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Prevalence of post-harvest fungal diseases of avocado in fruit markets in Embu County Kenya

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Abstract

Avocado fruit is highly valued for its nutritional benefits and industrial uses. Production of avocado contributes to poverty alleviation, food security, and economic growth in producing countries. However, production is hindered by fungal diseases such as anthracnose, scab and cercospora spot. This study assessed the prevalence of the aforementioned fungal diseases in various fruit markets in Embu County. A cross-sectional survey was done involving 120 fruit vendors across six markets. Avocado fruits were randomly selected and examined for the presence of anthracnose, scab and cercospora spot diseases. Symptomatic fruits were taken to the laboratory for further analysis. The *Hass* avocado was the most common variety, found in 39% of the sampled stores, followed by local varieties (24%), *Fuerte* (21%), and *Puebla* (4%). Most vendors (42%) used hooks to harvest avocados, while 39% picked the fruits by hand. For packaging, 54% used sacks, 21% used buckets, and 13% used crates. Fifty-four percent of vendors used synthetic fungicides for disease management, while others employed various cultural practices. Disease prevalence varied significantly ($P < 0.05$) across the markets. Kianjokoma market had the highest prevalence of anthracnose (35.0%), while Mutunduri had the lowest (21.3%). Siakago market recorded the highest prevalence of scab (36.3%), with Mutunduri again having the lowest (20.7%). Cercospora spot was most prevalent in Kianjokoma (35.0%). The study highlights widespread avocado fruit diseases, exacerbated by harvesting and post-harvest handling practices. Training farmers on post-harvest disease management could significantly reduce associated losses.

Key words: *Anthracnose, Avocado, Cultural practices, Post-harvest, Prevalence, Scab*

1. Introduction

Avocado (*Persea americana* Miller) is an important fruit crop grown in tropical and subtropical regions (Selladurai and Awachare, 2020). The fruit is grown in over three million hectares worldwide with the total global production estimated at about 8.06 million tonnes annually (Nyakang'i *et al.*, 2023). Mexico is the leading producer and exporter of the avocado fruit worldwide, accounting for about 30% of global output (Gómez-Tagle *et al.*, 2022; Herrera-González *et al.*, 2020). Africa is an emerging avocado fruit producer and exporter

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with countries such as Kenya, Ethiopia, South Africa, and Tanzania are leading the way (FAOSTAT, 2022). Kenya is the top avocado produce in Africa and number six in global rankings (FAO, 2020; Nyakang'i *et al*., 2023).

According to FAOSTAT (2022), approx. 28,000 ha of land in Kenya are under avocado production with an annual productivity of approx. 16.5 tonnes per ha. The country produced approximately 0.46 million tonnes of avocado in 2022 alone (FAOSTAT, 2022). About 70% of avocado fruits in Kenya are produced by small-holder farmers (Amare *et al*., 2019) scattered across the country but mainly in Murang'a, Bungoma, Migori, Kiambu, Kisii, Nyamira, Tharaka Nithi and Embu Counties of Kenya (Johnny *et al*., 2019). *Hass*, *Fuerte* and *Puebla* are the commonly produced avocado varieties for the local and export market. However, local avocado varieties are still popular among the growers, particularly for the domestic market and family consumption (Shivachi *et al.,* 2023).

Avocado is a highly nutritious fruit, packed with essential minerals like manganese, phosphorus, iron, and potassium. The fruit is rich with essential vitamins, including A, B, E, and â-carotene, among other vital nutrients (Dreher and Davenport, 2013). Consumption of avocado offers numerous health benefits, such as improving diet quality, providing rich nutrients, and reducing the risks of metabolic syndrome and cardiovascular diseases (Fulgoni *et al*., 2013; Nyakang'i *et al*., 2023). Additionally, avocados are valuable in the pharmaceutical, cosmetic, oil, and food industries (Nyakang'i et al., 2023). Moreover, the production and marketing of avocados have significantly boosted the rural economy by creating jobs and providing direct income in avocado-growing regions (Amare et al., 2019).

Avocado production is constrained by various biotic factors including diseases such as anthracnose, scab, cercospora spot, verticillium wilt, bacterial canker, among others (Ramírez-Gil *et al.*, 2021). The fruit is also attacked by pests such as false codling moth, thrips, scales and fruit flies (Dennill and Erasmus, 1992). Post-harvest fungal pathogen attacks various avocado fruits leading to lower shelf life and poor marketability (Ramírez-Gil *et al.*, 2021). The post-harvest fungal diseases are estimated to cause about 83% losses thus threatening the global avocado market (Fan *et al.*, 2017). Fungal infestation on avocado may often go unnoticed during growth period only to appear at maturity or during storage and marketing (Mekonnen *et al*., 2015). Nevertheless, there is little awareness among the producers and handlers about fungal disease control and management in the field and post-harvest care (Mekonnen *et al*., 2015).

