OCCURRENCE OF PASSION FRUIT DIEBACK IN UASIN-GISHU COUNTY AND REACTION OF PASSION FRUIT VARIETIES TO INOCULATION WITH DIEBACK PATHOGENS

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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DEDICATION

I dedicate this work to my beloved family; my husband Wemmic and sons Efraine and Roy.

Your prayers, patience and moral support made me come this far.

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ACRONYMS AND ABBREVIATIONS

AEZ	Agro-Ecological Zone
AUDPC	Area under Disease Progress Curve
CV	Coefficient of Variation
CRD	Completely Randomized Design
DAI	Days after Inoculation
GDP	Gross Domestic Product
GPS	Global Positioning System
KALRO	Kenya Agricultural and Livestock Research Organization
KPF 4	Kenya Passion Fruit No. 4
KPF 11	Kenya Passion Fruit No. 11
KPF 12	Kenya Passion Fruit No. 12
LH 2	Lower Highland 2
LH 3	Lower Highland 3
M a.s.l	Meters above sea level
NaOCl	Sodium Hypochlorite
PDA	Potato Dextrose Agar
PWD	Passion fruit Woodiness Disease
SDW	Sterile Distilled Water
SNA	Spezieller Nährstoffarmer Agar
UM 4	Upper Midland 4

ABSTRACT

Dieback is the most important fungal disease of passion fruits causing over 70% of crop loss. The disease is difficult and complex to manage due to the complexity of symptoms exhibited diversity of causal organisms and the dissemination pathways. The high preference of a highly susceptible variety by farmers aggravates the problem resulting in use of pesticides which lead to high cost of production and residues in the produce. Therefore, this study was carried out to determine the occurrence and distribution of dieback in Uasin-Gishu County and to evaluate passion fruit varieties for resistance to the disease.

A survey was carried out in 2019 after the short rain season to determine the occurrence and distribution of dieback of passion fruit in Uasin-Gishu County. A sample of 107 large and small scale farmers was taken in three agro-ecological zones (AEZs).A semi - structured questionnaire was administered to obtain information on socio-economic, passion fruit production, incidence, severity and prevalence of passion fruit diseases and their management. Disease incidence was determined as the percentage of plants with dieback symptoms in 25m^2quadrants, while severity was determined as the proportion of diseased plant area using a visual rating score chart. Twenty-five leaves and five stems of passion fruit with typical dieback symptoms were collected from five farms per AEZ for isolation of dieback causal agents. Pathogenicity of the isolated fungi was confirmed *in vitro* using detached passion fruit parts and on seedlings in a greenhouse. Evaluation of passion fruit varieties for resistance to dieback was carried out by inoculating three month old seedlings with four fungi isolated from passion fruit parts with dieback symptoms. The four fungal pathogens were inoculated singly and in combination into six passion fruit genotypes. Data on disease incidence and development was collected at a seven-day interval for nine weeks. Descriptive data was analyzed using SPSS version 20, while quantitative data was subjected to analysis of variance (ANOVA) using Genstat 15th Edition and the means separated using Tukey's test at $P \leq 0.05$.

The survey showed that purple passion fruit grafted on yellow variety was the most preferred by 94% of the farmers. Diseases (92%) and insect pests (71%) were the major constraints to passion fruit production. Majority (80%) of the diseases were fungal while 20% were viral. Dieback was the most prevalent fungal disease at 66%, while the least pervasive was Phytophthora blight with 15%. The disease distribution varied considerably across the ecological zones but was more severe in LH3 at 29% and lowest in LH2 at 8.6%. Majority (82%) of the respondents used fungicides as disease management intervention. Fungal pathogens isolated from the passion fruit dieback complex were *Fusarium oxysporum*, *Fusarium semitectum*, *Phytophthora nicotianae* and *Alternaria passiflorae*. *Phytophthora nicotianae* and *Alternaria passiflorae* were isolated in higher frequencies from the leaves as compared to *Fusarium oxysporum* and *Fusarium semitectum*, whose isolation frequency was higher in the stems. Inoculation experiments on purple passion fruit showed that *Alternaria passiflorae* was the most aggressive pathogen causing higher disease severity and incidence compared to *Fusarium oxysporum*, *Phytophthora nicotianae* and *Fusarium semitectum*. The six passion fruit genotypes inoculated with combined inoculum of the four dieback pathogens showed significant variation in the level of resistance with the mean AUDPC values ranging from 863 to 2683. Ester variety was the most resistant with the lowest AUDPC value of 863, while ordinary purple was the most susceptible with the highest AUDPC value. Based on the disease incidence and AUDPC values, the genotypes were grouped into four categories as resistant, moderately resistant, tolerant and susceptible.

The results indicate that passion fruit dieback is prevalent in Uasin-Gishu County and is widely distributed across all the agro-ecological zones but more severe in LH3. To reduce the spread and distribution, there is need to strengthen the capacity of farmers and nursery operators on best planting material propagation protocols. Farmers prefer to grow the ordinary purple passion fruit variety that is highly susceptible to the disease. The disease is caused by different fungal pathogens with *Alternaria passiflorae* being the most virulent. Farmers are therefore advised to adopt an integrated disease management approach in the management of dieback. Ester genotype, a purple variety with superior fruit characteristics than the ordinary purple, showed remarkable resistance to dieback under controlled conditions. Therefore, further field evaluation of ester passion fruit variety for reaction to dieback under varying climatic conditions and for acceptability by farmers is recommended.

CHAPTER ONE: INTRODUCTION

1.1 Background

Agriculture contributes 29.3 percent of the Gross Domestic Product (GDP) and provides livelihoods for more than 80 percent of the national population (AFA-HCD, 2018).The sector accounts for 65 percent of the country's total exports and domestically contributes to improved nutrition through the production of safe and diverse nutrient - dense food. Horticulture, the largest subsector, contributes 33 percent of the GDP and 38 percent of export earnings and has shown remarkable growth in the past years. According to the Kenya Economic Survey 2020, horticulture was the leading export earner followed by tea. In 2018, the domestic value of horticultural production amounted to KES 248.47 billion, out of which fruits contributed KES 54.39 billion, an 11.1 percent increase from the previous year, and accounting for 22 percent of the domestic value of horticultural produce. The area under fruit was 186,494 ha with a production of 3.1 million Metric tons. The area under fruits increased by 6.2 percent from the 2017 figure, and the production and value consequently increased by seven and eleven percent, respectively(AFA-HCD,2018).

In Kenya, passion fruit (*Passiflora edulis* Sims) is a prime commercial fruit and comes third in importance after avocadoes (84%) and mangoes (12%), in terms of foreign exchange earnings (AFA-HCD,2019).The demand for passion fruit is high both in local, regional markets as well as the export markets. It is popular in the cottage industry for fresh juice processing in Uganda, one of the leading market destinations. According to AFA-HCD (2019), the domestic value of both the purple and yellow passion fruit amounted to KES 2 billion accounting to 3% percent of the fruit's total value. Passion fruit is majorly grown by small-scale farmers as a diversification enterprise to secure income due to its short maturity period and high market value and therefore sustains livelihoods. The crop has great commercial potential in the country and export markets due to its increased demand for fresh fruits and processed juice. Nutritionally, the fruits are very rich in Vitamins A, C as well as carotenoids and alkaloids making it an essential food to human health (Cerqueira-Silva *et al.*, 2014). Unfortunately, despite the economic importance of this crop, the passion fruit value chain is presently under immense stress primarily due to reduced productivity leading to scarcity of fruits to feed downstream chain activities (Amata *et al.*, 2009). Worldwide, diseases are the main production constraints of passion fruits (Karim *et al.*, 2015). Soil- borne diseases are economically crucial since they reduce the life span of the passion fruit, turning it to a transient crop. The orchards are therefore renewed after every two years or less through the death of the whole stand (Amata *et al.*, 2009; Silva *et al.*, 2013). Farmers have resorted to the constant use of pesticides whose residuals in produce may negatively affect the country in the international market (KEPHIS, 2015). Additionally, pests are becoming largely resistant to the pesticides (KAPP and IIRR,2015;KEPHIS, 2015).Among the diseases limiting production, dieback has been noted as the most important causing over 70% crop loss(Amata *et al.*, 2009; Wangungu *et al.,* 2012;KAPP and IIRR, 2015). Susceptibility of the main passion fruit variety to dieback makes production unprofitable due to the frequent use of agrochemicals and reduced life span of the orchards. Therefore, there is a need to identify dieback resistant varieties that will lead to minimal use of pesticides, lower the cost of production, negate environmental hazards and eventually lead to increased productivity of safe passion fruits.

1.2 Problem Statement

Passion fruit has previously been ranked third among the export fruits at 8% (AFA-HCD, 2014) but currently, its contribution has declined to 1.4% despite increased demand for both fresh fruit and processed juice in domestic, regional and export markets. This trend is evidenced by the decline of its production and value by 12,499 tons and KES 109 million respectively in 2018, despite the area under production increasing by 139ha (AFA-HCD, 2018).

Dieback is listed as the most important fungal disease of passion fruit since its impact has crippled the highly lucrative passion fruit industry and its value chain, causing over 70% crop loss (Wangungu *et al.*, 2012). The disease is difficult and complex to manage due to the wide complexity of the symptoms exhibited, the number and diversity of causal organisms isolated from diseased materials and the diversity of dissemination pathways (Wangungu *et al.*,2011). It is a disease complex involving fungal pathogens caused by *Fusarium* spp. and *Phytophthora nicotianae* var. *parasitica* (Amata *et al.,* 2009).

As a result of the complexity of the disease, farmers resort to constant use of a myriad of pesticides to manage the disease. Consequently, this leads to an increase in cost of production as well as pesticide residual in the produce (KAPP and IIRR, 2015). Some of these pesticides include chlorothalonil, dithiocarbamates and carbendazim that were specifically detected in passion fruit (KEPHIS, 2012;KEPHIS, 2015).This may negatively affect Kenyan export significantly, especially in the international market who may impose stringent measures on Kenyan passion fruit produce. Incidentally, farmers are frequently using these pesticides in disease management. Apparently, the main variety grown by the farmers in the highlands has been documented as susceptible to most of the passion fruit diseases.

1.3 Justification

Despite the economic importance of purple passion fruit farming in Uasin-Gishu County, its productivity has been decreasing even with an increase in area under production. Pests and diseases are cited as the major production constraint with dieback recorded as the most important fungal disease. Considering the severity of the disease and narrow range of cultivated varieties most of which have been found to be susceptible to most passionfruit diseases, this research aims to identify varieties with greater resistance to dieback of passion fruit. This will result in minimal use of pesticides, reduction in cost of production and increased passion fruit productivity. By increasing passion fruit productivity through reduction of losses due to dieback, reduction of production cost due to minimal use of pesticides through use of dieback resistant varieties, Kenya can increase its national total production of safe passion fruits in an environmentally friendly way.

This, coupled with good marketing, will result in higher incomes and employment opportunities to the growers and the country, improving livelihoods thus alleviating poverty. Due to the consumption of safe fruits, a healthy nation will be created that will be highly productive in occasioning wealth for the nation.

The results of the study will inform policy recommendation such as management of dieback of passionfruit using resistant varieties and the relevant stakeholders like breeders communicated to through collaborative forums. Once the recommendations are availed to the farmers, it is expected that they will adopt them and consequently improve their yields narrowing the demand-supply gap along the value chain. Information generated from this study will also enrich the available literature on passionfruit, which is otherwise very scanty.

1.4 Objectives

The broad objective of this study was improved passion fruit productivity through improvement of dieback management by use of resistant varieties.

Specific objectives

The specific objectives were:

i. To determine the occurrence and distribution of dieback of passion fruit in Uasin Gishu County.
ii. To identify the causal agents of dieback of passion fruits and their pathogenicity.
iii. To evaluate various passion fruits germplasm for resistance to dieback.

Hypotheses

i. There is significant variation in levels and distribution of dieback of passion fruit in different AEZs of Uasin Gishu County.
ii. Dieback of passion fruits is caused by various pathogenic agents that act in synergism resulting in reduced passion fruit production.
iii. Passion fruit varieties in Kenya possess resistant traits to dieback therefore, its management by use of resistant varieties is possible.

CHAPTER TWO: LITERATURE REVIEW

2.1 Origin and distribution of Passion fruit

Passion fruit (*Passiflora edulis*) originated from the Southern part of Brazil and was extensively distributed in the tropics and subtropics in the 19th Century. The plant belongs to the family Passifloraceae and has a wide genetic diversity comprising of about 530 species out of which 60 species bear edible fruits with a few purple species (*Passiflora edulis var. purplar*) and the yellow species (*Passiflora edulis f. flavicarpa*)being of marketable value (Siddiq, 2012).

The purple passion fruit originated from Brazil to Northern Argentina through Paraguay. The yellow form is from an unknown region, perhaps Amazon (Joy, 2010).The yellow passion does well in humid tropical lowlands and is mostly grown in Brazil, Peru, Eucador, Venezuela, Fiji among others, while the purple passion is grown in higher areas and sub-tropical areas like Kenya, South Africa and Australia.

2.2 Characteristics of passion plant and fruits

Passion fruit is a perennial plant with a vine-like growth habit and shallow roots. It is propagated by seeds although cutting and grafting can be used. Passion fruit has quick financial returns since it takes only one year to maturity. Its life span exceeds three years, depending on the crop management. Cuttings and grafting propagation methods are practiced to obtain resistant plants and high yields (Siddiq, 2012).Grafting is often used as a disease management option, where the yellow passion fruit is used as a rootstock to confer resistance against nematodes and fusarium wilt (https://infonet-biovision.org).

The *Passiflorae edulis* Sims produces 30-45g leathery round fruits that turns to deep purple skin colour at maturity. The fruits are about 5 cm long and 4.5 cm in diameter. The pulp is less acidic and normally considered superior in both aroma and flavor than the yellow variety.

The *Passiflora edulis flavicarva* has large oval fruits, 6 cm long, weighing 60-90g and turn from green to yellow at maturity (Joy, 2010). The pulp is more acidic than the purple variety and usually used for juice extraction. This variety is usually resistant to nematodes and fusarium wilt and therefore used as rootstock for grafting onto the purple variety.

2.3 Production of passion fruits in Kenya

The global supply of passion fruit is estimated at 963,400 tons, with Brazil producing about 776,000 tons, followed by Ecuador and Indonesia (Tripathi, 2011;Cerqueira-Silva *et al.,* 2014). The yellow form is 95% used for juice extraction, while purple is majorly for fresh fruit consumption.

In Kenya, passion fruit (*Passiflora edulis* Sims) comes third in importance after avocadoes (84%) and mangoes (12%), in terms of foreign exchange earnings (AFA-HCD,2019).The most common varieties grown in Kenya are the purple passion fruit *(Passiflora edulis var. purplar)* and the yellow passion (*Passiflora edulis var. flavicarpa).*

The purple passion fruit *(Passiflora edulis var. purplar)* is the predominant variety as evidenced in Table 2.1 and Table 2.2. It does well in altitude regions of 1200m-1800m and has fast market returns from both local and export markets but it is susceptible to most passion fruit diseases. The yellow passion (*Passiflora edulis var. flavicarpa)* is most suited for the coastal lowlands (Table 2.2). Its juice is acidic hence less marketable. However, the variety is resistant to *Fusarium wilt*, tolerant to phytophthora blight, nematodes and brown spot (Amata *et al*., 2009)) and therefore widely used as root stock for purple passion fruit.

Though studies show that in Kenya passion fruit farming is done by small-scale farmers, it gave the country KES 2 billion from 3662 Ha as shown in Table 2.1 and 2.2 below. The production per unit area of purple passion fruit has been minimal averaging at 12 tons ha^{-1} (AFA-HCD, 2019).This is due to several limitations faced by the producers. However, the demand for passion fruits is high locally, in regional markets and in the export markets due to a shift in consumer preference from carbonated drinks.

Table 2.1: Production of purple passion fruit in selected counties 2018-2019

COUNTY	**2018**			**2019**		
	Area(Ha)	Vol(Tons)	Value (Kshs)	Area(Ha)	Vol(Tons)	Value (Kshs)
E.Marakwet	400.0	8720.0	448,800,000.00	462.0	9,486.0	554,436,000.00
Migori	108.0	1404.0	91,260,000.00	130.0	1560.0	79,560,000.00
Uasin Gishu	92.0	1104.0	84,280,000.00	187.0	1936.0	105,695,000.00
Bungoma	87.0	3149.0	163,900,000.0	126.0	2755.0	143,760,000.00
Kirinyaga	61.0	904.0	67,177,538.00	58.0	752.0	41,140,000.00
Others	1264.0	11,024.0	628,660,398.00	1193.0	8651.0	519,027,026.00
Total	**2,012.0**	**26,305.0**	**1,484,077,936.00**	**2,156.0**	**25,140.0**	**1,443,618,026.00**

Source:(AFA-HCD,2019)

Table 2.2: Production of sweet yellow passion fruit in selected counties 2018-2019

County	**2018**			**2019**		
	Area(Ha)	Vol(MT)	Value (KES)	Area(Ha)	Vol(MT)	Value (KES)
Kwale	528.0	7448.0	215,932,000.00	499.0	6,444.0	203,140,000.00
Lamu	236.0	3068.0	92,040,000.00	237.0	3270.0	98,100,000.00
Tana river	87.0	540.0	22,400,000.00	90.0	600.0	30,000,000.00
Meru	36.0	323.0	13,670,000.00	33.0	314.0	16,155,000.00
Machakos	26.0	65.0	3,250,000.00	26.0	65.0	3,250,000.00
Others	203.0	957.0	66,840,971.00	521.0	6,193.0	212,115,578.00
Total	**1,116.0**	**12,401.0**	**414,132,971.00**	**1,406.0**	**16,886.0**	**562,760,578.00**

Source:(AFA-HCD,2019)

2.4 Importance and utilization of passion fruit in Kenya

In Kenya, passion fruit (*Passiflora edulis* Sims) comes third in importance after avocadoes (84%) and mangoes (12%), in terms of foreign exchange earnings (AFA-HCD,2019). Passion fruit demand is high locally, regionally and in the export markets. The Ugandan market has shown tremendous growth for fresh fruit with consumption levels of up to 80% of Kenya's produce. Other markets include the UK, Holland, Germany and Belgium. According to AFA-HCD (2019), passion fruit contributed KES 2 billion by value, accounting to 3% of the total fruit value. Farmers prefer growing passion fruits due to its short maturity period and low labor intensity, thus its enormous potential to generate wealth and improve the livelihoods of its value chain actors.

