PREVALENCE AND CHARACTERIZATION OF AVOCADO FRUIT FUNGAL DISEASES IN EMBU COUNTY, KENYA AND THE EFFICACY OF *Aloe secundiflora* CRUDE EXTRACTS IN THEIR CONTROL

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented for any award in any other institution of higher learning or for any academic purpose.

Signature: ..... Date: 12/07/2023

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Recommendation

This thesis has been examined, passed and submitted with our approval as university supervisors.

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DEDICATION

I dedicate this thesis to my family, relatives and friends who were the source of motivation and encouragement.

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God bless you all.

ABSTRACT

Avocado production has increased exponentially worldwide due to increased demand for the fruit in the food, pharmaceutical, cosmetic, and oil industries. However, avocado production faces various constraints that include post-harvest losses due to pests and diseases, which contribute about 83% of yield losses. Fungal diseases are the most prevalent and are difficult to control using cultural methods, while continuous use of chemicals can lead to pathogen resistance and have adverse effects on humans and the environment. The main objective of this study was therefore to determine the prevalence of the fungal avocado fruit diseases in Embu County, isolate and characterize the causal pathogens and test the efficacy of *Aloe secundiflora* (Aloe) crude extract in the management of the fungal diseases. Aloe was selected because of its wide medicinal use and local availability. A descriptive cross-sectional survey design was carried out in six purposefully selected avocado markets in the study area to assess the prevalence of fungal diseases of avocado fruit. A structured questionnaire was administered to gather information on avocado production, marketing, and management. A total of 100 respondents were interviewed. The stores and avocado bags were randomly selected. The fruits in the bags were assessed to determine the prevalence of fungal avocado diseases. Experiments were then carried out in the laboratory to isolate and characterize the pathogens. Aloe leaves were collected in the field, and phytochemical analysis of the crude extracts was done. The efficacy of the crude extract against the pathogens was tested in the laboratory using a Complete Randomized Design (CRD). The data were subjected to an analysis of variance (ANOVA) using Scientific Analysis System version 9.4 Software. Significant means were separated using LSD at $p < 0.05$ significant level. Results on the demographic characteristics of the respondents showed that the majority were above 30 years of age, with more women than men, and had a primary level of education. More than 50% sourced avocado fruits from their own farms and were able to identify disease symptoms themselves. The main methods of harvesting were both handpicking and hook, and the product was mainly stored in sacks. More than 40% of the respondents used chemicals to control fruit diseases. Three fungal pathogens were isolated and characterized: *Colletotrichum gloeosporioides* (anthracnose), *Cercospora purpurea* (cercospora spot), and *Sphaceloma perseae* (avocado scab) and their mean percentage prevalence was 29.00%, 25.98% and 31.44%, respectively. The commonly sold varieties were *Hass, Fuerte*, *Puebla* and the local varieties, with *Hass* being the most preferred variety. *Fuerte* and *Puebla* were the most susceptible to these diseases. There were variations in the morphological characteristics of the pathogen isolates: *C. gloeosporioides* had a white to grey smooth mycelia, concentric zonation and sizes ranging from 3.0 - 5.0 µm in width and 10.3 - 18.2 µm in length. *Sphaceloma perseae* had a white and yellow layered mycelium with sizes ranging from 3.0 - 5.0 µm in width and 10.3 - 18.2 µm in length, while *C. purpurea* had a leathery grey to brown conidia of 2 - 5 µm in width and 20 -34 in length. Phytochemical analysis of the aloe-ethanol extract revealed the presence of steroids, phenolics, flavonoids, saponins, and terpenoids, but phenolics were only present in the hexane extract. The mean zone of inhibition for the ethanol and hexane extract against *C. gloeosporioides* was 13.50 mm and 9.36 mm, respectively, while for *Cercospora purpurea* was 4.72 mm and 4.32 mm, respectively, but no effect on *S. perseae* at any concentration. Vendors should therefore be trained on proper handling of avocado fruits to reduce post-harvest losses. *Aloe secundiflora* extracts can be integrated with other methods of managing avocado fungal diseases to reduce over-reliance on chemical fungicides.

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LIST OF ABBREVIATIONS

ACE	:	Angiotensin-Converting Enzyme
ANOVA	:	Analysis of Variance
BCA	:	Biological Control Agents
CRD	:	Complete Randomized Design
CT	:	Condensed Tannins
DMSO	:	Dimethylsulfoxide
FAO	:	Food and Agricultural Organization
FAOSTAT	:	Food and Agricultural Organization and Statistics
HCDA	:	Horticultural Crop Development Authority
HPLC-MS	:	High Power Liquid Chromatography-Mass Spectrometer
HT	:	Hydrolysable Tannins
LSD	:	Least Significant Difference
MIC	:	Minimum Inhibitory Concentration
NACOSTI	:	National Committee of Science Technology and Innovation
NaOH	:	Sodium Hydroxide
PDA	:	Potato Dextrose Agar
SAS	:	Scientific Analysis System
SDG	:	Sustainable Development Goals
WAC	:	World Agroforestry Centre

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CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

Avocado (*Persea americana* Miller) is a significant fruit crop grown in tropical and subtropical regions (Selladurai & Awachare, 2020). The crop is gaining acceptance worldwide and has received extensive marketing due to its uses in the pharmaceutical, cosmetic, oil and food industries (Kumar Gupta *et al.*, 2018). Avocado is grown in over three million hectares worldwide (Food and Agricultural Organization and Statistics [FAOSTAT], 2020). Mexico is the leading producer and exporter of the fruit worldwide, with an average of 10.7 tonnes of avocados per hectare (FAOSTAT, 2020). This translates to about 33.9% of avocados harvested worldwide. The crop thrives well in subtropical climates such as Pacific America, South East Asia and Africa. Africa is one of the largest avocado fruit exporters, mostly Kenya and South Africa (Wolstenholme, 2008).

Kenya has a conducive environment for avocado production. The country produces approximately 15.7 tonnes of avocados per hectare for local and for export (FAOSTAT, 2020), making it the sixth largest producer of avocado in the world and the leading in Africa (FAO, 2020). Kenya exports about 20% of avocado fruits produced every year (Wasilwa *et al.*, 2018). About 70% of avocado are produced by small-scale growers mainly in Murang'a, Bungoma, Migori, Kiambu, Kisii, Nyamira, Meru, Tharaka Nithi and Embu counties of Kenya (Johnny *et al.*, 2019). Embu County accounts for approximately 30% of total avocado production in Kenya (Hortinews, 2015). The popular avocado varieties grown in Kenya are *Hass*, *Fuerte*, *Puebla*, *Pinkerton*, *Duke*, *G6* and *G7* (Garrity, 2006).

Avocado is a nutritious fruit rich in essential minerals such as manganese, phosphorus, iron and potassium. It is rich in vitamins such as vitamin A, B, E and β-carotene (FAO, 2020). It is a high source of protein and carbohydrate for populations in avocado producing and non-producing countries (Fan *et al*., 2017). Avocados can be consumed in various forms and do not require processing, preservatives or taste enhancers (Chaves & Borges, 2016). The fruit has various uses in pharmaceutical, cosmetic and oil industries (Kumar Gupta *et al*., 2018).

Avocado production in Kenya is faced by various biotic constraints, for example, diseases such as avocado anthracnose, scab disease, *Cercospora* spot, Verticillium wilt, bacterial canker, among others (Kimaru, 2018). The fruit is also attacked by pests such as false codling moth, thrips, scales and fruit flies (Marais, 2016). Avocado fruit diseases of economic importance include anthracnose, scab and *Cercospora* spot that are caused by *Colletotrichum gloeosporioides*, *Sphaceloma perseae* and *Cercospora purpurea*, respectively (Marais, 2016). Characterizing these diseases is vital for developing and implementing effective control strategies.

There are several post-harvest fungal pathogens that infect various avocado cultivars, thus reducing the shelf life and marketability of the fruit. These post-harvest fungal diseases are estimated to cause about 83% losses in avocado production threatening the global avocado market (Fan *et al.,* 2017). Infection may go unnoticed during fruit development and appear at maturity in storage or while fruits are sold in the market (Mekonnen *et al.,* 2015). This poses a challenge for their management because disease presence is identified when fruits begin to soften or rot, symptoms that usually occur at the market stage when it is not possible to control the problem (Sharma *et al.,* 2017). Nevertheless, awareness among farmers and vendors about the protection of fruits from infection during growth in the field and postharvest care is limited or lacking (Mekonnen *et al.,* 2015). Assessment of the prevalence of these diseases is therefore important to map the geographic distribution and determine the status of the disease, in addition to providing baseline data to prioritize research problems (Eshte *et al.,* 2015).

Post-harvest fungal diseases are mainly controlled using synthetic chemicals (Yoon *et al.,* 2013). However, the use of fungicides especially those applied after harvest is becoming increasingly restricted particularly in many countries (Mekonnen *et al.,* 2015). For instance, regular copper fungicide sprays are applied in the postharvest control of avocado fruit diseases (Fesenko & Edwards, 2014). These chemicals contribute to toxicity in food and are thus not suitable for human consumption, in addition to polluting the environment (Plant *et al.,* 2005). Excessive use of chemicals also contributes to microbial fungal resistance to fungicides, which complicates fungal disease management in many countries (Roca *et al.,* 2015). Furthermore, losses due to fungal avocado diseases are still high about 83% despite the use of synthetic chemicals by avocado growers and traders (Fan *et al.,* 2017).

Cultural methods of controlling post-harvest fungal diseases include good sanitation procedures involving collection and disposal of diseased fruits (Agrios, 2005). Further, adequate postharvest handling practices are applied to prevent cuts and bruises to fruit surfaces (Wasilwa *et al.,* 2006). Fruits showing symptoms of postharvest diseases are not packed into cartons containing healthy fruits (Sarkhosh *et al.,* 2017). However, these methods are not effective, despite their use, market losses attributed to postharvest fungal diseases are still high (Agrios, 2005). It is therefore necessary to develop new and more antimicrobial substances against the pathogen for proper control and management (Carocho and Ferreira, 2013). The use of biopesticide control agents is a promising approach for the control of pathogens (Kesho, 2020), and plant extracts with secondary metabolites could be a good control measure against fungal pathogens.

Natural plant extracts have been widely used in the control of plant and animal diseases (Savoia, 2012). Plant extracts are easily obtained, economical, non-toxic and do not interfere with the biological balance compared to synthetic chemicals (Proestos, 2020). The antimicrobial properties of most plant extracts are a result of essential oils and other secondary metabolites in the plants, such as terpenoids, flavonoids, saponins, alkaloids, and tannins (Akinmoladun *et al.,* 2007).

Aloe is of economic importance in the control and management of diseases. It has been exploited since prehistoric times as a source of medicine for the treatment of various diseases. This is because Aloe has antibacterial, antifungal, anticancer, antiviral and immunomodulatory properties (Bashir *et al.,* 2011). Despite the wide usage of Aloe, there is little literature on the type of active ingredients within the plant's extracts (Bashir *et al.,* 2011). Aloe is tolerant to harsh environmental conditions and therefore able to reproduce continuously even during the dry season. As a way of surviving, it produces some metabolites that enable them to survive. Many of these metabolites have antimicrobial activities and have been used to control some plant diseases such as *Fusarium oxysporum* that cause wilt in several plant species (Rachuonyo, 2016).

Sixty-three species of Aloe occur in Kenya, of which around 50% are endemic (Bjorå *et al.,* 2015). They are widely distributed in the semi-arid areas and open grasslands of Kenya. Several of these species are threatened with extinction and knowledge about their use is important for their conservation strategies (Bjorå *et al.,* 2015). *Aloe*

secundiflora Engl. is among the most important species. There is limited information on the application of crude extracts of *Aloe secundiflora* in the control of post-harvest avocado fungal disease pathogens.

1.2 Statement of the Problem

Fungal avocado fruit diseases account for about 83% of avocado production losses and threaten the avocado market worldwide. They infect all cultivars of avocado, including the *Hass*, *Fuerte* and *Puebla* varieties grown in Kenya, and hence reduce the shelf life and marketability of the fruit. Information on the prevalence of these diseases in market stores and packing houses before entering the global market is very limited and could be utilized as an early control and management measure. Cultural methods of controlling pathogens are ineffective, while chemical methods are costly and unsafe for humans and the environment. In addition, the continuous use of the chemicals has led to pathogen resistance, creating a serious challenge in their management. Furthermore, despite their use, losses due to post-harvest avocado fungal infestations are still high. Natural plant extracts are an alternative and have been widely used to protect plants and animals against disease-causing pathogens. Among the plants is *Aloe secundiflora,* which has not been evaluated for its bioactivity against fungal avocado fruit diseases despite its antimicrobial activity against different fungal and bacterial pathogens.

1.3 Objectives of the Study

1.3.1 Broad Objective

To assess the prevalence of fungal diseases of avocado fruit in selected markets in Embu County, isolate and characterize the pathogens and test the efficacy of *Aloe secundiflora* crude extracts against the pathogens.

1.3.2 Specific Objectives

i. To determine the prevalence of post-harvest fungal diseases affecting avocado fruits in selected markets in Embu County.
ii. To isolate and morphologically characterize postharvest fungal pathogens infecting avocado fruits.
iii. To evaluate the *in-vitro* efficacy of *Aloe secundiflora* crude extracts against isolates of fungal avocado fruit pathogens.

1.4 Hypotheses

$H0_1$: There is no significant difference in the prevalence of postharvest fungal avocado fruit diseases in the selected markets of avocado in Embu County.

$H0_2$: There is no significant difference in the phenotypic characteristics of various fungal avocado fruit pathogen isolates from the infected avocado fruits.

$H0_3$: There is no significant difference in the efficacy of *Aloe secundiflora* crude extracts on isolates of fungal avocado fruit pathogens.

1.5 Justification of the Study

Avocado is a significant fruit crop in Kenya. It is a source of nutrition to human and it is widely used in pharmaceutical, cosmetic and oil industries as a raw material. Therefore, production of avocados is a source of livelihood to many households in Kenya. However, the yield and quality of avocado fruits is affected by several fungal diseases (Kimaru, 2018). This study therefore provides information on the prevalence of avocado fungal diseases as well as identifying the fungal species responsible for the diseases. Considering the challenges posed by control and management methods of fungal avocado fruit diseases, alternative plant fungicides are necessary in the face of disease resistance and environmental safety. Plant extracts are a better alternative due to biodegradability, effectiveness, multiple modes of action on pests, target specificity, lack of toxic residues and cost (Regnault, 2011). Plant extracts which are bio-pesticides have been used successfully to manage crop pests and diseases and hence minimize losses (Chandler *et al.*, 2011; Popp *et al.*, 2013).

Plant extracts have in the past studies yielded promising potentials in medicine. This study has added to the knowledge of the use of plant extracts such as *Aloe secundiflora* as antifungal agents in plant disease management. The study also aimed at providing a cheaper and available method of managing the most prevalent post-harvest avocado fruit fungal pathogens. Consequently, the results of this study have contributed to research goal of the Sustainable Development Goals (SDGs) to end hunger, achieve food security and improved nutrition, and promote sustainable agriculture in Kenya.

CHAPTER TWO
LITERATURE REVIEW

2.1 Overview of Avocado Production

2.1.1 Origin and Distribution of Avocado

Avocado (*Persea americana* Mill.) is a perennial fruit crop that belongs to family Lauraceae and originated in Central America and Southern Mexico (Galindo-tovar *et al.*, 2007). Avocado has three race sub-species; Guatemalan, West Indian and Mexican. Avocado plants were first introduced in Kenya in the 1930s by the Portuguese (Griesbach, 2005) for subsistence use, whereas commercial cultivation of avocado in Kenya started in the early 1960s (Griesbach, 2005).

Avocado is currently cultivated worldwide including the tropical and subtropical areas of Africa (Selladurai & Awachare, 2020). Mexico is the leading producer of Avocado in the world followed by Dominican Republic, Peru, Colombia, and Indonesia. Kenya was ranked number six worldwide in 2017 to 2020 (FAOSTAT, 2020). Kenya produces approximately 225,808 tonnes of avocado fruits worth KES 3.83 billion, which is 5% of the total value of the fruits sub-sector (FAOSTAT, 2020). The production is mainly by small-scale farmers (Galindo-tovar *et al.*, 2007).

Avocado trade contributes to the economy of the country, for example, more than 13% of the total production in Mexico is exported (FAO, 2019). In Chile, South Africa, Israel and Spain, avocado production is mainly intended for the overseas export market. In Kenya, avocado is the most important fruit at 62%, followed by mango at 26%, in terms of foreign exchange earnings from the mid 2000's to the year 2018 (Horticultural Crop Development [HCD], 2018). It is also one of the commercial fruits with about 20% being produced and exported per year (Wasilwa *et al.*, 2018). Mature avocado fruits are mostly available in large volume from March to September and the avocado production is for both local and export markets (Johnny *et al.*, 2019).

