part 2 The Cavendish Era and Beyond [07 Nov. 2019].

Ploetz, R. C. and Churchill, A. C. L. 2011. Fusarium wilt: the banana disease that refuses to go away. In: Bergh, I. V. D., Smith, M., Swennen, R., and Hermanto, C. (eds), *Proceedings of International ISHS-ProMusa Symposium on Global Perspectives on Asian Challenges*, 14-18 September 2009, Guangzhou. ISHS Acta Horticulturae, pp.519-526.

Ploetz, R. C. and Pegg, K. G. 2000. Fungal diseases of root, corm and pseudostem. In: Jones, D. (ed.), *Diseases of Banana, Abaca and Enset.* CABI Publishing, Wallingford, UK, pp. 143-159.

Ploetz, R.C., 2015. Management of Fusarium wilt of banana: A review with special reference to tropical race 4. *Crop Prot. 73*, pp.7-15.

Prasadji, K. J. 2006. Race and vegetative compatibility group of *Fusarium oxysporum* f. sp. *cubense* isolates in Andhra Pradesh. *Indian J. Mycol. Plant Pathol.* 36(2): 237-240.

Prashant, K. M., Roland, T. V. F., and Alastair, C. 2003. Development of a PCR-based assay for rapid and reliable identification of pathogenic Fusaria. *FEMS Microbiol. Lett.* 218: 329-332.

ProMusa. 2020. Fusarium wilt of banana [on-line]. Available: http ://www.promusa.org/Fusarium+wilt [12 January 2020].

Purwati, R. D., Hidayah, N., Sudjindro., and Sudarsono. 2008. inoculation methods and conidial densities of *Fusarium oxysporum* f.sp. *cubense* in Abaca. *J.Biosci.* 15(1): 1-7.

Qi, Y. X., Zhang, X., Pu, J. J., Xie, Y. X., Zhang, H. Q., and Huang, S. L. 2008. Race 4 identification of *Fusarium oxysporum* f. *sp. cubense* from Cavendish cultivars in Hainan province, China. *Plant Dis. Notes* 3: 46–47.

Ram, H. and Pandey, R. N. 2011. Efficacy of bio-control agents and fungicides in the management of wilt of pigeon pea. *Indian Phytopathol.* 64(3): 269-271.

Ramanathan, A., Vidhasekaran, P., and Samiyappan, R. 2000. Induction of defense mechanisms in greengram leaves and suspension-cultured cells by Macrophomina phaseolina and its elicitors. *J. Plant Dis. Prot.* 107(3):245-257.

Rana, D., Murmu, S., and Misra, D. K. 2017. *In vitro* studies on different isolates of *Fusarium oxysporum* f. sp. *cubense* causing Panama wilt of banana in lower gangetic plain. *Int. J. Curr. Microbiol. Appl. Sci.* 6(12): 1072-1081.

Rasera, J. B., Amorim, L., Marques, J. P. R., Soares, M. K. M., and Appezzato-da-Gloria, B. 2019. Histopathological evidences of early grapevine leaf senescence caused by *Phakopsora euvitis* colonisation. *Physiol. Mol. Plant Pathol.* 108: 101434.

Ravindran, A., Levy, J., Pierson, E., and Gross, D. C. 2012. Development of a loop-mediated isothermal amplification procedure as a sensitive and rapid method for detection of '*Candidatus Liberobacter solanacearum*' in potatoes and psyllids. *Phytopathol.* 102(9): 899-907.

Rekha, A., Rawal, R. D., and Prasad, M. B. N. V. 1996. Screening banana germplasm against Fusrium wilt. *Indian J. Hortic.* 53(1): 42-45.

Rishbeth, J. 1955. Fusarium wilt of bananas in Jamaica: I. Some observations on the epidemiology of the disease. *Annals Bot.* 19(3): 293-328.

Rodriguez-Romero, A. S., Guerra, M. S. P., and Jaizme-Vega, M. D. 2005. Effect of arbuscular mycorrhizal fungi and rhizobacteria on banana growth and nutrition. *Agron. Sustain. Dev.* 25 (3): 395-399.

Rogers, S. O. and Benedich, A. J. 1994. Extraction of total cellular DNA from plants, algae and fungi. In:Gelvin, S.B. and Schilperoort, R. A. (eds), *Plant Molecular Biology Manual*. Kluwer Academic Publishers, Dordrecht.

Sadasivam, S. and Manickam, A. 1996. *Biochemical Methods.* New Age Int. Ltd. Publishers, Coimbatore, 251p.

Saitou N. and Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*. 4:406-425.

Saravanan, T., Muthusamy, M., and Marimuthu, T. 2003. Development of integrated approach to manage the Fusarial wilt of banana. *Crop Prot.* 22(9): 1117-1123.

Saravanan, T., Muthusamy, M., and Marimuthu, T. 2004. Effect of *Pseudomonas fluorescens* on Fusarium wilt pathogen in banana rhizosphere. *J. Biol. Sci*. 4(2): 192-198.

Schippers, B. and Van Eck, W. H. 1981. Formation and survival of chlamydospores in Fusarium. In: Nelson, P.E., Tousson, T. A., and Cook, R. J. (eds), *Fusarium: Disease, Biology, and Taxonomy.* Penn State University Press, University Park, London, pp. 250-260.

Schreiter, S., Babin, D., Smalla, K., and Grosch, R. 2018. Rhizosphere competence and biocontrol effect of *Pseudomonas* sp. RU47 independent from plant species and soil type at the field scale. *Front.Microbiol. 9*:97.

Senthil, K. E. 2003. Integrated management of Fusarium wilt of vegetable cowpea (*Vigna unguiculata* sub sp. *sesquipedalis* (L) Verdcourt) M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 110p.

Shahnazi, S., Meon, S., Vadamalai, G., Ahmad, K., and Nejat, N., 2012. Morphological and molecular characterization of *Fusarium* spp. associated with yellowing disease of black pepper (*Piper nigrum* L.) in Malaysia. *J. Gen. Plant Pathol.* 78(3): 160-169.

Sharma, B. K. R., Singh, R. P., Saha, S., and Rai, A. B. 2010. Comparative evaluation of tebuconazole 250 ec along with other biorational fungicides in the management of Fusarium wilt of tomato in vitro. *Pestology* 34(10):51-53.

Sharma, G. and Pandey, R. R. 2010. Influence of culture media on growth, colony character and sporulation of fungi isolated from decaying vegetable wastes. *J. Yeast Fungal Res.* 1(8): 157-164.

