POST-HARVEST MANAGEMENT OF ANTHRACNOSE OF BANANA (*Musa spp*.)

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*This is to certify that the thesis entitled, "**POSR-HARVEST MANAGEMENT OF ANTHRACNOSE OF BANANA**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE** IN, PLANT PLTHOLOGY, embodies the result of a piece of bona fide research work carried out by **MD.YASIN ALI**, Registration No. **11-04328** under my supervision and my 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

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ABSTRACT

An investigation was carried out on post-harvest diseases of banana and evaluates the efficacy of some selected chemicals, botanicals and hot water treatment against the pathogen. The experiment was conducted in *in vitro* condition. In this study, the antifungal activity of six fungicides and hot water treatment were tested under *in-vitro* condition against *C. gloeosporioides*. The results of present study showed that three fungicides (Tilt 250 EC, Autostin 50 WDG, Rovral 50 WP) at 500 and 1000 ppm concentrations significantly checked the fungal growth. The lowest mycelial growth (1.10 cm) was found in case of Tilt 250 EC at 1000 ppm concentration at six day after inoculation. The lowest severity (2.50%) of banana was recorded in case of the same fungicide at same concentration (1000 ppm) and the reduction of infection was (97.08%) over control. On the other hand, hot water treatment of banana had significant effect in controlling anthracnose of banana over control. The severity of the disease gradually decreased with the increase of water temperature and came to the minimum infection (4.25%) at 55^{o}C temperature treated for 5 minutes. Application of Tilt 250 EC (1000 ppm) and 55^{0}C temperature for 5 minutes proved effective in management of anthracnose diseases of banana.

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LIST OF ABBREVIATED TERMS

ABBREVIATION	FULL WORD
et al.	And others
^{0}C	Degree centigrade
Etc.	Etcetera
Ed.	Edited
Eds.	Edition
cm	Centre meter
J.	Journal
No.	Number
PDA	Potato Dextrose Agar
%	Percent
LSD	Least Significant Difference
CRD	Completely Randomized Design
Res.	Research
Viz.	Namely
SAU	Sher-e-Bangla Agricultural University
BBS	Bangladesh Bureau of Statistics
BARI	Bangladesh Agricultural Research Institute
FAO	Food and Agriculture Organization

CHAPTER I

INTRODUCTION

Banana (*Musa sapientum* L.) fruit is one of the most important commercial fruit crops grown all over the world in the tropical and subtropical areas. It is the second largest fruit crop, belongs to family *Musaceae* in order *Scitamineae.* It can be grown round the year and it is widely adopted in Bangladesh. Banana is the cheapest as well as the nutritious fruit. The banana has become a very popular fruit in modern westernized diets for its flavor, texture and for its convenience value being easy to peel off and eat. Banana can make a useful contribution to the vitamin A, C and B_6 contents of the diet and is an important and immediate source of energy. It is often eaten by sportsmen and women during competition. A medium sized banana contains 280 kilojoules (Morton, 2001), which is more than that of deciduous or citrus fruits. The energy and nutritional status of banana and plantain are higher than other common tropical and sub-tropical fruits.

The banana and plantain which encompass a wide range of dessert and cooking varieties have their wild origins in Asia and the Pacific. The crop has spread throughout the tropical world, carried by traders and other travelers to Africa, South and Central America and the Caribbean islands. Now bananas are of major global importance in terms of food and income security to millions of' small farmers throughout the developing countries of the tropics. Apart from this it is considered as potential ‘Dollar Earning crop (INIHAP France, PROMUSA, 2001).

Exports of dessert bananas exceeded 12 million tons in 2000, mainly to the United States, the European Union, Eastern Europe and Japan. In the last 30 years, world production of dessert bananas has been doubled and export tripled. World production of banana is estimated to be 85 million tones, of which 30 million tones are plantains. Developing countries account for 98% of the

production of bananas and 100% of plantain production. The poorest countries provide 42% of total production. As for cooking bananas (plantains and other bananas), about 20 million tones are produced in Africa, which yields 50% of all plantains in the world (South America yields 25%, Asia 15% and Central America 10%) (INIHAP France, PROMUSA, 2001).

Banana and plantain have become very important for household food security globally and is recognized to be the fourth largest food crop. It also contributes significantly to the trade of horticultural crops in several countries particularly in developing countries (Srivastava and Emendra, 2002). The Banana is the most essential and important fruit crop which shares about 20% of total fruits production with 36% share in area (BBS, 2010). The average yield of Banana is 14 M.tones/hector, which is lower compared to other Banana-producing countries in the world. But in commercial orchard, yield is not less than 30 M.tones/hector (BBS, 2013).

Bananas are highly perishable commodities with post-harvest losses estimated to 25-30% (Kachhwaha, 1991).The post-harvest loss is 25-50% in Bangladesh (Amiruzzaman, 2001). In addition to infectious diseases, other factors are also responsible for this post-harvest loss of banana. Post-harvest diseases can cause serious losses of fruits (Banana) both in terms of quality and quantity. Fruits infected with disease have no market value. There are many post-harvest diseases of banana, cooking banana and plantain. The important diseases are crown rot, anthracnose, cigar end rot and finger rot etc. (Dadzie and Orchard, 1996).

Phytopathological losses arc the main single cause of avoidable post-harvest losses. Such losses are the results of microbial spoilage due to the infection by one or more pathogens. The average minimum loss reported by The National Academy of Sciences (NAS) for fruits and vegetables was 21%. Other estimates were 40 to 50% and above (Rames *et al*., 2002).

Banana undergoes a series of handling operation immediately after harvesting. During this harvest to consumption period, they receive various treatments (field hygiene, grading, packaging, storage in the transport, stack in the storage etc.). Post-harvest handling, if not properly done, brings loss to this perishable fruit. Post-harvest diseases of fruits are the results of combined effect of uncared handling and uncontrolled indigenous factors (Meah, 1995). Ripen banana suffers from various post-harvest diseases all over the world including Bangladesh. Due to climatic type of respiration leading to senescence and improper handling giving rise to physical injuries (e.g. Mechanical injuries, breakage, cuts, punctures, impact bruises, compression bruises, vibration bruises) the perishable banana fruits are affected by the post-harvest rot-diseases. These destructive diseases cause damage to quality, nutrient and the fruits become unlike for consumption, losing their market price. This brings loss to the producers, distributors, retailers and whole sellers.

In India, post-harvest diseases of fruits are responsible for causing losses up to 30% during harvest to consumption (Parpia, 1996). Post-harvest losses take a heavy toll on the harvested banana, which may vary from 20-80% (National Academy of Science, LISA, 1998).

Post-harvest losses to fruits in developing countries have been estimated to be in the range of 5-50% or more of the harvest (Salunkhe and Desai, 2000). Even in the countries with most advanced technologies available, the post-harvest losses are substantial (Cappellini and Ceponis, 1984; Eckert, 1995).

In Bangladesh a huge amount of banana is spoiled due to prevailing high temperature, humidity, inappropriate post-harvest handling and sub-optimal knowledge in the field of post-harvest technology as well as post-harvest pathology. Such spoilage can occur during transportation and /or in the wholesale market resulting considerable economic loss. These losses are caused partly by rotting due to pathogenic agents (fungi, bacteria etc.) and partly due to endogenous factors (respiration, senescence etc). Post-harvest diseases of fruits are the results of combined effect of uncared handling and

uncontrolled endogenous factors (Meah, 1995). Due to external agents (like fungi) banana fruit suffers from many post-harvest serious diseases such as anthracnose (*C. gloeosporioides*), Botryodiplodia rot, Crown rot (*Cephalosporiun* sp.), Fusarium rot (*F. semitectum*) and eye spot (*Paecilomyces variotii*). Among them Anthracnose *(C. gloeosporioides)* of banana is prominent. These diseases account for causing 10-15% post-harvest loss of banana (BARI, 2002). As per research findings 5-10% bananas are damaged due to post-harvest diseases in Bangladesh. It is approximately 44941.60 tons of bananas. The value stands at 35.95 core taka at the rate of 8000 Tk. per ton (BBS, 2001). It is necessary to identify the most prevalent pathogen causing post-harvest diseases and ultimately to reduce post-harvest loss of the banana fruit. Considering the apparent huge post-harvest loss of banana in Bangladesh it is imperative to embark on a comprehensive study on post-harvest diseases of banana. With this end in view, the present investigation was designed for achieving the following objective.

1) To collect the harvested banana from the local market of Dhaka city
2) To isolate and identify the causal pathogen of anthracnose of banana
3) To formulate the suitable management practices against anthracnose of banana.

CHAPTER II

REVIEW OF LITERATURE

2.1 Post harvest losses

In the Philippines, the banana growers incur a huge loss (30-40%) annually because of poor post-harvest techniques adopted by them (Flordeliza, 2002).

Banana and plantain are highly perishable commodities. A combination of high perishability, high ambient temperatures, slow marketing systems and poor market condition lead to huge losses Nigeria (IITA Res. Guide, 2002).

Post harvest losses of fruits and vegetables in Pakistan is about 35% (Hadi. 2001). According to him the factors responsible for post-harvest losses are adoption of poor pre-harvest measures, insect pest and disease infestation and biotic stresses; low technique harvesting procedures; non-application of pre-harvest recommended treatments/practices, harvesting at improper stage, improper care at harvest, non-removal of field heat, dumping procedure, moisture condensation causing pathogen infestation, packing in bulk without sorting and grading of produce, improper transportation and storage and distant and time consuming market distribution.

Srinivas *el al.* (1997) conducted a survey to assess post-harvest losses of mango in Karnataka and observed total post-harvest losses of 17.9%.

Surveys by Naqvi and Dass (1994) in several districts of Maharashtra India, indicated that 43-47% of the total losses of mandarins in truck and train transport, were due to post-harvest diseases such as stem end rot (*Botryodiplodia theobromae* Pat.). Daniels (1990) reported 20-40% post-harvest losses of fruits and vegetables in the tropical countries.

Meah and Khan (1997) surveyed the incidence of mango diseases throughout the country and reported an average loss of 15.2% due to diseases.

Subramanyam (1996) described that the post-harvest losses of fruits and vegetables in developing countries have been reported to vary between 15 and 50% with an estimated minimum loss of 20% at different stages of marketing.

In Kenya a case study of post-harvest losses of bananas was reported (FAO., 1997). The major factors responsible for the losses were mechanical damage and in transit ripening. Overall the losses from the defingering, bruising, broken fruits and in transit ripening were in the order of 22. 29. 15 and 30%, respectively. The position of the fruits in the truck affected the degree of damage with the total losses from the top, middle and bottom beinp, 21, 31 and 44% respectively. Estimates of post-harvest loss of *Musa* Crops in the traditional marketing systems ranged from 20-80% (FAO, 1997).

