MANAGEMENT OF POST HARVEST ANTHRACNOSE DISEASE OF MANGO CAUSED BY *Colletotrichum gloeosporioides*

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MANAGEMENT OF POST HARVEST ANTHRACNOSE DISEASE OF MANGO CAUSED BY *Colletotrichum gloeosporioides*

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CERTIFICATE

This is to certify that the thesis entitled **"MANAGEMENT OF POST HARVEST ANTHRACNOSE DISEASE OF MANGO CAUSED BY *Colletotrichum gloeosporioides*"** submitted to the Department of Plant pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **Master of Science** in**Plant Pathology**, embodies the result of a piece of *bonafide* research work carried out by, Registration No. **11-04335**under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

Dated:JULY, 2017

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Dedicated To
My Beloved Parents

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ABSTRACT

The present study has been conducted for the management of postharvest anthracnose of mango (*Mangifera indica*) at Plant Pathology Laboratory of Sher-e-Bangla Agricultural University during July-December 2017. The fruit market survey was conducted at three markets of Dhaka city *viz*. Kawran bazaar, Shyamoli and Mirpur-10. For the management of postharvest anthracnose disease, 13treatments were considered including control with three replications. The experiment was laid out in a Complete Randomized Design (CRD). The assigned treatments was as T_1 = Autostin 50WDG at 500 ppm concentration, T_2 = Autostin 50WDG at 1000 ppm concentration, T_3 = Tilt 250EC at 500 ppm concentration, T_4 = Tilt 250EC at 1000 ppm concentration, T_5 = Dithen M-45 at 500 ppm concentration, T_6 = Diathen M-45 at 1000 ppm concentration, T_7 = Rovral 50W at 500 ppm concentration, T_8 = Rovral 50W at 1000 ppm concentration, T_9 = Garlic extract 1:1 (Extract: water), T_{10} = Garlic extract 1:2 (Extract: water), T_{11} = Alamanda extract 1:1 (Extract: water), T_{12} = Alamanda extract 1:2 (Extract: water) and T_{13} = Control. Among the three fruit market, the highest disease incidence 18.62% and disease severity 14.78% of post-harvest anthracnose disease was recorded Kawran bazaar while the lowest disease incidence 12.52% and disease severity 9.32% was recorded Mirpur 10. In case of chemical and botanical fungicides, treatment, Tilt 250EC at 1000 ppm concentration showed the best performance in controlling mycelia growth of Anthracnose diseases. The highest growth inhibition of mycelia 82.12, 76.47 and 60.98% respectively at 3,5 and 7 DAI was reduced from the treatment Tilt 250 EC followed by Rovral and Autostin .Regarding percent (%) infected area, the lowest infestation 2.50, 3.00 and 4.50% at 3, 5 and 7 DAI, respectively and highest percent (%) reduction of infected area over control 92.13%, 92.06% and 92.80% at 3, 5 and 7 DAI, respectively was also achieved from the treatment, Tilt 250EC at 1000 ppm concentration. In terms of management practices using hot water treatment at 55°C treated mango showed the lowest mycelia growth of Anthracnose at 3, 5 and 7 DAI 3.78cm, 4.22cm and 5.60 cm, respectively and highest percentage of growth inhibition of mycelia 47.21cm, 45.76cm and 30.86cm at 3, 5 and 7 DAI, respectively. The lowest infected area 5.5%, 8% and 25.50% at 3, 5 and 7 DAI, respectively and highest by 81.67%, 85.45% and 72.28% at 3, 5 and 7 DAI, respectively.

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ABBREVIATIONS AND ACRONYMS

%	=	Percentage
AEZ	=	Agro-Ecological Zone
BBS	=	Bangladesh Bureau of Statistics
BCSRI	=	Bangladesh Council of Scientific Research Institute
Ca	=	Calcium
cm	=	Centimeter
CV %	=	Percent Coefficient of Variation
DAS	=	Days After Sowing
DMRT	=	Duncan's Multiple Range Test
e.g.	=	exempli gratia (L), for example
et al.,	=	And others
etc.	=	Etcetera
FAO	=	Food and Agricultural Organization
g	=	Gram (s)
GM	=	Geometric mean
i.e.	=	id est (L), that is
K	=	Potassium
Kg	=	Kilogram (s)
L	=	Litre
LSD	=	Least Significant Difference
M.S.	=	Master of Science
m^2	=	Meter squares
mg	=	Miligram
ml	=	MiliLitre
NaOH	=	Sodium hydroxide
No.	=	Number
^oC	=	Degree Celceous
P	=	Phosphorus
SAU	=	Sher-e-Bangla Agricultural University
USA	=	United States of America
var.	=	Variety
WHO	=	World Health Organization
µg	=	Microgram

CHAPTER I

INTRODUCTION

Mango (*Mangifera indica* L.) unarguably is one of the oldest and chooseful tropical fruits of the world and is rightly designated as "King" of all fruits. Mango belong to family Anacardiaceae is almost grown in all state of India. The name 'Mango' is derived from Tamil word 'Mangkay'. Mangoes originated in north-east India, Burma and Andaman Islands and bordering Bay of Bengal. According to Mukherjee (1958), the natural spread of the genus is limited to the Indo-Malaysian region, stretching from India to Philippines and New Guinea in the east. Mango is being cultivated for more than 4000 years (Candole, 1984).Among all types of fruits mango is considered as the class one fruit in the world. Popenoe (1964) mentioned mango as "the king of the oriental fruits". It is widely grown all over Bangladesh; while the quality mangoes solely concentrated in the north-west areas, especially greater Rajshahi, Dinajpur and Rangpur in Bangladesh (Karim, 1985). It ranks third among the tropical fruits grown in the world with total production of 30.147 million tons (FAO, 2010). In Bangladesh, mango ranks second position in terms of area cultivated and third in terms of production. The country produced 12 lac tons of mangos in 39.91 thousands ha of mango orchard during the period of 2015-16 (BBS, 2017)

In Bangladesh, there exists a wide variability in mango due to its cross pollination and seed propagation. Mango is a popular fruit of the country having some special organoleptic features such as excellent flavor, pleasant aroma, attractive color and test. It is a rich source of vitamins, minerals and total soluble solids (Pramanik, 1995). It is also a medium source of carbohydrate as ripe mango pulp contains 16.9% carbohydrate (Salunkhe and Deasai, 1984). The minimum dietary requirement of fruit/day/head is 85g, whereas our availability is only 30-35g,

which is much lower than recommended daily allowance (Siddique and Scanlan, 1995).

Mango fruit is very popular due to its wide range of adaptability, high nutritive value, richer in variety, delicious taste and excellent flavor. The fruit contain nearly 81 percent moisture, 0.4 percent fat, 0.6 percent proteins, 0.8 percent of fibers. It also contains nearly 17 percent of carbohydrates. The fruit is rich in important minerals like potassium, magnesium, sodium, phosphorus and sulphur (Anonymous, 2013).

A large number of diseases of mango were recorded by many researchers of Bangladesh (Sarkar, S. R, 2008; Choudhory, 2009; Choudhory *et al*. 2011) and in the world (Colon *et al*., 2002; Awasthi *et al*., 2005). Dey *et al*., (2007) reported anthracnose, stem end rot, powdery mildew, sooty mould, malformation and fruit rot complex were very common and destructive disease in Bangladesh. Out of these diseases anthracnose, die back, powdery mildew, leaf spot, sooty mould and red rust are important. They are caused by *Colletotrichum gloeosporioides*, *Diplodia natalenis*, *Oidium mangifera*, *Alternaria alternata*, *Capnodium ramosum* and *Cephaleurous virescencs* respectively are predominant diseases. Guogan and Bingquing (1999) observed that anthracnose disease usually affected the leaves, flowers, fruits and new shoots of mango trees. When young leaves were attacked, many small brown round spots with faint yellow margins appeared and the badly infected flower clusters turned black and rotted. Infected fruits were abnormal in shape, became black and then dropped. Post harvest anthracnose on mango appeared as round brown to black spots with an indefinite border on the fruit surface which could coalesce and cover extensive areas of the fruit (Arauz, 2000).Many physical and chemical treatments have been used for control of post-harvest losses in mango (Johnson *et al*., 1997). Wang (1989) suggested that low temperature storage is the most effective method for persevering the chemical composition of most perishable horticultural commodities because it retards

respiration, delays ripening besides imposing other undesirable metabolic changes. Low temperature handling and storage are the most important physical method of control post-harvest losses (Seyoum and Woldetsdik, 2004).Other than fungicides, hot water treatment is one of the physical methods for quality and shelf life of mango. It is an eco-friendly and quick exercise for maintain uniform temperature.It is reported that post-harvest anthracnose of mango effectively control by Neem leaf extract and Garlic extract for increasing for shelf time. The additional benefit of hot water treatment is that it can control postharvest diseases such as anthracnose and stem end rot (Couey, 1989). Sarkar (2012) observed the longer shelf life (14.87 days) in Neem leaf extract treated fruits followed by Garlic extract treated fruits having shelf life (14.25 days). Neem leaf extract and garlic extract treated mangoes appeared to be the best for extending shelf life (14.87, 14.25 days) in Langra mango fruits whereas the Control fruits had the shortest shelf life (10.00 days).

In context with above facts the present investigation was carried out to find out the proper postharvest management of anthracnose of mango.

Objectives:

1. To survey on the postharvest anthracnose disease of mango
2. To determine the disease incidence (DI) and disease severity (DS) of post-harvest anthracnose diseases of mango
3. To isolate and identify the causal organisms of post-harvest anthracnose diseases of mango
4. To formulate the suitable management practices against anthracnose of mango

CHAPTER II

REVIEW OF LITERATURE

Postharvest diseases of fruits are most severe causes of loss of production. Mango is susceptible to a number of diseases at all stages of its development from the seeding to the fruits. Post-harvest diseases result not only in substantial losses and reduction in mango production but also reduce quality of the fruits. In the present study an effort has been made to identify the different fungal diseases occurring in at orchard level and marketing channel after harvesting and to apply sustainable method of fruits handling to prevent postharvest fungal diseases and to find out proper management tactic to minimize the post-harvest losses and ultimately the farmers get benefited by utilizing the remedy. The information on postharvest diseases of mango available is reviewed and presented here.

2.1 Prevalence of post-harvest fungal diseases of Mango - Survey

2.1.1 Farm Level

Anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz.and Sacc. is the most important and widespread form of decay affecting mature mango fruit worldwide (Prusky and Keen, 1993). It is a widely distributed in the entire mango growing states of the India causing huge economic loss. Prabhakar *et al.* (2005) observed higher incidence of wound fungi such as *Aspergillus niger*(L.) Van, Tieghem and *Rhizopus arrizus* Fischer (0.90 percent) than other diseases *viz.* stem end rot, anthracnose at field level.

The total postharvest losses in variety Baganpalli of mango was 29.73 percent comprising 15.59 percent at the farm level and observed 23 percent fungal diseases resulted into losses at Bangalore (Murthy *et al.,* 2009). Mansour and his associates (2006) proved that *A. alternata*, *Botryodiplodia theobromae* Pat.

and*Botrytis cinerea* were highly pathogenic to mango cultivars Keitt, Kent, and Tommy Atkins at Egypt.

Percent disease incidence of four different postharvest diseases were observed in mango at different locations of Pakistan in which Multan has highest incidence of anthracnose (90.00%), stem end rot (86.60%), alternaria rot (76.60%), aspergillus rot (53.30%) while Khaniwal has the least incidence of anthracnose (40.00%), stem end rot (16.00%), alternaria rot (16.00%) and aspergillus rot (3.33%) as compared to Multan and Muzaffargarh with 100% prevalence of four postharvest disease i.e. anthracnose, stem end rot, aspergillus rot, alternaria rot at all locations (Malik *et al.*, 2016).

Tucho *et al*. (2014) studied the incidence and severity of mango anthracnose under farmers' field conditions and recorded that the incidence of mango anthracnose on leaf and fruit was significantly varied from district to district. The mean incidence on the fruit ranged from 36.20 to 74.00 percent. There was significant difference among districts in terms of mango anthracnose severity and it was highest in Gomma district and the lowest in Kersa district of Southwest Ethiopia. The mean severity values of mango anthracnose in the field ranged from 38.10 to 63.00 percent. The incidence of mango anthracnose can reach almost 100 percent in fruit produced under wet or very humid condition (Akem, 2006).

Malik *et al.* (2015) reported up to 25 percent post-harvest disease development by *A. alternata, Penicillium mangiferae, Botryodiplodia spp*. at farm level in Pakistan. Usually four fungal diseases viz. Stem end rot, Black rot, Rhizopus rot and Asperillus rot causes 1.5%, 1.0%, 1.0% and 0.7% fruit loss respectively with total average loss of 4.20% at orchard level (Sharma, 2014).

A comprehensive survey was undertaken in twelve mango orchards to record disease distribution for assessing the status of major post-harvest diseases of mango in the orchards of Punjab and Sindh province of Pakistan. In both

provinces at farmer block anthracnose disease incidence was ranged from 1.00 to 70.00 percent and stem end rot was from 15.00 to 80.00 while in demo block anthracnose disease incidence was 1.00 to 38.00 and stem end rot was from 13.00 to 60.00 percent (Iram *et al.*, 2014). Fateh *et al*. (2010) surveyed different districts of Punjab namely; Multan, Bhawalpur, Rahim yar Khan, Jhang, Lahore and Faislabad. During their survey they noticed that anthracnose is a common disease of mango with 30 percent disease incidence. While, In India during post harvest handling mango is attacked by twenty different genera of fungi (Pathak, 1980) of which anthracnose disease is the most important (Snowdon, 1990; Johnson and Coates, 1993).

