“COMPARATIVE PERFORMANCE OF FUNGICIDES WITH BIOAGENTS FOR MANAGEMENT OF ANTHRACNOSE DISEASE OF MANGO’’

THESIS

Submitted in partial fulfilment of the requirements
for the Degree of

MASTER OF SCIENCE
IN
AGRICULTURE

(PLANT PATHOLOGY)

By

Miss. ADSUL POOJA RAMESH

(ADPM/21/2807)

DEPARTMENT OF PLANT PATHOLOGY,

COLLEGE OF AGRICULTURE, DAPOLI

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DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretation of the Thesis entitled **"Comparative Performance of Fungicides with Bioagents for Management of Anthracnose Disease of Mango"** or part thereof has neither been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis/publication of any University or scientific organization. The source of materials used and all assistance received during the course of investigation have been duly acknowledged and that no part of the thesis has been submitted for any other degree or diploma.

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

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ABBREVIATIONS USED

%	:	Per cent
/	:	Per
@	:	At the rate of
spp.	:	Species
˚C	:	Degree Celsius
C.D.	:	Critical difference
CRD	:	Completely randomized design
CV	:	Coefficient of variation
cm	:	Centimeter
M	:	Molar
Conc.	:	Concentration
d.f.	:	Degree of freedom
Dist.	:	District
Dr.B.S.K.K.V.	:	Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth.
E.M.S.	:	Error mean sum of squares
et al.	:	And others
etc.	:	Etcetera
hrs.	:	Hour(s)
Fig.	:	Figures
g	:	Gram(s)
i.e.,	:	That is
Kg	:	Kilogram
m	:	Meter
M.S.S.	:	Mean sum of squares
mg	:	Milligram
Lit.	:	Liter
P.D.A.	:	Potato Dextrose Agar
Ppm	:	Parts per million
S.E.	:	Standard error
Tr./T	:	Treatment
viz.	:	Namely
w/w	:	Weight / weight
Sig.	:	Significant
BE	:	Biological Efficiency
RH	:	Relative Humidity
SS	:	Sum of squares
Sr.	:	Serial
&	:	And
No.	:	Number
PDI	:	Plant Disease Incidence

CHAPTER I

INTRODUCTION

1.1. Background Information

Mango (*Mangifera indica* L.*)* is an important fruit crop from Anacardiaceous family and believed to be originated from South East Asia, the Indo Burma Region, Myanmar, Bangladesh and India. By virtue of its wide adaptation, delicious taste, superb flavor, very high nutritive and medicinal value as well as its religious historical significance, it is called the "King of the fruits'' ranking eighth position in terms of production around the world (Uddin *et al*. 2018). It is fifth most widely produced fruit crop in the world after Banana, Citrus, Grapes and Apple and third most important fruit crop of tropics after Banana and Citrus. It is under cultivation in India for more than 4000 years and hence conspicuous bonds have been formed between the fruits and cultural history of country (Maske *et al.* 2022).

The fruit of mango is very popular with the masses due to its wide range of adaptability, high nutritive values and richness in variety, delicious taste and excellent flavour. It is a rich source of vitamins A and C. The fruit is consumed as raw or ripe. Good mango varieties contain 20 per cent of total soluble solids. Raw fruits of local varieties of mango are used for preparing various traditional products like raw slices in brine, amchur, pickles, murabba, chutneys, panhe (sharabat) etc. (Maske *et al.* 2022).

Mango fruits are known for their nutritional and commercial importance. They are indispensable food commodities across the globe. They 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 moisture content of ripe mangoes is 73.00- 86.70 per cent, carbohydrate 11.60-24.30 per cent, protein 0.50- 1.00 per cent, fiber 1.10 per cent, TSS 12.00-23.00^{o} B, acidity 0.12-0.38 per cen, vitamin A 6375-20750 µg/100 g, vitamin B1 40.00 mg/ 100 g and ascorbic acid 0.46 mg/100 g (Parthiban *et al*. 2020). It is adapted to a warm tropical monsoon climate with a pronounced dry season followedby rains. Mango typically produces flowers in the dry season and set fruits at the start of the wetseason. Therefore, it is considered as one of the oldest tropical fruits across the globe. Mango is a commercial horticultural crop in many countries of South-East Asia, India, Pakistan, Philippines,Malaysia, Thailand, Burma, Sri Lanka and Java. The main mango producing countries of the world are India, Pakistan, Mexico, Brazil, Haiti, Philippines and Bangladesh (Jenny *et al*. 2019).

Area production and productivity of mango in India

India produces about 50 per cent of world mango production with largest area. In India, mango is cultivated in more than 40 per cent of total fruit area. The area occupied by Mango in India is 16 lakh hectares, where the annual production and productivity is 10.80 lakh MT and 6.75 MT/ ha, respectively, as against a higher productivity of 30 MT/ ha in Israel (Parthiban *et al*. 2020).

Konkan region is one of the largest mango growing belts in the country occupying 182 000 ha area, which accounts for about 8 per cent of the total area under mango in the country with major contribution of over 35 per cent to the total export from India. It comprises of five mango growing districts *viz*. Palghar, Thane, Raigad, Ratnagiri and Sindhudurg, along the West Coast of India. The Konkan region is traditionally known as homeland for commercial cultivation of world-famous Alphonso which is the choicest variety of mango in the world occupying more than 95 per cent area in Konkan region . Mango being the major component of livelihood of this region, the multifarious improved technology for production of quality fruits under aberrant climate has been developed by DBSKKV, Dapoli (Haldankar *et al*. 2020).

Konkan region blessed with production of choicest mango variety Alphonso due to its favourable climate and soil type for mango production and productivity. There are more than 3000 named varieties such as Pairi, Alphonso, Dashehari, Langra, Fajali, Chausa, Totapuri, Neelam, Safeda, Rataul, Banganpalli (Baneshan), Mallika, Amrapali, Swarnarekha etc. are being cultivated for commercial purposes. Among them, Alphonso tops in the list. It is grown along west cost of India in Gujarat, Maharashtra, Goa and Karnataka which acclaimed as one of the best Indian mango varieties (Maske *et al.* 2022).

Alphonso has a great export potential due to its appreciable qualities like sugar acid blend ratio, attractive colour, pleasant aroma, tasty pulp without fibre and long keeping quality. Alphonso has more than 80 per cent share in export of mango products. The cv. Alphonso is poorest yielded with average productivity varying from 2.3 to 3 tons/ha which perhaps lowest in the country. Among the several factors ascribed for low yield, susceptibility to pests, diseases and occurrence of alternate bearing. (Maske *et al.* 2022).

Area, production and productivity of mango in the World, India and Konkan region

Particular	Area (lakh ha)	Production (mt)	Productivity (t ha-1)
World	37	26.34	9.3
India	16	10.80	6.75
Maharashtra	3.8	0.81	4.11
Konkan	1.47	0.27	2.5

(Haldankar *et al*. 2020)

1.2. Importance and need of the study

Due to tremendous increase in population and increased demand it is essential to improve the production with the available resources. The main reason for low productivity of mango in India can be attributed due to poor orchard management, dense canopies with wider spacing, poor sunlight interception and ventilation encouraging more pest and disease incidence. Mango is affected by a number of diseases at all stages of development, from seedling in the nursery to the fruit in storage or transit. There are several diseases which affect the mango yield in world and across the Konak region (Parthiban *et al*. 2020).

Sr.No.	List of disease/ Common Name	Causal organism
1	Powdery mildew	*Oidium mangiferae*
2	Anthracnose	*Colletotrichum gloeosporioides* Penz.
3	die back	*Botryodiplodia theobromae*
4	Mango Malformation	*Fusarium moniliforme*
5	Sooty mould or sooty blotch of mango	*Meliola mangiferae*
6	Pink disease	*Botryobasidium salmonicolor*
7	Grey blight or pestalotia leaf spot	*Pestalotiopsis mangiferae*
8	Leaf blight	*Macrophoma mangiferae*
9	Sclerotium rot	*Sclerotium rolfsii*
10	Root rot and damping off	*Rhizoctonia solani*
11	Red rust	*Cephaleuros virescens* Kunze

(Prakash and Misra. 2001)

Among them anthracnose disease of mango caused by *Colletotrichum gloeosporioides* have been tremendously affect the yield of mango (Parthiban *et al*. 2020). *Colletotrichum* species are one of the most important pathogens causing anthracnose disease in a wide host range including legumes, vegetables and fruit crops especially in tropical region. The genus *Colletotrichum* is recently designated as the world's eighth most important pathogen from pathogenic fungi based on perceived scientific and economic importance which cause fruit damage and production losses (Abera *et al*. 2016).

Colletotrichum corda corresponds to genus of phytopathogenic fungi that causes diseases and mainly anthracnose in different hosts. The genus *Colletotrichum* consists of approximately 600 species including most studied species like *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., *Colletotrichum capsici* (Schweinitz) Andrus & W. D. Moore, *Colletotrichum acutatum* J.H. Simmonds, *Colletotrichum dematium* (Persoon) Grove, *Colletotrichum nigrum* Ellis & Halsted and *Colletotrichum coccodes* (Wallroth) S. J. Hughes (Glomerellaceae). The species of this genus infect more than 3200 species of monocot and dicot plants (Arizpe *et al*. 2021)

The sexual stage of *Colletotrichum gloeosporioides* are *Glomerella cingulata*. The name *C. gloeosporioides* was proposed for the first time in Penzig, Poland in the year 1882 based on the type specimen *Vermicularia gloeosporioides*, collected from Citrus in Italy. This pathogen belongs to the Family*: Glomerellaceae*; Order*: Glomerellales*, Class: *Sordariomycetes*, Phylum Ascomycota and Kingdom Fungi. Globally, anthracnose is one of the most important pre-harvest diseases of mango (Jenny *et al*. 2019).

The symptoms of anthracnose appear at pre-harvest stages of mango. However, It is pathogenic to more than 470 different plants at various development stages such as mango, almond, apple, avocado, guava, Arabica coffee, cassava, dragon fruit, sorghum and strawberry. But, among these, anthracnose of mango is very important in commercial prospective. It is caused by two species where *C. gloeosporioides* is mainly responsible and *C. acutatum* plays very less

role in few locations. Another *C. gloeosporioides var. minor*, is no longer recognized. High humidity and moist condition are primary factors that helps in spread and development of anthracnose disease in mango.

The disease, also caused by C. *gloeosporioides* more prevalent on sun-exposed surfaces of fruit and pedicels. It has been called pepper spot and reduces fruit quality due to the presence of small, shiny, black lesions on the skin. The tear stain (also referred to as pepper spot in this paper) symptom on mango (*Mangifera indica* L.) fruit, also a result of infection by *C. gloeosporioides*, is thought to be a similar phenomenon.(Giblin et al 2010)

Anthracnose attacks, leaves, twigs, flowers and young fruits even this disease can also appear in the storage of mature fruits. Disease symptoms appear as slightly, black, sunken irregular shape lesions, which gradually enlarge and developed, leaf spotting, blossom blight, fruit staining and rot. In Bangladesh, about 25 to 30 per cent loses of the total production due to anthracnose and stem end rot (Jenny *et al*. 2019).

