

INCIDENCE AND SEVERITY OF ANTHRACNOSE (*Colletotrichum musae* Berk. & Curt. V. Arx.) AND EVALUATION OF POSTHARVEST HANDLING FACTORS AFFECTING DISEASE DEVELOPMENT ON MARKETED BANANA FRUITS IN ADDIS ABABA, ETHIOPIA

M.Sc. THESIS

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JANUARY, 2013

JIMMA, UNIVERSITY

**Incidence and Severity of Anthracnose (*Colletotrichum musae* Berk. & Curt.
V. Arx.) and Evaluation of Postharvest Handling Factors affecting Disease
Development on Marketed Banana Fruits in Addis Ababa, Ethiopia**

M.Sc. Thesis

**Submitted to the School of Graduate Studies
Jimma University College of Agriculture and Veterinary Medicine
In Partial Fulfilment of the Requirements for the Degree of Master of
Science in
Plant Pathology**

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JANUARY, 2013

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APPROVAL SHEET
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As thesis research advisors, we hereby certify that we have read and evaluated this thesis prepared, under our guidance, by Eyob Aysanew, entitled “**Incidence and Severity of Anthracnose (*Colletotrichum musae* Berk. & Curt. V. Arx.) and Evaluation of Postharvest Handling Factors Affecting Disease Development on Marketed Banana Fruits in Addis Ababa, Ethiopia.**” We recommend that it be submitted as fulfilling thesis requirement.

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DEDICATION

I dedicate this thesis to my beloved family.

STATEMENT OF AUTHOR

I declare that this is my original work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfilment of the requirements for M.Sc. degree at Jimma University College of Agriculture and Veterinary Medicine and is deposited at the library of Jimma University being accessible to borrowers under the rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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BIOGRAPHICAL SKETCH

The author was born on May 05, 1986 in Addis Ababa. He attended elementary and high school education from September 1992 - 2005 at Lideta Catholic Cathedral School and Miskaye Hizunan Medhanialem Monastery School. Then he joined and attended Department of Dry Land Crop and Horticulture Science at Mekelle University in September, 2005 and earned his B.Sc. degree in June, 2008. Soon after in October, 2008 he employed by Addis Ababa City Administration and worked for nine months at Department of Beauty and Park Development after that he joined Jimma University School of Graduate Studies to pursue his M.Sc. study in Plant Pathology in March 2010.

ACKNOWLEDGEMENTS

It would not have been possible to write this thesis without blessing of God, help and support of the kind people around me, to only some of whom it is possible to give particular mention here.

First of all I would like to thank my parents and younger brothers for given me their endless love, prayers and unequivocal encouragement throughout, as always, for which my mere expression of thanks likewise does not sufficient.

This thesis would not have been possible without the help, support and patience of my principal supervisor, Dr. Sethu Madhava Rao., not to mention his knowledge regarding this topic, support and good friendship advice. I would also like to express my deepest gratitude to my co-supervisor, Dr. Girma Adugna for his invaluable help of constructive comments and suggestions.

I would like to thank Dr. Eshetu Derso for his kindness and support during my stay in Plant Pathology Laboratory at Debrezeit Agrcultural Research Center. I would also like to thank Amsalu Nebiyu who helped me on statistical part of this thesis.

I would like to express my appreciation to the management of ET-fruit enterprise, administration office of Channo Dorga, Kibre fruit and vegetable sealers union and many other organizations who have not been mentioned here for their support and help towards my postgraduate affairs.

Last but not least, sincere thanks to all my friends for their kindness and moral support during my study. Thanks for the friendship and memories.

ABBREVIATIONS

| | |
|---------|---|
| ACPC | African, Caribbean and Pacific countries |
| AI | Anthraxnose incidence |
| ANOVA | Analysis of Variance |
| ASI | Anthraxnose severity index |
| B | Black |
| BLSD | Black leaf streak disease |
| BSI | Bruise severity index |
| BW | Blackish white |
| CHPHMPs | Customary Harvest and Postharvest Management Practices |
| CPHMPs | Customary Postharvest Management Practices |
| CRD | Complete Random Design |
| CRD | Complete Random Design |
| CSA | Central Statistical Authority |
| DO | Dark orange |
| FAO | Food and Agriculture Organization of the United Nations |
| FAOSTAT | Food and Agriculture Organization Statistics |
| FMG | Flat mycelium growth |
| I | Irregular |
| JUCAVM | Jimma University College of Agriculture and Veterinary Medicine |
| LO | Light orange |
| MoARD | Ministry of Agriculture and Ruler Development |
| NaOCl | Sodium Hypochlorite |
| NMSA | National Metrological Service Agency |
| PB | Pinkish black |
| PCFs | Physical condition of the fruits |
| PDA | Potato Dextrose Agar |
| PDI | Percent of disease index |
| RFMG | Raised fluffy mycelium growth |
| RI | Ripening index |
| RWS | Regular without sector |
| S | Smooth |
| SAS | Statistical Analysis System |
| SE | Standard error |
| SNNPR | Southern Nations, Nationalities and Peoples Region |
| SPSS | Statistical Package for Social Science |
| USD | United States Dollar |
| WP | White pinkish |

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ABSTRACT

*Anthracnose caused by Colletotrichum musae (Berk. & Curt. V.) Arx is one of the most important postharvest diseases of marketed banana fruits at the global level and often the disease is mentioned in relation to customary harvest and postharvest management practices (CHPHMPs) by growers and traders. However, in Ethiopia the status of the disease, characteristics of the pathogen, the reaction of detached banana genotypes against the pathogen and the effects of CHPHMPs on the disease are not well studied. Therefore, the current study was done with the objectives of determining the incidence and severity of anthracnose starting from farm up to retailer shops, surveying the CHPHMPs affecting the disease, characterizing Colletotrichum musae isolates and to determine the reactions of some banana genotypes against the disease. Survey and sampling of the study was conducted in farmers' field around Arba-Minch and major fruit markets in Addis Ababa and laboratorial works were conducted at Plant Pathology Laboratory of JUCAVM. Data on CHPHMPs of the farmers and traders were collected through single visit interviews using questionnaire format. Disease incidence was determined on the bases of totality of healthy and diseased fruits and severity was visually estimated and calculated by giving the scale from 0 to 4. Survey study revealed 9.8% and 4.1% incidence and severity of anthracnose, respectively at farmers' field. After transportation of the fruits to Addis anthracnose incidence was 12.2% with 3% of severity on assessed banana fingers. Disease incidence and severity were recorded as 30.4 and 2.6% at ripening rooms, 70.8 and 31.6% at retailer, 51.3 and 20.7% at ET-fruit shops, respectively. The CHPHMPs both by farmers and merchants were almost comparable however, some variations were observed. Among surveyed CHPHMPs variety, harvesting methods, fruit sources, the distance travelled, fruit covers during transportation, cleaning/grading, container type and from physical condition of the fruits (PCF) bruise severity on fruits surface and ripening level in general were factors significantly ($p < 0.05$) affected the disease at different phases of the study. The six isolates *C. musae* were also showed significant variation in some of their cultural and morphological characteristics on PDA and blackish white colony color, black substrate color, flat mycelium growth, regular colony shape with smooth colony margin were typical and dominant cultural characteristics of most of the isolates. In addition to these, some banana varieties were significantly ($p < 0.05$) different in their susceptibility to wound and quiescent anthracnose based on their rotted surface area among which William I, was found to be susceptible with rotted surface area of 3522.2 and 2521.9 mm² whereas butaza was the least susceptible for wound and quiescent anthracnose, respectively. Generally the study found anthracnose as an important postharvest disease of banana fruit causing qualitative and quantitative loss in many of the surveyed shops in Addis Ababa and the disease was found to be affected by CHPHMPs. Therefore, it is better to conduct further studies regarding the disease in relation to management practices and the loss need to be quantified. In addition to this it is important to study reactions of more number of commercial bananas to quiescent and wound anthracnose.*

Key words: Banana, Anthracnose, Incidence, Severity, CHPHMPs

1. INTRODUCTION

Bananas (*Musa* spp.) are one of the world's most important and yet inadequately studied crop. It is the fourth most important food crop in terms of gross value of production. Total world production of *Musa* is estimated at around 105 to 120 Mt per year (FAO, 2010), of which approximately one third is produced in sub-Saharan Africa. Total value of international trade was estimated around 7 billion USD per year, of which 83% of the export comes from Latin America and ACP countries accounts less than 10% of the world exports (FAO, 2011). There are two main groups: the sweetest banana or “fruit”, essentially the Cavendish varieties, which represents about 60 to 65 Mt, and cooking banana-including “plantain” in Africa or “pisang awak” in Asia consumed more as a vegetable, which represents some 40 to 50 Mt (FAO, 2009).

The fruit is widely grown in many developing countries around the world and is mainly distributed between 30^o North and 50^o South latitude (Morton, 1987) and is the most important food product within the least developed countries, being the staple food for some 400 million people. In Africa, the crop is particularly important in the humid forest and mid-altitude regions where it provides more than 25% of food energy requirements for around 70 million people. It is an easily produced source of energy and is rich in a number of important vitamins and minerals. In addition to being a staple food crop for rural and urban consumers, banana is also an important source of rural income. The crop is also environment friendly, combating soil erosion on hilly slopes, and readily lends itself to intercropping and mixed farming in most of growing areas of Africa (Frison *et al.*, 1998).

In Ethiopia banana is one of the most important horticultural crops grown at various agro ecological zones by small holders, private commercial farm and state owned large-scale plantations. It is produced by many small holders in permanent farming system which is common in most of east African countries where it often grown in association with tree crops such as coffee to supplement household income from their main crops. The very diverse agro-ecologies of the country are conducive to grow various tropical, subtropical and temperate fruits. According to the Ministry of Agriculture and Rural Development (MoARD, 2005),

there were about 3 million farmers involved in fruit production with a total area of about 43,500 ha and producing about 261,000 tons annually. From these much of cultivated land, bananas constituted the lion's share with about 60.6% of the fruit crop area followed by mangoes that contributed 12.6% of the area. A total of nearly 3.5 million quintals of fruits was produced in the country with bananas, papaya, mangoes and orange took up 55.32%, 12.53%, 12.78% and 8.35% of the fruit production, respectively (CSA, 2008).

Although the number of farmers seems high the area under cultivation is very small and mainly smallholder based. Each farmer grows very few trees of unimproved varieties/ cultivars which are poorly managed and mainly for domestic consumption. In addition to these, there has been considerable increase in pest and diseases pressures. As a result a well managed banana garden in east Africa, previously expected by the farmers to last up to fifty years now being to deteriorate after only four years, as is the case of parts of Uganda (Frison *et al.*, 1998). Considerable losses are being caused by black leaf streak disease (BLSD); black Sigatoka leaf spot caused by *Mycosphaerella fijiensis*, *Fusarium* wilt caused by *Fusarium oxysporum* spp. *cubense* and *Xanthomonas* wilt caused by *Xanthomonas vasicola* pv. *musacearum*. Among these diseases *Xanthomonas* wilt has been mentioned as endemic to Ethiopia infecting *Enset* and banana for over 50 years and was first reported and described in late 1960's (Mugenzi *et al.*, 2006; Ndungo *et al.*, 2006). Many of the commercial and smallholder fruit orchards in Ethiopia had been reported declining chiefly due to disease problems (Mohamed, 2002; Seifu, 2004).

In addition, to these constraints the post-harvest handling/ management practices are weak and have been causing significant loss of the produced fruits (Abreham, 2009). In Ethiopia it has been reported to present considerable postharvest losses of perishable fruits due to a complex of post-harvest fungal and bacterial pathogens, which seriously affects both the local and export markets (Abreham, 2009). Conservative estimates places losses of perishable commodities at 50% in under developing countries like Ethiopia and other tropical countries (Jeffries and Jeger, 1990).

Because fruits are highly perishable and susceptible to loss in quality or quantity more complex post harvest management practices in the marketing systems are required. However

the present situation in marketing system of the fruits is subjected to various limitations. Post harvest management practices such as harvesting, transportation and storage which are very important and requires attentions are easily seen by producers and merchants. Most of peasants have no adequate access to supply their fresh fruit products to the urban areas and also have no storage facilities at their localities and the products they harvest are usually exposed to the influence of diseases which are capable of attacking the fruits during some post harvest physiological changes until they are collected by the end users.

Among the postharvest diseases which cause significant loss of marketed banana fruits, anthracnose caused by *Colletotrichum musae* (Berk. & Curt. V.) Arx., is the major at the global level (Thangamani *et al.*, 2011). The disease had been noticed deteriorating the quality and nutritive value of the fruits and render them unfit for marketing and consumption, thereby causing severe loss to farmers and traders (Jeffries and Jeger, 1990). It was mentioned as the most important pathogen on wounded green and ripe banana fruits. On green fruits the fungus occasionally invades the necks of the fingers when damaged by flexing and lesions were described as sunken and covered with salmon coloured acervuli (Stover *et al.*, 1987; Anthony *et al.*, 2004) whereas, on ripening fruits the symptom is sunken brown spots developing with orange acervuli. Infection caused by the pathogen also mentioned to stimulate ripening of fruits and lesions were elongate with ripening (Meredith *et al.*, 1971). The disease was reported by Haque *et al.* (2003) in 5% and 49.9% of incidence and severity, respectively on marketed banana fruits. In addition to this Alvindia *et al.* (2000) and Hossain *et al.* (2010) reported 83% and 13% of anthracnose incidences on banana fruits at commercial ripening rooms and retailer shops, respectively.

Inflorescence tissues, flower bracts, leaves and fields with dead banana leaf were mentioned as significant sources of *Colletotrichum musae* inoculums (Fitzell and Peak, 1984; Dodd *et al.*, 1991) however, these and other factors such as field production practices and the general hygiene in storage facilities and pack (ripening)-house are not adequately developed and are being major constraints to improve production in quantity and quality for both local and export markets (Mohammed *et al.*, 2009).

Currently the government has put renewed emphasis on horticulture development. As a result, some large-scale commercial horticulture joint ventures with foreign companies have been established mostly to address the export market (EHDA, 2011). Therefore regarding the present constraints of the small scale banana producers and the promising expansion of fruit production in the country it is important to give attention and to study the status of incidence and severity of anthracnose beginning from the farm through the chain of commercialization and it is also important to determine the effect of customary pre and postharvest management practices by farmers and traders fruits on the disease. Thereby it is possible to mitigate the disease and resultant qualitative and quantitative losses between producers and merchants through demonstrating better management practices for the farmers/ traders to improve production and their management practices.

Objectives of the study

General objective

- To determine the incidence and severity of anthracnose caused by *Colletotrichum musae* Berk. & Curt. V. Arx., and assess harvest and postharvest factors affecting the disease development and quality loss on marketed banana fruits in Addis Ababa

Specific objectives

- To determine the incidence and severity of anthracnose on banana fruits at major fruit and vegetable markets of Addis Ababa.
- To assess harvest and post-harvest management practices customarily performed by growers and merchants and determine their effect on disease (anthracnose) development.
- To isolate and characterize the pathogen *Colletotrichum musae* Berk. & Curt. V. Arx.
- To evaluate the reactions of some banana genotypes to the disease (anthracnose) using fruit test method.

2. LITERATURE REVIEW

2.1. Banana Production and Importance

Banana (*Musa*. spp) is monocotyledonous plant belonging to the family of *Musaceae* of Zingiberales order. It is widely grown in many countries around the world and has traditionally been a cash crop for banana companies in Central America, Northern South America, and the islands of the Caribbean however, today it is grown in 123 countries of the world. It is considered as the prime fruit commodity for several developing countries. The FAO (2010) statistics denotes that total world production was estimated from around 105 to 120 Mt per year, of which India being the major producer followed by China, Philippines, Brazil and Ecuador ranking from 1 to 5 however, less than 20% is exported, with 15% are exported to the United States, Europe and Japan.

Table 1. Worlds' top ten banana producers (FAO, 2010)

| Rank | Country | Production (tons) | Amount (\$) |
|------|-------------|-------------------|-------------|
| 1 | India | 3736184 | 26217000 |
| 2 | China | 1146165 | 8042702 |
| 3 | Philippines | 1114265 | 8687624 |
| 4 | Brazil | 997306 | 6998150 |
| 5 | Ecuador | 954980 | 6701146 |
| 6 | Indonesia | 818200 | 5741352 |
| 7 | Uganda | 498785 | 3500000 |
| 8 | Mexico | 307718 | 2159280 |
| 9 | Costa Rica | 295993 | 2127000 |
| 10 | Colombia | 283253 | 1987603 |

In Africa different banana types and cultivars are grown in diverse eco-regions. East African bananas are mainly produced in the East African highlands around homesteads and in permanent fields. They are cultivated primarily for their fruit and used for food, brew and household incomes. In a number of other countries and in the East African Great Lakes

region, banana is the most important staple food crop (FAO, 2004). Approximately one-third of the bananas produced globally are grown in Sub-Saharan Africa, where the crop provides more than 25% of food energy requirements for more than 100 million people, Uganda being the leading producer and consumer (Tripathi *et al.*, 2009).