Gerini (1988) observed 5% mean weight loss of banana due to mechanical damage and fungal disease.

2.2 Post-harvest banana diseases and pathogenicity

Alam and Meah (2000) recorded five diseases of bananas on vars. Sabri and Amrita Saar during the survey in the two markets of Bangladesh in 1996. The diseases were fruit anthracnose (*Colletotrichum gloeosporioides*), *Botryodiplodia* rot, Crown rot (*Cephalosporitun sp.), Fusarium* rot (*F. semitectum*) and eye spot (*Paecilomyces variotii*). An average of 3.5% fruit rots were recorded.

Fresh Fruits are susceptible to attack by many plant pathogens after harvest because they are rich in nutrients and have lost most of the intrinsic resistance that has protected them during their development while attached to the plant (Eckert, 2000).

Post-harvest diseases of banana fruits are considered as a major threat to the banana industry. Fruit rot by *C. musae* occupies leading position among the post-harvest diseases (Sapiah *et al*, 2001).

East and Kenyon (1998) isolated fungi from treated and untreated banana fruits imported into the UK from Jamaica, Windward Islands, Costa Rica and Dominican Republic and established a collection of 7150 pathogenic fungal isolates. These were identified, and the frequencies of 1 occurrence of the main crown rot pathogens from different areas were recorded. The most common fungi isolated were *Colltotrichum* spp. and *Fusarium* spp.

Ploetz and Galan (1998) reported that post-harvest fruit rot diseases of banana were anthracnose (*Colletotrichum musae*), Cigar-end rot (*Trachvsphaeria fructigena* and *Verticillium theobromae*), crown rot (*Ceratocystis paradoxa, C. musae, Fusarium pallidoroseum, Lasiodiplodia theobromae and V. theobromae*), finger rot (*L. theobromae*), Johnsson spot (*Magnaporthe grisea*) and squirter diseases (*Nigrospora spaerica*).

Chillet *et al.* (1996) described anthracnose caused by *Colletotrichum musae* as the main factor responsible for post-harvest decay of West Indian banana.

In Nigeria, Odebode and Sanusi (1996) observed that *Botryodiplodia theobromae* Pat is a major organism causing spoilage of banana in southwest part of Nigeria. They described that *B. theobromae*, *Rhiopus oryzae*, *Aspergillus niger, A. flavus and Fusarium equisetti* were found to be associated with the ripening of bananas and also caused rot during storage.

In Caribbean, India, Taiwan and the Philippines, fully mature fruit is more susceptible to infection and the affected clusters ripen earlier. The disease develops faster during ripening and can spread to adjacent fingers (Slabaugh, 1994a, Slabaugh, 1994b).

Finlay and Brown (1993) observed that low numbers of conidia of *C. musae* (10-50) applied directly to the surface of freshly cut banana crowns caused extensive rot development. At least 20 times more conidia of *Fusarium pallidoroseum* required to cause comparable levels of crown rot. Examination of the relative pathogenicity of the major fungal species involved in development of crown rot in the Windward Islands, West Indies, (*C. musae, F. pallidoroseum, Fusarium moniliforme (Gibberella fujikuri), F. moniliforme var. subglutinans* and *Botryodiplodia theobromae*) using a standard inoculum of 2 x 10^3 conidia per crown, showed *C. musae* to be the most aggressive species. *C. musae* produced a distinctive soft, dry fibrous rot while the fruit was still green, and on ripening further rotting, softening and blackening of crown tissues occurred. The other crown-rot pathogens tested did not rot green fruit. Fruit inoculated with *C. musae* for 0, 1, 2 and 3 days at ambient temperature before ripening sustained increasingly more severe crown rot. *C. musae* was isolated from increasing rotting tissue that longer exposed for longer period under ambient conditions.

Singh and Prasan (1993) observed the effect of temperature and relative humidity on banana cultivars China and Malbhog inoculated with *Botryodiplodia theobromae, Fusarium oxysporum and Aspergillus flavus*. . They noted that deterioration of fruit or fruit pulp in both varieties at 30°C was due to *B. theobrotnae* followed by *F. oxysporurn*. Higher R^H (96%) promoted fruit spoilage in storage with maximum disease caused by *Botryodiplodia theobromae* followed by *F. oxysporum and A. flavus*.

In Australia, Wade et al. (1993) observed that banana (Musa AAA), bunch sections consisting of one hand attached to a short section of main stalk when inoculated with cultures of fungi isolated from diseased fingers, crown or main stalks and stored in sealed polythene bags containing 3-7% 1 oxygen, 10-13% CO_2, then ripened with ethylene in air for 24 hrs., fungi like *Colletotrichum musae*, *Fusarium moniliforme (Gibberella fujikuroi*) var. *subglutinans* caused high infection incidence in unripe fingers during storage. These fungi also

caused crown rot, but only *G. fujikuroi* var. *subglutinans* caused main stalk rot. *Alternaria alternata* and *F. pallidoroseum* both caused infection in unripe fingers during storage.

Lutchmeah and Santchurn (1991) observed that crown rot, pedicel rot, anthracnose, brown spherical lesions and blossom-end rot were the most common post-harvest diseases of banana cv. Naine in Mauritius during 1989-90, with percentage occurrences of 100, 98, 3,100 and 87.3 respectively. Black soft rot was observed less frequently. All diseases except brown spherical lesions, which were thought to be a physiological disorder, were caused by fungal pathogens. *Colletotrichum muscle* was found most frequently followed by *Fusarium spp., F. palliodoroseum, Botryodiplodia theobromae* and *C. gloeosporioides (Glomerella cingulatal).*

Adisa (1984) listed different fungi causing diseases of banana. He observed *Botryodiplodia theobromae* and *Rhizopus stolonifer* causing soft rot while *Aspergillus flavus* caused dry rot of banana. He mentioned further that the incubation period decreased with increase in temperature and relative humidity. Chilling disorders occurred at low temperature.

Bhangale and Patil (1994) first recorded a rot of immature banana caused by *Verticillium theobromae* in Maharashtra, India.

Wallbridge and Pinegar (1995) reported the development of crown rot of packing banana in fibreboard boxes. Each hand was severed from the main stalk leaving a portion of the crown attached to the fingers. The newly exposed tissue was a suitable substrate for colonization by microorganisms. *Colletotrichum musae, Fusarium semitectum, Verticillium theobromae, F. oxysporum* and other *Fusarium* spp. were the main fungi associated with crown rot of untreated fruit cv. Robusta.

Griffee and Burden (1984) studied the incidence and control of Colletotrichum musae on bananas. They isolated Colletotrichum musae from banana hand one week after bunch emergence and in subsequent weeks both from fingers and from crown tissue. Other fungi, including *Fusarium semitectum* and *Nigrospora sphaerica* were also isolated.

Stover (1992) reported that *Botryodiplodia* finger rot was one of the most common rots of bananas that were in transit in boxes for more than 14 days. However, it rarely occurs in fruit transported for 10 days or less. The disease has been reported on fruit from Central and South America.

Tongdee and Pataragetvit (1990) in Thailand isolated *Botryodiplodia theobromae* and *Gloeosporium musarum,* associated with crown rot of banana fruits during storage, which were highly pathogenic. Infected bananas showed premature ripening and severe rotting of crown tissue and finger stalk. Wounds were required for initial fungal penetration, out of four banana varieties; the two sweet ones were more susceptible.

Meredith (1985) isolated *Verticillium theobromae* and *Deigtoniella torulosa* from tip rot of banana fruits in Jamaica. Finger tip rot in Jamaica. rarely yielded *Fusarium moniliforme* var. *subglutinans* and other *Fusarium* spp.

After harvest, the banana fruit is subject to a range of fungal diseases with potential to cause significant damage. These diseases and their importance in international trade were described in the earlier texts of Wardlaw (1991), Meredith (1971) and Stover (1992) and more recently by Slabaugh and Grove (1982), Stover and Simmonds (1987), Jeger et al. (1995) and Thompson and Burden (1969).

Infection of the fruit through injuries made during or just after harvest is a major source of post-harvest decay. Several investigators have demonstrated

that most of the tropical fruits are laden with spores of pathogenic microorganisms awaiting entrance to establish infections in the host tissue (Baker and Wardlaw, 1977 , Barkai, 1996).

2.3 Chemical Control

Nath, K. *et al.* (2015) reported that banana fruits were dipped in fungicides solution for 2 minutes and kept for ripening results showed that minimum PDI was observed in propicanazole and SAAF (1.00%) treated fruits with 25 maximum reduction of fruit rot disease (98.76%) followed by carbendazim (2.50%) with 96.79 percent reduced fruit rot disease

Wade *et al.* (2003) used benomyl and prochloraz (500 mg) per litre for controlling post harvest banana diseases like crown rot, main stalk rot etc. They found that the two fungicides controlled most diseases at inoculation sites and naturally occurring main stalk rot. Prochloraz controlled more diseases than benomyl, and it was usually more effective.

Rippon *et. al.* (2003) reported that in trials against squirter (*Nigrospora sphaerica*) and blackened (*Gloeosporium musarum*) diseases of banana packed as singles wooden boxes, box lots of 100, in 3 grower sheds, were dipped in fungicide under conditions that could be expected to differ from those in acentralized packing shed. There was an apparent depletion of TBZ, depletion rate was highest in the initial stages of dipping but after topping each 25 cases, a sudden rise occurred. No phytotoxic effects were observed on either green or ripe fruit and ripening abnormalities were absent. Results indicated that with an initial concentration of Carbon 200 ppm and using a recommended procedure, a grower should be able to treat 100 boxes in any one dip and effectively control the major post-harvest fruit rots. *Pallidorayeum sp. and VerticiIlium* sp. were frequently isolated from diseased crowns of untreated fruit.

Frosard and Laville (2003) commented that dipping of banana in carbendazim two or more min at 200-300 ppm resulted control of fungal diseases and Long (1971) reported that benomyl was more effective than thiabendazole of methyl thiophenate in controlling banana stem end rot diseases.

Rippon (2002) compared efficacy of thiophanate-methyl with that of S. P. F. (Japanese formulation), benomyl and thiabendazole at different dosages for post-harvest treatment of banana hands artificially inoculated with *Gloeosporium musarum.* All the treatments provided some control but differed in their effective concentrations. Benomyl (up to 200 ppm) was about 23 times as effective as thiabendazole and 64 times as effective as thiophanates methyl.

