IDENTIFICATION OF STEM END ROT FUNGAL PATHOGENS ON AVOCADO FRUITS AND EFFICIENCY OF FUNGICIDES AND *TRICHODERMA* SPP. IN MURANG’A COUNTY, KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree award in any other university or any other award.

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We confirm that the work reported in this thesis was carried out by the student under our supervision and has been submitted with our approval as the University supervisors.

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DEDICATION
To my husband and family
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ABBREVIATIONS AND ACRONYMS

AEZ - Agro Ecological Zones

AGAK - Avocado Growers Association of Kenya

a.s.l - Above sea level CFUs

- Colony forming units DAP -

Diammonium phosphate GDP -

Gross Domestic Product

IPM - Integrated Pest Management

KALRO - Kenya Agricultural and Livestock Research Organization

LH1 - Lower Highlands (I) Agro-Ecological Zone

LM4 - Lower Midlands (4) Agro-Ecological Zone

LM5 - Lower Midlands (5) Agro-Ecological Zone

mg - Milligram

PCPB - Pest Control Products Board

PCR - Polymerase Chain Reaction

PDA - Potato Dextrose Agar

PDI - Percentage diseases index

SER - Stem End Rot

UH0 - Upper Highlands (0) Agro-Ecological Zone

UH1 - Upper Highlands (I) Agro-Ecological Zone

UM1 - Upper Midlands (I) Agro-Ecological Zone

UM2 - Lower Midlands (2) Agro-Ecological Zone

UM3 - Upper Midlands (3) Agro-Ecological Zone
UM4 - Upper Midlands (4) Agro-Ecological Zone
µl - Microliters
WA - Water Agar
ABSTRACT

Avocado (Persea americana Mill.) is a commercially important fruit cultivated in tropical and sub-tropical climates. In Kenya the fruit is cultivated for both local and export market, significantly contributing to the country’s GDP. The production of avocado fruits in Kenya is limited by fruit diseases among others. Stem end rot (SER) is an economically important post-harvest disease of avocado fruits contributing to losses of the fruits at the farm level, during storage and on the shelves. The causal agents of the disease in Kenya are not clear, but Botryosphaeriaceae spp. are most important causal agents of avocado SER in other parts of the world where the fruit is grown. This study was conducted to evaluate avocado production and SER disease management; determine the incidence of SER on avocado fruits in Murang’a County; identify the SER fungal pathogens, determine the efficacy of selected fungicides and the inhibitory effect of selected Trichoderma species against the SER fungal pathogens of avocado fruits. A structured questionnaire was administered by face-to-face interview with farmers while colony growth and conidia characteristics were used in morphological identification of the pathogens. Two fungicides, (Green Cop 500WP (Copper oxychloride 500g/Kg) and Milraz 76 WP (Propineb 70% + Cymoxanil 6%) were used in the in vitro inhibitory study while four Trichoderma spp. (T. asperellum, T. harzianum, T. atroviride and T. virens) were tested against the most isolated SER pathogens on post-harvest ‘Hass’ avocado fruits. The survey revealed that 84% of the farmers preferred ‘Hass’ avocado fruits, 85% intercropped the avocado fruits with food crops, while 88% of the farmers used organic manure only to replenish the soils. Majority of the farmers (62%) used grafted seedlings while 27% of the farmers top worked the old trees. Most farmers (84.6%) did not adopt the recommended field management practices, and none of the farmers used fungicide in the management of SER. Avocado fruits from the market and orchard had SER disease incidence of 52.08% and 45.70% respectively. Lasiodiplodia theobromae, Neofusicoccum parvum, Nectria pseudotrichia, Fusarium solani, Fusarium oxysporum, Fusarium equisetii and Geotrichum candidum were identified as the causal agents of SER on avocado fruits. Lasiodiplodia theobromae was the most frequently isolated pathogen followed by, N. parvum, N. pseudotrichia and F. solani respectively. All the fungicides significantly (P≤0.05) inhibited mycelial growth of the four pathogens. In both in vitro study and on post-harvest avocado fruits T. atroviride had highest efficacy against N. parvum, N. pseudotrichia and F. solani while T. harzianum had the highest efficacy against L. theobromae. This study has identified the causal agents of stem end rot disease of avocado fruits in Kenya. Trichoderma atroviride and T. harzianum present a potential alternative to synthetic fungicides against SER diseases of avocado fruits, and the possibility of using them at a commercial level should be explored.
CHAPTER ONE
INTRODUCTION

1.1 Background to the study

Globally, avocado fruit is cultivated in different ecological zones for both domestic and commercial purposes (Bower & Cutting, 1988). In Kenya Avocado cultivation began in the 1930s when the Portuguese introduced the fruit. Commercial production began in the 1960s, and in 1970 the first export to Europe was made (Wasilwa et al., 2006). The avocado fruit is grown in several agro-ecological zones by both small scale and large-scale farmers in Kenya (Wasilwa et al., 2006). The main bearing season is from September to January. Currently, Murang’ a County is leading in production (53%), followed by Kisii (11%), Kiambu (9%) and Nyamira (4%) counties (HCD, 2016).

Avocado fruits are an important source of income for both farmers and the business community involved in local and export trade. In 2016, avocado production in Kenya was 246,067 metric tons, which contributed to Ksh.4.63 billion, accounting for 8% by value of the fruit sub-sector (HCD, 2016).

The avocado fruit is of high nutritional value due to the presence of several minerals (potassium, iron and phosphorus) and vitamins (B, C, and E) (Schaffer et al., 2013). The fruit has a higher concentration of lipids and phytochemicals compared to other fruits (Siddiq, 2012). Consumption of the fruit is associated
with improved overall diet quality and heart protection benefits due to its ability to reduce cholesterol in the body (Bergh, 1992).

‘Hass’ and ‘Fuerte’ are the main varieties cultivated in Murang’a County for commercial purposes. Puebla, Duke and G6 varieties are also cultivated in small quantities within the County and traded locally (HCD, 2016). In the early years of avocado cultivation in Kenya, ‘Fuerte’ formed the bulk of export; however, changes in consumer preference have seen ‘Hass’ avocado being more popular. Currently, ‘Hass’ makes over 80% of the fruits exported from Kenya (Nyambati & Kioko 2018).

The common fruit rot diseases reported in most of the avocado growing regions in the world include; Anthracnose (*Colletotrichum* spp), Cercospora spot (*Pseudocercospora purpurea*), Dothiorella fruit rot (*Botrysphaeria* spp.), Scab (*Sphaceloma perseae*), Stem end rot (*Dothiorella* spp., *Thyronectria pseudotrichia*, *Phomopsis perseae*, *Diplodia mutila*, *D. pseudoserpis*, *D. seriata*, *Dothiorella iberica*, *Lasiodiplodia theobromae*, *Neofusicoccum australe*, *Neofusicoccum nonquaesitum*, and *Neofusicoccum parvum*) and Bacterial soft rot (*Erwinia carotovora*) (Twizeylimana et al., 2013; Whiley et al., 2002; Darvas, 1987).

Stem end rot (SER) is a significant post-harvest disease with a vast host range among tropical and subtropical fruits which includes avocado, citrus, mangoes, among others (Galsurker et al., 2018). The disease advances from the stem end as the fruit ripens, and it can be severe in orchards where anthracnose
disease (*Colletotrichum* spp.) is controlled (Ploetz, 2003). Stem end rot disease is associated with avocado losses in all major growing regions in the world (Twizeyimana et al., 2013). Botryosphaeriaceae species are the most common pathogens associated with SER of avocado fruits compared to other fungi. Different species of Botryosphaeriaceae are reported as the causal pathogen in different geographical regions. For instance in Israel *Lasiodiplodia theobromae*, in Australia and New Zealand, *Neofusicoccum luteum* and *N. ribis*, in South Africa *N. luteum* and *Nectria pseudotrichia* and in United States, *B. dothidea* (Valencia et al., 2019; Twizeyimana et al., 2013).

In Kenya, fungal pathogens that cause fruit rots remain the main constraint of avocado production (Nyambati & Kioko, 2018). Of economic importance are anthracnose and stem end rot diseases. According to Kimaru et al., 2018) *C. gloeosporioides*, and *Pestalotiopsis* spp. are the leading cause of anthracnose on avocado fruits in the country. Stem end rot pathogens of avocado fruits in Kenya are not clear. In Murang’a County, the main avocado fruit production region in Kenya, diseases, pests and poor agronomic practices are the main constraints facing avocado production (Wasilwa et al., 2006).

Management of avocado fruit rots has been through practicing orchard hygiene which involves; removal of deadwood, removal of leaves entangled in the tree canopy and tree pruning to improve aeration and create unfavorable conditions for the inocula. At the end of the harvesting season, all fruits infected and not infected are removed from the trees to reduce sources of inocula in the
next season (Ploetz, 2013). An integrated approach involving the use of resistant varieties, use of Copper-based fungicides and Pre-harvest sprays of biological agents such as *Bacillus subtilis*, are some of the methods used to control avocado fruit rots (Whiley et al., 2002; Marais, 2004; Demoz & Korsten, 2006; Pérez-Jiménez, 2008).

There are no fungicides or biological agents registered in Kenya for the control of avocado SER (PCPB, 2016). Consequently, farmers have been using fungicides registered for use on other crops, for instance, flowers while some farmers do not control the rots at all.

1.2 Statement of the problem

Avocado fruit is one of the major fruit crops grown for both export and local market in Kenya and contributes significantly to the country’s GDP (HCD 2016). Kenya overtook South Africa in the production and export of avocado in 2018, making the country Africa's biggest producer and exporter and third producer in the world behind Mexico and Peru (Amare et al., 2019). The increased export was attributed to increased production due to government policies that promote production of the avocado fruits, coupled with access to new markets such as the United Arab Emirates, Saudi Arabia, and Russian federation among others (unpublished data). The area under avocado production in Kenya increased by 41% and production volumes increased by 118 % between year 2005 and 2014 (FAO, 2017).
Increased production of the avocado fruits indicates the importance of the fruits as a contributor to the economic development of the rural households that are involved in the production. However, only 10% of total avocado fruits produced in Kenya are exported as compared to South Africa that exports 60% and Chile 55% (Amare et al., 2019). Low exportation of the avocado fruits despite the high production, is attributed to poor quality fruits due to fungal fruit rots coupled with poor production and harvesting procedures (Amare et al., 2019; Wasilwa et al., 2006). Stem end rot is one of the main fungal fruit rot reported to cause rots on avocado fruits (Twizeyimana et al., 2013).

The management of stem end rot on avocado fruits in Kenya has not been established; neither have conclusive studies to identify the causal pathogens been done. Studies on biological agents and fungicides that can effectively control SER on avocado fruits have also not been done. This study therefore aimed at identifying the causal agent(s) of avocado SER in Murang’a County and evaluating selected fungicides and bio control agents for their inhibitory effect on SER pathogens.

1.3. Justification

Avocado fruits are an important source of minerals, vitamins and oils. Consumption of the fruit is associated with an improved overall diet (Schaffer et al., 2013; Bergh, 1992). There has been a continued increase in the production of avocado fruits in Kenya, and in the year, 2018 exports from Kenya superseded
South Africa (Amare et al., 2019; HCD, 2017). Stem end rot is associated with avocado losses in all major avocado growing region in the world (Twizeyimana et al., 2013). In Kenya conclusive studies on SER pathogens of avocado fruits have not been done. The knowledge of the causal agent of SER from this study will be useful in deploying disease management strategies in the avocado orchards in the country. Several methods are identified in the management of SER, which includes, harvesting of avocado fruits with short pedicel and spraying the avocado plant with copper-based fungicides during flowering as well as post-harvest fungicidal treatment. Pre-harvest application of Bacillus subtilis effectively reduces SER of avocado. At the same time, post-harvest application of Trichoderma harzianum and Trichoderma viridae is reported to reduce SER of mangoes (Galsurker et al., 2018). Plant extracts are also effective in the reduction of SER of fruits. For instance combination of prochloraz® (500 μg mL⁻¹; P50) with 0.1% v/v thyme oil was reported to significantly reduce SER on avocado fruits in South Africa (Obianom & Sivakumar, 2018). There are no fungicides or biological control agents registered in Kenya for the control of avocado SER. The antifungal activity of selected fungicide and antagonistic activity of selected Trichoderma species in this study could help in identifying commercial fungicides as well as biological control agents which could be used in the management of SER on avocado fruits.
1.4 Objectives

1.4.1 General objective

The main objective of this study was to evaluate orchard management practices associated with stem end rots on avocado fruits, determine the incidence of stem end rot, and identify fungal pathogens associated with stem end rot disease and to evaluate the in vitro efficacy of selected fungicides and inhibitory effect of *Trichoderma* spp. against stem end rot pathogens of avocado fruits.

1.4.2 Specific objectives

i. To evaluate avocado production and orchard management practices by farmers in Murang’a County.

ii. To determine the incidence of stem end rot infections on avocado fruits grown in Murang’a County.

iii. To identify the causal agent(s) of stem end rot on avocado fruits in Murang’a County.

iv. To evaluate the in vitro efficacy of selected fungicides and inhibitory effect of *Trichoderma* spp. against identified stem end rot pathogens on avocado fruits.

1.5 Hypotheses

i. Poor production and management practices contribute to increased stem end rots infections on avocado fruits.
ii. Avocado fruits in Murang’a County are affected by stem end rot caused by different fungal species.

iii. Fungicides and *Trichoderma* spp. are effective against avocado stem end rot pathogens.

### 1.6 Conceptual Framework

Kenya is the highest producer of avocado fruits in Africa and third in the world, however, the country only exports 10% of the production due to poor quality fruits that fail to meet the export market requirements (Amare et al., 2019). Fruit disease among them SER contribute to production of poor-quality fruits. During harvesting most avocado fruits appear free from rots and other internal disorders, however in the process of ripening which commences after harvesting, the rots may appear (Hopkirk et al., 1994). Probably the infections occur before harvesting, during or even after harvesting and remain quiescent on the fruit until fungal inhibitor in the fruit reduces. The reduction is caused by physiological and biochemical changes that occur when the fruit is separated from the plant (Coates & Johnson, 1997; Hartill, 1991).

Poor field hygiene coupled with poor management practices help to maintain the inocula in the field. Although *Botryosphaeriaceae* spp. are known to cause SER on avocado fruits in other regions where the fruits are cultivated, there are no conclusive studies that have been done to determine the causal agents of the SER of avocado fruits in Kenya. Study on the orchard management practices and
the incidence of stem end rot needed to be carried out in order to determine the
level of infection of the disease. By identifying the SER fungal pathogens, testing
selected fungicides and biological agents against the SER pathogens, it is expected
that the study will contribute to a great extent, the control of SER on avocado
fruits in Kenya resulting to production of high-quality fruits. Figure 1.1 shows a
schematic representation of the conceptual framework.
Figure 1.1 Diagrammatic representation of the conceptual framework (Source; Author 2021)
CHAPTER TWO

LITERATURE REVIEW

2.1 Avocado production in the world

The avocado (*Persea americana* Mill) has been cultivated in the neotropics since ancient times and seems to have been domesticated in America before other annual crops (Galindo et al., 2008). The fruit crop has its origin in the eastern and central highlands of Mexico and the adjacent areas of Guatemala to the pacific coast (Paull & Duarte, 2011). Presently avocado is distributed worldwide in both the tropical and sub-tropical climate, and it is among the top six contributors to the human diet (Bergh, 1992). The fruit is not widely cultivated in most South east Asian countries due to the preferences for other fruits (Paull & Duarte, 2011).

The avocado fruit was introduced in Australia in the 19th century and commercial production began in the 1930s. In Israel, it was introduced in 1908 and Chile 1928 when commercial cultivars were imported into the country (Bost et al., 2013). In Africa, the avocado fruit crop was first introduced to South Africa in the 19th Century where it was grown for subsistence until 1920s when more productive varieties that were better adapted to the climatic conditions were imported from California prompting commercial production (Schaffer et al., 2013). It was not until the late 1970s that the fruit crop was cultivated in Ethiopia in a few orchards, later it was adopted by the small scale farmers, and currently it
is a significant source of livelihood to farmers and traders in the country (Shumeta, 2010).

Avocado production in the world has continued to increase over the years, and by 2018, Mexico was the largest producer followed by Chile and Peru, respectively. In the last decade, global avocado production has increased annually at an average rate of 6% (Altendorf, 2019). Naamani (2011) showed that Kenya is among the leading countries in the export of avocado fruits to the European market. In the year 2017, Kenya exported approximately 300 MT of avocado fruits (HCD 2017). The export market is dominated by the ‘Hass’ avocado fruits that make about 90% of the total export (Naamani, 2011).

2.2 Avocado production in Kenya

In the 1960s, farmers were encouraged to grow ‘Fuerte’ and ‘Hass’ In Kenya, avocado fruit crop was introduced by the Portuguese in the 1930s when local varieties like ‘Puebla’, ‘Duke’ and ‘G6’ were cultivated for subsistence purposes (Krishnan, 2016)fruits for commercial production. The year 1970 marked the beginning of avocado fruit export from Kenya to the European market when 23 tons of avocado fruits were exported (Wasilwa et al., 2006). By the year 2011, ‘Fuerte’ occupied 80% of the total land on avocado fruits in the country (Mutui et al., 2011), this trend has since changed and currently, ‘Hass’ avocado fruit is more widely grown than ‘Fuerte’ due to its export demand (Krishnan, 2016).

