OCCURRENCE AND MANAGEMENT OF *ALTERNARIA* LEAF SPOT IN SPINACH USING EXTRACTS FROM GINGER AND TUMERIC PLANTS

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DECLARATION

Declaration by the Candidate

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

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Declaration by Supervisors

This thesis has been submitted for examination with our approval as University Supervisors.

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DEDICATION

I dedicate this thesis to my cousins and siblings who have been really supportive of me and my parents who have committed to do whatever it takes to ensure that my siblings and I attain quality education.

ABSTRACT

Spinach (*Spinacia oleracea*) is an economically important vegetable crop in Kenya cultivated by small-scale farmers for domestic consumption. Alternaria leaf spot caused by *Alternaria alternata* is one of the diseases of spinach which cause low yields and poor quality crop in Kenya. The pathogen has been traditionally controlled using synthetic fungicides which are expensive and harmful to both humans and environment. The present study aimed at establishing the occurrence of *Alternaria alternata* in the farmer's fields, characterize the causal pathogen as well as test the efficacy of the extracts of two plants; *Curcuma longa* and *Zingiber officinale* against *Alternaria alternata* both *in vitro* and *in vivo* conditions. Extensive surveys were conducted in September 2018 in seven selected sub-counties namely; Mosop, Chesumei, Aldai, Nandi hills and Emgwen of Nandi county, and Kapseret and Moiben in Uasin Gishu county. In each farm the disease incidence was established and diseased samples collected for isolation, identification and characterization in the laboratory. Absolute methanol, ethanol water and ethyl acetate were the solvents used in extraction of *Curcuma longa* and *Zingiber officinale* rhizome. The extraction technique used was the modification of the homogenization in solvent. The solvent-to-sample ratio of 10:1 (v/w) solvent to dry weight ratio was used. The decoctions were screened for antimycotic activity using the poisoned food technique. The study revealed that the disease occurred in all the sub-counties surveyed. Out of the forty farms surveyed 57.50% had leaf spot disease. The highest disease incidence was observed in Mosop sub-county (41.31%). Overall disease incidence ranged between 25.79% and 50.51% among the farmer's fields. When isolates were studied in the laboratory, their morphological characteristics varied from fairly compact to luxuriant mycelial growth, texture varied from feathery to cottony. The growth rate was between 2.98 and 4.05 mm/day, and the spore yield after 12 days was between $4.68\times10^4$and 7.4×10^4 conidia/ml. *Curcuma longa* and *Zingiber officinale* extracts displayed varying degree of antifungal activity against the *Alternaria alternata* depending on the solvent used for extraction and the concentration. Methanolic extracts of *Curcuma longa* (64.56%) and *Zingiber officinale* (57.37%) demonstrated the highest antifungal activity against *Alternaria alternata*, which was significantly different ($p\leq0.05$) as compared to ethanolic, ethyl acetate and aqueous extracts at the concentration of 50 mg/ml. At the concentration of 25 mg/ml and 50 mg/ml the percent inhibition on the fungal growth was not significant ($p\leq0.05$) from the 8^{th} day for both tumeric and ginger. The results further showed that percentage inhibition increased with increasing concentration of the extracts. The findings indicated that methanol is the best solvent for extraction. The extracts from *Curcuma longa* displayed the highest percent decrease reduction in comparison to *Zingiber officinale* of 57.70% and 53.84%, respectively. These results have demonstrated that the use of *Curcuma longa* and *Zingiber officinale* extracts in control of *Alternaria alternata* associated with leaf spot of spinach is possible. The extracts can be incorporated into integrated disease management to reduce/eliminate spread of Alternaria as an alternative to synthetic fungicide application.

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LIST OF ABBREVIATIONS

ALS-	Alternaria Leaf Spot
ANOVA-	Analysis of variance
CRD-	Completely randomized design
CV-	Coefficient of variation
D.F-	Degrees of freedom
E-	Ethanol
E.A-	Ethyl acetate
F. pr-	F. probability
f.sp-	*Forma specialis*
FAOSTAT-	The Food and Agriculture Organization Corporate Statistical Database
G-	Ginger
GPS-	Geographical position system
I-	Inhibition zone
L-	Length
LSD-	Least significant difference
m.a.s.l-	Meters above sea level
M.S-	Mean square
M^2-	Metre squared
MIC-	Minimum inhibitory concentration
Ml-	Milliliters
MoA-	Ministry of Agriculture
PDA-	Potato dextrose agar
PDI-	Percent desease index
Psc-	Pascal
Rpm-	Revolutions per minute
S.S-	Sum of squares
T-	Tumeric
V.R-	Variance
W-	Water
W-	Width

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Spinach (*Spinacia oleracea*) is an edible flowering plant in the family Amaranthaceae and is native to central and western Asia (Sato *et al.,* 2010). It is one of the most common vegetable cultivated worldwide (Wachira *et al*., 2014). In Kenya Spinach is grown mostly by small scale holders for home consumption and for the market. The crop favours well fertile and well drained sandy loam soil. The rising demand of spinach has contributed to an increment in cultivation of this vegetable in Kenya (MoA, 2016). The total area under spinach production in Kenya was 5,615 hectares producing 75,563 tonnes in 2016 (FAOSTAT, 2016).

Raw spinach contains 91% water, 4% carbohydrates, 3% protein and negligible fat. It is a rich source of magnesium, manganese, iron, potassium, calcium, dietary fiber and folate (Ball, 2006). Koren (2007) reported that spinach is rich in vitamin A, B2, B6, B9, C, E and K. Spinach has health benefits that include improving blood glucose control in people with diabetes, lowering the risk of cancer, reducing blood pressure, improving bone health and lowering the risk of developing asthma.

1.2 Constraints to production of spinach

Spinach like other vegetables is infested by pests and diseases. Some of the major diseases infecting spinach are bacterial soft rot, cucumber mosaic virus, downy mildew, fusarium wilt, white rust and alternaria leaf spots which cause huge losses (MoA, 2000; Singh *et al.,* 2015). Thomma (2003) noted that the genus *Alternaria* is a significant plant pathogen causing foliage infections and is also credited for post-harvest spoilage of crops and other plant products. The sum losses caused by *Alternaria* sp. rank amongst the highest caused by any plant pathogen (Agrios, 2005).

Leaf spot disease on spinach caused by *Alternaria* sp. has been documented to cause vast economic losses in Saudi Arabia (Marraiki *et al*., 2012), Polland (Czajka *et al.,* 2015) and Pakistan (Aslam *et al.,* 2019) where the mortality due to the disease was found to range between 20-80%**.**

1.3 Statement of problem

Spinach provides a good source of mineral and vitamin elements (Ball, 2006). Spinach has become one of the staple vegetables after kales and cabbage in Kenya (Anon, 2017). In recent past, leaf spot disease on spinach caused by *Alternaria* sp. has become a threat to the production leading to crop mortality (Marraiki *et al*., 2012). Further, the sensitivity to *Alternaria* sp. is a key factor in inducing asthma and allergic rhinitis on immune-depressed individuals (Kuna *et al*., 2011). Leaf spot and Leaf blight caused by *Alternaria* species have been reported to cause yield loss both in quantity and quality. Leaf spot diseases have been generally managed by chemical fungicides. Continuous and wide use of these fungicides has caused serious threats to both human and environmental health. Due to increased awareness about the risk involved in the fungicides, the use of other alternative bio approaches in the management of plant diseases is gaining importance. Several findings have noted the ability of decoctions from various sections of plant as an exceptional bio-control method in managing plant pathogenic fungi (Shafique *et al.,* 2011).

1.4 Justification

In natural environment, spinach is exposed to a myriad of microorganisms of which they compromise the quality and safety of products. The mortality due to leaf spot disease has been reported to be very high (Waghmare *et al.,* 2010). It is important to establish the extent of occurrence and the damage that *Alternaria* sp. causes on spinach. The occurrence and extend tend to vary from region to region, with different

management options adopted. The most used management of *Alternaria* sp. has been by chemicals which have been known to cause harm to humans and environment, because of the residual effect. Mustapha *et al.,* (2017) noted that extensive use of fungicides and pesticides causes reproduction disorders, skin infections, endocrine disruption, cancer, respiratory diseases, infertility and birth defects when these chemicals get into human body through inhalation, dermal exposure or orally through consumption of chemically contaminated plant products. In addition, wide use of chemical fungicides results to destruction of biodiversity, killing of non-target species, water, soil and air pollution (Bourguet *et al.,* 2016). Further, these pathogens have been known to develop resistance to these fungicides (Humaira, 2015). This therefore necessitated the identification and further exploration of alternative botanicals for their management of *Alternaria* species causing leaf spot. Ginger and tumeric plants are rich in bioactive compounds that are antimicrobial and antimycotoxigenic as well as being safe on humans and environment (Ciqiong *et al.*, 2018; Olubunmi *et al.,* 2018).

1.5 Objectives

1.5.1 Main objective

To determine the occurrence of leaf spot and test the efficacy of extracts from *Zingiber officinale* (ginger) and *Curcuma longa* (tumeric) plants against *Alternaria* species causing leaf spot on spinach in Nandi and Uasin Gishu Counties of Kenya.

1.5.2 Specific objectives

(i) To determine the prevalence and incidence of Alternaria leaf spot on spinach in selected sub-counties of Nandi and Uasin Gishu counties.

(ii) To characterize *Alternaria* species responsible for leaf spot disease complex of spinach using morphological and cultural characteristics.

(iii) To evaluate the antifungal potential of *Zingiber offinale* and *Curcuma longa* in the management of *Alternaria* leaf spot in spinach.

1.6 Hypothesis

(i) Leaf spot disease caused by Alternaria species was present infecting spinach in Nandi and Uasin Gishu counties.

(ii) *Alternaria alternata* causing leaf spot disease in spinach grown in Nandi and Uasin Gishu counties, differ culturally and morphologically.

(iii) Extracts from selected medicinal plants have antifungal potential against *Alternaria* species infecting spinach.

CHAPTER TWO

LITERATURE REVIEW

2.1 Spinach production in Kenya

Spinach are widely grown and consumed as vegetable (Wachira *et al*., 2014). In Kenya the main producing areas are Nyandarua, Elgeyo-Marakwet and Nandi Counties where they are mostly grown under naturally prevailing rain conditions (Anon, 2014). These areas are at least 1300 m above sea level and have well drained clay or sandy loam soils, temperatures of between 15-22^{0}C and receive rainfall of over 600 mm per annum (FAOSTAT, 2010). Spinach in Kenya is grown for different markets which dictate the different varieties that are grown (Wachira *et al*., 2014). The processing varieties produced include Ford Hook Giant, King of Denmark, New Zealand, Giant Noble, Bloomsdale Long Standing and Early Hybrid No. 7 (Anon, 2017). Some of these are produced for the international markets, but most of the produce ends up in the local prime markets such as supermarkets and hotels while the remainder ends up in the open air markets (Anon, 2017).

2.2 Diseases of spinach

Sherf and Macnab (1986); MoA (2000) and Mohammed *et al.,* (2019) noted that spinach can be infected by diverse fungi species that cause foliage diseases. Some of these pathogens include *Alternaria* sp.*, Ramularia spinaciae, Cercospora beticola, Heterosporium variable Ascochyta spinaciae* and *Cercospora bertrandii* (Spinach leaf spots), *Colletrotichum spinaciae* (anthracnose), *Peronospora spinaciae* (downy mildew), *Albugo occidentalis* (white rust), *Fusarium oxysporum* f.sp. *spinaciae* (fusarium decline), *Pythium* spp. *Fusarium* sp. *Phytophthora* sp. and *Rhizoctonia solani* (damping off). Correll *et al.,* (1994) also reported spinach to be infected by Cucumovirus and Cucumber Mosaic Virus (spinach blight), Beet Mosaic Virus and

Tobacco Mosaic virus (spinach mosaic), Phytoplasma (Asters yellows). Hasan *et al.*, (2016); Gowen and Premachandra (2015) reported that spinach can be infested by nematodes such as *Meloidogyne incognita* (galling of spinach roots), *Pratylenchus* sp. (root lesion) and *Heterodera trifoli* (cyst nematode). Bazzi *et al.*, (1988) found spinach to be infected by *Pseudomonas syringae* p.v *spinacea* (bacterial leaf spot) and *Erwinia carotovora* (Bacterial soft rot).