The management of post-harvest avocado fungal disease relies heavily on synthetic chemicals (Yoon *et al.*, 2013). For instance, regular copper-based fungicide sprays, such as benomyl, are widely used by farmers (Fesenko and Edwards, 2014). However, use of synthetic fungicides is becoming increasingly restricted in many countries (Mekonnen *et al.*, 2015). Persistent use or misuse of synthetic fungicides may have detrimental effects on human health besides causing environmental pollution and loss of biodiversity (Ndayambaje *et al.,* 2019; Silva *et al*., 2022). Excessive use of fungicides can lead to microbial resistance, making it much harder to manage fungal diseases effectively. This resistance occurs when fungi are repeatedly exposed to fungicides, allowing them to evolve and develop mechanisms to survive the treatments (Corkley *et al*., 2022). Despite the use of fungicides in the management of post-harvest fungal diseases, losses attributed to pathogens have remained high (Fan *et al.*, 2017). Other management strategies against avocado post-harvest fungal diseases include use of cultural practices such as proper sanitation during harvesting, sorting and packaging (Kuru *et al*., 2016). Use of proper post-harvest handling practices to minimize cuts and bruises to fruit as well as proper disposal of diseased fruits, use of biological control among other methods (Agrios, 2005).

This study was done in Embu County, in central Kenya, to assess the prevalence of anthracnose, scab and cercospora spot diseases of avocado fruits. This is essential in mapping the geographical distribution and determining the status of these diseases in the region. The data provides baseline information to prioritize research and training in their control and management.

2. Materials and methods

This study was carried out in Embu Country, Kenya. A descriptive cross-section survey was done in six fruit markets to assess the prevalence of fungal diseases affecting avocado fruits. A structured questionnaire was used to collect data from the fruit vendors. Additionally, avocado samples were randomly taken from vendors and examined for symptoms associated with anthracnose, scab, and cercospora spot diseases. Questionnaires were administered to twenty (20) randomly selected avocado fruit vendors in each of the six selected fruit markets. A total of 120 fruit vendors took part in the study.

2.1. Determination of fungal disease prevalence

During sampling, the prevalence of avocado fungal fruit diseases was assessed on avocado fruit among vendors identified. For every vendor, all avocado fruits in the storage containers were thoroughly inspected and the disease identified based on the symptoms. Both infected and healthy fruits were enumerated and the data used to compute the percentage prevalence of the anthracnose, scab and cercospora spot disease as shown below (Aslam *et al.*, 2015).

$$\text{Disease Prevalence} = \frac{\text{No. of fruits infected}}{\text{Total number of fruits assessed}} \times 100$$

2.2. Isolation of Avocado fruit fungal pathogens

The infected avocado fruits were first washed with distilled water to remove any dust adhering to the fruit surface and then dried using sterile blotting papers. The diseased fruits were surface sterilized using 70% ethanol and double rinsed in distilled water to remove traces of ethanol. A sterile scalpel was used to cut a section of the diseased parts from the advanced margins of the lesions. The diseased parts were aseptically placed into petri dishes containing potato dextrose agar (PDA). The plates were covered, sealed with parafilm, labelled and incubated in the laboratory at room temperature. Cultures were inspected regularly for the fungal growth. Pure cultures of the fungal pathogens were obtained using the single spore isolation technique, where a sterilized inoculating loop was used to scrape a single colony from the isolated fungi and grown on PDA media. Morphological features of pure fungal isolated were recorded for a period of ten days.

2.3. Phenotypic characterization of the Avocado fruit fungal pathogens

The identification of the avocado fruit fungal pathogens was carried out as described by Fawole and Oso (1995). A drop of lactophenol cotton blue stain (LPCB) was placed on a clean microscopic slide and a small portion of the mycelium from the fungal cultures placed on the stain using a sterile wire loop. The hyphae were spread on the slide using a sterile wire loop. A cover slip was gently placed over the slide and examined under a compound microscope at x400 magnification. Phenotypic characteristics of the isolates such as colour of the colonies, texture, shape of mycelia, hyphae septation were recorded.

To visualize the fungal spores, pure cultures of fungal isolates were grown on PDA for 10 days at room temperature. The plates were flooded with sterile distilled water and filtered through double layer cheese cloth to remove mycelia. The filtrate was collected in clean universal bottles. Using a pipette, a tiny drop of the filtrate was placed on a microscopic slide and stained using lactophenol cotton blue stain, covered with a cover slip and assessed under a compound microscope. The shape of the spores was described and the size (length and width) of the three spores per isolate was measured using calibrated ocular slide and stage micrometre.