There is about 11,000 ha of land under avocado crop in Kenya, spreading in several agro-ecological zones within the country (Amare et al., 2019). Small-
scale farmers (85%) grow the fruit for subsistence, local and export market and dominate production of avocado fruits in Kenya. The main bearing season is from September to January (Mutui et al., 2011). Seventy percent (70%) of production in Kenya comes from the Central and Eastern regions of the country. Murang’a and Kiambu Counties are the main producers in the central region while Meru and Embu Counties are the main producers in the eastern region. Rift-valley and Coast regions contribute 15% of the total production in the country (Wasilwa et al., 2006).

2.2.1 Economic importance of avocado fruit in Kenya

Farmers in Kenya produce avocado fruits for consumption, local and export market, as well as for oil extraction (Muigai & Mwaura, 2003). Avocado fruit is the most important fruit in Kenya followed by mangoes, bananas, passion fruits and pawpaw, respectively (Nyambati & Kioko, 2018). In Murang’a County, avocado fruits are regarded as the third major cash crop after tea and coffee (unpublished data). The fruit is an important source of income for both farmers and the business community involved in local and export trade. In 2016, Kenya produced 246,067 metric tons of avocado fruits, worth Ksh.4.63 billion, accounting for 8% by value of the fruit sub-sector (HCD, 2016). The quantity of avocado fruits exported from Kenya has also continued to increase and in the year 2018 avocado export from Kenya accounted for 2.1% of the world exports similar to south Africa (Munhuweyi et al., 2020)
2.2.2 Constraints of avocado production in Kenya

Avocado production in Kenya is faced with several challenges, which include poor quality planting materials, poor crop management practices, limited access to market information by the farmers, erratic weather patterns, pests and diseases infestation, poor pre and post-harvesting handling practices and lack of technical expertise (Gyau et al., 2016; Mutui et al., 2011). Marketing of the fruits is also a challenge to the small-scale farmers who cannot directly export the fruits. Marketing companies who are able to export in bulk like Kakuzi, Vegpro and Sunripe buy the produce from the farmers (Wasilwa et al., 2006).

2.3 Nutritional importance

Avocado is a nutritious high calories fruit, with distinguishing characteristics of high content of unsaturated fatty acids. It is also rich in vitamin E, ascorbic acid, B-carotene, potassium and vitamin B₆ (Ozdemir & Topuzal, 2004). The fruit contains at least nine essential amino acids in different proportions (Shumeta et al., 2010). The fruit pulp contains bioactive phytochemicals like Carotenoids, Terpenoids, D-Mannoleptulose, Persenone A & B, Phenols and Glutathione all known to contain anti-carcinogenic properties (Ding et al., 2009; Dreher & Davenport 2013).

Consumption of avocado in the diet helps to improve cardiovascular health, assist in weight management and healthy ageing (Dreher & Davenport, 2013). Avocado fruit is hardly cooked because the pulp gets bitter when subjected
to high temperatures (Dorantes-Alvarez et al., 2012). When the pulp is applied on the skin, it rapidly penetrates the skin providing superior natural sunscreen and is therefore referred to as a natural cosmetic (Shumeta et al., 2010). The fruit is used in the cosmetic industry as well as in the preparation of various types of salads (Dorantes-Alvarez et al., 2012). Avocado fruit is a good source of natural oil which is light, delicate in character, contains no cholesterol and blends well with almost all foods (Swisher, 1988).

### 2.4 Botany of avocado plant

The avocado plant is an evergreen medium to large tree with rapid growth and a rounded canopy with dense foliage, especially when the tree is free spreading (Griesbach, 2005). Some cultivars may defoliate during the time of flowering. Grafted plants may remain low and spreading while those established from seeds may reach a height of about 25 meters. The plant has a relatively shallow rooting system that does not spread beyond the drift line of the tree canopy. Climate, soil and cultural practices greatly influence the rooting. The leaves are light green to reddish when young and turn dark green thick and leathery at maturity (Chanderbali et al., 2013).

The flowering of the vegetatively propagated trees starts 3-4 years after planting. The inflorescences that carry over a hundred yellowish to greenish flowers appear from August to September, and off-season flowering often appears in March to April. Some cultivars may flower at different times, depending on the
location (Griesbach, 2005). A large, heavy flowering tree plant produces over a million flowers, but only about 1% may mature to produce fruits (Bender, 2002).

2.4.1 Botanical classification of avocado fruit

The avocado (Persea Americana Mill) belongs to the Lauraceae family (Seymour & Tucker, 1993). The plants are classified as type A and B according to the unique flowering behaviour (Griesbach, 2005). For type A, the flowers open in the morning to present the female flower and close at noon. The following day at noon, it reopens to release the spores (male flower). For type B, it opens at noon to present the female flower and closes at five and reopen the flowing day at 6 am to release the pollens (male flower) (Bender, 2012). Type A cultivars include; Hass, Reed, Lula, Puebla, Simmonds, Choquette, Waldin, Sama and Pinkerton while type B cultivars are Zutano, Fuerte, Bacon, Nabal, Linda, Booth 7, Booth 8, Tonnage and Winter Mexican (Schaffer et al., 2013).

Botanically, avocado is classified into four strains West Indian, Guatemalan, Guatemalan hybrid and Mexican strain. Avocado varieties are derived from the four strains; for instance, 'Hass' is a Mexican strain (Chen et al., 2009). The fruit matures on the plant but ripens after harvesting. Several indices are used to determine optimal harvest point, and this includes the size of the fruit, appearance of the skin and the dry matter content. For the 'Hass' fruit, the skin changes from green to black when ripening and have dry matter content of 22% while the ‘Fuerte’ remains green with dry matter content of 21% (Siddiq, 2012).
2.5 Avocado cultivars

The avocado plant belongs to the genus *Persea*. The genus has two subgenera, subgenera *Eriodaphne* and subgenera *Persea*, which are both in the Lauraceae family. The commercial avocado belongs to the genus *Persea* in the subgenera *Persea* and the species *Persea americana* (Bergh, 1992). The subgenera *Persea* has about 50 species and avocado fruit is botanically designated *Persea americana* Mill. Many different kinds of fruit differ in maturity time, the thickness of the skin, size of the pulp and tolerance to cold (Bergh & Ellstrand, 1986). From the species, *P. americana* there are three ecological races/subspecies Mexican, Guatemalan and West Indians. The Mexican race is thought to be the ancient race of the commercial avocado (Storey et al., 1986).

The avocado cultivars grown in the world are grouped into two; cultivars grown in the tropics and cultivars grown in the sub-tropics. Those in the tropics include Lulu, Collinson, Tonnage, Taylor, Choquette, Pollack, Booth 7, Booth 8, Hall, Walden and Nabal. In the subtropics, cultivars grown commercially include Fuerte, Hass, Bacon, Zutano, Reed, Nabal, Ettinger (Israeli variety), Mexican seedlings, Sharwill' (Australian variety) (Gustafson, 1976). In Kenya ‘Fuerte’ and ‘Hass’ are the most widely cultivated. However, there are other cultivars like Pinkerton, Ettinger, Reed, Simmonds and Puebla (Wasilwa et al., 2006).
2.5.1 Cultivar Fuerte

It is the world’s best-known commercial cultivar. Cultivar Fuerte is thought to be a hybrid between a Mexican and a Guatemalan type. The cultivar produces a pear-shaped fruit of average size, which weighs between 290 – 380g with a comparatively small seed in the cavity. The fruit has thin leathery slightly rough skin with a pale yellow flesh which is almost free from fiber and with an oil content of 16 - 25 % (Griesbach, 2005). The fruit peels easily, have great taste and remain green when ripe (Dorantes-Alvarez et al., 2012). The plant has an extended picking season and the fruits have a long shelf life, which makes it favourable, especially in Kenya. The cultivar is, however, susceptible to cercospora (Pseudocercospora purpurea) and anthracnose (Colletotrichum spp.) diseases which are major causes of rots on avocado fruits (Griesbach, 2005).

2.5.2 Cultivar Hass

It is a Guatemalan cultivar with excellent adaptability to different climatic conditions. The cultivar produces a small fruit of about 340g, which is green at first but changes to dark blue to black when ripe (Bender, 2012). The fruit has a tough pebble skin, good shelf life and oil content of 18-23 %. The plant has a long harvesting season. However, it tends to produce small fruits when bearing heavily and exposed fruits to the sun often suffer from sunburn, reducing the quality and marketability of the fruits (Dreher & Davenport, 2013). Currently, the Hass cultivar is the most widely cultivated and dominates the commercial market due to
its characteristics that make it attractive to the global market (Kader & Yahia, 2011).

2.5.3 Cultivar Pinkerton

It is a hybrid of Mexican and a Guatemalan type and is quite tolerant to low temperatures. The tree is medium size but spread more than the Hass cultivar; it bears early, heavily and regularly. The fruits are long, pear-shaped with a dark green skin, which is medium-thick, leathery and pebbled and weighs about 250 - 410g. The fruit has a good shelf life with a skin that is excellent peeling, its small-seeded with large pulp and its resistant to cercospora disease (Griesbach, 2005).

2.6 Ecology requirements

The avocado plant grows in a wide range of ecological conditions from tropical to sub-tropical conditions that lie between latitude 40°N and 40°S. This is due to the presence of broad genetic differences in the ecological races (Shumeta, 2010). The plant does well in moderate rainfall of between 1250–1750 mm per annum that is well distributed throughout the growing season (Paull & Duarte, 2011), however, dry conditions are required during harvesting (Wolstenholme, 2013). The crop is grown in a wide range of temperatures globally which varies with races. The West Indian race has an optimum temperature of 25°C - 28°C and a minimum temperature of 1.5°C; however, it is susceptible to frost conditions. The Guatemala race, on the other hand, is adapted to cool tropical climate and it is not tolerant to low temperature. The Mexican–Guatemala hybrids like the Fuerte
are more tolerant and can survive in a temperature of up to -4°C, although fruit production will be reduced (Paull & Duarte, 2011). Prolonged exposure to a temperature of above 35°C - 40°C combined with low relative humidity results in fruits and leaves damage due to stress (Griesbach, 2005).

Avocado fruits grow in a wide range of soils ranging from deep, fertile well-aerated volcanic soils to sandy loam soils. It has a wide pH range of 5-7 but requires well-drained soil since the roots are susceptible to rots. The soil should have a moderate water table and if grown in soils with low water table the plant show dieback (Wolstenholme, 2013). Avocado plant is not a heavy feeder and makes little demands to the soil, however its sensitive to salts and does not tolerate saline conditions (Schaffer et al., 2013). Nitrogen is the most important element. Deficiency of nitrogen results in small pale leaves that drop very early, while the fruits become small and few especially in ‘Hass’. Excess nitrogen, on the other hand, results in reduced fruit set, especially in ‘Fuerte’ avocado. (Lahav, 1998). Potassium is required for growth and fruit bearing, Boron for growth and Zinc for fruit development. When Boron and Zinc are deficient, there is a reduction of the fruit size. Other nutrients required include Phosphorous for metabolism, Calcium for root development and Magnesium, Sulfur, Iron and Manganese for the health of the plant (Wolstenholme & Whiley, 2003).
2.7 Avocado production constraints

2.7.1 Avocado arthropod pests

Information on avocado arthropod pests is scanty, especially in parts of Africa, Central and South America. Most of the information available is from Australia, Israel, Mexico and South Africa (Schaffer et al., 2013). The major arthropod pests include thrips, scales, mites and fruit flies (Paull & Duarte, 2011). However, according to Odanga et al. (2017) the common avocado arthropod pests found in Kenya include Asian fruit fly (Bactrocera dorsalis), False codling moths (Thaumatotibia leucotreta), blossom thrips (Frankliniella schultzei) and the greenhouse thrips (Heliothrips haemorrhoidalis).

Cosmopolitan greenhouse thrips (Heliothrips haemorrhoidalis) is found in most avocado growing regions, especially Mexico, California, South Africa and Israel. When the population is low, thrips pose no significant threat to the avocado fruit production; however, when there is a disruption of the natural enemies the population increases. Thrips cause cracking of the skin, and this allows infection by the fungus Sphaceloma perseae (Peña et al., 2013).

There are about forty-two species of soft scales reported to affect avocado fruits around the world, but only six are said to be of economic importance. They affect the leaves and fruits, causing the development of sooty moulds (Paull & Duarte, 2011). Fruit flies do not cause a significant threat to avocado fruit production, especially where standard export conditions are observed. However,
they have occasionally been reported to cause considerable damage that prompt control in some avocado growing regions (Ware et al., 2016). The female fly lay eggs on the under-skin of the avocado fruit causing star-shaped cracks that lower the fruit quality and this may prohibit the export of the fruit (Paull & Duarte, 2011).

Mites of the genus oilgonychus infest the upper surface of the mature avocado leaves. When the population is high, the colonies spill over to the underside and new leaf flushes resulting in a reduction of the photosynthetic activity of the leaves (Peña et al., 2013). The avocado red mite Oligonychus yothersi (McGregor) causes leaf damage resulting in the reduction of photosynthesis and transpiration which has direct effect on fruits quality and yield (Rioja & Vargas, 2009). Other insect pests of avocado fruits include Mealybugs Pseudococcus longispinus, loppers Anacamptodes deflectaria (Guenee), Avocado lace bug, Pseudacysta perseae (Heidemann) and beetles (Peña, 2003).

In most of the avocado growing countries, an integrated pest management (IPM) approach is adopted as the preferred method in pest control. To evade the disruption of the natural enemies, chemical insecticides are avoided. Integrated pest management (IPM) is well developed in Israel with biological control being a major component of the IPM program (Schaffer et al., 2013).
2.7.2 Avocado diseases

2.7.2.1 Root diseases

Several diseases are reported to affect the avocado crop. The diseases of the roots include Phytophthora root rot \( (Phytophthora\ cinnamomi) \) Rands which affects the roots of the plants causing reduced fruit production (Dann et al., 2013). Avocado fruit trees display gradual decline where the leaves become small yellow-green, and then they shed off. In case of a severe attack, dieback occurs on the twigs. The causal organism thrives best under wet conditions with a temperature of between 21° to 31°C and a soil pH of 6.5 (Paull & Duarte, 2011).

Armillaria root rot is associated with \( Armillaria \) spp. and \( A.\ mellea \) is the most prevalent species (Pérez-Jiménez, 2008). Symptoms of the disease are only observed when the fungus colonizes most of the roots. Saprophytic behaviour is observed on the fungus, and it can survive on stump or dead roots buried in the soil for many years. The symptoms may include loss of vigour of the tree, yellowing of leaves, slow growth rate and sometimes the tree suddenly wilts and die (Dann et al., 2013). On the root surface, abundant mycelia strands are observed with naked eyes. Other diseases include rosellinia root rot \( (Rosellinia\ necatrix) \) (Paull & Duarte, 2011).

2.7.2.2 Fruit and foliage diseases

Diseases of the fruits and the foliage include anthracnose \( (Colletotrichum \) spp.), Phytophthora cankers \( (Phytophthora\ citricola) \), stem-end rot
(Botryosphaeria spp.), pseudocercospora spot, (Pseudocercospora purpurea), scab (Sphaceloma perseae) and sun blotch (avocado sun blotch viroid). Those of minor importance are; Dothiorella stem canker (Botryosphaeria spp.), Dothiorella fruit rot (Botryosphaeria spp), Verticillium wilt (Verticillium dahlia), Sooty blotch (Akarapeltopsis spp.), Sooty mould (Capnodium spp.) Alga leaf spot (Cephaleuros virescens), and Bacterial soft rot (Erwinia herbicola; E. carotovora). Although some of the diseases are regarded as minor, they can be significant in certain regions of the world or if introduced in new areas (Ploetz, 2003).

At the point of harvesting most avocado fruits appear free from rots and other internal disorders, however during ripening which commences after harvesting, the rots disorders may appear (Hopkirk et al., 1994). According to Coates & Johnson, (1997) and Hartill (1991), probably the infections occur before harvesting, during or even after harvesting and remain quiescent on the fruit until fungal inhibitor in the fruit reduces. The reduction is caused by physiological and biochemical changes that occur when the fruit is separated from the plant. The major fruit rot diseases on avocado fruits include; anthracnose, Pseudocercospora spot, stem end rot and scab (Griesbach, 2005). The pathogens associated with avocado disease include; Colletotrichum gloeosporiodes, Dothiarella gregaria, Botryodiplodia theobromae (Lasiodiplodia theobromae), Alternaria spp. and Phomopsis spp. Other pathogens of less significance include Pestalotiopsis versicolor, Rhizopus stolonifer, Pseudocercospora purpurea, Trichotheicum roseum and Penicillium spp. (Barkai, 2001; Coates & Johnson, 1997).
Stem end rot (infection through the stem of the fruit) and body rots (infection through the body of the fruit) can be caused by any of the pathogens above (Everett, 2002). Anthracnose alone causes about 36% loss of the harvested fruits, while stem end rots cause about 13% loss in South Africa (Korsten et al., 1992).