2.1.2 Market level (Retail and wholesale)

Prabakar *et al*. (2005) observed that the post-harvest fungal spoilage was higher in retail market than other stages of marketing. The total loss in mango by different pathogens at retail market was 31.20 to 51.70 percent. Of these, anthracnose accounted for 17.10 to 31.80 percent, stem end rot 10.80 to 13.10 percent, mixed infection 3.70 to 8.00 percent and Aspergillus and Rhizopus rot together accounted for 0.60 to 0.90 percent loss. The total loss was recorded as 2.30to 3.60 percent in which anthracnose contributed to 0.30 to 1.00 percent, stem end rot 0.90 to 1.50 percent, mixed infection 0.30 to 0.50 percent and *A. niger* and *R. arrhizus* together accounted for 0.70 to 0.80 percent loss. Among thirteen varieties surveyed, Neelum showed higher decay due to anthracnose in the consumer level (17.3 to 21.0) and retailer level (31.8 to 41.3) while in respect of stem end rot, Shenduram variety recorded 12.5 to 14.5 percent at consumer level and 19.6 to 27.3 percent at retail market.

Post-harvest losses up to 31.00 percent due to fungal diseases at retail level in Bangladesh reported by Murthy *et al*. (2009). Rathod (2010) observed that mango fruits were commonly infected by fungal disease like anthracnose,

Alternaria rot,*A. niger* rot, Blue mould rot, Botryodiplodia rot, Rhizopus rot and Phomopsis rot during the survey of different fruit markets of Marathawada regions of Maharashtra.

Survey in Junagadh markets revealed maximum incidence of Lasiodiplodia rot i.e. 5.20 percent followed by 4.0 percent of *Aspergillus* rot, *Rhizopus* rot, *Macrophomina* rot and *Colletotrichum* rot were 0.45, 0.15 and 0.1 percent respectively. At food processing unit also same trend was found with higher incidence in which maximum incidence of 22.60 percent of Lasiodiplodia rot followed by Aspergillus rot with 6. 95 percent.Incidence of remaining rots were comparatively low and below 1.00 percent. Thus at both the survey places, fungal pathogens viz., *L. theobromae* causing stem end rot and *niger* causing black rot were found to be major post harvest pathogens on Kesar mango fruits at Junagadh. (Jadeja and Vaishnav, 2000).

The prevalence of post harvest diseases *viz*. stem-end rot and anthracnose at the wholesalers and retailers level was investigated in twelve mango varieties among them the highest disease incidence was recorded in Fazli, Aswina, Gopalbhog and Langra at Bangladesh (Naznin *et al.,* 2007). Terna *et al.*,(2015) recorded the highest rot diameter in Apple mango (38.00 mm) followed by Peter (35.33 mm), Paparanda (26.83 mm) and Maijijia (25.83 mm).

Meer *et al.,* (2013) found during the survey of five major markets of the Punjab (Pakistan) that anthracnose and stem end rot diseases were 1 00 percent prevalent in the mango fruits of all markets of five locations while Alternaria rot and Aspergillus rot were absent in mangoes of Rawalpindi and Faisalabad markets and less prevalent (80%). At Multan the disease severity range of anthracnose, stem end rot, Alternaria rot and Aspergillus rot was 1 -5 severity scale while at Shujabad the Alternaria rot was also in 1-5 severity scale. The incidence of anthracnose (63.33%) at Faisalabad was high as compared to stem end rot (30%)

and Aspergillus rot (16.66%) at Rawalpindi and alternaria rot (23.33%) at Multan. Percent disease index of anthracnose (12.6%) was high at Faisalabad followed by Rawalpindi, Multan and Shujabad. Stem end rot (6.00%) percent disease index was high at Rawalpindi and low at Shujabad. Percent disease index of alternaria rot (5.33%) was high at Multan and absent at Rawalpindi. Aspergillus rot (3.33%) was high at Rawalpindi and absent at Faisalabad while Sharma (2014) recorded that the dominating diseases are aspergillus rot, black rot, anthracnose, rhizopus rot, stem end rot, alternaria rot, fusarium rot and scab causing average fruit loss 3.66 percent, 3.50 percent, 2.27 percent, 0.66 percent, 1.50 percent, 1.50 percent, 1.14 percent and 0.43 percent respectively at wholesale level While at retailer level the losses are mainly due to stem end rot and black rot causing 3.21 percent and 0.30 percent loss respectively whereas over ripening and rotting cause average loss of 1.50 percent and 2.00 percent respectively. As regards the overall assessment of fruit loss the results indicates that the total average loss is 15.66 percent at wholesale level and 9.00 percent at retailer level in U.P., India.

The incidence and severity of mango anthracnose significantly varied across the surveyed markets of South west Ethiopia. The incidence and severity of mango anthracnose ranged from 70.6 to 95.3 percent and 64.00 to 82.00 percent in the market, respectively. They found higher incidence of mango anthracnose in the market than at field condition. This could be attributed to fruit softening during the ripening process which causes break down of the natural defense mechanisms and enhances latent infections of anthracnose. The highest incidence and severity of the disease were recorded in Agro market (the big local market in Gomma district) which suggest that the presence of a strong relationship between farm and market indicating that fruits in the market are largely brought from the farmers' fields and those fruits are already infected in the field and disease development rapidly increased when brought in the market. Therefore, the highest disease incidence and severity recorded in the market places could be due to latent

infections which occurred before harvest and then remain quiescent until some point during ripening and poor post harvest handling practices (Tucho *et al.*, 2014). Anthracnose that potentially infects and causes significant loss on a wide range of tropical and sub-tropical fruits i.e. mango, banana, papaya and avocado is a good example of a disease arising from quiescent infections (Akem, 2006; Sanders and Korsten, 2003).

Post harvest disease development elsewhere is reported to be one of the major constraints to the quality and shelf life of mango fruit limiting its domestic and export marketing (Bally *et al.*, 2009; Chala *et al.*, 2014). During the survey of domestic markets of Punjab and Sindh in Pakistan, it was found that anthracnose and stem end rot diseases were 100 percent prevalent in mango fruits of all markets (Iram *et al.*, 2014). Hassan *et al.* (2010) reported that damage occurred to mango fruits by post harvest diseases were 48.00 to 56.00 percent at wholesalers' level in Dhaka and Rajshahi markets of Bangladesh.

2.2 Symptomatology

The symptoms of anthracnose disease vary a little between different hosts. The disease is characterised by the formation of distinctive lesions on leaf, twig, branch, stem flower and fruit often accompanied with 'wither tip' or 'die back' symptoms (Jefferies *et al.*, 1990).

Prakash and Srivastava (1987) reported that the fruit could be attacked at any stage of development. Young fruits if attacked drop down in large numbers. On older fruits, black spots were produced which not only made them undesirable for consumption but also spoiled their keeping quality. Initially the spots were few and round but often became numerous and formed large irregular blotches covering the entire fruit. The spots frequently coalesced over large areas, had large deep cracks, penetrated deeply in to the fruits causing extensive rotting under moist conditions; the blackened areas were coverd with minute pinkish pustules or

fruiting bodies and produced large number of minute spores, each of which was capable of causing fresh infection.

Anthracnose caused by *C. gloeosporioides* (*G. cingulata*), was the most important fungal disease of mango attacking flowers, fruits and leaves. The symptoms appeared as black lesions when the fruit was mature (Hahn, 1999).

Guogan and Bingquing (1999) observed that anthracnose disease usually affected the leaves, flowers, fruits and new shoots of mango trees. When young leaves were attacked, many small brown round spots with faint yellow margins appeared and the badly infected flower clusters turned black and rotted. Infected fruits were abnormal in shape, became black and then dropped.

Post harvest anthracnose on mango appeared as round brown to black spots with an indefinite border on the fruit surface which could coalesce and cover extensive areas of the fruit (Arauz, 2000).

Gupta and Sharma (2000) reported that on half matured mango fruits, *C. gloeosporioides* produced slightly sunken black spot. On severe infection, surface cracks may appear on fruits. Many of these spots coalesce to form bigger spots involving the whole fruit. On the ripening fruit, the disease occurred in sunken, blackish brown blotches upon which salmon buff masses of spores developed.

Misra *et al*. (2002) reported that the post harvest infection starts from the field as latent infection. On stored fruits, black spots are produced. Initially the spots are round but later coalesce to form larger irregular blotches. Sometimes, it covers the entire fruit surface. The spots have large deep cracks and the fungus penetrates deep into the fruit causing extensive rotting. Under moist conditions, the blackened areas become covered with minute pinkish reproductive bodies of the fungus. Staining, russetting and tear streaking, involving only the skin of the fruit, are attributed to the same fungus.

Ripe fruits affected by anthracnose develop sunken, prominent, dark brown to black decay spots before or after picking. Fruits may drop from trees prematurely. The fruit spots can and usually do coalesce and can eventually penetrate deep into the fruit, resulting in extensive fruit rotting. Most green fruit infections remain latent and largely invisible until ripening. Thus fruits that appear healthy at harvest can develop significant anthracnose symptoms rapidly upon ripening. A second symptom type on fruits consists of a "tear stain" symptom, in which are linear necrotic regions on the fruit that may or may not be associated with superficial cracking of the epidermis, lending an "alligator skin" effect and even causing fruits to develop wide, deep cracks in the epidermis that extend into the pulp (Scot, 2008).

2.3 Isolation, Identification, confirmation and characterization of postharvest fungal diseases of mango

Isolation of target fungi from plant material is influenced or affected by factors including the method of surface sterilization, the plating procedures, the culturing medium, the incubation conditions and most importantly the nature of the diseased host tissue. Sample diseased plant tissues with typical symptoms of the disease or signs of the pathogen being investigated are used for plating and isolation of target fungus. Soon after sterilization or prior to plating of surface sterilized samples, drying under a filtered air flow, often in a sterile hood, on sterile paper tissues is very much recommended (Waller *et al.*, 2002). Potato dextrose agar has also been identified by Kausar *et al*. (2009) to give best mycelium growth of *B. theobromae* and it is strongly recommended for the isolation of slow-growing fungi.

B. theobromae forms its spores endogenously within pycnidia (Mascarenhas *et al*., 1996) from the inner layers of cells lining the pycnidial cavity and exudes spore or conidiophores as pink pigmented fluid (Meah *et al*., 1991) or blackish

fluid into the culture on potato dextrose agar media (PDA) . The exudates are visible to the naked eye. The conidiophores are rarely branched, cylindrical, translucent, simple and sometimes having septations. Matured conidia are one-septate (two-celled) and cinnamon to dark brown, thick walled, ellipsoidal and often longitudinally striated and 20-30 x 10-15 jum in size were observed when cultured on PDA (Phipps *and* Porter, 1998).

Philips (2007) studied that *B. theobromae* colonies were often greyish to mouse grey to black, fluffy with abundant aerial mycelium. Matured cultures on PDA have black pigmentation and it can develop pycnidia and further sporulate. In PDA, the pycnidia may be found scattered, grouped or centered and visible. Pycnidia can also be found underneath or within the mycelium and may often be covered by bristles. Matured pycnidia can be up to 5 mm wide (Pitt and Hocking, 2009). Alam *et al.* (2001) recorded highest mycelium growth of *B. theobromae* on PDA and maximum pycnidia on Czapek's Dox agar. Similarly Quroshi and Meah (1991) observed fastest linear growth of *B. theobromae* on Richard's agar, mango leaf extract agar and on PDA. They recorded highest number of pycnidia on mango leaf extract followed by PDA. Conidia were also noted to be initially unicellular and translucent and matured conidia are one-septate (two-celled) and cinnamon to dark brown (Phipps and Porter, 1998; Alvarez and Nishijima, 1987). Conidia are granulose, sub-ovoid to ellipsoid oblong, thick-walled, base-truncate, ellipsoidal and often longitudinally striated and 20-30x 10-15 jum in size. When paraphysis are present they are often translucent, cylindrical and sometimes septate and up to 50 jum long (Alvarez and Nishijima, 1987).

Archana *et al.,* (2014) demonstrated that the conidia were hyaline with oil globules and the size ranges from 8.29 to 11.52 um x 2.60 to 6.30 jum. The basic growth pattern and colony type for all sixteen isolates remained constant when grown out on PDA. The majority of the conidia of *C. gloeosporioides* were oblong with obtuse ends and was generally shorter and broader than conidia of *C.*

fragariae and *C. acutatum* (Gunnell and Gubler, 1992). The conidia of *C. gloeosporioides* were straight with obtuse apex, hyaline, cylindrical to clavate and sometimes fusiform like *C. acutatum*. Clavate and lobed appressoria were mainly present in matured colonies. The spore measurement of *C. gloeosporioides* varied between 3.94-12.14 jum long and 1.43-2.14 um wide (Yun *et al.,* 2009).