The fruits are not only used for table purposes but also possess unique flavor, aroma and high nutritional and medicinal properties(Joy, 2010). They are processed to make fruit juice and concentrate. The juice is delicious with an excellent flavor hence known for its blending quality. The fruits contain an excellent proportion of reducing and non-reducing sugars and acids. They are rich in Vitamin A, C and minerals. Passion fruit is used for the treatment of many diseases like urinary infections and as a mild diuretic, digestive stimulant, heart tonic, asthma, and gastric cancer (Tripathi, 2011). Passion fruits have also been used in the extraction of oils for the manufacturing of cosmetic products like soaps, creams, shampoos, among others (Cerqueira-Silva *et al.*, 2014). Despite the different potential uses of *Passiflora* as a genetic resource, it is the commercialization of the fresh fruit and produced juice that justifies its cultivation in Kenya, and this has led to increased academic and economic interest.

2.5 Challenges facing passion fruit production in Kenya

In Kenya, passion fruit has previously been ranked third among the export fruits at eight percent (AFA-HCD,2014), but currently, its contribution has declined to 1.4% (AFA-HCD,2019) despite increased demand for both fresh fruit and processed juice in domestic, regional and export markets. Various abiotic and biotic factors constrain the enterprise, however, pests and diseases are the major factors that limit passion fruit productivity (AFA-HCD,2018). Fungal pathogens cause major diseases among which include dieback, Fusarium wilt, Phytophthora blight and brown spot. Among all these, dieback is the most important fungal resulting in over 70% fruit loss(Amata *et al.*, 2009; Wangungu *et al.*, 2012; KAPP and IIRR,2015*)*. The combination of dieback, root-rot causing pathogens and nematodes accounts for the reduction of passion fruit lifespan from five to two years.(Amata *et al.*, 2009). To control these diseases, farmers frequently apply pesticides leading to increased cost of production. Low access to quality healthy planting materials has also led to farmers using seedlings from uncertified nurseries, contributing to the spread of diseases. Farmers lack adequate knowledge on post-harvest handling, value addition and marketing. As a result, the full economic potential of the value chains is never achieved *(*KAPP and IIRR, 2015*)* hence many industrial processors are operating below installed capacity while others import pulp. Other challenges include lack of varieties that are adapted to local climatic conditions, (Cerqueira-Silva *et al.*, 2014), high initial capital, declining soil fertility, poor agronomic practices, insufficient water and high cost of labor.

2.6 Diseases affecting passion fruit

2.6.1 Brown spot

Brown spot, caused by *Alternaria passiflorae* has a wide distribution, having been first reported in Australia and also recorded in India, South Africa, Tanzania, Angola, Kenya, among others (Fischer & Rezende, 2008). In Kenya, the disease is found in all passion fruit growing areas such as Western, Nyanza, Central, Eastern and Rift valley (Amata *et al.*, 2009).

The major symptoms on leaves are reddish brown circular spots that are 5 mm in diameter though in high humidity, the spots usually grow larger than 2 cm in diameter. The spots centers become brittle and may tear apart. With the disease progress, foliar lesions merge and large surfaces of the leaf die, leading to abscission. Elongated dark brown lesions are observed on the twigs, and at times cause girdling and death of terminal portions (Joy & Sherin, 2012). On the fruit, the spots appear light brown, round and sunken and often join to cover large areas and produce red-brown circular spots (Fig 2.1).

Wind, water, rain and infected seedlings spread the conidia. The disease is favored by high humidity, high rainfall, crowded leaf canopy together with rising temperatures(Fischer & Rezende, 2008; Joy and Sherin, 2012).

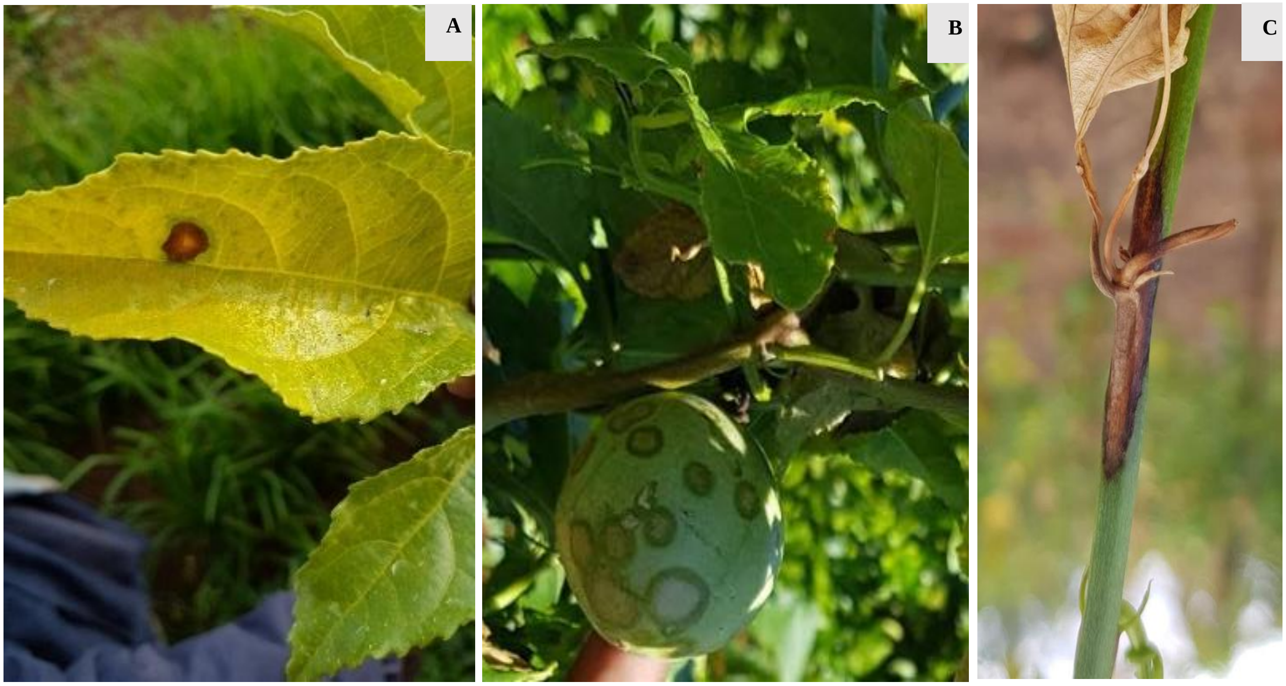

Figure 2.1: Typical symptoms of brown spot of passion fruit (A) brown circular spots on leaves (B) light brown round spots on fruits (C) elongated brown spot on stem.(Source: Peninah Munyao,2019)

2.6.2 Fusarium wilt

It is caused by *Fusarium oxysporum* f.sp. *Passiflorae.*Major hosts include *Acacia melanoxylon* (Australian blackwood), *Passiflora edulis* (passion fruit) and *Samanea saman* (rain tree) (Plantwise, 2018). The disease characteristically affects the vascular system, leading to impermeability of vascular walls therefore preventing movement of water to other plant parts. The vascular tissues show brown discoloration when dissected (Fintrac, 2019). In young plants, the glossy green leaves show a pale colour and a mild dieback, lower leaves drop, general plant wilting sets and death of the seedling occurs as the disease progresses. In mature plants, the disease presents as yellowing of young leaves, and consequently wilting and death of the plant (Manicom, *et al.*, 2003).Disease development may be unilateral or affect the whole plant (Joy & Sherin, 2012). The disease is favored by high temperature, dry soil condition, acidic soil and high relative humidity. The presence of root knot nematodes encourages the disease. The fungi survive in the soil for many years as chlamydospores (Orr and Nelson, 2018; Fintrac, 2019).The disease spreads through movement of pathogen in water, infected soil, infected planting materials and infected debris (Pegg *et al.*, 2019).

2.6.3 Phytophthora root and crown rot (Phytophthora Blight)

This disease is caused by *Phytophthora nicotianae*. The pathogen causes diverse symptoms on passion fruit. Affected plants first exhibit water soaked leaves which later appear scorched throughout the canopy (Fischer & Rezende, 2008). The vine apices die turning black. Necrosis of leaf veins, wilting and defoliation subsequently occurs. Affected areas of the stem are first purple and later brown above the graft union. They may completely girdle the stem causing wilting and vine collapse. Fruit symptoms comprise of large water soaked areas. Green grey water-soaked lesions develop on immature fruit and later cause abscission. In wet weather, the fruits and leaves may be covered with white fluffy material (Fintrac, 2019). Temperature and moisture are most important environmental factors affecting the disease spread. The disease is favored by warm wet conditions. In wet conditions, sporangia releases zoospores which swim to the host plant surface and infect the plant. These are easily disseminated through infected soil and water. In warmer condition, the sporangia function as a single spore and germinate directly infecting the host plant. The pathogen is also able to reproduce sexually, forming thick- walled oospores that may persist in the soil playing a key role in epidemiology of the disease. Under unfavorable conditions, the pathogen produces chlamydospores, which are responsible for long-term survival in soil and plant tissues. (Panabières *et al.*, 2016; Fintrac, 2019).

2.6.4 Passion fruit woodiness disease

In Kenya, passionfruit woodiness disease (PWD) is associated with cowpea aphid borne mosaic virus (Kilalo *et al.*, 2013). The main symptoms of PWD include strong mosaic, stunted growth, leaf rugose, reduced size and distortion, inhibition of fruiting, hard fruits of reduced size with thick pericarp with little or no pulp that end up splitting (Joy & Sherin, 2012).The disease is widely spread in all regions where passionfruit is grown, reducing fruit quantity and quality. The intensity of the disease is determined by the presence of the aphids, crop susceptibility, virus strain and environmental conditions.

2.7 Dieback of passion fruit

Among the passion fruit diseases, dieback has been listed as one of the major fungal diseases that require the highest priority in management since its impact has crippled the highly lucrative passion fruit industry. The disease causes over 70% of total fruit losses in the country (Wangungu *et al.*, 2012). The disease is difficult and complex to manage due to the wide complexity of symptom exhibited, the number and diversity of causal organisms isolated from diseased materials and the diversity of possible dissemination pathways (Wangungu *et al.*, 2011). According to Amata *et al.* (2009) and Wangungu *et al.* (2012), dieback is a disease complex involving fungal pathogens *Fusarium* spp. (*Fusarium oxysporum, Fusarium semitectum, Fusarium solani,*) and *Phytophthora nicotianae* var *parasitica*.

The pathogens survive as chlamydospores and oospores. These are resistant spores that can survive in soil and plant tissues for long. In the presence of a host and favorable environmental conditions, the chlamydospores and oospores germinate and produce large numbers of zoospores, micro and macrospores that infect the plant triggering spread. In the orchards, the pathogen is spread by runoff, soil movement, and infected pruning tools and through farming implements.

2.7.1 Symptoms of passion fruit dieback

The disease is characterized by plant's death from the tip or middle of the branches towards the root region. The affected leaves turn brown, later develop into fire scorch appearance, and eventually drop. The twigs die from the tips backwards, and later the branches dry out one after another leading to the death of the whole plant during the terminal phases of the disease (Fig 2.2). On the stem, brown spots are observed and vascular discoloration is evident when stem is cut longitudinally. Where grafted seedlings (yellow rootstock and purple scion) are planted, the rootstock remains alive while the purple passion fruit dies (Wangungu *et al.*, 2012). The dieback symptoms seem to overlap with other passion fruit disease symptoms documented under other diseases like brown spot, Fusarium wilt and Phytophthora blight. It therefore implies that if the dieback complex management is found, then a major passion fruit production constraint will have been resolved.

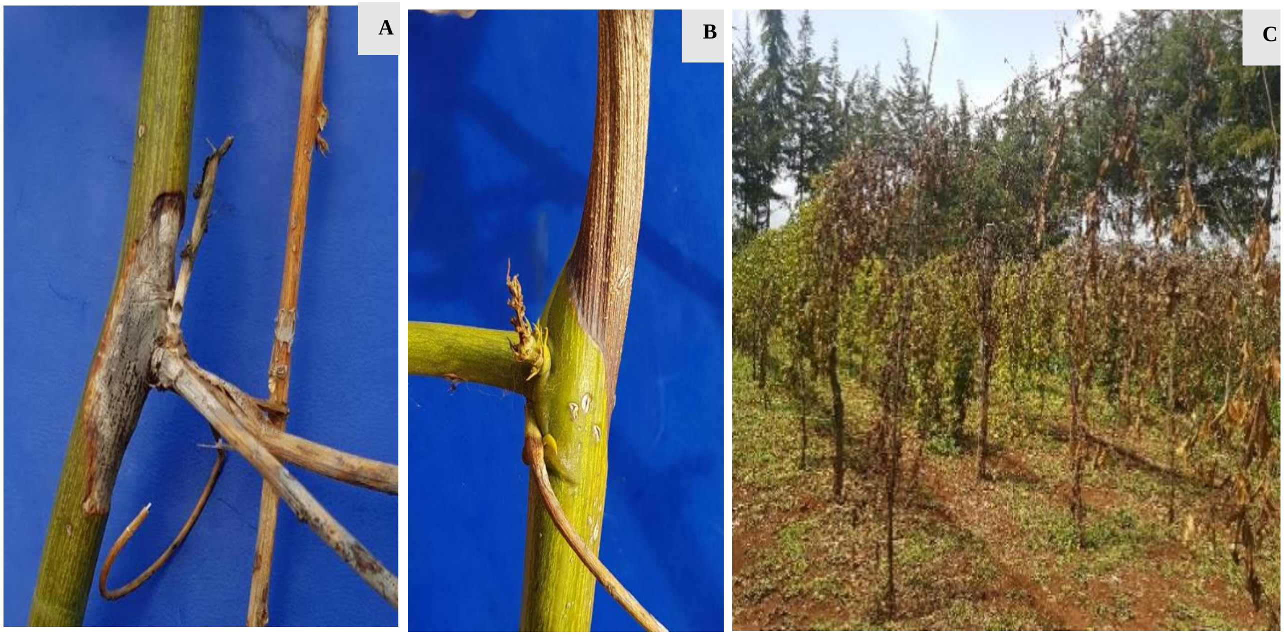

Figure 2.2: Dieback symptoms on passion fruit (A) brown stem lesion at initial phases of the disease (B) stem death towards root region (C) death of whole crop with characteristic fire scorched leaves at the terminal phase of the disease.(Source: Peninah Munyao,2019)

2.7.2 Distribution of dieback of passion fruit in Kenya

Dieback was first recorded in 2004 in Central Kenya (Wangungu *et al.*, 2014). However, according to previous research by Amata *et al.* (2009), dieback was found in all the main passion fruit growing regions in Kenya. These are Eastern, Nyanza, Central, Rift valley and Western regions. The disease incidence was an average of 55%, a mean severity of two (2) in reference to a score chart used and prevalence of above 70%. Western region had less magnitude of the disease and thus much production was encouraged in this region. In the coastal region, dieback has not been widespread since the yellow varieties grown in the region offer some tolerance to the pathogen.

Disease development is favored by drought (Wangungu, 2013). Other factors that have been noted to contribute to the success the disease include continuous planting of passion fruit that increases inoculum, poor field hygiene, especially using contaminated tools for pruning and poor canopy management. Injury of stems or branches during normal practices like weeding and pruning serves as avenues for pathogen entry.

2.7.3 Management of dieback of passion fruit

Cultural practices have been integral in management of passion fruit diseases. These are not particularly directed towards dieback since most of the farmers cannot single out the disease but only identify it as a fungal infection. Most of the practices employed by farmers include irrigation, fertilizer application, crop rotation, avoiding injuring plants to limit pathogen entry avenues, destroying all affected plant parts by burying or burning and ensuring fields are free from weeds which serve as reservoirs for the pathogens (Amata *et al*., 2012).

Apart from the cultural practices, fungicides are generally used in passion fruit production, which include chlorothalonil, dithiocarbamates and copper sulphate (Amata *et al.,* 2012). For dieback, these fungicides suppress the pathogens or slow down the disease progress momentarily according to Wangungu (2013).Worse still, these pesticides have been detected in higher levels in passion fruits than required thus posing a challenge with export markets (KEPHIS, 2012). For the severely infected plants, uprooting the whole plant remains the only viable option.

2.7.4 Use of resistant materials in management of dieback of passion fruit

Resistant materials has been used in the management of various passion fruit diseases, however, none of these have been effective against dieback (Silva *et al*., 2013). The yellow variety is usually tolerant to diseases such as brown spot, Fusarium wilt and Phytophthora blight. The only challenge posed by the yellow varieties is that they are slightly acidic and do well in low altitude areas. The purple varieties are sweet and juicy and much preferred but are less tolerant to most of the diseases especially soil borne pathogens (Cerqueira-Silva *et al*., 2014). To boost their resistance, they are grafted to yellow variety rootstocks since it is resistant to most diseases and nematodes hence found to be the best rootstock (Tripathi, 2011).

