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Distribution and Occurrence of Mango Anthracnose (*Colletotrichum gloesporioides* Penz and Sacc) in Humid Agro-ecology of Southwest Ethiopia

Ayantu Tucho, Fikre Lemessa and Gezahegn Berecha
Department of Horticulture and Plant Sciences, Jimma University, P.O. Box 307, Jimma, Ethiopia

Abstract: Mango (*Mangifera indica* L.) is grown in different agro-ecologies of Ethiopia and its production and productivity is limited by several biotic and abiotic factors. Mango anthracnose, caused by *Colletotrichum gloeosporioides* is considered as the most important mango disease in the country that contribute significantly to pre and post harvest fruit losses. However, the distribution and occurrence of mango anthracnose both in the field and at market in mango producing areas of southwestern Ethiopia is not yet documented. In this study, distribution and occurrence of mango anthracnose in three potential mango producing districts and one urban area in Jimma region, SW Ethiopia were assessed. At the same time knowledge and attitude of farmers against mango anthracnose was also assessed. The results showed that mango anthracnose was 100% prevalent in the study area. Anthracnose incidence and severity varied across farmer's field and market places. The disease incidence under farmer's fields ranged from 41-72.1% on leaf and from 36.2-74% on fruit. We found higher (95.3 vs. 82%) and lower (70.7 vs. 64%) incidence and severity in the market, respectively. The disease was more severe in the market place than in the farmer's fields. It was confirmed that the identified fungus was *C. gloeosporioides*. So, for better understanding of the prevalence and distribution of this disease and to design appropriate management options, similar assessments across different mango growing agro-ecologies and along mango value chain is crucial.

Key words: Incidence, severity, disease prevalence, mango, *Colletotrichum gloeosporioides*

INTRODUCTION

Mango (*Mangifera indica* L.) is grown throughout the tropics and subtropics of the world (Bally, 2006; Prusky *et al*., 2009) and it belongs to the family Anacardiaceae. It is considered to be the king of fruits due to its wide ecological range, delicious taste, excellent flavor, very high nutritive and medicinal value as well as great religio-historical significance (Lakshmi *et al*., 2011). The crop is grown in over 87 countries in the world with developing countries account for about 98% of total production while developed countries account for 80% of world import trade. Mango is the most popular and commonly consumed fruit among millions of people in tropical areas including Ethiopia. It is a highly nutritious fruit containing carbohydrates, proteins, fats, minerals and vitamins, in particular vitamin A (beta carotene), vitamin B_1, vitamin B_2 and vitamin C (ascorbic acid) (Bally, 2006). With estimated production of 26 million tons per annum (FAO, 2010), mango is ranked second only to banana both in quantity and value and fifth in total production among major fruit crops worldwide.

Mango is the leading fruit produced in most parts of eastern, southern and south-western Ethiopia both in area coverage and quantities produced (Yeshitela and Nessel, 2004; Chala *et al*., 2014). One can find ample garden mango trees in different parts of the country at farmer's holdings. As a result the livelihood of most of these farmers is highly supplemented by the sale of mango fruits. According to FAO (2010) the total cultivated area for mango in Ethiopia is 12,000 ha. However, current export share of mongo in Ethiopia is very small mainly due to low productivity (Chala *et al*., 2014).

Growing and marketing of fresh produce in Ethiopia is limited by post harvest losses both in terms of quantity and quality between harvest and consumption. According to Kader (2009) post-harvest loss of mango fruits in Ethiopia exceeds 26.3%. Mango production and quality among other, are limited by pre and post harvest diseases caused by bacteria, fungi and Nematodes (Chowdhury and Rahim, 2009). Moreover, there is a declining trend in yield and quality of mango in Ethiopia due to tree age and poor agronomic management. The most common diseases limiting mango productivity and

Corresponding Author: Ayantu Tucho, Department of Horticulture and Plant Sciences, Jimma University, P.O. Box 307, Jimma, Ethiopia

quality in Ethiopia are anthracnose, stem end rots, powdery mildew and mango malformation (Chala *et al*., 2014).