In Ethiopia, banana is major fruit crop accounting 51.01 % of the total 4,089,115 quintals annual fruit production (CSA, 2010). The main banana growing areas are located at Arba-Minch, 1, 200 masl in Southern Ethiopia, and in South Western Ethiopia along the Kaffa-Bench Maji axis (1,050 - 1,700 masl) (Addis *et al.*, 2004). Throughout the main *Enset* growing areas, and at below 2,100 masl, a few banana plants can be found on most *Enset* farms. There are an estimated four major and widely growing commercial varieties of banana fruits; Payo, Dwarf Cavendish, Giant Cavendish and Ducasse (Kenya) (Derbew, 2000). Currently various cultivars of plantain are being evaluated by the Ethiopian Agricultural Research Organization (at Melkassa and Jimma Centres) for their adaptation to different agro ecological zones of the country.

2.2. Postharvest Diseases of Perishable Fruits

Many of fungal pathogens which cause postharvest diseases belong to the phylum Ascomycota and these associated Fungi anamorphic (Fungi Imperfecti). In the case of the Ascomycota, the asexual stage of fungus (the anamorph) is usually encountered more frequently in postharvest diseases than the sexual stage of the fungus (the teleomorph). Important genera of anamorphic postharvest pathogens include species of *Penicillium*, *Aspergillus*, *Geotrichum*, *Botrytis*, *Fusarium*, *Alternaria*, *Colletotrichum*, *Dothiorella*, *Lasiodiplodia* and *Phomopsis* (Nelson, 2008).

From the above mentioned postharvest fungal pathogens *Colletotrichum* spp. which is our intent to deal with, had been reported to cause significant post harvest rots and the consequent loss of marketed banana fruit in many countries. The pathogen was responsible to cause a range of 2.04 to 5.1% incidence of anthracnose rot with a range of 24.7 to 50.1% severity on marketed banana fruits (Haque *et al.*, 2003). The disease was also observed in a range of 11 to 13% of rot incidence (Hossain *et al.*, 2010), in addition to this Alvindia *et al.* (2000) found

incidences of 86% crown rot, 83% anthracnose, 56% finger stalk rot, 11% finger rot and 3% finger end rot in non-chemical bananas imported into Japan from the Philippines (Self, 2002).

2.3. Etiology (*Colletotrichum* spp.)

Colletotrichum species cause anthracnose, which can lead to considerable damage in a large number of crops such as cereals, coffee and legumes (Prusky and Plumbly, 1992) and also been mentioned to cause even greater economic losses as a postharvest disease on tropical and subtropical fruit such as avocado, banana and mango (Jeffries and Jeger, 1990). The fungus had been reported to be found on decayed wild fruits (Photita *et al.*, 2001b) and also commonly isolated as endophytes from healthy plants, and identified as saprobes on dead plant material (Photita *et al.*, 2001a, 2004, 2005; Promputtha *et al.*, 2002). Endophytic, saprobic and many pathogenic strains in the genus had been frequently classified as *Colletotrichum gloeosporioides* or *Colletotrichum* spp. (Promputtha *et al.*, 2002). In addition to these Alvindia and Natsuaki (2008), reported that the pathogen was common and isolated from healthy leaves and roots of *M. acuminata* as an endophyte.

Environmental factors such as moisture and high humidity were mentioned as primary factors in anthracnose disease development and dispersed by wind, rain-splash, insects, birds and rats. The conidia which existed/ produced on inflorescence tissues, flower bracts, leaves and fields on dead banana leaf (most important) was found to be significant sources of inoculums (Fitzell and Peak, 1984; Dodd *et al.*, 1991).

2.4. Mode of Infection

Postharvest diseases are often classified according to how infection is initiated. The so-called 'quiescent' or 'latent' infections are those where the pathogen initiates infection of the host at some point in time (usually before harvest), but then enters a period of inactivity or dormancy until the physiological status of the host tissue changes in such a way that infection can proceed (Lassois *et al.*, 2010). The dramatic physiological changes which occur during fruit ripening are often the trigger for reactivation of quiescent infections. Examples of postharvest diseases arising from quiescent infections include anthracnose of various tropical fruit caused

by *Colletotrichum* spp. and grey mould of strawberry caused by *Botrytis cinerea* (Nelson, 2008).

The other major groups of postharvest diseases are those which arise from infections initiated during and after harvest. Often these infections occur through surface wounds created by mechanical or insect injury. Wounds need not be large for infection to take place and in many cases may be microscopic in size. Common postharvest diseases resulting from wound infections include blue and green mold caused by *Penicillium* spp. and transit rot caused by *Rhizopus stolonifer*.

Postharvest diseases of banana fruits such as, Crown rot caused by a complex of pathogens; *Colletotrichum musae* (Berk.& Curt.) v. Arx., *Fusarium* spp., mainly *Fusarium pallidroseum* (Cooke) Sacc., *Verticillium theobromae* (Turc.) Mason & Hughes, *Botryodiplodia theobromae* Pat. and *Nigrospora sphaerica* (Sacc.) & Mason; Anthracnose incited by *Colletotrichum musae*; Cigar end tip rot caused by *Verticillium theobromae* (Turconi) and *Gloeosporium musarum* and Fruit rot caused by *Botryodiplodia theobromae*, are most important diseases (Rivka, 2001).

Crown rot, infections by the various causal fungi was reported to be occurred in the fresh wounds created by severing the banana hands from the bunch stalks (Marin *et al.*, 1996; Anthony *et al.*, 2004). Presence of the pathogens had been mentioned on flowers and leaf trash in banana fields and to end up in the water used to wash banana fruits and to remove latex from the cut surfaces of the banana fruit crowns (Rivka, 2001). The rot affects tissues of the crown which joins the peduncles and it is not visible during the fingers are boxed. The symptoms and consequent rot generally occur after transportation or storage under favourable conditions of temperature and relative humidity for the pathogen and it will develop rapidly during ripening of the fruits (Lassois *et al.*, 2010).

Anthracnose which is the most important postharvest disease on banana, cooking banana and plantain is common on wounds/ bruise on fruit surface created during harvesting, transportation and storage; and it was also mentioned with its ability of attacking green fruits through invading the necks of the fingers when they are damaged during detaching of hands

from bunch through wounds (Dadzie, 1997). On green fruit it can cause an infection called latent infection in which it starts early in the season when the fruit is still on the tree but the pathogen remains dormant as a sub-cuticular hypha until the fruit approaches maturity. When the pathogen resumes activity on ripening, the infection causes the formation of typical brown spots on ripe fruits. It can also develop into destructive finger rots of green fruits in cold storage at 12 to 14°C. Spots on the fruits are at first water-soaked, usually irregular in shape and yellowish. The spots enlarge, may become lens-shape or spindle-shaped and turn dark brown to black with a water-soaked yellowish margin. Several spots may coalesce and affect large areas of the finger. Orange masses of spores develop at the centre of the spots under moist conditions. The disease is common on injured peel, is aggravated by bruises and wounds encountered during subsequent handling. Long storage and fluctuations to high storage temperatures had been noticed to favours anthracnose development (Meredith, 1971; Snowdon, 1990; Ploetz *et al.*, 1994).

2.5. Factors Affecting Postharvest Disease Development

Fruits and vegetable are characterized by high metabolic activities and known to possess short shelf life. Their physical condition and the pre and postharvest management practices influence the postharvest shelf life owing to physical, physiological, mechanical and hygienic conditions and cause losses during handling (marketing). Among the causes, pathological rots are the most serious followed by mechanical injury. Pathological rots together with mechanical injury had been mentioned to cause maximum damage to the perishables like banana (Oubahou and Otmani, 1995). Furthermore, proper postharvest processing and handling is an important part of agricultural production to increase the shelf life of produce and decrease the loss. The processes include the integrated functions of harvesting, cleaning, grading, cooling, storing, packaging, transporting and marketing. Therefore efforts to control these factors often are very important in reducing the incidence and severity of disease for example reducing mechanical damage during harvesting, grading and packaging greatly decreases the probability of postharvest decay due to of many disease-causing organisms (pathogens) must enter through wounds (Charles and New, 1996).

2.5.1. Variety

Immature fruits can be infected by microorganisms while they are attached to the plant or during harvesting and subsequent handling and marketing operations. The pre-harvest infection of fruits by microorganisms may occur either by direct penetration of the skin, through natural openings such as stomata, lenticels and growth cracks on the produce or through surface injuries. Favourable weather conditions at the time of maturation or ripening of the crop, or both, can lead to major loss due to diseases (Palipane and Rolle, 2008).

It is obvious that implementation of appropriate cultural practices and postharvest management practices used to combat diseases. The most effective way to limit the risks of a plant disease is to choose cultivars that exhibit the highest resistance or tolerance levels. Organic farmers depend primarily on selecting appropriate cultivars and on applying wide rotations. However, cultivars that often have characteristics that are desired by consumers or the processing industry are often highly susceptible to several pathogens. If alternative control measures are absent, farmers shift to growing lower-value but more tolerant cultivars (Waller *et al.*, 2001).

2.5.2. Harvesting methods

Method of harvest can significantly impact upon the postharvest quality and shelf life of fresh fruits (Palipane and Rolle, 2008). Mechanical injuries such as bruising, surface abrasions and cuts can accelerate loss of water, vitamin C and causing rotting fungi and bacteria to penetrate the produce, thus causing rapid deterioration (Kader and Rolle, 2004). Most fresh fruits and vegetables and all flowers are harvested by hand. The impact of drop height of the fruit to the ground could be minimized by using harvesting tools, hand picking, using picking poles and breaking the fall of fruit by spreading a crocus bag supported by hand.

Mechanical damages/ bruises created in poor handling techniques of banana bunches during harvesting had been described as major factors leading to post-harvest deterioration of banana, cooking banana and plantain (Dadzie, 1997). Damage by scarring fruits can be created during harvesting if bunches are allowed to fall down to the ground. This also could suffer the fruits to contaminate by microorganisms present as entophytes on plant debris of

unhygienic farm fields. Drop height of greater than 30 cm onto hard surface or 60 cm onto other fruits had been mentioned to cause bruise and/ or cracking particularly if the fruit are turgid (Bryant *et al.*, 2001).

2.5.3. Transportation

Transportation had been mentioned as serious problem faced by fruit growers in developing countries, where vehicles used in transporting bulk raw fruits to markets are not equipped with good refrigeration systems. In most developing countries, transportation of perishable commodities is in the most precarious stage. For local market, the produce mainly brought either by animal cart or human labour (Gustavo *et al.*, 2003) that can be listed as poor transport conditions and are major problem. The main limitations, includes rough roads, lack of refrigeration and poor truck suspension, are out of the control of growers and are important factors affecting the fruits quality (Gustavo *et al.*, 2003).

After transportation from farm fields fruits may suffer mechanical damage/ bruise during loading on vehicles and transportation to city markets due to impact of over loading, compression and vibration (Palipane and Rolle, 2008). In addition to this the speed of transportation and type of suspension can cause mechanical damage/ bruise on fruit surface. Following mechanical damage/ bruise on fruits surface results in physical/ colour change, softening of fruits tissue from breaking down of individual cell walls, early ripening due to an increasing in respiration rate and production of ethylene, and invasion by micro-organisms (Dadzie, 1997).

2.5.4. Container type

Fruits often have to be transported over considerable distances after harvest in many developing countries, in order to reach the market. To handle the produces several types of packaging materials are used from these materials baskets and other traditional containers are made from bamboo, rattan, straw and palm leaves, are common throughout the developing world (Adugna *et al.*, 2011). However, besides their advantages of being least cost and availability fruits often results in a serious qualitative and quantitative losses owing to physical or mechanical damage such as bruising and wounding, and subsequent rotting of the

produce (Palipane and Rolle, 2008). These packaging materials are poor in protecting of the fruits due to difficulties to clean when contaminated with decay organisms; lack rigidity and bend out of shape when stacked for long-distance transport; load badly because of their shape; cause pressure damage when tightly filled; they often have sharp edges or splinters causing cut and puncture damage.

Wooden and plastic crates are the other and widely used for handling of fruits for local markets in developing countries like Ethiopia. They are advantageous because they rigid, reusable can be packed flat and are inexpensive (Gustavo *et al.*, 2003). However, they have disadvantages of providing little protection from mechanical damage to the produce during transport; difficult to clean adequately for multiple uses; heavy and costly to transport; often have sharp edges, splinters and protruding nails, requiring some form of liner to protect the contents (Palipane and Rolle, 2008).

On the other hand, plastic boxes are comparatively durable and can last many years, easy to clean due to their smooth surface and are hard in strength, giving protection to products from mechanical damage in addition to this the bottom of containers are perforated to allow proper ventilation for avoidance of suitable environment for rot development. However they have disadvantages of higher cost relatively to wooden boxes (Palipane and Rolle, 2008).

2.5.5. Cleaning and grading

Cleaning and grading involves the removal of overripe, the removal of diseased, insect infested and mechanically damaged commodities and other unmarketable produce (Palipane and Rolle, 2008). Close attention to detail and good lighting are required at this stage. Damage extending to the aril rapidly leads to rots by micro-organisms, which may spread to sound fruit within the package. For this reason, fruit with pulled stems, splits, cracks and insect damage usually rejected at this stage (Gustavo *et al.*, 2003).

2.5.6. Storage

Storage is assumed to mean the holding of fresh fruit and vegetables under controlled conditions. Controlled temperature in storage is one of the main tools for extending the

postharvest life of fresh fruit. Low temperatures slow the rate of produce metabolism and the growth of microorganisms responsible for quality deterioration. Low temperature, in addition, minimizes the vapour pressure between the produce and the external environment, reduces water loss and thereby contributes toward maintaining freshness (Palipane and Rolle, 2008).

Good hygiene in the storage/ pack house is also required to avoid the spread of diseases so as pathogens can build-up on packing surfaces and fruit crates. These surfaces had been indicated to be washed with sanitizing agents such as chlorine every day. Water and fungicide dips also require frequent replacement or sanitizing. Waste fruit need to be regularly removed from the packing area to reduce the spread of spores (Gustavo *et al.*, 2003).

Tropical fruits had been advised to be stored at a temperature of 10⁰C with high humidity condition in order to prevent moisture loss and to preserve the freshness of fruit during low temperature storage. Given the fact that most fungi cease to grow under relative humidity conditions of less than about 90 % and only a few can grow at 85 % relative humidity, a relative humidity of 90 % is usually the best compromise condition for the storage of fruit (Palipane and Rolle, 2008).

2.5.7. Mechanical injury or bruise

Mechanical bruise on the fruits are the results of careless handling during harvesting, packing, transportation, storage etc. Some insects and birds are also responsible for the mechanical injury in fruits and vegetables (Dadzie, 1997). Mechanical injuries like bruising and cracking of fruits and vegetables render them more prone to attack by organisms and significantly increase the rate of water loss and gaseous exchange. Many a times the mechanical injury received by fruits, and adds to the loss of commodity. Puncturing of the containers and defective seals also leads to mechanical injury (Palipane and Rolle, 2008).

2.5.8. Ripening

Ripening is a natural stage of development which occurs when the fruit has ceased growing and is said to be mature. The process includes several noticeable changes that take place simultaneously. The peel and flesh tissues become soften when starch, the storage nutrient of

the fruit, is converted into sugar, some of which is subsequently used to provide energy which is necessary for metabolic processes, while the rest remains in the fruit, making it sweet and the flesh loses its firmness and becomes brown and gelatinous (Siriboon and Banulusilp, 2008).

It is obvious that during ripening increases fruit softness results in susceptibility for mechanical wound/ bruise and resultant microbial decay. It also had been described that disease resistance in fruit decreases during ripening as physiological and biochemical changes increases fruit susceptibility to pathogen infection, including quiescent disease (Zheng *et al.*, 2007), and the linkage between fruit ripening and increasing disease susceptibility is very strong (Yoruk, 2002).

3. MATERIALS AND METHODS

3.1. Study Area and Period

The field study was conducted in Arba-Minch of Gamu-Gofa zone and the postharvest chains were studied in Addis Ababa, capital city of Ethiopia, between July, 2011 and June, 2012 for twelve months. Arba-Minch is located in the southern corner of Southern Nations, Nationalities and Peoples Region (SNNPR) which is a major zone for banana production and supplier to the capital city, transporting about 505 km. It is situated at 6°2'N and 37°33'E at an altitude of 1,285 masl, receiving average annual rainfall of 900 mm with an average temperature of 29°C (Abebe, 2000). Addis Ababa is located at 9°1'48"N 38°44'24"E at an altitude of 2,400 masl. It receives annual average rain fall of 1180 mm with mean maximum and minimum temperature of 22.8°C and 10.6°C, respectively (NMSA, 2010).

All experiments were carried out in Plant Pathology Laboratory of Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) located at 355 km south-western of Addis Ababa. The area lies between of 7°41'N and 36 ° 50'E and elevation of 1704 meters above sea level. The area is characterized by a humid tropical climate of heavy annual rain fall that ranges from 1200 - 2000 mm per year with the mean annual ranges of maximum 25°C - 30°C and minimum 7°C - 12°C temperature (OPEDJZ, 2001).

