PREVALENCE AND INCIDENCE OF ANTHRACNOSE OF PAWPAW (*Carica papaya*), CHARACTERIZATION OF *Colletotrichum gloesoporoides* AND CONTROL USING PLANT EXTRACTS IN BARINGO AND ELGEYO-MARAKWET COUNTIES (KENYA)

BY

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DECLARATION

Declaration by the student

This thesis is my original work and has not been submitted for any academic award in any institution; and shall not be reproduced in part or full, or in any format without prior written permission from the author and/or University of Eldoret.

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DEDICATION

This thesis is dedicated to my children Sharon Jepchirchir, Ian Kibiwott, Lampard Kipkemoi and Sandra Jepkoech, my uncle Elijah Kiptoon Toroitich, my mother Linah Tarkok Toroitich and my father John Rotich Arap Chebet.

ABSTRACT

Papaya is the fourth most important fruit crop in Kenya after oranges, mangoes and bananas. It has also become an important export crop particularly in the arid and semi-arid lands. Anthracnose caused by *Colletotrichum gloeosporioides* results in huge economic losses. Therefore information on the epidemiology of the disease and its associated vector as well as control methods are vital in the management of the disease. The aim of the study was to determine the epidemiology of anthracnose in pawpaw, characterize the pathogen and determine the efficacy of plant extracts in controlling the pathogen. Specifically the study determined: the prevalence, incidence and severity of anthracnose in *Carica papaya* fruits; characterization of *Colletotrichum gloeosporoides* isolates infecting *Carica papaya* fruits; evaluated the *in-vitro* efficacy of plant extracts against isolates of *Colletotrichum gloeosporoides*; and determine the *in-vivo* efficacy of plant extracts against *Colletotrichum gloeosporoides* on anthracnose infected papaya fruits in Baringo and Elgeyo-Marakwet Counties in Kenya. To determine the prevalence, incidences and severity of anthracnose disease, field surveys were conducted in major pawpaw-growing areas of Baringo and Elgeiyo Marakwet Counties from March 2015 to June 2015, during fruiting stage, using the line transect method. A total of 32 farms in 8 areas (4 farms per area), in two counties with pawpaw at fruiting stage were sampled. Characterization of the *Colletotrichum gloeosporoides* was done at the Kenya Medical Research Institute laboratory using morphological, cultural, pathogenicity and aggressiveness methods. *In vitro* efficacy test of the plant extracts was done at the University of Eldoret to determine the effectiveness of the plant extracts against the fungi. *In vivo* tests were done on papaw plants in the field. To determine the efficacy range of the chemical dosages, a logistic regression model was applied. During the study, pawpaw infected with anthracnose disease and those that were not infected were observed. The symptoms of infection were observed on the leaves, initially occurred as small angular, brown to black spots while on the fruits the symptoms were exemplified as tear strain by occurrence of linear necrotic regions. The prevalence of anthracnose in *Carica papaya* in the farms was 95% in Baringo and upto 83% in Elgeyo-Marakwet County and differed significantly with altitude. The overall incidence of anthracnose infections on fruits was 9.23 ± 1.22% in Baringo County and 4.5 ± 1.1% in Elgeyo Marakwet County resulting in an overall of 7.5 ± 1.0% in the region. The severity of anthracnose disease was found to positively correlate with the incidence. In regions where lower incidences were reported (<8%), there was low severity of the anthracnose disease basically at level 2 followed by level 1. Meanwhile when incidences of anthracnose disease were higher, the severity of the disease increased mainly to levels 4 and 5. The use of *Fuerstia africana*, *Solanum incanum*, *Carisa edulis*, *Azadirachta indica* and *Aloe chiliensis* in Baringo and Elgeyo-Marakwet Counties as natural fungicides against pawpaw anthracnose were confirmed by laboratory results from this study. *Fuerstia africana* plant extracts were the most effective in the bioassay, while *Solanum incanum* and *Aloe chiliensis* extracts were the least effective. There were differences in the number of days to healing of *Carica papaya* infected with anthracnose, with *Fuerstia africana* extracts taking the shortest time to healing and *Carisa edulis* extracts taking the longest time. Therefore, the exploitation of one or more of these botanical fungicides for the control of one or more of these pathogens would be biodegrable, cheaper to obtain, environmentally safer and could serve as a good alternative to synthetic fungicides.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of varience
GENSTAT	GenStat Release 4.24DE
Df	degrees of freedom
P	Probability
DNA	Deoxyribonucleic acid
GC-MS	Gas Chromatography-Mass Spectrometer
HCDA	Horticultural Crops Development Authority
ITS	Internal Transcribed Spacer
KEMRI	Kenya Medical Research Institute
MIC	Minimum inhibition concentration
MIZ	Maximum inhibitory zone
MM	Minimum agar media
MMC	Minimum agar medium with chlorate
*Nit*M	*nit* mutants
χ^2	Chi square
PDA	Potato Dextrose Agar
SC	Czapek Dox Agar
PDI	Percentage Disease Incidence
RAPID	Random Amplified Polymorphic DNA
SC	Czapex Dox Agar
EU	European Union
WHO	World Health Organization
µg/mL	Micrograms per mililitre

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CHAPTER ONE

INTRODUCTION

1.1 Papaya

Papaya (*Carica papaya* L.) is one of the most popular fruit plants grown widely under tropical and sub-tropical climates (Silva *et al.,* 2007). The genus *Carica* is one of the four genera of Caricaceae family represented by one species of a herbaceous perennial fruit tree of economic importance worldwide – pawpaw (*Carica papaya* Linn.) (Yogiraj *et al*., 2016). Although the crop is native of tropical America; (Ross, 2013: Hardison *et al*., 2017), it's popularity especially the fruit has made it ubiquitous in all the tropical and subtropical regions of the world (Sancho *et al*., 2011). The popularity of the plant as agricultural crop stems from the recognized nutritional, medicinal and pharmacological properties beneficial to man (Gunde and Amnerkar, 2016; Shahid and Fatima, 2018). In this regard, *C. papaya* fruit contain vitamins A, B and C (Rivera-Pastrana *et al*., 2016: Milind and Gurditta, 2017), minerals such as potassium, calcium, magnesium, iron and sodium (Jiménez *et al*., 2018), essential amino acids (Nwofia and Ojimelukwe, 2012), and significant amounts of caretenoids, phenols, niacin, phosphorus and zinc (Sancho *et al*., 2011; Carvalho and Renner, 2015). The raw fruits also contain a commercially important alkaloid or proteolytic enzyme "Papain", that has found use in medicine and food additive (Saran and Choudhary, 2013; Chukwuemeka and Anthonia, 2016). It is these attributes that has enabled many people around the world to elect growing pawpaw as a food and cash crop (Milind and Gurditta, 2017).

1.2 Papaya production in Kenya

The production of *C. papaya* varies widely across the world. The most recent statistics available for 2016 estimate that the world production of the crop was approximately 7.2 million metric tonnes, where the Asian Region led in production accounting for 52.55% of the global (FAOSTAT, 2016; Evans and Ballen, 2017). In terms of the crop produced from individual countries production, followed by South America (21.09%) and Africa (13.16%) (India tops the figure with over 5.5 million metric tonnes, followed by production from Brazil (1.6 million metric) and Indonesia (1.2 million metric tonnes), with a substantial global production accounted for by Nigeria (0.8 million metric tonnes) (FAOSTAT,2016.https://www.worldatlas.com/articles/top-papaya-producing-countries.html).

In Africa, *C. papaya* was introduced over 100 years ago from Central and South America (Silva *et al*., 2007) and currently Nigeria is the leading African country producing *C. papaya* production (Olubode *et al*., 2017). The fruit of *C. papaya* is among the most consumed in many parts of Africa by humans (Oboh *et al*., 2015; Oyedemi *et al*., 2016) while other parts of the plant consumed by domestic animals (Imran *et al*., 2009). The crop has also widely found uses in ethnomedicine (Bamisaye *et al*., 2013; Sumathy *et al*., 2017). In Sub Saharan Africa (SSA), the fruit of *C. papaya* forms the most important fruit crop for many poor households (Mumba *et al*., 2012; Kehlenbeck *et al*., 2015; Oduola *et al*., 2016), and contributes to the national economy as food commodity, serving as a source of income and employment to a large supply chain (Milind and Gurditta, 2016). An overview of four years' data from 2012 to 2016 indicates that more than 3

million hectares were dedicated to production of between 3.2 to 4.2 million metric tones of *C. papaya* in the SSA region (FAOSTAT, 2016).

In Kenya, *C. papaya* was introduced into Kenya during the colonial times and is an important fruit crop grown in many parts of the country for local consumption and sales in the local market economies (Rimberia and Wamocho, 2011; Asudi *et al*., 2015). The area under *C. papaya* ranges between 300,000–500,000 hectares producing about 60,000 –150,000 metric tones/year (FAOSTAT, 2016). It is grown in almost all areas but the major provinces are Eastern, Rift Valley, Nyanza and Western. Growth of the *C. papaya* is characterized by a large number of smallholdings of no more than one ha per household (Mumo *et al*., 2013), mainly for subsistence with approximately 10% of production for commercial purposes (Asudi *et al*., 2016). There are basically six to eight varieties with different expected yields including: Solo, which produces small round sweet fruits with uniform sizes and shape; *Vega F1* that produces medium sized fruits with an attractive red flesh; *Sunrise*, produces smooth pear shaped fruits of high quality, weighing about 400-650 g; Mountain, produces small fruits only suitable for preserves and jam; *Red royale F1* which is an improved breed that gives good quality fruits weighing 1.7-2.3 kgs with red attractive colour and are very sweet and finally *Sinta* F1– female fruits are round while hermaphrodite fruits are oblong with an average weight of 2 kg (https://www.farmerstrend.co.ke/complete-guide-papaya-pawpaw-Date). Although Kenya currently is ranked the 8th largest producer of pawpaw worldwide, a major constrain in the production of *C. papaya* is often lower yield than the projected leading to economic losses (Horticultural Crops Development Authority, 2009). Therefore

understanding the causes of the low yield will form the cornerstone for management measures aimed at increasing the yields of *C. papaya*. Other crops of commercial importance that are produced in Kenya are avocados (Wasilwa *et al*., 2005; Kimaru *et al*., 2018), snap beans (Wagara and Kimani, 2007; Chemining'wa *et al*., 2011; Wahome *et al*., 2011), sorghum (Ngugi *et al*., 2000, 2001; 2002; Akosambo-Ayoo *et al*., 2011), mango (Njuguna *et al*., 2006), citrus (Njoroge *et al*., 2004), passion fruits (Amata *et al*., 2009).

1.3 Papaya production constraints in Kenya

A major constraint in the farming of *C. papaya* in the last five decades in Kenya has been the continuous attack of the crop by a variety of pathogens and/or diseases ascribed mainly to fungal, bacterial, nematodes and viral leading to heavy loss in yields (Persley and Randy, 2017). While most viral infection used to be common feature in *C. papaya* in 1950s to 1970s (Kulkarni, 1970; Seshadri, 1978), they have now tremendously declined (Ombwara *et al*., 2011). The most common, widespread and devastating disease affecting *C. papaya* is anthracnose caused by fungi (*Colletotrichum gloeosporioides*, Penz.) (Wanzala *et al*., 2010). Fruits attacked by the disease display two remarkable symptoms. The commonest is a dark-brown necrotic lesion on the young or matured fruits which is slightly sunken with raised rims (Vieira *et al*., 2013). The second type of symptom is the tear strain symptom described by occurrence of linear necrotic regions on the fruit that may or may not be associated with superficial cracking of the fruit epidermis causing an alligator skin effect on the fruit surface (Saini *et al*., 2016; Aktaruzzaman *et al*., 2018). Later, these lesions enlarge, become rounded, sunken and brown to black in colour (Saini

et al., 2016). Regardless of the type of symptom, the centers of these lesions often become covered with pink, gelatinous masses of spores especially during moist, warm weather. In Kenya, the prevalence of anthracnose among farmers is unknown despite several reports in the media about the occurrence of the disease (Persely and Randy, 2017).

1.4 Anthracnose disease of papaya

Anthracnose disease is caused by several fungal species classified under the genus *Colletotrichum,* the main ones being: *Colletotrichum gloeosporioides*, *C. capsici*, *C. acutatum* and *C. gloeosporioides* var. *minor* (Tapia-Tussell *et al*., 2008; Torres-Calzada *et al*., 2013; Stracieri *et al*., 2016; Tarnowski and Ploetz, 2016). For a long time, these species complexes were all classified as *C. gloeosporioides* (Hyde *et al*., 2009). The *C. gloeosporioides* var. *minor* has been described as a variant form of *C. gloeosporioides* and has so far never been reported in Kenya (Weir *et al*., 2017). The *C. gloeosporioides*, *C. capsici* and *C. acutatum* are difficult to distinguish and the disease symptoms they elicit on pawpaw are indistinguishable (Cai *et al*., 2009). Although infection of plants or plants parts by these pathogens occurs in the field, they remain quiescent until the fruit reaches the climacteric phase. The fungus is spread by wind and rain while disease emergence is favoured by high temperature and humidity. In Kenya, there are numerous studies that have identified *Colletotrichum* in coffee (Waller *et al*., 1993; Omondi *et al*., 2000; Hindorf and Omondi, 2011), sorghum (Ngugi *et al*., 2000; Ngugi *et al*., 2002; Tesso *et al*., 2012), beans (Ombiri *et al*., 2002; Wagara and Kimani, 2007; Kimutai, 2018), mango (Griesbach, 2003), passion fruits (Amata *et al*., 2009) and avocado (Wasilwa *et al*., 2005; Kimaru *et al*., 2018a,b). However, to date, as far as we know, there

is no study that has identified *Colletotrichum* in *Carica papaya* and therefore identification of its causal agent remains rather speculative. This has persisted despite the knowledge that identification of *Colletotrichum* spp. is a fundamental criterion in the development of more control measures of the disease.

Traditional identification and characterization of *Colletotrichum* species has relied primarily on differences in morphological features such as colony colour, size and shape of conidia and appressoria, growth rate, presence or absence of setae, and existence of the *Glomerella teleomorph* (Cano *et al*., 2004; Li *et al*., 2007; Mo *et al*., 2018). Studies of characterization of these features on *Colletotrichum* species have not yet been conducted in Kenya to the best of my knowledge. Moreover, the identification of the causal agent of pawpaw anthracnose as *C. gloeosporioides* is not specific enough since it could refer to any of the distinct species in the *C. gloeosporioides* complex (Schena *et al*., 2014). Furthermore, differentiation between *Colletotrichum* species based on host range or host of origin may not be a reliable criterion for fungi of this genus, since taxa such as *C. gloeosporioides, C. dematium* and *C. acutatum*, and others infect a broad range of host plants (Yan *et al*., 2015; Lei *et al*., 2016). Some taxa even appear to be restricted to host families, genus or species within those families, or even cultivars, whereas others have more extensive host ranges (Bragança *et al*., 2016; Sharma *et al*., 2017). It is necessary; therefore to determine which of the distinct species in the *C. gloeosporioides* complex is the causal agent of the disease. Apart from being genetically different compared to the strains of the pathogen from other fruit crops, the pawpaw biotype is readily distinguished from the other strains by virtue of its limited infectivity on other fruit crops (Honger *et al*., 2016; Keuete Kamdoum *et al*., 2018). In Kenya, it is not clear which

strains are involved in the anthracnose aetiology of *Carica papaya*, that may hinder the current protocol developed for controlling the occurrence of the disease.

1.5 Control of anthracnose

1.5.1 Synthetic fungicides and bacteriacides

Fungicide application at regular intervals has been generally recognized as the most effective method of controlling anthracnose (Ishii *et al*., 2016; Cao *et al*., 2017; Moral *et al*., 2018). However, there are several demerits that have been reported against their use (Forcelini and Peres, 2018). Synthetic chemicals often lead to increased resistance to diseases, weeds and insect pests alike, as well as the appearance of other diseases formerly unknown (dos Santos Diniz, 2018; Valarmathi, 2018; Kang *et al*., 2019; Savary *et al*., 2019; Zhao *et al*., 2019). Accumulation of harmful chemical residues in fruit, water and soil has also been reported after continuous use of synthetic chemicals (Syed *et al*., 2014; França *et al*., 2019; Martínez-Piernas *et al*., 2019). Fungicide food residues pose more carcinogenic risks than the insecticide and herbicide residues, where in some cases, fungicides affect the ripening process, and the use of fungicides and bactericides for extended periods may lead to the emergence of fungicide/bactericide-resistant strains of fungi and bacteria (Sanders *et al*., 2000; Wanyera *et al*., 2009; Vinod *et al*., 2009; Phoulivong, 2011). In addition, the use of some compounds to prevent the plant infection could cause undesirable attributes either to human and animal health, or to the environment (Verweij *et al*., 2009; Komárek *et al*., 2010; Przemieniecki *et al*., 2019). For this reason, it is therefore necessary to develop safer alternatives to synthetic fungicides that are effective and economically feasible as well as environmentally friendly.

1.5.2 Botanical fungicides and bacteriacides

Botanical fungicides and bactericides (often referred to as simply “botanicals”) are products that are derived from natural plant compounds used to control diseases in various ways (Serra *et al*., 2018; Khaliq *et al*., 2019). Botanicals are reported to be superior to synthetic fungicides because these natural compounds are usually biodegradable, environmentally safer and they are easier, and cheaper to obtain than the synthetic insecticides (Gao *et al*., 2018; Sarfraz *et al*., 2018; Song *et al*., 2017). Several plants and their extracts possess insecticidal (Boulogne *et al*., 2018), antifungal (Yi *et al*., 2016; Kumar and Kumari, 2019), bactericidal (Karim *et al*., 2017; Wang *et al*., 2018; Kumar and Kumari, 2019), as well as virucidal properties (Singh and Singh, 2019) in their leaves, seeds, stems and even roots. Once applied, botanicals leave no or little harmful residues (Khaliq *et al*., 2019; Meela *et al*., 2019). As a result, there are numerous research that have tested traditional medicinal plants for the control of plant diseases (Al-Samarrai *et al*., 2012; Gurjar *et al*., 2012; Jagtap *et al*., 2013; Yoon *et al*., 2013; Ma *et al*., 2019). Efficacies of different plant species on fungal infections vary widely and therefore there has been continued research to establish suitable plants species that can aid in the overall improvement of the antifungal action against anthracnose causing pathogens.

There are several plant species that are known and others recommended to control disease in plants in Kenya (Kigondu *et al*., 2009; Omwenga *et al*., 2009; Gakuya *et al*., 2013) and animals (Njoroge and Bussmann, 2006; Okitoi *et al*., 2007; Wanzala *et al*., 2012). These plants contain several active ingredients required as biological agents against disease that infect plants and animals. Among the numerous plants available,

Azadiracta indica (Nguta *et al*., 2010; Oyoo-Okoth *et al*., 2011; Aliero, 2017), *Fuerstia africana* (Muthaura *et al*., 2007; Keter and Mutiso, 2012; Okach *et al*., 2013), *Solanum incanum* (Njoroge *et al*., 2004; Kiringe, 2006; Keter and Mutiso, 2012) *Carissa edulis* (Koch *et al*., 2005; Tolo *et al*., 2006) and *Aloe chiliensis* (Tolo *et al*., 2010) among others have received more research attentions. They contain mosquitocidal, antiparasitic, larvicidal, bactericidal and viricidal properties and therefore have been tested and are being used in treatment of diseases in humans and other plants. Nevertheless they have rarely been tested against fungi causing anthracnose in plants. In light of the foregoing, this study determined the extent of anthracnose infections among farmers and evaluated the antifungal efficacy of *A. indica, F. africana, S. incanum, C. edulis* and *A. chiliensis* against *C. gloeosporioides*.

1.6 Statement of the problem

Anthracnose in crops results in huge losses in plants and therefore proper management measures should be determined before the problem escalates into irreparable damage. Several unpublished reports have indicated that the disease is particularly severe in most parts of Baringo and Elgeyo-Marakwet Counties due to the warm and humid conditions in the regions. However, the lack of study within the country on anthracnose in *C. papaya* implies that the prevelance and severity of the disease remains relatively unknown.

For some time now, the control of anthracnose has involved synthetic chemical use. The obvious disadvantages of chemical fungicides (Mitran *et al*., 2018) have resulted to suggestions for alternative use of plant compounds which are in abundant supply in the

region (Kipkore *et al*., 2014). This requires information on screening and bioassays of the efficacious doses of the compound from each of the plants and specific response of the pathogen to doses of the plant compounds. There has been no study on the use the stated plant extracts to control pawpaw anthracnose in Baringo and Elgeyo-Marakwet despite the observable cases of anthracnose among farmers.

Understanding of the severity of the disease will be simplified if the identity of the causative agent is known. Yet for many *C. papaya* growing areas, characterization of the pathogens remains superficial, which may lead to lack of any tangible effort to allocate resources to control pathogens.

1.7 Justification of the study

There is consistent lack of research on diseases affecting pawpaw in Kenya, despite the obvious signs that pawpaw has a number of diseases. There is minimal research on the occurrence of anthracnose in *C. papaya* except in grey literature sources. The major constraint in the farming of *C. papaya* in the last five decades in Kenya has been the continuous attack of the crop by a variety of fungal diseases, mainly anthracnose caused by *Colletotricum gloeosporoides*. This has resulted in reduced ability to control and manage this disease. This study will therefore provide information on the prevalence, incidences, severity and control of the disease, apart from identifying the fungal species responsible for the disease in pawpaw within the country through a laboratory setting.

The study will also provide information on ways to control anthracnose in pawpaw and reduce the yield losses in the crops in Kenya. Currently the only effective way of reducing anthracnose disease incidence and severity is by the use of synthetic chemical fungicides. Considering the challenges posed by synthetic insecticides, alternative plant fungicides are welcome in the face of disease resistance by the fungi and environmental safety concern over chemical use. Moreover, smallholder farmers need to be given cheap technologies that are safe, ecologically sound and at the same time very effective in controlling *Colletotrichum* spp. and that are similar to synthetic chemical fungicides that reduce disease losses and in turn realize increased production per unit area.

This study will add to the knowledge of the use of plant extracts as antifungal agents. Plant extracts from *Azadiracta indica, Fuerstia africana, Solanum incanum, Carissa edulis* and *Aloe chilensis* have in past studies yielded promising potentials as human medicine. Efficacies of plant extracts are known to be affected by among other factors location, amount of active compounds in the plants, extraction procedure and the species of organism under study. Therefore this study will add knowledge to the effects of plant biocides from the tropical region which may form a base for comparison with plant species derived from other regions. It will also identify the fungal species responsible for papaya anthracnose.

The results of this study will be of relevance because, by demonstrating the diversity, virulence and sensitivity to fungicides of *Colletotrichum* species infecting pawpaw fruits, will facilitate the advances and realization of disease management practices and in so

doing allow producers to reduce the economic losses in pawpaw production caused by anthracnose.

1.8 Objectives of the study

1.8.1 Main objective

The main objective of this study was to determine the prevalence of anthracnose of pawpaw (*Carica papaya*), its pathogenicity and biocontrol using plant extracts in Baringo and Elgeyo - Marakwet Counties in Kenya.

1.8.2 Specific objectives

The specific objectives of the study were to:

1. Assess the prevalence, incidence and severity of anthracnose in *Carica papaya* fruits in Baringo and Elgeyo-Marakwet Counties in Kenya
2. Characterize *Colletotrichum gloeosporoides* isolates infecting *Carica papaya* fruits in Baringo and Elgeyo-Marakwet Counties in Kenya
3. Evaluate the *in-vitro* efficacy of plant extracts against isolates of *Colletotrichum gloeosporoides* in Baringo and Elgeyo-Marakwet Counties in Kenya
4. Determine the *in-vivo* efficacy of plant extracts against isolates of *Colletotrichum gloeosporioides* in Baringo and Elgeyo-Marakwet Counties in Kenya.

1.9 Hypotheses

H_{01}: The prevalence, incidence and severity of anthracnose in *Carica papaya* fruits in Baringo and Elgeyo-Marakwet Counties in Kenya are low

H_{02}: There are no differences in the characteristics of *Colletotrichum gloeosporoides* isolates infecting *Carica papaya* fruits in Baringo and Elgeyo-Marakwet Counties in Kenya

H_{03}: Botanical extracts are not efficatious against isolates of *Colletotrichum gloeosporoides in vitro* in Baringo and Elgeyo-Marakwet Counties in Kenya

H_{04}: Plant extracts have no effects against *Colletotrichum gloeosporoides in vivo* on anthracnose infected papaya fruits in Baringo and Elgeyo-Marakwet Counties in Kenya

1.10 Scope of the study

In terms of content, the study determined the prevalence, incidence and severity of anthracnose disease in *C. papaya*, evaluated the molecular, cultural and morphological methods of identification of the causative agent and finally tested the efficacy of crude extracts of five medicinal plants *in vivo* and *in vitro* against *Colletotrichum gloespoirodes* causing anthracnose in *C. papaya*. In terms of time scope the study was done between the periods 2016 to 2019. Geographically the study was conducted within *C. papaya* growing areas of Baringo and Elgeyo-Marakwet Counties. The results of the study can however be extrapolated to all parts of Kenya and in the tropical areas with similar environmental conditions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background of *Carica papaya*

There is a considerable research, publication, documentation, photographs and monographs on background and requisite information concerning pawpaw plant. As such, there is a broad consensus concerning the description of the fruit with only slight divergence of opinion on regional distribution. Pawpaw is a deciduous non woody plant, that may attain 5 to 10 m in height, often narrowly conical tree native to the temperate woodlands (Jones and Layne, 2009; Hormaza, 2014; Yogiraj *et al*., 2014) (Plate 2.1). The lower tree trunk is noticeably scarred where leaves and fruit were borne. Pawpaw plant has a hollow, green or purple stem and can grow 1.8 between 3 m (6 to 10 ft) in a year, eventually reaching heights of 6 to 9 m (20 to 30 ft) (Peterson, 2005). The long dark green, petioled, obovate-oblong leaves are about 30 to 105 cm long and 30 to 60 cm wide, are profoundly divided into 5 to 9 lobed segments. Stems are hollow, light green to tan brown in color with diameter of 8 inches and bear prominent of scars (Arvind *et al*., 2013). Pawpaw plant produces large, sweet, melon-like fruits, where individual often fruits weigh 140 to 453 g [converted from ounce historical measures] and are 3 to 6 inches in length (Chan, 2009). The flowers of the plant are sweet in scent, often open at night (Kubitzki, 2013). Once ripe, the fruit appear as a large berry weighing about 15–50 cm long and 10–35 cm in diameter, where it feels soft and its skin attain an amber to orange tinge (Boning, 2006). However, the above description of pawpaw may vary from one geographical zone to another and with several varieties of pawpaw.

