

**“MOLECULAR CHARACTERIZATION, EPIDEMIOLOGY AND MANAGEMENT OF BANANA FRUIT
ROT CAUSED BY *Lasiodiplodia theobromae* (Pat.) Griffth and Maubl.
UNDER SOUTH GUJARAT CONDITION”**

A

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**MOLECULAR CHARACTERIZATION, EPIDEMIOLOGY AND
MANAGEMENT OF BANANA FRUIT ROT CAUSED BY *Lasiodiplodia*
theobromae (Pat.) Griffth and Maubl. UNDER SOUTH GUJARAT CONDITION”**

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A B S T R A C T

Banana (*Musa paradisiaca* L.), fruit is one of the most important commercial fruit and vegetable crops grown all over the world in the tropical and subtropical areas. The genus *Lasiodiplodia theobromae* (Pat.) Griffth and Maubl., is geographically widespread but is most common in the tropics and subtropics areas . The present investigations on fruit rot of banana caused by *Lasiodiplodia theobromae* (Pat.) Griffth and Maubl. were carried out under South Gujarat conditions. Considering the seriousness of the present investigations were undertaken to generate scientific information on this serious disease and its management. For this various aspects were carried out in Department of Plant Pathology, Department of Plant Biotechnology and Research Farm of Soil and Water Management, Navsari Agricultural University, Navsari.

Field as well as market survey was carried out in Navsari and Surat district from January, 2008 to December, 2009, the maximum per cent disease index (PDI) was observed at field level in

the month of October (2.65- 2.78 %) and in market highest per cent disease index was observed in the month of September (16.90%).

The correlation of the individual weather parameters with disease development exhibited positive correlation with temperature. Morning relative humidity showed significant positive correlation but it highly significant with evening evening humidity .

Isolation of fungi were made from infected banana fruit by tissues isolation, associated fungi were identified as *Lasiodiplodia theobromae* (Pat.) Griffth and Maubl., *Fusarium moniliforme* Sheld, *Fusarium* sp., *Aspergillus niger* (Van.Teigh), *Acremonium* sp. and *Curvularia* sp., which were confirmed by Agharkar Research Institute, Pune.

Lasiodiplodia theobromae initially produced cottony white fluffy growth on potato dextrose agar, which covered the Petridish within 3 to 4 days at room temperature. After 2 days colour of the colony changed from white cottony to olivaceous dark. Under the lower surface of the dish, complete blackish background was observed due to pigmentation by the fungus in media.

The *Lasiodiplodia theobromea* was inoculated on healthy banana (var. Grand naine) fruits and produced brownish- black discoloration on the peel and softening of the pulp on unripe banana fruits. Under moist conditions white to light gray cottony mycelium covered the infected tissues.

Crown rot disease was the major post harvest disease due to fungal pathogens viz ; *L.theobromae*, *F. moniliforme* and *A. niger*. The frequency of occurrence was 72 per cent followed by ripe fruit rot due

to *L.theobromae* and *F. moniliforme* with 10 per cent occurrence. From these two *Lasiodiplodia theobromae* was of the most frequently (43 per cent) isolated pathogen in both the districts (Navsari and Surat) market surveys.

The genomic DNA was isolated by CTAB method from all the isolates of *Lasiodiplodia theobromae* and utilizing for genomic diversity analysis using RAPD primers.

The UPGMA based cluster analysis of RAPD patterns showed that isolate RID was more diverse from all other isolates and recorded highest genetic distance (84%) from other isolates. Isolates MIJ1 and MIT showed maximum similarity (73%) because they were isolated from same host, almost similar region and same year. While, isolate BIN and MIN showed more similarity (64.2%) probably because both were isolated from different host but same region. In our study BIN and BIB-1 isolate causing banana fruit rot showed only 40 % similarity, which might be due to different geographic region.

During ripening stages the level of total phenol gradually decreased in both infected and uninfected fruits, but it was more decreased in infected green mature fruits. The level of total sugar gradually increased during fruit ripening. PAL activity decreased in ripening stages but it increased in infected fruits. PPO and POD activity gradually increased in the ripening stage but degree of increase was higher in infected fruits.

Pycnidiospores germinated and produced ellipse-shaped appressorium after inoculation on unripe banana fruits but its further growth was suppressed and could not colonized due to presence of

biochemicals in banana green peel. But in semi-ripe and ripe fruits fungus colonized speedily very fast.

Hyaline branched mycelium produced pycnidia with in 48hrs which produced hyaline elliptical single celled pycnidiophores.

Efficacy of seven fungicides at three concentrations were evaluated for their comparative efficacy against mycelial growth of *L. theobromae* through "Poisoned Food Technique" under *in vitro* condition. Carbendazim, propiconazole, hexaconazole, carbendazim 12% + mancozeb 63% (SAAF, 75 WP) and mancozeb (2500 ppm) completely inhibited the fungal growth and proved statistically superior over the rest of fungicides tested. Copper oxychloride stimulated the growth of *L.theobromae*.

Carbendazim and propiconazole at 250ppm concentration gave cent per cent control the finger rot disease under field condition. The fruits harvested from treated plant kept natural ripening at room temperature. Maximum fruit rot disease control was observed in propiconazole (98.56%) treated fruits followed by carbandzim (95.64%) under storage condition up to eating ripe stage.

Maximum per cent disease control was observed in propiconazole (98.79%) followed by SAAF and carbendazim treated fruits.

Twelve phytoextracts of various plant species at two concentrations were screened by poisoned food technique *in vitro* to know their inhibitory effect on the growth of *L. theobromae*. The extracts of garlic clove and cinnamon leaf extract at 10 per cent concentration inhibited growth 47.09 and 33.86 per cent respectively.

Growth stimulation was observed in lantana leaf extract ,deshi babul, eucalyptus and barmasi over the control.

Antagonistic effect of five isolates of known bioagents were evaluated against the *L. theobromae* by dual culture technique. The maximum inhibition (75.83 %) was recorded in presence of *Pseudomonas fluorescense* which showed strong antagonism against the pathogen followed by *Bacillus subtilis* (70.56%) having moderate antagonistic effect. *Pseudomonas fluorescense* and *Bacillus subtilis* were good antagonist against pathogen. The inhibiting capacity of bioagent was directly correlated with the faster growth rate of antagonist.

Carbendazim @ 0.5 gL^{-1} , propiconazole @ 1mL^{-1} , garlic clove and cinnamon leaf extract @ 100mL^{-1} controlled cent per cent finger rot disease followed by *Bacillus subtilis* @ 1×10^7 CFU (92.71 %). However, the plants sprayed with propiconazole exhibited delayed fruit maturity.

Propiconazole treated fruit significantly inhibited cent per cent disease followed by Carbendazim (94.12%), Cinnamon leaf extracts (91.48%), and bunch covered with blue polythene bag (87.32%) under storage condition. Bunch covered with blue polythene bag directly reduced the inoculums which cause less disease under storage condition.



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CERTIFICATE

This is to certify that the thesis entitled **“MOLECULAR CHARACTERIZATION, EPIDEMIOLOGY AND MANAGEMENT OF BANANA FRUIT ROT CAUSED BY *Lasiodiplodia theobromae* (Pat.) Griffth and Maubl. UNDER SOUTH GUJARAT CONDITION”** submitted by Mr. **KEDAR NATH** in partial fulfillment of the requirement for the award of degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE)** in **PLANT PATHOLOGY** of **NAVSARI AGRICULTURAL UNIVERSITY** is a record of bonafide research work carried out by his under my guidance and supervision and the thesis has not previously formed on the basis for the award of any degree, diploma or other similar title.

Place : Navsari

(K. U. Solanky)

Date : 30 March, 2011

Major Advisor

DECLARATION

This is to declare that the whole of the research work reported in the thesis in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY (**AGRICULTURE**) in **PLANT PATHOLOGY** by the undersigned is the result of investigations done by me under direct guidance and supervision of **Dr. K. U. Solanky**, Retd., Professor & Head, Dept. of Plant Pathology, N.M. College of Agriculture, Navsari Agricultural University, Navsari and no part of the work has been submitted for any other degree so far.

Place: Navsari

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Place: Navsari

Date: 13March, 2011
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I. INTRODUCTION

Banana (*Musa paradisiaca L.*), fruit is one of the most important commercial fruit and vegetable crops grown all over the world in the tropical and subtropical areas. It is the second largest fruit crop, belongs to family Musaceae in order Scitamineae. It is indigenous to indo-Malayan region. Cultivation of edible bananas is believed to have been started in this region in prehistoric times. It is possibly the world's oldest cultivated crop. It can be grown round the year and it is widely adopted in India. Apart from this it is considered as potential 'Dollar Earning crop'. It is known since the dawn of ancient history as one of the delicious fruit in the world.

Geographical, climatic and genetic studies of bananas indicate that all edible banana are indigenous to the warm moist regions of the tropical and subtropical over a wide range from 20°N to 20°S of the equator (Shanmugavelu *et al*, 1992), through it prefers tropical climate. Major banana producing countries are India, China, Philippines, Brazil, Ecuador, Indonesia, Costa Rica, Mexico, Thailand and Colombia. It is cultivated on an area of 4.88 Mha. with an average production of 93.7 MT. in world and India produced 28.6% of total banana production of the world during 2008-09 (FAO).

It is grown in a wide range of soil having good drainage and humus content. Amongst the major banana growing states of India, Tamil Nadu, Maharashtra, Karnataka, Gujarat, Andhra Pradesh, Assam and Madhya Pradesh are the leading banana producing states. In India it was cultivated on an area of 0.709 M ha. (11.6 per cent of total fruit area) with a production of 26.22 MT (38.3per cent of total fruit production) with a productivity of 37.0 T/ha during 2008-09 and in Gujarat it was cultivated in 0.0609 M ha. with 0.357 MT production, it shared 13.6 per cent of total national banana production during 2008-09 (NHB, 2009). It ranks six in terms of area and third in production with a second in productivity of 58.7 T/ha. (NHB, 2009). India is the largest banana consumer and producing country in the world followed by Brazil.

The major banana growing districts in the Gujarat are Anand, Bharuch Junagadh, Kheda, Narmada, Navsari, Surat and Valsad. It covered an area of 0.034 M ha with production 14.03 Lakh tones. Largest area covered by Anand district with an area 110 Lakh hectares with highest production of 5.04 Lakh tones annually. It is the most important fruit crops of South Gujarat region. The farmers prefer it because of its high demand as a fresh fruit in the market.

In India banana is fourth important food crop in term of gross value exceeding only by paddy, wheat and milk products. It is also a desert fruit for million apart from a staple food owing to its rich and easy digestability. The ripe fruits are edible, delicious and very nutritious. The content of carbohydrates is very high with a calorific value of 67- 137mg/100g fruit. It is good source of vitamin A (190 IU/100g of edible portion) and vitamin C (100mg/100g pulp) and fair source of vitamin B and B2. The fruits are rich in magnesium, sodium, potassium and phosphorus. The food value is about three times that of wheat. It makes healthy and salt free balanced diet than many

fruits. Banana fruit powder made from dried green fruits can be used as baby food and in the manufacture of chocolate and biscuits. The ripe fruits are used to prepare wine, jams, jellies, puddings and halwa.

Banana plant produces the parthanocarpic fruit of commercial importance and propagated vegetatively from underground storage organ (rhizome or sucker) and surface level is the meristematic region pseudostem which gives rise to the leaves and finally to the inflorescence which produces the fruit. The leaves emerge in sequence with each rolled leaf pushing throughout the centre of an increasingly greater number of over lapping leaf sheath base which constitute a pseudostem. The pseudostem produces flower only once and it cut off after fruiting. The fruits are called fingers, which are borne in hands.

The usual practice is to harvest banana before it is ripe. The ripening is then done artificially in the various ways, such as exposing the bunches to the sun, placing them over a hearth, wrapping them with green leaves and piling them in a heap, storing them in a closed godowns or smoking them or treated with ethylene. The fruit take 48to72 hrs to ripe.

Cultivated banana is susceptible to many diseases, mostly fungal pathogen which attacks various part of the plant from root to fruit. Bananas are highly perishable commodities with post harvest losses estimated to the tune of 25-30 per cent (Kachwaha *et al.*, 1991). Banana fruit suffers from many serious diseases such as fruit rot, crown rot, finger rot, cigar –end rot and pitting disease. The current postharvest problems for bananas are mainly concerned with storage and marketing.

The genus *Lasiodiplodia theobromae*, is geographically widespread but is most common in the tropics and subtropics regions (Punithalingam, 1980). It is plurivorous and has been associated with approximately 500 hosts. This apparently unspecialized plant pathogen reported to cause numerous diseases, including dieback, root rot, fruit rots, leaf spot and witches' broom amongst many others (Punithalingam, 1980).

The causes of such losses during storage and marketing include : (i) The susceptibility of mature fruit to physical damage (ii) Decay of ripe banana fruit due to attack by pathogenic fungi and (iii) uneven and unpredictable ripening of the fruit. Reducing post harvest losses of banana is a critical area of research.

It is necessary to identify the pathogen causing above said diseases and ultimately to reduce the yield loss of the banana fruit.

The present investigations were carried out considering the above informations with the following objectives

- (i) Survey for the occurrence of banana fruit diseases.
- (ii) Molecular characterization of *L. theobromae*
- (iii) Biochemical studies of banana fruit rot incited by *L. theobromae*
- (iv) Management of *L. theobromae* banana fruit rot disease

The present investigation was carried out on the following aspects

1. Survey for occurrence of banana fruit rot diseases and its correlation with weather parameters under south Gujarat conditions.
2. Isolation, identification and pathogenicity of pathogens associated with banana fruit rot diseases.
3. Molecular characterization of major fruit rot pathogen (*L.theobromae*) using molecular markers
4. Biochemical studies of rotted banana fruits infected by *L. theobromae*
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- 6.2 Bio-efficacy of phytoextracts against fruit rot pathogen *L. theobroma* under *in vitro* condition
- 6.3 Antagonistic effect of known bioagents on growth of fruit rot pathogen *L. theobromae*
- 6.4 Development of Integrated disease management (IDM) module for management of banana fruit rot disease

II. REVIEW OF LITERATURE

Plant diseases are one of the major constraints in higher crop production, resulting in drastic crop losses by reducing quality and quantity of yield.

Crown rot and fruit rot are the most severe post-harvest disease of banana. It is caused by a complex of fungi with *Colletotrichum musae* (Berk. and Curt.) being the main pathogen. Additionally, *Fusarium moniliforme* Sheld., *Fusarium pallidoroseum* (Cooke) Sacc., *Nigrospora sphaerica* (Sacc.) Mason and *Botryodiplodia theobromae* Pat. are implicated with pathogenicity in the Windward Islands (Griffiee and Burden, 1976; Wallbridge, 1981; Finlay and Brown, 1993; Johanson and Blazquez, 1992;). *Lasiodiplodia theobromae* is a primary pathogen and can initiate infection from low initial inoculum levels. The other pathogens require much higher inoculum levels to elicit symptoms and are generally regarded secondary invaders under South Gujarat conditions.

When the banana bunches are harvested in the field, latex flows from the cut surface of the crown, and spores of the fungi may enter the wound and initiate disease development. Visual disease symptoms do not appear until fruit gets ripened in the ripening centers. Once infection initiated, it can progress from the crown to the pedicels and eventually the fingers and later pulp gets rotted, which renders the fruit unmarketable. It has been suggested that the latent infection can also contribute to crown rot and fruit rot if the

pathogen finds a window of opportunity in the withering, senescing crown region.

Disease levels fluctuate seasonally with the high crown rot and fruit rot season coinciding with the rainy season (June to November) with an average rainfall of 240 mm/month as opposed to 126 mm/month during the dry season (FAO, 1999). In the rainy season, losses over 45% have been recorded under Indian condition. However, *L. theobromae* has also been found responsible for crown rot and fruit rot of banana. Pertinent literature of *Lasiodiplodia theobromae* Pat. on banana have been presented here.

The genus *Lasiodiplodia*, *L. theobromae*, is geographically widespread but is most common in the tropics and subtropics regions which is associated approximately with 500 hosts (Punithalingam, 1980). In view of its widespread occurrence, the large number of hosts and its known morphological variability (Punithalingam, 1980) it is possible that *L. theobromae* is composed of a number of cryptic species.

Scientific classification of *Lasiodiplodia theobromae*

Kingdom	:	Fungi
Phylum	:	Ascomycota
Class	:	Dothideomycetes
Subclass	:	Incertae sedis
Order	:	Botryosphaeriales

Family : Botryosphaeriaceae

Genus : Lasiodiplodia

Species : *theobromae*

Binomial name : Lasiodiplodia *theobromae*(Pat.) Griffon & Maubl.

Synonyms :

Botryodiplodia ananassae, *Botryodiplodia elasticae* , *Botryodiplodia theobromae*, *Botryodiplodia tubericola*, *Chaetodiplodia grisea*, *Diplodia ananassae*, *Diplodia theobromae*, *Diplodia tubericola*, *Lasiodiplodia nigra*, *Lasiodiplodia triflorae*, *Lasiodiplodia tubericola*, *Lasiodiplodiella triflorae* and *Macrophoma vestita*.

2.1 Survey for occurrence of banana fruit rot diseases and its correlation with weather parameters

Goos *et al.* (1961) found association of *Botryodiplodia theobromae* (Pat.) with finger rot disease and reported that infection occurs through tissues at the flower-end of the fingers and wounds. The fungus grow very slowly at temp. below 20°C. Optimum growth and rotting occurs at 25-30°C temperature.

Chakrabarti *et al.* (1977) surveyed the fruit markets and store-houses of West Bangal, for the occurrence of soft rot disease in singapuri vatiety of banana due to *Fusarium solani* (Mart.). The first symptom appeared on the skin of the fruit as scattered circular to elongated dark brown spots and progressed further. The entire fruit become soft and got rotted.

Dharm Vir (1977) observed brown discoloration extending from top to bottom of the finger inside the pulp of the banana fruit, the advanced infected tissue turns soft and pulpy. Isolation made from the diseased tissues yielded *Fusarium moniliforme* Sheld.

Roy *et al.* (1989) observed 15-20 per cent of banana var. Singapuri (Green variety) fruits were ravaged every year by fungi viz., *Drechslera spicifera* (Bainair), *Acremonium strictum* Walter Gams, *Chalara paradoxa* (Deseynes) Sacc., *Pestalotiopsis disseminata* (Thum.) Stey., *Drechslera musae-sapientum* (Hansford) Ellis and *Alternaria alternata* (Fr.) Keissler in North-West part of Bhagalpur.

Kachwaha *et al.* (1991) surveyed fruit markets and store houses and reported a soft rot disease of banana fruit to the extend of 25-30 per cent in cv. Chitidar caused by *Botryosphaeria ribis* Grossenb and Duggar at Sagar district of Madhya Pradesh

Johanson and Blazquez (1992) isolated crown rot fungi viz., *Botryodiplodia theobromae* (Pat.), *Colletotrichum musae* (Berk & Curt.), *Fusarium moniliforme* Sheld., *Fusarium pallidoroseum* (Cook) Scardo, *Colletotrichum* spp., *Acremonium* spp., *cladosporium* spp., *Gliocladium roseum* Bainer, *Nigrospora* spp., *penicillium* spp., *Pestalotiopsis* spp., *Phoma sorghina* (Sacc.) Boerema Dorenbosch and Van Kest, *Verticillium theobromae* (Turconi) Mason and Hughes, which cause serious losses in banana fruits exported to UK from the Windward Islands (WI). Among these, the most common were first three which are associated with crown rot.

Wade *et al.* (1993) observed that *Colletotrichum musae*, *Fusarium moniliforme* var. *subglutinans*, *Nattrassia mangiferae* (Syd.&Syd.) Sutton and Dyko, *Alternaria alternata* and *Fusarium pallidroseum* (Cooke) Sacc. caused high infection incidence in unripe fingers during storage and also crown rot, but only *Fusarium moniliforme* var. *subglutinans* and *Alternaria alternata* caused main stalk rot. *Lasiodyplodia theobromae* and *Phomopsis musicola* (Stevens and Young) Comb. infect only ripening fingers and *F. culmorum* infected only crown and main stalk.

Slabaugh (1994) found that finger rot disease caused by *Botryodiplodia theobromae* has been reported from Central and South America, the Caribbean Islands, India, Taiwan, and Philippines. Symptoms produced at the lower-end of the finger or at a wound site resulted in to brownish- black discoloration of the peel a softening of the pulp, peel becomes wrinkled.

Sadhu *et al.* (1996) reported 40-45 per cent banana fruit rot in markets of Burdwan (West Bengal) caused by *Penicillium citrinum* Thom.

Ved Ram and Dharm Vir (1986) observed that none of the pathogens showed their activity at 10⁰C., as the storage temperature was enhanced to 25⁰C an increase in activity of various pathogens resulted in higher percentage of infection due to *Aspergillus niger* Van.Teigh, *A. flavus*, *A. fumigatus*, *Curvularia lunata* and *Colletotrichum musae* (Berk. & Curt.) Arx.. Relative humidity indicated that with increase from 40 to 70 per cent, there was

corresponding increase in the per cent rot, hundred per cent infection was observed due to *A. niger*, *A. flavus* and *C. lunata* at 100% RH. and complete decay of banana fruits occurred at 100% RH due to all these pathogens.

Williamson and tondon (1996) reported maximum rotting of bananan fruits due to *Botryodiplodia theobromae* at 25°C.

Ploetz (1998) found that anthracnose (*Colletotrichum musae*), cigar-end rot (*Trachysphaeria fructigena* and *Verticillium theobromae*), crown rot (*Ceratocystis paradoxa*, *C.musae*, *Fusarium pallidoroseum*, *Lasiodiplodia theobromae* and *Verticillium theobromae*), finger rot (*Lasiodiplodia theobromae*), Johnson spot (*Magnaporthe grisea*) and squirter disease (*Nigrospora sphaerica*) were the most important diseases which reduced the quality and post harvest life of banana fruits.

Bagwan and Meshram (2003) observed highest banana fruit rot severity at 25°C temperature due to *Gloeosporium musarium* Cooks and Masee and *Fusarium solani* (Martius) and at 30°C temperature by *Rhizopus oryzae* Went and Cerlings, when fruits were stored at 100 per cent RH. There was no disease symptom on fruits kept at 10°C temperature. The severity of storage rots due to these three pathogens decreased with increase in storage temperature to 35°C.

Cherian and Mani Varghese (2007) recorded the severity of the banana fruit rot disease at various temperature due to *Thielaviopsis paradoxa* (De Seynes) which was highest at 35°C temperature. Highest severity of *Fusarium semitectum* was recorded in the fruits, kept at

30⁰C temperature with 100 per cent relative humidity. Both Fungi were unable to produce disease at 10⁰C temperature. Injury of the banana fruits caused more infection together with higher temperature and relative humidity.

2.2 Isolation, identification and pathogenicity of organisms associated with banana fruit rot disease

Srivastava and Tondon (1971) reported *Botryodiplodia theobromae* Pat., which produced brownish and water soaked discolouration leading to rotting of the pulp under moist conditions. Later on, white to light gray cottony mycelium covered the infected tissue. Pycnidial formation take place rather late. *Verticillium theobromae* (Turc.) was reported to be associated with ‘cigar-end’ specimens collected from Bhusawal and Hyderabad locations. Conidiophores are usually solitary or in group on the surface of peel, conidia 4-9x2-3.4 µm borne at the end of the tapering phialides . *Trichothecium roseum* associated with pink mould rot of banana fruits.

Dater and Ghule (1985) isolated *Cylindrocarpon tonkinensis* (Bugnic.) from severely rotted fruit of the banana Cv. Basrai and proved to be pathogenic on inoculation.

Wade *et al.* (1993) isolated fungi from diseased fingers, crowns and main-stalk viz., *colletotrichum musae*, *Fusarium moniliforme* var. *subglutinans* and *Nattrassia mangiferae* which caused high incidence in unripe fingers during storage. *Lasiodiplodia theobromae* and *phomopsis musicola* infected only ripening fingers, and *F. culmorum* infected only crowns and main-stalk. Storage

diseases of fruits became more severe when treated with ethylene and new disease arising from pre-storage inoculation appeared, such as *L. theobromae* rot of fingers and *F. pallidroseum* rot of crown.

Marin *et al.* (1996) isolated five fungi viz , *Fusarium semitectum*, *F. moniliforme*, *Penicillium sp.*, *Gliocladium roseum* and *Gliocladium sp.* associated with crown rot of banana fruits which were collected from markets of Mexico, Guatemala, costa Rica and Ecaudor.

Ramma *et al.* (1999) reported crown rot disease of banana was due to a complex of fungal pathogens, namely *Fusarium sp.* *Colletotrichum sp.* *Verticillium sp.* and *cladosporium sp.* It was found to develop rapidly during fruit ripening thereby reducing the quality and marketability of fruits.

Prasad and Singh (2002) observed hollow fruits of banana cv. Singapuri infected by *Fusarium moniliforme* (*Gibberella moniliforme*) from Magadh and Patna districts of Bihar state.

Cordeiro and Matos (2005) recorded pre-harvest and post-harvest banana pathogens. Among pre-harvest pathogens viz., *Pyricularia grisea* (*Magnaporthe grisea*), *Cercospora hayi*, *Fusarium solani*, *F. roseum*, *Deightoniella torulosa*, *Verticillium theobromae*, *Trachysphaera fructigena*, *Chloridium musae* and among post-harvest pathogens viz. *Fusarium roseum*, *Verticillium theobromae* and *Colletotrichum musae*. *C. musae* caused tip rot and anthracnose disease of banana fruits.

Morphology of Pathogen

While working with lucern crop, Ribaldi (1959) provisionally named the organism as *Botryodiplodia medicaginis* which was originally known as *Diplodia natalensis*. Siradhana and Jain (1962) isolated *Botryodiplodia theobromae* from rotted papaya fruit and observed that young mycelium was hyaline, profusely branched, sparsely septate, 2.0-4.8 µm in diameter. At later stage, the mycelium turned greenish to brown and ultimately black with close septation. The pycnidia on the host were black, ostiolate, stromatic, erumpent and confluent measuring 87.7-300.8 µm in diameter. Griffiths (1967) suggested *Botryodiplodia theobromae* as synonymous of *Diplodia natalensis*.