2.3 *Alternaria alternata*

2.3.1 Classification and history

Simmons (2007) classified Alternaria as follows: Kingdom: Fungi, Phylum: Ascomycota, Sub-division: Pezizomycotina, Class Dothideomycetes, Order: Pleosporales and Family; Pleosporaceae. Nees in 1817 first honoured the genus Alternaria with *Alternaria tenuis* as the type species. He noted that the conidial features of the genus were consistent, catenulate and attenuated. Fries (1832) suggested the renaming of *Alternaria tenuis* as *Torula alternate* Pers. More studies on the taxonomy of *Alternaria* sp. was done by Keissler (1912) and Elliot (1917) who gave a more detailed morphological characteristics of the genus with proposal of change from '*tenuis*' to specific epithet '*alternata*'. Simmon (1965) and, Srinath and and Sarwar (1965) gave the reasons why the specific epithet '*alternata*' should be adopted instead of the commonly accepted one '*tenuis*'. Neergaard (1945) studied the taxonomy, parasitism and economic importance of Alternaria in detail with Joly (1959) clearly stated the morphological differences of *Alternaria* species. Further, the genus was divided into three sections and a simple key for identification and determination of the most common species was suggested (Joly, 1959). From then, more detailed studies have been done by several scientists with an ultimate goal of producing a worldly accepted identification key for the genus Alternaria to avoid

confusion and mix up among the mycologists and other scientists at large (Simmons, 2007).

2.3.2 Economic importance of *Alternaria alternata*

Alternaria species cause plant diseases on many crops, infecting the leaves, stems, flowers and fruits. Agrios (2005) noted that total losses caused by this genus rank among the highest caused by any plant pathogen. Marraiki *et al*., (2012) noted *A. alternata* as the cause of leaf spot diseases in spinach in Saudi Arabia, similarly (Czajka *et al.,* 2015) reported *Alternaria alternata* as the pathogen causing leaf spot diseases in spinach and Aslam *et al.*, (2019) too noted *Alternaria alternata* as the cause of leaf spot disease of spinach in Pakistan.

Marraiki *et al*., (2012) noted that foliage diseases of spinach such as leaf spot disease caused by *Alternaria alternata* is of economic importance as it results in significant loss in yield and reduction in quality of leaves. Alternaria leaf spot of spinach has been recognized as a major biotic stress of single origin limiting yields causing a yield loss of 20-80%. Saha *et al.,* (2012) documented that *Alternaria* species produce non-host specific toxins; some of these include tenuazonic acid, alternariol, alternariol monomethyl ether, brefeldin A, tentoxin, zinniol as well as host-specific toxins which contaminate the product.

Further, Thomma (2003) noted that consumption of foodstuff contaminated with Alternaria toxins have been implicated in increased incidence of esophageal carcinoma in humans. The wide distribution and high variability of *Alternaria* spp. on crops and humans necessitates precise understanding of the causal agents for application of effective control and management strategies (Hong *et al.,* 2005).

2.3.3 Cultural and morphological characteristics of *Alternaria alternata*

Nees (1817) first described the morphology of the *Alternaria* species as cosmopolitan, surviving both as saprophytes as well as weak parasites where they form polymorphous conidia either singly or in short or longer chains. It has longitudinal as well as oblique septa and longer or short beaks with the spores occurring in the atmosphere and also in the soil. The telomorphs are known in very few species and placed in the genus Pleospora of class Loculoascomycetes of the phylum ascomycota, in which sleeper-shaped, muriform ascospores are produced in bitunicate asci. Ellis (1971) and Simmons (2007) reported that the spores of *Alternaria* species are dark, borne singly or in chains, multi-celled and mostly beaked with the cells being longitudinally and transversely separated.

The pure culture of the *Alternaria alternata* colony appears as grayish white at first and became black later (Czajka *et al.,* 2015; Aslam *et al.,* 2019). The fungus produces abundant, conidia having 3-8 transverse septations and 1-2 longitudinal septation. Conidia are solitary or in short chains, mostly ovoid with short conical or cylindrical apical beaks and smooth walled (Humaira, 2015; Chethana *et al.*, 2018). Gilardi *et al.,* (2019) noted that pure cultures of *Alternaria alternata* had multicellular, obpyriform to obclavate conidia measuring 14.7 to 41.0 µm in length and 7.2 to 12.1 µm in width, with two to five transverse septa and zero to three longitudinal.

2.3.4 Alternaria leaf spot symptoms manifested on spinach

The symptoms manifested on spinach start as small and circular spots with concentric rings at first which later became irregular lesions appearing on the upper surface of the lower and middle leaves. These circular spots appear as dark black coloured along the margins which encircle the necrotic region. With the spread of the disease, these necrotic spots turn to appear as blight (Marraiki *et al*., 2012; Czajka *et al.,* 2015;

Aslam *et al.,* 2019). Each spot was surrounded by a chlorotic halo and as the disease progressed, lesions enlarged; covering the entire leaf surface (Aslam *et al.,* 2019; Gilardi *et al.,* 2019). Other effects on the plant include extensive defoliation, reduced photosynthetic leaf area, loss of plant vigour, loss of reproductive capacity and loss of seed or plant death (Humaira, 2015).

2.3.5 Epidemiology and disease cycle of *Alternaria* leaf spot

Rotem (1994) noted that *Alternaria* sp. are cosmopolitan found in America, Europe, Africa, Asia and Mediterranean countries. It affects crops such as beans, carrots, watermelon, gourds, muskmelon, peppers, cucumber, pumpkin, wheat, pears, sesame, cauliflower, *Brassica* sp., tomatoes, potatoes, squash and parsleys (Singh *et al.,* 2015). Ellis (1968) and Singh *et al.,* (2015) noted that *Alternaria* sp. causing leaf spots overwinter as a saprophyte in diseased/decaying plant debris and on alternate wild perennial hosts where they can survive in debris for at least two years. Rotem (1994) reported the spread of *Alternaria* sp. from plant to plant by splashing water which carries conidia from infected plants to susceptible tissues, wind and field equipment. Alternaria species are dispersed from region to region through various pathways which include air-borne conidia and adherence on infected soil, seedlings, farm equipment or animals (Ojiambo, 1997).

Slavov *et al.,* (2014) reported that the spores of *Alternaria* sp. germinate in the water film on leaf surfaces within 16-24 hours under high humidity conditions. Upon germination more than one germ tube is produced. The less virulent species enter through the wounds and stomata while the more virulent species penetrate the leaf cells directly. As the fungus continue with establishment and colonization of more plant tissues, leaf lesions appear 3-21 days and later serve as source of inoculum (Sami *et al.,* 2012). Ellis (1968) and Rotem (1994) reported that increasing the

duration of leaf wetness results to increase in diseases severity. Kohla *et al.,* (2010) reported that the spores of *Alternaria* sp. persist in the soil as long as the plant debris has not fully decomposed. Further, they noted that the rate at which the disease spread in the field depends on the present initial inoculum, contaminated seeds, prevailing environmental temperatures, irrigation and wetness of the leaf.

2.4 Management of *Alternaria* diseases on spinach

Management of spinach diseases is more effective through integrated approach due to reasons such as environmental concerns, cost effectiveness and attainment of the ultimate production goals (Mizubuti *et al*., 2007). An amalgam of cultural and physical approaches is effective in the management of disease in any field (Massawe, 2010). Control of Alternaria is a big challenge and hence prevention is the best strategy. There are a number of strategies which have been applied for this purpose.

2.4.1 Cultural control

Use of certified and disease free planting materials is highly advisable and this ensures that the farmer does not introduce a pathogen in a pest-free area (infonet-biovision, 2015). Field sanitation helps to remove possible hosts which act as sources of inoculum for the disease and it also leads to starvation of the disease causing microorganism and eventual death. *Alternaria* sp. survives on the plant debris until favourable hosts and establishment conditions are available. Weeding is vital since some weeds are alternate hosts of the diseases (Hassan *et al*., 2010).

A minimum of a two year crop rotation helps in decomposition of crop residue, reduction of inoculum build up and helps in killing the already present sources of inoculum in a particular field. Using nitrogenous fertilizers is advisable as they reduce disease severity (Akrami and Yousefi, 2015). Harvesting crops on time reduces crop loss. Immediate ploughing in of the crop debris reduces inoculum build-up and the

survival of leaf spot pathogens (Kumar *et al.*, 2013). Practices like wider crop spacing and breaking compacted soil layer helps in reducing leaf wetness duration and soil moisture. The best way to proactively reduce the severity of the Alternaria leaf spot disease is to use healthy and treated seeds as well as eliminating potential inoculum sources (Singh *et al.*, 2015).

2.4.2 Chemical Management

Alternaria leaf spot pathogens can be controlled by various effective agrochemicals. Proper timing for first fungicide application is the best for effective control of the Alternaria pathogens. Once the first fungicide application has been made, subsequent sprays may be determined based on: Foliar application according to calendar schedule, based on recommended intervals for the specific fungicide used and by continual monitoring of disease progress and observation of temperature and forecasted rainfall (Singh *et al.*, 2015). Sinha and Prasad (1989) noted that Thiram 75% (5000ppm) was effective fungicide against *Alternaria* sp. whereas Thiram (80%) and Arasan 50% led to total inhibition of Alternaria at 10,000 ppm. Dithane M-45, Blitox, Bordeaux mixture, Difoltan, Captafol and Bavistin are also effective in the control of *Alternaria* spp. pathogens infecting various crops.

Prasad and Naik (2003) found that the application of Mancozeb followed by Bavistin, Iprodione and Thiram proved to be effective as seed dresser against Alternaria pathogens. Under *in vitro* conditions, non-systemic fungicides such as Mancozeb, and Iprodione, while among the systemic fungicides thiophanate methyl was found to be effective. Singh and Singh (2006) tested the efficacy of seven fungicides; Chlorothalonil, Propineb, Azoxystrobin, Mancozeb, Copper hydroxide, Copper oxychloride at ppm of 2500, 500, 1000, 2000 and 250 ppm and Hexaconazole against *A. alternata* at 50, 100, 200, 500 and 1000 ppm on the *Alternaria* sp. causing blight of

tomato. They found that the mycelial radial growth of the fungus greatly decreased by the application of all the fungicides. Hexaconazole was very effective as it lead to 100% growth deterrence.

Kumar *et al.,* (2013) tested synthetic fungicides such as carbendazim, vitavax, mancozeb, Thiram and chlorothalonil which proved to completely deterred *Alternaria alternata* pathogen. Ginoya and Gohel (2015) carried out *in vitro* screening of fungicides among them, Hexaconazole, tebuconazole, difenconazole (11.40%) and azoxystrobin (18.20%) + at three concentrations (500, 1000 and 1500 ppm) against the *Alternaria alternata* for which it was revealed that the fungicides actively deterred the mycelial growth of the pathogen and showed to be the highly effective.

2.4.3 Use of bio fungicides to control plant diseases

Biofungicides are derivatives of natural products including plants, microorganisms and animals which are used to manage pests in a non-toxic manner (Mizubuti *et al*., 2007; Kumar, 2015; Mishra *et al*., 2015). These products are important because unlike the synthetic fungicides they are easily degradable, they are non-toxic to humans and the environment, they are specific in their target, easily available and do not have residual effects on the produce (Kimani, 2014; Kumar, 2015).

2.4.4 Use of Bio-control agents

A number of bacteria and fungi have antagonistic properties where they either inhibit or kill other fungi and bacteria. Bio agents such as *Trichoderma harzianum, Trichoderma koningii* and *Fusarium* sp. are effective in controlling seed-borne *Alternaria* sp. (Singh *et al.,* 2015). Babu *et al.,* (2000) noted that *Trichoderma viridae* and *Bacillus subtilis* have high efficacy in deterring mycelial growth of *A. solani.* Yu *et al.,* (2008) noted that the secondary metabolites of an actinomycete strain, A19 were active in its inhibitory effect against *Alternaria alternata* and other fungi *in*

vitro. Di *et al.,* (2007) found that the chitosanase strain BS-0409 of *Bacillus megatherium* have antimycotic activity against *Alternaria solani* both in *in-vitro* and *in-vivo* conditions. It was also found that Sphingomonas and Curtobacterium have antimycotic activities only under *in-vitro* conditions against *Alternaria solani* isolated from tomato.

2.4.5 Use of natural plant extracts

Several studies have noted the ability of decoctions from different parts of medicinal plants as effective bio control method in controlling plant pathogenic fungi. The botanicals are eco-friendly besides being less hazardous (Shafique *et al.,* 2011). Ginger is known for its ethanomedicinal and nutritional values. Ginger rhizome has antimicrobial and antifungal effects (Tatsadjieu *et al.,* 2009).