2.1.2 Importance of Avocado

Avocado is a source of food in many households (Kathuri & Ireri, 2019). The fruit can be consumed without need for processing, use of preservatives or taste enhancers. It is eaten raw (ripe), as flavour soups, ice cream and milkshakes. It has high protein, minerals (Zinc & Selenium) and Vitamins (A, C & E). Avocado oil is easily digestible,

largely unsaturated and has low sugar content (Carvalho & Velásquez, 2015). The fruit contains fatty acids, like oleic acid (86 mg g−1 oil), palmitic acid (32 mg g−1 oil), linolenic acid (19 mg g−1 oil) and palmitoleic acid (14 mg g−1 oil) (Thierry & Tchatchou, 2012).

Avocado fruit contains more protein than any other fruit. It is rich in minerals, such as manganese, phosphorous, iron and potassium, with low sodium. It also contains vitamins; E and C, ß-carotene, thiamin, riboflavin, nicotinic acid and folate (Dreher & Davenport, 2013). The fruit is utilized in different ways; it is a source of oil when pressed, used as dessert drinks when blended, making guacamole, given as wedding gifts, it can be eaten with bread and ice cream, as a component in salads, or eaten alone (Crane *et al.,* 2019). Domestic consumption of avocado in Kenya is about 1-2 kg /person/year. However, consumption increases as the people become aware of the nutritional importance (Wasilwa *et al.,* 2004).

Avocado plant is also used as a source of medicine by different communities. For example the leaf extract is used as an antibiotic against disease such as dysentery (Fesenko & Edwards, 2014). Leaves are heated and applied on the forehead to relieve neuralgia while leaf decoction is used to cure ailment like diarrhoea, sore throat and haemorrhage. In Cuba, a decoction of the new shoots is used as a cough remedy. The roasted seed is used to cure diarrhoea and dysentery while powdered seed is used to treat hypertension (Weschenfelder *et al.,* 2015). Further, seed decoction, is used to relieve toothache due to tooth cavity (Henry *et al.,* 2015). Its nutritive composition contributes positively to the health of those who consume it including the maintenance of good cholesterol levels (Naveh *et al.,* 2017).

In cosmetics industry, avocado oil is considered to be superior to other vegetable oils due to its higher penetration ability and its nourishment to the glands beneath the skin with vitamins (Dreher *et al.,* 2013). Avocado oil is rich in vitamins A, B, D and E and is good quality oil. It has healing, regenerative and moisturizing properties hence beneficial in reducing age spots and the healing of scars (Eyres *et al.,* 2005). Avocado oil is used in hairdressing and is used in making hand lotions, facial creams and fine soap. The oil is also used to filter out the tanning rays of the sun and is similar to lanolin

in its penetrating and skin softening action. The pulp residue after oil extraction can be used as stock feed (Lu *et al.* 2007).

2.1.3 Common Avocado Varieties Grown in Kenya

Avocado varieties commonly grown in Kenya include *Puebla, Pinkerton, Hass* and *Fuerte* (Fig. 1) (Johnny *et al.*, 2019). Other cultivars include the local variety, *Booth 7* and *Booth 8* (Mexicana de Fitopatología *et al.*, 2003). Several studies have been devoted to characterize avocado varieties but have shown dissimilar results due to geographical differences, climatic conditions and harvest season (Gómez-López, 2002).

The *Fuerte* avocado variety is often recognized as the archetypal avocado, green in color, pear-like in shape, and medium to large in size ranging from six to twelve ounces in size (Eyres *et al.,* 2017). It has smooth, medium-thin skin that peels easily, with dense, pale green flesh. It is marginally 12 to 17% oily with a rich, creamy flavor and it is still considered by many due to its good taste. *Fuerte* is an early mid-season bearer which is susceptible to fungal diseases which include avocado fruit scab, anthracnose disease (Eyres *et al.,* 2017). This variety is popular among the producers worldwide (Johnny *et al.,* 2019).

Figure 1: Common avocado varieties grown in Kenya (A: *Fuerte*, B: *Hass* and C: *Puebla*)

Hass variety is pear to ovoid shaped. When ripe, it has tough, leathery, dark purple or nearly black skin (Wangithi *et al*., 2022). The flesh contains 18 to 22% oil with a small seed. *Hass* is a mid-late season, medium sized fruit with good shipping qualities but its shelf life is shortened due to its susceptibility to fungal avocado fruit diseases, post-harvest diseases (Wangithi *et al*., 2022).

Puebla variety is medium-sized, mid-season variety with ovate shape. It has a thin and smooth skin, which is glossy at maturity (Wasilwa *et al.,* 2004). The light green flesh is juicy, melting, and of good flavor with an oil content of nearly 20%. The ovate seed is medium to large and tightly fixed in its cavity. Flesh peels from the skin has few fibres and good eating quality (Thierry & Tchatchou, 2012). This cultivar is also susceptible to postharvest fungal avocado diseases such as avocado scab, *Cercospora* spot and anthracnose.

2.2 Fungal Pathogens that Cause Avocado Fruit Diseases

Avocado fruit is highly affected by several bacterial and fungal diseases. During growth and at harvesting, the fruits are exposed to diseases of economic importance such as scab (*Sphaceloma persea* Jenk.), anthracnose (*Colletotrichum gloeosporioides* Penzig) and Cercospora spot (*Cercospora purpurea* also known as *Pseudocercospora purpurea* (Cooke) Deighton). These result in unsightly blemishes or the development of post-harvest fruit rots and decay; both of which are unacceptable in the export market (Marais, 2016).

2.2.1 *Colletotrichum gloeosporiodes*

Colletotrichum gloeosporiodes is a filamentous fungus that occur on a single host or on a single species on multiple hosts causing anthracnose disease worldwide. The fungus thrives best in warm humid environmental conditions (Farr *et al.,* 2006). The fungus causes anthracnose disease on various fruits such as avocado, apple, citrus, papaya, passion fruit, mango, guava and grapes (Zakaria, 2021). When cultured in the media, the fungus generally produces circular, woolly or cottony colonies with characteristic colour, pale brown or greyish white (Kwodaga, 2018). The fungus primarily invades plants tissues where it produces various specialized structures during infection process (Kaingu *et al.,* 2012).

The penetration into host tissues relies on formation of specialized infection structures known as appressoria. These appressoria allow the fungus to penetrate the host cuticle and epidermal cell wall directly by a narrow penetration peg that emerges from the base of appressorium (Agrios, 2005). Insect wounds also can increase entry of the pathogen and make fungal disease development worse. Long distance transport is possible

through fruit movement but most likely in infected propagation material. On leaves lesions are often red, and the centre can fall out to result in holes (Lu *et al.*, 2009).

2.2.2 *Sphaceloma perseae*

Sphaceloma perseae is a fungal pathogen that cause avocado fruit scab disease. It not only infects avocados but also citrus fruits such as lemons and oranges. *Sphaceloma perseae* rupture the epidermis of avocado fruit and produce a hyaline conidia and conidiophores that form a dense covering in a dark-brown mass (Everett *et al.*, 2007). Spots are scattered or may coalesce to form irregular, rusted area on the entire fruit surface (Range, 2008). Avocado scab mostly attacks to the epidermis of the fruit.

Sphaceloma perseae generates asexual fruiting body that erupts from the injuries, on the fruit as small cream and olive-coloured masses of clustered conidiophores and spores. Scab infected fruit is more susceptible to avocado anthracnose which increases postharvest fruit rot (Range, 2008). Regions with prevailing moist conditions, rainy weather and low temperatures promote the sporulation of conidia spores of *Sphaceloma perseae* (Everett *et al.*, 2007).

In terms of pathogenicity, symptoms on fruit initially appear as corky, raised, oval, and irregular in shape with brown to purple spots. As the infection continues, the spots enlarge and coalesce to form large rough masses over the fruit epidermis. Cracking of the rough areas may allow penetration of secondary organisms and cause rotting of the fruit. *Sphaceloma* species produces potential secondary metabolite with phytotoxic properties called elsinochromes which is a variety of red/orange pigments (Fesenko & Edwards, 2014).

Sphaceloma perseae produces phytotoxins which kill the cells of the host organism and even form necrotic lesions on the tissue of the host plant by reactions of a singlet oxygen species from the pigment (Fesenko & Edwards, 2014). Proteins, lipids, and nucleic acids are broken down by the singlet oxygen in the plant cells. Addition of singlet oxygen quenchers decreases the toxicity of the species by preventing the interaction of the singlet oxygen species with the plant cells (Mexicana de Fitopatología *et al.*, 2003).

Diagnosis of *Sphaceloma perseae* is done through observations of symptoms on the fruits, culture morphology and molecular characterization of fungus isolated from infected avocado fruit (Trucks *et al.,* 2011). When cultured on potato dextrose agar (PDA) media, *S. perseae* grows slowly resulting in raised lumpy colonies. Cultures are dark brown on the underside of the plates, and mycelia are pink (Marais, 2016). It attacks the host producing acervuli that originate from fruit wounds as small, white, cream and olive masses of clustered spores and conidiophores. Conidia are colourless, aseptate and ovoid to strongly curved (Al-Jaradi *et al.,* 2018). They are carried to infection sites by wind, rain and insects. Thrips cause injuries that create entry sites for *Sphaceloma perseae* and greatly cause scab development in the host plant fruit (Trucks *et al.,* 2011).

2.2.3 *Cercospora purpurea*

Cercospora purpurea is a pathogenic organism that causes Cercospora spot in various plant fruits. It is an ascomyceteous fungus which causes small angular purple spots on avocado fruits. The spots enlarge and coalesce affecting most or whole fruit (Pérez-jiménez, 2008). This may crack and permit the ease entry of other pathogens just like anthracnose disease. Infections occur through conidia which develop on infected fruits and spread by wind and insects to infection points. Penetration can be direct or through wounds. The fungus may be isolated from symptomatic fruit tissues on PDA. The colony is leathery with tuffs of grey-brown conidiomata on it (Everett & Siebert, 2018).

Cercospora spot poses a primary problem to the quality of avocado and other plant fruits. The severity of disease is seasonal and depends on climatic conditions. The infection can cause losses of up to 60% (Pérez-jiménez, 2008). Cercospora spot cause lesions that appear on fruits and leaves of plants as small light-yellow spots (Pérez-jiménez, 2008). These lesions become red-brown eventually becoming hard leading to cracks. Mature plant leaves and fruits are resistant to the disease. Humid conditions and high temperatures favour the development of Cercospora spot disease (Far *et al.,* 2006). The fungal spores are wind-borne and the fungus is essentially spread by water splash.

2.3 Management of Fungal Avocado Fruit Diseases

Infection of fruits by pathogens can either occur before harvesting under field conditions or during harvesting and transit, storage and marketing (Kader *et al.,* 2011).

Actual disease occur only when the attacking pathogen starts to actively grow in the host. The primary cause of post-harvest diseases are pathogenic fungi especially in sub-tropical and tropical regions. Since the diseases are common and spread the infections, there is a necessity to identify and characterize the causative agents and to develop effective control measures for the diseases (Kader *et al.,* 2011).

2.3.1 Chemical Control of Fungal Avocado Fruit Diseases

Growers majorly use chemical control strategy to reduce the occurrence of postharvest diseases in avocado fruits (Barrera-Necha *et al.,* 2008). Control of these fungal fruit diseases in avocados has been through application of chemicals like prochloraz® at commercial level (Smith *et al.,* 2011). This is a non-systemic fungicide that is used as a first-defence mechanism in the packing line in the control of post-harvest diseases such as avocado scab and avocado anthracnose. Prochloraz® inhibits the mycelial growth of the pathogenic organism and also acts as a sterol inhibitor that enhance the fatty acid synthesis. The sterol inhibitor is an important constituent of the fungal cell wall (Malick *et al.,* 2014). Other fungicides used to control fungal avocado fruit diseases include Thiabendazole®, copper oxychloride®, Mancozeb®, Metiram® and Propine® (Agrios, 2005).

The development of fungicidal resistant strains has however made the use of fungicides unsustainable (Ippolito & Nigro, 2000). The use of fungicides is becoming increasingly restricted particularly in post-harvest diseases in most of the countries (Mekonnen *et al.*, 2015). Fungicides are also a source of environmental pollution and hazards due to the disposal of large quantities of fungicidal containing solutions, majorly in soil and water resources. Due to the risks associated with the use of post-harvest fungicides in avocados, the farmers and vendors need to find an alternative strategy to fungicidal application. The alternative strategies include; controlled and modified atmospheric conditions in storage (Kader, 1994), biocontrol agents (Janisiewicz *et al.,* 2001), high heat treatments (Fallik *et al.,* 1999), microwaves, chitosan and native products such as natural plant extracts and oil essentials (Gonelimali *et al.,* 2018).

2.3.2 Cultural Management of Fungal Avocado Fruit Diseases

This entails control of fungal avocado fruit diseases through putting in place good sanitation measures that involve collection and proper disposal of infected fruits

(Agrios, 2005). In addition, adequate post-harvest handling practices are applied to prevent injuries and bruises to the fruit and leaf surfaces (Wasilwa *et al.,* 2006). Those fruits showing symptoms of post-harvest diseases are not packed into storage vessels with healthy and uninfected fruits (Sarkhosh *et al.,* 2017). However, these methods are not effective and must be integrated with other methods.

2.3.3 Biological Control of Fungal Avocado Fruit Diseases

Biological control entails the use of an organism to influence the activities of a plant pathogen that is responsible for causing diseases. Biological control also applies to the use of the natural products extracted or fermented from various plants (Korsten, 2015). The natural products may be made by simple mixtures of native components which have specific activities to pathogenic species or complex mixtures with multiple impacts on the host as well as the target pathogenic organism or pest (Yoon *et al.*, 2013). Such natural plant extracts may mimic the living organisms' activities. Non-living inputs are more broadly referred to as bio-pesticides or bio-fertilizers. This depends on the essential benefit provided to the plant host (Morrone, 2019). Bio-control organisms known as bio-control agents include bacteria, fungi, and nematodes, among others. Some plants contain botanical pesticides or botanical components that are toxic to pathogenic organisms when extracted from the plant and applied on infected plants (Korsten, 2015).

The disadvantages and hazards of synthetic chemicals has led to the search for alternative plant disease control measures although there is considerable progress in the control of the fungal diseases by the use of fungicides (Cheloti *et al.,* 2010). The continuous use of synthetic chemicals against the pathogenic organisms that cause diseases has led to severe impacts on the living organisms such as animals and plants and their environment (Cheloti *et al.,* 2010). Plant extracts can be a safe alternative for controlling most plant pathogenic diseases since they are natural products. Further, the natural plant products have displayed positive results in experiments done in animals and plants on antibacterial and antifungal activities as they contain secondary metabolites which react differently to pathogens (Waithaka *et al.,* 2018).

2.3.3.1 Applications of Tannins in Control of Plant Fungal Diseases

Tannins are non-crystallisable substances that exist as pronounced astringent properties and are colloidal in nature. They show a characteristic of binding and precipitating gelatin in a solution (Ajao & Moteetee, 2017). They form insoluble compounds with tissue that yield gelatin. This aids in converting raw skin and hiding into the leather. Tannins coordinate the dermal network of hiding into drier and firm structures which are durable, thermally stable and water-resistant than the original hiding (Muchuweti *et al.,* 2006). Unripe fruits or wine have the acidic taste due to the presence of tannins in them.

Flowers as well as the autumn leaves have constituents of tannins which are responsible for the attractive colours. Tannins have three main sub-divisions of include pro-anthocyanidins which are also called condensed tannins (CT), mixed tannins and hydrolysable tannins (HT) (Bradley, 1990). They bind and deposit proteins which form tannin-protein complexes which are soluble or unsolvable. In addition, they can significantly affect the food quality that is consumed by humans and livestock (Mlambo & Mapiye, 2015). Tannins act as a biological antioxidant and a bioactive food component hence there is a growing interest in them (Belemkar & Ramachandran, 2015).

Tannins usually dissolve in water to form a solution and are uniformly distributed in most organs of the plant according to Sampaio and Da Costa (2016). The main functions of tannins are to provide defence against pathogenic organisms and herbivory (Ajao and Moteetee, 2017). They have also been used as preservatives in the tanning industry as well as manufacture of adhesive superglue (Belemkar & Ramachandran, 2015). Tannins have also been used in the preservation of vegetables to increase their shelf life in cans and storage vessels (Saxena *et al.,* 2013). It has also been used as an antimicrobial agents to various microorganisms. They have antibacterial activity (Agyare *et al.,* 2013) and also act as an important anticancer agent (Shan *et al.,* 2011). Extracts of *Aloe secundiflora* contain tannins that have been used as an antibacterial agent against bacterial disease (Nabatanzi *et al.,* 2020). They have shown antioxidant properties, helps in healing of wound process (Ajao & Moteetee, 2017).

Several studies have shown a possible biological effects of tannins. The effects include radical scavenging activities, anti-mutagenic, inhibiting the lipid peroxidase, antimicrobial, lipo-oxygenases *in vitro*, and anti-mutagenic, as well as anti-diabetic properties (Gupta & Abu-Ghannam, 2011). The antioxidant property of tannins is attached to the free radical and reactive singlet oxygen species-scavenging properties and the chelation of transition metal ions that change its oxidation (Kannan & Rajan, 2020). Existing reports show that the antioxidants property play a significant role in treatment of diseases like diabetes due to their ability to enhance insulin. In addition, antioxidant plays a crucial role in the inhibition of bacterial and fungal growth through their reaction with the molecules of protein located within the cell wall (Rasouli *et al.*, 2017).

