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ASSOCIATED FUNGAL PATHOGENS AND CAUSES OF POSTHARVEST LOSSES IN THE AVOCADO VALUE CHAIN IN HOSSANA TOWN MARKET, ETHIOPIA

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ABSTRACT

A significant proportion of post-harvest loss of agricultural produce is experienced in Ethiopia, especially in perishable horticultural commodities like fruits (mango, banana, papaya, avocado, sweet orange) and vegetables. Postharvest diseases derived from biotic sources such as microbial pathogens, or physiological disorders caused by abiotic sources contribute to shortening the postharvest life of the avocados, leading to loss in fruit quality. This study aimed to assess post-harvest losses and identify post-harvest pathogens associated with the decay of avocado fruits in Hossana town shops and a market which opens once a week. Random sampling method was used to select shops for survey and sample collection. Avocado fruits with different damage levels and disease symptoms were collected from 14 shops and the Hossana Market, packed using cartons then transported to the laboratory. A survey was conducted in selected shops and the market and identification of disease-causing pathogens was done through incubation of the samples taken from different parts of the fruits using potato dextrose agar (PDA) for seven days. Data was analyzed using SPSS, statistical software. Results from the survey revealed that postharvest loss of avocado varies across different post-harvest handling stages and the highest loss was recorded during storage (40%) followed by harvesting (26.7%). The most mechanical damage was observed in the MS shop (100%) followed by the HM (83.3%). This may be due to poor harvesting practices: unsuitable field or marketing containers and crates, which may have fractured wood, sharp edges, poor nailing or stapling. Disease incidence assessment showed that in three shops (AD2, MN2 and MS), 100% of fruits were infected. The highest disease severity index rated as 5 (>75%) was also observed in the same three shops (AD2, MN2 and MS) and the HM, followed by 3 shops (AD3, GM3 and NR) rated as 4 (51-75%). A total of 82 fungal colony growths were observed with *Colletotrichum gloeosporioides* being the most frequently isolated species (60 %) followed by Fusarium spp (26.67 %). Appropriate post-harvest handling technology and disease management strategy should be implemented from farm to fork to reduce post-harvest losses.

Key words: Avocado, *Colletotrichum gloeosporioides*, Fungal Pathogens, Post-Harvest Loss, Public market, Ethiopia

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INTRODUCTION

Losses of horticultural produce are a major problem in the postharvest chain. Post-harvest loss of fruits and vegetables is estimated to be 30-40% in developing countries [1, 2]. According to Food and Agriculture Organization (FAO), post- harvest loss has been estimated to be about 50% of the perishable commodities in Ethiopia [3]. Avocado fruits incur large (about 40%) losses during post- harvest handling [4].

Both quantitative and qualitative losses of variable magnitudes occur at all stages in the post-harvest system [5]. Postharvest loss in horticultural produce can be caused by a wide variety of factors, ranging from growing conditions to handling at the retail level. Not only are losses clearly a waste of food, but they also represent a similar waste of human effort, farm inputs, livelihoods, investments and scarce resources such as water [6]. The losses are due to high moisture, high sugars (in fruits) and low pH that promote fungal growth leading to fruit deterioration and decay. All these reduce the quality and sometimes result in completely unmarketable produce that fail to meet the standards for exports. Besides, fungal pathogens can attribute to quality loss and health hazards through the production of toxins [7, 8].

Various efforts have been implemented by policy planners in ensuring food security in the country over the years with emphasis mostly on production techniques, crop improvement through breeding, intensive arable land utilization, extension service and effective marketing strategies [9]. Horticultural crops are known for their high return per unit time and area which is one major tool to achieve food security in the country. However, post-harvest behavior and post-harvest management of horticultural produce have not been given sufficient attention over the years, most specifically, perishable horticultural produce.

This is due partly to inadequate studies on causes of losses along the food value chain. As a result of less attention given to this sector, a significant proportion of postharvest loss of agricultural produce is experienced in Ethiopia, especially in perishable horticultural commodities like fruits and vegetables accounting for as high as 30 to 40% loss [10].

Avocado is one of the most economically important fruits not only in Ethiopia but also in the world [11]. It is a highly perishable commodity [12] and yet valued for export. Avocados continue respiring even after harvest, commencing the ripening process almost immediately due to their climacteric characteristic of high respiration rates [13]. To delay the ripening process it is of importance to lower the temperature, eliminate mechanical damage and reduce ethylene production [14]. The ripening phase is essential for the eating quality but the post-harvest life of the fruit is shortened as soon as the ripening starts. Because of perishable nature and high moisture content, it is susceptible to post –harvest factors like rough handling, use of inadequate packaging materials, improper cooling, poor sanitation and improper harvesting method which leads to loss in quality and quantity. Anthracnose, Dothiorella/Colletotrichum fruit rot complex and stem-end rots are the most severe post-harvest diseases of avocado [15]. Literatures suggest proper handling, good sorting and cleaning, good packaging,

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adequate transportation and good storage facilities can reduce post-harvest losses of horticultural crops [16, 17, 18].

In Hossana town, avocado fruit retailers and shops are confronted with challenges of shelf life and marketable quality of fruits because of different factors. There are some studies reported in literature [19, 20, 21] about causes of post-harvest losses of avocado in some parts of Ethiopia. However, there are no previous studies on causes of post-harvest losses of avocado and fungal pathogen identification in Hossana market. Considering this, this study was initiated to asses causes of postharvest losses in avocado fruits and identification of associated fungal pathogens in Hossana town market.

MATERIALS AND METHODS

Description of the study areas

The assessment was carried out in Hossana town shops and market, Hadiya zone of Southern Nations, Nationalities and Peoples' Regional State (SNNPRS) of Ethiopia. The laboratory work was conducted at Wachemo University College of Agricultural Science Pathology Laboratory.