2.7.2.2.1 Anthracnose

The Colletotrichum species cause anthracnose. The most common species are C. gloeosporioides, Glomerella cingulata, G. acucata (C. acutatum) and Pestalotiopsis spp. (Kimaru et al., 2018; Marais, 2004). Anthracnose is an important post-harvest disease of avocado fruits. It causes reduction of fruit quality, shelf life and marketability of the fruits during transportation, storage and marketing (Bill et al., 2014). Anthracnose infections are promoted by high humidity above 80% and warm temperature between 18° - 26 °C (Pérez-Jiménez, 2008).

Large brown necrotic lesions characterize the disease at the center and on the margin of the leaves. On young and mature fruits, small dark lesion <5mm are observed on the skin surface and around the lenticels, which usually result in reduced fruit quality and fruit drop (Ploetz, 2003). When the fruits start to ripen, large spreading, sunken necrotic lesions, dark brown to black appear, and with time they penetrate to the pulp. The lesions may coalesce to the entire surface and pulp is affected (Freeman et al., 1998). Fungal spores germinate on developing
fruits and enter a dormant stage and remain hidden until the ripening of the fruit when it causes the rot (Griesbach, 2005).

Antifungal compounds called dienes found on the peal of the un-ripened fruits contribute to the dormancy. As the fruits ripen the concentration levels of the dienes decrease below the fungistatic level and the infecting hyphae that was on the skin surface spread to the surrounding tissues causing the disease (Marais, 2004).

The fungus is isolated by picking tissues from the edges of fruit rot and plating them on potato dextrose agar (PDA). The colony colours for *C. gloesporioides*, range from pale grey to salmon or dark grey. Specific primers CgInt/ITS4 for *C. gloesporioides* and CaInt2/ITS4 for *C. acutatum* are used in molecular identification (Pérez-Jiménez, 2008).

2.7.2.2.2 *Pseudocercospora* spot

*Pseudocercospora* spot which also is known as cercospora spot is one of the most common diseases in warm, humid, rainy climates where the fruit is produced (Cooke et al., 2009). The disease is caused by *Pseudocercospora purpurea* (Cooke) and can cause up to 70% of the fruit loss if not controlled (Marais, 2004). Cerescospora spot is a significant disease in Florida, Mexico, Australia and South Africa where it causes up to 69% loss in untreated orchards (Pérez-Jiménez, 2008). The ‘Fuerte’ avocado fruit is more susceptible to the disease than the ‘Hass’ fruit (Ploetz, 2003).
The disease affects the leaves and stem of the fruit. Angular purple to brown spots first appears on the leaf margins, while chlorotic haloes surround older spots. The individual lesions then coalesce to form large, brown, dead areas of leaf resulting in deformed, curled leaf, which eventually falls off (Crane et al., 2007). The infections also progress to the stem and young leaves may be defoliated (Pérez-Jiménez, 2008). On the fruits, it's characterized by small, raised, dark spots on the skin surface that are more or less circular. The spots later coalesce into large circular lesion which becomes slightly sunken when the infected cells dry up. Often small cracks appear within the lesions and allow the penetration of pathogens like C. gloesporioides that causes the rot (Marais, 2004). When the disease is arrested temporarily minute raised shiny black spots appear on the skin surface. It is usually superficial, but during advanced stages, it invades the flesh (Ploetz, 2003).

The fungus is isolated from fresh symptomatic tissues and inoculated on PDA. The colony appears leathery with tufts of conidiomata which are initially grey but change to brown with age (Pérez-Jiménez, 2008).

2.7.2.2.3 Scab

Scab disease is caused by Sphaceloma persea (Quezada et al., 2003). It is more prevalent in areas with high rainfall and high humid conditions resulting in severe losses due to fruit drop and lowered market value of the affected fruits (Pérez-Jiménez 2008). Emerging young tissues are most susceptible, but the leaves
become resistant as they mature (Quezada et al., 2003). On the fruits, the disease is characterized by oval to irregular shaped lesions which are slightly raised with brown to purplish-brown colour. As the disease progresses, the lesions enlarge and coalesce forming large rough, corky areas with intersecting raised ridges over the fruit surface. Scab does not cause rot, but if its invaded by secondary organisms like *C. gleosporiodes* substantial fruit decays occur (Ploetz, 2003). The fungus *S. perseae* is isolated from the infected tissues and inoculated on PDA, the colony colours vary from olive to brownish olive colour (Pérez-Jiménez, 2008).

### 2.7.2.2.4 Dothiorella fruit rot

Dothiorella fruit rot causes economically important post-harvest decay, but its loss is usually lumped together with anthracnose and stem-end rot (Dann et al., 2013). It is a disease of the packing house but on rare occasions, it may be found on over mature fruits in the orchard. Members of *Botryosphaeria* species cause Dothiorella fruit rot. The common species are *Botryosphaeria ribis* (Israel and South Africa) *Dothiorella aromatica* (Australia) and *Botryosphaeria parva* (New Zealand) (Kader & Yahia, 2011). The disease affects other parts of the fruits other than the stem end hence it is possible to distinguish it from the stem end rot. Superficial irregular amber to reddish-brown lesions appears on the fruit peel as it softens during ripening. The lesions become black and sunken as it enlarges rapidly. Watery decay spreads throughout the fruit, it shrivels and an unpleasant odour develops (Ploetz, 2003).
2.7.2.2.5 Stem end rot

Stem end rot occurs in various tropical and sub-tropical fruits which include avocado, mangoes, citrus and others (Galsurker et al., 2018). Diverse pathogenic fungi endophytically colonize the stem end during fruit development and remain quiescent until the onset of fruit ripening when it starts to develop. Stem end rot affects the stem end of the fruits, while Dothiorella fruit rot affects other portions of the fruits (Prusky et al., 2009).

All major avocado production regions in the world have reported the presence of stem end rot on avocado fruits (Guarnaccia et al., 2016). Losses due to stem end rot are more when harvesting occurs during the rainy season. Shriveling around the stem marks the beginning of SER, this is followed by decay, fruit discolorations and softening (Twizeyimana et al., 2013). Mycelial growth is often observed on the abscission scar when the stem button is removed. As ripening progresses dark brown to black lesions with well-defined margins advance from the stem end to the other parts of the fruits. Eventually, the fruit becomes shriveled, the pulp water-soaked and brown and as the decay progresses the vascular bundles become browned (Marais, 2004; Ploetz, 2003). The decay may then spread to the whole fruit. The symptoms of the rots rarely appear before harvesting, and the rot mostly occurs during packing, transit to the market, during or even after marketing (Twizeyimana et al., 2013).

The common pathogens associated with stem end rot include; *Lasiodiplodia theobromae*, *Glomerella cingulata*, *B. dothidea* among others
(Pérez-Jiménez, 2008). However, throughout the world anamorphs of species in the Botryosphaeriaceae are believed to be the most important causal agents of avocado SER (Valencia et al., 2019).

Different Botryosphaeriaceae spp. associated with severe SER are limited to specific geographic regions. For instance, in Israel, the leading cause of SER is Botryosphaeria rhodina while in South Africa Fusicoccum luteum and Nectria pseudotrichia (anamorph Tabercularia laterita) are the main causes of the disease. In Australia and New Zealand and Botryosphaeria ribis and Fusicoccum luteum are the leading causes of SER while in the United States, B. dothidea is the main cause of SER on avocado fruits (Twizeyimana et al., 2013). Other pathogens include Colletotrichum gloeosporiodes in combination with others or alone, Altanaria species, Drechslera setaria, Gibberella pulicaris (anamorph Fusarium sambucinum), Pestatotiopsis vesicolar, phomopsis persea and Rhizopus stolonifer, Dothiorella mangiferae synomised with Fusiococcus aesculi and Fusiococcus parvum (Ploetz, 2003). The pathogens are found on living and dead branches, leaves and living pedicels and in the soil. Probably most infections occur during harvesting when the cutting tools distribute the pathogen from already contaminated stems (Hartill & Everett, 2002).
2.8 Management of fruit rots disease on avocado fruits

2.8.1 Cultural practices

Cultural practices aimed at reducing the inocula and creating unfavorable conditions for disease development are useful in the reduction of fruit diseases on avocado fruits. They include eliminating mummified fruits before the onset of a new season and removing dead twigs and branches that could be a source of inocula before flowering. Selective pruning of the plant to open up the canopy, improve air circulation and sunlight penetration in the fruit tree canopy also effectively helps to control the infections. The pruned materials are also removed from the fields since they could be a source of inocula (Pérez-Jiménez, 2008). Stem end rot is also reduced by maintaining optimal nutrient value in the soil and avoiding water stress that decreases resistance to the infection through irrigation (Rees et al., 2012). Snapping of pedicel during harvesting should be avoided and instead clipping using frequently sterilised clippers be adopted (Hartill & Everett, 2002). Further, the length between harvest and consumption should be reduced, while harvesting should be avoided when wet since moisture encourages germination of spores (Marais, 2004).

2.8.2 Chemical control

Over the years, synthetic fungicides have been used effectively in the control of post-harvest diseases of fruits (korsten, 2006). For instance, prochloraz, a non-systemic fungicide is widely used in the control of fungal disease of
avocado fruits. (Obianom & Sivakumar, 2018). Copper-based fungicides are also extensively used in reduction and control of anthracnose and cercospora spot diseases on the avocado fruits (Duvenhage, 2002). Chemical control is widely developed where copper fungicides are applied at an interval of once a month alone or alongside other systemic fungicides to control Pseudocercospora spot (Pérez-Jiménez, 2008). Besides treating the fruits before harvesting with copper fungicides, dithiocarbamate, promyl and triadimefon fungicides effectively control anthracnose (Ploetz, 2003).

Application of copper fungicides at the end of the flowering stage helps to control scab while pre-harvest sprays with copper fungicides help to reduce stem end rot infection (Marais, 2004 ). To minimise damage and economic losses caused by scab, integrated approach that includes the use of resistant varieties and fungicidal sprays like copper and Benomly is advocated. Thrips are correlated to scab disease and their elimination help to control the disease (Pérez-Jiménez, 2008)

2.8.3 Biological control

Utilizing natural fungicides, microbial antagonist and bio-control agents (yeast, bacteria and antagonistic fungi), offers a potential alternative to the use of fungicides in the management of post-harvest disease of fruits (Geetanjli, 2017; Ferramola et al., 2013). Biological control approach involves the use of microorganisms to reduce or maintain the postharvest fungal pathogens below
economic loss. This is a widely explored approach in the control of postharvest fruit diseases in the world (Carmona-Hernandez et al., 2019). One of the most effective biological control method against the post-harvest disease of fruits is the introduction of artificial microbial antagonists on a fresh wound on fruits or before the inoculation of the pathogen (Sharma et al., 2009).

2.8.3.1 Biological agents used against post-harvest diseases of avocado fruits

2.8.3.1.1 Bacillus subtilis

Bacillus subtilis is widely researched on and established to have the potential to control avocado post-harvest diseases (Demoz & Korsten, 2006). The pathogen naturally inhabits and dominates fruit and leaf surfaces of the avocado plant where it competes with other organisms for nutrients and space (Irtwange, 2006). Bacillus subtilis B246, isolated from the avocado leaf surface and commercially registered as Avogreen in South Africa effectively controlled stem end rot pathogens on avocado fruits (Janisiewicz & Korsten, 2002). Avogreen is effective against Lasiodiplodia theobromae, Fusarium solani, Diethorella aromatic, Colletotrichum gloesporiodes stem end rot pathogens (Sharma et al., 2009).

The effectiveness of Bacillus subtilis is associated with the pathogen mode of action which includes ability to attach to the surface of the pathogen, mycoparasitism and competitive colonization (Demoz & Korsten, 2006). Bacillus subtilis has also demonstrated ability to control pathogens associated with fruit
rots on a wide range of fruits. For instance, anthracnose (*Colletotrichum musae*) on bananas, gray mold (*Botrytis cinerea*) on grapes, rot on melon fruits (*Altenaria alternata*) and brown rot of peach (*Monilinia fructicola*) (Carmona-Hernandez et al., 2019; Janisiewicz & Korsten, 2002).

Harvest and pre-harvest handling operations predispose avocado fruits to mechanical injuries which may result in infection by pathogens. Near harvest treatment of the avocado fruits with *Bacillus subtilis* also effectively control anthracnose and stem-end rot associated with colonization of the wounds by pathogens (Parthasarathy et al., 2017).

### 2.8.3.1.2 Yeasts species

Certain yeast species have antagonistic ability against fruit rot, for instance, *Candida aleophila* is a registered antagonist for control of post-harvest decay of fruits (Sten et al., 2016; Carmona-Hernandez et al., 2019). *Candida aleophila* competes for space, nutrients and inhibits the growth of the pathogen but do not cause death of the pathogen. The antagonist has less likely hood of being hazardous to consumers, therefore, regarded as a good biocontrol agent. *Candida aleophila* effectively controls rots caused by *Botrytis cinerea* and some *Fusarium* spp. on fruits which also causes stem end rot on avocado fruits (Sten et al., 2016).

### 2.8.3.1.3 Trichoderma species

Several *Trichoderma* spp. are widely tested against post-harvest fruit pathogens that also causes stem end rot on avocado fruits. Studies indicate that
*Trichoderma* spp. have great potential to control plant pathogens that have a wide host range (Sharma et al., 2009; Yahia, 2011) Table 2.1.

**Table 2.1** *Trichoderma* species and the post-harvest fruit pathogens they control

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Pathogen</th>
<th>Disease</th>
<th>host fruits</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. viride,</em></td>
<td><em>Lasiodiplodia</em></td>
<td>crown rot</td>
<td>Banana</td>
<td>(Sangeetha, et al., 2009)</td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td><em>theobromae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. koningii</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichoderma</em></td>
<td><em>Botrytis cinerea</em></td>
<td>Gray mold</td>
<td>Grape fruit</td>
<td>Sharma et al., 2009</td>
</tr>
<tr>
<td><em>harzianum</em></td>
<td></td>
<td></td>
<td>Kiwi fruit</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pear</td>
<td></td>
</tr>
<tr>
<td><em>Trichoderma</em></td>
<td><em>Colletotrichum</em></td>
<td>Anthracose</td>
<td>Rambutan</td>
<td>Sharma et al., 2009, Yahia, 2011</td>
</tr>
<tr>
<td><em>harzianum</em></td>
<td><em>gloeosporioides</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichoderma</em></td>
<td><em>Botryodiplodia</em></td>
<td>Stem end rot</td>
<td>Rambutan</td>
<td>Sharma et al., 2009, Yahia, 2011</td>
</tr>
<tr>
<td><em>harzianum</em></td>
<td><em>theobromae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichoderma</em></td>
<td><em>Lasiodiplodia</em></td>
<td>Stem-end rot</td>
<td>Mango</td>
<td>Sharma et al., 2009</td>
</tr>
<tr>
<td><em>viride</em></td>
<td><em>theobromae</em></td>
<td></td>
<td></td>
<td>2009</td>
</tr>
</tbody>
</table>
2.8.4 Literature summary and research gap

Avocado fruits are affected by several post-harvest diseases, stem end rot is one of the post-harvest diseases that affect the fruits in all regions where the fruits are grown. Fruits are infected while in the field or during pre-harvest and post-harvest handling but the infection remain quiescent on the fruit until ripening commences when the symptoms appear on the fruits. In most cases the avocado fruits are usually on transit or on the shelf resulting to loss of revenue. In many countries the extent of loss due to SER is not clear however, in South Africa stem end rot causes up to 13% loss of avocado fruits (Korsten et al., 1992). Pathogens associated with stem end rot in many countries where avocado fruits are grown have been identified but in Kenya losses of avocado fruits due to stem end rot are not quantified and the information on the pathogens that cause the disease is lacking.

Synthetic fungicides are effectively used in the control of post-harvest diseases of fruits. Copper based fungicides are widely used in the field to control post-harvest diseases on avocado fruits for instance in South Africa (Obianom & Sivakumar, 2018). There are no fungicides registered in Kenya for the control of post-harvest diseases of avocado fruits.

Substantial progress has been made towards biological control of postharvest disease of avocado fruits (Sharma et al., 2009; Demoz & Korsten, 2006), despite the progress, no attempt has been made towards biological control of post-harvest disease of avocado fruits in Kenya. *Bacillus subtilis* is a promising
biocontrol agent against a wide range of postharvest pathogens of avocado fruits. The potential of biocontrol strains of the same species isolated from the same plant in different locations may vary (Janisiewicz & Korsten, 2002), therefore its of paramount importance to pursue isolation of *Bacillus subtilis* from avocado fruits grown in different agro-ecological zones in Kenya and test for their effectiveness against identified fruit pathogens within the country.