Tannin is found in several plants as an organic component and it can be poisonous to humans and animals. It is used as a constipating agent to treat diarrhoea and dysentery. It can also be used as a counteract in alkaloid poisoning (Borokini & Omotayo, 2012). The tannins can precipitate alkaloids that are polar and dissolve in water and it has an inhibitory action on the angiotensin-converting enzyme (ACE) since they are polyphenols by nature. The angiotensin-converting enzyme inhibitory nature of these tannins may explain the hypotensive impacts of some traditional herbals (Huang *et al.*, 2013).

2.3.3.2 Applications of Flavonoids in Control of Plant Fungal Diseases

Flavonoids are phytochemicals that have a general structure made up of two phenyl groups and a heterocyclic ring. They are a broad class of secondary metabolites of plants that are characterized by nuclei of flavone consisting of a heterocyclic ring system. The ring is obtained from polyketide biosynthesis which is also referred to as ring A and it's linked via pyrone ring, which has oxygen molecule (Baikar & Malpathak, 2010). The compounds have low solubility in water and they are pale yellow in colour (Baikar & Malpathak, 2010). These compounds are majorly found in consumables in the form of O-glycosides. The most common sugar residue is the D-glucose with other sugars including arabinose, galactose, rhamnose, xylose, and glucuronic acid (Park *et al.*, 2011).

There are several types of flavonoids that have been identified from different plants according to Saxena *et al.,* (2013). Flavonoids contain the following components; flavanones catechins, chalcones, flavones is- flavones and anthocyanin (Park *et al.,* 2011). Flavonoids inhibit most bacterial and fungal pathogenic growth (Daglia, 2010). The active components from flavonoids have antifungal, antibacterial and insecticidal properties according to Orhan *et al*. (2010). Previous research studies have indicated that when they are mixed with antibiotics they suppress several disease- causing microorganisms in the laboratories and also in field experiments (Orhan *et al.,* 2010).

A. secundiflora flavonoid components have been used as an oxidant, used as a curative agents for malaria and antimicrobial to various fungi and bacteria pathogens (Nabatanzi *et al.,* 2020). The components of flavonoids have also been used for pharmaceutical activities for example used as antioxidant, wound healing, and anti- malarial, anticancer and anti –inflammatory agents (Ajao & Moteetee, 2017). Components that are recognized are more than 4,000 and majorly occur in fruits, fruit drinks, beverages such as coffee and tea, and vegetables. These different groups of native compounds normally inhibit the oxidation (Tanwar & Modgil, 2012). The biological activities affiliated to these components are antibacterial antifungal, antiviral, anti-inflammatory, and anti-carcinogenic activities (Orhan *et al.,* 2010). These biological activities are also known for their free radical scavenging properties and antioxidant properties (Karak, 2019).

Flavonoid components have anti-cancerous, antimalarial properties and anti-inflammatory effects (Li & Jiang, 2018). Flavonoids show antiradical property, which is majorly arranged toward hydroxyl as well as radicals of alkoxyl and peroxyl (Sieg and Kubanek, 2013). In addition, as these components indicate a very strong attraction for iron ions, their anti-oxidant activities may also be attributed to chelating iron's concomitant capacity (Paulis *et al.,* 2017). Components of flavonoids have a significant role in protecting plants from bacterial and fungal agents of diseases from attacking most plants as well as polyphenols (War *et al.,* 2012).

Flavonoids have the ability to inhibit spores germination of microbial agents of diseases in plants and humans (Gao *et al.,* 2010). Application of flavonoids in the treatment of most human diseases, more so in monitoring HIV, which causes AIDS has developed an increasing interest (Mehla *et al.,* 2011). Most of the components of flavonoids that prevent the growth of most plant fungal pathogens include flavanones,

flavans and isofavonoids (Gao *et al.,* 2010). There is an acknowledgment of flavone glycoside, namely luteolin, which is an antifungal component of the marine plants especially the angiosperms (Björn *et al.,* 2013).

2.3.3.2 Applications of Alkaloids in Control of Plant Fungal Diseases

Alkaloids are native products comprising the nitrogen atoms as the basic elements. The name alkaloids originates from the word "alkaline" and was used in describing any nitrogen-rich base. Alkaloids are natural synthetics from living organisms like plants, fungi, animals, and bacteria. Alkaloids from crude extract can be purified through acid-base extraction method (Lebrini *et al.,* 2011). The components of alkaloids include mineral elements such as nitrogen, oxygen, sulphur, and some of rare minerals for instance chlorine, bromine, and phosphorus (Schardl *et al*., 2010). These are secondary metabolites that can be secreted by both plants and some microorganisms such as bacteria (Kittakoop *et al.,* 2014). They are polar organic compounds hence they are soluble in both inorganic and organic solvents (Liang *et al.,* 2013).

Although alkaloids are beneficial, there are several of them that are harzadous to other organisms. They possess medicinal properties such as antitumor and antimalarial such as quinine, antibacterial like berberine and analgesic such as morphine (Bribi, 2018). In addition, these phytochemical compounds are characterized by a stimulants such as nicortine and caffeine. Almost all the alkaloids have a bitter taste since they are alkaline (Bribi, 2018). They are used in recreational drugs and in disease treatment (Bribi, 2018). They are also of economic importance due to their medicinal properties such as antiasthma, antimalarial, anticancer and antimicrobial activities (Hadi *et al.,* 2010).

Alkaloids act as antimicrobial agents against different plant pathogens and can be utilized to control pathogenic fungal and bacterial microorganism that cause plant diseases (Maatalah *et al*., 2012). Morphine and codeine are some of the bioactive components of alkaloids that act against bacterial and fungal pathogens, trypanosomes and plasmodia (Garba & Okeniyi, 2012; Kumar and Pandey, 2014). Alkaloid constituent of *Aloe secundiflora* can been used in the treatment of malaria and has also been used as an antibacterial and antifungal agents against various microorganisms (Chenia, 2013). Alkaloids are also used as a protein synthesis stimulant reservoir, a growth regulating agent, internal metabolite, or reproduction and final agent's

detoxifier and simple transformative agent of other substances although their build up can cause harm to the plant cells (Martins *et al.*, 2019).

2.3.3.4 Applications of Saponins in Control of Plant Fungal Diseases

Saponins are secondary plant metabolites that consist of a skeleton that results from a 30-carbon oxydosqualene precursor to which glycosyl residues are joined along with it and exists as glycoside (Moses *et al.,* 2014). Steroids exist mainly in monocotyledonous plants including families like Liliaceae, Droscoraceae, and Agavaceae. They can also be found in some dicotyledonous plants like foxglove (Bhatla, 2018). Plants with saponins are used as traditional medicines in many countries in the world, mainly in Asia.

Saponins are utilized in medicine, food and veterinary industries (Khalid *et al.*, 2012). They offer defence against microbial organisms in plant cells (Berezin *et al.,* 2010). Saponins are known to act by breaking the plasma membranes of the microorganisms according to Deshpande *et al*. (2013). They have anticancer or antitumor activity as well as antibacterial and antifungal activities (Li *et al.,* 2012). They are the most abundant and bioactive constituent of the natural plant extracts and they are used as anticancer and for lowering the high cholesterol content of fat in the human body (Fagbohun *et al.,* 2020). Saponin phytochemicals of *Tithonia diversifolia* have been utilized in the treatment of inflammatory diseases (Ajao and Moteetee, 2017). Although saponin components are hazardous to cold-blooded animals, they are considered to have no harm to warm blooded organisms such as mammals (Bhatla, 2018). Plant extracts with a high content of saponins are mainly used in treatment of water in Africa, making them safe for consumption by humans (Ugboko *et al.,* 2020). Plant extracts with a higher concentration of saponins are also known to boost the immune system in living organisms (Sirohi *et al.,* 2014).

2.3.3.5 Applications of Phenolics in the Control of Fungal Plant Diseases

Phenolics are organic phytochemical compounds that naturally found in plants. They are made of hydroxyl groups attached to an aromatic ring. The organic compounds mainly exist as conjugates with disaccharides or polysaccharides that are mainly joined together with the aromatic ring (Mandal *et al.,* 2010). Among the phytochemical

compound, phenol is one of the majorly spread secondary plant metabolite. These metabolites act as antioxidants that are crucial in the plant development.

The antioxidatory activities of phenolic compounds are associated with the radical scavenging ability of phenol compounds and their potential to inhibit lipid peroxidatives under oxidative stress (Shukla *et al.,* 2012). Phenols are used in food preservation. For instance, phenols have been used to control and suppress the growth of pathogens such as *Escherichia coli*, S*taphylococcus aureus* and *Bacillus cereus,* which are thedisease causing agents of various diseases (Chan *et al*, 2018)

2.3.3.6 Applications of Terpenoids in Control of Plant Fungal Diseases

Terpenoids are phytochemical compounds produced by plants that are responsible for various plant scents. Classification of these secondary metabolites is based on the number of isoprene units present according to Sahu *et al*. (2011). They include: Hemi terpenoids which consists of one isoprene unit and is the only hemiterpene. Although it's the only hemiterpene, it has derivatives of isoprene that contain oxygen molecule like prenol and isovaleric which are classified as hemi terpenoids (Sahu *et al*., 2011). The other terpenoid consists of two units of isoprene. Mono-terpenoids are produced from the biochemical change of mono-terpenes (Sahu *et al.*, 2011).

These phytochemicals are characterized by antibacterial and antifungal activities (Abdallah & Quax, 2017). They are active against living organisms like termites, insects, worms and mollusks that cause injuries and bacteria and fungi that cause infections (Okwute, 2012). The dark color in the bark and other parts of the plant are accounted for by the oxidation decomposition products and the derivatives from naphtha quinones (Abdallah & Quax, 2017).

2.4 Biopesticide Potential of *Aloe secundiflora* Against Fungal Fruit Diseases

Plants such as Aloes usually synthesize phytochemical compounds that include essential oils, flavonoids, saponins, alkaloids, tannins and terpenoids (Saxena *et al.*, 2013). These metabolites have antimicrobial activities to some pathogens that infect both plants and animals (Oyelana *et al.,* 2011). Analytical High Performance Liquid Chromatography- Mass Spectrometry (HPLC- MS) studies of the *Aloe secundiflora* exudate revealed presence of saponins, flavonoids, terpenoids, steroids which is a

mixture of phenolic metabolites, mainly anthrones, chromones and phenylpyrones with a very low concentration of polysaccharides and the compounds of aliphatics (Rebecca *et al.,* 2003). The phytochemicals could be responsible for various antifungal activities (Madike *et al.,* 2017). The high percentages of anthrones in the plant extract give a first line of evidence for the use of the natural plant extracts in veterinary practices (Rukenya, 2014).

Several phytochemical constituents from *A. secundiflora* have been isolated and include phenyl propanoids and phenyl ethanoids, saponins, tannins, furanone derivatives, flavonoids and sterols (Atawodi & Olowoniyi, 2015). The extracts from the *Aloe secundiflora* are active against *Fusarium oxysporum*, a fungal plant pathogen that causes tomato wilt (Cheloti, 2010). Micheni (2015) tested anti-fungal activity of *A. secundiflora* against *Pythium ultimum* that attacks potatoes and *Fusarium oxysporum* in bananas and reported a high level of mycelial growth inhibition. The efficacy has also been tested on management of *Erwinia carotovora* which is causes a post-harvest potato soft rot disease (Ndivo *et al.,* 2018). Naphthoquinones have also been isolated from the root extract of *Aloe secundiflora* with antimicrobial activity against *Mycobacterium tuberculosis* (Induli *et al.,* 2012). Therefore *A. secundiflora* is a natural source of phytochemicals that can be used in the management of plant fungal infections.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was carried out in different markets in Embu County, Kenya (Figure 2). The County is located at 0° 31' 58.80" North, 37° 27' 0" East and covers 2821 km^2. Embu County borders Kitui to the East, Kirinyaga to the West, Tharaka Nithi County to the North and Machakos to the South. It experiences an annual mean temperature of approximately between 18.7 °C to 22 °C with a bimodal annual rainfall of 1300 mm; the long rains begin from April and end in June while short rains begin from October and end in December. The County is divided into 11 agro-ecological zones. It has a potential for agricultural production of crops such as maize, beans, avocadoes, bananas, yams, arrow roots, *khat*, as well as livestock production.

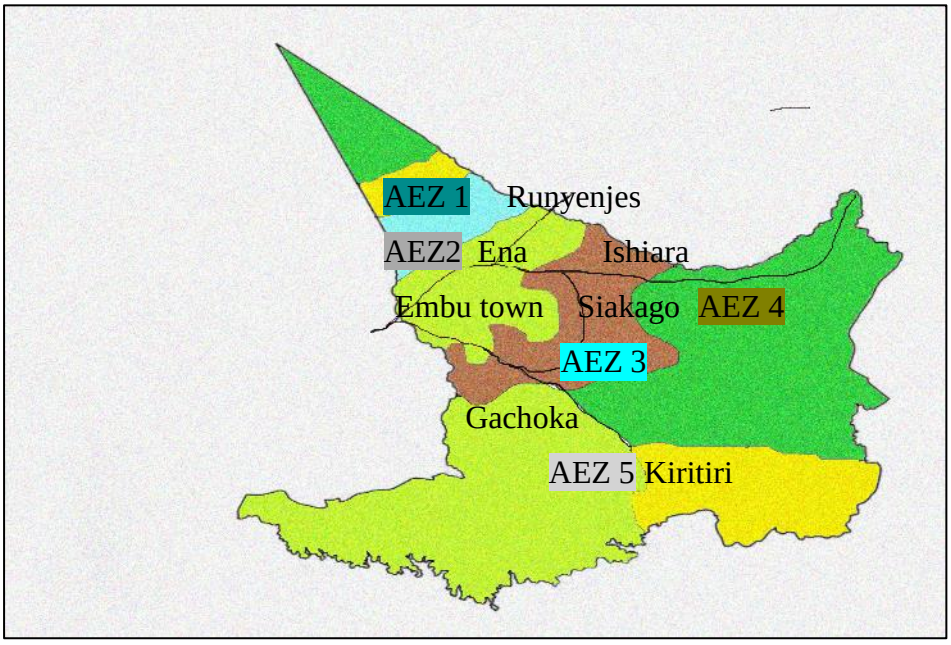

Figure 2: Map of Embu County showing different markets where study samples were collected

3.2 Research Designs

3.2.1. Survey Design

A descriptive cross-sectional survey design was adopted for this study. The survey was carried out in the six purposively selected avocado markets in the study area to assess the prevalence of fungal diseases of avocado fruit. A structured questionnaire (appendix 1) was administered to avocado fruits sellers to gather information on challenges of

avocado marketing, incidences of fungal diseases of avocado fruit, management strategies used and perceived success of such management options.

3.2.2 Experimental Design

In the laboratory, a 3×4 factorial experiment was laid out in a Complete Randomized Design (CRD). The isolates of the conidia were in three different levels. The concentrations of the plant extracts were at four different levels (0mg/ml, 2.5mg/ml, 5.0mg/ml, and 10.0mg/ml). Treatment with 0mg/ml concentration of Dimethylsulfoxide (DMSO) served as the negative control and Copper oxychloride was used as a control experiment. The experiment was replicated three times.

3.3 Target Population and Sampling Procedure

3.3.1 Target Population

The target population was avocado fruit sellers in the six selected markets; Runyenjes (RM), Siakago (SM), Kiritiri (KM), Embu town (ETM), Kianjokoma (KJM) and Mutunduri (MM) markets within Embu County.

3.3.2 Sample Size

A total of 120 avocado sellers participated in this study (Table 1). The sample size was determined using the formula below:

$$n = \frac{N}{1 + N(e)^2}$$

$$n = \frac{170}{1 + 170(0.05)^2}$$

Where n = estimated sample size, N = estimated population size, and e = Margin of error (Mo E), e = 0.05 according to Israel (2009).

Table 2: Sample distribution among the six markets

Markets	No. of Vendors	Sample size
Runyenjes (RM)	31	20
Siakago (SM)	25	20
Kiritiri(KM)	29	20
Embu town (ETM)	35	20
Mutunduri (MM)	25	20
Kianjokoma (KJM)	25	20
Total	170	120

3.3.3 Sampling Procedure

From a list of 18 markets that are in Embu County, six markets were purposively selected for the survey by the virtue of being located in avocado growing areas. The markets that were surveyed were: Kiritiri, Siakago, Embu town market, Kianjokoma, Mitunduri, and Runyenjes. In each of the six markets, twenty avocado vendors were randomly identified. For each vendor, avocado containers were randomly picked depending on the type (buckets, sacks or crates) and all the avocados in each of the container were counted and assessed for the presence of fungal pathogens. Purposive sampling was used to pick infected avocado fruits. Infected fruits samples showing symptoms of fungal avocado fruit diseases were aseptically packed in sterile containers and well labelled for further laboratory examination analysis.