The morphology of the fungus was described by Palo (1932) where in the spore of fungus was found to be 8.3 to 27.4 jum in length and 2.0 to 6.6 jum in width. They were irregular and appear as brown to black dots. The conidia were straight, cylindrical or oval 8-20 x 5-7 um hyaline usually with two rarely one oil drops and the acervuli (80250 jum) when mature exude pink masses of conidia under moist conditions (Sattar and Malik, 1939). The conidia were born on distinct well developed hyaline conidiophores. The size of conidia varied from 11-16 x 4-6 um and acervuli measured 115-467 x 95-22 um (Bose *et al.,* 1973). Simmonds (1965) reported conidia 11.9-17.0 x 3.6-5.8 um broad oblong with rounded ends 11.1-17.7 x 3.1-5.0 jum for *C. gloeosporioides* .

Gautam (2014) found the growing fungal colonies of *C. gloeosporioides* on culture media may be circular, wooly or cottony with characteristic colour. Vegetative hyphae observed were hyaline, simple, septate and branched. Conidiophores were long, hyaline, septate and unbranched. Conidia are straight, oblong or cylindrical with rounded or bulbous ends, hyaline, aseptate, one celled and dumbbell shaped. Setae are brown. Colonies of the *C. gloeosporioides* on potato dextrose agar showed whitish to dark grey with thick to sparse lawns of aerial mycelium when viewed from the top of petri dishes whereas, they had greenish to orange or dark brown centre bordered by creamy surrounding when viewed from the reverse side of the petri dish and conidia with hyaline, single celled and cylindrical with obtuse ends (Tucho *et al.,* 2014).

Meer *et al*. (2013) isolated different types of post harvest fungi *viz*., *C. gloeosporioides* (anthracnose), *Lasiodiplodia theobromae* (stem end rot), *Aletrnaria alternata* (alternaria rot) and *Aspergillus niger* (Aspergillus rot) from diseased mangoes. They observed that all pathogens are dominant except *A. alternata* and also recorded colony characters, spore characters and spore size of all the pathogens. They found that *C. gloeosporioides, L. theobromae, A. niger* producing whitish colony, grey to black colony and black colony respectively. *C. gloeosporioides* produced smooth, hyaline and sub cylindrical with round end spores (Length=18-30 ìm, Width=10-18 ìm), *L. theobromae* produced thick walled, bicelled and dark brown colored spores (Length=18-30 µm, Width=10-18 µm), *A. niger* produced sporangiophores were prominent under microscopes with short circular to semicircular sporangispores (Length=900-1600 µm, Width= 40-60 µm).

Manjula and Gupta (1964) isolated *Colletotrichum gloeosporioides* Penz. by plating the surface sterilized diseased tissue on plain agar and incubated at room temperature yielded a fungus, which was further purified by single spore technique by growing over Potato Dextrose Agar (PDA). In culture, the fungus forms a whitish mat of creeping hyphae on the agar surface with little or no aerial mycelium and later turns as smoke-gray.

Leaves and fruits collected from fields were taken back to the laboratory, washed and air dried. Then, they were dipped into 240 µg/ml of paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride, ICI Agrochemicals, U. K.) solution and left to dry in a designated place to avoid contamination. After the leaves were air dried, they were kept in a misted container and sealed within plastic bags in order to retain the moisture necessary for endophytic fungi to sporulate. It took about 2-4 days for leaves and fruits to show anthracnose lesions and to start to produce conidia mass. Single spore isolation was used for collecting isolates. Each isolate was collected

from each individual pustule from different leaves or fruits (Ker-chung kuo, 2001).

The pathogen *C. gloeosporioides* was isolated from infected mango leaves and flowers. Isolation was made by cutting a small section of anthracnose infected portion, which was surface sterilized with 0.1 percent $HgCl_2$ solution and rinsing in sterilized distilled water. It was then placed on PDA (Potato Dextrose Agar) medium, in sterilized Petri plates and incubated at 28 ± 2°C. The pure culture was maintained in PDA slants (Sundravadana *et al*., 2007).

Anu Mathews *et al*. (2009) isolated the pathogen *C. gloeosporioides* from infected Baneshan mango fruits collected from mango orchards at Agricultural Research Station, Anantharajupeta, Kadapa (Dt), Andhra Pradesh. Isolation was done by tissue segment method on potato dextrose agar medium (PDA) at 28±2°C and purified by single spore isolation method. The isolates were identified and maintained on PDA for further studies.

2.4 Pathogenicity

Darvas *et al*. (1987) reported that *C. gloeosporioides* was capable of penetrating the avocado fruit through uninjured rind. The fungi caused severe post harvest fruit decay when inoculated on the cut pedicel, the pedicel scar and in to wounds.

Subiros *et al*. (1987) inoculated healthy seedlings of Annona muricata with a conidial suspension of *C. gloeosporioides*, the first sign of cell collapse was observed after 48 hours and the lesions were fully developed 50 days after inoculation. Fungal infection was also detected at the node level, resulting in total necrosis of the apex and leaf primordial on the axillary buds, the vascular tissues being reached through the leaf trace.

The development of anthracnose symptoms only on wounded fruit on inoculation was reported by Jagadish *et al*., (1992) and the disease severity was found highest

on ripe fruit. Pathogenicity of the fungus *C. gloeosporioides* was proved by using pin prick and swab method for all the five isolates namely annona, ber, citrus, pomegranate and straw berry.

Ker-chung kuo (2001) re-isolated the pathogen *C. gloeosporioides* from leaves and stem of mango variety Irwin, 7 days after inoculation and confirmed the pathogen to prove pathogenecity.

Anu Mathews *et al*. (2009) tested the pathogenecity of *C. gloeosporioides* by using wound inoculation method on Baneshan mango fruits. Mango fruits were washed thoroughly under tap water and surface sterilized using 0.1 per cent $HgCl_2$ for 30 seconds and air dried. A circular inoculation site with 1cm diameter was marked on the fruit surface and wounds were made by puncturing the rind to a depth of 2mm using sterile needle. A drop of conidial suspension (1.0×10^4 conidia/ml) of the pathogen prepared from seven days old culture was transferred to the marked area and left for air drying. The mangoes were covered with polythene bags and incubated at 28±2°C to ensure high humidity for establishing favorable conditions for conidial germination and infection. After 5 days of incubation, pathogen was reisolated and its identity was confirmed.

2.5 Mango postharvest disease management

2.5.1 Varietal screening of mango against anthracnose.

Rathod (1994) screened eight cultivars of mango for studying their reaction against *C. gloeosporioides* under control condition. He reported that none of the cultivars was found to be completely free from disease. Cultivars Neelum and Totapuri showed moderate type of reaction while, Alphonso and Kesar were highly susceptible.

Paez Redondo (1995) tested seven mango varieties for their responses to *C. gloeosporioides*. He observed that Tommy Atkins and Keitt were highly resistant.

Banik *et al*. (1996) evaluated 28 cultivars of mango against *C. gloeosporioides* (G. *cingulata*) during May-July of 1992 and 1993. He reported wide range of variation in respect of disease reaction among the test cultivars. Alphonso, Langra, Banarasi, Chandankusa, Dasheri, Lakshmanbhog, Lalphuli, Langra, Mohanbhog, Piaraphuli, Saradamonibhog, Sarikhas, Totapuri and Zardalu were resistant while cultivars namely, Amrapalli, Fazli, Gulabkhas and Sardar Pasand were moderately resistant to C gloeosporioides.

Sharma and Badiyala (1998) screened twenty mango cultivar against *C. gloeosporioides* and observed that none of the mango cultivar resistant to anthracnose disease. The cultivar viz., Amrapali, Totapuri, Safeda, and Mallika were highly susceptible, whereas Alphonso, Baramasi, Samer Bahista, Rampur, Samer Bahista Chausa and Sindhuri were moderately susceptible.

Gud (2001) screened 21 cultivar of mango against fruit rot fungi under natural condition at different location and reported that Goa Mankur as susceptible and Sindhu, Kesar, Ratna, as highly susceptible.

Paez (1995a) tested seven mango varieties for their response to C. gloeosporioides infection in Colombia during 1991 and found that Tommy Atkins and Keitt were the most resistant. In trials conducted during 1991-94 in Colombia, mango cultivars Tommy Atkins, Keitt, Early gold, James Saigon and Vandyke were resistant to anthracnose caused by C. gloeosporioides and Irwin, Azucar, Rosa and Mariquita were highly susceptible (Paez, 1995b).

Banik et al. (1996) observed that among 28 cultivars, 13 (Alphonso, Langra, Banarasi, Chandankosa, Dushehari, Lakshmanbhog, Lalphuli, Langra, Mohanbhog, Piaraphuli, Saradamonibhog, Sarikhas, Totapuri and Zardaly) were resistant while seven (Amrapalli, Biswanath Chatterjee, Bombay Yellow, Fazli, Gulabkhas, Khohinoor and Safdar Pasand) were moderately resistant to C.

gloeosporioides during May-July of 1992 and1993 when inoculation was done to mango fruits using hypodermic needle method.

Some cultivers of mango were screened for disease resistance (Sharma and Badiyala, 1998) and found highest number of spots on culivar Amarapali (62.66) followed by Topapari (62.56), Lala Da Amb (54.39), Safeda (52.10) and Mallika(40.19) and were rated as highly susceptible and seven (Alphonso, Baramasi, Samar bahisht Rampur, S B Chausa, Shindhuri, Haryana, Khangi and Kukian Di chhalli) showed moderately susceptible reaction.

Sharma and Badiyala (1999) screened 20 mango cultivars against anthracnose disease caused by C. gloeosporioides both under field and laboratory conditions. They observed that Amrapalli (50.55 spots per leaf) was highly susceptible followed by Totapuri (44.40 spots) while the lowest disease intensity was recorded on Alphonso (12.86 spots) followed by Krishan Bhog (14.13 spots) respectively. Other cultivars were either susceptible or moderately susceptible and none was resistant.

Tiwari and Singh (1999) evaluated 23 mango cultivars for resistant to red rust (*Cephaleurus virescence*) and anthracnose *(C. gloeosporioides*) during 1989-91 and reported that Barbalia, Bombay green and Dilpasand were moderately resistant to both diseases.

2.5.2 Evaluation of hot water dip treatments against the disease development

Tandon and Singh (1968) reported that hot water treatment alone control the post harvest mango anthracnose and they observed that 55°C and 60°C temperature of water found to be best for control of this disease.

Prakash and Pandey (2000) reported that mango fruit after harvest treated with hot water treated at 52°C for 5, 15 and 30 minutes alone and in combination with

fungicides for the control of post harvest anthracnose disease and they observed that 52°C alone for 30 minutes was found to be effective in controlling the disease.

Sopee and Sangchote (2005) recorded that anthracnose disease incidence on mango fruits inoculated with heat-treated conidia at 55°C for 5 min was reduced by 93 percent. One day after inoculation, at the depth of 1 mm, fungal colonization was reduced by 80%. Disease incidence on fruit treated with HWT, VHT and HWT+VHT was reduced by 0.24, 0.26 and 0.14 per cent, respectively.

Waskar and Gaikwad (2005) observed that mango fruits after harvest were given hot water treatment at 52°C for 10 minutes alone and in combination with Bavistin (0.1%) and Captan (0.2%) for the control of post harvest anthracnose disease and also observed that hot water treatment combined with Bavistin (0.1%) was found to be best in controlling the incidence of anthracnose.

Le Thi-N *et al*., (2010) reported that the hot water treatment at 55°C for 3 min was decreased total spots of anthracnose disease for 6 days as compared to control. The disease incidences of the Alternaria alternata and Colletotrichum gloeosporiodes were decreased by application of hot water and vapour heat treatment followed by storage at 3oC for 3 weeks.

Jabbar *et al*., (2011) observed that hot water treatment was significantly reduced the anthracnose incidence on fruit as compared to control.

2.5.3 Evaluation of botanicals against the disease development

Hasabnis (1984) reported the treatment of plant extract of Allium sativum (bulb 10%), Azadiracta indica, Pongamia pinnata and Vitex negundo (leaves 20%) against mango fruit rots and observed that the per cent disease incidence of storage rots was 23.3, 30.0, 40.0 and 46.7, respectively as compared to 63.3 in undipped control.

Chauhan and Joshi (1990) studied the efficacy of 14 plant extracts (botanicals) and found that fruit dip treatment with Eucalyptus oil (1% and 2%) and castor oil (5% and 10%) inhibited infection for more than two weeks.

Prakash and Chauhan (2003) studied the efficacy of 13 plant extracts at different concentration against mango anthracnose, both in vivo and in vitro and found that clove oil is the best among these.

Bolivar et al. (2009) recorded the treated fruits with different phyto-extract was less affected by the presence of C. gloeosporioides, compared to the control.

Onyeani et al.(2012) revealed that, the least disease index of mango anthracnose (0.27) was recorded in both 10% (aqueous extract) and 30% (alcoholic extract) concentrations of Annona squamosa treatments and this was superior to 0.33 disease index recorded in benomyl treated fruits.

2.5.4 Efficacy of chemical fungicides

Poison agar tests were employed to determine the efficacy of nine fungicides against Colletotrichum gloeosporioides [Glomerella cingulata] isolated from mango. The fungicide concentrations ranged from 0 to 0.1 per cent and the diameter of fungal colonies was measured over five days. Benlate (benomyl), Bavistin (carbendazim), Topsin M (thiophanatemethyl), Champion (copper hydroxide), TopCop (sulfur and tribasic copper sulfate), Dithane (a dithiocarbamate), and Daconil (chlorothalonil) shown 100 per cent inhibition over control at 0.1 per cent (Elliott and Patterson, 2000).