The capital city of Hadiya zone, Hosanna town, is located 232 Kms away from Addis Ababa. Avocado selling shops are retailers who receive from wholesalers and sell their avocados to consumers. They are found in the town in different direction and smaller than big retail supermarket. On the other hand, Hossana market is one major local market which is opened once a week, every Saturday to the public to bring in fresh produce for sale.

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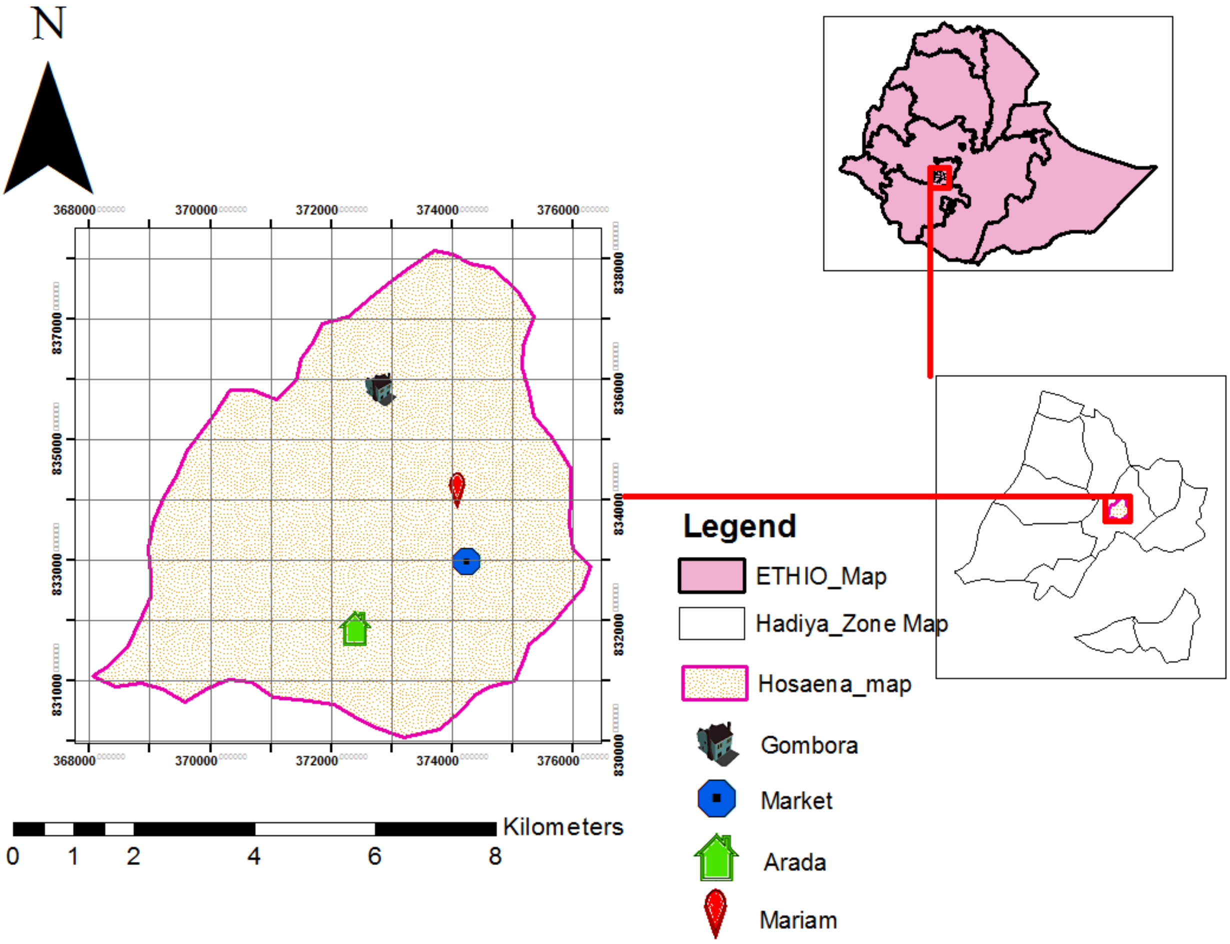

Figure 1: Map of the study area

Research design

The research design of qualitative and quantitative method was employed to collect data from a survey in order to reveal the existing causes of post-harvest losses of avocado in the market. For laboratory study, factorial design was used to identify post-harvest fungal pathogens.

Survey procedures and sample collection methods

Random sampling method was used to select avocado selling shops found in Hossana. A questionnaire was used to conduct a survey in 14 randomly selected avocado selling shops and Hossana market. Thirty farmers sold their avocados in open market and about 30 percent were selected randomly and participated in the interview. Six kilograms of avocados were collected from each shop and seller. Survey and data collection on causes of post-harvest losses in avocado was conducted by actual observation and through interviewing of retail sellers of avocados at shops and the market. Using questionnaires, the major points where the losses occurred were addressed. Sample size used was number of shops and market itself since from all

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interviewed shops and market sample was taken to laboratory for identification of fungal pathogens.

Fruit damage assessment

During survey, mechanically damaged avocado fruits were assessed from fourteen randomly selected retailer shops and Hossana market. From each retail seller, six kilograms of avocado samples and 30 fruits per site were assessed and the percentage of fruit damage was calculated using the following formula [22]:

$$\text{Percentage of fruit damage} = \frac{\textbf{Damaged sample}}{\textbf{Total sample}} * \mathbf{100}$$

Disease incidence and severity assessment assay

The infection was identified on basis defects observed on fully ripe avocados, such as sunken, water soaked, round to oval, regular to irregular and brownish red to black spots. Disease incidence (DI) was calculated as number of fungal infested fruits showing a defect out of total number of avocado fruits stored. Thirty (30) avocados were used for disease incidence evaluation and it was calculated using the following formula [23]:

$$\text{DI (Incidence Disease)} = \frac{\boldsymbol{Number\ of\ infected\ fruit}}{\boldsymbol{Total\ number\ of\ fruit}} * \mathbf{100}$$

Culture media preparation

The media used for this experiment was potato dextrose agar which is most commonly used for fungal pathogen identification. Potato dextrose agar (PDA) was prepared by dissolving commercially formulated powder PDA. The PDA was mixed with sterilized water in a flask at the rate of 39 g/L and the mixture was boiled while stirring with a magnetic stirrer for 15 minutes to completely dissolve the powdered agar until formation of foam. The solution was then autoclaved (Pressure Steam Sterilization, FM-280A, China) at 121°C at 15lb pressure for 15 minutes to sterilize the media. The liquid media was maintained under aseptic condition and allowed to cool. The media was poured into sterilized petri dishes and the PDA was then allowed to cool completely and solidified before being used for maintaining fungal cultures [24].