3.2. Study Design

Multi stage cross-sectional survey was conducted in different levels of banana marketing from Arba- Minch to Addis Ababa started from harvesting up to the retailers shop. Banana fruits collected from the ripening (*chella*) room, retails and ET- fruit shops in the capital were considered as a sampling unit.

3.3. Survey and Sampling Methods

3.3.1. Phase I: Banana Fruit Sampling and Gathering Relevant Information at Farm Gate

The first phase of the study was performed at *kebeles* of *Lante, Chanoo, Chanoo Dorga* and *Chelba* representing the major banana production areas around Arba-Minch. In each *kebele*, based on the data received from the above listed *kebeles* administration offices an estimated of 511 (N) small-scale banana growing farmers were randomly selected for customary harvest and postharvest management (CHPHM), 5% (n=25) of the population were covered in the survey.

Information were gathered regarding the CHPHMPs including varieties grown, harvesting methods, harvesting equipments, cleaning and grading, type of containers used, methods of transportation, storage condition and loading/ unloading practices were considered as harvest and postharvest management factors (HPHMFs) affecting development of quiescent and wound anthracnose in each banana farm, during transporting and marketing of the fruits.

After surveying the CHPHMPs, banana fruit samples soon after harvested and transported to the road side (farm gates) were randomly collected from 25 surveyed farms around Arba-Minch. Two bunches and the 2nd, 6th and 10th hands/ bunch were randomly selected from each farmer's banana fruit lots before loading and transporting to Addis Ababa. Following this, collected samples of banana fruits were packed in hard cartons, labeled with detailed information such as date, time of sampling, farmers name and locations (farm) *kebele*, variety etc. and transported to Laboratory of Plant Pathology at JUCAVM.

3.3.2. Phase II: Banana Fruit Sampling and Gathering Relevant Information Related to Transportation

The second phase of the study was conducted on 20 vehicles arrived to ripening (*Chella*) rooms which were found at ET-fruit enterprise and *Piassa Atekelt Tera* in Addis Ababa. In order to study the customary postharvest management practices (CPHMPs) during and after transportation of the fruits from the source, data were collected on variety, fruit source, types

of vehicles, container types, fruit loading/ unloading, fruit composition, fruit covers, distance traveled (Km) and road type were considered as CPHMFs affecting incidence and severity of anthracnose.

After surveying the CPHMPs banana fruit samples were randomly selected from the vehicles at ripening rooms. Two banana bunches at different positions were arbitrarily selected from each vehicles, next to these; the 2nd, 6th and 10th hands were carefully detached and collected from bunches. Collected specimens were then transported to Laboratory of Plant Pathology at JUCAVM, in similar procedures of sample collection in the first phase.

3.3.3. Phase III: Banana Sampling and Data gathering in the Markets/ ripening rooms, retailer and ET-fruit shops

The third phase of the study was conducted at major fruit and vegetable markets in Addis Ababa. According to the data collected from four fruit and vegetable sellers union, Trade and Industry Offices of two woredas (*Addis ketema* 01 and *Aradda* 07/08/09) and ET-fruit enterprise, it is estimated a total (N) of 95 ripening (*chella*) rooms, 383 retailer and 60 ET-fruit shops which sell banana fruits, were present at *Piassa Atikelt Tera*, *Kera Atekelt Tera*, *Merkato Atikelt Tera* and ET- fruit enterprise. To study the CPHMPs and incidence and severity of Anthracnose, sellers (shops) were stratified as ripening rooms (wholesalers), retailer and ET-fruit shops (wholesalers and retailers) based on characteristics they shared. Five percent of the shops at each stratum were covered in the study hence, in order to determine the sample size of each stratum the following formula by Mark (1998) was used;

$$nh = \frac{Nh}{N} \times n$$

Where:

nh is the sample size for stratum *h* (ripening room, retailer & ET-fruit)

Nh is the population size for stratum *h*;

N is total population size, and

n is total sample size.

After sample size determination data was gathered regarding the CPHMPs of the merchants (employees) at each stratum. Fruit variety, fruit source, container type, cleaning & grading, fruit composition and storage condition were considered as CPHMFs affecting incidence and severity of anthracnose.

After surveying the CPHMPs banana samples were randomly selected from boxes or hands and collected from 19 ripening rooms, 12 ET-fruit and 71 retailers' shops. A total of 95 boxes were randomly picked from surveyed ripening rooms. Five boxes each containing around 14 banana hands were selected and three hands were arbitrarily chosen from each box and a total of 1710 banana fingers were sampled.

To determine the incidence and severity of Anthracnose at retailers' level, 71 shops were surveyed from a total of 383 shops. Samples of 20 banana fingers were randomly removed from displays at each retailer shop and a total of 1420 banana fingers were sampled.

Based on the sample size 12 ET-fruit shops were surveyed and sampled from the total of 60 ET-fruit shops in the town. Two boxes containing around 7 kg (46 fingers) of banana fruits were randomly picked and 23 banana fingers were arbitrarily chosen from each box and a total of 552 banana fingers were sampled. Collected samples from each stratum were taken to Plant Pathology Laboratory of JUCAVM in similar procedures with the first and second phases of the study.

3.4. Isolation and Identification of the Causal Pathogen

The suspected pathogen *Colletotrichum musae* were isolated from infected fruits following the technique described by Jinyoung *et al.* (2002). Samples collected and stored in refrigerator were taken and the fresh banana fruits that were recently infected (symptomatic) bordering on healthy tissues were cut into small pieces of 3 mm diameter. The small pieces were washed with tap water and surface sterilized with 10% NaOCl for 30 to 60 sec, again rinsed with sterile water and bolted to dry on clean tissue paper. After drying, three pieces were aseptically placed into Petri dishes containing potato dextrose agar (PDA). Next, the inoculated Petri dishes marked with name of the sample and date of inoculation and incubated at a temperature of 25°C for 5 days, until fungal proliferation on medium surface. When the

fungus has grown 1 to 2 cm away from the tissue piece, it was sub-cultured from hyphal tips on to a suitable nutrient medium PDA.

Fungal identifications were carried out as described by Jinyoung *et al.* (2002) on their study of isolation and identification of *Colletotrichum musae* from imported bananas. The appearance on culture as seen by the naked eye, the color and texture of colony, culture medium and time of incubation were considered for identification. The other and the most significant fungus characteristics (*C. musae*) used for identification were spores and spore-bearing structures (sporophores) (Damm *et al.*, 2010; Thangamani *et al.*, 2011). These characteristics therefore examined under a compound microscope in 100X magnification power from the minute sample taken from cultures grown on PDA.

3.5. Characterization of the Fungus

3.5.1. Cultural Characteristics of the Isolates

Cultural characteristics of six *C. musae* isolates (three from farms at *kebeles* of Arba-Minch specifically; ALC1 from *Lante*, ACC2 from *Channo*, ACDC3 from *Channo Dorga* and three from fruit and vegetable markets in Addis Ababa; ARRC4 from ripening rooms, ARC5 from retailers and AET6 from ET-fruit shops) were studied on potato dextrose agar (PDA) as recommended for its suitability for growth of *C. musae* (Latiffah *et al.*, 2009; Thangamani *et al.*, 2011). The colony color, colony substrate, colony texture, shape, margin and radial colony growth (mm) were considered as cultural characteristics. The culture media was prepared in one liter of sterilized water or according to the amount needed and autoclaved at 120°C for 20 min. After that the media were let to be cooled and supplemented with 100µg/ml streptomycin to alleviate microbial contaminants and poured onto Petri dishes for solidification as described by Latiffah *et al.* (2009) and Thangamani *et al.* (2011).

Pure mother cultures of the isolates preserved on slant PDA were transferred to prepared medium according to the procedures of Latiffah *et al.* (2009). Five replicates were retained for each isolates and cultural characteristics were recorded from the colony of cultured plates incubated for 7 days at 25°C (Jinyoung *et al.*, 2002).

3.5.2. Morphological Characteristics

Morphological characteristics of six isolates from Arba-Minch and Addis Ababa were studied on minute samples taken from grown cultures on PDA after incubation for 7 days at 25°C and then the shape and size of 10 conidia and appressoria were studied. For studying morphological characteristics of appressoria, conidial suspension of 1×10^5 conidia/ml was inoculated on cellophane membrane on a slide glass and incubated for one day at 25°C to induce appressorial formation. Then the shape and size were assessed under compound microscope from the replicates of each isolates (Jinyoung *et al.*, 2002 and Shenoy *et al.*, 2007).

3.6. Pathogenicity of *Colletotrichum musae* Isolate

Pathogenicity was tested on Dwarf Cavendish banana variety which is susceptible to *C. musae* (Mohamed and Asmmare. MARC, personal communication, 20011). Healthy green matured Dwarf Cavendish banana were brought from Jimma Agricultural Research Center and washed with running tap water, surfaced sterilized with 10% NaOCl, and washed again with sterilized water. The fruits were blot dried intended for wounding and inoculation of the pathogen. Fruits were wounded with a sterile 3 mm cork borer, and inoculated with mycelia disc (3 mm in diameter) of the fungal isolates as described by Baiyewu *et al.* (2007) aseptically in a laminar flow chamber. Controlled fruits were wounded with the sterilized cork borer but inoculated with distilled sterile water. The inoculated banana fruits were placed in clean polyethylene bag (one fruit per bag) each moistened with wet balls of absorbent cotton wool to create humid environment and were incubated at 25°C for 7 days. The treatment (isolate) was replicated five times and disease causal agent was re-isolated from artificially inoculated fruits which manifest anthracnose symptoms to compare with the original isolate.

3.7. Evaluation of Banana Varieties to Wound and Quiescent Anthracnose

To study fruit reaction to wound and quiescent anthracnose, five different varieties: Poyo, William I, Butaza, Dwarf and Giant Cavendish banana fruits were collected from Jimma Agricultural Research Center. Matured fruits were harvested from the inner hands of bunches

to get healthy fruits. After that fruits were properly placed inside carton box and transported to Plant Pathology Laboratory at JUCAVM.

A total of 75 banana fingers, 15 for each variety were retained and combination of procedures for Pathogenicity test of *C. musae* described in Jinyoung *et al.* (2002), Shenoy *et al.* (2007) and Latiffah *et al.* (2009), and procedures for evaluating banana susceptibility to wound anthracnose by Chillet *et al.* (2002) were used with a little modifications. The fruits surface were then disinfected with 10% of NaOCl, washed with sterilized water and blotted to dry inside laminar flow on soft paper. From 15 fruits fingers of each variety 5 fruits for wound anthracnose, 5 for quiescent Anthracnose and the other 5 for control were prepared for the experiment.

For wound anthracnose test each fruit were wounded at three pointes on the surface (top, middle and bottom) with sterilized needle whereas, for quiescent anthracnose test fruits were unwounded. Conidial suspension was prepared from 7 days old PDA culture by grinding it with sterile distilled water in a pestle and mortar and the distilled water containing concentrated fungal spore was filtered through layers of cheesecloth (Thangamani *et al.*, 2011). Conidial suspension was then prepared by adjusting in 1×10^5 conidia/ml using haemocytometer and sprayed over the wounded and unwounded fruits (Latiffah *et al.*, 2009). Fruits were inoculated with only sterile distilled water served as control. All the inoculated fruits were kept inside clean plastic bag (one fruit per bag) each moistened with wet balls of absorbent cotton to create a humid environment and incubated at 28°C for 5 days. After incubation and symptom development on inoculated fruits data on length and width of necrotic tissue (mm^2) were collected and calculated to determine which variety is severely rotted (Chillet *et al.*, 2006).

Treatments (inoculation with wound, inoculation without wound and control) were replicated five times. The experiment was done in Complete Randomized Design (CRD) with factorial arrangement.

3.8. Data Collection and Analysis

3.8.1. Qualitative data

A structured questionnaire was designed to capture qualitative information on CHPHMPs by the farmers and sellers. Variety, harvesting methods & equipments, cleaning and grading, storage types, loading/ unloading methods, vehicle types, fruit composition, distance travelled, road types and containers types were surveyed and data were collected through single visit interviews to individual farmers and sellers in similar fashions by Mbaka *et al.* (2006) and Adugna *et al.* (2011). Field visits were undertaken to verify descriptions of the CHPHMPs and diagnose other possible observations on the farms and markets. Then the collected data were tabulated in the form of tables and descriptive statistics including percentages was used to determine the CHPHMPs at each surveying point.

Therefore percentage of customary harvest and post-harvest management practices at each survey phases were calculated as described by Mbaka *et al.* (2006):

$$\%CHPHMPs = \frac{NRSMPs}{TNR} \times 100$$

Where:

% CHPHMPs = Customary harvest and post-harvest management practices

NRSMPs = Number of respondents with similar management practices

TNR = Total number of respondents

Other qualitative data such as, colony color, texture, shape & margin, the shape of conidia and appressoria and characteristics of the disease symptom (lesion and their color) were collected through visual and microscopic observations.

3.8.2. Quantitative Data

Data regarding the ripening level, bruise severity, incidence and severity of anthracnose, conidial size, colony diameter and the rate of colony growth were collected. Ripening level (changes in color of peel) were evaluated and determined by color chart with a number from 1

to 7 as described by Cheng *et al.* (2009): 1 = hard and green; 2 = green with a trace of yellow; 3 = more green than yellow; 4 = yellow with a green hint; 5 = all yellow with green tip on the crown; 6 = all yellow; 7= yellow with brown sugar spots. From this the ripening index (RI) was calculated by the formula:

$$RI = \frac{\sum(Nx \times X)}{\sum Nx}$$

Where: x = ripening stage (1-7)

N_x =represents the number of fruit at the corresponding stage

Severity of mechanical bruise was measured in all stages of the study. To determine the area of bruise on sampled fruits surface a method developed by Dadzie *et al.* (1997) was used, bruise was circled and the diameter of bruised part measured using a ruler.

Bruised area of the fruit was calculated using a formula:

$$\text{Bruise area (A) as: } \pi \times (d/2)^2$$

Where: A = area of the circle

$$\pi = 3.14159$$

d = diameter of circle (Dadzie *et al.*, 1997).

After calculating the bruise surface area, percentage bruise severity (%BS) was calculated using the formula given by Jeger (1997).

$$\%BS = \frac{\text{Sum of bruised area}}{\text{Total number of assesed fruits} \times \text{Maximum area of bruise}} \times 100$$

The extent of disease occurrence/ disease incidence during survey was calculated on the bases of totality of healthy and diseased fruits as described by Ogbo and Oyibo (2008), using the formula:

$$\%I = \frac{\text{Number of infected fruit}}{\text{Total number of assesed fruits}} \times 100$$

For estimation of disease severity on individual sampled fruits, the whole fruit surface area was considered as 100% then the diseased area was visually estimated. Next, scale from 0 to 4 was given for percentage of rotten fruit surface and percent of disease index (PDI) or disease severity was calculated with the formula described in Cheng *et al.* (2009) where; 0 = no disease infection; 1 = the area of decay occupied < 25% of the fruit surface; 2 = the area of decay occupied 25 to 50% of the fruit surface; 3 = the area of decay occupied 50 to 75% of the fruit surface; and 4 = the area of decay occupied > 75% of the fruit surface. Percentage of Disease Index (PDI), i.e. severity (S) was calculated as:

$$\%DI = \frac{[\sum(N_y \times Y)] \times 100}{4 \sum N_y}$$

Where; Y= disease severity from (0 - 4)

N_y = represents the number of fruit with the corresponding severity score

Data on conidial size (μm) and colony diameter (mm) were measured using ruler and compound microscope (stage and ocular micrometer).

3.8.3. Data Analysis

Percentage data collected in the field survey and from laboratory examination of the fruits for ripening level, bruise severity, incidence and severity of Anthracnose were transformed using the Arc Sine transformation. Subsequently transformed data were subjected to Analysis of multiple linear regression to determine the CHPHMPs affecting disease development on the fruits. Independent sample *t* test was performed in order to determine whether these management practices were different in their effect and its size on disease incidence and severity. Collected data from laboratory examination or field survey were analyzed using Microsoft office excel 2007, SAS version 9.2 and SPSS version 16.0.

4. RESULTS

4.1. Phase I. Banana Anthracnose and Factors affecting the Disease at Farm in Arba-Minch

4.1.1. Incidence and Severity of the Disease

At farm mean percentage of ripening index was 1 (hard green) and bruise severity was 5.9% on a total of 1650 assessed fruit surfaces during purchased by brokers/ merchants. Mean percentage of anthracnose incidence and severity were 9.8 and 4.1%, respectively at surveyed farms around Arba-Minch. Anthracnose incidence was varied from 7.4 to 13.4% and severity was varied from 2.6 to 5.1% among farms (Table 2).