The use of resistant hybrids from crosses between purple and yellow passion fruits like KPF4, KPF11 and KPF 12 have been implemented as a control measure towards passion fruit disease. However, according to Wangungu (2013) and KAPP & IIRR (2015), KPF 4 and KPF 12 have been found to be susceptible to some of the passion fruit diseases. Further, these hybrids are mostly adapted to lowlands, therefore limiting the adaptation in higher regions. However, according to Matheri *et al*. (2016), the use of more tolerant hybrids together with fungicides have shown improved fruit yield than the use of fungicides alone on susceptible varieties.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Determination of occurrence and distribution of dieback of passion fruit in Uasin – Gishu County

3.1.1 Description of the study area

The study was conducted in Uasin-Gishu County in 2019 after the short rain season. The county covers a total area of 3,345.2 Km^2 and geographically, the region extends between longitudes 34^0 50' East, and 35^0 37' West and latitudes 0^0 03' South and 0^0 55' North. The altitude ranges from 1500m a.s.l to 2700m a.s.l with high and reliable rainfall experienced throughout the year. The average rainfall ranges between 624.9mm-1560.4mm and occurs between March and September with two distinct peaks in May and August. The dominant crops grown in the county are maize, wheat, beans and horticultural crops include passion fruits and vegetables. With the high infestation of Fall Armyworm (FAW) that has continually led to huge maize losses in the area, passion fruit may be the next frontier taking over the large chunks of land under maize.

3.1.2 Selection of farms and administration of questionnaires

The surveyed area covered five sub-counties namely Soy, Moiben, Ainabkoi, Turbo and Kapseret that altogether lied in LH3, LH2 and UM4 agro-ecological zones. Different farm sizes, both large and small scale, were systematically sampled in each AEZ by selecting the third passion fruit growing farm along the main road to give 107 farms. The sample size was determined using the following formula by Nassiuma (2000).

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

Where; n is the sample size, N is the population Size; CV is Coefficient of Variation and e is the margin of error. For this study, N =9,648. Since Nassiuma (2000) recommends a Coefficient of variation (CV) ranging between 20%-30% and a margin of error (e) of between 2%-5%, this study used a CV of 21% and a margin of error of 2%.

The sample size was calculated as shown below;

N=9648, CV=21% and e=0.02

$$n = \frac{9648\,(0.21^2)}{0.21^2 + (9648 - 1)\,0.02^2}$$

$$n = \frac{425.4768}{3.959}$$

n=107

A semi structured questionnaire was administered to obtain information on key passion fruit practices in the county, varieties grown, acreages, agronomic practices, diseases encountered, the incidence and severity, economic losses caused by the diseases and management options deployed by the farmers. Visual observation to determine the presence of disease through symptomatology was used to determine the disease incidence and severity. The GPS coordinates of each farm visited for data collection were obtained to generate a map showing localities of the farmers in the county and the hotspots of the various diseases.

3.1.3 Determination of prevalence, incidence and severity of dieback disease of passion fruits

Prevalence was recorded as the percentage of fields in which the disease symptoms were observed in relation to the total number of fields visited in each sub-county. The incidence of dieback was determined by marking 25m^2 quadrants and the number of plants with visible dieback symptoms counted in relation to total number of the plants captured in the quadrant (Manandhar *et al.*, 2016). Five quadrants were assessed in each farm using the disease incidence formula;

$$\%\ \text{Disease incidence} = \frac{\text{No. of infected plants}}{\text{Total no. of plants assessed}} \times 100$$

Five quadrants of 5x5 m were done, one at each of the four corners of the field and one at the middle and disease assessment was done on plants within the quadrants (Zarafi & Abdulkadir, 2013). On average, the plants captured in one quadrant were five. Disease severity was determined by observing the sizes of the lesion and spread in the diseased plant parts. The assessment was done on the same plants captured by the quadrants during the assessment of disease incidence.

A visual rating score chart (Table 3.1) that has 1-5 levels corresponding to the intensity of the expression of the disease was used to determine severity of dieback in the farms.

Table 3.1: Dieback on passionfruit severity scale modified from Wangungu *et al.*, 2011

Severity rating	Range of disease severity(%)	Symptom description	Pictorial
1	0	Symptomless;all plant parts free from any visual sypmtoms	 
2	0-15	Disease at initial stage of establishment;spots starting to appear on vines,tendrils withering from tip of atleast one vine,spots appearing on fruits (<0.25cm)	 
3	16-40	Elongated spots at multiple sites of the vines,more than 5 spots per fruit(>0.5 cm),atleast 1 auxilliary vine completely blighted	 
4	41-75	Expanding lesions on infected vines show distinct phases of diseased tissue , wounds with dead bark and and vine tips dying rapidly.Fruits on infected plants wither and start dropping off prematurely	 
5	76-100	The plant is completely dead or rapidly dying. Infection on both main vines (where 2 are retained) >75% plants defoliated . >50% of fruits shriveled and dropping prematurely.	 

3.2 Identification and pathogenicity of the causal agents of dieback of passion fruit

3.2.1 Collection, packaging and storage of infected specimens

The samples were collected from affected orchards in fifteen different farms during the survey. Five farms in each of the three Agro-Ecological Zones (LH2, LH3 &UM4) were randomly selected for sample collection. A sample collection data sheet was used to gather details about the sample i.e. location of the sample, collection date, part of the plant collected, description of the symptoms on the plant part and the disease incidence and severity.

The plant part samples collected included leaves and stems with typical symptoms of dieback of passion fruit. These included mild to moderate symptoms of death starting from the tip or middle of the branches towards the root region and leaves showing brown coloration and eventually developing to fire scorch appearance. Fresh and newly active lesions were collected such that there was a clear border between the healthy and diseased region. An average of twenty-five affected leaves and five stems were collected from each of the fifteen farms in the three AEZs. The samples were packaged in khaki sampling bags, tagged with respective sample data collection sheet and carried in a cool box to the laboratory where they were stored temporarily in a fridge at 4^0C awaiting isolation.

3.2.2 Isolation and frequency of fungi associated with dieback of passion fruit

The collected diseased passion fruit samples were sorted separately according to the plant part, symptom expressed and agro-ecological zone of collection. The symptoms observed on stems were discoloration and death from tips towards the root region while the leaves showed browning and wilting. The samples were washed under running tap water to remove all the dust, surface soil and other contaminants. For the leaves, the tissues with young lesions were chosen and carefully cut from the edges of the lesion using a scalpel. The stems were cut into 2-5mm fragments at the edge of the lesion margin and those that were thick were split longitudinally. These tissue fragments were surface sterilized in 1% sodium hypochlorite (NaOCl) for three minutes after which they were rinsed in three changes of sterile distilled water (SDW) and blot dried on filter papers (Al-Jaradi *et al*., 2018).

Five pieces of each surface-sterilized passion fruit samples from each farm were aseptically incubated on Potato Dextrose Agar (PDA) amended with antibiotics (40 ppm tetracycline and 50 ppm streptomycin) and replicated three times. The plates were incubated at room temperature for seven days under 12hr daylight and 12hr darkness cycle for growth. The most occurring fungi isolates were subcultured onto freshly prepared PDA.To purify the cultures, small mycelia bits from actively growing margins of the fungal colony were aseptically transferred onto the freshly prepared PDA and incubated for seven days at room temperature.

Isolation was done to determine causal agents of dieback, frequency of each fungal isolate from the plant parts and establish their prevalence in the various agro-ecological zones. The occurrence of each isolate was recorded as a percentage using the formula:

$$\%\text{Frequency} = \frac{\text{No. of isolate for each fungi}}{\text{Total no. of fungi isolated}} \times 100$$

3.2.3 Identification of fungal isolates

Identification of the fungal isolates was done on Spezieller Nahrstoffarmer Agar (SNA) and potato dextrose agar (PDA) on 7-14 days old cultures. The PDA was used to establish growth rate, colony morphology and pigmentation while SNA was to help stimulate the development of micro and macro conidia in the aerial mycelia. The isolates were characterized to species level based on cultural properties like mycelial colour, structure, texture as well as microscopic characteristics such as reproductive structures like spore shape and type. Isolates within the genus *Fusarium* were identified as described by Leslie and Summerel (2006) and Nelson *et al.* (1983). *Phytophthora* species were identified according to Martin *et al.* (2012) and Drenth & Sendall, (2001) while *Alternaria* spp was identified as described by Ellis and Holliday, (1970).

3.2.4 Evaluation of pathogenicity of isolated fungi on detached passion fruit *in vitro*

The passion fruit plant tissues used for the pathogenicity test were healthy and fresh detached stems, leaves and unripe fruits. A fresh stem of six-month old ordinary purple passion fruit was cut into segments of 15-16 cm length from the shoot tip. The stems, unripe fruits and leaves were surface sterilized in 1% sodium hypochlorite and rinsed in three changes of distilled water and blot dried with filter paper. The tissues were gently pricked with a sterile scalpel to create a wound and a single mycelial plug (4 mm in diameter) for each pathogen isolate (*Fusarium oxysporum, Fusarium semitectum, Phytophthora nicotianae* and *Alternaria passiflorae*) was cut from respective actively growing culture (seven-day old) and carefully placed upside down on the freshly cut plant tissue (Goussous *et al.*, 2009).

The inoculated site was wrapped with parafilm strips to retain moisture. Three replicates per fungal isolate were done. In the control experiment, the passion fruit part tissues were inoculated with a 4mm in diameter sterile mycelia agar plugs. All the inoculated plant part tissues were individually placed on sterilized sealed plastic boxes with moist filter papers and incubated at 25^{o}C . Symptom development and progress was assessed on alternate days from three days. Pathogen re-isolation was performed to compare the colonies obtained with those used as initial inoculum and microscopy done to confirm the pathogen's identity.

3.3 Evaluation of passion fruit germplasm for resistance to dieback

3.3.1 Description of plant materials

Six passion fruit varieties namely ester, ordinary purple, grafted purple, ordinary yellow, brazil and sweet yellow (KPF 12) seeds obtained majorly from Kenya Agricultural and Livestock Research Organization (KALRO) and other sources were raised and evaluated for resistance to dieback.

Table 3.2: List of passionfruit genotypes and their characteristics evaluated for resistance to dieback.

Variety	Characteristics
Ester	It is a South African purple variety, which is a cross between the yellow and purple granadilla. It is a bigger weight, better marketing quality, sweet and promises better yields. It takes time before it shrivels or loses moisture compared to existing ones (Louw, 2020).
Yellow passion(ordinary)	It is a yellow variety, with more vigorous growth and it is well adapted to tropical lowlands than purple passion fruit. Its pulp is quite acidic but very aromatic. The fruit turns from green to yellow at maturity and it is slightly larger than the purple. Due to its resistance to soil borne pathogens eg Fusarium wilt, it is extensively used as rootstock for grafting purple variety (Infonet- biovision, 2019)
Purple passion***(Passiflora edulis var.purplar)***	The fruits are round/oval 4-5cm in diameter and change colour from green to purple when ripe. Are sweeter than the yellow passion fruits with a superior aromatic flavor. Are sold locally or in the export markets as fresh fruits or extracted into juice. Does well in cooler temperatures at an altitude of 1200m-1800m. The variety is susceptible to soil borne pathogens (Infonet- biovision, 2019)
Sweet yellow (KPF 12)	The variety is a hybrid of the coastal yellow and purple variety. It is a yellow-skinned, sweet yellow variety with a slightly sour taste and mainly produced for high juice yield. It does well in low to mid altitudes (0 – 1,500m a.s.l). Yields 700 – 1,400 Kg per acre per week under good agronomic practices (KARI, 2014).

Brazil	Is a yellow passion fruit cultivar usually used as grafting rootstock due to its tolerance to soil borne diseases (Matheri *et al.*, 2016)
Grafted purple passion	It's the purple variety grafted on to yellow rootstock. The fruits are round and change colour from green to purple when ripe. It is termed to be more resistant to soil borne pathogens compared to the ordinary purple (Tripathi, 2011).

3.3.2 Raising of the seedlings

Passion fruit seedlings were raised in black polythene potting tubes of size 6"x9" and maintained in a greenhouse. Sterile soil was used to curb the detrimental effects of diseases. The potting media was prepared by mixing the sterile soil, manure and planting fertilizer. Each potting tube was filled up to ¾ full with the potting media. Two seeds were sown per tube at a depth of 1 cm, and the vigorously growing of the two selected for retention after two months. Watering was once after two days to ensure adequate but not excessive water. The seedlings were ready for inoculation at 3 months.

3.3.3 Inoculum preparation

Spore suspension for each fungal isolate was prepared by flooding the petridishes containing 14 day pure cultures with ten (10) ml of sterile distilled water. The mycelia were then aseptically scraped off from the surface of the media with a microscopic glass slide and the suspension transferred to a sterile glass beaker (Silva *et al.*, 2013). This procedure was repeated twice. The resultant suspension was filtered through a sterile muslin cloth to avoid agar residues. Sterile distilled water was then added to obtain a final volume of 100ml.The spore concentration was further adjusted to $1x10^6$ conidial/ml using a hemocytometer in readiness for inoculation.

3.3.4 Experimental design and layout

Two experiments were carried out at Kenya Agricultural & Livestock Research Organization (KALRO) Kabete in two separate greenhouses. For both experiments, three-month old passion fruit seedlings were arranged randomly in plots using Completely Randomized Design (CRD) with each plot having ten plants.

Experiment one was to assess symptom expression in ordinary purple variety that was the susceptible check. Four dieback fungi isolates namely *Fusarium oxysporum*, *Fusarium* *semitectum, Phytophthora nicotianae* and *Alternaria passiflorae* were inoculated independently in ten seedlings (plot) of ordinary purple variety and replicated five times. A control was also set up and inoculated with sterile distilled water.

Experiment two was to evaluate dieback resistance on passion fruit genotypes. Six passion fruit varieties namely brazil, ester, yellow passion, grafted purple, KPF 12 and ordinary purple were evaluated. Each plot (10 seedlings) of a genotype was inoculated with one level of combined dieback pathogens and replicated three times.

3.3.5 Inoculation of the seedlings

The inoculum was prepared as described in section 3.3.3 and the spore suspension concentration used for all the isolates was $1x10^6$spores/ml. The seedlings were inoculated with the inoculum of each of the fungal isolate both individually and in combination. For the individual isolates, (*Fusarium oxysporum, Fusarium semitectum, Phytophthora nicotianae, Alternaria passiflorae*), inoculation was done by spraying each of the experimental plants with the inoculum until all the external surface was well covered and 10ml of each isolate spore suspension added to the potting media (Wangungu, 2013).

For inoculation of the pathogens in combination, the four fungal isolates (*Fusarium oxysporum, Fusarium semitectum, Phytophthora nicotianae, Alternaria passiflorae*) were all inoculated in to seedlings (plots) of each genotype. This was achieved by spraying the spore inoculum of each isolate at a time on the study plants until the entire external surfaces were well covered and further 10mls added on to the growth media to enhance the activity of the soil borne pathogens. All the control treatments were sprayed with sterile distilled water but all other factors remained the same.

3.3.6 Assessment of disease development

Disease development and progress was assessed once every week and data was recorded for a period of nine weeks. Disease incidence was determined by using the disease incidence formula:

$$\%\text{Disease incidence} = \frac{\text{No. of infected plants}}{\text{Total no. of plants assessed}} \times 100$$

Disease severity was visually recorded using a scoring chart for dieback (Table 3.1). Disease progress and area under the disease progress curve (AUDPC) were calculated for each treatment in the experiment using the incidence and severity values obtained using the following equation of Shaner and Finney (1977).

$$\mathrm{AUDPC} = \sum_{i=1}^{n} \left[\frac{(y_{i+} y_{i+1})}{2} \times (t_{i+1} - t_i) \right]$$

Where: yi is the disease rating at the ith observation; ti is days after inoculation at the ith observation and n is the total number of observation.

3.4 Data analysis

Survey data that was primarily frequencies of variables as reported by respondents was analyzed using SPSS (Statistical Package for Social Sciences) version 20. Data on disease incidences and severity both from the field and greenhouse experiments were subjected to analysis of Variance (ANOVA) using Genstat 15th Edition Statistical Software and the means separated using Tukey's test at 5 % probability level. The analysis determined both significant and insignificant difference in the variables under study to provide evidence to support or reject the research hypothesis.

CHAPTER FOUR: RESULTS

4.1 Distribution of dieback of passion fruit in Uasin Gishu County

4.1.1 Demographic characteristics of passion fruit farmers in Uasin-Gishu County

Majority (78%) of the passion fruit farmers in all the AEZs were males. Many of the interviewed farmers were characterized by young age in the category of 40-50 years and had attained secondary level of education (48%) while a few had attained college education (14%).Nearly all the respondents were farmers however, some doubled as businessmen. (Table 4.1). Majority owned zero to two hectares of land followed by those owning two to four ha while a few (2%) had more than ten hectares.

Table 4.1: Demographic characteristics of passion fruit farmers in Uasin-Gishu County.