Muthomi *et al.,* (2017) reported that garlic extracts was most active on *A. solani* and *Rhizoctonia solani* while lemon decoctions were more active on *Pythium* and *A. solani*, with complete deterrence on growth of *A. solani* by turmeric. Decoctions from *Citrus limon* and *Curcuma longa* demonstrated high efficacy on both young and old cultures of *Rhizoctonia, Alternaria, Fusarium and Pythium.* Decoctions from *Zingiber officinale, Azadirachta indica, Rosmarinus officinalis* and *Aloe vera* demonstrated activity only on the young cultures of *Rhizoctonia solani.*

Mamunu*r et al., (2015)* noted that plant extracts from *Azadirachta indica, Carissa carandas, Swietena mahogany* and *Allium sativum* were significantly effective compared to Ridomil 50 WP (positive control) in inhibiting *Colletotrichum capsici* of *Capsium annuum.* Chethana *et al.,* (2012) found that neem, turmeric and garlic extract deterred growth of *Alternaria porri*. Further, plant decoctions from thorn apple (*Datura stramonium*), garlic (*Allium sativum*), rosemary (*Rosmarinus officinalis*) and neem (*Azadirachta indica*) have been noted to reduce early blight in tomato (Abo-

Elyousr and Nashwa, 2012; Rodino *et al.,* 2014). Singh *et al.,* (2011) reported that *Zingiber officinale* and *Capsicum frutescence* had inhibitory effect on the key post-harvest pathogens in citrus fruits.

Wongkaew and Sinsiri (2014) noted that ethanolic decoctions of turmeric showed high efficacy against *A. alternata*, *Fusarium oxysporum* f. sp. *lycopersici* And *Pythium sp.* Extracts from neem, garlic, black cumin, clove, white cedar, Brazillian pepper and anthi mandhaani had high antifungal activity against the spore germination of *Puccinia triticina* causing wheat leaf rust with neem extract recording a 98.99% inhibition (Shabana *et al.,* 2017). Ahmed *et al.,* (2016) noted that decoctions from *A. indica, Parthenium hysterophorus, Eucalyptus camaldulensis, Allium sativum* and *Datura stratmonium* were active against the mycelial growth of *Alternaria solani* causing Early blight of tomato with percentage inhibition increasing with increase in extract concentration.

Hassanein *et al.,* (2008) reported that *A. indica* is rich in phytoconstituents such as tetra terpenoids and phenolic compounds that are the active antimycotic compounds. Acqueous extracts of *Allium sativum*, *Bidens pilosa*, *Camelia sinensis*, *Aloe vera*, Annona muricata, *Chrysanthemum coccineum*, *C. Arabica*, *Datura stratmonium*, *Zingiber officinalis* and *Nicotiana tabacum* has significant antifungal activity against Pyricularia grisea causing Rice blast of rice under both *in vitro* and *in vivo* conditions *C. arabica* at concentrations of 10% and 25% (v/v) displayed the highest inhibition of 81.82% and 89.40% respectively followed by *N. tabacum* (Hubert *et al.,* 2015).

Taiga and Friday (2009) noted that *N. tabacum*, *A.indica* and *A. vera* decoctions contains alkaloids, flavonoids and tannins which are the active antifungal compounds. Acqueous extracts from *Jatropha curcas* and A. indica had high antifungal activity against *Aspergillus flavus, Fusarium oxysporum* and *Alternaria alternata* with *A.*

inidica displaying the highest percentage inhibition of mycelial growth of *Aspergillus flavus*, Further, *A. indica* reduced the spore size and sporogenesis of *A. alternata* (Abd El-Ghany *et al.*, 2015).

Anjum *et al.*, (2016) noted that *A. indica* (neem), *Tagetes* sp. (marigold) and *Melia azadrach* (chinaberry) are the natural plant products used in the manufacture of bio-fungicides and bio-insecticides since they possess high amounts toxic phytochemicals against a wide range of fungi and insects. Biswas and Ghosh (2018) observed that extracts from *Lantana camera, Allium sativum* and *Zingiber officinale* at the concentrations of 10% significantly inhibited the mycelial growth of *Alternaria brassicae* and *A. brassicola* under in vitro and in vivo conditions where *L. camera* showed 80 % inhibition, *A. sativum* (54.44%) and *Z. officinale* (17.78%) under *in vitro* conditions in comparison to control.

i. *Zingiber officinale* (Ginger)

Mehdi *et al.*, (2017) in their review reported that *Zingiber* spp. plants are aromatic herbs growing horizontal or fibrous rhizomes. They grow best in environments with moist well drained soils like sandy loam, red loam or clay loam having a pH of 6.0 to 6.5 and rich in humus. The crop prefers a temperature range of 19-28^{o}C and humidity of 70-90%. Ginger is a rhizome with antimicrobial and anti-mycotoxigenic importance (Rahmah *et al.*, 2013). Traditionally, ginger is used to treat a number of diseases such as pains, sore throats, nausea, vomiting, constipation, hypertension and infectious diseases (Tatsadjieu *et al.*, 2009). Rahmah *et al.*, (2013) reported from their study that ginger is rich in phenolic amalgams such as gingerol, cendrene, zingeberene, phellandrene, geranial, neral, β-bisabolene and α- curcumene which serve as the active fungitoxic phytochemicals.

Olubunmi *et al.,* (2018) screened methanolic extracts of ginger for phytochemicals which showed the presence of alkaloids, cardiac glycosides, tannin, saponins, flavonoids and terpenoids. They further screened for the efficacy of the extracts against *Aspergillus* spp., *Penicilllium* spp., *Fusarium* spp., *Trichoderma harzianum, Beauveria bassiana* and *Alternaria* spp. and noted that the extracts had varying degrees of antifungal activity against the fungal isolates. Nadia *et al.,* (2014) noted the difference in fungitoxicity of the various ethanolic extracts of ginger on the test pathogen which may be due to the fact that distinct compounds dissolve in separate organic solvents as a result of varying polarities.

Humaira (2015) found out that ethanolic extracts from ginger at various levels of concentrations deterred the growth of *A. alternata.* The inhibition percentage increased with increasing concentration for the management of leaf spot disease of spinach. Their results coincided with the findings of Sharma *et al.,* (2016) and Manoharan *et al*., (2010). Harbant *et al.,* (2011) in their study on the inhibitory effects of plant extracts on *Penicillium digitatum, Aspergillus niger* and *Fusarium* sp. reported that *Zingiber officinale* and *Capsicum frutescence* caused significant reduction on the growth of the pathogen with increasing concentration. Stangarlin *et al.,* (2015) in their review reported that decoctions of ginger at concentrations of 10, 15, 20 and 25% inhibited the growth of *Sclerotinia sclerotiorum* mycelia with increasing inhibition as the extract concentration increases, *in vitro*.

Curcuma longa and *Zingiber officinale* rhizomes crude extracts were found active against *Phythopthora infestans, Fusarium solani* and *Pyricularia oryzae* (Malkham *et al.,* 2012). Mudyiwa *et al.,* (2016) tested antifungal properties of ginger, garlic and onion against *Alternaria solani* at 50, 75 and 100% concentrations. They found that the ginger extracts suppressed the mycelial growth by a very high percentage. The

inhibition efficiency increased with an increase in plant extract concentration. Herivory *et al.,* (2016) found that ginger essential oils extracted from ginger rhizomes strongly inhibited the growth of *Fusarium spp*., *Alternaria* sp. *Escherichia coli*, *Bacillus cereus* and *Plasmodium falciparum*.

ii. Tumeric (*Curcuma longa*)

Curcuma longa is a perennial herbaceous rhizomatous crop belonging to the family of Zingiberaceae. It has green, petiolate, lanceolate and long leaves with white yellow flowers (Gairola *et al.,* 2010). The plant growth ranges from 55-85 cm with short stem. The plant has large leaves approximately one metre long and 8.5 cm wide (Patel, 2015). Pankaj and Richa, (2017) on their study found that *Curcuma longa* and *Zingiber officinale* were effective in controlling human dermatophytic fungi, *Trychophyton mentagrophytes* and *Microsporum auodinii*. Suruchi and Vikas, (2015) noted that turmeric rhizome is rich in two major secondary metabolites, the phenolic curcuminoids and hydrophobic essential oils which act as the active antifungal agents. Aqueous decoctions of turmeric are rich in thanatin and antimicrobial peptides, hence can be employed as an alternative to control fungal plant diseases since it has no side effects on humans (Mamarabadi *et al.,* 2018).

Madhusanka *et al.,* (2018) studied the phytochemical content of *Curcuma longa* and noted that turmeric has high content of phenolic compounds such as alkaloids, terpenoids, steroids, flavonoids, tannins and saponins. These phenolic compounds act as the active antifungal agents. A mixture of tumeric and ginger extracts showed excellent antifungal activity against *Malassezia furfur* compared to individual extracts (Ikpeama *et al.,* 2014). It also showed a higher antifungal efficacy compared to Gentamycin and Streptomycin hence providing scientific prove for the use of these important oils in controlling both human and plant infections (Richa *et al.,* 2011).

Richa and Meenakshi (2011) reported that a mixture of turmeric and ginger oils were more effective in inhibiting the growth of *T. rubrum* and *M. gypseum* than individual oils. Hence the oils of *Curcuma longa* and *Zingiber officinale* and their amalgams can serve as strong natural antifungal agents.

Stangarlin *et al.*, (2015) noted in their review that aqueous decoctions of turmeric rhizomes at concentrations of 5, 10 and 15% deterred the mycelial growth and sporulation of *Alternaria solani*, the curcumin homogenous mixture hindered the mycelial growth *in vitro*. The decoctions of *C. longa* showed inhibitory potential on over five pathogenic fungi, including *Alternaria alternata, Fusarium oxysporum, Botrytis cinera, Penicillium pallidum, Verticillum dahlia and Aspergillus terreus.* Eight chemical constituents of *C. longa* namely; curdione, isocurcumenol, curcumerol, curene, β-elemene, curcumin, germacrone and curcumol were reported as the active compounds against the mycelial growth, with curdione showing the best suppression rate (Ciqiong *et al.*, 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Determination of incidences and collection of leaf spot diseased leaves of spinach

Field surveys were conducted in September 2018, during the rainy season in the different agro ecological zones of Nandi county (0.34^{o}N, 35.25^{o}E); Mosop, Engwen, Chesumei, Aldai and Nandi Hills sub-counties with annul mean temperature range of between 14^{o}C- 29^{o}C, altitude range of between 1300- 2500 metres above sea level (m.a..s.l) and, annual mean rainfall of between 1280- 2100 mm and Uasin Gishu county (0.55 ^{o}N, 35.30^{o}E); Kapseret and Moiben sub-counties with annual mean temperature range of between 15 ^{o}C- 20.5 ^{o}C, altitude range of between 1500- 2700 m.a.s.l. and annual mean rainfall of between 1000- 1500 mm (MoALF, 2017 and NCScAO, 2018).

In the six sub-counties, forty farmers' fields at approximately five kilometers apart having spinach at different stages of growth and development (4-10 developed leaves) were sampled. Averagely, five farmer's fields were sampled from each sub-county where upon sampling the first farm in each sub-county, the preceding sampled farm was at approximately five kilometers from the starting farm. A structured Alternaria leaf spot survey questionnaire was used during the survey (Appendix I). The data gathered in each farm included, spinach variety (local or improved), cropping system (intercrop or monocrop) and methods of pest and disease control. Altitude of the surveyed area was recorded as read from Geographical Position System (GPS) equipment.

The leaf spot incidence on each farm surveyed was recorded as the mean per cent leaf spot by drawing three (3) quadrants of 1 m^{2}. Spinach plants showing symptoms such

as small, circular dark black coloured spots along the margins encircling the necrotic spots with concentric ring were counted and used to determine the percentage incidence over total spinach plants in the quadrant. The incidence from each farm was calculated as described by Bdliya and Gwio-Kura (2007).

$$\text{Disease Incidence (\%)} = \frac{\text{Total plants infected}}{\text{Total plants in the quadrant}} \times 100$$

Collection of diseased leaves was randomly done from infected spinach in the fields during survey. Three leaves (two leaves with moderate disease symptoms and one with early stage of disease infection) were sampled from each quadrant translating to three samples per farm. The collected leaves were then kept in a keep cool box containing ice (4^{o}C) and brought to the Microbiology laboratory at the University of Eldoret for further studies.