This disease spread occurs through rain drops. Thus, a proper knowledge of this diseaseis essential for its proper management to ensure the fruitful yield to increase the return from the fruit crop to the farmers. Several spray and dip treatments of chemicals are used in anthracnose disease management. This study has been carried out to investigate the anthracnose disease of mangoes, pre-harvest management of anthracnose and the efficacy of fungicides and bioagents.

1.3. Objectives of the study

1. Collection of disease sample from susceptible mango cultivar and isolation of pathogen.
2. Comparative performance of fungicide and bioagents against the pathogen *in vivo*.

1.4. Hypothesis

Comparative study of hazardous plant protection chemicals and ecofriendly bioagents in agriculture is a need of time. *Trichoderma* spp. are promising bio-control agents against many pathogens. Use of plant protection chemicals and *Trichoderma* under field conditions might be worthwhile in reducing inoculum density of *C. gloeosporioides* and thereby minimize postharvest losses. The present investigation will help to reduce the disease and prove the effective management strategies.

1.5. Scope and Limitation of study

The problem of using hazardous chemicals for disease management is drawing attention on a globally. The pathogen resistance to chemical fungicides, environmental contamination, and ecological imbalances have to be tested time to time. Accordingly comparative study of plant protection with chemicals and for ecofriendly biological agents is evaluated to know, which one is better for management of anthracnose disease with maintaining environmental approach.

Limitation

1) Availability of proper fungicides and live culture of specific bioagents.
2) Proper knowledge of bioagents, fungicides and its spray schedule along with optimum dose.
3) Methods of application of fungicides
4) Knowledge of pathogen, its perpetuation, dissemination and its vector.

CHAPTER II

REVIEW OF LITERATURE

Colletotrichum spp. are one of the most polyphagous fungal plant pathogens causing anthracnose disease to more than 470 different crops such as mango, chilly, papaya, beans, grapes, banana, avocado and strawberry etc. There are several diseases which affect the mango orchard across the Konkan region *viz*., anthracnose, die back, powdery mildew and mango malformation. Among them anthracnose disease of mango caused by *Colletotrichum gloeosporioides* have been tremendously affect the yield of mango (Parthiban *et al*. 2020). *C. gloeosporioides* severely damage the mango crop, causing anthracnose disease that ultimately result in preharvest fruit rot. The goal of the current research was to set disease management through, using different fungicides and *Trichoderma* species at optimum concentrations.

The literature reviewed on the management of anthracnose of mango with fungicides and bio-agent has been presented in succeeding pages of this chapter.

2.1. Collection of disease sample and isolation of the pathogen

Freeman *et al*. (1996) collected and isolated one hundred twenty specimens of *Colletotrichum gloeosporioides* from avocado (6 U.S. and 57 Israeli isolates) and almond (57 Israeli isolates) fruits.

Anthracnose fungus, *C. gloeosporioides*, obtained from mango fruits and leaves from Taiwan. A total of 1375 isolates was collected by Ker Chung Kuo., 2001.

Jayasinghe and Fernando. (2009) isolated the pathogen, causing anthracnose disease of mango, from symptomatic infected leaves collected from several locations in the Kalutara district in Sri Lanka.

Sangeetha and Rawal. (2009) collected the samples of leaves affected by anthracnose of mango (*C. gloeosporiodes*) from Arambakam, Dapoli, Hassan, Hessarghatta, Lucknow, Raichur, Tiruvur and Tumkur and isolated *C. gloeosporioides* from infected mango leaves.

Giblin *et al*. (2010) obtained eighty isolates of *Colletotrichum gloeosporioides* from avocado and mango fruit showing anthracnose and pepper spot symptoms from New South Wales.

A total twenty-five isolates of *C. gloeosporioides* causing mango anthracnose were collected from *M. indica* cv. desheri from different agroclimatic zones of India by Gupta *et al*.,

2010).

Mukherjee *et al*. (2011) collected infected mango fruits (*Mangifera indica*) showing typical anthracnose symptoms of *Colletotrichum* from local market of Khulna University campus, and isolated *Colletotrichum gloeosporioides*.

Awa *et al*. (2012) collected infected mango fruits with anthracnose symptoms from mango orchards in Ayetoro, Ibadan, Ogbomosho. Two hundred and thirty-one fungi isolated from diseased fruits. This isolation was carried out in both the Nigeria Agricultural Quarantine Service and in International Institute of Tropical Agriculture.

Phoulivong *et al*. (2012) isolated *Colletotrichum* strains from the infected fruits of chilli, coffee, longan, mango, papaya and rose apple, from orchards and local markets in Laos and Thailand.

Zakaria *et al*. (2015) collected mango fruits with anthracnose symptoms from several fruits' stalls, markets and supermarkets in Penang Island and state of Kedah, Peninsular Malaysia. Thirty-five *Colletotrichum* isolates were obtained from anthracnose lesion of two mango cultivars Chokanan and Harum Manis.

The mango leaves or twigs showing typical anthracnose symptoms were collected in polythene bags from Raichur, Yadgir, Kalaburgi and Bidar districts of North Eastern Karnataka and a total sixteen isolates of *C. gloeosporiodes* were obtained by Shivakumar *et al*., 2015.

Abera *et al*. (2016) collected samples of infected anthracnose of mango leaves, panicles and fruits from home gardens in nine districts and brought to the Jimma University College of Agriculture and Veterinary Medicine for isolation of the pathogen.

Zainab *et al*. (2016) collected total 125 leaves of mango anthracnose from the six locations *viz*., Arkilla, Dambuwa, Gagi, Illela, More and permanent site of the Usmanu Danfodiyo University, Sokoto and were isolated for identification.

Zainab and Shinkafi. (2016) collected mango fruits from different parts of Sokoto metropolis, Nigeria at large including other tropical regions of the world where mango was grown and the isolated pathogen was *C. gloeosporioides*.

Nattawut. (2016) isolated one hundred and twelve isolates of *C. gloeosporioides* from infected leaves and fruits of mangoes from Thailand. Out of 112 isolates, eight isolates exhibited at least 60 per cent inhibition.

Kumari *et al*. (2017) collected pathogen mango anthracnose infected leaves from the experimental orchard and isolated pathogen on PDA showing creamy white, regular and fluffy fungal growth after 24 hours of inoculation.

Twelve isolates of *C. gloeosporiodes* were obtained from mango leaves and twigs from Rewa (4 isolates), Sidhi (3 isolates), Satna (4 isolates) and Jabalpur (1 isolate) districts of Madhya Pradesh and screened in the greenhouse for its virulence using spray inoculation method on a year-

old mango graft and observed considerable variation (Kumar *et al*., 2017).

Majumdar and Mandal (2018) collected naturally infected ripe mango fruits with anthracnose from local Bolpur market and Palli Siksha Bhavana orchard.

Monosporic strains *Gro, Col, Oax, Sin* and *Tux* were isolated from infected mango Leaves, flowers, juvenile fruits and annual vegetative branches with anthracnose symptoms, were collected from commercial mango orchards located in the states of Oaxaca, Guerrero, Colima and Sinaloa, Mexico, by Ojeda *et al*. 2018.

Ansari *et al*. (2018) collected ripe mango fruits showing typical symptoms of anthracnose rot from local markets and Department of Plant Protection, Sindh Agriculture University Tandojam, Pakistan and used for isolation of causal pathogen.

Islam *et al*. (2018) studied fungi associated with mango fruits and their pathogenic potentiality. A total of ten isolates were isolated from the anthracnose from cv. Himsagar, Langra and Amrapali. The samples were collected from five different markets of Dhaka city, namely Farmgate, Kollayanpur, Kawran bazaar, Mirpur-10 and Pirerbagh bazaar.

Lurwanu and Sunusi. (2018) collected samples of infected mango leaves and fruits from four orchards which were brought to the Vegetable Pathology Laboratory in the Department of Crop Protection, Ahmadu Bello University, Zaria and isolated *C. gloeosporiodes, Alternaria alternata, Botrytis cinerea* and *Curvularia* spp.

Sayiprathap *et al*. (2018) collected mango leaves infected with anthracnose from different districts of Karnataka (India) *viz*., Bengaluru Rural, Chitradurga, Chikkaballapur, Dharwad, Haveri, Kolar, Raichur, Ramanagara, Shivamogga and Tumakuru during survey and used for isolation of the fungus, *C. gloeosporioides*.

Zainudin and Sattar. (2019) obtained infected anthracnose of mango fruits from several orchard in Peninsular, Malaysia *viz*.; Perlis, Penang, Perak, Selangor, Pahang and Melaka states. Five samples of fruits and leaves showing the symptom of anthracnose fruit rot were collected. A total of 33 isolates of *Colletotrichum* spp. were obtained.

Silva *et al*. (2019**)** studied 260 *Colletotrichum* isolates, associated with necrotic lesions on leaves and fruits, which were collected from chilli producing areas of Asia, Indonesia, Malaysia, Sri Lanka, Thailand and Taiwan.

Ten mango fruits were collected from each mango exported orchard in Phitsanulok, Nakhon Ratchasima, Sakeao, Prachinburi, Chiang Maiand Chachoengsao provinces for isolation of anthracnose of mango caused by *C. gloeosporioides*. A total forty-four isolates of *Colletotrichum* spp was obtained by Laksanaphisut *et al*., 2019.

Li *et al*. (2019) samples of mango leaves, fruits and branches with symptoms of anthracnose were gathered from six provinces *viz*; Fujian, Guangdong, Guizhou, Hainan, Sichuan and Yunnan in China were collected for fungal isolation. Based on morphological observation and ITS sequences, a total of 205 *Colletotrichum* isolates were obtained, and 128 representative

isolates were selected for further study *viz*.; 34 from Yunnan, 23 from Hainan, 18 from Guizhou, 13 from Sichuan, 16 from Guangdong and 24 from Fujian province.

The mango leaves infected with anthracnose were collected from mango orchard and used for isolation of the causal fungus by Ranjitha *et al*., 2019.

Total of 19 fungal colonies were isolated from the naturally infected mango fruits and leaves collected from 6 locations in Yola Adamawa state by Haruna *et al*., 2020. There was highly significant difference between the frequency of occurrence of isolates in both mango fruit and leaves.

Neto *et al*. (2020) studied 28 isolates of infected mango fruits in Rio Grande do Norte state, Brazil. Based on colony color, size of conidia, appressoria and growth rate, isolates were identified as C. *gloeosporioides*.

Wu *et al*. (2020) collected Mango fruits, stems and pedicels from diseased and healthy plants from 33 orchards in Taiwan, Chiayi, Tainan, Kaohsiung and Pingtung. The *Colletotrichum* species were isolated from these plant tissues. A total of 682 isolates from 560 samples were obtained.