Nascimento et al. (2000) evaluated prochlaraz, azoxystrobin and sodium bicarbonate to control C. gloeosporioides in mangoes. They observed that prochloraz and azoxystrobin gave 100 percent mycelia growth inhibition in vitro.

Prabakar et al. (2008) reported that among the fungicides tested in vitro, carbendazim and thiophanate-methyl reduced the mycelial growth up to 88.76 per

cent and 85.39 per cent over control, respectively. Mancozeb (0.2%) and Ziram (0.3%) were less effective against the mycelial growth of C. gloeosporioides. A similar trend was also observed in the case of conidial germination.

The efficacy of azoxystrobin against mango anthracnose pathogen C. gloeosporioides was tested under in vitro conditions. Azoxystrobin significantly reduced mycelial growth both in solid and liquid medium. Azoxystrobin at 0.25 and 0.5 ppm slightly inhibited the mycelial growth whereas 1.0 and above ppm completely inhibited the mycelial growth of C. Gloeosporioides (Sundravadana et al., 2007).

Anu Mathews et al. (2009) tested the in vitro efficacy of systemic fungicides viz., carbendazim (50 ppm), hexaconazole (25 ppm), propioconazole (25 ppm), thiophanate-methyl (50 ppm), prochloraz (50 ppm), thiram (750 ppm), captan (750 ppm), and non-systemic fungicides viz., mancozeb (1000 ppm) and copper oxychloride (1000 ppm) on the test pathogen C. Gloeosporioides revealed that all fungicides inhibited 100 per cent growth of the pathogen except mancozeb (inhibited 61.91 %). Significant reduction in radial growth of C.gloeosporioides at different concentrations of the tested fungicides was observed when compared to control. The results showed similar degree of inhibition of the pathogen with all the fungicides. From the data, it is evident that the pathogen C.gloeosporioides is highly sensitive to different concentrations of fungicides except mancozeb which inhibited only 61.91 per cent compared to control.

Suvarna et al. (2009) collected 20 isolates of C. gloeosporioides from mango leaves showing anthracnose symptoms in Chittoor and Kadapa districts of Rayalaseema regions of Andhra Pradesh (India) and designated those isolates as Cg1 to Cg20. The efficacy of commonly used ystemic fungicides viz., carbendazim (50 & 100 ppm), hexaconazole (25 and 50 ppm), thiophanate-methyl (50 and 100), propioconazole (25 and 50 ppm), and non-systemic fungicides viz.,

mancozeb (500 and 1000 ppm) and copper oxychloride (500 and 1000 ppm), was investigated in vitro against C. gloeosporioides [Glomerella cingulata] isolates by poisoned food technique. All the fungicides inhibited the growth of the pathogen. A significant reduction in radial growth of the isolates at the 2 concentrations of the tested fungicides was observed compared to the control. These results showed variation in the degree of sensitivity to the different fungicides among the isolates. All the isolates were classified under highly sensitive to sensitive categories both at 50 and 100 ppm concentrations for carbendazim. The same results were observed with thiophanate-methyl at 50 and 100 ppm and propioconazole at 25 and 50 ppm concentrations. Isolates Cg2, Cg6, Cg10, Cg13, Cg19, and Cg20 exhibited moderate resistance to hexaconazole at 25 and 50 ppm concentrations. Cg9 was resistant to mancozeb and Cg12 was resistant to copper oxychloride at 500 ppm and moderately resistant at 1000 ppm. The non-systemic fungicides mancozeb and copper oxychloride were less effective than the systemic fungicides.

Sohi *et al.* (1973) reported that instantaneous fruit of mango dip in benomyl (500 ppm) or thiobendazole (900 ppm) reduced the incidence of post harvest mango anthracnose to about 5 percent from 29 percent.

Chauhan and Joshi (1990) observed that post harvest application of carbendazim (0.05%) was most effective in controlling mango fruit anthracnose than phytoextracts.

Sharma *et al.* (1994) observed that mango fruit were dip in the carbendazim (0.1%) solution resulted in reduction of fruit decay (*C. gloeosporioides)* to the extent of 93.80 percent.

Gajbhiye *et al*. (2000) opined that when mango fruits treated with 0.1 percent carbendazim, the anthracnose disease did not appear upto 10 days and also noticed that residue level was below the MRL value.

Prakash and Pandey (2000) reported that mango fruits subjected to hot water (52°C for 15 min.) together with carbendazim (0.1%) treated mango fruits (cv. Dashehari) could be stored for 26 days at 12°C without any anthracnose incidence.

Mortuza *et al*. (2003) evaluated the effectiveness of pre- and post harvest application of eight fungicides to control post harvest anthracnose and stem end rot of mango in Bangladesh. The treatment comprising fruit dip in warm suspension (55°C) of carbendazim (0.1%) for 5 minutes was more effective in controlling anthracnose and stem end rot diseases of mango.

A hot – benomyl dip of 850 mg per liter a.i. at 52-55°C for 10 min completely eradicated anthracnose on fruits treated on the day of harvest and on the day after. There was no significant difference, however, between hot-benomyl dips or prochloraz dips (500 mg/l a.i. for 10 min) at ambient temperatures when fruit were treated on the third day after harvest (Dodd et al., 1991).

Thiabendazole (0.1 %) was the most effective fungicide for the control of C. gloeosporioides on mango cv. Dushehari giving a reduction in decay index of 95.7 per cent (Sharma et al., 1994).

Hot water dip at 50°C for 5 minutes and prochlaraz added to the hot water bath (180 ml Omega per 100 liters water) resulted in a significant and marked reduction in anthracnose disease in mango cultivars Zill and Kent. Hot benomyl treatments (5 minutes at 50°C; 200 gm benlate per 100 liters of water) were as effective in controllinganthracnose as was hot water treatment alone (5 minutes at 50°C) (Oosthuyse, 1997).

Nguyen et al. (1998) reported that hot water treatment at 520C for five minutes or ten minutes, reduced the incidence of anthracnose caused by C. gloeosporioides on Buoi mangoes.

Nascimento et al. (2000) evaluated prochlaraz, azoxystrobin and sodium bicarbonate to control C. gloeosporioides in mangoes. They observed that post harvest treatments with prochloraz and azoxystrobin gave 100 percent disease control.

Following harvest, mango (cv. Dashehari) fruits were treated with hot water at 52°C for 5, 15 and 30 min alone and in combination with fungicides to control postharvest anthracnose (Colletotrichum gloeosporioides [Glomerella cingulata]) disease. Hot water (52°C) alone for 30 min was very effective in controlling the disease. Treated fruits could be stored at 120C for up to 26 days (21 days at 120 + 5 days at ambient temperature for ripening). However, the duration of hot water treatment could be reduced to 15 min by supplementing with carbendazim or thiophanate-methyl (each @ 0.1%). Hot water (52°C for 15 min) together with carbendazim treated fruits could be stored for 26 days at 12°C without any anthracnose infection, while with thiophanate-methyl fruits could be stored for only 19 days at 120. Fungicides applied at ambient temperature did not control anthracnose. Hot water treatments did not show any adverse effect on fruit ripening (Om Prakash and Pandey, 2000).

Prakash and Pandey (2000) observed that hot water 52°C alone for 30 minutes was very effective in controlling anthracnose on mango cv. Dushehari.

Waskar and Gaikwad (2005) treated the mango fruits with hot water at 52°C for 10 minutes alone and in combination with fungicidal dips viz., Bavistin (0.1 %) and Captan (0.2 %). The mango fruits were then packed in corrugated fibre board box and then stored in two storage environments viz., at room temperature (28.12 to 36.180C temperature and 46.18 to 71.25 % RH) and in cool chamber (21.47 to 27.10°C temperature and 91 to 95 % RH). It was observed that hot water treatment combined with Bavistin (0.1 %) was found to be the best in controlling the incidence of anthracnose and stem-end rot. It was observed that shelf-life of

mango fruits could be extended for more than 28 days when given hot water treatment coupled with fungicides and stored in cool chamber. The untreated (control) fruits were found to have infected with Colletotrichum gloeosporioides and Diploidia natalensis.

Youngmok Kim et al. (2007) reported that visible appearance of anthracnose of mango during ripening was effectively inhibited by the hot water treatment (46°C for 75 min) combined with controlled atmosphere (CA) (CA1- 3% O2 + 97% N2 and CA2- 3% O2 + 10% CO2 + 87% N2), after 2 weeks at 10°C.

In general, storage temperatures of around 10°C can delay decay development. Bagging of mango fruit before harvest and postharvest treatment for 10 min with hot water (52–55°C) was reduced the anthracnose infection by 83 per cent and stem-end rot by 100 per cent (Dharini Sivakumar et al., 2010).

2.5.6 Integrated disease management against post-harvest mango disease

Prakash and Pandey (2000) observed that mango fruit after harvest were hot water treated at 52^0C for 5, 15, and 30 min. alone and in combination with fungicides for the control of anthracnose (post harvest) disease and they observed that hot water (52^oC for 15 minutes) together with carbendazim (0.1%) treated fruits could be stored for 26 days at 12^oC without any anthracnose infection, while with thiophanate-methyl (0.1%) treated fruits could be stored only for 19 days at 12^oC.

Govender *et al*. (2005) revealed that mango fruits were treated with biocontrol agent *(Bacillus licheniformis*) applied in hot water (45 ^{0}C) followed by a quarter strength prochloraz dip was lowest anthracnose incidence(20%) than chemical treatment, biocontrol treatment and untreated (30%, 40% and 60%), respectively.

Waskar and Gaikwad (2005) observed that mango fruit after harvest were given hot water treatment at 52 ^{0}C for 10 min. alone and in combination with Bavistin

(0.1%) and Captan (0.2%) for the control of anthracnose (post harvest) disease and observed that hot water treatment combined with Bavistin (0.1%) was found to be best in controlling of this disease.

Govender and Korsten (2006) studied different formulations of *Bacillus licheniformis* were evaluated on their own and in combination with prochloraz and stroburilin for their ability to reduce mango post-harvest fruit diseases (anthracnose and stem-end rot) when applied as a dip treatment in a mango pack house. Among them antagonist in combination with the commercial chemical were more effective comparable to that obtained with the commercial chemical control.

Jabbar *et al*. (2011) studied effect of combined application of fungicides and hot water on post harvest diseases of mango. In this case minimum anthracnose incidence score (0.03) was recorded in fruit subjected to T_6 (Topsin-M @1 g L-1 for 1 min + HWQT @ 48°C for 60 min) followed by T_2 (HWQT @ 45°C for 75 min) and T_8 (HWQT @ 48°C for 60 min + carbendazim @ 0.4 g $10L^{-1}$ at 52°C for 5 min) as compared to control. Singh (2011) observed the effect of heat treatment in combination with fungicides and plant extracton storage rotof mango. She observed that propiconazole and *Cannabis sativa* extract were most effective against anthracnose in both pre and post-inoculations.

2.5.7 Eco-friendly management of mango post-harvest diseases

Sangchote (1989) found that dipping fruit in hot water at 55°C for 5 min gave good control of stem-end rot of 'Nam Dorkmai' mango without heat injury. The efficacy and persistence of 14 plant extracts against *C. gloeosporioides* studied by Chauhan and Joshi (1990) and found that eucalyptus oil (2%) and castor oil (10%) solutions inhibited infection for more than two weeks when fruit were inoculated and were significantly better than the other plant extracts tested. Castor oil (5%), eucalyptus oil (1%), garlic bulb, mango, turmeric and lantana leaves also

significantly controlled the disease. Post harvest hot water treatment is now accepted worldwide to satisfy quarantine requirements for control of anthracnose in mango (McIntyre *et al.* 1993; Suhardjo *et al.* 1994). Among the post harvest treatment, hot water treatment is one of the heating methods for quality and shelf life mango. It is an effective heat transfer medium and within a short time, a uniform temperature profile will be maintained. The additional benefit of hot water treatment is that it can control postharvest diseases such as anthracnose and stem end rot (Couey, 1989). For hot water treatment, different dipping conditions have been recommended to control anthracnose effectively but a general conclusion is that the water temperature should be between 50°C and 55°C and the dipping time must be at least 5 min (Dodd *et al.* 1997). Angasu *et al.*, (2014) found that the mango fruits treated by hot water at 52°C for 5 minutes and 52°C for 10 minutes did not show any remarkable symptoms of anthracnose infection after 5, 10 and 15 days in storage as compared to control. This indicated that hot water treatment is the most favorable treatment for controlling anthracnose; the similar observations were observed by Chaplin *et al.* (1991) who found that hot water treatment is effective against fungal infection in fruits.

Opara *et al.*, (2000) reported that hot water treatment at 52°C for 5 or 10 minutes gave good control of anthracnose and stem-end rot diseases of 'Buoi' mango. Imtiaj *et al.* (2005) noticed that the extracts derived from *Curcuma longa* L. (leaf and rhizome), *Tagetes erecta* L. (leaf) and *Zingiber officinales* Roscoe (rhizome) were shown to have antifungal activities against fungal anthracnose by completely inhibiting conidial germination of *C. gloeosporioides*.

Msogoya and Kimaro (2011) showed the incidence of microbial decay decreased from 15.5 percent in untreated fruits to 2.3 percent in hot water treated mango fruits. Naz and Bano (2012) tested the water leaf extract of ***R. Communis*** L. showed inhibition by 55.7 percent and 51.3 percent respectively against ***A. fumigatus*** and ***A. flavus***. Sahi *et al.*, (2012) *in vitro* evaluation of the

effectiveness of the extract of neem (*Azadirachta indica* A. Juss.), garlic (*Allium sativum* L.), onion *(Allium cepa* L.) and safeda *(Eucalyptus camaldulensi* Dehnh.) against the mycelial growth of *B. theobromae* revealed that safeda and neem extracts were the most effective while garlic and onion extracts were comparatively and statistically less effective in inhibiting the vegetative growth of the fungus.