3.4 Research Instrument

A structured questionnaire (appendix 1) was administered to avocado sellers to gather additional information on challenges of avocado marketing, incidences of fungal diseases, management strategy and perceived success of such management options. The prevalence of the fungal avocado fruit diseases was assessed by observation of symptoms in the avocado vendors' groceries.

3.4.1 Pilot Survey

A pilot study was conducted prior to collection of the actual data to enhance validity and reliability of the research instrument. A sample of 10 avocado vendors from Chuka town was used as the pilot sample. Pilot sample was adequate to effectively represent the study sample.

3.4.2 Validity Test

The content validity of the questionnaires was ensured through expert judgment by the supervisors and experts in the area.

3.4.3 Reliability of the Instrument

The instrument was pilot tested to enhance reliability. Cronbach alpha was used to determine the reliability of the instruments using the pilot survey data. Cronbach's Coefficient determines how items correlate among themselves and tests internal consistency of the instruments. The instruments were considered reliable since the coefficients obtained were above 0.7.

3.5 Data Collection Procedures

3.5.1 Field Pre-visits and Determination of Sample Size

Reconnaissance field visit was made to establish the administrative network and authority to conduct research, study the market and distribution of avocado in Embu County specifically in Runyenjes, Kiritiri, Siakago, Embu town, Mutunduri and Kianjokoma markets. Further studies were conducted to establish the nature and management of the fungal fruit diseases of avocado after harvesting. This was done through interaction with 120 vendors by administration of the questionnaire, face-face interviews and also discussions. The pre-visit was also used to identify vendors that formed part of the sample used in the study of disease prevalence.

3.5.2 Prevalence of Fungal Avocado Fruit Diseases

Data on the prevalence of fungal avocado fruit diseases were obtained during market survey in avocado stores per the storage containers. All avocado fruits in the storage containers, both infected and uninfected were counted and recorded to determine the prevalence as shown in equation 1 (Aslam *et al.*, 2015). Symptoms used in the identification of the fungal diseases included dark brown lesions, grey spots on the epidermis of the fruit and rotten avocado fruits.

$$[\text{Equation 1}]\ \%\ \text{Prevalence} = -\frac{\text{No.of fruits infected}}{\text{Total number of fruits assessed}}\ x\ 100$$

3.6 Isolation of Avocado Fruit Fungal Pathogens

3.6.1 Preparation of Potato Dextrose Agar Media

Potato Dextrose Agar (PDA) media was prepared based on the manufacturer`s instruction. Thirty-nine grams of PDA media was placed in a sterilized conical flask and 1000 ml distilled water was added and stirred. The conical flask was then plugged using cotton and aluminium foil. The media was heated to boil till it formed a uniform solution. Thorough sterilization was done by autoclaving at 15 lbs. pressure (121 $^{\circ}$C) for 15 minutes in an autoclave (model X280A). The media was then left to cool to 50 $^{\circ}$C after which 25 mg/L of antibiotic-amoxicillin was added to prevent or alter the multiplication of bacterial contaminants. The PDA media was then mixed well before spreading into Petri dishes (Sheringham and Brightwell, 2012).

Fungal pathogen were isolated from fruit samples as described by De Carolis *et al*. (2012). Infected avocado fruits were cleaned with distilled water to remove dust and dried using a soft cloth. The diseased fruits were surface sterilized with 70% ethanol using cotton wool. The scalpel was then flame sterilized and used to cut a section of the diseased parts from advanced margins of the lesion. The section was aseptically transferred into a petri plate containing PDA media and another containing Corn meal agar. The petri plates were covered, sealed with parafilm and properly labelled with a permanent marker. The plates were then incubated at 25°C and monitored for fungal growth. To acquire pure culture, sub-culturing was done by single spore isolation technique whereby an inoculating loop was sterilized and used to scrap a single colony from the isolated fungi and grown on PDA media.

3.6.2 Phenotypic Characterization of the Avocado Fruit Fungal Pathogens

Identification and characterization of the avocado fruit fungal pathogens was carried out according to Fawole & Oso (1995). A drop of Lactophenol cotton blue stain was placed on a clean microscopic slide. A small part of the mycelium from each of the fungal cultures was picked using a sterile wire loop and placed in the drop of the stain. Two wire loops were used to spread the mycelium well on the slide and a cover slip gently placed over the slide.

Microscopic examination was carried out under high power objective lens (×40). Phenotypic characteristics of the fungus such as color, texture, shape of mycelia, hyphae septation were observed and recorded. The most prevalent fungal pathogen was also determined from the growth of colonies. One isolate from each species was in addition done through Koch‘s postulate where the pure cultures of the isolates growing on PDA were inoculated to healthy avocado fruits at different surfaces.

3.6.3 Determination of Conidia Morphology and Size

Pure cultures of fungal isolates grown on PDA for 10 days at room temperature were flooded with sterile distilled water and filtered through double layer cheese cloth to remove mycelia. The filtrate was collected in clean universal bottles and then diluted serially. A drop of the filtrate was placed on a slide hsing a pipette, covered with a cover slip and then placed on microscope stage. Lactophenol cotton blue stain was added to stain the spores to improve visibility. The shape of the spores was noted and the size

(length and width) of the 3 spores per isolate was measured using calibrated ocular slide and stage micrometer.

3.7 Efficacy of *Aloe secundiflora* on Avocado Fruit Fungal Pathogen

3.7.1 Collection of *Aloe secundiflora* Leaves and Preparation of Crude Extract

Mature Aloe leaves were collected from the field in Mbeere sub-county, a semi-arid region in Embu County. A knife was used to cut the Aloe leaves at the base. The leaves were then put in sterilized buckets and taken to the laboratory for analysis. In the laboratory the leaves were thoroughly cleaned with running water and then washed with distilled water to remove dust particles and any other debris. They were then cut into tiny pieces and spread on a clean and sterilized bench to dry under shade for seven days.

The dried Aloe leaves samples were ground into fine powder using a blender. Two samples, each of 112 g, were then soaked in separate organic solvents; hexane and ethanol just enough to cover the mass of the samples. The samples were then left for 72 hrs to allow enough time for absorption. Each sample was filtered using Whatmann filter papers and the filtrates poured into a round bottomed flask. The round bottomed flask with the contents was then connected to a rotary evaporator to evaporate the solvents under vacuum using a vacuum pump. This was carried out until maximum solvent had been removed. The extracts were then transferred into different beakers, kept in an oven and set at the respective boiling points of the solvents (for hexane 68 ^{o}C and for ethanol 78 ^{o}C) to fully evaporate the remaining amount of the solvents in the extract and remain with solid phytochemicals.

Different concentrations of 2.5 mg/ml, 5.0 mg/ml and 10.0 mg/ml of the extracts were prepared. For each concentration, a volume of 50ml was prepared for the separate extracts. To prepare 2.5 mg/ml, 5.0 mg/ml and 10.0 mg/ml concentration, 0.125g, 0.25g and 0.50g of the plant extracts, respectively, were measured and transferred into a 50 ml volumetric flask. Dimethylsulfoxide (DMSO) solvent was then added to dissolve the extract before topping it to the mark. They were then transferred to reagent bottles ready for use. The plant crude extracts were then stored in the refrigerator awaiting antimicrobial assay.

3.7.2 Phytochemical Analysis of *Aloe secundiflora* Extract

Phytochemicals in the *A. secundiflora* leaf extracts for the two solvents were examined using standard procedures according to Parekh and Chanda (2007). The phytochemicals tested were tannins, alkaloids, terpenoids, saponins, flavonoids, phenolics and steroids.

In the test for tannins, 0.5 g of the plant extract was swirled with about 10 ml of distilled water and then filtered. Three drops of 1% ferric chloride solution was added to 2 ml of the filtrate. The appearance of a green colour indicated the absence of tannins. Wagner test was used to determine the presence of alkaloids (Ghosh *et al.,* 2010). Five grams of the plant extract was added to 1 ml of ethanol to enhance dissolution and then filtered. To the filtrate, 1M Hydrochloric acid was added and then the solution boiled for about two minutes. Three drops of the Mayor's reagent solution were also added. Absence of red- brown precipitate indicated absence of alkaloids.

In the test for terpenoids, 0.2 g of the plant extracts were measured separately and 2 ml of propanoic acid was added. The solution was left to cool well in ice and then careful addition of concentrated sulphuric acid (H_2SO_4) was done (Yadav & Agarwala, 2011). Formation of red-brown layer indicated terpenoids present. Froth test was used to test for saponins which was carried out in reference to Adomi & Umukoro, (2010). Five millilitres of the plant extract was dissolved in one-millilitre of ethanol and then filtered. Three drops of 4.2 % w/v sodium bicarbonate solution were then added to 2 ml of the plant extract and thoroughly shaken. The plant extract was then allowed to settle for 15 minutes. Formation of froth was the positive test for saponins.

Test for flavonoids was carried out according to Hossain *et al*. (2013). It was achieved through adding 2 ml of concentrated sodium hydroxide solution (NaOH) to 4 ml aliquot of the plant extract. Dilute Hydrochloric acid was then added to the resulting mixture and observation made. Appearance of golden yellow precipitate indicated presence of flavonoids. Phenolic compounds test was done according to Kumar *et al*. (2010), where 2 ml of the plant extract was added to 1 ml of ferric chloride solution. Observation on colour change was observed. The colour was retained in hexane solvent extract hence indicated absence of phenolics.

In the test for steroids, two millilitres of the plant extract was added to 2 ml of methanol to dissolve the extract; three millimetres of liquid sulphuric VI acid was then added to the solution. Change in colour was then observed. A brown layer at the interface showed the presence of steroids.

3.8 Antimicrobial Activity of *A. secundiflora* on Avocado Fungal Pathogens

3.8.1 Preparation of Standard Inoculums for Test Pathogens

Preparation of stardard innoculum was carried out according to Alaniz *et al.,* (2011). Spore suspension of *Colletotrichum gloeosporioides* was prepared from a seven day old media cultures grown on PDA media. The colonies were sprayed with sterilezed distilled water. The spore suspensions were etracted using a sterilized inoculation loop to remove the spores from the surface of the PDA medium. The sterilized cork borer was hard pressed on the surface of PDA medium to remove a lamp of the PDA medium together with the *C. gloeosporioides* test pathogen.

After the removal of the spore suspensions, the pathogen was estimated using the fungal spores of 1×10^6, 2×10^6 and 3×10^6 for an antimicrobial test. The estimation was based on the size of the lamp of the PDA medium containing the *C. gloeosporioides* that was placed on the plate that was used for antimicrobial testing. This procedure was repeated for the other two pathogens; *C. purpurea* and *S. perseae*. The cultures were stored for use whereby the spore suspensions of the microorganisms were put into vials and then kept in a refrigerator at 5 $^{\circ}$C ready for culturing into the PDA medium for antimicrobial testing (Ruiz-Marine *et al*., 2010).

3.8.2 Preparation of Antimicrobial Test Discs

This was carried out according to Arunkumar (2009) procedure. Paper discs of 6 mm in diameter were cut off from a sheet of Whatman filter paper of size three using a paper punch. The test discs were then placed in a clean, dry capped bottle and sterilized in the autoclave for 15 minutes. The bottle containing the paper disc was kept in a cool, dry clean cupboard until the time for use. Antimicrobial test disc was made by taking two ml of each of the concentration of the stock solution of each plant extract and pipetted on a sterile paper disc of 6 mm diameter in a sterile Petri- dish, this was to give a concentration of approximately 2 mg/disc. The preparation of the test discs was

repeated three times to make sure each of the concentrations has three replicated test discs. The extract soaked discs were left to dry for 24hours on a sterile bench.

The test for antimicrobial activity was carried out according to Assob *et al*. (2011). Petri-dishes were dispensed with PDA media for the three most common pathogens: *C. gloeosporioides, C. purpurea and S. perseae*. The spore suspensions were cultured by transferring a lump of the picked fungal pathogen spores by use of a cork borer into sterile PDA medium on each plate. Whatman paper disc soaked with the plant extract was then placed on to the media at four different positions on the Petri-plates; the media was placed in an inverted position and incubated at room tempereature in an incubator for one week in the Laboratory to allow for the *in vitro* fungal growth. The zones of inhibition in which no microbial growth had occurred were measured in millimetres. The negative control was done with water while the positive control was a fungicide (copper oxychloride).

The minimum inhibitory concentrations (MIC) of the extracts was determined through broth tube dilution procedure using two-fold dilution in dextrose broth as described by Rukenya (2014). Sterile screw capped test tubes were used for serial dilutions. About 0.1 ml of standardized inocula of each of the three fungal pathogens were poured onto test tubes and incubated at 37 °C. After incubation, MIC values were determined by observing the suppression of growth of fungi colonies.

3.9 Data Analysis

Descriptive statistics was used to summarize data into means and percentages. Data on prevalence of post-harvest fungal avocado diseases recorded in mean percentages was subjected to a one-way analysis of variance (ANOVA). Data of diameters of zones of inhibitions at different concentrations obtained from efficacy of *A. secundiflora* crude extracts against the three most prevalent fungal avocado pathogens was subjected to a one way analysis of variance using Scientific Analysis System (SAS) version 9.4 software. Significant means were separated using Least Significance Difference (LSD) at $\alpha = 0.05$.

3.10 Ethical Consideration

The research permit was obtained from Chuka University Ethical Committee and the National Committee of Science, Technology and Innovation (NACOSTI). In addition, permission from the office of the Ministry of Agriculture and Trade in Embu County was obtained. The participants were first explained about the project and how the collected data would be used and they were assured that the data collected would remain confidential. The consent of market elders, the stores and grocery owners where the study was conducted was obtained. During collection care was taken to prevent infecting of uninfected fruits in the storage. Proper attribution of credit and acknowledgment has been given to all the contributors of this research. Lastly, the policy regarding plagiarism was adhered to in the process of data collection, analysis and in the write up of the document.

CHAPTER FOUR

RESULTS

4.1 Preliminary Analysis

On the preliminary analysis, the target sample for the study was 120 respondents but only 83.3% (100) responded, which was found to be adequate.

4.1.1 Demographic Characteristics of the Respondents

Majority (52%) of the respondents in the study were 60 years and above while respondents that were below 30 years were few at 20%. Most of those below the age of 30 years were young ladies. Overall, the number of female and male vendors was 63% and 37 %, respectively (Table 2). Majority (89 %) of the avocado fruit vendors in the study market sites were on full time basis fruit vendors, while the rest were involved with formal employments and other economic activities. Majority (34 %) of the vendors had primary level of education while 29% and 21% had secondary school and tertiary levels, respectively. However, 16 % of the vendors had no formal education.

Table 3: Socio-demographic characteristics of avocado fruits vendors in Embu County Markets

Demographic characteristics of vendors		Frequency	Percentage
Age	60yrs and above	52	52
	Between 30 and 60	28	28
	Below 30yrs	20	20
Sex	Females	63	63
	Males	37	37
Education level	Primary level	34	34
	Secondary level	29	29
	Tertiary level	21	21
	Illiterate	16	16

4.1.2 Source of the Avocado Fruits Sold

Avocado fruit vendors obtained the fruits from various sources within Embu County and beyond. Majority (50%) of the vendors sourced avocado fruits from their own farms which are within the county while 25% obtained avocado fruits from market places within the county (Fig. 3). Further, 10% of the vendors sourced the avocado fruits from farmers in the neighbouring Counties. However, 10% of the vendors had no idea on the source of the avocado fruits they were selling (Figure 3).

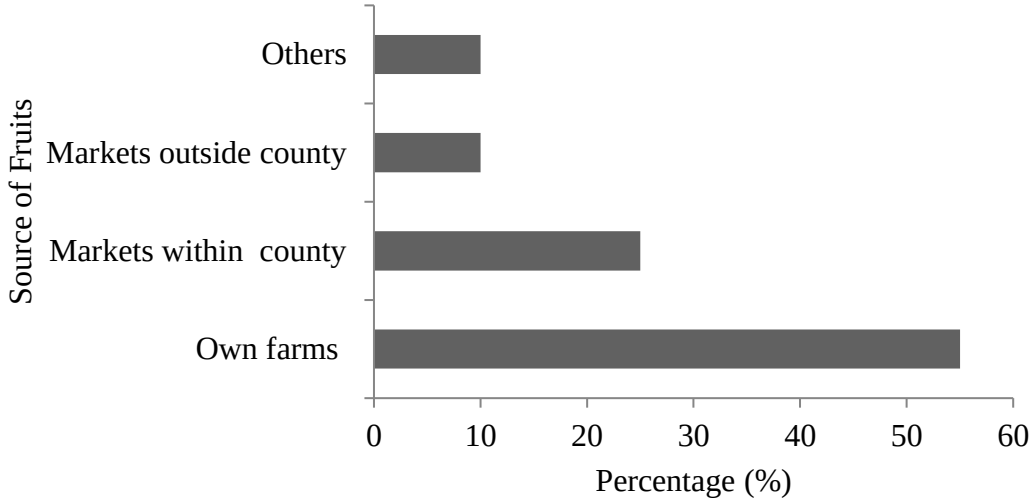

Figure 1: Sources of avocado fruits sold by vendors in various markets in Embu County

4.1.3 Identification of Avocado Fruit Diseases by Vendors in Embu County

Identification of the disease in the avocado fruits sold in the study area was mainly by vendors themselves, consumers and brokers. Some vendors (45%) were able to identify disease symptoms themselves while 30% identified through their customers. Others (15%) identified the symptoms through the brokers while 10% were not able to identify the symptoms through any means. Among the manifestated diseases symptoms identified in the market groceries were black spots, darkly colored lesions, brown spot and fruit rots.