Plate: 2.1: Photograph showing Carica papaya (a) plant and (b) flowers. Source: Yogiraj *et al.* (2014).

2.1.1 Origin of papaya

There is no general consensus among researchers about the origin of pawpaw. Although the exact area of origin is unknown, the papaya is believed native to Tropical America, perhaps in Southern Mexico and neighbouring Central America countries of Costa Rica (Pomper *et al*., 2010). Other researchers just point the native America as the origin of wild form of the plant (Kew, 2015) while others refer to its origin as tropical America (Bellini *et al*., 2003). It is possible that that it appeared first in those parts of Central America where the species was found, but on the other hand it may have resulted from several hydbridizations, some perhaps having occurred in Mexico (Croteau, 1986).

2.1.2 Taxonomy of papaya

Taxonomically, *Carica papaya* L. belongs to the Sub Kingdom: Tracheobionta, Class: Magnoliopsida, Subclass: Dilleniidae, Superdivision: Spermatophyta, Phyllum: Steptophyta, Order: Brassicales, family Caricaceae, Genus: *Carica*, (Pomper *et al*.,

2010). The family is currently divided into six genera of which *Carica* is one, with the only species *C. papaya* (Badillo, 2000).

2.1.3 Geographical distribution of papaya

The papaya family (Caricaceae) has an amphi-Atlantic distribution with two species in tropical Africa and ca.33 in Central and South America (Figure 2.1, page 17). Both African species are large trees, one (*Cylicomorpha solmsii*) in West Africa, the other (*C. parviflora*) in East Africa (Figure 2.1 E and F, page 22). The monotypic genus Horovitzia (*H. cnidoscoloides*), endemic to Mexico, is a small tree with spongy thin stems covered with stinging hairs (Badillo, 1993, 2000). The likewise Mexican (to Guatemalan) genus Jarilla (A and B) comprises three species of perennial herbs (Kubitziki, 2013). Jacaratia (Figure 2.1C, page 22) currently has eight tree species (including a suspected new species) occurring from southern Brazil to Mexico, and finally, Vasconcellea consists of 20 species, 19 of them trees (Figure 2.1 D, page 22) or shrubs, and one a climber. The sister group of Caricaceae is the Moringaceae, a family of 13 species of trees and shrubs from dry habitats in the Horn of Africa (seven species), Madagascar (two species), southwestern Africa (one species), and tropical Asia (three species; Badillo, 2000).

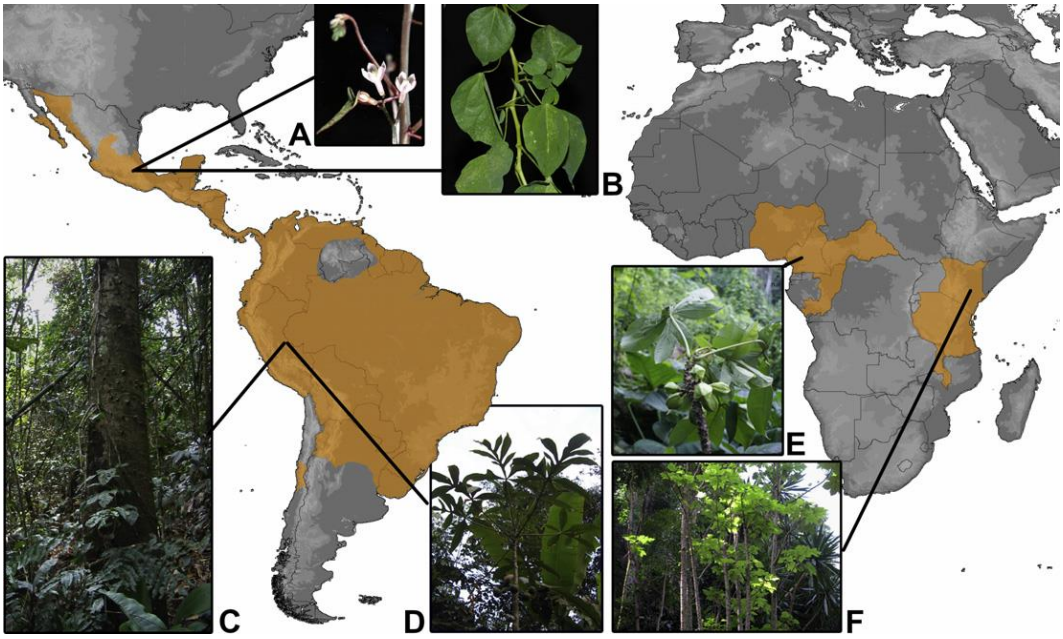

Figure 2.1: Distribution of the Caricaceae in Mexican/Guatemala (A/B), South America (C/D) and Africa (D,E). Source: Modified from Carvallo and Renner (2012).

2.1.4 Economic importance of papaya

The commercial interest in papaya is reflected in more than 3300+ papers published on this species between 1970 and 2018 (Web of Science, accessed 25 October 2018). Successful commercial production today is primarily in Hawaii, Tropical Africa, the Philippines, India, Ceylon, Malaysia and Australia, apart from the widespread but smaller scale production in South Africa, and Latin America (Arvind *et al*., 2013). Pawpaw fruit is a good source of dietary fiber, folate, vitamin A, C and E. The fruit also has calcium, iron, riboflavin, thiamine and niacin (Anonymous, 2011b). Pawpaw leaves are used as a meat tenderizer and are also eaten as vegetables (Millind and Garditta, 2017). Pawpaw leaf and bark extracts are reported to be employed as a remedy to mouth sores and toothaches by some cultures around the world (Anonymous, 2015b). Woods - Danzaru (2009) reported that a root infusion of pawpaw is used as a remedy for gonorrhoea in

Mozambique and syphilis in East Africa. Roots are also used as a remedy for kidney and bladder problems (Koneman and Roberts, 1985). The fresh seeds cause abortion if eaten by a pregnant woman (Chan *et al.*, 2009). Pawpaw crude latex is applied as in injection therapy in damaged intervertebral cartilage and is also applied to the skin to clean wounds (Imran *et al*., 2009). Unripened pawpaw fruit have properties of reducing blood sugar indicating that they can be used to control diabetes. The latex is reported to have antifungal properties and is used by the Indians of New Guinea to treat fungal infections of the skin (Draelos, 2001). *C. papaya* latex sap inhibits the growth of *Candida albicans* (Robin) Berkhout under *in vitro* conditions. Apart from controlling human diseases, studies have also showed that extracts of pawpaw leaves can be used to control plant diseases (Yoon *et al.,* 2013). Pawpaw extracts inhibited the growth of *F. oxysporum* mycelium (Jones and Layne, 2009). Cold and hot water leaf extracts of papaya are effective in reducing the growth of powdery mildew fungi *in vitro* and in reducing the spread of powdery mildew disease on pepper (*Capsicum annuum*) plants (Bellini *et al*., 2003). The extract of pawpaw leaves also reduces the incidence of seed-borne fungi of African yam bean seed (Tarnowski and Ploetz, 2016).

Pawpaw is a great source of proteolytic enzymes that are very important in digesting food. The most important of these proteolytic enzymes found in pawpaw is papain, which breaks down proteins in food, allowing for better digestion (Sepiah, 1993). Papain is used in prescription of digestive enzymes to treat individuals with cystic fibrosis or pancreatic conditions, producing for them what the body cannot produce naturally (Chitwood, 2002). Eating papaya is also a benefit because papain taken orally treats less serious

disgestion disorders such as bloating and chronic indigestion (Deba *et al*., 2008). In these cases papain is extracted, dried and sold as tablets (Woods-Panzaru *et al*., 2009). Another great benefit of papaya is to boost male virility (Koneman and Roberts, 1985). These blood vessels then dilate and increase blood flow. A more concentrated form of arginine is used to treat erectile dysfunction (Tasiwal *et al*., 2009). Many alternative medical practitioners believe that one of the benefits of papaya is to control premature aging (Sepiah, 1993). Papaya helps the body to properly digest food and when the body digests all the nutrients it needs, the body will remain vital for a long time (Horticultural Crops Development Authority, 2009). The antioxidants in papaya prevent cholesterol from oxidizing, courtesy of (Chau *et al*., 1983). When cholesterol becomes oxidized it forms plaque in the blood vessel walls that can eventually build up and causes a heart attack or stroke (Imungi and Wabule, 2017). Papaya treats inflammation: papain and chymopapain, protein-digestive enzymes found in papaya lowers inflammation and improve healing from burns (Sadhu and Chattopadhyay, 2001). This benefits the body as it helps heal skin injuries, relieves psoriasis, removes warts, treats ringworms and cold scores (Canzaez, 1993).

2.1.5 Anthracnose disease of papaya

Anthracnose is a fungal disease that attacks all plant parts at any growth stage (Torres-Calzada *et al*., 2012). It is generally caused by a fungus called *Colletotrichum gloeosporioides* (Torres-Calzada *et al*., 2012). Symptoms consist of small, brownish spots with light-coloured centers on the leaves and twigs (Ryan *et al*., 1994). Tiny cracks on the leaf spots indicate fruiting structures of the fungus. Considerable defoliation can result. The disease is a problem during cool, wet springs (McGinnis, 2005). Variegated

varieties are more susceptible (Hernandez-Albiter *et al*., 2007). At first, anthracnose generally appears on leaves as small and irregular yellow, brown, dark-brown, or black spots. The spots can expand and merge to cover the whole affected area (Webster *et al*., 2008). The colour of the infected part darkens as it ages. As it ages, the center of an older spot becomes blackish and emits gelatinous pink spore masses (Martton *et al*., 1984). Host plants are onion, banana, pawpaw, mango, lettuce and all other fruit trees (Woods-Panzaru *et al*., 2009). The affected plant parts are leaves, stems, petioles, pods, fruits and roots (Woods-Panzaru *et al*., 2009).

The fungus survives the winter dormant primarily in cankers on infected branches and twigs (Kwon *et al*., 2002). To a small extent, the fungus also survives in fallen leaves (Hashem, 2011). Wind carries the fungal spores from the cankers to developing leaf and twig tissue (Buwa and Staden, 2006). Infected young twigs are girdled and killed (Buwa and Staden, 2006). The fungus forms new spores on the infected leaves and twigs. These spores are then blown or splashed to nearby foliage where they germinate, penetrate and cause additional spots if weather conditions are favourable (Alvarez and Nishijima, 1987). Infected leaves shrivel and fall (Paull *et al*., 1997).

2.1.6 Epidemiology and aetiology of anthracnose disease

There is wide knowledge of the occurrence of anthracnose infecting several plants especially in warm, humid areas commonly infect the developing shoots and leaves (Thomas, 2009). The origin of the disease can be tracked back to the 1970s in North America (Jones *et al*., 2000), but then it differes from the current disease due to occurrence in cool and temperate climates (Deba *et al*., 2008). Since then the disease has

been reported in several plants of commercial importance. However, the causative agent, the fungus, was not described until 1991 (Tasiwal, 2009). Among these plants, it causes huge economic losses and therefore its importance as economically significant disease is widely acceptable in literature.

It is a major pre and postharvest disease of the crop throughout the tropics (Silva, 2007). The disease affects both leaves, twigs, petioles, panicles and fruits. It also causes blossom blight of flowers and results in poor fruit set (Estrada *et al*., 2000).

Pawpaw anthracnose is reported to be the most important disease affecting pawpaw worldwide (Bamisaye *et al*., 2013). Anthracnose disease also causes latent infection on developing fruits which generally remain quiescent until the fruit ripens (Meela *et al*., 2019) except on young fruits (Bamisaye *et al*., 2013). Symptoms develop on fruits in transit or storage and reduce their marketability (Chitwood, 2002). Anthracnose symptoms on leaves initially occur as small angular, brown to black spots that can coalesce to form large extensive lesions on the leaf. This is particularly common around the edges of the leaves. On panicles, the symptoms first appear as small black or dark brown spots which may enlarge or coalesce to kill the flowers before fruits are produced. Blighted flowers are dry and their colour varies from brown to black (Rivera – Pastran, 2016). Petioles, twigs and stems, are also susceptible and the typical black expanding lesions found on leaves can be found on them (Deba *et al*., 2008). Two types of symptoms are found on fruits. The commonest is a dark-brown lesion which is slightly sunken with raised rims (Nwofia and Ojimelukwe, 2012). This can be found on very

young fruits or matured fruits in storage or transit. The lesions can enlarge on the fruit surface and eventually penetrate the fruit and infected young fruits usually drop (Chattopadhyay, 2011).

These black necrotic lesions may or may not be accompanied by bright orange acervuli which are the fruiting bodies of the pathogen (Deba *et al*., 2008). The second type of symptom is commonly referred to as tear strain symptom in which are linear necrotic regions on the fruit that may or may not be associated with superficial cracking of the fruit epidermis causing an alligator skin effect on the fruit surface (Singh and Singh, 2019). Anthracnose causes premature fruit drop and direct reduction in quality of ripe fruits, shortening storage life time (Sumathy *et al*., 2017. In areas where rain is prevalent during flowering and fruit set, anthracnose can cause destruction of the inflorescence and infection and drop of young fruits.

Infection of the blossom or young fruits can result in total crop failure (Jones *et al*., 2000). Fruits smaller than 4 cm or of pea size usually aborts when blossoming (Przemieniecki *et al*., 2019). Incidence of pawpaw anthracnose has been reported between 32% in South Africa and 64.6% in Costa Rica and can reach 100% when fruits are produced under wet or very humid conditions. Similarly, post-harvest anthracnose incidence on pawpaw can also reach as high as 100% on fruits produced in wet or high humid conditions (Keuete Kamdoum *et al*., 2018).

2.1.7 Symptomatology of anthracnose of papaya

Anthracnose spots on green fruits are generally dark brown to black with a pale margin and lenticular in shape (Dickman and Alvarez, 1983; Sadhu and Chattopadhyay, 2011; and Yogiraj *et al.*, 2016). The affected areas increased in size and become sunken and coalesce to form large spots. On ripening fruits, the symptoms were as numerous small dark circular spots, which enlarge, coalesce and become sunken. Although the disease usually appears on the ripening portions of the fruit, occasionally the green portions of the fruit may become infected (Dickman and Alvarez, 1983). The disease symptoms are in the form of brown to black depressed spots on the fruits. The first symptoms of papaya anthracnose were round, water soaked, and sunken spots on the surface of the ripening fruit (Sheshadri, 1978). Lesions may become as large as 5 cm in diameter. Pinkish-orange areas are formed by the conidial masses that cover the lesion center and are frequently produced in a concentric ring pattern. The first symptoms are small well defined dried pink spots on the surface of ripening fruit (Thomas, 2009). Later, these lesions grow to 5 cm diameter, become rounded, sunken with depth of 3 to 5 mm and brown to black in colour. The lesions can be water-soaked or dried and hard. In the centre of the lesions, the fungus produces dark acervuli, frequently in a concentric pattern and orange to pink gelatinous mass of conidia can be observed. The whole lesion can be easily separated from the flesh of the fruit as a corkscrew, using a knife, leaving a well defined hole in the fruit. Symptoms on immature fruits and leaves are uncommon. Post-harvest infection usually produces stem-end rot. Spores caused infection only on unwounded detached, mature fruits (Meela 2019). It can cause latent infection of the fruits (Singh and Singh, 2019).

The first symptoms appear as brown superficial discolorations of the skin and then develop into circular, slightly sunken area, 1- 3 cm in diameter; usually they appear watersoaked (Teixeira da Silva *et al*., 2007). Gradually the lesions coalesce and sparse white mycelial growth often appears on the margin of such spots under humid condition an encrustation of salmon pink spores. Often arranged in a concentric pattern may develop on the surface of some of the older spots. In senescing papaya petioles the perfect stage of the anthracnose fungus, *Glomerella cingulata* (Ston.) Spauld and Schrenk, produces many ascospores (Duran *et al*., 1999). These become air borne and lodge on the fruit surface where they form appressoria upon germination. Isolation from petioles and from healthy papaya fruit surface usually resulted in recovery of colony type different from that resulting from isolation from anthracnose lesion. The part of the fruit near the stem was the most severely affected by the disease and *Glomerella cingulata* causes symptoms earlier to other pathogen and incidence was high after 48 hours of harvesting (Duran *et al*.,1999). Inoculations with *Colletotrichum gloeosporioides* resulted in complete rotting within 5 days (Mohan and Lakshmanan, 1987). The symptoms caused by *C. gloeosporioides* occur only when the fruit is fully ripened (Sepiah, 1993). The fungus attacks primarily the fruit of papaya, with mature fruit being more susceptible (Janisiewiez and Jeffers, 1997) and Marston *et al*., 1984). Petioles and leaves may be infected, but this is thought to be important only as a source of the fungus for further fruit infection. The disease signs begin as small, water-soaked spots on ripening fruit. As the spots develop, they become sunken, turn brown or black, and may enlarge to a few inches. The fungus may produce a pink mass of spores in the middle of the older spots. The pathogen grows into the fruit, resulting in softening of the fruit and an off flavor.

Growers practice a prophylactic program for this disease. The affected tissues become dirty brown, soft and finally rot (Sharma *et al*., 2017)). Infection may also occur when the fruit is immature and mummification and deformation of fruits take place. As the disease develops, it frequently produces large mass of spores in the central portions of the lesion causing them to turn light orange or pink (Chukwuemeka and Anthonia, 2016).

The fruit rot can also be initiated at the pre-harvest stage when inoculum load is very heavy on the stem and petioles (Cano *et al*., 2004). The initial symptoms are watersoaked, sunken spots one-fourth to one inch in diameter on fruit. The centers of these spots later turn black and then pink when the fungus produces spores. The flesh beneath the spots becomes soft and watery, which spreads to the entire fruit. Small, irregular-shaped watersoaked spots on leaves may also be seen. These spots eventually turn brown (Anonymous, 2003).

Three different species of *Colletotrichum* have been associated with pawpaw anthracnose worldwide. These are *Colletotrichum gloeosporioides* (Gujar *et al*., 2012), *Colletotrichum capsicum* var minor (Wilson and Wisniewski, 1999) and *Colletotrichum acutatum* (Ragsdal and Sister, 1944; Murray *et al*., 1999; Diehl, 2008; Tarnowski and Ploetz, 2008). *Colletotrichum gloeosporioides* var minor was regarded as a variant form of *C. gloeosporioides* and was reported only in Australia. Currently, it is no more recognized as the causal agent of the disease (Paull *et al*., 1997).

In tropical areas, *Colletotrichum gloeosporioides* has been reported as the major cause of

the disease while *C. acutatum* predominates in sub-tropical areas. In Florida and Brazil, both species were found on Pawpaw (Waller *et al*., 1993; Griesback, 2003). Though the two belong to separate species, the symptoms they develop on pawpaw fruits are indistinguishable. On other crops where the two occur simultaneously, diagnosis of the disease in absence of molecular characterization of the pathogens has been questioned (Walker *et al*., 1991).

2.2 Characterization of *Colletotrichum gloeosporoides*

Colletotrichum is one of the most important genera of pathogenic fungi worldwide causing economically important diseases of cereals, grasses, legumes, vegetables and perennial crops including trees (Carvallo and Renner, 2012; Woods - Panzaru *et al*., 2009). *Colletotrichum* was originally spelt as *Colletothricium* in 1831 but was later changed to its present spelling in 1837 (Sutton, 1966). Glomerella is the anamorph of *Colletotrichum* (Sancho *et al*., 2011). The fungi attack all parts of the plant, at all stages of development, from seedlings to mature plant and seed, causing disease symptoms commonly known as anthracnose (Gunde and Ammerka, 2016). Although *Colletotrichum* spp. are known as plant pathogens recent reports have indicated that there are some species of *Colletotrichum* that are associated with human and animal diseases. These infections have mostly been found in immunosuppressed people (Cano *et al*., 2004). *C. dematium* is responsible for corneal ulcers called keratitis in humans (Mendiratta *et al*., 2005). Other *Colletotrichum* species that are also reported to cause infection in humans are *C. gloeosporioides*, *C. coccoides* (Wallr.) S. Hughes and *C. graminicola* (Cesati)

Wilson (De Hoog *et al*., 1995; Cano *et al*., 2004). *Colletotrichum* spp. are also reported to be associated with animals and fish (Sanders *et al*., 2000; Mendiratta *et al*., 2005).

Colletotrichum spp. are the ethyologic agent of anthracnose disease and play an important role on agricultural subsistence economies world wide. These pathogens infect different crops, from monocotyledons plants, such as papaya and turf grass to higher dicotyledons, such as cashew trees. Nevertheless, despite the fact that *Colletotrichum* affect a wide spread number of crops, its pathogenic range increases caused by a rising number of species identified under these Genera that were classified as anthracnose's agent (Schena, 2014). Like many other fruits, the papaya fruits are also affected by various postharvest diseases like, anthracnose, stem-end rot, chocolate rot, *Fusarium* rot, *Aspergillus* rot and Rhizopus rot etc. The present investigation is on the anthracnose of papaya which is caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc, is responsible for the postharvest losses during storage, transit and marketing.

2.2.1 Characteristics of the *C. gloeosporoides*, the causal agent of papaya anthracnose

The *C. gloeosporioides* is a group species (Appendix I) made up different genetically distinct species brought together by similar conidial morphology and rDNA-ITS nucleotide sequences. It has been reported that the taxa *C. musae*, *C. kahawae*, *C. xanthorrhoeae*, *C. nupharicola*, *C. fragariae*, C. *gloeosporioides sensu stricto*, *C. horii*, *C. theobromicola*, *C. ignotum*, *C. tropicale*, *C. asianum*, *C. siamense*, *C. fructicola* and *C. hymenocallidis* as well as many putative undescribed species are all part of the *C.*

gloeosporioides sensu lato complex (Pomper *et al*., 2010). In several instances, the term *C. gloeosporioides* has been used interchangeably to refer to both *C. gloeosporioides sensu stricto* and the group species (Brown, 1995). However, the distinction was made very clear by Phoulivong (2011) who restricted the name to *C. gloeosporioides sensu stricto*, the original strain obtained from citrus which has recently been identified also on orchids. There exists some amount of confusion in the naming of isolates of *C. gloeosporioides* when differences in the nucleotide sequences of the ITS1 region were found among different isolates named as *C. gloeosporioides*. To overcome this confusion, Phoulivong (2011) proposed up to 3.6% variations in sequence homology as the ceiling for the naming of variants as *C. gloeosporioides*. The consequence of these ceiling was that other species especially, *C. kahawae* which showed a percent variation of less than 3.6% with some isolates of *C. gloeosporioides* had to be renamed. To solve the problem, it was proposed that *C. gloeosporioides* must be defined to cater for the *C. kahawae* isolates. However, Phoulivong (2011) showed that some isolates which have wrongly been assumed to be variants of *C. gloeosporioides* especially on tropical fruit crops are distinct *Colletotrichum* isolates whose species status has not been ascertained yet. The authors also reported that the anomalies detected in the identification of *C. gloeosporioides* isolates were due to the comparison of unknowns to wrong type strains (Phoulivong, 2011).

Despite the usefulness of the ITS region in resolving systematic issues, the region represent a small portion of the total genome and hence it was not possible to use the region to determine the species of isolates within the *C. gloeosporioides* complex

(Mendiratta *et al*., 2005). To achieve this, different gene regions were combined to obtain sufficient information to achieve such aim. These DNA regions include the intron of the glyceraldehyde-3–phosphate dehydrogenase gene, the intron of the beta tubulin gene and the actin gene (Liu *et al*., 2007 and Phoulivong 2011). Based on the results of these analyses it has been reported that the pathogen infecting pawpaws in Thailand and earlier on reported as *C. gloeosporioides* is rather *C. asianum* (Phoulivong, 2011). They also suggested that the wrong identification of the pathogen may not be restricted to Thailand alone but may be worldwide One method of resolving systematic issues within species complexes is the use of the Genealogical Concordance Phylogenetic Species Recognition (GCPSR). According to the method, speciation point is the point at which different gene trees show concordance (Chitwood, 2002). The method has been used to resolve systematic issues in the *Gibberella fujikori* complex (Tasiwal *et al*., 2009) and to identify different species of *Aspergillus flavus* (Kew, 2015). Among Morphological Species Recognition (MSR), Biological Species Recognition and Phylogenetic Species Recognition (PSR) (Tasiwal *et al*., 2009), PSR comes closer than the others to recognizing species consistent with Evolutionary Species Concept. This is because once progeny evolutionary species have formed from an ancestor, changes in gene sequences occur and can be easily recognized before changes have occurred in mating behavior or morphology. The method involves the construction and analysis of several gene trees (Koufopanou *et al*., 1997) and this makes it more robust in the identification of species compared to the use of a single gene (Cano *et al*., 2004).

2.2.2 Variability in *Colletotrichum gloeosporoides* species

Ninety-five isolates of *Colletotrichum* including 81 isolates of *C. acutatum* and 14 isolates from *C. gloeosporioides* were characterized by various molecular methods and pathogenicity tests (Torres-Calzada *et al*., 2012). Results based on random amplified polymorphic DNA (RAPD) polymorphism and internal transcribed spacer (ITS) 2 sequence data provided clear genetic evidence of two subgroups in *C. acutatum* (Asudi, 2010). The first subgroup, characterized as CA-clonal, included only isolates of strawberry and exhibited variable RAPD patterns and nearly identical ITS2 sequence analysis (Morrisey and Osbourn, 1999). A larger genetic group, CA-variable, included isolates from various hosts and exhibited variable RAPD patterns and divergent ITS2 sequence analysis, where no correlation could be drawn between genetic and pathogenicity groups (Adwan and Mhanna, 2008).