Anthracnose caused by *C. gloeosporioides* is the most serious mango disease worldwide (Smooth and Segall, 1963; Sangeetha and Rawal, 2009). Disease incidence as high as 32% in South Africa (Sanders *et al*., 2000) and 64.6% in Costa Rica during 1990 (Arauz *et al*., 1994) was reported. The incidence can reach almost 100% in fruit produced under wet or very humid conditions (Arauz, 2000). Anthracnose causes 30-60% yield losses on mango across different countries of the world (Akem, 2006; Chowdhury and Rahim, 2009). Fruit anthracnose disease has been found associated with mango fruits produced in the humid region of Southwestern Ethiopia. This disease has made mango production non-attractive to farmers and home gardeners in the study area and beyond. Therefore, understanding of the crop-pathogen system and distribution and prevalence of the anthracnose has paramount importance to design appropriate control measures (Akem, 2006; Chowdhury and Rahim, 2009; Chala *et al*., 2010). Fruit losses after harvest are also expected to be high due to poor transportation, handling practices and extended storage periods (Chala *et al*., 2014). However, empirical study addressing the distribution and occurrence of mango anthracnose both in the field and in postharvest environment particularly at market in this part of the country is lacking. Therefore, the aims of this study were: To determine the distribution and occurrence of mango anthracnose (*C. gloeosporioides*) both in the farmer's fields and local markets around Jimma areas in southwestern Ethiopia and to assess the knowledge and attitude of framers against mango anthracnose disease and its management.

MATERIALS AND METHODS

Study area: The study was conducted in the potential mango producing districts of Jimma region, south western Ethiopia from April to June, 2013. We selected three districts and one urban area mainly based on their mango production potential and area covered by mango cultivation (Table 1 and Fig. 1).

Table 1: Location and climatic characteristics of the study districts pre and post harvest

				Mean temperature (°C)	
Study districts	Location	Altitude (m.a.s.l)	Annual rain fall (mm)	Min.	Max.
Seka-Chokorsa	7036'41"N and 36044'12"E	1580-2560	1800-2300	15.00	25.0
Kersa	7038'-7054'30"N and 36038'-36053'E	1600-2400	1587	10.00	32.0
Gomma	750'35"-751'00"N and 36"35'30"E	1387-2870	800-2000	12.40	28.4
Jimma (urban)	70138056N and 3505237037E	1700-1730	1637	11.43	26.2