Alasoadura (1970) studied on *Botryodiplodia theobromae* and reported that stroma formed earlier when the mycelium injured by cutting, while Patel (1971) observed that pycnidia were formed in the group of 2-6. Pycnidia aggregated in botyose clusters which produced thin walled, single celled and hyaline immature conidia. Later it turned into 2-celled dark brown, dolliform to shortly cylindrical mature conidia (Ainsworth *et al.*, 1973). Barnett and Hunder (1972) described the asexual fruiting bodies measuring 120-155 x 370-465 µm.

Olunloyo and Esuruoso (1975) isolated organism from cashew, *Lasiodiplodia (Botryodiplodia) theobromae* and reported that it is the imperfect stage of *Botryodiplodia rhodina*. *Botryodiplodia theobromae* is an important and ubiquitous fungus mostly in the tropics and sub tropics. Because of its association with numerous diseases and its wide spread distribution, both geographically and on

numerous host plants, the fungus is referred in the literature by a multiplicity of names (Punithalingam, 1976).

Botryodiplodia theobromae is pleomorphic and member of the sphaeropsidales with immersed to erumpent or superficial, pilose or glabrous, simple or often aggregated pycnidia reaching upto 5 mm width, with or without stroma. The pycnidia are usually papillate with prominent ostiole. Conidia are initially hyaline, unicellular, ellipsoid to oblong, thick walled with granular contents. On maturity the conidia became 2-celled, dark brown, usually 20-30 X 10-15 μm , with differentially pigmented longitudinal bands resembling striation (Punithalingam, 1980).

Sabalpara (1983) measured length and breadth of pycnidia and pycnidiospores of *Botryodiplodia theobromae* (average 291.41 x 232.03 and 19.05 x 10.86 μm , respectively) from different sources. Kivati (1984) reported drought wilt of coconut palm incited by *Botryodiplodia theobromae* produced buff white mycelial mat, numerous pycnidia, two types of conidia, immature conidia, hyaline and aseptate. While mature conidia bi-celled, dark brown and thick walled with longitudinal striation,

Verma and Cheema (1984) isolated *Botryodiplodia theobromae* from die-back of pear and described that cottony white mycelium turning sooty black, producing abundant carbonous pycnidia in and on the culture medium (PDA). The pycnidia were ovoid to elongate, dark brown and one septate conidia measured 21.6-25.2 x 10.0-12.5 μm . Ekundayo and Haskins (1989) reported that pycnidia of *Botryodiplodia theobromae* originated from a network of vegetative

hyphae. Ram (1993) isolated *B. theobromae* from eight hosts and observed that spore size was similar in all isolates, but spores were smaller in mango isolates. Vegetative growth rate, mycelial dry weight and sporulation were lower in coconut isolate.

Cedeno *et al.* (1995) confirmed that *Lasiodiplodia theobromae* was the cause of passion fruit vine die-back and studied the cultural characters. The conidia were initially hyaline, unicellular and had thick cell walls, but later on became dark brown pigmented, developed one septum, showed longitudinal striation, measuring 23.4-26.1 x 12.3-3.9 μm .

2.3 Molecular characterization of *L. theobromae* using molecular markers

The RAPD patterns were obtained from DNA of the 10 *Fusarium oxysporum* isolates from *Argyranthemum frutescens* (L.) Sch. Bip. (Paris daisy). The molecular weight of amplicons ranged from 0.2 to 4.0 kb, and the 30 10-mer primers generated a total of 348 polymorphic bands which could be used to distinguish isolates from different host plants. Seven primers (OPB-11, OPM-06, OPM-08, OPE-17, OPT-06, OPT- 17, and OPT-18) were selected among 30 tested, as generating the most informative markers in the identification of the new isolates from Paris daisy. Representative results obtained with primers OPE-17 and OPM-08 gave good amplification. All the *F. oxysporum* isolates obtained from diseased *A. frutescens* in Liguria showed very similar RAPD profiles, and some of the tested primers (e.g. OPE-17, OPM-06 and OP-T17) generated a strong amplification

signal which enabled their clear distinction from representatives of other formae specialis (Pasquali *et al.*, 2003).

Analysis of the *Trichoderma* isolates using molecular markers revealed a high level of genetic diversity among the isolates. *T. virens* isolate, showed only 6 per cent similarity with *T. harzianum* isolate. Out of 41 isolates classified as *T. harzianum*, five isolates PBAT-34, PBAT-42, PBAT-27, PBAT-9 and PBAT-40 formed separate clusters and showed only 13 to 54 per cent genetic similarity with the remaining *T. harzianum* isolates. Out of the remaining *T. harzianum* isolates, only one-half of isolates showed 90 per cent genetic similarity with *T. virens* (Singh *et al.*, 2006).

Choudary *et al.* (2007) isolated thirteen species of *T. harzianum* from the rhizosphere soil samples collected from field grown crops and assessed the genetic variability in *T. harzianum*, using random amplified polymorphic DNA markers. These thirteen isolates showed 78-80 per cent similarity with standard culture of *T. harzianum* (MTCC 2050).

Prasad *et al* (2007) analysed the molecular variability in 45 isolates of the genus *Fusarium* using PCR based RAPD, ISSR and ITS-RFLP markers. RAPD analysis of 12 random primers amplified 1145 loci of size ranging from 0.4kb to 5.5 kb. Significant differences in RAPD profiles of 45 isolates of various *Fusarium* species was observed with two primers OPA-02 and OPC-11 and found genetic similarity coefficient ranged from 5 to 100 per cent.

Saharan *et al.* (2007) used RAPD to study genetic variation in 15 isolates of *Fusarium graminearum* caused head scab of wheat collected from naturally infected wheat from Punjab, Tamil Nadu and high ranges of Himachal Pradesh during 2000–02. A screening of sixty-one 10-mer oligonucleotide primers (OPAA 1-20, OPAC 1-20, OPAD 1-20, OPV 14), revealed 19 RAPD primers which produced strong and reproducible DNA amplicons by PCR. The amplification products were in the range of 300 bp to 1.2 kb. Maximum number of bands (11) obtained with primer OPAD 12 followed by ten bands with OPAA 12. Punjab isolates of *F. graminearum* from Gurdaspur (G 31) and Ludhiana (L23) were found genetically most similar (91.38%), whereas Wellington isolates of *F. graminearum* (W 5 and W 7) were found genetically most dissimilar (14.92%). Cluster analysis of band-sharing coefficients separated isolates of *F. graminearum* into four clusters. Lahaul valley isolates of *F. graminearum* (D 3, D 4 and D 5) grouped together (Group I), while *F. graminearum* isolates of Punjab (G 31 and L 23) formed a separate group (Group II). *F. graminearum* isolates of Wellington, viz. W 1, W 2, W 5 and W 3, W 4, W 6, W 8 clustered in groups III and IV respectively.

2.4 Biochemical studies of rotted banana fruits

Sugar content

Biale (1960) found that banana and mangoes at the unripe stage have a relatively higher starch content which is almost completely hydrolysed during ripening to simple sugars, glucose, fructose and sucrose. Biochemical changes observed in carbohydrates are predominant chemical transformations occurring during ripening of climacteric fruit with a decrease in starch and an increase in sugar content occurring during the ripening of most fruits (Biale,1964).

Lizana (1976) analysed banana fruits and found that the glucose and fructose are predominant sugars in post-climacteric bananas. John *et al.* (1981) studied the changes in ripening plantains of three cultivars, two horn type and one french type and compared with ripened bananas under same conditions. Banana contained 1 per cent starch when fully ripe and none when over ripe, whereas plantains contained 9 per cent starch when fully ripe and 3 per cent when over ripe. Total sugar content was 23 per cent in fully ripe and over ripe banana but in plantains it increased from 20 per cent when fully ripe to 27 per cent when over ripe fruits. The ratio of glucose to fructose was approximately in the unity with bananas and plantains at all stage of ripening. Sucrose was more than 70 per cent of the total sugars in fully ripe bananas and plantain and about half of the total sugars in over ripen fruits.

Terra *et al* (1983) reported the transformation of starch to glucose in bananas during ripening. The soluble sugar content gradually increased from the first week of the development to the

end of the ripening periods of cherry laurel (Kadioglu and Yavru, 1998).

Janave (2008) observed that the total sugar content also increased in ripe non-spongy fruits. However, in spongy-white pulp, a decrease in both reducing and non-reducing sugars was observed.

Phenol content

Janave (2008) observed the initial total phenolics content of unripe non-spongy fruits decreased by about 30 per cent. Spongy-white pulp caused by *Staphylococcus* bacteria, the phenolics content decreased still further. However, as the spongy tissue affected pulp turned brownish black, the levels increased by about 16 per cent over that in the non-spongy ripe fruits.

Enzyme activity

Polyphenol oxidase (o-diphenol: oxygen oxidoreductase, E.C.1.10.3.1.) has been found in most higher plants, and is responsible for enzymatic browning of raw fruits and vegetables (Mathew and Parpia, 1971). This reaction is important in food preservation and processing, and is generally considered to be an undesirable reaction because of the unpleasant appearance and concomitant development of an off flavour.

Chakraborty and Nandi (1978) reported increased peroxidase activity and polyphenol oxidase in banana fruit infected by *Botryodiplodia theobromae* pat.

Singh and Saxena (1991) observed gradual decrease in the catalase activity in healthy as well as in the fruits pulp inoculated with *Aspergillus flavus* and increase invertase activity which helped in accumulation of soluble sugars making pulp more soft.

Aydin and Kadioglu (2001) found that polyphenol oxidase and peroxidase activity gradually decreased during the development of the fruits followed then by an increase during the ripening period. The level of sugar gradually increased during fruit development and ripening which affects the taste of medlar fruits. These observations suggest that the increase in PPO and POD activities as well as in sugar and protein contents has an important role in reducing the astringent taste of the medlar fruits.

Shijiang Zhu and Baocheng Ma (2007) reported that activities of the defense related enzymes peroxidase (POD), catalase (CAT) polyphenol oxidase (PPO), phenylalanine ammonia Lyase (PAL), β -1,3- glucanase and chitinase were enhanced in benzothiadiazole (BTH) and in Methyl jasmonate (MeJA) treated banana fruits irrespective of inoculated with *Colletotrichum musae* or not. The results suggest that post harvest decay in banana fruits can be controlled by BTH and MeJA.

Janave (2008) observed changes in peroxidase levels in the crude enzyme extract at various stages of ripening and spongy fruits. In unripe non-spongy fruits, a very low POX activity was observed. POX activity increased by several fold in non-spongy fruits upon ripening, however, in spongy fruits there was a further 1.5-fold

increase in activity over that in ripe nonspongy fruits. The development of black color indicated changes in phenolics content either by POX or polyphenol oxidase (PPO) enzymes.

2.5 Histopathological study of banana fruits infected by *L. theobromae*

Simmonds (1965) stated four hypothesis to explain the resistance of unripe banana fruits, which were the limitations of the fungus penetration by the shortage of nutrients, the failure in degradation of cell wall components, the inhibition of fungal growth by toxins, and metabolic changes in the unripe banana fruits.

Microscopic examination carried out by Sarbjeet *et al.* (2007) revealed that in section of wheat grains inoculated with paired compatible monosporidial *Tilletia indica* cultures, the two layers of pericarp were not fused with each other. A cavity was present between the inner and outer layers of pericarp which was filled with teliospores of *T. indica*.

2.6 Management of banana fruit rot disease

2.6.1 Chemical control of banana fruit rot disease caused by *L. theobromae*

2.6.1.1 *In vitro* efficacy of fungicides against *L. theobromae*

The attempts made earlier to test fungicides against the fungus *L. theobromae* under laboratory conditions by different workers is reviewed here as under, Sabalpara (1983) reported bavistin and benlate effective against *B. theobromae*, a cause of mango die-

back. Thakore (1983) reported bavistin (0.025%), benlate (0.025%), calixin (0.025%), dithane M-45 (0.05%) and brestanol (0.2%) were most effective for the growth inhibition of *B. theobromae*, a cause of post-harvest rot of sapota. Li *et al.* (1995) also found that thiophanate-methyl suppressed mycelial growth and conidial germination of *L. theobromae*.

Ahmad *et al.* (1995) examined *in vitro* efficacy of seven fungicides viz., aureofungin, carbendazim, captan, benomyl, mancozeb, copper oxychloride and thiophanate-methyl against *L. theobromae* using solid and liquid Richards' medium. The results stated that benomyl (0.1%), captan (0.2%), carbendazim (0.1%), mancozeb (0.25%) and thiophanate-methyl (0.1%) were highly effective against the fungus in both solid and liquid media. Similarly, Sheler *et al.* (1997) reported that thiophanate-methyl (0.1%) and benlate (0.1%) could completely suppress the growth of *L. theobromae*.

Banik *et al.* (1998) observed the complete inhibition of mycelial growth of *C. gloeosporioides* and *B. theobromae* causing mango fruit rot by carbendazim (400 ppm), followed by captan (450 ppm), thiophanate methyl (450 ppm), ziram (600 ppm) and chlorothalonil (650 ppm).

Mahmood *et al.* (2002) reported that benlate @ 100 ppm and topsin-M (thiophanate-methyl) @ 50 ppm completely inhibited the mycelium growth of *L. theobromae*.

Yadav and Majumdar (2004) reported effectiveness of carbendazim and mancozeb against *L. theobromae* (Guava isolate).

Muhammad *et al.* (2005) observed significant inhibition of mycelial growth of *Lasiodiplodia theobromae* with carbendazim and thiophanate-methyl when used @ 1 ppm a.i. or more. Alliete was effective at relatively high concentrations i.e., @ 1000 and 10000 ppm a.i., whereas, copxykil, cuprocaffaro and thiovit failed to inhibit the mycelial growth of *L. theobromae*.

2.6.1.2 Pre-harvest application of fungicides for control of banana fruit rot disease

Khanna and Chandra (1976) reported that benomyl and aretan were highly toxic as they completely checked the banana (Var. Harichal) fruits rot caused by *Fusarium moniliforme* and *F. roseum* as pre and post inoculation treatment up to 8 days.

Ved Ram and Dharam vir (1984) got complete control of banana fruits rot decay caused by *A. flavus* and *A.fumigatus* by treating the fruits with thiophanate methyl, benlate, thiabendazole, bavistin, propionic acid and sodium metabisulphite at 2000 ppm up to 8 days of storage.

Patel (1989) *in vivo* tested seven systemic and nonsystemic fungicides for the control of twig blight of sapota caused by *B. theobromae* and revealed that bavistin (500 ppm) was significantly superior for disease control.

Naqvi (1993) reported that benomyl and carbendazim at 1000 ppm concentration were effective against *B. theobromae* and *C. gloeosporioides* causing post harvest disease of mandarin. Godara (1994) reported that bavistin (500 ppm) completely inhibited spore germination of *Penicillium italicum* and *Botryodiplodia theobromae*. Lowest severity of both rots were observed in ripe and semi-ripe fruits treated with bavistin (500 ppm) in both pre and post-inoculation treatments.

The gummosis disease in Japanese apricot and peach orchards caused by *L. theobromae* was controlled by spraying thiophanate-methyl @1g/lit.(Li *et al.*, 1995).

Rawal (1998) observed that die-back of mango caused by *L.theobromae* was controlled by spray of carbendazim @ 0.1%, methylethiophanate @ 0.1% or chlorothalonil @ 0.2% at fortnightly interval.

Mahmood *et al.*, (2002) found that 1st foliar spray of topsin-M (thiophanate-methyl) @1 g/lit. reduced the infestation of *L. theobromae* to 10% and 2nd spray of the same fungicide completely inhibited the fungus as no tissue yielded this fungus.

2.6.1.3 Post-harvest application of fungicides for control of banana fruit rot disease

Latchmeah & Santchurn (1991) gave fungicidal treatments to banana fruits after harvest before keeping for ripening in 500 ppm of ethrel (ethephon at pH 8 and prochloraz (1, 1.75 and 2.5 ml

a.i./litre, thiophanate-methyl (0.7, 0.88 and 1 ml/litre) and benomyl (100, 300 and 500 mg/litre) which gave control efficiency ranging from 89.6 to 100 per cent against pedicel rot, anthracnose and blossom-end rot, caused by *Colletotrichum masae*, *F.pallidoroseum*, *Fusarium spp.*, *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides*.

Ramma *et al.* (1999) reported complete control of crown rot pathogen (*Fusarium sp.*, *Colletotrichum sp.*, *Cladosporium sp.*, and *Verticillium sp.*) by application of benomyl @ 500 ppm and thiabendazole @ 1000 ppm up to 9 days after harvest.

2.6.2 Bio-efficacy of phytoextracts against fruit rot pathogen *L. theobromae* under *in vitro* condition

Sabalpara (1983) tried 21 phyto-extracts against *B. theobromae* (Mango isolate) and observed that none of phyto-extract caused significant inhibition of mycelial growth and sporulation of pathogen except eucalyptus.

Patel (1989) tested 21 plant extracts against *B. theobromae* (Sapota isolate) among them, garlic bulb, turmeric rhizome, garlic leaves and dhatura leaves extracts showed more than 50 per cent inhibition of mycelial growth.

Singh *et al.* (1993) evaluated eleven leaf extracts of medicinal plants, the extract from *Azadirachta indica* and *Ocimum sanctum* were the most effective in inhibiting the mycelia growth of *B. theobromae*, *Fusarium oxysporum*, *Helminthosporium speciferum*, *Curvularia lunata*, *Aspergillus flavus* and *Trichothecium roseum*.

Aqueous garlic clove extract at 30 and 50 ml /lit. concentration completely inhibited the mycelial growth of *B. theobromae*, while 10 ml /lit. concentration inhibited the conidial germination of the pathogen (Andrade *et al.*, 1996).

2.6.3 Antagonistic effect of known bioagents on growth of fruit rot pathogen *L. theobromae*

Florence and Sharma (1990) showed that *Bacillus subtilis* can be used as potential bio-control agent against the *B. theobromae*.

Aranguren *et al.* (1994) tested antagonistic potentials of different *Trichoderma* spp. *in vitro* against *B. theobromae* and achieved highest inhibition with *T. longibrachyatum* and *T. viride*.

Gupta *et al.* (1999) studied mycoparasitic activity of various bio-agents *in vitro* against *B. theobromae* and concluded that *T. viride*, *T. harzianum* and *T. pseudokoningii* inhibited the growth of *B. theobromae*.

Mortuza and Ilag (1999) Observed that *Trichoderma harzianum* exhibited the greatest inhibition in dual culture. *Trichoderma viride* reduced rotting by 29.07 to 65.06 per cent in artificially inoculated banana fruits before 4 hours with *L. theobromae* provided better protection than simultaneous application or treatment 4 hours after inoculation.

Alvindia and Natsuaki (2009) Found that *Bacillus amyloliquefaciens* DGA14 inhibited test crown rot pathogens (*Lasioidiplodia theobromae*, *Thielaviopsis paradoxa*, *Colletotrichum*

musae, and *Fusarium verticillioides*) in culture. Post-harvest application of *B. amyloliquefaciens* DGA14 in the packing house reduced the incidence of crown rot to a level significantly lower than in fungicidal treatment.

2.6.4 Development of Integrated disease management (IDM) module for management of banana fruit rot diseases

Bedimo *et al.* (2003) observed good control of banana anthracnose caused by *Colletotrichum musae* through deflowering and bunch covering at horizontal fingers stage (Hfing). Fruit contamination rate was significantly higher on late-covered bunches (Hfing +7 days, Hfing+ 14 days) and highest in uncovered bunches.

Win *et al* (2007) found that disease development was least (25 per cent) on cinnamon extract (@ 5.0 ml/lit.) treated fruit after inoculation of crown rot fungi (*Colletotrichum musae*, *Fusarium spp.* *Lasiodiplodia theobromae*) compared to carbendazim (@ 0.75g/lit.) but rotting was higher when cinnamon extract was applied before inoculation.

III. MATERIALS AND METHODS

3.1 Survey for occurrence of banana fruit rot diseases and its correlation with weather parameters under south Gujarat conditions.

To study distribution and prevalence of banana fruit rot pathogen(s) in main banana growing areas of South Gujarat, so as to develop an effective integrated management package with biological control as its main component, it was pre-requisite to undertake field survey for collection of different isolates of fruit rot pathogen in the highly humid area of South Gujarat. For this purpose, field surveys were conducted in the main banana growing districts of South Gujarat viz., Surat and Navsari.

The fruits were examined regularly during growth stages in different seasons in order to find out the per cent disease index (PDI) of banana fruit rots and its correlation with weather parameters. Monthly fixed plot surveys were carried out in fields and markets of Surat and Navsari districts from January, 2008 to December, 2009.

3.1.1 Field level survey

Banana fields were surveyed for the prevalence of diseases such as cigar end rot, finger rot banana pre-mature ripening which have impact on fruit quality. Although the damage caused by these diseases on banana fruits, can be downgraded or even rendered fruits unmarketable.

Five fields of banana, each from soil and water management project, Department of plant pathology and Gandevi farm, NAU, Navsari, Bardoli and Vyara were selected and 10 bunches

from each field were randomly selected for the fixed plots survey. Per cent disease index (PDI) was recorded and infected fruits were brought to laboratory for isolation of causal organisms. The per cent disease index (PDI) was also work out to correlate with weather parameter.

The severity was recorded on the basis of per cent fruit area infected under following assessment key (Plate-I)

Scale for Per cent disease index (PDI)

The disease severity was recorded using 0-5 scales as mentioned below

Scale	Description
0	Fruit completely healthy.
1	Disease present only near the crown or tip with a little browning of pericarp portion and fruit completely healthy.
2	< 10 percent pulp rot with browning of pericarp
3	10- 25 percent pulp rot with browning of pericarp
4	25- 50 percent pulp rot with browning of pericarp.
5	> 50 percent pulp rot with browning of pericarp

Per cent disease index (PDI) was calculated using to the formula (Rose,1974).

$$\text{Per cent disease index} = \frac{\text{Sum of all numerical ratings}}{\text{-----}} \times 100$$

Total number of fruit examined X maximum rating

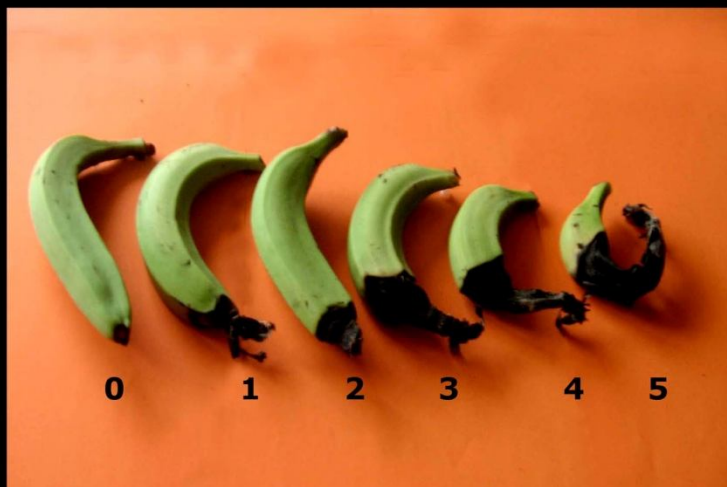
Monthly meteorological data of Navsari and Surat districts were collected from Meteorological Department, N.M. College of Agriculture, N.A.U., Navsari. The Interpretation and correlation of weather parameters with disease development was carried out.

3.1.2 Consumer/ Retail level survey

The sale of high quality fruit to consumers is an important challenge for the Indian fruit markets. Monthly surveys have consistently identified a number of quality defects in banana fruits displayed for retail sale, with postharvest diseases being one of the major quality problems.

Market survey was carried out from 5 locations viz, Navsari, Gandevi, Surat, Bardoli and Vyara. From each market 100 fruits (10 in each hand) were randomly selected and per cent disease index (PDI) at each visit was calculated. Infected fruits representing different symptoms were collected at each visit and brought to the laboratory for isolation and symptomatological studies. The per cent disease index (PDI) was work out to correlated with weather parameter as mention earlier in point 3.1.1.

Plate-I (A & B) : Assessment key for banana fruit rot disease



A = Finger rot (unripe immature fruits)



B = Ripe fruit rot

3.2 Isolation, identification and pathogenicity of microorganisms associated with banana fruit rot diseases

3.2.1 Collection of sample

Diseased samples of banana fruit (cv. Grand naine) with pulp rot, crown rot, tip end rot, red spots, peeling injury/bruising and finger rot were collected from fruit markets, fruit stalls and domestic store-rooms of Navsari, Gandevi, Surat, Bardoli and Vyara and from field of soil and water management project, Department of plant pathology and Gandevi farm and brought to laboratory, examined visually and microscopically. The symptoms observed were recorded up to complete rotting of banana fruits.

3.2.2 *Isolation of pathogens*

(A) Sterilization of equipment and culture media

The laminar flow was sterilizes with 95% alcohol and U.V. light radiation

The Petri-dishes, culture tubes and conical flasks were sterilized in an electrical hot air oven at 180⁰C for 1hrs.

The culture media were sterilized in an autoclave at 121⁰C at 15 pound pressure for 20 minutes. In case of pathogen which was isolated from heavily rotted fruits, a pinch of streptocycline was added prior to sterilization to prevent bacterial contamination.

The glass-wares were cleaned by cleaning solution and washed with tap water before sterilization (Appendix-I)

The inoculation loop and needle were sterilized by direct

heating (incineration) over spirit lamp flame and dipped in absolute alcohol.