3.1.1 Data analysis

The data on Alternaria leaf spot incidence per farm was first calculated into percentage per quadrant and transformed to normalize the data before analysis and the mean of the three quadrants in each farm was calculated and taken as the disease incidence of the farm. The obtained percentage incidence was then entered into excel and were analysed for Analysis of Variance (ANOVA) procedure using the GenStat computer Software 14^{th} Edition, release 14.10.5943, 2013 (VSN International Ltd) to verify the level of significance. Means separation was by Fishers unprotected least significant difference (LSD) at 5% probability level.

3.2 Isolation of *Alternaria* species

One centimetre portion of the diseased section with early stages of infection was sliced from diseased leaves and washed in running tap water for thirty seconds and thereafter surface sterilized in 1% sodium hypochlorite for three minutes. They were then rinsed in three changes of sterile distilled water and plated onto potato dextrose

agar (PDA) media in a 90 mm diameter petri plate under aseptic lamina conditions. The inoculated petri dishes were incubated at room temperature (25 ± 2^{o}C) and observations made from twenty four hours onwards. After five days, hyphal tip was transferred onto sterilized potato dextrose agar (PDA).

Pure cultures of sporulating fungi were obtained by single spore isolation technique as described by Noman *et al.,* (2018): The fungi isolates grown on PDA were sub-cultured onto a new PDA medium and incubated at 25 ± 2^{o}C for seven days. One colony was selected and spot inoculation of fungal spores was transferred into PDA (the media had been prepared two days earlier and dried at 28^{o}C for twenty four hours to enable absorption of excess water film). 0.1 ml of the sterilized distilled water was added into each plate medium then evenly spread on the surface by glass spreaders. The petri plates were left in the safety cabinet for fifteen minutes to dry. The inoculated media were incubated at 25^{o}C for 18-24 hours to establish germination. Thereafter, one germinating spore on the media was located with the help of a compound microscope and a small piece of agar medium containing one germinated spore was cut out aseptically and placed on new PDA medium and incubated at 25^{o}C for 10 days.

The isolates were designed as follows: NM01, NM02 … to NM09 for isolates from Mosop sub-county of Nandi, NC01, NCO2…to NC06 for isolates from Chesumei sub-county of Nandi, NA01, NA02…to NA05 for isolates from Aldai sub-county of Nandi, NE01, NE02…to NE05 for isolates from Emgwen sub-county of Nandi, NH01, NH02…to NH05 for isolates from Nandi Hills sub-county of Nandi, UK01, UK02… to UK06 for isolates from Kapseret sub-county of Uasin Gishu, UM01, UM02…to UM04 for isolates from Moiben sub-county of Uasin Gishu.

3.3 Identification of the *Alternaria* species isolates

This study aimed at identification of the 23 isolates of *A. alternata* from various farms in Nandi and Uasin Gishu counties. The pathogens, *Alternaria alternata*, were identified using taxonomic keys, cultural and morphological reference as described by Ellis (1971) and Simmons (2007) and confirmed by pathogenicity tests to fulfill Koch's postulates; the pure cultures of the fungal colony appears as grayish white at first and became black later while the spores are dark, borne singly or in chains, multi-celled and often beaked with the cells being transversely and longitudinally separated. The fungus produces abundant conidia having 3-8 transverse septations and 1-2 longitudinal septation. Conidia are solitary or in short chains, mostly ovoid with short conical or cylindrical apical beaks and smooth walled.

3.3.1 Pathogenicity Test

The pathogenicity test was done on growing spinach in sterile soils. Approximately twenty five kilograms of loam soils were sterilized in the autoclave at 121^{o}C and 15 psc for 45 minutes, and left to cool for six days then transferred to the nursery at the greenhouse. *Alternaria alternata* susceptible spinach variety (Fort Hook) was grown in the nursery containing sterile soils. Regular watering of the seedlings was done once a day, late in the evening until when the seedlings were approximately 10-15 cm in height. The seedlings were transplanted onto the plastic pots measuring sixteen centimeters (16 cm) in diameter, containing 1.5 kgs of sterile loam soil. Regular watering and monitoring of plants was done daily.

3.3.2 Inoculum preparation and inoculation

The inoculum was yarked from each isolate by flooding 10 day old PDA cultures with 10 milliliters of sterile distilled water, gently rubbed with a sterile glass rod then put in sterile universal bottles and shaken for twenty minutes in a shaker (Aerotror Infoors

AG CH-4103 Bottmingen, Switzerland) at 150 revolutions per minute (rpm) to dislodge the spores. The spore suspension was then filtered through two layers of sterile cheese cloth then conidial concentration estimated with the help of haemocytometer. A mean of five counts was calculated per colony and then adjusted to 1 x 10^7 conidia per milliliter using the formula; $C_1V_1=C_2V_2$ where, C-concentration and V-volume.

Six weeks old plants (3-5 leaves) were inoculated with the adjusted (1 x 10^7 conidia per milliliter) conidial suspension by spraying with simple atomizer. Each isolate was replicated thrice. Control plants were sprayed with sterile distilled water. Plants were covered for 24 hours with plastic bags to maintain 100% relative humidity as described by Marraiki *et al.,* (2012). Plants were kept in completely randomized design in the greenhouse at the University of Eldoret and monitored daily for the appearance of initial leaf spots symptoms.

3.3.3 Assessment of leaf spot

The plants were monitored daily for the appearance of initial leaf spots symptoms; small and circular spots with concentric rings at first which later became irregular lesions appearing on the upper surface of the lower and middle leaves. These circular spots appear as dark black coloured along the margins which encircle the necrotic region. With the spread of the disease, these necrotic spots turn to appear as blight with each spot surrounded by a chlorotic halo and as the disease progressed, lesions enlarged, covering the entire leaf surface (Marraiki *et al*., 2012; Czajka *et al.,* 2015; Aslam *et al.,* 2019; Gilardi *et al.,* 2019).

The records on per cent leaf spot severity on leaves due to *A. alternata* were taken weekly and last recording taken four weeks after leaf inoculation. Three leaves were

taken from each plant. The disease severity scale used was a modification of the one used by Biswas and Ghosh (2018);

Disease grade	Disease scale (%)	Description of isolates
0	0	Non-pathogenic isolates
1	1-10	Less severe isolates
2	11- 15	Moderately severe
3	16 - 50	Highly severe
4	51 - 100	Very severe

The percent disease index/severity (PDI) was calculated by the techniques of Mc.Kinney (1923).

$$PDI = \frac{\text{Sum of all numerical ratings}}{\text{Number of leaflets observed} \times \text{maximum rating used}} \times 100$$

3.4 Determination of cultural and morphological characteristics

A disc of 10 mm in diameter was cut with a cork borer from 10 day old single spore cultures grown on PDA and transferred onto the centre of freshly prepared PDA plates. Three replications for each of the twenty three isolates were maintained. The plates were incubated at 25^{o}C in a 24 hour dark cycle for ten days. The experimental design was a completely randomized design. The experiment was repeated once. The cultures in these plates were then used to study the nature of aerial mycelium growth, pigmentation (substrate), conidial size and growth rate.

The nature of aerial mycelium was studied by visual observation while pigmentation on the mycelium and the substrate studied using mycological colour chart as described by Rayner (1970).

The conidial measurement was done by taking a block of 0.5cm^3 of agar from ten days old PDA culture and mounted directly on a slide with a drop of in lactophenol cotton blue stain and covered with a cover slip and viewed under a light microscope. Morphology of conidia was described and measurements made in micrometres (μm).

Fifteen conidia per isolate were measured to determine the length and the width using a light microscope fitted with a micrometre. Determination of the width was done by measuring the widest section of the conidia.

The growth rate of each of the isolates was taken after every 24 hours and further the growth calculated by taking the mean on a 90mm diameter petri plate divided by 14 (number of days the fungus grew).

3.4.1 Data analysis

The data on growth rate were analysed for Analysis of variance (ANOVA) procedures using the GenStat computer Software 14th Edition, release 14.10.5943, 2013 (VSN International Ltd). Means separation was by Fishers unprotected least significant difference (LSD) at 5% probability level.

3.4.2 Determination of sporulation and spores in *Alternaria alternata*

A disc of 10 mm in diameter was cut using a cork borer from 12 day old single spore cultures, grown on PDA and transferred onto the middle of freshly prepared PDA plates, in three replicates for each of the twenty three isolates. The plates were incubated at 25^{o}C in a 24 hour dark cycle for ten days. The cultures from these plates were then used to estimate sporulation. The experimental design was a completely randomized design. The experiment was repeated once.

To estimate sporulation, conidial suspension was prepared from each culture plate using a modification of Jeff and Janet (2012) method: Ten day old single spore cultures were flooded with ten milliliters of sterile distilled water, gently rubbed with a sterilized glass rod and, suspended in sterile universal bottles and put in a rotary shaker at 150 revolutions per minute (rpm) for twenty minutes. The spore suspension was then filtered through two layers of sterile cheese cloth and the conidial suspension calculated using haemocytometer: 1 ml of the spore solution was placed

on each side of the haemocytometer and the spores in zones A, B, C, D and E on both sides of the haemocytometer counted (spores which fell on the left or bottom line were not counted while spores that fell on the right or top line were counted). A mean of five counts was taken per colony.

3.4.3 Data analysis

The number of spores obtained from five counts on the haemocytometer was taken as the mean number of spores produced by each isolate in three replicates. The data from these three replicates was then analysed for Analysis of variance (ANOVA) procedures using the GenStat computer Software 14th Edition, release 14.10.5943, 2013 (VSN International Ltd). Means separation was by Fishers unprotected least significant difference (LSD) at 5% probability level. Pearson correlation coefficient was used to determine the correlation between the growth rate and sporulation of the isolates.

3.5 Testing antifungal potential of ginger and tumeric plant extracts for the management of *Alternaria alternata*

3.5.1 Collection of ginger and tumeric plant samples

Ginger and tumeric (rhizomes) plant samples were randomly purchased from vendors at the local market in Eldoret town. The guiding principle in selection of the plant was the antifungal history from herbalists and documented reports from different studies worldwide (Goufo *et al*., 2010 and Al-Samarrai *et al*., 2012). The plants tested for antifungal potential were rhizomes of turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*).

3.5.2 Preparation of tumeric and ginger plants for extraction

The collected plant parts were washed in running tap water to get rid of dirt, insects and any other unwanted foreign matter. They were chopped into small pieces then

dried on the sun (22^{o}C) for three days up to moisture content of 13%. The dried plant parts were then ground into powder using an electric grinder. The ground parts were then preserved in sealed labeled glass bottles until use.

3.5.3 Extraction of crude extracts from plant samples

The extraction technique used was the modification of the homogenization in solvent as described by Malkhan's *et al.,* (2012). The solvent-to-sample ratio of 10:1 (v/w) solvent to dry weight ratio was used. Forty grams of each of the sun dried and ground powdered material from *Zingiber officinale* **(**Ginger) and *Curcuma longa* (Tumeric) were dissolved in 400 ml of absolute solvents (methanol, ethanol, water and ethyl acetate) in clean conical flasks with constant shaking on a shaker at a speed of forty revolutions per minute for 24 hours. After stirring, the solution was filtered through 2 layers of sterile cheese-cloth gauze and centrifuged at 5000 rpm for 20 minutes in a centrifuge (6000 series centrifuge, Centurion Scientific Ltd, Switzerland) to remove suspended fine particles before subjecting the filtrates to evaporation in Rotary Evaporator (Buchi Rotavapor R-3000, Switzerland) at 50^{o}C for solvent evaporation and purification of the extract. The oily materials from the rotary evaporator was removed and dried in the water bath at 40^{o}C for 12 hours. The dried material from the water bath was stored in small, sterilized 5 ml screw-capped glass bottles and kept in the refrigerator (4^{o}C) until further usage.

3.6 Evaluation of antifungal activity of crude extracts of tumeric and ginger

3.6.1 Preparation of plant extracts dilutions

The turmeric and ginger powder extracts were removed from the refrigerator and diluted. Aliquots of 0.05 g, 0.025 g and 0.0125 g of each extract (tumeric and ginger) were weighed into sterilized universal bottles and kept until use.

