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## The importance of avocado (*Persea americana* Mill.) fruits anthracnose and factors influencing the disease in Mana district, south-western Ethiopia

Misale Kuru<sup>a</sup>, Girma Adugna<sup>b</sup>  and Gezahegn Berecha<sup>b</sup>

<sup>a</sup>Department of Postharvest Management, College of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia; <sup>b</sup>Department of Horticulture and Plant Sciences, College of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia

### ABSTRACT

Anthracnose disease surveys were conducted in 25 farmers' orchards, wholesaler and retailer shops in south-west Ethiopia. In addition, harvesting and postharvest practices, and storage conditions influencing disease development were studied with observation and questionnaire. The assessment results indicated significant variation among farmers' orchards with the highest incidence ( $84.0 \pm 16.7\%$ ) and severity index ( $26.0 \pm 5.4\%$ ). Anthracnose damage of fruit was higher at retailers ( $76.7 \pm 20.8\%$ ) than in the wholesalers shop ( $56.7 \pm 32.5\%$ ). The total number of isolates identified was 249 and *Colletotrichum gloeosporioides* was the predominant pathogen proved by pathogenicity test. Among the major factors, harvesting avocado fruits with children (88%) and climbing on the tree (72%) resulted in fruit dropping that caused substantial injury and bruise. Generally, anthracnose caused by *C. gloeosporioides* of avocado fruit was prevalent in producer orchards that aggravated by traditional harvest and postharvest practices coupled to inadequate transportation and storage facilities at wholesaler and retailer shops with subsequent decay and loss of avocado fruits.

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Avocado; *Colletotrichum gloeosporioides*; *Persea americana*; postharvest disease

## Introduction

Avocado (*Persea americana* Mill.) is a tree fruit that belongs to the family *Lauraceae* and grows under diverse climatic conditions of the tropics and subtropics. Fifty-seven countries are involved in avocado production but Mexico is the major producer and 97% is consumed locally (Maria 2007). Ethiopia is the 10th major producer and the 6th leading consumer of this extremely nutritious fruit crop (FAOSTAT 2010). Avocado is only of a relatively recent introduction into the backyards of small-scale farmers in the south and south-western parts of

Ethiopia (Edossa 1997). Over the past 20 years, there is a great effort to produce and supply avocado to local markets with high demand and return values in the south-western part of the country (Zekarias 2010).

Mana district is one of the major avocado fruit producers and suppliers (47%) from Jimma zone; other districts producing this fruit are also Sekachekorsa (33%) and Goma (20%) (Woyessa & Berhanu 2011; Brehanu & Alemu 2013). Nevertheless, avocado production is hampered mainly by diseases such as Phytophthora root rot and Verticillium wilt in the field and fruit anthracnose (Muhammad et al. 2004; Brehanu & Alemu 2013). Anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz & Sacc. is considered to be universal disease that leads to spoilage of almost all kinds of mature avocado fruits. *C. gloeosporioides* is a postharvest pathogen of avocado that attacks fruit during growth in the orchard. Infections remain quiescent until the fruit ripens, causing symptom development and substantial decay losses during storage and marketing (Prusky & Plumbly 1992; Prusky & Keen 1993; Beno-Moualem & Prusky 2000).

Despite the nutritional and economic impotence of this potentially emerging fruit crop to farmers and traders in Ethiopia, little is understood about the impact of anthracnose disease and associated factors at various stages of avocado fruit. Therefore, the present study was conducted (1) to assess the incidence and severity of avocado fruit anthracnose at farm gate and market place, (2) to isolate and identify associated causal pathogen(s) and (3) to determine the influence of harvesting and postharvest handling practices on the disease development.

## Materials and methods

### Description of the study site

This study was conducted in Jimma Zone which is located in Oromia regional state 356 km away from Addis Ababa in south-west direction at the latitude of 07°15' N and 08°45' N, and longitudes 36°00' E and 37°40' E. The zone, in general, is divided into three agro-ecologies, namely, lowland (1000–1500 m), intermediate (1500–2500 m) and highland (2500–3300 m above sea level). The zone receives annual average rainfall of around 1000 mm for 8–10 months with temperature varying from 8 to 28 °C (FAO 2009). Survey on the distribution of the postharvest diseases of avocado was conducted in farmers' orchards of sample *kebeles* (peasant associations) of Mana district in Jimma Zone, and wholesalers and retailers in the market in Jimma town. The laboratory activities were conducted in Plant Pathology Laboratory, College of Agriculture and Veterinary Medicine, Jimma University.