2.4. Data analysis

The disease prevalence was recorded as the percentage number of avocado fruits showing the symptoms of specific avocado disease divided by the total number of fruits asses. One-way analysis of variance was done for data on disease prevalence between fruit markets, prevalence of avocado fungal disease by varieties and the morphometrics of fungal spores. Means that were significantly different ($p < 0.05$) were separated using Tukey's HSD test. All statistical analyses were done using R-software version 4.3.2 (R Foundation for statistics computing).

3. Results

To assess the prevalence of fungal pathogens in avocado, questionnaires were administered to 120 avocado traders in Embu Country. Fifty-five percent of the traders who participated in the study doubled as avocado farmers. Forty-five percent of the avocado traders in various fruit markets were able to positively identify fungal disease symptoms on the fruit surface while 10% lacked the knowledge to identify fungal symptoms. Other traders were only able to identify fungal diseases with the help of their customers (30%) or the middle men (15%) within the market.

Hass avocado variety was the most common among the fruit vendors. Thirty-nine percent of the fruits across the markets were *Hass* variety (Figure 1). Twenty-one percent of the fruits were *Fuerte* variety while *Puebla* had 16% of the market share. The rest of the avocado fruits in the market (24%) were the local avocado varieties at 24%.

The vendors in the study area used varied methods for picking mature avocado fruit during harvesting. Majority of the vendors (42%) used special hooks to detach the fruits from the tree which fell down, and they

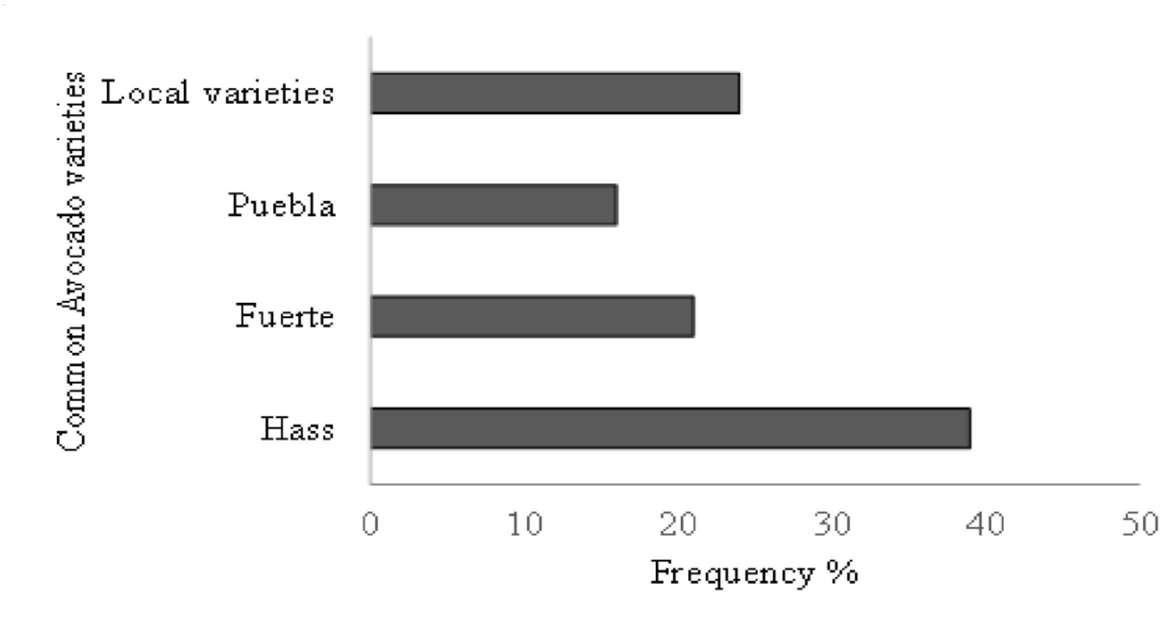

Figure 1: Common avocado varieties in various markets within Embu Country

were collected. Thirty-nine percent of the vendors used hand picking method to select mature fruits from the trees (Figure 2). The rest of the farmers either shook the tree for the mature fruits to fall (15%) or waited for mature fruits to fall down after ripening (4%).