(B) Methods of isolation for fungal pathogens

(i) Direct isolation

Nichrome wire/needle duly sterilized were used and the fungus growth from fruit surface was directly transferred to PDA (Appendix-III)

(ii) Tissue isolation method

Repeated isolations were carried out from the crown portion, rotted pulp, reddish spot on pericarp and dried tip end rot, after washing thoroughly with tap water. The infected tissue were cut into small bits, surface sterilized with 0.1 per cent mercuric chloride solution (1 g/litre) (Appendix-II) for 20 second followed by three subsequent washings of sterilized distilled water and then transferred aseptically on potato dextrose agar (PDA) medium in Petriplates. The Petriplates were incubated at room temperature for development of fungus growth. The plates were observed daily, the initial growth observed was picked up aseptically and it was transferred to PDA slants. The pure culture thus obtained was further purified by arial mycelia tip technique. The pure culture now stored safely and used for further investigations.

3.2.3 Morphological and cultural studies of pathogen

After purification, each fungus was allowed to sporulate. The sporulating cultures were identified on the basis of morphological characters of somatic and reproductive structures including spores.

Morphological characters of the fungus were studied by observing cotton blue stained slides under compound microscope (Appendix-IV). After calibrating the microscope, measurements of pycnidia and pycnidiospores were made with the help of ocular micrometer. On the basis of morphological characters, the causal fungus was found to be a species of *Lasiodiplodia* sp. and *Fusarium* sp. However, for detail identification the purified cultures were also sent to the Agharkar Research Institute, Pune.

3.2.4 Pathogenicity

To prove the Koch's postulate, mature and semi ripen healthy banana fruits (cv. Grand naine) were collected from field as well as from fruit market of Navsari and brought to the laboratory. The fruits were then surface sterilized by 0.1 per cent mercuric chloride solution(Appendix-II) for 30 seconds followed by three washings with sterilized water and separately inoculated with each of the isolated fungus by Pin- Pricking method. Each fungus was grown on PDA for seven days and fungal growth was taken from the periphery and was used for inoculations.

Five fruits were separately inoculated with each of the isolated fungus. The inoculated as well as uninoculated fruits were placed in sterilized, loosely tied polythene bags. A piece of sterilized wet absorbent cotton was placed inside each bag and the bag was kept at room temperature (24-28°C) in an incubation room. After development of symptoms, inoculated fruits were observed regularly. Reisolation of pathogen from the diseased fruits was done. Morphological as well as cultural characters of reisolated fungi were

compared with those of previously isolated from diseased banana fruits.

Determination of per cent occurrence of fungal pathogens

A preliminary survey of banana fields and markets in Surat and Navsari districts of South Gujarat showed that the disease is becoming severe in the area. Studies were, therefore, carried out to study the occurrence of the fungi which are associated with fruit rots.

The frequency of the fungi in the collected specimens was recorded using the following formula (Muhammad *et.al.*, 2004):

$$\text{Per cent Colonization} = \frac{\text{Number of pieces colonized by a pathogen}}{\text{Total number of pieces}} \times 100$$

Determination of percent occurrence of fungal frequency

This study was done to determine the frequency of occurrence of the different fungal isolates. Isolations were made from ten different rotted banana fruits and were cultured differently. The number of occurrence for each of the isolates in ten different samples were recorded and calculated as a ratio of occurrence and was then expressed in per cent using the below given formula.

$$\text{Per cent frequency} = \frac{X}{N} \times 100$$

X = Total number of individual organism in all the samples.

N = Total number of all the organism in all the samples assessed.

Symptomatological studies :

Symptomatology and severity of disease caused by each pathogen was determined by artificial inoculation of each pathogen in mature semi ripe healthy fruits (cv. Grand naine) which were collected from market. The fruits were then surface sterilized with 0.1 per cent mercuric chloride solution for one minute followed by three washings with sterilized water and separately inoculated with each of the isolated fungus in different fruit portion by Pin- Pricking method. Each fungus was grown on PDA for seven days and the fungal growth was taken from periphery was used for inoculations. Symptoms development for all the disease was recorded and severity was work out.

3.3 Molecular characterization of *L.theobromae* using molecular markers.

The use of DNA polymorphism has added a new dimension to the recognition and classification of genetic variability in fungi. In theory, DNA-based Random Amplified Polymorphic DNA (RAPD) was used to overcome all the limitations associated with the identification of sub-species taxas using morphology, pathogenicity, vegetative compatibility. If correlated to pathogencity, they could also be useful in identifying virulence groups (Woo *et al*, 1996). DNA based techniques are also valuable for use in phylogenic studies.

Considering the above information PCR based technique performed to know the genetic variability in *L. theobromae*.

RAPD has several advantages as a mean of characterizing genetic variability. These include speed, low cost, low DNA concentration, lack of radioactivity and these are not need for prior sequence information about the target DNA. It is also applicable to large numbers of isolates and enables analysis of variation at more than one locus (Bentley *et al*, 1995). As a result, this technique has been used extensively in the molecular characterization of *Lasiodiplodia theobromae*.

3.1 Experimental Materials

3.1.1 Fungal Materials

Total 9 accessions of *Lasiodiplodia species* were used for molecular study (Table-3.1).

3.1.2 Chemicals and instruments used for biochemical analysis and for DNA extraction and PCR:

All the chemicals used were of analytical reagent grade/molecular grade and were obtained from Sisco Research Lab. (SRL), LOBA Chemicals, Sigma, Hi-Media, E-Merck, Qualigens, Biogene USA, Bangalore Genei.

3.1.2.1 Instruments

Laminar flow	: Klenzflo
Weighing balance	: Sartorius, Anamed
pH meter	: Thermo Orion, Model-420
Spectrophotometer	: Nano photometer (Implene) UV-vis double beam Spectrometer (system)

Table 3.1 *Lasiodiplodia* accessions used in the present study

Sr. No.	Name of species	Host crop	Isolated from	Year of isolation	Name of accession
1	<i>Lasiodiplodia theobromae</i>	Banana	Navsari	2008	BIN
2	<i>Lasiodiplodia theobromae</i>	Mango	Navsari	2009	MIN
3	<i>Lasiodiplodia theobromae</i>	Mango	Junagadh	2006	MIJ1
4	<i>Lasiodiplodia theobromae</i>	Mango	Junagadh	2009	MIJ2
5	<i>Lasiodiplodia theobromae</i>	Mango	Talaja	2006	MIT
6	<i>Botryodiplodia theobromae</i>	Banana	Bangalore	2007	BIB1
7	<i>Botryodiplodia theobromae</i>	Banana	Bangalore	2007	BIB2
8	<i>Botryodiplodia theobromae</i>	Mango	Bangalore	2007	MIB
9	<i>Botryodiplodia theobromae</i>	Rose	Dantiwada	2007	RID

Waterbath	:	Nova, Cintex
Electrophoresis unit		
Submerine (Horizontal)	:	Genei
Micro-pipettes	:	Finnpipette, eppendorf
Ice maker	:	Icetronic
Centrifuge	:	Eppendorf refrigerated centrifuge Eltek cooling Centrifuge
Orbital shaker	:	Remi
Oven	:	Nova
Gel documentation	:	Gene genics
Cyclomixer	:	Remi (CM 101)
Deionised Water system	:	Millipore
PCR (Thermal cycler)	:	Eppendorf gradient

Total thirty primers from OPE-1 to OPE-10, OPF-1 to OPF-10 and OPG-1 to OPG-10 were used for initial screening but only four primers gave good amplification with *L. theobromae* genomic DNA (Table-3.2).

Table 3.2. Details of the RAPD primers amplified in the present study

S.No.	Primer code	Primer sequence (5'-3')
1.	OPE-3	CCAGATGCAC
2.	OPE-10	CACCAGGTGA
3.	OPF-9	CCTACGTCAG
4.	OPG-5	CTGAGACGGA

3.2.1 Fungus culture

Nine isolates of *L. theobromae* (Table 3.1) were grown in 250 ml flasks containing 50 ml of potato dextrose broth (PDB) and incubated at room temperature for 5–7 day. Mycelium was collected by filtration. Mycelial mats were washed with sterile distilled water and blotted dried. Deoxyribonucleic acid (DNA) was isolated and purified from each isolate according to Bowen *et. al.* (1996).

3.2.2 DNA extraction:

Extraction of total genomic DNA was carried out using CTAB method given by Saghai-Marroof *et al.* (1984). Chemicals, reagents and instruments are described in the (APENDIX –V).

Mycelium of each fungus samples 100 mg of mycelium material (wet weight) were ground to fine powder in pre- cooled mortar and pestle in liquid nitrogen. Powdered mycelium was quickly transferred to 1.5 mL microcentrifuge tubes containing 1 ml pre-heated at 65°C CTAB (Appendix -V). The tubes were then placed into

a TurboMix™ adapter (Scientific Industries, INC.) attachment for a Vortex Genie 2 (Fisher Bioblock Scientific) and homogenized for 10 min at maximum speed. Then, the tubes were centrifuged for 10 min at 11,000 g. After centrifugation, the supernatants were decanted into new tubes and the extraction procedure was repeated. An equal volume of phenol: chloroform:isoamyl alcohol (25:24:1) (Amresco) was added to each sample; the samples were then vortexed briefly, and centrifuged for 5 min in a microcentrifuge. The supernatant aqueous layer, containing nucleic acids was pipetted out into fresh centrifuge tubes. Nucleic acids were precipitated by addition of 0.6 volume of chilled isopropanol and letting it stand for a few hours at 4°C. To aid the precipitation, the tubes were spun at 10°C for 10 minutes at 10,000 rpm. The resulting supernatant was discarded and DNA pellet was given two 70% ethanol washes, dried and dissolved in 1 ml TE buffer (10:1)

3.2.3 DNA Purification

The dissolved DNA was transferred to 2ml eppendorf tubes and incubated for 1 hour with 150 micro litre stock. RNase (50 ng/ml) at 37°C and extracted subsequently with an equal volume of phenol by spinning in a micro centrifuge at 12,000 rpm for 15 minutes at 25°C. This step was repeated with phenol: chloroform (1:1 mixture) and chloroform: isoamyl alcohol (24:1 mixture). RNA- free, purified DNA was precipitated by addition 0.1 volume sodium acetate and 0.5 volumes chilled absolute ethanol to the aqueous layer and then pelleted by spinning at 12,000 rpm for 15 minutes. The precipitate was

washed twice with 70% ethanol, air-dried and dissolved in TE buffer (APENDIX –V).

3.2.4 DNA Quantification

DNA concentration was determined by using Nano photometer at 260 nm. The ratio of 1.7 to 1.9 at 260 and 280 nm was used.

3.2.5 DNA Dilutions

Part of each DNA sample was diluted with sterilized distilled water to yield a working concentration of 50ng/μl. The diluted samples were stored at 4°C for immediate use while the original stock was kept for long term storage at –20°C.

3.2.6 Randomly Amplified Polymorphic DNA (RAPD)

The genomic DNA amplified using random primers of OPE, OPF, and OPG series (Operon Tech., California, USA). The PCR reactions for RAPD were carried out in a 25 μl of reaction mixture as described by William *et al.* (1990).

3.2.6.1 Optimisation of conditions

PCR conditions were standardized for each individual primer combination by varying concentration of MgCl₂, dNTP and template DNA along with, optimisation of annealing temperatures. The concentrations of different components of PCR master mixture given

in Table 3.3 and cycle conditions in Table 3.4 under which clear, consistent bands of expected sizes were observed.

PCR amplified products were run in 2.5% agarose gels. For this 6.25g agarose was added to 250.0 ml of 0.50 X TBE buffer. The contents were mixed thoroughly and boiled for 3 minutes to dissolve the contents. The mixture was cooled to 45° C. Ethidium bromide 3µl per 100 ml gel was added to the molten gel. Molten gel was cast into a gel tray with appropriate comb. Polymerisation of the gel was allowed for 30 minutes after which the combs were taken out carefully. The gel was transferred to an electrophoresis tank having an appropriate quantity of 0.5X TBE buffer.

Table 3.3 Concentration of different components of master mixture for different primers for RAPD amplification.

Name of RAPD Primer	Mgcl₂ (mM)	Buffer (10X) (µl)	Primer (10pmol) (µl)	dNTPs 10mM (µl)	DNA (ng)	Taq DNA Pol. (U)
OPE-3	2.50	2.5	1.0	0.25	20.0	0.5
OPE-10	2.50	2.5	2.0	0.25	20.0	0.5
OPF-9	3.00	2.5	1.0	0.25	20.0	0.5
OPG-5	2.50	2.5	1.0	0.25	25.0	0.5

3.2.7.2 Electrophoresis

Loading dye (2µl) was added to each PCR tube containing amplified products and loaded in the slots of the agarose gel. Gene Ruler™ (3.0 µl) 100bp and 50bp was loaded in the first and last well respectively, of each gel to determine the size of amplified bands. Electrophoresis was carried out at 4.0V /cm for 2.5 hours.

Photography

The separated bands were seen under UV light and photographed by Gel documentation system and analyzed by Gene tool.

Table 3.4 : Table showing steps of PCR amplification for 4 primer pair

Primer No.	Initial denaturation (1)	Denatura-tion (2)	Anne-aling (3)	Extension (4)	Final Extension (5)	Remarks
OPE-3 OPE-10 OPF-9 OPG-5	94° for 4 min	94 ° for 45 sec	37 ° for 1 min	72 ° for 75sec	72 ° for 7 min	Steps 2 to 4 repeatition 30cycles
OPE-3 OPE-10	94° for 2 min	94 ° for 1 min	37 ° for 1 min	72 ° for 75sec.	72 ° for 7 min	Steps 2 to 4 repeatition 40cycles
OPF-9 OPG-5	94° for 2 min	94 ° for 1 min	38 ° for 1 min	72 ° for 1 75 sec.	72 ° for 7 min	40 cycles

3.2.8 Scoring & Data Analysis

Scoring was done manually for each marker. Allele sizes were determined based on the position of the bands relative to the ladder. Comparison of each profile for each primer was done on the basis of the presence versus absence (1/0) of RAPD products of the same length. Bands of the same length were scored as identical. Analyses were based on the simple matching index (Sokal and Michener, 1958), which measures the proportion of common data (either 0 or 1) between the isolates of scoring from gel and photograph of amplified product.

In every accession the different size of bands are consider as marker and scored across all 9 *L. theobromae* accession.

A dendrogram was derived from the distance matrix by the UPGMA (Sneath and Sokal, 1973) contained in the software package NTSYS 2.02i (Rohlf, 1997).

3.4 Biochemical studies of rotted banana fruit.

Biochemical studies were carried out to observe the post harvest changes in inoculated and uninoculated banana fruit with *L. theobromae*.

Pulp with pericarp samples from healthy and rotted fruits were collected at three ripening stages: green mature fruit, semi-ripe, and ripe fruit with and without inoculated at respective stages and changes in biochemicals were studied after 24, 48 and 72 hrs. The samples were either used immediately or kept frozen in liquid nitrogen and stored at -20 °C until further use for analysis of

Metabolites constituents viz., total phenol content, total sugar and defense related enzymes such as Phenylalanine ammonia lyase (PAL), Polyphenol oxidase (PPO) and peroxidase assay in relation to disease development.

3.4.1 Biochemical constituents

3.4.1.1. Total Soluble Sugar content

Homogenised pulp and pericarp (100 mg) were extracted with 5 ml of 80% ethanol and centrifuged at 3000 rpm for 10 minutes. Extraction was repeated 3 times with 80% ethanol and supernatants were collected into 25 ml volumetric flasks. Final volume of the extract was made to 25 ml with 80 % ethanol. The extract (0.3 ml) was pipetted from each treatment into separate test tubes and the tubes were placed in a boiling water bath for 3 minutes to evaporate the ethanol. One ml of MillQ water and 4 ml of 0.2% anthrone reagent (200 mg in 100 ml H₂SO₄) was added in each test tube and placed in ice cold water. Reagent blank was prepared by adding 1 ml of distilled water and 4 ml of anthrone reagent. The intensity of colour was read at 600 nm on spectrophotometer. A standard curve was prepared using 10 mg glucose per 100 ml distilled water (Franscistt *et al.*, 1971).

Total soluble sugar (mg/g) = Sample O.D. x Standard O.D. x Dilution factor

3.4.1.2 Extraction and estimation of Phenolic Compounds

One gram of fruit tissue homogenized with a pre-chilled mortar and pestle in 10 ml of 80 per cent methanol and the extracts left for 24 hr at room temperature before centrifuge at 15,000 rpm for 10 minutes (Swain and Hillis, 1959). One ml methanolic extract was added to 5 ml of distilled water and 250 μ L of Foline-ciocalteu reagent and the solution was kept at 25⁰C for 3 min. Then 1ml of a saturated solution of Na₂Co₃ (20per cent solution of Na₂Co₃) and 1 ml of distilled water were added and the mixture were incubated for 1hr. at 25⁰C. The absorption of the developed blue color was measured using spectrophotometer at 725nm of single wavelength. The total phenol content was calculated by comparison with a standard curve obtained from using pyrocatachol ranging between 0-25 μ g concentration.

The amount of phenols present in the sample was calculated as :

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

3.4.2 Defense related enzymes

3.4.2.1 Phenylalanine ammonia lyase (PAL)

Three hundred milligram of fruit tissue homogenized with a pre-chilled mortar and pestle in 3 ml of extraction buffer containing 50 mM borare-HCL buffer (pH 8.5) and 0.04 per cent β -mercaptoethanol. The homogenate was centrifuge at 10,000 rpm for 15 min. at 4⁰C. The

clear supernatant was used as the enzyme source for the assay of Phenylalanine ammonia lyase (Mahadevan and Sridhar, 1996).

The reaction mixture containing 3.0 ml of 0.1 M sodium borate buffer (pH 8.8), 0.5 ml of 0.1 M phenylalanine (dissolved in 0.1 M sodium borate buffer, pH 8.8). The reaction was initiated by the addition of 0.1 ml enzyme extract. The tubes were incubated at 37⁰C for 2 hr. The blank was also set with substrate (containing 3.0 ml of 0.1 M sodium borate buffer, pH 8.8 and 0.5 ml of 0.1 M phenylalanine). The O.D. read at 290 nm after 2 hr. The enzyme activity was expressed as U/hr/g protein.

3.4.2.2 Polyphenol oxidase (PPO)

Three hundred milligram of fruit tissue were grounded with a pre-chilled mortar and pestle in 3 ml of 0.1 M sodium phosphate buffer, pH 6.0. The homogenate was centrifuge at 10,000 rpm for 15 min. at 4⁰C. The clear supernatant was used for enzyme assay.

The reaction mixture contained 2.9 ml of catechol (0.01M catechol in 10mM phosphate buffer, pH 6.0) and reaction was initiated by the addition of 0.1 ml enzyme extract. The changes in the colour due to the oxidized catechol was read at 490 nm for one minute at an interval of 15 seconds. The enzyme activity was expressed as change in O.D./min/g protein (Malik and Singh, 1980).

3.4.2.3 Assay of peroxidase (POX)

The banana fruit tissue samples (1 g) were homogenized with a pre-chilled mortar and pestle in 2 ml of 0.1M phosphate buffer, pH7.0 at 4°C. The homogenates were centrifuged at 12,000 rpm at 4°C for 15 min. and the supernatant were used as enzyme source. The reaction mixture consisted 2 ml of 50 mM phosphate buffer (pH7.0) and 0.1mM EDTA, 10mM guaiacol 450µl, 10Mm H₂O₂ 450µl and 100 µl enzyme extract. POX activity was determined in the homogenates by measuring the changes in absorbance at 420 nm due to the formation of tetraguaiacol ($\epsilon = 26.6 \text{ mM}^{-1} \text{ cm}^{-1}$) in a reaction mixture were recorded at 15 seconds intervals for 1 minute. The enzyme activity was expressed as changes in the absorbance of the reaction mixture $\text{min}^{-1}\text{g}^{-1}$ on fresh weight basis(Hammerschmidt *et al.*, 1982).

3.5 Histopathological study of banana fruits infected by *L. theobromae*

Banana fruits (var., Grand Naine) after blooming unripe and green were allowed to ripe naturally and after attaining a greenish yellow to yellow colour were inoculated *in situ* with seven days old pure culture of *L. theobromae* by pin-pricking method and fruits were kept for symptom development. The inoculated banana fruits were kept at saturated relative humidity at 22-25°C for 48 hrs. Then Cross section was cut from infected as well as healthy fruit following microtomy. The sections were stained by HCL- Giemsa and mounted in DPX (Appendix- VI). The

slides were observed under light microscope for presence or absence of of fungal structures in banana pulp at 100 × magnification.

3.6 Disease management strategies

The development of new technologies for the control of banana fruit rot diseases, must take into account the changing face of the fruit markets. With reduced returns becoming an unfortunate reality for many producers in the present time, disease management strategies must be able to demonstrate a favourable cost-benefit relationship. The trend towards large scale production also needs to be taken into account, as this may impact on the application of disease management strategies.

3.6.1 Chemical control of banana fruit rot disease caused by *L. theobromae*

Seven different systemic, non-systemic and combination product (systemic + non systemic) fungicides were tested against *Lasiodiplodia theobromae* under *in vitro* and *in vivo* conditions.

3.6.1.1 *In vitro* efficacy of fungicides against *L. theobromae*

The objectives of the present studies were, to determine the infection levels of *L. theobromae* associated with different fruit rot in South Gujarat, characterization of isolates of ecological proximity or distant origins, their virulence and determine minimum inhibitory

concentration (MIC) values of different fungicides to find out the most suitable for field applications. The results of the present work will be helpful in managing the disease under field conditions in future.

Seven different fungicides were bioassayed against fruit rot pathogen *in vitro* by poisoned food technique (Nene and Thapliya, 1993). The fungicides with their respective concentrations are used as mentioned in Table 3.5

The measured quantities of fungicides were incorporated in the melted sterilized PDA medium aseptically to

Table-3.5 : The list of fungicide with their respective concentration

Sr. No.	Common name	Trade name	Chemical name	Concentration		
				I	II	III
1	Mancozeb	Dithane M-45 75 WP	Zinc ethylene bis + manganese ethylene bisdithiocarbamate	1500	2000	2500
2	Copper oxychloride	Fytolan 50 WP	Basic cupric chloride	1500	2000	2500
3	Chlorothalonil	Kavach 75 WP	Tetrachloro isophthalonitrite	1500	2000	2500
4	Carbendazim	Bavistin 50 WP	2-methoxy carbamoyl (1-benzimidazole)	250	500	1000
5	carbendazim + mancozeb (12 % + 63 %)	Sixer 75 WP (combination product)	carbendazim + mancozeb	250	500	1000
6	Propiconazole	Tilt 25 % EC	1-[(2-(2,4-dichlorophenyl)-4-propyl-1, 3-dioxolan-2-yl) methyl] – 1H-1, 2, 4-triazole	250	500	1000
7	Hexaconazole	Contaf 5 % EC	2,4-dichloro phenyl-1(1h-1, 2, 4-triazole-1-yl) hexane 2-D	250	500	1000

obtain desired concentration (minimum inhibitory concentrations, MICs; 250 to 2500 ppm) of different fungicides at the time of pouring into borosil glass Petri plates (Ø 90 mm). The 60 ml medium with fungicide was shaken well to give uniform dispersal of fungicides. Then the 20 ml medium with fungicides were poured in each of the Petri plates. After solidification, 5 mm discs of 7 days old culture of *L.theobromae* was placed in the centre of test plates and arranged in completely randomized design with three repetition. The plates were incubated at $25 \pm 2^{\circ}\text{C}$ temperature. The plates without fungicides served as control. Data on mean colony growth (mm) were recorded after 48 and 72 hrs of inoculation. After 48 and 72 hrs of incubation, diameter of fungal growth was measured in each case, by averaging two diameter of fungal colony at right angle to one another and the per cent inhibition was calculated by using the formula given by (Bliss, 1934).

$$\text{PGI} = \frac{C - T}{C} \times 100$$

Where,

PGI = Per cent growth inhibition

C = Colony diameter in control (mm)

T = Colony diameter in treatment (mm)

3.6.1.2 Pre harvest application of fungicides:

The use of crop protection chemicals prior to harvest has been referred to as a means of reducing the pre-harvest infections that lead to post harvest deterioration.

Field experiment

The field experiments were carried out at a commercial banana field “Soil and Water Management Project”, situated at Navsari Agricultural University, Navsari, district Navsari of the Gujarat. Mancozeb, carbendazim, propiconazole, mancozeb + carbendazim (SAAF) that gave the best inhibition of *L.theobromae in vitro* were further evaluated in the field conditions. Mancozeb @ 3.33 g/litre, carbendazim @ 0.5 g/litre, propiconazole @ 1ml/litre and mancozeb + carbendazim (SAAF) @ 0.33 g/litre were dissolved in water to get a final concentration of 2500, 250, 250 and 250 ppm a.i. There were four plants per fungicidal treatment. The plants were thoroughly sprayed two times, first at bunch emergence and second spray before 15 days of harvest. Before the first spray the plants were tagged and all dead leaves were removed by cutting. Fertilizer application was done as per the recommended dose @ 200:90:200 NPK g/ plants. Irrigation was given as and when required with tube well water. Isolation from the bunches of treated and control plants was made before each spray to determine the infection on each plant. The effect of fungicides on banana bunch was also evaluated by assessing the per cent disease index before and after treatment. Per cent disease index was evaluated with the help of a model of Rose (1974). Per cent disease index (PDI) was recorded as procedure followed and mentioned earlier in point 3.1.1. Per cent disease index was evaluated before the harvesting and 10 days after 2nd spray of each fungicide.

One hand containing ten fruits were selected from each bunch brought to laboratory, kept for ripening under natural condition

at room temperature up to eating ripening stage. Untreated fruits from each bunches served as control. Each treatment was repeated four times, containing forty fruits. All treatments of the uninoculated fruits as well as untreated fruits were packed in sterilized polythene bags and stored at 25-28°C and 90-95% RH. After 12 days, PDI was calculated for all treatments as mention earlier in point 3.1.1 and the Efficacy (E) of each chemical treatment was calculated as under

$$E = \frac{\text{PDI of control fruits} - \text{PDI of treated fruits}}{\text{PDI of control fruits}} \times 100$$

Data obtained from the experiments on per cent disease index (PDI) in field and after storage of fruits under natural condition at eating stage were subjected to analysis of variance technique. Treatment means were compared by employing critical difference (CD) test at 5% probability using MSTATC statistical computer package (Michigan State University, East Lansing, MI).