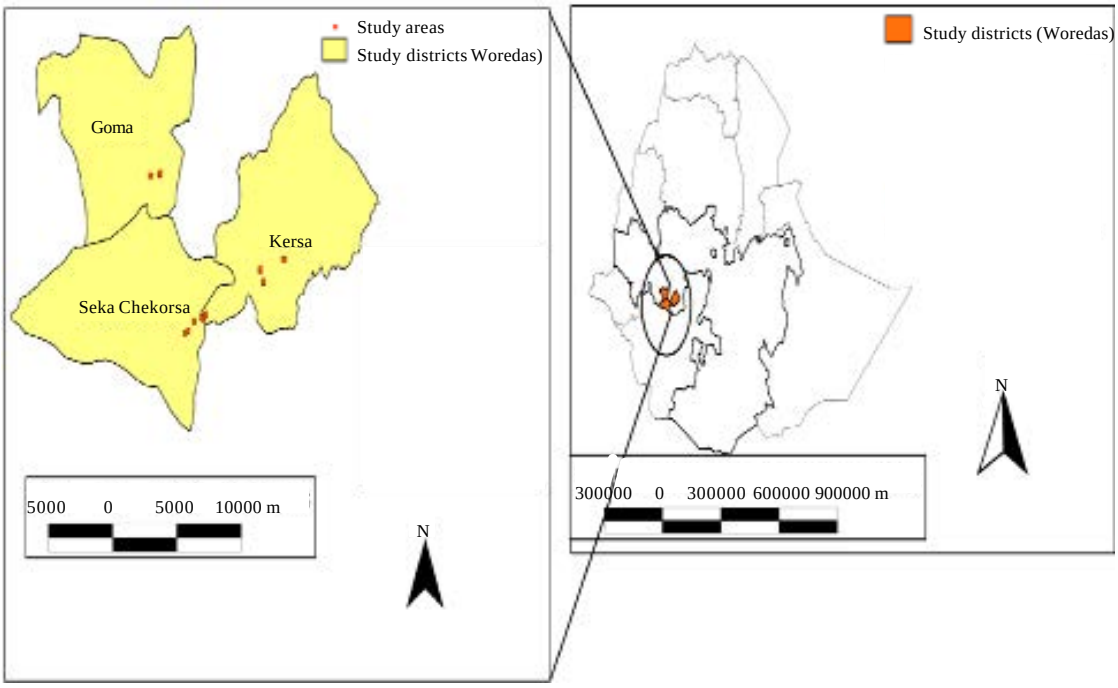

Fig. 1: Map of the study area

Survey and sampling method: The purposive sampling method was used for selecting districts, kebeles within district and mango orchard. Then we employed random sampling for selecting mango tree within orchard. Three kebeles per each district and six trees per plot were assessed. In addition, cultural practices such as age of crops and cropping pattern were noted. For the post-harvest assessment, four markets, (one big local market in each selected districts and urban area) were assessed and traders were the source of the sampled fruits. The assessed market places were Bishishe in Jimma, Agaro in Gomma district, Sarbo in Kersa and Seka in Seka Chekorsa district.

Assessment of mango anthracnose disease incidence, severity and prevalence: Disease assessment was conducted along the direction from Jimma town to the respective districts. Six trees per plot were randomly selected and used for the disease incidence and severity assessment on leaves and fruits in each kebele. Assessments were performed at three positions of the tree (upper, middle and lower). The post harvest disease incidence and severity assessments were conducted at market places. For this fifteen fruits from five traders were randomly selected and replicated three times for each market.

In addition, using structured questionnaires, the knowledge and attitude of farmers against mango diseases and its management was assessed. Six farmers per Kebele and 72 farmers in total were interviewed by contacting the farmers face to face.

Disease incidence and prevalence: Disease incidence on the fruit and leaves was assessed using the equation:

$$\text{Disease incidence (I)} = \frac{\text{No. of infected fruits (leaves)}}{\text{Total No. of assessed fruits (leaves)}} \times 100$$

$$\text{Percent of occurrence (Prevalence)} = \frac{\text{No. of field with infected mango}}{\text{Total}} \times 100$$

Disease severity: Disease severity was recorded using a five point rating scale (Corkidi *et al*., 2006; Fig. 2). The assessment was performed at physiological maturation stage of the fruit. The numerical ratings were converted to Percent Disease Index (PDI) using the following equation (Mayee and Datar, 1986):

$$\text{PDI} = \frac{\text{Sum of numerical ratings}}{\text{No. of plant scored} \times \text{Maximum score on scales}} \times 100$$