	Agro-Ecological Zones			
Farmer characteristics	**LH3**	**LH2**	**UM4**	**Mean**
Sex Male	74.6±0.5	75.0±0.1	85.0±0.8	78.2
Female	25.4±0.5	25.0±0.1	15.0±0.8	21.8
Age of farmer (years)				
20_30	07.0±0.1	00.0±0.2	10.0±0.3	5.6
30_40	15.5±0.1	18.8±0.2	05.0±0.3	13.1
40_50	31.0±0.1	37.5±0.2	40.0±0.3	36.1
50_60	32.4±0.1	37.5±0.2	30.0±0.3	33.3
Above 60	14.1±0.1	06.3±0.2	15.0±0.5	11.8
Education level				
Primary	38.0±0.8	37.5±0.2	45.0±0.2	40.2
Secondary	49.3±0.8	43.8±0.2	40.0±0.2	44.3
College	12.7±0.8	18.8±0.2	15.0±0.2	15.5
Occupation				
Farmer	90.1±0.7	93.8±0.1	90.0±0.1	91.3
Farmer/Bs.Man	9.9±0.7	6.3±0.1	10.0±0.1	8.7
Land size owned (Ha)				
0.0_2.0	63.4±0.1	62.5±0.3	70.0±0.2	65.3
2.0_4.0	31.0±0.1	18.8±0.3	15.0±0.2	21.6
4.0_8.0	4.2±0.1	6.3±0.3	15.0±0.2	8.5
8.0_10.0	0.0±0.1	6.3±0.3	0.0±0.2	2.1
Above 10.0	1.4±0.1	6.3±0.3	0.0±0.2	2.5

4.1.2 Passion fruit production in Uasin-Gishu County

The most preferred variety by passion fruit farmers in Uasin-Gishu County was grafted purple. (Table 4.2). According to the study results, passion fruit was predominantly a commercial crop by majority of the smallholder farmers on a piece of land less than 0.4 ha and the lifespan of most orchards was utmost two years. Across all the agro-ecological zones, the source of most planting material was within own households (26%) and other local farmers (47%) with a small proportion (31%) sourcing from commercial suppliers. A greater proportion (64%) of the respondents were in passion fruit production for less than five years though in LH3 farmers were in production for a longer period relative to other zones. The average productivity of the crop was 10 tons ha^{-1}with a higher production recorded in LH2.

Table 4.2: Proportion of farmers in relation to passion fruit production practices and characteristics across AEZs

Passion fruit production aspects	Category	Agro ecological Zone			Mean
		LH3	LH2	UM4	
Variety	Purple	93.0±0.0	100.0±0.0	90.0±0.6	94.3
	Yellow & purple	7.0±0.0	0.0±0.0	10.0±0.6	5.7
Source of planting materials					
	Own Nursery	25.4.±0.5	18.8±0.1	35.0±0.9	26.4
	Private Nurseries	28.2.±0.1	37.5±0.2	30.0±0.3	31.3
	Other Farmers	53.5.±0.1	43.8±0.2	45.0±0.3	47.4
Production duration (years)					
	0-5	53.5.±0.1	68.8±0.2	70.0±0.2	64.1
	06-10	22.5.±0.1	12.5±0.2	15.0±0.2	16.7
	>10	23.9.±0.1	18.8±0.2	15.0±0.2	19.2
Reason for production	Commercial	71.8.±0.2	68.8.±0.2	70.0.±0.2	70.2
	Comm.&Subsistence	28.2±0.1	31.3.±0.2	30.0.±0.2	29.8
Orchard Lifespan (years)					
	≤1	2.8±0.1	0.0±0.1	15.0±0.2	5.9
	1.5	11.3±0.1	43.8±0.1	30.0±0.2	28.4
	2	32.4±0.1	56.3±0.1	55.0±0.2	47.9
	>2	53.5±0.1	0.0±0.1	0.0±0.2	17.8
Area under passion (Ha)					
	0.0-0.4	80.3±0.1	68.8±0.2	95.0±0.1	81.3
	0.4-0.8	15.5±0.1	25.0±0.2	5.0±0.1	15.1
	0.8-1.2	1.4±0.1	6.3±0.2	0.0±0.1	2.6
	1.2-1.6	2.8±0.1	0.0±0.2	0.0±0.1	0.9
Yield	Tonnes/Ha	10.9±0.2	12.7±.0.1	8.1±.1.5	10.1

4.1.2.1 Farmer trainings on good agricultural practices on passion fruit production

From the survey results, 27% of the respondents had carried out soil test with minimal variation within the zones (Table 4.3) Training, a key component in crop production showed minimal variance within the zones. On average, 50% of the passion fruit farming population had received some general training on passion fruit production. The farmers also benefitted from tailored trainings and from the results, it was evident that disease management and production of planting materials were the most sought after by majority (31%) of the respondents across ecological zones. Only a few (3%) farmers had received trainings on all aspects along passion fruit value chain within the zones. The two major difficulties faced by respondents in accessing agricultural training services were distance to the training facilities (37%) and limited knowledge about the training opportunities (33%).

Table 4.3: Proportion of farmers' trained on passion fruit production across three AEZs of Uasin-Gishu County

Category	Agro-Ecological zones			Mean
	LH3	LH2	UM4	
Soil test done	25.4±0.1	31.3±0.2	25.0±0.1	27.2
Farmers trained on passion fruit production	45.1±0.6	50.0±0.1	45.0±0.2	46.7
Specific training aspects				
Pest & disease Management	4.2±0.2	12.5±0.1	5.0±0.4	7.2
Post-Harvest handling	5.6±0.2	6.3±0.1	10.0±0.4	7.3
Planting materials/Dse Mgt	32.4±0.2	31.3±0.1	30.0±0.4	31.2
All training Aspects	4.2±0.2	0.0±0.1	5.0±0.4	3.0
Reasons for non-attendance at trainings				
Far locations	38.0±0.1	50.0±0.1	25.0±0.2	37.6
Not invited	39.9±0.1	27.3±0.1	33.0±0.2	33.0
Experienced	0.0±0.1	6.3±0.1	0.0±0.2	2.1

4.1.3 Challenges facing passion fruit production in Uasin-Gishu County

Passion fruit production was found to be constrained by both abiotic and biotic factors. Across all the ecological zones, the respondents ranked diseases (91%) and insect pests (70%) as the most significant limitation to passion fruit production. Other notable constraints mentioned included insufficient water for production, poor market structures leading to fluctuation of produce prices, labor unavailability and lack of planting materials as well as others that contributed to 10%. According to the survey, LH2 experienced less drought (56%) relative to other ecological zones while LH3 had sufficient market outlets for sale of their produce. (Table 4.4)

Table 4.4: Challenges facing passion fruit growers in Uasin-Gishu County

Challenges	Agro-Ecological Zone			Mean
	LH3	LH2	UM4	
Diseases	94.5	89.8	91.3	91.8
Insect pests	75.6	66.1	69.6	70.5
Insufficient water	67.1	56.0	65.5	62.8
Market	40.9	3.1	26.1	23.3
Labor	18.5	4.1	0.0	11.3
Maintenance costs	12.3	10.5	6.7	9.6
Planting Material	9.5	7.5	6.7	7.9
*Others	8.1	13.1	10.0	10.4

*Price fluctuations, high initial capital and lack of trellising post

4.1.4 Prevalence, incidence and severity of passion fruit diseases in Uasin Gishu County

4.1.4.1 : Prevalence of passion fruit diseases in Uasin Gishu County

Among the sub-counties surveyed, the most commonly observed passion fruit diseases were fungal (Table 4.5). Woodiness was the most prevalent in the county with minimal variation within the sub-counties with the disease increasing as much as 100% in certain areas. Dieback was the most important fungal disease in the county with a mean prevalence of 66%. All the diseases affecting passion fruit were recorded in all Sub-Counties, the exception to this was Fusarium wilt and Phytophthora blight, which were not observed in Kapseret sub-county. Passion fruit diseases were in high frequency in Soy Sub-County (51%), followed by Moiben (48%) while the least was 41% in Kapseret sub-county.

Table 4.5: Prevalence of passion fruit diseases in Uasin Gishu County

Diseases	Disease Prevalence in Uasin -Gishu County					Mean
	Soy	Kapseret	Moiben	Turbo	Ainabkoi	
Woodiness	81.0	100.0	93.8	87.5	100.0	92.5
Dieback	66.7	71.4	50.0	62.5	81.3	66.4
Brown Spot	38.1	35.7	46.9	50.0	43.8	42.9
Fusarium wilt	19.0	0.0	31.3	29.2	6.3	17.2
Phytophthora blight	52.4	0.0	18.8	4.2	0.0	15.1
Mean	51.4	41.4	48.2	46.7	46.3	46.8

4.1.4.2 Prevalence of pathogens isolated in three AEZs in Uasin-Gishu County

The greater proportion of all the pathogens isolated across the Agro-Ecological Zones was *Alternaria passiflorae* with highest frequency in LH2 (42%) and lowest in UM4 (36%). *Fusarium oxysporum* occurrence showed significant ($P \leq 0.05$) difference in UM4. Percentage occurrence of *Phytophthora nicotianae* was least in UM4 and highest in LH3.In contrast, *Fusarium semitectum* was found only in samples from LH3.However, occurrence of *Alternaria passiflorae*, *Phytophthora nicotianae* and *Fusarium semitectum* was non-significant across the three agro-ecological zones (Table 4.6)

Table 4.6: Pathogens isolated from various Agro-Ecological zones

Zones	Pathogen isolated from various Agro-Ecological Zones			
	A.passiflorae	*F. oxysporum*	*P. nicotianae*	*F.semitectum*
LH3	37.1a	2.3b	11.0a	0.5a
UM4	36.5a	7.7a	4.4a	0.0a
LH2	41.7a	2.0b	6.2a	0.0a
Mean	38.4	3.4	7.4	0.2
LSD	16.2	4.7	7.0	1.0
P value	0.765	0.037	0.096	0.455

(*In columns, values followed by different letters are significantly ($P \leq 0.05$) different by Tukey's test)

4.1.4.3 Incidence and severity of passion fruit diseases in the Agro-Ecological Zones

All the five passion fruit diseases were recorded across the three agro-ecological zones with significant (P≤0.05) difference in disease intensities (Table 4.7). The data indicated that woodiness was widely distributed with a mean incidence of 20.5, followed closely by dieback with a mean incidence of 14.4 while Phytophthora blight recorded the lowest mean incidence of 2.11. Amongst the agro-ecological zones, maximum incidence ratings for all the diseases was observed in LH3 except brown spot whose maximum incidence was in LH2. Woodiness incidence differed significantly (P≤0.05) among the agro-ecological zones while for dieback, no significant difference was observed in LH2 and UM4. Fusarium wilt and brown spot incidences showed significant difference in LH2 while Phytophthora blight showed non-significant incidence difference across all the zones.

The survey revealed a marked significant (P≤ 0.05) difference in the passion fruit disease severity across the agro-ecological zones (Table 4.7). Woodiness virus had the highest mean severity with 26.5 as compared to Phytophthora blight, which had the lowest.LH3 agro-ecology recorded highest severity means for all diseases except for brown spot that had its maximum mean severity in LH2.

Table 4.7: Percentage incidence and severity of passion fruit diseases in the various AEZs

Incidence					
AEZ	**Woodiness**	**Dieback**	**Fusarium wilt**	**Brown spot**	**Phytophthora blight**
LH2	20.5b	5.9b	0.1b	10.7a	2.2a
LH3	33.3a	28.6a	4.5a	6.4b	3.7a
UM 4	7.6c	8.7b	4.7a	3.3b	0.5a
Mean	20.5	14.4	3.1	6.8	2.1
LSD (P≤0.05)	7.4	8.0	3.7	3.8	3.8
Severity					
AEZ	**Woodiness**	**Dieback**	**Fusarium wilt**	**Brown spot**	**Phytophthora blight**
LH2	31.5a	8.7b	0.1b	12.0 a	1.6a
LH3	36.2a	29.1a	5.8a	7.1b	2.6a
UM 4	11.9b	10.7b	7.0a	6.2b	0.5a
Mean	26.5	16.2	4.3	8.4	1.6
LSD (P≤0.05)	8.2	8.2	4.2	4.3	2.9

(*In columns, values followed by different letters are significantly (P≤0.05) different by Tukey's test)

4.1.4.4 Spatial distribution of dieback of passion fruit in Uasin-Gishu County

The dieback disease map illustrating the disease epidemic pattern levels across the agro-ecological zones showed that dieback of passion fruit was concentrated at the central part of the county at the time of the survey (Fig 4.1 and Fig 4.2). The highest disease incidence was recorded in LH3 at 28.6%, which is not only a wet humid region but also covers the largest area of the ecological zones.The cooler LH2 agro-ecology had relatively lower incidence of 5.9% compared to UM4 that 8.7% diseases incidence.

In terms of severity, LH3 showed significant difference in severity at 29.1% compared to LH2 at 8.7% and UM4 at 10.7%. The survey work was carried out at the main passion fruit growing areas at the time of study and this explains why the disease pattern is not widely distributed in the whole county.

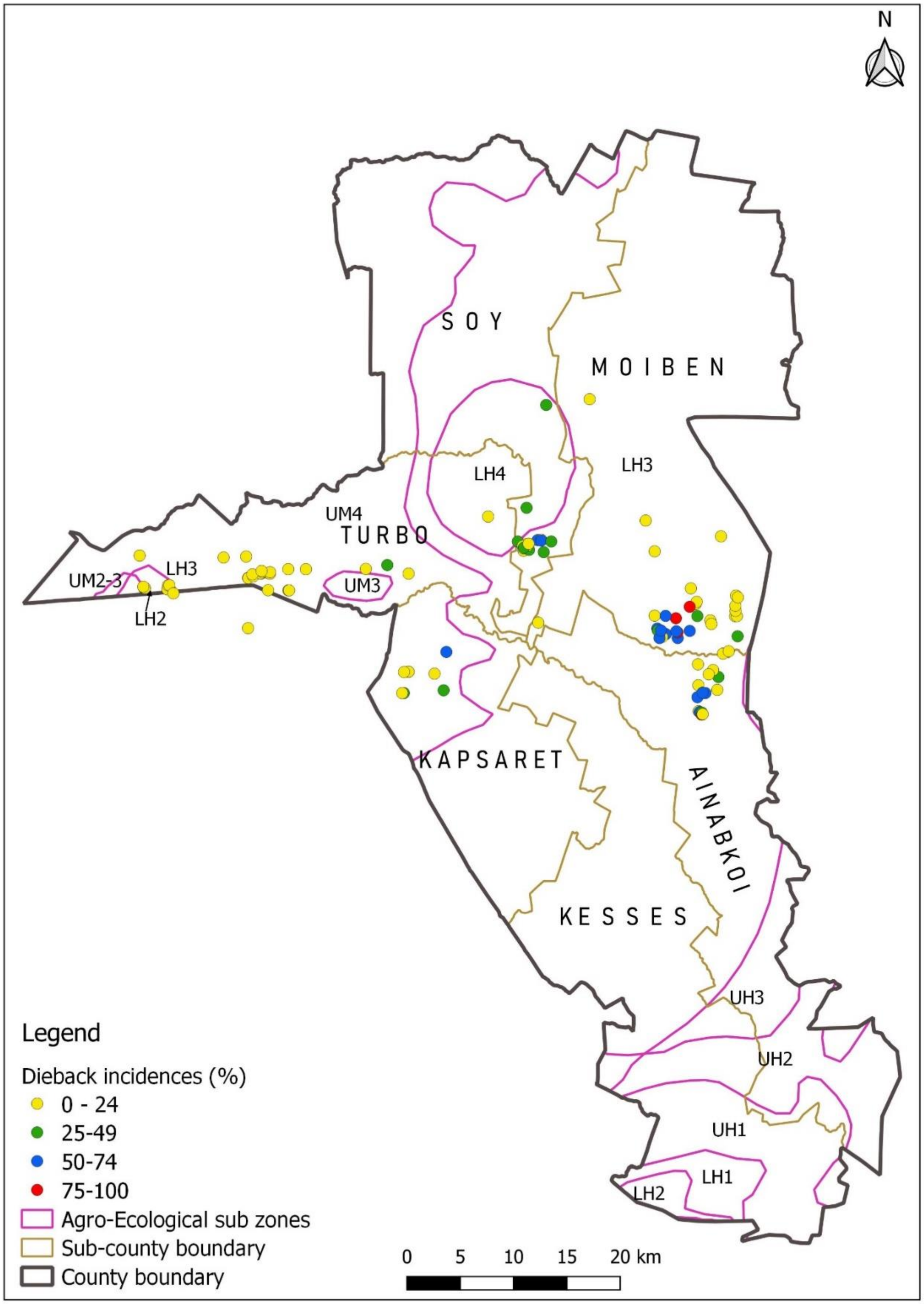

Figure 4.1: Disease map showing dieback of passion fruit incidence patterns in AEZs of Uasin-Gishu County. The variations in colour shades are used to typify disease intensity in the study area.