### Assessment of factors influencing disease development

The survey was conducted in avocado orchards of five sample peasant associations, namely, Doyo Bikila, Gube Mulata, Gudeta Bula, Somodo and Buture and then

five avocado growing farmers were considered from each association in Mana district of Jimma Zone during 2012–2013. The district was chosen first because of its high production and second it's the nearest to the Jimma town; therefore, it is easier to follow the market trend from producer to wholesalers and retailers. In addition to data collected from farmers (producers), samples of wholesalers and retailers in the fruit market chain were considered for the study. Simple random sampling method was followed and in total, (31) respondents that represented producers (25), wholesalers (3), and retailers (3) were interviewed on harvesting and postharvest handling and storage practices of avocado fruits at each surveyed area using the self-administrated questioners approach.

### ***Samples from farmers' orchards***

To test for infection, fungal pathogen contamination and damage, 3–6 injured and healthy avocado fruits were taken per orchard in labelled and perforated sterile (70% alcohol) plastic bags during harvesting season. Samples were collected from a total of 25 farmers in the five peasant associations; observed to grow different avocado cultivars such as Hass, Pinkerton and Fuerte although samples were collected from Fuerte, the most common cultivar across the orchards.

### ***Samples from wholesalers and retailers***

In the avocado market chain, representative samples of wholesalers and retailers in Jimma town were visited to assess anthracnose attack and to collect avocado fruit samples for further examination of quiescent (latent) infection of the pathogen. A total of 36 apparently healthy looking fruits were sampled from the wholesalers ( $n = 18$ ) and retailers ( $n = 18$ ). All the fruit samples were transported to the laboratory for isolation and identification of the associated pathogens.

The infection of anthracnose was identified on the basis of symptoms i.e. dark sunken lesion on avocado fruits, and disease incidence was calculated as number of infected fruits showing any single symptom out of total number of avocado fruits sampled (Ogbo & Oyibo 2008).

$$\text{Disease incidence(\%)} = \frac{\text{Number of infected fruits}}{\text{Total number of fruit samples}} \times 100$$

The disease severity was measured on a 0–4 scale where, 0% = no symptom, 1–25% = slight, 26–50% = moderate, 51–75% diseased and 76–100% = heavily diseased, and then per cent severity index of anthracnose infection was estimated from the numerical ratings of the total samples using the following formula.

$$\text{Percent severity index} = \frac{\text{Sum of numerical ratings}}{\text{Total number of fruits examined} \times \text{maximum grade}} \times 100$$

### ***Isolation and identification of fungal pathogens associated with avocado fruit***

The collected avocado fruits ( $n = 186$ ) were first washed in tap water and then the fleshy part cut into pieces with sterilised scalpel. The pieces were surface sterilised in 10% sodium hypochlorite solution and rinsed thrice with sterile distilled water. Soon after blotting, three–four sterilised pieces were placed on potato dextrose agar (PDA), Oxoid, in Petri dishes and incubated at room temperature ( $25 \pm 1$  °C) for 7 days. Single-spore cultures of the fungus were prepared on PDA slants in test tubes and stored in refrigerator at 4 °C for further study. The identification and characterisation of the fungal isolates were carried out based on cultural and morphological structures described by Sutton (1992).

### ***Pathogenicity test***

In total, five types of fungal isolates were retrieved from the collected samples and *C. gloeosporioides* was identified to species level. The pathogenicity of *C. gloeosporioides*, the most frequently isolated fungus and known to be associated with anthracnose (nine isolates, three from each group, namely orchard, wholesaler and retailer) was tested by inoculating into 15 healthy avocado fruits collected from Melkasa Agricultural Research Centre. The fruits were first surface sterilised with 70% ethanol and superficially wounded with a sterile needle. Cotton plug ( $5 \text{ cm}^2$ ) was dipped in spore suspension,  $2 \times 10^6$  conidia/ml, of each isolates and swabbed over the injured surface of the fruits. Wounded avocado fruits (five) but treated with sterile water in a similar fashion were included as negative control. The inoculated and control fruits (a fruit/box) were placed in disinfected small polyethylene box and incubated at room temperature for seven days while checking for symptom appearance every day. Finally, re-isolation was made from the inoculated avocado fruits with anthracnose symptom and compared with the original isolates (Baiyewu et al. 2007).

