VARIABILITY STUDIES IN POST HARVEST PATHOGENS INFECTING MANGO FRUITS IN MAHARASHTRA

by

Miss. Suryavanshi Nikita Nitin
(Reg. No. 019/221)

A thesis submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA

in partial fulfilment of the requirements for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

in

PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY AND AGRICULTURAL MICROBIOLOGY

POST GRADUATE INSTITUTE
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MAHARASHTRA, INDIA
2021

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part
there of has not been submitted
by me or another person to any
other University or Institute
for a Degree or
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Date : / /2021

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This is to certify that the thesis entitled, **"VARIABILITY STUDIES IN POST HARVEST PATHOGENS INFECTING MANGO FRUITS IN MAHARASHTRA"** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (Maharashtra) in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY**, embodies the results of a piece of bonafide research work carried out by **Ms. SURYAVANSHI NIKITA NITIN,** under my guidance and supervision and that no part of the thesis has been submitted to any other University for Degree or Diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.

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Place : MPKV, Rahuri.

Date : / /2021 **(N. N. Suryavanshi)**

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LIST OF ABBREVIATIONS

@	:	At the rate of
°C	:	Degree Centigrade (S)
C. D.	:	Critical Difference
Cm	:	Centimetre (s)
cv.	:	Cultivar
EC	:	Emulsifiable concentrates
et al.	:	And other (et alli)
etc.	:	Et cetera
Fig.	:	Figure
f.sp.	:	Forma species
G	:	Gram (s)
hrs.	:	Hours
i.e.	:	That is
Kg	:	Kilogram (s)
Lbs	:	Pounds
Ltd.	:	Limited
µm	:	Micrometer
Mm	:	Millimeter
Ml	:	Milliliter
No.	:	Number
/	:	Per
%	:	Per cent
S. E.(m)	:	Standard Error of mean
spp.	:	Species
viz.	:	Namely

ABSTRACT

VARIABILITY STUDIES IN POST HARVEST PATHOGENS INFECTING MANGO FRUITS IN MAHARASHTRA

by

Ms. SURYAVANSHI NIKITA NITIN

A candidate for the degree

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2021

Research Guide	**:**	**Dr. S. V. Kolase**
Department	**:**	**Plant Pathology**

Mango (*Mangifera indica* L.) fruit, which ranks fifth in production worldwide, is cultivated throughout the tropics as well as subtropical areas such as Spain, Florida, North Africa, Israel and Japan. Mango is generally considered as “King of fruits” as it has wide adaptability, high nutritive value, richness in variety, delicious taste, excellent flavor, attractive appearance and popularity among the masses. Mango is cultivated in an area of 2.29 million hectares with a production of 20.07 million tonnes and a productivity of 9.7 MT/ha. As per (Anonymous 2019) data base mango occupies 34.89 of total fruit area, 20.7 % of total fruit production.

Fungi are the prime importance for storage rots. Among the fruit rots, anthracnose caused by *Colletotrichum gloeosporioides* is the most prevalent in humid growing areas. *Lasiodiplodia theobromae* is recently becoming one of the major diseases of mango both in pre and post harvest conditions. Alternaria rot caused by fungus *Alternaria alternata* can become serious when anthracnose and stem end rot are well controlled. Mango decay caused by the plant pathogenic fungus *Aspergillus niger* is one of the most dangerous post harvest disease, leading to the losses of fruit quality during storage.

The present investigation was carried out on the “Variability Studies in Post Harvest Pathogens Infecting Mango Fruits in Maharashtra” with a view to assess organisms responsible for post harvest spoilage, their pathogenicity, cross infectivity potential in order to suggest suitable control measures in respect of fungicides and bioagents for the management of post harvest diseases of mango.

Isolation carried out from infected mango fruits recorded association of four fungal pathogens *viz*., *Colletotrichum gloeosporioides* Penz., *Alternaria alternata* Groves and Skolko, *Lasiodiplodia theobromae* Pat. and *Aspergillus niger* and pathogenicity of all the four pathogens was proved.

Contd…

The pathogen *Colletotrichum gloeosporoides* produced brown to black sunken lesion on fruit and further caused fruit rot. *Alternaria alternata* produced black spots on fruits which on progresses as rot. *Lasiodiplodia theobromae* produced blackening on fruits and thereafter severe rotting from stem end and *Aspergillus niger* produced brown to black discoloured lesions on fruits which further caused fruit rot.

When isolates of pathogens were inoculated on three different fruit hosts it was observed that there was maximum diversity with respect to host preference and the degree of infection to a particular fruit type. *Alternaria alternata* possessed highest degree of infectivity on guava fruit.

Among the six fungicides tested, carbendazim + mancozeb, thiophanate methyl and carbendazim were most effective with maximum inhibition radial mycelial growth of all the four pathogens *in vitro*.

Under *in vitro* condition *Trichoderma viride* was found to inhibit maximum growth of all post harvest pathogens followed by *Trichoderma harzianum* and yeast (*Saccharomyces cerevisiae*) while among the bacterial bioagents *Pseudomonas fluorescens* was found effective in inhibiting the mycelial growth of the pathogens than *Bacillus subtilis*.

1. INTRODUCTION

The mango (*Mangifera indica* L.) fruit which ranks fifth in global production is grown in the tropics and subtropics including Spain, Florida, North Africa, Israel, and Japan. Mango is a drupe type fruit that belongs to the Anacardiaceae family. It is native to India and South East Asia. Hundreds of cultivated varieties have been introduced and successfully grown to other warm regions of the world. They are erect and fast growing with sufficient height and the canopy can be broad and rounded, or more upright, with a relatively slender crown. The mango tree is medium to large 10 to 40 m in height, evergreen with symmetrical, rounded canopy ranging from low and dense to upright and open. Bark is usually dark brown to black, rather smooth, superficially cracked or inconspicuously fissured, peeling off in irregular, rather thick pieces. It attains great age can live well over 100 years. Trees in cultivated orchards are kept at 6-9 m height. Mango is known as the "King of Fruits" because of its adaptability, high nutritional value, delicious taste, excellent flavour, attractive appearance and widespread popularity. Fruits are known for their nutritional and commercial importance. They are indispensable food commodities across the globe.

Mango play a vital role in human nutrition by supplying essential growth elements such as vitamins, minerals, amino acids, carbohydrates, fats and many other essential nutrients in daily diets; therefore, help to keep good and normal health. The fruit contains nearly 81 per cent moisture, 0.4 per cent fat, 0.6 per cent protein, 0.8 per cent of fiber. It also contains nearly 17 per cent of carbohydrate. The fruit is rich with important minerals contains important minerals like Potassium, magnesium, Sodium, Phosphorus, and Sulphur. Mangoes are an excellent source of vitamins A and C which both are important antioxidant nutrients.

For its high palatability and nutritive value, it is marketed in many parts of the world and its pulp is consumed by large number of people. It is also consumed as preserved product in various forms like jam, juice, salted pickle, spiced pickle, halva, chutney, slices, and pulp by many people.

India accounts for 40.4% of world’s mango production. Mango is cultivated in an area of 2.29 million hectares with a production of 20.07 million tonnes and a productivity of 9.7 MT/ha. As per (Anonymous 2019) data base mango occupies 34.89 of total fruit area, 20.7% of total fruit production. India ranks first in production of mango. Total annual production estimated about 8.21 million tones. Area under Mango crop in Andhra Pradesh is the highest in the India.

Mango is grown in India in tropical and subtropical regions from sea to an altitude of 1500 meters. It is grown almost in all states of India. However, it is mainly cultivated in Andhra Pradesh, Bihar, Gujarat, Karnataka, Kerala, Maharashtra, Orissa, Tamil Nadu, Uttar Pradesh and West Bengal. Indian mango is being exported to various foreign countries including Gulf, USA, China and England getting significant amount of foreign currency to India. Therefore, farmers in India in general while in Andhra Pradesh, Maharashtra and Goa in particular are growing mango on

large scale. The farmers of Maharashtra, since last two decades have inclined to grow mango on large area.

More than thousand varieties of Mango are grown in India. However, only about 30 varieties are grown on commercial scale in different states. The popular varieties of mango that are being cultivated and transported from other markets are Dasheri, Langra, Mulgoa, Neelum, Pairi, Benisan, Kesar, Alphanso, Lalbagh, Totapuri and many local varieties.

Though, the India is second largest producer of fruits, neither the Indians are able to consume the required quantity of fruits for healthy diet nor they are able to grab their share in the international market in proportion to their production. One of the main reasons for this is the losses of fruits due to post harvest diseases as it reduces quality and quantity of marketable fruits. The losses due to post harvest diseases of mango during storage and transport result in considerable financial loss to the sellers as well as the consumers.

Post harvest management means the handling of an agricultural product after harvest to prolong storage life, freshness and an attractive appearance. In order to deliver a quality product to the market and ultimately to the consumer to command buyer attention and gives the grower a competitive edge, proper post-harvest management is the need of the hour. Post harvest diseases can cause severe losses, sometimes because they yield completely unmarketable fruit and in many cases because blemished fruit does not meet the cosmetic standards for first quality fruit in the major import markets. Although blemished fruit can often be sold in the less demanding local markets, this practice results in important economic losses due to the considerable differences in export and local prices.

There are many plant pathogenic organisms such as bacteria, fungi which causes different types of diseases of fruits during storage periods and symptoms caused due to fungal diseases vary greatly that depend on type of pathogen, host (fruit) and environmental factors. Fungi are of prime importance for storage rots. Major mango diseases are anthracnose (*Colletotrichum gloeosporioides*); alternaria fruit rot *(Alternaria alternata* and *A. tenuissima*); stem end rot (*Lasiodiplodia theobromae* and *Dothiorella spp*.); fruit rot (*Phoma mangifera, Pestalotia mangifearae, Macrophomina spp*.). In Maharashtra the environment during mansoon period is most congenial for the development of fruit rot in the late harvested varieties like Dasheri, Neelum, Alphanso, Totapuri, Ratna and Langra.