Although several yeast species and *Trichoderma* species and have been reported to effectively control post-harvest diseases of fruit, none has been tested against the post-harvest disease of avocado fruits. The Possibilities of identifying *Trichoderma* species that are effective against stem end pathogens should also be explored.
CHAPTER THREE
MATERIALS AND METHODS

3.1 Description of the study area

Murang’a County lies between latitudes 0° 34’ South and 1° 7’ South and Longitudes 36° East and 37° 27’ East. It is also found between altitude 914m a.s.l in the east and 3,353m a.s.l in the west with temperature range of 13° C to 22°C (Jaetzold et al., 2007). The County occupies a total area of 2,558.8 square kilometers (Murang’a County Government, 2017).

The County has nine Agro-Ecological Zones (AEZ): UH0 zone, which is a forest reserve and no farming activities take place here; UH1 and LH1 zones that are suitable for dairy, sheep and tea farming. UM1 is a tea and coffee zone while UM2 and UM3 are coffee zones, and UM4 is a sunflower maize zone (Jaetzold et al., 2007). Farmers in UM1, UM2, UM3 and UM4 practice mixed farming and avocado fruit cultivation is concentrated in these zones. Zone LM3 and LM4 is a marginal area suitable for cotton growing but a few farmers grow avocados. (Murang’a County Government 2017; Jaetzold et al., 2007).

More than 75% of the County is covered with fertile andosol soils, especially UM1 to UM4 explaining why the County enjoys highly productive small scale farming (Jaetzold et al., 2007). The County experiences two rainy seasons, long rains from March to May with the highest rainfall recorded in April and short rains from October and November (Murang’a County Government 2017; Jaetzold et al., 2007).
Figure 3.1: Map of the study area (adopted from Murang’a County Government official website)
3.2 Production and management practices associated with stem end rot on avocado fruits

3.2.1 Field survey

A survey was carried out in the avocado growing areas of Murang’a County from September 2017 to March 2018. Four out of the seven Sub-Counties that have high concentration of avocado farmers were selected for the survey. The selected Sub-Counties were Gatanga, Kandara, Murang’a South and Kiharu.

A structured questionnaire (Appendix 1) was developed and pre-tested with 10 avocado farmers. After pre-testing, the questionnaire was adjusted. The questionnaire captured information on demographic characteristics of the farmers, sources of planting materials, land use system, production levels, orchard management, occurrence and management of fruit diseases and income from the avocado fruits. A stratified random sampling method was used to identify the farmers included in the study. The stratum was a farmer with at least five fruiting variety Hass Avocado trees. The data was collected by interacting with 162 farmers through administration of the questionnaire by face-face interviews (Appendix 2 and 3).

Enumerators recruited from the sub-county offices helped to conduct face-to-face interviews using the questionnaires. Target respondents were the household head, but in case of absence, the spouse of the household head or a close relative/next of kin was interviewed. The population size of farmers undertaking avocado production in the county is not known. Consequently, the required sample
size of the farmers was determined using Naing et al., 2006) method as indicated below;

\[ n = \frac{p q Z^2}{E^2} \]

Where;

n - Sample size; p - Proportion of the population growing avocado fruits; q =1- p;
Z- Confidence level and E- Allowable error since the proportion of the population was not known.

\[ P = 0.5, q = 1 - 0.5 = 0.5 \text{ Z} = 1.96 \text{ and } E = 0.09 \]

\[ N = \frac{0.5 \times 0.5 \times 1.96^2}{0.09^2} = 118.567 = 119 \text{ Farmers} \]

One hundred and nineteen (119) farmers was the minimum sample size expected. However, 162 farmers (Gatanga 40, Kandara 69, Murang’a South 34 and Kiharu 19) were included in the survey.

### 3.2.2 Data analysis

Information from the survey questionnaire was coded and entered in a spreadsheet and analyzed using Statistical Package for Social Sciences (SPSS) version 22. Descriptive statistics were expressed by means, standard error of the mean, frequencies and percentages. Kruskal - Wallis test, Chi-square test, Pearson correlations test were used to determine the relationships between variables and one-way ANOVA used to determine significant differences between variables.
3.3 Determination of the incidence of stem end rot infections on avocado fruits in Murang'a County

3.3.1 Sample collection

3.3.1.1 Avocado fruits from the orchards

To determine SER disease incidence on ‘Hass’ avocado fruits, a survey was carried out during the avocado fruiting season, September 2017 to March 2018. Gatanga, Kandara, Murang'a South and Kiharu sub- counties were selected for the survey. One hundred and six two orchards (162) with more than five fruiting trees were selected at random along an identified all-weather road transient through the four sub counties. Using secateurs, six asymptomatic mature avocado fruits of ‘Hass’ were harvested arbitrarily from each five randomly selected avocado fruit trees in every orchard, thirty fruits (30) per orchard and pooled. Part of the pedicel was left on the fruit to maintain freshness. Four hundred and fifty-three avocado fruits were randomly selected from the 4860 fruits obtained.

To determine the sample size, an assumption of 60% prevalence in reference to anthracnose diseases was adopted and the sample size was determined using the following formula (Naing et al., 2006).

\[ n = \frac{pqz^2}{d^2} \]

n= minimum sample size required

p = proportion of the target plants estimated to be infected

q = 1 – p
\[ z = \text{level of precision (1.96) which corresponds to 95 \% confidence level} \]
\[ d = \text{degree of accuracy desired set at 0.05} \]

\[ n = \frac{0.6 (0.4) (1.96^2)}{0.05^2} \]
\[ = 368.79 \]

Three hundred and sixty-nine (369) fruits were required for the assessment of stem end rot infections. However, 453 fruits from 4,860 fruits harvested from the orchards were sampled, packed in cartons (15 fruits per carton) and transported to KALRO – Kandara, Mycology Laboratory. The fruits were washed with clean tap water, surface sterilized using 0.5 \% sodium hypochlorite for 30 seconds and rinsed in distilled water. The fruits were placed on sterilized trays, covered with sterile cotton material and left in the laboratory at room temperature (22° – 25°C) for 7-14 days to allow the development of stem end rot. Fruits that developed SER disease were separated from the healthy fruits and both clusters of fruits counted. The data was used to calculate the disease incidence using the equation below, adopted from Awa et al. (2012).

\[
\text{Incidence (\%) = } \frac{\text{Number of avocado fruits developing stem end rot} \times 100}{\text{Total number of Avocado fruits examined}}
\]

3.3.1.2 Avocado fruits from the markets

‘Hass’ avocado fruits at different stages of ripening were bought from different traders within the three main markets in the county, Kandara, Kirwara
and Maragwa market (ten from each) at weekly intervals for two months. The Sample size table was used to determine the number of fruits from the market at 95% confidence and a margin error of 5% (Research advisor, 2006). Two hundred and forty (240) fruits were sampled, packed in cartons and taken to KALRO - Kandara Mycology Laboratory for analysis. The fruits were washed with clean tap water, surface sterilized using 0.5% sodium hypochlorite for 30 seconds and rinsed in distilled water. The fruits were placed on sterilized trays, covered with sterile cotton material and left in the laboratory at room temperature (22° – 25°C) for 7-14 days to allow the development of stem end rot. Fruits that developed SER disease were separated from the healthy fruits and both clusters of fruits counted. The incidence of the disease was calculated as stated in 3.3.1.1.

3.3.3 Data analysis

Information on SER development on the fruits was recorded in a spreadsheet and exported to MedCal statistical software v19.1.6. Descriptive statistics were generated where the data was expressed in frequencies (n) and percentages and the confidence interval (CI) calculated. Comparison of rates (Sahai & Khurshid, 1995) was used to determine the significant difference between the incidence in the farm and market at p value ≤ 0.05.
3.4 Isolation and identification of stem end rot fungal pathogens from avocado fruits.

3.4.1 Fungal isolation

Four hundred and fifty-three ‘Hass’ avocado fruit were collected from four sub-counties and 240 fruits of the same variety were collected from three markets in Murang’a County, respectively. The fruits were washed with clean tap water, surface sterilized using 0.5 % sodium hypochlorite for 30 seconds and rinsed in distilled water. The fruits were placed on sterilized trays, covered with sterile cotton material and left in the laboratory at room temperature (22° – 25°C) for 7-14 days to allow the development of stem end rot. The symptomatic ‘Hass’ avocado fruits were separated from the health ones. Small pieces of flesh from the margins of symptomatic flesh were placed aseptically in 9 cm diameter Petri dishes containing potato dextrose agar (PDA) amended with streptomycin sulfate (0.2%) to discourage bacterial growth. The inoculated plates were sealed with parafilm and incubated at 24°C ±1 for 3 to 5 days to allow fungal growth (Djeugap et al., 2015).

3.4.2 Preparation of pure cultures

Single spore isolation was done to obtain pure cultures from the sporulating isolates. Cultures that had grown for 7 days were flooded with sterile distilled water. A sterile wire loop was used to scrap the conidia and place them in 1ml of sterile distilled water. Fifty microliter of conidia suspension was spread on
1.5% (wt/vol.) Water agar (WA) and incubated at 24°C overnight on a sterilized lamina flow bench. Germinated conidia were transferred to PDA amended with streptomycin sulfate (0.2%) using sterile glass needle and incubated at 25°C, to induce growth and sporulation.

For non-sporulating isolates, pure cultures were obtained by transferring hyphal tips of 7-day old colonies on 1.5% (wt/vol.) Water Agar (WA) and allowed to grow for 24 hours. Tips of the mycelial growth in the WA were transferred into 9 cm petri dish containing PDA amended with 0.2% of streptomycin sulfate. Slant universal bottles were used to preserve the pure cultures of the isolates and stored in the refrigerator at 4°C for later use.

3.4.3 Morphological identification

The isolates were identified based on cultural characteristics on PDA media and spore characteristics observed on the microscope as described by Valencia et al. (2019), Phillips et al. (2013) and Watanabe (2010). The cultural characteristics used in identification included growth rate, colony colour, texture margin and elevation. To induce conidia production, stored isolates were plated on PDA amended with autoclaved avocado wood chips and incubated at 24± 1°C for 14 – 21 days. The conidia were harvested by flooding the cultures with sterile distilled water. The conidia were scraped off using a sterile wire loop and later filtered through doubled layer muslin cloth to remove the mycelia. The collected filtrate was diluted serially in sterile universal bottles to 10^5 and the length, width
and shape of the conidia noted. Lactophenol blue was used in microscopic observations. The length and width of conidia (N=50) from each isolate were measured using light microscope Zeiss - Primo Star, coupled to an AxioCam ERc 5s camera.

Further, to support the morphological identification of the isolates, 11 isolates from fruits sourced from both the markets and the orchards were selected for pathogenicity test and molecular identification.

3.4.4. Pathogenicity test

To establish Koch’s postulates, the 11 isolates were subjected to pathogenicity test as described by Freeman et al. (1998) and Twizeyimana et al. (2013). Each of the isolates was subjected to two methods of inoculation.

3.4.4.1 Mycelia disc inoculation

A sterile cork borer (5 mm diameter) was used to wound the stem end of each fruit and mycelial discs of equivalent diameter obtained from the edge of actively growing pure cultures and placed on the wound. Six inoculated fruits for each pathogen and six control fruits inoculated with plain PDA were arranged on individual trays and covered with cling film to conserve moisture and avoid contamination. The fruits were incubated at room temperature of 24°C ± 1 for 12 days.
3.4.4.2 Spore suspension inoculation

After snapping the pedicel of sterilized, air-dried fruits, 50 μl of conidial suspension (5×10⁵ conidial/ml) was sprayed on stem end opening. The six inoculated fruits of each isolate and six control fruits inoculated with distilled water were arranged on individual trays and covered with cling film. The inoculated fruits were incubated at 24 ± 1°C. Evaluation was done after 12 days by cutting the fruits lengthwise and the following scale of 0 to 4 used to rate the stem-end rot: 0 = no visible rot; 1 = 1 to 25% rot; 2 = 25% to 50% rot; 3 = 50% to 75% rot and 4 = > 75% rot (Freeman et al. (1998) and Twizeyimana et al. (2013). At the end of the pathogenicity test, re-isolation from the infected fruits was done, inoculated on PDA and re-isolated fungal colonies compared morphologically to the original isolates. Stem end rot severity on avocado fruits was calculated using the formula described by Lakshmi et al. (2011).

\[
\text{Percent disease index (PDI)} = \frac{\text{Sum of numerical ratings}}{\text{No. of fruits examined}} \times 100 \times \text{Maximum grade}
\]

3.4.5 Molecular identification of the isolates

3.4.5.1 DNA extraction

An improved fungal extraction protocol described by Innis et al. (2012) was used to extract DNA from three representative isolates of each species. Pure fungal cultures derived from the single spores incubated in PDA were used. Forty
milligram (mg) of mycelia was placed in a microcentrifuge tube containing 300 μl of extraction buffer (Tris-HCl, 200mM Ph 8.5; EDTA, 25mM; 1 M NaCl 250mM; SDS, 0.5%) with glass beads. The tubes were placed in a fastprep®-24 genegrinder for one minute at 2000 rpm. Two hundred microlitre (μl) of 3mM sodium acetate pH5.2 was added and refrigerated at -20°C for 10 minutes. After incubation, the samples were centrifuged for 10 minutes at 13000 rpm. After that, the supernatants were transferred into fresh 1.5 ml microcentrifuge tubes. Equal amounts of isopropanol were added to the supernatants and allowed to stand for 5 minutes at room temperature. After five minutes, the samples were centrifuged for 10 minutes at 13000 rpm and supernatant discarded. Five hundred μl of 70% ethanol was then added to the pellets and centrifuged at 13000 rpm for 10 minutes to wash the pellet. The nucleic acid pellets obtained were air dried and then resuspended in 50 μl of low salt TE buffer (Tris-HCl, 1 mM, pH 8; EDTA, 0.1 mM) and stored at -20°C for later use.

3.4.5.2 Determination of quality of DNA

The quality of DNA was determined by use of agarose gel electrophoresis and quantified using a NanoDrop ND-1000 Spectrophotometer. Agarose gel (1%) was prepared by mixing 1g of agarose powder into 100mg of 1x TBE buffer and stained with 3μl of Ethidium Bromide. The mixture was poured into casting tray with a comb to solidify. A 1.5μl of 1KB marker was loaded on the first well of solid gel followed by 2μl of each DNA isolate. An electric voltage of 100 volts
was connected to the gel for 30 minutes to facilitate the migration of the DNA. Formed DNA bands were visualized under UV light using ENDURO™ GDS (Innis et al., 2012). Later the DNA was normalized to 20 ng/μl for Polymerase Chain Reactions (PCR).

3.4.5.3. Polymerase Chain Reactions and Gel Electrophoresis

3.4.5.3.1 Polymerase Chain Reactions

The extracted DNA from eleven representative isolates was used as templates for Polymerase Chain Reactions (PCR). Two set of primers, **ITS1**(*TCCGTAGGTAACCTGC GG*) and **ITS4** (*TCC TCC GCT TAT TGA TAT GC*), **ITS5** (*GGA AGT AAA AGT CGT AAC AAG G*) and **ITS1** sourced from Inqaba Africa Genomic Platform, South Africa, were used in the amplification of the internal transcribed region rDNA of the fungal isolates (Phillips et al., 2013). The PCR reaction volumes of 25 μl containing 2.5 μl of 0.2 μM of each primer, 5 x My Taq reaction buffer, 0.25 μl Taq polymerase (Bioline, Meridian Life Science, Memphis, USA), 40 ng/μl of each DNA template and 12.75 μl of molecular water was used. For amplification, the GeneAmp 9700 DNA Thermal Cycler (Perkin-Elmer) was used. The process involved an initial denaturing step at 94°C for 30 seconds; followed by 35 cycles: denaturing at 94 °C for 30s, annealing at 55°C for 30s followed by extension for 1 minute at 68 °C; and a final extension step of 5 min at 68 °C (Borneman & Hartin, 2000). Agarose Gel Electrophoresis was used to confirm amplification of the DNA.
3.5.5.3.2 Agarose Gel Electrophoresis

To confirm amplification, the PCR products were run on agarose gel. A 1.5% Agarose gel was prepared by mixing 1.5g of agarose powder into 100mg of 1x TBE buffer and stained with 3μl of Ethidium Bromide. The mixture was poured into casting tray with a comb to solidify. A 1.5μl of 1KB marker was loaded on the first well of solid gel followed by 2μl of each amplified product. An electric voltage of 100 volts was connected to the gel for 45 minutes to facilitate the migration of the amplified PCR products. Formed DNA bands were visualized under UV light using ENDURO™ GDS. The clear DNA bands that were visualized confirmed the amplification of DNA (Innis et al., 2012).

3.4.5.4 DNA cleaning and sequencing

The PCR products were cleaned using the QIAquick PCR Purification Kit from Qiagen according to manufacturer instructions and submitted for Sanger sequencing in both directions at Inqaba Africa Genomic platform, South Africa.