Lutchmeah and Santchurn (2001) evaluated benomyl, prochloraz and thiophanate methyl (Topsin M) at different doses for control of 4 common post-harvest diseases (crown rot, pedicel rot, anthracnose and blossom-end rot) of banana cv. Naine in Mauritius. Prior to fungicidal treatment, the fruits (Banana) were subjected to a ripening treatment in 500 ppm of ethrel at pH 8. Prochloraz (1, 1.75 and 2.5 nil a.i/litre) and thiophanate methyl (0.7, 0.88 and 1 ml/litre) gave good control, efficiency ranging from 89.6 to 100% against pedicel rot, anthracnose and blossom-end rot. All 3 conc. of benonyl used (100,300 and 500 mg/litre) gave similar results against pedicel rot and anthracnose, however, only the highest conc. gave comparable results to prochloraz and thiophanate methyl against blossom-end rot. Control efficiency of approx. 70% was obtained against crown rot with only the highest cone.of the 3 fungicides tested.

Eckert (2000) studied on the recent developments in chemical control of post-harvest diseases of banana in USA. Mainly benzimidazole fungicides (thiabendazole, benomyl and carbendazim) and sterol inhibitors (imazalil, prochloraz and propiconazole) and their efficacy in controlling anthi-acnose

and stem end rot on citrus, banana, mango, papaya, pineapple and avocado are discussed. Benzimidazole tolerant biotypes of *Botryodiplodia* and *Colletotrichum* have been recognized in the harvested mango and bananas treated preharvest with this group of fungicides.

Alam and Meah (2000) found that Tilt (2000ppm) was most effective in controlling infection by *Colletotrichum gloeosporioides* in banana.

Johanson *et al*. (1999) reported that highly absorbent cellulose pad coated with TBZ were applied to the cut crown surface of bananas harvested in St. Lucia to control crown rot caused principally by *Fusarium* sp. and *Colletotrichum musae.* The bananas were held at ambient temp for 24 hr then at 15°C. After I, 5, and 10 d, samples were taken from the crown and analyzed for TBZ using HPLC. TBZ did not move into the crown tissue from the pad to any great extent.

Michail *et al*. (1998) tested five chemicals and systemic fungicides in vitro and in vivo for control of *Botryodiplodia theobromae* and *Gloeosporium musarum (Colletotrichum musae)*. Control was achieved by dipping bananas in 1% acetic or propionic acid in 10% H_2O_2. In alternat the disease was controlled by dipping bananas for 15 min. in Benlate (200 PIN") and/or TBZ (400 ppm). Acetic and propionic acids and H_2O_2 do not leave chemical residues and may protect post-harvest bananas.

East and Kenyon (1998) assessed the sensitivity of some banana rot, pathogens to thiabendazole. Putative biocontrol agents were also isolated from banana material and their potential for control was determined using a previously developed bioassay.

Jiang *et al.* (1997) observed that after harvest, fruits when soaked in a solution of carbendazim containing 500 mg/litre for 3 minutes before packing in PVC bags containing ethylene absorbent and storing at room temperature (24°C). The treatment greatly reduced the occurrence of bitter rot and improved keeping quality.

Jiang *et al.* (1997) reported that triazole fungicides (Propicinazole, myclobutanil, flusilazole and bitertanol) used at low concentration (50 ppm) to avoid accumulation of residues in fruits, successfully controlled post-harvest disease of banana caused by *Colletotrichum musae* and *Fusarium* spp. Fruit dipping and shower-spraying were more efficient than low-or high-volume spraying.

According to Edwin (1994) commercially prepared fungicide or insecticide should be used as preventive measures before symptoms appear. The choice of the right fungicide, thoroughness in spraying and right timing of sprays are essential to the successful control of diseases.

Johanson and Blazquez (1992) investigated banana crown rot on field packed fruit from the Windward Islands and assessed their sensitivity to the fungicide thiabendazole, prochloraz and immazalil. Isolations of pathogens were made from rotted banana crowns from the Windward Islands over 2 years. Most of the fungi isolated showed expected levels of sensitivity to thiabendazole (TBZ), but some isolates of *Colletotrichum* were resistant. They suggested that these fungicides might also be considered as alternatives to TBZ. Pathogenicity of *Colletotrichum* and *Fusarium* were confirmed. Both species were sensitive to prochloraz in vitro, but only *Fusarium pallidoroseum* was sensitive to benomyl. *Verticillium* sp. was frequently isolated from diseased crowns of benomyl treated fruit and it was suggested that the failure of benomyl to control crown rot in Queensland might have been due to the presence of benomyl tolerant crown rot fungi in banana. Other fungi isolated from diseased crowns were *Penicillium cotylophilum, Alternaria triticina, Cladosporium oxysporum, Acremonium* sp, *Fusarium* spp. and *Bipolaris* sp.

Jones (1991) tested a number of fungicides for control of post-harvest crown rot of bananas which was assessed on fruit harvested in Oct-Nov. from North, Northeastern Queensland, Australia. Prochloraz and flusilazole were most effective. Prochloraz also reduced disease incidence during storage, the

levels of crown rot on prochloraz treated fruit remained low even after a slight initial increase, while crown rot levels of water-treated fruit significantly increased during 16 days in storage. All other fungicides tested significantly reduced disease incidence compared with an untreated control. Benomyl, propiconazole, imazalil and myclobutanil were less effective than prochloraz and flusilazole ($p<0.05$) while triforine and hymexazol were least effective.

Vir and Sharma (1995) observed that dipping the fruit (Banana) in triforine (2500 ppm) for 5 min gave good protection against rot and deterioration caused by *Colletotrichum musae* and enhanced the shelf life.

Ram and Vir (1993) found that benzimidazole fungicides controlled A. niger in inoculated fruit (Banana) and prolonged its shelf life up to 8 days. Among the other fungicides tested, only sodium metabisulphite gave equal control. Propionic acid and salicylic acid were effective for only 4 days.

Ram and Vir (1993) reported that Bavistin (carbendazim), Benlate (benomyl), thiabendazole and Panactin dip- treatment effectively controlled II *C. musae* post-harvest decay. Propionic and salicylic acids were also effective 111. but the latter caused skin discoloration.

Khanna and Chandra (1997) reported that benomyl and Aretan were highly toxic to the growth of *F. moniliforme* (*Gibberella fujikuroi*) and *F. roseum.* Banana rots caused by both pathogens were checked by benomyl at II 100 ppm but Aureofungin was effective only at a high cone. (2000 ppm).

Shillingford (1997) described his findings that the severe fruit rotting occurred when washing water was deliberately contaminated with withered banana blossom colonized by *Fusariuin* spp, *Botryodiplodia theobromae*, and occasionally *Colletotrichum nusae*. A 400 mg/ml dip of thiabendazole alone or following immersion of banana hand in a water suspension of withered banana blossoms did not adequately control wound anthracnose or crown rot. In vitro tests showed that the quaternary ammonium chlorides and formalin were superior to sodium hypochlorite in eradicating conidia of *C. musae*,

Bortyodiplodia theobromae and *F. semitectum*. The in vitro sensitivity of these fungi indicates a possible role for disinfectants other than chlorine in the washing water of Jamaican boxing plants.

Knight *et al.* (1997) studied the crowns of green banana hands treated at harvest with benomyl which were inoculated with *F. semitectum* and *Colletotrichum musae* during ripening, rots developed at the points of inoculation and there was little contamination by other organisms. Control usually remained entirely or fairly free from rotting. The extent of rotting following inoculation with either fungus or without fungus did not differ significantly. *F semitectum* was, therefore, considered to be a primary wound pathogen.

Frossard *et al.* (1996) reported that the emulsifiable concentrate formulation of imazalil (R23979) controlled post-harvest rots but it scorched the fruit skin at concentrations of 200 ppm and above and was therefore not recommended. The sulphate (R 27180) and nitrate (R18531) formulations at 500 and 1000 ppm were compared with 250 ppm benomyl. The results confirmed the efficacy of imazalil, particularly the sulphate form, against crown rots but control of finger rots was slightly less than that of benomyl.

Temkin *et al* (1996) found that the main causal agents of banana tip rots were *Fusarium* spp. *Deightoniella torulosa* in the coastal region, and *Colletotrichum musae* and *Fusarium* spp.in Jordan valley. Spraying with 0.2% benomyl (50%) controlled banana tip rot in both regions if applied 7-14 days after bunch emergence. They also observed that when young banana bunches were sprayed once with 0.2% benomyl (50%) 7-14 days after emerrcncc, fruit rots caused by *Fusarium* spp. and *Deightoniella torulosa* were controlled. Treatment immediately after emergence was not effective against the fungi and the treatment 28 days after emergence gave no control of *Fusarium* spp.

Wallbridge and Pinegar (1995) found that *C. musae, F. dimerum, Gliomastix cerealis, Curvularia senegalensis, Drechslera sacchari, Curvularia*

verrucloisa and *Drechslera* sp. were associated with I13Z treated fruit. The in vitro sensitivity to TBZ and henomyl of all isolates from TBZ treated fruit and some isolates from untreated fruit was determined. Fungi shown to be insensitive to both TBZ and benomyl belonged mainly to the genera *Curvularia* and *Drechslera* and were isolated from TBZ treated fruit except an isolate of *Curvularia pallescens*.

Takano. *et at* (1995) reported that soaking of banana clusters in 1000 ppm of N-benzoyl-L-Leucine after inoculation with *Gloeosporiun musarum (Colletotrichun musae*) was more effective than preinoculation treatment with the fungicide. The effect was due to inhibition of hyphal elongation.

Griffee and Pinegar (1994) used benomyl and TBZ as standards against 10 test fungicides for comparison. In the *in vivo* studies thiophanatemethyl and benzimidazole derivatives (bavistin and DAM 18654) at 250 mg /ml a.i. gave a similar degree of control as benomyl at the same dosage and TBZ at 100 mg / ml. Tridemorph, pimarcin, 1:3 butylene glycol, cufraneb, triforine and experimental fungicides R 70881 and DS 9073 were much less effective. When tested *in vitro*, Bavistin and DAM 18654 were active at similar concentration to the standards, thiophanate methyl and DS 9073 were also active but at slightly higher concs. tridemorph and pimaricin were less active and the others inactive.

Griffee and Burden (1994) discovered machine, designed for the control of the fungal crown rot complex, involved an open circulation system and a small centrifugal pump. Bananas in trays as are passed by gravity along a conveyor onto a cascade of fungicide (benomyl). The effect of depletion of fungicide, particularly by foaming was decreased and the rate of replenishment of benomyl at an initial cone method gave better control than dipping.

According to Shillingford (1900), Benomyl and Thiabendazole were very effective in minimizing decay of banana fruits even at low concentration of 200 to 300 ppm.