Sharma (2014) conducted experiment on hot water treatment of mango fruits at 55°C and recorded that Rhizopus rot was reduced to 19.84% as compared to control (76.91%) at 5 minutes exposure. Stem end rot and Fusarium rot were reduced to 22.11 percent and 22.59 percent as compared to 83.05 percent and 83.89 percent in control respectively at 10 minutes exposure. Scab, Anthracnose, Aspergillus rot and Alternaria rot were reduced to 27.14 percent, 28.07 percent, 28.64 percent and 29.65 percent as compared to 80.08 percent, 82.47 percent, 76.73 percent and 79.79 percent in control at 10, 15, 20 and 10 minutes respectively. Disease incidence of anthracnose and stem- end rot in Philippine 'carabao' mango fruits decreased significantly when dipped in hot water at 53°C for 10 minutes (Buganic Jr. *et al.* 1997). Recent efforts have focused on the development of environmentally safe, long-lasting, and effective biocontrol methods for management of anthracnose diseases. The utilization of natural products especially the plant extracts has been shown to be effective against many plant pathogens and considered to be safe for consumers and environments (Hernandez-Albiter *et al.,* 2007). A number of plant species have been reported to possess natural substances that are toxic to a variety of plant pathogenic fungi (Spencer *et al.,* 1957; Fawcett and Spencer, 1970).

The extracts of plants exhibited marked effect on germination of fungal spores as well (Singh and Singh, 1981; Singh *et al.,* 1983 and Dubey, 1991) and it inhibited the fungal growth (Khair *et al.,* 1995). Alam *et al*. (2002) tested the effect of ten plant extract as fungicides on conidial germination of *C. gloeosporioides* and

found *T. erecta* (leaf) and *A. indica* extracts were most effective in inhibition of conidial germination after immersing 5 to 30 minutes in 5:1.5 (w/v) concentration. Singh *et al.* (1993) reported the antifungal activities of leaf extracts against *B. theobromae, F. oxysporum, Helminthosporium spiciferum, Curvularia lunata, A. flavus* and *Trichothecium roseum*. They used some medicinal plants such as, *Calotropis procera, Vitex negundo, Lantana camara, A. indica, Ficus religiosa, Ocimum sanctum*, *Typha orientalis*, *Argemone mexicana*, *Achyranthes aspera*, *Datura fastuosa* and *Ricinus communis* and observed good control against these pathogens. Among the eleven leaf extracts, those of *A. indica* and *O. sanctum* were most effective in controlling the fungi.

Islam (2003) tested ten plant extracts were as inhibitor against conidial germination of *A. flavus, A. niger* and *A. fumigatus.* All of the extracts were showed more or less inhibitory effect against conidial germination of the tested fungi after immersing 5 to 30 minutes. The conidial germination (95%) of *A. flavus* was inhibited (or reduced) by the extract of *Lawsonia inermis*, while that of *A. niger* was most inhibited by *A. indica*. Inhibitory effect was observed on germ tube formation of *A. flavus, A. niger* and *A. fumigatus,* when the fungi were immerged in *L. inermis* and *A. indica* extracts.

Mandhar *et al.,* (2000) have found the mangoes treated at 46^{o}C for 65 minutes could disinfest both fruit fly and anthracnose and 52°C for 5 minutes could disinfest anthracnose only. Efficacy of neem oil found to be effective against rot may be due to presence of *Nimbicidin* an antifugal substance (Rawat, 1993) was reported to inhibit the growth of *A. niger* causing fruit rots in mango.

Prakash and Pandey (2000) reported that hot water treatment at 52^{0}C for 30 minutes was very effective in controlling the anthracnose in mango fruits. Hot water treatment at 52^{0}C for 15 minutes effective against the post-harvest fungal diseases of mango fruits (Prasanna Kumar *et al.*,2005). Thi-Nghiem (2010) found

that the disease incidences of the *A. alternata* and *C. gloeosporioides* on mango fruits were decreased by application of hot water and vapour heat treatment followed by storage at 3^0C for 3 weeks. Heat treatments such as hot water dipping vapour heat, hot dry air or combinations of these have been increasingly used as a quarantine treatment in several studies to retard postharvest fungal damage to fruits and vegetables (Mansour *et al.,* 2006). Particular attraction of heat treatment is that no use of chemicals (Couey,1989; Fallik *et al.,* 1996; Lopez *et al.,* 1998; Rodov *et al.,* 2000; Tohamy, 2004). Jacobi *et al.,* (2000) observed the Kensington mangoes were more resistant to postharvest disease and of higher quality after treatment with hot air, hot water or both. Jacobi and Wong (1992) and Jacobi and Giles (1997) found that at 53°C hot water dipping for 5 minutes was successful treatment for quality of Kensington mango fruit. Also found that vapor heat treatment (VHT) at 47°C for 15-20 minutes was right for disease management of Kensington mango. Results of Nguyen *et al.* (1 998) reported that HW treatment of Buoi mango at 52°C for 10 minutes induced higher shrivel incidence while at 52°C for 5 minutes had potential for reducing postharvest diseases with minimal fruit mass loss and shriveling compared with untreated fruits. Similarly heat protocols have been successfully developed for treating a wide range of mango varieties, including Carabao from the Philippines (Merino *et al.,* 1985), Nang Klangwan from Thailand (Unahawatti *et al.,* 1986), Harumanis from Malaysia (Mohamed *et al.,* 1994) and Buoi from New Zealand (Nguyen *et. al.,* 1998). *In vitro* evaluation of the effectiveness of the extract of neem (*A. indica*), garlic (*A. sativum),* onion (*A. cepa)* and safeda (*Eucalyptus camaldulensis)* against the mycelial growth of *A. theobromae* revealed that neem extracts was the most effective while garlic and onion extracts were comparatively and statistically less effective in inhibiting the growth of the fungus (Sahi *et al.,* 2012). Sarkar (2012) observed the longer shelf life (14.87 days) in Neem leaf extract treated fruits followed by Garlic extract treated fruits having shelf life (14.25 days). Neem leaf extract and garlic extract treated mangoes

appeared to be the best for extending shelf life (14.87, 14.25 days) in Amrapali mango fruits whereas the Control fruits had the shortest shelf life (10.00 days).

Singh *et al*. (2000) studied the effect of $GA_3$and plant extract, castor oil and Neem oil on storage behaviour of mango (*M. indica*) cv. Langra and reported that the treatment of Neem oil (10%) showed the minimum physiological weight loss when compared to other treatments and controls, wherein, the maximum physiological weight loss (17.28%) was recorded on the 12^{th} day of storage. Bagwan (2001) stated that treatment of banana fruits with Neem extract (*A. indica*) for 5 minutes were most effective for controlling various postharvest diseases of banana. Many plant and plant products have been reported to be antimicrobials against plant pathogenic fungi (Bowers and Locke, 2000). Plant extracts might be a substantial alternative of chemical pesticides in controlling plant diseases.

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted during July- December 2017 at the Department of Plant pathology, Sher-e-Bangla Agricultural University. Mango varieties Langra, was selected for the study of post-harvest anthracnose of mango in this chapter.

3.1. Survey on the postharvest diseases of mango at different market place

3.1.1. Survey sites

A survey was conducted at following wholesale and retail markets of Dhaka district.

Table 1. Sampling structure for estimation of post-harvest diseases

Sl. No.	Survey area	Sample size
1.	Kawran Bazar Dhaka	3retailer(1wholesaler considering a replication)
2.	Mirpur-10 Dhaka	3retailer(1wholesaler considering a replication)
3.	Shyamoli Dhaka	3retailer(1wholesaler considering a replication)

Figure1. Infected mango with Anthracnose

3.1.2. Fruit sampling

The methodology of sampling was adopted as suggested by Diedhiou *et al*., (2007). Mango fruits were sampled (20 fruits per sample) and 5 samples in each replication. The percent disease incidence and severity was calculated as follows:

Figure2.Healthymango

% Disease incidence (% DI),

$$DI\ (\%) = \frac{Y}{X} \times 100$$

Here, DI = Disease incidence (%)

Y =Number of infected fruits

X = Total number of fruits observed

% Disease severity (% DS):

$$DS\ (\%) = \frac{A_1}{A_2} \times 100$$

Here, A_1 = Area of fruit tissue infected by diseases

A_2 = Total fruit area inspected

3.2. Collection, isolation and identification of pathogen

3.2.1. Collection of diseased specimens

The fruits of mango having typical symptoms of anthracnose were collected from the market, the pathogen was isolated and identified as per the key characteristics of *Colletotrichum gloeosporioides*under microscope.

3.2.2. Microscopic observation of diseased specimen

The anthracnose infected fruits were collected and microscopically examined for confirmation of the fungus. Sections of the diseased fruits were made with the help of a sharp blade on a clean glass slide having a drop of lactophenol. The slide was then covered with a cover slip and observed under microscope. After confirmation of fungal spores, isolation was done in the laminar air flow chamber under aseptic conditions following the standard tissue isolation procedure.

3.2.3. Isolation

Disease mango samples were separated and brought to the laboratory for isolation. The mango fruits were disinfected by dipping in sterilized distilled water until all dust particles were removed and dried with sterilized paper towel. Diseased surface of mango fruits with typical symptom were separated and cut into small pieces, surface sterilized with $HgCl_2$ (0.1%), rinsed three times in sterilized distilled water, dried on sterile blotting paper and plated on PDA media. The plates were incubated at room temperature (27°C ± 2). The pathogens was purified by single spore isolation technique and maintained on slants in refrigerator.

3.2.4. Preparation of medium

The potato dextrose agar medium was used for isolation of the pathogen and also in most of the experimental studies. The composition of potato dextrose agar medium used was as follows:

Potato	200 g
Dextrose	2 g
Agar-agar	20 g
Distilled water	1000 ml

Two hundred grams of peeled potatoes were cut into small pieces and boiled in distilled water and then the extract was collected by filtering through muslin cloth and 20.00 grams of dextrose was dissolved in the extract. Later 20.00 grams of agar-agar was melted in half litre of distilled water separately. Both the solutions were mixed and final volume was made to 1000 ml with distilled water, later it was sterilized in an autoclave at 121º C at 15 lbs pressure for 20 minutes and preserved for further use.

3.2.5. Identification of the causal fungus

After sporulation spore suspension was made in sterile water and the dilution was adjusted such that in one loopful 25-30 spores could be counted under the low power of microscope. One such loopful was mixed with 25 ml of melted sterilized Plain Agar (2%) and poured in sterile Petri dish. After 12 hours of incubation, the germinating spore was located under the microscope and transferred to PDA slants. This was subsequently allowed to grow and sporulate. Monoconidial culture established in this way was maintained by periodical transfer on P.D.A. slants. The identity of pure culture of the test fungus thus obtain was confirmed for their species from Division of Plant Pathology, SAU, Dhaka.

3.2.6. Maintenance of culture of the pathogen

The pathogen was sub-cultured on potato dextrose agar slants and allowed to grow for one week at 28±1ºC. Such slants were stored in a refrigerator at 4°C and again sub-cultured at regular intervals during the course of investigation under aseptic conditions to maintain viability of the pathogen.

3.2.7. Pathogenicity test

The pathogenicity of the isolated fungus *Colletotrichum gloeosporides* was tested on mango fruit. To prove the Koch's postulate, mature and semi ripen healthy mango fruits were collected from field as well as from fruit market and brought to the laboratory. The fruits were then surface sterilized by 2% sodium hypochlorite solution for 2 minute followed by three washings with sterilized water and air dried then separately inoculated with each of the isolated fungus by Pin- Pricking method. Five fruits were separately inoculated with each of the isolated fungus. The inoculated as well as non-inoculated fruits were placed in sterilized, loosely tied polythene bags. A piece of sterilized wet absorbent cotton was placed inside

each bag and the bag was kept at room temperature (24-28^{0}C) in an incubation room for symptoms development. Inoculated fruits were observed regularly. Reisolation of pathogenic fungi from the diseased fruits was done. Morphological as well as cultural characters of reisolated fungi were compared with those of previously isolated from diseased mango fruits.

3.3. *In vitro* screening of fungicide, botanicals and hot water treatment against *C. gloeosporides* in the laboratory

3.3.1.a. *In vitro* screening of fungicide against *C. gloeosporides* in the laboratory

Four fungicides reported effective against different plant pathogens including *C. gloeosporides* were evaluated by Growth inhibition technique (cup method)

Table 2. Fungicides used in the bio-assay against *C. gloeosporides*

SI No.	Trade Name	Chemical Name	Active Ingredient	Concentration (ppm)
1	Dithane M-45	Manganous ethylene bisdithio carbamateion	80% Mancozeb	500,1000
2	Rovral 50 wp	3(3,5dichloropheny1)-N-(1methylethyl)-2,4dioxuimidazol-idene Carboxamide	50% Iprodione	500,1000
3	Tilt 250 EC	1-[2-(2,4-Dicholorophenyl)- 4-propyle- 1,3-diooxalane-2 EI-Methy1)-IH,1,2,4-Triyazole	25% Propiconazole	500,1000
4	Autostin 50WDG	Mythy1-2-Benzimidazole Carbamate	50% Carbendazim	500,1000

3.3.1.b. Preparation of fungicide solution

Fungicide solutions were prepared dissolving required amount of fungicide in water for each concentration in 100 ml Erlenmeyer flask. Flasks were labeled appropriately and shaken thoroughly before use.