3.6.2 *In vitro* screening of ginger and tumeric rhizome extracts against the *A. alteranata*

Poisoned food technique as described by Grover and Moore (1962) was used. PDA media was weighted into fifty glass flasks of 60 ml and autoclaved for 15 min at 121^{o}C with a pressure of 15 psi. After autoclaving, the media was cooled to about 45^{0}C. The 0.05 g, 0.025 g and 0.0125 g of the ginger and tumeric extracts were aseptically diluted with the prepared PDA media to make dilutions 50 mg/ml, 25 mg/ml and 12.50 mg/ml for each plant extract. Shaking was done gently for 1 minute to allow for a proper mixing of extract. Approximately 15 ml aliquots of the amended media were dispensed into sterilized 90 mm petri-dishes replicated thrice. A cork-borer was used to cut 10 mm agar discs from 10 day old pure *Alternaria alternata* cultures and placed at the centre of the plate and incubated at room temperature (25±2^{o}C). Control plates with media not amended with plant extracts was inoculated and incubated in the same conditions. Observations were made at day 4, 8, 12, 16 and 20 after plating as until control filled a 90 mm petri plate. The experimental design was a completely randomized design with three replications.

Colony diameter was determined by measuring the mean radial growth in millimeters (mm). The inhibition zone (I), was calculated using the standard formula of Francisco (2010) as follows:

$$\% \text{ Inhibition} = \frac{C-T}{C} \times 100$$

C= Pathogen radial growth in mm in control

T=Radial growth in mm in treated plates

3.6.3 Data analysis

The data on percentage inhibition of *Alternaria alternata* culture by plant extracts was first calculated into mean percentage inhibition from the three replicates of each

extract before analysis and the mean of the three replicates taken as the percentage inhibition. The obtained percentage inhibition was then entered into excel and were analysed for Analysis of Variance (ANOVA) procedure using the GenStat computer Software 14th Edition, release 14.10.5943, 2013 (VSN International Ltd) to verify the level of significance. Means separation was by Fishers unprotected least significant difference (LSD) at 5% probability level.

3.6.4 Efficacy of plant extracts against Alternaria leaf spot of spinach

Susceptible spinach (Fort Hook) plants in the greenhouse (3-5 leaves) were sprayed with spores of *Alternaria altermata* at the concentration of 1 x 10^7 conidia per milliliter. After the appearance of the first symptoms (9th day), methanol extracts of turmeric and ginger (best effective extract in *in vitro* screening) were sprayed at the concentration of 50 mg/ml. Control plants were sprayed with sterile distilled water. The experiment was conducted in a complete randomized design with three replications.

To study the disease severity a scorecard technique following a 5 point scale (0-5) for scoring leaf spot disease as described by Biswas and Ghosh (2018) was adopted. From each plant, 3 leaves were selected randomly and marked. Disease scoring was done at 7, 14, 21 and 28 days after planting. The rating scales used for the study of disease severity were as follows;

Disease grade	Disease scale (%)	Description
0	0	No symptoms
1	1-10	Leaf area covered
2	11- 25	Leaf area covered
3	26 - 50	Leaf area covered
4	51 - 75	Leaf area covered
5	>75	Leaf area covered

The percent disease index (PDI) was calculated by the techniques of Mc.Kinney (1923).

$$PDI = \frac{\text{Sum of all numerical ratings}}{\text{Number of leaflets observed} \times \text{maximum rating used}} \times 100$$

Percent decrease in PDI

The percent decrease in PDI was calculated by using the formula given by Biswas and Ghosh (2018)

$$\frac{C-T}{C} \times 100$$

Where; C- PDI observed in control treated

T-PDI observed in different treatments

CHAPTER FOUR

RESULTS

4.1 Prevalence and incidence of Alternaria leaf spot.

Out of 40 farms surveyed, 23 (57.50%) farms confirmed the presence of spinach infected with Alternaria leaf spot as shown in Plate 1. The Alternaria leaf spot disease incidence ranged from 25.79% to 50.51% among the farmers visited, with a mean of 21.37% across all the areas surveyed. The highest leaf spot incidence was 50.51% in Mosop sub-county of Nandi county and the lowest disease incidence was at 25.79% in Chesumei sub-county of Nandi county (Table 1). The prevalence did not differ significantly ($P \leq 0.05$) among the various sites surveyed except at Kapkong'ony and Kabiyet (Table 1; Appendix II).

Plate 1: Diseased spinach plants showing leaf spot symptoms (a), diseased leaf showing the characteristic black spots on the front side (b), characteristic black spots on the back side (c) of a spinach plant leaf.

Table 1: Incidence of Alternaria leaf spot of spinach from various sites of Nandi and Uasin Gishu counties during 2018.

County	Sub-county	Altitude (m.a.s.l)	*A. alternata* isolate	Mean incidence (%)
Nandi	Chesumei	2038.67	NC05	0a
Nandi	Chesumei	2018.84	NC06	0a
Nandi	Aldai	1784.34	NA04	0a
Nandi	Aldai	1925.67	NA05	0a
Nandi	Nandi Hills	2041.65	NH02	0a
Nandi	Nandi Hills	2103.45	NH03	0a
Nandi	Nandi Hills	1538.81	NH04	0a
Nandi	Nandi Hills	1918.22	NH05	0a
Nandi	Emgwen	2016.72	NE02	0a
Nandi	Emgwen	1976.54	NE03	0a
Nandi	Emgwen	2001.16	NE04	0a
Nandi	Emgwen	1998.68	NE05	0a
Uasin Gishu	Kapseret	1996.56	UK05	0a
Uasin Gishu	Kapseret	1992.67	UK06	0a
Uasin Gishu	Moiben	2156.63	UM02	0a
Uasin Gishu	Moiben	2196.41	UM03	0a
Uasin Gishu	Moiben	2070.62	UM04	0a
Nandi	Chesumei	1978.32	NC04	25.79b
Nandi	Emgwen	1984.78	NE01	30.04bc
Nandi	Chesumei	1946.51	NC03	31.73bcd
Nandi	Aldai	1803.25	NA01	31.79bcd
Uasin Gishu	Kapseret	2146.65	UK02	32.03bcde
Uasin Gishu	Kapseret	2134.54	UK03	32.64bcde
Nandi	Chesumei	2044.48	NC01	33.11bcde
Nandi	Mosop	1977.03	NM01	33.7bcde
Uasin Gishu	Kapseret	1894.69	UK01	33.77bcde
Nandi	Aldai	1925.78	NA03	34.72bcdef
Nandi	Mosop	1924.65	NM02	35.39bcdef
Uasin Gishu	Kapseret	2038.67	UK04	35.75bcdef
Nandi	Mosop	1838.55	NM06	37.57cdefg
Nandi	Aldai	1824.14	NA02	38.21cdefg
Nandi	Mosop	1878.89	NM05	38.55cdefg
Uasin Gishu	Moiben	2154.08	UM01	40.7cdefgh
Nandi	Chesumei	2023.62	NC02	41.23defgh
Nandi	Nandi Hills	1998.24	NH01	41.53defgh
Nandi	Mosop	1951.84	NM03	41.62defgh
Nandi	Mosop	1946.82	NM08	42.65efgh
Nandi	Mosop	1864.65	NM09	45.08fgh
Nandi	Mosop	1898.72	NM04	46.7gh
Nandi	Mosop	1872.03	NM07	50.51h
			Mean	21.37
			CV	31.2
			LSD	10.844

Values with the same letter(s) along the same column are not significantly different (P≤0.05).

Alternaria leaf spot occurred in all the seven sub-counties surveyed. There was no significant difference (P≤0.05) among Emgwen, Nandi Hills and Mosop sub-counties but they differed significantly from Chesumei, Aldai and Kapseret. Mosop sub-county differed significantly (P≤0.05) from all the sub-counties (Figure 1: Appendix III). Alternaria leaf spot prevalence was 100% in Mosop sub-county, 66.67% in Chesumei, 66.67% in Kapseret, 60% in Aldai but the lowest occurrence in Nandi Hills 20%.

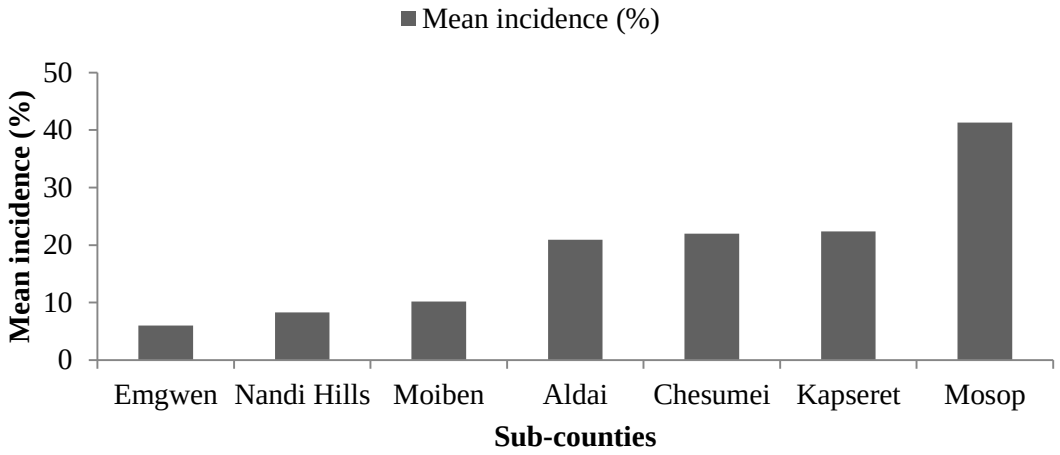

Figure 1. Incidence of Alternaria leaf spot of spinach at various sub-counties of Nandi and Uasin Gishu counties.

The mean disease incidence was found higher in the altitudes ≤1900 metres above the level (m.a.s.l). There was no significant difference (P≤0.05) on disease incidence at altitudes of 1901-2000 and altitudes greater than 2000m.a.s.l but differed significantly from the disease incidence at altitudes less than 1900m.a.s.l (Figure 2; Appendix IV).

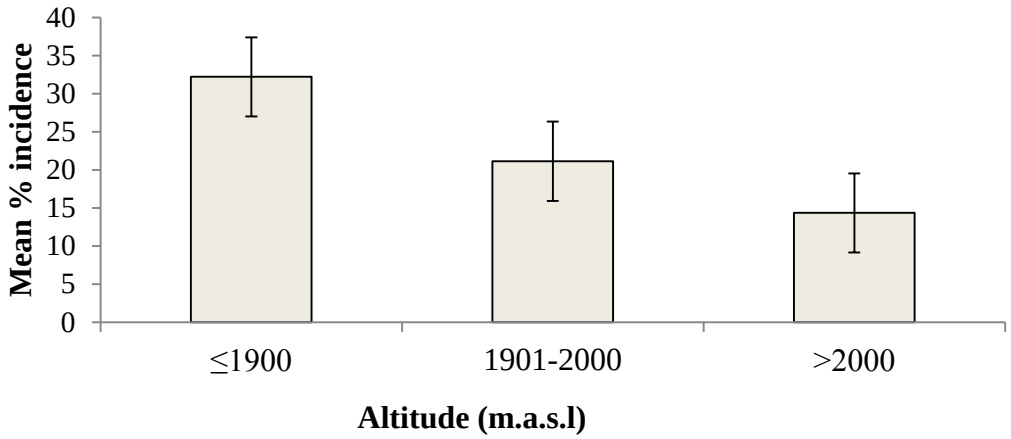

Figure 2: Incidence of Alternaria leaf spot at various altitudes in Nandi and Uasin Gishu counties.

During the survey, a study of the spinach cultivation and cropping systems was done and it was noted that the mean acreage under spinach cultivation per farmer was 0.4 acres. Majority of the farmers grew local variety as either a monocrop or as an intercrop. Further, some farmers grew improved variety (Fort hook). Seventy two per cent of the farmers had local varieties while 28% had improved varieties (Table 2). The improved varieties were mostly grown in Nandi Hills, Emgwen and some parts of Kapseret (Tuiyo and Kapkagaron) sub-counties. The local varieties were grown mostly in Mosop, Chesumei and Aldai sub-counties. Intercropping with kales (*Brassica* sp.) was practiced by 67.7% of the farmers. Other intercrops included black night shade, cabbages and a few Irish potatoes. On the farms which the spinach had been intercropped with *Brassica* sp. (kales and cabbages), the disease incidence was higher than the farms which had spinach grown as a pure crop stand (Table 2) suggesting the potentiality of these intercrops as alternate hosts or cross-infectivity of the leaf spot pathogens.