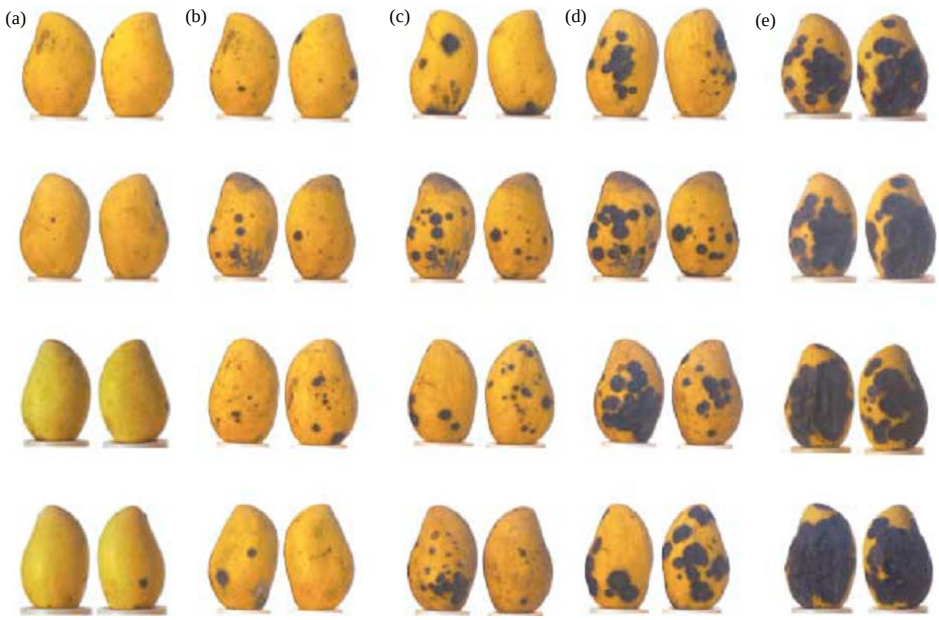

Fig. 2(a-e): Scale used for the assessment of mango anthracnose severity (Corkidi *et al*., 2006). (a) 0-1% infected area (no disease), (b) 1-5% infected area (slightly diseased), (c) 6-9% infected area (moderate disease), (d) 10-49% (severely diseased) and (e) 50-100% infected area (very severely diseased)

Isolation of the causal pathogen: Isolation was done to confirm whether the causal pathogen was anthracnose or other fungus. Isolation was performed by cutting several small sections 3-5 mm^2 from the margin of the infected lesion so that they contain both diseased and healthy looking tissue of mango and surface sterilize by sodium hypo chlorate (NaOCl) for about 15-30 sec. Next, the sections were taken out aseptically one by one and at regular intervals to surface sterilize each at different times (Agistini and Timmer, 1992). The sections were washed in three changes of sterile water and blotted dry on clean sterile paper towels. Then three pieces of tissue were placed per petr idish on a freshly-prepared Potato Dextrose Agar medium (PDA). Finally, the petridishes were incubated for 7 days at 28°C. Fungal growth was examined using binocular and compound microscope.

Pathogenicity tests

Preparation of spore suspension: Pathogenicity test of the identified *C. gloeosporioides* was tested on detached leaf and fruit of mango. The suspension of conidia was prepared by suspending mycelia scraped from 7 days old culture of *C. gloeosporioides* in 3 mL sterile distilled water and shaking vigorously for 3 min (Onyeani *et al*., 2012). The resulting suspension was filtered through 2-layer cheese cloth. The concentration of spore suspension was adjusted to 1×10^6 spores or conidia/millimeter using haemacytometer.

Fruit wounding technique (pin-prick inoculation): Three mango cultivars commonly growing in the country (Tommy Atkins, Apple mango and local cultivar) were used. Inoculation was performed following the method of Than *et al.* (2008). Fifteen green matured mango fruits, five fruits from each variety were randomly collected, thoroughly washed and disinfected in 70% ethanol and 1% NaOCl. The disinfected fruits were then rinsed in four changes of sterile distilled water and air before inoculation. The fruits were each pierced with sterilized needle in three places. Then after, 0.02 mL containing 1×10^6 mL^{-1} spore suspension of fungal isolates was dropped on the wounded portion of the fruit using pipette, sealed in moist plastic box with sponge sprayed with sterilized water to maintain at least 95% relative humidity (Than *et al*., 2008) and incubated for 7 days at 28°C. Control fruits were inoculated with sterile distilled water. Anthracnose symptoms were evaluated after 7 days.

Detached leaf technique: Detached new leaves free from anthracnose symptom were collected, washed and surface sterilized. The leaves were then sprayed with the spore solution of fungal isolate and placed on five larger plastic petri dishes lined on the inside with moist tissue paper, covered with moist paper towels and incubated for 7 days at 28°C until symptom appearance.

Re-isolation of isolated fungal pathogens: The causative organisms in the diseased parts were re-isolated on potato dextrose agar as described above. The characteristics of the re-isolated pathogens were compared with their original isolates.

Statistical analysis: Data was first checked for various ANOVA assumptions. The field survey data for mango anthracnose (incidence and severity) was analyzed using three stage nested design. The post harvest mango anthracnose data was analyzed using one way ANOVA. The main and interaction effects of anthracnose disease response variables across location were determined using the proc GLM of SAS software version 9.2 (SAS, Inc., 2008). Mean separation was carried out using LSD test at 5% level of significance.