Diseased leaves and mature fruits were collected from seven mango cultivated districts in the north of Cote d'Ivoire to identify and characterize *Colletotrichum* species isolates associated with mango leaves and fruits. A total of 70 isolates were collected by (Dembele *et al*., 2020).

Pedraza *et al*. (2020) studied a total of 118 *Colletotrichum* isolates obtained from symptomatic mango tissues *viz*.; 110 from leaves, 5 from fruits and 3 from twigs collected from 59 orchards distributed in the states of Chiaps, Sinaloa, Nayarit, Veracruz, Michoacan, Colima and Guerreo in Mexico.

Arizpe *et al*. (2021) isolated the pathogen *C. gloeosporioides* from fruits of Tommy Atkins and Ataulfo mango cultivars showing advanced degrees of black spots collected from open markets in Saltillo, Coahuila, Mexico.

Mangoes were collected from orchards and markets in several cities of Senegal in July and August of 2015 both in the North (Pout, Notto Gouye Diama), and in the South (Kolda, Sedhiou, and Ziguinchor) by (Diallo *et al*., 2021). A total thirty isolates of *Colletotrichum* were isolated from anthracnose lesions of mango from several regions in Senegal.

Maske *et al*. (2022) collected anthracnose infected mango fruits. A Thirty-five *Colletotrichum* isolates were isolated from anthracnose lesion of two mango cultivars, Chokanan and Harum Manis.

The *C. gloeosporioides* was isolated from mango fruit showing typical symptoms of anthracnose in Ziguinchor (Cisse *et al*., 2022).

Ortiz *et al*. (2022) obtained fungal isolates from leaves and fruit of mango, in which seven

isolates belong to the *C. gloeosporioides* complex, the causal agent of anthracnose in mango. Among the isolates the *Colletotrichum* was isolated from fruit, while the rest were found in infected leaves.

Mango fruits (*Mangifera indica* L. and other *Mangifera* species) were collected from the Department of Agriculture and Fisheries (DAF) ANMG sites located at the Ayr, Southedge and Walkamin Research Facilities (Grice *et al*., 2023).

Rattanakreetakul *et al*. (2023) collected inflorescence, leaves and fruits of mango cultivar Nam Dok Mai See Thong (NDMST) showing typical anthracnose symptoms from orchards located in Chachoengsao, Phichit and Ratchaburi. A total of 37 fungal isolates were resembling *Colletotrichum* spp.

2.1.1. Pathogenicity of the test pathogen

Spray inoculation and Pin prick method

Giblin *et al*. (2010) carried out pathogenicity of isolated pathogen on mango fruit (*cv*. 'Brooks') from the orchard of C. Jacobs near Bundaberg, Queensland. Fruit was inoculated by soaking a filter paper disc in the spore suspension of an isolate (5×10^6 conidia/ml) and then the disc was removed from the suspension with forceps and placed on the surface of the fruit on the allocated area. Isolates were inoculated in series followed by a water control disc. Fruit were assessed for presence or absence of a visible lesion, and the diameter of the lesion was measured at ripen stage 12 days after inoculation.

Gupta *et al*. (2010) pathogenicity assay tests with 25 *C. gloeosporioides* isolates were performed on the mango seedlings of Desheri under greenhouse condition. Pin prick inoculation technique was employed with spore suspension to prove pathogenicity.

The variation in pathogenicity of different isolates of *C. gloeosporioides* was tested by pin prick method. For this purpose, one year old Alphonso grafts were chosen and wounds were made by pin prick method. The spore suspension was made through sterile distilled water. Spore suspension containing a load of 2×10^4 conidia/ ml was inoculated on the wounded areas. After inoculation, the seedlings were covered with polythene bags to ensure high humidity by (Shivakumar *et al*., 2015).

Langra mango grafts were used to prove the pathogenicity of different isolates of *C. gloeosporioides* by spray inoculation method (Kumar *et al*., 2017). It was observed that lesions were developed on the inoculated leaves. Numerous browns to black irregular spots appeared on the surface of the leaves later when sprayed with spore solution. The spots rapidly increased in size and coalesced to form elongated brown necrotic areas. To confirm the pathogen, *C. gloeosporioides* was re-isolated from the infected leaves and verified that it was as the same as original culture and there by proved pathogenicity of all the twelve isolates of *C. gloeosporioides*.

Kumari *et al*. (2017) leaves and fruits of highly susceptible variety Dashehari were artificially inoculated by pinprick method with conidia suspension of *C. gloeosporioides*. The symptoms of the disease appeared after 4-5 days of inoculation. The infected leaves exhibited the

brown necrotic spots (20-25 mm in diameter) and dark brown to black depressed necrotic areas on fruits. These diseased leaves and fruits were used for reconfirmation of the pathogen associated with this disease.

The upper axial leaf surface of the mango seedlings was inoculated with the conidia of *C. gloeosporioides* using three inoculation methods infusion, pinpricking and droplet. The infusion method involved injecting 0.1 ml spore suspension of the inoculum into the midvein using a sterile hypodermic syringe, the inoculum concentration used was 10^6 spores/ml. For the Pinprick method, the mango leaf was injured by creating pricks with a sterile needle, 0.1 ml spore suspension was dropped on injured pricks. Droplet method involves dropping 0.1 ml spore suspension on an intact leaf surface using a micropipette. These seedlings were observed daily for symptoms, data on lesion size was also taken daily for seven days after inoculation. Pathogen were re-isolated from the leaves when showed symptoms and the cultures obtained were compared with the original culture to confirm Koch's postulates (Lurwanu and Sunusi., 2018).

Islam *et al*. (2018) reported pathogenicity test of the test isolates. Healthy mangoes were collected from the selected market and washed thoroughly in running tap water and dipped in one liter of 10 per cent Chlorox solution for surface sterilization. Sterilized wet cotton was placed in a corner of box to maintain humidity. Before spraying, the fruits were scratched with sterilized niddle and inoculated by spraying the conidial suspension (10^5 conidia/ml) of isolated fungi separately with an atomizer and placed into separate plastic boxes. In control set, sterilized distilled water was used instead of conidial suspension on the sterilized mango surface. The inoculated mango fruits with plastic boxes were incubated at 25 ± 2°C for 7 days. Observation were recorded after 7 days, when the fruits showed characteristic symptoms. The fungi were isolated from artificially inoculated fruits and compared with the previous one which showed the disease symptoms naturally infected fruits.

Zainudin and Sattar. (2019) harvested matured and healthy mango fruits (cv Chokanan, MA224) of uniform size and age from an orchard in Melaka and were used for the pathogenicity test. Each fruit was inoculated using non-wounded method by placing it directly to 5 mm diameter of mycelium plug on the mango surface. All the inoculated mangoes were incubated in covered containers at same condition, 27 ± 1°C in the dark. All treatments and control were replicated twice with four mangoes for each fungal isolate. After eight days of inoculation, the fungal colonies from lesion were subculture onto PDA and compared with the original isolates to confirm the Koch's postulates.

Laksanaphisut *et al*. (2019) were used fruits of mango for artificial inoculation**.** The unwounded fruit was artificially inoculated with 25 µl of conidial suspension ($3x10^6$ conidia/ml) of each individual *Colletotrichum* isolate and was subsequently covered with sterile Whatman No.1 filter paper disc (0.5x0.5 cm) maintain moisture of the inoculum. Each fruit was inoculated

at 3 positions including 2 cm from stem end, in the middle and 2 cm from stylar end on one side of the fruit. For control treatment, each fruit was dropped with 25 µl of sterile distilled water. All inoculated fruits were maintained under humid condition at 25± 5° C for 24 h. The plastic bag was then removed and inoculated fruits were further incubated at 25-28º C for 10 days.

Virulence of isolates was assessed in Tommy Atkins mango fruits purchased from growing areas in Rio Grande do Norte, Brazil**.** Three wounds (7 mm in diameter) were made on fruit epidermis using a sterile metal rod and PDA disks (5 mm in diameter) from the edge of the colony were cut out aseptically from the 7 day old growth PDA plates and kept on each wound. The control group consisted of fruits inoculated with PDA disks without fungal growth. After inoculation, fruits were placed in plastic trays (previously disinfected with 70% alcohol) containing cotton wool moistened with sterile distilled water, which was then covered with plastic wrap to maintain moisture in fruits. Subsequently, the trays were kept at room temperature (30±1 °C) under natural photoperiod to prove the Koch's postulate by (Neto *et al*., 2020).

Haruna *et al*. (2020) collected freshly harvested healthy matured mango fruits from Kasuwan gwari market for proving pathogenicity of fungal isolates, and spray then 50 µL of 10^5 spore /ml suspension of *C. gloeosporioides*. *C. gloeosporioides* was able to produce the dark brown sunken lesions (anthracnose symptom) on both mango fruits and leaves, hence proved to be the etiological agent of the disease. Accordingly, *C. gloeosporioides* was the causal agent of mango anthracnose in South Western Nigeria.

Pathogenicity tests were carried out using green matured mango fruits of cv. Kent randomly collected from four orchards Sinematiali. Inoculations of fruits were done by wound inoculation method. Fruits were pierced with sterilized needle in three portions and pipette 20µL containing $1x10^6$ ml^{-1} spores suspension for each isolate on the three wounded fruits. Control were inoculated with 20µL of sterile distilled water. Inoculated fruits were incubated at 25–28°C in cartons lined with moist laboratory wipes and covered with plastic bags and daily sprayed with sterilized distilled water to maintain at 95 per cent relative humidity. Five days after incubation, lesions were measured and compared with the original isolates to prove Koch's postulates (Dembele *et al*., 2020).

Pedraza *et al*. (2020) carried out pathogenicity tests on mango fruits (cv. Manila) at the stage of ripening. The fruits were inoculated using the test pathogen. Fruits were wounded in two allocated areas with a sterile toothpick. A mycelial plug was removed from the margin of 6-day old PDA culture and placed onto the wounded surface of fruits. A noncolonized agar plug was placed on the wounds of 10 fruits which used as a the control. The fruits were incubated at 25 °C in the dark on plastic trays lined with two layers of paper towel moistened with sterile distilled water and enclosed in a plastic bag. Six days after inoculation, the virulence of each isolate was assessed by measuring lesion diameter.

For artificial inoculation Lombok variety of mango was used. The inoculum was adjusted

to a concentration of 1–3×10^6 conidia per ml with an Improved Neubauer hemocytometer. For each cultivar, one 25 µl droplet of sterile distilled water was placed in the center of each of the two marked spots on the single control fruit, while for all other fruit one droplet of 25 µl of conidial suspension was placed in the center of each of the two marked spots. All fruits were then incubated in plastic containers for 48 h. Fruits were assessed every second day, post inoculation, for their stage of ripeness, lesion development and disease severity by (Grice *et al*., 2022).