## **Results and discussion**

### ***Incidence and severity of anthracnose on avocado fruits***

The disease incidence and severity varied along farmers orchards and avocado value chain (Table 1). The incidence was higher with 84% in farmers' orchards at Gube Mulata followed by Buture (48%) peasant associations. The mean per cent severity index ranged from 22% at Somodo and Buture to 26% at Gube mulata (Table 1). The differences in disease intensity can be attributed mainly to variations in avocado cultivars grown by farmers and prevailing weather conditions mainly rainfall and temperature. In general, "Fuerte" is believed to be more susceptible to anthracnose than other avocado cultivars; while high humidity (>80%), warm temperature (18–26 °C) and heavy rainfall favour anthracnose infections (Maria 2007). In Australia, it has been found that Fuerte, Nabal, Wurtz and Rincon

**Table 1.** Incidence and severity of avocado anthracnose (mean  $\pm$  SD) in farmers' orchards of Mana district and at wholesaler and retailer shops in the market at Jimma town, south-west Ethiopia during 2013.

Location	Sample group	Incidence (%)	Severity (%)
Doyo Bikila	Farmers orchard <sup>a</sup>	28.0 $\pm$ 22.8	18.0 $\pm$ 10.9
Gudata Bula		20.0 $\pm$ 14.1	16.0 $\pm$ 8.9
Gube Mulata		84.0 $\pm$ 16.7	26.0 $\pm$ 5.4
Somodo		28.0 $\pm$ 22.8	22.0 $\pm$ 4.4
Buture		48.0 $\pm$ 33.4	22.0 $\pm$ 4.4
Jimma town	Wholesalers	56.7 $\pm$ 32.5	30.0 $\pm$ 17.3
Jimma town	Retailers	76.7 $\pm$ 20.8	36.7 $\pm$ 11.5
Overall mean $\pm$ SD		48.7 $\pm$ 25.0	24.3 $\pm$ 7.1

<sup>a</sup>n = 5 avocado orchards per location, SD = standard deviation.

cultivars are more susceptible (Manicom 2001). Hass is also susceptible but the anthracnose spots are not clearly visible against the background of the black peel of ripe fruit (Pegg 1991; Prusky 1996; Faber & Ohr 1999; Havenga et al. 1999; Manicom 2001).

The result of survey in the market also indicated more anthracnose incidence of 76.7% in retailers' shops than in wholesalers (56.7%), with respective severity index of 36.7 and 30% (Table 1). Similarly, study by Ogbo and Oyibo (2008) showed that anthracnose (28%) and *Dothiorella* rots (29%) were the second important postharvest disease of avocado fruit next to *Cercospora* spot (36%) at a local market in Nigeria.

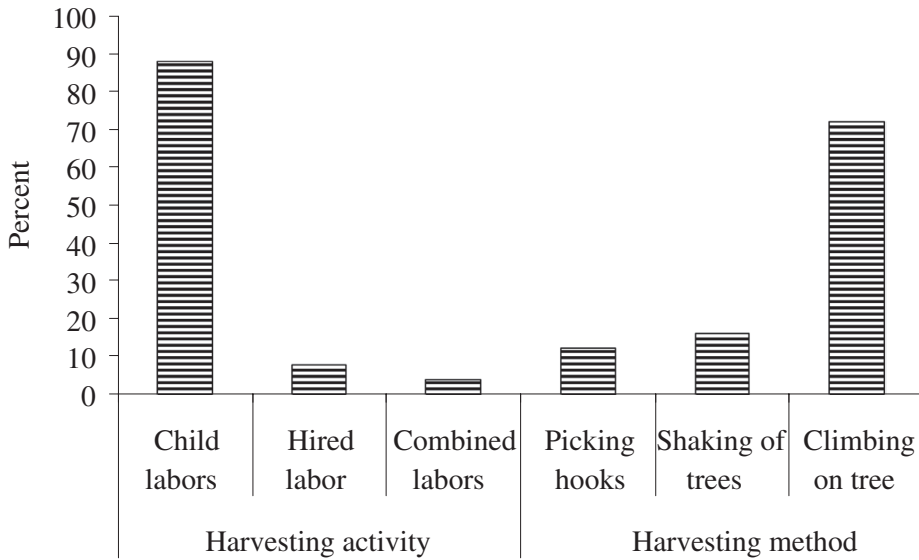
## Effect of harvesting and postharvest handling practices on anthracnose disease development