Sharma, M., Rathore, A., Mangala, U. N., Ghosh, R., Sharma, S., Upadhyay, H. D., and Pande, S. 2012. New sources of resistance to Fusarium wilt and sterility mosaic disease in a mini-core collection of pigeon pea germplasm. *Eur. J. Plant Pathol.* 133(3): 707-714.

Sharma, N., Pant, B. D., and Mathur, L. 2018. *Fusarium oxysporum* induced defense response in resistant and susceptible cultivars of *Eruca sativa* (Miller). *Int. J. Agric. Res.*13 (1): 26-38.

Shukla, D. N. 2019. Studies on variability and management of *Fusarium oxysporum* f. sp. *cubense* isolates causing Panama wilt of banana. Ph.D. thesis , Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, 142 p.

Simmonds, N. W. 1966. *Bananas.* Longmans, London, 512 p.

Simmonds, N.W. and Shepherd, K., 1955. The taxonomy and origins of the cultivated bananas. *Bot. J. Linnean Soc. 55*(359):.302-312.

Singh, A., Singh, A. K., and Singh, A. 1998. Screening of chilli germplasm against Fusarium wilt. *Crop Res.* 15: 132-133.

Singh, H. V. 2000. Biochemical basis of resistance in *Brassica* species against downy mildew and white rust of mustard. *Plant Dis. Res.* 15(1): 75-77.

Singh, R., Sindhu, A., and Singal, H. R. 2003. Biochemical basis of resistance in chickpea (*Cicer arietinum* L.) against Fusarium wilt. *Acta Phytopathol. Entomol. Hung.* 38(1-2): 13-19.

Singha, I. M., Kakoty, Y., Unni, B. G., Das, J., and Kalita, M. C. 2016. Identification and characterization of *Fusarium* sp. using ITS and RAPD causing Fusarium wilt of tomato isolated from Assam, North East India. *J. Genet. Eng. Biotechnol.* 14(1): 99-105.

Sivamani, E. and Gnanamanickam, S. S. 1988. Biological control of *Fusarium oxysporum* f.sp. *cubense* in banana by inoculation with *Pseudomonas fluorescens. Plant Soil* 107 (1): 3-9.

Sivamani, E. 1987. Wilt diseases of banana in Tamil Nadu. Studies on the biology and control of Panama wilt caused by *Fusarium oxysporum* f. sp. *cubense* (E.F. Smith) Snyder ed. Hansen. Ph.D. Thesis. University of Madras, Chennai, India.

Sivamani, E. and Gnanamanickam, S. S. 1987. Distribution of panama wilt pathogen in Tamil Nadu. *Indian J. Agri. Sci.* 57: 854-857.

Smith, E. F. 1910. A Cuban banana disease. *Sci.* 31: 754-755.

Smith, L. J. 2006. Developing healthier banana roots with mycorrhizae and rhizobacteria. Ph.D. thesis, Brisbane School of Agronomy and Horticulture, University of Queensland, 318p.

Snyder, W. C. and Hansen, H. N. 1940. The species concept in Fusarium. *Am. J. Bot.* 27(2): 64-67.

Solaiman, A. R. M. and Rabbani, M. G. 2006. Effects of NPKS and cow dung on growth and yield of tomato. *Bull. Inst. Trop. Agr. Kyushu Univ. 29*(1): 31-37.

Soma, D., Sapana, S., and Ashok, A. 2008. Efficacy of fungicides on mycelial growth and enzyme production on *Rhizoctonia solani* and *Fusarium oxysporum*. *Ann. Plant Prot. Sci.* 16(1): 135-138.

Somu, R. 2012. Studies on Panama disease of banana caused by *Fusarium oxysporum* f. sp. *cubense* (E. F. Smith) Synd. and Hans. M.Sc. (Hort) thesis, Unversity of Horticultural Sciences, Bagalkot, 138p.

Somu, R., Thammaiah, N., Kulkarni, M. S., Swamy, G. S. K., and Devappa, V. 2013. Roving survey on panama disease (*Fusarium oxysporum* f. sp. *cubense*) in banana growing areas of Karnataka. *Int. J. Plant Prot.* 6(1): 56-58.

Somu, R., Thammaiah, N., Swamy, G. S. K., Kulkarni, M. S., and Devappa, V. 2014. In vitro evaluation of fungicides against *Fusarium oxysporum* f. sp. *cubense*. *Int. J. Plant Prot*. 7(1): 221-224.

Sonakar, P., Kumar, V., and Anoop, S. 2013. Studies on cultural and morphological characters of tomato wilt (*Fusarium oxysporum* f. sp. *lycospersici*). *Int. J. Biol. Sci*. 10: 1637-1640.

Sowmya, G. S. 1993. Studies on Panama disease of banana caused by *Fusarium oxysporum* f. sp. *cubense*. M.Sc. (Ag) thesis, University of Agricultural Sciences, Bangalore, 120p.

Sreeja, S. J. 2014. Integrated management of Fusarium wilt and anthracnose of vegetable cowpea (*Vigna unguiculata* sub sp. *sesquipedalis* (L) Verdcourt) using new generation fungicides. Ph.D.(Ag) thesis, Kerala Agricultural University, Thrissur, 182p.

Stokes, M. E., Chattopadhyay, A., Wilkins, O., Nambara, E., and Campbell, M. M. 2013. Interplay between sucrose and folate modulates auxin signaling in Arabidopsis. *Plant Physiol. 162*(3):1552-1565.

Stover, R. H. 1962. *Fusarium Wilt (Panama Disease) of Banana and Other Musa species.* Commonwealth Mycological Institute. Kew, 117p.

Stover, R. H. 1972. *Banana, Plantain and Abaca Diseases.* Commonwealth Mycological Institute, Kew, UK.316p.

Stover, R. H. and Waite, B. H. 1960. Studies on Fusarium wilt of bananas: V. Pathogenicity and distribution of *F. oxysporum* f. sp. *cubense* races 1 and 2. *Can. J. Bot*. 38(1): 51-61.

Su, H. J., Hwang, S. C., and Ko, W. H. 1986. Fusarial wilt of Cavendish bananas in Taiwan. *Plant Dis.* 70(9): 814-818.

Tauzin, A. S. and Giardina, T. 2014. Sucrose and invertases, a part of the plant defense response to the biotic stresses. *Front. Plant Sci.* 5: 293.