A mycelial plug of a 5-day-old culture was inoculated on wounded fruit and leaves. Typical anthracnose lesions were observed around the inoculation sites. The anthracnose lesions on fruit enlarged faster than those on leaves on 5 days after inoculation. No lesions were observed on the control fruit or leaves. After the pathogenicity test, all the *Colletotrichum* spp. were re-isolated from the infected tissues and confirmed by Koch's postulate by (Rattanakreetakul *et al*., 2023).

Leaf detached Technique

Jayasinghe and Fernando. (2009) was tested pathogenicity of the two *Colletotricum* spp. (MA 1, MA 2, & MG 1, MG 2) on young mango leaves. Drops (0.02ml) of an aqueous conidial suspension (1X10^5 spores' ml -1) prepared from 7-day-old cultures of each isolate were placed on copper brown six leaves inoculated with each isolate. Later cross infection ability of the two isolates was tested on young detached leaves of Hevea rubber (clone RRIC 121). Six drops of conidial suspension (0.02 ml, 1X10^5 spores' ml -1 prepared from 7-day-old cultures) were placed on either side of the midrib on the lower surface of each leaf. Inoculated leaves were incubated at 28±2^0C in humid chambers. Specimens inoculated with sterile distilled water drops served as controls.

Awa *et al*. (2012) were collected new leaves free from anthracnose symptom, washed, and surface sterilized. Which were then, sprayed with the spore solution of the test fungal isolates and placed in plastic trays and incubated in a moist chamber for 5 days at 28ºC.

Abera *et al*. (2016) carried out pathogenicity tests on detached fruits, and leaves of mango on Kent variety. Eight isolates were selected for inoculation on detached mango fruit, and leaves. Surface sterilized fruits and leaves were placed in a plastic box with tissue paper then sprayed with sterilized water to maintain at least 95% relative humidity. The samples were inoculated using the wound/drop inoculation method which included pin-pricking on leaves, the fruits to a 3 mm depth with a sterile needle in the middle portion of fruit and then placing 20µl of conidia suspension onto the wound. Control fruits were inoculated with 20µl of sterile distilled water. The inoculated samples were incubated in the plastic containers at 25°C under controlled conditions. The characters of the re-isolated pathogens were compared with their original isolates to prove pathogenicity.

Hoz *et al*. (2016) To prove pathogenicity of mango cv. Tommy Atkins were used for artificial inoculation on leaves and fruits. Leaves were placed inside Petri plates containing a plastic mesh, sterile paper and 1 ml sterile distilled water. Fruits were placed inside a plastic box

(one fruit per box) which contain sterile paper soaked in sterile distilled water to maintain humidity. Leaves placed inside Petri plates, were inoculated with 10 µl of the conidial suspension at six different spots, and fruits were inoculated with 100 µl of the conidial suspension on a wound of approximately 1 mm in diameter. Controls were inoculated with the corresponding volume of sterile distilled water**.** Each isolate was evaluated on its original host and on the alternate host. Inoculated samples were incubated at room temperature with alternate light and dark periods of 12 hrs. each. Symptoms and reisolated test pathogen were examined and proved pathogenicity. Photographs of the symptoms were taken every other day for 15 days.

128 isolates were used for pathogenicity and virulence tests on detached leaves and fruits of mango (cv. Tainong) under controlled conditions. Freshly harvested mango fruits and young leaves without visible disease were used for the tests. After air drying, detached young healthy leaves (12–15 cm) were placed into plastic containers (9 cm ×17 cm ×25 cm) lined with paper toweling, and six stab wounds were made forming a 5-mm-diam circle. Mycelial plugs (6mm diameter) from margins of PDA cultures were placed on each wound. For controls, sterile agar plugs were used. The containers were sealed and incubated in the dark at 25 °C. Aggressiveness was assessed after one-week inoculation by measuring lesion diameter by (Li *et al*., 2019).

Udhayakumar *et al*. (2019) proved pathogenicity of all the twenty isolates of *C. gloeosporioides* tested by detached leaf technique in mango seedlings of var. Neelam. The young detached leaves of mango were inoculated with conidial suspension of *C. gloeosporioides* isolates containing 1×10^5 conidia ml-1 and incubated in transparent polyethylene bags. The bags were placed at room temperature (28 ± 2^oC) for 4 days and observed for appearance of symptoms. For comparison, control treatment was sprayed with sterile water. Four polythene bags (5 leaves per bag) were maintained.

Wu *et al*. (2020) conducted pathogenicity tests on detached young leaves and mature fruits of mango (*Cv*. Irwin). A stab wound was made on each fruit with a sterilized needle, and a hyphal disc was placed on the wound. Ten fruits were inoculated for each isolate, and PDA discs were used as a negative control. The lesion diameter was measured at 7 days post-inoculation to assess the virulence of different isolates. Small, to large spots emerged on leaves near appeared 5-6 days after inoculation. Other four *Colletotrichum s*pecies caused black lesions with clear edges on leaves 5 days post-inoculation, and pathogens were re-isolated from the edges of the lesions, proves Koch's postulates.

2.2. *In vitro* comparative performance of fungicides

Jagtap *et al*. (2015) studied *in vitro* evaluation of new synthetic fungicides against *C. gloeosporioides* causing anthracnose of pomegranate. Among the fungicides Carbendazim + Mancozeb @ 3 per cent showed 82.10 per cent inhibition of mycelial growth of fungus followed by Chlorothalonil with 75.80 per cent and least inhibition of mycelial growth was recorded in captan 63.48 per cent.

Jayalakshmi *et al.* (2015) tested the efficacy of six non systemic (one combi) fungicides against *C. gloeosporioides* under *in vitro* condition at 0.1, 0.2 and 0.3 per cent concentration. Among non-systemic (one combi) fungicides, Carbendazim + Mancozeb @ 0.3 per cent concentration showed 81.88 per cent inhibition of mycelial growth of fungus followed by Captan with 73.88 per cent and least inhibition of mycelial growth was recorded in Copper Oxychloride with 20.32 per cent.

Ramani *et al.* (2015) studied *in vitro* evaluation of various fungicides to check the colony growth of the fungus *C. gloeosporioides* causing anthracnose of banana. The results of combination of fungicides revealed that Carbendazim 12 per cent WP + Mancozeb 64 per cent WP were proved most effective and gave cent per cent growth inhibition at all concentration *viz*; 100 ppm, 250 ppm, 500 ppm and 1000 ppm respectively.

Ahamad *et al*. (2018) conducted a evaluation of the effectiveness of different concentration of four different fungicides *viz*; Carbendazim, Chlorothalonil, Hexaconazole and Mancozeb against anthracnose of soybean caused by *C. truncatum* under *in vitro* condition. Among the fungicide, Carbendazim was found most effective and recorded highest inhibition in growth (98%) which was at par with Hexaconazole (97%) whereas Mancozeb (42.67%) and Chlorothionil (33.67%) were appreciably ineffective in mycelial inhibition when compared with control, after 7 days of incubation.

Burgute and Magar. (2019) studied *in vitro* efficacy of seven non-systemic fungicides against *C. gloeosporoides* for radial growth inhibition using poisoned food technique. The result showed that Carbendazim 12% + Mancozeb 63% 75 WP at 2000 ppm concentration showed 94.00 per cent mycelial growth inhibition followed by Propineb 70 WP (51.48%), Chlorothalonil 75 WP (48.14%), Mancozeb 75 WP (42.77%) Metalaxyl 8% + Mancozeb 64% 72 WP (33.07%) and Cymoxanil 8% + Mancozeb 64 % 72 WP (19.73%) respectively. The least inhibition of fungus was recorded in combi fungicides Cymoxanil 8% + Mancozeb 64 % 72 WP (19.73%).

Mahesh *et al*. (2020) screened the five combi fungicides against *C. gloeosporoides* under field condition. Among them (Carbendazim 12 + Mancozeb 63 WP) inhibited maximum mean mycelial growth of 91.23 (73.14) and 88.40 (73.63) per cent respectively followed by (Cymoxanil 8 + Mancozeb 63 WP) with 86.54 per cent and (Iprovalicarb 5.5 + Propineb 61.25 WP) with 86.17 per cent mycelial inhibition. Least per cent inhibition was observed in (Metalaxyl 8 + Mancozeb 64 WP) with 83.21 per cent.

Bagade *et al*. (2020) assayed fungi toxic activities of six different fungicides against *C. lindemuthianum* causing anthracnose of common bean. The result indicated that, Carbendazim+ Mancozeb @ 0.25 per cent and Carboxin + Thiram @ 0.3 per cent was the most effective for arresting 100 per cent mycelial growth followed by Pyraclostrobin (91.48 per cent), Copper Oxychloride (88.55 per cent) and Propineb (72.22 per cent). Least mycelial growth inhibition

observed in Azoxystrobin (54.44 per cent).

Poonacha *et al*. (2020) studied five contact, six systemic fungicides and five combi products against *C. lindemuthianum* under *in vitro* conditions. The contact fungicides were evaluated at 1000, 2000 and 3000 ppm concentrations. The systemic fungicides were evaluated at 500, 1000 and 1500 ppm concentrations whereas combi products were evaluated at 500, 1000 and 2000 ppm concentration. Among the combi product (Carbendazim + Mancozeb), (Tricyclazole + Mancozeb) and (Carboxin + Thiram) recorded cent per cent inhibition of mycelial growth at all the concentrations. Least inhibition of 74.07 per cent was observed in (Trifloxystrobin + Tebuconazole).

Vandana *et al*. (2021) evaluated seven combi products for their efficacy against *C. gloeosporioides* at 1000, 2000, 2500 and 3000 ppm concentration. Mean per cent inhibition of mycelia growth were recorded for all the different combi products, among which Tricyclazole 18 per cent + Mancozeb per cent, Carbendazim per cent + Mancozeb per cent and Carboxin per cent + Thiram per cent showed complete inhibition. The efficacy of Captan per cent + Hexaconazole 5 per cent (79.07 per cent) was much the same as Tricyclazole 45 per cent + Hexaconazole 10 per cent (77.40 per cent). Hexaconazole 4 per cent + Zineb 68 per cent were least effective, which recorded 70.45 per cent mycelia growth inhibition.

Divya *et al*. (2022) studied *in vitro* efficacy of new generation (Propiconazole 25 EC, Difenconazole 25 EC, and Azoxystrobin 23 SC) and combination fungicides (Carbendazim 12 per cent + Mancozeb 63 per cent WP and Trifloxystrobin 25 per cent + Tebuconazole 55 per cent WP) were tested at four different concentrations, namely 10, 25, 50, and 100 ppm, using the poisoned food technique. The result showed that propiconazole 25 EC at 100 ppm and combination fungicides, Carbendazim 12 per cent + Mancozeb 63 per cent WP at 25 ppm and Trifloxystrobin 25 per cent + Tebuconazole 55 per cent WP at 100 ppm, resulted in 100% inhibition of the mycelial growth of the pathogen. At 100 ppm, Difenoconazole 25 EC, Azoxystrobin 23 SC, and Captan 50 percent WP + Hexaconazole 5 percent WP inhibited mycelial growth by 69.33, 73.33, and 79.10 per cent, respectively.