4.1.4 Harvesting Methods of Avocado Fruits

Since most of the vendors with groceries in the markets had their own avocado farms, the method of harvesting of avocado fruits was a major concern. The vendors used different harvesting methods. Thirty-nine percent of the vendors used handpicking during harvesting to minimize fruit damages. Forty-two percent of vendors applied both handpicking and hooks in harvesting of the avocado fruits. A small percentage of the number of vendors (15%) shook the trees for fruits to drop as a method of harvesting. Others (4%) waited for some of the fruits to drop as a method of harvesting.

4.1.5 Packaging of Avocado Fruits

A large number of the vendors (54%) packed their avocado fruits in sacks mainly for local market. Avocado fruits that were meant for export markets were however packed in crates, buckets and sometimes left on the floors for pick up by vehicles as they were transported to the export company (Fig. 4). This accounted for 11% of the vendors. Twenty-one percent of the vendors used buckets alone to package their fruits. However, 13% of the vendors utilized crates only to pack their avocado fruits in the markets. A small number (1%) did not use any packaging material.

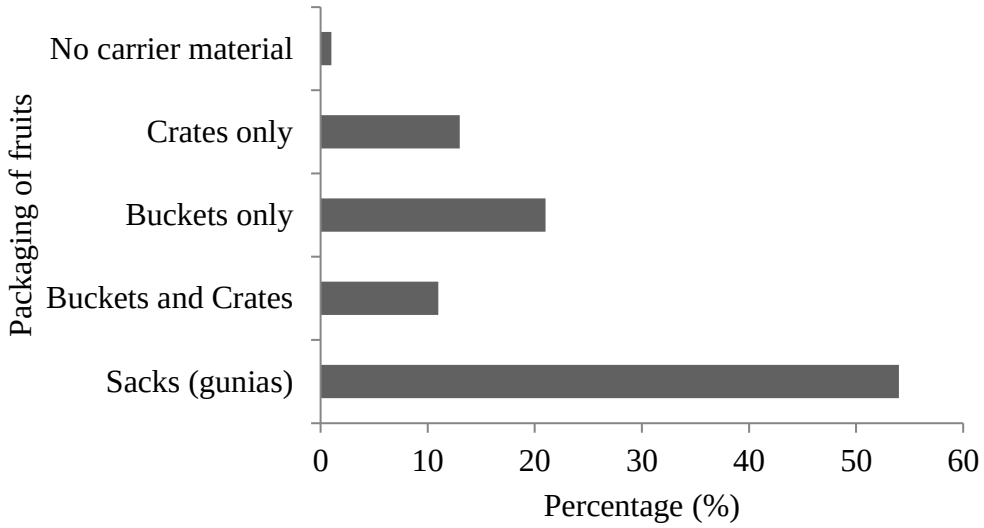

Figure 4: Packaging material for the harvested avocado fruits

4.1.6 Management of Avocado Diseases

4.1.6.1 Chemical Control

Vendors reported to use various chemicals to control diseases affecting their avocado fruits (Fig. 5). The most commonly used fungicides were GreenCop which contain Copper oxychloride, Milraz with 70% of Propineb and 6% of Cymoxaxil, Bayleton that contain 250g/Kg Triadimefon. About 40% of the vendors used Copper Oxychloride, 4% used Bayleton while 3% used Milraz to manage the diseases. About 30% of the respondents did not use any chemicals. Some vendors (16%), used chemicals to control the diseases although they could not remember the fungicide used since they had not kept records.

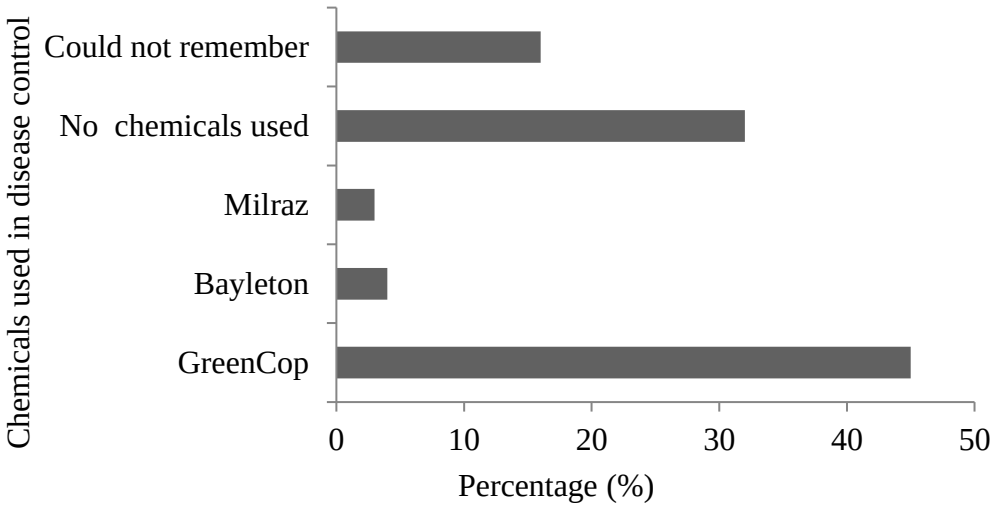

Figure 2: Chemicals used to control avocado fungal pathogens in Embu County

4.1.6.2. Cultural Methods of Controlling Avocado Fruit Fungal Diseases

Many of the vendors (55%) did not use any strategy of disease management. About 25% of the vendors who owned farms applied pruning of the avocado trees to improve aeration in the avocado tree canopies. Some vendors (22%) improved grocery hygiene procedures by collecting the avocado infected fruits, and collected fallen avocado leaves to make compost. Other avocado vendors (3%) used hand weeding for their avocado crops in the field and separated the infected avocado fruit from the uninfected to reduce the spread of diseases.

4.1.7 Avocado Varieties Sold in Different Markets of Embu County.

Different avocado varieties were sold in different markets. The commonly sold varieties were *Hass, Fuerte, Puebla* and the local varieties. Although all the markets had different varieties, the mostly preferred variety was *Hass* (39%), followed by local varieties (24%), *Fuerte* (21%) and *Puebla* (16%).

4.2 Prevalence of Avocado Fruit Fungal Diseases

The avocado fruits sold were infested by different fungal diseases and the infection rates varied significantly in the markets.

4.2.1 Prevalence of *C. gloeosporioides* (Anthracnose) on Avocado Fruits

The prevalence of *C. gloeosporioides* (anthracnose) avocado fruit infections differed significantly ($P < 0.05$) among the markets. The highest mean prevalence infection of the avocado fruits was recorded in Runyenjes (35.0%), followed by Kianjokoma (34%), Siakago (29.30%), Kiritiri 27.95% and Embu town market with 26.45% (Table 3). Mutunduri market however recorded the lowest prevalence of 21.30%. Fruits from the six markets were infected with *C. gloeosporioides* with an average of 29.00% rate of infection.

Table 3: Percentage Mean Prevalence of *C. gloeosporioides* **(**anthracnose) on avocado fruits

Markets	Mean estimate*
Runyenjes	35.00a
Kianjokoma	34.00ab
Siakago	29.30ab
Kiritiri	27.95bc
Embu town market	26.45c
Mutunduri	21.30c
LSD	6.2951
Mean	29.00
CV%	34.57

*****Means followed by the same letter do not differ significantly

4.2.2 Prevalence of *Sphaceloma perseae* (Avocado Scab) on Avocado fruits

There was a significant difference ($P < 0.05$) in the prevalence of *S. perseae* (avocado scab) in the different markets. The highest and the lowest prevalence of disease by the avocado scab disease were recorded in Siakago and Mitunduri market with a mean prevalence value of 36.40% and 20.70%, respectively (Table 4).

Table 4: Percentage Mean Prevalence of avocado scab disease on avocado fruits

Markets	Mean estimate*
Siakago	36.40a
Kianjokoma	35.85a
Kiritiri	34.25ab
Runyenjes	32.75ab
Embu town market	28.70c
Mutunduri	20.70d
LSD	12.51
Mean	31.44
CV%	32.71

*Means followed by the same letter do not significantly differ.

4.2.3 Prevalence of *Cercospora purpurea* (Cercospora Spot) on Avocado Fruits

There was a significant difference ($P < 0.05$) in the prevalence of *Cercospora purpurea* (Cercospora spot) in the different markets. The highest and the lowest prevalence of disease by the Cercospora spot was recorded in Kianjokoma market (35.15%) and Mutunduri market (18.95%) (Table 5).

Table 5: Percentage Mean Prevalence of Cercospora spot disease on avocado fruits

Markets	Mean estimate*
Kianjokoma	35.15a
Runyenjes	28.20b
Siakago	26.23bc
Embu town market	25.70bc
Kiritiri	21.65cd
Mutunduri	18.95d
LSD	11.42
Mean	25.975
CV%	38.35

*Means followed by the same letter do not significantly differ.

4.2.4 Prevalence of the Avocado Fruit Fungal Diseases in Each Market

All the markets recorded different rates of prevalence of the three diseases. In Runyenjes market, there was a significant variation ($P < 0.05$) in the mean prevalence of the three diseases. Anthracnose disease (*C. gloeosporiodes*) and avocado scab (*S. perseae*) were the most prevalent (28.05%) while Cercospora spot (*C. purpurea*) had a

mean prevalence of 22.05%. There was a significant difference ($P < 0.05$) in the mean prevalence of the three fungal diseases in Siakago market with avocado scab recording the highest mean prevalence (35.16%) and Cercospora spot the lowest mean prevalence (20.52%). Kiritiri market had a significant difference (P<0.05) with avocado scab having highest mean prevalence (27.68%) and Cercospora spot lowest mean prevalence of 16.16%.

There was no significant difference in the mean prevalence of the diseases in Embu town market with anthracnose showing the highest prevalence (21%) and Cercospora spot showing the lowest (16.16%). In Mutunduri market there was no significant difference ($P > 0.05$) in the prevalence of the diseases. Kianjokoma market had a significant difference (P< 0.05) where *Cercospora* spot disease recorded the highest prevalence of 35.79 % and anthracnose recording the lowest prevalence of 18.68 % (Fig. 6).

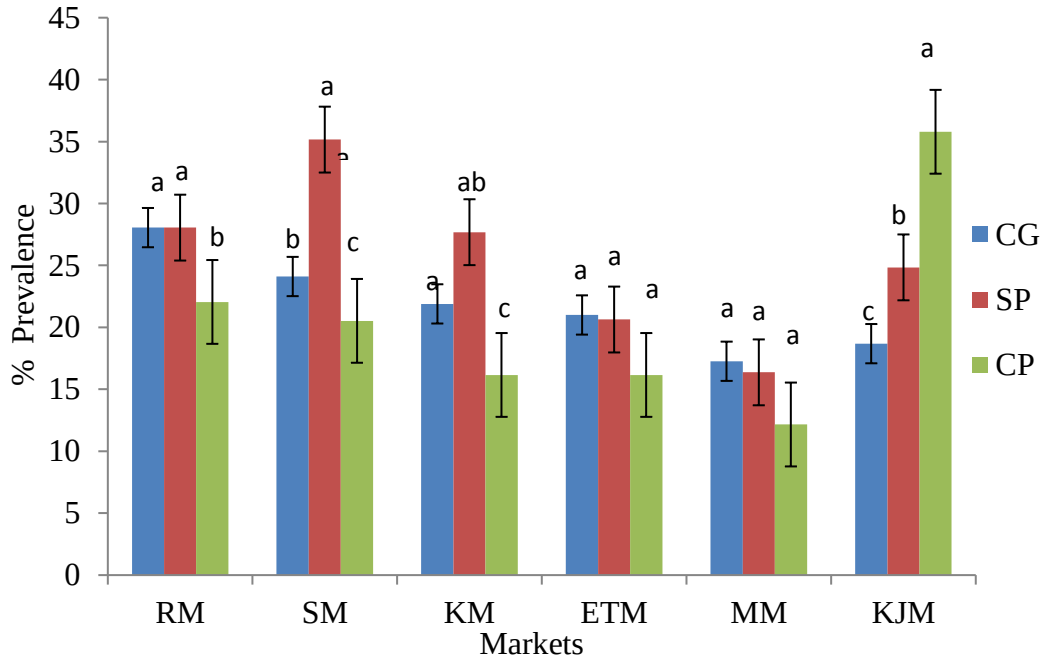

Figure 6: Prevalence of fungal avocado fruit pathogens in different avocado markets. Where CG-*Colletotrichum gloeosporioides*; SP-*Sphaceloma perseae*; CP-*Cercospora purpurea;* SK-Siakago; KJM-Kianjokoma; KM-Kiritiri; RM-Runyenjes; ETM-Embu town market; MM-Mutunduri*Bars followed by the same letter in each market are not significantly different.

4.2.5 Prevalence of the Avocado Fruit Fungal Disease in Different Varieties

The mean prevalence of infection in the avocado fruits differed among the avocado varieties assessed (Fig. 7). *Fuerte* and *Puebla* varieties were highly infected with Cercospora spot.

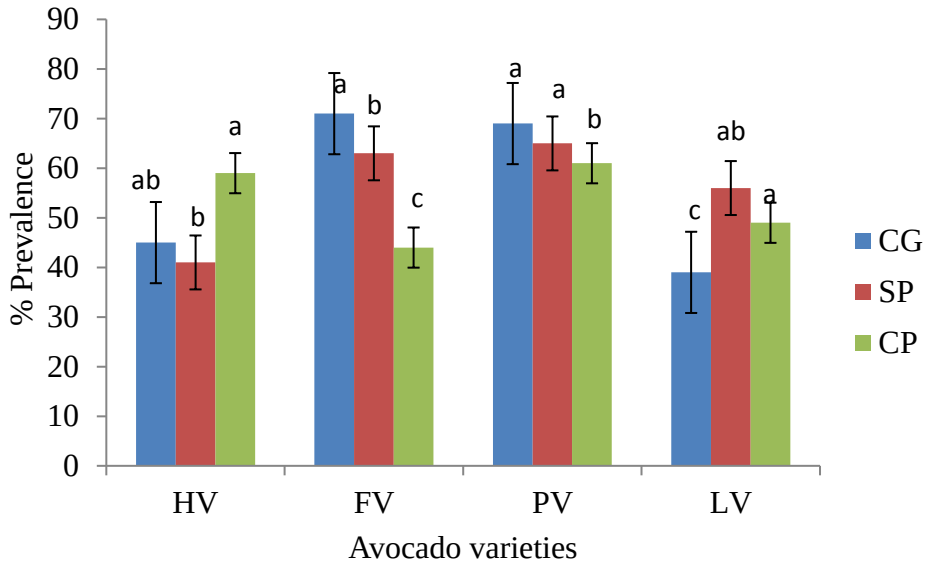

Figure 7: Prevalence of the fungal avocado fruit pathogens in different avocado varieties. Where CG-*Colletotrichum gloeosporioides*; SP-*Sphaceloma perseae*; CP-*Cercospora purpurea;* HV-Hass; FV-Fuerte; PV-Puebla; LV-Local variety. *Bars followed by the same letter in each market are not significantly different.

Hass and local varieties were sold in all the markets and their infection rate was comparatively low compared to the other varieties. *Fuerte* and *Puebla* were not common in some markets. In terms popularity of the varieties, Embu town market had the highest number of vendors selling *Hass*, *Fuerte,* local and Puebla at 51%, 37.5%, 49% and 40.6%, respectively. The more popular variety was *Fuerte* compared to the other varieties. However, promotion of *Hass* variety through the Ministry of Agriculture and Livestock had resulted to wide popularity.

4.2.6 Disease Symptoms Observed on Avocado Fruits in the Markets

Infected fruits showed different symptoms (Fig. 8). Fruits showing symptoms of anthracnose (*C. gloeosporioides*) showed circular lesions of different diameters (2–10 mm) in the epidermis, of light brown colour in the surface, getting depressed and change to dark colour with time (Figure 8 (A). Those of avocado scab disease

(*Sphaceloma perseae*) were characterized by dark-brown lesions (Figure 8 B) while those of Cercospora spot (*Cercospora purpurea*) were characterized by sunken brownish black spots (Figure 8 C).