Different packaging materials were used for transportation and storage of the harvested fruits. Use of sacks (gunny bags and nylon sacks) was the most common method of avocado packaging (54%) while twenty-one

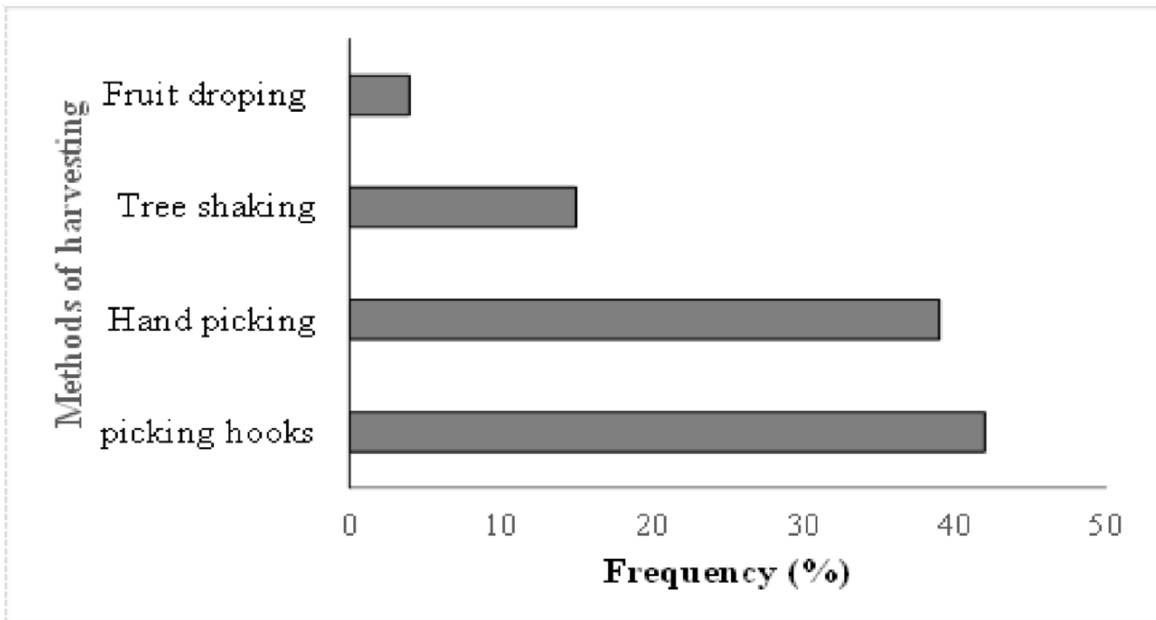

Figure 2: Methods used for harvesting Avocado fruits during harvesting

percent of fruit vendors preferred the use of buckets (Figure 3). Crates were used by 13% of the vendors while 1% of vendors did not use any packaging.

The study highlighted a significant dependence on chemical methods for managing fungal diseases. Seventy percent of respondents reported using fungicides as part of their agronomic practices. Among these, 47% used

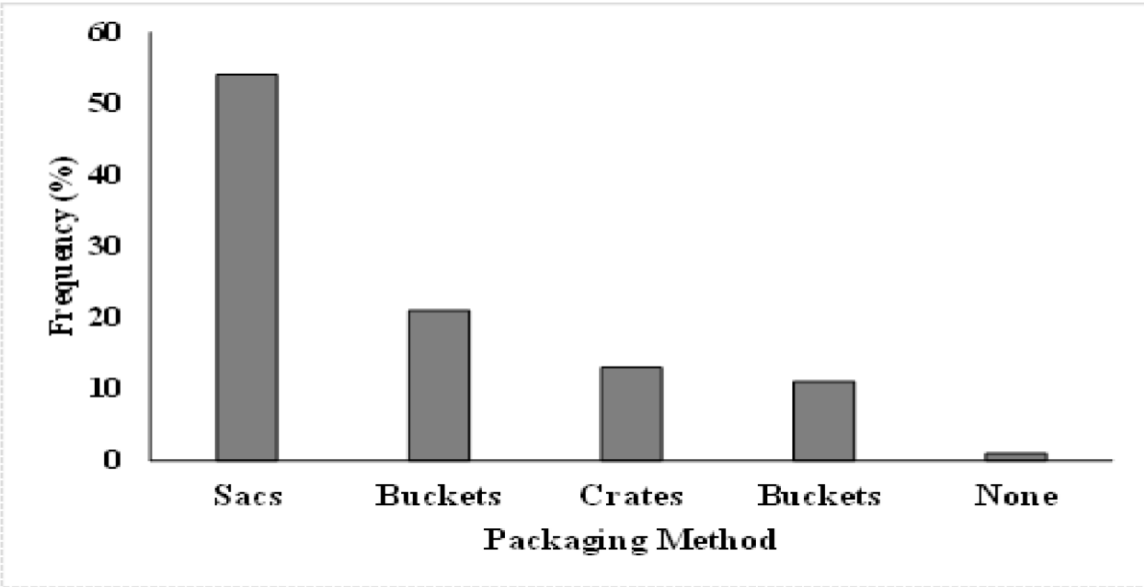

Figure 3: Methods of packaging avocado fruits after harvest

Green Cop® 500WP, which contains copper oxychloride. Additionally, 4% of vendors used Milraz®, containing propineb and cymoxanil, while 3% used Bayleton®, which contains triadimefon. Sixteen percent of respondents used fungicides they could not identify.