3.4.5.5 Bioinformatics analysis

Sequenced data was analyzed by assigning reads to samples, indexes, primers and adaptors. The primers were marked using Picard (https://broadinstitute.github.io/picard/index.html). Bam2fastq (https://gsl.hudsonalpha.org/information/software/bam2fastq) to convert the resultant bam files to fastq. The overall sequencing quality of the reads was

51
evaluated visually using the Fast QC program [http://www.bioinformatics.babraham.ac.uk/projects/fastqc/]. The quality parameters used in filtering the reads included a minimum length of 250bp and a minimum QC value of 30. Trimming was done corresponding to the adapters and low-quality sequences from all the reads. Subsequent, analysis and processing of the reads was done in the CLC Genomic Workbench 11.0 where the overlapping reads were merged. The de novo assembly of the unassembled reads as well as the alignment of the raw reads was performed using CLC Genomics Workbench 11.0 with default parameters (minimum contig = 100 bp, 23 K-mer, similarity fraction = 80% and length fraction = 50%).

BLAST analysis of the ITS rDNA sequences was done to support the morphological identification of the samples, whereby the closest match (98-100% similarity) in the NCBI Gene Bank database was extracted from the database for each of the sample. The IT SrDNA sequences of the samples were deposited in the NCBI GenBank database using BankIt. ClustalW and MegaX software was used to construct a dendrogram based on the ITS sequences by the Neighbor-Joining method to demonstrate the genetic relationship of fungal species.

3.4.5.6 Data analysis

The Sanger sequenced data was analyzed and processed using CLC Genomic Workbench version 11.0, while phylogenetic analysis to determine
maximum likelihood algorithms was done using Mega X as described by Kumar et al. (2018).

Analysis of Variance (ANOVA) followed by Tukey's post-hoc was used to compare the mean percentage growth rate of inoculated fungi, while Student's t-test was used to compare SER lesions on fruits under different methods of inoculation. Statistical analysis was performed using Min tab v8 (Minitab, LLC).

3.5 Efficacy of selected fungicides and biological agents against stem end rot pathogens

3.5.1 In vitro antifungal assay of selected fungicides

The four most frequently isolated pathogens \textit{Lasiodiplodia theobromae}, \textit{Neofusicoccum parvum}, \textit{Nectria pseudotrichia} and \textit{Fusarium solani} were used in the inhibitory test.

3.5.1.1. Poisoned food technique

The antifungal activity of Green Cop 500WP (Copper oxychloride 500g/Kg) and Milraz 76 WP (Propineb 70% + Cymoxanil 6%) fungicides registered in Kenya by Pest Control Products Board (PCPB) for use against fungal pathogens of other crops like flowers were determined using poisoned food technique as described by Das et al. (2010).

The manufacturers’ recommended doses on the fungicide label were used to determine the concentrations used in this study. For each of the fungicides, the concentrations were prepared as follows; half the recommended rate (0.25g/l
Green Cop and 0.87g/l Milraz), the recommended rate (0.50g/l Green Cop and 1.75g/l Milraz) and twice the recommended rate (1.00g/l Green Cop and 3.50g/l Milraz) (Table 3.1). Fungicides at different dilutions were thoroughly mixed with sterile PDA agar at 40 - 45°C. The amended PDA with different concentration of the fungicide was poured into 9 cm diameter sterile Petri dish and replicated in four plates. Actively growing mycelium of 7-day old fungal cultures was cut at the edges using 5mm diameter cork borer and placed at the center of each plate. Control plates were prepared by placing the fungal growth on PDA that was not amended with fungicides. The inoculated plates of both amended and non-amended PDA were arranged in a sterile hood at a temperature of 24 ±1°C. For each plate, the diagonal measurements of mycelia growth were taken daily for eight days and recorded.

Inhibition of the mycelia growth by the fungicides was calculated based on the colony diameter of the untreated plates using the following formula described by Kimaru et al. (2018).

\[
\% \text{ inhibition} = \frac{X-Y}{X} \times 100
\]

Where:

X - Growth in the control plate

Y - Growth in fungicide treated plates.
Table 3.1 Concentrations of fungicides used in the inhibitory trials against stem end rot isolates of avocado fruits

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Active ingredients</th>
<th>Manufacture recommended dose (g/l)</th>
<th>Formulated concentration (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GreenCop 500WP</td>
<td>Copper</td>
<td>0.50</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>oxychloride</td>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>500g/Kg</td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>Milraz 76 WP</td>
<td>Propineb 70% and</td>
<td>1.75</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>Cymoxanil 6%</td>
<td></td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.50</td>
</tr>
</tbody>
</table>

3.5.2 Antagonistic activity of *Trichoderma* spp. against stem end rot fungal pathogens *in vitro*

3.5.2.1 Dual culture technique

The inhibitory activity of four *Trichoderma* species; *T. atroviride*, *T. virens*, *T. asperellum* and *T. harzianum* against the four SER fungal isolates (*Lasiodiplodia theobromae*, *Neofusicoccum parvum*, *Nectria pseudotrichia* and *Fusarium solani*) was determined using dual culture technique.

3.5.2.1.1 Source of the antagonists

Four *Trichoderma* spp. were used in this study. *Trichoderma harzianum* was obtained from the biological fungicide, TRIANUM P (*T. harzianum* Rifai strain T22, 1 x 10^9 colony forming units (cfu)/gram of dry weight) from Koppert
Biological Systems. *Trichoderma asperellum* was obtained from the biological fungicide MAZAO SUSTAIN (TRC900 1.7 x 10^9 cfu/gram of dry weight) from real IPM. *Trichoderma atroviride* (KRI) and *T. virens* (BMLT54P1), were obtained from the Department of Agriculture Science and Technology, Kenyatta University. The spore suspension of each *Trichoderma* spp. was inoculated on sterile PDA on separate petri dishes and allowed to grow for 7 days. The mycelial culture of the *Trichoderma* spp. was used in the dual culture test.

Sterile PDA was poured into 9 cm diameter petri dishes. Myelia disc (5 mm in diameter) from the edge of actively growing cultures of the pathogens were placed at one side of the petri dish and 5 mm mycelial disc of *Trichoderma* isolates placed at the opposite side of the petri dish. Petri dishes inoculated at one edge with a 5 mm mycelial disc of fungal pathogens served as control. All the treatments were replicated six times. The mycelial growth of the test pathogen in the control plate and in the dual culture plate was recorded. Percentage inhibition was calculated using the following formula as described by Rajendiran et al. (2010).

\[
\% \text{ inhibition} = \frac{C-T}{C} \times 100
\]

Where:

C- Mycelial growth of the pathogen in control

T- Mycelial growth of the pathogen in dual culture plate
3.5.3 Efficacy of *Trichoderma* spp. against stem end rot fungal pathogens on post-harvest avocado fruits

3.5.3.1 Effect of pre - inoculation with *Trichoderma* spp.

Mature ‘Hass’ avocado fruits with no apparent symptoms of a disease and no physical damage were harvested from a farm in Murang’a County. The fruits were washed with clean tap water, surface sterilized by washing with 0.5 % sodium hypochlorite and rinsed in distilled water. The fruits were then air-dried at room temperature on sterilized trays in the laboratory. Sterilized, air-dried ‘Hass’ avocado fruits were individually inoculated at the stem end with 50µl of spore suspension (5×10^4 conidial/ml) of each antagonist. After 24 hours, each of the avocado fruits was inoculated with 50µl of fungal spore suspension (5×10^4 conidial/ml) of each SER fungal isolates. Each of the treatments was replicated four times. The inoculated avocado fruits were placed in sterilized sealed plastic containers (separate container for each fruit). Evaluation was done after 12 days by cutting the fruits lengthwise. A category scale of 0 to 5 was used to rate the severity of SER development on the avocado fruits as:

<table>
<thead>
<tr>
<th>% rot on avocado fruit</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>No rot</td>
<td>0</td>
</tr>
<tr>
<td>0 –10%</td>
<td>1</td>
</tr>
<tr>
<td>11 –25%</td>
<td>2</td>
</tr>
<tr>
<td>26 –50%</td>
<td>3</td>
</tr>
</tbody>
</table>

57
51 –75% 4
≥76% 5

Percent disease index for SER on avocado fruits was calculated using the formula described by Lakshmi et al. (2011).

\[
\text{Percent disease index (PDI)} = \frac{\text{Sum of numerical ratings}}{\text{No. of fruits examined}} \times 100
\]

3.5.3.2 Effect of concurrent inoculation of avocado fruits with *Trichoderma* spp. and stem end rot fungal pathogens

Sterilized air dried ‘Hass’ avocado fruits were individually treated at the stem end with 50µl of spore suspension (5×10^4 conidial/ml) of each antagonist. Immediately each of the treated fruits was treated with 50µl of fungal spore suspension (5×10^3 conidial/ml) of the SER fungal isolates. Each of the treatments was replicated four times. The inoculated avocado fruits were placed in sterilized sealed plastic containers (separate container for each fruit). Evaluation was done after 12 days by cutting the fruits lengthwise. Evaluation of disease severity was analyzed as indicated in section 3.5.3.1.

3.5.3.3 Effect of Post-inoculation with *Trichoderma* spp.

Sterilized ‘Hass’ avocado fruits were individually treated at the stem end with 50µl of fungal spore suspension of the SER pathogens (5×10^4 conidial/ml).
After 24 hours, each of the treated fruits was inoculated at the stem end with 50μl spore suspension (5×10^3 conidial/ml) of each antagonist. Each of the treatments was replicated four times. The inoculated avocado fruits were placed in sterilized sealed plastic containers (separate container for each fruit). Evaluation was done after 12 days by cutting the fruits lengthwise, and evaluation of disease severity was analyzed as indicated in section 3.5.3.1.

3.5.3.4 Data analysis

The experimental data on the inhibitory effect of Milraz and Green Cop fungicide and the antagonistic activities of *Trichoderma* spp. was recorded and tabulated in spreadsheet and later exported to MinTab 17.0 software. Descriptive statistics were generated upon which the data was expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was used to analyze the statistical significance difference among treatment groups. Tukey’s post-hoc tests was used for pairwise separation and comparison of means. The hypothesis for significant differences was tested at P ≤ 0.05.
CHAPTER FOUR

RESULTS

4.1 Avocado production and management of stem end rot in Murang’a County

4.1.1 Demographics of avocado growers

4.1.1.1 Age of the farmers

All age groups within the county, from the youth to the old above 70 years were involved in avocado production. However, older farmers above 56 years were the majority (81.1%). Farmers below 35 years were the least (0.6%) followed by farmers aged 36 - 45 (4.9%). Majority of the farmers were within the age bracket of 66 -75 years (35.8%) while the oldest farmers were within the age bracket of 76 - 85 years (21.6%) (Figure 4.1)

![Age distribution of avocado farmers in Murang’a County](image)

**Figure 4.1:** Age distribution (%) of the avocado farmers in Murang’a County
4.1.1.2 Gender of the farmers

Majority of the avocado farmers were male (69.8%) while 30.2% were female (Figure 4.2).

Figure 4.2: Gender distribution (%) of the avocado farmers in Murang’a County

4.1.1.3 Education level of the farmers

Forty-seven-point five percent (47.5%) of the farmers had primary education while 34% of the farmers had acquired secondary education. Only 3.1% of the farmers had tertiary education, while 15.4% of the farmers, aged 66 and above, had no formal education (Figure 4.3).
Figure 4.3: Education level of the avocado farmers in Murang’a County

4.1.2 Avocado Farmer’s categories

Majority of the avocado farmers (84%) were fulltime farmers, 9.9% were retirees cultivating avocado fruits as the main source of income. The others were part-time avocado farmers with either other occupations (3.7%) or civil servants’ jobs (2.5%) respectively (Figure 4.4).
Figure 4.4: Categories of interviewed avocado farmers in Murang’a County

4.1.3 Avocado varieties and production system adopted by farmers in Murang’a County

Most of the avocado fruit trees cultivated (84.24%) were ‘Hass’ while 14.08% were ‘Fuerte’. Others, ‘Pinkerton’ and ‘Puebla’ were 1.68% (Table 4.1). Majority of the farmers (85.8%), intercropped avocado fruit trees with food crops (bananas, maize, beans and irish potatoes) and only 14.2% of the farmers practiced mono-cropping (Table 4.1). Farmers practiced mixed farming where they reared livestock (dairy cattle and goats) and cultivated different types of crops.
**Table 4.1:** Avocado varieties and production system adopted by farmers in Murang’a County

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cropping system</strong></td>
<td></td>
</tr>
<tr>
<td>Intercropping</td>
<td>85.8</td>
</tr>
<tr>
<td>Monocropping</td>
<td>14.2</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Fruit variety</strong></td>
<td></td>
</tr>
<tr>
<td>Hass</td>
<td>84.24</td>
</tr>
<tr>
<td>Fuerte</td>
<td>14.08</td>
</tr>
<tr>
<td>Others</td>
<td>1.68</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

### 4.1.4 Sources of planting materials

Farmers planted grafted seedlings from unregistered nurseries (private nurseries, neighbors, own farm), registered nurseries owned by the institutions (KALRO Kandara and Jomo- Kenyatta University nurseries) or top worked avocado trees in the field. During the establishment of the orchards most of the farmers (40.1%) purchased grafted seedlings, 22.2% grafted their own seedlings while 27.2% top worked old trees. Farmers had a poor perception of the best growth stage for a good scion; hence, occasionally flowering would occur at the seedling stage after grafting.
4.1.5 Income earned by avocado farmers

Majority of the avocado farmers (58%) earned the highest income from avocado fruits, while 14.8% and 6.8% of the avocado farmers earned the highest income from bananas and coffee, respectively. Only 1.9% of the avocado farmers earned the highest income from dairy farming (Figure 4.5)

![Bar chart showing farming activities earning highest income for farmers in Murang’a County](image)

**Figure 4.5:** Farming activities earning highest income for farmers in Murang’a County
4.1.6 Avocado plants owned by the farmers

The number of avocado plants owned by the farmers was significantly influenced by education \((P \leq 0.03)\), source of planting materials \((P \leq 0.02)\), cropping system \((P \leq 0.02)\) and avocado being the highest income crop \((P \leq 0.02)\) (Table 4.3). Age \((P \leq 0.26)\), gender \((P \leq 0.14)\) and farmer’s category \((P \leq 0.05)\) did not significantly influence the number of avocado plants owned by the farmers (Table 4.2).

Table 4.2: Demographic and production characteristics, influencing the number of avocado plants owned by the farmers

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>P- values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.264</td>
</tr>
<tr>
<td>Gender</td>
<td>0.146</td>
</tr>
<tr>
<td>Farmer’s category</td>
<td>0.057</td>
</tr>
<tr>
<td>Education</td>
<td>0.037*</td>
</tr>
<tr>
<td>Cropping system</td>
<td>0.022*</td>
</tr>
<tr>
<td>Source of planting materials</td>
<td>0.027*</td>
</tr>
<tr>
<td>Highest income crop (avocado)</td>
<td>0.027*</td>
</tr>
</tbody>
</table>

*Significant at \((P \leq 0.05)\)
4.1.7 Land size and number of avocado plants owned by farmers

According to the Pearson correlations test, land size significantly influenced the number of avocado plants owned by the farmers (P < 0.0001) as well as the number of ‘Hass’ avocado plants planted (P < 0.001) (Table 4.3). There was an insignificant negative correlation between land size and the number of ‘Fuerte’ avocado and other varieties planted (Table 4.3).

<table>
<thead>
<tr>
<th>Land on:</th>
<th>r- value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avocado fruits</td>
<td>0.600**</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Hass variety</td>
<td>0.903**</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Fuerte variety</td>
<td>0.108</td>
<td>P &lt; 0.170</td>
</tr>
<tr>
<td>Other varieties</td>
<td>0.009</td>
<td>P &lt; 0.908</td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level (2-tailed).

Most avocado farmers planted ‘Hass’ as compared to ‘Fuerte’ and other varieties (Table 4.4).
Table 4.4: Number of avocado plants owned by farmers

<table>
<thead>
<tr>
<th>Plants</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hass</td>
<td>23.86</td>
<td>2.64&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fuerte</td>
<td>3.99</td>
<td>0.44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Others</td>
<td>0.475</td>
<td>0.19&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean followed by the same letter in each column are not significantly different at (P≤0.05)

4.1.8 Cultural practices adopted by the farmers

Most farmers (84.6%) had not adopted the recommended field management practices neither prioritized field hygiene practices (Table 4.5). Farmers lacked knowledge on best pruning practices and randomly cut the fruit tree branches to create space for food crop production. Irrigation water was not available and only 0.6% of the farmers irrigated their orchards. Weeding of the orchards was not prioritized by most of the farmers.

Table 4.5: Cultural practices adopted by the farmers

<table>
<thead>
<tr>
<th>Management practice</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pruning</td>
<td>9.3</td>
</tr>
<tr>
<td>Weeding</td>
<td>2.5</td>
</tr>
<tr>
<td>Irrigation</td>
<td>0.6</td>
</tr>
<tr>
<td>Pruning &amp; weeding</td>
<td>3.0</td>
</tr>
<tr>
<td>None</td>
<td>84.6</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>
Majority of the farmers were not aware of the required spacing for different avocado varieties and in most of the orchards, the plants were very closely spaced forming a thick canopy that interfered with light penetration. Some of the avocado plants were very tall hence complicating harvesting.