It was further noted that 35% of the farmers obtained the seeds and/or seedlings from either the neighbor or local markets (28%) without the knowledge of purity and quality of these seeds/seedlings. Thirty seven per cent of the farmers reported to have

obtained certified seeds from Agro-dealers in Eldoret, Kapsabet or Nandi Hills. Most farmers left crop residues on the farm after harvesting which could be a source of inoculum for the next growing season. Crop rotation was practiced by only 35% of the farmers. Spinach was rotated with maize, *Brassica* sp., beans or irish potatoes. Monocropping of spinach was practiced by 65% of the farmers. Soil amendments through the addition of organic manure especially cow and sheep dung was practiced by 75% of the farmers and a few farmers applied chicken waste on the soil. Twenty per cent of the farmers reported to applying inorganic fertilizers such as Diammonium Phosphate and foliar fertilizers during planting and leafy stage respectively. Twenty five per cent of the farmers did not apply any kind of fertilizers to their crops at any stage while 55% applied organic fertilizers either during land preparation time or during planting or some days after the crops have established. Some farmers still practiced traditional way of disease control like dusting the diseased crops with ashes without exactly knowing the cause of the disease (12.5%). Only 25% of the farmers used pesticides in the management of diseases and pests.

Table 2: Spinach cultivation and cropping system adopted by farmers in Nandi and Uasin Gishu counties.

Cultivation/crop system		Percentage of farmers practicing
Spinach variety;	Improved variety (Fort hook)	28
	Local variety	72
Cropping system;	Pure stand	32.3
	Intercropping	67.7
	Crop rotation	35
	Mono cropping	65
Source of seeds or seedlings;	Agro-dealers	37
	Local market	28
	Neighbor	35
Soil amendments;	Addition of organic manure	75
	No soil amendments	25
Use of fertilizers;	Organic fertilizers	55
	Inorganic fertilizers	20
	No use of fertilizer	25
Disease/pest control;	Use of synthetic pesticides	25
	Traditional ways	12.5

4.2 Virulence of *Alternaria alternata* on susceptible spinach variety

Twenty one (91.30%) of the twenty three (23) isolates were pathogenic to spinach variety (Fort Hook), while two isolates were non-pathogenic. The Alternaria leaf spot symptoms on spinach were noted from the ninth day of inoculation. There was variation among the isolates on virulence and were grouped into highly aggressive (16%> in severity), moderately aggressive (10-15% in severity) and less aggressive (<10% in severity) based on the time they took to initiate disease symptoms after inoculation and disease severity score. The highly aggressive isolates induced the disease 8-12 days after inoculation on spinach and it constituted 52.12% isolates. Moderately aggressive isolates induced the disease 12-15 days after inoculation and it constituted 30.43% isolates and less aggressive isolates induced the disease over 15 days after inoculation and it constituted 8.70% isolates (Table 3). Most of the highly aggressive isolates were from Mosop and Kapseret sub-counties. Isolates collected from the same sub-county showed variation in pathogenicity with isolates demonstrating different levels of aggressiveness.

Table 3: Virulence of *Alternaria alternata* on susceptible spinach variety.

Aggressiveness level of the isolates	Number (in brackets) and percentage of isolates showing the features
Non-pathogenic isolates	(2)8.7
Less aggressive isolates	(2) 8.7
Moderately aggressive isolates	(7)30.43
Highly aggressive isolates	(12)52.12

4.3 Cultural and Morphological characteristics of *Alternaria alternata*

Alternaria alternata showed significant variability in cultural and morphological characteristics on potato dextrose media (PDA) (Table 4). The predominant aerial mycelia growth was moderately compact which was observed in 60.87%, 26.09% had fairly compact and 13.04% had moderately luxuriant (Table 5). The isolates had

grayish white cottony compact aerial mycelium (Plate 2a), rough margins and dark reverse with dark black substrate (Plate 2b). Isolates had grayish white compact aerial mycelium with black centre (Plate 2c) and wavy margins with dark green substrate (Plate 2d). Further, other isolates had dense raised grayish white aerial mycelium with small dark concentric rings and circular margins. The conidia varied in shape from mostly muriform to ellipsoidal having smooth walls and 1-3 longitudinal and 2 to 10 transverse septations with cylindrical curved beaks (Plate 2e). The conidial length ranged between 24.76 µm for the shortest to 75.14 µm for the longest and the width ranged from 6.82 µm to14.78 µm (Table 4). The conidia were multi-celled born in chains of up to 10 or more on conidiophores varying colours from light olivaceous to dark brown as shown in (Plate 2g). The conidiophores were unbranched, arising singly or in clusters, long or short (Plate 2f and h). Conidiophores were olivaceous to olivaceous brown with majority being straight and a few curved. Further, the conidiophores were slightly swollen at the apex having terminal scars.

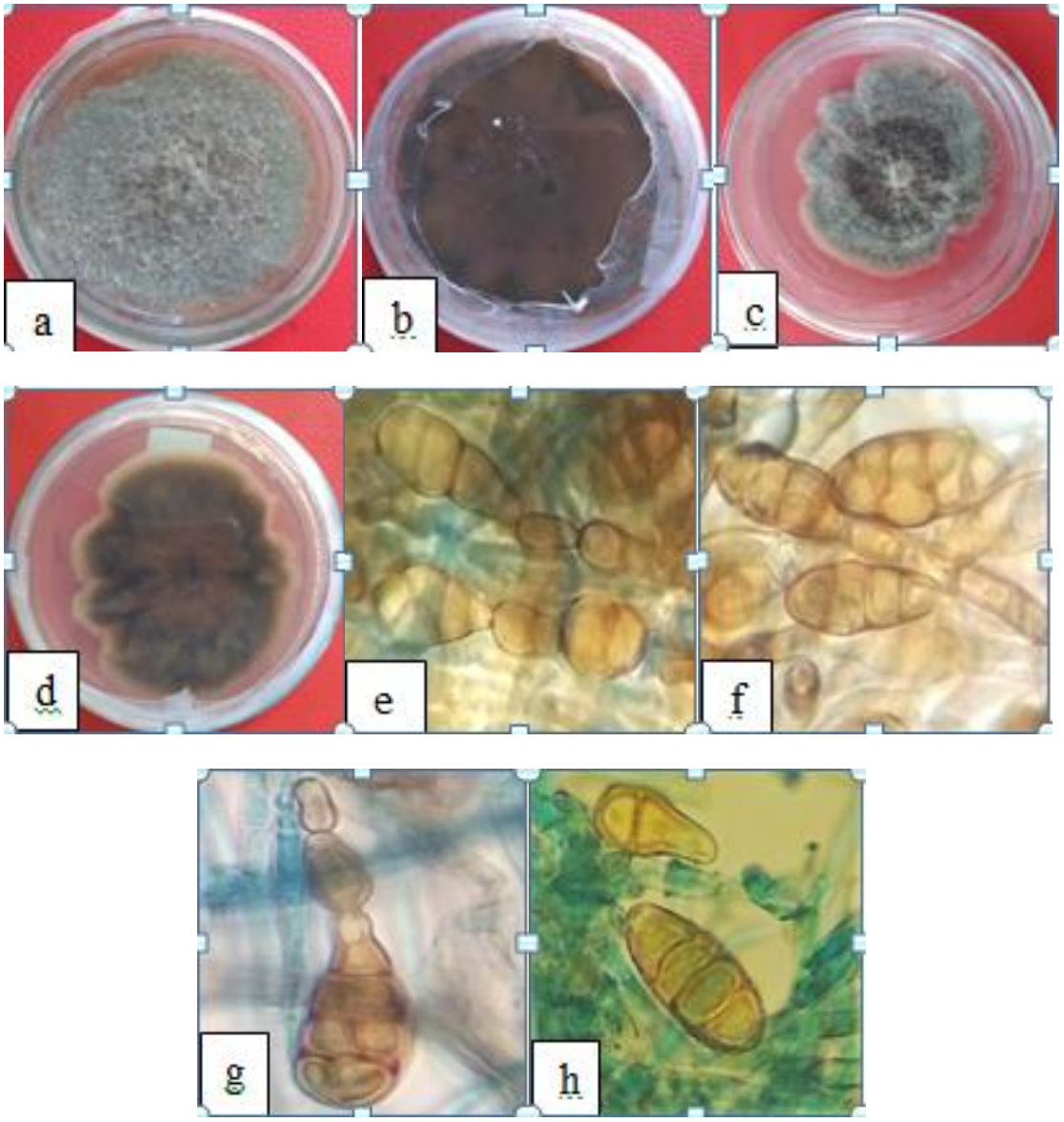

Plate 2: The pure cultures of *Alternaria alternata*, front and back (a and b) and (c and d) respectively showing colour and texture of mycelia. Micrographs of *A. alternata*; (e) long conidiophores; (f) multi-celled conidia; (g) long smooth walled multi-cell conidia with both transverse and longitudinal septations, and (h) short single smooth walled conidiophores with cylindrical curved beaks.

Table 4: Cultural and Morphological characteristics of *Alternaria alternata* isolates collected from Nandi and Uasin Gishu counties.

Isolate	Aerial mycelium growth	Mycelial texture	Mycelial colour	Substrate colour	Growth rate mm/day	Mean spores per ml	L (µm)	W(µm)
NM01	Moderately compact	Cottony	Gray white	Black	3.735	5.6×10^4	64.66	9.64
NM02	Moderately compact	Cottony	Gray white	Black	3.635	6.16×10^4	65.46	9.68
NM03	Moderately compact	Aerial	Black gray	Dark green	3.85	4.68×10^4	68.24	9.18
NM04	Moderately luxuriant	Feathery	Black	Black	3.685	6.64×10^4	42.04	7.82
NM05	Moderately compact	Cottony	Black gray	Dark black	3.615	5.96×10^4	62.42	9.21
NM06	Fairly compact	Feathery	Green with black center	Dark green	3.65	4.95×10^4	71.52	13.95
NM07	Fairly compact	Feathery	Gray green	Gray	3.735	6.2×10^4	69.62	14.62
NM08	Moderately compact	Velvet	Gray green	Dark green	3.535	7.2×10^4	58.80	11.16
NM09	Moderately compact	Feathery	Black gray	Black	4.03	5.4×10^4	69.76	10.41
NC01	Moderately compact	Velvet	Black gray	Gray	3.93	7.4×10^4	70.32	11.82
NC02	Fairly compact	Cottony	Gray white	Black	3.7	5.16×10^4	60.34	12.61
NC03	Moderately compact	Cottony	Gray white	Gray	3.05	6.76×10^4	38.65	7.02
NC04	Moderately luxuriant	Velvet	Black gray	Black	3.485	4.84×10^4	40.46	9.46
NA01	Moderately compact	Cottony	Gray white	Black	3.565	5.72×10^4	36.82	14.78
NA02	Fairly compact	Aerial	Black gray	Dark green	4.015	5.38×10^4	72.48	12.34
NA03	Moderately compact	Feathery	Olivaceous green	Dark green	4.05	6.56×10^4	73.02	10.78
UK01	Moderately luxuriant	Feathery	Black gray	Dark black	3.685	4.8×10^4	44.81	10.34
UK02	Moderately compact	Feathery	Gray with black center	Black	2.98	5.12×10^4	28.12	6.82
UK03	Moderately compact	Cottony	Gray green	Gray	3.7	6.4×10^4	24.76	8.43
UK04	Fairly compact	Cottony	Gray white	Black	4.05	5.0×10^4	75.14	13.98
NH01	Moderately compact	Velvet	Gray green	Gray	3.6	5.28×10^4	49.61	10.88
NE01	Moderately compact	Aerial	Black gray	Gray	4	4.96×10^4	68.90	12.86
UM01	Fairly compact	Aerial	Olivaceous green	Dark green	3.5	5.68×10^4	36.78	7.06
					3.686086957			

r= -0.0833, P<0.05=0.7055, 'L' and 'W' represent the conidial size in Length and Width respectively as measured in micrometers (µm)

On mycelial colour; 26.09% of the isolates had gray white, 34.78% had black gray, 3.35% had gray with black centre, 3.35% had black, 3.35% had green with black centre, 8.70% had olivaceous green and 17.39% had gray green (Table 5). Majority (39.0%) of the isolates studied, coloured the substrate black, 26.09% had coloured the substrate dark green, 8.70% dark black and 26.09% had gray (Table 5). The predominant mycelial texture was cottony and feathery which was observed in 30.43% of the isolates, while 17.39% had velvet and aerial growth each (Table 5).