Fungal isolation procedures

Avocado fruits that displayed symptoms of fungal infection were collected from retail shops and market of Hossana town. The fungi were isolated from decaying avocado fruits and identified in the laboratory. Diseased avocado fruit tissues were cut from active lesion surfaces under aseptic conditions. The tissues were surface sterilized by soaking the sections in freshly prepared Sodium hypochlorite (3% w/v) for 3 minutes. After three serial washings in sterile distilled water, avocado tissues were placed (4 pieces per plate) on (PDA) and incubated around 25^{o}C for 7 days. Pure cultures of the individual isolates were made by taking small pieces from the mother culture and placed on sterile PDA. The colonies that emerged from each plated samples were purified and sub-cultured on the PDA media by taking small pieces from the cultured

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fungi. The plates were incubated at 25°C under similar conditions and the setups were observed daily until the organisms became fully grown [25].

Identification of isolated and purified fungi assay

The identification of isolated and purified fungi was done based on colony characteristics (mainly color on both sides of the plates and shape) and morphological appearance of conidiophores and conidial appearance under a microscope by using a method described in Valencia *et al.* [26]. To identify morphological appearance of conidiophores and conidial under the microscope, a drop of water was placed on a slide plate and a small portion of the mycelium from the cultured fungal was removed and placed on the drop of the water with the aid of needle. The mycelium was spread very well on the slide with the aid of two needles. The slide was then examined under the microscope [27].

Frequency of fungal pathogen colony determination assay

The frequency of fungal disease of avocado from 14 retail shops and a market was assessed after fungal colonies emerged from each plated avocado samples were purified and sub-cultured on PDA by the use of the following formula:

$$\text{Frequency (\%)} = \frac{\text{number of fungal colony in the sample}}{\text{Total number of sample}} \times 100$$

Frequency of fungal pathogen was done from the growth of colony in the sample. A total of 82 fungal colony growths were observed from fourteen (14) shops and market in Hossana. These 82 isolates were identified based on their mycelial colony and microscopic features into five fungal species belonging to three genera (Table 3).

Data Analysis

The collected data from survey and laboratory were categorized, coded and summarized into numeric value and analyzed using SPSS. Descriptive data analysis: frequency and percentages were computed, presented in tables and bar chart graphs.

RESULTS AND DISCUSSION

Socioeconomic characteristics of respondents in the study area

Among the respondents, 73.3% were male and 26.7% were female. In terms of age, 54.3 % ranged from 31-35 years, 26.7% ranged from 36-40 years and 20% ranged from 18-25 years. Most, 73.3% were educated to certificate level and 26.7% were uneducated (Table 1). The data are used to describe socioeconomic characteristics of respondents who participated in the survey.

Cause of post-harvest loss of avocado fruit

Post-harvest loss assessment results of avocado fruits indicate that a number of factors play important roles in the deterioration of fruits from farm to fork. Post –harvest losses of avocado fruits were recorded across different postharvest handling points (Figure 2). The highest loss was recorded during storage (40%) followed by harvesting 26.7% with the lowest loss recorded at the point of packaging 13.3 %. Knowing where the losses

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occur in the food supply chain is important to determine potential causes and implement the best post-harvest practices [28].

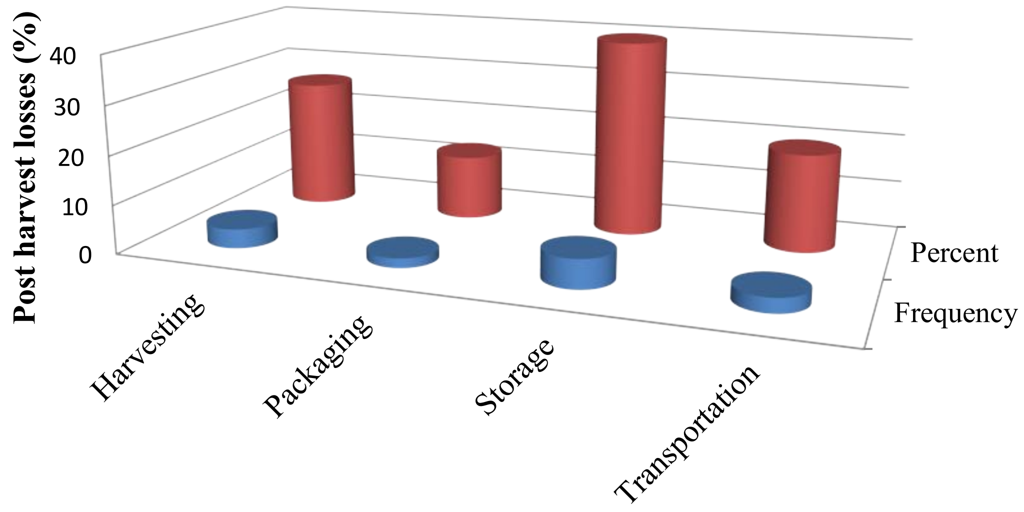

Figure 2: Sites of post-harvest loss of avocado fruit at different supply chain

Poor harvesting methods such as shaking trees or picking fallen fruits contribute largely to the postharvest losses. Scratches, cuts, punctures and bruises to the fruit also accelerate mechanical injuries. This trend brings major decay problems after harvest, especially when the fruit is ripened off the tree. To prevent mechanical damage Humble and Reneby [19] suggest the farmers to use baskets for collection of the avocado fruits from the tree. When the avocado is transported from the field, the vibration should be minimized and bouncing of the fruits should be avoided [29]. This finding is in line with Kasso and Bekele [30] who reported that harvesting technique is one factor that causes fruit losses.