3.6.3 Post-harvest application of fungicides for control of banana fruit rot disease

Chemical treatments applied to the post-harvest phase to prevent losses due to diseases are also important. They may be applied as dips, dusts, sprays and fumigants. They must only, however, be used in conformity with recognized legal standards.

Mancozeb, carbendazim, propiconazole, mancozeb + carbendazim (SAAF) that gave the best inhibition of *L.theobromae* *in vitro* were used as the post-harvest dips treatments. Mancozeb @ 3.33 g/lit., carbendazim @ 0.5 g/lit., propiconazole @ 1ml/litre and

mancozeb + carbendazim (SAAF) @ 0.33 g/lit. were dissolved in water to get a final concentration of 2500, 250, 250 and 250 ppm a.i. Banana c.v. Grand Naine. fruits were harvested at uniform maturity stage and were treated by dipping for 2 minutes in the respective fungicides solutions.

A randomized complete block design with four replicates considering one hand as one replication having 10-12 healthy fruits. The fruit samples were subject to the above treatments and placed in trays to natural ripening at ambient temperature (25-32⁰C) up to full ripening stages. Per cent disease index (PDI) was worked out as procedure followed earlier as mentioned in point 3.1.1. The Efficacy (E) of each chemical treatment was calculated as followed 3.6.2.

3.7 Bio-efficacy of phytoextracts against fruit rot pathogen *L. theobromae* under *in vitro* condition

Bioefficacy of twelve phytoextracts of different plant species having medicinal value were tested *in vitro* condition by poisoned food technique. The list of phytoextracts tested were as mentioned in table 3.6.

Table 3.6: Plants extracts tested against banana fruit rot pathogen

Sr. No.	Plant species	Botanical name	Plant part/Plant product used
1.	Acalypha	<i>Acalypha indica</i> L.	Leaves
2.	Barmasi	<i>Catharanthus roseus</i> L.	Leaves
3.	Garlic	<i>Allium sativum</i> L.	Clove
4.	Turmeric	<i>Curcuma longa</i> L.	Rhizome
5.	Lantana	<i>Lantana camara</i> L.	Leaves
6.	Neem	<i>Azadirachta indica</i> Juss.	Leaves
7.	Black Tulsi	<i>Ocimum basilicum</i> L.	Leaves
8.	Deshi babul	<i>Acacia nilotica</i> L.	Leaves
9.	Eucalyptus	<i>Eucalyptus citriodora</i> Hoch.	Leaves
10.	Aloevera	<i>Aloe barbadensis</i> L.	Leaves
11.	Cinnamon	<i>Cinnamomum zeylanicum</i> L.	Leaves
12.	Ardusi	<i>Adhatoda vasica</i> Ness	Leaves
13.	Control	-	-

Method of Extraction preparation (Shekhawat & Prasad, 1971)

The fresh and healthy leaves/ bulb were brought to laboratory and thoroughly washed with tap water and then surface sterilized with 2 per cent sodium hypochloride solution for 5 minutes followed by three washing with sterilized distilled water and kept in sterilized beaker. 100 g air dried plant part was crushed in grinder mixture (Sumeet) by adding 100 ml of distilled sterilized water. The phytoextracts thus obtained were then filtered through double layered sterilized muslin cloth and filtrate was centrifuged at 10,000 rpm for 10 minutes and the clear extract was collected in sterilized conical flask. The clear extracts were made to volume of 1: 1 (w/v). The flasks were labeled and stored in refrigerator for further use. This was considered as 100 per cent concentration for the study of efficacy of plant extract (Sinha and Saxena, 1989). Two different concentrations 5 and 10 per cent were used to study the inhibitory effect on growth by using “poisoned food technique” (Schemitz, 1930).

100 ml of PDA was taken in flasks of 250 ml capacity, plugged and sterilized by autoclaving at 121⁰C temperature (1.2 kg/cm²) for 20 minutes. After autoclaving and cooling to about 45⁰C, 5 ml and 10 ml of the respective extracts were mixed thoroughly in the flasks containing 100 ml of PDA medium. Medium without phytoextracts served as control. Twenty ml of media was poured in each sterilized Petri plate under aseptic conditions. All these poured Petri plates were replicated four times. After solidifying, the plates

were inoculated with 10 days old culture block of pathogen in the centre with the help of sterilized 4 mm cork borer and were incubated at $28 \pm 2^{\circ}\text{C}$ temperature. The observation on radial growth of pathogen was measured by averaging two diameters of colony at right angle to one another at 24 hour interval and per cent growth inhibition (PGI) of the mycelia growth was calculated by using the formula of (Vincent, 1947).

$$\text{PGI} = \frac{C - T}{C} \times 100$$

Where,

PGI = Per cent growth inhibition

C = Colony diameter in control (mm)

T = Colony diameter in treatment (mm)

3.8 Antagonistic effect of known bioagents on growth of fruit rot pathogen *L. theobromae*

The test organism (Table 4) and *L. theobromae* were grown on PDA and from 6 days old culture of respective antagonist and pathogen, 4mm diameter disc of the test organism was cut aseptically and placed at one end of the Petri plate containing 20 ml PDA. In the opposite end a similar disc of the pathogen was aseptically placed (Dual culture technique, Dennis and Webster, 1971).

Four repetition of each treatment were maintained and the plate with only pathogen served as control. The plates were incubated at $28 \pm 2^{\circ}\text{C}$ temperature and after seven days of incubation, radial

growth of test organism and *L. theobromae* was measured. Per cent growth inhibition of the pathogen in each treatment in comparison to control was calculated by the following equation (Asalmol *et al.*, 1990).

$$\text{PGI} = \frac{C - T}{C} \times 100$$

Where,

PGI = Per cent growth inhibition

C = Colony diameter in control (mm)

T = Colony diameter in treatment (mm)

Table 3.7: Antagonists tested against banana fruit rot pathogen

Treatment	Bioagents	Place of isolates
T ₁	<i>Trichoderma viride</i> Pers	IARI (New Delhi) isolates
T ₂	<i>Trichoderma harzianum</i> Rifai	Junagadh (Gujarat) isolates
T ₃	<i>Trichoderma longibrachyatum</i> Rifai	IARI(New Delhi) isolates
T ₄	<i>Bacillus subtilis</i>	Anand (Gujarat) isolates
T ₅	<i>Pseudomonas fluorescens</i>	Junagadh (Gujarat) isolates

3.9 Development of Integrated disease management (IDM) module for management of banana fruit rot diseases

The aim of this investigation was to present the latest information on the biology and management of banana fruit diseases

based on an integration of field and postharvest approaches, including cultural practices, phytochemicals, bioagents and fungicide usage.

The necessity of anti-microbial agents which are cheap, easier to prepare and that would be available for the control of post-harvest rot of banana fruits makes this research a necessity. The study therefore tries to investigate the effects of extracts from Garlic and Cinnamon on post-harvest fruit rot of banana.

Field experiment

The integrated disease management experiment were carried out at a commercial banana field of “Soil and Water Management Project”, situated at Navsari Agricultural University, Navsari, district Navsari of the Gujarat. Chemicals carbendazim and propiconazol @250ppm ,phytoextracts garlic and cinnamon @ 10 per cent and bioagents (*Bacillus subtilis* and *Pseudomonas fluorescens*,) that gave the best inhibition of *L.theobromae in vitro* were used in the field trials. carbendazim @ 0.5 g/litre, Propicanazole @ 1ml/litre, garlic and cinnamon @ 100ml/litre and bioagent 1×10^7 CFU/ml were dissolved in water to get a final concentration. Covering the bunch after emergence with blue polythene bag (50µm LLDPE) and green leaves of the same banana plant was also carried out (farmers practice).

There were five plants in each treatment. The plants were thoroughly sprayed before 15 days of harvest. Before the spray the plants were tagged and all dried leaves were removed by cutting. At the time of planting fertilizer dose was given @ 200:90:200 NPK g/

plants to all treated and control plants, plants were irrigated regularly with tube well water. Isolation from the bunches of treated and control plants were made before spray to determine the infection level on each plant. The effect of each treatment on banana bunch was also evaluated by assessing the diseases severity before and after treatment. Disease severity was evaluated with the help of a model proposed for evaluating disease severity of banana fruit rot caused by *L. theobromae*. Per cent disease index (PDI) was recorded as procedure followed in earlier point as mention in 3.1.1. The Efficacy (E) of each treatment was calculated as followed in previous point 3.6.1.2. Per cent disease index (PDI) was evaluated before the harvesting in each treatment.

One hand containing ten fruits were selected from each bunch brought to laboratory kept for ripening under natural condition at room temperature up to full ripening stages. Remaining procedure was followed as described in previous point 3.6.1.2. Per cent disease index (PDI) was worked out by following the same procedure as earlier mentioned in point no. 3.1.1 of this chapter. The Efficacy (E) of each treatment was calculated as followed the point in 3.6.1.2 of this chapter.

IV. RESULTS AND DISCUSSION

The present investigations on fruit rot of banana caused by *Lasiodiplodia theobromae* were carried out under South Gujarat conditions. The results obtained in different studies are presented and discussed as below.

4.1 Survey for occurrence of banana fruit rot disease and its correlation with weather parameters under south Gujarat conditions

The pathogens that caused post harvest spoilage were isolated and their extent of loss on banana fruits were assessed by systematic monthly surveys at Navsari and Surat districts from field to consumer level (market) were carried out during 2008 and 2009. Percent disease index (PDI) was recorded as per procedure as mention earlier in 3.1.1. Banana fruits were predominantly infected by *Lasiodiplodia theobromae* pat. and *Fusarium moniliforme* Sheld. The mixed infection of *Lasiodiplodia theobromae* Pat., *Fusarium moniliformae* Sheld, *Fusarium* sp., *Aspergillus niger* VanTiegh., *Acremonium* sp. and *Curvularia* sp. at different stages of marketing and storage was also observed.

The magnitude of loss varied from place to place due to post harvest diseases on banana variety “Grand naine” at different stages of storage and marketing. The fungal spoilage of banana fruit was higher at retail and consumer level and minimum at wholesale and field levels, since the symptoms of the disease on unripe banana fruit manifested only upon ripening.

4.1.1 Field level survey

The data of Navsari district field survey presented in Table 4.1 revealed that maximum per cent disease index (PDI) during 2008 was observed during October (2.87 %), during this month maximum temperature 34.30°C and minimum temperature 21.70°C , morning relative humidity 88.80 and 55.00 per cent and rain fall 0.20 mm followed by April (2.38%) and March (2.28%). Minimum per cent disease index observed in month of May (0.11%). During 2009, (Table 4.2) maximum per cent disease index was observed during October (2.43%), at this month max. temperature 33.90°C and min. temperature 21.50°C , morning relative humidity 85.10 and evening 47.00 per cent, rain fall 2.70 mm followed by November (2.24%) and December (2.23%). Minimum per cent disease index was observed in month of May (0.10%).

The correlation of the weather parameters with disease development led to the conclusion that per cent disease index exhibited significant weak positive correlation ($r= 0.18$) with avg. max. temperature in the year 2008 but in 2009 the correlation was highly positive significant ($r= 0.82$) and negative correlation ($r= -0.66$ and -0.74) with minimum temperature in the year 2008 and 2009 respectively. Morning relative humidity showed significant positive correlation ($r= 0.31$), evening relative humidity was highly significant positive ($r= 0.80$) in the year 2008. In the year 2009 correlation was highly significant positive ($r=0.64$ and 0.85) with morning and evening humidity respectively.

Table 4.1: Effect of weather parameters on occurrence and development of banana fruit (finger) rot disease at Navsari field during the year 2008

Sr. No.	Month	Temperature		Relative humidity (%)		Rainfall (mm)	Per cent disease index
		Max.	Min.	Morn.	Even.		
1	January	29.20	12.50	81.10	32.30	0.00	1.53
2	February	29.70	13.00	73.10	34.70	0.10	2.08
3	March	34.00	18.60	85.30	40.50	0.00	2.28
4	April	35.10	22.60	83.20	46.50	0.00	2.38
5	May	33.40	26.90	84.20	63.00	0.00	0.11
6	June	31.80	26.70	88.60	75.80	4.70	0.45
7	July	29.50	25.60	93.50	84.60	24.20	0.48
8	August	29.10	24.80	93.90	83.90	23.60	1.03
9	September	30.60	24.50	93.20	78.10	13.00	1.63
10	October	34.30	21.70	88.80	55.00	0.20	2.87
11	November	33.60	18.80	81.60	44.30	0.00	2.25
12	December	32.0	17.20	80.40	40.20	0.20	2.20
Correlation Coefficient (r)							
PDI		0.18	-0.66	0.31	0.80	0.43	<i>P= 0.05</i>

Table 4.2: Effect of weather parameters on occurrence and development of banana fruit (finger) rot disease at Navsari field during the year 2009

Sr. No.	Month	Temperature		Relative humidity (%)		Rainfall (mm)	Per cent disease index
		Max.	Min.	Mon.	Evn.		
1	January	31.30	15.60	80.50	32.70	0.00	1.42
2	February	33.00	15.00	81.80	28.60	0.00	2.05
3	March	35.70	19.60	82.10	29.80	0.00	2.13
4	April	37.70	22.90	68.70	31.90	0.00	2.18
5	May	34.20	26.40	77.20	55.40	0.00	0.10
6	June	33.20	27.50	83.80	65.40	1.40	0.25
7	July	29.50	25.30	92.00	82.60	33.90	0.38
8	August	30.70	26.00	86.50	74.00	6.30	1.01
9	September	32.40	25.30	89.00	66.40	8.00	1.52
10	October	33.90	21.50	85.10	47.00	2.70	2.43
11	November	32.20	19.50	76.40	42.90	0.70	2.24
12	December	31.20	16.30	80.00	36.40	0.00	2.23

Correlation Coefficient (r)						
PDI	0.82	-0.74	0.64	0.85	0.88	$P=0.05$

But rainfall had positive correlation ($r=0.43$) in the year 2008 and highly positive correlation ($r=0.88$) in 2009.

In case of survey of district Surat fields (Table 4.3), maximum per cent disease index was observed during October to December (2.68-2.04%), during this period max. temperature ranged from 35.40 to 32.80°C and min. temperature ranged from 18.70 to 22.50°C, morning relative humidity 82.60- 79.60 and evening 50.70 - 62.00% and rain fall 0.00 to 17.00 mm. The highest per cent disease index was observed in month of October (2.68 %) and minimum per cent disease index was observed in month of May (0.09%). During survey of the year 2009 (Table 4.4), maximum per cent disease index was observed during October to December (2.13-3.03%), at this period max. and min. temperature ranged from 32.22-34.68 to 18.92 -22.98°C respectively, morning and evening relative humidity 59.50-77.34 to 34.86 -42.68 per cent respectively and rain fall 0.00-0.79 mm. The highest per cent disease index was observed in month of November (3.03 %) and minimum in month of May (0.10%).

The correlation of the individual parameters with disease development led to the conclusion that per cent disease index exhibited significant weak positive correlation ($r=0.12$) with avg. max. Temperature in the year 2008 but in 2009 the correlation was positive highly significant ($r= 0.85$) and negative correlation ($r= -0.59$ and -

0.74) with minimum temperature in the year 2008 and 2009 respectively. Morning relative humidity showed significant positive correlation ($r = 0.47$) but evening RH was

Table 4.3: Effect of weather parameters on occurrence and development of banana fruit (finger) rot disease at Surat field during the year 2008

Sr. No.	Month	Temperature		Relative humidity (%)		Rainfall (mm)	Per cent disease index
		Max.	Min.	Morn.	Even.		
1	January	29.60	14.30	78.30	45.30	0.30	1.10
2	February	29.70	16.00	74.80	33.70	0.00	1.00
3	March	34.00	19.60	72.40	28.50	0.00	0.87
4	April	36.50	24.80	70.60	26.50	0.00	0.14
5	May	34.70	27.60	70.80	34.30	0.00	0.09
6	June	32.70	26.80	84.60	70.30	13.30	0.21
7	July	30.90	26.20	88.6	69.50	11.50	0.78
8	August	30.50	25.60	83.20	72.90	27.40	1.23
9	September	31.60	25.30	81.60	72.40	7.15	1.65
10	October	35.40	22.50	82.60	62.00	17.00	2.68
11	November	34.10	20.20	80.80	52.30	0.20	2.33
12	December	32.80	18.70	79.60	50.70	0.00	2.04

Correlation Coefficient (r)							
	PDI	0.12	-0.59	0.47	0.70	0.48	$P=0.05$

Table 4.4: Effect of weather parameters on occurrence and development of banana fruit (finger) rot disease at Surat field during 2009

Sr. No.	Month	Temperature		Relative humidity (%)		Rainfall (mm)	Per cent disease index
		Max.	Min.	Morn.	Even.		
1	January	31.56	17.63	62.30	35.46	0.00	1.12
2	February	33.48	19.30	55.28	37.98	0.00	1.05
3	March	34.22	21.60	53.20	34.68	0.00	1.43
4	April	39.08	25.40	57.40	28.20	0.00	0.18
5	May	32.73	27.43	74.00	51.25	0.00	0.10
6	June	35.05	28.18	71.75	53.00	6.60	0.25
7	July	30.90	25.82	88.40	79.4	7.17	0.68
8	August	32.03	26.60	80.75	68.75	10.00	1.42
9	September	32.88	26.34	82.25	66.00	5.01	1.93
10	October	34.68	22.98	77.34	42.68	0.79	2.88
11	November	32.90	20.92	67.63	45.55	0.00	3.03
12	December	32.22	18.92	59.50	34.86	0.00	2.13
Correlation Coefficient (r)							
PDI		0.85	-0.74	0.54	0.74	0.65	<i>P=0.05</i>

highly significant positive with evening ($r = 0.70$) during 2008. In the year 2009 significant positive correlation with morning ($r=0.54$) and evening humidity (0.74). Avg. max. temperature had non significant positive correlations ($r = 0.31$). Rainfall had positive correlation with disease development in the year 2008 and 2009.

The average results for year 2008 and 2009 presented in Table 4.5 and fig.1. Indicated that at Navsari the finger rot per cent disease index was higher in the month of November (2.65%) followed by April (2.28%) and November (2.24%) and minimum in the month of May (0.10%) in both the years. At Surat higher per cent disease index was observed in the month of October (2.78%) followed by November (2.68%) and December (2.09%) and minimum in the month of May (0.09%). These results more or less collaborated with earlier worker of Goos *et al.* (1961) who found association of *Botryodiplodia theobromae* (Pat.) with finger rot disease which occurred at 25-30°C temperature.

4.1.2 Consumer level/ Retail level Survey

The extent of loss due to different diseases at consumer level is presented in Table 4.6, indicated that during the year 2008 in Navsari market, maximum per cent disease index was observed during July to November (10.2 to 18.2%), during this period max. temperature ranged from 29.50- 34.30°C and min. temperature 18.80- 25.60°C, morning and evening relative humidity 93.90-81.60 and

84.60-44.30 per cent respectively and rain fall 24.20 to 0.00 mm. The highest per cent disease index was observed in the

Table 4.5: Mean occurrence of banana fruit (Finger) rot disease at Navsari and Surat field during 2008 and 2009

Sr. No.	Month	Per cent disease index					
		Navsari			Surat		
		2008	2009	Pooled	2009	2008	Pooled
1	January	1.53	1.42	1.47	1.10	1.12	1.11
2	February	2.08	2.05	2.07	1.00	1.05	1.03
3	March	2.28	2.13	2.20	0.87	1.43	1.15
4	April	2.38	2.18	2.28	0.14	0.18	0.16
5	May	0.11	0.10	0.10	0.09	0.10	0.09
6	June	0.45	0.25	0.35	0.21	0.25	0.23
7	July	0.48	0.38	0.43	0.78	0.68	0.73
8	August	1.03	1.01	1.02	1.23	1.42	1.33
9	September	1.63	1.52	1.58	1.65	1.93	1.79
10	October	2.87	2.43	2.65	2.68	2.88	2.78
11	November	2.25	2.24	2.24	2.33	3.03	2.68
12	December	2.20	2.23	2.22	2.04	2.13	2.09
Correlation Coefficient (r)							
Per cent disease index (PDI)		Max. temp.	Min. temp.	RH. Morn.	RH. Evn.	Rainfall	P= 0.05
2008		0.53	-0.63	0.33	0.65	-0.45	

2009	0.31	-0.60	0.34	0.43	0.46	
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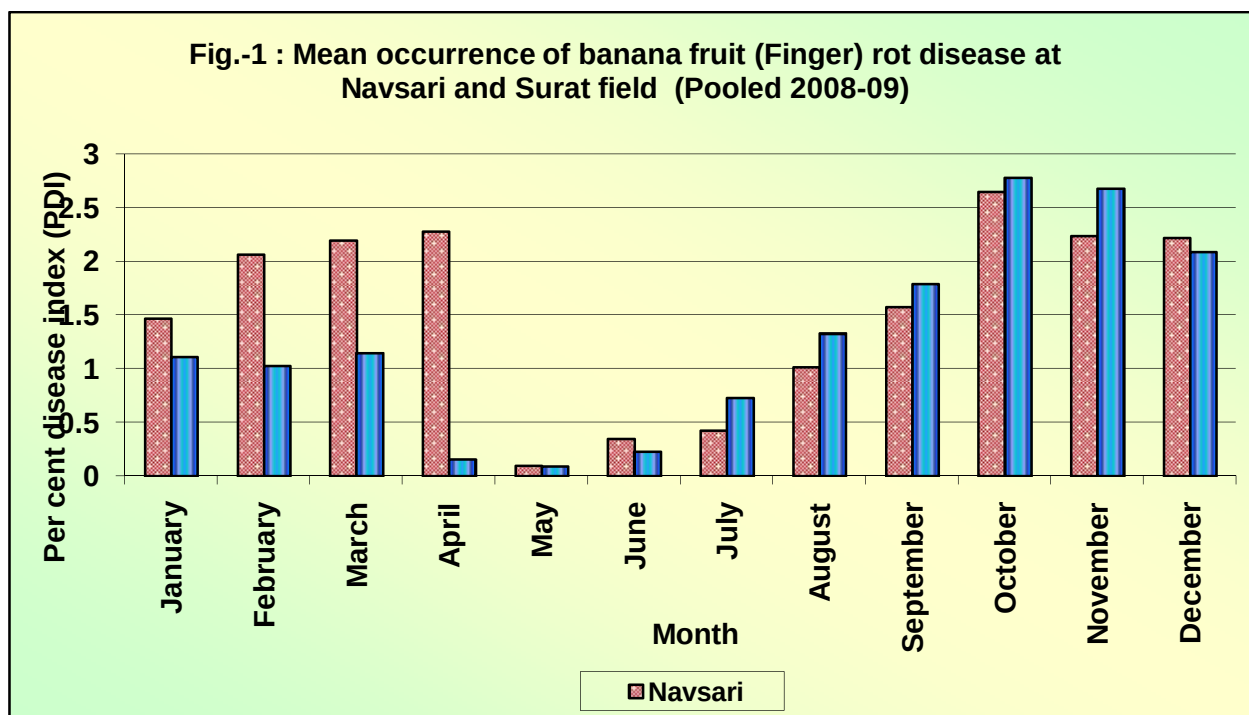


Table 4.6: Effect of weather parameters on occurrence and development of banana fruit rot disease at Navsari market during 2008

Sr. No.	Month	Temperature		Relative humidity (%)		Rainfall (mm)	Per cent disease index
		Max.	Min.	Morn.	Even.		
1	January	29.20	12.50	81.10	32.30	0.00	4.4
2	February	29.70	13.00	73.10	34.70	0.10	3.6
3	March	34.00	18.60	85.30	40.50	0.00	5.6

4	April	35.10	22.60	83.20	46.50	0.00	7.0
5	May	33.40	26.90	84.20	63.00	0.00	8.8
6	June	31.80	26.70	88.60	75.80	4.70	9.6
7	July	29.50	25.60	93.50	84.60	24.20	10.2
8	August	29.10	24.80	93.90	83.90	23.60	13.4
9	September	30.60	24.50	93.20	78.10	13.00	18.2
10	October	34.30	21.70	88.80	55.00	0.20	17.4
11	November	33.60	18.80	81.60	44.30	0.00	11.2
12	December	32.00	17.20	80.40	40.20	0.20	6.4
Correlation Coefficient (r)							
PDI		-0.02	0.49	0.51	0.59*	0.47	<i>P=0.05</i>

month of September (18.2 %) and minimum in the month of February (3.6%). During the year 2009, (Table 4.7) maximum per cent disease index was observed during August to December (10.2-15.6%) in this period max. and min. temperature ranged from 30.70-33.90 and 16.30 -26.00⁰C respectively, morning and evening relative humidity 89.00-76.40 to 74.00 -36.40 per cent respectively, and rain fall 6.30-0.00mm. The highest per cent disease index was observed in the month

of September (15.6 per cent) and minimum in the month of March (4.6%).

The correlation of the individual parameters with disease development led to the conclusion that per cent disease index exhibited negative correlation ($r = -0.02$) with avg. max. temperature in the year 2008. In the year 2009 the correlation was positive ($r = 0.36$). Morning relative humidity showed significant correlation ($r = 0.51$) and it was significant with evening RH ($r = 0.59$) but in case rainfall had positive correlation ($r = 0.47$). During the year 2009 Av. max. temperature had significant positive correlations ($r = 0.36$), morning and evening relative humidity had positive correlation ($r = 0.51$ and 0.59) in the year 2008, and in 2009 correlation was also positive ($r = 0.17$ and 0.58) in both the years.