4.1.9 Soil replenishment

Most farmers (88.9%) applied 2-3 handful of farmyard manure during planting while 9.3% applied both farmyard manure and diammonium phosphate (DAP) (Table 4.6). Majority of the farmers (80.2%) did not replenish the soil in the subsequent seasons while 14.2% applied farmyard manure only in the subsequent seasons of fruit growth (Table 4.6). The avocado fruit buying companies discouraged the application of inorganic fertilizers and encouraged organic farming of the fruits. Farmers preferred to use the little organic fertilizer available on food crop production.
Table 4.6: Fertilizer used by farmers and time of application

<table>
<thead>
<tr>
<th>During planting</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farmyard manure only</td>
<td>88.9</td>
</tr>
<tr>
<td>Farmyard manure &amp; DAP</td>
<td>9.3</td>
</tr>
<tr>
<td>DAP only</td>
<td>1.8</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subsequent application</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Farmyard or DAP</td>
<td>80.2</td>
</tr>
<tr>
<td>Farmyard manure only</td>
<td>14.2</td>
</tr>
<tr>
<td>Farmyard manure &amp; DAP</td>
<td>5.6</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

4.1.10 Harvesting and marketing of avocado fruits

Avocado fruits do not mature at the same time on a given plant; therefore, farmers adopted selective harvesting of the fruit. Moreover, availability of market influenced harvesting of the fruits. The main harvesting season was from March to May and minimal harvesting is done in the month of September and October. Harvesting was done manually by climbing the trees and a combination of handheld pole terminated with a hook and a basket are used to avoid dropping the fruits (Table 4.7). Farmers used assorted containers to transport the fruits from the orchard to the parking area within the farm (Table 4.7)
Table 4.7: Methods of harvesting and carrier containers used from the orchards to packing area within the farm

<table>
<thead>
<tr>
<th>Methods of picking</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using Hooked sticks</td>
<td>90.1</td>
</tr>
<tr>
<td>Hand-picking and hooked sticks</td>
<td>7.4</td>
</tr>
<tr>
<td>Hand-picking</td>
<td>2.5</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Carrier containers</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Containers used</td>
<td>Percentage (%)</td>
</tr>
<tr>
<td>Sisal woven baskets</td>
<td>37.0</td>
</tr>
<tr>
<td>Sacks</td>
<td>24.7</td>
</tr>
<tr>
<td>Sacks &amp; Sisal woven baskets</td>
<td>29.0</td>
</tr>
<tr>
<td>Buckets</td>
<td>6.2</td>
</tr>
<tr>
<td>Crates</td>
<td>3.1</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

Fruits were sorted and graded at the farm during harvesting. Disease-free fruits within the preferred size were picked for the export market while the rest were sold in the local market. To prepare the fruit trees for the next growing season, farmers harvested all the fruits from the trees regardless of size and disease attack (anthracnose, scab) and sold to the local market traders at the end of the harvesting season.
Farmers did not maintain production records; hence, it was not possible to establish the average number of fruits harvested per avocado plant per season from the farmers.

Farmers sold their fruits to buying companies specializing in the export market, intermediaries and local market traders. Some farmers were organized into marketing groups and buying companies bought the fruits through the groups. Kakazi Limited graded the fruits per group not per farmer lowering the prices for some individual farmers. Enrollment was voluntary and not all farmers belonged to a marketing group. Intermediaries also bought avocado fruits on behalf of the export companies from the farmers. Prices were determined through negotiations between the farmers and the traders. Payment by the avocado buying companies was not prompt and this contributed to farmers selling the fruits to the intermediaries who paid on delivery.

4.1.11 Availability of extension services

The majority (88.3%) of the farmers did not receive extension services from extension officers, while 11.7% received extension services from the fruit buying companies’ extension staff.

4.1.12 Presence of stem end rots on avocado fruits

Most of the farmers (99%) indicated that avocado fruit in their farms were affected by fruit rot infections. Although fruits showing different disease (anthracnose, scab, cercospora spot) symptoms were observed in all orchards,
farmers did not use chemicals or any other method to control the infections. According to the farmers, avocado fruit exporting companies only bought organically grown fruits. Majority of the farmers (97.5 %) were not able to differentiate stem end rot disease on the avocado fruits from anthracnose diseases and assumed all rots on avocado fruits were due to anthracnose disease.

4.1.13 Cost of harvesting and orchard accessibility

Farmers paid one shilling per fruit harvested. The harvesting cost increased the cost of production and lowered the profit. In some regions, poor road network made some farms inaccessible during the rainy season, resulting in delayed fruit harvesting. Consequently, fruits remained un-harvested beyond the maturity time leading to quality reduction and poor sales.

4.2 Incidence of stem end rot disease and stem end rot fungal pathogens on avocado fruits in Murang'a County

4.2.1 Stem end rot infection on mature avocado fruits in Murang'a County

Healthy mature fruits from the orchards and fruits at different stages of ripening from the markets were infected with stem end rot diseases at an average of 47.9 % (Table 4.8). Avocado fruits from the orchard had lower SER disease incidence compared with those from the market although there was no significant difference (Table 4.8).
The confidence interval at 95% confidence level, of avocado fruits infection from the orchard, ranged between 0.41 and 0.55 while the confidence interval of avocado fruits from the market, ranged between 0.43 and 0.62 (Table 4.8).

**Table 4.8:** Incidence of stem end rot on avocado fruits from Murang'a Country

<table>
<thead>
<tr>
<th>Source of avocado fruits</th>
<th>Percentage incidence</th>
<th>Confidence Interval at 95% confidence level</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orchards</td>
<td>45.70</td>
<td>CI; 0.41 to 0.55</td>
<td>0.43</td>
</tr>
<tr>
<td>Markets</td>
<td>52.08</td>
<td>CI; 0.43 to 0.62</td>
<td></td>
</tr>
</tbody>
</table>

**4.3 Morphological and molecular identification**

**4.3.1 Morphological characteristics of the SER isolates**

Based on colony and conidial features (Table 4.9, Plate2) seven species of stem end rot fungal pathogens were identified from the isolates.

*Lasiodiplodia theobromae* (132 fungal isolates), the colony on PDA was round and smooth. At first white aerial filamentous mycelia with grey center developed. With age, colony turned grey, then dark grey to black (Plate 1, a & b). The pycnidia were grey in colour, either simple or aggregated.
*Neofusicoccum parvum* (81 fungal isolates), the colony on PDA was rough with irregular margins. Initially, white dense filamentous aerial mycelia developed and turned dark to black with time (Plate 1, c & d). Pycnidia were black, globose and simple or aggregated.

*Nectria pseudotrichia* (61 fungal isolates), the colonies on PDA were white, cottony, with filamentous, aerial mycelia growth. The colony growth was regular and rough, with smooth margins (Plate 1, e & f).

*Fusarium solani*, colonies on PDA with white, cottony with floccose mycelium growth was observed on 24 fungal isolates. The colony margins were regular and smooth. The rate of growth was low. The underside was pale to brown in colour (Plate 1, g & h).

*Fusarium oxysporum* (17 fungal isolates), the colonies on PDA had abundant white to creamy aerial mycelia. The colony margins were smooth and sometimes slightly looped. The reverse side of the colony was pale red to peach violet in colour (Plate 1, i & j).

*Fusarium equiseti* (13 fungal isolates), the colonies on PDA were white, with abundant cottony mycelium that browned with age. Pale to dark brown diffusible pigmentation observed (Plate 1, k & l).

*Geotricum candidum* (4 fungal isolates), the colonies on PDA were not dense, white to beige colonies appressed onto culture medium with smooth margins were observed (Plate 1, m & n).
Plate 1: Characteristics of colony (reverse and front) of pathogenic isolates of SER on PDA. (a) and (b) represent reverse and front of *Lasiodiplodia theobromae* (GA11); (c) and (d) *Neofusicoccum parvum* (GA7); (e) and (f) *Nectria pseudotrichia* (GA13); (g) and (h) *Fusarium solani* (1GEF8); (i) and (j) *Fusarium oxysporum* (MS4a); (k) and (l) *Fusarium equiseti* (MS16(a)); (m) and (n) *Geotrichum candidum* (GA6).
Table 4.9: Conidia characteristics of pathogenic SER isolates

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Conidia appearance</th>
<th>Conidia Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lasiodiplodia</em> theobromae</td>
<td>Subovoid to ellipsoid. Initially, they were aseptate, thick walled and hyaline, with time they formed single medium septum and became dark brown</td>
<td>17.35 to 29.31  11.23 to 14.91µm (mean 22.68  5.70µm) (Plate 1, a) (Plate 2).</td>
</tr>
<tr>
<td><em>Neofusicoccum</em> parvum</td>
<td>Bluntly round to subovoid, aseptate, hyaline with granular content, with time they turned light brown to black.</td>
<td>19.77 to 15.25  4.10 to 7.5 µm (mean 17.01  5.70µm) (Plate 2, a) (Plate 2).</td>
</tr>
<tr>
<td><em>Nectria</em> pseudotrichia</td>
<td>Ovoid to subovoid with greenish granular content</td>
<td>6.27 to 12.50  2.20 to 9.40µm (mean 8.49  4.95µm) (Plate 3, a) (Plate 2).</td>
</tr>
<tr>
<td><em>Fusarium</em> solani</td>
<td>Microconidia were hyaline oval and some cylindrical with smooth edges.</td>
<td>Microconidia 5.02 to 8.52  2.91 to 5.50 µm (mean 6.88  3.79µm), Macroconidia were 13.05 to 34.18  2.10 to 5.50 µm (mean 18.85</td>
</tr>
<tr>
<td></td>
<td>Macroconidia were hyaline slightly curved broad with 2-3 septa</td>
<td></td>
</tr>
</tbody>
</table>
| **Fusarium oxysporum** | Microconidia ovoid to kidney-shaped without septa  
Macroconidia - thin walled, falcate to almost straight, both ends almost pointed with 2-3 septa. | Microconidia 11.2 to 19.9 μm  
4.5 to 8.4 μm (mean 15.4 μm),  
Macroconidia 22.1 to 43.9 μm  
5.1 to 12.5 μm (mean 28.4 μm 7.5 μm)  
(Plate 5, a) (Plate 2). |
|-----------------------|-------------------------------------------------|-------------------------------------------------|
| **Fusarium equiseti** | macroconidia - long and slender  
slightly curved at the ends | macroconidia - 25.3 to 46.7 μm  
3.5 to 4.6 μm (mean 37.2 μm 3.24 μm)  
were observed (Plate 6, a) (Plate 2). |
| **Geotrichum candidum** | Arthroconidia - smooth margined, hyaline, one celled, subglobose or cylindrical with either rounded or truncated apices | Arthroconidia - 6.1 to 19.7 μm  
2.3 to 10.3 μm (mean 11.38 μm 5.56 μm)  
(Plate7, a) (Plate 2). |
Plate 2: Conidia of the pathogenic isolates of SER (a). Lasiodiplodia theobromae (x400), (b). Neofusicoccum parvum (x400), (c). Nectria pseudotrichia (x400), (d). Fusarium solani (x400), (e). Fusarium oxysporum (x400), (f). Fusarium equiseti (x400), (g). Geotrichum candidum(x400).
4.3.1.1 Stem end rot fungal pathogens on mature avocado fruits in Murang’a County

The avocado fruits from the orchards and the markets were infected with several fungal pathogens. There was no significant difference in the infection of the avocado fruits from the orchards and the markets by the identified stem end rot fungal pathogens (Table 4.10). *Lasiodiplodia theobromae* had the highest incidence on fruits from both the orchards and the markets at 39.61% and 40.00% respectively, followed by *Neofusicoccum parvum* (26.57% & 20.80%) and *Nectria pseudotrichia* (18.84% & 17.60%), respectively. The other fungal pathogens; *Fusarium solani, Fusarium oxysporum, Fusarium equiseti* and *Geotrichum candidum* had a lower incidence of between 6.76% and 3.320% (Table 4.10).

Table 4.10: Stem end rot fungal pathogens on avocado fruits from Murang’a County

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Orchard % Incidence</th>
<th>Market % Incidence</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. theobromae</em></td>
<td>39.61</td>
<td>40.00</td>
<td>0.96</td>
</tr>
<tr>
<td><em>N. parvum</em></td>
<td>26.57</td>
<td>20.80</td>
<td>0.06</td>
</tr>
<tr>
<td><em>N. pseudotrichia</em></td>
<td>18.84</td>
<td>17.60</td>
<td>0.80</td>
</tr>
<tr>
<td><em>F. solani</em></td>
<td>6.76</td>
<td>8.00</td>
<td>0.68</td>
</tr>
<tr>
<td><em>F. oxysporum</em></td>
<td>4.83</td>
<td>5.60</td>
<td>0.76</td>
</tr>
<tr>
<td><em>F. equiseti</em></td>
<td>3.38</td>
<td>4.80</td>
<td>0.70</td>
</tr>
<tr>
<td><em>G. candidum</em></td>
<td>0</td>
<td>3.20</td>
<td>0.01</td>
</tr>
</tbody>
</table>

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4.3.2 Pathogenicity of the isolated fungi

The ‘Hass’ avocado fruits inoculated with mycelial agar as well as those inoculated with spore suspension developed characteristic symptoms similar to initial symptoms on the fruits from the orchards and the market (Figure 4.6), therefore confirming the Koch’s postulates. However, the SER development on the fruits differed across the different fungal species as well as the method of inoculation (Table 4.11).

When ‘Hass’ avocado fruits were inoculated with mycelia plugs, *N. pseudotrichia* caused the most severe SER (90.83%) on the cut open fruit surface, followed by *L. theobromae* (81.67%) and *N. parvum* (80.83%). However, the stem end rot development on the fruits due to *N. pseudotrichia*, *L. theobromae* and *N. parvum* did not differ significantly. *Fusarium solani*, *F. oxysporum*, *F. equiseti* and *G. candidum* caused less severe SER on the avocado fruits. There was a significant difference in stem end rot development when fruits were inoculated with *F. solani* and *F. equiseti* as compared to inoculation with *F. oxysporum* and *G. candidum*. Nevertheless, when conidial suspension of the stem end rot pathogens was inoculated on the stem end of the avocado fruits, *N. parvum* caused the most severe SER (97.50 %), followed by *L. theobromae* (84.17%) and *N. pseudotrichia* (74.17%). *Fusarium solani*, caused 60.80% SER while *F. equiseti*, *F. oxysporum* and *G. candidum* had 41.67%, 19.2% and 16.67%, respectively (Table 4.11).
Stem end rot development on the ‘Hass’ avocado fruits, when inoculated with mycelia plugs and conidial suspension of *N. parvum*, *N. pseudotrichia*, *F. solani*, *F. equiseti*, *F. oxysporum* and *G. candidum* differed significantly. However, there was no significant difference in symptoms development when the fruits were inoculated with mycelia plugs and conidial suspension of *L. theobromae* (Table 4.11). No symptoms were observed on the control fruits (Figure 4.6).
Figure 4.6: Symptoms of stem end rot on inoculated ‘Hass’ avocado fruits. a-b control c. *Geotricum candidum* d. *Fusarium equiseti* e. *F. oxysporum* f. *F. solani* g-h. *Neofusicoccum parvum* i. *Nectria pseudotrichia* j. *Lasiodiplodia theobromae*
Table 4.11: Severity of stem end rot on the cut open ‘Hass’ avocado fruit surface, after inoculation with mycelial plugs and conidial suspension of SER fungal pathogens

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Pathogen</th>
<th>Mycelia plugs % severity</th>
<th>Conidial suspension % severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ka 6</td>
<td><em>L. theobromae</em></td>
<td>81.67±4.4\textsuperscript{Aa}</td>
<td>84.17±9.70\textsuperscript{ABa}</td>
</tr>
<tr>
<td>Ga 7</td>
<td><em>N. parvum</em></td>
<td>80.83±2.39\textsuperscript{Ab}</td>
<td>97.50±1.12\textsuperscript{Aa}</td>
</tr>
<tr>
<td>Ga13</td>
<td><em>N. pseudotrichia</em></td>
<td>90.83±4.55\textsuperscript{Aa}</td>
<td>74.17±5.39\textsuperscript{Bb}</td>
</tr>
<tr>
<td>(1)G3F8</td>
<td><em>F. solani</em></td>
<td>28.33±3.80\textsuperscript{Bb}</td>
<td>60.80±11.2\textsuperscript{Ca}</td>
</tr>
<tr>
<td>MS4a</td>
<td><em>F. oxysporum</em></td>
<td>5.00±0.00\textsuperscript{Ca}</td>
<td>19.2±6.7\textsuperscript{Eb}</td>
</tr>
<tr>
<td>(1)GF17</td>
<td><em>F. equiseti</em></td>
<td>24.17±0.83\textsuperscript{Bb}</td>
<td>41.67±6.91\textsuperscript{Da}</td>
</tr>
<tr>
<td>Ga 6</td>
<td><em>G. candidum</em></td>
<td>6.67±1.05\textsuperscript{Cb}</td>
<td>16.67±3.07\textsuperscript{Ea}</td>
</tr>
<tr>
<td>Control</td>
<td>Control</td>
<td>0.00±0.00\textsuperscript{C}</td>
<td>0.00±0.00\textsuperscript{F}</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± standard error of mean. Values followed by different lowercase superscript across the rows and different uppercase superscript along the columns are statistically significant at P≤0.05.