2.3. *In vitro* comparative performance of bio-agents

Devamma *et al*. (2015) evaluated the seven antagonistic fungal isolates by dual culture technique for their antagonistic effect against *C. gloeosporioides* causing anthracnose of pomegranate under *in-vitro* conditions. After 7 days of inoculation, maximum inhibition of mycelial growth 73.50 per cent was observed in *T. harzianum* (Madanapale field isolate MDPF-1), followed by *T. viride* (Palamner field isolate PLRF-5) 60.43 per cent. Least inhibition was recorded in *T. harzianum* (Punganur field isolate PGRF-3)29.70 per cent respectively.

Pandit and Kausha. (2017) studied the antagonistic activity of two fungal bioagents *viz*., *Trichoderma viride*-H (Hydrabad strain), *T. harzianum*-H (Hydrabad strain) and one bacterial bioagents *Pseudomonas fluorescens*-TNAU against *C. truncatum* causing anthracnose of horse

gram using dual-culture technique. Results showed that maximum inhibition of mycelial growth was observed with *T. viride*-H (58.81 per cent) followed by *P. fluorescens*-TNAU (46.03 per cent) and *T. harzianum*-H (35.47 per cent).

Musheer and Ashraf. (2017) studied the *in vitro* efficacy of bio-control agents *viz*; *Trichoderma viride*, *T. harzianum*, *T. virens*, *T. koningii* and *T. hamatum* against the *C. gleosporiodes* causing turmeric leaf spot disease**.** Among five *Trichoderma* sp. tested, *Trichoderma viride* recorded highest per cent of mycelial growth inhibition (67.79 per cent) with a colony diameter of 27.19 mm. It was followed by *T. harzianum*, *T. koningii*, *T. virens* and *T. hamatum* showed 65.07 per cent,54.96 per cent, 52.46 per cent and 38.87 per cent inhibition with 31.43mm, 40.53mm, 42.79mm, and 55.02mm colony diameter growth, respectively.

Ahamad *et al*. (2018) evaluated three bio-agents *T. viride, T. harzianum* and *T. asperallum* against *C. truncatum* causing anthracnose of soybean under *in vitro* by using dual culture technique. Among the three bioagents, *T. harzianum* was found to be most effective and recorded 67 per cent inhibition of the test pathogen of soybean followed by *T. viride* (58 per cent) and *T. asperallum* (50 per cent).

Rana *et al*. (2020**)** studied the effect of five different species of *Trichoderma viz*; *Trichoderma viride, Trichoderma hamatum, Trichoderma harzianum, Trichoderma koningii* and *Trichoderma virens* were evaluated under *in vitro* condition against *C. falcatum* causing red rot of sugarcane. The result showed that *Trichoderma harzianum* gave best result in dual culture with maximum growth inhibition of 84.4 per cent followed by *T. hamatum* with growth inhibition of 83.3 per cent, *T. viride* with 82.2 per cent growth inhibition, *T. virens* with 77.2 per cent growth inhibition whereas *T. koningii* showed minimum growth inhibition of 76.2 per cent.

2.4. *In vivo* comparative performance of fungicides

The effectiveness of Azoxystrobin 23 per cent SC was observed by Sundravadana *et al*. (2006) in a field experiment at Tamil Nadu Agricultural University, India, on 15–20 year old mango orchard. Azoxystrobin treatments were used with five doses *viz*; 0.25, 0.5, 1.0, 2.0, and 4.0 ml/l respectively. Disease severity were recorded at 7 and 15 days interval. Among these doses, 0.25 and 0.5 ml/l slightly reduced the leaf anthracnose i.e., 28.72 and 27.58 PDI respectively. whereas other higher doses 1.0, 2.0 and 4.0 ml/l significantly suppressed the leaf infection i.e., 17.58, 17.02 and 16.31 PDI respectively.

Adhikary *et al.* (2013) evaluated efficacy of Azoxystrobin against mango anthracnose under field conditions. Azoxystrobin @ 25 per cent and 50ppm concentrations reduced the leaf anthracnose i.e., 50.60 per cent and 54.91 per cent disease reduction over control. Whereas other high doses 100, 200, 300 and 400 ppm significantly suppressed the leaf anthracnose i.e., 71.26 per cent, 73.45 per cent, 74.64 per cent and 75.29 per cent disease reduction over control respectively.

So, the efficacy of Azoxystrobin increased with increase in the concentrations.

Chand *et al*. (2013) investigated nine fungicides *viz*.; Carbendazim (@0.1 per cent) Thiophanate methyl (@0.1 per cent), Mancozeb (@0.2 per cent), Chlorthalonil (@0.2 per cent), Propineb (@0.2 per cent), Carbendazim 12 per cent + Mancozeb 63 per cent (@ 0.2 per cent) Tricyclazole (@0.1 per cent), Hexaconazole (@0.1 per cent) and control with three replications in RBD on most popular mango variety Lagra (Malda) to manage anthracnose of mango. The result showed that all the fungicides were effective in the suppression of the disease in the five years average of incidence of mango on Langra (Malda) germplasm. Carbendazim @ 0.1 per cent was more effective (PDI value of 4.48). It was followed by Thiophanate-methyl (0.1%), Tricyclazole (0.1%), Campanion (0.2%), Mancozeb (0.2%), Propineb (0.2%), Chlorothalonil (0.2%) and Hexaconazole (0.1%) with PDI value 6.18, 7.50, 10.32, 12.07, 13.22, 14.46 and 17.34 respectively. Carbendazim @ 0.1% was the most effective (< 4.48 PDI) in the five years.

Three systemic fungicides (Thiophanate methyl, Azoxystrobin and Myclobutanyl) and a contact fungicide (Mancozeb) were tested in the field to assess their efficacy in the control of field infection and postharvest rot of mango due to anthracnose. The result showed that preharvest treatment with Thiophanate methyl 450 g/l dose kept 80 per cent of mangoes from foliage covered by the fungicide free. In the same tree, mangoes from foliage parts not covered by the fungicide exhibited 46.6 per cent of healthy fruits. Treatment with Azoxystrobin 250 g/l dose gave respectively for mangoes from the treated and untreated foliage portion 44.4 per cent and 13.2 per cent disease free ripe fruits. Treatment with Myclobutanyl 240 g/l application dose was the least effective, with only 26.6 per cent and 6.6 per cent of uninfected mangoes from the treated and untreated tree portion respectively. From the non-treated control trees, no ripe disease-free fruit was recorded by (Diedhiou *et al*., 2014).

Pandey and Gupta. (2015) conducted a field trial on chilli variety LCA-355 against anthracnose of chilli. It comprises nine fungicides *viz*.; Mancozeb (@0.3 per cent), Propineb (@0.3 per cent), Myclobutanil (@0.1 per cent), Hexaconazole (@0.1 per cent), Triadimefon (@0.1 per cent), Azoxystrobin (@0.3 per cent), Carbendazim (@0.1 per cent), Cymoxanil + Mancozeb (@0.3 per cent), Flusilazole (@0.1 per cent), two bioagent *viz*.; *A. niger* -V(@0.5 per cent + sticker (@0.1 per cent) and *T. viride* (@0.5 per cent), one antibiotic Kasugamycin with cultural practice hand picking of initial set of green chilli fruits with one control. The findings clearly indicated that among the 14 treatments, thrice hand picking of initial set of green chilli fruits was significantly superior with the lowest PDI (13.33 per cent). However, the highest PDI (59.0 per cent) in red chilli fruits was recorded in control. Further, revealed that foliar sprays of *Aspergillus niger* -V (@0.5 per cent) along with sticker (@0.1 per cent) at 10 days interval resulted in 29.83 per cent PDI which was found to be the second-best treatment followed by spray of combined fungicide Cymoxanil (8 per cent) + Mancozeb (64 per cent) @0.3 per cent with 30 per cent PDI. The PDI of

anthracnose on red chilli fruits were recorded as 35 per cent, 35.83 per cent and 36 per cent with the spray of Azoxystrobin (@0.1 per cent), Mancozeb (@0.3 per cent) and Triademefon (@0.1 per cent) respectively. The PDI on red chilli fruits ranged from 38.16-43.50 per cent in the treatment with foliar spray of *T. viride* (@0.5 per cent). The other tested fungicides included in the study such as Propineb, Carbendazim, Hexaconazole, Myclobutanil, Flusilazole and Kasugamycin showed less effectiveness after three sprays.

The effectiveness of Azoxystrobin (23 per cent) SC was showed by Ravikumar *et al*. (2017). The experiment was conducted at humid tropical regions of Karnataka, India with six treatments of different fungicides, *viz*., Azoxystrobin (23 per cent) SC @ 1ml/l of water, Azoxystrobin (23 per cent) SC @ 2ml/l of water, Standard Azoxystrobin 25 SC (market sample) @ 1ml/l of water, Standard Hexaconazole (5 per cent) SC @ 2ml/l of water, Standard Copper oxy chloride (50 per cent) WG @ 2.4g/ l of water along with one untreated Control. Two sprays were taken up at 20 days interval during flowering stage. The result showed that Azoxystrobin (23 per cent) SC @ 2ml/l significantly reduced PDI on leaves (6.94 per cent) as compared to control (14.85 per cent). Azoxystrobin @ 1ml/l and standard Copper oxy chloride (50 per cent) WG @ 2.4g/l were the next best treatments for managing anthracnose disease in mango.

The effectiveness of Azoxystrobin (23 per cent) SC was showed by Thammaiah and Swamy (2017) at farmer's field of Gokak taluk, Belgaum district. Susceptible variety Kesar was selected for the evaluation of efficacy of Azoxystrobin against anthracnose disease. There were seven treatments *viz*., Azoxystrobin (23 per cent) SC @ 0.05 per cent, Azoxystrobin (23 per cent) SC @ 0.1 per cent, Azoxystrobin (23 per cent) SC @ 0.15 per cent, Azoxystrobin (23 per cent) SC @0.2 per cent, Amistar (25 per cent) SC @0.1 per cent, Carbendazim (0.1 per cent) and control. Results revealed that, two sprays of Azoxystrobin (23 per cent) SC @ 0.2 per cent, (12.94% per cent as against 59.86 per cent in control) effectively controlled the anthracnose disease followed by Azoxystrobin (23 per cent) SC @0.15 per cent (13.49 per cent), Azoxystrobin (23 per cent) SC @ 0.1 per cent (20.20 per cent), Azoxystrobin (23 per cent) SC @0.05 per cent (27.22 per cent) and Amistar (@0.1 per cent) (26.86% per cent). The disease intensity was the highest (59.86 per cent) in control.