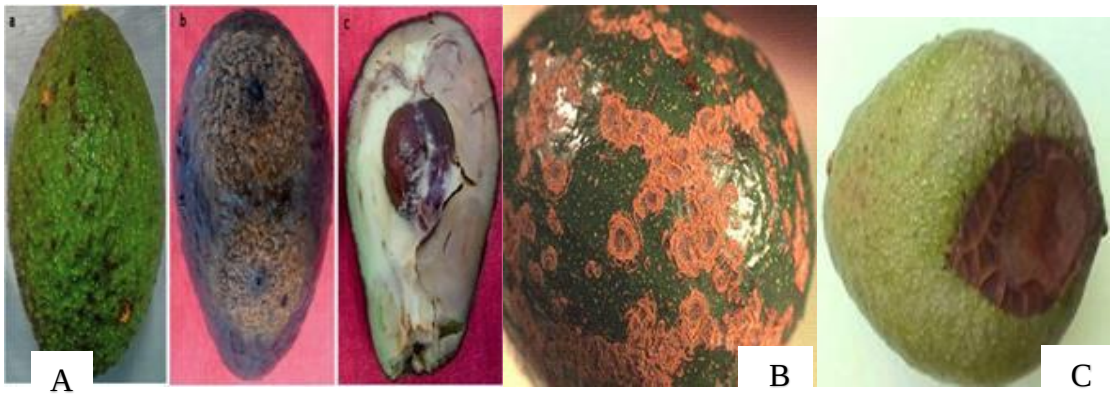

Figure 8: Disease symptoms of anthracnose disease (A), avocado scab (B) and Cercospora spot (C) in avocado fruits

4.3 Morphological Characterization of Fungal Avocado Fruit Pathogens

A total of 72 fungal isolates from infected avocado fruits in all the markets were identified.

4.3.1 Morphological Characterization of *Colletotrichum gloeosporioides*

A total of 40 isolates had whitish to greyish colour and cottony smooth mycelia on the upper side and grey to cream colour (Fig. 9a) and on the bottom side showing creamish grey colour (Fig. 9b). On isolation and inoculation into a healthy avocado fruit, a characteristic darkly coloured, rounded lesion was formed on the fruit epicarp while inside the fruit formed mesocarp soft rot of light colour.

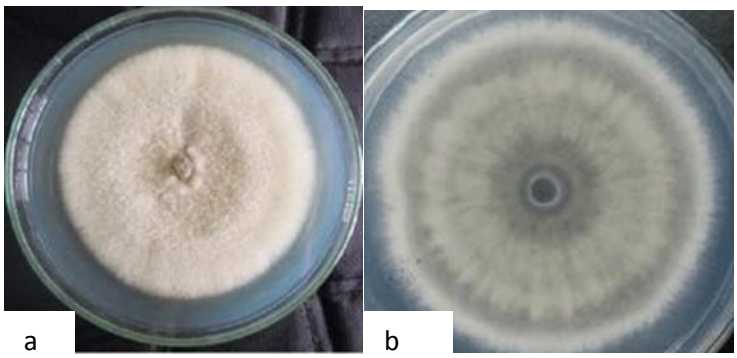

Figure 9: Pure Cultures Isolates of *C. gloeosporioides* showing the upper side of the culture (a) and the bottom side of the culture (b).

Colletotrichum gloeosporioides grew rapidly on the PDA media and covered the whole surface of the petri plate after 9 days of inoculation. The mycelial colour of the isolates ranged from white- grey, white-cream and grey on the topr side of the culture. Similarly, the bottom side of the cultures was creamish grey in colour. In terms of mycelia structure, cottony was observed in 24 isolates as compared to velvety observed in 16 isolates. It had a fast growing mycelium that formed concentric zonations. All the *C. gloeosporioides* spores observed were cylindrical and straight with smooth round ends, of large masses that were scattered over the colony and lacked a perithecia with size ranges between 3.0-5.0 μm in width and 10.3 – 18.2 μm in length (Table 6).

Table 6: The mean width and length (μm) of spores produced by 10 days-old *Colletotrichum gloeosporioides* isolates

*Isolate	Width**	Length**
AR	5.1a	18.2a
BS	4.7a	17.1ab
CK	4.7a	16.2bc
DE	3.6bc	15.0cd
FM	3.5cd	14.5d
GJ	3.0d	10.3e
LSD	0.1396	0.434
Mean	4.044	15.23
CV	1.897	3.645

**Means on the same column followed by similar letter(s) are not significantly different at 5% probability level. *FM-isolate from Mutunduri; RM from Runyenjes, DEfrom Embu town, CK from Kiritiri, BS from Siakago and GJ from Kianjokoma markets.

4.3.2 Morphological Characterization of *Sphaceloma perseae*

A total of 18 isolates had yellow colonies. Cultures were white on the underside of the plates, and mycelia had white and yellow layers (Fig. 10 a & b). On isolation and inoculation into a healthy avocado fruit, a dark brown lesion was confirmed in the epidermis of the fruit. All the 18 isolates of *Sphaceloma perseae* typically formed a slow-growing mycelium, raised and gummy to mucoid colonies on PDA medium. The mycelial growth rate was very slow.

Figure 10: Pure culture of *Sphaceloma perseae* isolates.

Sphaceloma perseae isolates developed masses of conidiophores bearing hyaline conidia ovoid or elongated coloured conidia with septation and 5-8 x3-4 µm (Table 7). The spore sizes of the isolates did not differ significantly ($P > 0.05$) in terms of width and length among the isolates.

Table 7: The mean width and length (µm) of spores produced by 10 days - old *Sphaceloma perseae* isolates

*Isolate	Length**	Width**
FM	4.0[a]	8.0[a]
AR	3.8[ab]	7.9[a]
DE	3.8[ab]	7.5[a]
CK	3.4[b]	6.4[b]
BS	3.1[c]	5.5[c]
GJ	3.0[c]	5.0[c]
LSD	0.4301	0.455
Mean	3.543	6.7166
CV	6.76	3.71

**Means on the same column followed by similar letter(s) are not significantly different at 5% probability level. *FM-isolate from Mutunduri; RM from Runyenjes, DE from Embu town, CK from Kiritiri, BS from Siakago and GJ from Kianjokoma markets.

4.3.3 Morphological Characterization of *Cercospora purpurea*

Fourteen isolates had colonies which were white leathery with tuffs of grey-brown conidiomata on them, initially grey becoming brown with age (Fig. 11 a & b). On inoculation into a healthy avocado fruit, it was characterized by brownish black spots. The 14 isolates of *Cercospora purpurea* formed a tufted, leather-like mycelium with a slow mycelial growing rate.

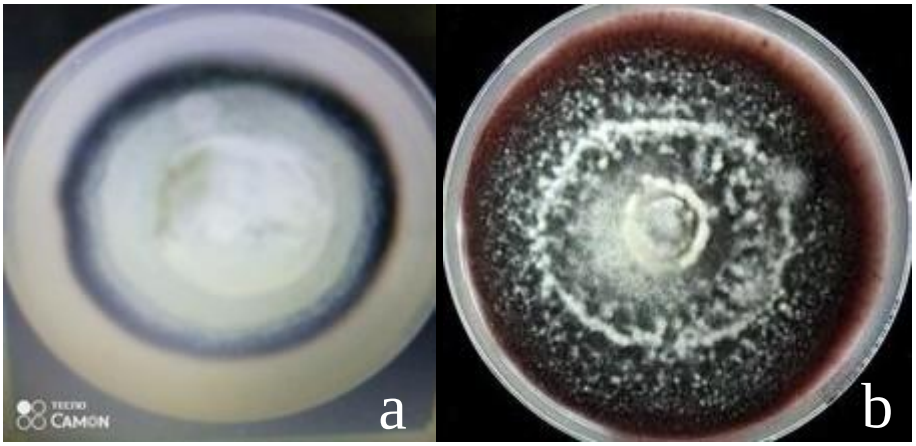

Figure 11: A pure culture of *Cercospora purpurea* isolate, (a) for the upper side while (b) on the lower side

Cercospora purpurea conidia were long rod shaped to cylindrical, with a blunt end, pale olive, 9 – 11 septate, straight or curved and 20 –34 x 2 – 5 µm (Table 8) were cylindrical in shape with a truncate base.

Table 8: The mean width and length (µm) of spores produced by 10 days-old *Cercospora prpurea* isolates

*Isolate	Width**	Length**
GJ	5.0[a]	34.0[a]
BS	4.1[b]	31.3[b]
FM	3.9[b]	30.8[bc]
DE	3.1[bc]	29.2[c]
CK	2.5[cd]	24.0[d]
AR	2.1[d]	20.1[e]
LSD	0.5673	0.1369
Mean	3.45	28.23
CV	2.203	1.107

**Means on the same column followed by similar letter(s) are not significantly different at 5% probability level. *FM-isolate from Mutunduri; RM from Runyenjes, DE from Embu town, CK from Kiritiri, BS from Siakago and GJ from Kianjokoma markets.

4.4 Efficacy of *Aloe secundiflora* Crude Extracts on the Avocado Fungal Pathogens

4.4.1 Qualitative Analysis of Phytochemicals in *Aloe Secundiflora* Crude Extract

Several phytochemical compounds of the plant extracts were identified from *Aloe secundiflora*. A total of five phytochemicals were found using ethanol extract and four

using hexane extract (Table 9). Phenolics were only detected in ethanol solvent extract. Tannins and Alkaloids were not present in any of the crude extract.

Table 9: Results of phytochemical analysis of *Aloe secundiflora* extract

Phytochemical Present	Plant Crude Extract	
	Ethanol solvent extract	Hexane solvent extract
Terpenoids	+	+
Tannins	-	-
Alkaloids	-	-
Flavonoids	+	+
Saponnins	+	+
Phenolics	+	-
Steroids	+	+

* (+) signifies presence of the phytochemical compound while (-) signifies the absence of the phytochemical compound.

4.4.2 Antimicrobial Activity of *Aloe secundiflora* Crude Extracts

The inhibitory activities of *A. secundiflora* on avocado fruit fungal isolates were determined by measuring the diameter of zones of inhibition in millimetres. There were observable zones of inhibition for only *C. gloeosporioides* (Figure 12) and *C. purpurea* in different concentrations. However, the extract did not inhibit *S. perseae* in-vitro.

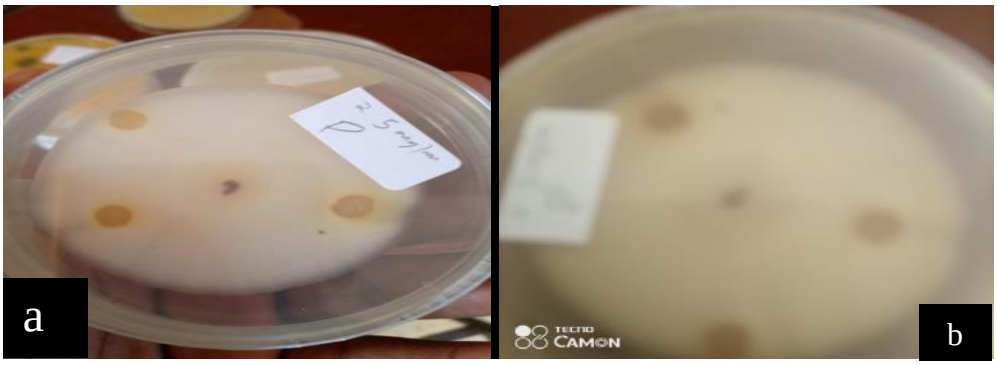

Figure 12: Zones of inhibition for *Colletotrichum gloeosporioides* isolates for *Aloe secundiflora* crude extract at 25mg/ml for (a) ethanol solvent extract and (b) for hexane.

4.4.2.1Antimicrobial Activity of the Plant Extract against *Colletotrichum gloeosporioides*

Various concentrations of plant extracts obtained using ethanol and hexane had inhibitory activities against *C. gloeosporioides*. There was an increase in the diameter of zone of inhibition as the *Aloe secundiflora* extract concentration was increased. The

effect of the different concentration of the plant extract was statistically significant ($P < 0.05$). The mean zone of inhibition for the ethanol extract ranged from 15.59 mm to 11.67 mm (Table 10).

Table 10: Mean Zones of inhibition recorded on Petri dishes treated with different concentrations of *A. secundiflora* ethanol extract against *Colletotrichum gloeosporioides* isolates

Concentration (mg/ml)	Mean Zones of Inhibition (mm)*
10	15.59[a*]
5	13.20[b]
2.5	11.67[c]
LSD	0.49
Mean	13.50
CV	3.71

*Means followed by different letter are not significantly different at 5% probability level. Values are means of three isolates each with three replicates.

Aloe secundiflora ethanol *extract* concentration of 10.0 mg/ml had more microbial inhibitory activity on the *C. gloeosporioides* growth than the lower concentration of 5.0 mg/ml. The concentration of 2.5 mg/ml had the lowest zone of inhibition on the pathogen growth (Table 11). However, *Aloe secundiflora* hexane crude extract showed lower mean diameters of zones of inhibition (10.53mm to 8.01mm) on *Colletotrichum gloeosporioides* than that of ethanol extract.

Table 11: Mean zones of inhibition recorded on Petridishes treated with different concentrations of *A. secundiflora* hexane extract against *Colletotrichum gloeosporioides* isolates

Concentration (mg/ml)	Mean Zones of inhibition (mm) *
10	10.53a
5	9.63b
2.5	8.01c
LSD	0.21
Mean	9.36
CV	2.29

*Means followed by different letter are significantly different at 5% probability level. Values are means of three isolates each with three replicates.

4.4.2.2 Antimicrobial Activity against *Cercospora purpurea*

Increase in the concentration of *Aloe secundiflora* extract increased the inhibition of *C. purpuea*. The effect of the different concentration of the plant extract was statistically significant ($P < 0.05$) The mean zone of inhibition for ethanolic extract ranged from 7.22 mm to 2.80 mm (Table 12) while that of hexane extract ranged from 5.27 mm to 3.38 mm (Table 13). The plant extract concentration of 10.0 mg/ml had more microbial inhibitory activity on the *C. purpurea* growth compared to the concentration of 2.5 mg/ml which had the lowest zone of inhibition. The inhibitory activity against *C. purpurea* increased linearly with an increase in the concentration of the *A. secundiflora* crude extract.

The statistical analysis showed that the extract at different concentrations significantly produced zones of inhibition ($P < 0.05$) against the growth of *Cercospora purpurea*. The zone of inhibition on the positive control was significantly higher than that of the plant extract. The zone of inhibition for concentration at 10.0 mg/ml was lower than the zone of inhibition of the positive control.

Table 12: Zones of inhibition recorded on Petri dishes treated with different concentrations of *A. secundiflora* ethanol extract against *Cercospora purpurea.*

Concentration (mg/ml)	Mean Zones of Inhibition (mm) *
10	7.22a
5	4.31b
2.5	2.80c
LSD	0.37
Mean	4.77
CV	7.81

* Means followed by the different letter are significantly different at 5% probability level. Values are means of three isolates each with three replicates.

Table 13: Zones of inhibition recorded on Petri dishes treated with different concentrations of *A. secundiflora* hexane extract against *Cercospora purpurea.*

Concentration (mg/ml)	Mean Zones of inhibition (mm) *
10	5.27[a]
5	4.32[b]
2.5	3.38[c]
LSD	0.15
Mean	4.32
CV	3.56

* Means followed by different letters are significantly different at 5% probability level.

4.4.2.3 Antimicrobial Activity against *Sphaceloma perseae*

There were observable zones of inhibition for positive control against *S. perseae* isolates (Fig. 13) with a mean diameter of 17.3mm. However, *A. secundiflora* crude extracts did not inhibit the growth of the pure cultures of *S. perseae* at any concentration. There was no significant difference in the results between the negative control and all the concentrations of *A. secundiflora* crude extract.

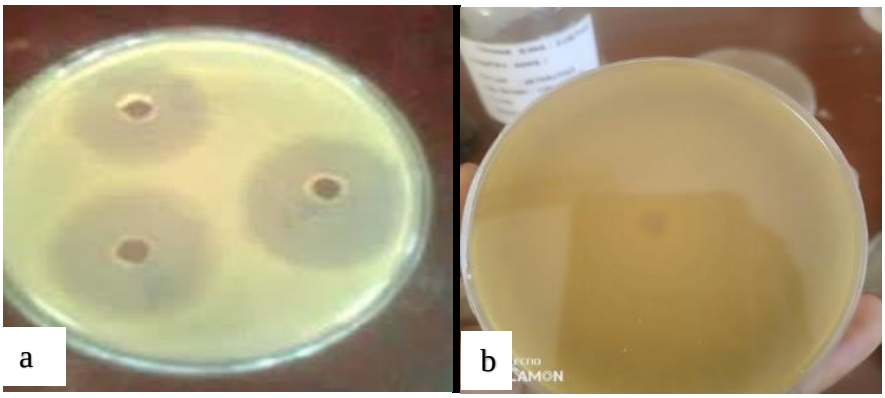

Figure 13: Zones of inhibition for positive control (a) and no inhibition of *Sphaceloma perseae* (b)

CHAPTER FIVE

DISCUSSION

5.1 Prevalence of Avocado Fruit Fungal Diseases

5.1.1 Data from Vendors' Response

The demographic characteristics of the respondents interviewed included age, gender and the level of education. Majority of the respondents were above 30 years of age. This is similar to a study conducted on post-harvest losses of fruits at Tshakhuma fruit market in Limpopo Province in South Africa where majority of the vendors (74%) were of middle age (Mashau *et al*., 2012). In a related study, harvesters tended to be unemployed women, mostly aged between 30 and 49 (Venter & Witkowski, 2013). In the current study, the age group below 30 years was relatively low, probably due to migration from rural to urban areas in search of formal employment and business opportunities in urban centres (Nyamweri & Gorran, 2011).