RESULTS AND DISCUSSION

Constraints of mango production in the study areas: The present study survey results revealed several constraints associated with mango production in the study areas. The most prominent constraints were extreme environmental conditions (erratic rainfall, prolonged drought due to delay in onset of rain), mango anthracnose and bacterial blight. About 64.4% of the respondants indicated mango anthracnose as the major challenge to mango production which blackens the fruits thereby predisposes them to pre-mature dropping before harvest. About 27.7 and 2.7% of the respondents said environmental condition and bacterial blight, as major problems of mango production in the region, respectively (Table 2).

Types of variety grown and management practices against mango anthracnose: Although, different varieties of mango were produced in the study areas, about 83.3% respondents produce local variety, 6.9% of respondents produce Tommy Atkins and 9.7% of the respondent

Table 2: Problems associated with mango production in the study areas pre and post harvest

Constraints	Frequency of respondents	Percentage
Mango anthracnose	50	64.4
Bacterial blight	2	2.7
Environmental condition	20	27.7
Total	72	100.0

produce Apple mango variety. These results showed most of the respondents grow local varieties of mango that have been under cultivation for more than 20 years old. Besides, the sources of the different local mango varieties introduced by the community are unknown. As a result constraints such as failure to set fruit, extended periods on fruits setting, susceptibility to diseases and pests, are very common. All respondents said that mango anthracnose disease is more prominent during humid and wet condition than hot and dry condition.

For the management of mango anthracnose disease, 6.9% of the respondents use combinations of inter-cropping, timely planting and removing infected plants, 16.6% of the respondents use chemicals and 76.4% of the respondents did not use any kind of measures to control the disease (Table 3). Generally, according to the responses of the farmers, cultural practices such as sanitation, pruning and different cropping pattern to control mango anthracnose disease was lacking. Particularly, there was no practices of removing dead or diseased wood, additional growth flushes to allow more light penetration into the leaf canopy and control of tree height to facilitate cultural management practices such harvesting.

Incidence and severity of mango anthracnose under farmers' field: The incidence of mango anthracnose on leaf and fruit was significantly varied from district to district (Fig. 3). The mean incidence on the fruit ranged from 36.2-74%.

There was significant difference among districts in terms of mango anthracnose severity ($p<0.0001$). The mean severity values of mango anthracnose in the field ranged from 38.1-63.0%. The severity of mango anthracnose was highest in Gomma district and the lowest in Kersa district (Fig. 4).

The high incidence and severity of mango anthracnose disease on fruit in Gomma district could be attributed to the prevalence of rain during flowering and fruit set (personal observation). Among the different environmental factors, rainfall is known to play significant role in releasing of condia from acervuli and their subsequent spreading in the field (Agrios, 2005). Compared to the other districts, the landscape of Gomma district is characterized by matrix of shade grown coffee and small agricultural lands and wetlands. Nearly all households grow coffee under shade tree or intercropped with different fruits such as mango, avocado and banana. The presence of different shade tree for nursing coffee

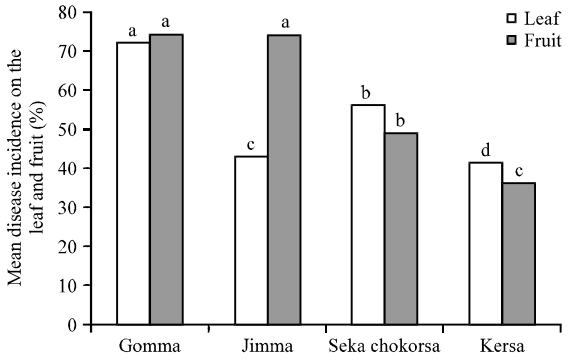

Fig. 3: Mean disease incidence of mango anthracnose on the leaf and on the fruit across the surveyed districts. Bars copped with the same letter(s) are not significantly different at $p<0.05$