Fungicides are a primary means of controlling postharvest diseases of fruits and vegetables (Eckert and Ogawa, 1985).

2.4 Botanical control

Cruz *et al* (2013) reported that citric extract at 4% was the most efficient treatment because the disease incidence was 19.44%, the disease severity was 9.34% and the disease control was 90.16%. Less severity and, consequently, more disease control were achieved by immersing the berries into the emulsion of essential oil of garlic (*A. sativum*) followed by treatments with *C. langsdorfii*, *E. caryophyllata* and *C. zeylanicum.*

Islam and Meah (2011) observed that garlic (Allium cepa L) bulb extract and Allamanda (*Allamanda cathertica* L.) leaf extract were found promising arresting mycelia growth and spore germination of Phomopsis vexans in vitro and in vivo.

Misra and Dexit (1997) reported that the fungitoxicity of bulb of garlic and they showed that garlic could be used as a potent fungicide against plant pathogens in vitro.

2.5 Non-Chemical Control

Amin and Hossain (2012) reported that the best hot water treatment was 54 °C for 8 minutes. Shelf-lives of BARI Kola 1 and Sabri Kola treated with hot water increased by 26 and 27.5%, respectively against untreated fruits. Post-harvest loss (decay and crown rot) of these varieties was reduced, respectively by 95% and 70% against untreated fruits.

Mirshekari *et al* (2012) observed that hot water dip for 10 min at 50°C inhibited conidia germination (100 %) of *C. musae* better than application of fungicide alone (55.92 %).

Costa, and Erabadupitiya (2004) reported that hot water treatment at 50 ◦C for an optimum period of 3 min also achieved significant control of anthracnose and crown rot in banana without loss of fruit quality.

Alam and Meah (2000) found that washing bananas after harvest in water brought a considerable reduction of fruit-anthracnose.

Heat treatments as a beneficial method for control of postharvest problems especially diseases on various horticultural produce have been reported (Afek *et al.*, 1999).

Shillingford (1997) observed that *Fusarium spp.*,*Verticillium theobromae* and *Colletotrichum musae* were the major causes of post-harvest fruit rots of bananas. Inoculums present in the water in wash tank was more important as a source of infection than airborne inocultini. Control could be achieved by washing and deflowering hunches before dehanding, accompanied by other sanitation methods.

Burden (1999) reported that 6 min. immersion in hot (55°C) water before ripening controlled *Colletotrichum musae* in inoculated fruits and destroyed the conidia of the fungus completely.

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted in *in vitro* condition at the Plant Pathology laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 to study the Post-harvest Anthracnose disease of Banana during the period of March to September, 2017. This chapter maps out the methods utilized this work. It describes the key method, use of different chemicals, Botanicals and eco-friendly component for the management of Anthracnose disease of banana.

3.1Experimental Site

The Experiment was conducted at the Plant Pathology laboratory under the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207.

3.2 Experimental period

The experiment was conducted during the time of March to September, 2017.

3.3 Selection of variety

The most popular commercial banana variety ‘Sabri’ was inspected. Disease samples were collected from Kawran bazer wholesale market and different retail shop at Sher-e-Bangla Nagor.

3.4 Isolation, Purification and identification of the causal organisms

3.4.1 Isolation of the pathogens

Banana fruits with characteristic diseased samples were surface sterilized with cotton swab (70% alcohol). Small pieces (2-17. cm) of fruit tissues at the junction of diseased and healthy portion were cut out aseptically. Inocula thus prepared were placed into Potato Dextrose Agar (PDA) plates following Tissue

Plating Method and the plates were placed at room temperature for 7-8 days. Daily observation was made for the fungal growth.

3.4.2 Purification of the Pathogen/organisms

The fungi growing out of inocula on PDA were transferred to fresh PDA plates from where sub-cultures were made for the purification by transferring fungal block with conidia.

3.4.3 Identification of Pathogen/organisms

After purification, the growth characters and morphology of the fungi were studied. The organisms were identified following available literatures (Kulwant *et al.* 1991; Kulshrestha, *et a1*. 1976 ; Frost, 1964).

3.5 Pathogenicity test

The pathogenicity of the isolated fungus *Colletotrichum gloeosporioides* was tested on banana fruit. To prove the Koch's postulate, mature and semi ripen healthy banana 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 banana fruits.

3.6 *In vitro* screening against *C. gloeosporioides* in the laboratory

3.6.1 *In vitro* screening of fungicide against *C. gloeosporioides* in the laboratory

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

Table 1. 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

3.6.2 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.

3.6.3 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 hr. interval until the control plates were filled in (Islam and Akhter, 2001).

3.6.4 *In vitro* screening of Botanicals against *C. gloeosporioides* in the laboratory

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

3.6.5 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.

Table 2. 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

3.6.6 Bioassay following growth inhibition technique

3.6.7 Groove/Cup method

Procedures of this method have been described in 3.6.3.

3.7 Dipping test in fungicides and Botanicals solution

Four fungicides (Dithane M-45, Rovral 50 WP, Tilt 250 EC, Autostin 50WDG) and two botanicals (Garlic and Allamanda extract) were tested for their efficacy against post- harvest diseases of banana 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 bananas were soaked in the fungicide and botanicals solutions. The control treatments concerned bananas dipped in sterile water only. Each treatment was repeated 4 times, with a repetition consisting of 5 bananas. Fruits were thereafter incubated at room temperature in the laboratory. The bananas 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.

3.8 Hot water treatment of banana

Green matured bananas were 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 bananas dipped in sterile normal water. Each treatment was repeated 4 times, with each repetition consisting of 5 bananas.

3.9 Data collection

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

3.10 Data Analyses

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

CHAPTER IV

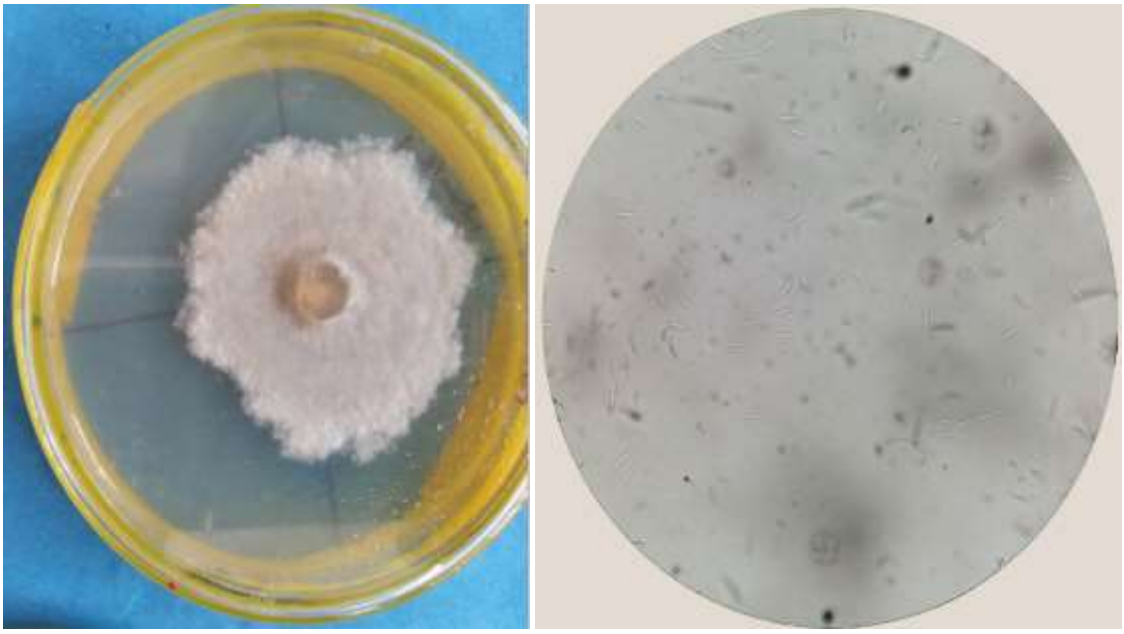
RESULTS

4.1 Isolation and identification of causal organism

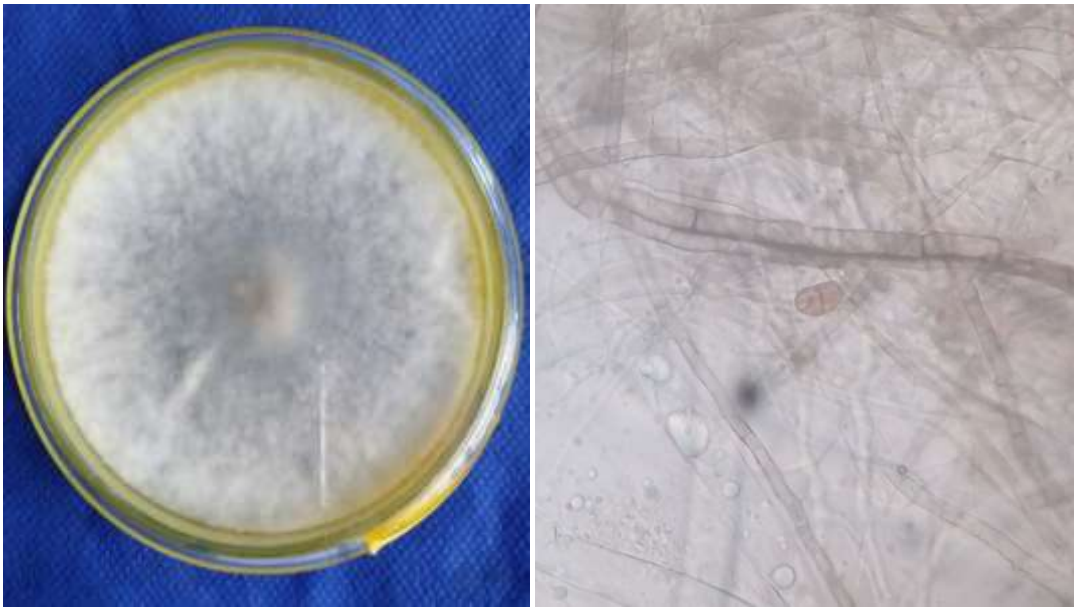
The harvested banana variety ‘Sabri’ collected from local markets of Dhaka city showed typical anthracnose symptoms.

The fungi isolated from the anthracnose was identify as *C. gloeosporioides* (plate 2a). Other associated fungi were *Fusarium sp*, *Rhizopus sp*, and *Aspergillu flavus* with anthracnose disease (plate 3). Small black specks were found to extend to end of banana hands. The lesions were increased in size and later became sunken and coalesced forming spots on the surface with characteristic bright salmon colored dot like masses of conidia (plate 1). The pathogen *C. gloeosporioides* was identified based on their key characteristic (CMI description).