Thakker, J. N., Patel, S., and Dhandhukia, P. C. 2013. Induction of defense-related enzymes in banana plants: effect of live and dead pathogenic strain of *Fusarium oxysporum* f. sp. *cubense*. *ISRN Biotechnol.*

Thakker, J.N., Patel, N., and Kothari, I.L. 2007. *Fusarium oxysporum* derived elicitor-induced changes in enzymes of banana leaves against wilt disease. *J Mycol Plant Pathol*. 37:510-513.

Thangavelu R, Sundararaju P, Sathiamoorthy S, Reghuchander T, Velazhahan R, Nakkeeran S, Palanisamy A. 1999. Status of Fusarium wilt of banana in India. In: *Proceedings of the International Workshop on Banana Fusarium Wilt Disease*, 18 – 20 October 1999, Malaysia. INIBAP- ASPNET, Philippines, pp. 58 – 63.

Thangavelu, R. and Mustaffa, M. M. 2010a. First report on the occurrence of a virulent strain of Fusarium wilt pathogen (Race-1) infecting Cavendish (AAA) group of bananas in India. *Plant Dis.* 94(11): 1379-1379.

Thangavelu, R. and Mustaffa, M. M. 2010b. A potential isolate of *Trichoderma viride* NRCB1 and its mass production for the management of fusarium wilt disease in banana. *Tree For. Sci. Biotech.* 4(2): 76-84.

Thangavelu, R., Kumar, K. M., Devi, P. G., and Mustaffa, M. M. 2012. Genetic diversity of *Fusarium oxysporum* f. sp. *cubense* isolates (Foc) of India by inter simple sequence repeats (ISSR) analysis. *Mol. Biotechnol.* 51(3): 203-211.

Thangavelu, R., Loganathan, M., Arthee, R., Prabakaran, M., and Uma, S. 2020. Fusarium wilt: a threat to banana cultivation and its management. *CAB Rev.* 15(4): 1-24.

Thangavelu, R., Mostert, D., Gopi, M., Devi, P. G., Padmanaban, B., Molina, A. B., and Viljoen, A. 2019. First detection of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (TR4) on Cavendish banana in India. *Eur. J.Plant Pathol. 154*(3): 777-786

Thangavelu, R., Sundararaju, P., Sathiamoorthy, S., Raghuchander, T., Velazhahan, R., Nakkeeran, S., and Palaniswami, A. 2001. Status of Fusarium wilt of banana in India. In: Molina, A. B., Nikmasdek, N. H., and Liew, K. W. (eds), *Banana Fusarium wilt management: Towards sustainable cultivation*. Proceedings of an international workshop, Los Banos, Philippines. INIBAP- ASPNET, Philippines, pp. 58-63.

Tomita, N., Mori, Y., Kanda, H., and Notomi, T. 2008. Loop-mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products. *Nat. Protocols* 3(5): 877-882.

Ubaud, L. T. and Requina, J. D. 2016. Vesicular arbuscular mycorrhizae and *Trichoderma harzianum* as biological control agents against panama disease o

f ‘Cavendish’ banana caused by *Fusarium oxysporum* f. sp. *cubense* tropical race 4. *Asian. Res. J. Agric.* 1(2): 1-11.

Vandana, V. V., Bhai, S., and Azeez, S. 2014. Biochemical defense responses of black pepper (*Piper nigrum* L.) lines to *Phytophthora capsici*. *Physiol. Mol. Plant Pathol.* 88: 18-27.

Vanitha, S. C., Niranjana, S. R., and Umesha, S. 2009. Role of phenylalanine ammonia lyase and polyphenol oxidase in host resistance to bacterial wilt of tomato. *J. Phytopathol.* 157(9): 552-557.

Veeramohan, R., Govindarajalu, T., and Ramassamy, V. 1994. Biochemical and physiological changes in chilli leaves inoculated with *Alternaria solani*. *Ad. Plant Sci*. 7: 29-34.

Vincent, J. M. 1947. Distortions of fungal hyphae in the presence of certain inhibitors. *Phytopathol.* 48: 268–270.

Vinit, K. S., Prem, N., Biswas, S. K., and Gajendra, P. S. 2010. Efficacy of fungicide for management of wilt disease of lentil caused *by Fusarium oxysporum* f. sp. *lentis*. *Ann. Plant Prot. Sci.* 18(2): 411-414.

Visentin, I., Tamietti, G., Valentino, D., Portis, E., Karlovsky, P., Moretti, A., and Cardinale, F. 2009. The ITS region as a taxonomic discriminator between *Fusarium verticillioides* and *Fusarium proliferatum*. *Mycol. Res.* 113(10): 1137-1145.

Vishwanath, S., Chandrashekar, S. C., Rajanna, M. D., and Tejavathi, D. H. 2011. Histopathology of Banana Infected with *Fusarium oxysporum* f. sp. *cubense* (EF Sm.) Synd. & Hans. *Pest Technol.* 5(1): 63-66.

Visser, M., Gordon, T., Fourie, G., and Viljoen, A. 2010. Characterization of South African isolates of Fusarium oxysporum f. sp. cubense from Cavendish bananas. *S. Afr. J. Sci.* 106(3-4): 1-6.

Waite, B. H. 1963. Wilt of *Heliconia* spp. caused by *Fusarium oxysporum* f. sp. *cubense* race 3. *Trop. Agric. Trin.* 40: 299-305.

Waite, B. H. and Dunlap, V. C. 1953. Preliminary host range studies with *Fusarium oxysporum* f. sp. *cubense. Plant Dis. Rep. 37:*79-80.

Waite, B. H. and Stover, R. H. 1960. Studies on Fusarium wilt of bananas: vi. Variability and the cultivar concept in *Fusarium oxysporum* f. sp. *cubense*. *Can. J. Bot.* 38(6): 985-994.

Waman, A. A., Bohra, P., Sathyanarayana, B. N., Chandrashekar, S. C. and Thilaka Rani, R., 2013. Are bananas (*Musa* spp.) really safe from their aesthetic relatives? Screening potential alternative hosts of *Fusarium oxysporum* f. sp. *cubense*. *J. Hortic. Sci. Biotechnol.* 88(5): 559-562.

Wani, A.H. and Mir, R.A., 2011. Fusarium rot of potato and its management in Kashmir valley. *J. Plant Dis. Sci. 6*(2), pp.107-113.

Wani, S. A, Mohiddin, F. A., Hamid, B., Rizvi, G., Bhat, K. A., Hamid, A., Alam, A., Baba, Z. A., Padder, S. A., and Bhat, M. A. 2014. Incidence of Fusarium wilt of chilli (*Capsicum annum* L.) in Kashmir valley and its management by *Trichoderma* spp. *Mycopathol.* 12(1): 1-8.