The largest loss recorded during storage can be attributed to failure to control temperature and relative humidity of storage environment. The places where most of the respondents stored their avocados for selling were directly exposed to sunlight, which increases temperature of the produce. An increase in temperature causes an increase in the rate of natural respiration of avocado fruits, therefore food reserves and water content become depleted [31]. These activities accelerate the loss of avocado fruit and shorten the shelf life of the fruit. Lallu *et al*. [32] reported that exposing a fruit to sunlight leads to water loss through transpiration which causes the fruit's quality change. Lack of proper storage materials at retail market is another important factor which affects quality and shelf life of avocado fruit. This study is in line with FAO [33] who indicted that lack of proper storage facilities in developing countries is seen as the main cause of post-harvest losses. Humble and Reneby [19] reported that post-harvest losses contribute to undernourishment and food insecurity. Use of improved storage facility as well as packing is required at retail level. Local collectors and wholesalers need training on post -harvest handling, maintaining quality and marketing of avocado

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fruits. Another interesting observation made during the interview was that injured, diseased and healthy avocado fruits were mixed up during storage.

Transportation is another important factor which causes post- harvest losses of avocado fruits in Hossana shops and market which accounts for 20 % of the losses (Fig. 2). Avocados sold in the shops and market are transported without cold chain for long distances. Poor transportation roads, overriding and contact of the fruits with bins cause huge losses during transportation. According to Bantayehu *et al.* [34] the major causes of fruit loss during transportation are inappropriate transportation means, poor roads, damage injury during loading and unloading, poor market infrastructure (high temperature, low relative humidity and sun burning), market price failure, long distance transport, mixing different fruits during transport and mixed transportation of ripe/unripe fruit and diseased/disease free fruits.

In developing countries, loading and unloading operations are carried out by unskilled and uneducated workers who generally do not carry products carefully which causes mechanical damage in agricultural produce [35]. Mixed loads of bulk commodity is again a serious concern as the agricultural produce have different responses to temperature, transpiration, dehydration and ethylene production. All together, these factors affect durability of the commodities through enhancing physiological, mechanical, biological and chemical losses [36].

The packaging materials used in the study area for transportation were sacks. The sacks allow for proper aeration but do not protect the fruits from mechanical damage causing fruit loss by crushing. Fruits are also hipped into the sacks generating a lot of heat as a result of metabolic activities. These reactions in turn accelerate mechanical damage and aid microbial attack [37, 38]. On the other hand, an increment of moisture content supports the growth of microorganisms especially fungi which speed up the deterioration rate of avocado fruits. Poor quality packages which provide little or no protection during handling, transport and storage accelerate the loss of avocado fruits. Olayemi *et al.* [39], stated that if the sacks used for handling/packaging of produce have no palletizing and large mass of commodity is tightly packed, gas exchange between commodities become decreased, which leads to quality deterioration and shortened shelf life of the fruit.

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Mechanical damage

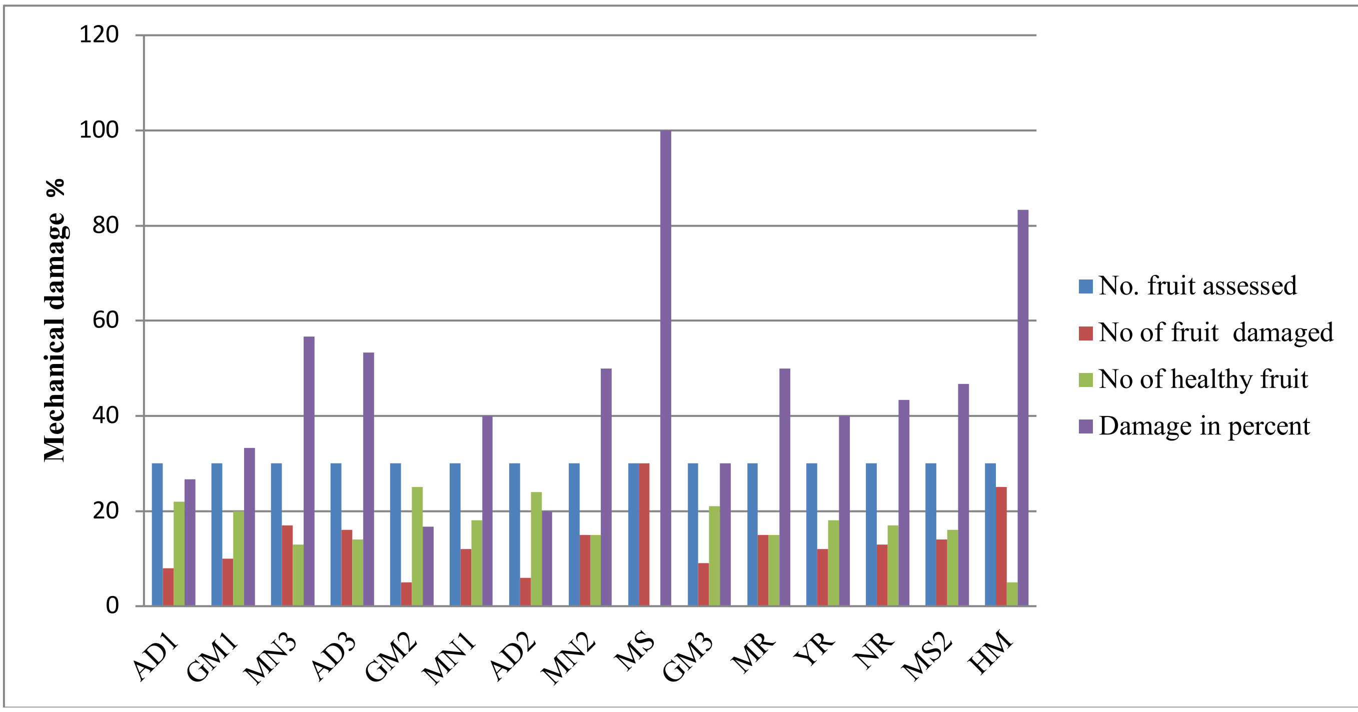

Figure 3: Number of fruit assessed, No of fruit damaged, No healthy fruit and damage in percent in different shops and Hossana market