Plate 1: Disease symptoms of anthracnose of banana



2a.Culture and conidia of *Colletotrichum gloeosporioides* (Anthracnose)

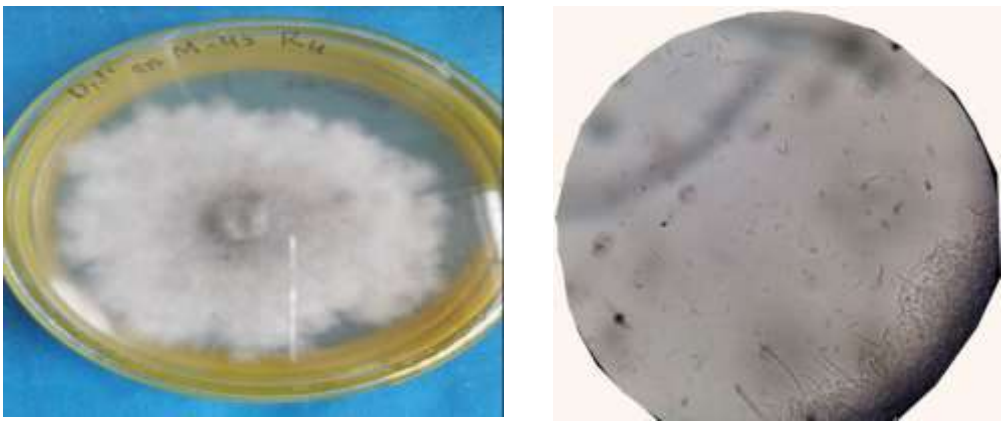


2b. Culture and conidia of *Botryodiplodia theobromae* (Crown rot)

Plate 2: Major Fungi associated with post-harvest Anthracnose of banana

3a. Culture and conidia of *Aspergillus flavus*

3b. Culture and conidia of *Rhizopus sp*



3c. Culture and conidia of *Fusarium sp*

Plate 3: Fungi (Associated as secondary infection) with anthracnose of banana

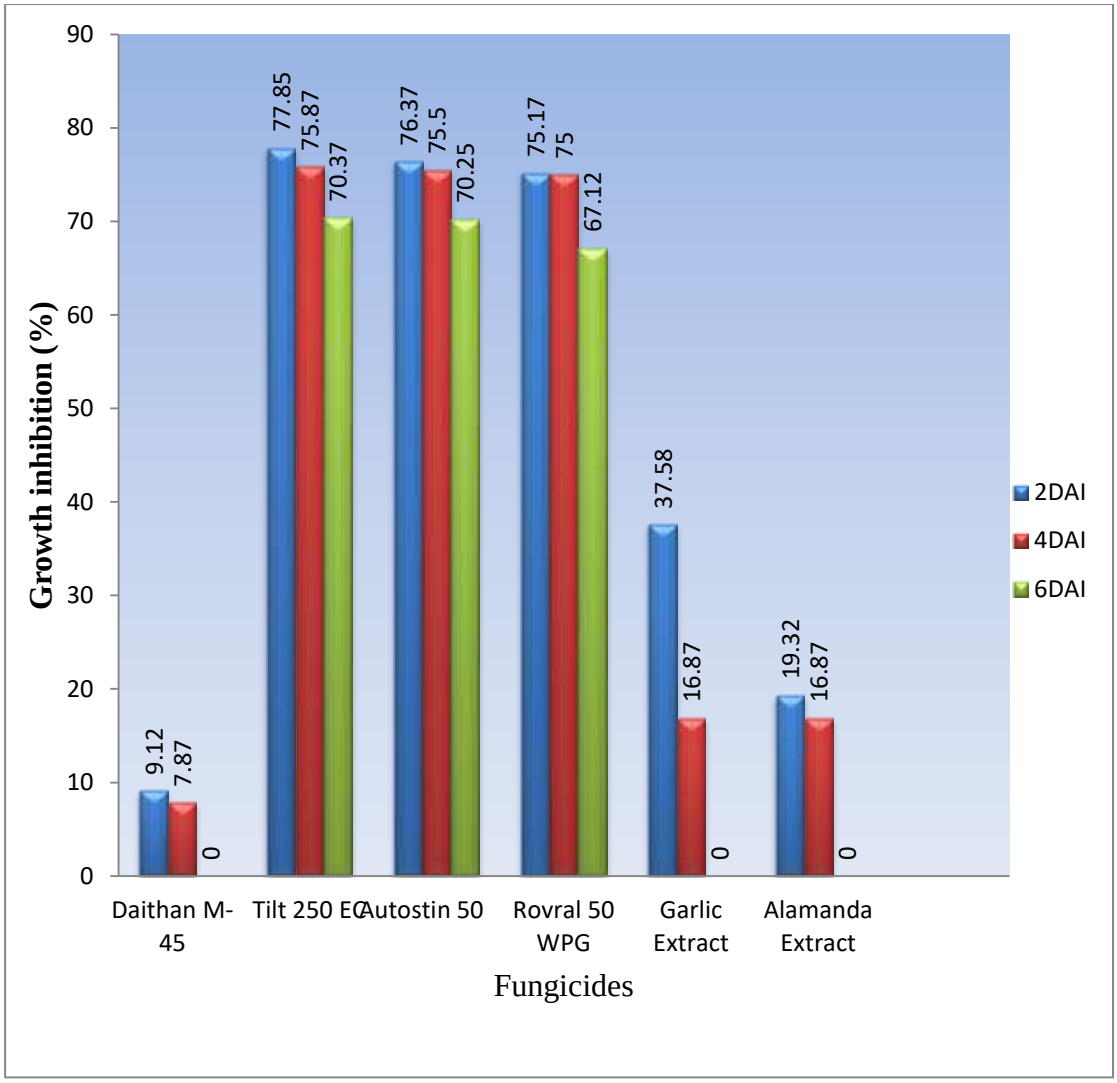
4.2 *In vitro* evaluation of selected fungicides against isolated fungi of banana

Effect of the treatments in controlling post-harvest anthracnose of banana (*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, Alamanda extract along with control.

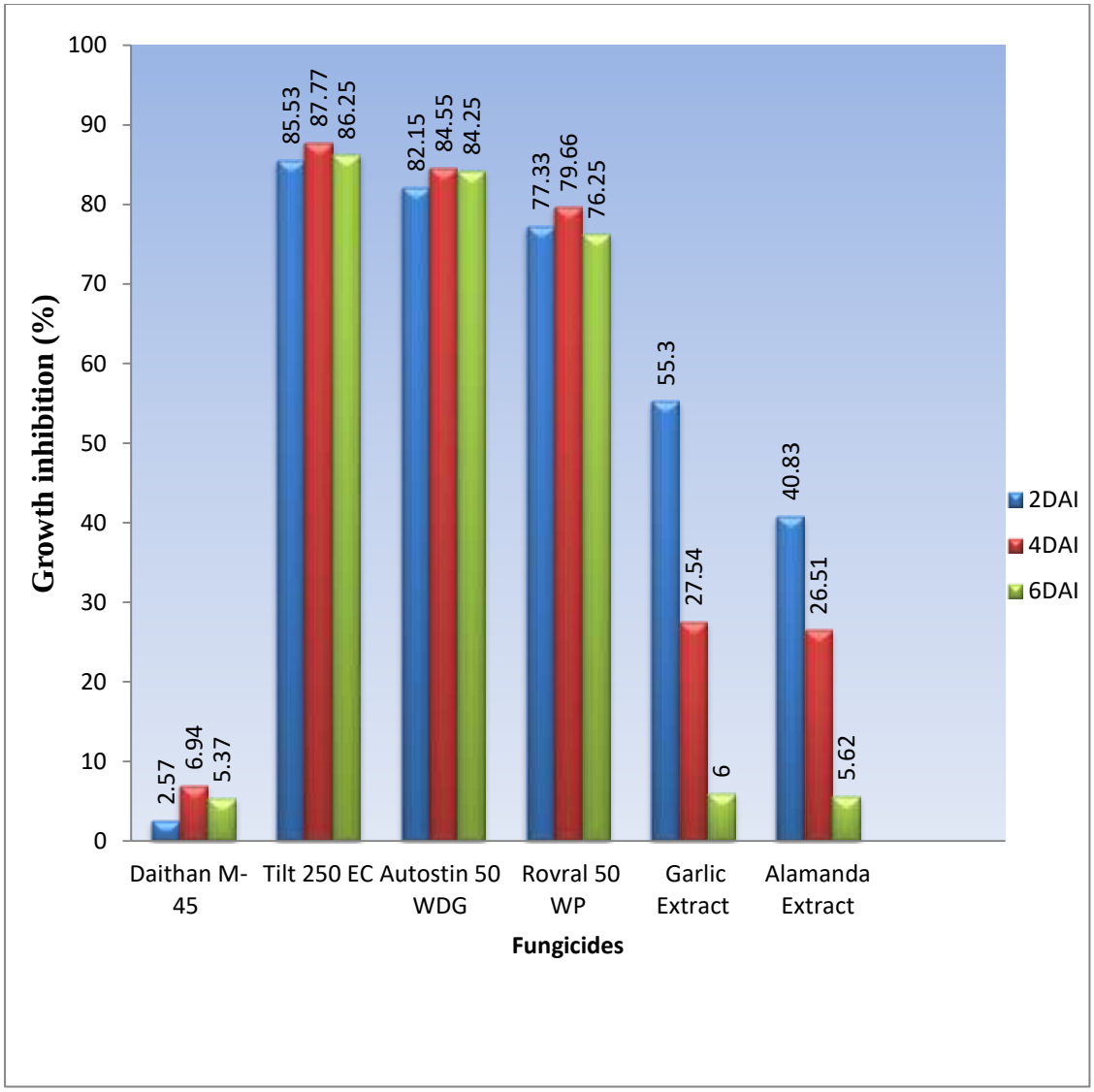
4.2.1. *In vitro* efficacy of different fungicides in different concentration on inhibition of mycelial growth of *C. gloeosporioides* in poison food technique

The efficacy of different fungicides and botanicals in different concentration on radial mycelial growth of pathogen were determined *in vitro* condition and presented in table 3. The efficacy of the test fungicides and botanicals were found to varied significantly on inhibition of mycelial growth of *C. gloeosporioides* in respect of different concentration. In all the cases the fungicides and botanicals showed in greater efficiency in inhibiting of mycelial growth in higher concentration. Among the fungicides, Tilt 250 EC showed the highest performance against the mycelial growth of the pathogen that inhibited 70.37% mycelial growth in 500 ppm and 86.25% mycelial growth in 1000 ppm at 6 days after inoculation. The second height performance against mycelial growth of the pathogen was record in case of Autostin 50 WDG that inhibition the mycelial growth by 70.25%, 84.25% respectively by 500 ppm and 1000 ppm followed by rovral (67.12%, 76.25%) in comparison to control.