Table 2. Mean percentage of ripening index (RI), bruise severity index (BSI), anthracnose incidence (AI) and anthracnose severity index (ASI) at farm level around Arba-Minch, South Ethiopia

| Surveyed Kebeles | No. of surveyed farms | No. of inspected fingers | RI | BSI | AI | ASI |
|------------------|-----------------------|--------------------------|----------|------------|------------|------------|
| <i>Lante</i> | 8 | 528 | 1 | 4.9 | 7.4 | 5.1 |
| <i>Channo</i> | 6 | 396 | 1 | 3.6 | 9.4 | 2.6 |
| <i>C. Dorga</i> | 6 | 396 | 1 | 6.2 | 10.5 | 3.6 |
| <i>Chelba</i> | 5 | 330 | 1 | 8.8 | 13.4 | 4.7 |
| Mean | | | 1 | 5.9 | 9.8 | 4.1 |

4.1.2. Factors affecting the Disease at Farm

The survey results showed that 80% of the farmers grow Cavendish (giant and dwarf) banana variety and the others grow varieties locally named *Ambowuha* and *Ye-Eretria Muz*. Most farmers grow banana for the market and their own household consumption. All of the interviewed farmers (100%) in the survey use big sharp edged knife known as *Konchera* to harvest bunches; from these only 32% of them prop up bunches during harvesting. After harvesting, 28% of the farmers graded their product as suitable and unsuitable for market quality on the physical appearance such as mechanical wounds and rot caused by microorganisms. They tie 2 to 4 bunches of banana on strong wooden stick to be carried by two men or animal drawn cart to transport the bunches to the road side where brokers and/ or

merchants are available to purchase their products during the late afternoon. Among the surveyed farmers 28% used human labour and the other 72% used animal drawn carts for transportation of bunches from farm to road side or their houses.

Farmers usually keep their products on the fields around farm gate or aside the main road by covering with banana leaves. All of the interviewed farmers (100%) practiced this storage or preservation method until they sell. After the deal between farmers and brokers/ merchants, banana bunches were loaded on vehicles surface wrapped with banana leaves (Table 3).

Table 3. Percentage of Customary Harvest and Postharvest Management Practices around Arba-Minch, South Ethiopia

| CHPHMPs | Character given | Number of farmers at four kebeles | | | | Percent age (%) |
|----------------------|-----------------------|-----------------------------------|--------------|-----------------|---------------|-----------------|
| | | <i>Lante</i> | <i>Chnno</i> | <i>C. Dorga</i> | <i>Chelba</i> | |
| Variety grown | Cavendish | 5 | 4 | 6 | 5 | 80 |
| | Other local varieties | 3 | 2 | 0 | 0 | 20 |
| Harvesting equipment | <i>Konchera</i> | 8 | 6 | 6 | 5 | 100 |
| | Other equipments | 0 | 0 | 0 | 0 | 0 |
| Harvesting methods | With support | 3 | 3 | 1 | 1 | 32 |
| | Without support | 5 | 3 | 5 | 4 | 68 |
| Cleaning/ grading | Yes | 3 | 5 | 5 | 5 | 72 |
| | No | 5 | 1 | 1 | 0 | 28 |
| Transportation | Human labor | 4 | 0 | 0 | 3 | 28 |
| | Animal cart | 4 | 6 | 6 | 0 | 72 |
| Storage | Field store | 8 | 6 | 6 | 5 | 100 |
| | Controlled room | 0 | 0 | 0 | 0 | 0 |
| Loading/ unloading | Staking of fruits | 8 | 6 | 6 | 5 | 100 |
| | Other methods | 0 | 0 | 0 | 0 | 0 |

To study if CHPHMPs at farm level affects the incidence and severity of anthracnose on banana fruits multiple regression analysis was carried out. Based on this the regression analysis (Appendix A. Table 1) predicting farm level incidence of anthracnose from CHPHMPs and physical conditions of the fruit (PCF) was statistically significant, $p < 0.05$.

The strength of correlation between the predictors and disease incidence was found to be positive strong relationship ($r = 0.99$) and adjusted $R^2 = 0.98$ indicates that 98% of the variance in disease incidence was accounted for the predictors. CHPHMPs and PCF such as harvesting method, cleaning/ grading and bruise severity were significantly ($p < 0.05$) affected disease incidence on banana fruits (Table 4).

Table 4. Multiple linear regression analysis for farm level anthracnose incidence

| Variables | Std. coefficients β_j | t | p |
|--------------------|--------------------------------|-------|-------|
| Harvesting methods | 0.19 | 2.13 | 0.05 |
| Cleaning/ grading | 0.16 | 2.03 | 0.05 |
| Ripening level | -0.03 | -0.65 | 0.52 |
| Bruise severity | 0.52 | 5.76 | 0.000 |

The regression analysis to determine the predictors (CHPHMPs and PCF) effects on farm level anthracnose severity was statistically significant, $p < 0.05$ (Appendix A. Table 2). The strength of correlation between the predictors and disease severity was positively strong ($r = 0.99$) relationship and adjusted $R^2 = 0.99$ indicates that 99% of the variance in disease severity was accounted by the predictors. Harvesting method, bruise severity and disease incidence were significantly ($p < 0.05$) affected anthracnose severity (Table 5).

Table 5. Multiple linear regression analysis for farm level anthracnose severity

| Variables | Std. coefficients β_j | t | p |
|--------------------|--------------------------------|------|-------|
| Harvesting methods | -0.21 | -3.7 | 0.002 |
| Cleaning/ grading | 0.03 | 0.53 | 0.600 |
| Ripening level | 0.03 | 1.08 | 0.290 |
| Bruise severity | 0.48 | 4.83 | 0.000 |
| Incidence | 0.67 | 5.4 | 0.000 |

After determining the CHPHMPs which were significantly affected anthracnose incidence and severity, independent sample *t* test was carried out and the result revealed significant difference in mean percentages of incidence and severity among farmers which harvested banana bunches with support and without support. Incidences were 11.1% and 24%; and severity were 10.7% and 22.8% among the two groups of farmers that harvest bunches with support and without support, respectively (Table 6).

Mean percentage of incidence and severity were also significantly different among the two groups of respondents that did grade and didn't grade their fruits to avoid decayed, immature and mechanically injured fruits (Table 6). Incidence was highly affected by farmers that did grade or didn't grade their fruits in 0.83 and 0.96 of effect sizes (Eta²), respectively. Form evaluated PCF, bruise severity on the fruit surface was significantly affected both incidence and severity of anthracnose with the respective effect sizes of 0.8 and 0.8Eta², and incidence by itself was found to be one of the factors significantly affected anthracnose severity with the effect size of 0.6 Eta².

Table 6. Independent sample *t* test of predictors (mean ± SE) at farm level

| CHPHMPs | Group | Incidence | Eta ² | Severity | Eta ² |
|-------------------|-----------------|------------------------|------------------|------------------------|------------------|
| Harvesting method | Without support | 24.02±1.2 ^a | 0.96 | 22.79±1.4 ^a | 0.96 |
| | With support | 11.06±1.1 ^b | 0.84 | 10.71±1.3 ^b | 0.83 |
| Cleaning/ grading | Yes | 10.45±1.2 ^a | 0.83 | - | - |
| | No | 23.53±1.3 ^b | 0.96 | - | - |

Means followed by different letters at each group of CHPHMPs are significantly different at (*p* = 0.05)

Eta², indicates the Cohen's effect sizes of 0.2-0.4 are small, 0.5-0.7 are medium and 0.8-1.0 are large

4.2. Phase II: Banana Anthracnose and Transportation Factors affecting the Disease

4.2.1. Incidence and Severity of the Disease

Up on arrival of the vehicles to the ripening rooms all of the fruits were at 1.1 ripening index and mean percentage of bruise severity that could arise because of vibration and/ or

compression during transportation was 8%. The mean percentage of banana anthracnose incidence and severity were 4.9% and 1.6%, respectively. Diseased severity on fruit surface was ranged from 0 to 3.1% and the highest 3.1% of anthracnose severity was recorded on vehicles surveyed at *Piassa Atekelt-Tera* however, no severe anthracnose rot on fruits surface were observed on vehicles surveyed at ET-fruit enterprise (Table 7).

Table 7. Mean percentage of ripening index (RI), bruise severity index (BSI), anthracnose incidence (AI) and anthracnose severity index (ASI) after transportation to Addis at two locations

| Survey location | No. of sampled vehicles | No. of inspected fingers | RI | BSI | IA | SAI |
|---------------------|-------------------------|--------------------------|------------|------------|------------|------------|
| ET-fruit enterprise | 2 | 216 | 1.1 | 7.1 | 1.5 | 0.0 |
| Piassa Atekelt-Tera | 19 | 864 | 1.0 | 8.9 | 8.2 | 3.1 |
| Mean | | | 1.1 | 8.0 | 4.9 | 1.6 |

4.2.2. Transportation Factors influencing the Disease

From surveyed 20 vehicles 90% were Isuzu trucks which had no adequate provisions such as cooling system and proper loading condition for conveying perishable fruits similar to banana whereas ET-fruit enterprise used modern vehicles possessed with cooling system. The Cavendish banana variety was dominant in 75% of sampled vehicles and 95% of the fruits were brought from Arba-Minch and the other 5% was from Asebe-Teferi. During arrival of the vehicles at the ripening rooms, 90% fruits that were transported by Isuzu, were covered with banana leaves to mitigate air drying during transportation. In all surveyed vehicles bunches were unloaded sliced into hands by skilled labors, placed inside wooden (90%) and plastic box (10%) covered with news paper and stored in ripening (*Chella*) rooms (Table 8).

Table 8. Percentage of customary postharvest management practices during arrival of the fruits to ripening rooms in Addis

| CPHMPs | Characters given | No. of surveyed vehicles at | | Percentage |
|------------------------|-----------------------------|-----------------------------|----------|------------|
| | | Piassa Atikelt-Tera | ET-fruit | |
| Varity | Only Cavendish | 13 | 2 | 75 |
| | Cavendish + Other varieties | 5 | 0 | 25 |
| Fruit source | Arba Minch | 17 | 2 | 95 |
| | Asebe-Teferi | 1 | 0 | 5 |
| Type of vehicle | Isuzu Trucks | 18 | 0 | 90 |
| | Other vehicles | 0 | 2 | 10 |
| Container type | Wooden box | 18 | 0 | 90 |
| | Plastic box | 0 | 2 | 10 |
| Fruit unloading | Cutting / staking hands | 18 | 2 | 100 |
| | Other methods | 0 | 0 | 0 |
| Fruit composition | Only banana | 18 | 2 | 100 |
| | Banana + other varieties | 0 | 0 | 0 |
| Fruit covers | Banana leaves | 18 | 0 | 90 |
| | Other covers | 0 | 2 | 10 |
| Distance traveled (Km) | 505 km | 17 | 2 | 95 |
| | Other | 1 | 0 | 5 |
| Type of road | Metallic + non metallic | 18 | 2 | 100 |

The regression analysis (Appendix A. Table 3) showed significant ($p < 0.05$) effect of predictors on anthracnose incidence and the correlation between predictors and disease incidence was found to be positive strong ($r = 0.98$) and adjusted $R^2 = 0.96$ indicates 96% of the variance in disease incidence was explained by the predictors. From evaluated predictors; fruit source, fruit cover and bruise severity were significantly ($p < 0.05$) affected anthracnose incidence after transportation (Table 9).

Table 9. Multiple linear regression analysis for anthracnose incidence after transportation

| Variables | Std. coefficients β_j | <i>t</i> | <i>p</i> |
|-----------------|--------------------------------|----------|----------|
| Variety | -0.04 | -0.59 | 0.560 |
| Fruit source | 0.24 | 3.49 | 0.004 |
| Fruit cover | -0.67 | -8.79 | 0.000 |
| Ripening level | 0.32 | 2.63 | 0.200 |
| Bruise severity | 0.30 | 2.69 | 0.020 |

After transportation, anthracnose severity on banana fruits was significantly ($p < 0.05$) affected by predictors (Appendix A. Table 4). The strength of correlation between predictors and anthracnose severity was positive strong ($r = 0.98$) and adjusted $R^2 = 0.93$ indicates that 93% of the variance in anthracnose severity was explained by predictors such as the distance in which the fruits were travelled by vehicles and mechanical bruise created on the fruit surface (Table 10).

Table 10. Multiple linear regression analysis for anthracnose severity after transportation

| Variables | Std. coefficients β_j | <i>t</i> | <i>p</i> |
|-----------------------|--------------------------------|----------|----------|
| Variety | 0.09 | 0.97 | 0.35 |
| Fruit cover | -0.24 | -1.66 | 0.12 |
| Distance travelled | -0.16 | -2.06 | 0.05 |
| Ripening level | 0.28 | 1.22 | 0.24 |
| Bruise severity | 0.78 | 4.12 | 0.001 |
| Anthracnose incidence | -0.25 | -0.97 | 0.351 |

Independent sample *t* test was evident that mean percentage of anthracnose incidence was significantly ($p < 0.05$) different among banana fruits brought from Arba-Minch and Asebe-Teferi (Table 11). Fruits were transported in two types of vehicles, in open Isuzu trucks wrapped with banana leaves and in modern vehicles to transport perishables like banana fruits

and mean percentage of anthracnose incidence was significantly ($p < 0.05$) different between fruits covered with banana leaves during transportation and fruits brought in modern vehicles (other covers).

Table 11. Independent sample *t* test of the predictors (mean \pm SE) after transportation

| CPHMPs | Group/ Mean | Incidence | Eta ² | Severity | Eta ² |
|-------------------------|---------------|------------------------------|------------------|------------------------------|------------------|
| Fruit source | Arba-Minch | 20.32 \pm 6.2 ^a | 0.17 | - | - |
| | Asebe-Teferi | 8.53 \pm 2.5 ^b | 0.26 | - | - |
| Fruit cover | Banana leaves | 21.45 \pm 2.8 ^a | 0.68 | - | - |
| | Other covers | 4.27 \pm 4.4 ^b | 0.93 | - | - |
| Distance travelled (Km) | 505 | 19.39 \pm 6.6 ^a | 0.26 | 8.04 \pm 4.48 ^a | 0.05 |
| | 238 | 25.99 \pm 4.1 ^b | 0.31 | 19.55 \pm 5.1 ^b | 0.11 |

Means followed by different letters at each category of CPHMPs are significantly different at ($p= 0.05$)

Eta², indicates the Cohen's effect sizes of 0.2-0.4 are small, 0.5-0.7 are medium and 0.8-1.0 are large

4.3. Phase III: Banana Anthracnose and Factors affecting the Disease at Ripening, Retailer and ET-fruit Shops

4.3.1. Incidence and Severity of the Disease

A total of 1710, 1420 and 552 banana fingers were inspected from ripening rooms, retailer and ET-fruit shops, respectively. Based on this mean percentage of 1.5, 6.1 and 6.1% ripening index at ripening rooms, at retailers and ET-fruit shops were recorded, respectively. Mean percentage of bruise severity were 10.7, 26.9 and 21.6%; mean percentage of anthracnose incidence were 28.3, 68.2 and 51.3%; and severity were 3.1, 31.7 and 20.7% at ripening, retailer and ET-fruit shops, respectively (Table 12). Generally from surveyed shops the highest 6.8% of anthracnose incidence and 31.7% of severity were recorded at retailer shops and the least 28.3% of anthracnose incidence and 20.7% of severity were recorded at ripening rooms.

Table 12. Mean percentage of ripening index (RI), bruise severity index (BSI), anthracnose incidence (AI) and anthracnose severity index (ASI) at ripening rooms, ET- fruit and retailer shops

| Shop type | Survey location | No. of shops | No. of inspected fingers | RI | BSI | IA | ASI |
|----------------|----------------------|--------------|--------------------------|------------|-------------|-------------|-------------|
| Ripening rooms | Piassa Atekelt Tera | 14 | 1260 | 1.5 | 11.3 | 30.2 | 2.1 |
| | ET-fruit enterprise | 2 | 180 | 1.1 | 1.7 | 8.5 | 0.1 |
| | Merkato Atekelt Tera | 3 | 270 | 1.7 | 19.2 | 46.2 | 6.7 |
| Mean | | | | 1.5 | 10.7 | 28.3 | 3.1 |
| Retailers shop | Piassa Atekelt Tera | 37 | 740 | 6.2 | 29.6 | 78.1 | 33.1 |
| | Merkato Atekelt Tera | 21 | 420 | 5.9 | 23.2 | 61.4 | 25.8 |
| | Kera Atekelt Tera | 13 | 260 | 6.1 | 28 | 65 | 37.1 |
| Mean | | | | 6.1 | 26.9 | 68.2 | 31.7 |
| ET- fruit | Addis Ababa | 12 | 552 | 6.1 | 21.6 | 51.3 | 20.7 |
| Mean | | | | 6.1 | 21.6 | 51.3 | 20.7 |

4.3.2. Factors affecting the Disease at Ripening Rooms, Retailer and Et-fruit Shops

From surveyed ripening, retailer and ET-fruit shops 79%, 79% and 100% of them were mainly seal Cavendish (giant and dwarf) variety, respectively (Table 13). All (100%) of ET-fruit shops and 79% of ripening rooms chiefly obtained the fruits from Arba-Minch whereas, for retailers *Piassa Atekelt-Tera* was the main source of banana fruits accounting for 72% and the other 28% of retailers obtained the fruits from ripening rooms found at ET-fruit enterprise. Wooden box was widely used by 84% of the ripening rooms and 69% of retailers for handling the fruits although fruit were handled in plastic box in all surveyed ET-fruit shops. Cleaning/grading of diseased, severely wounded and physically abnormal fruits were done in all (100%) surveyed ET-fruit shops however, only 64% of ripening rooms and 37% of retailer shops grade their fruits as suitable/ unsuitable for the market (Table 13). All of surveyed retailer and ET-fruit shops were stored/ handled banana fruits under room temperature while

in all ripening rooms fruits were kept inside controlled room. The fruits were usually kept in these rooms only for 24 hrs for induction (ethylene) of artificial ripening. Fruits were seen kept alone in all ripening rooms however, since the fruits at ET-fruit and retailer shops were ripe and ready to be sold for consumers, it was kept inside wooden/ plastic boxes and/ or occasionally displayed together with other fruit or hanged with a hook.