2.2.2.1 Pathogenicity and aggressiveness

C. gloeosporioides is ubiquitous pathogen infecting a wide range of crops including pawpaws. Colonies on potato dextrose agar are grayish white to dark grey with aerial mycelia which ranged from a thick mass to sparse tufts associated with fructifications. The pathogen produces short, hyaline, unicellular conidia that are either cylindrical with obtuse ends or ellipsoidal with a rounded apex and a narrow truncated base. The conidia form on hyaline to faintly brown conidiophores in acervuli which are irregular in shape and approximately measure 500 µm in diameter. Setae (4-8 × 200 µm) which are found on the acervuli are one to four septated, brown, slightly swollen at the base and tapered at the apex (Deba *et al*., 2008; Jagtap *et al.*, 2013).

C. gloeosporioides infecting pawpaw is both genetically and pathologically distinct from other isolates of the pathogen infecting other tropical fruit crops and has been described as the pawpaw bio-type of the pathogen (Waller *et al*., 1993). The pawpaw biotype of the pathogen is restricted to the pawpaw crop and unlike the other strains of the pathogen it has not been found on any other crop naturally (Mitran *et al*., 2018; Waller *et al*., 1993). Isolates of the pawpaw bio-type from different locations are genetically uniform and hence is conjectured to be clonal and distributed worldwide from a single source. Irrespective of where they were found, isolates of the pawpaw bio-type of pathogen had been identified based on two substitutions at the 78th and 138th position of the ITS1 nucleotide sequences which corresponds to the 73rd and 133rd position beginning from the first nucleotide of the region. At the 78th position, the isolate possess an 'A' while the strains from the other tree crops possess a 'C' and at the 138th position this pawpaw bio-type possess a 'G' while the others posses a 'T'. They also posses the same mtDNA and rDNA restriction digestion profile which is unique in comparison to the other isolates from the other tree crops (Sepiah, 1991; Croateau, 1986; Mitran *et al*., 1994; Gupta *et al*., 2012). These characteristics of the pathogen had been utilised in the demonstration of cross infection of the strains of the pathogen from other tree crops onto pawpaw (Ragsdal and Sister, 1994). Currently, it has been reported that the pawpaw bio-type of *C. gloeosporioides* may be *Colletotrichum asianum*, a pathogen previously found on coffee (Phoulivong 2011).

2.2.2.2 Colony characteristics

Traditionally, plant-associated fungi are identified based on morphological features such as colony colour, size and shape of conidia, optimal temperature, growth rate, presence or absence of setae and the existence of the teleomorph, *Glomerella* (Jones *et al*., 2000; Sutton, 1966). However, due to the effect of environment on the stability of morphological traits and the existence of intermediate forms that can be associated with storage and frequent sub-culturing, and the overlap of morphological traits such as conidial morphology and cultural characteristics, the use of these morphological and physiological characteristics is limited in the identification of members of the genus (Koneman and Roberts, 1985; Bailey and Jager, 1992 and Brown *et al*., 1995). In certain cases, using these methods has generated confusion as to the causal agent of a disease where both *C. gloeosporioides* and *C. acutatum* were suspected (Bailey and Jager, 1992). In fact it has been reported that many disease symptoms attributed to *C. gloeosporioides* before 1965 may have been caused by *C. acutatum* (Waller *et al*., 1993). It is necessary for an accurate diagnosis of diseases suspected to be caused by members of the genus *Colletotrichum,* to apply a combination of both morphological and molecular data to identify the pathogen (Bellini *et al*., 2003).

2.2.2.3 Genetic characteristics

Several molecular methods have been employed for species delineation within the genus *Colletotrichum* and this has enabled the accurate identification of *C. gloeosporioides* infecting fruit crops in general. These include the use of abitrarily primed polymerase chain reaction (ap-PCR), Analysis of A+T-rich DNA associated with mtDNA and

Nuclear DNA polymorphisms and Ribosomal DNA analysis (Freeman *et al*, 1998). Ribosomal DNA (rDNA) genes appear as multiple copies in the genome and are conserved. In contrast the non-coding internal transcribed spacer region (ITS) between the small and large nuclear rDNA regions is suitable targets for detection of recent evolutionary divergence within *Colletotrichum* (Freeman *et al*., 1998). Additionally, species specific primers have been designed based primarily on the sequence dissimilarities of the ITS region of representative *Colletotrichum* species and have been used successfully and sequences have been used to characterize the pathogen from a wide range of fruit crops including almond, avocado and strawberry (Gupta *et al*., 2011 and Griesback, 2003). Sequence analysis of the region has been used to accurately differentiate between and identify several species within the *Colletotrichum* genus including *C. gloeosporioides* (Hyde *et al*., 2009). Recently, the intron of the glutamine synthetase gene was evaluated for its suitability as a rapid diagnostic tool for identification of *C. gloeosporioides* and *C. acutatum* infecting a wide range of crops. It was found that restriction fragment length polymorphism of the 1-kb intron and the presence or absence of poly-T chains at the beginning of the intron nucleotide sequence could be used to accurately distinguish between the two species (Liu *et al*., 2014). Other gene introns found useful for characterizing members of the *Colletotrichum* genus include the beta tubulin, glyceraldehyde-3-phosphate dehydrogenase gene and the beta tubulin genes (Chan, 2009; Alexopoulos and Mims, 2001). Multiple species of *Colletotrichum* are known to infect the same host. For example, *C. gloeosporioides* and *C. acutatum* are known to cause disease on citrus (Brown *et al*., 1995), while coffee is affected by *C. fructicola*, *C. asianum* and *C. siamense* (Vinod *et al*., 2009). In Florida and

Brazil both *C. gloeosporioides* and *C. acutatum* were found to be the causal agent of anthracnose on pawpaw (Komarek *et al*., 2010: Wanyera *et al*., 2009). In most cases, *C. gloeosporioides* and *C. acutatum* produce symptoms on the same host that are indistinguishable and especially on pawpaw where only the two have been reported as causing pawpaw anthracnose, accurate diagnosis of the disease relies primarily on distinguishing between the two. This strategy has been employed in Florida (Honger *et al*., 2016) and in Brazil (Arvind *et al*., 2013). To accurately distinguish between the two, conidial morphology, growth rate and susceptibility to fungicides have been combined with the use of one or more of the several molecular methods on several crops including pawpaw. Generally, *C. gloeosporioides* produce conical spores that are rounded at both edges while *C. acutatum* produces fusiform conidia with sharp edges and grows at a slower rate than the *C. gloeosporioides* (Diehl, 2008). Also *C. acutatum* is reported to be resistant to benomyl and extremely tolerant to carbendazim which contrasts with *C. gloeosporioides* which is extremely susceptible to both fungicides (Jagtab *et al*., 2013).

Anthracnose fungi may cause defoliation of most pawpaw, maple, oak, elm, walnut and sycamore species, which occurs after unusual cool, wet weather during bud break (Vashissta and Sintia, 2000). Single attacks are seldom harmful to the tree, but yearly infections will cause reduced growth and predispose the tree to other stresses (Smith and Onions, 1994). Damage may be in the form of killing of buds, which stimulates the development of many short twigs which may spoil the shape of the tree girding and killing of small twigs, leaves, and branches up to an inch in diameter repeated early loss of leaves, which over several successive years weakens the tree and predisposes it to

borer attack and winter injury, premature leaf drop which lessens the shade and ornamental value of the tree. Specific symptoms of anthracnose vary somewhat depending on the tree species infected (Ali *et al*., 1999).

2.2.2.4 Cultural characteristics

During cultural characteristics of *C. gloeosporioides*, fungus grows slowly on malt or potato dextrose agar (Robert *et al*., 1999). Colonies are appressed, granular and white, and darken with age (Shaikh *et al*., 2017). Sporulation of *Colletotrichum* sp in culture is highly variable (Kariba *et al*., 2001), and is enhanced by amending media with dogwood tissue or extract, according to Hernandez-Albiter *et al* (2007). Conidiomata are dark, globose, 150-290 um in diameter. Conidia are hyaline, elliptical to fusiform, 5.5-10 x 1.5-10 um (Torres-Calzada *et al*., 2012).

There are two types of *C. gloeosporioides*, A and B, which have been described on the basis of distinct symptoms on different *Stylosanthes* species (Peraza-Sanchez *et al*., 2005). Type A isolates, which are absorbed on a wide range of *Stylosanthes* species, induce restricted tan-coloured lesions surrounded by a dark margin on leaves and stems (Peraza-Sanchez *et al*., 2005). Type B isolates affecting *S. guianensis*, cause extensive brown necrotic areas on plants (Diehl, 2008). Furthermore, type A isolates produce more conidia with obtuse apices, and grow more quickly than type B isolates on solid media (Shaikh *et al*., 2017). The two types were partially distinguished by the molecular level by the analysis of dsRNA, rDNA, genomic DNA and chromosomal number and arrangement (Kiprop *et al*., 2002).

2.2.2.5 Morphological characteristics

In morphological characteristerization, *C. gloeosporioides* vary widely but two distinct groups are separated mainly by conidium uniformity (Peraza-Sanchez *et al*., 2005). This is borrowed from examination *in vitro* for colony growth and characteristics, conidium morphology, and growth rates at a range of temperatures (New *et al*., 2000). One group comprises isolates from anthracnose blight lesions on *S. guianensis* while the other is from more discrete anthracnose symptoms on a range of *Stylosanthes* spp (McGinnis, 2005). Conidia from the former group are more variable in both size and shape than the latter, as reported by Hashem (2011). The described conidium size range of *C. gloeosporioides* causing *Stylosanthes anthracnose* is to be extended to between 5 and 35 um long x 2.5 and 7.5 um wide (Paull *et al*,. 1997). Growth rates also vary between the two groups within global regions supporting the subdivision of this *C. gloeosporioides* complex (Murray *et al*., 1999).

There are large variations in cultural and morphological characteristics where conidia, cultural characteristics, appressoria, and setae of 24 isolates of *Colletotrichum* species from strawberry were compared (Shaikh *et al*., 2017). The virulence of each isolate on plants of 14 strawberry cultivars and one breeding clone was evaluated (Sahito *et al*., 2003). Thirteen isolates identified as *C. fragarie* produced cylindrical conidia; developed beige to olive to gray colonies, generally with dark olive to dark gray reverse colony colors; and did not form the ascigerous state in culture (Barry and Thornsberry, 1991). Two isolates identified as *Glomerella cingulata* (anamorph: *C. gloeosporioides*) developed gray or olive-gray colonies with dark gray to dark olive reverse colony colors,

produced cylindrical conidia, and formed the ascigerous state in culture (McGinnis, 2005). Nine isolates identified as *C. acutatum* produced fusiform conidia and developed pink, orange, rose, or beige colonies with predominantly cream, pink, or rose reverse colony colors; none formed an ascigerous state in culture (Paull *et al*., 1999). Disease severity ratings after plant spray inoculations resulted in a highly significant isolate x cultivar interaction, suggesting that some isolates may represent different races among the tested isolates of *C. fragarae* and *C. acutatum*. Overall, *C. fragariae* isolates caused more severe petiole and crown symptoms than did *C. acutatum* isolates, which in turn caused more severe symptoms than did *C. glorosporiodes* isolates (Vashista and Sintia, 2000). However, some cultivars were more susceptible to certain *C. acutatum* isolates than to some *C. fragariae* isolates, for example the cultivar Sunrise was susceptible to *C. acutatum* isolates Goff and Mil-1 but resistant to *C. fragariae* isolate Fla-2 (Barry and Thornsberry, 1991).

2.3 Control of anthracnose disease of papaya

Several options exist for the control of pawpaw anthracnose which include siting of pawpaw orchards in drier areas, the use of resistant cultivars, farm sanitation and the application of fungicides (Diehl, 2008). These methods are usually applied together in an integrated pest management programme (Paull *et al*., 1999).

2.3.1 Physical control

Farm sanitation which involves the removal of excess foliages and their subsequent removal from the field is aimed at reducing the inoculum level in the field prior to the

production season. However, despite the importance of the method, it is rarely practised by farmers due to the cost and difficulty (Tolo *et al*., 2010). Choice of resistant cultivars has been described as the most important control measure after site selection. The resistance of pawpaw cultivars appears to depend on the geographical location at which the crop is cultivated and this may be linked to the identity of the pathogen within the locality (Ali *et al*., 1999). Several pawpaw cultivars have been found with moderate resistance to the disease, but due to the high market cosmetic standards, no commercial cultivar is sufficiently resistant to be produced in humid areas without fungicide application as the levels of the disease on such cultivars are unacceptable in commercial situations (Peraza – Sanchez *et al*., 2005) Therefore, the most profitable production of cultivars which are very susceptible but important in international trade would be the establishment of the orchards in arid areas (Deba *et al*., 2008). In recent times, the possibility of boosting the natural resistance of susceptible fruits has been investigated by the application of salicylic acid and acibenzolarS-methyl.

2.3.2 Chemical control

Fungicide application to control pawpaw anthracnose has been done both at the pre-and postharvest periods. On the field, application of fungicides to control pawpaw anthracnose can reach as high as 25 sprays of both contact and systemic fungicides within a production season in environments favourable to the development of the disease (New *et al*., 2000). In drier areas or areas where the production season completely escapes the rains, the crop can be grown without the application of fungicides (Fenner *et al*., 2006). Different strategies have been developed for the application of fungicides in different

locations for the management of the disease. In Florida, a comprehensive field spray programme involving the weekly application of fungicides from flowering to fruit set followed by weekly application of systemic or biweekly application of contact fungicides has been developed (MacMillan, 2019). A similar comprehensive programme has been developed to be used in Australia (MacMillan, 2019). In The Philippines, fungicides are applied at specific periods of the production system and this results in a fewer application of fungicides compared to the Florida programme (Hashem, 2011). The use of disease forecasting system which advises the application of fungicides only when the model predicts the advent of infections has resulted in a fewer applications of fungicides compared to the calendar based programme to achieve the same level of control (Smith *et al*, 1998). The models incorporate the effects of temperature and humidity on the pathogen and hence may be specific to a locality (Asudi, 2010).

Very few fungicides have been approved to be used on pawpaw both at the pre- and postharvest stages of production. The fungicide, prochloraz has been listed to be applied on fruits destined for the US markets. Others include Benomyl, Prochloraz, Captan, Ferbam, Thiabendazole and Copper fungicides. Currently, benomyl is not permitted while prochloraz is the only synthetic fungicide approved to be used on the fruits after harvest (Arauz, 2000). However, there are differences in the types of fungicides approved to be used on fruits destined for the European market or the United States markets. For example, dithiocarbamate fungicides which include mancozeb are allowed in the EU but not in the US markets (Arauz, 2000). Generally, copper fungicides are allowed in both markets and chemical residues of these fungicides are not a problem. However, they have been reported as being less effective under high disease pressure (Arauz, 2000) and may

also be phytotoxic to flowers (Barry and Thornsberry, 1991).

Sodium bicarbonate associated with the yeast *Candida oleophila* was found to show anti fungal activity against *C. gloeosporioides* in the stored and shipped papaya fruits (Marston *et al*., 1984). Rios and Recio (2005) demonstrated that the crude aqueous extracts from garlic, peppermint, castor bean, and pepper showed inhibitory effects on the same fungus, thus suggesting that the plant extracts have a potential use as an efficient control in relation to the physical or chemical methods. Fenner *et al*. (2005) analyzed the raw methanolic extracts and fractioned the aerial parts of seven plants species for their *in vitro* antifungal activity against a panel of standardized and clinical opportunistic pathogenic yeasts and filamentous fungi.

2.3.3 Cultural practices

When feasible, infected twigs and branches should be pruned and destroyed during fall or winter (Jones *et al*., 2000). Severe pruning of large diameter branches is not good practice for most types of trees, because it triggers the bushy growth of water sprouts, which are poorly attached to the trunk and become susceptible to diseases such as powdery mildew (Rios and Recio, 2005). To stimulate vigorous growth of trees suffering severe effect of anthracnose, the fertilizing is done after the leaves open and spring rains have stopped (Marston *et al*., 1984). Irrigation systems that wet leaves are avoided (Aliero *et al*., 2006).

While pollarding-severe pruning that removes all the year's growth-is not recommended for most trees (Oddy, 2000), the method on London plane trees is used to control

anthracnose, because it stimulates the growth of new shoots and foliage (Murray *et al*., 1999).

2.3.4 Biological control

Streptomyces sp. strain MJM5763 is effective in bio controlling anthracnose in pawpaw caused by *C. gloeosporioides* (Torres-Canzada *et al*., 2012). The strain is potential alternative to chemical fungicides for reducing losses to anthracnose in pawpaw (Valarmathi, 2018).

In recent years, the biological properties of plant extracts have been receiving attention. Many studies have pointed out the possibility to use the plant essential oils in plant pathology for the control of pathogenic microorganisms (Smith *et al*. 2000; Fokialakis *et al*., 2006). These essential oils contain a variety of chemicals, including terpenoids which are known to be involved in the plant defense mechanism against the plant enemies (Galeffi, 1980). For this reason, these compounds have been tested against several phytopathological agents. Janisiewiez and Jeffers (1997) evaluated the essential oils from mustard, neem, clove, and cinnamon for their effectiveness in reducing the populations of *Phytophthora nicotianae*. Chabbra and Uiso (2017) demonstrated that the essential oils from several Australian plants have significant effect against the growth of some bacteria such as *Escherichia coli* and *Staphylococcus aureus*, and the yeast *Candida albicans*.

2.3.5 Resistant varieties to *Colletotrichum gloeosporioides*

The papaya, *Carica papaya* L, scarcely develops varieties resistant to either fungi, bacteria or viruses (Griffiths, 1999). ‘Pusa Majesty (‘Pusa22-3’), Improved Petersen,

‘Wilder’, ‘Hortus Gold’, ‘Cedro’(highly resistant), ‘Waimanalo-23’, ‘Line 8’ and ‘Line 40’ are resistant to *C. gloeosporioides,* and ‘Kapoho Solo’ and ’45-T_{22}’ are moderately resistant to this fungi (Teixeira da Silva *et al*., 2007).

2.4 Use of plant biocides in controlling diseases in plants

Medicinal plants are the most ancient source of drugs for curing various sorts of diseases in plants (Akosombo-Ayoo *et al*., 2011). Their recognized biological actions led to their cultivation, even in antiquity, in Egypt, Greece, along the Mediterranean and in China. Almost one quarter of all medicines are derived from the 250,000 flowering plants on the earth's surface (Murray *et al*., 1999). Some of the secondary metabolites there-in may be toxic to lower beings or man, or indeed both. Their use in the crude or refined form is of utmost interest in the efforts aimed at integrating herbal with orthodox medicine. With the understanding that only one in eight of the potential drugs has been found, it is estimated that more than 300 drugs are yet to be discovered in the rainforests worldwide (Farnsworth, 1990; Kumar, 2019). The efficacy of plant extracts is due to the presence of one or more biologically active principle. Pharmacological assays have shown that the activity is not always due to the main components, but the minor ones, or even to the synergism of all the active principles (Galeffi, 1980). With modern advances in the techniques for isolation and structure determination of active principles, even minute amounts of them can be isolated and their structures determined ((Aliero, 2017 and Shaikh *et al*., 2017).

The African continent has not only an extraordinary diversity of plant species but also a large number of traditional healers who exploit the vegetable material at their disposition.

Based on careful observation and judicious choice of plants, it is possible to discover very interesting new natural products (dos Santos Diniz, 2018). These biologically-active compounds are isolated from African medicinal plants by bioassay-guided fractionation procedures in which various screening methods are employed to locate the desired activities in the crude extracts and in the fractions issuing from the different separation steps (Hormaza, 2014). A large number of medicinal plants exist, key among which a dozen have been evaluated for use as disease prevention agents.

Fuerstia africana is a terrestrial herb that belongs to the family Labatiae (Sancho *et al*., 2011). It wildly grows in most climates and ecological zones of the world (Oddy, 2000). In Kenya (Kokwaro, 1976), *F. africana* grows fairly well in savannah grassland and to a small extent in semi-arid areas. It has been used for centuries by many communities worldwide for medicinal purposes (Rotich, 2010). For example, the Ameru community of Kenya has been using it as an anti-anthracnosel herb (Kumar, 2019). The Kalenjin of Kenya has been using it to cure skin infections (Webster *et al*., 2008). Also pastoral communities of Central Africa (Harbone, 2000) are still using *F. africana* to treat skin infection. *Solanum incanum* is a terrestrial herb or soft wooded shrub up to 1.8m in height with spines on the stem, stalks and calyces and with velvet hairs on the leaves (Chabbra and Uiso, 2017). The leaves are alternate, egg-shaped in outline with broad end at base (ovate) with slightly wavy margins, with a grey-green upper surface and a green-white lower surface and used for treating cattle against diarrhoea (Pinn, 2010). *Carissa edulis* is a spiny, much branched, small tree, shrub or scramble up to 5m in height, with a milk sap. *C. edulis* is widespread in many parts of Africa, (Hernandez-Albiter *et al*., 2007). It grows at forest edges, in forests and woodlands where Euphorbia, Acacia and

Croton commonly occur, especially on rocky hill sides on clay soils and used in Tanzania for treating general ailments in human beings (Pinn, 2010).

Aloe chilensis is a succulent plant species of the genus *Aloe.* It is an evergreen plant that originates from the Arabian Peninsula (Hardison *et al*., 2015). It grows wild in Tropical climates around the world. It is mainly used in agriculture, medicinal production and in making toiletaries (Abebe, 2019).

Azadirachta indica is a tree in the mahogany family Meliaceae and native to the Indian subcontinent. *A. indica* bark is used to extract neem oil, and also for controlling skin infections in children in East African countries. It is an invasive plant in parts of Kenya, Uganda and Tanzania (Kokwaro, 1976).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

3.1.1. Background, location and size

This study was conducted in Elgeyo-Marakwet and Baringo Counties (Figure 3.1). Both Baringo and Elgeyo-Marakwet Counties are in the former Rift Valley Province of Kenya. Baringo County is bordered by Turkana County and West Pokot County to the North, Samburu County and Laikipia County to the East, Nakuru County and Kericho County to the South, Uasin Gishu County to the South West and Elgeyo Marakwet County to the West. It covers an area of 8,655 km^2. Baringo County lies between Latitudes 0°13" South and 1°40" north and Longitudes 35°36" and 36°30" East. Elgeyo-Marakwet County extends from latitude 0°20′ to 1°30′ North and longitude 35°0′ to 35°45′ East and borders West Pokot County to the North, Baringo County to the East, Trans Nzoia County to the Northwest and Uasin Gishu County to the West. Elgeiyo Marakwet County covers an area of 3050 Km^2.

3.1.2 Climate

The regions receives bimodal annual rainfall ranging between 800 mm to 1100 mm. Long season occur from March to June with the peak period in April and May while short rainy season occurs between August to November. The average temperature is 23°C where the minimum value is 15ºC and maximum is 30°C.

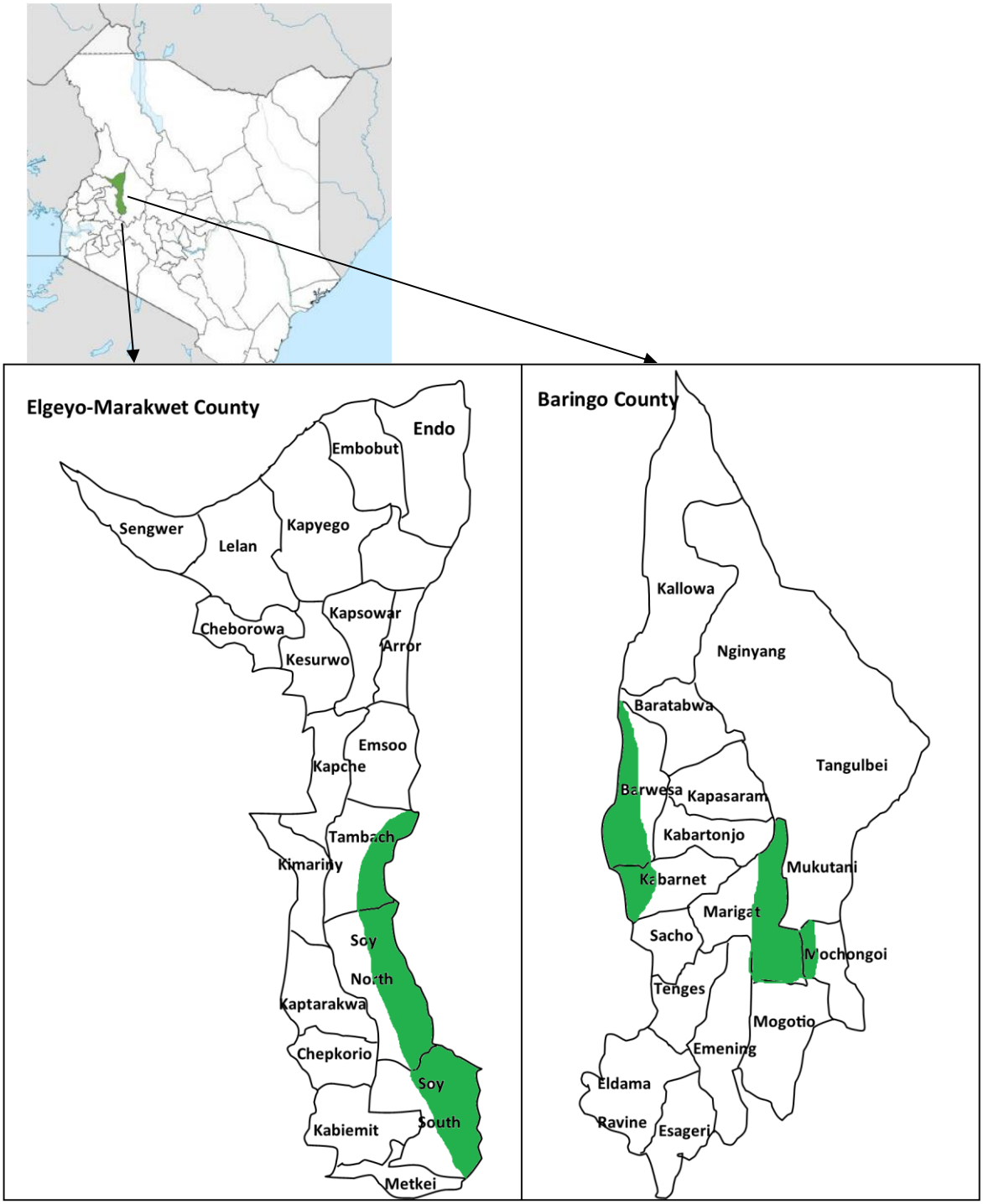

Figure 3.1: Location of the study area of Baringo and Elgeyo-Marakwet Counties showing the administrative divisions

Key: Areas that were sampled for symptoms of papaya anthracnose
Areas that were not sampled for symptoms of papaya anthracnose

3.1.3 Soils and hydrology

In Baringo County, the soil pH ranges from moderately acid (5.0) to moderately alkaline (7.5) while in Elgeyo Marakwet it ranges from strongly acid (4.43) to moderately alkaline (6.86). Soils in the region have low fertility due to leaching and loss of humus through continuous cultivation. They are heavy in texture and are mainly orthic ferrasols and humic aerosols (Jaetzold *et al*., 2005).