Among the post harvest diseases of mango, anthracnose is the most prevalent in humid growing areas. Arauz (2000) reported that *Colletotrichum gloeosporioides* pathogen causing mango anthracnose is responsible for fruit drop in mango. The incidence of this disease can reach almost 100% in fruit produced under wet or very humid conditions. Globally, anthracnose is one of the most important post-harvest diseases of mango. The symptoms of anthracnose appear at

both pre- and post-harvest stages of mango. However, post harvest anthracnose is responsible for severe losses both qualitatively and quantitatively.

Pandey *et al*. (1984) reported that premature fruit drop of mango was caused by *Alternaria alternata* in Allahabad. Lonsdale and Kotze (1993) reported that blossom spot of mango was caused by *Alternaria alternata* which ultimately resulted in drop of flowers and fruits.

Alternaria rot caused by the fungus *Alternaria alternata* can become serious when anthracnose and stem end rot are well controlled. *Alternaria* infects the mango fruits through the lenticel on the skin. After infection on the tree the fungal hyphae remain dormant until fruit ripening when it starts to grow intercellularly. The symptom includes small black circular spots 0.5 to 1.0 mm in diameter which develop around the lenticels. The fungus develops in the lenticels and penetrates the fruit resulting in darkening of the intercellular spaces and cell collapse. Initially spots are concentrated around the stem end of the fruit where high number of lenticels are present. The spots grow and coalesce to become a single spot that can cover whole fruit. Later the disease progresses into the flesh which darkens and becomes practically soft.

Mango decay caused by the plant pathogenic fungus *Aspergillus niger* is one of the most dangerous post harvest diseases, leading to the losses of fruit quality during storage. The symptoms were first observed as small, light yellow colour suppressed lesions around the stem end region. The lesions increased in size resulting in depressed mesocarp and a soft rot condition. The centre of the lesion became sunken and was covered with brownish black spores.

Stem end rot is caused by *Lasiodiplodia theobromae*. It is recently becoming one of the major diseases of mango both in pre and post harvest conditions. The disease is characterized by wilting of branches and twigs and as stem end rotting of fruits. The optimum temperatures for the growth of pathogen is 20-30 °C, while light intensity has no effect on growth. These fungi are water borne and spread through rain splash from dead twigs. The *Lasiodiplodia* is very active in warmer seasons and its symptoms are characteristic.

For reducing the post harvest loss it is essential to start protecting it in the field and then careful harvesting, hygienic handling, packaging and storage, temperature regulated transportation and finally intelligent marketing. Since, it is impossible to completely control all diseases it is necessary to keep them at a level that does not result in significant economic loss. The adoption of integrated disease management practices for their control is desired for this purpose.

Post harvest diseases are conventionally controlled by fungicides. There has been increased awareness among the people about the risk associated with the use of fungicides in controlling post harvest diseases. This imposed the development of alternative strategies for controlling post harvest diseases, which are ecologically safe and risk free to human beings. Biological control is one of such approach.

In view of the post harvest spoilage due to various fungal pathogens indicated above, it was thought worthwhile to undertake the study of the pathogens associated with the post-harvest diseases of mango and their management with the following objectives:

1. To collect samples and isolate different pathogens infecting mango fruits from Local market.
2. To study pathogenicity of isolated pathogens from mango fruits.
3. To ascertain the host specificity behaviour of isolates by cross inoculation.
4. To identify the level of sensitivity of isolates of various pathogens to fungi-toxicants and bio-agents under *in vitro* condition.

2. REVIEW OF LITERATURE

Post harvest infection of the fruits are the most common causes of production loss. Mangoes are susceptible to a variety of diseases throughout their life cycle from planting to harvest. Not only post harvest infections cause significant losses and a drop in mango production but they also lower the quality of the fruits. Handling of fruits is the most important step in preventing post harvest losses. The study of post harvest diseases and their prevention in the marketing channel contributes significantly to increasing fruit production even more by minimizing post harvest losses. Anthracnose, stem end rot, black rot, and soft rot are the most common post harvest diseases of mango in India. Anthracnose and stem end rot are two of the most common diseases that affect crops both before and after harvest. To meet the targets of successful mango delivery to distant markets fruit quality must be improved.

One of the primary challenges facing the mango sector is post harvest management. In this study an effort was made to identify the various fungal diseases that occur after harvesting at the field level and in the marketing channel. There is a need to apply the sustainable method of fruit handling to prevent post harvest fungal diseases and to find out proper management strategies to reduce post harvest losses so that the farmers get benefited through the use of the remedy. The available information on mango post harvest diseases is examined and presented here.

2.1 Pathogens Isolated from Diseased Fruits of Mango

2.1.1 Association of *Colletotrichum gloeosporioides*

Ranly *et al.* (1995) reported that *C. gloeosporioides* causing anthracnose of mango from South Florida.

Ekbote *et al*. (1997) reported that *C. gloeosporioides* causing anthracnose of mango from Karnataka in India, Akhtar *et al*. (1998) reported that *C. gloeosporioides* causing anthracnose of mango from Pakistan and Arauz (2000) reported that *C. gloeosporioides* causing anthracnose of mango from USA. Patil (2001) reported the association of *C. gloeosporioides* on mango fruits.

Peres *et al*. (2002) reported that *Colletotrichum* spp. cause anthracnose in various fruits post-harvest and are a particularly important problem in tropical and subtropical fruits. The disease in fruits of avocado, guava, papaya, mango and passion fruit has been reported to be caused by *C. gloeosporioides* and in banana by *C. musae*. In subtropical and temperate crops such apple, grape, peach and kiwi, the disease is caused by *C. acutatum*.

Prashanth (2007) described typical anthracnose symptoms on inflorescence, leaves and fruits as black to brown necrotic depressed spots appeared on the leaves with circular margin, in advanced stage, spots coalesced and resulted in bigger patches leading to defoliation. On fruits, brown spherical depressed spots occurred in scattered form on the pericarp and in advanced stage spots coalesced to form necrotic rotten patches.

Sangeetha and Rawal (2008) isolated *C. gloeosporioides* from infected mango leaves.

Jayasinghe and Fernando (2009) reported the association of the pathogen *C. gloeosporioides* with symptomatic mango leaves.

Awa *et al.* (2012) proved that mango (*Mangifera indica* L.) fruit rot caused by anthracnose is the most economically important postharvest disease limiting shelf life and export of fresh mango fruits in Nigeria. This study investigated the etiology, disease incidence and disease severity of mango fruit anthracnose in Southwestern Nigeria. The result of the investigation revealed that 96 isolates out of 231 fungi isolates recovered from symptomatic mango fruits were *Colletotrichum gloeosporioides* isolates based on their whitish orange colony, septated hyphae and capsule like appearance and pathogenicity test conducted. Other 14 fungi species encountered, accounted for 135 isolates.

Sharma and Kulshrestha (2015) reported that *Colletotrichum* species are present in both tropical and subtropical regions of the world. *C. gloeosporioides* is most important pathogen and belongs to order melanconiales. It is usually inactive in dry season but during favourable conditions it causes anthracnose disease to large number of economic crops amongst which mango anthracnose is important as far as losses caused by pathogen is concerned.

2.1.2 Association of *Alternaria alternata*

Ranly *et al*. (1995) reported that mango decline in South Florida was due to *A. alternata.*

Patil (2001) recorded association of *A. alternata* with diseased fruits of mango.

Kobiler *et al*. (2011) reported that black spot caused by *A. alternata* is the main post harvest factor that impairs the quality and reduces the storability of persimmon fruit (*Diospyros kaki* cv. triumph). The fungus infects the fruit in the orchard and remains quiescent until harvest.

Troncoso-Rojas and Tiznado-Hernandez (2014) studied that *A. alternata* causes black spot in many fruits and vegetables around the world. It is a latent fungus that develops during the cold storage of fruits, becoming visible during the marketing period thereby causing large post harvest losses.

2.1.3 Association of *Aspergillus niger*

Jadeja and Vaishnav (2000) observed the *A. niger* causing black rot was found to be major post harvest pathogen on ‘Kesar’ mango fruits.

Singh and Sumbali (2000) studied mycoflora on the surface of jujube fruits, among them *A. niger* was consistently recorded during the entire period of fruit development. Fifty isolates of *A. niger* isolated from the pre harvest fruits were observed to cause extensive post harvest rot of mature jujube when inoculated artificially.

Prabhakar *et al*. (2005) observed higher incidence of wound fungi such as *A. niger* (L.) Van, Tieghem and *Rhizopus arrizus* Fischer (0.90%) than other diseases *viz*., *Penicillium* and *Trichothecium* were the common and frequent.

Joshi and Vaidya (2007) isolated 17 generations and 19 species from deteriorating fruits and vegetables, out of these species *Aspergillus, Alternaria, Lasiodiplodia, Colletotrichum, Penicillium* and *Trichothecium* were the common and frequent.

Bhujbal (2008) reported the association of *A. niger* as a causal agent of post harvest diseases in grapes.

Bennett (2010) reported the characteristics of *A. niger*, the hyaline, septate mycelia with black colour conidia and the spore bearing structures.

Krishnapillai and Wilson Wijeratnam (2012) observed that in July 2009 approximately 10% of the harvested mangoes var. Ambalavi from home garden at Thirunevely, Shrilanka were observed with different symptoms light yellow colour suppressed lesions around the stem end region. The lesions increased in size resulting in depressed mesocarp and a soft rot condition. The centre of the lesion became sunken and was covered with brownish black spores of *Aspergillus*.