In case of survey of Surat market (Table 4.8), maximum per cent disease index was observed in the month of September to November (14.4-12.6%), during this period max. and min. temperature ranged from 31.60-35.40 to 20.20-25.30°C respectively, morning and evening relative humidity 80.80-82.60

Table 4.7: Effect of weather parameters on occurrence and development of banana fruit rot disease at Navsari market during 2009

Sr. No.	Month	Temperature		Relative humidity (%)		Rainfall (mm)	Per cent disease index
		Max.	Min.	Morn.	Even.		
1	January	31.30	15.60	80.50	32.70	0.00	9.8

2	February	33.00	15.00	81.80	28.60	0.00	6.4
3	March	35.70	19.60	82.10	29.80	0.00	4.6
4	April	37.70	22.90	68.70	31.90	0.00	5.4
5	May	34.20	26.40	77.20	55.40	0.00	6.6
6	June	33.20	27.50	83.80	65.40	1.40	7.0
7	July	29.50	25.30	92.00	82.60	33.90	7.4
8	August	30.70	26.00	86.50	74.00	6.30	10.2
9	September	32.40	25.30	89.00	66.40	8.00	15.6
10	October	33.90	21.50	85.10	47.00	2.70	15.0
11	November	32.20	19.50	76.40	42.90	0.70	13.8
12	December	31.20	16.30	80.00	36.40	0.00	13.2
Correlation Coefficient (r)							
PDI		0.36	0.10	0.17	0.58*	0.08	<i>P=0.05</i>

Table 4.8: Effect of weather parameters on occurrence and development of banana fruit rot disease at Surat market during 2008

Sr. No.	Month	Temperature		Relative humidity (%)		Rainfall (mm)	Per cent disease index
		Max.	Min.	Morn.	Even.		
1	January	29.60	14.30	78.30	45.30	0.30	5.6
2	February	29.70	16.00	74.80	33.70	0.00	4.6
3	March	34.00	19.60	72.40	28.50	0.00	5.4
4	April	36.50	24.80	70.60	26.50	0.00	6.4
5	May	34.70	27.60	70.80	34.30	0.00	7.0
6	June	32.70	26.80	84.60	70.30	13.30	7.8
7	July	30.90	26.20	88.60	69.50	11.50	8.0
8	August	30.50	25.60	83.20	72.90	27.40	9.8
9	September	31.60	25.30	81.60	72.40	7.15	14.4
10	October	35.40	22.50	82.60	62.00	17.00	13.8
11	November	34.10	20.20	80.80	52.30	0.20	12.6
12	December	32.80	18.70	79.60	50.70	0.00	7.2
Correlation Coefficient (r)							

PDI	0.12	0.65*	0.70*	0.55	0.64*	<i>P=0.05</i>
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to 52.30 -72.40 per cent respectively and rain fall 0.20 to 7.15 mm. The highest per cent disease index was observed in the month of September (14.4%) and minimum in the month of February (4.6%). During in the year 2009 (Table 4.9), maximum per cent disease index was observed in the month of August to November (10.6-13.4%), during this period max. and min. temperature ranged from 32.03-34.68 to 20.92 -26.34⁰C respectively, morning and evening relative humidity 82.25-67.63 to 42.68 -68.75 per cent respectively and rain fall 0.00-10.00 mm. The highest per cent disease index was observed in month of September (13.4 %) and minimum in the month of February (3.6 %).

The correlation of the weather parameters with disease development led to the conclusion that per cent disease index exhibited significant weak positive correlation ($r=0.12$) with avg. maximum temperature in the year 2008. In the year 2009 the correlation was significant positive ($r= 0.53$) and it was highly significant ($r= 0.65$) with min. temperature in the year 2008 and weak positive correlation ($r=0.20$) in the year 2009. Morning and evening relative humidity showed significant positive correlation ($r= 0.70$) ($r= 0.55$) in the year 2008 and in the year 2009 (Table 4.9) significant positive correlation ($r=0.07$ and 0.08) with morn. and even. RH. Rainfall had slight positive correlation in both the years.

The average results of the year 2008 and 2009 presented in Table 4.10 and fig.2 revealed that post harvest rot was higher in the month of September (16.9%) followed by October (16.2%) and November (12.5%) and minimum in the month of February (5.0%) at Navsari market in both the years. In surat market higher per cent disease index was in month of September (13.9%) followed by October (13.2%) and November 12.1%) and minimum in the month of February (4.1%).

Table 4.9: Effect of weather parameters on occurrence and development of banana fruit rot disease at Surat market during 2009

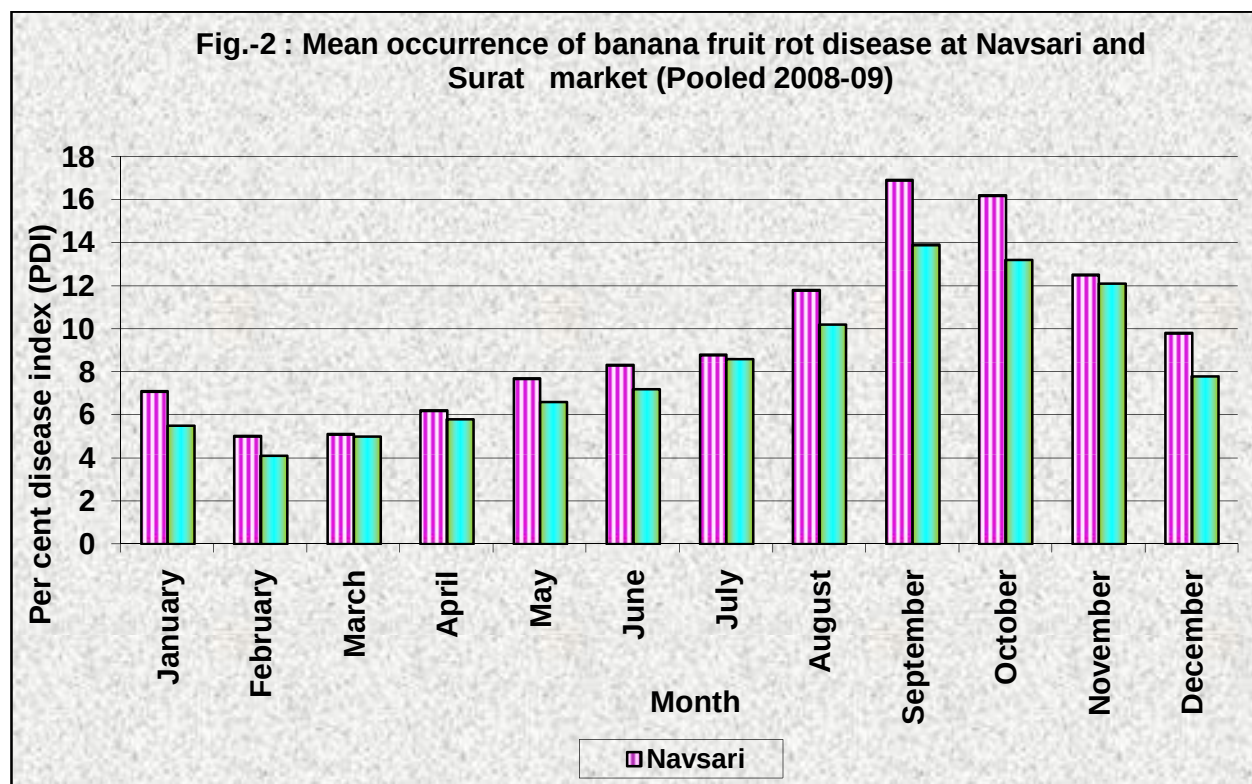
Sr. No.	Month	Temperature		Relative humidity (%)		Rainfall (mm)	Per cent disease index
		Max.	Min.	Morn.	Even.		
1	January	31.56	17.63	62.30	35.46	0.00	5.4
2	February	33.48	19.30	55.28	37.98	0.00	3.6
3	March	34.22	21.60	53.20	34.68	0.00	4.6
4	April	39.08	25.40	57.40	28.2	0.00	5.2
5	May	32.73	27.43	74.00	51.25	0.00	6.2
6	June	35.05	28.18	71.75	53.00	6.60	6.6
7	July	30.90	25.82	88.40	79.40	7.17	9.2
8	August	32.03	26.60	80.75	68.75	10.00	10.6
9	September	32.88	26.34	82.25	66.00	5.01	13.4

10	October	34.68	22.98	77.34	42.68	0.79	12.6
11	November	32.90	20.92	67.63	45.55	0.00	11.6
12	December	32.22	18.92	59.50	34.86	0.00	8.4
Correlation Coefficient (r)							
PDI		0.53	0.20	0.07	0.08	0.19	<i>P=0.05</i>

Table 4.10: Mean occurrence of banana fruit rot disease at Navsari and Surat market during 2008 and 2009

Sr. No.	Month	Per cent disease index					
		Navsari			Surat		
		2008	2009	Pooled	2009	2008	Pooled
1	January	4.4	9.8	7.1	5.6	5.4	5.5
2	February	3.6	6.4	5.0	4.6	3.6	4.1
3	March	5.6	4.6	5.1	5.4	4.6	5.0
4	April	7.0	5.4	6.2	6.4	5.2	5.8
5	May	8.8	6.6	7.7	7.0	6.2	6.6
6	June	9.6	7.0	8.3	7.8	6.6	7.2
7	July	10.2	7.4	8.8	8.0	9.2	8.6
8	August	13.4	10.2	11.8	9.8	10.6	10.2
9	September	18.2	15.6	16.9	14.4	13.4	13.9
10	October	17.4	15.0	16.2	13.8	12.6	13.2
11	November	11.2	13.8	12.5	12.6	11.6	12.1
12	December	6.4	13.2	9.8	7.2	8.4	7.8
Correlation Coefficient (r)							
Per cent disease index (PDI)		Max. temp.	Min. temp.	RH. Morn.	RH. Evn.	Rainfall (mm)	P= 0.05
2008		-0.15	0.30	0.51*	0.42*	0.22	

2009	-0.04	0.29	0.56*	0.59*	0.23	
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The correlation of the weather parameters with disease development led to the conclusion that per cent disease index exhibited negative correlation with avg. maximum temperature and significant positive with minimum temperature in both the years. Morning and evening relative humidity showed highly significant positive correlation and Rainfall had slight positive correlation in both the years.

This results collaborated with earlier workers namely Ved Ram and Dharm Vir (1986) observed that none of the pathogens showed their activity at low temperature ($10^{\circ}\text{C}.$), but storage temperature enhanced to 25°C an increase in activity of various pathogens resulted in higher percentage of infection due to *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Curvularia lunata* and *Colletotrichum musae*. Relative humidity indicated that increase in RH from 40 to 70

per cent, there was corresponding increase in the per cent rot, hundred per cent infection was observed due to *A. niger*, *A. flavus* and *C. lunata* at this RH. Complete decay of banana fruits occurred at 100% RH due to all these pathogens. Williamson and Tondon (1996) reported maximum rotting of banana fruits due to *Botryodiplodia theobromae* at 25⁰C. Bagwan and Meshram (2003) observed highest banana fruit rot severity at 25⁰C temperature due to *Fusarium solani* (Martius) and at 30⁰C temperature, when fruits were stored at 100 per cent RH. There was no disease symptom on fruits kept at 10⁰C temperature. The severity of storage rots due to these three pathogens decreased with increase in storage temperature up to 35⁰C. Cherian and Mani Varghese (2007) recorded the highest severity of *Fusarium semitectum* in the fruits kept at 30⁰C temperature with 100 per cent relative humidity. Disease was not produced at 10⁰C temperature. Injury of the banana fruits caused more infection together with higher temperature to gather with relative humidity.

The present results showed that the highest per cent disease index was observed in month of October to December and minimum in the month of May. The highest per cent disease index was observed in the month of October in both years and in case of field survey at Surat district field.

Maximum per cent disease index was observed in the month of August to November, but it was highest in the month of September in both the years at both places at market level survey (consumer level). Disease development was positively correlated with temperature, relative humidity and rainfall.

Looking to the average per cent disease index of the fruit rots in markets and field level survey, maximum per cent disease index was due to *L. theobromae*, both the year and surveyed places, *L. theobromae* rot ranked first followed by *F. moniliformae*. Their damage was high at consumer level as compared to fields, hence the further work on different aspects of the fruit rot of banana caused by *L. theobroma* Pat. was carried out.

4.2 *Isolation, identification and pathogenicity of microorganisms associated with banana fruit rot disease*

4.2.1 **Isolation**

Diseased samples of banana fruit (cv. Grand naine) with pulp rot, crown rot, tip end rot, red spots, peeling injury/bruising and finger rot were collected from fruit markets, fruit stalls and domestic store-rooms of Navsari, Gandevi, Surat, Bardoli and Vyara and from banana field of Soil and Water Management Project, Department of plant pathology and Gandevi farm and brought to laboratory and were subjected to tissue isolation. The fungus growth on fruit surface was directly transferred to PDA. Total six fungal pathogens were found associated with rotting and differentiated on the basis of cultural characteristics. Two cultures were isolated by direct fungus growth transfer to potato dextrose agar medium which was commonly found in tissue isolation also. These cultures were further purified by hyphal tip method and were maintained on PDA slants for further studies. The periodical sub-culturing and multiplication were done on the PDA to keep cultures fresh and were used throughout the investigations

4.2.2 Pathogenicity

The fruit rot pathogens were able to infect the inoculated banana fruits (cv. Grand naine) by Pin- Pricking method as mentioned in "Materials and Methods" and symptoms were developed on fruits (plate-II). The re-isolation was carried out from these inoculated fruits, which yielded the same pathogens matched with the original isolates.

4.2.3 Identification of pathogens

Cultural and morphological characters of isolated fungus were studied on PDA medium.

These cultures were also sent to Agharkar Research Institute, Pune for identifications and confirmation. The isolates were identified as *Lasiodiplodia theobromae* Pat., *Fusarium moniliformae* Sheld, *Fusarium* sp., *Aspergillus niger*, *Acremonium* sp. and *Curvularia* sp. (Plate-III).

The *Lasiodiplodia theobromae* Pat. is more prevalent pathogen was isolated and purified for study purposes, artificially inoculated on healthy banana (var. Grand naine) fruits and produced similar symptoms of fruit rot those found in naturally infected fruits. Reisolations from infected inoculated fruits yielded cultures which were identical with the cultures which are used for inoculation of the fruits.

Morphological characters of *L. theobromae*

Colony:

The fungus initially produced cottony white fluffy growth on potato dextrose agar, which covered the Petridish (90 mm) within 3 to 4 days at room temperature. The growth was depressed in the centre (around the point of inoculation) and fluffy, raised in the rest of the area, which touched to lid of the dish. After 2 days, colour of the colony changed from white cottony to olivaceous dark. Under the lower surface of the dish, complete blackish background was observed due to pigmentation by the fungus in the medium.

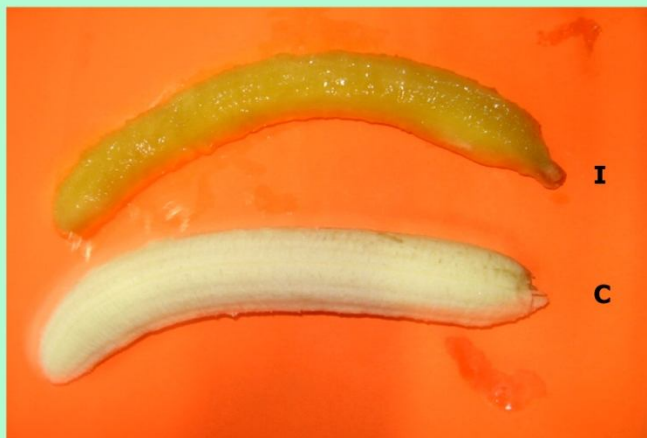
Plate-II (A & B) : Pathogenicity test



A = Banana pulp rot symptom by *L. theobromae*

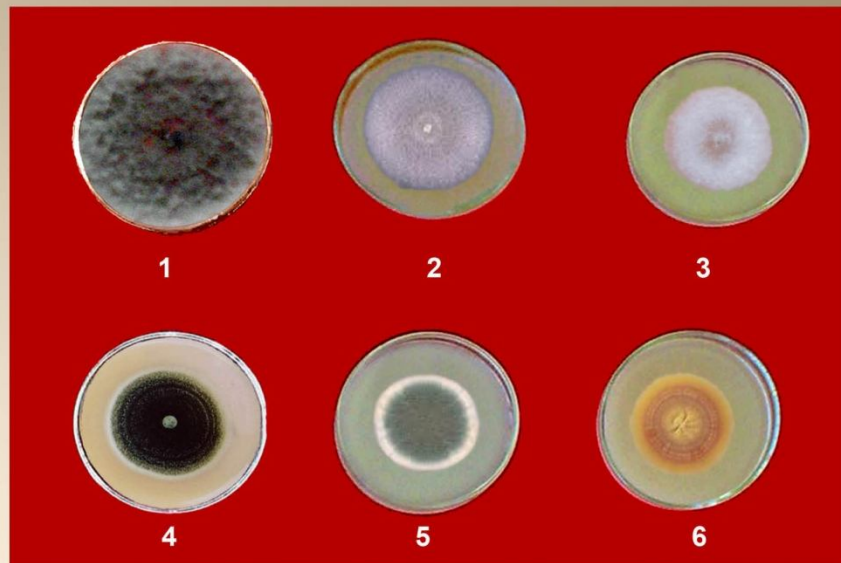
I = Inoculated

C = Control



B = Banana pulp rot symptom by *F. moniliforme*

Plate-III : Pure culture of fungi associated with banana fruit rot



1. *L. theobromae*
2. *F. moniliforme*
3. *Fusarium* sp.
4. *A. niger*
5. *Acremonium* sp.
6. *Curvularia* sp.



Plate-IV : Micro photograph of *L. theobromae* showing mycelium and mature conidia

Mycelium

Young hyphae were hyaline, aseptate, which turned to septate and dark brown on maturity on potato dextrose agar within 3 to 4 days of inoculation (plate-IVA).

Pycnidial bodies

Seven days after growth, few black pycnidial bodies were observed clearly on the surface, while from the lower surface, it became visible by holding the dish against the light. After ten days, fruiting bodies were clustered and produced stromata. Pycnidia were brown to black, sub-globose to globose, erumpent and ostiolated. Pycnidial bodies measured 216.62 X 209.89 μm size.

Pycnidiospores

Initially pycnidiospores oozed out from pycnidia were immature, hyaline, thin walled, globose to subglobose and single celled measured 22.49 X 13.69 μm . Mature spores became bi-celled, dark brown, thick walled, dolliform to shortly cylindrical. The mature pycnidiospores were 20.11 X 13.65 μm in size at 100x magnification (plate- VIII A&B).

Microphotography of the pycnidia and pycnidiospores from various parts viz. naturally infected and inoculated banana fruits, was done and same are shown in plate- IXB.

On the basis of morphological characters, the fungus was identified as *Lasiodiplodia theobromae* Pat. All the morphological characters matched the characters of *Lasiodiplodia theobromae* Pat. described by various workers (banana and other isolates). Ribaldi (1959) provisionally named the organism as *Botryodiplodia medicaginis* which was originally known as *Diplodia natalensis* of (lucern isolate). Siradhana and Jain (1962) isolated *Botryodiplodia theobromae* from rotted papaya fruit and observed that young mycelium was hyaline, profusely branched, sparsely septate, 2.0-4.8 μm in diameter. At later stage, the mycelium turned greenish to brown and ultimately black with close septation. The pycnidia on the host were black, ostiolate, stromatic, erumpent and confluent measuring 87.7-300.8 μm in diameter. Griffiths (1967) was of the opinion that *Botryodiplodia theobromae* is a synonym of *Diplodia natalensis*.

Alasoadura (1970) carried out studied on *Botryodiplodia theobromae* and reported that stroma formed earlier when the mycelium injured by cutting, while Patel (1971) observed that pycnidia were formed in the group of 2-6. Barnett and Hunder (1972) described the asexual fruiting bodies measuring 120-155 x 370-465 µm in size. Pycnidia aggregated in botyose clusters which produced thin walled, single celled and hyaline immature conidia. Later it turned into 2-celled dark brown, dolliform to shortly cylindrical mature conidia (Ainsworth *et al.*, 1973). Olunloyo and Esuruoso (1975) isolated pathogen from cashew, named it as *Lasiodiplodia (Botryodiplodia) theobromae* and reported that it is the imperfect stage of *Botryodiplodia rhodina*.

Botryodiplodia theobromae is an important and ubiquitous fungus mostly in the tropics and sub tropics. Because of its association with numerous diseases and its wide spread distribution, both geographically and on numerous host plants, the fungus is referred in the literature by a multiplicity of names (Punithalingam, 1976). *Botryodiplodia theobromae* is pleomorphic and member of the sphaeropsidales with immersed to erumpent or superficial, pilose or glabrous, simple or often aggregated pycnidia reaching upto 5 mm width, with or without stroma. The pycnidia were usually papillate with prominent ostiole. Conidia are initially hyaline, unicellular, ellipsoid to oblong, thick walled with granular contents. On maturity the conidia became 2-celled, dark brown, usually 20-30 X 10-15 µm, with differentially pigmented longitudinal bands resembling striation (Punithalingam, 1980). Sabalpara (1983) measured length and breadth of pycnidia and pycnidiospores of *Botryodiplodia theobromae*

(average 291.41 x 232.03 and 19.05 x 10.86 µm, respectively) from different sources. Kivati (1984) reported drought wilt of coconut palm incited by *Botryodiplodia theobromae* produced buff white mycelial mat, numerous pycnidia, two types of conidia, immature conidia, hyaline and aseptate. While mature conidia bi-celled, dark brown and thick walled with longitudinal striation. Verma and Cheema (1984) isolated *Botryodiplodia theobromae* from die-back of pear and described that cottony white mycelium turning sooty black, producing abundant carbonous pycnidia in and on the culture medium (PDA). The pycnidia were ovoid to elongate and dark brown one septate conidia measuring 21.6-25.2 x 10.0-12.5 µm. Ekundayo and Haskins (1989) reported that pycnidia of *Botryodiplodia theobromae* originated from a network of vegetative hyphae. Ram (1993) isolated *B. theobromae* from eight hosts and observed that spore size was similar in all isolates, but spores were smaller in isolates from mango. Vegetative growth rate, mycelial dry weight and sporulation were lower in coconut isolate. Cedeno *et al.* (1995) confirmed that *Lasiodiplodia theobromae* was the cause of passion fruit vine die-back and studied the cultural characters. The conidia were initially hyaline, unicellular and had thick cell walls, but later became dark brown pigmented, developed one septum, showed longitudinal striation, measuring 23.4-26.1 x 12.3-3.9 µm.

The identification of fungus (*Lasiodiplodia theobromae* (Pat.) Griff. & Maubl.) has also been confirmed by mycologist, Agharkar Research Institute. Pune (No.3/426-2008)

Rotting symptoms

The fungal rotting agent that infested the banana fruits were composed mainly of *Lasiodiplodia theobromae* Pat., *Fusarium moniliforme* Sheld, *Fusarium* sp., *Aspergillus niger*, *Acremonium* sp. and *Curvularia* sp. as well as fungi that did not sporulate and therefore were not identified (plate-III).

Dynamics in symptom development by fungal Species

Finger rot by *L. theobromae*

Mature unripe healthy banana fruits were inoculated with seven days old culture of *L. theobromae* and left for symptom development. Symptoms usually begin at the lower-end of the finger at a inoculated site. The decay spreaded uniformly causing brownish-black discoloration of the peel and softening of the pulp. The affected area of the peel becomes wrinkled and encrusted with pycnidia. The pulp was reduced to a soft, rotten mass and a dark-gray mold grew on the peel surface under high humidity. The rate of disease development increased with maturity and spreaded to adjacent fingers. Infected clusters tend to ripe prematurely and fully mature fruits are most susceptible to infection. The infection occurred through tissues at the flower-end of the fingers and wounds created by the insects. The disease developed slowly below 20°C and low humidity and maximum rotting occurred at 24-28°C with high humidity(> 80%). Thus, results of our study corroborate with the results reported by Goos *et al.* (1961). They found association of *Botryodiplodia theobromae* (Pat.) with finger rot disease and reported that infection occurs through

tissues at the flower-end of the fingers and wounds. Slabaugh (1994a) found that finger rot disease caused by *Botryodiplodia theobromae* has been reported from Central and South America, the Caribbean Islands, India, Taiwan, and the Philippines. Symptoms produced at the lower-end of the finger or at a wound site resulted in to brownish- black discoloration of the peel and a softening of the pulp and peel becomes wrinkled. Our findings are in agrément with the above these workers.

Ripening rot by *Lasiodiplodia theobromae*

Mature semi ripe healty banana fruits were inoculated with seven days old culture of *L. theobromae* and incubated at room température for symptom development. Banana rotting began at the second day of incubation. The disease menifested as brownish and water –soaked discoloration (plate-II). It proceeded irregularly on either end; diseased fruits assumed a dark brown colour. In general, the rotting of the pulp is comparatively faster than that of the rind. Under moist conditions white to light gray cottony mycelium covered the infected tissue. Pycnidiospore formation takes place rather late. These results are more or less similar with earlier workers reported by Srivastava and Tondon (1971) observed that *Botryodiplodia theobromae* Pat., produced brownish and water soaked discolouration leading to rotting of the pulp under moist conditions. Later on, white to light gray cottony mycelium covered the infected tissue. Wade *et al.* (1993) isolated fungi from diseased fingers, crowns and main-stalk viz. *colletotrichum musae*, *Fusarium moniliforme* var. *subglutinans* and *Nattrassia mangiferae* and caused high infection incidence in unripe fingers during storage. *Lasiodiplodia theobromae* and

phomopsis musicola infected only ripening fingers, and *F.culmorum* infect only crowns and main-stalk. Storage disease of fruits became more severe when treated with ethylene and new disease arising from pre-storage inoculation appeared, such as *L. theobromae* rot of fingers and *F.pallidoroseum* rot of crown.