3.3.1.c. Bioassay following growth inhibition technique

Groove/Cup method:

From a PDA plate three 5 mm discs of the medium were scooped from three places maintaining an equal distance from the centre by a sterilized disc cutter. One ml of fungicide solution was put into each hole and the plates were stored overnight in refrigerator for diffusion of the input in the medium around the hole before resumption of fungal growth. One 5-mm block of 7 days old fungal culture (pathogen) cut by sterilized disc cutter was placed at the centre of the plate. The linear growth (cm) of mycelium of *C. gloeosporides* was recorded at 24 hours interval until the control plates were filled in (Islam and Akhter, 2001).

3.3.2. *In vitro* screening of Botanicals against *C. gloeosporides* in the laboratory

Two botanicals reported to having fungicidal properties were evaluated against *C. gloeosporides* using growth inhibition technique. The indigenous plants used in the experiment are listed below.

3.3.2.a. Preparation of plant extracts

For extraction of juice, required amount of respective parts of each plant was taken, washed in tap water, crushed in a mortar and pestle. The crushed materials were blended in an electric blender adding equal amount of sterile water for 1:1 solution. The blend was filtered through sterile cheesecloth. The supernatant was diluted in equal amount of sterile water for 1:2 solutions.

Table3. Indigenous plant extracts assayed against *C. gloeosporides*

Local Name	Scientific Name	Plant parts used
Garlic	*Allium sativum L*	Bulb/ Clove
Allamanda	*Allamanda cathertica* L	Leaf

3.3.2. b. Bioassay of plant extracts against *C. gloeosporides* in growth Inhibition technique

Groove/Cup method

Procedures of this method have been described in 3.6.3.

3.4. Dipping test in fungicides and Botanicals solution

Four fungicides (Dithane M-45, Rovral 50 wp, Tilt 250 EC, Autostin 50WDG) and two botanicals (Gaelic and Allamanda extract) were tested for their efficacy against post- harvest diseases of mango resulting from infection in the field. All the four fungicides were evaluated at 500 ppm and 1000 ppm and the botanicals extract were evaluated at 1:1and 1:2 concentration.

Green mature mango was soaked in the fungicide and botanicals solutions. The control treatments concerned Mango dipped in sterile water only. Each treatment was repeated 4 times, with a repetition consisting of 5 mangos. Fruits were thereafter incubated at room temperature in the laboratory. The mango were monitored every 2 days for diseases development until ripening of the fruits. Disease incidence and severity were assessed on the basis of fruit infection and fruit surface affected.

3.5. Evaluation of different fungicides, botanicals and hot water treatment against anthracnose

Green matured mango was washed in fresh water to remove dirt and latex. These fruits were treated in hot water using the 'Hot-water plant' for 5 minutes at 45^0,50^0,52^0 and 55^0 temperatures. The treated fruits were shade dried and kept on an aseptic wooden table for study at room temperature. The control treatments concerned mango dipped in sterile normal water. Each treatment was repeated 4 times, with a repetition consisting of 5 mangos.

3.6. Data collection

Disease incidence and severity were recorded at market level. In *in vitro* bioassay of fungicides and botanicals, length and breadth wise mycelial growth were recorded. On treated mango, data collection was started after the untreated fruits expressed symptoms. The symptoms of disease appearance were recorded. Percent fruit infection and diseased of fruit were recorded. Percent fruits infection was calculated on the basis of totality of healthy and diseased fruits. Fruit surface area diseased of fruit was calculated as the portion of an individual fruit diseased considering the total surface of the fruit as 100%.

3.7. Data Analyses

Data were analyzed statistically to determine differences between treatments. All data were subject to F-test and analyzed in MSTAT-C statistical package programme and treatment measure were compared with DMRT.

CHAPTER IV

RESULTS AND DISCUSSION

Mango is susceptible to a number of diseases at all stages of its development from the seeding to the fruits. Post-harvest diseases result not only in substantial losses and reduction in mango production but also reduce quality of the fruits. The study of postharvest anthracnose disease and their prevention in marketing channel make a significant contribution by reducing the postharvest losses. In the present study, an effort has been made to determine the occurrence in marketing channel after harvesting and to apply ecofriendly treatments to prevent this post-harvest fungal disease. The results and discussion of present investigation on various aspects are presented in this chapter.

4.1. Survey on anthracnose diseases of mango at different market places

From the survey, anthracnose disease was identified by visual observation as described by Reddy and Murti (1990); Rajput and Haribabu (1985). Postharvest anthracnose diseases of mango was found on green fruit, tiny brown spots developed that enlarge on a ripening fruit and found on the peel in tear-shaped patterns. Eventually, the whole fruit rots and fungal fruiting bodies are formed on the rotten surfaces.

It is obvious from the mean data (Table 2), percent disease incidence of post-harvest anthracnose diseases varied significantly in different market. Three locations *viz*. Kawran bazaar, Shyamoli and Mirpur-10 were selected for the survey to identify anthracnose disease of mango and their incidence and severity. Under the survey, anthracnose disease was marked as the most serious fungal disease identified by visual observation at all three locations. Popular mango varieties Langra was selected for % disease incidence and % disease severity affected by anthracnose diseases. It was found that among the three locations, the

highest anthracnose disease incidence and severity (18.62% and 14.78%, respectively) was observed in Kawran bazaar where the lowest (12.52% and 9.32%, respectively) was in Mirpur 10.

Table4. Incidence of mango anthracnose diseases in the different fruit market in Dhaka city

Variety	Name of diseases	Name of market in Dhaka city	% Disease incidence	% Disease severity
Langra	Anthracnose	Kawran bazar	18.62 a	14.78 a
		Shyamoli	16.71 b	7.26 d
		Mirpur 10	12.52 d	9.32 b
$LSD_{0.05}$			0.362	0.257
CV (%)			7.288	5.274

4.2. Isolation, identification and pathogenocity test of the pathogen

The isolated organism from was identified as anthracnose disease of mango *Colletotrichum gloeosporioides* (Penz.) Penz.& Sacc. It produced whitish to pinkish colony and conidia were smooth, hyaline and sub cylindrical with round end.

To prove Koch's postulate, the isolated organism from diseased mango fruit was inoculated by employing cork borer wounding method as mentioned in chapter 3. The symptoms observed on inoculated fruits were similar to those seen in naturally infected fruits. Re-isolations made from artificially inoculated fruits consistently yielded the culture which was identical to one used for inoculation of the fruits. Uninoculated fruits did not produce any symptom. Thus, pathogenocity was successfully proven by artificial inoculation techniques exploited. The

isolation, identification and Koch's postulate described by Daquioag and Quimio (1973), Hasabnis (1984) and Akhtar *et al*. (1998) are matching with our observations and hence the disease was confirmed as anthracnose caused by *Colletotrichum gloeosporioides*.

Table5. Characterization of isolated fungal pathogens from diseased mangoes

Disease	Fungus(identified)	Colony characters	Spore/conidia characters	Spore/conidial size
Anthracnose	*Colletotrichum gloeosporioides*	Whitish to pinkish colony formed	Smooth, hyaline and sub cylindrical with round end spores	Length= 3.90 to 12.10 µm Width = 1.40 to 2.11 µm

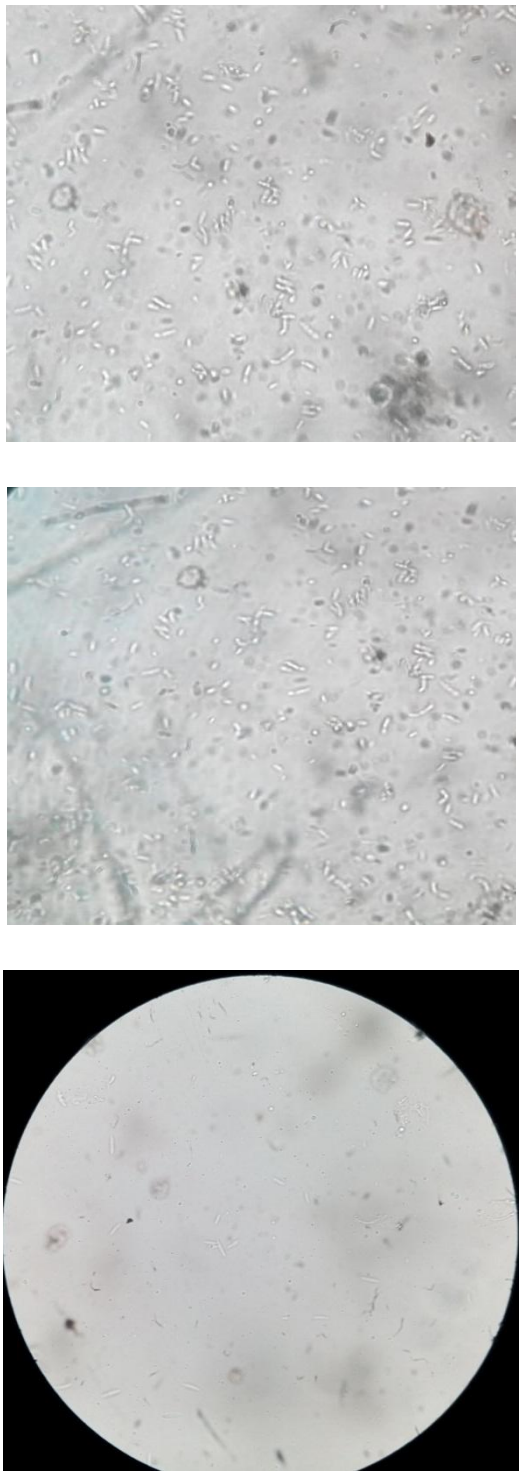


Fig3.Isolated*Colletotrichum gloeosporioides*

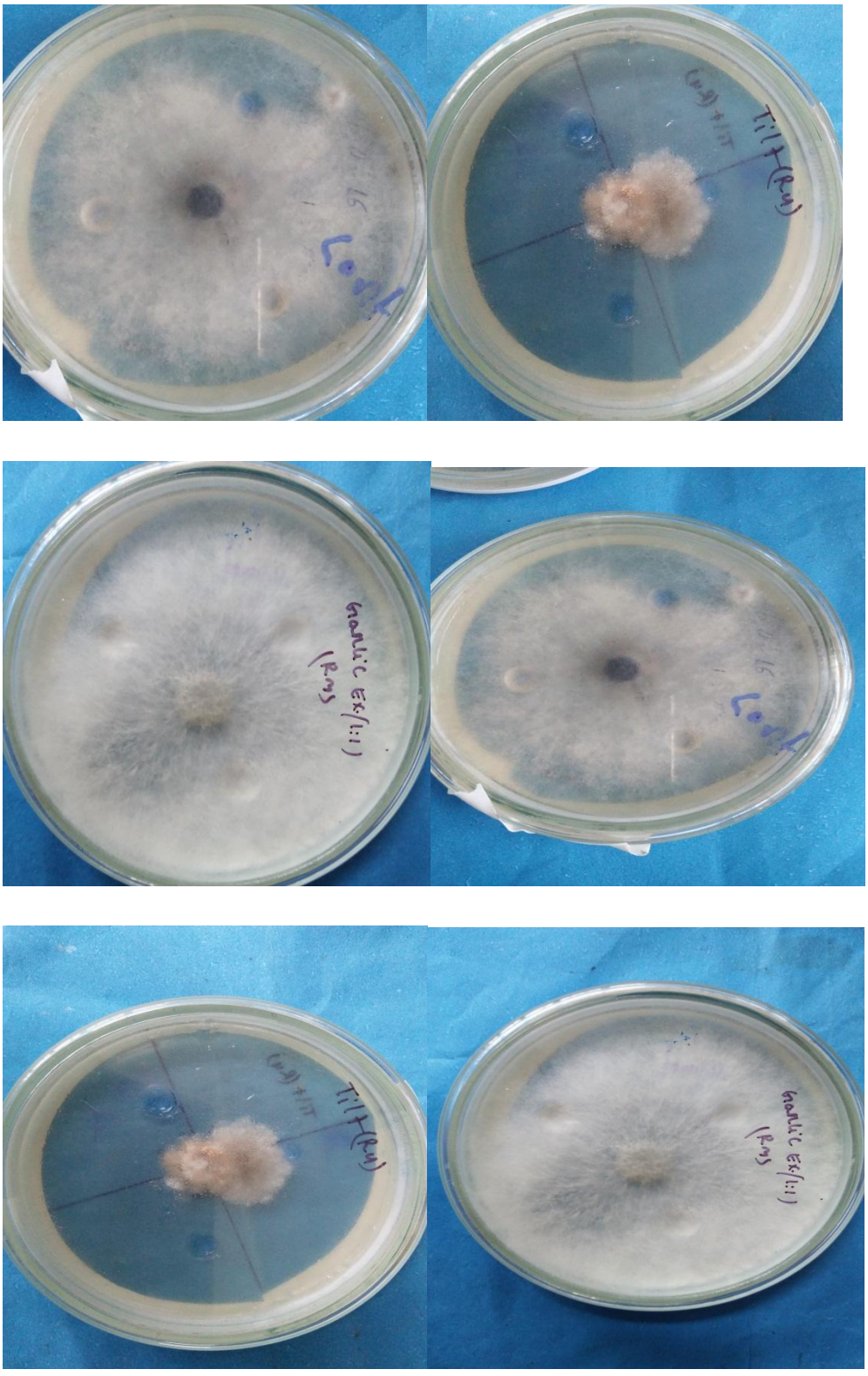


Fig4. Reduction of mycelial (*C. gloeosporioides)* growth using different treatments