4.3.3 Molecular identification of the isolated stem end rot pathogens

4.3.3.1 DNA amplification of the stem end rot pathogens

To support the morphological identification of the samples, markers ITS5 & ITS4, and ITS1 & ITS5 were used for identification and consistently yielded high levels of species discrimination. The PCR amplification for the ITS yielded products of 526 to 550 bp. A single band was obtained when the PCR products were run on agarose gel (Figure 4.7 & 4.8).
Figure 4.7: Agarose gel amplification of PCR products for ITS region of *Fusarium oxysporum* 1, *Fusarium solani* 2, 6, 7, *Neofusicoccum parvum* 3, *Lasiodiplodia theobromae* 4, 9, *Fusarium equiseti* 5, 8, *Nectria pseudotrichia* 10, *Geotricum candidum* 11, 12

Figure 4.8: Agarose gel amplification of PCR products for ITS region of *Fusarium oxysporum* 1, *Fusarium solani* 2, 6, 7, *Neofusicoccum parvum* 3, *Lasiodiplodia theobromae* 4, 9, *Fusarium equiseti* 5, 8, *Nectria pseudotrichia* 10, *Geotricum candidum* 11, 12
4.4 Phylogenetic of isolated stem end rot pathogen

The molecular identification of the fungal pathogens was inferred from 12 sequences. They included *F. equiseti* 2 sequence (MK922072, MK922069), *F. oxysporum* 1 sequence (MK922065), *F. solani* 3 sequence (MK922070, MK922071 and MK922066), *G. candidum* 2 sequence (MK215811, MK922075), *L. theobromae* 2 sequence (MK922068, MK922073), *N. parvum* 1 sequence (MK922067) and *N. pseudotrichia* 1 sequence (MK922074). The closest match between isolates from this study and those mined from the GeneBank ranged from 99-100% similarity and as shown in Table 4.12.
**Table 4.12:** GeneBank accession numbers obtained for isolates in this study and those from the NCBI GenBank database used in species identification from ITS sequences

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate</th>
<th>ITS Sequence from this study</th>
<th>ITS Reference sequence from GeneBank</th>
<th>Percentage similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. equiseti</em></td>
<td>2MS16a</td>
<td>MK922072</td>
<td>MG274307</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>1GF17</td>
<td>MK922069</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>F. oxysporum</em></td>
<td>MS4a</td>
<td>MK922065</td>
<td>MK590412</td>
<td>99</td>
</tr>
<tr>
<td><em>F. solani</em></td>
<td>MS47b</td>
<td>MK922070</td>
<td>GQ229075</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>MS37a</td>
<td>MK922071</td>
<td>KX688164</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>1G3F8</td>
<td>MK922066</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. candidum</em></td>
<td>GA6a</td>
<td>MK215811</td>
<td>HG936031</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>GA6</td>
<td>MK922075</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. theobromae</em></td>
<td>GA11</td>
<td>MK922068</td>
<td>KP872340</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>KA6</td>
<td>MK922073</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>N. parvum</em></td>
<td>GA7</td>
<td>MK922067</td>
<td>HQ832811</td>
<td>99</td>
</tr>
<tr>
<td><em>N. pseudotrichia</em></td>
<td>GA13</td>
<td>MK922074</td>
<td>MG800781</td>
<td>99</td>
</tr>
</tbody>
</table>
4.5 Sensitivity of stem end rot pathogens against selected fungicides and
*Trichoderma* species

4.5.1 Effects of Green Cop 500WP (Copper oxychloride 500g/Kg) and Milraz
76WP (Propineb 70% and cymoxaxil 6%) fungicides on the mycelia growth
of *F. solani, L. theobromae, N. pseudotrichia* and *N. parvum*

The mycelial growth of all the pathogens (*F. solani, L. theobromae, N.
pseudotrichia* and *N. parvum*) was inhibited by both Copper oxychloride and
milraz. The percentage inhibition increased with increase in concentration of the
fungicides. The inhibition percentage of *F. solani* increased with increase in
concentration of Copper oxychloride up to 74%. There was no significant
difference in inhibition of *F. solani*, between the three concentrations (0.25g/l,
0.5g/l and 1.0g/l) of milraz. There was a significant difference in percentage
inhibition between 0.131g/l, and 0.625g/l, concentrations of Copper oxychloride.
In all the concentrations of Copper oxychloride there was no mycelial growth of *N.
pseudotrichia*. The mycelial growth inhibition of *N. parvum* and *L. theobromae*
increased with increase in concentration of Copper oxychloride and at 1.25g/l,
there was no mycelial growth. Percentage inhibition of *N. parvum* was
significantly different among the three concentrations of Copper oxychloride
(Table 4.13). The highest percentage inhibition was attained at 1.0g/l of milraz
concentration where *N. parvum* was the most inhibited at 78% and *N.
pseudotrichia* least inhibited at 45%. The mycelial growth of *N. pseudotrichia* and
*L. theobromae* was significantly inhibited between 0.5g/l and 1.0g/l concentration of Milraz. The mycelial growth *N. parvum* was significantly inhibited between 0.25g/l and 0.5g/l concentration of milraz fungicide (Table 4.13).

**Table 4.13:** Percentage inhibition of Green cop and Milraz on the mycelia growth of *Neofusicoccum parvum, Lasiodiplodia theobromae, Nectria pseudotrichia* and *Fusarium solani*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(g/l)</th>
<th><em>F. solani</em></th>
<th><em>L. theobromae</em></th>
<th><em>N. pseudotrichia</em></th>
<th><em>N. parvum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Green cop Milraz</td>
<td>0.131</td>
<td>70.51±0.51aC</td>
<td>12.80±3.94cD</td>
<td>100.00±0.00aA</td>
<td>82.62±1.35bB</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>50.45±1.60bA</td>
<td>27.13±9.37cC</td>
<td>28.05±2.77cB</td>
<td>73.48±1.52aB</td>
</tr>
<tr>
<td></td>
<td>0.625</td>
<td>71.61±0.38aB</td>
<td>46.34±5.57bC</td>
<td>100.00±0.00aA</td>
<td>89.63±3.54bA</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>49.76±0.89bA</td>
<td>48.48±4.52bB</td>
<td>32.32±5.02cAB</td>
<td>78.66±0.61aA</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>74.86±1.66aB</td>
<td>100.00±0.00aA</td>
<td>100.00±0.00aA</td>
<td>100.00±0.00aC</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>55.94±0.86bcA</td>
<td>60.37±4.30bA</td>
<td>45.27±3.64cA</td>
<td>78.35±1.26aA</td>
</tr>
</tbody>
</table>

Values are expressed as Means ± SEM. The comparison was done among the pathogens on same day. Means within respective columns followed by different upper-case superscripts are significantly different at P ≤0.05; means within respective rows followed by different lowercase superscripts letters are significantly different at P ≤ 0.05

### 4.5.2 Growth inhibition of SER pathogens by *Trichoderma* spp. in dual culture

All the *Trichoderma* species reduced the mycelial growth of the four (*L. theobromae, N. parvum, N. pseudotrichia* and *F. solani*) avocado stem end rot pathogens. The highest mycelial growth inhibition of *L. theobromae* was given by
T. harzianum followed by T. atroviride. Trichoderma asperellum and T. virens gave the least radial growth inhibition against L. theobromae (Table 4.14).

Trichoderma atroviride had the highest mycelial growth inhibition against N. parvum (48%), N. pseudotrichia (55%) and F. solani (32.95%). Trichoderma atroviride significantly (p ≤ 0.05) inhibited the mycelial growth of N. parvum, N. pseudotrichia and F. solani compared to the other antagonists. Trichoderma asperellum was the least effective in inhibiting the mycelial growth of all the pathogens (L. theobromae (29.88%), N. parvum (14.50%), N. pseudotrichia (25%), and F. solani (14 %) (Table 4.14). Although T. virens inhibited the mycelial growth of all the pathogens, the highest inhibition was on N. pseudotrichia at 45% inhibition.

Table 4.14: Antagonistic activity of Trichoderma spp. against SER fungal pathogen in vitro.

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>% Mycelial growth inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T. asperellum</td>
</tr>
<tr>
<td>L. theobromae</td>
<td>29.88±3.94&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>N. parvum</td>
<td>14.50±2.22&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>N. pseudotrichia</td>
<td>25.00±0.58&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>F. solani</td>
<td>14.00±4.76&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM for four replicates per group. Statistical comparison were made within a row and values with the same superscript are not significantly different by one-way ANOVA followed by Tukey’s post hoc test (p ≤ 0.05).
4.5.3 Effect of *Trichoderma* spp. against SER on ‘Hass’ avocado fruits, post-harvest

All *Trichoderma* spp. inhibited the development of SER on avocado fruits. Fruits treated with *T. asperellum* at the three inoculation stages (pre-inoculation, concurrent inoculation and post-inoculation) remained free from SER caused by *F. solani*. Similarly, the severity of SER by *L. theobromae* was significantly (*p < 0.05*) reduced up to 10, 7.5 and 5% in the three tests, respectively. During the three inoculations, *T. asperellum* reduced SER on avocado fruits by *N. parvum* up to 30, 55 and 40% respectively. There was no development of SER by *N. pseudotrichia* during concurrent inoculation with *T. asperellum*, however, during pre-inoculation and post inoculation SER severity reduced to 20 and 7.5% respectively (Table 4.15).

All fruits remained free from SER due to *N. parvum*, *N. pseudotrichia* and *F. solani* during concurrent and post-inoculation with *T. atroviride*. *Trichoderma atroviride* did not inhibit development of SER on the fruits by *L. theobromae* during concurrent and post-inoculation. During pre-inoculation, the fruits remained free from SER development due to *N. pseudotrichia*. Similarly, the severity of SER due to *L. theobromae*, *N. parvum*, and *F. solani* was reduced to 5, 7.5 and 7.5% respectively during pre-inoculation with *T. atroviride* (Table 4.15).

When *T. harzianum* was applied post-inoculation with *L. theobromae*, *N. parvum*, *N. pseudotrichia* and *F. solani*, no SER developed on the avocado fruits. Besides, when *T. harzianum* was applied concurrently with *N. parvum*, *N.
*pseudotrichia* and *F. solani* the fruits remained free from SER. *Trichoderma harzianum* did not inhibit development of SER on the avocado fruits caused by *L. theobromae* during concurrent inoculation and *N. pseudotrichia* during pre-inoculation (Table 4.15).

All fruits remained free from SER when *T. virens* was inoculated 24 hours after the fungal pathogen. Similarly, during pre-inoculation, the avocado fruits remained free from SER caused by *N. pseudotrichia* and *F. solani* while in concurrent inoculation no SER developed on the fruits due to *N. parvum* and *F. solani*. The severity of SER due to *L. theobromae* was reduced up to 42.5% in both pre-inoculation and concurrent inoculation with *T. virens* (Table 4.15). *Trichoderma atroviride* was most effective in controlling the development of SER by *N. parvum*, *N. pseudotrichia* and *F. solani* in all treatments, while, *Trichoderma virens* and *T. harzianum* were most effective when applied post-inoculation (Table 4.15).
Table 4.15: Efficacy of *Trichoderma* spp. against SER on ‘Hass’ avocado fruits post-harvest

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Disease severity index %</th>
<th>Pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>N. pseudotrichia</em></td>
<td><em>N. parvum</em></td>
</tr>
<tr>
<td>Pre-inoculation</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. asperellum</em></td>
<td>20.00±20.0b</td>
<td>30.00±10.00b</td>
</tr>
<tr>
<td><em>T. atroviride</em></td>
<td>0.00±0.00c</td>
<td>5.00±5.00c</td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>90.00±0.00a</td>
<td>30.00±15.0b</td>
</tr>
<tr>
<td><em>T. viridans</em></td>
<td>0.00±0.00c</td>
<td>35.00±35.0a</td>
</tr>
<tr>
<td>Concurrent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>inoculation</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. asperellum</em></td>
<td>0.00±0.00a</td>
<td>55.00±0.00a</td>
</tr>
<tr>
<td><em>T. atroviride</em></td>
<td>0.00±0.00a</td>
<td>0.00±0.00c</td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>90.00±0.00a</td>
<td>17.50±2.50b</td>
</tr>
<tr>
<td><em>T. viridans</em></td>
<td>7.50±7.50b</td>
<td>0.00±0.00c</td>
</tr>
<tr>
<td>Post-inoculation</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. asperellum</em></td>
<td>7.50±7.50a</td>
<td>40.00±0.00a</td>
</tr>
<tr>
<td><em>T. atroviride</em></td>
<td>0.00±0.00b</td>
<td>0.00±0.00b</td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>0.00±0.00b</td>
<td>0.00±0.00b</td>
</tr>
<tr>
<td><em>T. viridans</em></td>
<td>0.00±0.00b</td>
<td>0.00±0.00b</td>
</tr>
<tr>
<td>Control</td>
<td>90.00±0.00a</td>
<td>60.00±0.00a</td>
</tr>
</tbody>
</table>

Values are expressed as Means ± SEM for four avocado fruits per group. Means within respective columns followed by different lower-case superscripts are significantly different at p ≤0.05

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CHAPTER FIVE

DISCUSSION

5.1 Avocado fruits production and management practices in Murang'a County

Since year 2000, the acreage under avocado production in Kenya has increased significantly, supporting increased export of the avocado fruit from Kenya (Johnny et al., 2019). Increased production is fueled by high demand for avocado fruits in the global market due to consumer awareness of the fruit's dietary value (Magwaza & Tesfay 2015). Gender is a significant factor in Africa and culturally land tenancy and utilization is associated with men who are the household heads (Abang et al., 2014; Blackden et al., 2007). Consequently, land ownership in Kenya is controlled mainly by the household heads who are male in most cases. They manage production and income from the farm.

According to Njuki et al. (2004), in Kenya's central highlands, men are more involved in cash crop production, while women are more involved in food crop production. Currently, avocado is the main cash crop in Murang'a County since coffee has declined in production and sale (Njenga, 2016). The decline in income from coffee could explain the shift to avocado farming. Migration of men to urban areas in search of jobs might have contributed to a considerable number of women managing the orchards (Njuki et al., 2004). Nkari & Kibera (2016) reported that farmer's age, gender and education influence production performance. However, age and gender did not influence the production and
marketing of the avocado fruits. These corroborate reports by Ngeywo et al. (2015), where age and gender did not affect coffee production.

Farmers of all age groups were involved in avocado production. However, most farmers (81.1%) were above 56 years of age, representing the retired population. The findings contrasted Megerssa & Alemu, (2013) reports, where avocado farmers from Ethiopia had a mean age of 41.33 years. The presence of high number of avocado growers who are advanced in age was attributed to fear of losing a livelihood, failure of the farmers to hand over farms to their offspring as well as migration of the young generation to the urban areas in search of jobs and business opportunities.

The farmers' lack of proper record keeping could explain their inability to give the average number of fruits they harvested per tree per season. Poor record keeping could have been contributed by lack of training by extension service providers on necessary farm management skills.

According to Nkari & Kibera (2016), academic qualification influences the farmer’s managerial skills. Further, Saina et al. (2012) established that farmers that had at least secondary school education were more efficient, self-reliant, and are capable of solving farming problems. This is in agreement with the findings of this study. Education significantly influenced the number of avocado plants owned by the farmers and, in return, influenced the quantity of production. However, education did not significantly influence the sale of the avocado fruits in both export and local markets. According to Amare et al. (2019), poor quality fruits,
high regulatory standards in the export markets, and weak organization of avocado markets are the key factors that influence the export of avocado fruits from Kenya. The report could probably explain the insignificant contribution of education to marketing of the avocado fruits in this study.

The flowering of seedlings soon after grafting suggests that farmers used old scions and pointed to the inaccessibility of reputable nurseries that could supply certified seedlings at affordable cost for commercial farming of avocado fruits. The findings were similar to results in other avocado growing areas in the world. For example, in Australia, the avocado industry is regulated, and the country has well developed nursery scheme for the production of true – to – type and disease-free seedlings since 1970 (FAO 1999). The lack of specialized nurseries comes with a share of challenges; plants fail to breed true to their parental line resulting in heterogeneity of production. These could affect flowering, fruiting, maturity time, and size of the fruit and to a large extent, marketability of the fruit.

According to Wertheim-Heck, (2010), intercropping avocado with annual crops is a common practice in Sub-Sahara Africa as a way of utilizing the empty spaces before the plant establishes itself. A similar trend was observed in the study area. Farmers preferred intercropping avocado plants with food crops. Small land sizes, food security considerations and diversification to avoid total loss contributed to the trend. The findings were consistent with Shumeta, (2010)
reports where avocado farmers in Ethiopia intercropped avocado fruits with food crops.

‘Hass’ was more popular than ‘Fuerte.’ The findings differed with Wasilwa et al. (2006) who reported high popularity of ‘Fuerte’ in Kenya. The difference in prevalence was attributed to change in global market demand where consumers prefer ‘Hass’ fruit due to its keeping quality and taste. A similar trend has been reported in Israel, Australia and California, where ‘Hass’ avocado fruit is increasing in production (Lazicki et al., 2016; Hofshi, 1996).