Table 13. Percentage of customary postharvest management practices at ripening rooms, retailer and ET-fruit shops around Addis Ababa, Ethiopia

| CPHMPs | Category | Surveyed stratum | | |
|-------------------|------------------------|------------------|----------|----------|
| | | Ripening room | Retailer | ET-fruit |
| Variety | Cavendish | 79 | 79 | 100 |
| | Cavendish+ local Var. | 21 | 21 | 0 |
| Fruit sources | Arba-Minch | 79 | 0 | 100 |
| | Piass Atekelt Tera | 0 | 72 | 0 |
| | ET- fruit enterprise | 0 | 28 | 0 |
| | Other production areas | 21 | 0 | 0 |
| Container type | Plastic box | 16 | 31 | 100 |
| | Wooden box | 84 | 69 | 0 |
| Cleaning/ grading | Yes | 64 | 37 | 100 |
| | No | 36 | 63 | 0 |
| Fruit composition | Only banana | 100 | 32 | 100 |
| | Banana + other fruits | 0 | 68 | 0 |
| Storage type | Controlled room | 100 | 0 | 0 |
| | Room temperature | 0 | 100 | 100 |

4.3.2.1. Ripening Rooms

The regression analysis (Appendix A. Table 5) shows the customary postharvest management practices by the merchants and physical condition of the fruits were significantly ($p < 0.05$) affected anthracnose incidence at ripening rooms. The correlation between the predictors and disease incidence was positive strong ($r = 0.98$) and adjusted $R^2 = 0.95$ indicates 95% of the variance in disease incidence among ripening rooms were explained by predictors such as clanging/ grading, ripening level and bruise severity (Table 14).

Table 14. Multiple linear regression analysis for anthracnose incidence at ripening rooms

| Variables | Std. coefficients β_j | <i>t</i> | <i>p</i> |
|-------------------|--------------------------------|----------|----------|
| Variety | 0.01 | 0.18 | 0.86 |
| Fruit source | 0.11 | 2.08 | 0.45 |
| Cleaning/ grading | 0.09 | 0.78 | 0.05 |
| Ripening level | -0.06 | -0.39 | 0.03 |
| Bruise severity | 0.94 | 7.10 | 0.00 |

Regression analysis (Appendix A. Table 6) showed that predictors were significantly ($p < 0.05$) affected anthracnose severity at ripening rooms. The correlation between the predictors and disease severity was found to be strong and positive ($r = 0.99$) and adjusted $R^2 = 0.99$ showed that 99% of the variance in disease severity was accounted for the predictors, such as container types, cleaning/ grading, ripening level, bruise severity and anthracnose incidence (Table 15).

Table 15. Multiple linear regression analysis for anthracnose severity at ripening rooms

| Variables | Std. coefficients β_j | <i>t</i> | <i>p</i> |
|-------------------|--------------------------------|----------|----------|
| Variety | -0.04 | -1.51 | 0.16 |
| Container type | 0.11 | 3.44 | 0.00 |
| Cleaning/ grading | 0.16 | 2.99 | 0.01 |
| Ripening level | 0.19 | 2.47 | 0.02 |
| Bruise severity | -0.6 | -4.69 | 0.00 |
| Incidence | 1.24 | 10.31 | 0.00 |

Alike the previous analysis, independent sample *t* test was performed, in order to determine the effect of variation in CPHMPs among ripening rooms on anthracnose incidence and severity. As a result 23.6% and 47.9% of anthracnose incidence was recorded among the two groups of ripening rooms that clean/ grade and didn't clean/ grade their fruits with the effect

size of 0.69 and 0.8 Eta^2 , respectively. Mean percentage of disease severity was 2.8% and 14.6% among the two groups with 0.79 and 0.86 Eta^2 effect size, respectively. Ripening level and bruise severity were also affected both anthracnose incidence and severity with the respective effect size of 0.77 and 0.25 on incidence and 0.53 and 0.50 on severity (Table 16). The ripening index and bruise severity on the fruits surface were factors which significantly affected anthracnose incidence and severity with the respective effect sizes of 0.8 and 0.25 Eta^2 , and severity was also found to be affected by incidence with the effect size of 0.8 Eta^2 .

Table 16. Independent sample *t* test of predictors (mean \pm SE) at ripening rooms

| CPHMPs | Groups/ means | Incidence | Eta^2 | Severity | Eta^2 |
|-------------------|---------------|------------------------------|----------------|------------------------------|----------------|
| Container type | Plastic box | - | - | 5.51 \pm 3.8 ^a | 0.12 |
| | Wooden box | - | - | 11.87 \pm 5.5 ^b | 0.43 |
| Cleaning/ grading | Yes | 23.61 \pm 3.9 ^a | 0.69 | 2.78 \pm 1.5 ^a | 0.79 |
| | No | 47.92 \pm 3.5 ^b | 0.80 | 14.6 \pm 1.7 ^b | 0.86 |

Means followed by different letters at each category of CPHMPs are significantly different at ($p= 0.05$)

Eta^2 , indicates the Cohen's effect sizes of 0.2-0.4 are small, 0.5-0.7 are medium and 0.8-1.0 are large

4.3.2.2. Retailer Shops

The regression analysis (Appendix A. Table 7) showed anthracnose incidence was significantly ($p < 0.05$) affected by predictors at retailer shops. The correlation between predictors and disease incidence was found to be positive strong ($r = 0.97$) and adjusted $R^2 = 0.95$ indicates that 95% of the variance in disease incidence was accounted for the predictors such as variety, cleaning/ grading, container type and ripening level (Table 17).

Table 17. Multiple linear regression analysis for anthracnose incidence at retailer shops

| Variables | Std. coefficients β_j | <i>t</i> | <i>p</i> |
|-------------------|--------------------------------|----------|----------|
| Variety | -0.16 | -3.74 | 0.000 |
| Fruit source | -0.14 | -1.93 | 0.08 |
| Container type | 0.31 | 3.53 | 0.001 |
| Cleaning/ grading | 0.18 | 2.61 | 0.011 |
| Fruit composition | -0.12 | -1.47 | 0.146 |
| Ripening level | 0.94 | 9.07 | 0.000 |

The regression analysis (Appendix A. Table 8) showed that anthracnose severity at retailer shops was significantly ($p < 0.05$) affected by the predictors such as variety, cleaning/ grading and ripening index and the correlation between predictors and disease severity was found to be positive strong relation ($r = 0.98$) and adjusted $r^2 = 0.92$ indicates that 92% of the variance in disease severity was accounted for predictors.

Table 18. Multiple linear regression analysis for anthracnose severity at retailer shops

| Variables | Std. coefficients β_j | <i>t</i> | <i>p</i> |
|-------------------|--------------------------------|----------|----------|
| Variety | 0.314 | 4.27 | 0.000 |
| Fruit source | -0.14 | -1.78 | 0.08 |
| Cleaning/ grading | -0.07 | -0.45 | 0.005 |
| Ripening level | -0.25 | -3.07 | 0.003 |
| Incidence | 0.373 | 1.66 | 0.102 |

The independent sample *t* test (Table 19) evident that mean percentage of anthracnose incidence and severity were significantly different among retailer shops. Anthracnose incidence were 70.9 and 26.4%; and severity were 37.7 and 19.6% at retailer shops that sale only Cavendish and other different banana varieties, respectively. Form this it was observed that incidence and severity were higher at retailers that sale only Cavendish banana and the

effect of Cavendish banana on mean percentage of incidence and severity were 1.0 and 0.96Eta², respectively which are large affect sizes.

Cleaning/ grading was also other factor significantly affected anthracnose incidence and severity. The mean percentages of incidence were 34.04 and 76.32%; and severity were 22.44 and 40.14% among retailers which did and didn't clean/ grade their fruits and it was evident that anthracnose incidence and severity were significantly different between the two groups of retailers (Table 19).

Table 19. Independent sample *t* test for predictors (mean ± SE) at retailer shops

| CPHMPs | Groups/ means | Incidence | Eta ² | Severity | Eta ² |
|-------------------|-----------------|--------------------------|------------------|--------------------------|------------------|
| Varity | Cavendish | 70.86 ± 4.0 ^a | 1.0 | 37.74 ± 2.8 ^a | 0.96 |
| | Cavendish+local | 26.38 ± 3.7 ^b | 0.92 | 19.64 ± 2.1 ^b | 0.86 |
| Container type | Wooden box | 83.94 ± 4.5 ^a | 0.99 | - | - |
| | Plastic box | 50.46 ± 3.2 ^b | 0.98 | - | - |
| Cleaning/ grading | Yes | 34.04 ± 2.8 ^a | 0.95 | 22.44 ± 2.2 ^a | 0.89 |
| | No | 76.32 ± 3.1 ^b | 0.99 | 40.14 ± 1.9 ^b | 0.96 |

Means followed by different letters at each category of CPHMPs are significantly different at (*p*= 0.05)

Eta², indicates the Cohen's effect sizes of 0.2-0.4 are small, 0.5-0.7 are medium and 0.8-1.0 are large

4.3.2.3. ET-fruit Shops

At ET-fruit shops anthracnose incidence was predicted from physical conditions of the fruits such as, ripening level and bruise severity on the fruit surfaces and the regression analysis (Appendix A. Table 9) showed that disease incidence was significantly (*p* < 0.05) affected by ripening level of the fruits. The correlation between ripening level and the disease incidence was found to be positive and strong (*r*= 0.98) and adjusted R²= 0.94 indicates that 94% of the variance in disease incidence was explained by ripening level of the fruits (Table 20).

Table 20. Multiple linear regression analysis for anthracnose incidence at ET-fruit shops

| Variables | Std. coefficients β_j | t | p |
|-----------------|--------------------------------|------|-------|
| Ripening level | 0.624 | 0.01 | 0.012 |
| Bruise severity | 0.363 | 1.82 | 0.103 |

Anthracnose severity was also predicted from its level of incidence and physical conditions of the fruits and the regression analysis (Appendix A. Table 10) showed that severity was significantly ($p < 0.05$) affected by the predictors. The correlation between predictors and the disease severity was found to be positive and strong ($r = 0.99$) and adjusted $R^2 = 0.97$ indicates that 97% of the variance in disease severity was explained by the predictors such as ripening level and anthracnose incidence.

Table 21. Multiple linear regression analysis for anthracnose severity at ET-fruit shops

| Variables | Std. coefficients β_j | t | p |
|-----------------|--------------------------------|-------|-------|
| Ripening level | 0.56 | 2.78 | 0.024 |
| Bruise severity | -0.07 | -0.43 | 0.676 |
| Incidence | 0.50 | 2.14 | 0.050 |

4.4. Isolation and Identification of the Causal Pathogen

Isolation of fungi was done from banana samples with anthracnose lesions collected from the farm, after transportation, ripening room, retailers and ET-fruit shops found in fruit and vegetable markets at *Piassa Atekelt Tera*; *Merkato Atekelt Tera*; *Kera Atekelt Tera* and ET-fruit enterprises in Addis Ababa, Ethiopia. Table 22 evident that from the prepared plates *C. musae* was dominant in association to anthracnose lesions on banana fruits. However, other fungal species such as *Penicillium*, *Fusarium*, and *Aspergillus* were identified with a total of 13, 7 and 7 percentage frequency, respectively.

Table 22. Fungal species and frequency recorded from diseased banana fruits at different stages of the study

| Sampled location/ shop | Name of identified pathogen | Percentage frequency |
|------------------------|-----------------------------|----------------------|
| Farm | <i>C. musae</i> | 96 |
| After transportation | <i>C. musae</i> | 78 |
| Ripening rooms | <i>Fusarium</i> | 6 |
| | <i>C. musae</i> | 91 |
| | <i>Penicillium</i> | 7 |
| | <i>Aspergillus</i> | 2 |
| Retailer shops | <i>C. musae</i> | 92 |
| | <i>Aspergillus</i> | 5 |
| | <i>Penicillium</i> | 2 |
| | <i>Fusarium</i> | 1 |
| ET-fruit shops | <i>C. musae</i> | 96 |
| | <i>Penicillium</i> | 4 |

From result above it is clear that *C. musae* was found to be an important pathogen responsible to cause banana anthracnose in all stages of the current study. Occasionally the fungus was seen invading the green fingers, when damaged by flex and the lesions were sunken and covered with salmon-colored. On ripening fingers lesions with sunken and brown spots were developed with orange acervuli.

4.5. Pathogenicity of *Colletotrichum musae* Isolate

In order to confirm whether the pathogen is associated to banana anthracnose, pathogenicity test was conducted on Dwarf Cavendish banana variety. As a consequence lesions were developed on inoculated fruit but not on un-inoculated fruits. Symptoms produced by the pathogen were appeared as black sunken lesion circling the wounded and inoculation spots often symptom was distributed all over the outer part of the fruits. The fungus was developed as acervuli with concentric rings, sporulating with masses of pinkish conidia on some of inoculated fruits.

After lesions are developed and characterize on artificially inoculated fruits the pathogen was re-isolated and was studied for its colony characteristic and conidial morphology. From this colony of the isolate was observed as loose and white aerial mycelium, which became orange with age. The mean length and width of conidia were $13\mu\text{m} \times 6\mu\text{m}$ with aseptate cylindrical shape. Based on these morphological characteristics, the pathogen associated with banana anthracnose identified as *C. musae*.

4.6. Characterization of the fungus

4.6.1. Cultural Characteristics of the Isolates

Cultural characteristics of six *C. musae* isolates three from Arba-Minch and three from Addis Ababa were studied on PDA. The results confirmed that the highest 80 mm of radial colony growth was recorded on isolate ACI2 and the lowest radial colony growth of 48 mm was observed on isolate AET6. Regardless of isolate ACDI3 and AET6 the four isolates of *C. musae* exhibited significant ($p < 0.05$) difference in colony color on PDA. Blackish white colony color was dominantly produced by 71.6, 90, 63.4 and 90% of replicates of ALI1, ACDI3, ARSI5 and AET6 isolates, respectively. All the replicates of isolate ACDI3 which was obtained from Arba-Minch *channo dorga kebele* and AET6 which was obtained from ET-fruit shop in Addis Ababa were produced blackish white colony color with black substrate color and were characterized by flat mycelium growth and they were significantly ($p < 0.05$) different in colony color, substrate color and colony texture from the other isolates. All the replicates of each isolates were regular in their colony shape and they were not significantly ($p > 0.05$) different from each other (Table 23).

Table 23. Frequency of Cultural characteristics of six *C. musae* isolates on PDA

| Isolates | Colony color | | | Substrate color | | | Colony texture | | Colony shape | Colony margin | | Colony diameter |
|--------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------------------|
| | BW | WP | LO | B | PB | DO | RFMG | FMG | RWS | S | I | (mm) |
| ALI1 | 71.6 ^b | 18.4 ^d | 0.0 ^b | 71.6 ^b | 18.4 ^a | 0.0 ^b | 18.4 ^c | 71.6 ^b | 90.0 ^a | 90.0 ^a | 0.0 ^c | 65.0 ^{bc} |
| ACI2 | 26.6 ^c | 59.8 ^a | 26.6 ^a | 26.6 ^c | 59.8 ^d | 26.6 ^a | 63.4 ^a | 26.6 ^d | 90.0 ^a | 26.6 ^c | 63.4 ^a | 80.0 ^a |
| ACDI3 | 90.0 ^a | 0.0 ^e | 0.0 ^b | 90.0 ^a | 0.0 ^b | 0.0 ^b | 0.0 ^d | 90.0 ^a | 90.0 ^a | 90.0 ^a | 0.0 ^c | 51.0 ^d |
| ARR4 | 39.2 ^d | 39.2 ^b | 26.6 ^a | 39.2 ^d | 39.2 ^c | 26.6 ^a | 50.8 ^b | 39.2 ^c | 90.0 ^a | 39.2 ^b | 50.8 ^b | 61.0 ^c |
| ARSI5 | 63.4 ^c | 26.7 ^c | 0.0 ^b | 63.4 ^c | 26.7 ^d | 0.0 ^b | 63.4 ^a | 26.6 ^d | 90.0 ^a | 90.0 ^a | 0.0 ^c | 72.0 ^b |
| AET6 | 90.0 ^a | 0.0 ^e | 0.0 ^b | 90.0 ^a | 0.0 ^d | 0.0 ^b | 0.0 ^d | 90.0 ^a | 90.0 ^a | 90.0 ^a | 0.0 ^c | 48.0 ^d |
| Means | 63.39 | 24.02 | 8.85 | 63.39 | 24.02 | 8.85 | 32.68 | 57.32 | 90.0 | 70.97 | 19.04 | 62.83 |

Colony color BW= blackish white, WP= white pinkish, LO= light orange

Substrate color B= black, PB= pinkish black, DO= dark orange

Colony texture RFMG= raised fluffy mycelium growth, FMG= flat mycelium growth

Colony shape RWS= regular without sector

Colony margin S= smooth, I= irregular

Means followed by common letters are not significantly different at 5%, LSD=7.04

4.6.2. Morphological Characteristics of the Isolates

Morphological characteristics of the six isolates were studied from cultures grown on PDA for the reason that the medium supports maximum colony growth and it was recommended in most research outputs. The shape and size of the isolates were examined under microscope and the conidial masses were generally coalesced together. Table 24 evident that conidia were aseptate, cylindrical or ellipsoid and the length ranged from 7 to 19 μm . The highest length of conidia which is 19 μm was observed on ARRC4 isolate obtained from Addis Ababa. Width of conidia ranged from 4 to 9 μm and ARSC5 isolate from Addis was observed with the highest conidium width of is 9 μm .