2.1.4 Association of *Lasiodiplodia theobromae*

Johnson *et al*. (1992) reported that the stem end rot pathogens of mango (*Dothiorella dominicana, Dothiorella mangiferae, Lasiodiplodia theobromae, Diplodia natalensis, Phomopsis mangiferae, Cytosphaera mangiferae, Pestalotiopsis spp*. and *Dothiorella* 'long'), as well as other fungi (including *Alternaria alternata*) were found to occur endophytically in the stem tissue of mango trees prior to inflorescence emergence.

Shelar (1994) reported *L. theobromae* causing dieback in mango.

Mascarenhas *et al*. (1996) isolated a major post harvest pathogen of mango (*Mangifera indica*) and identified as *L. theobromae*. Its morphological characteristics, growth profile and fruit spoilage potential were studied.

Dodd *et al*. (1997) reported that *L. theobromae* and *Dothiorella spp*. were responsible for stem end rot.

Kaur and Verma (2002) reported that during periodical survey of local fruit markets of Ludhiana and subsequent microscopic examination of the samples of diseased orange fruits drawn at regular interval revealed the prevalence of several rots *viz*., green mould rot (*P. digitatum*), stem end rot (*L. theobromae*) with maximum incidence of green mould on orange fruits.

Sanchez *et al*. (2013) studied stem end rot of fruits and dieback of branches in mango in Mexico and found that *L. theobromae* and *Neofusicoccum parvum, Neofusicoccum spp*. and *L. pseudotheobromae* were associated with the disease.

2.2 Isolation And Pathogenicity

Om Prakash and Raoof (1979) proved the pathogenicity of *L. theobromae* causing stem end rot disease of mango. Padule (1990) proved pathogenicity of *A. niger* on grapes.

Patil (2001) proved the pathogenicity of *C. gleosporioides* and *A. alternata* on mango fruits.

Venkataravanappa (2002) and Prashanth (2007) purified the culture by single spore isolation after isolating *C. gloeosporioides* from infected tissues of mango and pomegranate using two per cent water agar media.

Bangar (2003) and Bhujbal (2008) proved pathogenicity of *A. niger* on grape fruits.

Daniel *et al.* (2008) proved the pathogenicity of *A. alternata* on citrus fruits.

Shahbaz *et al*. (2009) conducted pathogenicity test of *L. theobromae* by stem inoculation method. The artificial inoculations were done by cutting a small flap on the basal portion of the mango stem and inserting a 5 mm potato dextrose agar piece containing viable culture of the fungus (*L. theobromae*). After 30 days, lesion development was measured distal to the point of inoculation. Reisolation were made from diseased tissue to confirm pathogenicity of the fungi.

Krishnapillai and Wilson Wijeratnam (2012) observed that Koch's postulates were satisfied by transferring mycelial discs of 5 mm diameter of *Aspergillus niger* from a 5 days old culture grown on PDA to the surface sterilized, wounded, mature Ambalavi mangoes. Non inoculated Ambalavi mangoes were kept as control. The control and the inoculated mangoes were kept at ambient temperature (30-34 °C) typical symptoms were developed in the inoculated mangoes within five to six days while there was no rotting observed in the control fruits. Koch's postulate were confirmed by reisolating pathogen from inoculated fruits.

Li *et al*. (2013) conducted a pathogenicity test of the five isolates in the field on healthy tissues. Five green twigs and five 3 year old branches were used. Three wounds were made on each twig or branch with a sterilized needle. Mycelial plugs were placed on wounds and covered with parafilm. Uncolonized PDA plugs were used as controls. Two weeks later, typical brown lesions were observed on inoculated branches and gum exuded from infected wounds. No symptoms were seen on the control. Koch's postulates were fulfilled by reisolation of *L. theobromae* from diseased branches.

Ajay Kumar (2014) described that *Colletotrichum* is one of the major plant pathogen causing anthracnose, a plant disease on variety of hosts from trees to grasses. Pathogenicity of *C. gloeosporioides* as plant pathogenic, saprophytic and endophytic fungi was also discussed.

Maqsood *et al.* (2014) reported that *in vitro* studies to identify fungal pathogens responsible for rotting and decaying mango fruits during storage along with isolation and testing their pathogenicity on healthy fruits. Results revealed that all selected commercial mango varieties infected by stem end rot.

2.3 Cross Inoculation Studies

Simmonds (1965) demonstrated that *C. gloeosporioides* isolates from fruit hosts could readily cross-infect over a wide host range, however isolates were more aggressive in attacking the host from which they were originally isolated. Isolates of *C. musae* from banana and *C. gloeosporioides* from mango displayed very restricted activity towards hosts other than those from which they originated.

Quimio and Quimio (1975) tested the pathogenicity of mango anthracnose *C. gloeosporioides* isolated from mango, citrus and papaya. Isolates proved to be cross pathogenic with varying degrees. *C. musae* only could infect banana but *Gloeosporium psidii* could infect mango, guava and banana as well *Glomerella cingulata* infected all aerial parts of mango except the bark of the main trunk. Mature leaves required wounding for infection. Infection progressed faster in wounded tissues and in ripe fruits. Lesions were first observed 12 hr after inoculation on wounded, ripe fruits as compared with 24 hr on wounded, unripe ones. On unwounded ripe and unripe fruits lesions appeared after 48 hr on the surface of intacted fruits, and penetration occurred after 24 hr in intact ripe fruit tissues and 48 hr in unripe one.

Bhat and Hedge (1987) indicated the possibility of physiological specialization from the cross inoculation test with *C. gloeosporioides* (*G. cingulata*) isolated from different fruit crops.

Lima Filho *et al*. (2003) cross infectivity studies were carried out by *Colletotrichum* spp. isolates from cashew, mango, papaya and passion fruit and *C. musae* from banana which produced necrotic and depressed lesions on fruits, except on passion fruit, which was susceptible to its isolates only. The isolates of *C. gloeosporioides* produced amylolytic, lypolytic, proteolytic, and cellulolytic enzymes, whereas *C. musae* did not produce any detectable cellulases.

Twumasi *et al.* (2014) reported that the cocoa, mango, banana and yam isolates of *L. theobromae* were found to be virulent with similar pathological effects in the experimental crops, that is cocoa and mango. It is concluded from this work that *L. theobromae* isolates from the four different crops, that is cocoa, mango, banana and yam in Ghana`s forest agricultural zone are infectious and have damaging effects on other neighbouring crops with economic consequences.

Archana *et al*. (2014) reported that the aim of the work was to analyse virulence nature of *C. gloeosporioides* in the differentiation of isolates obtained from mango fruits and their potential to cause disease in different varieties. From the survey twenty six isolates of *C. gloeosporioides* were isolated and identified using morphological characters. Among the five artificial inoculation methods tested, pinprick plus spore suspension spray was the best suitable method. By this method out of twenty six isolates used, MCG 16 was identified as virulent isolate based on lesion diameter, per cent disease incidence and virulence index produced on inoculated fruits.

2.4 Fungitoxicants

Prusky (1989) reported that the most dangerous diseases are: mango malformation, bacterial canker, mildew, anthracnose, stem end rot and Alternaria rot. Benzimidazole fungicides (benomyl and carbendazim) and sterol inhibitors (imazalil and prochloraz) have given excellent control of anthracnose and stem end rot.

Suseela Bhai *et al*. (2003) reported that *in vitro* studies using different fungicides against *Colletotrichum* spp. responsible for premature yellowing and bean shedding in vanilla showed that Thiophanate methyl even at very low concentration *i.e*. 100 ppm was highly inhibitory to the fungus followed by carbendazim (250 ppm) or carbendazim + mancozeb mixture (2000 ppm).

Ashoka (2005) tested seven systemic and four non- systemic fungicides at three concentrations under *in vitro* conditions against *C. gloeosporioides*. Systemic fungicides *viz*., bayleton, benomyl, prochloraz and Saaf (Combined formulations) were successful in completely (100%) inhibiting the growth of *C. gloeosporioides* at all three concentrations (0.025, 0.005 and 0.1%) whereas, non-systemic fungicide mancozeb was found to be effective in inhibiting the growth of fungus (77.65%).

Gud and Raut (2008) reported that thiophanate methyl and propiconazole were most effective against *C. gloeosporioides* followed by hexaconazole and carbendazim.

Prashant *et al.* (2008) studied four systemic fungicides and reported that maximum per cent inhibition of growth of *C. gloeosporioides* was observed in difenconazole (90.78%) and propiconazole (90.78%) followed by carbendazim (88.89%) while least per cent inhibition of fungus was recorded in iprobenfos (75.99%) at 0.1 per cent concentration.

MeiJiao *et al*. (2009) studied the efficacy of 23 fungicides against *L. theobromae*. The results revealed that spergon, propiconazole, flusilazole, prochloraz, iprodione, difenoconazole, tebuconazole, myclobutanil, pyraclostrobin, validamycin, carbendazim, chlorothalonil and mancozeb are effective for the management of *L. theobromae*.

Vinod (2009) reported that carbendazim was effective among all the tested chemicals and gave cent per cent mycelial inhibition of *C. gloeosporioides.*

Devamma *et al.* (2012) reported that among all the six fungicides evaluated against *C. gloeosporioides* the cause of mango anthracnose, the systemic fungicide thiophanate methyl and the non-systemic fungicide mancozeb (100%) proved to be effective in inhibiting the mycelial growth of the highly virulent pathogen at 50 ppm and 500 ppm concentrations, respectively.

Sahi *et al*. (2012) revealed the effectiveness of Topsin M and Daconil against the mycelial growth of *L. theobromae* and mancozeb was found least effective in inhibiting the mycelial growth of *L. theobromae*.