Note: AD1=Adebabay1; GM1=Gombora 1; MN3=Meneharia 3; AD3=Adebabey 3; GM2=Gombora 2; MN1=Menaharia 1; AD2=Adebabay 2; MN2=Meneharia 2; MS=Mesalemia; GM3=Gombora3; MR=Mariam; YR=Yerusalem; NR=Naramo; MS2=Mesalemia 2; HM=Hossana Market

As indicated in Figure 3, the highest damage of avocado fruits was observed in MS shop (100%) followed by HM (83.3%), while lowest damage was observed from GM2 shop (16.7%). The results obtained indicated that the maximum damage may be due to poor harvesting practices; unsuitable field or marketing containers and crates which may have fractured wood, sharp edges, poor nailing or stapling, over packing or under packing of field or marketing containers, careless handling, such as dropping or throwing or walking on produce and packed containers during the process of grading, transport or marketing. The study [40] found that mechanical damage is one factor which initiates or increases defects of avocado fruits because of inadequate handling of the fruits from harvest to packaging.

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Disease incidence

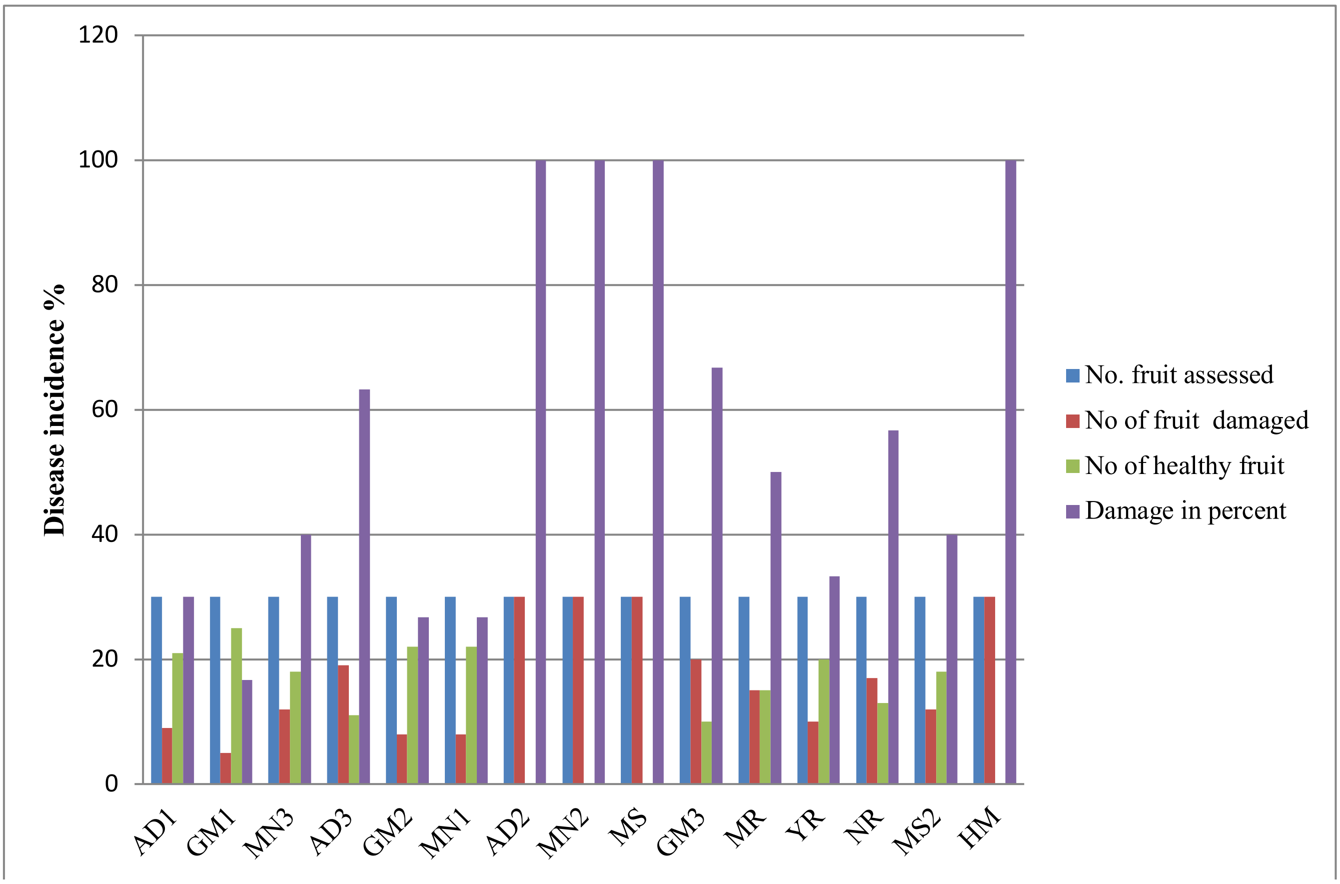

Figure 4: Number of fruits assessed, No. of fruits damaged, No healthy fruits and damage in percent in different shops and Hossana market

Disease incidence assessment results showed that the maximum percentage of infected fruits was observed in three shops (AD2, MN2 and MS) and HM at 100%, followed by GM3 at 66.7% and the minimum percentage of fruit damage was detected in MN1 and GM2 shop at 26.7% (Fig 4). The largest disease incidence recorded in those shops was due to placement of avocados by exposing directly to sunlight, lack of good sanitation and poor post-harvest handling practice. The mechanical damage during handling at different stages along postharvest chain predisposes the fruits to fungal pathogens [41]. On the other hand, MN1 and GM2 shops stored their fruits on shelves under a shade preventing sun burning of the fruits. They also properly cleaned the storage areas all the time.