Table 24. Morphological characteristics of *C. musae* isolates on PDA

| Isolates | Conidia | | | Appressoria | | |
|----------|-------------|------------------------|------------------|-------------|------------------------|---------------------|
| | Shape | Size (μm) | | Shape | Size (μm) | |
| | | L | W | | L | W |
| ALC1 | Ellipsoidal | 12.2 ^b | 5.8 ^a | Irregular | 7.1 ^b | 8.20 ^c |
| ACC2 | Cylindrical | 12.6 ^b | 6.7 ^a | Round | 7.1 ^b | 9.0 ^{bc} |
| ACDC3 | Cylindrical | 12.9 ^{ab} | 6.2 ^a | Irregular | 7.0 ^b | 9.1 ^{bc} |
| ARRC4 | Ellipsoidal | 12.5 ^b | 7.0 ^a | Irregular | 10.3 ^a | 11.0 ^{ab} |
| ARSC5 | Cylindrical | 13.3 ^{ab} | 7.6 ^a | Irregular | 10 ^a | 12.2 ^a |
| AEC6 | Cylindrical | 15.5 ^a | 7.5 ^a | Round | 10 ^a | 10.4 ^{abc} |
| Mean | | 13.17 | 6.8 | | 8.58 | 9.98 |

Mean morphological characteristics of ten conidia and appressoria

Means followed by common letters are not significantly different at $p=0.05$ by DMRT LSD for length and width of conidia and appressoria, 2.6

The shape and size of appressorium were characterized by having round and/ or irregular shape developed from the mycelia and/ or directly from germ tubes. The length of appressorium was ranged from 5 to 14 μm and its width was ranged from 5 to 16 μm . The highest length of appressorium, 15.5 μm was recorded on AEC6 isolate obtained from Addis.

4.7. Evaluation of Banana Varieties to Wound and Quiescent Anthracnose

Reactions of different detached banana varieties to wound and quiescent anthracnose was evaluated through inoculating suspension of anthracnose conidia on artificially created wounds and on the surface of the fruit as a result, regardless of the giant and dwarf Cavendish varieties, all tested varieties were significantly ($p < 0.05$) different from each other in their rotted surface area for wound anthracnose (Table 25). The so-called William I variety was severely rotted or susceptible to wound anthracnose with rotted surface area of 3522.2 mm², followed by the two varieties from Cavendish banana (giant & dwarf) with 2303.4 and 2346.5 mm² of rotted surface area. The rest of the varieties, Butaza and Poyo were manifest anthracnose symptom comparatively on a slighter surface of 1505.7 and 1604.6 mm² rot area to wound anthracnose. On the other hand, from tested banana fruits Poyo and Dwarf Cavendish varieties were not significantly ($p > 0.05$) different for quiescent anthracnose with 1347.1 and 1322.7 mm² of rotted area however, William I and Butaza were recorded with 2521.9 mm² and 732.8 mm² rotted surface area for quiescent anthracnose.

Table 25. Mean rotted surface area of banana fruits for wound and quiescent anthracnose (Mean±SE)

| Variety | Rotted area (mm ²) | | |
|-----------------|--------------------------------|----------------------------|----------------------------|
| | Control | Inoculation with wound | Inoculation without wound |
| Poyo | 241.9 ^b ±15.6 | 1604.6 ^c ±7.5 | 1347.12 ^c ±29.5 |
| William I | 414.6 ^a ±11.6 | 3522.2 ^a ±109.8 | 2521.9 ^a ±24.1 |
| Butaza | 178.6 ^c ±13.7 | 1505.7 ^d ±14.7 | 732.8 ^d ±25.5 |
| Giant Cavendish | 243.9 ^b ±13.3 | 2303.4 ^b ±11.2 | 1517.8 ^b ±14.2 |
| Dwarf Cavendish | 227.7 ^b ±8.9 | 2346.5 ^b ±59.5 | 1322.7 ^c ±31.3 |

F value = 3851.39 p value < 0.0001 CV = 2.74 R² = 0.99 LSD = 46.13

Means followed by the same letter/s are not significantly ($p < 0.05$) different from each other

5. DISCUSSION

Incidence and severity were used as a tool for measuring the status of anthracnose at farm, after transportation, ripening rooms, retailer and ET-fruit shops found in fruit and vegetable markets of Addis Ababa. During farm level study, anthracnose incidence and severity were 9.8 and 4.1%, respectively. The ripening index of the fruits and bruise severity were 1.0 and 5.9%, respectively. These results agreed with the report by Self *et al.* (2002) who found 3.1% and 1.6% of anthracnose incidence and severity, respectively on green banana fruits. In addition to this, the fungus mentioned to start quiescent infection at farm on green banana fruits however, the previous studies by Jegger *et al.* (1995) evident that successful penetration of the fungus is restricted by accumulation of phytoalexins as the fruit ripen and it was particularly mentioned to be become important on wounded green fingers with scratches and wounds caused by handling processes (Haque *et al.*, 2003; Alvindia *et al.*, 2008; Chang *et al.*, 2009 and Elsalam *et al.*, 2010).

Surveyed farmers were employed almost similar traditional management methods (Table 3) and the fruits were subjected to physical damages/ bruises which are important factors for the disease development and this was in agreement with the findings of Mbaka *et al.* (2006) who observed farmers with similar traditional pre and postharvest management methods affecting the incidence and severity of major diseases of passion fruit in Kenya. From evaluated CHPHMPs by the farmers and physical damages on the fruits, the study evident that harvesting methods, cleaning/ grading and bruise severity were significantly ($p < 0.05$) affected the disease at farm (Table 4 and 5). The independent sample *t* test evident that, mean percentages of anthracnose incidence and severity were significantly different among farmers harvested bunches with support and without support (Table 6). This result was supported by Bryant *et al.* (2001) who found significant difference in severity of mechanical wound and disease incidence among the fruits dropped form a height of $< 30\text{cm}$ and $\geq 30\text{cm}$ onto hard surfaces, or $< 60\text{cm}$ and $\geq 60\text{cm}$ onto other fruit.

The study which was done after the fruits transported to the markets showed that fruits were brought from two sources Arba-Minch and Asebe-Teferi (Table 8). Open Isuzu trucks were the major means of transportation to convey the fruits and travelled a distance of 238 to

505Km from production areas. Percentage of ripening index of the fruit was 1.1 and bruise severity index was 8%. Anthracnose incidence and severity were 4.9 and 1.6%, respectively (Table 7). Fruit source, fruit cover, the distance travelled and bruise severity on the fruits surface were factors significantly ($p < 0.05$) affected banana anthracnose after transportation. The current results were in line with the report by Llyas *et al.* (2007) that showed 13% and 9% of banana loss due to mechanical injuries and anthracnose disease, respectively during transportation from production area to whole sale markets and it was concurred by Elsalam *et al.* (2010) which indicate significant rots on bananas due to anthracnose under conducive temperature, relative humidity and mechanical injuries in relation to postharvest handling methods during long distance transportation even when the fruits are green. In addition to this Magalhaes *et al.* (2004) reported that the internal and external quality of banana fruits to be affected when ripe due to mechanical injuries created by compression and vibration (shaking) of the vehicle especially on bad parts of the road and wounds induced ethylene production and the subsequent hastening of ripening and anthracnose infection during transportation.

The study at ripening rooms, retailer and ET-fruit shops showed that the mean percentage of disease incidence were 30.4, 70.8 and 51.3%; and severity were 2.6, 31.7 and 20.7%, respectively. The result obtained was similar with the report by Haque *et al.* (2003) who found 5% and 49.9% of incidence and severity anthracnose, respectively on marketed banana fruits. In addition to these, Hossain *et al.* (2010) reported 13% and 83% incidences of banana anthracnose at commercial ripening rooms and retailer shops, respectively. At this point of the study it was evident that the highest 51.8% of bruise severity and 6.1 of ripening index were recorded at retailer shops which were scored the highest disease incidence and severity (Table 12) and this was agreed with the reports by Jinyoung *et al.* (2002) that indicates anthracnose to become more severe when the fruits are ripened (ripening index of 5 to 7).

From the CPHMPs (Customary Harvest and Postharvest Management Practices) of the merchants and PCF (Physical condition of the fruits) variety, fruit source, container type, cleaning/ grading, ripening level and bruise severity were factors significantly ($p < 0.05$) affected anthracnose on banana fruits. Independent sample *t* test was evident significant difference in mean percentage of anthracnose incidence and severity among merchants that clean/ grade and didn't clean/ grade their fruits (Table 16 and 19). Cleaning/ grading had been

mentioned as an important task to avoid injured, over ripen and diseased fruits (Palipane and Rolle, 2008) thereby can reduce disease incidence and severity through eliminating fungal propagule on wounded or diseased fruit surface and the current result is in agreement with the report by Gustavo, (2003) who found 7.1 and 11.8% of anthracnose severity on cleaned and didn't cleaned collections of banana fruits, respectively.

Types of containers used by merchant at ripening and retailer shops were other factors significantly ($p < 0.05$) affected anthracnose. At ripening rooms the mean percentages of anthracnose severity were significantly different (Table 16) and incidence was significantly different among merchants that used plastic and wooden boxes at retailer shops (Table 19). The highest anthracnose incidence and severity were recorded at merchants that used wooden boxes to handle banana fingers. Although using the plastic or wooden boxes to handle perishable fruits have their own disadvantages, the previous studies by FAO (1989) showed that using wooden box could increase anthracnose severity through increasing mechanical wound/ bruise because of its' rough surfaces, sharp edges, splinters and protruding nails in comparison to plastic boxes which have smooth surface without any sharp edges that could create mechanical bruise on the fruits surface in addition to this plastic boxes can be washed or cleaned whenever they are dirt or contaminated which is vital to eliminate microorganisms including *C. musae*. Furthermore, Da Costa *et al.* (2010) ranked cardboard, torito + cardboard and plastic boxes from one up to three in their effect of creating mechanical injury/ bruise on banana pulp.

Bruise severity was physical factor on the fruit that was significantly ($p < 0.05$) affected anthracnose incidence and severity at farm, after transportation and ripening rooms. The bruise created on the fruits surface was estimated to be created through pre and postharvest mismanagement practices at farm before harvesting, during harvesting, transportation from production area to ripening rooms by farmers and merchants. Similar study output was reported by Chillet *et al.* (2006) that indicate mechanical bruises on banana fruits created due to management practices were the major factor for wound anthracnose development throughout the chain of commercialization from producing areas to the sales markets. In addition to this (Panhwar, 2006) reported that the rate of water loss/ respiration, heat

production, ripening and infection by *C. musae* were found to be enhanced by mechanical bruise on banana fruits.

Ripening level was major physical factor significantly ($p < 0.05$) affected anthracnose at ripening, retailer and ET-fruit shops. The current study evident that anthracnose incidence and severity were greater on ripen fruits at retailer and ET-fruit shops than unripe once at farm and ripening rooms. This result was in agreement with the reports by Jinyoung *et al.* (2002) and Elsalam *et al.* (2010) that anthracnose was major disease of ripening and ripe bananas at markets and they found the disease to be more severe when the bananas were ripened and they were notice that ripening to be stimulated by anthracnose infection on green fruits and lesions were elongated with ripening.

Anthracnose incidence by itself was significantly ($p < 0.05$) affected anthracnose severity at farm, ripening rooms and ET-fruit shops. During survey, farm fields and ripening rooms were seen with unhygienic and careless handling of the fruits and residuals of banana plant were much enough to cause more incidences thereby severity. This was similar with observations made by Dadzie, (1997) and Palipane and Rolle, (2008) in which the pathogen was found to be common in ware houses/ storages and ripening room with poor hygiene/ sanitation. Since *C. musae* is commonly found as endophytes on healthy plants and as saprobes on dead plant material it is obvious that the pathogen was capable of contaminating the fruits and cause severe infection by the fact that the more virulent, abundant, and active the pathogen, the longer the pathogen side would be and the greater the potential amount of disease and this was also agreed with observations made by Promputtha *et al.* (2002) and Photita *et al.* (2004). Generally, it had been indicated that the more abundant the primary inoculum and the closer it is to the fruits, the more severe the disease and the losses that result (Agrios, 2005).

At retailer shops anthracnose incidence and severity were significantly ($p < 0.05$) affected by the fruits variety. During survey at the markets identification of banana variety was very difficult because of absence of recorded data regarding the fruit varieties however, it was identified considering the morphology, fruit source and reply obtained from sealers. There were two groups of retailers that soled Cavendish (dwarf and giant) and Cavendish together with other local varieties (Table 13) and the independent sample *t* test evident significant ($p <$

0.05) difference in mean percentages of anthracnose incidence and severity among the two retailers groups (Table 19). The result obtained was supported by Bellaire *et al.* (2008) who reported genotypes of bananas differed in their resistance to anthracnose caused by *C. musae*.

The study of identification of causal pathogen of banana anthracnose evident that anthracnose of banana fruit caused by filamentous fungal pathogen *Colletotrichum musae* was the main postharvest disease at farm (after harvest), after transportation, ripening rooms, retailer and ET-fruit shops (Table 22) on lesions of anthracnose. The result obtained in the current study was agreed with the reports by Latiffah *et al.* (2009), Hossanin *et al.* (2010), and Thangamani *et al.* (2011) who reported *C. musae* in relation to anthracnose lesions on banana fruits and it had been mentioned as the main disease on wounded green and ripe banana fruits. However, other fungal species such as *Fusarium*, *Penicillium* and *Aspergillus* were identified from anthracnose lesions on diseased fruits. Among the *Fusarium* spp: *Fusarium semitectum*, *Fusarium roseum* and *Fusarium pallidoroseum* were reported as postharvest diseases associated with crown rots of banana and plantains (Dadzie, 1997).

In the pathogenicity test symptoms produced by the pathogen were appeared as black sunken lesion circling the wounded and inoculation spots. Often symptom was distributed all over the outer part of the fruits. The fungus was developed as black acervuli with concentric rings, sporulating with masses of pinkish conidia on some of inoculated fruits. In this study the results obtained on artificially inoculated fruits were agreed with the results obtained by Jinyoung *et al.* (2002), Photita *et al.* (2005) and Thangamani *et al.* (2011). The colony of isolate taken from inoculated fruit was loose with white mycelium, which becomes orange in age that was culturally similar to that previously described by Photita *et al.* (2005). The mean length and width of conidia were $13\mu\text{m} \times 6\mu\text{m}$ with aseptate cylindrical shape and this result was also in agreement with the reports by Jinyoung *et al.* (2002) and Elsalam *et al.* (2010).

The study of cultural characteristics of *C. musae* isolates from Arba-Minch and Addis Ababa showed presence of significant difference in their colony color, substrate color, colony texture, colony margin and radial colony growth on PDA. Isolates were frequently observed with blackish white and white pinkish colony color (Table 23). The present findings are in similarity with the report by Amarjit *et al.* (2006) who observed isolates with blackish white,

light pink and dark orange coloured colonies. Isolates were produced black, pinkish black and dark orange substrate colors and significant difference was observed among isolates except ACDI3 and AET6. Similar results were obtained by Thangamani *et al.* (2011) in which the isolates produced pinkish black, black and dark orange substrate colors. Isolates were recorded with raised fluffy and flat mycelium growth and some of them were significantly different in their colony texture however, colony of all the isolates were characterized by regular shape without sector and no significant difference was observed (Table 23). Similar observations were made by Kuramae *et al.* (1997), Manjunath (2009) and Thangamani *et al.* (2011).

Morphological characterization of *C. musae* isolates was revealed that the shape of conidia were aseptate, mostly cylindrical sometimes ellipsoidal and the mean length and width of conidia were 13.2 X 6.8 µm and significant difference was observed among some of the isolates (Table 24). These characteristics agreed with the descriptions given by Jinyoung *et al.* (2002) and Latiffah *et al.* (2009) *C. musae* with short conidiophores bearing single cylindrical conidia in size of 14.7 x 7.1 µm. In the present study appressorium were characterized by round and irregular shapes and was developed directly from germ tubes and the mean length and width were 8.6 x 10.1 µm and significant difference was observed between isolates. The result obtained was in harmony with those reported by Thangamani *et al.* (2011).