Kolase *et al*. (2014) reported that under *in vitro* studies carbendenzim (0.1%) was beneficial for inhibiting the growth of *C. gloeosporioides.*

Gupta *et al.* (2014) found that carbendazim and mancozeb were most effective at higher doses (1000 ppm) against *A. niger* under *in vitro* condition.

Singh and Singh (2006) found that carbendazim and mancozeb completely inhibited the fungal growth *in vitro* at higher concentration (1000ppm).

Suresh *et al*. (2016) revealed that among the 10 fungicides tested at different two concentrations (250 and 500 ppm) carbendazim, carbendazim + mancozeb and propiconazole, completely inhibited the growth of *L. theobromae* concentrations whereas pyraclostrobin was least effective.

2.5 Bioagents

The diseases of mango are generally controlled through several rounds of fungicide application, thereby generating disadvantages such as environmental contamination, development of resistance in pathogens and residual contamination in fruits. To combat this problem and to meet the demand for residue-free foods, identifying alternative methods has been under investigation over the recent past. One such alternative is the use of biocontrol agents, which have given promising results in control of post harvest diseases.

Biocontrol is an environmentally sound and effective means of reducing or mitigating diseases through the use of antagonistic microbes. Effective biocontrol agents offer great potential to develop alternative methods that are economical and suited for adoption by the small-scale mango industry.

Okigobo and Ikediugwu (2000) proved the antagonistic nature of *Trichoderma viride* to *A. niger, L. theobromae* and *P. axalicum*, causal organism of post harvest rot in yams.

Adekunle *et al.* (2001) reported that *Trichoderma harzianum* was efficient in control of several pathogens.

Bhuvaneshwari and Subba Rao (2001) reported that, mango fruits inoculated with *T. viride* remained free from the infections of *A. niger, A. flavus* and *L. theobromae* indicating their suppression by *T. viride*.

Prasanna Kumar (2001) reported that *T. viride* spore suspension was effective in reducing the post harvest diseases of mango.

Shirshikar (2002) reported the efficacy of *T. viride* spore suspension against post harvest diseases of mango.

Yadav and Mujumdar (2004) revealed that among three method of bacterial antagonist (*Bacillus subtilis*) inoculation maximum inhibition of mycelium growth of *L. theobromae* was obtained by flooding method compared to streaking and disc method.

Kota *et al*. (2006) revealed that *T. viride* spore suspension was very effective in reducing the post harvest diseases of mango caused by *A. niger* and *L.theobromae in vivo*.

Bhadraiah (2007) proved antagonistic nature of *T. viride* against *A. niger*, *A. fungatus, F. oxysporum* and *F. solani.*

Koomen and Jeffries (2007) screened 648 microorganisms, including bacteria, yeasts and filamentous fungi isolated from blossom, leaves and fruits of mango against *C. gloeosporioides*. Results yielded two potential candidates for further trials, isolate 204 (identified as *Bacillus cereus*) and isolate 558 (identified as *P. fluorescens*).

Nallathambi *et al*. (2009) described that isolates of *Trichoderma* species from hot arid regions, fungicides and their combinations were evaluated for the management of ber fruit rot at post-harvest stage. Out of 16 isolates of *Trichoderma* species, six isolates checked growth of mycelia of *A. alternata* by more than 55 per cent.

Saju *et al.* (2012) reported *Pseudomonas fluorescens, Bacillus subtilis* and *Trichoderma harzianum* showed more than 70 per cent inhibition in mycelial growth of *C. gloeosporioides*, the causative of blight in large cardamom.

Suhannaa *et al*. (2013) observed the 47 isolates of *Trichoderma spp*. against mango stem end rot caused by *L. theobromae*. They revealed that the isolates T46 and T9 exhibited maximum inhibition of growth of pathogen at day 6 (77.65%) and (87.45%) respectively.

Sahdev and Chaudhari (2015) reported that *T. viride* (sardarkrushinagar isolate) was observed as potential antagonist agent against *A. alternata* , followed by *T. viride* (Hyderabad) and *T. viride* (Anand). Ten different known antagonists were screened *in vitro* for their antagonism to *A. alternata* by dual culture method.

Konsue *et al.* (2020) described that select antagonistic yeasts for the control of fruit rot caused by *L. theobromae* and anthracnose caused by *C. gloeosporioides* in post harvest mango fruit, 307 yeast strains isolated from plant leaves were evaluated for their antagonistic activities against these two fungal pathogens *in vitro*.

3. MATERIALS AND METHODS

The present investigation entitled “Variability Studies in Post Harvest Pathogens Infecting Mango Fruits in Maharashtra” was carried out during 2019-2021, conducted at Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri. The details of the material used and methods followed during the present investigations are present in this chapter.

3.1 Materials

The following material was used during the present investigation.

3.1.1 Diseased and Healthy Mango Fruits

To study the different fungi responsible for various kinds of post harvest diseases of mango, matured ripe diseased and healthy fruits of mango were collected from local markets at Rahuri and Ahmednagar, Maharashtra.

3.1.2 Glasswares

Common standard glasswares used during investigation were petriplates, test tubes, glass slides, conical flasks and beakers of various capacities, glass rods, bowls, volumetric flasks, funnel, measuring cylinders of different capacities, pipettes, desiccator *etc*. were used for study.

3.1.3 Equipments

Common laboratory equipments used for laboratory experiments were BOD incubator, refrigerator, research microscope with microscopic camera, autoclave, electronic top pan balance, inoculation chamber, laminar air flow cabinet (LAF), physical balance, sprayer, centrifuge machine, mixer, *etc*.

3.1.4 Cultural Media

The common laboratory medium PDA was used for isolation of organisms responsible for the spoilage of mango fruits and cultures of isolated organisms were maintained on PDA slants for further investigation.

Potato Dextrose Agar (PDA)

i.	Potato (peeled)	200 gm
ii.	Dextrose	20 gm
iii.	Agar	20 gm
iv.	Distilled water	1000 ml

3.1.5 Chemicals

The chemicals used for preparation of media and laboratory use were obtained from Department of Plant Pathology and Agril. Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri.

Table 3.1 Fungicides Used for Sensitivity Study Against Post Harvest Pathogens.

Sr. No	Common name	Trade name	Active Ingredient	Source
1	Azoxystrobin 23 % SC	Amistar	Azoxystrobin	Sygenta Pvt.Ltd
2	Carbendazim 50 % WP	Bavistin	Carbendazim	BASF Pvt. Ltd.
3	Mancozeb 75 % WP	Dithane M-45	Mancozeb	Dow Pvt. Ltd.
4	Propineb 70 % WP	Antracol	Propineb	Bayer Pvt. Ltd.
5	Thiophanate methyl 70 % WP	Hexastop	Thiophanate methyl	Coromandal Pvt. Ltd.
6	Carbendazim 12 %+ Mancozeb 63 % WP	Saaf	Carbendazim+Mancozeb	UPL Pvt. Ltd.

3.1.6 Bioagents

The pure culture of *Trichoderma viride*, *Trichoderma harzianum,* yeast (*Saccharomyces cerevisiae), Pseudomonas fluorescens* and *Bacillus substilis* were obtained from the Culture Bank, Department of Plant Pathology and Agril. Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri were utilized for further experimental studies.

3.1.7 Miscellaneous Materials

These included non absorbent cotton, polypropylene and polyethylene bags, paper bags, rubber bands, mercuric chloride ($HgCl_2$), blotting paper, denatured spirit, ethyl alcohol, hand instruments (Scissor, cutting blade, inoculating needle, forcep, cork borer), spirit lamp, test tube stand, adhesive labels, glass marker *etc*.

3.2 Methodology

3.2.1 Collection of Diseased Samples

Diseased specimens of mango fruits were collected on the basis of symptoms from local markets of Rahuri and Ahmednagar in the Maharashtra state by personal visit.

3.2.2 Isolation of Pathogens Associated with Post Harvest Diseases of Mango

3.2.2.1 Glassware cleaning

During the experimental studies, glasswares were kept for a day in the cleaning solution containing 60g potassium dichromate ($K_2Cr_2O_7$), 60 ml of concentrated sulphuric acid (H_2SO_4) dissolved in one lit. of water. Then they were cleaned by washing with detergent soap solution followed by rinsing several times in tap water and finally with distilled water.

3.2.2.2 Sterilization

The glasswares and different media were sterilized in an autoclave at 1.1 kg/cm^2 pressure for 20 minutes.

3.2.2.3 Isolation of the pathogen

The microorganisms responsible for spoilage of mango fruits were isolated on PDA medium by Agar plate technique protocol from diseased fruits of mango collected from different markets of Rahuri and Ahmednagar. The infected portion of mango fruit was cut into small pieces. These pieces were disinfected by surface sterilization with 1:1000 mercuric chloride solution for one minute followed by three washing with sterilized water in order to remove traces of corrosive sublimate. These small pieces of infected mango fruits were transferred aseptically to sterilized Petri plates containing PDA medium (3 to 4 pieces/plates). These plates were then incubated at room temperature (27 ± 2 °C) for seven days. The plates were critically observed for the typical growth of the fungus. The fungus colonies showing different colouration and sporulation were separated and sub-cultured in separate plates by single spore isolation method. The fungal colonies were then transferred on PDA slants for further investigation.

3.2.3 Maintenance of Pure Culture

Pure culture of different fungal pathogens were obtained by following hyphal tip method. Isolated and purified pathogen was sub-cultured on potato dextrose agar slants and kept at 27 ± 2 °C for seven days for incubation in incubator. After observing the growth such slants were preserved in refrigerator, the isolates were sub-cultured once in month and used for further studies.