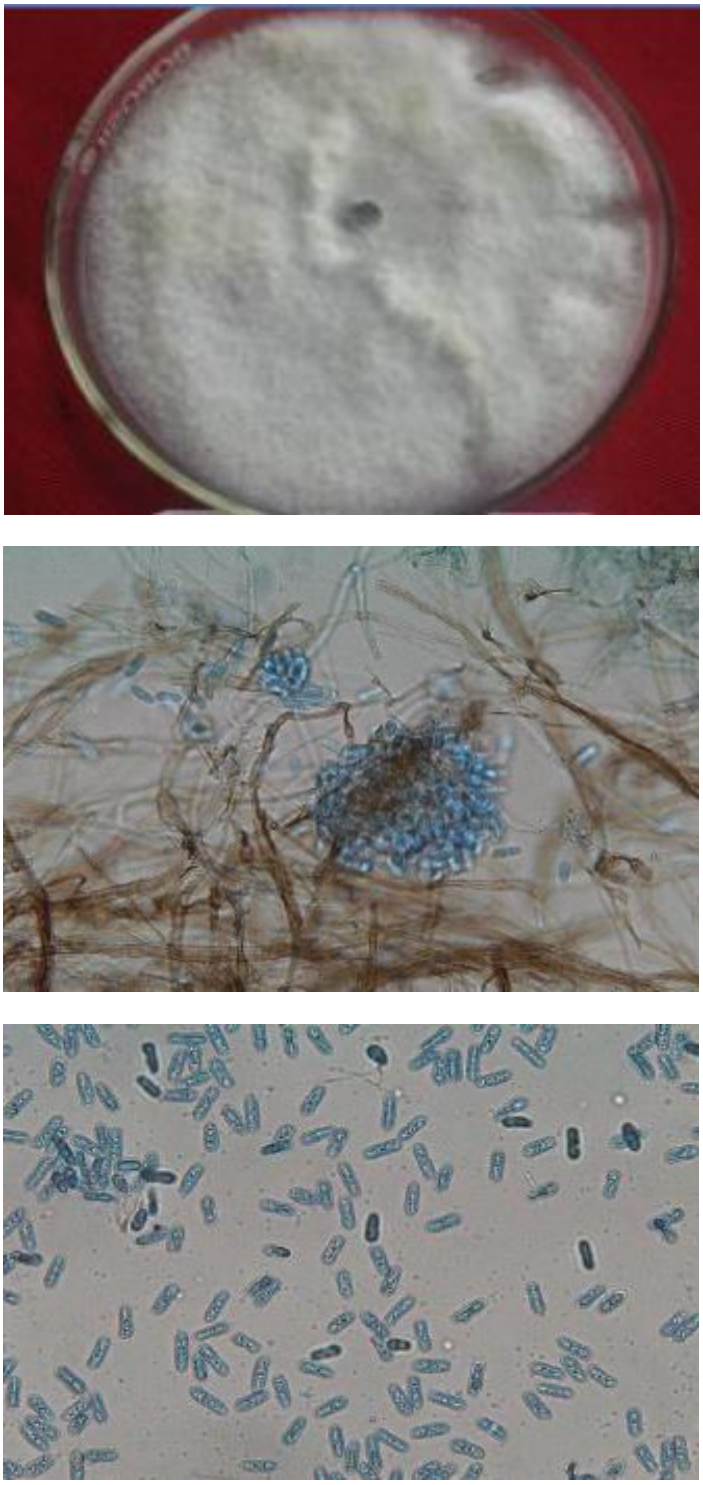


Figure5. Structural presentation of *C. gloeosporioides*

4.3. *In vitro* evaluation of selected fungicides against *C. gloeosporioides* of mango

Effect of the treatments in controlling post-harvest anthracnose of mango (*C. gloeosporioides*) was evaluated in *in vitro* condition. The results were compiled based on the inhibition of radial mycelium growth of pathogen against 6 treatments viz. Dithane M-45, Tilt 250 EC, Autostin 50 WDG, Rovral 50 WP, Garlic extract, Allamanda extract along with control.

4.3.1. *In vitro* efficacy of different fungicides in different concentration against mycelial growth of c. *gloeosporioides* in poison food technique method

Significant influence was found in controlling mycelia growth of anthracnose in mango at different days after inoculation affected by different fungicides and botanicals at different concentrations (Table 4). Results revealed that at 3 days after inoculation (DAI) of pathogen, the lowest mycelia growth (1.28 cm) was found from Tilt 250EC at 1000 ppm concentration which was statistically identical with Tilt 250EC at 500 ppm concentration and Autostin 50WDG at 1000 ppm concentration. Similar trend was also observed at 5 and 7 DAI. The lowest mycelia growth at 5 DAI (1.48 cm) and at 7 DAI (3.20 cm) was also observed from Tilt 250EC at 1000 ppm concentration followed by Tilt 250EC at 500 ppm concentration. Rovral 50W and Autostin 50WDG at 1000 and 500 ppm concentration also showed comparatively lower mycelia growth at 3, 5 and 7 DAI compared to Tilt 250EC at 1000 ppm concentration. Among the botanical fungicide, Garlic extract showed lower mycelia growth compared to Alamanda extract both at 1:1 and 1:2 concentration. Control treatment showed highest mycelia growth (7.24 and 7.16 cm) at 3 DAI. The highest mycelia growth at 5 DAI (7.88 and 7.82 cm) and at 7 DAI (8.12 and 8.20 cm) also observed from control treatment. Among the different chemical and botanical fungicides, the highest mycelia growth (6.52, 7.48 and 7.80 cm at 3, 5 and 7 DAI respectively)

was found from Alamanda extract at 1:2 concentration followed by Dithen M-45 at 500 ppm concentration.

In terms of percent growth inhibition of mycelia, the best performance (60.98%) was achieved by Tilt 250 EC at 1000 ppm concentration at 7 DAI. The lowest % growth inhibition (3.94%) of mycelia over control was recorded from Allamanda extract at 1:2 concentrations at 7 DAI respectively followed by Dithen M-45 at 500 ppm concentration.

Table6. Effect of different fungicides and botanicals at different concentrations on inhibition of mycelia growth of *Colletotrichum gloeosporioides*

Treatments	**Concentration**	**Radial mycelial growth**			**% growth inhibition**
		3 DAI	5 DAI	7 DAI	7 DAI
Autostin 50WDG	500	3.12 e	5.68 e	6.32 de	22.17
	1000	2.20 g	2.96 h	4.50 f	45.12
Tilt 250EC	500	2.10 g	2.66 hi	3.72 g	54.19
	1000	1.28 g	1.84 k	3.20 h	60.98
Diathen M-45	500	6.20 b	7.22 b	7.40 bc	8.87
	1000	4.66 cd	6.10 d	6.36 de	22.44
Rovral 50W	500	2.60 ef	3.16 gh	3.80 g	53.20
	1000	1.58 ef	2.14 j	3.48 gh	57.56
Garlic extract	1:1	2.80 ef	3.36 g	4.75 f	42.07
	1:2	4.78 cd	5.34 ef	6.60 d	18.72
Allamanda extract	1:1	5.18 c	6.74 c	7.76 b	5.37
	1:2	6.52 b	7.48 b	7.80 b	3.94
Control	--	7.16 a	7.82 a	8.20 a	--
$LSD_{0.05}$	--	0.368	0.384	0.402	--
CV (%)	--	3.514	3.817	4.215	--

4.4. Efficacy of fungicides and botanicals against anthracnose disease in dipping test method

4.4.1. Percent fruit infection and infection reduction over control using fungicides and botanicals at 3 DAT

Significant variation was recorded in terms of infected area of postharvest mango caused by *Colletotrichum gloeosporioides* in response to different chemical and botanical fungicides at 3 days after treatment (DAT) (Table 5). The lowest infected area (2.50%) was recorded from the treatment, Tilt 250EC at 1000 ppm concentration which was followed by the results obtained from the treatments Rovral 50W at 1000 concentrations and Tilt 250EC at 500 ppm concentration. Control treatment showed the highest infected area (31.75%) followed by Allamanda extract at 1:2 concentration.

Considering % reduction of infected area over control, the highest reduction (92.13%) was recorded from Tilt 250EC at 1000 ppm concentration followed by Rovral 50W at 1000 ppm concentration and Tilt 250EC at 500 ppm concentration. Autostin 50WDG at 1000 ppm concentration and Rovral 50W at 500 ppm concentration also showed comparatively higher % reduction of infected area over control. The lowest % reduction of infected area over control (16.67%) was recorded from Alamanda extract at 1:2 concentration followed by Daithen M-45 at 500 ppm concentration.

Table7. Effect of different fungicides and botanicals with different concentrations on reduction of infected area (%) against anthracnose of mango caused by*Colletotrichum gloeosporioides* at 3 days after treatment (DAT)

Treatments	Concentration (ppm)	Infected area (%) at 3 DAT	Reduction (%) over control
Autostin 50WDG	500	8.00 f	73.33
	1000	5.50 h	82.68
Tilt 250EC	500	3.25 i	89.17
	1000	2.50 j	92.13
Diathen M-45	500	20.25 c	32.50
	1000	15.50 d	51.18
Rovral 50W	500	4.50 hi	85.00
	1000	3.00 i	90.55
Garlic extract	1:1	7.25 fg	77.17
	1:2	10.00 e	66.67
Alamanda extract	1:1	20.50 c	35.43
	1:2	25.00 b	16.67
Control	--	30.00 a	--
	--	31.75 a	--
$LSD_{0.05}$	--	1.142	--
CV (%)	--	4.527	--

4.4.2. Percent fruit infection and infection reduction over control using fungicides and botanicals at 5 DAT

At 5 days after treatment (DAT), the infected area of postharvest mango caused by *Colletotrichum gloeosporioides* affected by different chemical and botanical fungicides showed significant variation (Table 6). Results denoted that the lowest infected area (3.00%) was recorded from the treatment, Tilt 250EC at 1000 ppm concentration which was followed by the results obtained from the treatments Tilt 250EC at 500 ppm concentration and Rovral 50W at 1000 ppm concentration. Control treatment showed the highest infected area (52.00 and 50.50%) followed by Alamanda extract at 1:2 concentration. Among the different chemical and botanical fungicides, highest infected area (42.00%) at 5 DAT was obtained from Alamanda extract at 1:2 concentrations.

Considering % reduction of infected area over control, the highest reduction (94.06%) at 5 DAT was recorded from Tilt 250EC at 1000 ppm concentration followed by Tilt 250EC at 500 ppm concentration and Rovral 50W at 1000 ppm concentration. Rovral 50W at 1000 ppm concentration, Autostin 50WDG at 1000 ppm concentration and Garlic extract at 1:1 concentration also showed comparatively higher % reduction of infected area over control. The lowest % reduction of infected area over control (16.67%) at 5 DAT was recorded from Alamanda extract at 1:2 concentration followed by Diathen M-45 at 500 ppm concentration.

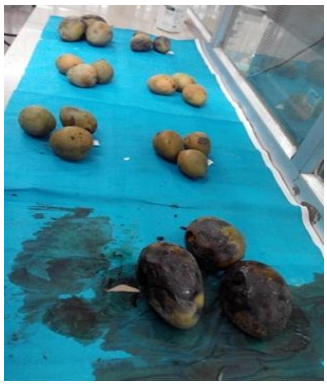
Table8. Effect of different fungicides and botanicals with different concentrations on reduction of infected area (%) against anthracnose of mango caused by *Colletotrichum gloeosporioides* at 5 days after treatment (DAT)

Treatments	Concentration (ppm)	Infected area (%) at 5 DAT	Reduction (%) over control
Autostin 50WDG	500	25.00 f	51.92
	1000	18.50 gh	63.37
Tilt 250EC	500	5.50 jk	89.42
	1000	3.00 k	94.06
Diathen M-45	500	36.75 c	29.33
	1000	26.50 f	47.52
Rovral 50W	500	10.75 i	79.33
	1000	6.50 j	87.13
Garlic extract	1:1	20.00 g	60.40
	1:2	30.25 de	41.83
Alamanda extract	1:1	33.00 d	34.65
	1:2	42.00 b	19.23
Control	--	52.00 a	--
	--	50.50 a	--
$LSD_{0.05}$	--	2.314	--
CV (%)	--	5.736	--

4.4.3. Percent fruit infection and infection reduction over control using fungicides and botanicals at 7 DAT

Significant influence was also observed at 7 days after treatment (DAT) on the infected area of postharvest mango caused by *Colletotrichum gloeosporioides* in response to different chemical and botanical fungicides (Table 7).The lowest infected area (5%) was recorded from the treatment, Tilt 250EC at 1000 ppm concentration followed by the treatments Tilt 250EC at 500 ppm concentration. Control treatment showed the highest infected area (98.00% and 88.00%) followed by Alamanda extract at 1:2 concentration.