Plate-VI (A & B) : Symptoms development by *L. theobromae*



A = Green fruits inoculated



A = Ripe fruits inoculated

Fusarial rot

Banana fruits rotting Started from second day of incubation, water soaked brown discoloration appeared later on turn to dark brown pulp rotted and in high humidity condition rotting was faster. Results of our study corroborate with earlier worker viz., Dharm Vir (1977) who observed brown discoloration extending from top to bottom of the finger inside the pulp of the banana fruit, the advanced infected tissue turns soft and pulpy. Isolation made from the diseased tissue yielded *Fusarium moniliforme* Sheld. Marin *et al.* (1996) isolated five fungi viz , *Fusarium semitectum*, *F. moniliforme*, *Penicillium sp.*, *Gliocladium roseum* and *Gliocladium sp.* associated with crown rot of banana fruits which matched with our study. Prasad and Singh (2002) observed hollow fruits of banana (*Musa paradisiacal*) cv. Singapuri, were infected by *Fusarium moniliforme* (*Gibberella moniliformis*). Symptom development differed in present results with study of Prasad and Singh (2002) as where we did not found hollowness in fruits.

Importance of the fungal pathogens in banana fruit rotting

Fruit incubated at room temperature allowed to infect post harvest banana pathogens. The percentage fruit colonization as well as the prevalence of individual fungal species was determined by using method of Muhammad *et.al.* (2004). Results are presented in Table 4.11A. Crown rot disease was the major post harvest disease caused by fungal pathogens viz ; *L.theobromae*, *F. moniliforme* and *A. niger* frequency of occurrence was 72 per cent. followed by ripe rot due to *L.theobromae* and *F. moniliforme* with 10 per cent occurrence and

blossom end rot due to *Fusarium* sp. and *Acremonium* sp. were found during 2008 and 2009 in Navsari and Surat market.

The number of occurrence for each of the isolates in ten different samples were recorded and calculated as a ratio of occurrence and was expressed in per cent by using the formula mentioned in 3.2.4. Crown rot was found to be caused by a complex of fungal pathogens viz., *Lasiodiplodia theobromae*, *Fusarium moniliforme*, *Fusarium* sp., *Aspergillus niger* and fruit rot was due to *Lasiodiplodia theobromae* and *Fusarium moniliforme*. From these two diseases *Lasiodiplodia theobromae* was the most frequently (43 per cent) isolated in both diseases followed by *Fusarium moniliforme* (21.5 per cent) and *Acremonium* sp. occurred at very little frequency (3.25 per cent) which associated with blossom end rot disease from Navsari and Surat markets (Table 4.11,B). It was found to develop rapidly during fruit ripening thereby reducing the quality and marketability of banana fruits. Our results corroborate with Cordeiro and Matos (2005), who have recorded the same pre-harvest and post-harvest banana pathogens.

Fruits inoculated with isolated fungus and incubated at room temperature allowed to express the post harvest banana rot

Table 4.11 A : Occurrence of disease and disorders of banana fruits during market surveys of Navsari and Surat districts during the year 2008 and 2009

Disease/ Disorders	(%) Frequency of occurrence avg. of two years	Microorganisms isolated
Crown rot	72	<i>Lasiodiplodia theobromae</i> , <i>Fusarium moniliformae</i> , <i>Fusarium</i> <i>sp.</i> , <i>Aspergillus niger</i>
Ripe rot	10	<i>Lasiodiplodia theobromae</i> , <i>Fusarium moniliformae</i> ,
Blossom end rot	4	<i>Fusarium sp.</i> , <i>Acremonium sp.</i>
Red spots	24	<i>Curvularia sp.</i>
Peelinjury/bruising	8	<i>Fusarium sp</i>

All fruit assessments were made at the “eating ripe” stage.

Table 4.11B: Frequency of fungal pathogens associated with banana fruit rot collected from Navsari and Surat market during the year 2008 and 2009

Microorganisms	Frequency (%)*				Average
	2008		2009		
	Navsari	Surat	Navsari	Surat	
<i>Lasiodiplodia theobromae</i>	48	42	43	39	43.00
<i>Fusarium moniliformae</i>	18	24	21	23	21.50
<i>Fusarium sp.,</i>	10	12	13	13	12.00
<i>Aspergillus niger</i>	6	8	4	7	6.25
<i>Curvularia sp.</i>	7	7	9	8	7.75
<i>Acremonium sp.</i>	3	5	2	3	3.25

*Isolated from ten infected fruit collected from markets

symptoms. The percent fruit colonized as well as the prevalence of individual fungal species was determined.

The result presented in Table-4.11C, shows that severe crown rot, light pulp rot and moderate brown symptom on or near to crown skin were developed when *L. theobromae* was inoculated in crown portion, but when it was inoculated in fruit pulp showed severe fast pulp rotting with brownish, water-soaked discoloration and fruits were completely rotted within 4-5 days after inoculation at room temperature at >80 per cent RH. *F. moniliformae* showed moderate crown rot and pulp rot but when it was inoculated in crown portion not showed pulp rotting. *Aspergillus niger* caused late rot when fruits were over ripened. *Curvularia* sp. produced symptom when fruit fully ripened or at over ripened stage, it caused infection up to pericarp only, but not caused pulp rot. *Acremonium* sp. produced internal rotting. The present study also found that when green mature fruits inoculated with pathogen did not show any diseased symptoms up to 5 days of storage.

4.3 Molecular characterization of *L. theobromae* using molecular markers

Intact high molecular weight *Lasioidiplodia* genomic DNA is essential for successful RAPD analysis. Earlier methods of DNA isolation involving sodium dodecyl sulphate and various enzymes were not only costlier but also cumbersome time consuming and yielded less plant and fungal DNA. Saghai-Marouf *et al.* 1984 used the detergent CTAB (hexadecyl trimethyl

Table 4.11 C : Symptoms development on banana fruits inoculated with isolated pathogens

Pathogen	Inoculated area	Symptoms produced on banana fruits			
		Crown rotting	Internal rotting (Pulp)	Brown/red spot on skin	Remarks
<i>Lasiodiplodia theobromae</i>	Crown	3	1	2	Brown discoloration developed fast
	Fruit	1	3	3	Pulp rotted very fast with watery exudates
<i>Fusarium moniliforme</i>	Crown	2	0	1	Moderate brown discoloration with mycelium on crown
	Fruit	2	2	1	Pulp rotted at moderate rate
<i>Fusarium sp.</i> ,	Crown	2	1	1	Moderate brown discoloration with mycelium development
	Fruit	1	2	2	Pulp rotted with moderate rate
<i>Aspergillus niger</i>	Crown	0	1	1	light rotting symptoms developed
	Fruit	0	2	1	Slow pulp rotting
<i>Curvularia sp.</i>	Crown	0	0	0	No symptoms developed
	Fruit	0	0	3	Pinkish red spot developed on fruit skin, never pulp rotted
<i>Acremonium sp.</i>	Crown	1	0	0	Initial internal rotting occurred at very slow rate
	Fruit	1	1	0	Internal rotting occurred from tip at slow rate

0= No. symptoms, 1= very light, 2= moderate, 3= sever

ammonium bromide), to eliminate polysaccharides generally known to effect the quality and thus digestibility of DNA.

The DNA isolated using the CTAB method from all the isolates of *Lasiodiplodia theobromae* has occupied a position in the gel corresponding to λ DNA (50kb) indicating high molecular weight DNA. The DNA bands were observed to be sharp, no or less streaking is suggestive of negligible DNA shearing (plate VII A)

The absorbance were recorded at 260nm (A_{260}) and 280nm (A_{280}) after diluting the DNA 300 times in TE buffer. The final concentration of DNA was kept 50 ng/ μ l for RAPD analysis. Absorbance at 260nm has been reported to be equivalent to 50 μ g of DNA/ml. A ratio of A_{260}/A_{280} between 1.8-2.0 was considered to be indicative of pure DNA. Most of DNA has got a ratio between 1.8-2.0, hence the DNA isolated was pure enough to be use for RAPD analysis (Table 4.12).

Total thirty random decamer primers belonging to OPE, OPF and OPG series obtained from IDT were used for initial screening. Only four primers i.e. OPE-3, OPE- 10, OPF-9 and OPG-5 gave good amplification and polymorphism and also generated significant information. Most of the primers produced fragments below 850 bp. The pattern of amplification has been found to be isolates and primer dependent. Not only the size of fragments were variable but number of bands generated by different primers were also much variable (PlateVII B&C).

RAPD analysis using 4 decamer primers generated a total of 122 bands, all are polymorphic. The number of amplified products varied between 4 and 7 and the size of the products ranged from 320 – 850 kb (Table 4.13).

Table 4.12. DNA yield based on absorbance (260nm) obtained from various isolates of *Lasiodiplodia theobromae*

Sr. No.	Isolates	OD at 260 nm	OD at 280 nm	Ratio of A260/A280	Conc. of DNA µg/nl
1	BIN	0.065	0.032	2.0	0.97
2	MIN	0.063	0.033	1.9	0.94
3	MIJ1	0.037	0.020	1.8	0.56
4	MIJ2	0.019	0.010	1.9	0.29
5	MIT	0.042	0.022	1.9	0.63
6	BIB1	0.022	0.011	2.0	0.33
7	BIB2	0.017	0.009	1.9	0.26
8	MIB	0.02	0.010	2.0	0.35
9	RID	0.014	0.008	1.8	0.30

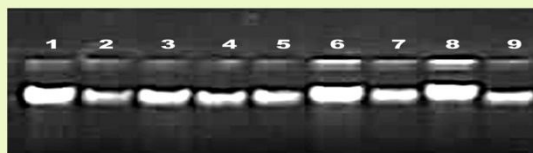
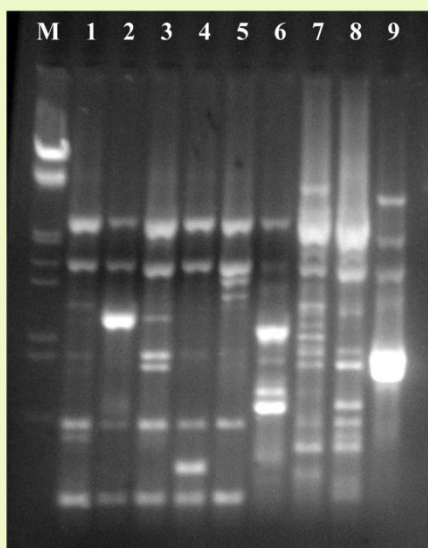
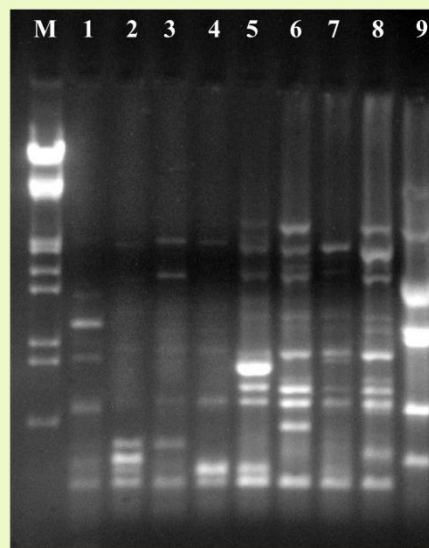


Plate-VII (A) : Genomic DNA of different isolate of *Lasiodiplodia theobromae*



OPE-3



OPE-10

Plate-VII (B) : RAPD patterns generated by OPE-3 and OPE-10 in different isolates of *Lasiodiplodia theobromae*

M - DNA molecular marker

1 - BIN

2 - MIN

3 - MIJ1

4 - MIJ2

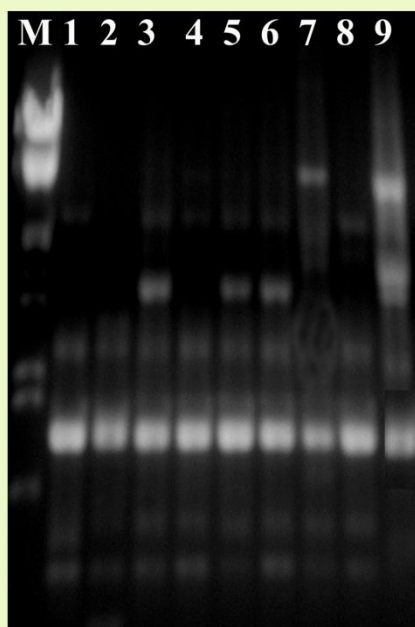
5 - MIT

6 - BIB1

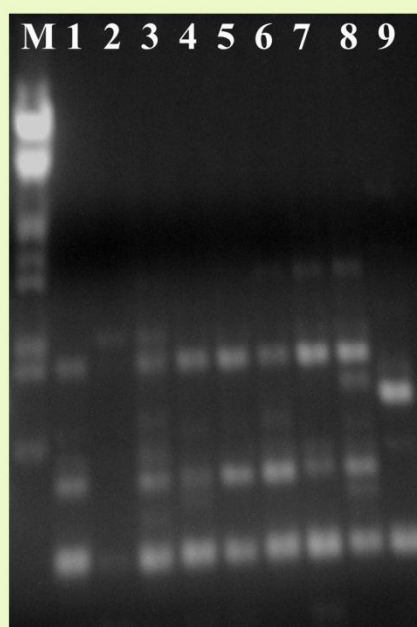
7 - BIB2

8 - MIB

9 - RID



OPE-9



OPG-5

**Plate-VII (C) : RAPD patterns generated by OPE-3 and OPE-10
in different isolates of *Lasiodiplodia theobromae***

M - DNA molecular marker

- 1 - BIN**
- 2 - MIN**
- 3 - MIJ1**
- 4 - MIJ2**
- 5 - MIT**
- 6 - BIB1**
- 7 - BIB2**
- 8 - MIB**
- 9 - RID**

The RAPD profiles showed the variable banding pattern of four primers and clustered the isolates by UPGMA analysis (Fig. 3). The all isolates form two major clusters (A, and B). Cluster-A isolates (BIN,MIN,MIJ1,MIT,MIJ2,BIB1,BIB2 and MIB) of *Lasiodiplodia* spp. caused rot in banana as well as mango fruits isolated and collected from the Gujarat (South Gujarat and Saurashtra) and Bangalore (Karnataka) Regions. Cluster- B isolate (RID) caused die beck disease in rose collected from North Gujarat (Dantiwada) region. The cluster-A further divided in to two cluster (I and II) and four isolates i.e. BIN,MIN,MIJ1 and MIT were laid on first sub- cluster of cluster-I , these were isolated from Gujarat regions, while MIJ2 placed in second sub cluster of cluster- II and showed maximum diversity among Gujarat isolates it might be due to old culture (isolated in the year 2006) and periodical sub culturing up to 2010 so that fungus may change their genetic variability as well as pathogenic variability. Isolates MIJ1 and MIT showed maximum similarity (73%) because they were isolated from same host, almost similar region and same year. Isolate BIN and MIN showed more similarity (64.2%), both were isolated from different host but same region. In cluster-II, three isolates (BIB1, BIB2 and MIB) were found on two sub-cluster. Isolate BIB1 and BIB2 was closely similar because they were isolated from same region and same host. The Dendrogram constructed on the basis of RAPD patterns showed that isolate RID was different from all other isolates and recorded highest genetic distance from all other isolates.

The isolates were first separated into two clusters based on the similarity value, lowest similarity(16.6%) was observed between MIB and RID. Moreover, RID exhibited maximum 33.3% similarity with all other isolates and shows more diversification among the isolates collected from different parts of India as well as from different host (Table 4.14)

Furthermore, group- A consisted of more diverse isolates based on RAPD and showed more broader range of pathogenicity compared to group- B, least pathogenic culture of Dantiwada (RID). Isolate RID did not cluster with any of the isolates and remained independent in dendrogram at a much higher level of diversity. In our results BIN and BIB1 isolate causing banana fruit rot showed only 40 % similarity but it was higher than MIB isolate (Mango fruit rot), which might be due to different geographic region.

Similarly, Saharan *et al.* (2007) analysed 15 isolates of *Fusarium graminearum* collected from Punjab, Tamil Nadu and Himachal Pradesh and found that Punjab isolates of *F. graminearum* from Gurdaspur (G 31) and Ludhiana (L23) were found genetically most similar (91.38%), whereas Wellington isolates of *F. graminearum* (W 5 and W 7) were found genetically most dissimilar (14.92%). Cluster analysis of band-sharing coefficients separated isolates of *F. graminearum* into four clusters. Lahaul valley isolates of *F. graminearum* (D 3, D 4 and D 5)

Table 4.13: List of primer used in the study along with their sequences and some characteristics of the amplification products in *Lasiodiplodia* isolates

Sl. No.	Primer code	Total No. of PCR Products	Polymorphism (%)
1	OPE-3	42	100.0
2	OPE- 10	35	100.0
3	OPF-9	26	100.0
4	OPG-5	19	100.0

TABLE- 4.14 : Jaccard's similarity coefficient between the *Lasiodiplodia* spp. based on the RAPD data

	BIN	MIJ2	MIN	MIJ1	MIT	BIB1	MIB	BIB2	RID
BIN	1.000								
MIJ2	0.357	1.000							
MIN	0.642	0.428	1.000						
MIJ1	0.642	0.428	0.600	1.000					

MIT	0.562	0.375	0.625	0.733	1.000				
BIB1	0.400	0.250	0.380	0.450	0.550	1.000			
MIB	0.227	0.142	0.217	0.333	0.304	0.320	1.000		
BIB2	0.291	0.217	0.391	0.391	0.416	0.541	0.500	1.000	
RID	0.210	0.176	0.333	0.200	0.238	0.208	0.166	0.280	1.000

grouped together (Group I), while *F. graminearum* isolates of Punjab(G 31 and L 23) formed a separate group (Group II). *F. graminearum* isolates of Wellington, viz. W 1, W 2, W 5 and W 3, W 4, W 6, W 8 clustered in groups III and IV respectively.

Different workers have been grouped different fungal population in different regions by using RAPD analysis (Pavlic *et al.*, 2004.; Melania *et al.*,2006, Singh *et al.*, 2006, Choudary *et al.*,2007, Alves *et al.*,2008, Huang *et al.*,2009).

4.4 Biochemical studies of rotted banana fruit

In this study, the changes in the levels of total phenol and total sugar as well as the activities of Phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD) enzymes during banana fruit rot infection and ripening were determined.

The results of present investigation showed that there was appreciable changes in the total soluble sugar and total phenol contents as well as in the activities of PAL, PPO and POD enzymes. Especially, the increase in sugar content and PPO and POD activities

may reduce the astringent taste of the banana fruits in the ripening stage.

4.4.1 Total soluble sugar

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

The perusal of data presented in Table 4.15(b), showed that the total soluble sugar varied in uninoculated and inoculated banana fruits with *L. theobromae* at green mature, semi ripe and ripe stages. Total soluble sugar content was higher (85.84 mg/g fresh weight) in ripe fruits while lowest (2.01 mg/g fresh weight) in green mature fruits at initial time. Total sugar content was increased in the beginning of the fruit ripening with increase of storage period. It was higher (211.57 mg/g fresh weight) in ripe uninoculated fruits and still further decreases (203.99 mg/g fresh weight) in inoculated fruits and lowest in uninoculated green mature fruits after 72hrs of storage. A nine-fold increase in total soluble sugar content was observed in green mature inoculated fruits (18.28 mg/g fresh weight) followed by green mature uninoculated fruits (178.54 mg/g fresh weight) and semi ripe fruits (166.14 mg/g fresh weight) after 72hrs of storage. The total soluble sugar content was increased with ripening in inoculated as well as uninoculated banana fruits, but it was lower in semi ripe and ripe inoculated fruits than uninoculated fruits after 48 and 72 hrs of incubation. These results are corroborated with earlier worker of Terra *et al.* (1983), they found that unripe fruits of banana and mangoes

have a relatively higher starch content which is almost completely hydrolysed during ripening to simple sugars, glucose, fructose and sucrose (Biale,1960). Similarl results indicated that decreasing of total soluble sugar content in inoculated fruits related with disease development because it utilized by the pathogen as food for their growth and development(Biale, 1964, Lizana, 1976, John *et. al.*,1981, Kadioglu and Yavru, 1998).

4.4.2 Total phenols

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

Total phenol contents was measured in banana tissue at three maturity stages i.e., green mature, semi ripe and ripe fruits in uninoculated and inoculated with *L. theobromae* after 0,48 and 72 hrs of storage. The results presented (Table 4.15a) that phenol content was higher in green mature fruits (14.80 mg/g fruit weight) than semi ripe (13.38 mg/g fruit weight) and ripe fruits (10.98 mg/g fruit weight) at initial time. The phenol content was decreased with increasing storage period in uninoculated and further decreased in inoculated in all three stages. However, total phenol content was decreased as the fruits ripened, and in rotted fruits decrease further. Our results are consistence with earlier worker of Janave (2008). He found that as the mango fruits ripened, the initial total phenolics content of unripe non-spongy fruits decreased by about 30 per cent.

4.4.3 Phenylalanine ammonia lyase (PAL) activity

The results demonstrated (Table 4.15c) that enzyme activity was higher in green mature inoculated fruits (75.33 Units $\text{h}^{-1} \text{g}^{-1}$ protein) than uninoculated (59.33 Units $\text{h}^{-1} \text{g}^{-1}$ protein) which was decreased in ripe inoculated fruits (9.11 Units $\text{h}^{-1} \text{g}^{-1}$ protein) and uninoculated (3.56 Units $\text{h}^{-1} \text{g}^{-1}$ protein) after 72hrs of storage. These results suggest that constitutive level of PAL was higher in green mature fruits then decreased with the ripening stages. It was first decrease then increase in inoculated green mature fruits. The PAL activity was increased after 24 and 72 hrs storage in green mature and semi-ripe fruits irrespective of infection, while decreased in ripe fruits as compared to 0 hrs. Moreover, PAL activity was 2.5 fold higher in inoculated ripe fruits as compared to uninoculated ripe fruits at 72 hrs incubation. This suggested that during ripening in healthy fruits phenol synthesis is decreased.

4.4.4 Polyphenol oxidase (PPO) activity

The PPO activity was found (Table 4.15d) lower in green mature inoculated and uninoculated fruits at all storage period of determination although, the enzyme activity of uninoculated (2.4 Units $\text{min}^{-1} \text{g}^{-1}$ protein) was more or less similar to inoculated (3.08 Units $\text{min}^{-1} \text{g}^{-1}$ protein) after 72hrs of storage. However, in uninoculated semi ripe fruits PPO activity was increased about 14.72 fold after 72 hrs incubation, while in inoculated semi-ripe fruits it was higher (19.33 fold). At the 0 hrs incubation the PPO activity was 4.69 fold higher in ripe fruits as compared to semi-ripe fruits but it was increased after 72 hrs in both inoculated and uninoculated semi ripe

fruits. Our results suggests that PPO activity was increased beginning the fruit ripening and further increased with the increasing storage time but increament was more pronounced in the inoculated fruits. These results are consistant with Chakraborty and Nandi (1978), they also observed increased polyphenol oxidase in banana fruit infected by *Botryodiplodia theobromae*. Polyphenol oxidase and peroxidase activity gradually decreased during the development of the fruits followed then by an increase during the ripening period. The level of sugar gradually increased during fruit development and ripening which affects the taste of medlar fruits (Aydin and Kadioglu, 2001). These observations suggest that the increase in PPO and POD activities as well as in sugar and protein contents has an important role in reducing the astringent taste of the fruits.

4.4.5 Peroxidase (POX) activity

Peroxidase (POX) activity was found (Table 4.15e) higher in ripe fruits (49.75-121.9 $\mu\text{mol guaiacol min}^{-1} \text{g}^{-1}$ protein) than in semi ripe fruits (8.73-63.8 $\mu\text{mol guaiacol min}^{-1} \text{g}^{-1}$ protein) up to 72hrs of storage. The POX activity was enhanced after inoculation with *L.theobromae* in all ripening stages. The degree of increase in infected green mature, semi ripe and ripe was 12.33, 7.30 and 2.45 fold higher respectively than uninoculated fruits. These results are corroborated with earlier findings of Lee *et al.*(2000),they indicated the green and red pepper fruit with *Colletotrichum gloeosporioides*, the peroxidase genes were strongly activated in the green fruit by anthracnose disease but not in red fruit resistant to anthracnose disease. These results suggest that the peroxidase genes may be inducible during the

pathogenesis of the anthrachnose disease rather than in the disease resistance response. Similar results were observed by Chakraborty and Nandi (1978) in banana fruit infected by *Botryodiplodia theobromae*. Similarly, Janave (2008) observed the change in peroxidase levels in mango fruits at various stages of ripening. The development of black color in ripened fruits indicated increased activity of POX or polyphenol oxidase (PPO) enzymes. In present study reduction in PAL activity and enhancement in PPO and POX activity in ripe fruits may be correlated with reduction of phenol content in ripe fruits. Higher activity of these enzymes in inoculated fruits as compared to uninoculated fruits reflects the invasion of pathogen.