3.2.4 Identification of Isolates

Pathogens isolated from diseased samples of mango fruits were identified on the basis of morphological and conidial characters observed under microscope and on the basis of pathogenicity test (Barnett and Barry, 1972).

3.2.5 Pathogenicity

3.2.5.1 Inoculation

The pathogenicity of the pathogen obtained from diseased fruits was tested and established according to Koch’s postulates in experiment. Pathogenicity test of the isolated fungi was conducted by undertaking the inoculation experiments on healthy fruits of mango. These mango

fruits (healthy) were washed in sterilized water and disinfected with 1:1000 mercuric chloride solution for one minute and were immediately washed in sterile water to remove the traces of disinfectant. Fruits were slightly punctured at two to three equidistant points to create wounds of 1 mm diameter and about 1.5 mm deep, seven days old cultures of each isolate having good conidial and mycelial growth were used separately for preparation of spore suspension in sterile water. Already injured fruits were sprayed with this spore suspension with the hand atomizer or sprayer under aseptic conditions. After that fruits were allowed to dry.

After inoculation with test fungi by above method, moist sterile cotton wool was placed into the moist chamber to create maximum humidity to develop favourable environment for disease development condition. These inoculated fruits were kept separately into moist chamber for 8 days for the development of symptoms and the symptoms were critically examined daily after inoculation. The type of symptom expressed were recorded eight days after inoculation.

3.2.5.2 Reisolation

Reisolation of inoculated fungal pathogens were carried out from the artificially inoculated parts of the mango fruits *viz*., neck of the fruits, skin peel, pulp and tip of fruits on PDA medium. The fungi obtained were transferred and maintained separately on PDA slants for identification.

3.2.6 Cross Inoculation Studies of Post Harvest Pathogens Isolated from Mango Fruits

Host specificity study was undertaken in order to determine the host range of the pathogens within the host genera under study. The respective parts of each host fruit were inoculated with all isolates from various pathogens. Inoculations were in a similar fashion as described in the pathogenicity test. The degree of cross infectivity of each isolate on different hosts was confirmed by observing symptoms.

The observations on latent period and severity of lesion development on fruits of banana, sweet orange and guava were recorded and average virulence index of each isolates on different fruits was determined. The data obtained were subjected to statistical analysis by Factorised Completely Randomised Design (FCRD).

The virulence index (VI) was calculated by modifying the formula of Mathur *et al*. (2001) to suit the requirement at present studies. The formula is based on the lesion diameter and the invasion of the pathogen. The invasion index (I) was rated as given in the Table 3.2.

The virulence index (VI) is given as :

VI = 3.14 X A/2 X I X L

Where, A = Aggressiveness (lesion diameter in mm)

I = Invasion index

L = Latent period in days (10)

3.14 = Area constant

The pathological variation within the isolates of sweet orange, banana and guava was studied as sufficient number of isolates obtained from these hosts.

Table 3.2 Ratings for Invasion Index (I)

Rating	Invasion Index
1	Necrotic lesions developing superficially on the fruit peel/ skin
2	Necrotic lesions completely invading the peel/skin extending to the inner side of the peel and touching the pulp or aerials
3	Invasion of the fungus in the pulp / aerials and causing its discoloration
4	Invasion deep seated reaching to the stone/heart of the fruit initiating the rotting symptoms

Inoculated fruits were kept in the humid chamber for 10 days. The temperature at 28 °C and 90 per cent relative humidity was maintained inside the humid chamber throughout the experimentation. Mango fruits were also inoculated with each isolated culture. Intensity of disease was recorded ten days after inoculation. The virulence index (VI) was calculated as per the given formula. The data obtained was subjected to statistical analysis. The statistical analysis was carried out by factorial completely randomized design (FCRD).

3.2.7 Sensitivity of Pathogens Against Fungicides

Experimental Details:

For laboratory condition Design- CRD

Fungicide treatment – i. Treatments- 7

ii. Replication- 3

Fungicide name	Concentration(%)
Azoxystrobin 23 % SC	0.04 %
Carbendazim 50 % WP	0.1 %
Mancozeb 75 % WP	0.25 %
Propineb 70 % WP	0.2 %
Thiophanate methyl 70 % WP	0.1 %
Carbendazim 12 % + Mancozeb 63 % WP	0.15 %

The experiment was conducted to study the sensitivity of isolates of post harvest pathogens of mango collected from local markets of Rahuri and Ahmednagar in Maharashtra. Sensitivity of these isolates to carbendazim and mancozeb commonly used fungicides was tested by farmers. The pathogen was grown on potato dextrose agar medium prior to the setting of the experiment.

The fungicide suspension was made by adding required quantity of fungicides to the melted potato dextrose agar medium to obtain the desired concentration on the basis of active ingredient

present in the chemical. 20 ml of poisoned medium was poured into each sterilized petriplate and suitable checks were maintained without addition of fungicides. 5 mm of ten days old fungal disc was taken from the periphery of the culture and was placed in the centre of the poisoned medium aseptically and incubated at 28 °C for seven days. Three replications were maintained for each treatment and the diameter of the colony was measured in two directions and the average was recorded after incubation for seven days. Per cent inhibition of the fungus was calculated by using the formula suggested by Arora and Upadhyay (1978).

$$\% \text{ Growth Inhibition} = \frac{\text{Colony growth in control plate} - \text{Colony growth in interacting plate}}{\text{Colony growth in control plate (mm)}} \times 100$$

Based on mean radial growth, the isolates were classified as highly sensitive, sensitive, moderately resistant, resistant and highly resistant to each fungicide as given below:

Class	Per cent inhibition over control
Highly sensitive	> 90
Sensitive	> 80 – 90
Moderately resistant	> 70– 80
Less sensitive	> 50 – 70
Non sensitive	< 50

3.2.8 Sensitivity of Pathogens Against Bioagents

Bioagent treatment - i. Treatments - 6
ii. Replication – 3

Table 3.3 Bioagents used for treatment

Sr. No.	Bioagents
1	*Trichoderma harzianum*
2	*Trichoderma viride*
3	Yeast (*Saccharomyces cerevisiae*)
4	*Bacillus subtilis*
5	*Pseudomonas fluorescens*

Bioagents – *Trichoderma viride* and *Trichoderma harzianum.*

The biological agents *i.e*. *Trichoderma viride* and *T. harzianum* were tested for their antagonistic properties against fruit rotting fungi by “Direct Bit Placement Method” (Broadbent *et al*. 1971).

The 1000 ml PDA (6.5 pH) was prepared, sterilized in autoclave at 1.05 kg/cm^2 pressure for 15 minutes and 50 plates were poured. Each pathogen was replicated 3 times separately for both the antagonists *i.e. T. viride* and *T. harzianum.*

Dual culture inoculation method was followed in which seven days old cultures of *C. gloeosporioides, A. alternata, A. niger, L. theobromae, T. viride* and *T. harzianum* were used. Culture discs of 5 mm of each of the antagonists potential and target pathogens were taken with the help of sterilized cork borer and transferred to 90 mm diameter PDA culture plates. The 5 mm disc of antagonists and target pathogens was placed opposite to each other *i.e.*1 cm away from the edges at equidistance. The Petri plate inoculated with disc of test pathogen alone served as control. The inoculated plates were then incubated at 27 ± 2 °C in BOD incubator for seven days. The radial mycelial growth of the test pathogen was measured to assess the antagonistic potential of *Trichoderma spp*. against test pathogens.

The per cent growth inhibition of pathogens in presence of antagonists was calculated as per the formula outlined by Arora and Upadhyay (1978).

$$\% \text{ Growth Inhibition} = \frac{\text{Colony growth in control plate} - \text{Colony growth in interacting plate}}{\text{Colony growth in control plate (mm)}} \times 100$$

Bioagents – Yeast (*Saccharomyces spp.), Pseudomonas fluorescens* and *Bacillus subtilis.*

Dual culture inoculation method was followed in which seven days old cultures of *C. gloeosporioides, A. alternata, L. theobromae* and *A. niger* grown on PDA and two days old culture of yeast *S. cerevisiae* grown on yeast extract peptone agar medium and *P. fluorescens* and *B. subtilis* grown on nutrient agar medium were used in this experiment.

A mycelial disc of 5 mm of each of the four isolated pathogen was cut from colony margin with the help of sterilized 5 mm cork borer and placed into the centre of a Petri dish containing PDA separately. Then strains of *S. cerevisiae, P. fluorescens* and *B. subtilis* were streaked circular, zig-zag around the pathogen disc at the centre of PDA plate. Plates containing test pathogen without these bioagents served as control. Then inoculated plates were incubated at 27 ± 2 °C in BOD incubator for seven days and observations were recorded 2, 4 and 7 days after inoculation.

3.3 Statistical Analysis

Result of the all experimental treatments will be statistically analysed by Completely Randomized Design (CRD) using analysis of variance (ANOVA).To compare different numerical observations, the data was statistically analysed by using the appropriate statistical methods (Panse and Sukhatme, 1978).

4. EXPERIMENTAL RESULTS

Mango (*Mangifera indica* L.) is one of the most significant economically important fruit crops in India and is regarded as the "King of Fruits." It suffers from significant post-harvest losses with fungal infections being one of the major causes of high losses in Maharashtra and India. During the course of the laboratory experiment a series of experiments were carried out to investigate the variability of post harvest pathogens infecting mango fruits in Maharashtra.

The study focused on mango post harvest infections which have a significant role in the spoilage of mango fruits during storage and transportation. Mango fruits are highly perishable, makes them susceptible to spoiling after harvest. The pathogens associated with the deterioration of mango fruits after harvest were studied *in vitro*. The observations recorded and the results obtained are as follows.