### Harvesting

All avocado farmers (respondents) performed harvesting after the fruit started dropping. The harvesting activity was carried out by hired labour (8%) and largely by children (88%) (Figure 1). Among harvesting practices, picking hooks (12%), shaking of trees (16%) and climbing on the tree (72%) were exercised (Figure 1) that usually resulted in internal and external injury caused by dropping of avocado fruits to the ground. Besides wounding, microbial contamination of the fruits is quite common due to direct contact with muddy or dusty ground.

### Transportation

The respondent pointed out that 66.7% of the traders used open "Isuzu" trucks to transport the fruits to town markets usually in the late afternoon (Figure 2 (B)), whereas 33.3% of the respondents transported to produce using different local means of transportation such as on horseback (Figure 2 (B)) and minibus vans together with the people. Brehanu & Alemu (2013) noted that donkeys and horses are used principally to transport avocado from the farm gate to accessible



**Figure 1.** Avocado harvesting practices and methods in Mana district of Jimma zone, south-western Ethiopia.



**Figure 2.** (A) Avocado fruits ready for market at farm gate, (B) transportation on horseback and "Isuzu" truck, (C) fruits partly on floor and in wooden basket at wholesaler shop (D) after unloaded and weighing upon arrival.

roads and markets. Therefore, most of the harvested avocado fruits did not usually reach market on time due to weak transportation facilities including poor roads in most rural areas of south-western Ethiopia.

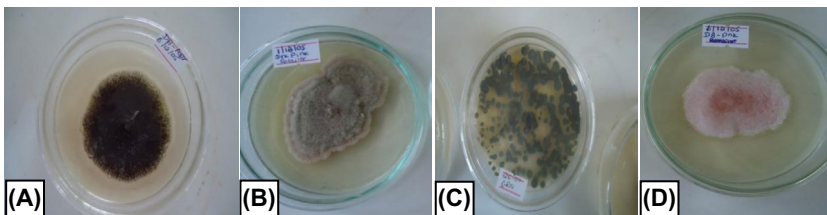
## Storage

Avocado producers used different packaging materials to transport the fruits and 66.7 and 33.3% of the interviewed farmers use plastic sacks (Figure 2 (A)) and baskets in the farm yard, respectively. Brehanu and Alemu (2013) denoted that various types of fibre sacks are popular containers used to transport fruits from farm gate to primary procurement centres. It is observed that avocado fruits are piled on cemented and/or bare ground with or without plastic sheets underneath (Figure 2 (C and D)) until retailers show up, whereas the latter actors (retailers) displayed the fruits on benches and kept in baskets for more than 10 days while waiting for consumers. Baiyewu et al. (2007) found that such improper handling of fruits increased microbial infection.

Our study showed that harvesting activities, postharvest handling including the containers and transportation and storage facilities of avocado fruit are inadequate along the value chains from farm gate to retailer shops. This poor handling created more favourable conditions for microbial infection that subsequently resulted in enormous loss of avocado fruits in the market.

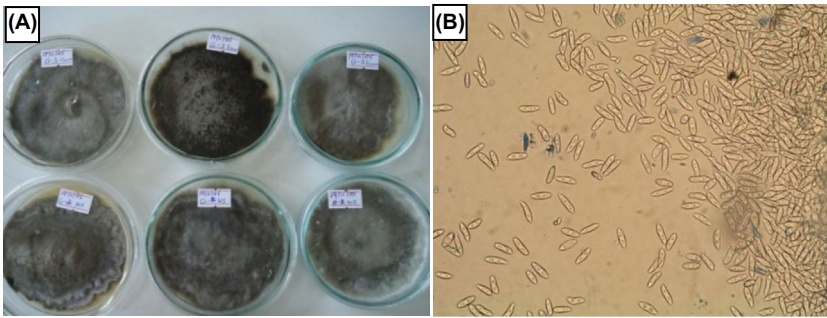
## Identification and characterisation of postharvest fungal pathogens of avocado fruits