Conversely, 30% of respondents did not use synthetic chemicals for managing fungal diseases. Instead, they employed various cultural practices such as regular tree pruning (25%), proper farm hygiene like disposing of diseased fruits (22%), and regular weeding (3%). The remaining respondents did not use any fungal control methods.

3.1. Prevalence of Anthracnose, Scab and Cercospora spot on Avocado Fruits

There was a significant difference ($p < 0.05$) in the prevalence of the avocado fungal fruit diseases in various markets in Embu County (Table 1). Kianjokoma (KJM) market had the highest prevalence of Anthracnose (35.0%) while Mutunduri market (MTM) had the lowest (21.3%). The highest prevalence of scab was recorded in Siakago market (SKM) (36.3%) while Mutunduri (MTM) recorded the lowest prevalence (20.7%). Cercospora spot was more prevalent in Kianjokoma market (KJM) with a mean prevalence of 35.5% while Kiritiri (KRM) and Mutunduri (MTM) reporting the lowest prevalence of 18.7% (Table 1).

Table 1: Prevalence of Avocado fruit diseases in Embu County

	Anthracnose*	Scab	Cercospora spots
ETM**	27.93 ± 13.34ab	28.7 ± 2.45ab	25.75 ± 3.34ab
KJM	35.00 ± 2.52a	35.85 ± 3.42a	35.50 ± 1.82a
KRM	26.45 ± 2.22ab	34.35 ± 2.57a	18.65 ± 1.61b
MTM	21.3 ± 2.22b	20.70 ± 2.20b	18.65 ± 1.61b
RNM	34.00 ± 1.86a	32.75 ± 1.98a	20.20 ± 2.46ab
SKM	29.3 ± 2.67ab	36.34 ± 2.94a	26.20 ± 3.35ab

Note: *Mean prevalence ± standard error of avocado fruit fungal diseases (n = 20). Means in the same column followed by the same letters are not significantly different $P \leq 0.05$; ** SKM-Siakago; KJM-Kianjokoma; KRM-Kiritiri; RNM-Runyenjes; ETM-Embu town market; MTM-Mutunduri.

The prevalence of anthracnose, scab and cercospora spot disease of avocado differed significantly with the fruit variety ($p < 0.05$). *Fuerte* variety recorded the highest prevalence of anthracnose disease at 71% while local avocado varieties recorded a prevalence of 39% which was the lowest (Figure 4). *Puebla* had a higher scab prevalence (65%) compared to *Hass* that had the lowest (41%). Generally, the prevalence of Cercospora spot was lower in all fruit varieties compared to anthracnose and scab avocado diseases. *Puebla* had the highest prevalence of Cercospora spot (61%) with *Fuerte* recording 44% which was the lowest (Figure 4).