4.1 Collection of Diseased Mango Fruits

The infected mango fruits on the basis of symptoms like small circular spots on the fruits, suppressed lesions around the stem region used in this study were collected from local markets of Ahmednagar and Rahuri.

4.2 Isolation of Post Harvest Pathogens Infecting Mango Fruits

Over the course of three months, the following four isolates of fungal pathogens were obtained from infected mango (*Mangifera indica* L.) fruits collected from fruit markets of Rahuri and Ahmednagar (Plate 2).

1) *Colletotrichum gloeosporioides* Penz.

2) *Alternaria alternata* Groves and Skolko

3) *Aspergillus niger*

4) *Lasiodiplodia theobromae* Pat.

The results obtained are in confirmation with the results obtained by Joshi and Vaidya (2007) who isolated 17 genera and 19 species from deteriorating fruits and vegetables, out of these species *Aspergillus, Alternaria, Lasiodiplodia, Colletotrichum, Penicillium* and *Trichothecium* were the most common and frequent.

Several workers have reported the pathogens associated with the post harvest diseases of mango. Shelar (1994), Mascarenhas *et al*. (1996), Jadeja and Vaishnav (2000), Patil (2001), Prashanth (2007), Bhujbal (2008) and Jayasinghe and Fernando (2009) had pointed out the association of fungal pathogens like *Penicillium, Aspergillus, Fusarium, Colletotrichum, Alternaria* and *Lasiodiplodia* responsible for spoilage of mango fruits.

4.3 Identification of the Cultures

The pathogenic fungi which were isolated from the rotted mango fruits were identified as

1. *Colletotrichum gloeosporioides* Penz.
2. *Alternaria alternata* Groves and Skolko
3. *Aspergillus niger*
4. *Lasiodiplodia theobromae* Pat.

The pathogenic fungi identified on the basis of morphology and pathogenicity test carried out at Department of Plant Pathology and Agril. Microbiology, MPKV, Rahuri.

4.4 Purification of the fungal culture

The fungal culture of isolated pathogen was purified and pure culture of isolated pathogens were maintained for carrying out investigation. The hyphal tip of isolated fungus grown on PDA was cut and shifted to slant containing PDA as basal medium and kept for incubation. After incubation this slant were transferred to refrigerator and sub-cultured at regular interval of time to study pathogen variability of spores.

4.5 Pathogenicity

Mango fruits were artificially inoculated with the pathogens that would have previously been isolated from infected fruits. All of the pathogens were found to be pathogenic and symptoms showed on the fruits within 3-6 days of inoculation which was as similar to symptoms observed on infected mango fruits (Plate 3).

Patil (2001) proved the pathogenicity of *C. gloeosporioides, A. alternata, F.oxysporum* and *C. herbarum* on mango fruits. Om Prakash and Raoof (1979) proved the pathogenicity of *L. theobromae* causing stem end rot disease of mango. Padule (1990) proved pathogenicity of *A. niger* on grape fruits. The results obtained are also in agreement with Bangar (2003) and Bhujbal (2008), Ajay Kumar (2014) and Maqsood *et al.* (2014).

4.6 Cross Infectivity Potential of Isolated Pathogens

The perusal of the Table 4.1 revealed that, the *A. alternata* possessed the highest degree of infectivity. The average mean virulence index was 3.28 and was maximum among all the pathogens. *C. gloeosporioides* possessed highest degree of infectivity on guava fruit (mean VI 5.23) and followed by banana (mean VI 1.66) and sweet orange(mean VI 0.96).While *A. alternata* showed greater infectivity on guava fruits(mean VI 6.63) and then less infectivity on sweet orange (mean VI 1.46) and banana (mean VI 1.76). Isolates *C. gloeosporioides* and *A. alternata* , these are referred as highly virulent pathogens of guava fruits.

The *L. theobromae* showed greater infectivity on guava fruits(mean VI 5.94) and then less infectivity on sweet orange (mean VI 1.74) and banana (mean VI 0.98) These are grouped as virulent isolates. The third group designated as moderately virulent isolates, showed very close

linkage with respect to virulence index. *A. niger* possessed high infectivity on guava fruits (mean VI 5.03) and then less infectivity sweet orange (mean VI 1.45) and banana (mean VI 1.20).

Quimio and Quimio (1975) tested the pathogenicity of mango anthracnose *C. gloeosporioides* isolated from mango, citrus and papaya.

These result are confirmed with Simmonds (1965), Bhat and Hedge (1987), Lima Filho *et al.* (2003) and Twumasi *et al.* (2014).

Table 4.1 Variation in Virulence index of isolates and its cross infectivity potential on different fruits

Virulence Index					
Isolates	**Sweet orange**	**Guava**	**Banana**	**Mean**	
Colletotrichum gloeosporioides	0.96	5.23	1.66	2.61	
Alternaria alternata	1.46	6.63	1.76	3.28	
Lasiodiplodia theobromae	1.74	5.94	0.98	2.89	
Aspergillus niger	1.45	5.03	1.20	2.56	
Mean	1.40	5.71	1.40	2.83	
				S.E.(m)±	**CD @ 1%**
Isolates				0.05	0.16
Host fruits				0.04	0.12
Isolates x Host fruits				0.16	0.47

4.7 Evaluation of Fungicides

4.7.1 *Colletotrichum gloeosporioides*

The data presented in Table 4.2 revealed that, among the six fungicides maximum 100 per cent and 98.33 per cent inhibition of mycelial growth was recorded in carbendazim 12%+ mancozeb 63 % WP and thiophanate methyl 70 % WP respectively. It was followed by carbendazim 50 % WP (95.37%), mancozeb 75 % WP (83.89%), azoxystrobin 23% SC (80.96%) and propineb 70 %WP (78.51%) (Fig.1 and Plate 4).

All fungicides were found effective in inhibiting the growth of test pathogen. No mycelial growth of test pathogen was observed in carbendazim + mancozeb (00 mm), the next effective treatments were thiophanate methyl (1.50 mm) and carbendazim (4.16 mm) which were found superior and most effective treatments followed by mancozeb (14.50 mm) with mycelial growth of pathogen followed by azoxystrobin (17.13 mm) and propineb (19.33 mm) which were least effective treatments. Among the different fungicides evaluated azoxystrobin and propineb were found comparatively less effective against *C. gloeosporioides* with maximum mycelial growth of test pathogen. (Table 4.2, Plate 4 and Fig 1).

Suseela Bhai *et al*.(2003) reported that thiophanate methyl even at very low concentration *i.e.* 100 ppm was highly inhibitory to the fungus followed by carbendazim (250 ppm) or carbendazim + mancozeb mixture (2000 ppm).

The results obtained during present investigation are in confirmation with results reported by earlier scientists *viz*., Ashoka (2005), Gud and Raut (2008), Prashant *et al.* (2008), Vinod *et al.* (2009), Devamma *et al.* (2012) and Kolase *et al*. (2014).

Table 4.2 *In vitro* evaluation of fungicides against *Colletotrichum gloeosporiodes*

Tr. No.	Treatments	Conc.(%)	Mean Colony diameter(mm)	Per cent inhibition rate
T_1	Azoxystrobin 23 % SC	0.04 %	17.13 (24.43)	80.96
T_2	Carbendazim 50 % WP	0.1 %	4.16 (11.74)	95.37
T_3	Mancozeb 75 % WP	0.25 %	14.50 (22.36)	83.89
T_4	Propineb 70 % WP	0.2 %	19.33 (26.07)	78.51
T_5	Thiophanate methyl 70 % WP	0.1 %	1.50 (6.97)	98.33
T_6	Carbendazim 12 % + Mancozeb 63 % WP	0.15 %	00 (00)	100
T_7	Control	-	90 (71.54)	
		S.E(m)±	0.49	
		CD @ 1%	1.51	

***Mean of three replications; Figures in parenthesis are arc sine transformed values.**

4.7.2 *Alternaria alternata*

The data presented in Table 4.3 showed that among six fungicides tested against the pathogen, carbendazim 12 % + mancozeb 63 % WP inhibited maximum growth of the pathogen which was 98.33 per cent followed by carbendazim 50 % WP (97.41%) which was statistically at par with most significant and superior treatment in carbendazim 12 % + mancozeb 63 % WP, thiophanate methyl 70 % WP (95.74%), mancozeb 75 % WP (85.37%), azoxystrobin 23 % SC (81.67%) and propineb 70%WP (78.67%) (Fig. 2 and Plate 5).

The radial mycelial growth of pathogen ranged from 1.50 mm to 19.20 mm over the control. The different fungicides were found effective least mycelial growth of test pathogen was observed with carbendazim + mancozeb (1.50 mm), carbendazim (2.33 mm) and thiophanate methyl (3.83 mm) which was found most effective and superior over the other fungicides followed by mancozeb (13.17 mm) also found effective followed by azoxystrobin (16.50 mm) and propineb (19.20 mm) had maximum mycelial growth of pathogen compared other fungicides. Amongst different fungicides tested against *A. alternata* carbendazim, carbendazim + mancozeb, thiophanate methyl were found most effective treatment with least mycelial growth of pathogen and mancozeb was comparatively less effective with moderate mycelial growth of pathogen. Azoxystrobin and propineb were found least effective fungicide which showed maximum mycelial growth of test pathogen. (Table 4.3, Plate 5 and Fig 2).