Women were the majority, and it has been reported that income generated from the sales of fruits helps them to raise their status in the community, as they make a contribution to household income and improve their standards of living (Venter & Witkowski, 2013). Vendors who had no formal education and those with basic primary level education were involved in farming and marketing throughout the year. Education has the potential to improve understanding and communication in post-harvest technology (Mashau *et al*., 2012).

The study indicated that avocado vendors had difficulties in conceptualizing various agronomic, production and disease management practices. Most of the fruit vendors are reported to lack knowledge on disease and pest management (Abang *et al.,* 2014). Poor management of diseases and pests may also be attributed to the inability to interpret critical information and instructions on pest and disease control on fungicide labels according to Panuwet *et al.* (2012) and Mengistie *et al*. (2015). Overall, the number of female vendors was higher than that of men. Generally, there are more women involved in fruit marketing than men (Busari *et al.,* 2015).

A small percentage of the avocado vendors in the study area in the markets were not in a position to identify diseases associated with avocado possibly due to ignorance, lack of knowledge and awareness on the diseases provided by agricultural farming and marketing extension officers (Auwal *et al.,* 2015). However, about 45% of the vendors could identify

the fungal avocado fruit diseases due to ecoomic losses of up to 60% during marketing due to quality considerations and fruit rejection by customers and severe fruit rots.

The level of maturity of the avocado fruits during harvesting may have contributed to fungal diseases. About 40% of the retail vendors who sourced their avocado fruits from their farms and within the county recorded that they waited for the avocado fruit drops as a method of harvesting after fruit maturity. Such fruits were damaged and infested by microorganisms causing fruits to rot while still in the farm. Mezgebe *et al*. (2016) showed similar findings on post-harvest losses of perishable fruits when harvesting, on handling procedures and storage. Such diseased fruits in the farms during harvesting act as the source of inocula for new infections (Agrios, 2005). In addition, some of the vendors shook the avocado trees for fruits to drop and collect the fruits for sale. Shaking the tree during harvesting can lead to 100% fruit rot on storage (Omolo *et al.,* 2011; Dessalegn *et al.,* 2016).

The vendors stored their avocado fruits in crates, sacks, buckets and open pick up within the markets. According to Seid *et al.* (2013), use inappropriate packaging material such as sacks and crates in fruit transport and packaging is a basic factor related to high levels of post-harvest losses. The use of sacks does not protect freshly harvested commodities from damage (Kereth *et al*., 2013). Furthermore, they create high heat because of metabolic reaction which ultimately hastens mechanical damage and microbial attack. Mechanical damage during loading, unloading and the exposure of the avocado fruits to high temperature in the open pick during transport can lead to physiological changes in the fruit and in favour of post-harvest diseases (Dessalegn *et al.,* 2016).

The management of the fungal avocado fruit diseases was mainly through the use of fungicides as well as cultural practices. Use of these fungicides was limited because the fungicides are not registered for use in control of the fungal avocado fruit diseases in Kenya. Farmers and vendors therefore have been using the fungicides due to their effectiveness in the management of diseases on other crops upon which they are registered for (Pest Control Products Board [PCPB], 2016). Continued use of chemical pesticides to control pests and diseases can be hazardous human, animals and environment due to their toxic inherent nature (Abang *et al.,* 2014; Megistie *et al.,* 2017).

The avocado varieties that were common in the market included *Fuerte*, *Hass*, *puebla* and local variety. *Hass* and *Fuerte* variety were the most commonly preferred by the vendors. *Hass* variety is also preferred in other avocado producing areas such as Murang´a County (Kimaru, 2020). This is because of its disease resistance, yield, good bearing habit, good fruit size and quality, and early time of maturity (Evans and Halmiton, 1999). The findings also concur with that of Coit (1968) who described *Fuerte* as a preferred avocado variety in Chili market and nearest farms due to its marketable size, an attractive green colour, oil content ideal, excellent flavour, unique and survival for a long season.

5.1.2 Prevalence of Avocado Fruit Fungal Diseases in the Market Stores

The percentage mean prevalence of anthracnose disease of avocado caused by *C. gloeosporioides* was high in Runyenjes and Kianjokoma market stores (41% and 36% respectively). This could be attributed to the mild temperature and high humidity of these regions which favour the growth of the pathogen. Handiso *et al* (2019) found that the highest and lowest anthracnose disease prevalence was observed in Alaba and Shashogo markets with cumulative prevalence of 41.88% and 36.81%, respectively. These results were different to the findings of Kugui *et al* (2020) who reported very high prevalence of papaya anthracnose in Baringo (95%) and Elgeyo marakwet markets (83%). These results also concur with Mezgebe *et al*. (2016) findings on post-harvest losses of perishable fruits during harvesting, handling and storage in Ethiopia due to high prevalence of anthracnose disease.

The highest and lowest mean prevalence of infection by the avocado scab disease (*Sphaceloma perseae*) were recorded in Siakago market and Mitunduri market with a mean prevalence value of 35.16% and 16.37%, respectively. A study carried out by Trucks *et al* (2011) showed a high prevalence of *Sphaceloma perseae* pathogen of avocado which is carried to infection sites by wind, rain and insects. He did also report that damages caused by insects create entry sites for *S. perseae* and greatly encourage development of scab disease and its prevalence. This was also similar to the findings by Omolo *et al*. (2011) and Dessalegn *et al*. (2016) on high prevalence of avocado scab whereby 100% avocado dark brown lesions and fruit rots on storage was identified on avocado fruits harvested through tree shaking method.

There was high prevalence of infection by the Cercospora spot (*Cercospora purpurea*) recorded in Kianjokoma market and Mitunduri market with the lowest mean prevalence value

among the selected markets. The same high prevalence of Cercospora spot was recorded by the findings of Darvas *et al.* (1987). They reported that one of the most important pre and post-harvest fruit disease of avocado at Westfalia Estate was Cercospora spot, caused by *Cercospora purpurea*. The high percentage prevalence may have been due to presence of humid and rainy conditions in Kianjokoma region. High prevalence of *Cercospora* spot disease in commonly avocado producing and marketing regions with warm, humid and rainy conditions persist in South Africa (Kallideen, 2020).

5.1.3 Prevalence of the Avocado Fruit Fungal Diseases per Market

The prevalence of fungal avocado fruit diseases was different in different markets. Anthracnose and Cercospora spot diseases were highly prevalent in Embu town, Kianjokoma and Mutuduri markets. Related findings were also reported by Wasilwa *et al*. (2004) in their study on the status of production and marketing of avocado in Embu County. They did report that the most prevalent post-harvest fungal avocado diseases in Embu district were Anthracnose and Cercospora spot which are a threat to avocado production and marketing. Kimaru (2020) also reported high prevalence of Anthracnose diseases in different avocado growing areas in Murang´a County.

Avocado scab (*Sphaceloma perseae*) was highly prevalent in Siakago and Kiritiri markets compared to the other two fungal avocado fruit pathogens. These findings were similar to a report by Vidales Fernandez, (1996) where avocado scab had a high prevalence in the sub-counties of Michoacan State. Vidales (1996) also reported that the disease attack was more severe in areas with warm sub-humid climates and its occurrence increased fruit price losses from 27 up to 53%.

5.1.4 Prevalence of the Fungal Avocado Fruit Disease in Different Varieties

Four main varieties of avocado fruits were found to be sold in the selected markets (*Hass, Fuerte, Puebla* and the local variety). *Fuerte* and the *Puebla* varieties were the most susceptible to anthracnose disease and avocado scab in all the markets as reported earlier by Wasilwa (2006). Perez (2008) also reported that *Fuerte, Puebla* and local variety were more susceptible to anthracnose, scab and Cercospora spot disease due to their light epidermis which is easily damaged. *Hass* variety, which is the most preferred by customers, had a low susceptibility due to its leathery nature making it resistant to diseases (Hoddle & Hoddle, 2008). The variety has a long shelf life and highly marketable (Aktar *et al*., 2019).

5.1.5 Disease Symptoms Observed on Avocado Fruits in the Market

The symptoms observed for Anthracnose were similar to the ones reported by Nelson (2008) that the fungus *C. gloeosporioides* usually form a round, darkly-coloured, depressed lesion that spread rapidly on the fruit surface and into the pulp site and causes fruit rots. The damages may develop salmon-coloured, sticky mass of spores similar to anthracnose diseases. These symptoms were also observed by Kimaru (2020) on his study on characterization of *C. gloeosporioides*.

Cercospora spot disease symptoms observed on avocado fruits were similarly reported by Kallideen (2020) that on avocado fruit, the first sign of disease attack is formation of a dark epidermis followed by swollen underlying fruit tissues which form a small dark spot. As the cells die and dry out, the spots become sunken. Avocado scab has symptoms of dark brown lesions on the epidermis of the fruit skin. These symptoms were similar to those observed by Vidales (1996).

5.2 Morphological Characterization of Fungal Avocado Fruit Pathogens

5.2.1 *Colletotrichum gloeosporioides*

The isolates of *Colletotrichum gloeosporioides* from diseased avocado fruits that showed symptoms of anthracnose disease varied in their morphological characteristics in terms of conidial lengths. These morphological characteristics concurred with those of *C. gloeosporioides* isolates observed by Pallem *et al*. (2012) in avocado fruits with wide cultural variations. The spores produced by the cultures were straight with rounded end, ranging within 3.0-5.1 micron in width and 10.3-18.2 micron in length as also reported by Kimaru (2020). The mycelia colour of the isolates observed was mainly white to grey compared to cream grey on the upper side of the culture. The variances observed in cultural and morphological characteristics of the fungus could be affiliated to genetic variations and different growth conditions such as temperature, light quality, light duration and wavelength and repeated laboratory sub-culturing (Vidyalakshini & Divya, 2013).

5.2.2 *Sphaceloma perseae*

Cultures were white on the underside of the plates, and mycelia had white and yellow layers. *Sphaceloma perseae* isolates developed masses of conidiophores bearing hyaline conidia ovoid or elongated coloured conidia with septation and a range of 5-8 x 3-4 µm. These

findings were similar to those made by Fan *et al.* (2018), whose report stated that the colour of the *S. perseae* pathogen ranges from white to yellow and that the mycelia of the pathogen grow at a very slow rate when grown on PDA media.

5.2.3 *Cercospora purpurea*

The isolates had colonies that were white leathery with tuffs of grey-brown conidiomata on them, initially grey becoming brown with age. These cultural characteristics were typical to those of *C. purpurea* reported by Khallideen (2020). Marais (2004) reported similar results that once the pathogen is isolated it grows rapidly on ordinary nutrient media and produce a tufted leathery growth, which grey in colour at first, later become brown or dark-brown. The conidia were long rod-shaped to cylindrical, with blunted ends, pale olive, 9 – 11 septate, straight or curved and 20 – 180 x 2 – 5 µm were cylindrical in shape with a truncate base. Similarly, Groenewald *et al* (2013) reported almost similar conidia characteristics with a wide range of the measurement of the length and width of the spores. Van Eeden & Korsten (2006) reported that *C. purpurea* remains dormant and can survive harsh climatic conditions such like extreme temperatures, unavailability of nutrients and it is favoured by low humidity.

5.3 Efficacy of *Aloe secundiflora* Crude Extracts on the Fungal Avocado Pathogens *in vitro*

5.3.1 Phytochemicals in *Aloe secundiflora* Crude Extract

The study revealed the presence of various phytochemicals present within the *Aloe secundiflora* crude plant extracts prepared using Ethanol and hexane solvents. The two are organic solvents whereby ethanol is a polar solvent while hexane is non-polar and they are able to dissolve some phytochemicals (Gupta *et al.,* 2010). Different solvents are required in crude extraction and phytochemical analysis. The extraction output and biological activity of the plant extracts is not only affected by the extraction solvent but also affected by the extraction technique (Ajana *et al.,* 2012).

Several solvents such as ethanol, methanol, acetone, and water, have frequently been used for extracting useful secondary metabolites from the plant material. Due to the many and different bioactive compounds contained in plant materials and their different solubility properties in various solvents, the suitable solvent for extraction depends on the particular plant organ and the bioactive components to be isolated (Mahdi *et al.,* 2012). Therefore, to recommend an optimal extraction solvent for individual plant materials is generally a challenge.

Aloe secundiflora crude extract contains phytochemicals such as flavonoids, terpenoids, , saponins, phenolics and steroids when extracted using ethanol solvent (Mariita, 2011). However, Kaingu *et al.,* (2013) revealed the absence of phenolics in hexane solvent extract of *A. secundiflora* when used to treat *Ascaridia galli* and other bacterial diseases. The variation in the phenolic content could be attributed to the nature of the solvent, technique of extraction and extraction duration which could have an effect on the concentrations of the secondary metabolites.

The results of this study collaborated to a study done by Fasola and Iyamah, (2014) who found the presence of terpenoids, flavonoids, saponins and steroids in *A. secundiflora* that was tested in treatment of malaria. The findings were also similar to those established by Mbithi *et al.* (2018) from Zambia who confirmed that *A. secudiflora* crude extract have terpenoids, flavonoids and saponins which were used to treat different human microbial infections. These results also concurred with the findings established by Ogoti *et al.,* (2015) who identified the presence of saponins, flavonoids and terpenoids in the crude extract of *A. secundiflora*. A study carried out by Odeyemi *et al.,* (2014) showed the presence of alkaloids, monoterpene, phenol, flavonoids, sesquiterpene and diterpenes in the leaf extract of *A. secundiflora* using solvent extraction technique.

However, Waithaka *et al.* (2018) revealed the presence of tannins and alkaloids in *A. secundiflora* crude extract. This could be associated to the nature of solvent used, the extraction method or the concentration level of the bioactive compound in the plant was low. The phytochemical screening showed the presence of saponins, tannins, glycosides, terpenoids, alkaloids and flavonoids. The results were however dissimilar to a study done in India by Cheloti *et al.,* (2010) who revealed the absence of glycoside and terpenoids from an ethanolic plant extract of *Aloe secundiflora.*

5.3.2 Antimicrobial activity of *Aloe secundiflora* Crude Extracts

5.3.2.1 Antimicrobial Activity of the Plant Extract against *C. gloeosporioides*

The plant extract had an inhibitory effect against fungal microbes infesting avocado fruits. The inhibition zone ranged from 15.59 mm to 11.67mm and for hexane extract ranged from10.59 mm to 8.01 mm. These findings were similar to those reported by Micheni (2015) on the anti-fungal activity of *A. secundiflora* against *Pythium ultimum* that attacks potatoes and *Fusarium oxysporum,* a fungal pathogen that infects bananas with the highest mean inhibition of 16.01 mm

for both organisms using ethanol solvent. Similar results were also posted by Itonga, (2011) that the crude extract of *A. secundiflora* was effective against eight different fungal species.

According to Al-Mujammaa'e (2008), *A. secundiflora* extracts act by inducing a disruption in fungal cell metabolism, increasing permeability of fungal cell membrane and also destroy the cell wall structure. The more the *A. secundiflora* extract is concentrated the higher the inhibition levels. Increase in the concentration of bioactive component in the plant extracts has been shown to inhibit the mycelia growth of fungal pathogens (Ilondu, 2012). The negative control plates treated with DMSO alone showed no inhibition, which confirms that *A. secundiflora* extract has antifungal compounds that inhibited the growth of *C. gloeosporioides*.

5.3.2.2 Antimicrobial Activity against *Cercospora purpurea*

The finding showed that the crude extract of *A. secundiflora* resulted in a significant mean zone of inhibition (7.22 mm-2.80mm) of the growth of *Cercospora purpurea*. This could be attributed to the various phytochemical components such as; glucorunic acid, p- coumaric acid, ferulic acid, phenylpropanoids, dehydro α lapachone and lapachol, β-sitosterol, ferulic acid and iridoids that could be causing the zone of inhibition (Waithaka *et al.,* 2018). The study also revealed that as the concentration of *A. secundiflora* extract was increased, the zone of inhibition by the plant extract also increased. This is similar to the findings by Micheni (2015) and Wokocha & Wekereke (2005) who established that the activity of the crude extracts was very effective on increase in their concentration.

5.3.2.3 Antimicrobial Activity against *Sphaceloma perseae*

Sphaceloma perseae pathogen mycelial growth was not inhibited by crude extracts of *A. secundiflora.* There were observable zones of inhibition when treated with copper oxychloride fungicide as the positive control. Contrary to the behaviour of this pathogen, fungicides are more effective against various fungi and they inhibit the active division of the cells and mitosis in fungi (Yin *et al*., 2011).

5.3.3 Inhibition Comparison between the Plant Extract and Positive Control

This study revealed that the copper oxychloride fungicide showed the highest inhibitory effect compared to the plant extract. Although the chemicals are an effective and faster way for fungal disease management since they are easily obtained in the markets, their consequences have posed healthy problems to humans, livestock and the environment. A study done by

Kesavachandran *et al*. (2009), reported that statistically about one million people die as a result of the chronic infections brought about by fungicide poisoning. Although the pesticides are a source of job opportunities to those who formulate and manufacture the chemicals, they pose a risk to their lives because they interact with harmful extraction solvents and chemicals in the process of manufacturing (Tornero & Hanke, 2016). Farmers and vendors are also at risk since they inhale the toxic fumes during spraying and mixing of the fungicides.