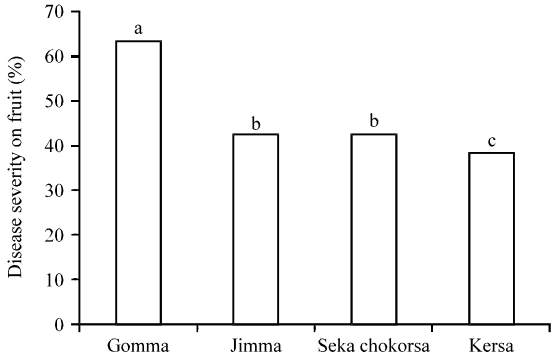

Fig. 4: Mean anthracnose severity on fruit across assessed districts. Bars copped with the same letter(s) are not significantly different at $p<0.05$

Table 3: Control strategies used by farmers to control mango anthracnose in surveyed area pre and post harvest

Control	Frequency of respondents	Percentage
Cultural practice	5	6.90
Chemical	12	16.60
No management	55	76.38
Total	72	100.00

could possibly alter the microclimates (humidity and rainfall) in the surrounding. Generally, high forest cover increases relative humidity (Aerts *et al*., 2011) and this usually favor the development of fungal diseases such as anthracnose of mango. Studies have already indicated that anthracnose cause significant impact in areas where rainfall is prevalent (Arauz, 2000; Onyeani *et al*., 2012; Chala *et al*., 2014). The incidence of this disease can reach almost 100% in fruit produced under wet or very humid condition (Akem, 2006). The highest disease incidence observed in this study could also be attributed to poor

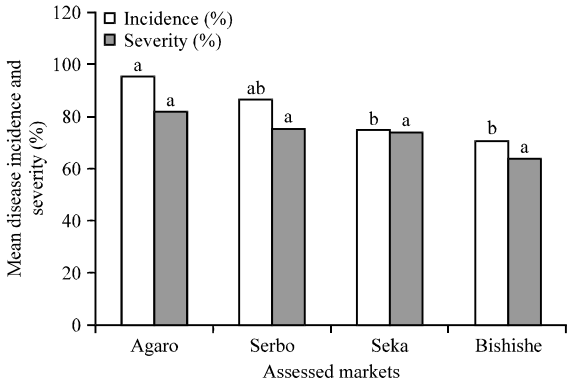

Fig. 5: Incidence and severity of mango anthracnose disease across different markets in SW Ethiopia. Bars copped with the same letter(s) are not significantly different at $p<0.05$

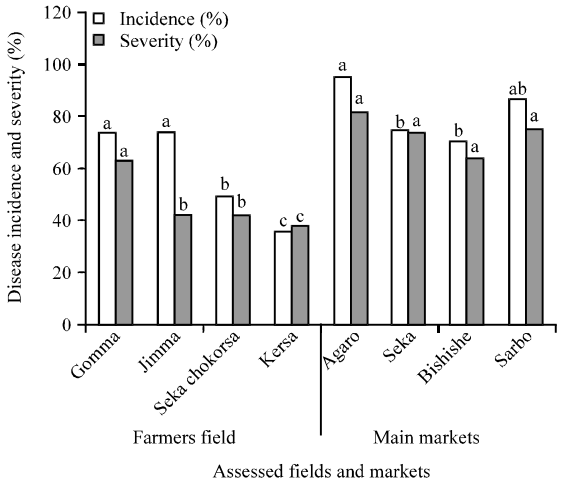

Fig. 6: Mango anthracnose disease incidence and severity in farmers' field and in main markets. Bars copped with the same letter(s) are not significantly different at $p<0.05$

farm sanitation. In the surveyed areas, farmers did not prune the damaged stems from infected plants and remove debris of diseased stems and fruits around the farm. Studies have reported conidia produced from debris or dead leaves as the main source of *C. gloeosporioides* inoculums which could rapidly initiate an epidemic once favorable condition for dispersal and infection occurred (Fitzell and Peak, 1984; Estrada *et al*., 2000; Ploetz, 2003).