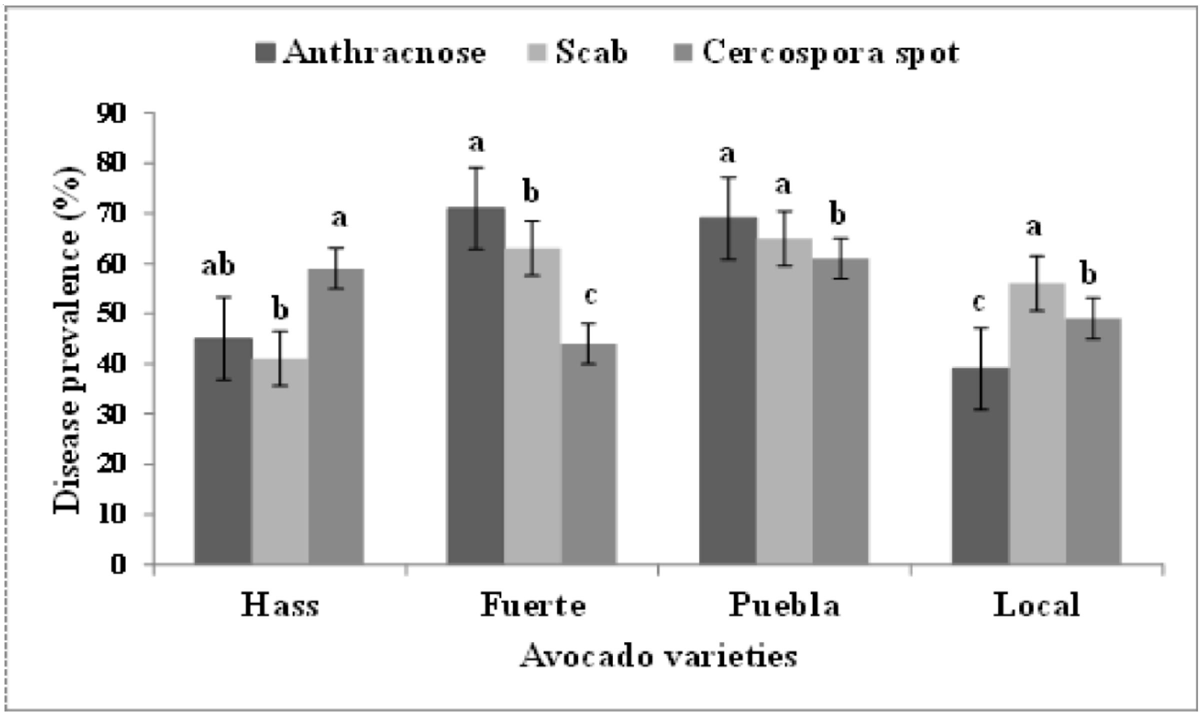

Figure 4: Prevalence of the fungal avocado fruit disease in different avocado varieties

Note: Bars followed by the same letter in within the same group are not significantly different at P $\leq$ 0.05

3.2. Morphology of avocado fungal isolates

A total of forty *Colletotrichum* spp. isolates were obtained from avocado fruits having anthracnose symptoms. The isolates had whitish to greyish colour and cottony smooth mycelia on the upper side and grey to cream colour on the bottom side (Figure 5A and B). The fungal colony grew rapidly on the PDA and covered the entire surface of the petri dish by the 9th day after inoculation. The mycelial colour of the isolates ranged from white-grey, white-cream and grey on the upper side of the culture. Similarly, the bottom side of the cultures was creamish grey in colour. The spores of the isolates were cylindrical and straight with smooth round ends, of large masses that were scattered over the colony. The size ranged between 3.00-5.07 μm in width and 10.20- 18.13 μm in length (Table 2).

Eighteen *Sphaceloma* spp. isolates were isolated from avocado fruits with scab symptoms. On PDA culture media, the isolates formed a slow-growing mycelium, with raised and gummy to mucoid colonies. The mycelial appeared whitish in colour on the upper side of the plates with yellowish mycelia (Figure 5 C and D). The fungal isolates developed masses of conidiophores bearing hyaline conidia. The conidia were septate ovoid or elongated. The spore width measured from 3.0-4.0 µm and a length ranging from 5.0 - 8.0 µm (Table 2). The sizes of the spores differed significantly ($p < 0.05$) in terms of width and length among the isolates from different fruit market.

Fourteen *Cercospora spp.* isolates were obtained from fruits showing scab symptoms. The isolates had white leathery colonies with tuffs of grey-brown conidiomata on them, initially grey becoming brown with age (Figure 5 E and F). The isolates formed a tufted, leather-like mycelium with a slow mycelial growing rate. The conidia were pale olive in colour, they were long rod shaped to cylindrical, with a blunt end or straight-curved and with 9-11 septa. The spore measure between 2.0-4.9 µm and 19.9-33.9 µm in length (Table 2).