Considering % reduction of infected area over control, the highest reduction (92.80%) was recorded from Tilt 250EC at 1000 ppm concentration followed by Tilt 250EC at 500 ppm concentration. Rovral 50W at 1000 ppm concentration, Autostin 50WDG at 1000 and 500 ppm concentration also showed comparatively higher % reduction of infected area over control. The lowest % reduction of infected area over control (11.11%) was recorded from Alamanda extract at 1:2 concentration followed by Alamanda extract at 1:1 concentration and Diathen M-45 at 500 and 1000 ppm concentration.



a. Treated Mango

b. Tilt 250 EC

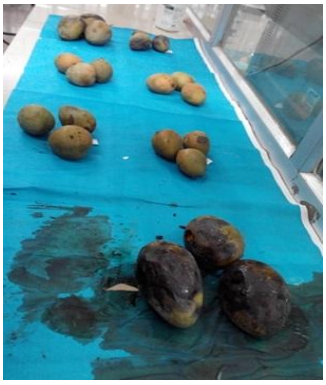
c. Rovral 50 W

d. Infected Mango

e. Austin 200 WGD

f. Control

Fig 7: Effect of different fungicides (500 ppm conc.) against anthracnose of mango at 7 days after treatments.



a. Treated Mango

b. Rovral 50 W

c. Tilt 250 EC

d. Control

e. Infected Mango

f. Austin 200 WGD

Fig 8: Effect of different fungicides (1000 ppm conc.) against anthracnose of mango at 7 days after treatments.

Table9. Effect of different fungicides and botanicals with different concentrations on reduction of infected area (%) against anthracnose of mango caused by *Colletotrichum gloeosporioides* at 7 days after treatment (DAT)

Treatments	Concentration (ppm)	Infected area (%) at 7 DAT	Reduction (%) over control
Autostin 50WDG	500	53.55 g	39.39
	1000	43.66 h	50.37
Tilt 250EC	500	10.00 k	88.86
	1000	6.3 l	92.80
Diathen M-45	500	75.25 c	16.39
	1000	71.50 d	18.75
Rovral 50W	500	25.00 i	71.59
	1000	19.33 j	78.03
Garlic extract	1:1	60.00 f	31.82
	1:2	65.50 e	27.22
Allamanda extract	1:1	76.50 c	13.07
	1:2	80.00 b	11.11
Control	--	90.00 a	--
	--	88.00 a	--
$LSD_{0.05}$	--	2.512	--
CV (%)	--	6.377	--

4.5. Efficacy of hot water treatment against anthracnose of mango

4.5.1. Percent fruit infection and infection reduction over control using hot water treatment at 3 DAT

Significant variation was recorded in terms of infected area of postharvest mango disease caused by *Colletotrichum gloeosporioides* affected by hot water treatments (Table 9). The lowest infected area (5.50%) was recorded from the hot water treatment at 55°C followed by treatment at 50°C where the control treatment showed the highest infected area (30.00%).

Considering % reduction of infected area over control, the highest reduction (81.67%) was recorded from hot water treatment at 55°C where the hot water treatment at 45°C showed the lowest % reduction of infected area over control (65.83%).

Table10. Effect of hot water treatment at different temperature on reduction of infected area (%) against anthracnose of mango caused by *Colletotrichum gloeosporioides*at 3 days after treatment (DAT)

Hot water temperature (°C)	Duration of treatment (Minute)	Infected area (%) at 3 DAT	Reduction (%) over control
55	5	5.50 d	81.67
50	5	7.75 c	74.17
45	5	10.25 b	65.83
Control	5	30.00 a	--
$LSD_{0.05}$	--	1.069	--
CV (%)	--	3.644	--

4.5.2. Percent fruit infection and infection reduction over control using hot water treatment at 5 DAT

Significant variation was recorded in terms of infected area of postharvest mango caused by *Colletotrichum gloeosporioides* affected by hot water treatments (Table 10). The lowest infected area(8.00%) was recorded from the hot water treatment at 55°C followed by treatment at 50°C where the control treatment showed the highest infected area (55.00%).

Considering % reduction of infected area over control, the highest reduction (85.45%) was recorded from hot water treatment at 55°C where the hot water treatment at 45°C showed the lowest % reduction of infected area over control (44.55%).

Table11. Effect of hot water treatment at different temperature on reduction of infected area (%) against anthracnose of mango caused by *Colletotrichum gloeosporioides* at 5 days after treatment (DAT)

Hot water temperature (°C)	Duration of treatment(minute)	Infected area (%) at 5 DAT	Reduction (%) over control
55	5	8.00 d	85.45
50	5	25.75 c	53.18
45	5	30.50 b	44.55
Control	5	55.00 a	--
$LSD_{0.05}$	--	2.144	--
CV (%)	--	4.368	--

4.5.3. Percent fruit infection and infection reduction over control using hot water treatment at 7 DAT

Remarkable variation was recorded in terms of infected area of postharvest mango caused by *Colletotrichum gloeosporioides* affected by hot water treatments (Table 11). The lowest infected area (25.50%) was recorded from the hot water treatment at 55°C followed by treatment at 50°C where the control treatment showed the highest infected area (92.00%).

Considering % reduction of infected area over control, the highest reduction (72.28%) was recorded from hot water treatment at 55°C where the hot water treatment at 45°C showed the lowest % reduction of infected area over control (12.50%).

Table12. Effect of hot water treatment at different temperature on reduction of Infected area (%) against anthracnose of mango at 7 days after treatment

Hot water temperature (°C)	Duration of treatment(minute)	Infected area (%) at 7 DAT	Reduction (%) over control
55	5	25.50 d	72.28
50	5	75.00 c	18.48
45	5	80.50 b	12.50
Control	5	92.00 a	--
$LSD_{0.05}$	--	1.376	--
CV (%)	--	5.289	--

The present study has been designed to study the postharvest anthracnose disease management of mango (*Mangifera indica*) against pathogenic interruption using different chemical and botanical fungicides and also hot water treatments. The study was conducted in three phases *viz*. fruit market survey on anthracnose disease incidence and severity, identification of causal organism and management practices regarding diseases incidence and severity to reduce postharvest losses. The fruit market survey was conducted at three location of Dhaka city *viz*. Kawran bazaar, Shyamoli and Mirpur-10. Identification of pathogen was conducted in the laboratory from the survey samples. For the management of postharvest diseases, 13 treatments were considered including control with three replications.

Survey reports revealed that postharvest anthracnose pathogen is prevalent one. Prabakar *et al.,* (2005) observed that the post-harvest fungal spoilage was higher in retail market than other stages of marketing. The total loss in mango by different pathogens at retail market was 31.20 to 51.70 percent due to Anthracnose accounted for 17.10 to 31.80 percent. Post-harvest losses up to 31.00 percent due to fungal diseases at retail level in Bangladesh reported by Murthy *et al.,* (2009). Rathod (2010) observed that mango fruits were commonly infected by fungal disease like anthracnose, *Alternaria* rot, *A. niger* rot, Blue mould rot, Botryodiplodia rot, Rhizopus rot and Phomopsis rot. The prevalence of post-harvest diseases anthracnose at the wholesalers and retailers level was observed in variety Langra at Bangladesh (Naznin *et al.,* 2007). Postharvest anthracnose of mango is reported to be major constrain for quality and shelf life for domestic and export marketing.

In terms of efficacy of fungicides against disease development, results revealed that fungicides both chemical and botanical could be most effective for controlling postharvest diseases of mango. Mortuza *et al.,* (2003) evaluated the effectiveness of pre and post harvest application of eight fungicides to control post harvest anthracnose and stem end rot of mango in Bangladesh. The treatment

comprising fruit dip in warm suspension (55°C) of carbendazim (0.1%) for 5 minutes was more effective in controlling anthracnose and stem end rot diseases of mango. Sohi *et al*. (1973) reported that instantaneous fruit of mango dip in benomyl (500 ppm) or thiobendazole (900 ppm) reduced the incidence of post harvest mango anthracnose to about 5 percent from 29 percent.

Hot water dip treatments against the disease development could be an effective option in controlling postharvest anthracnose disease of mango Prakash and Pandey (2000) reported that mango fruit after harvest treated with hot water treated at 52°C for 5, 15, and 30 minutes alone and in combination with fungicides for the control of post harvest anthracnose disease and they observed that 52°C alone for 30 minutes was found to be effective in controlling the disease. Sopee and Sangchote (2005) recorded that anthracnose disease incidence on mango fruits heat-treated at 55°C for 5 min was reduced by 93 percent. Le Thi-N *et al*. (2010) reported that the hot water treatment at 55°C for 3 min was decreased total spots of anthracnose disease for 6 days as compared to control.

CHAPTER V

SUMMARY AND CONCLUSION

Post-harvest anthracnose disease of mangoes is vulnerable diseases in Bangladesh, but least concrete information regarding their distribution, incidence and severity and also management is available. Therefore, the present study has been conducted to manage the postharvest anthracnose of mango (*Mangifera indica*). The study was conducted in three phase's viz. survey on disease incidence and severity of anthracnose of mango fruit of different markets, identification of causal organism and management practices to reduce postharvest losses.

The survey was conducted at three location in Dhaka city viz. Kawran bazaar, Shyamoli and Mirpur-10. Identification of pathogen was conducted in the Plant Pathology Laboratory. For the management of postharvest diseases of anthracnose, 13 treatments of chemical and botanicals including control with three replications and hot water treatment were considered. The experiment was laid out in a Complete Randomized Design (CRD).

Three experiments were carried out throughout the study period in July-December, 2017. The disease was identified based on the observed symptoms in the infected fruit following the description of Reddy and Murti, (1990); Rajput and Haribabu, (1985).

Prevalence of Anthracnose of mango varied from location to location. Among the locations, the highest disease incidence (18.62%) and severity (14.78%) of post-harvest Anthracnose disease was recorded in the fruit market of Kawran bazaar where the lowest incidence (12.52%) and severity (9.32%) was recorded in the fruit market of Mirpur -10 in Dhaka city.

In terms of *in vitro* efficacy of fungicides and botanicals, among the treatments, Tilt 250 EC at 1000 ppm concentration showed the best performance in controlling mycelia growth of *C.gloeosporioides.* In case of percent growth inhibition of mycelia, Tilt 250EC at 1000 ppm concentration showed highest inhibition of mycelia (60.98%) at 7 days after inoculation (DAI) where the Allamanda extract 1:2 (Extract: water) concentration gave the lowest percent inhibition of mycelia (3.94%) at 7 DAI.

Treatments effect of different fungicides and botanicals on reduction of infected area of anthracnose of mango was varied. The highest percent reduction of infected area (92.80%) over control was achieved from Tilt 250EC at 1000 ppm concentration at 7 Days after treatment (DAT) where the lowest (11.11%) was obtained from Allamanda extract at 1:2 (Extract: water) concentration at 7 DAT.

In terms of management practices using hot water treatment, 55°C treated mango showed the best performance in controlling the growth of Anthracnose diseases. The lowest infected area (25.50%) of mango was obtained from the hot water treatment at 55°C where control treatment gave highest mycelia growth (92.00%) at 7 DAT. Again, percent reduction of infected area over control, the highest (72.28%) reduction was achieved from hot water treatment at 55°C at 7 DAI where the lowest reduction (12.50%) was obtained in treatment hot water at 45°C temperature at 7 DAT.

Finally, among the treated fungicides, Tilt 250EC at 1000 ppm concentration and physical treatment of hot water at, 55°C temperature can be considered as best treatment for controlling postharvest anthracnose disease of mango.

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APPENDICES

Appendix I. Significant effect on incidence of mango anthracnose diseases in the different fruit market in Dhaka city

Source of variation	Degrees of freedom	Mean square of plant height	
		% disease incidence	% disease severity
Replication	2	1.056	1.114
Factor A	2	8.386	10.512
Error	4	1.014	1.376

Appendix II. Significant effect on different fungicides and botanicals at different concentrations on inhibition of mycelia growth of *Colletotrichum gloeosporioides*

Source of variation	Degrees of freedom	Mean square of radial mycelia growth		
		3 DAI	5 DAI	7 DAI
Replication	2	0.052	0.113	0.147
Factor A	13	2.537	4.216	4.312
Error	26	0.078	0.114	0.132

Appendix III. Significant effect on different fungicides and botanicals with different concentrations on reduction of infected area (%) against anthracnose of mango caused by *Colletotrichum gloeosporioides* at different days after treatment (DAT)

Source of variation	Degrees of freedom	Mean square of infected area		
		3 DAI	5 DAI	7 DAI
Replication	2	0.036	0.087	0.109
Factor A	13	1.386	3.441	3.568
Error	26	0.018	0.066	0.314

Appendix IV. Significant effect on hot water treatment at different temperature on inhibition of mycelia growth of *Colletotrichum gloeosporioi*

Source of variation	Degrees of freedom	Mean square of radial mycelia growth		
		3 DAI	5 DAI	7 DAI
Replication	2	0.022	0.106	0.133
Factor A	2	2.614	3.886	3.055
Error	4	0.038	0.057	0.042

Appendix V. Significant effect on hot water treatment at different temperature on reduction of infected area (%) against anthracnose of mango caused by *Colletotrichum gloeosporioides* at different days after treatment (DAT)

Source of variation	Degrees of freedom	Mean square of infected area		
		3 DAI	5 DAI	7 DAI
Replication	2	0.014	0.028	0.044
Factor A	2	1.266	2.514	3.078
Error	4	0.056	0.081	0.072