The performances of the botanicals were not found satisfactory in inhibition of mycelial growth in comparison to control. In 1:2 concentration the garlic and alamanda extracts completely fail to inhibit the mycelial growth of the fungi. In 1:1 concentration, the mycelial growth inhibition by garlic and alamanda extracts were also insignificant which were 6.00% and 5.62% respectively at 6 days after inoculation.



Graph 1: Effect of different fungicides with 500 ppm conc. on inhibition of mycelial growth of *C. gloeosporioides*



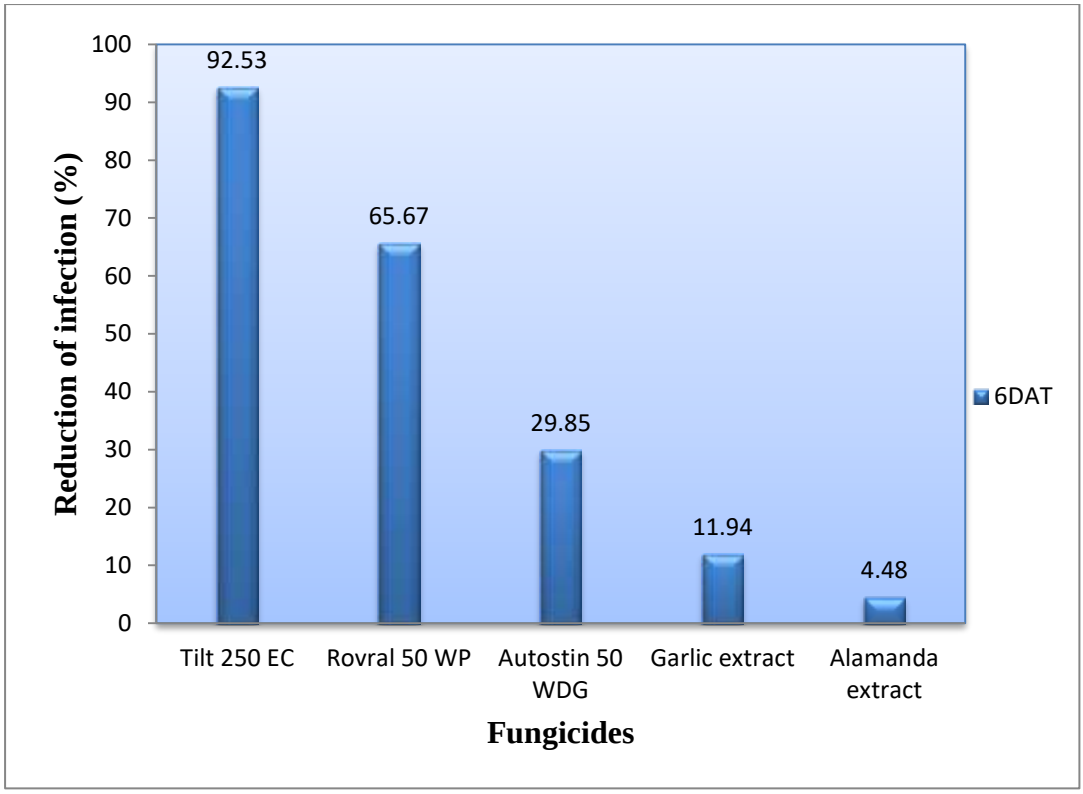
Graph 2. Effect of different fungicides with 1000 ppm conc. on inhibition mycelial growth of *C. gloeosporioides*

Table 3. Effect of different fungicides with different concentration on inhibition of mycelia growth of *C. gloeosporioides*

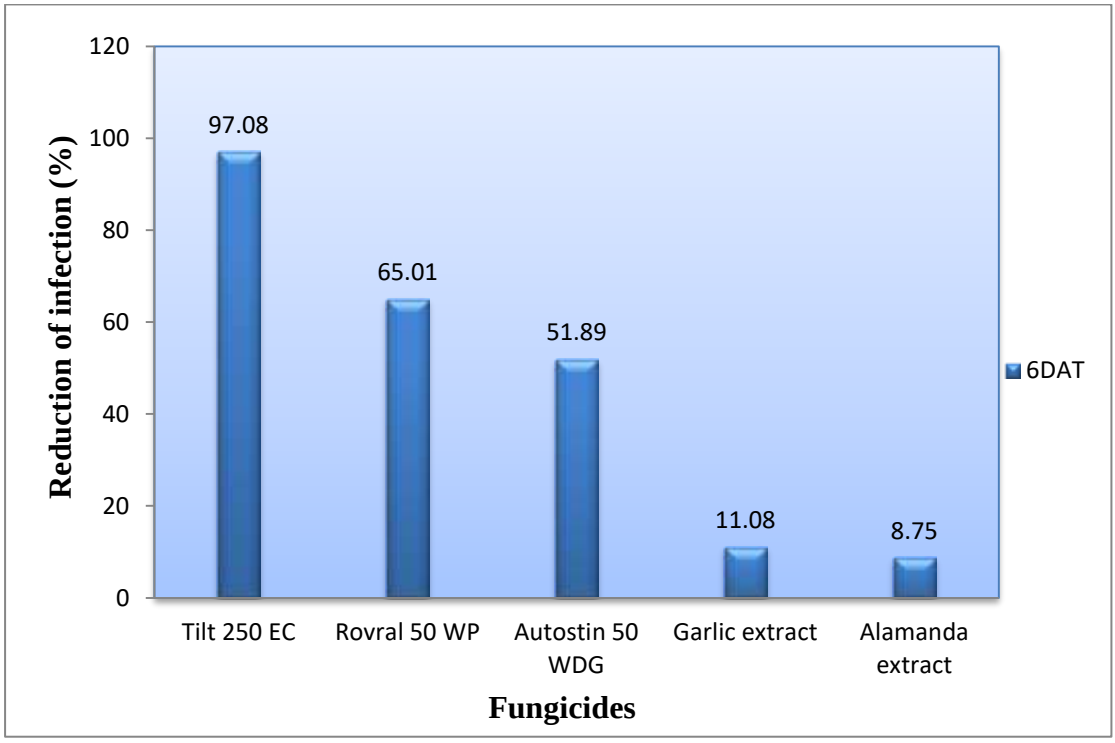
Fungicides	Concentration (ppm)	Radial mycelia growth					
		2DAI		4DAI		6DAI	
		Growth (cm)	Growth inhibition (%)	Growth (cm)	Growth inhibition (%)	Growth (cm)	Growth inhibition (%)
Daithan M-45	500	6.77	9.12	7.37	7.87	8.00	00
	1000	6.02	2.57	7.23	6.94	7.57	5.37
Tilt 250 EC	500	1.65	77.85	1.93	75.87	2.38	70.37
	1000	0.90	85.53	0.95	87.77	1.10	86.25
Autostin 50 WDG	500	1.76	76.37	1.96	75.50	2.42	70.25
	1000	1.11	82.15	1.20	84.55	1.26	84.25
Rovral 50 WP	500	1.85	75.17	2.00	75.00	2.63	67.12
	1000	1.41	77.33	1.58	79.66	1.90	76.25
Garlic Extract	1:2	4.65	37.58	6.65	16.87	8.00	00
	1:1	2.78	55.30	5.63	27.54	7.52	6.00
Alamanda Extract	1:2	6.01	19.32	6.65	16.87	8.00	00
	1:1	3.68	40.83	5.71	26.51	7.55	5.62
Control		7.45		8.00		8.00	
		6.22		7.77		8.00	

4.2.2 Effect of different fungicides and botanicals in controlling postharvest anthracnose disease of banana caused by *C. gloeosporioides*

Harvested banana fruit collected from Kawran Bazer wholesale market were treated with selected fungicides and botanicals and evaluated for their efficacy in controlling anthracnose disease of banana. Most of the fungicides showed promising effect in controlling the anthracnose disease (Table 4). The efficacy of the fungicides increased with the increase of concentration. Among the fungicides, Tilt 250 EC showed the promising performance in controlling post-harvest disease of banana that inhibited 92.53% and 97.08% disease severity respectively in 500 ppm and 1000 ppm concentration. The second highest performance was recorded by Rovral 50 WP that reduced the disease severity by 65.67% and 65.01% at 500 ppm. and 1000 ppm respectively. Among the fungicides, the lowest performance was showed by Autostin 50 WDG that inhibited 29.85% severity at 500 ppm and 51.89% in 1000 ppm concentration. The botanicals did not show any significant performance in controlling the post-harvest anthracnose disease of banana.



Graph 3. **Effect of different fungicides (500 ppm conc.) against anthracnose banana caused by *C. gloeosporioides* at 6 days after treatment**



Graph 4. Effect of different fungicides (1000 ppm conc.) against anthracnose of banana caused by *C. gloeosporioides* at 6 days after treatment

Table 4. Effect of different fungicides with different concentration on reduction of infection area (%) against anthracnose of banana caused by *C. gloeosporioides* at 6 days after treatment