Weller, D. M., Raaijmakers, J. M., McSpadden-Gardener, B. B., and Thomashow, L. S. 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Ann. Rev. Phytopathol.* 40: 309-48.

White, T. J., Bruns, T., Lee, S. J. W. T., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protoc. Guide Methods Appl.* 18(1): 315-322.

Yadeta, K. A. and Thomma, B. P. H. J. 2013. The xylem as battleground for plant hosts and vascular wilt pathogens. *Front. Plant Sci.* 4: 97.

Yano-Melo, A.M., Maia, L.C., Saggin, O.J., Lima-Filho, J.M., and Melo, N.F., 1999. Effect of arbuscular mycorrhizal fungi on the acclimatization of micropropagated banana plantlets. *Mycorrhiza 9*(2):119-123.

Yun, S. H., Arie, T., Kaneko, I., Yoder, O. C., and Turgeon, B. G. 2000. Molecular organization of mating type loci in heterothallic, homothallic, and asexual *Gibberella/Fusarium* species. *Fungal Genet. Biol.* 31(1): 7-20.

Zhang, N., Xin, H. E., Zhang, J., Raza, W., Xing-Ming, Y. A. N. G., Yun-Ze, R. U. A. N., Qi-Rong, S. H. E. N., and Huang, Q. W. 2014. Suppression of Fusarium wilt of banana with application of bio-organic fertilizers. *Pedosphere* 24(5): 613-624.

Zhang, X., Zhang, H., Pu, J., Qi, Y., Yu, Q., Xie, Y., and Peng, J. 2013. Development of a real-time fluorescence loop-mediated isothermal amplification assay for rapid and quantitative detection of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 in soil. *Plos One* 8(12): 82841.

Zuo, C., Deng, G., Li, B., Huo, H., Li, C., Hu, C., Kuang, R., Yang, Q., Dong, T., Sheng, O., and Yi, G. 2018. Germplasm screening of Musa spp. for resistance to *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4). *Eur. J. Plant Pathol.* 151(3): 723-734.

APPENDICES

APPENDIX I

Composition of culture medium used for the studies

Half strength potato dextrose agar (PDA) medium

Ingredients for a litre of distilled water.

Peeled pieces of potatoes - 100 g

Dextrose - 10 g

Agar - 20 g

APPENDIX II

Composition of reagents used for DNA isolation and agarose gel electrophoresis

1. **CTAB buffer (2X)**

   - 2 per cent CTAB (w/v)
   - 100mM Tris base (pH 8)
   - 20mM EDTA (pH 8)
   - 1.4 M NaCl
   - 1.0 per cent polyvinyl pyrrolidine
   - 0.2 per cent 2-β mercaptoethanol

2. **Chloroform: isoamyl alcohol (24:1 v/v)**

3. **TAE buffer (50X) for 1 litre**

   - Tris base -247g
   - glacial acetic acid -57.1 ml
   - 0.5 M EDTA (pH 8) -100 ml
   - Distilled water -1000 ml

4. **Loading dye (6X)**

   - 0.25 per cent Bromophenol blue
   - 0.25 per cent Xylene cyanol
   - 30 per cent Glycerol in water

APPENDIX III

Nucleotide sequences of ITS rDNA region of collected isolates during survey

>S1

ACATACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGA
CGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTGAGTA
AAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCA
TCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTC
AGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGC
GGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTG
GGACTCGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCT
TCCATAGCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACG
CCGTTAAACCCCAACTTCT

>S2

CAAACCCCTGTGACATACACACTTGTTGCCTCGGCGGATCAGCCCGCTCCC
GGTAAAACGGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATG
TAACTTTTGAGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCT
CTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTG
AATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCG
CCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCA
CAGCTTGGTGTTGGGACTCGCGTTAATTCGCGTTCCTCAAATTGATTGGCG
GTCACGTCGAGCTTCCATAGCGTAGTAGTAAAACCCTCGTTACTGGTAATC
GTCGCGGCCACGCCGTTAAACCCCAACTTCTGAATGTTGACCTCGGATCA
GGTAAA

>S3

TGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGAACATA
CCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCC
CGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTGAGTAAAACC
ATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGAT
GAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTG
AATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGC

ATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGGGAC
TCGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCA
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>S5

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>S6

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>S7

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>S8

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>S9

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>S10
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>S13

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>S14

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>S16

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>S17

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>S18

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>S25

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>S30
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GAACTTAAGCATATCAATAGG

APPENDIX IV

Nucleotide sequence of ITS rDNA region of *Fusarium redolens*

> *Fusarium redolens*

CTGCGGGAAGTACATATCATAAGCGGAGGAGCATATCAATAAGCGGAGA
GCATATCAATAAGCGGAGGAGCATATCAATAACCGGAGGAGCAATCAAT
AAGCGGATGATTCATCTGTAACTTCAGGATAAAACCATTAATCAGACGAA
ACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAA
AATGCAATAAGTAATGTGAATTGCAAAATTCAGTGAATCATCGAATCTTT
GAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCG
TCATTTCAACCCTCAAGCCCTCGGGTTTGGTGTTGGGG

APPENDIX V

Sequences deposition details of ITS rDNA region of collected isolates in NCBI

Fusarium oxysporum f. sp. cubense isolate Thiruvananthapuram -TVM 1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: MN519712.1

FASTA Graphics

Go to:

```
LOCUS       MN519712                422 bp    DNA     linear   PLN 05-OCT-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Thiruvananthapuram -TVM 1
            internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA
            gene, complete sequence; and internal transcribed spacer 2, partial
            sequence.
ACCESSION   MN519712
VERSION     MN519712.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 422)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-SEP-2019) Plant Pathology, Kerala Agricultural
            University, Mannuthy, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..422
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Fusarium oxysporum f. sp. cubense isolate Mudapuram - Thiruvananthapuram TVM 2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MN520842.1

FASTA Graphics

Go to:

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LOCUS       MN520842                460 bp    DNA     linear   PLN 05-OCT-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Mudapuram -
            Thiruvananthapuram TVM 2 internal transcribed spacer 1, partial
            sequence; 5.8S ribosomal RNA gene and internal transcribed spacer
            2, complete sequence; and large subunit ribosomal RNA gene, partial
            sequence.
ACCESSION   MN520842
VERSION     MN520842.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 460)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-SEP-2019) Plant Pathology, Kerala Agricultural
            University, Mannuthy, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..460
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Fusarium oxysporum f. sp. cubense isolate Nedumangad - Thiruvananthapuram TVM 8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MN752172.1

FASTA Graphics

Go to:

```
LOCUS       MN752172                523 bp    DNA     linear   PLN 08-DEC-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Nedumangad -
            Thiruvananthapuram TVM 8 internal transcribed spacer 1, partial
            sequence; 5.8S ribosomal RNA gene and internal transcribed spacer
            2, complete sequence; and large subunit ribosomal RNA gene, partial
            sequence.
ACCESSION   MN752172
VERSION     MN752172.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 523)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-DEC-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..523
                     /organism="Fusarium oxysporum f. sp. cubense"
                     /mol_type="genomic DNA"
                     /cultivar="Rasthali/Poovan (AAB)"
                     /isolate="Nedumangad - Thiruvananthapuram TVM 8"
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Fusarium oxysporum f. sp. cubense isolate Attingal - Thiruvananthapuram TVM 4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MN520598.1

FASTA Graphics

Go to:

```
LOCUS       MN520598                446 bp    DNA     linear   PLN 05-OCT-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Attingal -
            Thiruvananthapuram TVM 4 internal transcribed spacer 1, partial
            sequence; 5.8S ribosomal RNA gene and internal transcribed spacer
            2, complete sequence; and large subunit ribosomal RNA gene, partial
            sequence.
ACCESSION   MN520598
VERSION     MN520598.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 446)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-SEP-2019) Plant Pathology, Kerala Agricultural
            University, Mannuthy, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /organism="Fusarium oxysporum f. sp. cubense"
                     /mol_type="genomic DNA"
```

Fusarium oxysporum f. sp. cubense isolate Neyyattinkara - Thiruvananthapuram TVM 5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MN520604.1

FASTA Graphics

Go to:

```
LOCUS       MN520604                495 bp    DNA     linear   PLN 05-OCT-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Neyyattinkara -
            Thiruvananthapuram TVM 5 internal transcribed spacer 1, partial
            sequence; 5.8S ribosomal RNA gene and internal transcribed spacer
            2, complete sequence; and large subunit ribosomal RNA gene, partial
            sequence.
ACCESSION   MN520604
VERSION     MN520604.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 495)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-SEP-2019) Plant Pathology, Kerala Agricultural
            University, Mannuthy, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /organism="Fusarium oxysporum f. sp. cubense"
                     /mol_type="genomic DNA"
```

Fusarium oxysporum f. sp. cubense isolate Karyavattom - Thiruvananthapuram TVM 6 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MN520606.1

FASTA Graphics

Go to:

```
LOCUS       MN520606                475 bp    DNA     linear   PLN 05-OCT-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Karyavattom -
            Thiruvananthapuram TVM 6 internal transcribed spacer 1, partial
            sequence; 5.8S ribosomal RNA gene and internal transcribed spacer
            2, complete sequence; and large subunit ribosomal RNA gene, partial
            sequence.
ACCESSION   MN520606
VERSION     MN520606.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 475)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-SEP-2019) Plant Pathology, Kerala Agricultural
            University, Mannuthy, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /organism="Fusarium oxysporum f. sp. cubense"
                     /mol_type="genomic DNA"
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Fusarium oxysporum f. sp. cubense isolate Azhoor - Thiruvananthapuram TVM 7 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: MN520635.1

FASTA Graphics

Go to:

```
LOCUS       MN520635                414 bp    DNA     linear   PLN 05-OCT-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Azhoor -
            Thiruvananthapuram TVM 7 internal transcribed spacer 1, partial
            sequence; 5.8S ribosomal RNA gene, complete sequence; and internal
            transcribed spacer 2, partial sequence.
ACCESSION   MN520635
VERSION     MN520635.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 414)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-SEP-2019) Plant Pathology, Kerala Agricultural
            University, Mannuthy, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /organism="Fusarium oxysporum f. sp. cubense"
                     /mol_type="genomic DNA"
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Fusarium oxysporum f. sp. cubense isolate Karukutty - Ernakulam EKM 1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: MN527251.1

FASTA Graphics

Go to:

```
LOCUS       MN527251                436 bp    DNA     linear   PLN 08-OCT-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Karukutty - Ernakulam EKM
            1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal
            RNA gene, complete sequence; and internal transcribed spacer 2,
            partial sequence.
ACCESSION   MN527251
VERSION     MN527251.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 436)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2019) Plant Pathology, Kerala Agricultural
            University, Mannuthy, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /organism="Fusarium oxysporum f. sp. cubense"
                     /mol_type="genomic DNA"
                     /cultivar="Rasthali/Poovan (AAB)"
                     /isolate="Karukutty - Ernakulam EKM 1"
                     /isolation_source="Pseudostem"
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Fusarium oxysporum f. sp. cubense isolate Angamaly - Ernakulam EKM 2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: MN527254.1

FASTA Graphics

Go to:

```
LOCUS       MN527254                431 bp    DNA     linear   PLN 08-OCT-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Angamaly - Ernakulam EKM
            2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal
            RNA gene, complete sequence; and internal transcribed spacer 2,
            partial sequence.
ACCESSION   MN527254
VERSION     MN527254.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 431)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..431
                     /organism="Fusarium oxysporum f. sp. cubense"
                     /mol_type="genomic DNA"
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Fusarium oxysporum f. sp. cubense isolate Aluva - Ernakulam EKM 3 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA ...