3.1.4 Economic activities

The economy of the two Counties is mainly agro-based, where maize, beans, pigeon peas, Irish potatoes, sweet potatoes, sorghum, cassava and finger millet are the main food crops and coffee, cotton, macadamia and pyrethrum are cash crops. Livestock products include beef, mutton as well as hides and skins. Maize is the main food crop. Fruits grown include pawpaw, bananas, mangoes, oranges, lemons and water melons. This area is classified under low medium - agro – ecological zones (Jaetzold *et al*., 2005).

3.2 Field survey of anthracnose prevalence, incidence and severity in *C. papaya*

Survey of papaya anthracnose disease was carried out in selected farmer's fields and market centres in Baringo and Elgeyo Marakwet Counties along accessible routes. Some of the equipment used in the survey were sisal twine, sterirized scalpel blade, tape measures, wooden sticks and writing materials. Papaya plant parts in the field were inspected for the presence of symptoms of papaya anthracnose disease. A sample of papaya fruits was inspected for the presence of the disease in market centres.

The presence or absence of the disease in a County or Sub-County represented occurrence (Appendix II) of papaya anthracnose. Disease incidence (%) was determined as per cent anthracnose infected plants or fruits per field or market. Disease severity (%) on papaya fruits was rated on 1 to 5 scale, where 1=0% of fruit area affected, 2=1-25%, 3=26-50%, 4=51-75%, and 5=76-100% fruit area affected (Bautista-Banos *et al*., 2002). Diseased samples of papaya plant parts and fruits were collected and stored in a cool box and transported to Microbiology Laboratory, University of Eldoret (Appendix IX), for further experiments. The survey adopted exploratory survey research design to evaluate the occurrence, prevalence, incidence and severity of anthracnose in *C. papaya*. The design was conducted to establish priorities, develop operational definitions and improve the final research design (Kumar, 2019).

The target population for this study was the number of pawpaw farmers from where the number of pawpaw fruits was determined in Baringo and Elgeyo-Marakwet Counties. During reconneisance, there were 12,239 farms growing *C. papaya*. The numbers in Baringo County was 7,463 (60.9%) and 4,776 in Elgeyo-Marakwet (39.1%).

3.2.1 Sample size

The sample size for the number of farms was computed using sample using Yamane (1967) formula: thus $n = \frac{N}{1 + Ne^2}$.

Where n = required responses

N = Population

e^2 = error limit (0.1 for samples between 100 to 1000)

Number of households of papaya will be determined as:

$$\text{n for respondents} = \frac{12239}{1 + 12239 * 0.1^2} = 99.189 \approx 99$$

During the field surveys, only 20 farmers in Baringo and 12 in Elgeyo-Marakwet allowed (Appendix X) the researcher, through their cooperative office, to sample from their farms, the rest of the farmers were either hostile or were based in regions which had high insecurity.

Disease assessment was conducted in each farm selected from the two counties. During the assessment, a line transects of 9 m^2 (3 m × 3 m) were applied randomly in 8 areas (parts of the 2 Counties that have pawpaw plantations) and the number of pawpaw plants with fruits selected. Pawpaw fruits from each transect within the farms were visually inspected for the presence of symptoms of papaya anthracnose disease. Disease prevalence, incidence and severity were determined as:

$$\%\,\text{prevalence} = \frac{\text{Number of farms with anthracnose disease affecting fruits}}{\text{Total number of farms surveyed}} \times 100$$

$$\%\,\text{incidence} = \frac{\text{Number of fruits showing symptoms of anthracnose}}{\text{Total number of fruits surveyed}} \times 100$$

Disease severity was based on visual rating scales i.e. rated on 1 to 5 scale (Bautista-Banos *et al*., 2002), where 1 = 0% of fruit area affected, 2 = 1-25%, 3 = 26-50%, 4 = 51-75%, and 5 = > 75% fruit area affected.

3.2.2 Research design

The research design employed a 5x3x1 three factorial design, randomized design and replicated three times. Papaya belonging to Kapo solo, Shillong, Surya, Co-1 and Pink Fleshed Sweet varieties with no cracks, rots or no visible deformities were used in this study. Fruits were disinfected by immersing them in 1 % NaOCl solution for 1 min, washed twice with sterile distilled water and dried at room temperature.

3.2.3 Visual and microscopic determination of *C. gloeosporioides*

A total of 320 fruit samples that were randomly picked from the samples were packed in cool boxes and sent to the laboratory for further confirmation of the occurrence of *C. gloeosporioides*. The signs of anthracnose in papaya that were collected from the samples were stained and observed at the mechanical stage of a light microscope at a magnification of × 400.

3.2.4 Anthracnose incidence and collection of samples

Papaya fruits with visible symptoms of infection, for example chlorosis, were picked from 5 pawpaw trees in transect, from the 32 farms. The picked papaya fruits were placed in clean bags and transported to the KEMRI laboratory to establish presence or absence of *C. gloeosporioides* for possible isolation. The number of fruits having *C. gloeosporioides* that causes papaya anthracnose was taken as a percentage incidence in that farm. Microscopically, the anthracnose pathogen in each papaya fruit was recorded and classified into different *Colletotrichum* species depending on the morphological characteristics noted.

3.2.5 Spore concentration adjustment

Ten days old monoconidial cultures of *Colletotrichum gloeosporioides* grown on potato dextrose agar (PDA) were scraped with a sterilized scalpel and transferred into a conical flask containing 50 ml of sterilized distilled water under aseptic conditions. The spore concentration was adjusted to10^6 spores/ml after counting the number of spores in a haemocytometer with the aid of a lOx binocular microscope. Five fruits from each cultivar were used to screen for resistance against anthracnose disease in this experiment. Fruits were washed with tap water and dipped in a spore suspension of *C. gloeosporioides* for ten minutes.

3.3 Characterization of *Colletotrichum gloeosporoides* isolates infecting *Carica papaya* fruits in Baringo and Elgeyo – Marakwet County.

The characterization methods were based on microscopy. Characterizations of the *Colletotrichum* species were done using three methods: Morphology, aggressiveness and culture characteristics. The colour of the aerial mycelium was determined according to the morphological colour chart (Rayner, 2014). Identification of *Colletotrichum* species used synoptic keys of Riddell slide cultures (Riddel, 2019).

3.3.1 Morphological characterization of *C. gloeosporoides* isolates

The isolates were sub - cultured for 12 days at 26°C and filtered through muslin cloth. Drops of the filtrates were placed on microscope slides and mixed with lactophenol/cotton blue to stain the conidia. The length and width were measured for 50 conidia, and conidial shape (acute or obtuse apices) was recorded, at ×600 or ×1250

magnification.

Isolates representative of different sites were selected for morphological species identification. These isolates were cultured on PDA in darkness at 25^0C for 5 days. Mycelial disks (5mm) were taken from the growing edge of colonies and transferred onto plates of papaya leaf agar (PLA) (McGinnis, 2005). The PLA plates were placed in an incubator under continuous fluorescent light at 25^0C. Conidia were harvested after 14 days, and setae after 22-30 days, from acervuli produced on leaf pieces. Conidia was mounted in water, and examined for size and shape, using Zeiss Axiophot 2 microscope with an Axiocam CCD camera and AxioVisio digital imaging software (AxioVision software Release 3.1, version 3-2002; Carl Zeiss Vision Imaging systems. Colony colour was determined after 7 days on PDA at 25^0C under conditions of alternating cycles of 12 hour darkness.

In determining the conidial morphology, the length and width were measured for 50 conidia, and conidial shape (acute and obtuse apices) recorded at X600 or X1250 magnification.

3.3.2 Characteristics based on aggressiveness of *Colletotrichum gloeosporoides* isolates

Thirty two *C. gloeosporioides* isolates from papaya fruit from two counties of Baringo and Elgeiyo Marakwet showing anthracnose symptoms were screened for aggressiveness, by inoculating onto detached papaya fruit and branches of young, grafted nursery trees still attached to the tree. Aggressiveness was measured by comparing the disease severity

over time. In this study, conidia length was used to place the isolates *C. gloeosporioides* into distinct groups.

The positive control in this experiment was carbendazim, a standard antifungal. The negative control was performed using distilled water. *C. gloeosporoides* isolates obtained from different fields were inoculated into uninfected fruits. Disease development and severity were recorded as described by Gurjar *et al*., (2012), where 1 = 0% fruit area affected (very low aggressiveness), 2 = 1 – 25% fruit area affected (low in aggressiveness), 3 = 26 – 50% fruit area affected (moderately aggressive), 4 = 51 – 75% fruit area affected (aggressive) and 5 = above 75% fruit area affected (highly aggressive).

3.3.3 Characterization of *Colletotrichum gloeosporioides* isolates using cultural characteristics

Isolates of *Colletotrichum* species from two counties (Baringo and Elgeiyo Marakwet) were grouped using cultural characteristics, for example mycelial texture, sporulation and conidia pigmentation

Isolation of *Coletotrichum* was done at the Kenya Medical Research Institute (KEMRI), Nairobi. Culture of C. *gloeosporioides* was done as described by Smith and Onions (1994) using Potato Dextrose Agar (PDA) and Czapex Dox Agar (SZ). The *C. papaya* were removed from the coolboxes and transferred to a sterile bench and the suspected diseased part of the fruit cut and transferred into a beaker containing 70% ethyl alcohol for 5 minutes. The content was transferred to a laminar hood maintained at a temperature of 25°C. They were then rinsed 5 times in sterilized distilled water. The content was then

removed and transferred to the surface of sterilized hood and cut into smaller blocks of 5 mm × 5 mm. The cut pieces were transferred into a 9 cm petridish containing sterilized Potato Dextrose Agar (PDA) to culture the isolates. The PDA was sterilized by autoclaving at 15 PSI at 121°C for 20 minutes. After 4 days, hyphal tips transfer was made onto sterilized PDA medium containing streptomycin at 0.4 mg/ml. pure cultures of *Colletotrichum* spp. were obtained by single spore isolation (Robert *et al*., 1999). The pure cultures were then grown in PDA medium at room temperature (25^0C) for 7 days.

Plates containing SZ were inoculated with the specimens. The fungal isolates were sub-cultured into Potato Dextrose Broth from the primary culture and incubated at 25^0C for 48 hours. The fungal species were identified using colour charts comparison and by use of dichotomous key. Pure cultures were obtained by single spore isolation and maintained in PDA culture tubes at 4°C and used as stock culture throughout the study. Plugs (5mm) were cut from the margin of fresh colonies and stored in 20% glycerol in a freezer at -70^oC.

Ten days old monoconidial cultures of *C. gloeosporoides* grown on potato dextrose agar (PDA) were scraped with a sterilized scalpel and transferred into a conical flask containing 50 ml of sterilized distilled water under aseptic conditions. The spore concentration was adjusted to10^6 spores/ml after counting the number of spores in a haemocytometer with the aid of a lOX binocular microscope.

A method described by Robert *et al*.(1999) was used to preserve the pure form of *Colletotrichum* spp. isolates. Colonies produced from single spores were preserved as

pure colonies and stored in sieved loam soil placed in universal bottles at room temperature.

Each isolate of *C. gloeosporioides* was plated onto PDA at room temperature. Three 5-mm plugs were aseptically punched from actively sporulating areas near the growing edge of a 5 day-old culture of these isolates. Each plug was placed onto PDA dishes and incubated under the same conditions as starter cultures. After 7 days, colony size, shape, margin and colour was recorded. Colony diameter of every culture was recorded daily for 7 days. Growth rate was calculated as the 7-day average of mean daily growth (mm per day). Three cultures of each isolate were investigated and experiments were conducted twice. For examination of conidial morphology, all isolates were sub cultured as mentioned above. Cultures were washed with sterile water and drops of the suspension placed on microscope slides and mixed with lactophenol/cotton blue to stain the conidia. Length and width were measured for 30 conidia per isolate. Conidial shape (cylindrical or falcate) was also recorded. For acervuli and complex mutityphal structures producing conidia on conidiophore (complex conidiomata), sometimes mutilocolour or resulting from the confluence of acervuli, the presence of setae was assessed using a microscope at X600 magnification. Conidial and appressorial shape and size were evaluated using a microscope at X600 or X1250.

3.3.4 Pathogenicity tests

Pathogenicity tests (Appendices III and IV) were performed with representative set of isolates, from all pathogenic groups and locations, using Pink Fleshed Sweet papaya fruit

variety. An aqueous conidial suspension (1×10^6 spores ml^{-1}) was prepared from 7-day-old cultures of each isolate and then placed on the fruit by the wound/drop method (Kanchana-Udomkan *et al*. 2004). This method involved pin-pricking the surface of the fruit to a 1 mm depth and then placing 20 µl of conidial suspension over the wound. Three fruits were tested per isolate and experiments conducted thrice. The inoculated fruit, along with appropriate controls (fruit inoculated with sterile distilled water) were incubated at room temperature (25°C) in humid chamber. Symptoms were recorded 5 days after inoculation and re-isolation. According to Koch's postulates, they were made from all resulting lesions. The diseased pawpaw fruit that were taken to the laboratory (see section 3.1.3 above) from each farm was used for isolation and identification of the pathogens responsible for the pawpaw anthracnose infection. This was done microscopically, visually and by use of photographs for comparison purposes.

3.3.5 Inoculum preparation, plate inoculation and incubation

The *C. gloeosporioides* isolates were transferred into PDA medium, and incubated for 3 to 4 days at 25 $\pm$ 1°C. The cultures were then sub - cultured onto fresh plates containing the PDA medium. In determining the conidial morphology, the length and width were measured for 50 conidia, and conidial shape (acute and obtuse apices) recorded at X600 or X1250 magnification.

3.3.6 Data collection

After the plates containing inoculated PDA were incubated at 25 ± 1°C for 3 to 4 days, several developments were observed, for example, mycelial texture and colony

pigmentation. This experiment involved visual and not microscopic examination. The colony diameter was measured by vernier calipers.

3.4 *In vitro* efficacy tests against anthracnose in *C. papaya* using plant extracts against isolates of *C. gloeosporoides*

In vitro tests were conducted through antifungal assays, conidial germination test, Maximum inhibition zone (MIZ) and direct light Microscopy.

3.4.1 Collection and processing of plant materials

Selection of the plants (Table 3.1and Plate 3) was based on available ethnobotanical information from traditional health practitioners and literature. The leaves, flowers, fruits, roots and stems of *Aloe chiliensis* (Linn.), *Azadirachta indica* (A. Juss), *Carissa edulis* (Forssk.), *Fuerstia africana* (T.C.E. Frie) and *Solanum incanum* (Linn.) were collected from several areas in Baringo and Marakwet Counties. The plant parts samples were packed in clean bags and transported to University of Eldoret, Botany Department where sample voucher specimens were deposited in the University herbarium for identification. The plant parts samples were dried at room temperature (26°C) for two weeks before grinding them into powder using an electric grinder in the School of Agriculture and Biotechnology.

Table 3.1: Selected medicinal plants collected from Baringo and Marakwet Counties in Kenya

TABLE 1: Plant parts collected from Baringo and Marakwet Counties in Kenya used in the current study

Plant species	Family	Parts	Collection area	
Aloe chilensis	Asphodelaceae	Leaves, fruits and roots	Baringo	Kapsergong
Azadirachta indica	Meliaceae	Leaves, fruits and roots	Baringo	Marigat
Carissa edulis	Apocynaceae	Leaves, fruits and roots	E. Marakwet	Kimwarer
Fuerstia africana	Labiatae	Leaves, fruits and roots	E. Marakwet	Tambach
Solanum incanum	Solanaceae	Leaves, fruits and roots	Baringo	Ossen

(Source: Author)

3.4.2 Preparation of the plant crude extracts

To obtain the crude extracts from the tested plants, the method used by Cakir *et al* (2004) was applied with slight modifications. The powder of aerial parts (leaves, flowers, fruits, stems and roots) of dried plant materials was extracted separately with water, methanol and chloroform (100 g/L) at room temperature. Extraction with each solvent was carried out three times for 48 hours. After each time, the extract was filtered through filter paper (Wattman No. 1). After filtration, the extracts were collected and concentrated under low pressure at 40^{o}C using rotary agent disk planted into the center of the petri plate containing PDA in the bioassay experiment. The negative control was 6mm disk treated with distilled water and inoculated into the petri plate containing PDA in the bioassay. The positive control was carbendazim. The negative control was the extracts of the five plants (Plate 3.1).

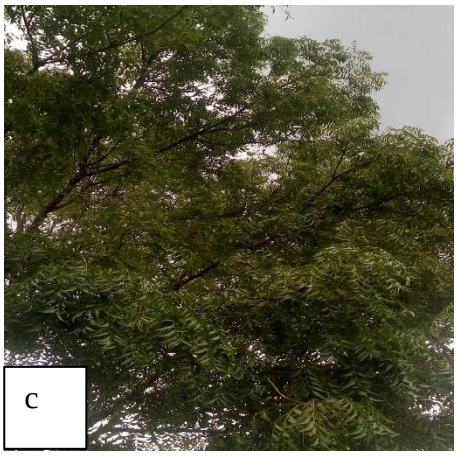

Plate: 3.1 Botanicals for *In vitro* analysis in Baringo and Elgeiyo-Marakwet Counties.

3.1 a: *Fuerstia africana* bushes in Tambach, Elgeiyo Marakwet

3.1 b: *Aloe chiliensis* plant in Kapsergong, Baringo

3.1 c: *Azadarachta indica* leaves in Marigat, Baringo.

3.1 d: *Solanum incanum* leaves in Ossen, Baringo

3.1 e: *Carisa edulis* plant in Kimwarer, Elgeiyo Marakwet

3.4.3 Inoculum

Colletotrichum spores that were used as inoculum were obtained from cultures grown on PDA for 7 days at 25°C. A 5 ml sample of sterile water was added to each PDA plate and was scrapped gently with the edge of a glass slide to obtain a spore suspension. The suspension was then sieved in 4 layers of cheesecloth to remove mycelia fragments. A 6 mm disc in diameter was obtained from these cultures then placed onto the center of fresh PDA-containing plate. Each of the isolates required a disc each. One fungus (*C. gloeosporioides*) was used in the bioassay experiment.

3.4.4 Antifungal assay

During antifungal assay, a paper disc was used where filter paper (6mm) in diameter was sterilized by dry heat for 1 hr at 1600 oven temperature and impregnated with each of the test extracts using a capillary pipette. Antifungal activity of water, methanol, and chloroform crude leaf {Appendix V (a)}, fruit {Appendix V (b)} and root {Appendix V (c)} extracts of *F. africana, S. incanum, C. edulis*, *A. indica* and *A. chiliensis* were evaluated, separately by the paper disk-agar diffusion method (Barry and Thornsberry, 1991). The concentrations from water extracts acted as negative control. Six concentrations were prepared (10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, and 10^{0}), using the method described by Robert *et al* (1999). Test plates (diameter: 6 cm) were prepared with PDA medium and inoculated in surface with as pore suspension in sterile dissolution of 0.9% saline. The concentration was adjusted to 10^{4} CFU/ml. The diameter of inhibited zone was measured in mm, and the degree of inhibition of fungal growth was recorded on a 1-5 scale, based on the table below:

Table 3.2: Scale on degree of inhibition of fungal growth of *C. gloeosporoides*

Scale	Degree of fungal growth
1.	No inhibition
2.	Inhibition zone barely distinct, fungal Growth
3.	Inhibition well distinct with sparse ca.50% of the control
4.	Inhibition zone with sparse (ca.25% of the control) fungal growth
5.	Inhibition zone free of visible fungal growth.

(Barry and Thornsberry. 1991)

3.4.5 Conidial germination tests

In conidial germination test, Conidia of *C. gloeosporioides* were adjusted using hemacytometer to a concentration of 10^5 conidia/mL. A 10 uL of plant extracts and 90 uL of the conidial suspension were mixed and the mixtures were added to the surface of dried depression slides. The slides were then placed on a glass rod in petri dish under moistened conditions and incubated at 25°C, for 24 h. Control conidia received an equivalent amount of the solvent. After incubation, slides were fixed in lactophenol cotton blue and observed microscopically for spore germination. The experiment was laid out in CRD with three replications. The number of conidia germinated was scored to calculate the percentage inhibition of conidial germination.

3.4.6 Minimum Inhibition Zone

In determining the Maximum Inhibition Zone (MIZ), the diameters of Maximum inhibition zone (MIZ) were determined according to Kariba *et al*., (2001). Paper disks (diameter: 6 mm) were impregnated with 60 µl of the reconstituted samples at a concentration of 25–150 mg/ml. The disks were transferred aseptically into PDA plates inoculated with the test fungi. MIZ was regarded as the lowest concentration that produced a visible zone of inhibition. Experiments were executed in triplicates. The plates were incubated at 27°C for 4–6 days. For each extract, three replicate trials were therefore conducted against each fungus.

3.4.7 Data collection by microscopy

Direct light Microscopy involved, culture of growing fungi was determined by light microscopy by suspending the colony in lacto phenol cotton blue ink at a magnification of × 100 (× eye piece and × 10 objective). A sample of mycelium of the fungal species was taken from the periphery of the colony grown (Appendix VI) on culture media after 6 days of incubation. The samples were fixed in lactophenol-cotton blue and examined under light microscope (Olympus 40, Germany) to examine structural abnormalities. Samples from control plates without crude extracts were also stained and observed. Microphotographs were taken using a microscope attached camera.

3.5 *In vivo* efficacy tests against anthracnose of *C. papaya* using plant extracts

Plant extracts that showed antifungal activity were further tested for their effects against papaya anthracnose on detatched fruit. Papaya fruits were obtained from Kaptich village

of Marigat farm. The experimental design in this section was 5x3x1x1. The undamaged, matured fruits of comparable size and colour class and free from pesticide were used. Aqueous extracts of *F. africana*, *S. incanum*, *A. indica*, *A. chiliensis* and *C. edulis* were evaluated (Appendix VII) at concentrations of 10 and 25% (w/v). Conidial suspensions of *C. gloeosporioides* was prepared from 10-day old culture and adjusted to 10^5 conidia/ml using hemacytometer. Papaya fruits were surface-sterilized by dipping in 1% potasium hypochlorite (KOH) solution for 10 minutes rinsed in sterile water and inoculated by dipping into spore suspension of *C. gloeosporioides*. After incubation for 15 h covered by plastic sheet until conidia germinated, fruits were dipped into aqueous extracts, while the control fruits were dipped into sterile water, as negative control. Carbendazim was used as positive control. Three fruits (replications) were used for each of the treatments. The experiments were done randomly. Disease severity was recorded using a scale developed by Gurjar *et al*., 2012, where 1=0% of fruit area affected, 2=1–25%, 3=26 – 50%, 4=51-75%, and 5 =76-100% fruit area affected.

3.6 Data analysis

All data was entered, organized and managed using EXCEL spreadsheet for Windows XP (Griffits, 1999). All statistical analyses were performed using a version of STATISTICA 10.0 statistical packages (Kumar, 2019). Normality of data distributions was checked by means of their skewness and kurtosis to determine any need for applying appropriate data transformation procedures as described by Agresti (2017).

Analysis of variance (ANOVA) was carried out with the statistical software SAS v. 9.0. Least Significant Difference (LSD) at 5% probability level was used for mean

comparison. Disease severity ratings were square root transformed while percent spore germination was arsine transformed before statistical analysis.

To determine the efficacy range of the chemical dosages, a logistic regression model, which can be used to identify non-linear responses to the range of concentrations, was fitted to the data. The logit model: $\text{logit}[\theta(x)] = \log\left[\frac{\theta(x)}{1-\theta(x)}\right] = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_i x_i$ is a general logistic model, which takes the form: $\log\left[\frac{\rho}{1-\rho}\right] = \beta_o + \beta_1 C + \beta_2 C^2 + \beta_3 C^3$ in dose response treatments, where denotes the probability of activity, β_0 is an intercept, β_1 is the coefficient of concentration C, and C_2 is the coefficient of quadratic response in C while β_3 is the coefficient of dose response in C. The model was fitted using GENSTAT (GenStat Release 4.24DE) statistical software program. Model fit was based on residual likelihood ratio chi-square statistic. For each parameter analyzed, differences in the inhibition zones among groups exposed to different *F. africana, S. incanum, C. edulis, A. indica* and *A. chiliensis* leaf {Appendix VIII (a)}, fruit {Appendix VIII (b)} and root {Appendix VIII (c)} extracts concentrations were tested by One-Way ANOVA. Analysis of variance (ANOVA) was carried out with statistical software V. 14 and Least Significant Difference (LSD) at 5% probability level was used for mean comparison. Similarity report (Appendix XII) was determined by use of turnitin originality software.

CHAPTER FOUR

RESULTS

4.1 Occurrence, incidence and severity of anthracnose on papaya fruits in Baringo and Elgeyo-Marakwet Counties in Kenya

During the study, pawpaw infected with anthrocanose and those that were not infected were observed (Plate 4.1). The symptoms of infection were clear on the leaves. They initially occurred as small angular, brown to black spots that coalesced to form large extensive lesions on the leaf. In fruits of pawpaw, the symptoms were exemplified as tear strain symptom by occurrence of linear necrotic regions on the fruit that was associated with superficial cracking of the fruit epidermis causing an alligator skin effect on the fruit surface. Later, these lesions become enlarged, rounded, sunken and brown to black in colour. The centers of these lesions were often covered with pink, gelatinous masses of spores especially during moist, warm weather.