Table 4.15: Biochemical changes in banana fruits infected by *L. theobromae*

(a) Total phenol content mg/g fruit weight								
Sr. No.	Sample	0 hrs.	48 hrs. after		72 hrs. after			
		Uninoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Fold *	Fold **
1	Green mature fruits	14.802±0.26	12.028±0.72	10.05±0.53	10.065±1.08	7.358±1.19	-1.47	-2.01
2	Semi ripe fruits	13.375±1.43	11.483±1.07	9.476±0.16	10.456±0.11	7.911±0.07	-1.28	-1.69
3	Ripe fruits	10.984±0.39	9.008±0.65	8.266±0.59	8.522±0.91	7.0547±0.73	-1.29	-1.56

(b) Total sugar content mg/g fruit weight								
Sr. No	Sample	0 hrs.	48 hrs. after		72 hrs. after			
		Uninoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Fold	Fold
1	Green mature fruits	2.016±0.79	7.514±0.79	11.176±0.59	9.327±0.34	18.28±0.23	+4.62	+9.07
2	Semi ripe fruits	50.819±2.90	89.139±0.79	86.14±1.32	178.54±2.01	166.14±5.40	+3.58	+3.27
3	Ripe fruits	85.841±1.06	197.32±2.87	181.05±0.99	211.57±56.42	203.99±5.21	2.46	+2.38

(c) PAL Activity U/hr/g fruit weight								
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Sr. No	Sample	0 hrs.	48 hrs. after		72 hrs. after			
		Uninoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Fold	Fold
1	Green mature fruits	51.551±3.44	20.331±2.70	3.777±0.77	59.328±4.91	75.326±2.0	+1.15	+1.61
2	Semi ripe fruits	8.777±0.34	7.1103±1.0	12.221±1.29	65.738±6.37	50.328± 3.28	+4.81	+3.68
3	Ripe fruits	13.666±1.39	5.444±0.27	10.221±1.05	3.555±3.01	.11±1.17	+3.27	+1.04

(d) PPO Activity u/mint/g fruit weight

Sr. No	Sample	0 hrs.	48 hrs. after		72 hrs. after			
		Uninoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Fold	Fold
1	Green mature fruits	0.253±0.13	0.319±0.05	0.660±0.23	2.398 ±1.03	3.08±0.28	+9.48	+12.17
2	Semi ripe fruits	0.330±0.09	0.429±0.10	0.726±0.10	4.862±1.35	6.380±0.46	+14.73	+19.33
3	Ripe fruits	1.551±0.31	2.046±0.41	2.409±0.50	3.619±1.02	4.565±1.57	+2.33	+2.94

(e) POX Activity u/mint/g fruit weight

Sr. No	Sample	0 hrs.	48 hrs. after		72 hrs. after			
		Uninoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Fold	Fold
1	Green mature fruits	3.4176±0.23	17.468±1.73	30.759±1.87	27.341±1.34	42.151±4.84	+8.0	+12.33
2	Semiripe fruits	8.734±0.74	40.632±2.73	37.214±2.40	53.923±3.95	63.796±3.98	+6.17	+7.30
3	Ripe fruits	49.746±3.41	66.454±4.21	95.314±5.40	111.64±6.98	121.90±4.12	+2.24	+2.45

Mean of three replication

*Change over time in uninoculated

**Change over time in uninoculated on inoculated

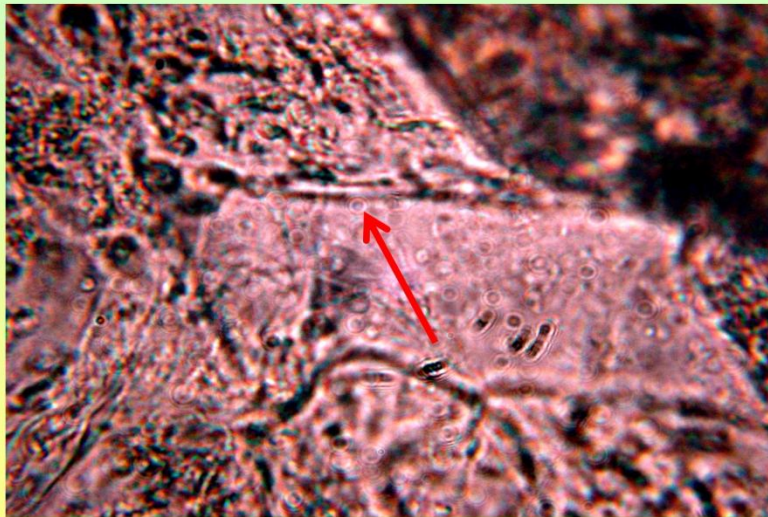
During ripening stages the level of total phenol gradually decreased in both infected and uninfected fruits, but it was more decreased in infected green mature fruits. The level of total sugar gradually increased during fruit ripening. PAL activity decreased in ripening stages but it increased in infected fruits. PPO and POD activity gradually increased in the ripening stage but degree of increase was higher in infected fruits.

4.5 Histopathological study of *L. theobromae* causing banana fruit rot

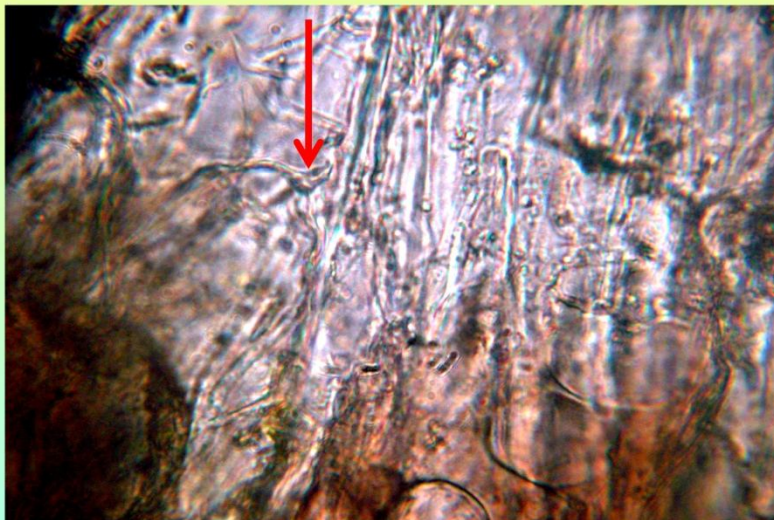
Seventy- five days old banana fruits (var., Grand Naine) were inoculated *in situ* with fungal culture of *L. theobromae* by prickle-pin method then the banana fruits were sliced from inoculated and uninoculated and prepared for microscopy, the microphotographs are present in plate VIII.

Pycnidiospores germinated 8 hr after inoculation. An ellipse-shaped appressorium was formed 20-24hr after inoculation

**Plate-V (A & B) : Histopathology of banana fruit indicating
presance of mycelium**

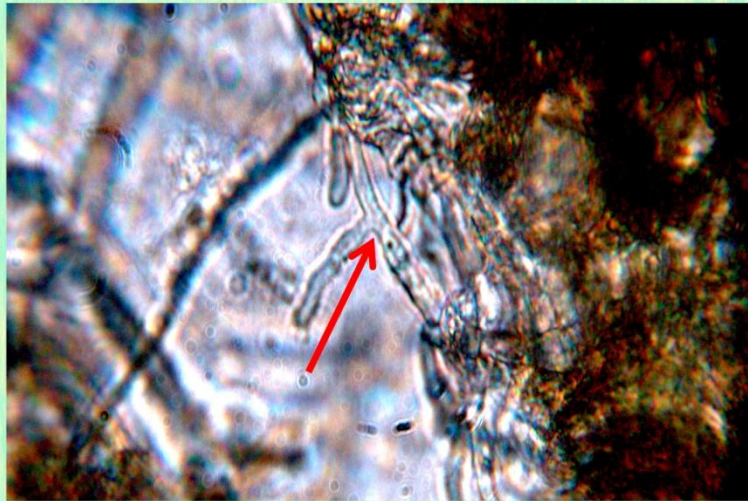


A = In green fruit (Undeveloped)



B = In ripe fruit (Fully developed)

Plate-VIII (A & B) : Microphotograph showing penetration of mycelium in the fruit pulp



A = Mycelium inside the fruit pulp (100 X magnification)



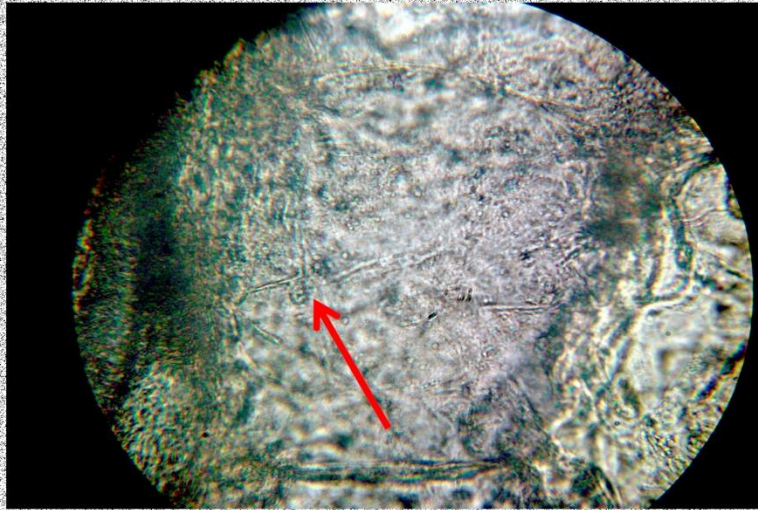
B = Formation of fruiting body like structure in pulp

B = Formation of fruiting body like structure in pulp

on unripe banana fruits but its further growth was suppressed and not colonized between or within the cells. The green fruits showed necrotic lesion with browning around the point of entry. But in case of semi-ripe and ripe fruits, fungus colonized speedily within 8-10hr and also colonized within the cells and between the cells (Plate IXA). Mycelium first aseptate hyaline branched between the cells then it enters the cell and produced pycnidia within 48hr become septate branched brownish colour and produced hyaline elliptical single celled pycnidiospores (Plate IX B). Globose Pycnidia produced after 48hrs of inoculation. From the present study it is observed that total phenol content and PAL activity was higher in green mature healthy fruits which is toxic to the pathogen and due to metabolic changes in the unripe banana fruits do not produce disease. The present investigation collaborated by earlier worker Simmonds (1965) who stated four hypothesis to explain the resistance of unripe banana fruits, shortage of nutrients was the limitations for the fungal penetration. The failure in degradation of cell wall components, the inhibition of fungal growth by toxins, and metabolic changes in the unripe banana fruits. Mulvena *et al.* (1969) isolated a fungistatic substance, 3, 4-dihydroxybenzaldehyde from the outer skin of the unripe Cavendish banana which inhibited the growth of *Colletotrichum musae*. Swinburne (1978) reported that latency may be caused by the production of antifungal compounds, phytoalexins. Chang *et al.* (1987) observed feather like deposits exclusively in the epidermal cell of the green unripe banana fruits. These deposits degraded and disappeared when the green unripe banana ripened, and the pathogen penetrated into the epidermal cells and then into the tissue. No

feathery deposits were observed on ripe banana fruits, in which the *Colletotrichum musae* could penetrate and colonized.

**Plate-IX (A & B) : Microphotograph of healthy fruit pynidiospores
in rotted pulp**



A = Microphotograph of healthy uninfected banana fruit (100 X)



**B = Microphotographs showing presence of immature pycnidiospores
in rot ted banana fruit pulp (450 X)**

4.6 Disease management strategies

The development of new technologies for the control of banana fruit rot diseases must be taken into account for the changing scenario of the fruit markets. The reduced returns becoming an unfortunate reality for many producers in the present time. Disease management strategies must be able to demonstrate a favourable cost-benefit relationship, as this may impact on the application of disease management strategies.

4.6.1 Chemical control of banana fruit rot disease caused by *L. theobromae*

Seven different systemic, non-systemic and combination product (systemic + non systemic) fungicides were tested against *L. theobromae* under *in vitro* and *in vivo* condition.

4.6.1.1 *In vitro* efficacy of fungicides against *L. theobromae*

The present studies was carried out to determine minimum inhibitory concentration (MIC) of seven fungicides at three different concentrations were evaluated against mycelial growth by "Poisoned Food Technique"(Nene and Thapliya, 1993) to find out the most inhibitory concentration for field applications as well as post harvest treatment.

The perusal of results presented in Table 4.16 revealed that all the fungicides tried were inhibitory to the fungal growth except copper oxychloride. Carbendazim (250, 500 and 1000 ppm), Propiconazole (250, 500 and 1000 ppm), Hexaconazole (500 and 1000 ppm), carbendazim 12% + mancozeb 63% (SAAF, 75 WP) [1500, 2000

and 2500 ppm] and mancozeb (2500 ppm) completely inhibited the fungal growth and proved statistically superior over the rest of fungicides tested. Next best fungicides were mancozeb at 2000 ppm (83.54 %) Hexaconazole at 250 ppm (80.65 %) and Chlorothalonil at 2000 ppm (76.97 %) inhibited the fungal growth. Copper oxychloride at higher concentration (2500ppm) was found to stimulate the growth of *L.theobromae*. as compared to control (Plate X). Present result showed that increase concentration of fungicides decreased the growth of pathogen but in case of copper oxychloride stimulated growth of *L.theobromae* after 48 hrs incubation at room temperature. After 72hrs of incubation carbendazim, propiconazole and SAAF completely inhibited the growth of pathogen in all tested concentration but mancozeb at 2500ppm conc. completely inhibited growth, than hexaconazole at 1000ppm (89.63%) and Chlorothalonil at 2500 ppm (87.59%) but in case of copper oxychloride at all test concentration pathogen over grew the petri plates i.e. growth was stimulated.

The present finding are in agreement with the results obtained by Sabalpara (1983) who reported that bavistin and benlate were effective against *B. theobromae*, a cause of mango die-back. Thakore (1983) reported bavistin (0.025%), benlate (0.025%), calixin (0.025%), dithane M-45 (0.05%) and brestanol

Table 4.16: Efficacy of fungicides on growth of *L. theobromae* under *in vitro* condition

Sr. No.	Fungicides	Conc. (ppm)	Growth after 48hrs of incubation		Growth after 72hrs of incubation	
			Growth	Growth	Growth	Growth

			(mm) *	inhibition (%)	(mm)	inhibition (%)
1.	Mancozeb (Dithane M-45 75%WP)	1500	16.00 (4.06) **	60.49 (10.99) ***	32.33 (5.73) **	64.07 (8.03)
		2000	6.67 (2.68)	83.54 (12.00)	20.67 (4.60)	77.04 (8.80)
		2500	0.00 (0.71)	100.00 (12.67)	0.00 (0.71)	100.00 (10.02)
2.	Copper oxychloride (Blue copper 50 WP)	1500	45.50 (6.78)	-12.40 (6.94)	90.00 (9.51)	0.00 (0.71)
		2000	51.83 (7.23)	-28.00 (5.69)	90.00 (9.51)	0.00 (0.71)
		2500	61.67 (7.88)	-52.30 (2.84)	90.00 (9.51)	0.00 (0.71)
3.	Chlorothalonil (Kavach 75 WP)	1500	19.33 (4.45)	52.27 (10.62)	41.17 (6.45)	54.26 (7.40)
		2000	9.33 (3.13)	76.97 (11.72)	25.67 (5.09)	71.48 (8.48)
		2500	0.00 (0.71)	100.00 (12.67)	11.17 (3.41)	87.59 (9.39)
4.	Carbendazim (Bavistin 50 WP)	250	0.00 (0.71)	100.00 (12.67)	0.00 (0.71)	100.00 (10.02)
		500	0.00 (0.71)	100.00 (12.67)	0.00 (0.71)	100.00 (10.02)
		1000	0.00 (0.71)	100.00 (12.67)	0.00 (0.71)	100.00 (10.02)
5.	Propiconazole (Tilt 25 % EC)	250	0.00 (0.71)	100.00 (12.67)	0.00 (0.71)	100.00 (10.02)
		500	0.00 (0.71)	100.00 (12.67)	0.00 (0.71)	100.00 (10.02)
		1000	0.00 (0.71)	100.00 (12.67)	0.00 (0.71)	100.00 (10.02)
6.	Hexaconazole (Contaf 5 % EC)	250	7.83 (2.88)	80.65 (11.88)	18.67 (4.38)	79.26 (8.93)
		500	0.00 (0.71)	100.00 (12.67)	12.33 (3.58)	86.30 (9.31)
		1000	0.00 (0.71)	100.00 (12.67)	9.33 (3.13)	89.63 (9.49)
7.	Carbendazim 12 % + Mancozeb 63 % (SAAF 75 WP)	1500	0.00 (0.71)	100.00 (12.67)	0.00 (0.71)	100.00 (10.02)
		2000	0.00 (0.71)	100.00 (12.67)	0.00 (0.71)	100.00 (10.02)
		2500	0.00 (0.71)	100.00 (12.67)	0.00 (0.71)	100.00 (10.02)
8.	Control	-	40.50 (6.40)		90.00 (9.51)	
S.Em.±			0.036	0.08	0.079	0.05
C.D. at 5%			0.10	0.22	.22	0.15

C.V. %		2.49	1.20	3.68	1.17

* Average of three repetition.

** Figures are $\sqrt{X} + 0.5$ transformed values

*** Figures are $\sqrt{X + 60}$ transformed values

Plate-X : Efficacy of fungicides against *L. theobromae*



Sr No.	Fungicides	Concentration		
		I	II	III
1.	Mancozab	1500	2000	2500
2.	Copper oxycchloride	1500	2000	2500
3.	Chlorothalonil	1500	2000	2500
4.	Carbondazim	250	500	1000
5.	Propiconazole	250	500	1000
6.	Hexaconazole	250	500	1000
7.	SAAF-75WP	1500	2000	2500
8.	Control	-	-	-

(0.2%) were most effective for the growth inhibition of *B. theobromae*, a cause of post-harvest rot of sapota. Godara (1994) found that bavistin (1000 ppm) completely inhibited fungus growth of *Botryodiplodia theobromae*. Ahmad *et al.* (1995) stated that benomyl (0.1%), captan (0.2%), carbendazim (0.1%), mancozeb (0.25%) and thiophanate-methyl (0.1%) were highly fungitoxic to *L. theobromae* in both solid and liquid media. Li *et al.* (1995) also reported that thiophanate-methyl suppressed mycelial growth and conidial germination of *L. theobromae*. Similarly, Sheler *et al.* (1997) reported that thiophanate-methyl (0.1%) and benlate (0.1%) could completely suppress the growth of *L. theobromae*. Banik *et al.* (1998) observed the complete inhibition of mycelial growth of *B. theobromae* causing mango fruit rot by carbendazim (400 ppm), followed by captan (450 ppm), thiophanate methyl (450 ppm), ziram (600 ppm) and chlorothalonil (650 ppm). Mahmood *et al.* (2002) reported that benlate @ 100 ppm and topsin-M (thiophanate-methyl) @ 50 ppm completely inhibited the mycelial growth of *L. theobromae*. Yadav and Majumdar (2004) reported effectiveness of carbendazim and mancozeb against *L. theobromae* (Guava isolate). Muhammad *et al.* (2005) observed significant inhibition of mycelial growth of *Lasiodiplodia theobromae* by carbendazim and thiophanate-methyl when used @ 1 ppm a.i. or more. Alliete was effective at relatively higher concentrations i.e., @ 1000 and 10000 ppm a.i., whereas, copxykil, cuprocaffaro and thiovit failed to inhibit the mycelial growth of *L. theobromae*.

4.6.1.2 Pre harvest application of fungicides for management of banana fruit rot disease

The fungicides found most effective in inhibiting the fungal growth of *L.theobramae in vitro* were selected for pre and post harvest application to control banana fruit rot diseases under field as well in storage condition. Mancozeb (2500 ppm), carbendazim (250 ppm), propiconazole (250 ppm), and SAAF (1500 ppm) were selected as pre and post harvest application treatment as mentioned in Materials and Methods (3.6.2). Per cent disease index (PDI) was recorded.

The results presented in Table 4.17 showed that all the tested fungicides found effective in controlling the finger rot disease over control under field condition. Carbendazim and propiconazole @250 ppm controlled cent per cent finger rot disease followed by SAAF @ 1500 ppm under field condition till the harvest.

The fruits harvested from treated plants were kept for ripening at room temperature were allowed to ripe naturally up to eating ripe stage and then observation on disease development was recorded.

The result presented in Table 4.18 revealed that propiconazole, carbendazim and SAAF significantly reduced the PDI under natural storage condition. Minimum per cent disease (1.50%) was observed in propiconazole treated harvested fruits followed by carbendazim (4.00%) and SAAF (5.50%). Maximum per cent control (98.20%) was observed in propiconazole treated fruits followed by

Table 4.17: Effect of pre-harvest application of fungicides on banana fruit (finger) rot disease development under field conditions

Sr. No.	Fungicides (ppm)	Per cent disease index (PDI)**	Per cent disease control
1.	Mancozeb (Dithane M-45 75%WP) (2500)	0.22 (4.86)*	91.48 (73.55)
2.	Carbendazim (Bavistin 50 WP) (250)	0.00 (4.05)	100.00 (90.00)
3.	Propiconazole (Tilt 25 % EC) (250)	0.00 (4.05)	100.00 (90.00)
4.	Carbendazim 12 % + Mancozeb 63 % (SAAF 75 WP) (1500)	0.08 (4.36)	97.36 (81.59)
5.	Control	2.23 (9.51)	0.00 (4.05)
S.Em.±		0.042	0.12
C.D. at 5 %		.013	0.37
C.V.%		9.09	3.01

* Figures in the parentheses are angular transformed ($X+0.5$) values.

** Average of four replication

carbendazim (95.16%), carbendazim+mancozeb (93.45%), mancozeb was moderate in controlling PDI (86.12%). Present study also indicated that propiconazole treated fruits take 2-3 day more time to ripe as compared to other treatments and pulp rotting was rare when

stored up to 16 days after harvesting. The present results suggested that propiconazole and carbendazim were the best fungicide to control the field as well as storage disease and also increased shelf life of banana fruits.

The present results are more or less in agreement with the results obtained by Khanna and Chandra (1976) who reported benomyl and aretan were highly toxic as they completely checked the banana (Var. Harichal) fruits rot pathogen viz., *Fusarium moniliform* and *F. roseum* as pre and post inoculation treatment up to 8 days. Ved Ram and Dharam vir (1984) got complete control of banana fruit rot decay caused by *A. flavus* and *A.fumigatus* by treating the fruits with thiophanate methyl, benlate, thiobendazole, bavistin, propionic acid and sodium metabisulphite at 2000 ppm up to 8 days of storage. Patel (1989) reported that bavistin (500 ppm) was significantly superior for disease control of twig blight of sapota caused by *B. theobromae* . Naqvi (1993) showed that benomyl and carbendazim at 1000 ppm concentration were effective against *B. theobromae* causing post harvest disease of mandarin. Godara (1994) reported lowest severity of post harvest rots of ripe and semi-ripe fruits treated with bavistin (500 ppm) in both pre and post-inoculation treatments. The gummosis disease in Japanese apricot and peach orchards caused by *L. theobromae* was controlled by spraying thiophanate-methyl @1g/lit. (Li *et al.*, 1995). Rawal (1997) observed that die-back of mango caused by *L.theobromae* was controlled by spraying at fortnightly interval with carbendazim @ 0.1%, methylethiophanate @ 0.1% or chlorothalonil @ 0.2%. Mahmood *et al.*, (2002) also reported the

effectiveness of two sprays of topsin-M (thiophanate-methyl) @1 g/lit. in reduction of infection caused *L. theobromae*.

4.6.1.3 Post-harvest application of fungicides for control of banana fruit rot disease

To know the efficacy of various fungicides, mature healthy banana fruits cv. Grand Naine of equal size having 10-12 fruits in one hand were first treated with respective concentration of fungicides and then kept for natural ripening as mentioned in 3.6.3. Per cent disease index (PDI) was recorded as described in chapter no 3.1.

The result presented in Table 4.19 minimum PDI was observed in propiconazole and SAAF (1.0%) treated fruit followed by carbendazim (2.5%). Maximum PDI (4.0%) was observed in mancozeb treated fruits (94.70%). Mancozeb and carbendazim produced brownish discoloration on fruit skin after 4-6 days of storage, but there was no effect on pulp. The present results are more or less in agreement with the results obtained by Latchmeah and Santchurn (1991). They gave fungicidal treatments at three different concentration of thiophanate-methyl and benomyl which inhibited the different rot in the range of 89.6 to 100.0 per cent. Godara (1994) found that Bavistin (1000 ppm) completely inhibited germination of spores of *Penicillium italicum* and *Botryodiplodia theobromae*. Lowest severity of both rots were observed in ripe and semi-ripe fruits treated with Bavistin (500 ppm) in both pre and post-inoculation treatments. Ramma *et al.* (1999) found benomyl 500 ppm and thiabendazole @ 1000 ppm which completely inhibited crown rot pathogens up to 9 days after harvest.

Table 4.18: Effect of pre-harvest application of fungicides on banana fruit rot disease development under storage conditions

Sr. No	Fungicides (ppm)	Per cent disease index	Per cent disease control
1.	Mancozeb (Dithane M-45 75%WP) (2500)	11.50 (20.27)	86.12 (68.55)
2.	Carbendazim (Bavistin 50 WP) (250)	4.00 (12.25)	95.16 (77.98)
3.	Propiconazole (Tilt 25 % EC) (250)	1.50 (8.13)	98.20 (83.45)
4.	Carbendazim 12 % + Mancozeb 63 % (SAAF 75 WP) (1500)	5.50 (14.18)	93.45 (75.76)
5.	Control	83.00 (67.62)	0.00 (4.05)
S.Em.±		1.52	1.39
C.D. at 5 %		4.57	4.29
C.V.%		13.05	9.68

* Figures in the parentheses are angular transformed ($X+0.5$) values.

** Average of four replication

Fruit assessment was done at the “eating” stage.

Table 4.19: Effect of post-harvest application of fungicides on banana fruit rot disease development under storage conditions

Sr. No.	Fungicides (ppm)	Per cent disease index	Per cent disease control
1.	Mancozeb (Dithane M-45 75%WP) (2500)	4.00 (11.53)	94.70 (77.34)
2.	Carbendazim (Bavistin 50 WP) (250)	2.50 (9.97)	96.79 (80.52)
3.	Propiconazole (Tilt 25 % EC) (250)	1.00 (7.03)	98.76 (85.06)
4.	Carbendazim 12 % + Mancozeb 63 % (SAAF 75 WP) (1500)	1.00 (7.03)	98.67 (84.77)
5.	Control	76.50 (61.34)	0.00 (4.05)
S.Em.±		1.83	1.03
C.D. at 5 %		5.51	3.11
C.V.%		20.22	9.95

* Figures in the parentheses are angular transformed ($X+0.5$) values.

** Average of four replication

Fruit assessment was done at the “eating” stage.

4.6.2 Bio-efficacy of phytoextracts against fruit rot pathogen *L. theobromae* under *in vitro* condition

Phytoextracts known to have inhibitory effect on the growth of various fungal and bacterial pathogens. This information is certainly useful in exploiting inhibitory principle for developing botanical fungicides in plant disease management. In the present investigations, unsterilized centrifuged twelve phytoextracts of various plant species (Table 3.6) at two concentrations were evaluated by poisoned food technique *in vitro* to know their inhibitory effect on the growth of *L. theobromae*

Data regarding the colony diameter (mm) and per cent inhibition of the fungal growth at two concentration of phyto extracts are presented in the Table 4.20. The comparative growth and colony diameter of treatments are depicted in the plate XI.