A total of 249 fungal isolates were retrieved from the avocado fruit samples collected from the study areas, and they were identified on the basis of their cultural and morphological structures as *C. gloeosporioides* ( $n = 169$ ), *Alternaria* spp. ( $n = 41$ ), *Fusarium* spp. ( $n = 23$ ), *Penicillium* sp. ( $n = 15$ ) and black *Aspergillus* sp. ( $n = 1$ ) (Figure 3 (A–D)). *C. gloeosporioides* (Figure 4) was the most frequent fungal isolates from avocado fruits sampled at retailers, farmers and wholesalers with 71, 68 and 65%, respectively (Figure 5). *Alternaria* sp. was the next dominant fungal pathogen at retailers (22%) and farmers (19%) while *Aspergillus* sp. was common (23%) at wholesalers (Figure 5). *C. gloeosporioides* is the primary postharvest pathogen of avocado that attacks fruit during growth in the orchard and that remain quiescent until the fruit ripens, causing symptom development and substantial decay losses during storage and marketing (Prusky & Plumbley

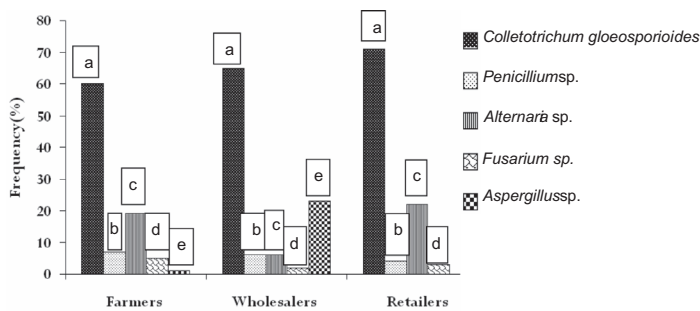


**Figure 3.** Identified fungi colony associated with avocado fruit collected from farmers' field and market place in Jimma zone, south-west Ethiopia (A) black *Aspergillus* sp. (B) *Alternaria* sp. (C) *Penicillium* sp. and (D) *Fusarium* sp.





**Figure 4.** (A) Colony features and (B) conidial appearance of *Colletotrichum gloeosporioides* isolates on potato dextrose agar after 10 days of incubation.



**Figure 5.** Frequency of fungal isolates associated with avocado fruit samples collected from farmers' fields, wholesalers and retailers shops in Jimma south-west Ethiopia.

1992). Ogbo and Oyibo (2008) also indicated that the occurrence of fungal isolates associated with avocado fruit surface was *Aspergillus niger* (40%), *Rhizopus stolonifer* (29.9%), *Fusarium* sp. (17.9%) and *Penicillium* sp. (11.9%).

The colony colour of *C. gloeosporioides* isolates appeared to be pale to dark grey on potato dextrose agar (Figure 4 (A)), with abundant conidia that varied in shape but cylindrical to straight tapered base and pointed ends were frequent (Figure 4 (B)).

### Pathogenicity test

A typical symptom of anthracnose, i.e. dark sunken lesions, was observed on all avocado fruits inoculated with three isolates of *C. gloeosporioides* after 3–5 days inoculation (Figure 6 (A)). The fruits treated with sterile water were apparently healthy during the same period (Figure 6 (B)). The fungus was re-isolated from such symptomatic lesions and found to be the same as that of original isolates and *C. gloeosporioides* was proved to be the causal pathogen of avocado anthracnose both in the field and after harvest.



**Figure 6.** (A) Infection symptoms of anthracnose (black lesions) on avocado fruits inoculated with *Colletotrichum gloeosporioides* and (B) apparently healthy appearance of wounded but noninoculated fruits.

In conclusion, the incidence and severity of avocado fruit anthracnose, caused by *C. gloeosporioides*, were as high as 84 and 26%, respectively, in the farmers orchards. The disease intensity had similar trend in the market but with more anthracnose infection and fruit rot recorded in retailer shop than in that of the wholesalers. The disease was found to be favoured and aggravated by improper harvesting and postharvest handling, and lack of good transportation and storage facilities. Therefore, producers and other stakeholders along the value chain should get awareness on proper handling of avocado fruit that circumvent mechanical injury and reduce microbial infection and losses.

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### Disclosure statement

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### ORCID

Girma Adugna  <http://orcid.org/0000-0001-5749-7546>

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