Disease severity

Disease severity assessment results showed that the maximum disease severity index rated as 5 (>75%) was observed from the sample taken from 3 shops (AD2, MN2 and MS) and HM followed by 3 shops (AD3, GM3 and NR) rated as 4 (51-75%) while minimum disease severity index was found in samples taken from GM1 shop rated as 2 (<5%) (Table 2). The highest severity rate may be due to keeping the fruit under

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normal ambient temperature after ripening and improper handling of fruit. The recommended storage temperature of avocado fruit is ranged from 4.5-13^{o}C but the mean air temperature of Hossana town is found between 22-30 ^{o}C. Exposure of the fruit to this temperature and lack of use of shade contributes to high fruit temperatures which favor the growth of microorganisms, high water losses and wilting of the fruit.

Frequency of fungal pathogen colony
Colletotrichum gloeosporioides was the most frequently isolated species (60 percent) followed by *Fusarium spp* (26.67 percent). On the other hand, the least frequent species found were *Colletotrichum truncatum* and *Pestalotiopsis spp* which recorded 6.67%. Anthracnose disease prompted by *Colletotrichum gloeosporioides* is one of the most severe post-harvest diseases on avocado in Hossana shops and markets which causes huge post-harvest losses. Ajay Kumar [42] reported that Anthracnose caused by *C. gloeosporioides* is a serious problem that results in a considerable loss by damaging fruits such as strawberry, mango, citrus fruits, avocado, bananas, vegetables and medicinally or ethno botanically useful plants.

Figure 5: Avocado sample showing symptom of anthracnose disease

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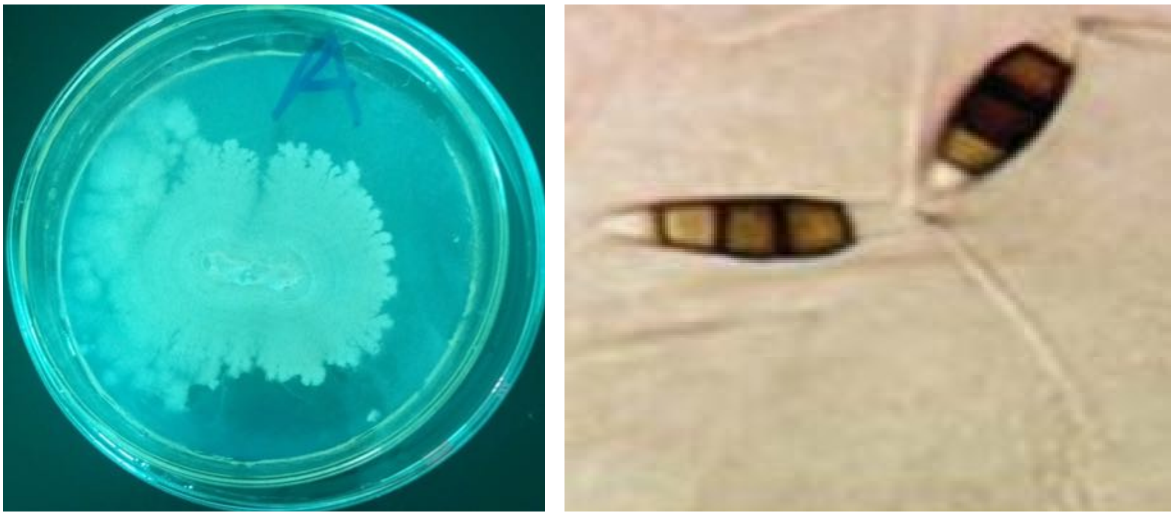

Figure 6: Colony characteristics of pathogenic isolates of *Pestalotiopsis clavispora spp* on PDA