Incidence and severity of mango anthracnose at the market: The incidence of mango anthracnose was significantly varied across the surveyed markets. The incidence of mango anthracnose ranged from70.6-95.3% (Fig. 5). We found higher incidence of mango anthracnose in the market than at field condition. This could be attributed to fruit softening during the ripening process which causes break down of the natural defense mechanisms and enhances latent infections of anthracnose. Post harvest anthracnose is the major reason for losses of mangos during storage and transport. The high postharvest incidence could also be attributed to poor handling and transportation to the market. In most cases farmers in the study areas transport fruits to the market using animal (e.g., donkey, mule) and human labor particularly women. Such traditional way of transportation can inflict mechanical damages on the fruits in the form of bruise and this will enhance fungal disease development. Postharvest disease development elsewhere is reported to be one of the major constraints to the quality and shelf life of mango fruit limiting its domestic and export marketing (Bally *et al*., 2009; Chala *et al*., 2014). Like other perishable fruits and vegetables, mango has also been found prone to postharvest fruit decay owing to rapid disease development during storage and ripening (Prusky *et al*., 2009).

There was higher severity of mango anthracnose on fruits in the surveyed markets but the difference among them was not significant ($p>0.05$). The mean values of mango anthracnose severity at the market ranged from 64-82%. Severity of the disease could also be higher at consumer hands where ripe fruits might be store one or more days after purchase because further ripening encourage pathogen development thereby cause significant losses. Akem (2006) stressed that postharvest losses of mango fruits could go upto 97%, depending on varieties, locations, cultural practices employed and prevailing environmental conditions.

Our result showed higher disease incidence and severity on fruits in the main markets than at farmer's fields (Fig. 6). For instance, the highest incidence and severity of the disease were recorded in farmers' fields at Gomma district and Agaro market (the big local market in Gomma district). This suggest that the presence of a strong relationship between farm and market indicating that fruits in the market are largely brought from the farmers' fields and those fruits are already infected in the field and disease development rapidly increased when brought in the market. Therefore, the highest disease incidence and severity recorded in the market places could be due to latent infections which occurred before harvest and then remain quiescent until some point during

ripening and poor postharvest handling practices. Anthracnose that potentially infect and cause significant loss on a wide range of tropical and sub-tropical fruits (mango, banana, papaya and avocado), is a good example of a disease arising from quiescent infections (Akem, 2006; Sanders and Korsten, 2003).

Isolation of the causal pathogen: Colonies of the fungus on potato dextrose agar showed whitish to dark grey with thick to sparse lawns of aerial mycelium when viewed from the top of petri dishes (Fig. 7a) whereas, they had greenish to orange or dark brown centre bordered by creamy surrounding when viewed from the reverse side of the petri dish (Fig. 7b). We observed conidia with hyaline, single celled and cylindrical with obtuse ends (Fig. 7c, d). The fungus was, morphologically identified to be *Colletotrichum gloeosporioides*. The present results agree with Onyeani *et al.* (2012).

Pathogenicity test: Pathogenicity test was carried out for mango anthracnose (*C. gloeosporioides)* isolated from symptomatic mango fruits. The inoculated fruits showed anthracnose disease symptom typical of those observed on both healthy leaf and fruits of mango (Fig. 8). Our result agree with earlier findings (Than *et al*., 2008; Sangeetha and Rawal, 2009; Jayasinghe and Fernando, 2009) who confirmed the pathogenicity of *C. gloeosporioides* on detached mango fruit.