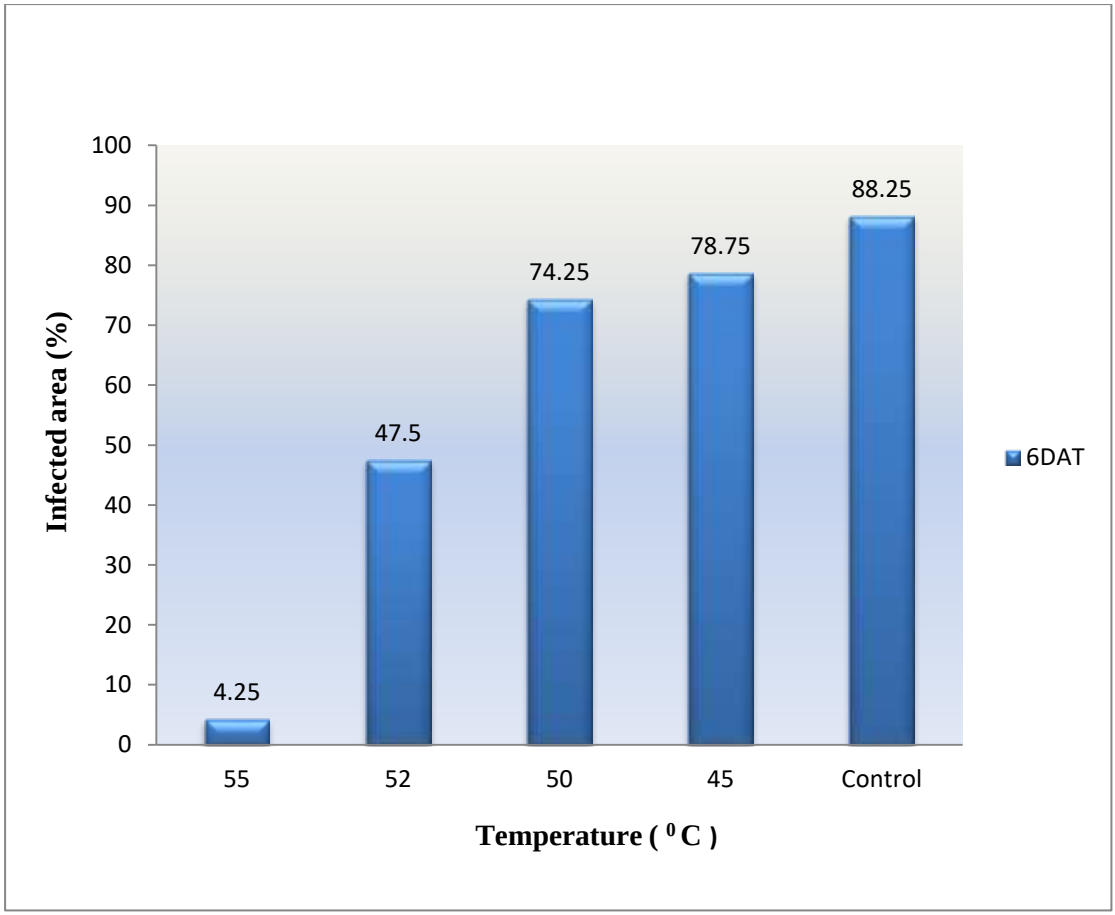
Fungicides	Concentration (ppm)	Infected area (%) at 6 DAT	Reduction of infection (%) over control
Tilt 250 EC	500	6.25	92.53
	1000	2.50	97.08
Rovral 50 WP	500	28.75	65.67
	1000	30.00	65.01
Autostin 50 WDG	500	58.75	29.85
	1000	41.25	51.89
Garlic extract	1:2	73.75	11.94
	1:1	76.25	11.08
Alamanda extract	1:2	80.00	4.48
	1:1	78.25	8.75
Control		83.75	
		85.75	

4.2.3 Effect of hot water treatment on the incidence of anthracnose disease of banana

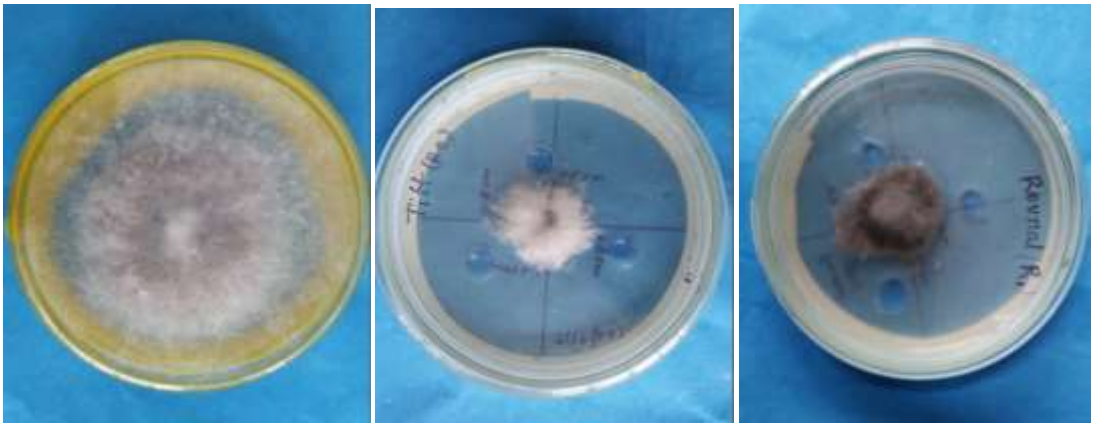
Infected banana fruits treated with different temperature ranged from 45^0 to 55^0C for 5 minutes were subjected to investigation of the effect on the incidence of *C. gloeosporioides* in *in vivo* condition. Hot water treatment of banana had significant effect in controlling anthracnose of banana over control (**Graph 5**). The infected area of banana gradually decreased with the increase of temperature and came to the minimum area of infection (4.25%) at 55^0C. The highest reduction of infected area 95.18% was recorded in case of banana treated with 55^0 C for 5 minutes followed by 52^0 C for 5 minutes (46.18%) and 50^0 C for 5 minutes (15.86 %).

Table 5. Effect of different Hot water treatment against anthracnose of banana caused by *C. gloeosporioides* at 6 days after treatment

SI. NO.	Temperature (^{0}C)	Duration of treatment	Infected area (%)	Reduction of infection (%)
1	55	5	4.25 c	95.18
2	52	5	47.50 b	46.18
3	50	5	74.25 a	15.86
4	45	5	78.75 a	10.76
6	Control	5	88.25 a	
	CV (%)		21.35	
	LSD		19.93	



Graph 5. Effect of hot water treatment of banana at different degree against anthracnose disease caused by *C. gloeosporioides* at 6 days after treatment

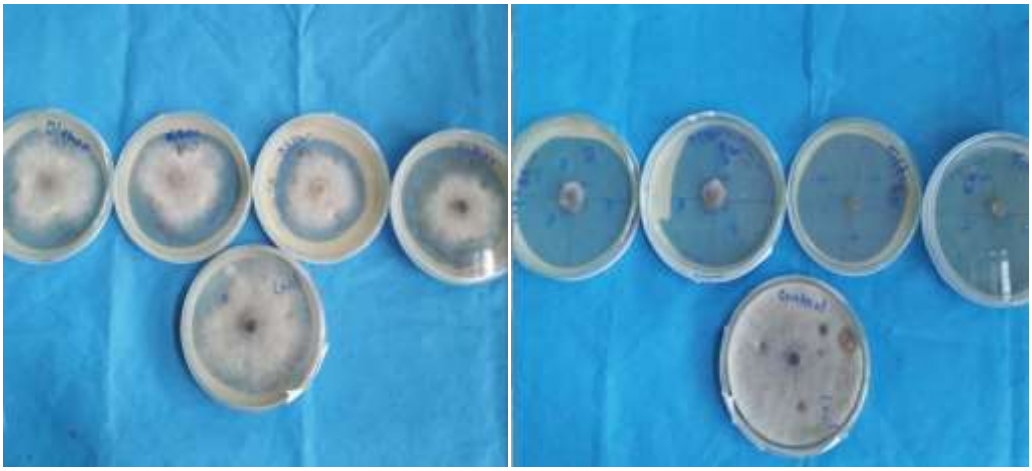


4a. Dithan M-45 4b. Tilt 250 EC 4c. Rovral 50WP

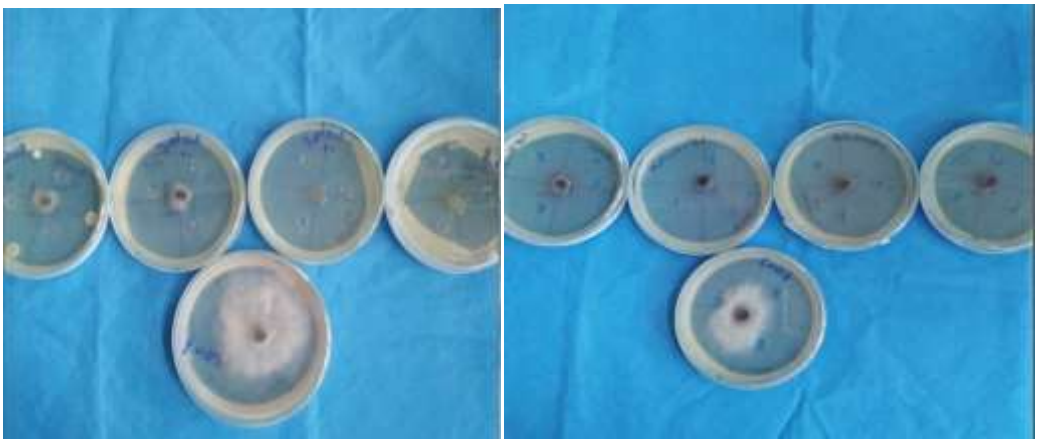
4d. Autostin 50 WDG 4e.Garlic extract (1:2) 4f.Alamanda extract (1:2)

4g. Control

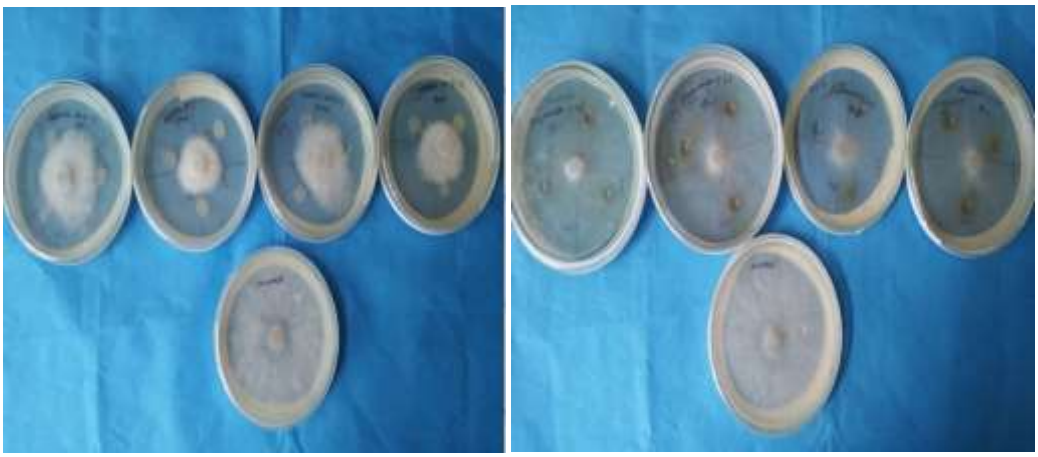
Plate 4. Mycelial growth of *C. gloeosporioides* against different fungicides at 500 ppm concentration at 4 DAT



5a. Dithan M-45 5b. Tilt 250 EC



5c. Rovral 50WP 5d. Autostin 50 WDG



5e. Garlic extract (1:1) 5f. Alamanda extract (1:1)

Plate 5. Mycelial growth of *C. gloeosporioides* against different fungicides at 1000 ppm concentration at 4DAI

6a. Tilt 250 EC

6b. Rovral 50 WP

6c. Autostin 50 WDG

6d. Garlic Extract (1:2)

6e. Control

Plate 6. Effect of different fungicides (500 ppm conc.) against anthracnose of banana caused by *C. gloeosporioides* at 6 days after treatment

7a. Tilt 250 EC

7b. Rovral 50 WP

7c. Autostin 50 WDG

7d. Garlic Extract (1:1)

7e. Control

Plate 7. Effect of different fungicides (1000 ppm conc.) against anthracnose of banana caused by *C. gloeosporioides* at 6 days after treatment

8a. **55^0 C**

8b. **52^0 C**

8c. **50^{0}C**

8d. **45^0 C**

8e. **control**

Plate 8. Effect of different Hot water treatment of banana against anthracnose disease caused by *C. gloeosporioides* at 6 day after treatment

CHAPTER V

DISCUSSION

Effect of different treatments in controlling post-harvest pathogen of banana was evaluated both in *in vitro and in vivo* 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, Alamanda extract along with hot water treatment control.