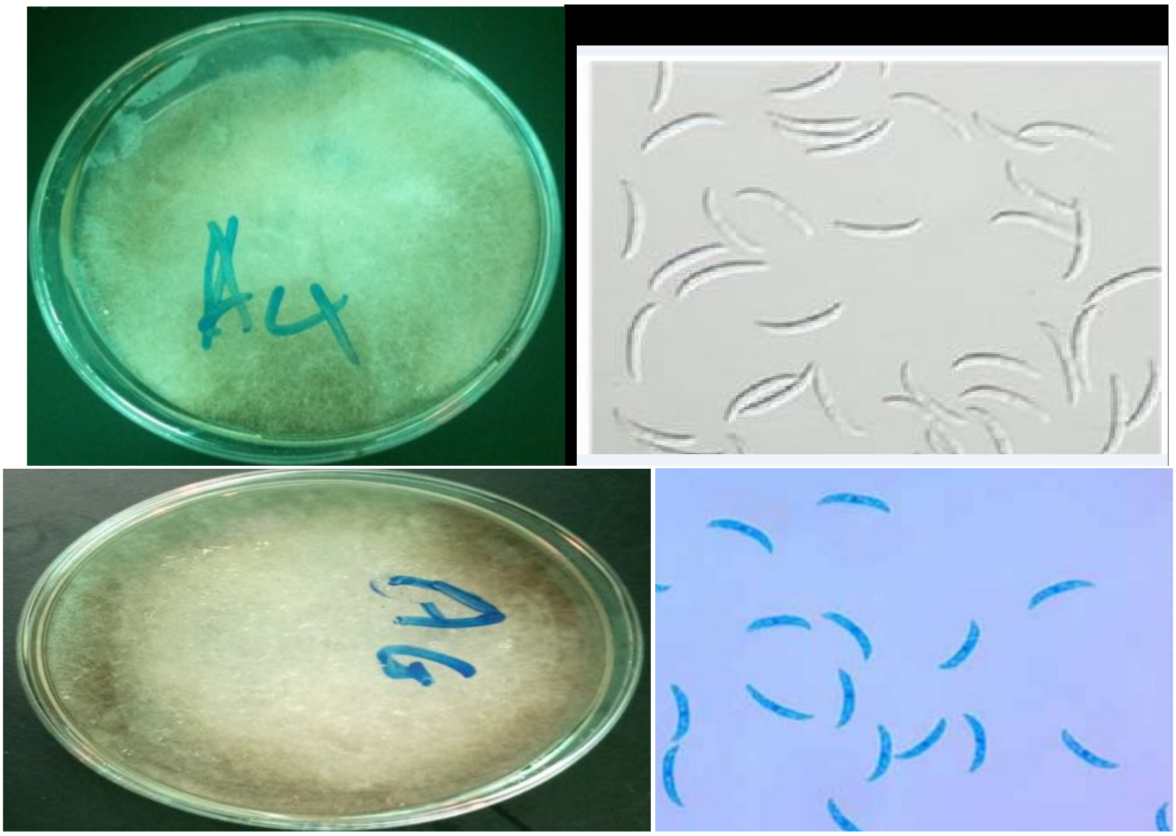

Figure 7: Colony characteristics of pathogenic isolates of *Colletotrichum truncatum spp* on PDA

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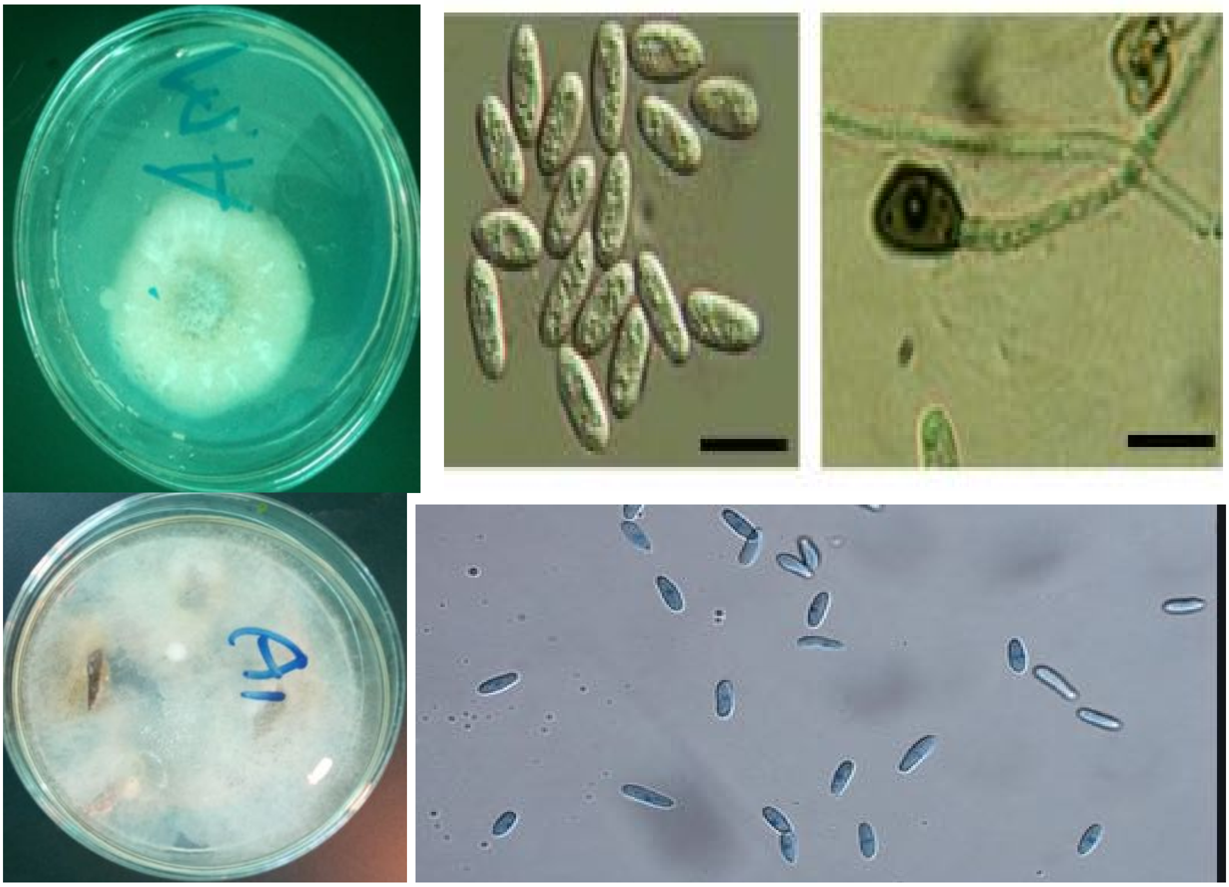

Figure 8: Colony characteristics of pathogenic isolates of *Colletotrichum gloeosporioides spp on PDA*