Manasa *et al.* (2018) Reported that Pre-harvest sprays of Azoxystrobin @0.1 per cent significantly minimised the latent infection and manifestation of the anthracnose disease (3.3 per cent) on fruits at postharvest stage. Fungicides were obtained from Karnataka Agro Chemicals, Dharwad. Fungicides namely, Thiophanate Methyl, Carbendazim, Tricyclazole at 0.1 per cent were prepared by dissolving 1 g of each fungicide in 100 ml of water and making up the volume to 1000 ml. For Azoxystrobin of 0.1 per cent, 1 ml of it was dissolved in water and made up to 1000 ml. Zineb of 0.2 per cent was prepared by dissolving 2 g of zineb in 100 ml of water and then

making up the volume up to 1000 ml. Among them Azoxystrobin at 0.1 per cent after first (20.71 per cent), second (22.33 per cent) and third sprays (24.54 per cent) assured greater protection to mango fruits in the field from anthracnose.

Sayiprathap *et al.* (2018) evaluated nine fungicide treatments and minimum per cent disease index (7.50 per cent) was recorded in Trifloxystrobin + Tebuconazole at 0.05 per cent concentration with 76.72 per cent disease reduction over control followed by Tebuconazole (9.72 per cent) PDI with 69.84 per cent disease reduction over control and Propiconazole (10.72 per cent) PDI with 66.73 per cent disease reduction over control at 0.1 per cent concentration while, maximum per cent disease index (24.96 per cent) with 22.55 per cent disease reduction over control was recorded in mancozeb at 0.3 per cent concentration. Whereas, Difenconazole (14.93 per cent) and Hexaconazole (13.29 per cent) were on par with each other with 53.67 per cent and 58.45 per cent disease reduction over control respectively at 0.1 per cent concentration. Similarly, propineb (21.75 per cent) at 0.3 per cent concentration and Azoxystrobin (20.71 per cent) at 0.1 per cent concentration were also on par with each other with 32.51 per cent and 35.74 per cent disease reduction over control respectively. The highest PDI (32.23 per cent) was recorded in untreated plot.

Kumar *et al*. (2020) tested efficacy of seven fungicides *viz*.; Carbendazim @ 0.1 per cent, Thiophanate Methyl @ 0.1 per cent, Mancozeb @ 0.2 per cent, Chlorothalonil @ 0.2 per cent, Tricyclazole @ 0.1 per cent, Carbendazim + Mancozeb @ 0.2 per cent and Propineb @ 0.2 per cent) was evaluated in RBD with three replications. The fungicides were sprayed thrice at 10 days interval, starting from the initiation of the disease symptoms in mango tree leaves and inflorescence. The result revealed that, among the different treatments, foliar spray with Carbendazim + Mancozeb (0.2 per cent) was the most effective for controlling anthracnose at Mohanpur, Paria and Rewa with a mean PDI of 6.16 per cent, 8.37 per cent and 6.06 per cent, respectively. At Sangareddy, Chlorothalonil (0.2 per cent) was found to be effective with a mean PDI of 8.80 per cent, whereas at Vengurle, Carbendazim (0.1 per cent) was found to be effective in controlling anthracnose of mango with a mean PDI of 9.81 per cent.

Das *et al*. (2020) carried out investigation in a RBD with five treatments and four replications in subtropical climatic condition of West Bengal at Regional Research Sub-Station, West Bengal, India. There were five treatments *viz*.; Azoxystrobin (23 per cent SC) @ 500 ml/ha, Picoxystrobin (22.52 per cent) SC @400 ml/ha, Kresoxim-methyl (44.3 per cent) SC @700 ml/ha, Hexaconazole (5 per cent) EC@ 1000 ml/ha. The data revealed that highest disease control was in Picoxystrobin (22.52 per cent) SC @ 400 ml/ha (75.89 per cent, 69.58 per cent and 72.97 per cent) followed by Azoxytrobin (23 per cent) SC @ 500 ml/ha (71.93 per cent, 63.83 per cent and 66.54 per cent), Kresoxim-methyl (44.3 per cent) SC @ 700 ml/ha (60.50 per cent, 52.70 per cent and 53.89 per cent) and Hexaconazole (5 per cent) EC @ 1000 ml/ha (46.71 per cent, 32.28 per cent and 37.58 per cent) on leaves, shoots and bunches respectively at 15 days after 2nd spray. All treatments

controlled effectively the anthracnose disease in grapes.

2.5. *In vivo* comparative performance of bio-agents

Naguleswaran *et al*. (2014) conducted a field experiment to investigate the effectiveness of *T. viride* against *C. gloeosporioides* and recorded that *T. viride* applied as a foliar spray resulted in relatively low disease incidence (1.08 per cent) as compared to the untreated control (11.3 per cent).

Saxena *et al*. (2015) evaluated the effectiveness of phyllosphere *Trichoderma* isolates for elevating the defence responses in chilli against *C. capsici* infection. A spore suspension of both the *Trichoderma* isolates at $2x10^7$ cfu/ml concentration was used for treating chilli plants. Treatment with *Trichoderm*a isolate BHUF4 recorded a 49.6 ± 7.74 per cent reduction in lesion development when compared to untreated plants while rhizospheric *Trichoderma* isolate T16A recorded a 44.44 ± 9.6 per cent reduction in the number of lesions.

Musheer and Ashraf. (2017) evaluated the efficacy of various *Trichoderma* species against *C. gloeosporioides* which causes the turmeric leaf spot disease. Three foliar sprays at concentration $4x10^6$ conidia/ml of *Trichoderma* spp. were administered. *T. viride* was superior to all the species in reducing the disease.

The effectiveness of pre-harvest sprays of bio-agents like *T. viride* and *T. harzianum*, each at a concentration of 0.5 per cent, was assessed in an experiment conducted by Manasa *et al*. (2018) at various growth stages of mango fruit (Alphonso), before the onset of flowering, at peanut and marble stages. When compared with control fruits (21.33 per cent index), it was found that *T. harzianum* and *T. viride* dramatically reduced the disease index of mango fruits to 17.00 per cent and 15.67 per cent, respectively.

Under field conditions, Lalhruaitluangi and Sinha. (2019) assessed the effectiveness of five isolates of *Trichoderma* in the form of foliar spray (5g/l of water) against the *C. truncatum*- the cause of soybean pod blight. Average disease incidence recorded was *T. harzianum* - KU933468 (7.33 per cent), T. *harzianum* - KU933474 (7.60 per cent), *T. atroviride* - KU933472 (8.75 per cent), *T. asperellum* - KU933475 (9.29 per cent), *T. ovalisporum* - KU904456 (9.69 per cent) where the average disease incidence in control was 14.31 per cent.

Isolates of T3, T4, T15 and T19 of *Trichoderma asperallum* and T6 isolate of *Trichoderma harzianum* were used for field evaluation against *C. gramincola* (Ces.). Foliar spray of talc-based formulation *Trichoderma* isolates @ 10g/lit of water was given after 30 DAS, 45 DAS, 60DAS and 75DAS after 15 days of interval to check the effect of different treatments on the progress of anthracnose of sorghum. The result showed that minimum PDI was recorded in T19 isolate (45.82 per cent) followed by T3 isolate (46.59 per cent) which was statistically at par with T3 isolate whereas maximum PDI was recorded in untreated control (71.27 per cent) at 75 DAS by (Manzar and Singh., 2020).

T. asperellum was applied as a foliar spray by Chien and Huang (2020) for the control bacterial spot of tomato. The effectiveness of *T. asperellum* CHF 78 (10^6 conidia/ml) decreased disease severity up to 27.7 per cent.

The usefulness of *T. harzianum* for management of mango anthracnose was investigated Sharma *et al.* (2021) Estimate the efficacy of the bio-control agents as spray application under field conditions showed that three successive sprays with *T. harzianum* isolate 1 at 5 per cent concentration, followed by other two sprays at an interval of fifteen days, were the most effective at both the locations and reduced the disease to an extent of 59.91 per cent, 54.77 per cent disease control on leaves and 56.57 per cent, 52.66 per cent disease control on fruits, respectively. It was followed by *T. virens* (53.93 per cent, 51.98 per cent on leaves, 51.74 per cent, 50.41% on fruits). At both locations on leaves, minimum percentages of disease index of 26.67 and 30.59 were recorded with *T. harzianum* isolate 1. Analogically, at both locations on fruit minimum percentages of disease indices of 27.69 and 32.46 were recorded with *T. harzianum* isolate 1.

Vandana *et al*. (2021) evaluated several fungicides and bio agents against *C. gloeosporioides*. Among systemic fungicides, Propiconazole 25 per cent EC, Hexaconazole 5 per cent EC, Iprobenfos 48 per cent EC and Kresoxim methyl 44.3 per cent EC recorded cent per cent mycelial inhibition of *C. gloeosporioides*. Among six contact and seven combi products (1000, 1500, 2000 and 3000 ppm), Mancozeb 75 per cent WP, Zineb 75 per cent WP, Tricyclazole 18 per cent + Mancozeb 62 per cent, Carbendazim 12 per cent + Mancozeb 63 per cent and Carboxin 37.5 per cent + Thiram 37.5 per cent completely inhibited the growth of *C. gloeosporioide*. Among different bio agents tested, *Trichoderma harzianum*, UAS, Dharwad isolate recorded significantly higher per cent mean inhibition followed by *Trichoderma harzianum*, UHS, Bagalkot isolate.

Three isolates of *Trichoderma asperellum* (S3M4ZIG, F3GP3 and F3GP3-5), one isolate of *Trichoderma viride* (F2KV3) and a fifth isolate of *Trichoderma* spp. (S5M5ZN), obtained from mango tree organs in Senegal, were used in direct confrontation against *C. gloeosporioides in vivo* tests on fruits, by Cisse *et al.,* (2022). The incidence and severity of anthracnose were evaluated after 20 days of incubation. It showed that fruits that received the isolates F2KV3 (*T. viride*), F3GP3 and S3M4ZIG (*T. asperellum*) after inoculation with *C. gloeosporioides* showed a low incidence of anthracnose, with a mean value around 22 per cent. At the same time the negative control treatment (T0) that was soaked in sterile water was displaying 44.44 ± 00.00 per cent incidence. The positive control (T+), inoculated with the pathogen presented the highest incidence with 88.88 ± 00.00 per cent.

Houndahouan *et al.* (2022) evaluated two strains of *Trichoderma viz*, *T. harzianum* and *T. pseudokoningii* against *C. gloeosporioides in vivo.* The results revealed that all the strains of both species of Trichoderma showed evident antagonistic activity against *C. gloeosporioides*. The strains of *T. pseudokoningii* and those of *T. harzianum* reduced the mycelial growth of *C. gloeosporioides* by 56.94 per cent and 47.48 per cent, respectively.