The result obtained from experiment of evaluating banana varieties to wound and quiescent anthracnose was evident that William I, was severely rotted or found to be susceptible to both types of anthracnose and it was significantly ($p < 0.05$) different from the other four varieties (Table 25). All the evaluated varieties showed significant ($p < 0.05$) difference in amount of rooted surface area due to wound and quiescent anthracnose regardless of the giant and dwarf Cavendish varieties for wound anthracnose. The result obtained was not possible be confirmed with other experimental outputs regarding the tested banana varieties because of limited accessibility of published information. However, Bellaire *et al.* (2008) reported that banana genotypes were varied in their resistance to anthracnose. In addition to this the current study revealed that all tested banana varieties were severely rotted in wound anthracnose and similar results were obtained by Chillet *et al.* (2006) and Lassosis *et al.* (2010).

6. SUMMARY AND CONCLUSIONS

6.1. Summary

Banana is one of the most important horticultural crops in Ethiopia grown at various agro-ecological zones by small holders, private commercial farms and state owned large-scale plantations. The fruit accounts for 60.6% of cultivated land in fruit production and in 2008, from a total of nearly 3.5 million quintals of fruits banana was constituted the lion share with about 55.3%. It is widely produced by small-scale farmers in Southern and South Western Ethiopia along the Keffa-Bench Maji axis and throughout the main *Enset* growing areas. The crop is mainly cultivated for their fruit and used for food and to supplement the household income.

However, production is suffering lots of problems of which diseases in plantations and after harvest are the major. The crop had been reported to decline in its productivity while in the field with *Xanthomonas* wilt; black leaf streak and *Fusarium* wilt diseases in many banana producing countries of Eastern Africa. In addition to these, postharvest diseases were reported causing significant loss of produced fruits in many developing countries due to poor postharvest management practices. From the postharvest diseases of banana fruit, anthracnose caused by *Colletotrichum musae* which attack the fruits during growth in the field is being the major problem. The infections remain quiescent until the fruit ripens, causing symptom development and substantial decay losses during storage and marketing. The disease was reported to cause a range of 2 to 5% and 11 to 13% rot in incidences with a range of 24.7 to 49.9% rot severity on marketed banana fruit in different countries around the globe. Furthermore, it was mentioned to cause crown rot on banana fruits together with other fungal pathogens.

Pre and postharvest management practices such as field sanitation, variety grown, harvesting equipments and methods, transportation, general hygiene in package houses and storages and methods of packaging by the growers and traders had been mentioned to play significant role on the disease. Physiological condition of the fruits such as wound/ bruise on the fruit surface and early ripening of the fruit caused by improper management practices are also provide suitable conditions for the disease infections.

Therefore the current study was conducted to address these gaps to some extents, through determining the status of incidence and severity of anthracnose along with customary pre and postharvest management practices starting from farms around *Arba-Minch* which is major zone of banana production in the country, after transportation up to fruit and vegetable markets found in Addis Ababa where nearly all types of fruits and vegetables produced around the country were sealed. Field visits were also undertaken at surveyed farms and markets and relevant information regarding the management practices were collected. And banana samples were collected and transported to Laboratory of Plant Pathology at JUCAVM for further studies.

Based on this the study revealed that anthracnose was an important postharvest disease of banana fruits and the result obtained in the current study was agreed with the reviewed literatures that showed the importance of anthracnose disease at commercial ripening rooms and retail markets of banana fruits. Surveyed farmers and merchants were employed traditional pre and postharvest management practices which were found to be more or less similar with other survey study outputs and it was evident that the disease incidence and severity were found to be significantly affected by management practices all the way through commercialization processes by the producers and merchants. The independent sample *t* test also evident banana fruits at farmers and merchants that employed better management practices were recorded with lower mechanical injuries and anthracnose incidence and severity. From evaluated CHPHMPs; harvesting methods, cleaning/ grading, sources of the fruits, fruit covers during transportation, the distance travelled, container type and from PCF; ripening level and bruise severity were in general factors significantly ($p < 0.05$) affected anthracnose development on banana fruits.

The previous study outputs of cultural and morphological characteristics reported that the presence of differences and similarities regarding colony colour, substrate colour, colony texture, colony margin, size of conidia and appressorium among collections of *C. musae* isolates grown on PDA. The present study obtained similar results which indicate significant variation among isolates from the two locations. However, no significant differences in cultural and morphological characteristics were observed among some of the isolates.

Banana varieties were reported to be different in their resistance to anthracnose disease caused through mechanical bruise and along with the fruit ripening. This was agreed with the current result which evident the variation in susceptibility/ resistance among the tested varieties. In general all the fruits tested for wound anthracnose were observed with higher disease severity (rotted surface area) than unwounded once. From evaluated varieties William I, was severely rotted/ susceptible for both wound and quiescent anthracnose on the other hand, Butaza was recorded with the least total rotted surface area for both types of anthracnose.

6.2. Conclusions

Anthracnose caused by *C. musae* was found to be an important postharvest disease causing significant rots and resultant loss of fruits in quality and quantity especially on marketed banana fruits in Addis Ababa. The disease was significantly affected by the CHPHMPs by farmers and traders and physical conditions of the fruits (mechanical bruise and ripening level). In addition to these evaluated banana varieties were found to be severely rooted to anthracnose caused by mechanical bruises than unwounded once however they were different in their resistance.

Therefore in order to alleviate the qualitative and quantitative losses of banana fruits due to wound and quiescent anthracnose:

- Further studies on banana anthracnose are required to be conducted and it would be better to quantify the consecutive loss of the fruit and determine the CHPHMPs that could affect the disease at farm and market levels.
- It would be better farmers (farmers' union) and merchants to be trained by the stalk holders to advance their awareness regarding the disease in relation to their CHPHMPs as the ET-fruit enterprise dose.
- Further studies in large scale are required on banana varieties which are being evaluated for adaptation and those which already are on farmers' hand, for their reaction to wound and quiescent anthracnose.

REFERENCE

- Abebe Belachew, 2000. Dry-spells analysis for studding the sustainability of rain-fed agriculture in Ethiopia: The Case Study of Arba Minch Area.
- Abreham Tadesse (ed.), 2009. Increasing crop production through improved plant protection. Vol. II. Plant Protection Society of Ethiopia (PPSE). PPSE and EIAR, Addis Ababa, Ethiopia. 542pp.
- Addis T., F. Handoro and G. Blomme, 2004. Bacterial Wilt (*Xanthomonas campestris* pv. *musacearum*) on *Enset* and Banana in Ethiopia. *Info Musa* **13(2)**: 44-45.
- Adugna, D., D. Gerba, B. Diriba and T. Kassaye, 2011. Identification of major causes of post-harvest losses among selected fruits in Jimma zone for proffering veritable solutions. *International Journal of Current Research*. **3(11)**: pp.040-043
- Agrios, G.N., 2005. Plant pathology. Elsevier Academic Press, Burlington. 5th ed. pp.81
- Alvindia, D. G., T. Kobayashi, Y. Yaguchi and K.T. Natsuaki, 2000. Symptoms and the associated fungi of postharvest diseases on non-chemical bananas imported from the Philippines. *Jpn. J. Trop. Agric.* **44**: 87-93.
- Alvindia, D.G. and K.T. Natsuaki, 2008. Evaluation of fungal epiphytes isolated from banana fruit surfaces for bio-control of banana crown rot disease. *Crop Protection*. **27**: 1200-1207.
- Amarjit, S., K.S. Verma and M. Chander, 2006. Effect of different culture media on the growth and sporulation of *Colletotrichum gloeosporioides* causing guava anthracnose. *Plant Disease Research*, 21: 224.
- Anthony, S., K. Abeywickrama, R. Dayananda, S.W. Wijeratnam and L. Arambewela, 2004. Fungal pathogens associated with banana fruit in Sri Lanka, and their treatment with essential oils. *Mycopathologia* **157**: 91-97.
- Baiyewu, R.A., N.A. Amusa, O.A. Ayoola and O.O. Babalola, 2000. Survey of the post-harvest diseases and aflatoxin contamination of marketed Pawpaw fruit (*Carica papaya* L) in south western Nigeria. *Afric. J. Agri. Rese.* **2(4)**, pp. 178-181.
- Bryant, P.H., C. McConchie and R. McConchie, 2001. Effect of fruit hydration on lychee response to impact injury. *Proceedings of the Australasian Post-harvest Conference*, Adelaide.
- Bussaban, B., S. Lumyong, P. Lumyong, T. Seelanan, D.C. Park, E.H.C. McKenzie, K.D. Hyde, 2005. Molecular and morphological characterization of *Pyricularia* and allied genera. *Mycologia*. **97**: 1002-1011.

- Charles, D. and J.H. New, 1996. Packaging for export from developing countries: developments in packaging. Wind ward Islands bananas. *Postharvest News and Information*. **7**: 25-30.
- Cheng, B.Ma, T. Wan-Li, Li-Yan Ma, Ling-Ling Li, Lu-Bin Zhang and Shi-Jiang Zhu, 2009. The role of chitinase gene expression in the defence of harvested banana against anthracnose disease. *J. Amer. Soc. Hort. Sci.* **134(3)**: 379-386.
- Chillet, M. and B.L. Lapeyre, 2002. Variability in the production of wound ethylene in bananas from the French West Indies. *Sci. Hortic.* **96**: 127-137.
- Chillet, M., O. Hubert, M.J. Rives and B.L. Lapeyre, 2006. Effects of the physiological age of bananas on their susceptibility to wound anthracnose due to *Colletotrichum musae*. *Plant Dis.* **90**:1181-1185
- Coates, L.M., G.I. Johnson and A.W. Cooke, 1993. Postharvest disease control in mangoes using high humidity hot air and fungicide treatments. *Ann. Appl. Biol.* **123**: 441-448.
- CSA (Central Statistics Agency), 2004. Biotechnology and food security in developing countries. Biotechnology and Molecular Biology Central statistics Agency. Agricultural sample survey, Statistical Bulletin Number **302**. Addis Ababa, Ethiopia.
- CSA (Central Statistics Agency), 2010. Area and production of crops: Private peasant holdings, meher season. Agricultural sample survey. **6**: pp 15.
- Da Costa, F.B., R. Puschmann, S.I. Moreira, J.I.R. Junior and F.L. Finger, 2010. Survey of mechanical injury in 'Prata Ana' banana during shipping. *Revista Verde (Mossoró-RN-Brasil)*. **5(1)**: pp.72-78.
- Dadzie, B.K. et J.E. Orchard, 1997. Routine postharvest screening of banana/ plantain hybrids: criteria and methods. INIBAP Technical Guidelines 2. International Plant Genetic Resources Institute, Rome, Italy; International Network for the Improvement of Banana and Plantain, Montpellier, France; ACP-EU Technical Centre for Agricultural and Rural Cooperation, Wageningen, The Netherlands.
- Damm, U., R. Baroncelli, Lei Cai, Y. Kubo, R. O'Connell, B. Weir, K. Yoshino and P.F. Cannon, 2010. *Colletotrichum*: specious, ecology and interactions. *Article info.* **1(2)**: 161-165.
- Dodd, J.C., A.B. Estrada, J. Matcham, P. Jeffries and M.J. Jeger, 1991. The effect of climatic factors on *Colletotrichum gloeosporioides*, causal agent of mango anthracnose, in the Philippines. *Plant Pathology* **40**: 568-575.
- Dodd, J.C., D. Prusky and P. Jeffries, 1997. Fruit diseases. In: R.E. Litz (ed.). *The Mango: Botany, Production and Uses*. CABI., pp. 257-280.

- EHDA (Ethiopian Horticultural Development Agency), 2011. Exporting fruit and vegetables from Ethiopia. Assessment of development potentials and investment options in the export-oriented fruit and vegetable sector. pp. 20-25. Ethiopian Horticultural Development Agency March 15, 2011.
- Elsalam, K.A., S. Roshdy, O.E. Amin and M. Rabani, 2010. First morphogenetic identification of the fungal pathogen *Colletotrichum musae* (Phyllachoraceae) from imported bananas in Saudi Arabia. *Genet. Mol. Res.* **9** (4): 2335-2342.
- FAO, 1989. Prevention of post-harvest food losses of fruits, vegetables and root crops training manual. Food and Agriculture Organization of the United Nations, Rome.
- FAO, 2004. Statistical database: Food and Agricultural Organization of United Nations, Rome.
- FAO, 2010. Statistics division of crops production. <http://faostat.fao.org>. Last accessed 2010-11-23.
- Fitzell, R.D. and C.M. Peak, 1984. The epidemiology of anthracnose disease of mango: inoculum sources, spore production and dispersal. *Annals of Applied Biology* **104**:53-59.
- Fitzell, R.D., 1979. *Colletotrichum acutatum* as a cause of anthracnose of mango in New South Wales. *Plant Disease Reporter* **63**:1067-1070.
- Frison, E.A., C. S. Gold, E. B. Karamura and R. A. Sikora, 1998. Mobilizing IPM for sustainable banana production in Africa. pp. 5-8. Proceedings of a workshop on banana IPM held in Nelspruit, South Africa 23-28 November 1998.
- Gustavo, V.B.C., J. Juan, M. Fernández, M.A. Stella, S.T. Maria, L.M. Aurelio, W.C. Jorge, 2003. Handling and preservation of fruits and vegetables by combined methods for rural areas. Technical manual FAO agricultural service bulletin 149, Rome.
- Haque, A.M., K.M. Khalequzzaman, M.I. Shariful and M.M. Hossain, 2003. Survey the prevalence of market diseases of banana. *Pakistan journal of plant pathology.* **2**(3): 169-173.
- Hilton, D.J. 1994. Impact and vibration damage to fruit during handling and transportation. In: B.R. Champ, E. Highley and G.I. Johnson, (eds.). Proceedings of the Post-harvest Handling of Tropical Fruits. Australian Centre for International Agricultural Research. pp.116-26.
- Hossain, M. T., S.M.M. Hossain, M.A. Bakr, A.K.M., M. Rahman and S.N. Uddin, 2010. Survey on major diseases of vegetables and fruit crops in Chittagong region. *Bangladesh J. Agril. Res.* **35**(3): 423-429

- Hyde, K.D. Cai L., P.F. Cannon, J.A. Crouch, P.W. Crous, U. Damm, P.H. Goodwin, H. Chen, P.R. Johnston, E.B.G. Jones, Z.Y. Liu, E.H.C. McKenzie, J. Moriwaki, P. Noireung, S.R. Pennycook, L.H. Pfenning, H. Prihastuti, T. Sato, R.G. Shivas, Y.P. Tan, P.W.J. Taylor, B.S. Weir, Y.L. Yang, J.Z. Zhang, 2009. *Colletotrichum*- names in current use. *Fungal Diversity*. **39**: 147–182.
- Jeffries, P. and M.J. Jeger., 1990. The biological control of postharvest diseases of fruit. *Biocontrol News Info*. **11**: 333-336.
- Jeger, M.J., S. Eden-Green, A. Johanson, J.M. Waller and A.E. Brown, 1995. Banana diseases. In: Chapman and Hall (eds.). *Banana and Plantains*. 317-381. London, UK:.
- Jinyoung Lim, Tae Heon Lim and Byeongjin Cha, 2002. Isolation and identification of *Colletotrichum musae* from imported bananas. *Plant Pathol. J.* **18(3)** : 161-164
- Jones, D.R. and W.R. Slabaugh, 1998. Anthracnose and fungal scald. In: R.C. Ploetz, G.A. Zentmyer, W.T. Nishijima, K.G. Rohrbach and H.D. Ohr (eds.). *Compendium of tropical fruit diseases*. Minnesota, USA: *American Pathological Society Press*. pp 4-5.
- Kader, A.A. and S.R. Rosa, 2004. The role of post-harvest management in assuring the quality and safety of horticultural produce. Food and Agriculture Organization, agricultural bulletin. **152**: pp7-12
- Kuramae, E.E., C.R. Lopes, N.L. Souza and Machado, 1997. Morphological and molecular characterization of *Colletotrichum* spp. from citrus orchads affected by post bloom fruit drop in Brazil. *European Journal of Plant Pathology*. **103**: 323-329.
- Labavitch, J.M., 1998. Fruit ripening and defense against pathogens-loss of resistance or gain of susceptibility. In: Johnson, G.I., Highley, E., Joyce, D.C. (eds.), *Disease Resistance in Fruit*, ACIAR Proceedings. Canberra, pp. 53-60.
- Lassois, L., M.J. Haissam, C. Marc, 2010. Crown rots of bananas. Preharvest factors involved in postharvest disease development and integrated control methods. The American Phytopathological Society. *Plant Disease*. Vol. **94**: No. 6
- Latiffah, Z., S. Shamsiah, Z. Maziah and S. Baharuddin, 2009. Characterization of *Colletotrichum* species associated with anthracnose of banana. *Tropical Life Sciences Research*. **20(2)**: 119-125.
- Lindy Coates and Greg Johnson, 2003. Post harvest disease of fruit and vegetables.
- Llyas, M. B., M.U. Ghazanfar, M.A. Khan, C.A. Khan and M.A.R. Bhatti, 2007. Postharvest losses in apple and banna during transportation and storage. *Pak. J. Agri. Sci.*, Vol. **44(3)**.