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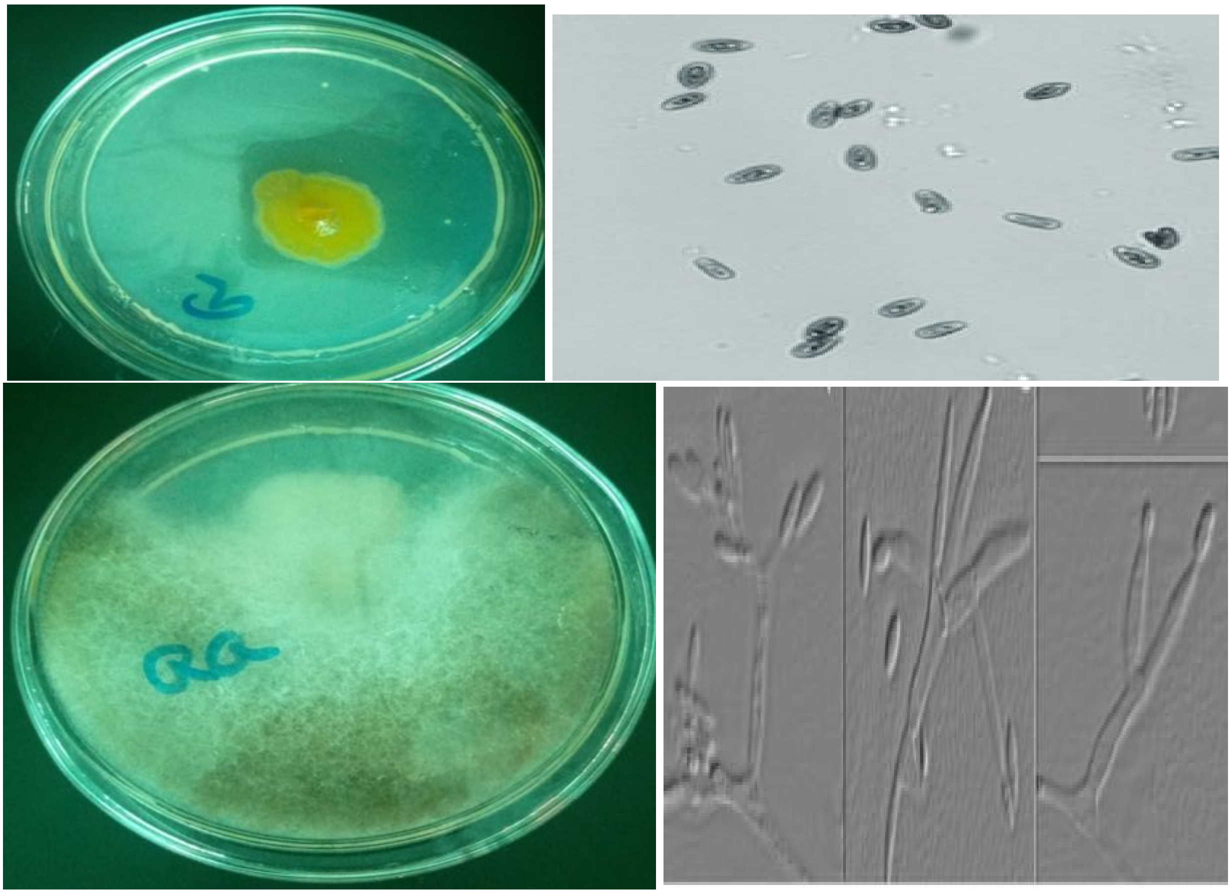

Figure 9: Colony characteristics of pathogenic isolates of Fusarium thapsinum spp. On PDA

Post-harvest diseases of avocado fruits were surveyed in fourteen (14) shops and Hossana market. Anthracnose and Fusarium spps were detected as the main cause of avocado rots. Of these, anthracnose is the most devastating disease which affects the avocado fruits while in the field as well as after harvest. Fungal isolates from diseased avocado fruits showing symptoms of anthracnose collected from the study area varied significantly in their cultural characteristics on PDA media in terms of texture and color. The isolates had whitish, grey to brown, creamish color and cottony, velvety mycelium on the top side and greyish cream with circular orange-pinkish color and dark brown on the reverse side. Four isolates of Fusarium recovered from avocado fruits were identified as *Fusarium thapsinum*. *Fusarium thapsinum* forms an abundant white mycelium which may darken (violet pigments) with age on PDA medium, as in sample taken from AD3 and orange- yellow in sample taken from GM1 and MN1 (Fig. 9).

CONCLUSION

The main points of postharvest losses in the supply chain of avocado fruits are during harvesting, packaging, storage and transport with a maximum loss recorded during storage. The highest mechanical damage of avocado fruits was observed in MS shop.

On the other hand, maximum disease severity index rated as 5 (>75%) was observed from the sample taken from 3 shops (AD2, MN2 and MS). The two most common diseases causing immense postharvest losses in avocado fruits found in Hossana town are anthracnose caused by *Colletotrichum gloeosporioides* and fusarium rot which is caused by *Fusarium thapsinum spp*. This study investigated only the causes of post-harvest losses and post-harvest fungal pathogens of avocado fruit in Hossana town Market. Therefore, further research is required to identify the causes of losses and associated fungal pathogen of avocado fruit along value chain in other part of the country.

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Table 1: Socio-economic characteristics of respondents in the study area

		Frequency	Percent
Sex	Male	11	73.3
	Female	4	26.7
Age	18-25	3	20.0
	31-35	8	53.3
	36-40	4	26.7
Educational status	un-educated	4	26.7
	Certificate	11	73.3

Table 2: Percentage of disease severity index of avocado fruit at different shops and Hossana market

Shops and market	No. fruit assessed	No of fruit damaged	No of healthy fruit	Damage in percent	scale
AD1	30	9	21	30.0	3
GM1	30	5	25	16.7	2
MN3	30	12	18	40.0	3
AD3	30	18	12	60.0	4
GM 2	30	8	22	26.7	3
MN1	30	8	22	26.7	3
AD2	30	23	7	76.7	5
MN2	30	25	5	83.3	5
MS	30	27	3	90.0	5
GM3	30	20	10	66.7	4
MR	30	15	15	50.0	3
YR	30	10	20	33.3	3
NR	30	17	13	56.7	4
MS2	30	12	18	40.0	3
HM	30	26	4	86.7	5

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Table 3: Frequency of postharvest fungal species of avocado

N	Genus	Species	Frequency in percent
1.	*Collectotrichum*	*Colletotrichum gloeosporioides*	60
2.	*Collectotrichum*	*Colletotrichum truncatum*	6.67
3.	*Fusarium*	*Fusarium thapsinum sp*	26.67
4.	*Pestalotiopsis*	*Pestalotiopsis* clavispora spp	6.67

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