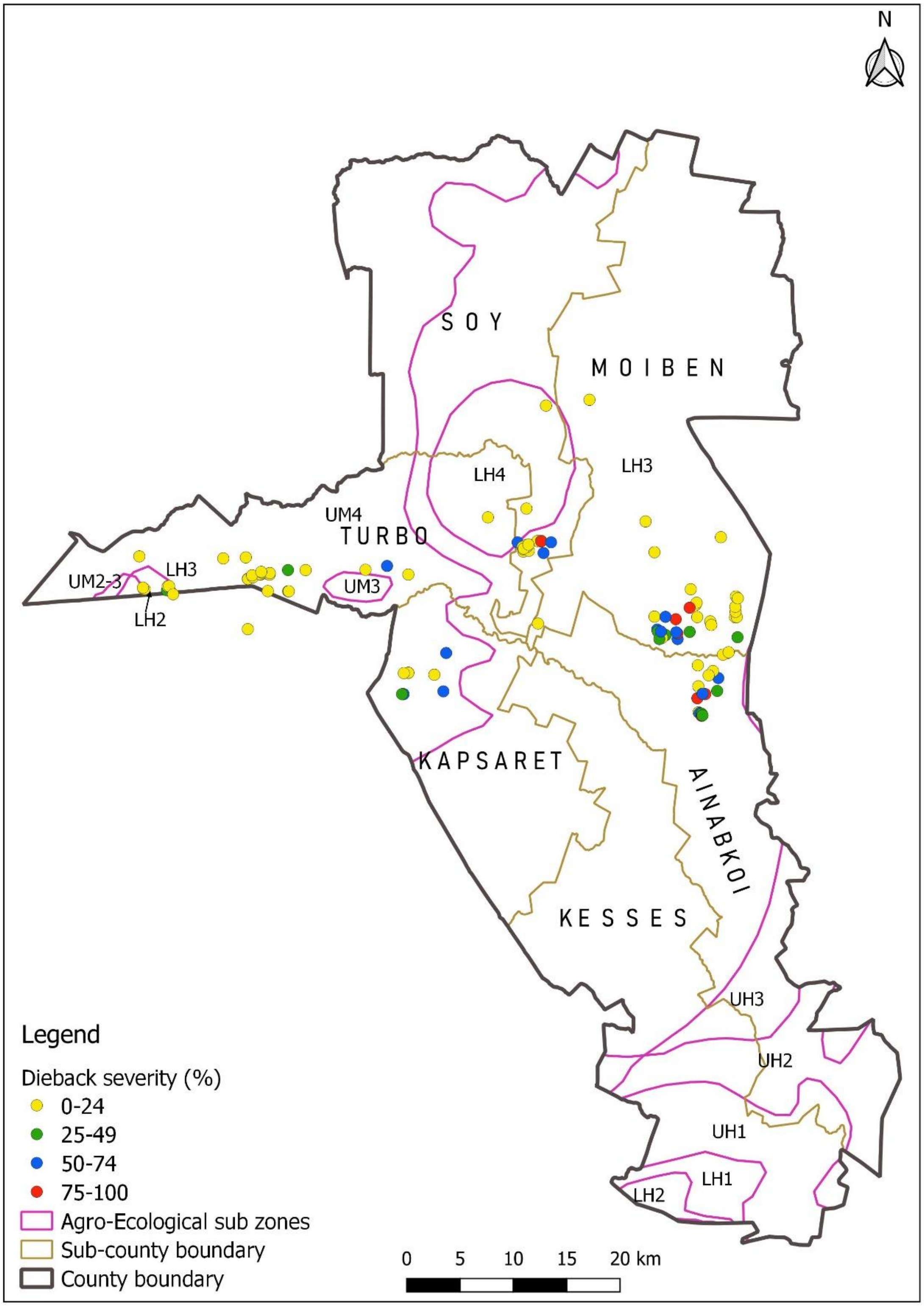

Figure 4.2: Disease map showing dieback of passion fruit severity patterns in AEZs of Uasin-Gishu County. The variations in colour shades are used to typify disease intensity in the study area.

4.1.5 Management of passion fruit diseases

The respondents used a myriad of strategies to manage diseases of passion fruit (Table 4.8). Across the ecological zones, vast majority of the respondents (82%) extensively used fungicides like mancozeb, metalaxyl and carbendazim as a key intervention to disease management. However, LH2 respondents used the chemical sprays minimally (70%) compared to LH3 where the intervention use was striking (90%).

Bio-control, particularly *Trichoderma harzianum* use varied minimally across the zones. Other cultural practices like pruning and manure application were also employed as mitigation strategies by a small percentage of the respondents. Notably, in LH2 integrated disease management approach was more adopted relative to other zones. In contrast, a substantial proportion of farmers (14%) did not apply any mitigation measures towards disease management in their orchards.

Table 4.8: Proportion of farmers applying various strategies to manage passion fruit diseases in Uasin- Gishu County

Disease Management Practice	Agro-Ecological zone			**Mean**
	LH3	**LH2**	**UM4**	
Fungicide application	90.5	70.0	86.0	82.2
Use of Trichoderma	9.5	14.3	4.3	9.4
Pruning	15.6	20.0	8.7	14.8
Manure application	6.3	7.1	4.3	5.9
No Management	18.8	8.2	17.4	14.8

**multiple responses*

4.2 Causal agents of passion fruit dieback and their pathogenicity

4.2.1 Isolated and identified passion fruit dieback causal agents

From the diseased passion fruit samples, the four most occurring isolates were identified. Variations in culture medium were noted in colour, spore types and mycelial growth pattern. The fungal pathogens were identified as *Phytophthora nicotianae, Fusarium oxysporum, Alternaria passiflorae* and *Fusarium semitectum.*

On PDA, *Phytophthora nicotianae* isolate (Fig 4.3) exhibited moderate growth with dense rosette growth pattern. The colony margin was smooth and uniform. When viewed under microscope, the mycelia were coenocytic with hyphal swellings. Sporangia produced were lemon shaped (ovoid), spherical or ellipsoid with some having a papilla. The non-caducious sporangia were formed singly or in a loose sympodium on long stalks. Chlamydospores were present, both terminal and intercalary. Reproductive structures were also observed with the oogonia notably smooth and spherical.

Isolate of *Fusarium oxysporum* (Fig 4.4) colony on PDA showed white aerial mycelia with a tinge of purple on the obverse and deep purple on the reverse. Microscopic examination showed abundant oval to kidney shaped single celled microconidia borne on false head on short monophialides. The macroconidia were abundant, mostly three septate, slender, sickle shaped with a foot-shaped basal cell and curved apical cell. Conidiophores were both branched and unbranched monophialides and chlamydospores were present, formed singly or in pairs.

All *Alternaria passiflorae* (Fig 4.5) isolates produced profuse mycelial growth on PDA, initially hyaline then turned to grey- brownish at the obverse and brown black on the reverse. When viewed under a microscope, conidiophores were brownish in colour, both short and long, straight or curved, and mostly occurred singly. The conidia were born either singly or in chains of up to five on the apex of distinctive conidiophores. They were light to dark brown in colour, with transverse and longitudinal septa and varied in shape from obclavate to mostly ellipsoidal and muriform having tapered apex with 1 to 3 longitudinal and 2-10 transverse septa.

Fusarium semitectum (Fig 4.6) isolate exhibited a slow culture growth, with off white center and orange brown edges at the obverse on PDA. Light orange sporodochia were also present. The reverse was brown colored with deep brown center. As the culture aged, it changed to carmine red. Microscopic analysis showed no microconidia. Macro-conidia were of two types; spindle shaped with a papilla at the basal cell with 3-5 septa and sickle-shaped with a distinct foot shape basal cell with 3-6 septa. There was abundant production of spindle shaped mesoconidia from polyphialides in the aerial mycelium. Few chlamydospores occurring singly were observed.

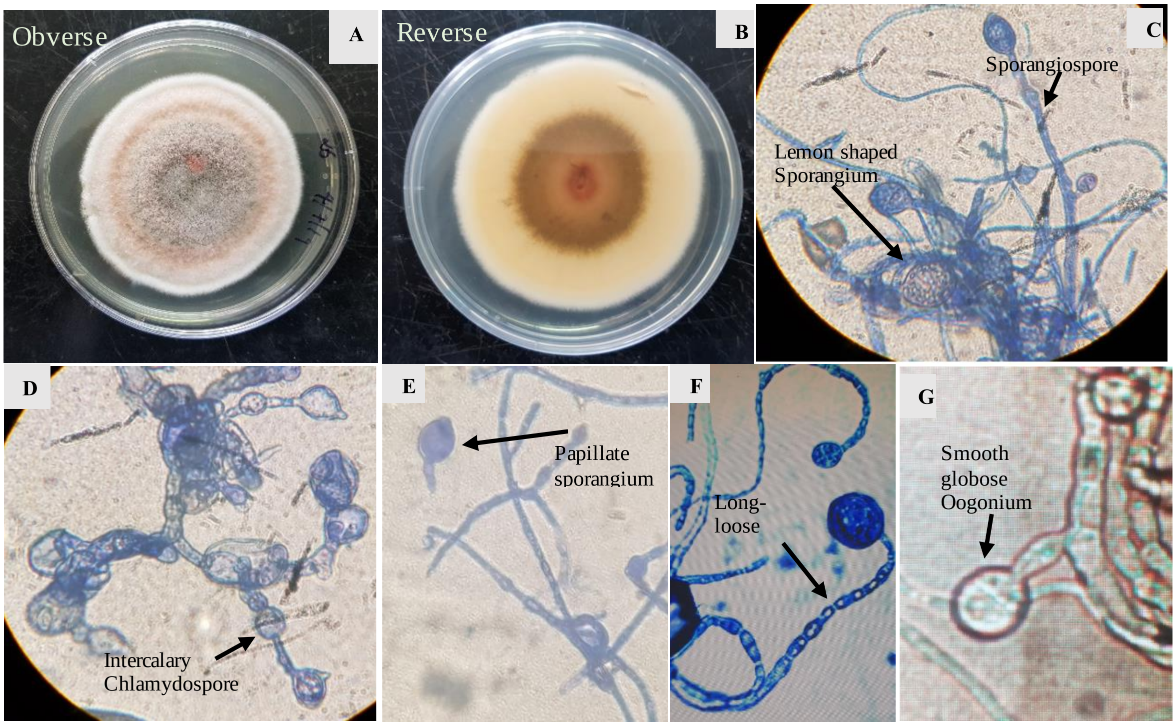

Figure 4.3: Cultural and morphological characteristics of *Phytophthora nicotianae*(X400): colony features on PDA (A, B), reproductive structures under microscope (C-G).

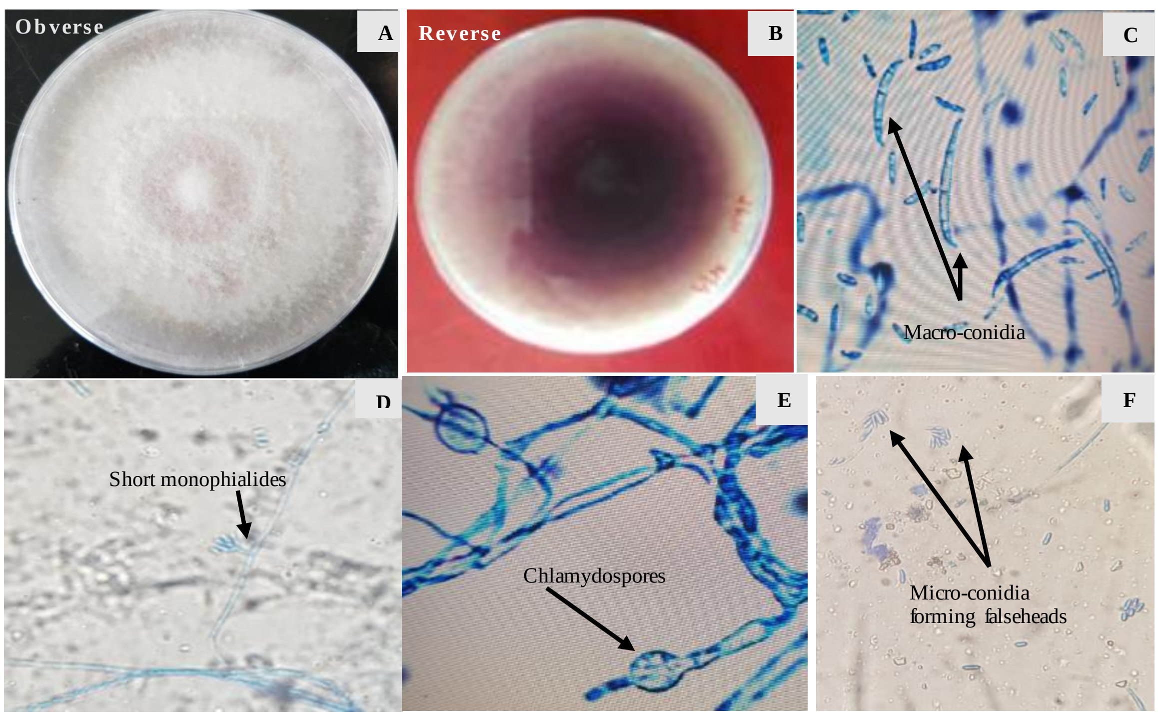

Figure 4.4: Cultural and morphological characteristics of *Fusarium oxysporum*(X400): colony aspects on PDA (A, B) Conidial structures(C-F).

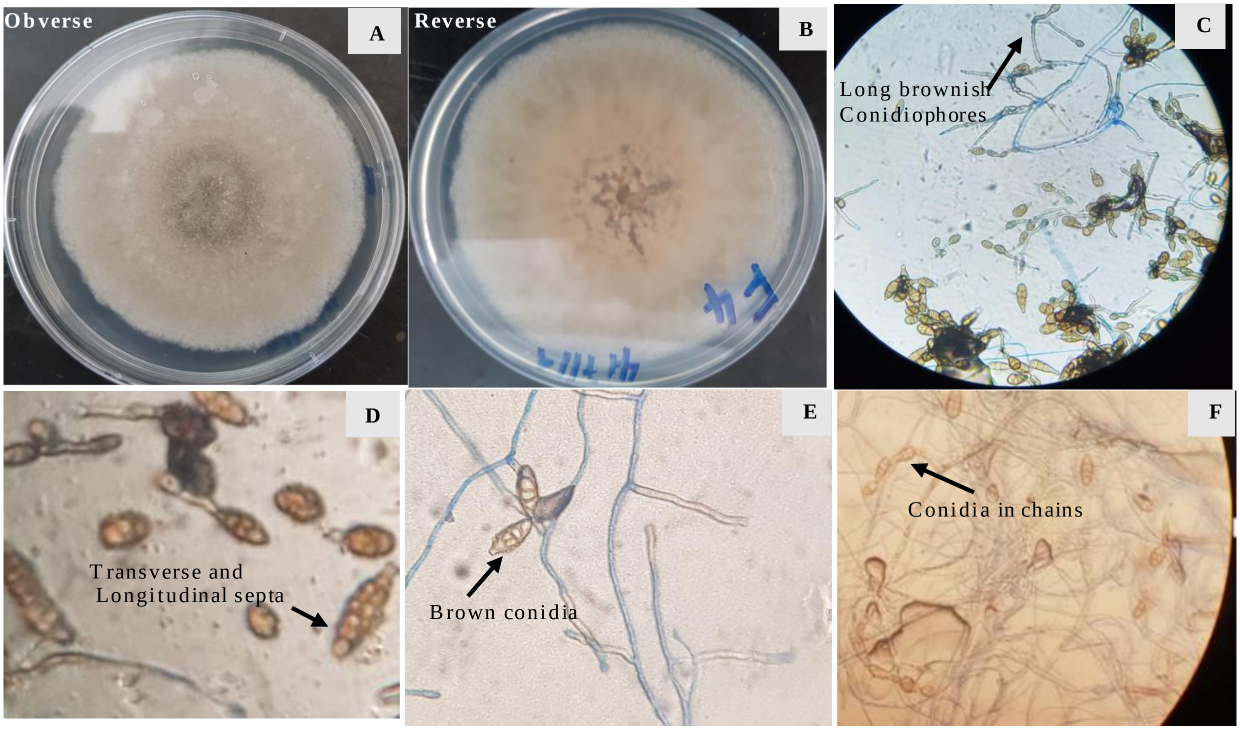

Figure 4.5: Cultural and morphological features of *Alternaria passiflorae*(X400): colony characteristics on PDA (A, B) conidia and conidiophores (C-F)

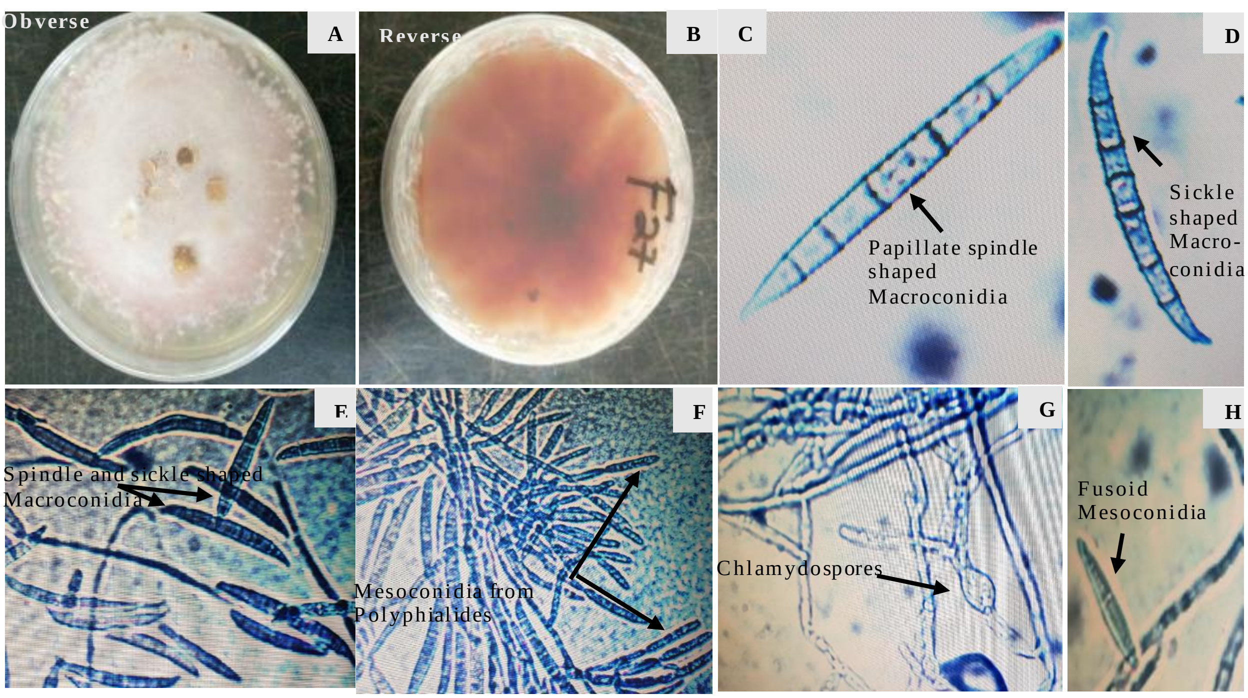

Figure 4.6: Cultural and morphological characteristics of *Fusarium semitectum* (X400): colony features on PDA (A, B) spore characteristics under microscope(C-H)

4.2.2 Isolation frequency of fungi associated with dieback from different passion fruit parts in the three AEZs

Across the Agro-Ecological zones, LH3 had the highest fungal isolation frequencies while LH2 had the lowest (Table 4.9). *Alternaria passiflorae* and *Phytophthora nicotianae* were significantly recovered from the leaves more than the stems with a mean of 46.1 and 8.6 respectively. In contrast, *Fusarium oxysporum* and *Fusarium semitectum* were isolated in higher frequencies from the stems than the leaves with a mean of 4.1 and 0.3 and mean of 3.8 and 0.0 respectively.