Table 5: Cultural and morphological characteristics of *A. alternata* isolates collected from Nandi and Uasin Gishu counties.

Character		Percentage of isolates showing the features
Aerial mycelium growth		
	Moderately compact	60.89
	Fairly compact	26.09
	Moderately luxariant	13.04
Mycelial texture		
	Cottony	30.43
	Aerila	17.39
	Feathery	30.43
	Velvet	17.39
Mycelial colour		
	Gray white	26.09
	Black gray	34.78
	Black	3.35
	Gray green	17.39
	Olivaceous green	8.70
	Gray with black centre	3.35
	Green with black centre	3.35
Substrate colour		
	Black	39
	Dark green	26.09
	Dark black	8.7
	Gray	26.09
Growth rate		
	Fast growth	21.74
	Moderate growth	65.22
	Slow growth	13.04
Sporulation		
	High sporulation	8.7
	Moderate sporulation	43.48
	Low sporulation	21.74

The isolates were grouped according to the growth rate into fast growth (≥4.0 mm/day), moderate growth (≥ 3.5-3.99 mm/day) and slow growth rate (< 3.5 mm/day). The growth rate ranged between 2.98 mm/day to 4.05 mm/day with a mean growth rate of 3.69 mm/day. The distributions of isolates into fast, moderate and slow growths were 21.74%, 65.22% and 13.04% respectively (Table 5). There was significant difference (P≥0.05) in growth rate among the isolates (Table 6; Appendix V).

Table 6: Growth rate of *A. alternata* isolates infecting spinach in Nandi and Uasin Gishu counties.

A. alternata Isolate	Growth rate (mm/day)
UK02	2.98a
NC03	3.05a
NC04	3.49b
UM01	3.5b
NM08	3.54bc
NA01	3.57bcd
NH01	3.6bcd
NM05	3.62bcd
NM02	3.64bcd
NM06	3.65bcd
NM04	3.69cde
UK01	3.69cde
NC02	3.7cde
UK03	3.7cde
NM01	3.74de
NM07	3.74de
NM03	3.85ef
NC01	3.93fg
NE01	4fg
NA02	4.02fg
NM09	4.03g
NA03	4.05g
UK04	4.05g
Mean	3.688
CV	2.9
LSD	0.1767

Values with the same letter(s) along the same column are not significantly different (P≤0.05).

4.3.1 Sporulation of *A. alternata* isolates on PDA

Alternaria alternata isolates showed significant difference in sporulation on PDA. Based on sporulation the isolates were classified into four categories. Two (8.70%) isolates had very high sporulation (>7.0×10^4); 26.09% of the isolates had high

sporulation ($6.0\text{-}7.0\times10^4$), 43.48% of the isolates had moderate sporulation ($5.0\text{-}6.0\times10^4$) and 21.74% of the isolates having low sporulation, ($<5.0\times10^4$). The sporulation of isolates was significantly different ($P\leq0.05$) in most sub-counties and/or sites. Some isolates from the same sub-county showed variation ($P\leq0.05$) with respect to sporulation (Table 7, Appendix VI). For example isolates NM03 and NM06, NC01 and NC04, and UK01 and UK03 were isolates from the same sub-counties but showed significant variation in sporulation. When the relationship between sporulation and growth rate was compared it was established that there was no correlation between the growth rate and sporulation. The correlation coefficient was negative and low.

Table 7: Sporulation of *A. alternata* isolates on PDA

A. alternata Isolate	Spores per ml
NM03	46800a
UK01	48000ab
NC04	48400bc
NM06	49500cd
NE01	49600cd
UK04	50000de
UK02	51200ef
NC02	51600fg
NH01	52800gh
NA02	53800h
NM09	54000h
NM01	56000i
UM01	56800i
NA01	57200i
NM05	59600j
NM02	61600k
NM07	62000k
UK03	64000l
NA03	65600m
NM04	66400mn
NC03	67600n
NM08	72000o
NC01	74000p
Mean	57326
CV	1.6
LSD	1476.6

Values with the same letter(s) along the same column are not significantly different ($P\leq0.05$). r= -0.0833, P<0.05=0.7055.

4.4. *In-vitro* antifungal activities of ginger and tumeric plants

4.4.1 Antifungal activities of Ginger (*Zingiber officinale*) rhizome extract against *Alternaria alternata.*

Extracts of ginger showed varying anti-fungal potential against *A. alternata* which depended on the solvent used during extraction and the concentration of the extract at which the fungi was exposed (Plate 3a to d). There were significant difference ($P \leq 0.05$) among the extracts of *Zingiber officinale* inhibition activity on *A. alternate* (Figure 3). Methanolic extracts of ginger rhizomes showed the highest antifungal potential against the *A. alternata* with a percentage inhibition of between 40.07% and 57.37% in the concentration of 12.50 mg/ml and 50 mg/ml of the extract. Acqueous extract of ginger rhizomes showed moderate antifungal potential against the *A. alternata* with percentage inhibition of between 31.21% and 56.56% in the concentration of 12.50 mg/ml and 50 mg/ml of the extract, similarly ethyl acetate extract of ginger demonstrated moderate antifungal activity against the *Alternaria alternata* with percentage inhibition of between 22.79% to 52.32% in 12.50 mg/ml and 50 mg/ml of the extract respectively (Figure 3). The lowest antifungal potential against the *A. alternata* was shown by ethanolic extracts which ranged between 25.37% and 43.02% on 12.50 mg/ml and 50 mg/ml respectively as shown in (Figure 3)

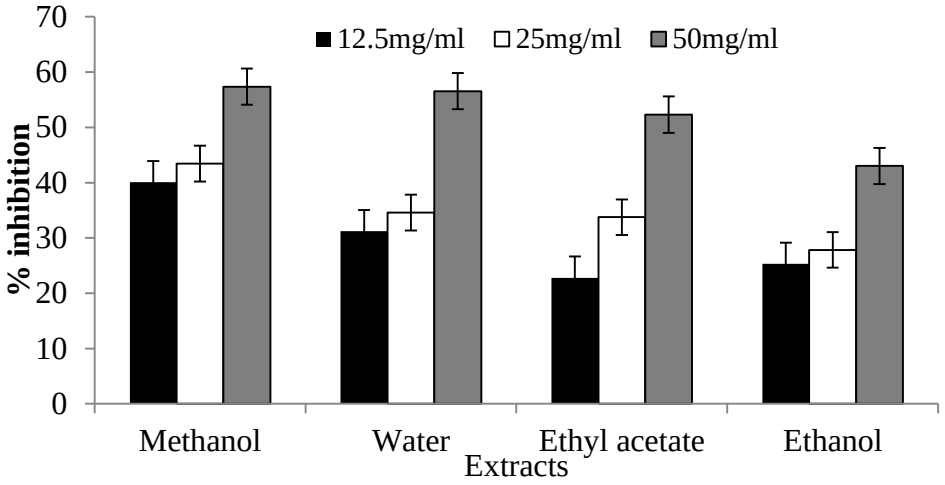

Figure 3: Percentage inhibition of *A. alternata* by ginger extracts at various levels of concentration at day 20 of incubation.

There were significant differences (P≤ 0.05) among the extracts in the ginger inhibition activity against *A. alternata* (Table 8; Appendix VII). Methanolic extracts of *Z. officinale* demonstrated the highest antifungal activity which was significant (P≤0.05) against *A. alternata* compared to ethanolic, aqueous and ethyl acetate extracts, but the percent inhibition was not significant (P≤0.05) from 8th day by all the solvents of the extraction for ginger. Percentage inhibition was not significantly different in aqueous and ethyl acetate extracts from day 4 to day 12 and from day 4 to day 16 respectively.

Table 8: Antifungal effect of ginger rhizome extracts against *Alternaria alternata* at different days.

Days of incubation	Methanol	Ethanol	Water	Ethyl acetate
Day 1	0.0a	0.00a	0.0a	0.0a
Day 4	22.88b	14.9b	19.75b	20.86b
Day 8	42.17c	24.61c	28.87bc	31.77bc
Day 12	43.61c	24.74c	30.2bc	32.13bc
Day 16	45.76c	27.8c	34.37c	33.64bc
Day 20	46.97c	32.06c	40.79c	38.68c
LSD (0.05)	9.668	8.988	14.181	15.668
CV	15.8	23.9	30.4	32.9

Values with the same letter(s) along the same column are not significantly different (P≤0.05).

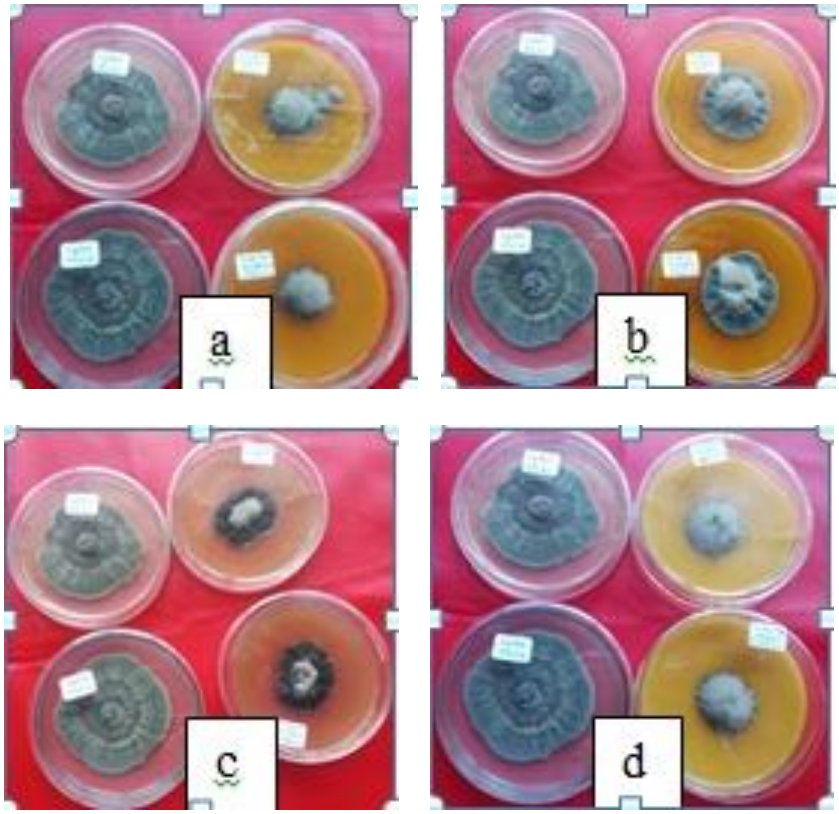

Plate 3: The suppressed colonies displayed by different ginger extracts at the concentration of 50 mg/ml, (a) Methanolic extract, (b) ethanolic extract (c) aqueous extract and (d) ethyl acetate extract. The plates in the left hand side in all the pictures represent the controls.

4.4.2 Antifungal activities of tumeric (*Curcuma longa*) rhizome extract against *Alternaria alternata*.

Extracts of turmeric showed varying levels of anti-fungal potential depending on the solvent used for extraction and the concentration at which the *Alternaria alternata* was exposed (Plate 4a to d). There was significant difference ($P \leq 0.05$) among the turmeric extracts against *A. alternata* (Table 9; Appendix 7). Methanolic extracts of tumeric rhizomes showed the highest antifungal potential against the *A. alternata* with a percentage inhibition of between 46.3% and 64.56% in the concentration of 12.50 mg/ml and 50 mg/ml of the extract. Turmeric rhizomes extracted by ethyl acetate showed moderate antifungal potential against the *A. alternata* with percentage

inhibition of between 33.74% and 62.88% in 12.50 mg/ml and 50 mg/ml of the extract respectively, Similarly, ethanolic extract of turmeric demonstrated moderate antifungal activity against the *A. alternata* with percentage inhibition of between 36.27% to 59.92% in 12.50 mg/ml and 50 mg/ml of the extract respectively (Figure 4). The lowest antifungal potential against the *A. alternata* was shown by aqueous extracts which ranged between 42.56% and 54.82% on 12.50 mg/ml and 50 mg/ml respectively as shown in Figure 4.