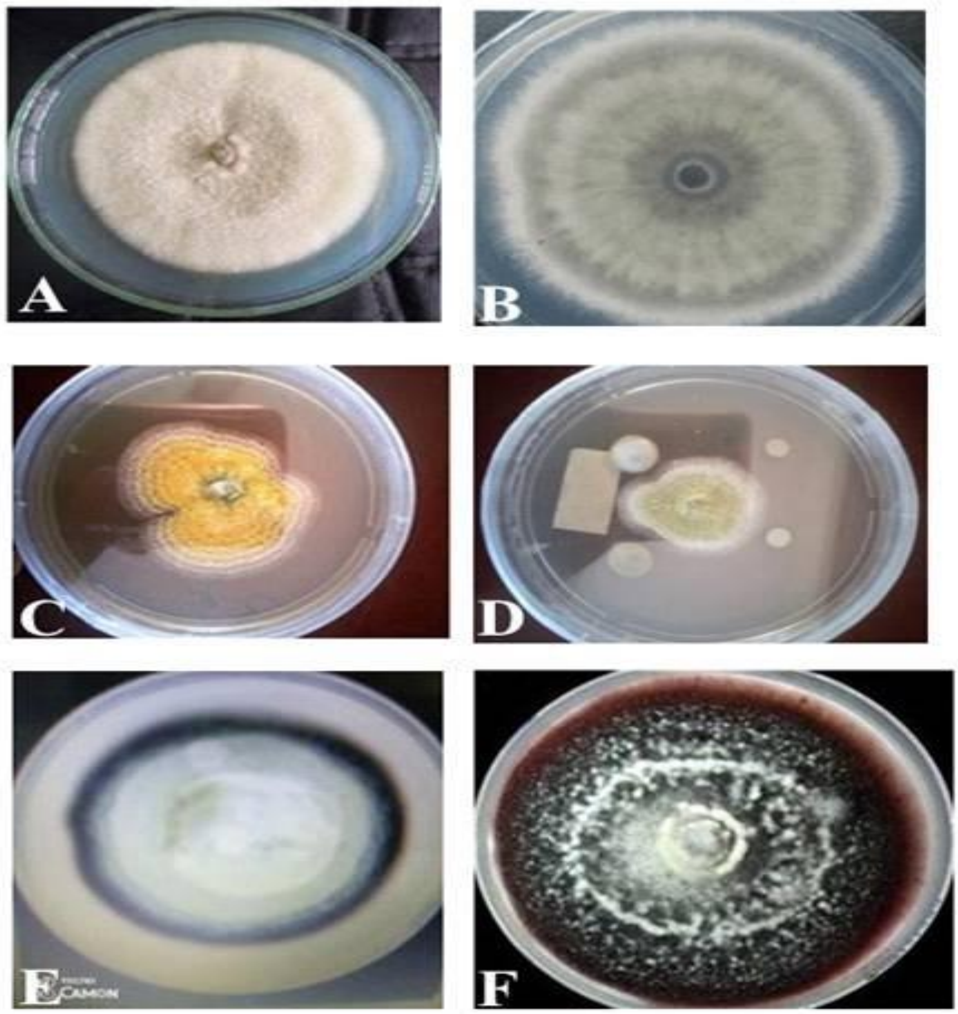

Figure 5: Avocado fungal isolates on PDA media ten days after inoculation

Note: A = *Colletotrichum* spp. upper surface, B = lower surface; C = *Sphaceloma* spp. upper surface, D = lower surface; E = *Cercospora* spp. upper surface, F = lower surface. fungal isolates

Table 2: The mean width and length (μm) of fungal spores from fungal spores

Isolate[a]	***Colletotrichum* spp.**		***Cercospora spp.***		***Sphaceloma spp.***	
	Width[b]	**Length**	**Width**	**Length**	**Width**	**Length**
BS	4.33±0.15[c]	17.03±0.12[a]	4.10±0.10[a]	31.07±0.21[b]	3.00±0.36[b]	5.33±0.25[c]
CK	4.73±0.12[b]	16.02±0.10[b]	2.50±0.10[b]	24.07±0.21[d]	3.40±0.10[ab]	6.40±0.40[b]
DE	3.57±0.06[d]	14.96±0.06[c]	3.03±0.15[b]	29.10±0.10[c]	3.77±0.06[a]	7.77±0.06[a]
AR	5.07±0.06[a]	18.13±0.15[d]	2.00±0.10[c]	19.93±0.83[e]	3.80±0.10[a]	7.93±0.21[a]
FM	3.57±0.06[d]	14.33±0.38[e]	3.93±0.06[d]	30.80±0.20[b]	4.00±0.36[a]	8.00±0.26[a]
GJ	3.00±0.10[e]	10.20±0.20[f]	4.93±0.06[a]	33.90±0.10[a]	3.00±0.26[b]	5.00±0.10[c]

Note: [a] Mean size (width and length) ± Standard error of *Colletotrichum* spp., *Sphaceloma* spp. and *Cercospora* spp. fungal spores 10 days after culturing. Means in the same column followed by the same letter are not significantly different at $p \leq 0.05$; [b] FM-isolate from Mutunduri; AR from Runyenjes, DE from Embu town, CK from Kiritiri, BS from Siakago and GJ from Kianjokoma fruit markets.

4. Discussion

This study aimed to assess the prevalence of avocado fungal diseases in Embu County, focusing on *Colletotrichum* spp. (causing anthracnose), *Sphaceloma* spp. (causing scab), and *Cercospora* spp. (causing cercospora spots). The research involved avocado vendors from six different fruit markets within the country. Majority of the participants could identify symptoms of these diseases, indicating a high level of awareness among the vendors. However, 10% of the respondents were unable to recognize fungal associated symptoms.

Hass variety was the most popular avocado variety in the local markets. This variety was stocked by 39% of the fruit's vendors. The variety is popular for export market (Herrera-González *et al.*, 2020). Twenty-four percent of the fruits in the market were local varieties. These varieties are larger in size and have low fat content compared to *Hass* variety. They grow slowly and can take a long time to mature. *Fuerte* and *Puebla* varieties were present in 21% and 16% respectively. *Fuerte* is the second most popular avocado variety in Kenyan market. Compared to Hass, *Fuerte* has a low-fat content and calorie count. This variety is preferred for processing (Nyakang'i *et al*., 2023).