Table 4.9: Fungal pathogens isolated from passion fruit stems and leaves from three Agro-Ecological zones in Uasin-Gishu County

	Isolated fungal pathogens											
	P.nicotianae			*A.passiflorae*			*F. oxysporum*			*F. semitectum*		
	UM4	**LH2**	**LH3**	**UM4**	**LH2**	**LH3**	**UM4**	**LH2**	**LH3**	**UM4**	**LH2**	**LH3**
Stem	3.7	2.8	11.0	32.2	29.8	30.2	5.1	2.7	4.6	0.0	0.0	1.0
Leaf	8.8	6.1	11.0	40.8	53.5	44.0	10.3	1.0	0.0	0.0	0.0	0.0
Mean	6.2	4.4	11.0	36.5	41.7	37.1	7.7	1.9	2.3	0.0	0.0	0.5
LSD	10.8	6.9	11.0	25.8	19.3	20.1	10.9	5.5	4.5	0.0	0.0	2.0
P value	0.335	0.331	0.999	0.355	0.017	0.204	0.325	0.34	0.08	0.0	0.0	0.326

4.2.3 Pathogenicity of the isolated fungi

All the fungal isolates recovered from diseased samples of passion fruit plant parts were further confirmed through Koch's postulates on detached leaf and stem assays *in vitro*. The isolates were found to be pathogenic, exhibiting typical symptoms of infection by the seventh day (7DAI) post inoculation. *Fusarium oxysporum* was found to be more aggressive in disease development compared to other isolates. Initially, the mycelia of the *Fusarium oxysporum* inoculant appeared dense-white with a tinge of purple and as the infection progressed, the mycelia became abundant, covering the stem and the leaf (Fig 4.7).On splitting the stem longitudinally, the vascular bundles showed brown coloration.

On inoculating *Fusarium semitectum* to the leaf and stem (Fig 4.8), the initial signs were sparse pink mycelia on the stem. As the infection became severe, the mycelia appeared carmine red, profuse and covered the stem and the leaves. When cut longitudinally, pink brownish coloration was observed on the vascular bundles.

Results obtained from inoculation of *Alternaria passiflorae* suggested the pathogen was the causal agent of brown spot of passion fruit. The samples inoculated with the isolate exhibited small circular to irregular spots (Fig 4.9) which further turned to light brown patches with characteristic concentric zonation as the infection became severe.

Inoculation of the detached passion fruit stems and leaves with *Phytophthora nicotianae* reproduced the symptoms caused by the pathogen (Fig 4.10). The fruit presented varying sizes of round shaped water soaked lesions that were light brown in colour. At an advanced stage, sporulation was observed and the lesion coalesced to cover the entire fruit. The leaves inoculated on the abaxial side exhibited sporulation on the underside of the leaf.

Each isolate was successfully recovered from the respective inoculated passion fruit part, exhibiting similar cultural, morphological and spore features as in the initial isolate.

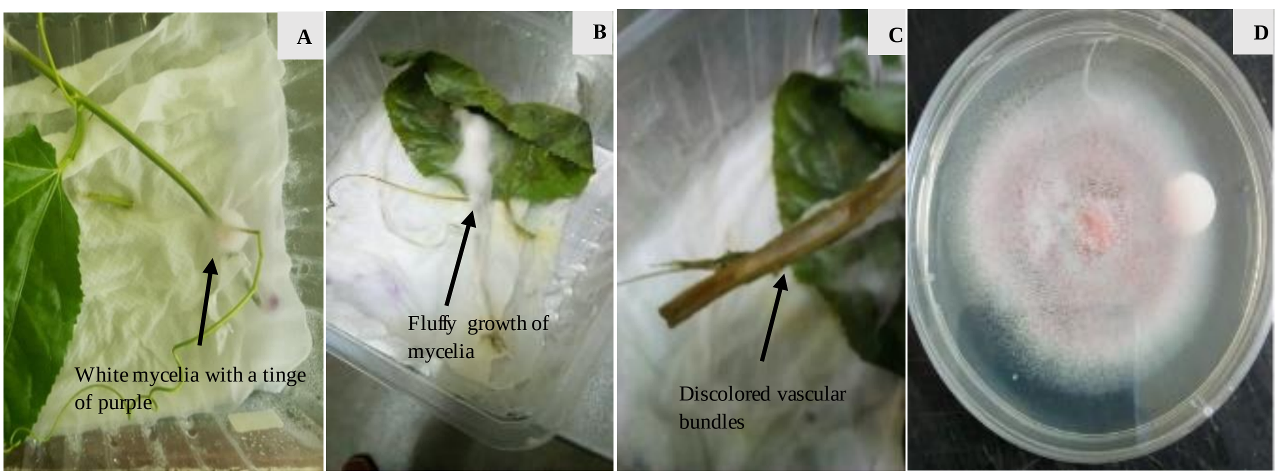

Figure 4.7: Pathogenicity results displaying morphology typical to *Fusarium oxysporum* on detached stem and leaf (A) symptoms on stem (B and C) symptom on leaf and longitudinal section of stem (D) re-isolated *Fusarium oxysporum* colony on PDA.

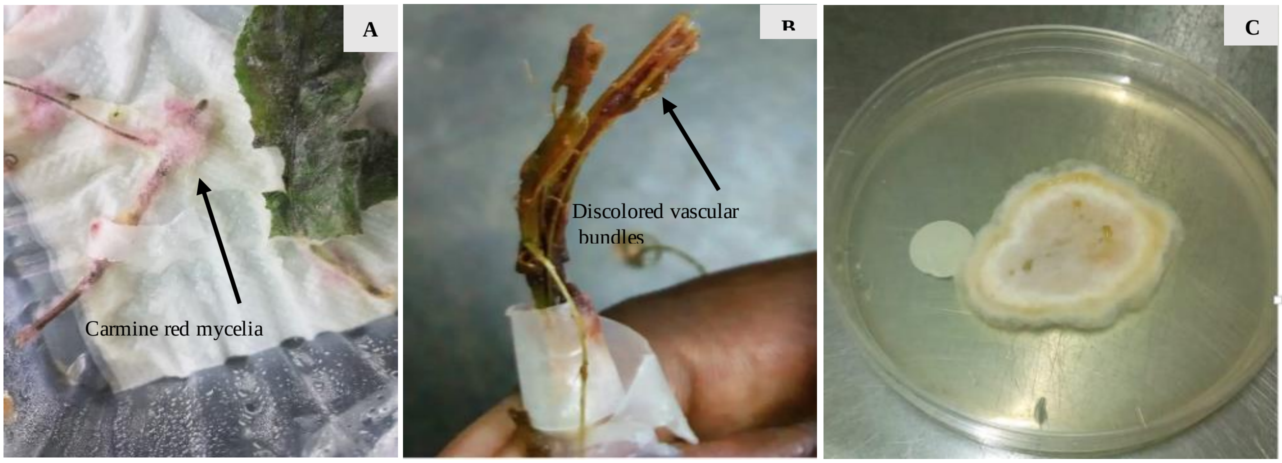

Figure 4.8: Pathogenicity test of *Fusarium semitectum* on detached stem and leaves of passion fruit (A) symptoms on stem (B) longitudinal section on stem (C) re-isolated *Fusarium semitectum* colony growing on PDA media.

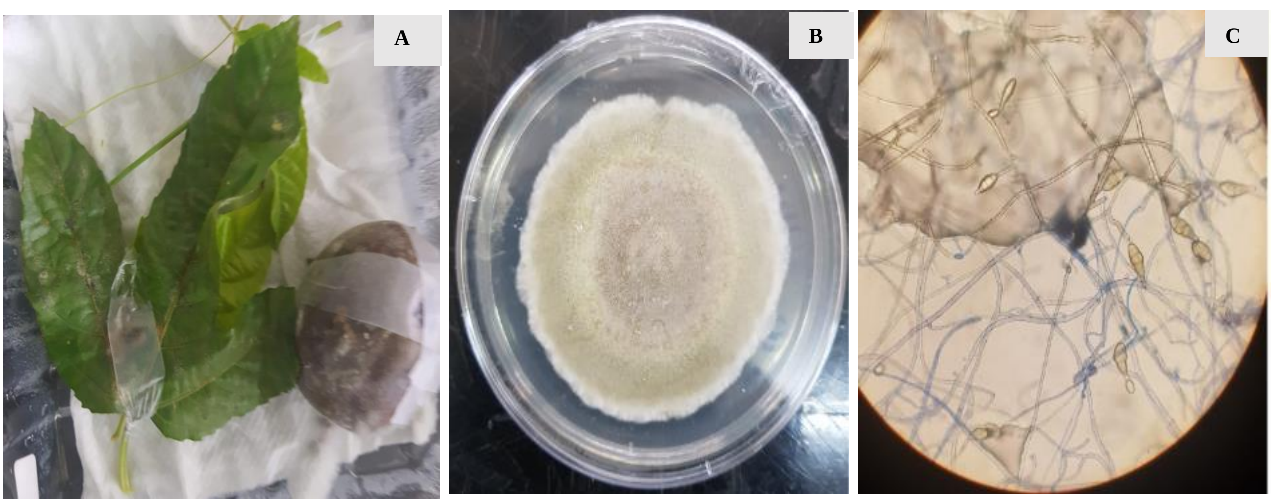

Figure 4.9: Symptoms of brown spot after pathogen inoculation in passion fruit leaf and fruit (A), (B) re-isolation from inoculated leaf (C) *Alternaria passiflorae* conidia under microscope.

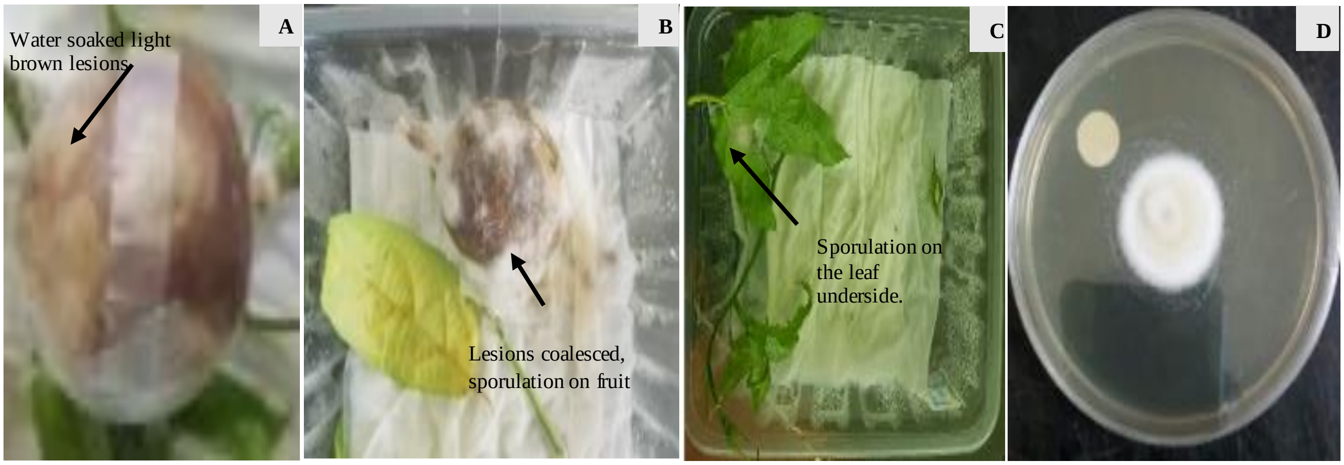

Figure 4.10: Sequence of *Phytophthora nicotianae* associated symptoms in detached passiflorae edulis sims parts, (A and B) symptoms on fruit (C) symptoms on leaves (D) re-isolated *Phytophthora nicotianae* colony growing on PDA.

4.3 Resistance of passion fruit germplasm to dieback

4.3.1 Symptoms caused by dieback associated fungi on susceptible purple passion fruit

Symptomatic seedlings inoculated with *Alternaria passiflorae* exhibited small circular dark brown spots that were up to 1cm in size and formed lighter central areas (Fig 4.11). Most of the affected leaves were chlorotic and readily defoliated. On the stems, elongated dark brown spots that could cause stem girdling with disease progress were observed. The un-inoculated seedlings showed no symptoms.

Typical symptoms of *Fusarium oxysporum* were observed in the passion fruit seedling leaves, roots and stems after inoculation with the pathogen (Fig 4.12). The older leaves turned yellow while the new ones had a glossy yellow appearance. Some stems developed progressive necrotic lesion that led to wilting and subsequent death of the vine or the whole seedling. On dissecting the affected seedling, vascular bundles showed brown discoloration. Detrimental effects like discoloration and reduced growth on root development were also observed.

Passion fruit seedlings inoculated with *Phytophthora nicotianae* exhibited irregular water soaked light brown lesions on the leaves which grew and merged into large brown to black necrotic spots (Fig 4.13). Some of the affected stems had progressive necrotic lesions at the collar region that were purple at first then turned to dark brown and subsequently covered with white fluffy fungal growth.

Fusarium semitectum was found to be pathogenic to passion fruit seedlings (Fig 4.14). The inoculated seedlings developed necrotic lesions on the stem, chlorosis and dark brown necrotic spots on the lower leaves. As the disease progressed, random seedlings wilted and eventually died. Longitudinal sectioning of the wilted seedlings showed extensive vascular discoloration. The root system of the wilted seedlings was highly inhibited and extensively discolored showing clear symptoms of decay.

Each pathogen species was successfully re-isolated from inoculated symptomatic seedling tissues thereby fulfilling Koch's postulates (Fig 4:15). However, all the uninoculated seedlings in the control experiments showed no symptoms of infection throughout the study period.

Figure 4.11: Symptomatic tissues of *Alternaria passiflorae* inoculated passion fruit seedlings (A)yellowing and defoliation of the older leaves(B) circular brown spots with lighter central part surrounded by green halo (C) dark brown lesion on stem.

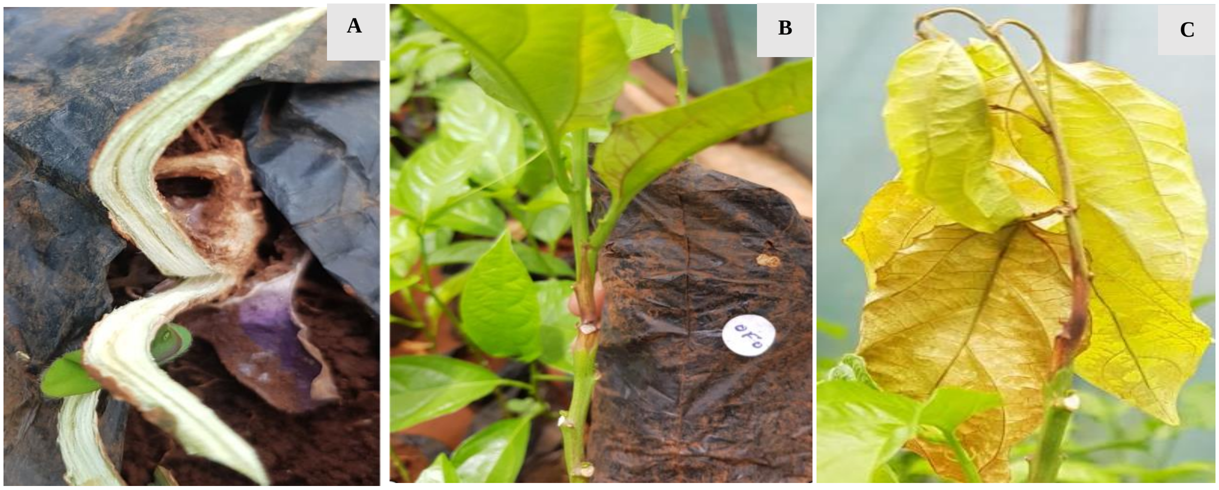

Figure 4.12: Symptoms caused by *Fusarium oxysporum* on passion fruit seedlings: (A) discoloration of vascular bundles (B) stem lesion (C) dieback of the seedling

Figure 4.13: Symptoms of infection by *Phytophthora nicotianae* (A) water soaked brown lesions on leaves (B) purple brown lesion on stem (C) white fungal fluffy growth on collar region.

Figure 4.14: *Fusarium semitectum* associated symptoms in purple passion fruit seedlings (A) dark brown necrotic spots on leaves (B) necrotic lesion on stem (C) blackened and reduced root system.