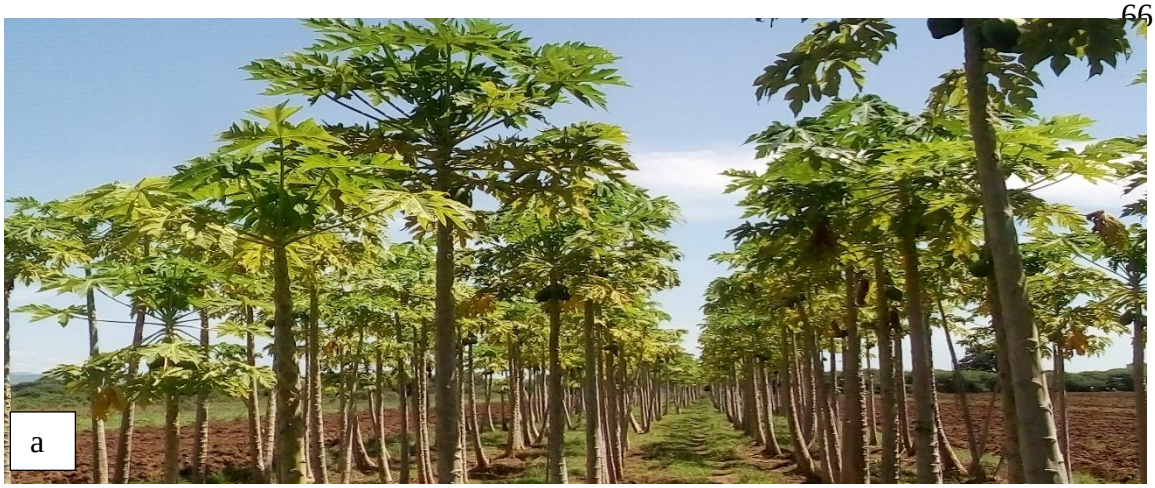

Plate: 4.1: *Carica papaya* farm, fruits and leaves in the field

4.1a: Papaya farm in the field
4.1b i: Fruit under normal growth condition 4 ii: when infected with anthracnose

4.1c i: health leaves of anthracnose 4c ii: when infected with anthracnose

The occurrence and incidence of anthracnose in *C. papaya* in Baringo and Elgeyo-Marakwet Counties is shown in Table 4.1. The occurrence of anthracnose in the farms was 100% in Baringo and 83.3% in Elgeyo-Marakwet County, with an average of 93.75% of the two Counties. There was no occurrence of anthracnose disease in high altitude area (> 1280 m asl) in Baringo. At altitude of 1062 meters above sea level, the occurrence of the disease started reducing in the affected farms. In Elgeyo Marakwet Couunty, there was no occurrence of the disease at altitude ranging between 1300 to 1400 m asl.

From an observation of 2682 pawpaw plants sampled from Baringo and Elgeyo-Marakwet, the highest incidence of anthracnose of *C. papaya* in Baringo was 44.6% and the lowest was 20.6% in Elgeyo-Marakwet. The mean incidence of the two Counties was 31.1%. In Elgeyo-Marakwet, the highest incidence was 41.7%, with the lowest mean being 0.0%. The mean incidence was 20.6%. The overall mean of anthracnose of *C. papaya* of the two Counties was 26. 7% (Table 4.1).

Table 4.1: Occurrence and incidence of anthracnose of *C. papaya* plants and fruits sampled from farmers in Baringo and Elgeyo- Marakwet Counties

County	Sub County	Area	Farm	Altitude (m.a.s.t)	Occurrence (+ or -)	Incidence (%)
Baringo	B. South	Marigat	Kaptich	1062	+	30.0
			R7	1060	+	38.0
			R1	1032	+	33.3
			L3	1057	+	34.5
	„	Koriema	Koriema	1100	+	34.5
			Kimalel	1150	+	28.9
			Patkawanin	1151	+	30.5
			Kibingo	1130	+	34.5
	B. Central	Kapkelelwa	Kapkelelwa	1280	+	20.6
			Kisok	1275	+	27.8
			Oinobmoi	1230	+	31.0
			Kurumbsoo	1012	+	38.9
	B. South	Mochongoi L	Sandai	1010	+	41.1
			Loboi	1008	+	37.6
			Molok	1012	+	24.3
			Kaptombes	1011	+	24.4
	B. North	Barwessa	Barwessa	1000	+	44.6
			Muchukwo	1009	+	38.9
			Likwon	1002	+	33.3
			Chesongo	1005	+	35.7
Mean BAR					**100%**	**33.1%**
Elgeyo Marakwet	K. South	Kimwarer	Kimwarer	1260	+	25.4
			Seko	1256	+	27.8
			Kapokpok	1250	+	41.7
			Emsea	1230	+	29.2
	K. South	Cheptebo	Cheptebo	1200	+	31.0
			Chekobei	1205	+	28.8
			Chepsigot	1230	+	22.5
			Biretwo	1300	-	0.0
	K. North	Tambach L	Nyawa	1400	-	0.0
			Kapshokwei	1380	+	9.3
			Kewapsos	1350	+	9.9
			Sangeta	1310	+	16.9
Mean EMC					**83.3%**	**20.2%**
Overal mean					**93.75%**	**26.7%**

Key: + = Presence of anthracnose, - = absence of anthracnose

The prevalence of anthracnose differed significantly (χ^2 = 12.2312, df = 42, P = 0.0004) among the sampling locations. Further analysis showed that prevalence of anthracnose disease was found to differ significantly within the county of occurrence and altitude (χ^2 = 45.2312, df = 42, P = 0.0000). At an altitude between 1310 and 1380 m asl, the prevalence of the anthracnose disease started to decline during surveys. The mean prevalence of anthracnose in Baringo County was 100%. In Elgeyo-Marakwet County, the mean prevalence was 83.3%. The overall mean prevalence of the two Counties was 93.75%.

Table 4.2: Prevalence of anthracnose of *C. papaya* plants and fruits sampled from farmers in Baringo and Elgeyo- Marakwet Counties

County	Sub County	Area	Farm	Altitude (m.a.s.t)	Prevalence (%)
Baringo	Baringo South	Marigat	Kaptich	1062	100
			R7	1060	100
			R1	1032	100
			L3	1057	100
	Baringo South	Koriema	Koriema	1100	100
			Kimalel	1150	100
			Patkawanin	1151	100
			Kibingor	1130	100
	Baringo Central	Kapkelelwa	Kapkelelwa	1280	100
			Kisok	1275	100
			Oinobmoi	1230	100
			Kurumbsoo	1012	100
	Baringo South	Mochongoi L	Sandai	1010	100
			Loboi	1008	100
			Molok	1012	100
			Kaptombes	1011	100
	Baringo North	Barwessa	Barwessa	1000	100
			Muchukwo	1009	100
			Likwon	1002	100
			Chesongo	1005	100
Mean BAR					**100%**
Elgeyo Marakwet	Keiyo South	Kimwarer	Kimwarer	1260	83.3
			Seko	1256	83.3
			Kapokpok	1250	83.3
			Emsea	1230	833
	Keiyo South	Cheptebo	Cheptebo	1200	83.3
			Chekobei	1205	83.3
			Chepsigot	1230	83.3
			Biretwo	1300	0.0
	Keiyo North	Tambach L	Nyawa	1400	0.0
			Kapshokwei	1380	83.3
			Kewapsos	1350	83.3
			Sangeta	1310	83.3
Mean EMC					**83.3%**
Overal mean					**93.75%**

The severity of anthracnose disease in *C. papaya* is shown in Table 4.3. Generally the severity of the anthracnose disease was more in Baringo than Elgeyo Marakwet and was found to differ according to the incidence. In regions where lower incidences were reported (<8%), there was low severity of the anthracnose disease basically at level 2 followed by level 1. Meanwhile when incidences of anthracnose disease were higher, the severity of the disease increased mainly to levels 4 and 5.

Table 4.3: Severity of anthracnose disease in *C. papaya*

				Anthracnose disease severity				
County	**Sub County**	**Area**	**Farm**	**1**	**2**	**3**	**4**	**5**
Baringo	Baringo South	Marigat	Kaptich	1.9	2.6	0.8	0.9	0.8
			R7	0.4	1.1	1.5	1.2	6.8
			R1	1.1	2.9	2.1	3.2	3.2
			L3	1.7	3.6	1.4	1.1	0.2
	Baringo South	Koriema	Koriema	0.8	0.5	0.2	3.1	3.1
			Kimalel	0.7	0.7	1.5	3.4	4.0
			Patkawanin	0.4	0.4	0.8	4.5	2.4
			Kibingo	0.3	0.3	0.4	2.2	3.1
	Baringo Central	Kapkelelwa	Kapkelelwa	0.7	0.7	0.9	3.3	4.7
			Kisok	1.1	1.1	1.4	1.7	2.1
			Oinobmoi	0.8	0.8	1.0	3.5	2.4
			Kurumbsoo	1.9	2.6	1.1	1.1	0.3
	Baringo South	Mochongoi L	Sandai	2.2	3.8	0.9	0.6	1.0
			Loboi	1.2	1.2	1.5	2.5	3.9
			Molok	0.2	0.2	0.2	5.4	5.4
			Kaptombes	0.4	0.4	0.5	4.3	5.8
	Baringo North	Barwessa	Barwessa	1.4	1.4	1.7	3.2	0.9
			Muchukwo	1.2	1.2	1.5	3.7	3.6
			Likwon	1.5	1.5	1.9	4.5	0.3
			Chesongo	2.5	3.2	1.0	1.2	1.1
Elgeyo Marakwet	Keiyo South	Kimwarer	Kimwarer	0.7	3.3	1.2	1.1	2.3
			Seko	0.9	2.9	1.3	1.1	1.3
			Kapokpok	0.8	2.1	0.5	1.2	1.5
			Emsea	1.3	3.4	1.1	0.9	0.7
	Keiyo South	Cheptebo	Cheptebo	1.5	2.4	0.6	0.2	0.3
			Chekobei	1.3	2.3	0.7	0.3	0.0
			Chepsigot	0.8	1.2	0.7	0.7	0.6
			Biretwo	0	0.0	0.0	0	0.0
	Keiyo North	Tambach L	Nyawa	0	0.0	0.0	0	0.0
			Kapshokwei	0.7	1.2	0.5	0.5	0.4
			Kewapsos	1.1	1.3	0.5	0.4	0.2
			Sangeta	1.1	1.3	0.6	0.5	0.4

Key: Low severity 1 - 2

Higher 3 - 4

Severer Above 5

Results showing the ranges of altitude verses symptoms of papaya anthracnose before maturity are shown in Table 4.4. Symptoms of papaya trees based on leaf defoliation and fruit yellowing were assessed on altitude ranges in meters from 1051 to 1400 (Table 4.4). High leaf defoliation of 23 was recorded in altitude range of 1251-1400, and lower leaf defoliation of 8 recorded in altitude range of 1251 - 1300. High fruit yellowing of 14 was recorded in altitude range of 1101–1150, with the lowest fruit yellowing of 3 was recorded in altitude range of 1351–1400. Leaf defoliation and fruit yellowing had means of 17.10 and 10.43 respectively. The rates of leaf defoliation and fruit yellowing symptoms were similar ($p<0.05$) and appear to drop with rise in altitude.

Table 4.4: Altitude range (m) and symptoms of papaya anthracnose before maturity in Baringo and Elgeyo-Marakwet Counties using 100 papaya trees

	Symptoms	
Altitude	**Leaf defoliation**	**Fruit yellowing**
1051 - 1100	16	12
1101 - 1150	21	14
1151 - 1200	19	11
1201 - 1250	22	13
1251 - 1300	23	10
1301 - 1350	11	05
1351 - 1400	08	03
Mean	**17.10**	**10.43**

4.2 Characterizations of Colletorichum gloeosporoides isolates infecting papaya fruits in Baringo and Elgeyo-Marakwet Counties

Results showing the variations in morphological characteristics of *Colletotrichum* isolates recovered from papaya fruits are shown in Table 4.5. Based on the morphological characteristics, most colonies of *C. gloesporoides* were grey either dark or olive, with conidium diameter ranging from 80 to 86 mm, length of 14.1 to 18 mm and width of 4.1 to 4.6. Meanwhile the appresoria were clavate or clavate irregular in shap, with length ranging from 9.5 mm – 11.0 mm and width of 5.1 to 6.5 mm. On the other hand, *C. capsici* had white grey, white or cotton grey colony, of diameter 78 mm to 84 cm, with a conidium length of 16.7 mm to 19.5 mm, conidium with of 4.4 mm to 5.0 mm. In the

shape of *C. capsici* was clavate or clavate irregular with length of 10.5 mm to 11.0 mm and width of 5.6 mm to 6.1 mm.

Table 4.5: Morphological characteristics of *Colletotrichum* isolates recovered from papaya fruits

		Colony		**Conidium**		**Appresorium**		
Isolate source	Species	Colour	Diameter (mm)	Length (mm)	Width (mm)	Shape	Length	Width
Kaptich	*C. gloesporoides*	Dark grey	80	14.1	4.2	Clavate	9.8	6.1
Koriema	*C. gloesporoides*	Dark	81	14.5	4.1	Clavate irregular	9.5	6.5
K'kelelwa	*C. gloesporoides*	Grey	80	18	4.5	Clavate irregular	9.9	5.1
Sandai	*C. gloesporoides*	Olive grey	86	14.5	4.9	Clavate	10.3	5.5
Barwessa	*C. Capsici*	White grey	83	17.3	4.6	Clavate	10.8	6
Kimwarer	*C. capsici*	White	78	18.1	4.4	Clavate irregular	10.8	5.8
Cheptebo	*C. capsici*	Cotton grey	80	19.5	5.0	Clavate irregular	11.0	5.6
Nyawa	*C. capsici*	White grey	84	16.7	4.8	Clavate	10.5	6.1

Other morphological characteristics traits based on the size of conidia of *C. gloeosporoides* isolates from papaya plants are shown in Table 4.6. The mean length of conidia ranged from 9.9 mm to 18.1 mm (Average = 12.6 mm), the width ranged from 2.8 mm to 4.9 mm (average = 4.1 mm), the average % Cylindrical conidia was 86% with the highest of 100% and lowest of 70%. 60% of the papaya plants had setae and majority had appresoria.

Table 4.6: Morphological characteristics and size of conidia of *C. gloeosporoides* isolates from papaya plants

Area	Length (mm) (Mean amplitude)	Width (mm) (Mean amplitude)	% Cylindrical conidia	Setae	Apressoria
Marigat	12.2(11.8-12)	4.0(3.5-4.5)	100	-	-
Koriema	11(10.3–11.5)	4.5(3.3-3.9)	70	+	…
Kapkelelwa	16.1(15.5-16.7)	3.5(3.2-4.1)	80	+	…
Mochongoi L	13.0(11.2-14.8)	3.7(3.4-4.3)	100	-	..
Barwessa	15.6 (14.4-17.08)	4.5(3.3-4.7)	100	+	..
Kimwarer	13.2(9.9-18.1)	3.0(2.8-3.9)	85	_	.
Cheptebo	15.0(12.5-17.5)	3.8(3.4-4.2)	90	+	.
Tambach L	11.6(9.1-14.1)	4.3(3.6-4.9)	70	+	.

The characteristics of the identified species of fungi causing anthracnose in papaya are shown in Plate 4.2. In terms of colony morphology, the surface morphology had fluffy white, velvet white with raised folds or pink traits. The underside morphology had pink to red brown, ffy white, velvet white with raised folds or pink traits. The underside morphology had pink to red brown, yellow to none brown or pink buff. In microcopy, macroconidia was cylindrical and abundant with thin, roughened walls, with terminal filament. Microconidia had small and round long hyphae, numerous irregular sizes with some attached to branched conidiophores that are usually sparse and cluster shaped.

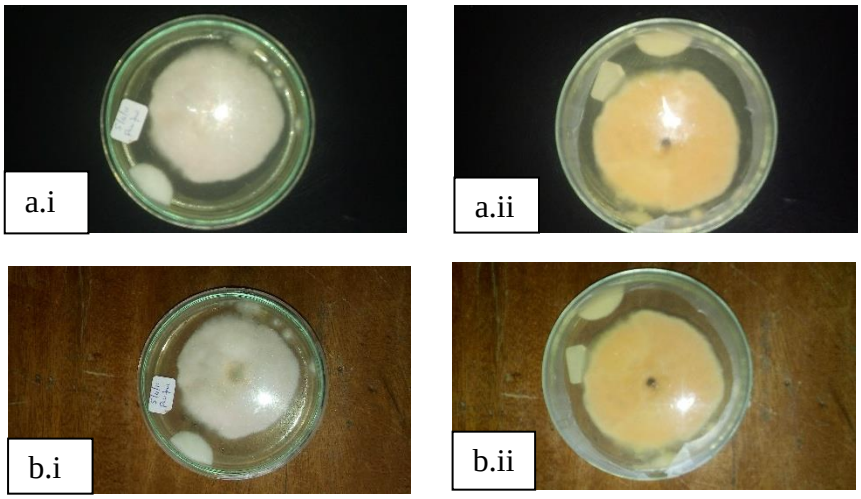

Plate: 4.2: *Colletotrichum gloeosporoides* (8 days) papaya anthracnose culture colony on Potato Dextrose Agar (surface and reverse)

4.2a.i and b.i: The surface of *C. gloeosporoides* (8 days) papaya anthracnose culture colony on Potato Dextrose Agar

4.2a.ii and b.ii: The reverse of *C. gloeosporoides* (8 days) papaya anthracnose culture colony on Potato Dextrose Agar

The cultural characteristics of *Colletotrichum* species are shown in Table 4.7. In terms of shape, all the species were circular in shape and smooth in margin. The cultural characteristics colour of *C. gloesporoides* was mainly grey, black or pale white in colour, with medium to thick texture while the acervuli were found to be dominated by distinct. Meanwhile C. *capsici* was dominated by white or pale white colour, the texture was dominated by low, medium and intermediate texture and the acervuli were mainly indistinct.

Table 4.7: Cultural characteristics of the *Colletotrichum* species

County	Area	Farm	Species	Colour	Texture	Acervuli
Baringo	Marigat	Kaptich	*C. gloesporoides*	Grey	Medium	Distinct
		R7	*C. gloesporoides*	Black	Thick	Distinct
		R1	*C. gloesporoides*	Grey	Thick	Indistict
		L3	*C. gloesporoides*	Grey	Medium	Distinct
	Koriema	Koriema	*C. gloesporoides*	Pale	Medium	Distinct
		Kimalel	*C. gloesporoides*	Black	Thick	Indistinct
		Patkawanin	*C. gloesporoides*	Pale	Medium	Distinct
		Kibingo	*C. gloesporoides*	Black	Medium	Distinct
	Kapkelelwa	Kapkelelwa	*C. gloesporoides*	Grey	Thick	Distinct
		Kisok	*C. gloesporoides*	Grey	Medium	Distinct
		Oinobmoi	*C. gloesporoides*	Pale	Medium	Indistinct
		Kurumbsoo	*C. gloesporoides*	Grey	Intermediate	Distinct
	Mochongoi L	Sandai	*C. gloesporoides*	Grey	Less	Indistinct
		Loboi	*C. gloesporoides*	Black	Medium	Distinct
		Molok	*C. gloesporoides*	Black	Medium	Distinct
		Kaptombes	*C. gloesporoides*	Grey	Smear	Indististinct
	Barwessa	Barwessa	*C. capsici*	White	Low	Distinct
		Muchukwo	*C. capsici*	White	Low	Distinct
		Likwon	*C. capsici*	White	Smear	Distinct
		Chesongo	*C. capsici*	White	Smear	Distinct
Elgeyo	Kimwarer	Kimwarer	*C. capsici*	White	Thick	Indistinct
Marakwet		Seko	*C. capsici*	White	Medium	Indistinct
		Kapokpok	*C. capsici*	White	Medium	Distinct
		Emsea	*C. capsici*	Pale	Smear	Indistinct
	Cheptebo	Cheptebo	*C. capsici*	White	Intermediate	Distinct
		Chekobei	*C. capsici*	White	Medium	Indistinct
		Chepsigot	*C. capsici*	Grey	Intermediate	Indistinct
		Biretwo	*C. capsici*	White	Low	Distinct
	Tambach L	Nyawa	*C. capsici*	Grey	Smear	Indistinct
		Kapshokwei	*C. capsici*	White	Smear	Indistinct
		Kewapsos	*C. capsici*	White	Thick	Indistinct
		Sangeta	*C. capsici*	White	Intermediate	Indistinct

The reaction of different papaya cultivars against anthracnose is shown in Table 4.8. Among the cultivars, pink fleshed took the longest time to show first sign of anthracnose, followed by Kapo Solo HS and Co-1 S each with 5 days. Meanwhile the remaining varieties took 4 days to first showing reaction to anthracnose causative agent. The pink fleshed swe*et al*so had the least PDI at 39 followed by Co-1 S at 46 while the largest PDI occurred in Surya and Kapo Solo HS.

Table 4.8: Response of different papaya cultivars against anthracnose during the study

Variety	Number of days to symptoms	Farm	PDI	Resistance level
Kapo Solo HS	5	Kimwarer Tambach Lower	58	HS
Shillong HS	4	Cheptebo Barwesa	52	HS
Surya HS	4	Mochongoi Lower	60	HS
CO-1 S	5	Koriema	46	S
Pink fleshed sweet	6	Marigat Biretwo	39	S

Key: PDI – Percentage Disease Index
Resistance level: HS – Highly Susceptible
S - Susceptible

The infectivity of *C. papaya* to *C. gloeosporioides* pathogen is shown in Figure 4.1. It took 3 days for upto 20% of the *C. papaya* to be infected and subsequently upto 13 days to achieve 50% infection and 24 days to achieve 75% infection of the *C. papaya* with *C. gloeosporioides*.

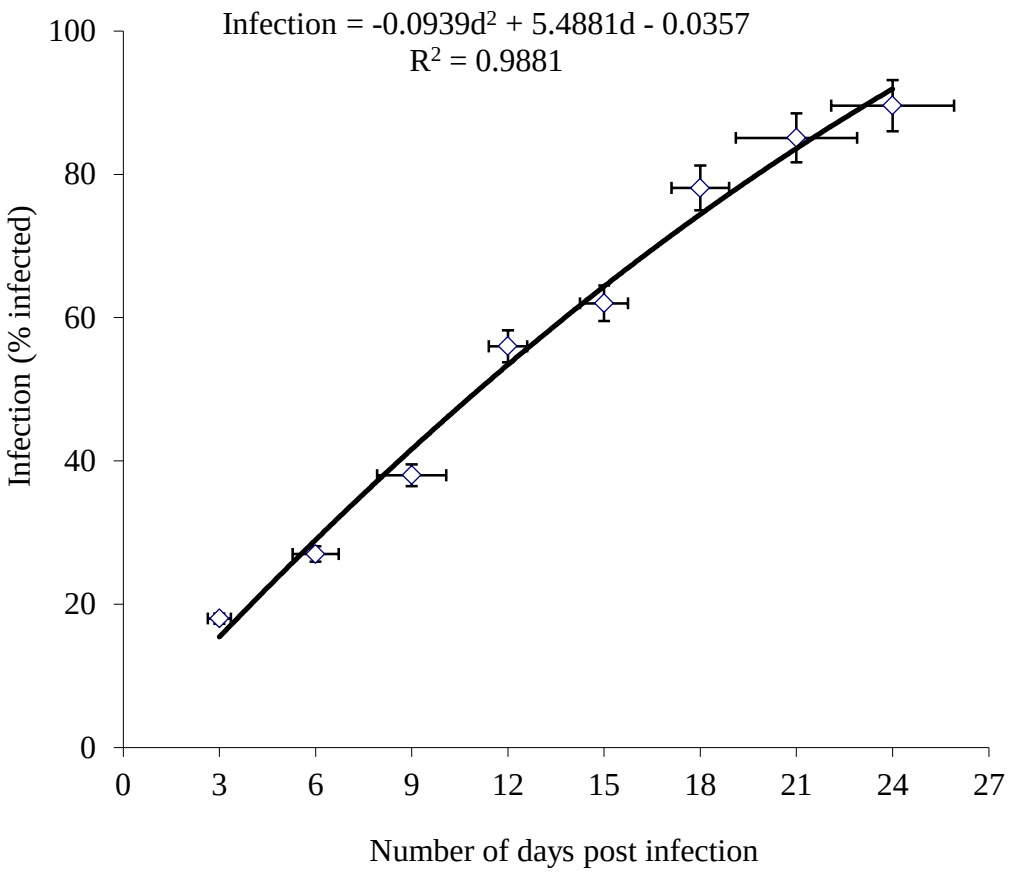

Figure 4.1: Host reaction of *Carica Papaya* fruits to isolates of *C. gloeosporioides* 9 days after wound/drop inoculation and 15 days after non-wound/drop inoculation

External and internal infected papaya fruits are shown in Plate 4.3. The externally infected fruit is soft and has an overflow characteristic on one part. It is yellow, brown and dark colour. It also has dark scars that appear to be a rot. The internally infected papaya fruit of anthracnose has visible infection routes that are brown in colour. It shows that *C. gloeosporoides* penetrated the papaya fruit through appresorium after establishment on the fruit surface, causing infection.