High fruit infection in this study area was similar to study reported by Nyambati & Kioko (2018) & Wasilwa et al. (2006). Fruit infection influenced both export and local markets. Therefore, controlling SER infections on avocado fruits could lead to increased export and local market sales, resulting in increased farmer’s income.

Farmers who had the highest income from avocado fruits sold more ‘Hass’ avocado fruits to the export market, suggesting that controlling fruit infections could result to improved export of the produced fruits. Farmers with large land sizes produced more fruits, indicating that if the avocado farmers had access to large pieces of land, they could increase avocado fruit production and sale to the export market. Avocado fruits that failed to meet the export market quality requirements were sold in the local market, explaining why increased sales in the local market did not increase the production of the avocado fruits.
Farmer's failure to adopt the recommended management practices resulted in a thick canopy that compromised the growth and production of the crop. The accepted spacing for most of the commercial avocado varieties is 5.5m by 3m. However, a slightly bigger spacing can be allowed for the vigorously growing trees (Stassen et al., 1999). Thick canopies created a conducive environment for the buildup of the fungal spores that cause avocado fruit diseases. Dead branches, leaves and infected fruits usually conserve many spores that infect healthy fruits (Coates et al., 2001). This could explain the presence of high fruit infections in the study area. Field hygiene practices, including pruning, removal of dead branches and leaves and infected fruits, is an essential strategy in managing fruit diseases (Twizeyimana et al., 2013).

5.2 Morphological and molecular characteristics of identified stem end rot pathogens

Lasiodiplodia theobromae, Neofusicoccum parvum, Nectria pseudotrichia, Fusarium solani, Fusarium oxysporum, Fusarium equiseti and Geotrichum candidum caused avocado SER in the study area. The identified pathogens have been associated with SER of avocado fruits in other avocado growing regions in the world such as North America (California), Chile, South Africa and Italy (Valencia et al., 2019; Guarnaccia et al., 2016; Twizeyimana et al., 2013; Darvas, 1987). From the current study, L. theobromae was the most frequently isolated pathogen followed by N. parvum and N. pseudotrichia. The findings are in
agreement with report by Galsurker et al. (2018) that identified *L. theobromae* as an emerging pathogen of fruits SER worldwide. The pathogen has been associated with SER of mangoes and pawpaw (Honger et al., 2015; da Silva Pereira, et al., 2012) and identified as a major pathogen that causes post-harvest disease of many fruits (Mohamed & Saad, 2009).

Further, results corroborate findings from other avocado growing regions of the world where members of *Botryosphaeriaceae* spp. are reported to cause SER of avocado fruits. For example in South Africa, Italy, California and New Zealand. In South Africa *N. pseudotruchia* was the most frequently isolated pathogen and occasionally, *L. theobromae* was isolated. In Italy, California and New Zealand *N. parvum* was the most frequently isolated pathogen (Valencia et al., 2019; Guarnaccia et al., 2016; Twizeyimana et al., 2013; Hartill, 1991; Darvas, 1987). Temperatures influence the SER pathogen predominance in an area. *Botryosphaeriaceae* spp. thrive in high temperature while water stress stimulates latent infections by the species ( Galsurker et al., 2018; Ploetz, 2003; ). The avocado fruit production in Murang'a County is concentrated on the lower region of the County that is characterized by temperature ranges of between 18.0 to 27.2 °C (Jaetzold et al., 2007). This could explain why *L. theobromae* and *N. parvum* were the most often isolated fungal pathogens in this study.

In California, *N. parvum* was associated with other species of *Botryosphaeriaceae* (*N. australre, N. luteum, Fusicoccum aesculi, Dothiarella iberica*) (Twizeyimana et al., 2013). However, in this study, only *Neofusicoccum*
parvum was isolated; similar to reports on stem end rots pathogen of avocado fruits in Italy (Guarnaccia et al., 2016). Fusarium species: F. solani, F. oxysporum, F. equiseti were found to be minor pathogens of SER in Kenya. Similar findings were reported in South Africa and New Zealand (Hartill, 1991; Darvas, 1987). Moreover, Fusarium spp. were reported to be causes of avocado rots in Ethiopia (Kebede & Belay, 2019). Geotrichum candidum was exclusively isolated from four avocado fruits from the market and none from the orchards. The pathogen has been associated with sour rots of tomatoes, citrus fruits and vegetables (Thornton et al., 2010). The avocado fruits were bought from the open-air market, where fruits are usually placed together with other fruits like citrus and bananas. The pathogen could have been from the citrus fruits and penetrated the avocado fruits through the stem end scar. Colletotrichum gloeosporioides previously recovered from SER of avocado in Italy and California (Twizeyimana et al., 2013; Darvas, 1987) was not isolated from stem end of the avocado, comparable to reports from Chile (Valencia et al., 2019). Moreover, Twizeyimana et al. (2013) identified C. gloeosporioides as a weaker avocado SER pathogen and is only important when in combination with other stem end rot pathogens.

Morphological characteristics together with DNA analysis were used to identify and differentiate L. theobromae, N. parvum, and N. pseudotrichia. Lasiodiplodia theobromae grew fast and colonized the Petri dish in two days. Neofusicoccum parvum and N. pseudotrichia colonized the Petri dish in four and five days, respectively. The three pathogens showed almost similar morphological
features. However, ITS sequences of these fungi clearly allowed the differentiation of the species. *Lasiodiplodia theobromae* was the most isolated pathogen from fruits from both orchards and markets, followed by *N. parvum* and *N. pseudotrichia*.

During pathogenicity studies, the three pathogens (*L. theobromae*, *N. parvum* and *N. pseudotrichia*) also caused the most severe SER on avocado fruits. The three pathogens are, therefore, identified as the main causal agents of avocado SER in Kenya.

### 5.3 Efficacy of fungicides and *Trichoderma* spp. against *L. theobromae*, *N. parvum*, *N. pseudotrichia* and *F. solani*

Fungicides are reported to control stem end rot fungal pathogens of fruits effectively, for instance, mangoes (Saeed et al., 2017; Javaid et al., 2008). However, post-harvest fungicides against SER pathogens of avocado fruits have hardly been tested. In Kenya, and there are no registered fungicides for use by farmers against SER pathogens of avocado fruits. Two fungicides Milraz 76WP (Propineb 70% and cymoxaxil 6%) and Green Cop 500WP (Copper oxychloride 500g/Kg), reported to control *C. gloeosporioides* effectively and also reported to be used by farmers against all avocado rots by Kimaru et al. (2018) were tested for their sensitivity against identified stem end rot pathogens *in-vitro*.

*Lasiodiplodia theobromae*, *N. parvum* and *N. pseudotrichia* were highly sensitive to Green Cop (Copper oxychloride 500g/L) and no mycelial growth was
observed at 1.25g/l concentration of Copper oxychloride. Similarly, Milraz 76WP (Propineb 70% and Cymoxanil 6%) was found to significantly inhibit the mycelial growth of the four pathogens that caused SER on avocado fruits. The findings were contrary to reports by Galsurker et al. (2018), where Prochloraz, a non-systemic imidazole, was less effective in controlling SER of avocado fruits but effective against anthracnose.

Application of copper oxychloride combined with mancozeb, from flowering to harvesting, was reported to reduce SER of mangoes caused by *L. theobromae*, a member of *Botryosphaeriacae* family (Galsurker et al., 2018). The report corroborates results from this study where there was no mycelial growth of the members of *Botryosphaeriacae* family (*Lasiodiplodia theobromae, N. parvum* and *N. pseudotrichia*) at 1.25g/l concentration of copper oxychloride. Similarly, copper oxychloride at 1.25 g/l concentration was the most effective (74% inhibition) against the mycelial growth of *F. solani*. In a similar study conducted against *F. solani* isolated from tea (*Camellia sinensis*), copper oxychloride was most effective at 85% inhibition (Kumhar et al., 2015). Similarly, Everett et al. (2005) reported high efficacy of copper oxychloride against *C. gloeosporioides, Botryosphaeria parva, B. dothidea* and *Phomopsis* sp. causing both the stem end and body rots.

Although Milraz 76WP (Propineb 70% and Cymoxanil 6%) fungicide was effective against the four pathogens, none of the pathogens was completely
inhibited and the highest inhibition was at 78% for *N. parvum* while the other pathogens had a lower inhibition percentage.

The ability of *T. harzianum* to inhibit the mycelial growth of *L. theobromae* reported in this study was in agreement with a study by Wijeratnam et al. (2008) where *T. harzianum* was reported to effectively control *L. theobromae* that caused SER of papaya and mangoes in Sri Lanka. Similarly, Bhadra et al. (2014) reported the greatest inhibition of *L. theobromae* by *T. harzianum* in concurrent inoculation. Moreover, *T. harzianum* was reported to significantly reduce stem end rot of Rambutan caused by *L. theobromae* (Galsurker et al., 2018).

In this study, *T. atroviride* was the most effective against *F. solani* as compared to *T. asperellum*, *T. harzianum* and *T. virens*, corroborating results by Kumar et al., (2017) that reported higher efficacy of *T. atroviride* against *F. solani* compared to *T. harzianum*. Rajendiran et al. (2010) also reported strong antagonistic activity of *T. atroviride* against *Fusarium* species that caused post-harvest rots of fruits. *Trichoderma atroviride* inhibited the mycelial growth of *L. theobromae* up to 36.28%, although the inhibition was lower than that of *T. harzianum* (54.57%), *T. atroviride* has been reported to be effectively control *L. theobromae* that cause stem end rot of mangoes. *Trichoderma virens* inhibited the mycelial growth of *L. theobromae* corroborating report by Buensanteai & Athinuwat (2012).
Trichoderma asperellum inhibited the mycelial growth of L. theobromae by 29.88%. Trichoderma asperellum strain NG-TI61 was previously reported not to have any antagonistic activity against L. theobromae in-vitro. However, the conidia and culture filtrates of T. asperellum controlled the rot caused by L. theobromae on the banana fruits (Adebesin et al., 2009), corroborating results in this study.

Trichoderma atroviride stood out in the control of N. parvum, N. pseudotrichia and F. solani while T. harzianum stood out in the control of L. theobromae during the in vitro test and post-harvest treatment of the avocado fruits. Similarly, studies conducted by Borges et al. (2018) on biocontrol of teak canker caused by L. theobromae showed a positive correlation between the in vivo and in vitro studies. Trichoderma atroviride showed higher efficacy than T. harzianum against L. theobromae, N. parvum and N. pseudotrichia during pre-inoculation. In the evaluation of biocontrol agents for grapevine pruning wound protection, Kotze et al. (2011) reported that T. atroviride was more effective than T. harzianum against L. theobromae and N. parvum when it was applied on the wound before the pathogens, corroborating results from this study. Similarly, Valenzuela et al. (2015), reported high efficacy of T. atroviride against C. gleosporiodes when it was inoculated 24 hours before the pathogen. The results could suggest that the bioactivity nature of the T. atroviride against fungal pathogens is protective.
*Trichoderma asperellum* showed high efficacy against *L. theobromae* on post-harvest avocado fruits, contrary to what was expected since in the *in vitro* test, *T. asperellum* displayed an inhibition percentage of only 29%. However, this is comparable to report by Borges et al. (2018) in the *in vivo* test of *T. asperellum* against *L. theobromae* causing teak canker, where *T. asperellum* showed complete control of *L. theobromae*.

When the fungal pathogens were applied on post-harvest avocado fruits 24 hours before the antagonists, *T. asperellum, T. harzianum* and *T. virens*, showed high efficacy against the four SER fungal pathogens. Likewise, *T. atroviride* showed complete efficacy against *N. parvum, N. pseudotrichia* and *F. solani* indicating the ability of *Trichoderma* spp. to control the fungal pathogens even when they have established on the fruits.
CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Farmers did not use any management strategy to control stem end rot infections on the avocado fruits. Most farmers were not able to differentiate stem end rot from other fruit rot diseases for instance anthracnose disease. The incidence of the stem end rot disease on avocado fruits from Murang’a County is generally high and the fruits from the orchards had slightly lower disease incidence compared to fruits sold in the local market.

Fungal pathogens *L. theobromae, N. parvum, N. pseudotrichia, F. solani, F. oxysporum, F. equiseti* and *G. candidum* were for the first time in Kenya identified to cause stem end rot of avocado fruits. *Lasiodiplodia theobromae* was the most frequently isolated pathogen from the avocado fruits from both orchards and markets, followed by *N. parvum* and *N. pseudotrichia*. The three pathogens are therefore, identified as the main causal agents of avocado SER in Kenya.

The SER fungal pathogens *L. theobromae, N. parvum, N. pseudotrichia* and *F. solani* were inhibited by Milraz76WP (Propineb 70% and Cymoxanil 6%) and Green Cop (copper oxychloride 500g/L) *in-vitro*. Green Cop (copper oxychloride 500g/L) was effective than Milraz76WP (Propineb7 0% and Cymoxanil 6%).

*Trichoderma* spp. *T. asperellum, T. harzianum, T. atroviride* and *T. virens* inhibited the mycelial growth of *L. theobromae, N. parvum, N. pseudotrichia* and
F. solani in dual culture. Trichoderma harzianum was more effective against L. theobromae while T. atroviridae was more effective against N. parvum, N. pseudotrichia and F. solani in vitro. Trichoderma asperellum, T. harzianum, T. virens and T. atroviride have the potential to control SER of avocado fruits.

6.2 Recommendations

- Pre-harvest and post-harvest management practices of avocado fruits in the country should be established and avocado farmers trained on crop husbandly practices.

- Field trials for the fungicides Milraz 76WP (Propineb 70% and Cymoxanil 6%) and Green Cop (copper oxychloride 500g/L) on the control of L. theobromae, N. parvum, N. pseudotrichia and F. solani in avocado are recommended. Agrochemical manufacturers to be involved in the field trials so that obtained data can be used to extend the label of the fungicides to include avocado disease.

- The conidia and culture filtrates of the Trichoderma spp. should be tested against the SER pathogens (L. theobromae, N. parvum, N. pseudotrichia and F. solani) on the avocado fruits. Field trials for the Trichoderma spp. (T. asperellum, T. harzianum, T. atroviride and T. virens) on the control of L. theobromae, N. parvum, N. pseudotrichia and F. solani in avocado are recommended.
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APPENDICES

Appendix 1: Survey questionnaire used to collect data on production and management practices adopted by avocado farmers in Murang’a County, Kenya.

SECTION A: Questionnaire identification

Date of the interview ……………… Enumerator code ……………

Location ………………………………………………………………………

GPS readings ………………… ………………………………………

SECTION B: Household information

1. Name of the farmer ………….Gender ……………… Age …………

2. Level of education ………. Occupation of the ……………………

BI: land use information

3. Size of your farm in acres ………………………………………

4. Out of the total land which area is under;

5. Food crop ……..Avocado fruits ……..Other cash crop ……………

6. Rank the top four (4) farm enterprise in your farm in terms of income generation and family food availability.

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<thead>
<tr>
<th>Income generation</th>
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3. ........................................ 3. ........................................

7. What are the other sources of income for the household?
   ........................................................................................................

**B2: Avocado varieties**

8. Which varieties of avocado fruits do you grow?

   ‘Fuerte’ ............ ‘Hass’ ............ others specify
   ........................................................................................................

9. Is it intercrop or monocrop? .................................................................

10. Where do you source your planting materials from?

**Section C 1: Production levels**

11. How long have you grown avocado fruits ...............................

12. How many avocado trees do you have in the farm ......................

13. Do you intend to expand your production .................................

14. If yes why? .................................................................

15. If no why? .................................................................

**C2: Income from avocado fruits and marketing**

16. How many avocado fruits do you harvest per plant?

17. What is the cost of production and income from Avocado fruits per year?

18. What percentage of your avocado fruits do you sell to the export market?

19. What percentage of your avocado fruits do you sell to the local market?

20. Do you process the avocado fruits ...............................
21. If yes how? 

22. What are the challenges you experience when marketing the fruits?

SECTION D: Orchard management

D1: Avocado nutrition

23. Do you apply fertilizers on your avocado fruits?

24. If yes which type of fertilizers do you apply?

25. At what stage do you apply your fertilizers?

26. How often do you apply the fertilizers?

27. If no give reasons for not using the fertilizers

D2: Fruit rot diseases incidence and their effect on income

28. Are your ‘Hass’ avocado fruits affected by stem end rots diseases?

29. What are the diseases symptoms you observe on fruits, stem and leaves?

30. Leaves

31. Fruits

32. Stem

33. How often do you experience the disease symptoms?

34. Do these diseases symptoms affect sale of your produce?

35. If yes how?

36. Do you control diseases during fruit development?
37. If yes how? ……………………………………………………………

38. Do you treat the avocado fruits after harvesting?

39. If yes how? ……………………………………………………………

40. What other production challenges do you experience during production

…………………………………………………………………………………

Appendix 2: Map showing distribution of interviewed farmers and main Markets
Appendix 3: Coordinates showing location of interviewed farmers

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