In *in vitro* condition, the efficacy of fungicides on radial mycelial growth of *C. gloeosporioides* were observed. All the tested fungicides significantly reduced radial mycelial growth of the fungus. The lowest radial mycelia growths were recorded in case of Tilt 250 EC, Autostin 50WPD and Rovral 50 WP both in 500 and 1000 ppm conc. The highest radial mycelia growth was recorded in control preceded by Dithane M-45, Garlic extract and Alamanda extract. Among the fungicides, Tilt 250EC at 1000 ppm conc. gave the highest inhibition of mycelia growth (86.25 %) followed by Autostin 50 WDG (84.25%) and Rovral 50 WP (76.25%) at 6 day after inoculation. From the results, it is observed that the inhibition percentage was increased with the increase of concentration of the fungicidal solutions irrespective of fungicides. These finding are similar to that of Alam and Meah (2000), Nath, K. *et al.* (2015) and Jiang *et al.* (1997) who reported that Tilt 250EC was found to be effective fungicide against *C. gloeosporioides.* However, the botanicals like Garlic and Alamanda extract did not show any remarkable performance in inhibition of mycelia growth of *C. gloeosporioides*. The findings regarding the effectiveness of botanicals are dissimilar with the findings of Misra and Dexit (1997), Cruz, M.E.S *et al* (2013) and Islam and Meah (2011) who reported that the mycelia growth of *C. gloeosporioides*.

In case of *in vivo* evaluation against post harvest anthracnose of banana, the efficacy of fungicides as well as botanicals were also found similar in controlling the disease. All the tested fungicides remarkably reduced the infected area of fruit. The highest reduction of infected area (92.53% and

97.08%) were recorded in case of Tilt 250 EC at the conc. of 500 and 1000 ppm, respectively. However the performance of Tilt 250 EC was far better than that of Rovral 50 WP and Autostin 50 WDG. The lowest performance was noticed in case of Garlic (11.94 and 4.48%) and Alamanda (11.08and 8.75%) at 1:1 and 1:2 concentration of the extracts, respectively. These finding are also similar to that of Alam and Meah (2000), Nath, K. *et al.*(2015) and Jiang *et al.* (1997) who reported that Tilt 250EC was found to be effective against *C. gloeosporioides* in controlling anthracnose both in *in vitro* and *in vivo* condition. Garlic and Alamanda extracts found to be ineffective in controlling the disease.

In case of hot water treatment, temperature ranged from 45^0 to 55^0C for 5 minutes were evaluated to find out the effect of hot water treatment in reducing the severity of anthracnose of banana caused by *C. gloeosporioides*. Hot water treatment reduced the severity of the disease promisingly. However, the effect of hot water treatment varied with the variation of temperature of water. The highest reduction of severity (95.18%) and the lowest fruit area diseased (4.25%) was found in case of 55^0C temperature treated for 5 minute. The highest fruit area diseased (78.75 %) was recorded in case of 45^0C temperature treated for 5 minute. The performance of the treatment increased with the increase of the temperature. The present findings are similar to that of Burden (1999) and Amin and Hossain (2012). However, these results are dissimilar to the report of Costa and Erabadupitiya (2004) who reported that hot water treatment at 50^0C temperature treated for 3 min reduce the anthracnose disease of banana.

CHAPTER VI

SUMMERY AND CONCLUSION

The experiment was conducted in Plant Pathology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka. The investigation was conducted to evaluate the efficacy of different treatments for the management of post harvest anthracnose disease of banana of Sabri variety.

The most frequently isolated fungi for the disease of anthracnose was *C. gloeosporioides.* Other associated fungi were *Fusarium sp*, *Rhizopus sp*, *Aspergillus* sp. The pathogen *C. gloeosporioides* was identified based on its key characteristics.

In *in vitro* condition, 4 fungicides with 500 ppm and 1000 ppm concentration and 2 botanicals with 1:1 and 1:2 concentration and hot water treatments with different temperature and time range were assayed against the pathogen of anthracnose of banana. Tilt 250 EC reduced the height radial mycelial growth (86.25%) while Autostin 50 WDG and Rovral 50 WP reduced 84.85% and 76.25% mycelial growth respectively with 1000 ppm concentration. Botanicals with 1:1 percentage concentration showed some effect but not at all remarkable.

Banana fruits treated with five fungicides at 500 ppm and 1000 ppm concentration and botanicals with 1:1 and 1:2 concentrations and also with hot water ranged from 45^0 to 55^0 C at 5 minutes. The lowest infected area (6.25%) and the highest reduction of fruit area infected (97.08%) was recorded by Telt 250EC at 1000 ppm concentration over control followed by Autostin 50 WDG (51.89%) and Rovral 50 WP (65.01%). The performance of botanicals were not found satisfactory.

Hot water treatment of banana (55^0C for 5 minutes) reduced the percent fruit area diseased by 95.18%. The infection of surface area gradually decreased with the increase of water temperature. The lowest fruit area infection (4.25%) was found in 55^0C for 5 minute while the highest fruit area infection 88.25% in control.

From the findings it may be concluded that the chemical fungicide Tilt 250 EC (1000 ppm) and hot water treatment (55^0C for 5 minute) have a significant impact on reduction of post-harvest anthracnose disease of banana. However, the commercial farmers are suggested to use hot water at 55^0 C for 5 minutes as eco-friendly treatment that have no health hazard.

Further experiment need to conduct following more eco-friendly components for the management of post-harvest anthracnose as well as crown rot (*Botryodiplodia theobromae)* of banana.

CHAPTER VII

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APPENDICES

Appendix I: Effect of different fungicides with 500 ppm conc. on inhibition of mycelia growth of *C. gloeosporioides*

Sl. No.	Fungicides	Radial mycelia growth					
		2DAI		4DAI		6DAI	
		Growth (cm)	Growth inhibition (%)	Growth (cm)	Growth inhibition (%)	Growth (cm)	Growth inhibition (%)
1	Daithan M-45	6.77 ab	9.12	7.37 ab	7.87	8.00 a	00
2	Tilt 250 EC	1.65 d	77.85	1.93 c	75.87	2.38 b	70.37
3	Autostin 50	1.76 d	76.37	1.96 c	75.50	2.42 b	70.25
4	Rovral 50 WPG	1.85 d	75.17	2.00 c	75.00	2.63 b	67.12
5	Garlic Extract	4.65 c	37.58	6.65 b	16.87	8.00 a	00
6	Alamanda Extract	6.01 bc	19.32	6.65 b	16.87	8.00 a	00
7	Control	7.45 a		8.00 a		8.00 a	
CV (%)		1.52		13.89		7.50	
$LSD_{(0.05)}$		1.377		1.02		0.628	

Appendix II. Effect of different fungicides with 1000 ppm conc. on inhibition of mycelia growth of *C. gloeosporioides*

SI. No.	Fungicides	Radial mycelia growth					
		2DAI		4DAI		6DAI	
		Growth (cm)	Growth Inhibition (%)	Growth (cm)	Growth inhibition (%)	Growth (cm)	Growth inhibition (%)
1	Daithan M-45	6.02 a	2.57	7.23 a	6.94	7.57 a	5.37
2	Tilt 250 EC	0.90 d	85.53	0.95 c	87.77	1.10 b	86.25
3	Autostin 50 WDG	1.11 cd	82.15	1.20 c	84.55	1.26 b	84.25
4	Rovral 50 WP	1.41 cd	77.33	1.58 c	79.66	1.90 b	76.25
5	Garlic Extract	2.78 bc	55.30	5.63 b	27.54	7.52 a	6.00
6	Alamanda Extract	3.68 b	40.83	5.71 b	26.51	7.55 a	5.62
7	Control	6.22 a		7.77 a		8.00 a	
	CV (%)	37.44		17.77		13.38	
	$LSD_{(0.05)}$	1.763		1.135		0.991	

Appendix III. Effect of different fungicides against anthracnose of banana caused by *C. gloeosporioides* at 6 days after treatment

SI. NO.	Fungicides	Concentration (ppm)	Infected area (%)	Reduction of infection (%)
1	Tilt 250 EC	500	6.25 d	92.53
2	Rovral 50 WP	500	28.75 c	65.67
3	Autostin 50 WDG	500	58.75 b	29.85
4	Garlic extract	1:2	73.75 ab	11.94
5	Alamanda extract	1:2	80.00 a	4.48
6	Control		83.75 a	
	CV (%)		19.06	
	$LSD_{(0.05)}$		15.88	

Appendix IV. **Effect of different fungicides against anthracnose of banana caused by *C. gloeosporioides* at 6 days after treatment**

SI. NO.	Fungicides	Concentration (ppm)	Infected area (%)	Reduction of infection (%)
1	Tilt 250 EC	1000	2.50 c	97.08
2	Rovral 50 WP	1000	30.00 b	65.01
3	Autostin 50 WDG	1000	41.25 b	51.89
4	Garlic extract	1:1	76.25 a	11.08
5	Alamanda extract	1:1	78.25 a	8.75
6	Control		85.75 a	
	CV (%)		25.05	
	$LSD_{(0.05)}$		19.63	
	Lebel of significance			

Appendix V. Effect of different Hot water treatment of banana against anthracnose of banana caused by *C. gloeosporioides* at 6 days after treatment

SI. NO.	Temperature (^{0}C)	Duration of treatment	Infected area (%)	Reduction of infection (%)
1	55	5	4.25 c	95.18
2	52	5	47.50 b	46.18
3	50	5	74.25 a	15.86
4	45	5	78.75 a	10.76
6	Control	5	88.25 a	
	CV (%)		21.35	
	LSD		19.93	