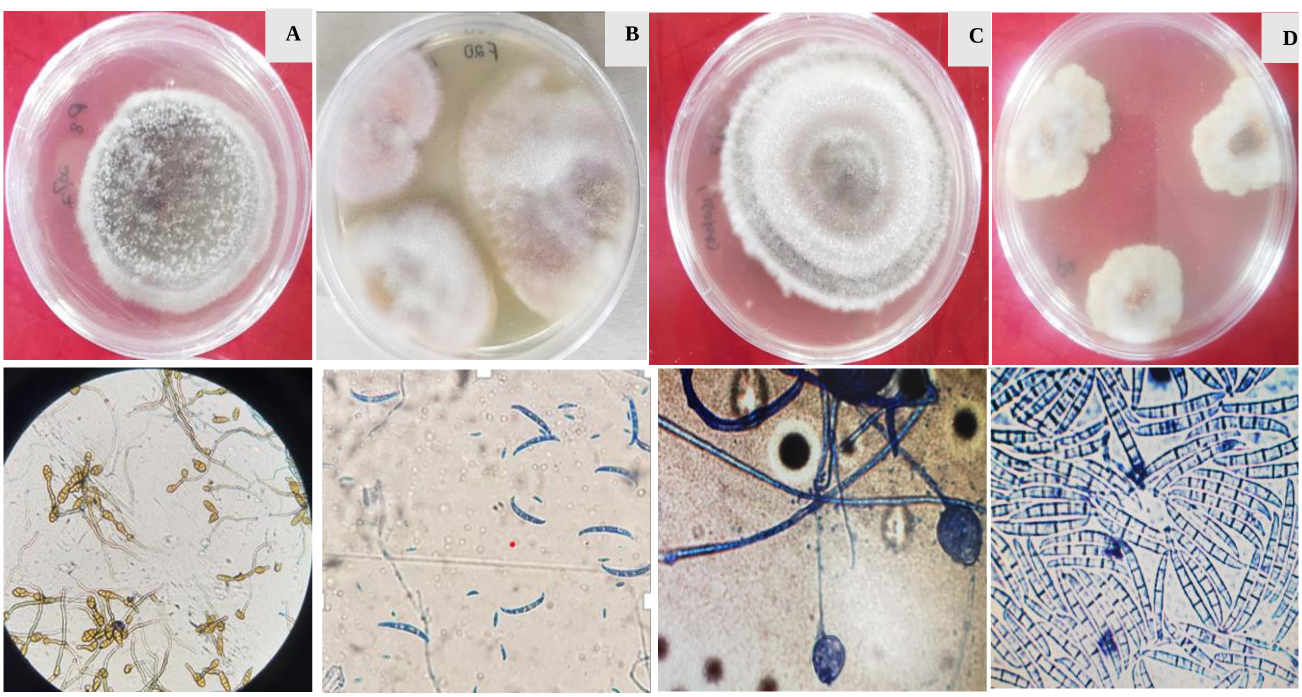

Figure 4.15: Pathogenicity test of the fungal isolates: colony morphology on PDA and microscopic (X400) examination of (A) *Alternaria passiflorae* (B) *Fusarium oxysporum* (C) *Phytophthora nicotianae* and (D) *Fusarium semitectum*.

4.3.2 Virulence of dieback associated fungi on susceptible purple passion fruit

The results showed that all the isolates studied were pathogenic to ordinary purple passion fruit, a species known to be highly susceptible. Disease symptoms development was evaluated 17 days after inoculation. The non-inoculated control plants did not exhibit symptoms of infection through out the study period(data not shown).All the pathogens showed slower disease progress at the initial stage of establishment with the diseases gradually intensifying over time until maximum severity and incidence was observed in the nineth week. Results of analysis of variance also showed significant (P≤0.05) difference among the isolates for the AUDPC values for both severity and incidence as shown in Fig 4:16b and Fig 4:17.

The disease severity progress curve (Fig 4.16 a) showed *Alternaria passiflorae* having a divergent progress curve as compared to the other pathogen curves however, the AUDPC severity results (Fig 4.16 b) showed non-significant difference in the aggressiveness and virulence of *Alternaria passiflorae*, *Phytopthora nicotianae* and *Fusarium oxysporum* isolates. However, *Alternaria passiflorae* recorded the highest AUDPC severity mean value of 147 while *Fusarium semitectum* had the lowest.

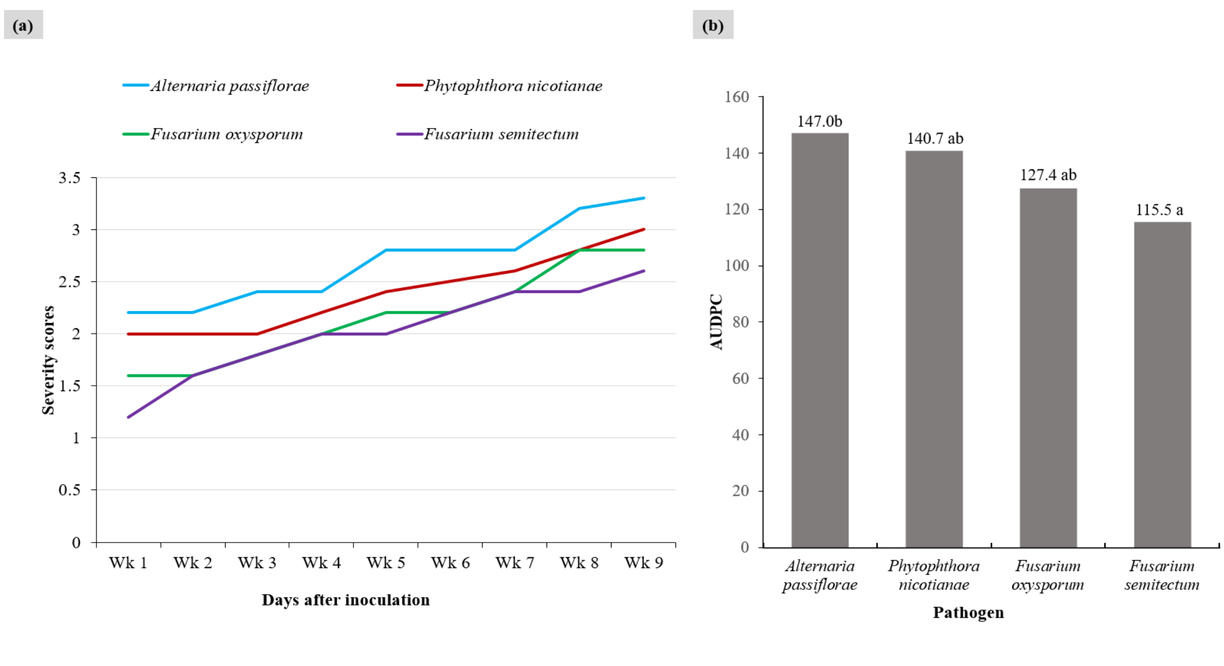

Figure 4.16: Disease severity curves (a) and Area under disease progress curve (b) of ordinary purple passion after inoculation with dieback causal pathogens. (*values followed by different letters are significantly (P≤0.05) different by Tukey's test).

Inoculation with *Alternaria passiflorae* resulted in highest values of disease incidence (72%) as well as the highest lowest mean of 30% demonstrating a highly significant disparity from the other pathogens. Although the overall mean values of *Fusarium semitectum*, *Fusarium oxysporum* and *Phytophthora nicotianae* were not significantly (P>0.05)different, *Phytophthora nicotianae* recorded a higher mean than *Fusarium oxysporum* and *Fusarium semitectum*.Despite *Fusarium semitectum* producing the least disease pressure, it elicited a dramatic increase in the pattern of disease response towards the end of the study period.

The AUDPC incidence values ranged from 1512 to 3101 with a mean of 1995. *Alternaria passiflorae* isolate was the most aggressive recording the highest mean AUDPC value of 3101 while *Fusarium semitectum* isolate caused the lowest AUDPC value of 1512 in the study. *Fusarium oxysporum* and *Phytophthora nicotianae* produced moderate disease infection scoring 1841 and 1526 AUDPC values respectively. However, only *Alternaria passiflorae* AUDPC mean value was found to be highly significant from the other pathogens at 5% level of significance. (Fig 4.17)

Table 4.10: Percentage incidence of passion fruit diseases on purple passion fruit seedlings inoculated with fungi isolated from diseased passion fruits tissues

	Weeks after inoculation									
Pathogen	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Mean**
F. semitectum	4.0a	6.0a	8.0a	20.0a	24.0a	36.0a	40.0a	48.0ab	64.0a	27.4a
F. oxysporum	8.0a	10.0a	12.0a	28.0a	32.0a	32.0a	36.0a	40.0a	48.0a	27.3a
P. nicotianae	16.0a	20.0a	20.0a	28.0a	32.0a	36.0a	42.0a	50.0ab	54.0a	33.1a
A. passiflorae	30.0b	40.0b	46.0b	54.0b	56.0b	58.0b	66.0b	72.0b	72.0b	54.9b
Mean	14.5	19.0	21.5	32.5	36.0	40.5	46.0	52.5	59.5	35.7
LSD	10.9	13.1	11.6	13.1	10.3	13.1	14.8	17.4	18.6	10.4

(*In the mean column, values followed by different letters are significantly ($P \leq 0.05$) different by Tukey's test)

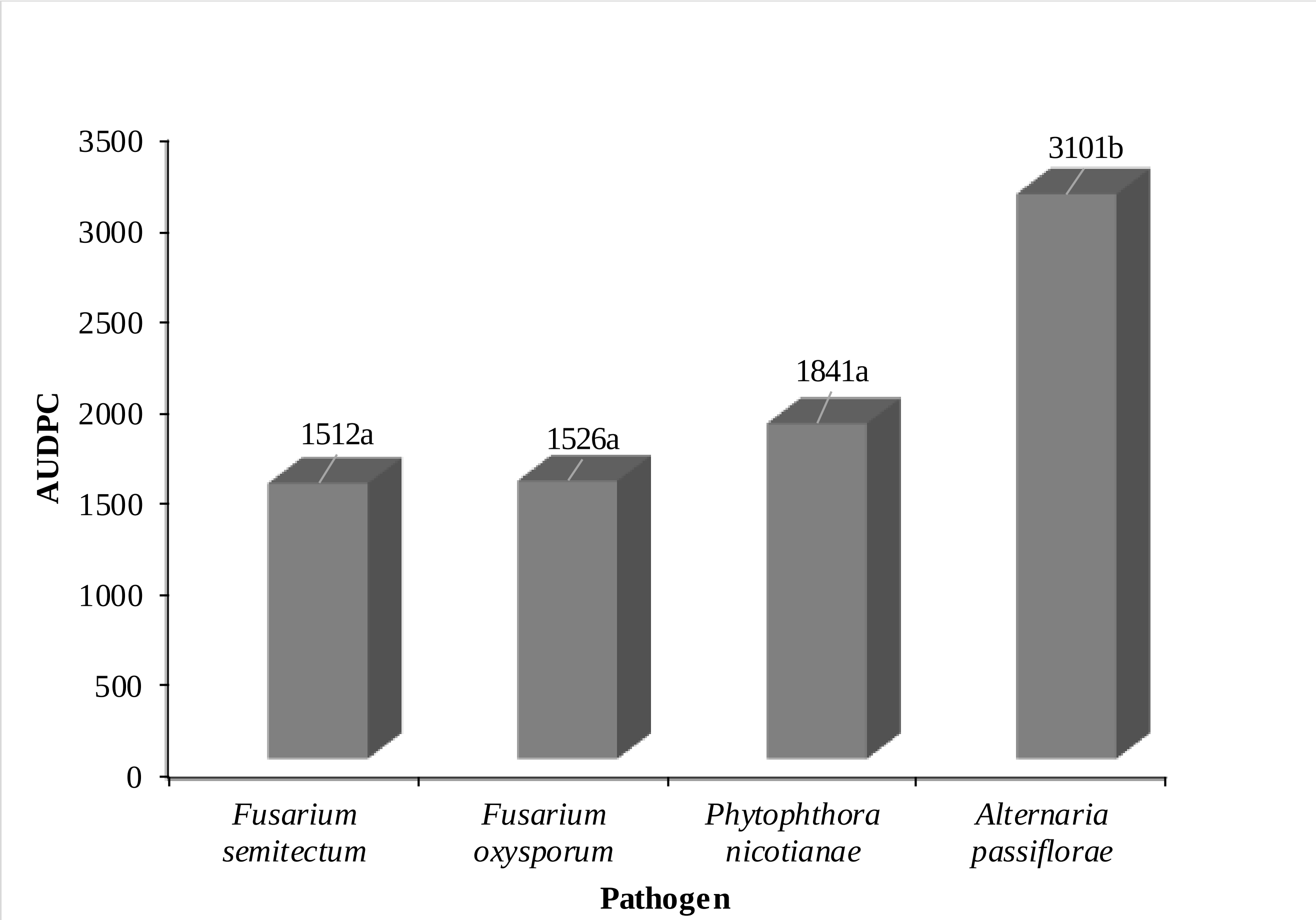

Figure 4.17: Area under disease progress curve for ordinary purple passion after inoculation with four test pathogens (*values followed by different letters are significantly ($P \leq 0.05$) different by Tukey's test).

4.3.3 Response of six passion fruit genotypes to dieback causal agents

In 180 inoculated seedlings, 86 seedlings expressed symptoms associated with the dieback of passion fruit test pathogens. The non-inoculated control plants did not exhibit symptoms of infection through out the study period .

In general, the incubation period of the germplasms that showed symptoms was 17 days except for ester variety that had an incubation period of 31 days. However, despite having almost the same incubation period, considerable variability in disease intensity was observed among the genotypes. Dieback symptoms in ordinary purple and grafted purple were evident in the first week at 20%

and 17%. Exponential increase in percentage infection was observed with both varieties recording the highest disease incidences of 73% and 53% respectively by the end of the experiment.

Brazil, a yellow variety showed a less rapid pattern of disease increase than ordinary and grafted purple. The variety recorded a mean disease incidence of 31% which was a higher value compared to ordinary yellow and sweet yellow varieties that were not significantly($P>0.05$) different from each other. In contrast, disease establishment in ester variety exhibited delayed less severe symptoms and proceeded more slowly to attain the least mean infection of 15% amongst all the genotypes (Table 4.11). When random inoculated seedling parts were taken for re-isolation, dieback causal agents were also recovered in some asymptomatic seedlings.

The area under the disease progress curve (AUDPC) calculated based on the disease incidence varied significantly amongst the genotypes with the mean AUDPC value ranging from 863 to 2683. Ordinary purple variety, which was the susceptible check recorded the highest AUDPC score at 2683 while ester variety recorded the lowest AUDPC value of 863 in the test. The grafted purple variety that is thought to be more resistant than ordinary purple showed no significant difference statistically though it recorded a higher AUDPC value from the ordinary purple. Mean AUDPC for brazil variety was slightly higher than for sweet yellow and ordinary yellow whose means were not significant ($P>0.05$) from each other. (Fig 4.18).

Based on the disease incidence and AUDPC values, the genotypes were grouped into four categories namely resistant (ester), moderately resistant (sweet yellow and ordinary yellow), (tolerant) brazil and susceptible (grafted and ordinary purple).