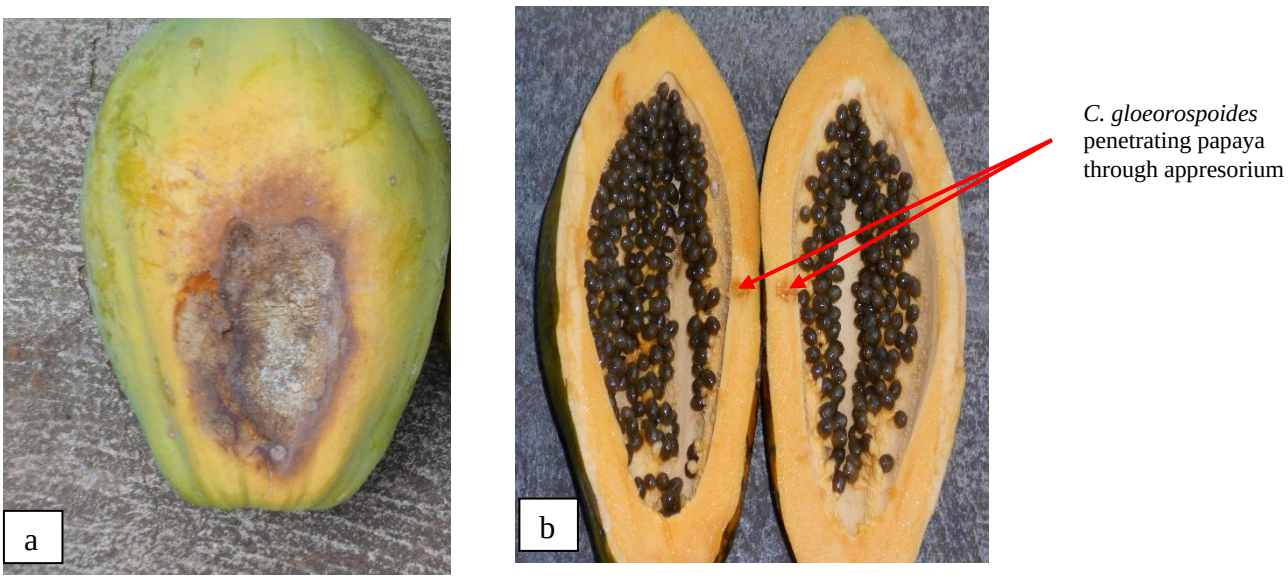

Plate 4.3: External and internal infected papaya fruit of anthracnose

4.3a: Externally infected papaya fruit of anthracnose with visible rot

4.3b: Internally infected papaya fruit of anthracnose with visible route

The pathogenicity tests of *C. gloeosporoides* on papaya are shown in Table 4.9. The total number of plants sampled was 100 in a range of 3 to 24 days. Percengae incidence ranged from 18 to 77%. The highest pathogenicity was achieved from a severity scale of 5 (highly virulent), followed by severity of 4 (moderately virulent) and severity of 3 (virulent). The lowest pathogenicity was recorded in a severity of 1 (very less virulent), followed by sevetity of 2 (less virulent).

Table 4.9: Pathogenicity tests of *C. gloeosporoides* on papaya

Days	Sampled plants	Infected plant	Incidence (%)	Severity	Pathogenicity
3	100	18	18	1	Less virulent
6	100	27	27	2	Less virulent
9	100	38	38	2	Less virulent
12	100	56	56	3	Virulent
15	100	62	62	3	Virulent
18	100	68	68	4	Mod. virulent
21	100	74	74	4	Mod. virulent
24	100	77	77	5	Highly virulent

Conidial characteristics of two groups of *Colletotrichum* species is shown in Table 4.10. The shape of most of the *C. gloeoporioides* were cylindrical with rounded ends with length ranging from 13.56 µm to 14.24 µm and width often around 4 µm. The growth rate of *C. gloeoporioides* ranged from 10.76 mm/day to 11.57 mm/day with lesion diameter from 22.18 mm to 24.17 mm. Meanwhile *C. capsici* were mainly falcate, with acute apex shape and the length was 22.83 µm to 23.84 µm. Width ranged 3.00 µm to 3.02 µm. The growth rate of *C. capsici* averaged 8.17 to 10.42 mm/day with lesion diameter of 18.78 to 19.31 mm.

Table 4.10: Conidial characteristics of aggressiveness of the *Colletotricum* species

Fungal species	Shape Length(um)	Length (µm)	Width (µm)	Growth rate (mm/day)	Lesion diameter (mm)
C. gloeoporioides	Cylindrical, with rounded ends	13.98	4.01	11.02	23.89
C. gloeoporioides	Cylindrical, with rounded ends	13.97	4.02	10.76	24.17
C. gloeoporioides	Cylindrical, with rounded ends	14.24	4.00	11.15	23.43
C. gloeoporioides	Cylindrical, with rounded ends	14.02	4.00	11.57	22.18
C. gloeoporioides	Cylindrical, with rounded ends	13.77	4.00	10.9	23.76
C. gloeoporioides	Cylindrical, with	13.56	4.00	10.93	24.34
C. capsici	Falcate, with acute apex	23.76	3.00	10.42	19.31
C. capsici	Falcate, with acute apex	22.83	3.02	8.17	18.78
C. capsici	Falcate, with acute apex	23.84	3.00	9.13	19.09

4.3 *In-vitro* efficacy of plant extracts against isolates of *Colletotrichum gloeosporoides* on anthracnose infected papaya fruits in Baringo and Elgeyo-Marakwet Counties in Kenya

The study determined the minimum inhibitory concentration of leaves crude extracts of five plants species against *C. gloeosporioides* for methanolic, chloroform and ethnanolic extract of the five plant species are shown in Plate 4.4 (ai, aii, bi and bii). The highest maximum zone of inhibition of *C. gloeosporoides* (12.4mm) was recorded in chloroform (10^0 ppm) extracts of *F. africana* followed by *A. indica* and *C. edulis* while the lowest maximum zone of inhibition occurred in ethanolic extracts obtained from *S. incanum* and *A. chiliensis*. The pawpaw fruit culture exhibited higher inhibition zones (level 4) than the leaf culture (level 3). The white discs represented varying treatments.

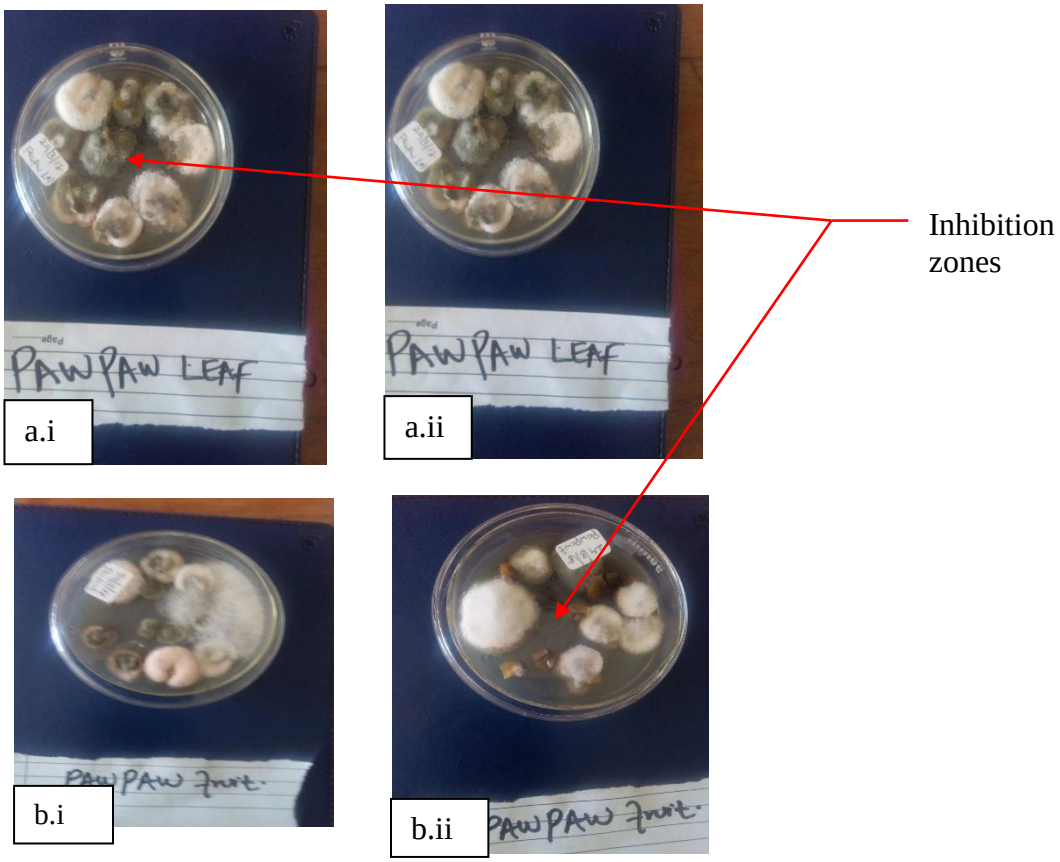

Plate 4.4: Inhibition zones of *C. gloeosporoides* treated with plant extracts

4.4a.i: *F. africana* leaf methanolic extracts showing zone of inhibition at 10^0 and 10^{-3} against *C. gloeosporoides*

4.4a.ii: *F. africana* leaf chloform extracts showing zone of inhibition at 10^0 and 10^{-3} against *C. gloeosporoides*

4.4b.i: *S. incanum* fruit methanolic extracts showing zone of inhibition at 10^0 and 10^{-3} against *C. gloeosporoides*

5b.ii: *S. incanum* fruit chloroform extracts showing zone of inhibition at 10^0 and 10^{-3} against *C. gloeosporoides*

The maximum zones of inhibition from a logistic regression of the bioassays performed on the leaves extracts of five plant species on *C. gloeosporioides* is shown in Figure 4.2. The response of *C. gloeosporioides* to the leaf extracts of the five species were significant ($P < 0.05$) with extracts from *F. africana*, *A. indica* and *C. edulis* displaying significant dose-response relationship. The trends of the estimated minimum zone of inhibition of the in the plates after treatment with leave extracts of the *F. africana*, *A. indica* and *C. edulis* fully fitted the logistic regression model describing a dose response treatment ($R^2 > 0.96$). The trends in curves followed the trend: *F. africana* > *A. indica* > *C. edulis* > (*A. chiliensis* = *S. incanum*).

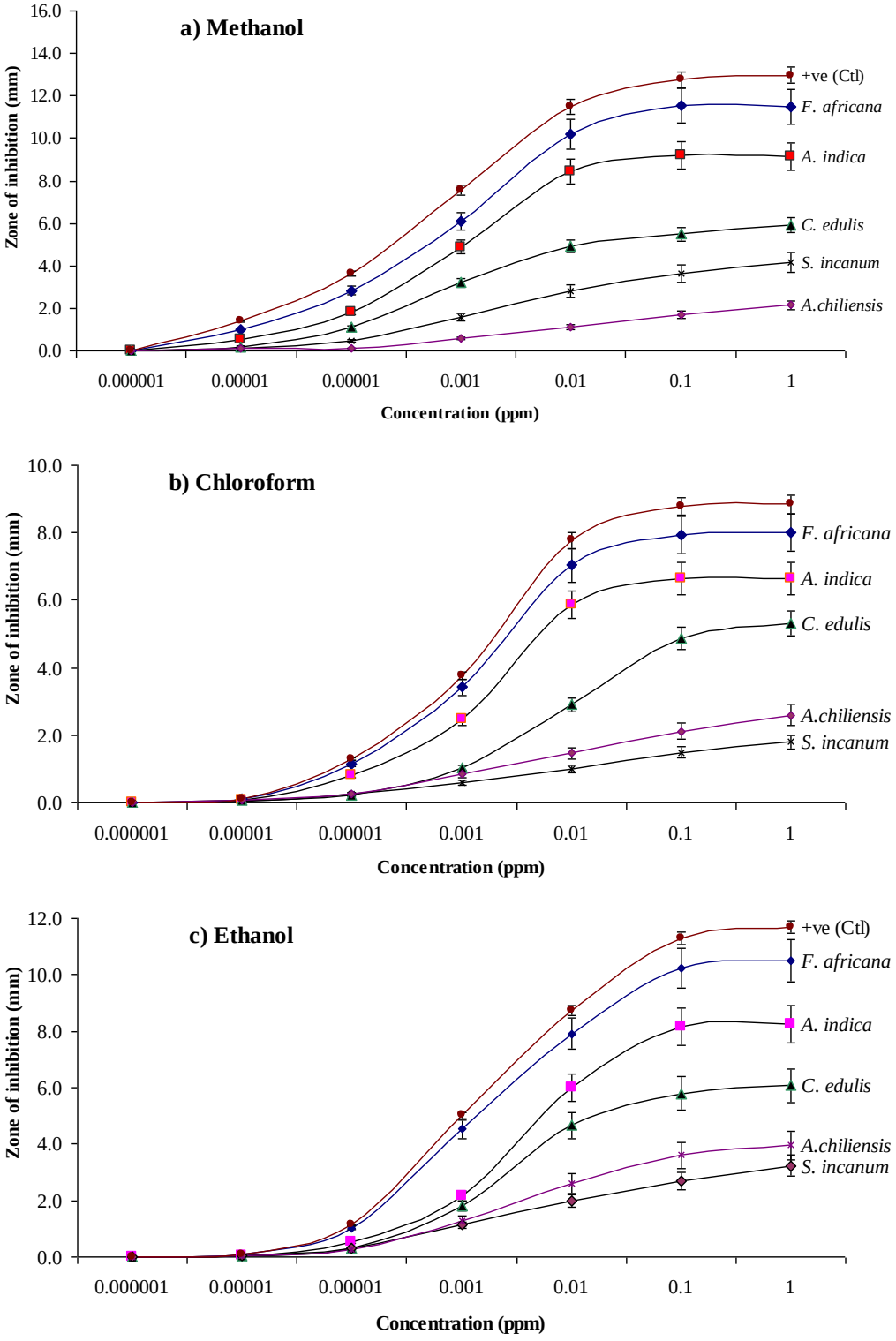

Figure 4.2: Efficacy (minimum zone of inhibition) of leaves crude extracts of five plants species against *C. gloeosporioides*

NB: The vertical bars denote the standard error of the mean calculated from ANOVA

The maximum zones of inhibition from a logistic regression of the bioassays using fruit extracts of the plant species on *C. gloeosporioides* are shown in Figure 4.3. The response of *C. gloeosporioides* to the fruit extracts of the five species were significant ($P < 0.05$) where the extracts from *S. incanum, F. africana, A. chiliensis,* and *A. indica* displayed significant dose-response relationship and fully fitted the logistic regression model describing a dose response treatment ($R^2 > 0.94$). The trends in curves followed the trend: *S. incanum* > *F. africana* > *A. chiliensis* > *A. indica* > *C. edulis*.

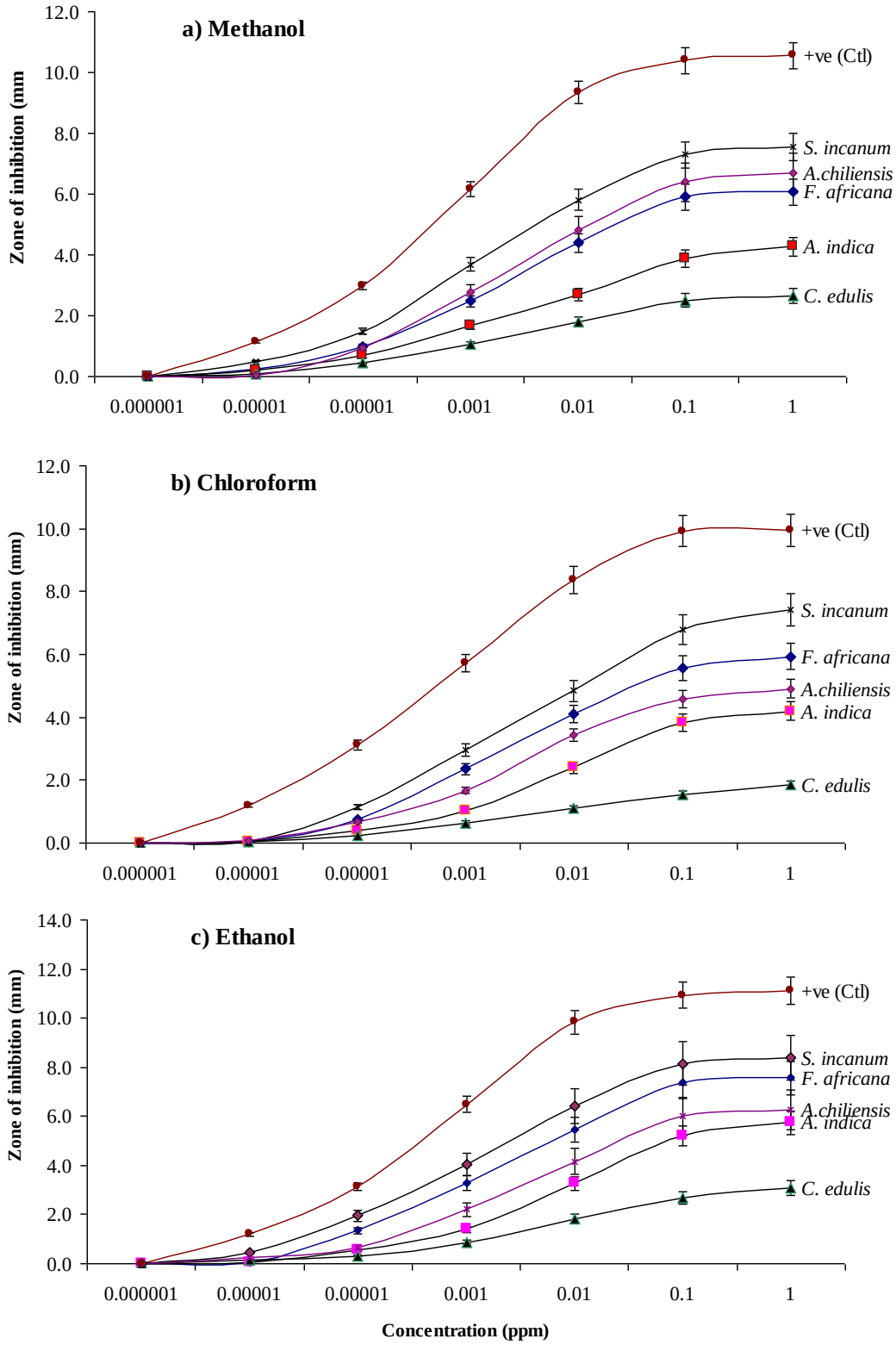

Figure 4.3: Efficacy (minimum inhibitory concentration) of fruits crude extracts of five plants species against *C. gloeosporioides*

NB: The vertical bars denote the standard error of the mean calculated from ANOVA

The minimum zones of inhibition from a logistic regression of the bioassays performed using root extracts of the five plant species against the fungi are shown in Figure 4.4. The response of *C. gloeosporioides* to the leave extracts of the five species were significant ($P < 0.05$) dose-response relationship and fully fitted the logistic regression model describing a dose response treatment ($R^2 > 0.95$). The trend curves of the minimum zone of inhibition followed the trend: *F. africana* > *A. indica* > *A. chiliensis* > *C. edulis* > *S. incanum*.

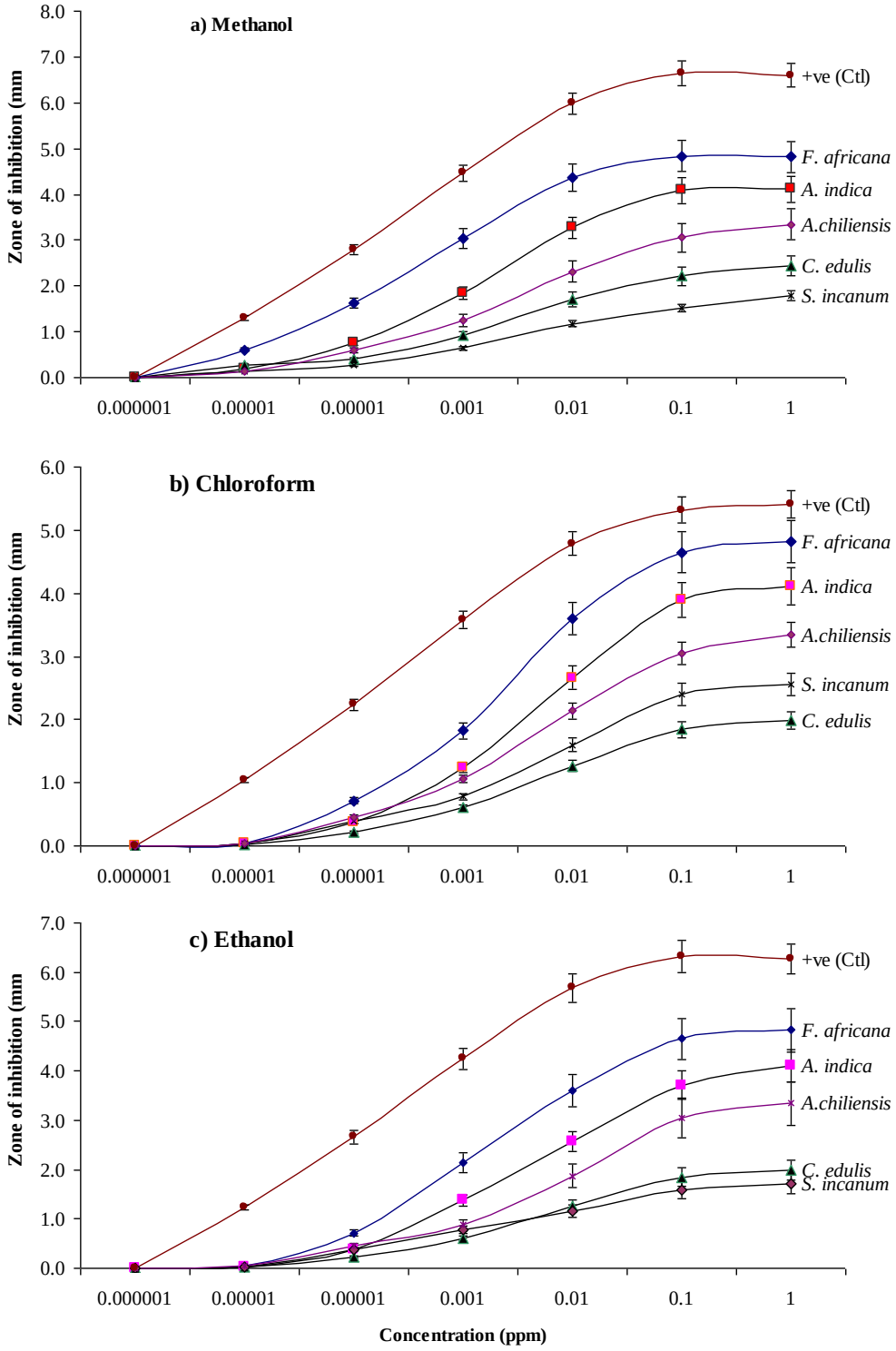

Figure 4.4: Efficacy (minimum inhibitory concentration) of roots crude extracts of five plants species against *C. gloeosporioides*

NB: The vertical bars denote the standard error of the mean calculated from ANOVA

4.4 *In-vivo* efficacy of plant extracts against isolates of *Colletotrichum gloeosporoides* on anthracnose infected papaya fruits in Baringo and Elgeyo-Marakwet Counties in Kenya

Percentage infection, severity of infection, soluble solid content and mass loss of papaya fruit were subjected to plant specimens for 8 days at 27^0C is shown on Table 4.11. The percentage infection was 36 – 82%, while the highest and lowest severity was 4 and 2 respectively. The range for the soluble soluble content was 7.0 – 9.5 with the highest being 9.5 and lowest 5.5. The highest percentage mass lose was 8.2% and the lowest was 5.5%, with the range being 5.5 – 8.2%. The means of infection percent, severity of infection, soluble solid content and mass loss percent are 41.17, 2.50, 7.8 and 6.63 respectively after papaya parts were treated by plant leaf extracts of *F. africana, S. incanum, C. edulis*, *A. indica* and *A. chiliensis*. However, the control was more effective in all cases.

Table 4.11: Effect of leaf extracts on infection percentages and severity, soluble solid content and fruit mass loss of papaya inoculated with *C. gloeosporioides*

Plant specimen	**Papaya Fruit**			
	Infection %	Severity of infection	Soluble solid content	Mass loss %
Control	82	4	9.5	8.2
F. africana	41	2	7.8	6.5
S.incanum	36	2	7.1	5.8
C. edulis	49	2	7.0	5.5
A.indica	45	2	7.5	6.2
A.chiliiensis	36	3	8.9	7.6
Mean	**41.2**	**2.5**	**7.8**	**6.6**

Number of days to healing of infected *C. papaya* after treatment with the leaf extracts is shown in Figure 4.5. There were differences in the number of days to healing of the *C. papaya* infected with anthracnose (χ^2 = 12.3433, df = 4, P = 0.0002) maximum of 10 days. The fruits of *C. papaya* infected with anthracnose healed faster when treated with the leaf extracts of *F. africana* followed by *A. indica* while treatment with *S. incanum* and *A. chiliensis* leaf extracts resulted in significantly the longest healing time of over 7 days.

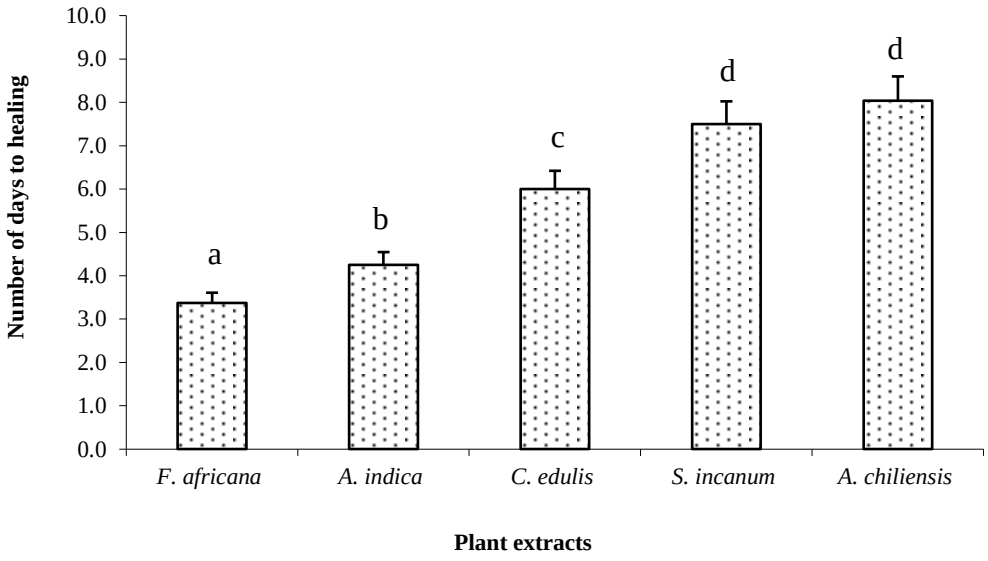

Figure 4.5: Number of days to healing of infected *C. papaya* after treatment with the leaf extracts of the plants

NB: The vertical bars denote the standard error of the mean calculated from ANOVA. Similar lettering above each indicates that the means are significantly different for each of the plant extracts ($p<0.05$).