Table 4.3 *In vitro* evaluation of fungicides against *Alternaria alternata*

Tr. No.	Treatments	Conc.(%)	Mean Colony diameter(mm)	Per cent inhibition rate
T_1	Azoxystrobin 23 % SC	0.04 %	16.50 (23.95)	81.67
T_2	Carbendazim 50 % WP	0.1 %	2.33 (8.70)	97.41
T_3	Mancozeb 75 % WP	0.25 %	13.17 (21.26)	85.37
T_4	Propineb 70 % WP	0.2 %	19.20 (25.97)	78.67
T_5	Thiophanate methyl 70 % WP	0.1 %	3.83 (11.25)	95.74
T_6	Carbendazim 12 % + Mancozeb 63 % WP	0.15 %	1.50 (6.97)	98.33
T_7	Control	-	90 (71.54)	
		S.E(m)±	0.57	
		CD @ 1%	1.76	

***Mean of three replications; Figures in parenthesis are arc sine transformed values.**

4.7.3 *Aspergillus niger*

The data presented in the Table 4.4 showed that among the six fungicides used against the *A. niger* maximum inhibition of mycelial growth was showed in carbendazim 12 % + mancozeb 63 % WP (98.33%), carbendazim 50 % WP (97.41%) and thiophanate methyl 70 % WP (96.44%), followed by mancozeb 75 % WP (83.22%), azoxystrobin 23 % SC (82.96 %) and propineb 70 % WP (82.26%) (Fig. 3 and Plate 6).

The radial mycelial growth of the test pathogen *A. niger* was recorded and it was ranged from 1.50 mm to 15.96 mm over the control (90 mm). All fungicides were found effective in inhibiting the growth of test pathogen. Least mycelial growth of test pathogen was observed with carbendazim + mancozeb (1.50 mm) found superior and most effective treatments and the next effective treatments were carbendazim (2.33 mm) and thiophanate methyl (3.20 mm) which were at par with the treatment of carbendazim + mancozeb. The next effective treatment was mancozeb (15.10 mm) mycelial growth of pathogen followed by azoxystrobin (15.33 mm) and propineb (15.96 mm) which were least effective treatments. Among the different fungicides evaluated azoxystrobin and propineb were found comparatively less effective against *A. niger* with maximum mycelial growth of test pathogen. (Table 4.4, Plate 6 and Fig 3).

Gupta *et al.* (2014) found that carbendazim and mancozeb were most effective at higher doses (1000 ppm) against *A. niger* under *in vitro* condition.

Table 4.4 *In vitro* evaluation of fungicides against *Aspergillus niger*

Tr. No.	Treatments	Conc.(%)	Mean Colony diameter(mm)	Per cent inhibition rate
T_1	Azoxystrobin 23%SC	0.04 %	15.33 (23.04)	82.96
T_2	Carbendazim 50 %WP	0.1 %	2.33 (8.65)	97.41
T_3	Mancozeb 75 % WP	0.25 %	15.10 (22.85)	83.22
T_4	Propineb 70 % WP	0.2 %	15.96 (23.54)	82.26
T_5	Thiophanate methyl 70 % WP	0.1 %	3.20 (10.20)	96.44
T_6	Carbendazim 12 % + Mancozeb 63 % WP	0.15 %	1.50 (6.97)	98.33
T_7	Control	-	90 (71.54)	
		S.E(m)±	0.69	
		CD @ 1%	2.11	

***Mean of three replications; Figures in parenthesis are arc sine transformed values.**

4.7.4 *Lasiodiplodia theobromae*

With the reference to data presented in Table 4.5 , it was revealed that among all the six fungicides tested maximum control of mycelial growth of the pathogen was recorded in carbendazim 12 % + mancozeb 63 % WP (98.15%) and carbendazim 50 % WP (97.78%) which was statistically at par with most significant and superior treatment carbendazim 12 % + mancozeb 63 % WP. It was followed by thiophanate methyl 70 % WP (95.41%), mancozeb 75 % WP (86.30%), azoxystrobin 23 % SC (84.07%) and propineb 70 % WP (82.78%) (Fig. 4 and Plate 7).

The radial mycelial growth of *L. theobromae* ranged from 1.67 mm to 15.50 mm over the control. Different fungicides were found effective for management of pathogen. The least mycelial growth of test pathogen was observed in the treatment of carbendazim + mancozeb (1.67mm), carbendazim (2.00 mm) and thiophanate methyl (4.13 mm) which was found most effective and superior over the other fungicides followed by mancozeb (12.33 mm), azoxystrobin (14.33 mm) and propineb (15.50 mm). Amongst different fungicides tested against *L. theobromae* carbendazim, carbendazim + mancozeb and thiophanate methyl were found most effective treatment with least mycelial growth of pathogen and mancozeb was comparatively less effective with moderate mycelial growth of pathogen. Azoxystrobin and propineb were found least effective fungicide which showed maximum mycelial growth of test pathogen *L. theobromae* (Table 4.5, Plate 7 and Fig 4).

The result of present investigation is in consonance with those results reported by previous workers as Prusky (1989) and Suresh *et al*. (2016).

Table 4.5 *In vitro* evaluation of fungicides against *Lasiodiplodia theobromae*

Tr. No.	Treatments	Conc.(%)	Mean Colony diameter(mm)	Per cent inhibition rate
T_1	Azoxystrobin 23%SC	0.04 %	14.33 (22.24)	84.07
T_2	Carbendazim 50 %WP	0.1 %	2.00 (8.08)	97.78
T_3	Mancozeb 75 % WP	0.25 %	12.33 (20.54)	86.30
T_4	Propineb 70 % WP	0.2 %	15.50 (23.16)	82.78
T_5	Thiophanate methyl 70 % WP	0.1 %	4.13 (11.73)	95.41
T_6	Carbendazim12%+ Mancozeb 63 % WP	0.15 %	1.67 (7.40)	98.15
T_7	Control	-	90 (71.54)	
		S.E(m)±	0.42	
		CD @ 1%	1.28	

***Mean of three replications;Figures in parenthesis are arc sine transformed values.**

4.8 Evaluation of Bioagents

4.8.1 *Colletotrichum gloeosporioides*

Five bio control agents were evaluated (*Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Bacillus subtillis*, *Saccharomyces cerevisiae*) to check their efficacy against *C. gloeosporioides* under *in vitro* condition by adopting dual culture method. Significantly maximum per cent growth inhibition was recorded with *T. viride* (75.91%) which found superior and best antagonist against *C. gloeosporioides.*

The next best treatment was *Trichoderma harzianum* (75.30%) per cent inhibition followed to this *S. cerevisiae* (55.94 %), bacterial antagonist *P. fluorescens* found superior over *B. subtillis* with per cent growth inhibition 51.80 % and 50.58 % respectively. (Fig. 5 and Plate 8).

From the five antagonistic bioagents treatment, *T. viride* was found superior and most effective in mycelial inhibition of pathogen and recorded minimum mycelial growth (21.67 mm) followed by *T. harzianum* with mean colony diameter (22.22 mm), Yeast (*S. cerevisiae*) (39.65 mm), *P. fluorescens* (43.37 mm) and *B. subtillis* (44.47 mm). Amongst the five bio control agents tested, *T. viride* found significant as it had less mycelial growth of pathogen followed by *T. harzianum* was found second best antagonist which was statistically at par with most significant and superior treatment *T. viride* , next to this *S. cerevisiae* found superior followed by *P. fluorescens* and *B. subtillis* which was least effective (Table 4.6 and Plate 8).

Table 4.6 Efficacy of bioagents against *Colletotrichum gloeosporiodes*

Tr. No.	Treatments	Mean colony diameter(mm)	Per cent inhibition rate
T_1	*Trichoderma harzianum*	22.22 (28.11)	75.30
T_2	*Trichoderma viridae*	21.67 (27.68)	75.91
T_3	Yeast (*Saccharomyces cerevisiae*)	39.65 (39.01)	55.94
T_4	*Bacillus subtilis*	44.47 (41.81)	50.58
T_5	*Pseudomonas fluorescens*	43.37 (41.17)	51.80
T_6	Untreated control	90 (71.54)	
	S.E(m)±	0.55	
	CD @ 1%	1.64	

***Mean of four replications; Figures in parenthesis are arc sine transformed values.**

4.8.2 *Alternaria alternata*

With the reference to data presented in Table 4.7, it was revealed that significantly maximum per cent growth inhibition of *A. alternata* was recorded with *T. viride* (65.19%) which was found superior and best antagonist followed by *T. harzianum* (61.83%), yeast (*S. cerevisiae*) (54.50%), bacterial antagonist *P. fluorescens* (50.80%) found superior over *B. subtilis* (48.86%).

Trichoderma viride was found superior and most effective in mycelial inhibition of pathogen and recorded minimum mycelial growth (31.32 mm) followed by *T. harzianum* with mean colony diameter (34.35 mm), Yeast (*S. cerevisiae*) (40.95 mm), *P. fluorescens* (44.28 mm) and *B. subtillis* (46.02 mm). Amongst the five bio control agents tested, *T. viride* found significant as it had less mycelial growth of pathogen followed by *T. harzianum* was found second best antagonist. next to this *S. cerevisiae* found superior followed by *P. fluorescens* and *B. subtilis* which was least effective (Table 4.7 and Plate 9).

Table 4.7 Efficacy of bioagents against *Alternaria alternata*

Tr. No.	Treatments	Mean Colony diameter(mm)	Per cent inhibition rate
T_1	*Trichoderma harzianum*	34.35 (35.86)	61.83
T_2	*Trichoderma viride*	31.32 (34.01)	65.19
T_3	Yeast (*Saccharomyces cerevisiae*)	40.95 (39.76)	54.50
T_4	*Bacillus substilis*	46.02 (42.70)	48.86
T_5	*Pseudomonas fluorescens*	44.28 (41.69)	50.80
T_6	Untreated control	90 (71.54)	
	S.E(m)±	0.24	
	CD @ 1%	0.72	

***Mean of four replications; Figures in parenthesis are arc sine transformed values.**

4.8.3 *Aspergilllus niger*

The data presented in Table 4.8 showed that among five bioagents tested against the pathogen, significantly maximum per cent growth inhibition was recorded in the treatment of *T. viride* (49.50%) followed by *T. harzianum* (45.30%) per cent inhibition followed to this *S. cerevisiae* (45.11%), bacterial antagonist *P. fluorescens* (41.91%) and *B. subtilis* (39.36%) (Fig. 7 and Plate 10).