The results revealed that all the plant extracts at two concentrations stimulated the growth of *L. theobromae* significantly as compared to control except garlic, aloevera and cinnamon. The extracts of garlic clove and cinnamon leaf extract at 10 per cent concentration gave 47.09 and 33.86 per cent growth inhibition respectively of *L. theobromae*. Garlic clove and cinnamon leaves extract at 5 per cent gave 17.34 per cent growth inhibition. The next effective phytoextracts in order of inhibition which differed significantly from each other were extracts of aloevera leaves at 10 and 5 per cent which gave 16.5 per cent and 8.23 per cent growth inhibition respectively.

Growth stimulation was observed in 10 per cent leaf extract of lantana (75.2%), deshi babul (60.39%), barmasi (45.49%) and eucalyptus (47.13%) at 5 per cent over the control. Increase in growth of fungus was more at 10 per cent than in 5 per cent conc., in case of eucalyptus higher growth stimulation was at 5 per cent concentration, it declined at 10 per cent concentration (Plate XI). This suggest that all phytoextracts stimulated the growth in respective concentration and are good substrate for the growth of *L. theobromae*. Most of post harvest diseases are of complex nature, *L. theobromae* have a wide host range which caused rotting in almost all fruit and vegetable crops alone or in association, where it is difficult to isolate and purify from other pathogens, in such cases basal medium having lantana, babul, eucalyptus or barmasi makes isolation and purification easier.

Thus, results of our study are in agreement with the results reported by earlier workers viz Sabalpara (1983) who tried 21 phyto-extracts against *B. theobromae* (Mango isolate) and observed that none of phyto-extracts caused significant inhibition of mycelial growth and sporulation of pathogen except eucalyptus. Garlic bulb extract which inhibited the spore germination and mycelial growth of some important fungal pathogen of jute (*Botryodiplodia theobromae*) which was major seed borne pathogen (Ahmed and Sultana, 1984).

Table 4.20: Bio-efficacy of phytoextracts on growth of *L. theobromae* under *in vitro* condition

Sr. No.	Common name of Phytoextracts		Growth after 48hrs of incubation	
		Conc. (%)	Growth(mm) *	Growth inhibition(%)
1.	Acalypha	5	45.67 (6.79) **	-13.25 (8.20) ***
		10	52.67 (7.29)	-30.59 (7.06)
2.	Barmasi	5	53.67 (7.36)	-33.07 (6.88)
		10	58.67 (7.69)	-45.49 (5.91)
3.	Garlic	5	33.33 (5.82)	17.34 (9.89)
		10	21.33 (4.67)	47.09 (11.30)
4.	Turmeric	5	42.33 (6.44)	-4.959 (8.69)
		10	46.67 (6.87)	-15.71 (8.05)
5.	Lantana	5	54.67 (7.43)	-35.57 (6.70)
		10	70.67 (8.44)	-75.20 (2.30)
6.	Neem	5	46.33 (6.84)	-14.90 (8.10)
		10	48.67 (7.01)	-20.69 (7.73)
7.	Black Tulsi	5	45.67 (6.79)	-13.25 (8.20)
		10	56.67 (7.56)	-40.49 (6.23)
8.	Babul	5	54.67 (7.42)	-35.67 (6.64)
		10	64.67 (8.02)	-60.39 (4.45)
9.	Eucalyptus	5	59.33 (7.73)	-47.13 (5.77)
		10	51.33 (7.20)	-27.30 (7.30)
10.	Aloevera	5	37.00 (6.12)	8.23 (9.42)

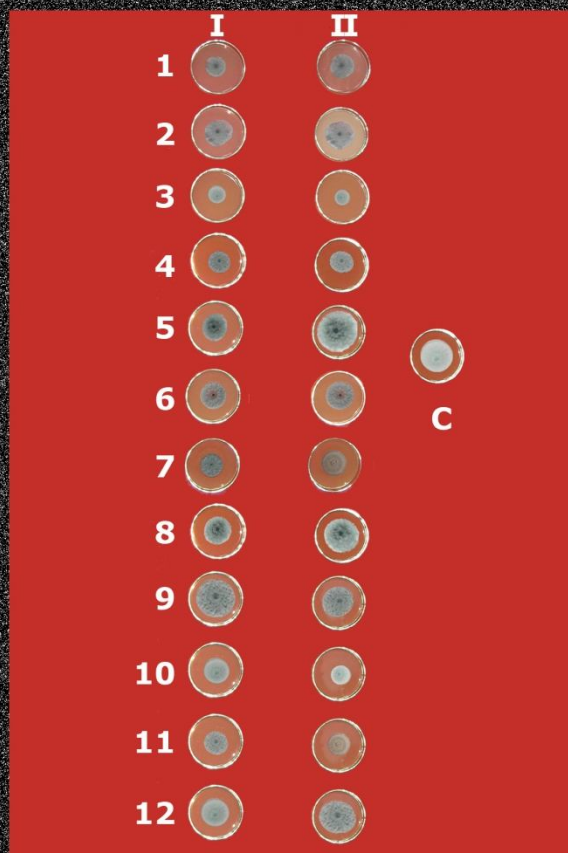
		10	33.67 (5.84)	16.50 (9.85)
11.	Cinnamon	5	33.33 (5.82)	17.34 (9.89)
		10	26.67 (5.21)	33.86 (10.70)
12.	Ardusi	-	50.67 (7.16)	-25.65 (7.40)
			57.33 (7.60)	-42.15 (6.20)
	Control		40.33 (6.39)	-
			S.Em.± C.D. at 5%	S.Em.± C.D. at 5%
Treatment			0.04 0.12	0.12 0.35
Concentration			0.02 0.047	0.05 0.15
Treatment X Concentration			0.06 0.16	0.18 0.50
C.V. %			1.44	4.06

* Average of three repetition.

** Figures are $\sqrt{X} + 0.5$ transformed values

*** Figures are $\sqrt{X + 80}$ transformed values

Plate-XI : Bioefficacy of phytoextract against *L. theobromae*



Concentration

I = 5%

II = 10%

Sr. No.	Common name	Scientific name
1.	Acalypha	<i>Acalypha indica</i> L.
2.	Barmasi	<i>Catharanthus roseus</i> L.
3.	Garlic	<i>Allium sativum</i> L.
4.	Turmeric	<i>Curcuma longa</i> L.
5.	Lantana	<i>Lantana camara</i> L.
6.	Neem	<i>Azadirachta indica</i> Juss.
7.	Black Tulsi	<i>Ocimum basilicum</i> L.
8.	Babul	<i>Acacia nilotica</i> L.
9.	Eucalyptus	<i>Eucalyptus citriodora</i> Hoch.
10.	Aloevera	<i>Aloe barbadensis</i> L.
11.	Cinnamon	<i>Cinnamomum zeylanicum</i> L.
12.	Ardusi	<i>Adhatoda vasica</i> Ness
C.	Control	

Patel (1989) tested 21 plant extracts against *B. theobromae* (Sapota isolate) among them, garlic bulb, turmeric rhizome, garlic leaves and dhatura leaves extracts showed more than 50 per cent inhibition of mycelial growth. Singh *et al.*, (1993) evaluated eleven leaf extracts of medicinal plants, the extract from *Azadirachta indica* and *Ocimum sanctum* were the most effective in inhibiting the mycelia growth of *B. theobromae*, *Fusarium oxysporum*, *Helminthosporium speciferum*, *Curvularia lunata*, *Aspergillus flavus* and *Trichothecium roseum*. Aqueous garlic clove extract at 30 and 50 g /lit. concentration completely inhibited the mycelial growth of *B. theobromae*, while 10 g /lit. concentration inhibited the conidial germination (Andrade *et al.*, 1996).

4.6.3 Antagonistic effect of known bioagents on growth of fruit rot *L. theobromae*

The antagonistic effect of five isolates of bioagent was evaluated against the *L. theobromae* by dual culture technique. Based on observations of radial growth of antagonist and test fungus, per cent inhibition was calculated. The results are expressed in table 4.21 and depicted in plate XII.

The results revealed that all bio agents were significantly superior in inhibiting the growth of *L. theobromae* over the control. The maximum inhibition (75.83 %) was recorded in presence of *Pseudomonas fluorescense* and showed strong antagonism with the pathogen. This was followed by *Bacillus subtilis* (70.56%), *T. longibrachyatum* (61.25%), *T. harzianum* (47.5%) and *T. viride* (31.39%) which showed moderate antagonism. *Pseudomonas*

fluorescence and *Bacillus subtilis* have showed good antagonism against pathogen. The inhibiting capacity of isolates was directly correlated with the faster growth rate of antagonist. Thus, results of our study are more or less corroborate with the results reported by earlier workers viz; Florence and Sharma (1990) who showed that *Bacillus subtilis* can be used as potential bio-control agent against the *B. theobromae*. Aranguren *et al.* (1994) tested antagonistic potentials of different *Trichodaerma* spp. against *B. theobromae* and achieved the highest inhibition with *T. longibrachyatum* and *T. viride*. Gupta *et al.* (1999) studied mycoparasitic activity of various bio-agents *in vitro* against *B. theobromae* and concluded that *T. viride*, *T. harzianum* and *T. pseudokoningii* inhibited growth of *B. theobromae*. Mortuza and Ilag (1999) Observed that *Trichoderma harzianum* exhibited the greatest inhibition the growth of *L. theobromae* in dual culture.

Alvindia and Natsuaki (2009) reported that *Bacillus amyloliquefaciens* DGA14 inhibited all test crown rot pathogens (*Lasiodiplodia theobromae*, *Thielaviopsis paradoxa*, *Colletotrichum musae*, and *Fusarium verticillioides*) in culture. Post-harvest application of *B. amyloliquefaciens* DGA14 in the packing house reduced the incidence of crown rot to such a lower level than in fungicidal treatment.

Table 4.21: Antagonistic effect of bioagents on growth of *L. theobromae* under *in vitro* condition

Sr. No.	Antagonists	Growth inhibition (%) [*]
1.	<i>Trichoderma viride</i>	31.39 (34.38) ^{**}
2.	<i>Trichoderma harzianum</i>	47.50 (43.56)
3.	<i>Trichoderma longibrachyatum</i>	61.25 (51.50)
4.	<i>Bacillus subtilis</i>	70.56 (57.37)
5.	<i>Pseudomonas fluorescense</i>	75.83 (60.55)
6.	Control	0.00 (4.05)
S.Em.±		0.028
C.D. at 5 %		0.083
C.V.%		0.86

^{*} Average of three repetition.

^{**} ^{*} Figures in parentheses are angular transformed (X+0.5) values.

Plate-XII : Effect of antagonist on growth of *L. theobromae*



1. *T. viridae*
2. *T. harzianum*
3. *T. longibrachyatum*
4. *B. subtilis*
5. *P. fluorescens*
6. Control

4.6.4 Development of Integrated disease management (IDM) module for management of banana fruit rots disease

The aim of this investigation is to present the latest information on the biology and management of banana fruit rot diseases based on integration of field and postharvest studies, including cultural practices, phytochemicals, bioagents and fungicide usage.

The results presented (Table 4.22) that Carbendazim @ 0.5 gL⁻¹, Propiconazole @ 1mL⁻¹, Garlic clove and Cinnamon leaf extract @ 100mL⁻¹ gave cent per cent disease control followed by *Bacillus subtilis* @1X10⁷ CFU (92.71 %) and bunch covered with blue polythene bag (80.89%). It was also observed that the bunches treated with propiconazole showed prolonged maturity of fruits as compared to other treatments. In untreated control bunches, per cent disease index was increased with increase in time. None of the treatments exhibited phytotoxic effect on fruits under the field conditions.

One hand having ten fruits were selected from each bunch brought to the laboratory kept for ripening under natural condition at room temperature up to full ripening stage and allowed disease to develop. Evaluation of each treatment was done with help of model, described in chapter point 3.1. All fruits assessments were made at the eating ripe stage, the results presented in Table-4.23. All the treatments significantly reduced

Table 4.22: Effect of Integrated management of banana fruit (finger) rot disease development under field condition

Sr. No.	Treatment	Disease development	
		PDI**	Inhibition(%)
1.	Carbendazim (Bavistin 50 WP) (250ppm)	0.00 (4.05)*	100.00 (90.0)
2.	Propiconazole (Tilt 25 % EC) (250 ppm)	0.00 (4.05)	100.00 (90.0)
3.	Galic bulb extract@ 10%	0.00 (4.05)	100.00 (90.0)
4.	Cinnamon leaf extract@10%	0.00 (4.05)	100.00 (90.0)
5.	<i>Bacillus subtilis</i> @1x10 ⁷ CFU/ml	0.18 (4.73)	92.71 (74.89)
6.	<i>Pseudomonas fluorescense</i> @ 1x10 ⁷ CFU/ml	0.33 (5.23)	84.53 (67.24)
7.	Bunch covered by folding grown leaf	1.17 (7.50)	42.21 (40.80)
8.	Bunch covered with blue LLDPE bags	0.39 (5.41)	80.89 (64.44)
9.	control	2.05 (9.19)	0.00 (4.05)
S.Em.±		0.06	0.34
C.D. at 5 %		0.18	1.02
C.V.%		11.41	7.06

* Figures in the parentheses are angular transformed values.

** Average of three replication

per cent disease index except bunch covered with leaf folding under field conditions. Propiconazole treated bunch significantly checked fruit rot disease followed by Carbendazim (94.12%), Cinnamon leave extracts (91.48%), bunch covered with blue polythene bag (87.32%) and Garlic clove extract (83.07%). Thus, results of our study corroborate with the results reported by Bedimo *et al.* (2003) they observed control of banana anthracnose (*Colletotrichum musae*) through deflowering and bunch covering at horizontal fingers stage. Win *et al.* (2007) found that disease development was least (25 per cent) on cinnamon extract (@ 5.0g/lit.) treated fruit after inoculation of crown rot pathogen (*Colletotrichum musae*, *Fusarium spp.* *Lasioidiplodia theobromae*) compared to carbendazim (@ 0.75g/lit.).

From the present results it is clear that preharvest treatment reduced the infecting propagules of the fruit rot pathogens there by reduces the post-harvest decay. *Bacillus subtilis* reduced fruit rot development under storage conditions as compared to field condition because it required time and suitable condition for colonization on fruit surface in field condition which was less as compared to storage condition. Antagonist also enhanced defence related enzyme activity. In case of bunch covered with blue polythene bag directly reduced the inoculum which is carried to storage condition, resulting in to less fruit rot disease.

Table 4.23: Effect of integrated management approaches on banana fruit rot disease under storage condition

Sr. No.	Treatment	Disease development	
		PDI**	Inhibition(%)
1.	Carbendazim (Bavistin 50 WP) (250ppm)	4.87(13.40)*	94.12 (76.58)
2.	Propiconazole (Tilt 25 % EC) (250 ppm)	0.00 (4.05)	100.00 (90.0)
3.	Galic bulb extract@ 10%	14.20 (22.54)	83.07 (66.08)
4.	Cinnamon leaf extract@10%	7.08 (15.43)	91.48 (73.55)
5.	<i>Bacillus subtilis</i> @10 ⁷ CFUml ⁻¹	17.00 (24.73)	79.55 (63.47)
6.	<i>Pseudomonas fluorescense</i> @ 10 ⁷ CFUml ⁻¹	19.17 (26.33)	77.15 (61.78)
7.	Bunch covered with leaf folding	67.50 (55.55)	19.34 (26.45)
8.	Bunch covered with blue polythene	10.55 (19.41)	87.32 (69.57)
9.	control	83.78 (66.64)	0.00 (4.05)
S.Em.±		1.38	0.12
C.D. at 5 %		4.09	0.38
C.V.%		8.89	3.01

* Figures in the parentheses are angular transformed values.

** Average of three hand, 10-12 fruits/ hand,

All fruit assessment was made at the “eating” stage.

V. SUMMARY AND CONCLUSION

Banana (*Musa paradisiaca* L.), fruit is one of the most important commercial fruit and vegetable crops grown all over the world in the tropical and subtropical areas. The genus *Lasiodyplodia theobromae*, is geographically widespread but is most common in the tropics and subtropics regions (Punithalingam, 1980). The present investigations on fruit rot of banana caused by *Lasiodyplodia theobromae* were carried out under South Gujarat conditions.

Field survey was carried out in Navsari and Surat district from January,2008 to December,2009 concluded that maximum per cent disease index (PDI) was observed in the month of October (2.65%) followed by April (2.28%) and November (2.24%) and minimum per cent disease index was recorded in month of May (0.10%) at Navsari in both the years. At Surat higher per cent disease index was observed in the month of October (2.78%) followed by November (2.68%) and December (2.09%) and minimum per cent disease index was recorded in the month of May (0.09%).

Post harvest rot was higher in the month of September (16.9%) followed by October (16.2%) and November (12.5%) and minimum in the month of February (5.0%) at Navsari market in both

the years. In Surat market higher per cent disease index was observed in month of September (13.9%) followed by October (13.2%) and November 12.1%) and minimum in the month of February (4.1%).

The correlation of the weather parameters with disease development led to the conclusion that per cent disease index exhibited negative correlation with avg. max. temperature and significant positive with minimum temperature in both the years. Morning and evening relative humidity showed highly significant positive correlation and Rainfall had positive correlation in both the years.

Isolation of fungi were made from infected banana fruit, by tissues isolation and associated fungi were identified as as *Lasiodiplodia theobromae* Pat., *Fusarium moniliforme* Sheld, *Fusarium* sp., *Aspergillus niger*, *Acremonium* sp. and *Curvularia* sp., which was further, confirmed by Agharkar Research Institute, Pune.

Lasiodiplodia theobromae initially produced cottony white fluffy growth on potato dextrose agar, which cover the Petridish within 3 to 4 days at room temperature. After 2 days, colour of the colony changed from white cottony to olivaceous dark. Under the lower surface of the dish, complete blackish background was observed due to pigmentation by the fungus.

The *Lasiodiplodia theobromae* was inoculated on healthy banana (var. Grand naine) fruits, produced brownish- black discoloration on the peel and a softening of the pulp on unripe banana fruits. On the ripe fruits brownish and water –soaked discoloration observed in the infected area. Under moist conditions white to light

gray cottony mycelium covered the infected tissues. Pycnidiospore formation takes place rather late.

Water soaked brown discoloration appeared then turned to dark brown and pulp got rotted slowly, rotting was fast in high humid condition due to *Fusarium moniliformae*. *Aspergillus niger* produced brown discoloration on peel and rotted pulp when fruits were over riped. *Curvularia* sp. produced minute pinkish spot when fruit riped, but when fruit on full to over ripe stage, it extended in size, gone up to pericarp, our can not infected pulp. *Acremonium* sp. produced brownish internal rotting at blossom end portion of fruits.

Crown rot disease was the major post harvest disease due to fungal pathogens viz ; *L.theobromae*, *F. moniliforme* and *A. niger* the frequency of occurrence of these pathogens was 72 per cent on ripe banana fruits of Grand Naine cultivar. The next fruit rot disease was due to *L.theobromae* and *F. moniliforme* with 10 per cent occurrence. *Lasiodiplodia theobromae* was the most frequently (43 per cent) isolated in both the diseases followed by *Fusarium moniliforme* (21.5 per cent) from both Navsari and Surat market places.

The genomic DNA was isolated by CTAB method from all the isolates of *Lasiodiplodia theobromae* and utilizing for genomic diversity analysis using RAPD primers.

Only four RAPD primers out of thirty i.e. OPE-3, OPE-10, OPF-12 and OPG-5 gave good amplification and polymorphism and also generated significant information. Most of the primers produced fragments below 850 bp and generated a total of 122 bands, all are polymorphic.

The UPGMA based cluster analysis of RAPD patterns showed that isolate RID was more diverse from all other isolates and recorded highest genetic distance (84%) from other isolates. Isolates MIJ1 and MIT showed maximum similarity (73%) because they were isolated from same host, almost similar region and same year. While, isolate BIN and MIN showed more similarity (64.2%) both were isolated from different host but same region. In our study BIN and BIB-1 isolate causing banana fruit rot showed only 40 % similarity but it was higher than MIB and MIJ1 isolates (Mango fruit rot), which might be due to different geographic region.

Total sugar was higher (211.57 mg/g fresh weight) in ripe uninoculated fruits and decreased (203.99 mg/g fresh weight) in inoculated fruits and lowest in uninoculated green mature fruits after 72hrs of storage. A nine-fold increase in total sugar content was observed in green mature inoculated fruits followed by green mature uninoculated and semi ripe fruits.

Total phenol content was higher in green mature fruits (14.80 mg/g fruit weight) than decline in semi ripe (13.38 mg/g fruit weight) and ripe fruits (10.98 mg/g fruit weight) at initial time. The phenol content was decreased with increasing storage period in uninoculated and further decreased in inoculated in all three stages.

Phenylalanine ammonia lyase (PAL) activity was higher in green mature inoculated fruits (75.33 Units $\text{h}^{-1} \text{g}^{-1}$ protein) than uninoculated (59.33 Units $\text{h}^{-1} \text{g}^{-1}$ protein) which was decreased in ripe inoculated fruits (9.11 Units $\text{h}^{-1} \text{g}^{-1}$ protein) and uninoculated (3.56 Units $\text{h}^{-1} \text{g}^{-1}$ protein) after 72hrs of storage.

Polyphenol oxidase (PPO) activity of uninoculated fruits (2.4 Units min⁻¹ g⁻¹ protein) was more or less similar to inoculated fruits (3.08 Units min⁻¹ g⁻¹ protein) after 72hrs of storage. However, in uninoculated semi ripe fruits PPO activity was increased about 14.72 fold after 72 hrs of incubation, while in inoculated semi-ripe fruits it was higher (19.33 fold).

Peroxidase (POX) activity was found higher in ripe fruits (49.75-121.9 µmol guaiacol min⁻¹ g⁻¹ protein) than in semi ripe fruits (8.73-63.8 µmol guaiacol min⁻¹ g⁻¹ protein) up to 72hrs of storage. The POX activity enhanced after inoculation with *L.theobromae* in all ripening stages. The degree of increase in infected green mature, semi ripe and ripe was 12.33, 7.30 and 2.45 fold higher respectively than uninoculated fruits.

Pycnidiospores germinated and produced ellipse-shaped appressorium after 20-24hrs of inoculation on unripe banana fruits but its further growth was suppressed and not colonized between or within the cells due to biochemical presence in banana green peel. In semi-ripe and ripe fruits fungus colonized speedily within 8-10hrs. Hyaline branched mycelium produced between the cells then it produced pycnidia within 48hrs which produced hyaline elliptical single celled pycnidiophores.

Efficacy of seven fungicides at three concentrations were evaluated for their comparative efficacy against mycelial growth of *L. theobromae* through "Poisoned Food Technique" under *in vitro* condition. Carbendazim, propiconazole, hexaconazole, carbendazim 12% + mancozeb 63% (SAAF, 75 WP) and mancozeb (2500 ppm)

completely inhibited the fungal growth and proved statistically superior over the rest of fungicides tested. Copper oxychloride was found to stimulate the growth of *L.theobromae*.

Mancozeb (2500 ppm), carbendazim (250 ppm), propiconazole (250 ppm), and SAAF (1500 ppm) were selected as pre and post harvest application treatment on the basis of their inhibitory effects under *in vitro* condition. Carbendazim and propiconazole gave hundred per cent control of finger rot disease. Fruits harvested from treated plant, which were kept for natural ripening at room temperature. Maximum inhibition (98.56%) of fruit rot was observed in propiconazole treated fruits followed by carbandzim (95.64%) under storage condition up to eating ripe stage.

Maximum per cent disease inhibition (98.79%) was observed in propiconazole followed by SAAF and carbendazim treated fruits. Mancozeb and carbendazim showed phytotoxic effect producing brownish discoloration after 4-6 days of storage, but their was effect on pulp.

Twelve phytoextracts of various plant species at two concentrations were screened by poisoned food technique *in vitro* to know their inhibitory effect on the growth of *L. theobromae*. The extracts of garlic clove and cinnamon leaf extract at 10 per cent concentration gave 47.09 and 33.86 per cent growth inhibition respectively of *L. theobromae*. Growth stimulation was observed in lantana at 10 per cent leaf extract (75.2%) followed by deshi babul (60.39%) 10 per cent, eucalyptus (47.13%) 5 per cent and barmasi (45.49%) 10 per cent concentration over the control.

Antagonistic effect of five isolates of known bioagents were evaluated against the *L. theobromae* by dual culture technique. The maximum inhibition (75.83 %) was recorded in presence of *Pseudomonas fluorescense* and showed strong antagonism against the pathogen followed by *Bacillus subtilis* (70.56%) which showed moderate antagonism. *Pseudomonas fluorescense* and *Bacillus subtilis* were showed good antagonism against the pathogen. The inhibiting capacity of bioagent was directly correlated with the faster growth rate.

Carbendazim @ 0.5 gL⁻¹, propiconazole @ 1mL⁻¹, garlic and cinnamon @ 100mL⁻¹ gave cent per cent disease control followed by *Bacillus subtilis* 1X10⁷ CFU (92.71 %) of finger rot disease. However, the plants sprayed with propiconazole exhibited delayed fruit maturity. None of the treatment elicited any phytotoxic response under the field conditions.

Propiconazole treated fruits gave cent per cent disease at 1mL⁻¹ concentration followed by Cinnamon leaf extracts (91.48%), Garlic clove extract (83.07%), Carbendazim (94.12%) and bunch covered with blue polythene bag (87.32%) under storage condition. Bunch covered with blue polythene bag directly reduced the inoculum which cause less disease under storage condition.

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CERTIFICATE

This is to certify that I have no objection to supply one copy of any part of this thesis at a time to any scientist or researchers through reprographic process for rendering reference services in a library or documentation centre.

Place : Navsari

Date : 13 March, 2011

(KEDAR NATH)