Number of days to healing of infected *C. papaya* after treatment of the fruits with plant extracts is shown in Figure 4.6. There were significant differences in the number of days to healing after treatment of the *C. papaya* infected fruits with the fruit extracts of herbal

plants (χ^2 = 7.2442, df = 4, P = 0.0007). Healing occurred in the shortest time in *C. papaya* treated with fruit extracts of *S. incanum* followed by healing from *F. africana* and *A. chiliensis* while the number of days to healing was delayed in *C. papaya* treated with fruit extracts of *A. indica* and *C. edulis*.

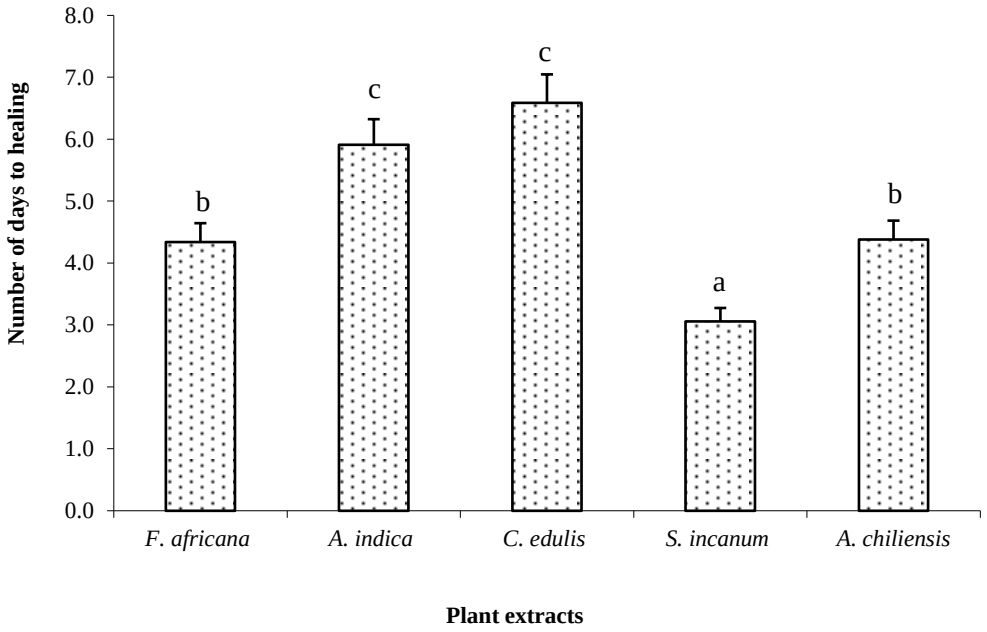

Figure 4.6: Number of days to healing of infected *C. papaya* after treatment with fruit extracts of the herbal plants

NB: The vertical bars denote the standard error of the mean calculated from ANOVA. Similar lettering above each indicates that the means are not significantly different for each of the plant extracts ($p<0.05$).

The number of days to healing of infected *C. papaya* after treatment with the roots extracts of the five medicinal plants is provided in Figure 4.7. There was a significant difference in the number of days to healing after treatment of the *C. papaya* infected fruits with the root extracts of herbal plants (χ^2 = 4.6674, df = 4, P = 0.0012). Healing of infected fruits occurred in the shortest time in *C. papaya* treated with root extracts of *F.*

africana and *A. indica* followed by *fruit* healing after treatment with *A. chiliensis* while extracts from *C. edulis* and *S. incanum* delayed healing of infected *C. papaya*.

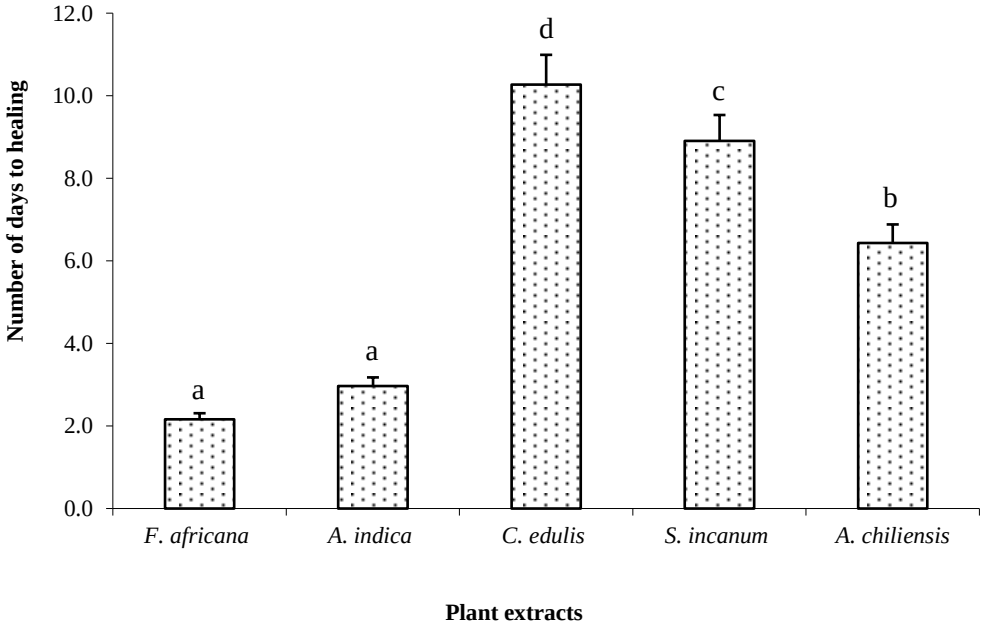

Figure 4.7: Number of days to healing of infected *C. papaya* after treatment with the roots extracts of the plants

NB: The vertical bars denote the standard error of the mean calculated from ANOVA. Similar lettering above each indicates that the means are not significantly different for each of the plant extracts ($p<0.05$).

The results of diameter of inhibition, inhibition effect and spore germination of antifungal activity of solvent extracts of some plant species from Kerio Region against *C. gloeosporoides* are shown on Table 4.11. Strong antifungal activity was exhibited by the extracts of *Fuesrtia africana* chloroform leaf extracts, with a diameter of inhibition of 4.5 mm and a growth inhibition score of 4; followed with a diameter of inhibition of 4.4 mm,

and a growth inhibition score of 3. *Aloe chiliensis*-ethanolic extracts diameter of inhibition of 1.3 mm and a growth inhibition score of 3 had the weakest antifungal activity. *A. chiliensis* chloroformfruit extracts showed spore germination of 35.7%, followed by *F. africana* methanolic leaf extracts (36.6%) and *F. africana* chloroform leaf extracts (35.5%). *A. chiliensis* ethanolic root extracts had the lowest spore germination of 0.7%.

TABLE 4.12: Antifungal activity of solvent ((1 x 10^{-3}) extracts of some plant species from Kerio Valley region against *C. gloeosporoides*

Plant species	Plant family	D1 (mm)[a]	1E[b]	Spore germination (%)[c]
F. africana	Labatiae	4.1	3	12.5
S. incanum	Solanaceae	13.1	4	3.1
C. edulis	Apocynaceae	2.0	1	20.0
A. indica	Meliaceae	1.6	3	1.1
A. chiliensis	Asphodelaceae	30.5	1	28.9
Control		0.0	4	1.4

[a]diameter of inhibition zone in mm measured after 4 days of incubation

[b]inhibition effect on a 0-4 scale, where 0 = none and 4 = strong

[c]spore germination 24 h after treatment

values are means of three replications

CHAPTER FIVE

DISCUSSION

5.1 Prevalence, incidence and severity of anthracnose in *Carica papaya* fruits in Baringo and Elgeyo-Marakwet Counties in Kenya

During the study it was positively determined that the pawpaw fruit were infected with anthracnose through visual observation. The symptoms of infection (Plate 4.1) were on the leaves, initially as small angular, brown to black spots that coalesce to form large extensive lesions on the leaf, which were common around the edges of the leaves. In fruits of pawpaw, the symptoms were exemplified as tear strain symptom by occurrence of linear necrotic regions on the fruit was associated with superficial cracking of the fruit epidermis causing an alligator skin effect on the fruit surface which is consistent with description of the same conditions in other studies (Saini *et al*., 2016; Aktaruzzaman *et al*., 2018). Anthracnose spots on green fruits are generally dark brown to black with a pale margin and lenticular in shape (Snowdon, 2017). The affected areas increased in size and become sunken and coalesce to form large spots. Later, these lesions become enlarged, rounded, sunken and brown to black in colour (Saini *et al*., 2016). The disease usually appears on the ripening portions of the fruit, occasionally the green portions of the fruit may become infected (Dickman and Alvarez, 1983). The disease symptoms are in the form of brown to black depressed spots on the fruits. The centers of these lesions were often covered with pink, gelatinous masses of spores especially during moist, warm weather. Symptoms first appear as brown superficial discolorations of the skin and then develop into circular, slightly sunken area, 1- 3 cm in diameter; usually they appear watersoaked (Srivastava and Tandon, 1971). Gradually the lesions coalesce and sparse

white mycelial growth often appears on the margin of such spots under humid condition an encrustation of salmon pink spores. Basically through visual observation it was easy to identify the infected fruits and leaves of the pawpaw plant which unfortunately occur when the disease has significantly progressed within the plant.

The field survey for papaya anthracnose (Tables 4.1 and 4.2) was conducted from February 2016 to December 2016, and microscopy studies carried out in 320 samples. The study showed that the prevalence of anthracnose in *C. papaya* in the farms was 95% in Baringo and upto 83% in Elgeyo-Marakwet County. These findings concur with those found in other studies in Europe (Torres-Calzada *et al*., 2013), Bangladesh (Hamim *et al*., 2014a) and Ethiopia (Mekonnen *et al*., 2015) of prevalence and incidence on banana. The differences in the prevalence of anthracnose disease were associated with the locality as well as altitude (Bellini *et al*., 2003).

The study also determined the overall incidence of anthracnose disease based on observation of 2682 fruits. The papaya fruit varieties that were surveyed against respective farm areas in the two counties were Kapo Solo in Kimwarer and Tambach Lower, Shillong in Cheptebo, Surya in Mochongoi Lower, Co.1 in Koriema and Barwessa and Pink Fleshed Sweet in Marigat and Biretwo. The study indicated that the incidence of of anthracnose was 9.23 ± 1.22% in Baringo County and 4.5 ± 1.1% in Elgeyo Marakwet County resulting in an overall of of 7.5 ± 1.0% in the region. The result indicates a higher incidence of anthracnose in fruits sampled from Baringo than Elgeyo Marakwet County, which is due to the differences in the weather conditions. Anthacnose occur more in more hot and humid conditions and incidence is often lower in areas that

are lower in temperature (Silva *et al*., 2007). In the current study, Baringo County was established to be hot and humid than Elgeyo Maraket and therefore one of the main causes of the higher incidence of anthracnose disease. The altitude was also found to affect the incidence of the anthracnose disease but this may be related directly to the variations in the temperature and other altitude related effects as established by McConnel *et al*. (2018).

The occurrence, prevalence and incidence of anthracnose were found to differ significantly ($P < 0.5$) with altitude. The differences in the prevalence of anthracnose disease were associated with the weather as well as altitude (Table 4.4). In an altitude range of 1051 – 1100m, the number of papaya leaves that were defoliated out of 100 papaya trees was 16. The number of leaves that underwent defoliation in an altitude range of 1351 – 1400m was only 8. Similarly, the number of papaya fruits that yellowed was 12, in an altitude range of 1051 – 1100m. The number of fruits, out of 100 pawpaw trees that yellowed in an altitude range of 1351 – 1400m was a paltry 3. This showed that the lower the altitude range, the lower or sometimes symptoms development of *C. papaya*, and the higher the altitude range, the lower the cases of anthracnose symptoms as in Biretwo and Nyawa in Elgeyo-Marakwet County. This means that leaf defoliation and fruit yellowing have similar ($p<0.05$) rates with change in altitude range (Olubode *et al*., 2017). In the two counties, higher altitudes showed decline in the incidence of the disease, which concurs with other known studies done elsewhere (Tasiwal, 2008; Oniha and Egwari, 2015).

The severity of anthracnose disease in *C. papaya* (Table 4.3) showed specific trends. Generally the severity of the anthracnose disease was more significant in Baringo than Elgeyo Marakwet and is attributed to the weather conditions and altitude. However, it was found that severity of the disease tended to correlate with incidence which concurs with other studies of the anthracnose disease in other plants (Hossain *et al*., 2010). In areas where incidences of the disease was low, there was less severe cases of the disease which was likely due to the exposure of the region to more aggressive forms of *C. gleospoiredes*. Meanwhile, when incidences of anthracnose disease were higher, the severity of the disease increased mainly to levels 4 and 5 suggesting that the pathogen had multiplied after long period of exposure to the plants. It is also probable that at higher incidences, the severity was higher due to the multiplicity of the pathogens infecting the plants. This may be associated with the econimics of the pathogen control where after prolonged exposure and attempts at controlling the pathogens, the local community members appear to accept that the problem is there (Ready *et al*., 2017). There is also a possibility that higher incidences causes more exposure of the fruits to pathogens and with the help of environmental conditions causes increased severity.

5.2 Characterization of *Colletotrichum gloeosporoides* isolates infecting *Carica papaya* fruits in Baringo and Elgeyo-Marakwet Counties in Kenya

Colletotrichum is one of the most important genera of pathogenic fungi worldwide causing economically important diseases of cereals, grasses, legumes, vegetables and perennial crops including trees (Wharton and Dieguez-Uribeondo, 2004; Turechek *et al*., 2006; Sutton, 1966; Than *et al*., 2008). The fungi attack all parts of the plant, at all stages

of development, from seedlings to mature plant and seed, causing disease symptoms commonly known as anthracnose (Bailey and Jager, 1992). Although *Colletotrichum* spesies are known as plant pathogens, recent reports have indicated that there are some species of *Colletotrichum* that are associated with human and animal diseases. These infections have mostly been found in immunosuppressed people (Cano *et al*., 2004). Mendiratta *et al*. (2005) reported that *C. dematium* is responsible for corneal ulcers called keratitis in humans. Other *Colletotrichum* species cause infection in fruits and include *C. gloeosporioides*, *C. coccoides* (Wallr.) and *C. graminicola* (De Hoog, 1995; Cano *et al*., 2004). Therefore their characterization is of utmost importance.

A combination of morphological characters, cultural characters, tests on pathogenicity and aggressiveness confirmed that *C. gloeosporioides* and *C. capsici* are the main causative agents for anthracnose disease of papaya fruit in Baringo and Elgeyo-Marakwet Counties in Kenya. *C. gloeosporioides* and *C. capsici* pathogens cause huge economic loses in fruits such as papaya, avocado, mango and oranges majorly in tropical areas (Meela *et al*., 2019).

Based on morphological characteristics (Tables 4.5 and 4.6), most colonies of *C. gloeosporoides* were dark grey, with few being olive grey, with conidium diameter, lenth and width ranging from 80 to 86 mm, 14.1 to 19.5 mm and 4.1 to 5.0 mm respectively. On the other hand, most colonies of *C. capsici* had white grey colony colour, with a diameter range of 78 mm to 84 cm, conidium length of 16.7 mm to 19.5 mm, and conidium with of 4.4 mm to 5.0 mm. Most colonies of *C. gloesporoides* and *C. capsici* had grey colony colour, with clavate irregular appresorium shape (Damm *et al*., 2010). The identification of the isolates based on morphological characteristics showed a

variation among *Colletotrichum* species. *C. gloeosporoides* isolates exhibited characteristics already described by several authors (Lei *et al*., 2016) allowing their identification. Moreover morphological characters of *C. gloeosporoides* described in this study are similar to the characters observed by Asudi (2010) on banana. *C. gloeosporioides* is a group species made up different genetically distinct species brought together by similar conidial morphology and rDNA-ITS nucleotide sequences (Ombiri *et al*., 2002). It has been reported that the taxa *C. musae, C. kahawae, C. xanthorrhoeae, C. nupharicola, C. fragariae,* C. *gloeosporioides sensu stricto*, *C. horii*, *C. theobromicola*, *C. ignotum*, *C. tropicale*, *C. asianum, C. siamense, C. fructicola* and *C. hymenocallidis* as well as many putative undescribed species are all part of the *C. gloeosporioides sensu lato* complex (Damm *et al*., 2010). In several instances, the term *C. gloeosporioides* has been used interchangeably to refer to both *C. gloeosporioides sensu stricto* and the group species (Sreenivasaprad *et al*., 1996). However, the distinction was made very clear by Phoulivong, (2011) who restricted the name to *C. gloeosporioides sensu stricto*, the original strain obtained from citrus which has recently been identified also on orchids. There exists some amount of confusion in the naming of isolates of *C. gloeosporioides* when differences in the nucleotide sequences of the ITS1 region were found among different isolates named as *C. gloeosporioides*. To overcome this confusion, Sreenivasaprad *et al*, (1996) proposed up to 3.6% variations in sequence homology as the ceiling for the naming of variants as *C. gloeosporioides*. The consequence of these ceiling was that other species especially, *C. kahawae* which showed a percent variation of less than 3.6% with some isolates of *C. gloeosporioides* had to be renamed. To solve the problem, it was proposed that *C. gloeosporioides* must be defined to cater for the *C.*

kahawae isolates. However, Phoulivong, (2011) showed that some isolates which have wrongly been assumed to be variants of *C. gloeosporioides* especially on tropical fruit crops are distinct *Colletotrichum* isolates whose species status has not been ascertained yet. The authors also reported that the anomalies detected in the identification of *C. gloeosporioides* isolates were due to the comparison of unknowns to wrong type strains (Phoulivong, 2011).

Colletotrchum gloeosporoides isolate representatives of the 32 farms evaluating cultural characteristics (Table 4.7) grown in the universal media (PDA) showed diverse response in attaining maximum growth (Braganca *et al*., 2016). The isolates varied significantly in cultural and conidial characteristics, produced grey to pale white colour (Cai *et al*., 2009). The colour of *C. gloesporoides* was mainly grey, black or pale white in colour, with medium to thick texture while the acervuli were found to be dominated by distinct. Sporulation also showed greater variation in different media, ranging from excellent to poor sporulation. The effect of the different culture media differered significantly. Meanwhile C. *capsici* was dominated by white or pale white colour, the texture was dominated by low, medium and intermediate texture and the acervuli were mainly indistinct.

Many isolates of *C. gloeosporoides* often exhibit extensive variation in culture and furthermore, the culture conditions, including the media, the age of the culture and temperature used, cannot ne standard between laboratories (Cai *et al*., 2009). Thus *C. gloeosporoides* showed different types of variability in colony morphology, colony coluor and radial growth in PDA, which was found to be the most suitable medium for

growth of the fungal isolates (Honger *et al*., 2016). However, according to Kanchana – Udomkan *et al*. (2004), no significant difference was noticed in shape and size of the conidia among the different isolates. Therefore, the results of the present study have revealed the existence of cultural variability among the fungal isolates. The study gives vital and basic information on the cultural characteristics of the *C. gloeosporoides* pathogen causing anthracnose of papaya.

Based on aggressiveness (Table 4.10), the shape of most of the *C. gloeoporioides* were cylindrical with rounded ends with length ranging from 13.56 µm to 14.24 µm and width often around 4 µm. The growth rate of *C. gloeoporioides* ranged from 10.76 mm/day to 11.57 mm/day with lesion diameter from 22.18 mm to 24.17 mm. Meanwhile *C. capsici* were mainly falcate, with acute apex shape and the length was 22.83 µm to 23.84 µm. Width ranged 3.00 µm to 3.02 µm. The growth rate of *C. capsici* averaged 8.17 to 10.42 mm/day with lesion diameter of 18.78 to 19.31 mm.

The papaya varieties did not demonstrate complete resistance in any of the *C. gloeosporoides* isolates. According to Aktaruzzaman *et al*. (2018), the variations of the quantitative measurements on disease development provided an idea of the diverse nature of the fungal population in their aggressiveness, that is, natural varience in virulence. The level of the aggressiveness of the isolates of a given pathogen is also an important consideration in disease control magaement (Asudi *et al*., 2016). In order to establish aggressiveness classes, and taking into focus the high correlation values observed from the papaya fruits, a headmap comprising of aggressiveness traits was produced (Duran *et al*., 1999). According to Kiprop *et al*. (2002), the isoltes were grouped in five different classes of aggressiveness that is high, moderate and low. Highly aggressive isolates are

able to reach the complete necrosis of papaya fruit tissues in 23.89 – 24.34 mm. Moderate aggressive isolates were able to reach the complete necrosis of papaya tissues in 22.18 – 23.76 mm and to reach the severity level 4. Finally, the low aggressive isolates are able to reach the complete necrosis of papaya fruit tissues in more than 19 mm and reach the severity level 4.

Among papaya cultivars (Table 4.8), pink fleshed took the longest time to show first sign of anthracnose, followed by Kapo Solo HS and Co-1 S each with 5 days. Meanwhile the remaining varieties took 4 days to first showing reaction to anthracnose causative agent. The pink fleshed sweet also had the least PDI at 39 followed by Co-1 S at 46 while the largest PDI occurred in Surya and Kapo Solo HS.

There was little variation observed among the five tested varieties when screened against anthracnose disease. All the varieties screened developed first symptoms in 4 days after inoculation except Kapo Solo, Co-1 and Pink Fleshed Sweet which showed symptoms after 5 and 6 days respectively. The results revealed that, there was no variety that was completely or resistant jn regard to the PDI (Jones and Layne, 2009). Co-1 and Pink Fleshed sweet varieties had some resistance to infection by *C. gloeosporoides*, more than than Kapo Solo, Shillong and Surya. No sources of resistance were identified during the screening of germplasm against anthracnose disease of papaya. The reaction of different cultivars against anthracnose disease of papaya was found only in the categories of susceptible or highly susceptible (Lustria *et al*., 2009). Co-1 and Pink Fleshed Sweet varieties were found to have a susceptible reaction while Kapo Solo, Shillong and Surya had a highly susceptible reaction (Mumo *et al*., 2013). In the current study, none of the

tested papaya varieties had resistance to *Colletotrichum gloeosporoides*.

Pathogenicity tests (Table 4.9) performed using Pink Fleshed Sweet papaya variety with the *Colletotrichum* species isolated, showed that all were able to infect and cause symptoms in wounded pawpaw fruit, proving that both species were causal agents of anthracnose infection on pawpaw. In this study This study, the total number of plants sampled was 100 in a range of 3 to 24 days. Percengae incidence ranged from 18 to 77%. The highest pathogenicity was achieved from a severity scale of 5 (highly virulent), followed by severity of 4 (moderately virulent) and severity of 3 (virulent). The lowest pathogenicity was recorded in a severity of 1 (very less virulent), followed by sevetity of 2 (less virulent). This is the first report in Côte d'Ivoire to highlight the pathogenicity of *C. gloeosporioides* on pawpaw. The results of Latiffah *et al* (2009) also showed the involvement of *C. gloeosporioides* in pawpaw anthracnose in Malaysia. The fact that *C. gloeosporioides* was a pathogen of pawpaw confirmed numerous reports about the cross-infection potential among different species of *Colletotrichum* on a multitude of hosts (Freeman *et al*., 1998). A difference in virulence was found depending on the inoculated *Colletotrichum* species. The severe symptoms observed in wounded leaves and fruits demonstrated the importance of epidemiological studies to verify the usefulness of pruning leaves before blooming so as to frustrate primary inoculum. All fungal isolates were pathogenic for the leaves and fruits in Baringo and Elgeyo-Marakwet Counties. However, there was variability in virulence among the 32 fungal isolates from the fruits. This showed significant virulence compared with other studies on *C. acutatum*. The highest virulence of *C. gloeosporoides* could be explained by the affinity of this species

to pawpaw fruit. *C. gloeosporoides* has been reported to be the most common causal anthracnose of many pawpaw cultivars (Jinyoung Lim *et al*., 2002, Priyadarshanie *et al*., 2015).

5.3 *In vitro* efficacy of plant extracts against isolates of *C. gloeosporoides* on anthracnose infected papaya fruits in Baringo and Elgeyo-Marakwet Counties in Kenya

In this study, the minimum inhibitory concentration of leaf crude extracts (Plate 4.4 and Figures 4.2, 4.3 and 4.4) of five plants species against *C. gloeosporioides* for methanolic, chloroform and ethnanolic extract of the five plant species were tested. It was reported by Cakir *et al*. (2004) that methanol, chloroform, ethanol and ethane extracts were effective in controlling post harvest diseases, while maintaining the fruit quality. It was established that the highest minimum inhibitory concentration was recorded in *C. gloeosporioides* treated methanolic leaf extracts of *F. africana* extracts followed by chloroform *A. indica* and *C. edule* extracts. The lowest minimum inhibitory concentration was recorded in ethanolic *S. incanum* and *A. chiliensis* extracts. This suggest that these plant contain bioactive compounds that have anti fungal efficacies as reported elsewhere (McConnel *et al*., 2017; Gregory *et al*., 2018; Christpher *et al*., 2019; Merub *et al*., 2019). The most probable compounds responseible for the observed phytochemical characteristics are sitosterol and β-sitosteryl-β-Dglucoside in *F. africana*, and in *A. indica* and *C. edule* are *Azadiractin*, lignans, pipenoiols, steroids, neolignans, alkaloids, propenylphenols, terpenes, piperolides, chalcones, flavanones, flavones, and amides bearing isobutyl, pyrrolidine, dihydropyridine, and piperidine moieties, all of which could exhibit high antimicrobial and antifungal properties (Navickiene *et al*., 2000; Villasenor *et al*., 2002;

Johann *et al*., 2019). The levels of plant bioactive compounds with antifungal activity could be influenced by many factors which include the age of plant, harvesting time point, extraction solvent, and method of extraction (Okigbo, 2005).

The minimum zones of inhibition of *C. gloeosporioides* when treated with extracts of fruit extracts showed that the extracts from *S. incanum*, *F. africana*, *A. chiliensis*, and *A. indica* displayed significant dose-response relationship and fully fitted the logistic regression model describing a dose response treatment ($R^2 > 0.94$). The trends in curves followed the trend: *S. incanum* > *F. africana* > *A. chiliensis* > *A. indica* > *C. edulis*.

The findings of this study are in tandem with previous reports on the antifungal activity of *F. africana*, *A. indica*, *A. chiliensis*, *C. edulis* and *S. incanum* (Sahito *et al*., 2003). Previous phytochemical investigation of *F. africana* resulted in isolation of different kinds of alkaloids and the active ingredients of this plant have antifungal properties that could prove beneficial to agriculture (Omwenga *et al*., 2009). Crude extracts of *Asparajus racemosus* exhibited antifungal activity and the groups of compounds present that were likely to be responsible for the fungitoxicity were identified to be glycosides, saponins and tannins (Sancho *et al*., 2011).