CHAPTER III

MATERIAL AND METHODS

All the laboratory experiments carried out to fulfill the defined objectives of the present research entitled "**Comparative Performance of Fungicides with Bioagents forManagement of Anthracnose Disease of Mango"** were carried out at the department of Plant Pathology, College of Agriculture, Dapoli and the field experiments were conducted at the Centre for Mango Excellence, College of Horticulture, Dr. B.S.K.K.V., Dapoli.

The detail description about various material, techniques and methods used during the pursuing of research work is given in the succeeding pages.

3.1. Materials and Methodology

3.1.1. Experimental material

3.1.1.1. Collection of disease samples

The diseased samples showing typical anthracnose symptoms collected from the Centre for Mango Excellence, College of Agriculture, Dr. B.S.K.K.V., Dapoli. Wrapped in a separate paper bag on which write the date, day, and time of sample collection and brought to the laboratory for further investigations.

3.1.1.2. Culture media

The common laboratory culture medium PDA will be used for isolation, purification, multiplication and maintenance of pure cultures of the test pathogen and also for various *in-vitro* studies.

3.1.1.3. Glassware

Sterilized glassware (Borosil, Corning, J-sil) *viz.,* Petri dishes, test tubes, conical flasks, volumetric flasks, measuring cylinder, glass rods, beakers, funnel, pipettes etc. will be used for various studies. obtained from the Department of Plant Pathology, College of Agriculture, Dapoli.

3.1.1.4. Equipment's

The laboratory equipment's *viz.*, autoclave, hot air oven, laminar air flow cabinet, BOD incubator, refrigerator, binocular research microscope, electronic balance, pH meter, etc. available at the Department of Plant Pathology, College of Agriculture, Dapoli and will be used asand when required.

3.1.1.5. Chemicals

Standard chemicals and reagents required for the experimentation will be obtained from the Department of Plant Pathology, College of Agriculture, Dapoli.

3.1.1.6. Fungicides

Various fungicides intended to be evaluated in different *in vitro* and *in vivo* studies will be obtained from the Department of Plant Pathology, College of Agriculture, Dapoli.

3.1.1.7.Glassware cleaning

The glassware was dipped in a solution containing 60 ml of potassium dichromate ($K_2Cr_2O_7$) and 60 ml of concentrated sulphuric acid (H_2SO_4) in 1000 ml of water for 24 hours followed by washing underrunning tap water and finally rinsed with distilled water.

3.1.1.8. Sterilization

The media were autoclaved at 1.054 kg/cm^2 pressure for 20 minutes. Blades, inoculation needles, cork borers, forceps, and other small metallic tools were routinely sterilized by incineration.

3.1.1.9. *Trichoderma* strains:

Three *Trichoderma* strains will be collected from Department of Plant Pathology, College of Agriculture, Dapoli.

3.1.1.10. Miscellaneous

Polypropylene bags, inoculation needle, forceps, blotter paper, paper bags, spirit lamp, labels, etc. were used during the course of present investigation.

3.2. Methodology

3.2.1 Examination of diseased samples

3.2.1.1. Visual Examination

The development of symptoms under natural conditions in the field were observed and recorded.

3.2.1.2. Microscopic Examination

Fresh leaf and twig samples showing typical anthracnose symptoms were collected from the field, brought to the laboratory and washed thoroughly with running tap water to remove extraneous material. With the help of sharp blade, free hand sections of the diseased leaf and twig sample were taken. Leaf tissue sections were placed in water drop on clean glass slides, covered with cover slip and mounted initially under low power objective lens of compound microscope and morphology of fungal mycelium, colour of the mycelium, and spores were observed.

3.3. Preparation of culture media

Potato dextrose agar medium was used for isolation, pathogenicity test, biocontrol agent and preserving stock culture of test pathogen.

Composition of PDA medium

Sr. No.	Ingredients	Composition g/ml

1	Peeled Potato	200.0 g
2	Dextrose	20.0 g
3	Agar	20.0 g
4	Distilled Water	1000.0 ml

3.4. Isolation of fungus causing anthracnose

Standard tissue isolation technique was employed to isolate the pathogens. Naturally infected mango leaves showing typical anthracnose disease symptoms were collected from the orchard of the Centre for Excellence of Mango. Diseased leaves were properly cleaned under running tap water; blot dried and cut with a sharp sterilized blade into small (5 mm) pieces with half healthy and half diseased portions keeping intact. A 0.1% solution of mercuric chloride was used to disinfect small pieces of leaf sample, which were submerged for 30 sec. Such bits were dried with a sterilized blotter and aseptically inoculated on sterilized solidified PDA in Petri plates. The plates were incubated at room temperature (27 ± 2^0C) for seven days and monitored for growth of the causal organism. The PDA slants with pure fungal growth were stored in refrigerator at 4^0C for future use.

3.4.1. Identification of the pathogen

Identification of the isolated organisms was done on the basis of morphological and colony characteristics. The recorded characters were compared with description available in standard books, research articles and authentic websites for fungal identification.

3.4.2. Inoculation

3.4.2.1. Spray inoculation and Pin prick method

Healthy seedlings of mango (Cv-Alphanso) were brought to the laboratory to prove Koch's postulates. Healthy, green, uniform sized leaves on such seedlings were chosen for artificial inoculation. Seven days old pure culture of the isolated fungus grown in PDA was used for inoculation. Mycelial plugs of such culture were cut with a 5 mm cork borer. The leaves, petioles and stems of selected mango seedlings were injured with a sterile needle. Then, the mycelial plugs were placed on the injured portion of the leaves, petioles and stems and covered with sterile non-absorbent cotton plugs to avoid drying of mycelium. One healthy, injured but non inoculated plant served as control. All the inoculated and non-inoculated plants were transferred to a humid chamber and routinely observed for symptom development. The pathogenicity was carried out by pin prick method. The leaves, petiole, stem was wounded with sterile needle and inoculated with 2 x 2 mm mycelial agar plugs of the fungus. And also, with the help of sprayed suspension on the crops. Seven days old culture of the isolated pathogens grown in potato dextrose agar was used for inoculation. One plant kept as it is as control. Both the control and treatment, the agar plug was covered with sterile nonabsorbent cotton and sprayed with sterile distilled water. The inoculated and uninoculated control plant were kept inside a big pathogenicity chamber separately to maintain

high humidity for two days at room temperature. The plant was routinely examined for the appearance of disease symptoms.

3.4.2.2. Leaves detached technique

The detached leave was washed under running tap water for 60 seconds followed by surface sterilization by immersing the fruits in 70% ethanol for 3 minutes, 2% sodium hypochlorite solution for 5 minutes and then rinsing three times in sterile distilled water for 2 minutes and drying with sterile tissue paper and then air drying. Surface sterilized leaves were placed in a plastic box with tissue paper then sprayed with sterilized water to maintain at least 95% relative humidity. The samples were inoculated using the wound/drop inoculation method which included pin-pricking on leaves, the fruits to a 3 mm depth with a sterile needle in the middle portion of leaves and then placing 20µl of conidia suspension onto the wound. Control fruits were inoculated with 20µl of sterile distilled water. The inoculated samples were incubated in the plastic containers at 25°C under controlled conditions. The plastic box removed after 48hr and leaves were kept at the same temperature. The plant was routinely examined for the appearance of disease symptoms.

3.4.3. Re-isolation

After the development of symptom on artificially inoculated plant, they were differentiated with naturally developed symptoms under field conditions. Samples taken from artificially infected plants were used for re-isolation of the organism on the PDA under *in vitro*. The fungal growth obtained on potato dextrose agar medium after re-isolation was compared with the original culture of the organism isolated from naturally infected anthracnose disease of mango, to obey Koch's postulate of pathogenicity.

3.5. Comparative performance of fungicide and bio-agents against the *C. gloeosporioides in vitro*

3.5.1. Comparative performance of fungicide against the *C. gloeosporioides in vitro*

The efficacy of three different fungicides *viz*; two systemic Thiophanate methyl 70% WP @ 0.05%, 0.075%, 0.1%, 0.125, 0.2%, and Azoxystrobin 23% SC@ 0.1% and one contact Carbendazium12%+Mancozeb 63%WP @ 0.15%, at different concentration were assessed by Poison food technique (Nene and Thapliyal, 1993). The pathogen *C. gloeosporioides* of mango was grown on PDA in Petri plates for seven days prior to setting the experiment. The experiment was planned to evaluate *in vitro* efficacy of three fungicides against the pathogen.

Based on the recommended dosages, to obtain the desired concentration on the basis of active ingredients and whole product present in the chemical, fungicide suspension was prepared in PDA by adding required quantity of fungicide. 20 ml of poisoned medium was poured in each of the sterilized Petri plates and each treatment was replicated thrice with one concentration. Mycelial disc of 5 mm was taken from the periphery of seven-day old culture and placed in the centre of Petri plate containing poisoned media and incubated at 27± 2 °C till growth of the fungus reached the peripheries in the control plate. Suitable checks were also maintained without addition

of any fungicide. The diameter of the colony was measured in three directions and average was worked out. Those petri plate were also observed for presence or absence of sporulation.

3.5.2. Comparative performance of bio-agents against the *C. gloeosporioides in vitro*

Antagonistic activity of *Trichoderma* strains against mango anthracnose pathogen was tested on PDA using dual culture technique (Huang and Hoes.1976). Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile petri plates and allowed to solidify. For evaluation of fungal biocontrol agents, mycelial discs of 5 mm in diameter of test fungus were inoculated at one end of the petri plates and antagonistic fungus was placed opposite to it on the other end. The experiments were performed thrice with seven replication and one plates kept as a control. The plates were incubated at 27± 2°C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947).

$$\text{Per cent Inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

C = Growth (mm) of the test fungus in untreated control plate

T = Growth (mm) of the test fungus in treated plates

Experiment details : Comparative performance of fungicide and bio-agents against the *C. gloeosporioides in vitro*

Design : CRD (Completely Randomized Design)

Replications : Three

Treatments : Eleven

List of fungicides and bio-agents evaluated *in vitro* against *C. gloeosporioides*

Tr.No.	Common names and formulation	Trade name	Manufacturing company	Conc. in (%) used
T_1	Thiophanate methyl 70% WP	Roko	Bio-Stadt	0.05%
T_2	Thiophanate methyl 70% WP	Roko	Bio-Stadt	0.075%
T_3	Thiophanate methyl 70% WP	Roko	Bio-Stadt	0.1%
T_4	Thiophanate methyl 70% WP	Roko	Bio-Stadt	0.125%
T_5	Thiophanate methyl 70% WP	Roko	Bio-Stadt	0.2%
T_6	Carbendazium12%+Mancozeb 63%WP	Crossman	Shivalik Crop Sciences	0.15%
T_7	Azoxystrobin 23% SC	Amistar	Syngenta	0.1%
T_8	*T. harzianum*		-	-
T_9	*T. longibrachiatum*		-	-
T_{10}	*T. koningii*		-	-
T_{11}	Control		-	-

3.6. Comparative performance of fungicide and bio-agents against the *C. gloeosporioides in vivo*

3.6.1. Comparative performance of fungicide against the *C. gloeosporioides in vivo*

The Field experiment was conducted at Centre for Mango Excellence, College of Horticulture, Dr. B.S.K.K.V., Dapoli. Sixty-four trees with uniform flowering were choosened (Cv-Alphanso). The efficacy of three fungicides *viz*; two systemic Thiophanate Methyl 70% WP @ 0.05%, 0.075%, 0.1%, 0.125, 0.2%, and Azoxystrobin 23% SC@ 0.1% and one contact Carbendazium12%+Mancozeb 63%WP @ 0.15%, at different concentration was evaluated in Randomized Block Design (RBD) with three replications. These fungicides were dissolved in 30 litre waters for each treatment and stirred with rod to make up mixture well. The fungicides were sprayed thrice at 15 days interval starting from the first appearance of disease. Percent Disease Intensity (PDI) was recorded on 50 leaves/tree that were randomly selected before the first spray of fungicides and two tree were selected for one treatment. Subsequent observations were recorded after 15 days of each spray.