The fungicides also interfere with the endocrine system by counteracting the production of the hormones from the endocrine glands; exposure of the fungicide to varied rates of dosage pose a negative implications to the immune system, reproductive system and finally result to cancer (Kesavachandran *et al*., 2009). Similar findings were revealed by Iyer and Makris, (2010) who reported a growing death rate due to the destruction of the cardio-vascular and respiratory disorders caused by pesticides in humans. Furthermore, gastrointestinal and the lymphatic tissues upon exposure to pesticides were at risk of having mutation thus leading to cancer. Singh & Sharma (2018) reported the presence of Copper oxychloride, Bromuconazole and Mancozeb component of fungicide in the pawpaw fruit. Due to the lack of awareness by the consumer and the farmer, they are exposed to these synthetics when they consume fruits that are treated with pesticides to manage pests (Singh and Sharma, 2018).

Previous studies showed that the pesticide inhibitory property usually is as a result of polymerization of β-tubulin in microtubules reducing their proliferation and dynamic instability (Ding *et al.,* 2016). Further, methyl benzim-idazole carbamate fungicides suppress the meeting of spindle microtubules owing to disturbed chromosomal alignment and microtubule-kinetochore interactions at the metaphase plate causing chromosome loss and chromatid loss in pathogenic fungi (Lebeda *et al.,* 2010).

The plant extract had differences in the zones of inhibition which could be due to the differences in nature, quality and quantity of the inhibitory substances present in the plant extracts (Ceylan & Fung, 2004). It is evident from the results that the zones of inhibition of the different pathogenic species by the plant extracts depends on the plant species and phytochemical compound concentration. Comparable findings reported by Veresoglou *et al.,* (2013) and Kumaran (2003) that the fungal susceptibility toward a plant extract was due to plant species, the solvent used for extraction and extract concentration, as well as the pathogen tested. The activity of plant crude extracts and essential oils as anti-spore agents have been

shown against a large number of fungal infections as established by other several studies (Singh *et al.*, 2017).

CHAPTER SIX

SUMMARY OF THE FINDINGS, CONCLUSION AND RECOMMENDATION

6.1 Summary of the Findings

On demographic characteristics of the respondents, majority were above 30 years of age with a higher number of women than men, and had primary level of education. More than 50% of the vendors sourced avocado fruits from their own farms and were able to identify disease symptoms themselves. The main method of harvesting of avocado fruits was both handpicking and hooks, and mainly stored in sacks. More than 40% of the respondents used chemicals to control fruit diseases. This was attributed to the easy accessibility of chemicals and the general public knowledge of inorganic chemicals. Some vendors used no control measures at all to curb the fungal pathogens. This might have resulted from a lack of understanding of the diseases and the cost of control methods. The commonly sold varieties were *Hass, Fuerte, Puebla* and the local varieties with the *Hass* being the mostly preferred variety. The mean prevalence of infection differed among the varieties.

Three avocado fruit fungal pathogens were identified; *C. gloeosporioides* (Anthracnose), *S. perseae* (Avocado Scab) and *C. purpurea* (Cercospora Spot) that contributed to huge losses in the markets in Embu County. The prevalence of these pathogens varied within and among the markets probably due to poor handling methods, inadequate knowledge about the diseases, variety of avocado fruits sold, resistance of some of the pathogens to chemicals applied and variation of climatic conditions within the study area.

The cultural and morphological characteristics of each of the pathogens obtained from the study area revealed a slight difference in conidia sizes in terms of length and width but all the sizes were within the usual range of the spores of each of the pathogens. In terms of colour, the isolates of each of the pathogens did not differ significantly from each other. Therefore, it was confirmed that the pathogens isolated from infected avocado fruits in the study area were actually the respective fungal pathogens.

Various phytochemicals from *Aloe secundiflora* crude extracts were identified using both ethanol and hexane extract. The extracts were tested for their inhibitory property on the test pathogens under in vitro conditions. The efficacy was at different

concentration and it was found that those isolates with higher concentrations of the crude extracts with ethanol solvent gave the highest mean inhibition diameter (15.59mm and 7.22mm) compared to the crude extract with hexane (10.53mm and 5.27mm) for *C. gloeosporioides* and *C. purpurea* respectively. On the other hand, isolates of *S. perseae* were only inhibited by the positive control and were stubborn to the crude extract.

6.2 Conclusion

Most vendors in Embu County have avocado trees in their farms. Avocado fruits produced are consumed as a source of nutrients and sold as source of income at the local market. Marketing and production of avocado in the County is mainly by vendors who have little knowledge and awareness on proper post-harvest avocado fruit management. Some use fungicides to control the diseases, though these chemicals are not registered for use in avocado fruit. Awareness creation is therefore important to successfully market avocado fruits that are of good quality and quantity.

Diseases affecting avocado fruits after harvest are anthracnose, cercospora spot and scab diseases that were noted in all the markets in the study site. There was higher prevalence of anthracnose and cercospora spot in Runyenjes and Kianjokoma markets while avocado scab was more prevalent in Siakago market. Identifying factors that contribute to this variation is important for proper control and management of the diseases. The causative agents were *Colletotrichum gloeosporioides, Cercospora purpurea* and *Sphaceloma perseae* of anthracnose, cercospora spot and avocado scab, respectively. Avocado varieties that were commonly grown and sold in the markets were *Hass, Fuerte, Puebla* and local varieties which were susceptible to the fungal avocado fruit pathogens.

The growth of *C. gloeosporioides* and *Cercospora purpurea* isolates were inhibited by *Aloe secundiflora* crude extract *in vitro* at varying concentrations. These extracts are relatively economical, safe and non-hazardous and can be used as antimicrobials against the characterized pathogens. They can be an alternative to reliance on fungicides and form an integral part of integrated pest management.

6.3 Recommendations of the Study

i. Vendors should be trained on proper handling and management of avocado fruits during harvesting, transport and storage to reduce post-harvest infections losses.
ii. The identified pathogens in this study should be integrated in research and studies for proper disease control and management.
iii. Extracts of *Aloe secundiflora* can be used alongside other methods in managing *C. gloeosporioides* and *Cercospora purpurea* to reduce over reliance on chemical fungicides.

6.4 Suggestions for Further Studies

I. Phytochemical analysis of other species of aloe and their potential in control of the studied diseases.
II. Molecular characterization of the pathogens for further identification.
III. Carrying out field experiments to test the efficacy of the plant extract on the avocadoes farms.
IV. Isolation and purification of the active compounds in *Aloe secundiflora* and their activity screened against a wide range of economically important plant fungal pathogens.

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APPENDICES

APPENDIX I

QUESTIONNAIRE FOR COLLECTING DATA ON AVOCADO PRODUCTION, MARKETING AND DISEASE MANAGEMENT PRACTICES IN EMBU COUNTY

Name……...Date……. Sheet No……. Market……..

RESPODENT IDENTITY

1.1 Age /__/__/

1.2. Sex M /__/ F /__/

1.3. Occupation: ……

1.4. Level of education: None /__/ Primary /__/ Secondary /__/ Tertiary

1.5. Literacy language: English /__/ Local language /__/

2. Avocado varieties, source of avocado fruits for sell

2.1. Which avocado varieties do you sell? .....

2.2 Where do you source your avocado fruits from? .....

2.3 Do you apply chemicals in managing them?

2.4 a) What is your total estimate avocado fruit no? …….

b) Approximately what Percentage of your total avocado fruits goes to waste during?

i) Harvesting …… ii) Storage……. iii)

Transportation……. iv) Grading stage for export ( ), At the local market ( )

2.5 Where and who do you sell your avocado fruits to? Local Mkt ( ), Export ( ), Broker ( ) other ( )

3 Fungicide used to manage avocado diseases

3.1. Who identify the diseases and pest affecting your fruits in the market? Self ( ), Extension officer () Family member ( ) other ( )

3.2. Which are the major diseases and which pesticides do you use? (Please specify names, target disease and their quantities) Name of pesticide: Disease: quantities

3.3. If the vendor does not know product names, ask her/him why? ....

3.4. How do you acquire pesticide products you are using? At the local market /__/ at a licensed retailer /__/ Neighbour---/, Exporter (_)

3.5. Do you spray the pesticides? Yes ( ) No ( ). If not who spray your crop? a) If yes do you think you incur risks of being exposed to those pesticides? Yes /__/ No /__/ and if yes, which risks……..

3.6. Do you have spray programs? Yes /__/ No /__/ If yes, do you spray during harvesting season? Yes ( ) No ( ): And which chemicals do you use? ..... Do you apply chemicals during storage and transport? Yes ( ) No ( ) If yes, which chemicals..

3.7. If there are unused products left, what do you do with them? ……

3.8. Are you trained on safe use and handling of pesticides? ……. By who and when? ……

3.9. What other disease and pest management practices do you practice other than use of fungicides? …….

4. Handling and storage of avocado fruits

4.1. How do you harvest your avocado fruits if you have a farm?

4.2 How do you determine the maturity of the avocado fruits before harvest?

4.2.1 Where do you put your fruits after harvest? Crates /__/ Bucket /__/ Sack / /

4.2.2. Do you treat your avocado fruits before storage? Yes ( ) No ( ). If yes what do you use?

4.3. Where do you store your harvested avocado before sale? ……

4.4. What do you do with the diseased fruits before and during storage? Leave/throw them ( ), Feed them to livestock ( ), bury them ( ) Dispose them along the road ( ) Sell at lower cost ( ) any other ( ).

4.5. Approximately what quantities of the fruits are lost due to decay? ½ ( ) ¼ ( ) 1/5 ( ) others ( )

5. Perception of environmental risks

5.1. Is there any water source (well, stream, river, forage,) in the vicinity or in your markets? Yes /__/ No /__/

5.1.1. If yes, specify ……

5.1.2. What is the distance between the water source and the area you are treating?

5.1.3. What is the water source used for? ……..

5.2. Have you noticed the death or disappearance of some insects or animals since you have been using the chemicals? Yes /__/ No /__/ 5.2.1. If yes, which ones?

5.3. Do you think that those products pose a risk to the environment? Yes /__/ No

5.3.1. If yes, why? ……

5.3.2. If not, why? …….

6. Suggestions and recommendations

6.1. Please provide your suggestions/recommendations concerning the use of pesticides in general

APPENDIX II
PERMIT LETTER FROM CHUKA UNIVERSITY ETHICS COMMITTEE

CHUKA UNIVERSITY INSTITUTION ETHICS COMMITTEE
Telephones: 0612304004
Fax line: 020 2310302
P.O. Box 109 - 60400
Chuka

14th December 2021

REF: CUIERC/ NACOSTI 205

TO: **Mulei Francisca Mwongeli**

Dear Sir/madam

RE: Prevalence and Characterization of Fungal Avocado Fruit Diseases and Their Control Using, Aloesecundiflora Crude Extracts

This is to inform you that *Chuka University IERC* has reviewed and approved your above research proposal. Your application approval number is *NACOSTI/NBC/AC-0812* .The approval period is 14thDecember 2021to 14th December 2022

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by *Chuka University IERC*..
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to *Chuka University IERC* within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to to *Chuka University IERC* within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to *Chuka University IERC*.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely

Dr. Benjamin Kanga...
SECRETARY CHUKA UNIVERSITY

APPENDIX III
RESEARCH PERMIT

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **863825**

Date of Issue: **02/April/2022**

RESEARCH LICENSE

This is to Certify that Ms. FRANCISCA MWONGELI MULEI of Chuka University, has been licensed to conduct research in Embu on the topic: PREVALENCE AND CHARACTERIZATION OF FUNGAL AVOCADO FRUIT DISEASES IN EMBU COUNTY AND THEIR CONTROL USING Aloe secundiflora CRUDE EXTRACTS for the period ending : 02/April/2023.

License No: **NACOSTI/P/22/16715**

863825

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

APPENDIX IV
ANALYSIS OF VARIANCE OF PREVALENCE OF ANTHRACNOSE DISEASE (*Colletotrichum gloeosporiodes)* IN ALL THE MARKETS

Source of variation	df	SS	MS	F- value	P-value
Model	24	6580.03333	274.16806	2.73	0.0003
Error	95	9551.96667	100.54702		
Corrected Total	119	16132.00000			

APPENDIX V
ANALYSIS OF VARIANCE OF PREVALENCE OF AVOCADO SCAB DISEASE (*Sphaceloma perseae*) IN ALL THE MARKETS

Source	df	SS	MS	F Value	Pr > F
Model	24	8178.43333	340.76806	2.86	0.0002
Error	95	11317.15833	119.12798		
Corrected Total	119	19495.59167			

APPENDIX VI
ANALYSIS OF VARIANCE OF PREVALENCE OF AVOCADO CERCOSPORA SPOT DISEASE (*C. purpurea*) in all the markets

Source of variation	df	Type III SS	MS	F Value	Pr > F
Market	5	3146.275000	629.255000	6.34	<.0001
vendor	19	4849.758333	255.250439	2.57	0.0014

APPENDIX VII
ANALYSIS OF VARIANCE OF WIDTH OF SPORES OF *C. gloeosporioides*

Source	df	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	9.45111111	1.89022222	200.14	<.0001
Error	12	0.11333333	0.00944444		
Corrected Total	17	9.56444444			

APPENDIX VIII
ANALYSIS OF VARIANCE OF LENGTH OF SPORES OF *C. gloeosporioides*

Source	df	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	116.2577778	23.2515556	597.90	<.0001
Error	12	0.4666667	0.0388889		
Corrected Total	17	116.7244444			

APPENDIX IX
ANALYSIS OF VARIANCE OF WIDTH OF SPORES OF *S. perseae*

Source	df	Sum of Squares	Mean Square	F Value	Pr > F

Source	df	Type III SS	Mean Square	F Value	Pr > F
Pathogen	2	0.75175556	0.37587778	8.21	0.0025
crude_extract	2	27.24935556	13.62467778	297.60	<.0001
Replicate	2	0.00026667	0.00013333	0.00	0.9971

Model	7	2.79055556	0.39865079	7.13	0.0031
Error	10	0.55888889	0.05588889		
Corrected Total	17	3.34944444			

APPENDIX X
ANALYSIS OF VARIANCE OF LENGTH OF SPORES OF *S. perseae*

Source	df	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	27.63722222	3.94817460	63.11	<.0001
Error	10	0.62555556	0.06255556		
Corrected Total	17	28.26277778			

APPENDIX XI
ANALYSIS OF VARIANCE OF WIDTH OF SPORES OF *C. purpurea*

Source	df	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	18.14833333	2.59261905	457.52	<.0001
Error	10	0.05666667	0.00566667		
Corrected Total	17	18.20500000			

APPENDIX XII
ANALYSIS OF VARIANCE OF ANTIMICROBIAL ACTIVITIES OF *Aloe* secundiflora (HEXANE EXTRACT) AGAINST *C. gloeosporioides*

Source	df	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	28.00137778	4.66689630	101.94	<.0001
Error	20	0.91562222	0.04578111		
Corrected Total	26	28.91700000			

APPENDIX XIII
ANALYSIS OF VARIANCE OF ANTIMICROBIAL ACTIVITIES OF *Aloe secundiflora* (Ethanol extract) against *C. gloeosporioides*

Source	df	SS	Mean Square	F Value	Pr > F
Model	6	72.1128444	12.01880741	48.13	<.0001
Error	20	4.99482222	0.24974111		
Corrected Total	26	77.1076667			

Source	df	Type III SS	Mean Square	F Value	Pr > F
Pathogen	2	1.97228889	0.98614444	3.95	0.0359
crude_extract	2	70.14046667	35.07023333	140.43	<.0001
Replicate	2	0.00008889	0.00004444	0.00	0.9998

APPENDIX XIV
ANALYSIS OF VARIANCE OF ANTIMICROBIAL ACTIVITIES OF *Aloe secundiflora* (Ethanol extract) against *C. purpurea*

Source	df	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	93.38573333	15.56428889	111.97	<.0001
Error	20	2.78006667	0.13900333		
Corrected Total	26	96.16580000			

Source	df	Type III SS	Mean Square	F Value	Pr > F
Pathogen	2	2.97680000	1.48840000	10.71	0.0007
crude_extract	2	90.38686667	45.19343333	325.12	<.0001
Replicate	2	0.02206667	0.01103333	0.08	0.9240

APPENDIX XV

ANALYSIS OF VARIANCE OF ANTIMICROBIAL ACTIVITIES OF *Aloe secundiflora* (Ethanol extract) against *C. purpurea*

Source		df	Sum of Squares	Mean Square	F Value	Pr > F
Model		6	16.84053333	2.80675556	118.81	<.0001
Error		20	0.47247407	0.02362370		
Corrected Total		26	17.31300741			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Pathogen	2	0.78267407	0.39133704	16.57	<.0001
Crude extract	2	16.05558519	8.02779259	339.82	<.0001
Replicate	2	0.00227407	0.00113704	0.05	0.9531