- Mahadthanapuk, S., M. Sanguansermisri, R.W. Cutler, V. Sardud and S. Anuntalabhochai, 2007. Control of anthracnose caused by *Colletotrichum musae* on *Curcuma alismatifolia* Gagnep. Using antagonistic *Bacillus* spp. *Amer. J. Agric. and Biolo. Sci.* **2**: 54-61.
- Manjunath, 2009. Morphological and molecular characterization of *Alternaria alternata* and *Colletotrichum gloeosporioides* incitants of leaf blight and anthracnose diseases of Noni and their management, M.Sc.(Ag) Thesis, Tamil Nadu Agricultural University, Coimbatore, India, pp: 222
- Marin, D.H., T.B. Sutton, S.M. Blankenship and W.H. Swallow, 1996. Pathogenicity of fungi associated with crown rot of bananas in Latin America on Grande Naine and disease-resistant hybrid bananas. *Plant Dis.* **80**: 525-528.
- Mbaka, J.N., M.N. Waiganjo, B.K. Chegeh, B. Ndungu, J.K. Njuguna, S. Wanderi, J. Njoroge and M. Arim, 2006. A survey of the major passion fruit diseases in Kenya.
- Meredith, D.S., 1971. Transport and storage diseases of bananas: Biology and control. *Trop. Agric.* **48**: 35-50.
- Mgenzi, S.R.B., D. Muchunguzi, T. Mutagwaba, F. Mkondo, R. Mohamed and V. Aritua, 2006. An outbreak of banana bacterial wilts disease in Muleba district, Kagera region, Tanzania. African Crops Net. [http:// www.africancrops.net/news/april06](http://www.africancrops.net/news/april06).
- MoARD (Ministry of Agriculture and Rural Development), 2005. Vegetables and fruits production and marketing plan (Amharic Version), Ministry of Agriculture and Rural development, Addis Ababa, Ethiopia.
- Ndungo, V., S. Eden-Green, G. Blomme, J. Crozier and J. Smith, 2006. Presence of banana *Xanthomonas* wilt (*Xanthomonas campestris* pv. *musacearum*) in the Democratic Republic of Congo (DRC). *Plant Pathology New Disease Reports* **55(2)**: 294.
- Nelson, S., 2008. Postharvest rots of banana. Cooperative Extension Service; College of Tropical Agricultural and Human Resources University of Hawaii.
- NMSA (National metrological service agency), 2010. National metrological agency of the federal democratic republic of Ethiopia. Last Retrieved at 2010-05-09.
- Ogbo, E.M. and A.E. Oyibo, 2008. Effects of three plant extracts (*Ocimum gratissimum*, *Acalypha wilkesiana* and *Acalypha macrostachya*) on post-harvest pathogen of *Persea Americana*. *Journal of Medicinal Plants Research.* **2(11)**, pp. 311-314.
- Okech, S., C.S. Gold, S. Abele, C.M. Nankinga, P.M. Wetala, P.V. Asten, A. Nambuye and P. Ragama, 2004. Agronomic, pests and economic factors influencing sustainability of banana-coffee systems of western Uganda and potential for improvement. *Uganda Journal of Agricultural Science* **9**: 432-444.

- OPEJZ (Office of Planning and Economic Development for Jimma Zone), 2002. Statistical Abstract. Jimma, Oromia, Ethiopia.
- Oubahou, A.A. and M. El-Otmani (eds.). 1995. Postharvest physiology, pathology and technologies for horticultural commodities: recent advances. Proceedings of an International Symposium, Agadir, Morocco. 520 pp.
- Palipane, K.B. and S.R. Rosa, 2008. Good practice for assuring the post-harvest quality of exotic tree fruit crops produced in Jamaica. Food and Agricultural Organization of the United Nations, Rome.
- Panhwar, F., 2006. Post harvest technology of fruits and vegetables. <http://www.eco-web.com/edi/060529.html>. Last Retrieved at 2011-08-01.
- Photita, W., S. Lumyong, P. Lumyong and K.D. Hyde, (2001a). Fungi on *Musa acuminata* in Hong Kong. *Fungal Diversity*. **6**: 99-106.
- Photita W., S. Lumyong, P. Lumyong and K.D. Hyde, (2001b). Endophytic fungi of wild banana (*Musa acuminata*) at Doi Suthep Pui National Park, Thailand. *Mycological Research*. **105**: 1508-1513.
- Photita, W., S. Lumyong, P. Lumyong, E.H.C. McKenzie and K.D. Hyde, 2004. Are some endophytes of *Musa acuminata* latent pathogens? *Fungal Diversity*. **16**: 131-140.
- Photita, W., Taylor, P.W.J., Ford, R., Hyde, K.D. and Lumyong, S., 2005. Morphological and molecular characterization of *Colletotrichum* species from herbaceous plants in Thailand. *Fungal Diversity* **18**: 117-133.
- Ploetz, R.C., G.A. Zentmeyer, W.A. Nishijima, K.G. Rohrbach and H.D. Ohr. (eds.), 1994. Compendium of tropical fruit diseases, banana. APS Press, the American Phytopathological Society, St. Paul, Minnesota.
- Prompttha, I., S. Lumyong, P. Lumyong, E.H.C. McKenzie and K.D. Hyde, 2002. Fungal succession on senescent leaves of *Manglietia garrettii* on Doi Suthep-Pui National Park, northern Thailand. *Fungal Diversity*. **10**: 89-100.
- Prusky, D. and R.A. Plumbly, 1992. Quiescent infections of *Colletotrichum* in tropical and subtropical fruits. In: J.A. Bailey and M.J. Jeger (eds). *Colletotrichum: biology, pathology and control*. Wallingford, U.K., CAB International, 289-307.
- Prusky, D., 1998. Mechanism of resistance of fruit and vegetable to postharvest diseases. In: Johnson, G.I., Highley, E., Joyce, D.C. (eds.), Disease Resistance in Fruit, ACIAR Proceedings. Canberra, pp. 19-33.
- Rivka Barkai, 2001. Postharvest diseases of fruits and vegetables: development and control.

- Self, G., 2002. Musa fruits pre- and post-harvest. In: Proceedings of the XV reunion. Realizada en Cartagena de Indias, Colombia. Association de bananeros de Colombia AUGURA.
- Shenoy, B.D., R. Jeewon, W.H. Lam, D.J. Bhat, P.P. Than, P.W.J. Taylor and K.D. Hyde, 2007. Morpho-molecular characteristics and epitypification of *Colletotrichum capsici* (Glomerellaceae, Sordariomycetes), the causative agent of anthracnose in chilli. *fungus diversity*. **27**: 197-211.
- Siriboon, N. and P. Banlusilp, 2008. Study on the ripening process of “Namwa” banana. Faculty of biotechnology, Assumption university Bangkok, Thailand. **17(4)**: 02
- Snowdon, A.L., 1990. A colour atlas of post-harvest diseases and disorders, Vol. **I**, General Introduction and Fruits. Wolfe Scientific, London.
- Stover, R. H. and N. W. Simmonds, 1987. Bananas. 3rd ed. Longman Scientific and Technical Publishing, U.K.
- Thangamani, P.R., P. Kuppusamy, M.F. Peeran, K. Gandhi and T. Raguchander, 2011. Morphological and physiological characterization of *Colletotrichum musae* the causal organism of banana anthracnose. *World Journal of Agricultural Sciences* **7(6)**: 743-754.
- Tournas, V.H., 2005a. Moulds and yeasts in fresh and minimally processed vegetables, and sprouts. *International Journal of Food Microbiology*. **99**: 71-77.
- Tournas, V.H., 2005b. Spoilage of vegetable crops by bacteria and fungi and related health hazards. *Critical Reviews in Microbiology*. **31**: 33-44.
- Tripathi, L., M. Mwangi, V. Aritua, W. K. Tushemereirwe, S. Abele and R. Bandyopadhyay, 2009. Xanthomonas wilt: A threat to banana production in East and Central Africa. *The American Phytopathological Society. Plant disease*. **93(5)**
- Waller, J.M., J.M. Lenne and S.J. Waller, 2001. Plant pathologist’s pocketbook 3rd edition. Biological farming systems, Wageningen University, Marijkeweg. pp. 318-327.
- Yoruk, R., M.O. Balaban, M.R. Marshall, S. Yoruk, 2002. The inhibitory effect of oxalic acid on browning of banana slices. In: Annual Meeting and Food Expo, Anaheim, California, **(30)18**.pp.74.
- Zheng, X.L., S. Tian, M.J. Gidley, H. Yue, B. Li, 2007. Effect of oxalic acid on ripening and decay incidence in mango fruit during storage at room temperature. *Postharvest Biology and Technology* **(45)**: pp.281–284

APPENDICES

APPENDIX: A

Appendix Table 26. ANOVA table for anthracnose incidence at farm

| Model | DF | SS | MS | F | <i>P</i> |
|-------------------------|----|---------|--------|--------|----------|
| Regression | 4 | 1018.48 | 254.62 | 260.03 | 0.000 |
| Residual | 20 | 19.58 | 0.98 | | |
| Total | 24 | 1138.1 | | | |
| $r = 0.99$ $R^2 = 0.99$ | | | | | |

Appendix Table 27. ANOVA table for anthracnose severity at farm

| Model | DF | SS | MS | F | <i>P</i> |
|-------------------------|----|---------|--------|--------|----------|
| Regression | 5 | 1004.79 | 200.95 | 681.03 | 0.000 |
| Residual | 19 | 5.606 | 0.29 | | |
| Total | 24 | 1010.39 | | | |
| $r = 0.99$ $R^2 = 0.99$ | | | | | |

Appendix Table 28. ANOVA table for anthracnose incidence after transportation

| Model | DF | SS | MS | F | <i>P</i> |
|-------------------------|----|--------|--------|-------|----------|
| Regression | 5 | 761.65 | 152.33 | 87.95 | 0.000 |
| Residual | 14 | 24.25 | 1.73 | | |
| Total | 19 | 785.89 | | | |
| $r = 0.98$ $R^2 = 0.96$ | | | | | |

Appendix Table 29. ANOVA for anthracnose severity after transportation

| Model | DF | SS | MS | F | <i>P</i> |
|-------------------------|----|--------|-------|-------|----------|
| Regression | 6 | 446.56 | 74.43 | 42.55 | 0.000 |
| Residual | 13 | 22.74 | 1.75 | | |
| Total | 19 | 469.29 | | | |
| $r = 0.98$ $R^2 = 0.93$ | | | | | |

Appendix Table 30. ANOVA for anthracnose incidence at ripening rooms

| Model | DF | SS | MS | F | <i>P</i> |
|------------|--------------|---------|--------|-------|----------|
| Regression | 5 | 3354.17 | 670.83 | 73.71 | 0.000 |
| Residual | 13 | 118.32 | 9.101 | | |
| Total | 18 | 3472.49 | | | |
| $r = 0.98$ | $R^2 = 0.95$ | | | | |

Appendix Table 31. ANOVA table for anthracnose severity at ripening rooms

| Model | DF | SS | MS | F | <i>P</i> |
|------------|--------------|--------|--------|--------|----------|
| Regression | 6 | 723.37 | 120.56 | 278.03 | 0.000 |
| Residual | 12 | 5.2 | 0.434 | | |
| Total | 18 | 728.57 | | | |
| $r = 0.99$ | $R^2 = 0.99$ | | | | |

Appendix Table 32. ANOVA table for anthracnose incidence at retailer shops

| Model | DF | SS | MS | F | <i>P</i> |
|------------|--------------|----------|---------|-------|----------|
| Regression | 6 | 36811.17 | 6135.19 | 244.5 | 0.000 |
| Residual | 64 | 1605.93 | 25.09 | | |
| Total | 70 | 38417.1 | | | |
| $r = 0.98$ | $R^2 = 0.95$ | | | | |

Appendix Table 33. ANOVA for anthracnose severity at retailer shops

| Model | DF | SS | MS | F | <i>P</i> |
|------------|--------------|----------|---------|--------|----------|
| Regression | 6 | 10061.37 | 1676.89 | 127.65 | 0.000 |
| Residual | 64 | 840.78 | 13.13 | | |
| Total | 70 | 10902.48 | | | |
| $r = 0.96$ | $R^2 = 0.92$ | | | | |

Appendix Table 34. ANOVA for anthracnose incidence at ET-fruit shops

| Model | DF | SS | MS | F | <i>P</i> |
|------------|--------------|----------|---------|-------|----------|
| Regression | 2 | 90.47.48 | 2820.62 | 52.82 | 0.000 |
| Residual | 9 | 770.78 | 32.77 | | |
| Total | 11 | 981.25 | | | |
| $r = 0.96$ | $R^2 = 0.90$ | | | | |

Appendix Table 35. ANOVA for anthracnose severity at ET-fruit shops

| Model | DF | SS | MS | F | <i>P</i> |
|------------|--------------|---------|--------|-------|----------|
| Regression | 3 | 1312.83 | 437.61 | 74.97 | 0.000 |
| Residual | 8 | 46.69 | 5.84 | | |
| Total | 11 | 1359.53 | | | |
| $r = 0.98$ | $R^2 = 0.97$ | | | | |

Appendix Table 11. ANOVA for evaluation of banana varieties to wound and quiescent anthracnose

| Source | DF | SS | MS | F | <i>P</i> |
|-----------------|----|--------------|------------|---------|----------|
| Model | 14 | 72238928.89 | 5159923.49 | 3851.39 | < .0001 |
| Error | 60 | 80385.32 | 1339.76 | | |
| Corrected total | 74 | 723119314.21 | | | |

APPENDICES: B



Figure 1. Banana farm at Arba-Minch



Figure 2. Bunch harvesting method



Figure 3. Transportation around Arba-Minch



Figure 4. Banana up on arrival to ripening rooms



Figure 5. Banana loaded vehicle at ET-fruit enterprise



Figure 6. Method of banana unloading



Figure 7. Banana fruit handling at ripening rooms



Figure 8. Banana fruit loss at ET-fruit enterprise

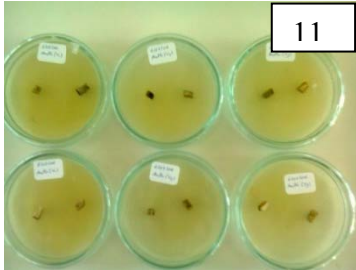


9



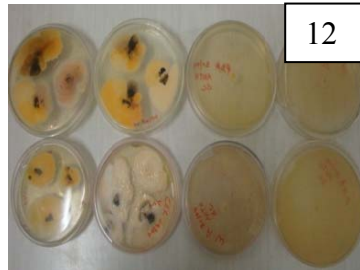
10

Figure 9 and 10. Method of sample collection



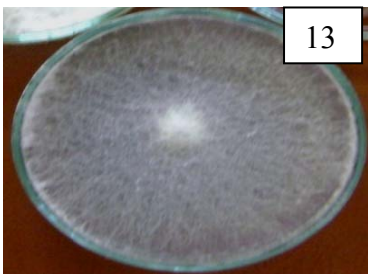
11

Figure 2. Prepared plates on PDA



12

Figure 3. Grown culture of *C. musae*



13

Figure 4. Pure culture of *C. musae*



14

Figure 5. Anthracnose symptom- pathogenicity test

Survey at Addis Ababa upon arrival of banana fruits from production area

Date of interview

Region.....Sub cityWoreda.....

Code of the respondent Sex..... Age.....

Education level of respondent

| Variety | | Fruit source | | Type of vehicle | | Container type | | Fruit unloading | | Fruit composition | | Fruit covers | | Distance traveled (km) | Type of road | |
|-----------|-----------------------|--------------|------------|-----------------|--------------------|----------------|-------------|-------------------|---------------|-------------------|-------------------|--------------|---------|------------------------|------------------------------|-----------|
| Cavendish | Other local varieties | Arba minch | Other area | Open lorry | Closed lorry/Isuzu | Woodn box | Plastic box | Staking of fruits | Other methods | Only banana | With other fruits | Banana leaf | Or else | | Metallic / Non metallic road | Otherwise |
| | | | | | | | | | | | | | | | | |

Survey at ripening rooms, retailer and ET- fruit shops found in Addis Ababa

Postharvest handling of banana fruit at ripening rooms, retailer and ET-fruit shops

Date of interview.....

Region.....Sub cityWoreda.....

Code of the shopSex.....Age.....

Owner’s education level

Shop type

Ripening room.....Retailer..... ET-fruit.....

| Variety | | Fruit source | | Container type | | Cleaning/ grading | | Fruit composition | | Storage type | |
|-----------|-----------------------|--------------|------------|----------------|-------------|----------------------|----|-------------------|-----------------|-----------------|------------------|
| Cavendish | Other local varieties | Arba Minch | Other area | Wooden box | Plastic box | yes | No | Only banana | Banana + others | Controlled room | Room temperature |
| | | | | | | | | | | | |