GenBank: MN527256.1

FASTA Graphics

Go to:

```
LOCUS       MN527256                540 bp    DNA     linear   PLN 08-OCT-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Aluva - Ernakulam EKM 3
            small subunit ribosomal RNA gene, partial sequence; internal
            transcribed spacer 1, 5.8S ribosomal RNA gene, and internal
            transcribed spacer 2, complete sequence; and large subunit
            ribosomal RNA gene, partial sequence.
ACCESSION   MN527256
VERSION     MN527256.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 540)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /organism="Fusarium oxysporum f. sp. cubense"
                     /mol_type="genomic DNA"
                     /cultivar="Rasthali/Poovan (AAB)"
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Fusarium oxysporum f. sp. cubense isolate Muppathadam - Ernakulam EKM 5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: MN527257.1

FASTA Graphics

Go to:

```
LOCUS       MN527257                419 bp    DNA     linear   PLN 08-OCT-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Muppathadam - Ernakulam
            EKM 5 internal transcribed spacer 1, partial sequence; 5.8S
            ribosomal RNA gene, complete sequence; and internal transcribed
            spacer 2, partial sequence.
ACCESSION   MN527257
VERSION     MN527257.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 419)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..419
                     /organism="Fusarium oxysporum f. sp. cubense"
                     /mol_type="genomic DNA"
                     /cultivar="Rasthali/Poovan (AAB)"
                     /isolate="Muppathadam - Ernakulam EKM 5"
                     /isolation_source="Pseudostem"
                     /host="Banana"
                     /db_xref="taxon:61366"
```

Fusarium oxysporum f. sp. cubense isolate Kadungallur - Ernakulam EKM 4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MN527356.1

FASTA Graphics

Go to:

```
LOCUS       MN527356                460 bp    DNA     linear   PLN 08-OCT-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Kadungallur - Ernakulam
            EKM 4 internal transcribed spacer 1, partial sequence; 5.8S
            ribosomal RNA gene and internal transcribed spacer 2, complete
            sequence; and large subunit ribosomal RNA gene, partial sequence.
ACCESSION   MN527356
VERSION     MN527356.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 460)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /organism="Fusarium oxysporum f. sp. cubense"
                     /mol_type="genomic DNA"
                     /isolate="Kadungallur - Ernakulam EKM 4"
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Fusarium oxysporum f. sp. cubense isolate Kannara - Thrissur TSR 1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: MN527468.1

FASTA Graphics

Go to:

```
LOCUS       MN527468                454 bp    DNA     linear   PLN 08-OCT-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Kannara - Thrissur TSR 1
            internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA
            gene, complete sequence; and internal transcribed spacer 2, partial
            sequence.
ACCESSION   MN527468
VERSION     MN527468.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 454)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /organism="Fusarium oxysporum f. sp. cubense"
                     /mol_type="genomic DNA"
                     /cultivar="Rasthali/Poovan (AAB)"
                     /isolate="Kannara - Thrissur TSR 1"
                     /isolation_source="Pseudostem"
                     /host="Banana"
                     /db_xref="taxon:61366"
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Fusarium oxysporum f. sp. cubense isolate Nadathara - Thrissur TSR 2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: MN527520.1

FASTA Graphics

Go to:

```
LOCUS       MN527520                420 bp    DNA     linear   PLN 08-OCT-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Nadathara - Thrissur TSR
            2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal
            RNA gene, complete sequence; and internal transcribed spacer 2,
            partial sequence.
ACCESSION   MN527520
VERSION     MN527520.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 420)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..420
                     /organism="Fusarium oxysporum f. sp. cubense"
                     /mol_type="genomic DNA"
                     /cultivar="Rasthali/Poovan (AAB)"
```

Fusarium oxysporum f. sp. cubense isolate Chalakudy - Thrissur TSR 3 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal R...

GenBank: MN527522.1

FASTA Graphics

Go to:

```
LOCUS       MN527522                547 bp    DNA     linear   PLN 08-OCT-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Chalakudy - Thrissur TSR
            3 small subunit ribosomal RNA gene, partial sequence; internal
            transcribed spacer 1, 5.8S ribosomal RNA gene, and internal
            transcribed spacer 2, complete sequence; and large subunit
            ribosomal RNA gene, partial sequence.
ACCESSION   MN527522
VERSION     MN527522.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 547)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..547
                     /organism="Fusarium oxysporum f. sp. cubense"
                     /mol_type="genomic DNA"
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Fusarium oxysporum f. sp. cubense isolate Thrikkur - Thrissur TSR 4 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RN...

GenBank: MN528143.1

FASTA Graphics

Go to:

```
LOCUS       MN528143                509 bp    DNA     linear   PLN 08-OCT-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Thrikkur - Thrissur TSR 4
            small subunit ribosomal RNA gene, partial sequence; internal
            transcribed spacer 1, 5.8S ribosomal RNA gene, and internal
            transcribed spacer 2, complete sequence; and large subunit
            ribosomal RNA gene, partial sequence.
ACCESSION   MN528143
VERSION     MN528143.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 509)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..509
                     /organism="Fusarium oxysporum f. sp. cubense"
                     /mol_type="genomic DNA"
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Fusarium oxysporum f. sp. cubense isolate Ollukkara - Thrissur TSR 5 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal R...

GenBank: MN528565.1

FASTA Graphics

Go to:

```
LOCUS       MN528565                543 bp    DNA     linear   PLN 08-OCT-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Ollukkara - Thrissur TSR
            5 small subunit ribosomal RNA gene, partial sequence; internal
            transcribed spacer 1, 5.8S ribosomal RNA gene, and internal
            transcribed spacer 2, complete sequence; and large subunit
            ribosomal RNA gene, partial sequence.
ACCESSION   MN528565
VERSION     MN528565.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 543)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (02-OCT-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..543
                     /organism="Fusarium oxysporum f. sp. cubense"
                     /mol_type="genomic DNA"
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Fusarium oxysporum f. sp. cubense isolate Nandikkara - Thrissur TSR 8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MN749627.1

FASTA Graphics

Go to:

```
LOCUS       MN749627                611 bp    DNA     linear   PLN 07-DEC-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Nandikkara - Thrissur TSR
            8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal
            RNA gene and internal transcribed spacer 2, complete sequence; and
            large subunit ribosomal RNA gene, partial sequence.
ACCESSION   MN749627
VERSION     MN749627.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 611)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-DEC-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..611
                     /organism="Fusarium oxysporum f. sp. cubense"
                     /mol_type="genomic DNA"
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Fusarium oxysporum f. sp. cubense isolate Anandapuram - Thrissur TSR 7 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MN663127.1

FASTA Graphics

Go to:

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LOCUS       MN663127                521 bp    DNA     linear   PLN 17-NOV-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Anandapuram - Thrissur
            TSR 7 internal transcribed spacer 1, partial sequence; 5.8S
            ribosomal RNA gene and internal transcribed spacer 2, complete
            sequence; and large subunit ribosomal RNA gene, partial sequence.
ACCESSION   MN663127
VERSION     MN663127.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocrεomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 521)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-NOV-2019) Plant Pathology, Kerala Agricultural
            University, Mannuthy, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /cultivar="Rasthali/Poovan (AAB)"
                     /isolate="Anandapuram - Thrissur TSR 7"
                     /isolation_source="Pseudostem"
                     /host="Banana"
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Fusarium oxysporum f. sp. cubense isolate Kottayi - Palakkad PKD 1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MN663130.1

FASTA Graphics

Go to:

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LOCUS       MN663130                494 bp    DNA     linear   PLN 17-NOV-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Kottayi - Palakkad PKD 1
            internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA
            gene and internal transcribed spacer 2, complete sequence; and
            large subunit ribosomal RNA gene, partial sequence.
ACCESSION   MN663130
VERSION     MN663130.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 494)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-NOV-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
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Fusarium oxysporum f. sp. cubense isolate Kuzhalmannam- Palakkad PKD 2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MN663148.1

FASTA Graphics

Go to:

```
LOCUS       MN663148                528 bp    DNA     linear   PLN 17-NOV-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Kuzhalmannam- Palakkad
            PKD 2 internal transcribed spacer 1, partial sequence; 5.8S
            ribosomal RNA gene and internal transcribed spacer 2, complete
            sequence; and large subunit ribosomal RNA gene, partial sequence.
ACCESSION   MN663148
VERSION     MN663148.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 528)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-NOV-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /cultivar="Rasthali/Poovan (AAB)"
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Fusarium oxysporum f. sp. cubense isolate Chethali - Palakkad PKD 3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MN663157.1

FASTA Graphics

Go to:

```
LOCUS       MN663157                534 bp    DNA     linear   PLN 17-NOV-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Chethali - Palakkad PKD 3
            internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA
            gene and internal transcribed spacer 2, complete sequence; and
            large subunit ribosomal RNA gene, partial sequence.
ACCESSION   MN663157
VERSION     MN663157.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 534)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-NOV-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
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            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /isolate="Chethali - Palakkad PKD 3"
                     /isolation_source="Pseudostem"
                     /host="Banana"
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Fusarium oxysporum f. sp. cubense isolate Kannadi - Palakkad PKD 4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MN749615.1

FASTA Graphics

Go to:

```
LOCUS       MN749615                454 bp    DNA     linear   PLN 07-DEC-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Kannadi - Palakkad PKD 4
            internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA
            gene and internal transcribed spacer 2, complete sequence; and
            large subunit ribosomal RNA gene, partial sequence.
ACCESSION   MN749615
VERSION     MN749615.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 454)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-DEC-2019) Plant Pathology, Kerala Agricultural
            University, Mannuthy, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
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                     /cultivar="Rasthali/Poovan (AAB)"
                     /isolate="Kannadi - Palakkad PKD 4"
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Fusarium oxysporum f. sp. cubense isolate Mukkom - Kozhikode KKD 1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GenBank: MN749616.1

FASTA Graphics

Go to:

```
LOCUS       MN749616                430 bp    DNA     linear   PLN 07-DEC-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Mukkom - Kozhikode KKD 1
            internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA
            gene, complete sequence; and internal transcribed spacer 2, partial
            sequence.
ACCESSION   MN749616
VERSION     MN749616.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 430)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-DEC-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
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            ##Assembly-Data-END##
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                     /cultivar="Rasthali/Poovan (AAB)"
                     /isolate="Mukkom - Kozhikode KKD 1"
                     /isolation_source="Pseudostem"
                     /host="Banana"
```

Fusarium oxysporum f. sp. cubense isolate Koduvally - Kozhikode KKD 2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MN749617.1

FASTA Graphics

Go to:

```
LOCUS       MN749617                493 bp    DNA     linear   PLN 07-DEC-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Koduvally - Kozhikode KKD
            2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal
            RNA gene and internal transcribed spacer 2, complete sequence; and
            large subunit ribosomal RNA gene, partial sequence.
ACCESSION   MN749617
VERSION     MN749617.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 493)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-DEC-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
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                     /cultivar="Kadali (AA)"
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                     /isolation_source="Pseudostem"
                     /host="Banana"
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Fusarium oxysporum f. sp. cubense isolate Omassery - Kozhikode KKD 3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MN752175.1

FASTA Graphics

Go to:

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LOCUS       MN752175                516 bp    DNA     linear   PLN 08-DEC-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Omassery - Kozhikode KKD
            3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal
            RNA gene and internal transcribed spacer 2, complete sequence; and
            large subunit ribosomal RNA gene, partial sequence.
ACCESSION   MN752175
VERSION     MN752175.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 516)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-DEC-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
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FEATURES             Location/Qualifiers
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Fusarium oxysporum f. sp. cubense isolate Ambalavayal- Wayanad WYD 12 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MN752174.1

FASTA Graphics

Go to:

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LOCUS       MN752174                502 bp    DNA     linear   PLN 08-DEC-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Ambalavayal- Wayanad WYD
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            RNA gene and internal transcribed spacer 2, complete sequence; and
            large subunit ribosomal RNA gene, partial sequence.
ACCESSION   MN752174
VERSION     MN752174.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
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            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 502)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-DEC-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
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Fusarium oxysporum f. sp. cubense isolate Kenichira - Wayanad WYD 2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MN749620.1

FASTA Graphics

Go to:

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LOCUS       MN749620                517 bp    DNA     linear   PLN 07-DEC-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Kenichira - Wayanad WYD 2
            internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA
            gene and internal transcribed spacer 2, complete sequence; and
            large subunit ribosomal RNA gene, partial sequence.
ACCESSION   MN749620
VERSION     MN749620.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
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            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 517)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-DEC-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
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Fusarium oxysporum f. sp. cubense isolate Sulthan Bathery - Wayanad WYD 3 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit riboso...

GenBank: MN749621.1

FASTA Graphics

Go to:

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DEFINITION  Fusarium oxysporum f. sp. cubense isolate Sulthan Bathery - Wayanad
            WYD 3 small subunit ribosomal RNA gene, partial sequence; internal
            transcribed spacer 1, 5.8S ribosomal RNA gene, and internal
            transcribed spacer 2, complete sequence; and large subunit
            ribosomal RNA gene, partial sequence.
ACCESSION   MN749621
VERSION     MN749621.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
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            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 681)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-DEC-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
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Fusarium oxysporum f. sp. cubense isolate Kakkavayal - Wayanad WYD 4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: MN749622.1

FASTA Graphics

Go to:

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LOCUS       MN749622                523 bp    DNA     linear   PLN 07-DEC-2019
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Kakkavayal - Wayanad WYD
            4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal
            RNA gene and internal transcribed spacer 2, complete sequence; and
            large subunit ribosomal RNA gene, partial sequence.
ACCESSION   MN749622
VERSION     MN749622.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 523)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-DEC-2019) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
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APPENDIX VI

Nucleotide sequence of ITS rDNA region of Foc isolated directly from infected banana rhizome and its submission details in NCBI

> MN953004
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ATACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACG
GCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTGAGTAAA
ACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATC
GATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCA
GTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCG
GGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGG
GACTCGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTT
CCATAGCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGC
CGTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCC
GCTGAACTTAAGCATATCAATAGG

Fusarium oxysporum f. sp. cubense isolate Vellanikkara -Rhizome internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gen partial sequence

GenBank: MN953004.1

FASTA Graphics

Go to:

```
LOCUS       MN953004                526 bp    DNA     linear   PLN 21-JAN-2020
DEFINITION  Fusarium oxysporum f. sp. cubense isolate Vellanikkara -Rhizome
            internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA
            gene and internal transcribed spacer 2, complete sequence; and
            large subunit ribosomal RNA gene, partial sequence.
ACCESSION   MN953004
VERSION     MN953004.1
KEYWORDS    .
SOURCE      Fusarium oxysporum f. sp. cubense
  ORGANISM  Fusarium oxysporum f. sp. cubense
            Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
            Sordariomycetes; Hypocreomycetidae; Hypocreales; Nectriaceae;
            Fusarium; Fusarium oxysporum species complex.
REFERENCE   1  (bases 1 to 526)
  AUTHORS   Lishma,N.P. and Anita,C.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-JAN-2020) Plant Pathology, Kerala Agricultural
            University, Vellanikkara, Thrissur, Kerala 680 656, India
COMMENT     ##Assembly-Data-START##
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            ##Assembly-Data-END##
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CHARACTERIZATION AND INTEGRATED MANAGEMENT OF *Fusarium oxysporum* f. sp. *cubense* (E. F. SMITH) SNYDER AND HANSEN CAUSING FUSARIUM WILT DISEASE OF BANANA

By

Lishma N. P.

(2017-21-018)

ABSTRACT OF THE THESIS

Submitted in the partial fulfilment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY IN

AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

2020

ABSTRACT

Fusarium wilt of banana caused by the soil borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc) is a serious constraint to banana cultivation in Kerala. The fungal species constitute four pathogenic races, of which Race 1 is the prevalent one in our country and Race 4 is one of the emerging threats, though not reported from Kerala yet. The present study was undertaken to characterize the associated pathogenic races and to develop an integrated package for the disease management.

The project initiated with purposive sampling surveys in various districts *viz*., Thiruvananthapuram, Ernakulam, Thrissur, Palakkad, Kozhikode and Wayanad representing different agroclimatic zones of Kerala. The per cent disease incidence (PDI) and the per cent disease severity (PDS) ranged from 1.52 to 43.65 per cent and 20.34 to 49.57 per cent. The correlation analysis of PDI with weather parameters showed a positive correlation with rainfall. However, it was negatively correlated with temperature.

The study on symptoms under natural as well as artificial conditions showed characteristic external and internal symptoms. The number of days taken for complete wilting under artificial inoculation was 29.67 in Rasthali (AAB), 47.99 in Njalipoovan (AB), 31 in Kadali (AA) and 37.67 in Chenkadali (AAA).

Among the thirty isolates of the Foc collected, twenty three isolates were from Rasthali variety, four isolates from Kadali, two isolates from Njalipoovan and one from Chenkadali.

Studies on identification of Foc races with the differential host assay revealed that the varieties such as Cavendish (assay host to Race 4), Nendran (assay host to Race 4), *Heliconia* sp. (assay to Race 3) and Monthan (assay to Race 2) did not produce any type of symptoms whereas, all the isolates produced symptoms on Rasthali (assay host to Race 1) variety. A non polymerase chain reaction (PCR) based quick molecular diagnostic technique with loop mediated isothermal amplification (LAMP) assay was developed for the detection of Races of the pathogen. All isolates showed positive reaction to the LAMP assay for Race 1 and negative for Race 4. A PCR was also

standardised for the confirmation of the races. It is concluded that all the isolates collected from different agroclimatic zones belonged to the Race 1 category of the pathogen only.

Cultural and morphological characterization of the isolates revealed white coloured aerial mycelium with pink pigmentation and cottony and fluffy mycelial mat. The mycelial growth rate in half strength potato dextrose agar (PDA) medium ranged from 0.83 to 2.40 cm/day and the length and breadth of macroconidia and microconidia measured about 15.01 - 20.20 µm x 2.14 - 5.07 µm and 4.49 - 7.42 µm x 1.35 - 3.13 µm respectively. The inter-septal length and breadth of hyphae ranged from 16.14 to 22.94 µm and 4.22 to 6.57 µm respectively and the size of chlamydospores varied from 5.68 to 9.58 µm in diameter. The PCR based molecular characterization of isolates using ITS (internal transcribed spacer) primers produced single bands of size approximately 580 bp. *In silico* analysis of the sequences showed 96 to 100 per cent homology to Foc. Based on cultural, morphological and molecular characters, the pathogen was identified as *Fusarium oxysporum* f. sp. *cubense*.

The screening of accessions maintained in the germplasm of Banana Research Station (BRS), Kannara was done to assess their disease resistance to Foc Race 1 and were grouped into six categories. Fifteen immune varieties *viz*., Attunendran, Zanzibar, Big Ebanga, Nedunendran, Nendran, BRS II, Thiruvananthapuram, Pachanadan I, Cultivar Rose, Pisang Lilin, Pisang Jari Buaya, Yangambi Km5, Grand Naine, Chinese Cavendish and Nendran Hybrid and four highly susceptible varieties *viz*., Cheriya Poovan, Valiya Poovan, Kadali and Rasakadali were identified.

The estimation of biochemical parameters for the assessment of host plant disease resistance against Foc Race 1 revealed that the activity of total phenols and defense related enzymes was more in resistant varieties compared to susceptible varieties and the activity of reducing and non reducing sugars was more in susceptible varieties.

An *in vitro* experiment was conducted for the evaluation of chemical fungicides, biocontrol agents and botanicals for control of the pathogen. The effective treatments

from *in vitro* evaluation were carried over to pot culture and field experiments for the disease management. Among the various treatments, an integrated package comprising of *Pseudomonas fluorescens* + arbuscular mycorrhizal fungi and *Trichoderma* enriched cow dung + tebuconazole (T6) was proved to be the best for yield and disease management. It is concluded that the present study has enlightened our knowledge on characterization, race identification and management of Fusarium wilt pathogen infecting banana.