On the field, application of fungicides to control Pawpaw anthracnose can reach as high as 25 sprays of both contact and systemic fungicides within a production season in environments favourable to the development of the disease (Dodds *et al*., 2000). In drier areas or areas where the production season completely escapes the rains, the crop can be grown without the application of fungicides (Arauz, 2000). Different strategies have been developed for the application of fungicides in different locations for the management of the disease. In Florida, a comprehensive field spray programme involving the weekly

application of fungicides from flowering to fruit set followed by weekly application of systemic or biweekly application of contact fungicides has been developed (MacMillan, 2019).

The minimum zones of inhibition from a logistic regression of the bioassays performed on using root extracts of the five plant species against the fungi showed that *C. gloeosporioides* to the root extracts of the five species were significant ($P < 0.05$) dose-response relationship and fully fitted the logistic regression model describing a dose response treatment ($R^2 > 0.95$). The trend curves of the minimum zone of inhibition followed the trend: *F. africana* > *A. indica* > *A. chiliensis* > *C. edulis* > *S. incanum*.

5.4 *In vivo* efficacy of plant extracts against *Colletotrichum gloeosporoides* on anthracnose infected papaya fruits in Baringo and Elgeyo-Marakwet Counties in Kenya

The fungitoxic effects of aqueous plant extracts of different plant species indicate the importance of many plant species as a possible natural source of fungicidal materials. Many workers have reported antifungal activities of different plant species and stressed the importance of plants as a possible source of natural fungicides (Tewari and Dath, 1984; Lakshmanan, 1990; Ogbebor *et al*., 2007).

The effect of leaf extracts on infection percentages, severity of infection, soluble solid content and fruit mass loss of papaya fruit (Table 4.11) were inoculated with *C. gloeosporoides* and stored for 8 days at 27^0C, *F. africana* was found to be most effective with 41 infection %, severity infection scale 4, soluble solid content of 7.8 and mass loss of 6.5%. *S. incanum*, *C. edulis*, *A. indica* and *A. chiliensis* followed in that order with

respect to infection %, severity, soluble solid content and mass loss %. This deduces that these papaya fruit parameters increases with the ability of plant extracts, and declines with those plant extracts with less ability of treatment of *C. gloeosporoides*. Infection % and severity of infection are similar ($p<0.05$) in that rising and declining behaviour. However, there is no correlation between soluble content and mass loss % because they do not have sharp rise or sharp decline. They have mixed results among plant extracts. Carbendazim, the control has the highest ability than any of the plant extracts.

During the *in-vivo* test, it was observed that there were differences in the number of days to healing (Figures 4.5, 4.6 and 4.7) of *C. papaya* infected with anthracnose using extracts from the leaves, fruits and roots. Healing was improved when the fruit was treated with the methanolic leaf and root extracts of *F. africana* followed by chloroform fruit *A. indica* extracts, while treatment with *S. incanum* and *A. chiliensis* ethanolic leaf extracts resulted in significantly the longest healing time of over 7 days. Meanwhile when the fruits of plants were used, *C. papaya* treated with fruit extracts of *S. incanum* followed by *F. africana* and *A. chiliensis*. One possible explanation for the superiority of the methanolic extracts is that the organic solvent might have reacted with the active ingredients in *F. africana* leaf extracts, forming inactive compounds (Estrada *et al*., 2000). That is, a chemical change might have occurred during extraction with organic solvents.

This study also demonstrated the possibility of using plant extracts to control mycelial growth as well as conidial germination (Table 4.12) of *C. gloeosporiodes*. For instance, methanolic extracts of *F. africana* was most effective in reducing the spore germination and growth of *C. gloeosporoides* when compared with the organic solvent for extracting

the fungitoxic principle in neem seed as earlier suggested by Hernandez – Albiter (2007). In this study, the plant part extracts of respective solvents of tested plants showed a higher inhibition on spore germination percentage compared to the untreated control. This agrees with the report of Vinod *et al*. (2009), which reported the *In vitro* evaluation of botanicals and bioagents on spore germination, and fungicides against anthracnose of papaya caused by *Colletotrichum gloeosporioides*. This study has demonstrated the possibility of using plant extracts to impair conidial germination of *C. gloeosporoides;* and thus may be effective in controlling Anthracnose in pawpaw fruits. *Fuerstia africana* chloroform leaf extracts exhibited a strong antifungal activity, followed by *F. africana* chloroform leaf extracts. Chloroform leaf extracts of *Aloe chilensis* ethanolic root extracts were the least effective in inhibiting conidial germination of *C. gloeosporoides*,

From previous reports, there are a variety of plant extracts that were used to control fungal anthracnose. For instance, crude methanol, chloroform, and acetone extracts of Piper betle leaves at the concentration of 10 μg/mL could inhibit the growth of *C. gleospoiroides* (responsible for anthracnose disease in pepper) mycelium at 85.25, 78.53, and 73.58%, respectively (Ayoub *et al*., 2018). Also, at the same concentration, crude methanol, chloroform, and acetone extracts of *F. africana* followed by *A. indica* leaves were found to prevent *C. capsici* spore germination at 80.93, 74.09, and 72.91%, respectively. Moreover, the leaf extracts of *S. incanum*, *O. bacillicum* and *Allium sativum* exhibited 100% inhibition of *C. gloeosporioides* (responsible for anthracnose in para rubber) mycelial growth when applying at 50 and 100% w/v, respectively, and both of these extracts could completely suppress spore germination when applying as minimal as 10% w/v (Ogbebor *et al*., 2007). Other plant pathogenic fungi could also be inhibited by

plant extracts. For example, the ethanol extracts of *O. gratissimum* and *A. chiliensis* leaves were also reported to prevent *Fusarium oxysporum* and *Aspergillus niger* spore germination at over 65%.

CHAPTER SIX

CONCLUSIONS AND RECOMMNEDATIONS

6.1 Conclusions

1. There was 100% occurrence of anthracnose in *C. papaya* among the sampled villages in Baringo and upto 80% of the villages in Elgeyo Marakwet County, with an overall occurrence of 93.75% occurence of the disease in the two counties. The prevalence of anthracnose in *C. papaya* in the farms was 95% in Baringo and upto 83% in Elgeyo-Marakwet County and differed significantly with county of occurrence and altitude. The overall incidence of anthracnose infections in fruits was 9.23 ± 1.22% in Baringo County and 4.5 ± 1.1% in Elgeyo Marakwet County resulting in an overall of 7.5 ± 1.0% in the region.

2. On morphological characteristics, most colonies of *C. gloeosporoides* were either grey, dark grey; white grey or white, with conidium diameter ranging from 80 to 86 mm, length of 14.1 to 18 mm and width of 4.1 to 4.6 mm. Severity of anthracnose disease was more in Baringo than Elgeyo Marakwet and was found to positively correlate with the incidence.

3. The crude extracts of the five plants had significant effects on the growth of *C. gloeosporoides* ($p < 0.05$) and was found to be the most effective when methanol was used for extraction. Methanolic and chloroform extracts were more effective than ethanolic extracts. The highest maximum inhibition was recorded in *C. gloeosporoides* treated extracts of *F. africana* followed by *A. indica* and *C. edulis* while the lowest

maximum zone of inhibition occurred in extracts obtained from *S. incanum* and *A. chiliensis*.

4. There were differences in the number of days to healing of the *C. papaya* infected with anthracnose. Overally, the strength of the botanicals during healing the papaya infected of anthracnose followed the trend: *F. africana* > *A. indica* > *S. incanum* > *A. chiliensis* > *C. edulis*.

6.2 Recommendations

From the study, the following recommendations were made:

1. Systematic and comprehensive survey of all the papaya growing areas of Baringo and Elgeyo-Marakwet, and all parts of Kenya and in the tropical areas with similar environmental conditions for anthracnose of papaya should be conducted, for the disease to be controlled.
2. *Fuerstia africana*, *Solanum incanum*, *Azadirachta Indica*, *Carisa edulis* and *Aloe chiliensis*, among other botanicals, should be used for antifungal control of papaya fruit anthracnose in Kenya to encourage herbal plant controls in management of fungal infections.
3. Herbal scientists, through the respective country or County goverments, should protect botanicals in their natural settings from human activities to ensure the sustainable control of food or cash crops and economics of production.

4. Further study should be conducted to determine the active compounds contained in the botanicals which have fungicidal activity against *C. gloeosporoides* due to emergence of drug resistant factors.

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APPENDICES

Appendix I: Some clinical species of *Colletotrichum*

Species	Host	Symptoms
1. *C. dermatium*	Mulberry	colonies variable with white to pale mouse
	Beech seedlings	grey or grey-vinaceous patches with abundant setae and black conical sclerotia.
2. *C. destructivum*	Cowpea	conidia and setae formed on pale brown, angular cells, 4-7um diameter.
3. *C. truncatum*	Soybean	Conidia and setae formed on pale brown, Conidiophores are ellipsoidal
4. *C. acutatum*	Tree tomato	Pink- orange colony subpopulations causing almond anthracnose
5. *C. trifolii*	Alalfa	Acervuli grouped across the colony area. Conidia was short, broad and cylindrical. Appresoria pale colour.
6. *C. gloeosporioides*	Mango	Optimum growth temperature is 25^0C. Mycelial growth, size of of conidia ranged from 9.5-10.6mm per day. Isolates were clustered into four distinct groups. Conidium size ranged four distinct groups. Conidium size ranged from2.5-7.5 um wide. Optimum temperature is 25^0C. Conidia were oblong with obtuse ends. Setae dark brown. No conidia. Colonies of isolates showed a dense, white mycelial growth

			turning to a dark olive colour.
7.	*C. fragariae*	Strawberry	Colony colour ranged from beige to dark grey. Mature setae dark brown. Uniform in width except for apical cells. Produced conidia in individual isolates.
8.	*C. graminicola*	Bluegrass	Presence of appressoria and sclerotia. Colony diameter varied from 8.3-7.1cm. Colony colour ranged from dity white grey to whitish grey.
9.	*C. coccodes*	Tomato	No conidia were found. The isolates belonging to the group B and C had the same morphology.
10.	*C. crassipes*	Hyacinth	Produces two primary toxins. Have dwarf, small colonies. Flat colonies with irregular surface and filamentous margins. 3 strains give rise to glossy and rough colony forms.
11.	*C. kahawae*	Coffee	Easily contaminated. Slow colony growth rate compared to other species. The cultures produced perithecia ascocarp. Conidial shape was straight, hyaline and cylindrical.
12.	*C. boninense*	Citrus	Colony characteristics varied widely among the isolates. Colonies grew rapidly at 25-28^{0}C. Average colony diameter was 51-52mm. Isolates formed

		colonies with white margins and dull orange centers.
13. *C. capsici*	Pepper	Cultural behavior ranged from cottony to fluffy. Colony colours ranged from white to grey. Growth rate of isolates was between 32.0 - 67.5mm. Average conidial size varied from 2.23-33.6 um. Average setae size varied from 4.48-177.21 um.
14. *C. simmondsii*	Safflower	Circular colonies which are slightly center raised and usually smooth, translucent and varied from pinpoint to 2-3 mm in diameter. The rest of the colony was dry and rough.

Appendix II: Occurrence (absence/presence) of anthracnose of *C. papaya* fruits sampled from farms in Baringo and Elgeyo-Maraket Counties

County	Area	Farms	Occurrence
Baringo	Marigat	Kaptich	+
Baringo	Marigat	R7	+
Baringo	Marigat	R1	+
Baringo	Marigat	L3	+
Baringo	Koriema	Koriema	+
Baringo	Koriema	Kimalel	+
Baringo	Koriema	Patkawanin	+
Baringo	Koriema	Kibingor	+
Baringo	Kapkelelwa	Kapkelelwa	+
Baringo	Kapkelelwa	Kisok	+
Baringo	Kapkelelwa	Oinobmoi	+
Baringo	Kapkelelwa	Kurumbsoo	+
Baringo	Mochongoi L	Sandai	+
Baringo	Mochongoi L	Loboi	+
Baringo	Mochongoi L	Molok	+
Baringo	Mochongoi L	Kaptombes	+
Baringo	Barwessa	Barwessa	+
Baringo	Barwessa	Muchukwo	+
Baringo	Barwessa	Likwon	+
Baringo	Barwessa	Chesongo	+
Elgeyo Marakwet	Kimwarer	Kimwarer	+
Elgeyo Marakwet	Kimwarer	Seko	+
Elgeyo Marakwet	Kimwarer	Kapokpok	+
Elgeyo Marakwet	Kimwarer	Emsea	+
Elgeyo Marakwet	Cheptebo	Cheptebo	+
Elgeyo Marakwet	Cheptebo	Chekobei	+
Elgeyo Marakwet	Cheptebo	Chepsigot	+
Elgeyo Marakwet	Cheptebo	Biretwo	-
Elgeyo Marakwet	Tambach L	Nyawa	-
Elgeyo Marakwet	Tambach L	Kapshokwei	+
Elgeyo Marakwet	Tambach L	Kewapsos	+
Elgeyo Marakwet	Tambach L	Sangeta	+

Appendix III: Pathogenicity of *C. gloeosporioides* using Pink Fleshed Sweet papaya variety inoculated in the crossed systems, and formation of aggressiveness groups of *C. papaya*

Isolates area	Pathogenicity diameter of the lesions (cm)	Aggresiveness group (AG)
Marigat	1.52 ± 0.05	1
Koriema	1.48 ± 0.03	1
Kapkelelwa	1.43 ± 0.04	2
Mochongoi L	1.35 ± 0.02	2
Barwessa	1.28 ± 0.03	2
Kimwarer	1.32 ± 0.01	3
Cheptebo	0.91 ± 0.04	3
Tambach L	0.62 ± 0.02	4

Key: 1 = Very low aggressiveness; 2 = low in aggressiveness; 3 = moderately aggressiveness; 4 = aggressive

Appendix IV: Pathogenicity and reaction of host species to *C. gloeosporoides* isolates recovered from papaya isolates from different farms in Baringo and Elgeiyo Marakwet Counties after 5 days of inoculation

	Lession diameter (cm)				
	Baringo		Elgeiyo Marakwet		
Isolates	Marigat	Koriema	Kimwarer	Tambach Lower	**Mean**
1	1.89	2.25	2.30	1.27	1.93
2	2.67	1.60	1.55	1.12	1.74
3	3.20	1.85	1.77	0.73	1.89
4	1.58	1.48	1.12	0.51	1.17
5	1.69	2.20	0.90	0.60	1.35
6	4.30	1.72	0.60	0.40	1.76
7	1.50	1.30	0.65	0.51	0.99
8	2.90	0.81	0.40	0.50	1.15

Appendix V: Plant extracts and maximum inhibitory concentrations

(a) Leaf extracts and maximum inhibitory concentrations

	Concentration	*F. africana*	*A. indica*	*C. edulis*	*S. incanum*	*A.chiliensis*
Methanol	0.000001	0	0	0	0	0
	0.00001	0.07	0.05	0.03	0.05	0.06
	0.00001	1.03	0.55	0.32	0.18	0.12
	0.001	6.09	4.56	2.62	1.13	0.56
	0.01	7.48	6.24	4.91	2.29	1.13
	0.1	7.74	6.71	5.49	3.29	1.69
	1	7.8	6.71	5.56	4.15	2.15
Chloroform	0.000001	0	0	0	0	0
	0.00001	0.07	0.05	0.03	0.05	0.06
	0.00001	1.81	1.24	0.32	0.4	0.26
	0.001	5.34	3.84	1.6	0.93	0.84
	0.01	10.99	9.16	4.53	1.9	1.46
	0.1	12.4	10.19	7.6	2.56	2.12
	1	12.25	10.09	8.29	3.45	2.6
Ethanol	0.000001	0	0	0	0	0
	0.00001	0.07	0.05	0.03	0.05	0.06
	0.00001	1.03	0.55	0.32	0.33	0.26
	0.001	4.53	2.17	1.8	1.13	1.27
	0.01	7.9	6	4.67	2	2.6
	0.1	11.01	8.16	5.8	2.69	3.6
	1	11.79	8.24	6.07	3.24	3.96

(b) Fruit extracts and maximum inhibitory concentrations

	Concentration	*F. africana*	*A. indica*	*C. edulis*	*S. incanum*	*A.chiliensis*
Methanol	0.000001	0.00	0.00	0.00	0.00	0.00
	0.00001	0.24	0.19	0.07	0.51	0.03
	0.00001	0.99	0.67	0.43	1.48	0.95
	0.001	2.48	1.69	1.05	3.69	2.34
	0.01	4.40	2.70	1.81	5.80	4.40
	0.1	5.90	3.88	2.50	7.29	6.02
	1	6.07	4.27	2.65	7.54	6.50
Chloroform	0.000001	0.00	0.00	0.00	0.00	0.00
	0.00001	0.05	0.04	0.02	0.04	0.06
	0.00001	0.76	0.40	0.23	1.13	0.66
	0.001	1.95	1.02	0.65	2.47	1.66
	0.01	3.83	2.39	1.11	4.40	3.43
	0.1	4.95	3.82	1.55	5.60	4.57
	1	5.27	4.20	1.85	5.82	4.91
Ethanol	0.000001	0.00	0.00	0.00	0.00	0.00
	0.00001	0.07	0.05	0.13	0.43	0.23
	0.00001	1.35	0.55	0.32	1.95	0.66
	0.001	3.30	1.39	0.88	4.06	2.20
	0.01	5.45	3.26	1.84	6.40	4.16
	0.1	7.40	5.20	2.68	8.15	6.00
	1	7.56	5.74	3.10	8.38	6.27

(c) Root extracts and maximum inhibitory concentrations

	Concentration	*F. africana*	*A. indica*	*C. edulis*	*S. incanum*	*A.chiliensis*
Methanol	0.000001	0.00	0.00	0.00	0.00	0.00
	0.00001	0.05	0.03	0.02	0.03	0.04
	0.00001	0.71	0.38	0.22	0.39	0.45
	0.001	1.83	0.96	0.75	0.65	0.88
	0.01	3.60	2.25	1.36	1.18	1.87
	0.1	4.65	3.71	1.85	1.52	3.05
	1	4.82	4.11	2.17	1.79	3.34
Chloroform	0.000001	0.00	0.00	0.00	0.00	0.00
	0.00001	0.05	0.03	0.02	0.03	0.04
	0.00001	0.71	0.38	0.22	0.39	0.45
	0.001	1.83	0.96	0.61	0.78	0.88
	0.01	3.60	2.25	1.27	1.36	1.87
	0.1	4.65	3.71	1.85	2.07	3.05
	1	4.82	4.11	1.98	2.31	3.34
Ethanol	0.000001	0.00	0.00	0.00	0.00	0.00
	0.00001	0.05	0.03	0.02	0.03	0.04
	0.00001	0.71	0.38	0.22	0.39	0.45
	0.001	1.83	0.96	0.61	0.78	0.88
	0.01	3.60	2.25	1.27	1.16	1.87
	0.1	4.65	3.71	1.85	1.60	3.05
	1	4.82	4.11	1.98	1.70	3.34

Appendix VI: *In vitro e*valuation of different plant extracts against mycelial growth of *Colletotrichum gloeosporioides* the causal agent of anthracnose of papaya

Fungicides Comcentrations	Per cent inhibition of mycelial growth			
	0.05	**0.1**	**0.15**	**Mean**
Benomyl (50% WP)	58.00	63.33	83.33	68.22
Propiconazole (25% EC)	68.14	75.48	100.00	81.21
Carbendazim (50% WP)	100.00	100.00	100.00	100.00
Hexaconazole (5% EC)	92.11	94.07	94.33	93.50
Triadimefon (25% WP)	71.10	81.10	92.14	81.57

Appendix VII: Effects of aqueous extracts on severity of anthracnose and quality of papaya fruits.

		Quality parameters[c]			
Treatments[a]	**Disease severity[b]**	PH	TSS	TA	AA
F. africana (10%)	2.2	5.79	9.27	0.160	58.37
S. incanum (25%)	1.3	5.57	7.80	0.190	64.72
C. edulis (10%)	2.8	5.76	9.60	0.153	53.07
A. indica (25%)	2.7	5.76	9.20	0.150	55.72
A. chiliensis (10%)	2.6	5.84	9.47	0.163	58.37

Appendix VIII: Statistical tests of efficacy for plant extracts

(a) Statistical test of significance for the efficacy of leaf extracts against fungal isolates

		Probit parameter estimates		Chi-square goodness of fit	
Test agent	R square	Z	P-value	χ^2	P-value
F. africana	0.9567	13.8104	0.0000	46.0452	0.0001
A. indica	0.9634	8.1735	0.0032	21.3411	0.0043
C. edulis	0.9876	10.1892	0.0002	54.4322	0.0001
S. incanum	0.8734	10.6501	0.0001	43.7517	0.0012
A.chiliensis	0.8342	9.3421	0.0002	45.2342	0.0002

(b) Statistical test of significance for the efficacy of fruit extracts against fungal isolates

		Probit parameter estimates		Chi-square goodness of fit	
Test agent	R square	Z	P-value	χ^2	P-value
F. africana	0.9911	23.9995	0.0000	43.12323	0.0000
A. indica	0.9773	12.1094	0.0003	9.4411	0.0002
C. edulis	0.9811	16.0023	0.0000	11.4339	0.0000
S. incanum	0.9477	15.34463	0.0000	23.7520	0.0000
A.chiliensis	0.9876	10.1892	0.0002	54.4322	0.0001

(c) Statistical test of significance for the efficacy of root extracts against fungal isolates

		Probit parameter estimates		Chi-square goodness of fit	
Test agent	R square	Z	P-value	χ^2	P-value
F. africana	0.9623	13.9995	0.0000	24.12323	0.0000
A. indica	0.9773	8.1033	0.0006	10.4459	0.0007
C. edulis	0.9811	11.0045	0.0000	13.4321	0.0000
S. incanum	0.9477	13.34463	0.0000	21.7520	0.0000
A.chiliensis	0.9876	9.1892	0.0002	13.4322	0.0004

Appendix IX: Recommendation Letter

University of Eldoret
Flame of knowledge and innovation

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DEPARTMENT OF BIOLOGICAL SCIENCES

REF: UOE/BIOLS/PCC/93 **DATE:** 28th October, 2015

TO WHOM IT MAY CONCERN

Dear Sir/Madam,

8.10 **RE: KUGUI SAMUEL KIPROP (SC/D.Phil/B/05/13)**

This is to certify that the above named is a bonafide student of the University of Eldoret in the Department of Biological Sciences, School of Science undertaking a D.Phil. course in Botany (Plant Pathology).

Mr. Kugui satisfactorily completed his coursework for the program named and is currently carrying out his research work. The topic of his research is '***Determination of Incidence of Anthracnose of Papaya, Pathogen Characterization, and its Biocontrol using Plant Extracts in Baringo and Elgeyo Marakwet Counties, Kenya***'.

Kugui is a hardworking, honest and dedicated person and we request you to allow him carry out his research in the concerned area.

Any assistance accorded to him will be highly appreciated.

Yours faithfully,

UNIVERSITY OF ELDORET HOD 28 OCT 2015 BIOLOGICAL SCIENCES P.O.BOX 1125-30100

Dr. J.A. Makwali,
AG. HEAD, DEPARTMENT OF BIOLOGICAL SCIENCES.

JAM/ear.

Appendix X: Recommendation Letter

University of Eldoret
flame of knowledge and innovation

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OFFICE OF THE DIRECTOR
BOARD OF POSTGRADUATE STUDIES

Our Ref: UOE/BPS/01 **DATE**: 2nd November, 2015

Your Ref:

The Chief Executive Officer
NACOSTI
P.O. BOX 30623 – 00100
NAIROBI.

Dear Sir/Madam,

8.9 **RE: RECOMMENDATION FOR SAMUEL KIPROP KUGUI – SC/DPHIL/B/05/13**

I write this in support of the application for **SAMUEL KIPROP KUGUI** for Research funding from National Commission for Science, Technology and Innovations (NACOSTI). The applicant is undertaking a thesis research entitled **"DETERMINATION OF INCIDENCE OF ANTHRACNOSE OF PAPAYA, PATHOGEN CHARACTERIZATION, AND ITS BIOCONTROL USING PLANT EXTRACTS IN BARINGO COUNTY, KENYA".**

The applicant is a registered PhD student in the **Department of Biological Sciences**, **School of Science in the University Of Eldoret**. The applicant has completed the coursework and has successfully defended his proposal in readiness for commencement of the research.

I therefore strongly support the application for funding for the student from your esteemed institution to enable him complete the postgraduate studies. Your support will be highly appreciated.

Yours faithfully,

DIRECTOR
Board of Postgraduate Studies
University of Eldoret.

DR. ELIZABETH W NJENGA (PhD)
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

Tel: +254 716980251/ +254 735925989
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Appendix XI: Permission to Collect Research Samples in the Field

University of Eldoret
P. O. Box 1125
ELDORET

17TH JUNE 2015

The Manager,
Marigat Farmers
Cooperative Union
P. O. Box 23,
MARIGAT

MARIGAT FARMERS CO-OP. SOCIETY LTD. MANAGER 15 JUNE 2015 Received

Dear Sir,

RE: PERMISSION TO COLLECT ANTHRACNOSE FUNGAL SAMPLES FROM YOUR PAWPAW FARMS

I am student to University of Eldoret taking a plant pathology course at PHD level.
I would like to collect the anthracnose fungal samples from your pawpaw farms under your management.

This will assist me in my research study entitled "Incidences of anthracnose Pathogenicity and control using plant extracts in pawpaw (*C. Papaya*) from Baringo and Elgeiyo Marakwet Counties (Kenya)"

Yours faithfully,

SAMUEL KIPROP KUGUI
PRINCIPAL INVESTIGATOR

Appendix XII: Similarity Report

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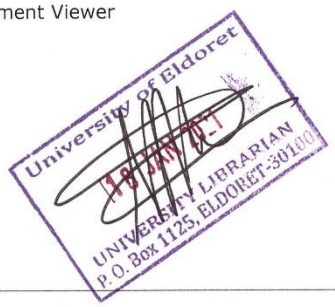

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