Table 4.11: Percentage incidence of dieback on passion fruit genotypes nine weeks after inoculation with fungi associated with dieback

	Weeks after inoculation									
Genotype	1	2	3	4	5	6	7	8	9	**Mean**
Ester	0.0a	0.0a	3.3a	6.7a	20.0a	23.3a	26.7a	26.7a	33.3a	15.2a
S. yellow	3.3a	13.3ab	16.7a	16.7ab	26.7ab	33.3a	33.3ab	33.3ab	33.3a	23.7ab
O. yellow	6.7a	16.7ab	16.7a	20ab	30.0abc	30.0a	33.3ab	33.3ab	33.3a	24.4ab
Brazil	3.3a	13.0ab	13.3a	26.7bc	33.3abc	36.7ab	46.7bc	50abc	56.7ab	31.1bc
G. purple	16.7a	30.0b	36.7b	40.0c	46.7c	53.3bc	53.3c	53.3bc	53.3ab	42.6cd
O. purple	20.0a	33.3b	36.7b	40.0c	43.3bc	56.7c	60.0c	66.7c	73.3b	47.8d
Mean	8.3	17.7	20.6	25.0	33.3	38.3	42.2	43.9	47.8	30.9
LSD	13.7	14.9	9.2	11.0	12.0	12.7	12.3	15.1	16.2	7.5
CV	90.3	46.1	24.6	24.2	19.7	18.0	16.0	18.9	18.6	13.7

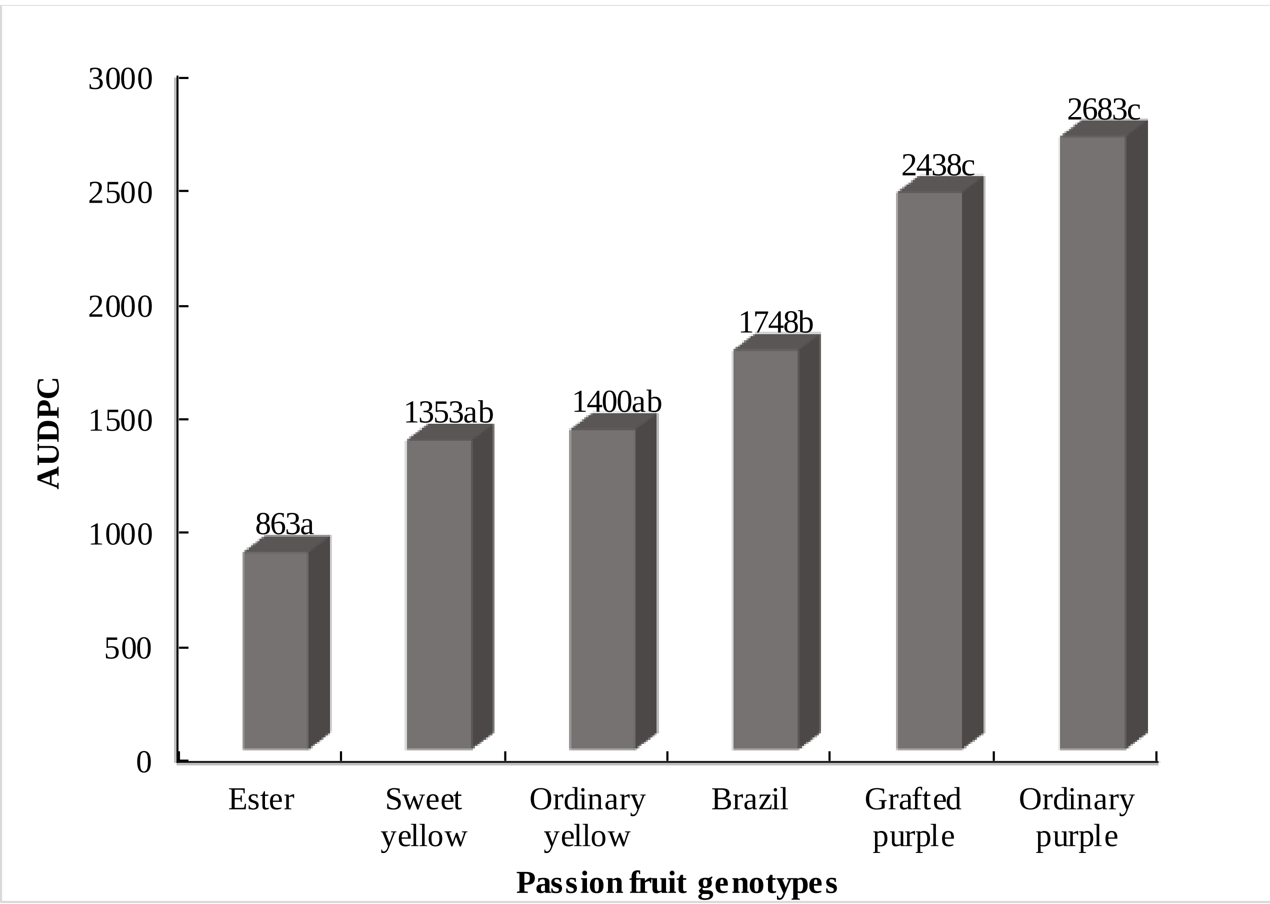

Figure 4.18: AUDPC of dieback of passion fruit in six passion fruit genotypes.
(*means followed by the same letter are not significantly (P>0.05) different based on Tukey's test)

CHAPTER FIVE: DISCUSSION

5.1 Distribution of dieback of passion fruits in Uasin Gishu County

Most passion fruit farmers in all AEZs were males in their active age group who had attained average education level and their primary occupation was farming. This finding was consistent with Mwirigi *et al*. (2013) and Atukunda *et al*. (2018) who found male dominance, active age groups involvement coupled with high educational profile as key features of passion fruit growers. This is an indicator that passion fruit farming is a priority enterprise with the potential to alleviate poverty and sustain livelihoods among farmers.

Despite its importance, passion fruit was predominantly small scale on farms less than 0.4 hectare even though most farmers had more land that could be put into production. Farmers grew a susceptible variety whose main source was informal nurseries. The lifespan of the orchards capped at two years with a productivity of 10 tons ha^{-1}. This confirms similar finding by Karani-Gichimu *et al*. (2015) who observed that passion fruit in the Kenyan highlands was allocated 0.26 ha; orchard life spanned for an average of 1.6 years and the production was 10.34 tons ha^{-1} against 24 tons ha^{-1}potential.

High potential for passion fruit production exists but the smaller farm sizes possibly undermine the capacity to generate surplus needed to improve productivity. According to Birch (2018) and Mohammed& Mehmet (2018), commercialization is positively associated with land size. However, Karani-Gichimu *et al*. (2015) assert otherwise, that an increase in farm size under passion fruit by 1% would on average reduce its yield by 1% since the farmers would not adequately cater for the required management practices.

The use of a susceptible variety, further reported by Wangungu (2013) could be influenced by the absence of resistant and productive varieties that are adapted to local conditions. However, the constant sourcing of the planting materials from informal sources comparable to Wangungu (2013) and Karani-Gichimu *et al*. (2015) implies that various intervention put in place by regulatory agencies have not yielded much and so there still a significant call for measures to address the practice.

Across the zones, farmers decried passion fruit production related constraints which in order of frequency, diseases were rated as the most important, majority being fungal while a few were viral.

High preference for a highly susceptible variety obtained from informal nurseries coupled with drought among other factors possibly accelerated the role of diseases as a major constraint affecting productivity (Wangungu *et al.,* 2012; Karani-Gichimu *et al.*, 2015; Atukunda *et al.*, 2018).

Dieback was the most prevalent passion fruit fungal disease across the AEZs. Brown spot, Phytophthora blight and Fusarium wilt whose causal agents are associated with dieback were also recorded in considerable variations in prevalence, incidence and severity. This finding is in conformity with previous works of Amata *et al.*, (2009) who found dieback most prevalent at 80% in respect to other passion fruit diseases. The observed distribution pattern of the diseases in the surveyed AEZs showed a correlation with variation in climatic condition of the ecozones that tended to influence the growth and development of the disease causal pathogens (Pegg *et al.*, 2019). According to Lamichane & Venturi (2015) and Yigrem *et al.* (2019), other factors apart from ecological preference of the pathogens that possibly influenced variation in disease incidences and severity across the ecozones include farmer management practices and other abiotic factors.

According to the study, the incidence and severity of Fusarium wilt which is favored by high temperature, light soils, dry soil condition and high relative humidity (Agrios, 2005; Orr and Nelson, 2018; Pegg *et al.*, 2019; Pelczar *et al.*, 2020) was highest in UM4 but lowest in LH2. This would be due to insufficient rainfall and soils with low water holding capacity in UM4 zone compared with the LH2 zone, characterized by high rainfall (1150mm-1220mm) with red clay soils and lower temperatures (Jaetzold and Schmidt, 1983).

Phytophthora blight was more severe in LH3 and LH2 that are characterized by high rainfall and relatively higher temperatures (Jaetzold and Schmidt, 1983). The high soil moisture content of these zones not only favor germination and spread of sporangia but also decreases oxygen in the soil, making plant roots more susceptible to the causal pathogen thus the disease is confined to warm wet areas (Panabieres *et al.*, 2016; Jung *et al.*, 2018; Mesta, 2018; Pelczar *et al.*, 2020). In addition, brown spot, favored by high humidity, abundant rainfall and warm temperatures (Fischer and Rezende, 2008; Joy and Sherin, 2012), was found higher in LH2 zone.

Dieback caused by *Fusarium* spp, *Phytophthora* spp, *Alternaria* spp and possibly others (Amata *et al.*, 2009; Wangungu, 2013) was recorded in all AEZs. It was more prevalent and severe in LH3 where the associated individual pathogens exerted maximum disease pressure and lowest in LH2 where the individual disease pressure was lowest. The mean severity of the diseases caused by the individual pathogen species across the zones was 4.5%, but when in complex, the severity of the co-infection surged to 16%. This indicates that the co-occurring pathogens interacted with each other through synergism. Vast majority of the respondents extensively used fungicides as a key intervention to disease management however for dieback, fungicides only stopped or slowed down the disease progress momentarily (Wangungu, 2013). While effective training of the orchards and other cultural practices have the potential to minimize incidence of dieback (Wangungu *et al.*, 2014;Abasi *et al.*, 2018), it was done by a few of the respondents. Therefore, a holistic management strategy of dieback must address the role of all co-occurring pathogens, pathogen-pathogen interaction, temporal order of host infection and their impact on plant defense system (May *et al.*, 2009; Lamichane and Venturi, 2015; Abdullah *et al.*, 2017).

5.2 Causal agents of passion fruit dieback and their pathogenicity

Isolation of passion fruit samples with typical symptoms of dieback showed that dieback is caused by several fungal species namely *Phytophthora nicotianae*, *Fusarium oxysporum, Alternaria passiflorae* and *Fusarium semitectum*. This finding is partially in concordance with similar work by Amata *et al*. (2009) who found the causal agents as *Phytophthora nicotianae, Fusarium oxysporum, F. pseudoanthophilum, F. subglutinans, F. solani* and *F. semitectum* and Wangungu (2013) too who reported the causal agents as *Fusarium solani, Fusarium semitectum, Fusarium oxysporum, Phytophthora nicotianae*, *Alternaria passiflorae* and *Ascochyta passiflorae*. This confirms that dieback is as a result of a network that involves a wide range of microbial interaction and the possibility that the synergism of the pathogen interaction leads to persistent disease severity in the field (Lamichane and Venturi, 2015).

The pathogens were isolated from both stems and leaves of symptomatic plants. However, *Fusarium oxysporum* and *Fusarium semitectum* were isolated in higher frequencies from the stem compared to the leaves. This is probably because they are termed as classical vascular wilt pathogens (Pegg *et al.*, 2019). This confirms previous finding by Rooney- Latham *et al*. (2011), Vicente *et al*. (2014) and Koyyappurath *et al*. (2016) that *Fusarium* spp. has been isolated more frequently from roots and stems than leaves. *Phytophthora nicotianae* and *Alternaria passiflorae* isolation frequencies were highest in the leaves perhaps because they cause various types of spots and blights on foliage (Taylor *et al*., 2008; Joy and Sherin, 2012; Aljaradi *et al*., 2018; Wu *et al*., 2018).

Across the AEZs, *Alternaria passiflorae* was the most prevalent isolate. Its highest mean was in LH2 presumably due to its preference to high humidity, abundant rainfall and warm temperatures but lowest in UM4 which is a drier zone. *Fusarium oxysporum* was highest inUM4 possibly due to the zone's high temperature, dry soil condition and high relative humidity (Orr and Nelson, 2018; Pegg *et al*., 2019; Pelczar *et al*., 2020). *Phytophthora nicotianae* favored by warm wet weather was high in LH3 whose ecological characteristics favor the pathogen. *Fusarium semitectum* was isolated only in LH3 and at very low frequency and therefore its role in the dieback complex needs to be investigated further.

Pathogenicity test done *in vitro* and greenhouse revealed that all fungal isolates were pathogenic but *in vitro*, the lesions were more severe than *in vivo* which according to Pacheco *et al*., (2012), could have occurred due to physiological changes in the detached plant parts consequently affecting their resistance level.

5.3 Resistance of passion fruit germplasm to dieback

Individual inoculation of dieback fungal pathogens on a susceptible purple variety exhibited diverse symptom expression in varying intensities. Symptoms produced by the isolates consisted of marked chlorosis, necrosis, leaf spots, stem lesions, wilts, but with a common end result of dieback. *Alternaria* spp., *Fusarium* spp. and *Phytophthora* spp. have been reported to cause dieback on annual and perennial plants (Ploetz, 2006).

Alternaria spp. causes dieback in passion fruit (Fischer and Rezende 2008; Joy and Sherin, 2012), kiwi fruit (Karakaya and Celik, 2012), chilli (Kumar *et al*., 2016), citrus (Timmer *et al*., 2003) among others. The pathogen causes dark brown spots that elongate and girdle branches, twigs, vines or leaf axils causing death of the plant parts resulting to dieback. Similarly, *Phytophthora* spp. completely girdles the stem and destroys the root system of a plant restricting water absorption and subsequent transportation to the upper plant parts (Joy and Sherin, 2012; Wangungu, 2013).

Fusarium spp. is among the most ubiquitous fungi in terrestrial ecosystem and a common associate of higher plants. Several authors have shown that *Fusarium* spp. induce dieback on olive trees (Trabelsi *et al*., 2017), avocado and passion fruit (Ploetz, 2006; Fischer and Rezende, 2008). The dieback results from the damage the disease causes on the stem and roots by clogging the xylem vessels with its mycelia, spores and tyloses reducing the host capacity to extract and conduct water and minerals to the upper parts (Manicom *et al.,* 2003; Agrios, 2005).

Amongst all the pathogens inoculated, *Alternaria passiflorae, Phytopthora nicotianae* and *Fusarium oxysporum* caused significantly highest disease severity. *Alternaria passiflorae* was the most aggressive causing highest disease severity mean and consequently highest area under disease progress curve. The pathogen also scored the highest isolation frequency from the diseased samples collected from the field suggesting this pathogen could be highly implicated in dieback of passion fruit. Study by Wangungu (2013) found both *Fusarium* spp. and *Phytophthora* spp. causing highest disease severity in relation to other species but the current study indicates that *Alternaria passiflorae* is becoming a pathogen of economic importance in passion fruit production.

Although *Fusarium semitectum* has been generally regarded as a saprophyte or a secondary colonist occurring on plant material and other substrates (Leslie and summerel, 2006; Maina *et al*., 2009), this study revealed that despite its low isolation frequency, its inoculation resulted to marked disease severity that was similar to *Fusarium oxysporum* and *Phytophthora nicotianae*. The finding corresponds with the results of Kim and Kim (2004) and Zakaria *et al*. (2016), who found *F.semitectum* pathogenic, causing pineapple fusariosis and related to fusarium wilt in melon respectively. It is evident that the pathogen is pathogenic but might have lower competitive ability in the presence of other pathogens.

Inoculation of the combined fungal isolates showed considerable variability in disease intensity among the six genotypes. The evaluation of resistance was under controlled conditions therefore the differences in symptom expression were almost exclusively of a genetic origin due to minimized environmental effect (Silva *et al*., 2013). This therefore suggests that the considerable differences in the disease incidence and AUDPC means were an evidence of genetic variability among the six genotypes under study.

Five of the genotypes had an average incubation period of 17 days while only one genotype showed an extended incubation period of 31days.A long incubation period is an indicator of partial resistance of host plants to a given pathogen (Van der Plank, 1963). It was observed that symptom initiation in all genotypes was slow at the initial stages and this could be related to the initial density of the test pathogen, however, the severity of the disease increased with time suggesting multiplication of the pathogen (Goncalves *et al.*, 2017).

Ester variety had the longest incubation period, lowest disease incidence and AUDPC value and therefore considered resistant. The variety is a cross between the yellow (*Passiflora edulis var. flavicarpa*) and purple granadilla (*Passiflora edulis var. edulis*) (Louw, 2020). This indicates that its superior resistant trait could be attributed to the high level genetic variability generated from the cross (Silva *et al.*, 2013). Since the genotype is a purple variety, it is adaptable to the climatic conditions of the study area.

Sweet yellow (KPF 12) and ordinary yellow were grouped as moderately resistant. This finding was consistent with that of Wangungu *et al*. (2014) who found that KPF 12 (hybrid) portrayed tolerance to dieback causal pathogens on inoculation through wounding. Yellow variety (*Passiflora edulis var. flavicarpa*) has been documented to be partially resistant to diseases like Fusarium wilt (Fischer and Rezende, 2008; Joy and Sherin, 2012) and passion fruit collar rot (Ssekyewa, 2010). This may indicate why the genotype exhibited moderate resistance to dieback, however, the yellow varieties are not adaptable to the ecology of the study area therefore limiting their adoption.

Grafted and ordinary purple genotypes were found to be most susceptible genotypes in the study. This is despite being the most preferred by the passion fruit growers in the region probably due to lack of a resistant variety adaptive to the ecological characteristics. However, the grafted variety recorded a slightly lower AUDPC value since grafting has been shown to reduce incidences of some diseases like Fusarium wilt. Data obtained is supported by KAPP and IIRR, (2015) whose on-station research established that purple passion fruit, C5 and KPF4 varieties were most susceptible to dieback. Similarly, Wangungu *et al.* (2014) also observed that the highly susceptible purple passion fruit variety was at a higher risk of dieback infection than KPF 12 variety.

All the genotypes used in this study were cultivated varieties but according to Cerqueira-Silva *et al*. (2014), *Passiflora* genetic resource is highly diverse in remarkable variability in both commercial and wild species that can be explored to increase significant genetic gains towards resistance to complex diseases like dieback without compromising the agronomic attributes.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The results from this study indicate that passion fruit production was majorly for commercial purpose in the study area. The dominant variety grown by farmers was the purple variety which is susceptible to most passion fruit diseases. The major source of the planting materials was informal nurseries. Diseases were the main constraint limiting production and were widely distributed in the study area.

Dieback of passion fruit was prevalent and widely distributed across all the agro-ecological zones but more severe in LH3. The disease is caused by a complex of different fungal pathogens (*Phytophthora nicotianae, Fusarium oxysporum, Alternaria passiflorae* and *Fusarium semitectum*) with *Alternaria passiflorae* being the most virulent. Majority of the farmers use fungicides to manage passion fruit disease therefore increasing cost of production and risk of residues in the produce. Amongst the passion fruit germplasm tested, ester genotype, a purple variety showed remarkable resistance to dieback under controlled conditions.

6.2 Recommendations

Based on this study's findings and conclusions, it is recommended that:

1. To reduce the spread and distribution of dieback, there is need to strengthen the capacity of farmers and nursery operators on best planting material propagation protocols.
2. To significantly curb the effect of the dieback associated pathogens, farmers are advised to adopt an integrated disease management approach in the management of dieback.
3. Further evaluation of ester variety that seemed resistant to dieback of passion fruit under a controlled environment is recommended under varying climatic conditions as the final confirmatory test before release and adoption by farmers.

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