3.6.2. Comparative performance of bio-agents against the *C. gloeosporioides in vivo*

Three local *Trichoderma* species *viz*.; *T. harzianum*, *T. longibrachiatum* and *T. koningii* each with 10% conc. were further evaluated under field conditions on randomly selected mango plants of cultivar 'Alphonso' at Centre for Excellence of Mango.

3.6.2.1. Mass multiplication of *Trichoderma* spp

All the three *Trichoderma* spp. *viz*. *T. harzianum, T. longibrachiatum* and *T. koningii* were grown on PDB (Potato dextrose broth) medium. The growth of these species on broth medium was inoculated on thick pohae (flat beaten rice) for mass multiplication. A quantity of 1000 g of thick pohae (flat beaten rice) was taked and then it was washed with tap water 3-4 times to remove dirt and other materials. Then it was allowed to air dry up to 70-75% moisture level. In order to added M_NSO_4 and $CaCl_2$ @ 0.4% and dextrose 10g then it was well mixed with pohae. These pohae were filled in 1000 ml of conical flask (each flask containing 300g thick pohae). Then all the flasks were autoclaved. Mycelial mat of respective *Trichoderma* species from PDB medium was transferred to the flasks containing pohae under aseptic conditions and plugged with non-absorbent cotton. Then these flasks were maintained at room temperature. After completion of growth of *Trichoderma* spp. On pohae, it was used for preparation of *Trichoderma* solution. In order to prepare spray solution, 300ml of distilled sterile water was added to the flask containing fully grown mycelium on pohae, after shaking well, the spore suspension of *Trichoderma* was obtained which was passed through muslin cloth to get grain free liquid. This solution was used as a stock solution and was used for spraying as per requirement.

3.6.3. Evaluation of *Trichoderma* species under field conditions against the *C. gloeosporioides*

In this disease, leaves, twigs and inflorescence initially infected and then the infection progresses to whole tree. Selected twigs of branches with initial symptoms in the canopy were tagged in such a way that there were 10 twigs per replication (per plant) and 2 trees per treatment.

Experiment was laid out in randomized block design (RBD) with three replications for each treatment. The spore suspension of each *Trichoderma* species was sprayed at a concentration of 10% (3 L of *Trichoderma* suspension in 30 litres of water), thrice starting with the initiation of symptoms of anthracnose disease sprays at an interval of 15 days. The water-sprayed trees served as control. The observations on per cent disease intensity on leaves, twigs and inflorescence were recorded by adopting standard disease scales proposed by the authors mentioned below. Disease index for anthracnose disease on leaves was recorded a day before first spray and 15 days after each spray.

Experiment Details : Comparative performance of fungicide and bio-agents against the *C. gloeosporioides in vivo*

Design : Randomized Blocks Design (RBD)

Replications : Three (3)

Treatments : Eleven (11)

Treatment details :

List of fungicides and bio-agents evaluated under field conditions against *C. gloeosporioides*

Tr.No.	Common names and formulation	Trade name	Manufacturing company	Conc. in (%) used
T_1	Thiophanate methyl 70% WP	Roko	Bio-Stadt	0.05%
T_2	Thiophanate methyl 70% WP	Roko	Bio-Stadt	0.075%
T_3	Thiophanate methyl 70% WP	Roko	Bio-Stadt	0.1%
T_4	Thiophanate methyl 70% WP	Roko	Bio-Stadt	0.125%
T_5	Thiophanate methyl 70% WP	Roko	Bio-Stadt	0.2%
T_6	Carbendazium12%+Mancozeb 63%WP	Crossman	Shivalik Crop Sciences	0.15%
T_7	Azoxystrobin 23% SC	Amistar	Syngenta	0.1%
T_8	*T. harzianum*	-	-	10%
T_9	*T. longibrachiatum*	-	-	10%
T_{10}	*T. koningii*	-	-	10%
T_{11}	Control	-	-	-

The Disease severity was assessed by using a 0 – 9 scale given by Jamadar and Desai. (1997).

Sr. No.	Rating	Description
1	0	No infection observed
2	1	1 – 10%

3	3	10.1 – 15.0%
4	5	15.1 – 25.0%
5	7	25.1 – 50.0%
6	9	More than 50%

The per cent disease intensity (PDI) was calculated using the formula developed by McKinney. (1923).

$$PDI = \frac{\text{Sum of individual disease ratings}}{\text{Total number of leaves assessed x Maximum disease grade}} \text{ X } 100$$

Further, per cent Disease Control (PDC) was calculated by following formula:

$$\% \text{ Disease control PDC} = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \text{ X } 100$$

3.5. Statistical analysis

The data generated of all the experiments will be statistically analyzed (Panse and Sukhatme.1967). The standard error and critical difference were work out and the results obtained were compared statistically.

3.6. Collaboration (if any)

No any collaboration work with other institutes.

CHAPTER IV
RESULTS AND DISCUSSION

Among the diseases of mango, anthracnose is a prominent and destructive disease caused by *Colletotrichum gloeosporioides.* The quiescent infection of anthracnose results in pre-harvest rot of mango fruit crop. The present research entitled "Comparative performance of fungicides with bio-agents for management of anthracnose disease of mango" was conducted at the Department of Plant Pathology, College of Agriculture, Dr. B. S. K. K. V., Dapoli and Centre for Excellence of Mango, College of Horticulture, Dr. D. B. S. K. K. V., Dapoli, during the year 2022-2023 on the aspects *viz*; collection of disease sample from susceptible mango cultivar and isolation of pathogen and comparative performance of fungicides with bio-agents against the pathogen *in vivo*. The results obtained from all the experiments conducted to fulfil the aforementioned objectives are presented here under.

4.1. Examination of diseased samples

4.1.1 Visual Examination

The occurrence of anthracnose disease was observed at mango orchard, Department of Horticulture, College of Agriculture, Dr. B. S. K. K. V., Dapoli. The typical symptoms of anthracnose disease on mango plants that were naturally occurred were visible on the leaves, petioles, twigs, stem and inflorescence. Plants infected with anthracnose exhibit the symptoms *viz*; leaf spot, blossom blight, wither tip, twigs blight and fruit rot. Pre-harvest anthracnose symptom was characterized by sub-cuticular and angular black lesions develop on stems, leaves and inflorescences which enlarged and coalesced to destroy the leaf edges or whole inflorescences. The lesions enlarge along the leaf margin affect the growth of leaves; when the lesions become dry, it fall out to form 'shot hole 'appearance.

4.1.2 Microscopic Examination

Temporary mounts of diseased leaf, inflorescence and twig tissues collected from naturally infected plants were prepared in lactophenol cotton blue by taking thinnest sections from samples and observed under compound microscope.

Microscopic examination of infected leaf sections showed the presence of septate mycelium with hyaline, cylindrical conidia.

The observations mentioned above showed the association of fungal pathogens with the disease.

4.2 Collection of disease sample and isolation of the pathogen

4.2.1 Isolation

The field samples showed typical anthracnose symptoms on leaves, petioles, stems and inflorescence were collected and brought to the laboratory and temporary mounts were observed under compound microscope to know the association of the pathogen. The fungus observed in association with disease samples was isolated on PDA by following standard procedure of tissue isolation. After seven days of incubation at ambient temperature, pure, cottony white, elevated, thick, compact mycelial colony grew in the petri plates.

The fungus isolated from diseased leaf tissue formed whitish to gray-colored mycelial growth and gray conidial mass in the center which turned black after incubation at 27± 2^0 C for a period of 10 days.

Similar results have been obtained by Jayasinghe and Fernando. (2009) from Sri Lanka for isolation of *C. gloeosporioides* from infected mango leaves. Also, the collection and isolation of *C. gloeosporioides* isolates were reported by Shivakumar *et al*. (2015) from Alphonso mango grafts in North Estern Karnataka, Zainab and Shinkafi (2016) from Sokoto, Kumari *et al*. (2017) from *cv*. Dashehari, Kumar *et al*. (2017) from Vindhya Region of Madhya Pradesh, Sayiprathap *et al*. (2018) from Karnataka.

The collection and isolation of *Colletotrichum gloeosporioides* from infected mango fruits were also reported by Awa *et al.* (2012) from Nigeria, Zakaria *et al*. (2015) from Malaysia, Majumdar and Mandal. (2018) from West Bengal, Ansari *et al*. (2018) from Pakistan. Similarly, Zainudin and Sattar. (2019) collected and isolated from Malaysia, Laksanaphisut *et al*. (2019) from different location from China, Neto *et al*. (2020) from Brazil, Arizpe *et al*. (2021) from Mexico. Also, similar results were obtained by Chung Kuo. (2001) from Tainan, Gupta *et al*. (2010) from India, Abera *et al*. (2016) from south west part of Ethiopia, Nattawut Rungjindamai. (2016) from Thailand for isolation of *C. gloeosporioides* from infected leaves and fruits of mango.

Most of the workers have reported *C. gloeosporioides* as the major cause of anthracnose of mango and present conclusions also confirm this.

4.2.2 Inoculation

Pathogenicity test of isolated fungus was carried out with two techniques *viz*; spray inoculation and pin prick method and leaves detached technique**.**

4.2.2.1 Spray inoculation and pin prick method

The pathogenicity test of isolated fungus was carried out on leaves, petioles and stem of eight-month-old Alphanso seedling of mango cultivar. The seedlings used in this experiment were watered when required and more than 80 per cent humidity and ambient temperature was maintained in the chamber to facilitate easy penetration of the host tissues. Initially, small, grey to brown specks appeared on artificially inoculated plant parts which gradually expanded into dark brown to black coloured lesions. These symptoms were identical to those seen in the field. No symptoms were observed on non-inoculated seedlings (Plate III A).