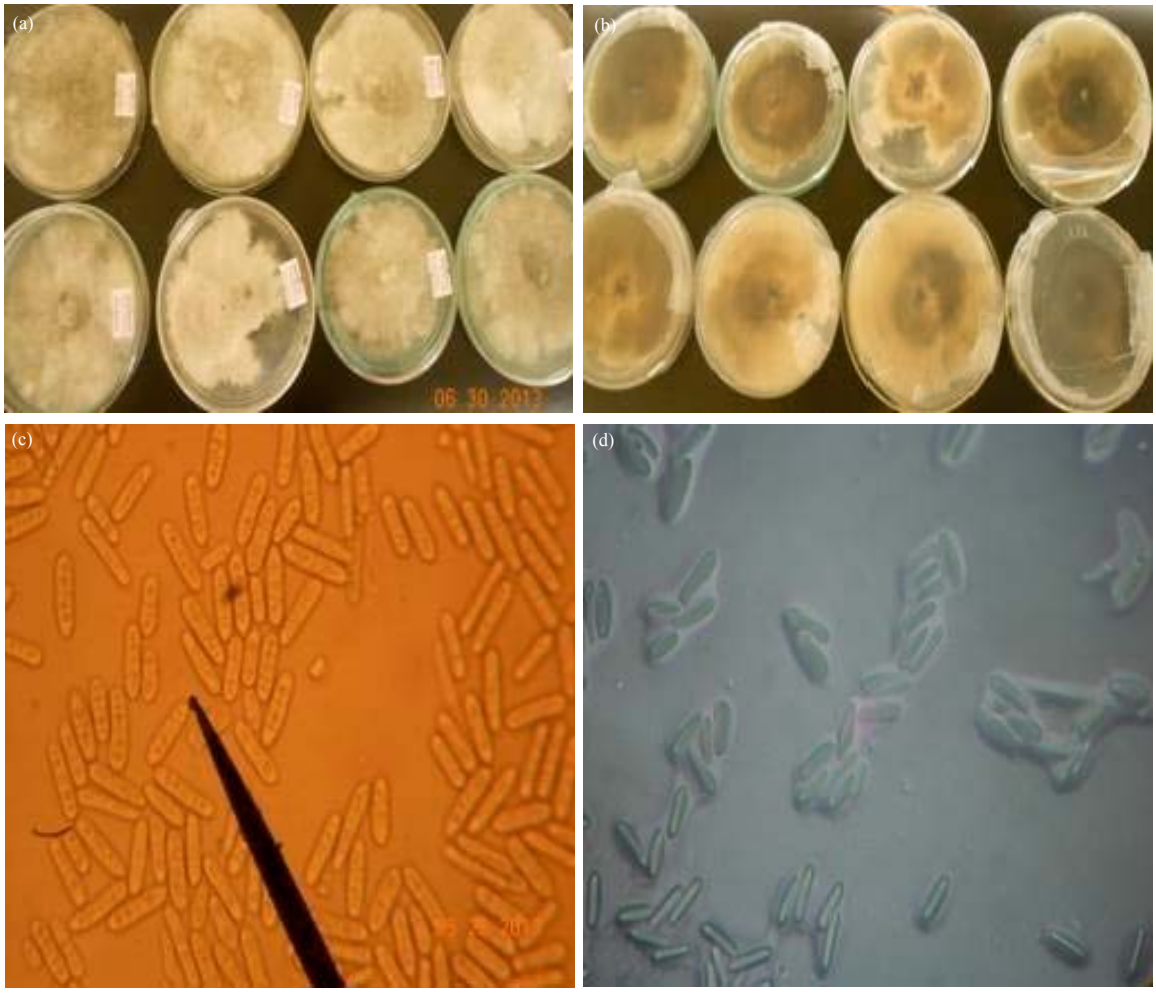

Fig. 7(a-d): *Colletotrichum gloeosporioides* the causal agent of mango fruit anthracnose disease, (a) Top view of colony in a Petri dish (b) Reverse view and (c, d) Microscopic view

Fig. 8(a-f): (a) Tommy Atkins variety, (b) Apple, (c) Variety before inoculation and Tommy Atkins variety (d) Apple variety and (e, f) Local variety after inoculation with symptom

CONCLUSION

Our results clearly showed the importance of mango anthracnose disease in the study area. The prevalence of manage anthracnose was 100%. Anthracnose incidence and severity varies from location to location and from market to market. The disease was more severe in the market than in the field. However, there was a strong relationship between the incidence and severity of mango anthracnose in the field and in the local markets of the respective survey areas, indicating the importance of considering the whole value chain while attempting to manage mango anthracnose disease in the study area and beyond. In this study, we only assessed incidence and severity on leaves and fruits. However, the fungus is known to invade panicle, twigs, leaves and fruits. To get better understanding of the distribution of this disease, studies that assess incidence and severity on the panicle and twigs are highly recommended. Furthermore, in order to get full picture of the prevalence of mango anthracnose disease and to design appropriate control methods, it is advisable to conduct similar assessments in different mango growing agro- ecologies of the country and mango value chain.

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