Fungal diseases in avocados cause significant yield losses globally (Mekonnen *et al*., 2015; Nelson Scot, 2008). High post-harvest losses are often due to mechanical injuries inflicted during harvesting (Mandemaker *et al.*, 2006). In this study, 42% of respondents used hooks to pick the fruits or shook the tree to make the fruits fall, which damages the fruits and predisposes them to infection. Bruising can serve as an infection pathway, leading to increased brown patches on the fruit (Mandemaker *et al*., 2006). Additionally, such damage can lower the quality of the fruits, making them unmarketable. Mechanical damage during harvesting and packaging, often due to improper handling, is a major challenge (Hurtado-Fernández *et al*., 2018). Most respondents reported the use of picking hooks, shaking the tree, or waiting for the fruits to drop naturally upon reaching physiological maturity. This common practice causes substantial injury and bruising (Kuru *et al.,* 2016), reducing fruit quality and marketability. To minimize damage, fruits should be hand-picked where possible, or the drop height should be kept minimal.

The method used to package avocado fruits after harvesting significantly determines their post-harvest quality. The packaging material should not compromise the fruits' shelf-life. A majority of traders (54%) use nylon sacks, while others use a variety of containers such as buckets (21%), crates (13%), or a combination of both. The choice of packaging material should aim to protect and preserve the fruits during transport to the market (Hurtado-Fernández *et al.*, 2018; Schmidt and Schmidt, 2019). However, sacks do not adequately protect freshly harvested commodities from damage (Kereth *et al*., 2013). They also accumulate heat from metabolic reactions, which accelerates mechanical damage and microbial attack. Mechanical damage during loading and unloading, as well as exposure to high temperatures during transport, can lead to physiological changes in the fruit, making them more susceptible to post-harvest diseases (Dessalegn *et al*., 2016).

The study revealed widespread use of fungicides to control avocado fungal diseases. Some of the fungicides are not approved for use in the management of avocado fungal pathogen. Fifty-four percent of the respondents used fungicides at some point during avocado production. Indeed avocado, are attacked by various pests and diseases (Ramírez-Gil *et al*., 2021) some of which are not approved for use in the management of avocado fruit fungal pathogen. However, persistent use of synthetic pesticides to control pests and diseases may be hazardous to human, animals and environment due to their inherent toxic nature (Abang *et al.*, 2014).

4.1. Prevalence of Anthracnose, Scab and Cercospora spot on Avocado fruits

The prevalence of the three fungal diseases varied across the fruit markets, with some markets recording higher incidences than others. This variation may be attributed to climate differences between the study areas. The six fruit markets are located in different agro-ecological zones, resulting in diverse climatic conditions (Kala *et al.*, 2012). Different fungal pathogens thrive in specific climatic conditions. For instance, Cercospora spot disease in avocados is common in warm, humid, and rainy conditions (Kallideen, 2020). High incidences of *Colletotrichum* spp., responsible for anthracnose, have been reported in leading avocado-producing countries (Kuru *et al.,* 2016). Additionally, fungal disease development may be influenced by harvesting and postharvest practices, as well as storage conditions, which vary among farmers (Kuru *et al.,* 2016). Improper harvesting and postharvest handling further exacerbate this issue (Kuru *et al.,* 2016).

The isolates of each of the three fungal pathogens differed in their morphological characteristics but matched those of *Colletotrichum* spp., *Sphaceloma* spp., and *Cercospora* spp. (Kimaru *et al.,* 2020). These differences in cultural and morphological features could be associated with genetic variations and varying growth conditions, such as temperature, light, and repeated laboratory sub culturing.

5. Conclusion

Post-harvest avocado fungal diseases; anthracnose, scab, and Cercospora spot diseases are widespread in various avocado markets within Embu County, with prevalence varying from one market to another. Common cultural practices among farmers and traders may have contributed to the severity of these diseases. The methods of harvesting, packaging, and transportation are crucial in avoiding the predisposition of fresh fruits to infection. While the losses attributed to anthracnose, scab, and Cercospora could be significant, the use of unregulated chemicals in their management may pose risks to human health and the environment. However, proper cultural practices, coupled with appropriate harvest and post-harvest handling, may reduce the incidence of avocado fungal diseases. Most vendors did not use any strategy to manage fungal avocado fruit diseases, highlighting the need for training on the management of post-harvest avocado diseases, especially the post-harvest handling.

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