From the five antagonistic bioagents treatment, *T. viride* was found superior and most effective in mycelial inhibition of pathogen and recorded minimum mycelial growth (45.45 mm) followed by *T. harzianum* (49.22 mm), Yeast (*S. cerevisiae*) (49.40mm), *P. fluorescens* (52.27 mm) and *B. subtillis* (54.57 mm). Amongst the five bio control agents tested, *T. viride* found significant as it had less mycelial growth of pathogen followed by *T. harzianum*, *S. cerevisiae*, *P. fluorescens* and *B. subtilis* which was least effective (Table 4.8 and Plate 10).

Table 4.8 Efficacy of bioagents against *Aspergillus niger*

Tr. No.	Treatments	Mean Colony diameter(mm)	Per cent inhibition rate
T_1	*Trichoderma harzianum*	49.22 (44.53)	45.30
T_2	*Trichoderma viridae*	45.45 (42.37)	49.50
T_3	Yeast (*Saccharomyces cerevisiae*)	49.40 (44.63)	45.11
T_4	*Bacillus substilis*	54.57 (47.60)	39.36
T_5	*Pseudomonas fluorescens*	52.27 (46.28)	41.91
T_6	Untreated control	90 (71.54)	
	S.E(m)±	0.19	
	CD @ 1%	0.57	

***Mean of four replications; Figures in parenthesis are arc sine transformed values.**

4.8.4 *Lasiodiplodia theobromae*

With the reference to data presented in Table 4.9, it was revealed that significantly maximum per cent growth inhibition of *L. theobromae* was recorded in the treatment of *T. viride* (63.94%) which was found superior and best antagonist followed by *T. harzianum* (60.00%), yeast (*S. cerevisiae)* (52.11%), Bacterial antagonist *P. fluorescens* (44.44%) found superior over *B. subtilis* (37.63%).

The *T. viride* was found superior and most effective in mycelial inhibition of pathogen and recorded minimum mycelial growth (32.45 mm) followed by *T. harzianum* with mean colony diameter (36.00 mm), Yeast (43.10 mm), *P. fluorescens* (50 mm) and *B. subtillis* (56.12 mm). Amongst the five bio control agents tested, *T. viride* found significant as it had less mycelial growth of pathogen followed by *T. harzianum* was found second best antagonist. next to this *S. cerevisiae* found superior followed by *P. fluorescens* and *B. subtilis* which was least effective (Table 4.9 and Plate 11).

Table 4.9 Efficacy of bioagents against *Lasiodiplodia theobromae*

Tr. No.	Treatments	Mean Colony diameter (mm)	Per cent inhibition rate
T_1	*Trichoderma harzianum*	36.00 (36.85)	60.00
T_2	*Trichoderma viride*	32.45 (34.71)	63.94
T_3	Yeast (*Saccharomyces cerevisiae*)	43.10 (41.07)	52.11
T_4	*Bacillus substilis*	56.12 (48.49)	37.63
T_5	*Pseudomonas fluorescens*	50.00 (44.98)	44.44
T_6	Untreated control	90 (71.54)	
	S.E(m)±	0.24	
	CD @ 5%	0.72	

***Mean of three replications; Figures in parenthesis are arc sine transformed values.**

Adekunle *et al.* (2001) reported that *Trichoderma harzianum* was efficient in control of several pathogens.

Bhuvaneshwari and Subba Rao (2001) reported that 70 mango fruits incubated with *T. viride* remained free from infection of *A. niger, A. flavus* and *L. theobromae* indicating their suppression by *T. viride*.

Konsue *et al.* (2020) described that select antagonistic yeasts for the control of fruit rot caused by *L. theobromae* and anthracnose caused by *C. gloeosporioides* in post harvest mango fruit, 307 yeast strains isolated from plant leaves were evaluated for their antagonistic activities against these two fungal pathogens *in vitro*.

The result obtained is in consonance with those results reported by previous workers *viz*., Okigobo and Ikediugwu (2000), Adekunle *et al.* (2001), Prasanna Kumar (2001), Shirshikar (2002), Bhadraiah (2007), Koomen and Jeffries (2007), Saju *et al.* (2012), Konsue, *et al.* (2020).

5. SUMMARY AND CONCLUSION

Mango is one of the most important crop in India. Though the India is second largest producer of fruits, neither the Indians are able to consume the required quantity of fruits for healthy diet nor they are able to grab their share in the international market in proportion to their production. One of the main reasons for this is the losses of fruits due to post harvest diseases and is reducing its quality and quantity. The present investigation entitled "Variability studies in post harvest pathogens infecting mango fruits in Maharashtra" was undertaken with a view to study these diseases with different aspects like identification, isolation of pathogens, pathogenicity, cross infectivity potential on various host fruits, effect of fungicides and the effect of bioagents. The results obtained during the study are summarized as below:

1. Isolation and Identification of Isolated Pathogens

Isolation of the causal fungi was made from the diseased fruits of mango from which four fungal cultures were obtained. The identifications of fungal cultures is done on the basis of morphological characters and pathogenicity test carried out at Department of Plant Pathology and Agril. Microbiology, MPKV, Rahuri, Dist. Ahmednagar.

The four fungal cultures isolated are :

1) *Colletotrichum gloeosporioides* Penz.

2) *Alternaria alternata* Groves and Skolko

3) *Aspergillus niger*

4) *Lasiodiplodia theobromae* Pat.

2. Pathogenicity

In this study, mango fruits were artificially inoculated with the pathogen that would have previously been isolated from infected fruits. All of the pathogens were found to be positive and symptoms showed on the fruits within 3-6 days after inoculation. The pathogen was reisolated from artificially inoculated diseased mango fruits that showed typical symptoms, and the reisolated pathogen was cultured on PDA media and produced a fungal culture that was identical to the original isolated test pathogen.

3. Cross Inoculation Potential of Isolates on Various Host Fruits

Cross inoculation potential of pathogens on various fruits *viz*., sweet orange , guava and banana were studied. When isolates of pathogens were inoculated on three different fruit hosts it was observed that there was maximum diversity with respect to host preference and the degree of infection to a particular fruit type. *Colletotrichum gloeosporioides* and *Alternaria alternata* possessed highest degree of infectivity on guava fruit (VI 5.23 and VI 6.63 respectively) followed by banana (VI 1.66 and VI 1.76 respectively) and sweet orange(VI 0.96 and VI 1.46 respectively). While *Lasiodiplodia theobromae* and *Aspergillus niger* showed greater infectivity on guava

fruits(VI 5.94 and VI 5.03 respectively) and then less infectivity on sweet orange(VI 1.74 and VI 1.45 respectively) and banana (VI 0.98 and VI 1.20 respectively).

4. *In vitro* Evaluation of Fungicides Against Various Post Harvest Pathogens Infecting Mango Fruit

In *in vitro* evaluation of different chemical fungicides against different isolates of *Colletotrichum gloeosporioides, Alternaria alternata, Lasiodiplodia theobromae* and *Aspergillus niger* was carried out at recommended concentrations. Among the six fungicides tested, Carbendazim 12 % + Mancozeb 63 % WP (0.15%) (SAAF) and Thiophanate methyl (0.1%) completely inhibited the mycelial growth of the fungus which was followed by Carbendazim (0.1%) and Mancozeb (0.25%), While, Propineb (0.2%) and Azoxystrobin (0.04%) recorded the least inhibition of the fungus growth.

5. Efficacy of Various Bioagents Against Post Harvest Pathogens Infecting Mango Fruit.

In *in vitro* evaluation of bioagents, it was observed that *Trichoderma viride* showed maximum inhibition of growth of pathogens followed by *Trichoderma harzianum*, yeast (*Saccharomyces cerevisiae*), *Pseudomonas fluorescens* and *Bacillus subtilis*. In case of bacterial antagonists *P. fluorescen*s found effective than *B.subtilis*.

Conclusions

1. The fungal pathogens *Colletotrchum gloeosporioides, Alternaria alternata, Lasiodiplodia theobromae and Aspergillus niger* were associated with post harvest diseases in mango.
2. The Koch's postulate was proved by pathogenicity test revealed that *C. gloeosporioides, A. alternata, L. theobromae* and *A. niger* were the pathogens causing post harvest diseases of mango.
3. *C. gloeosporioides* and *A. alternata* possessed highest degree of infectivity on guava fruit followed by banana and sweet orange. While *L. theobromae* and *A. niger* showed greater infectivity on guava fruits followed by sweet orange and banana.
4. Under *in vitro* conditions, all five fungicides tested against post harvest pathogens were found to be efficient in reducing the mycelial growth of test pathogen. Thiophanate methyl (0.1%) and carbendazim 12 % + mancozeb 63 % WP (0.15%) fungicides was highly sensitive against all of the isolates followed by carbendazim (0.1%) and mancozeb (0.25%) was sensitive while propineb (0.2%) and azoxystrobin (0.04%) were highly resistant against all isolates of pathogens.
5. *Trichoderma viride* was found to be the most effective in inhibiting the mycelial growth of the pathogen with the least amount of mycelial growth followed by *Trichoderma harzianum*, yeast (*Saccharomyces cerevisiae), Pseudomonas fluorescens*, and *Bacillus subtilis*. This eco-friendly method of management has great potential for commercial use in future.

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7. VITAE

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MASTER OF SCIENCE (AGRICULTURE)

IN

PLANT PATHOLOGY

2021

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