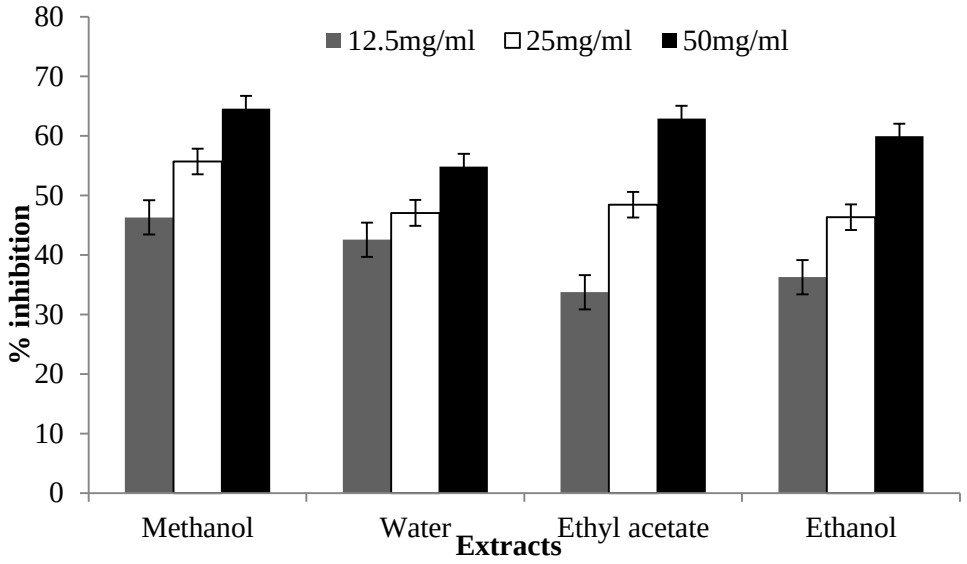

Figure 4: Percentage inhibition by tumeric extracts at various levels of concentration at day 20 against *A. alternata.*

It was established that from day 4 to day 8 the inhibition percentage showed significant difference ($P \leq 0.05$) in all the extracts against *A. alternata*. However, from day 8 onwards there was no significant difference except in the water extracts of turmeric as shown in (Table 9).

Table 9: Antifungal effect of tumeric rhizomes against *Alternaria alternata* at different days.

Days of incubation	Methanol	Ethanol	Water	Ethyl acetate
day 1	0.00a	0.00a	0.00a	0.00a
day 4	26.77b	20.3b	28.94b	32.22b
day 8	44.96c	38.92c	42.23c	44.96c
day 12	45.43c	39.63c	44.07c	45.76c
day 16	47.61c	40.75c	45.6cd	46.66c
day 20	50.92c	43.17c	50.49d	47.67c
LSD (0.05)	9.709	11.173	4.968	11.076
CV	14.8	20.2	7.8	16.8

Values with the same letter(s) along the same column are not significantly different (P≤0.05).

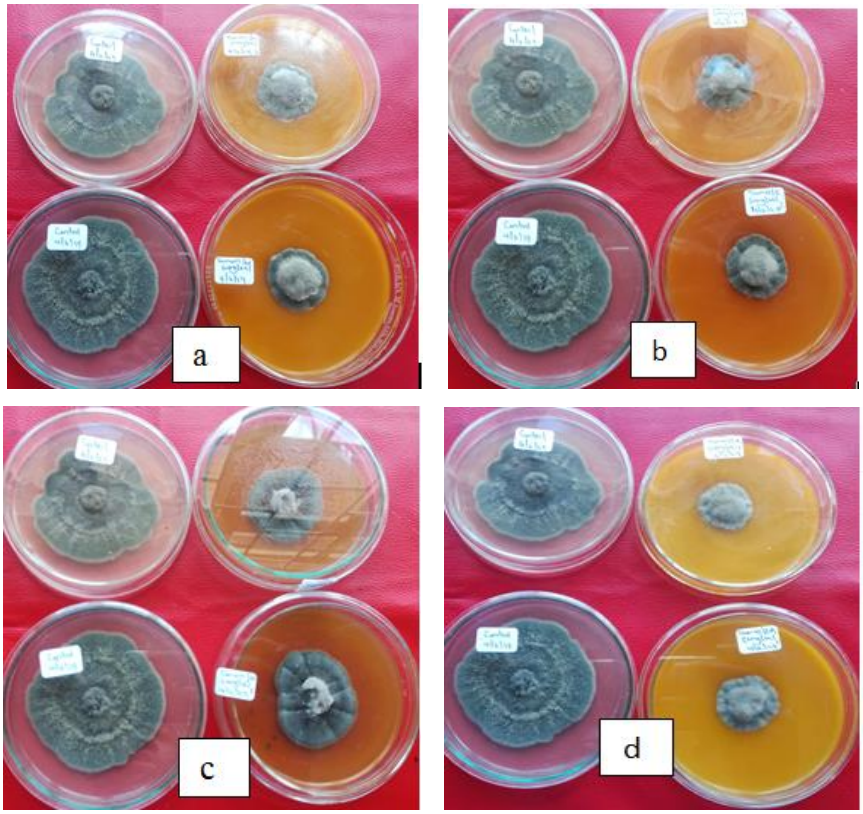

Plate 4: The suppressed colonies displayed by different tumeric extracts at the concentration of 50 mg/ml. (a) Methanolic extract, (b) ethanolic extract, (c) aqueous extract and (d) ethyl acetate extract. The plates in the left hand side in all the pictures represent the controls.

4.4.3 Comparison of antifungal activities of turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*).

For all the plant extracts (Methanolic extract, Ethanolic extract, Acqueous extract and Ethyl acetate extract) at different levels of concentrations the activity of extracts on the growth of the *A. alternata* was found to be active from day 8 after inoculation (Figure 5). There was significant difference (P≤0.05) among the extracts of ginger and turmeric inhibition activity on *A. alternata*. The growth of the *A. alternata* was actively inhibited with almost constant growth inhibition from day 8 onwards. With increasing concentration of the plant extract, the inhibition percentage increased. Methanolic extract of turmeric showed the highest inhibition at the concentration of 50 mg/ml followed by aqueous extract of turmeric. Ethanolic extract of ginger had the least inhibition percentage on the *A. alternata* in all the plant extracts tested. Extracts of *Curcuma longa* performed better than the *Zingiber officinale* extracts (Figure 5).

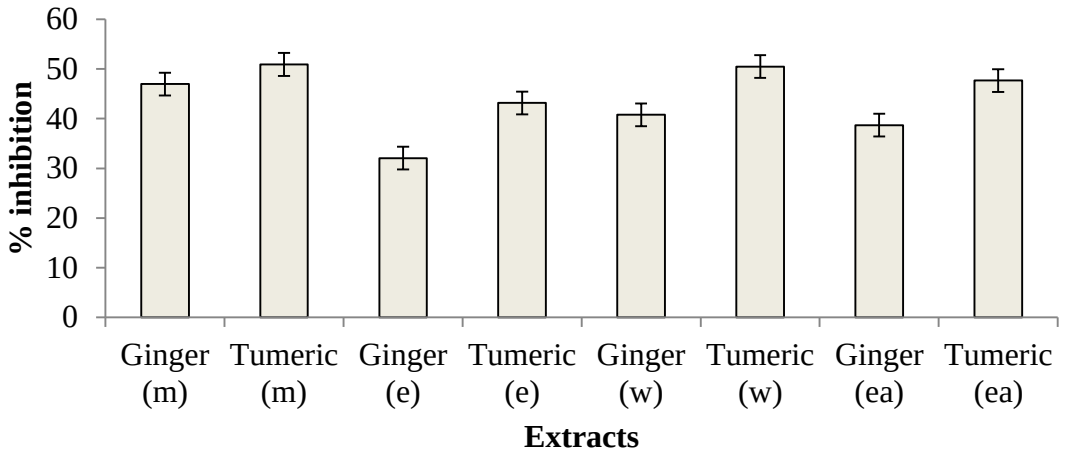

Figure 5: Comparison of antifungal activities of *Curcuma longa* and *Zingiber officinale.*

4.4.4. *In-vivo* evaluation of plant extracts against *A. alternata*

There was a percent disease index (PDI) of 18.3% and 20% on spinach treated with turmeric crude methanolic plant extracts and ginger crude methanolic plant extracts, respectively (Table 10). This was significantly different (P≤0.05) with the non-treated

infected plant. The disease decrease when compared with non-inoculated control was 57.70% for turmeric crude extracts and 53.84% reduction in disease from spinach treated with ginger plant extracts. However, from these results there was no significant difference ($P \leq 0.05$) in disease reduction between the crude extracts of the two plants in the management of Alternaria leaf spot disease in spinach.

Table 10: Alternaria leaf spot percent disease index (PDI) and percent disease decrease from crude plant extracts treatment.

Treatment (50mg/ml)	PDI	Percent disease decrease
Tumeric	18.33%	57.70%
Ginger	20.00%	53.84%
Control	43.33%	

CHAPTER FIVE

DISCUSSION

5.1 Prevalence and incidence of Alternaria leaf spot.

Different symptoms of Alternaria leaf spot on spinach were noted during the survey and these included dark black coloured spot margin circular spots starting on the lower leaves upwards. The upper surface leaves showed small circular dark spots encircling a necrotic region. The leaves showed loss of vigour, chlorosis and yellowing, drying of some leaves and in severe infection resulted to death of the whole plant.

These leaf spot symptoms were similar to the ones reported on spinach by Marraiki *et al.,* (2012); Anuj *et al.,* (2014); Czajka *et al.,* (2015) and Aslam *et al.,* (2019). The leaf spot prevalence varied among the sub-counties with Mosop recording the highest incidence (100%), followed by Chesumei and Kapseret. This report is the first to establish an incidence of Alternaria leaf spot in Uasin Gishu and Nandi. Which showed a mean of 21.37% which was lower than what Czajka *et al.,* (2015) reported in Poland (50%) in spinach fields. In similar study done in Pakistan (Aslam *et al.,* 2019) reported an approximate disease incidence of 45%.

The variations in the incidence of Alternaria leaf spot in the farms could have been due to variations in spinach varieties grown, the cropping system practiced, the stage of plant growth during the survey, the handling of the crop residue after harvest and control measures employed by farmers to control the leaf spot disease as reported elsewhere by Marcin *et al.,* (2012); Czajka *et al.,* (2015); Singh *et al.,* (2015) and Aslam *et al.,* (2019).

From the present study it was noted that spinach production is mainly done in small scale for domestic use and the surplus is sold at local markets. Further, it was noted

that most farmers do traditional farming with little improvement in spinach cultivars and cultivation systems. All these and other factors contribute to low production. Ivan *et al.,* (2014) noted that spinach yield varies with locations and seasons, agronomic practices and productive cultivars with desirable quality, uniformity, disease and pest resistance that are adaptable to variable environmental conditions. FAOSTAT (2010) reported that average spinach yield should be 26.3 t/ha. The findings from the current study failed to quantify the production since most farmers interviewed do not quantify their produce as its cultivation is mainly for domestic use coupled by low receptiveness of farmers to new production technologies and adoption of hybrid varieties that are highly productive.

Morelock and Correll (2008) noted that hybrid spinach varieties makes up 85% to 95% of the production in USA, Europe, Japan and China which has greatly resulted to yield increase. Similar findings were reported by Ivan *et al.,* (2014) who noted that increase in spinach yield is dependent on the improvement of spinach cultivars. These are not in tandem with the findings from the present study where most farmers grow local varieties. The findings from the present study showed that very few farmers use nitrogen rich fertilizers, whereas Bradley *et al.,* (1975) found increase in spinach yield when nitrogen fertilizers. Koike *et al.,* (2011) reported spinach grown on rows in standard raised beds and slightly raised beds are highly productive compared to spinach grown on rows on flat grown. From the present study, spinach cultivation was fully carried out on an open field and on a flat ground.

5.2 The virulence of *Alternaria alternata*

Alternaria leaf spot susceptible spinach variety test were used to confirm the pathogenicity of the 23 isolates of *A. alternata* to cause leaf spot. The initial symptoms of Alternaria leaf spot on Fort Hook variety appeared on the ninth day and

the pathogen re-isolated onto PDA medium from infected plants after 20 days. The resulting fungus from the inoculated plants was identical to that originally noted on the spinach leaves, confirming the Koch's postulates. Marraiki *et al.,* (2012) and Czajka *et al.,* (2015) re-isolated the pathogen after about 10 days from the infected spinach. The past and the present findings are similar despite having been done in different places at different times. The leaf spot symptoms observed in the greenhouse were similar to those observed in the field.

Variations in the virulence of the *A. alternata* to cause leaf spot on variety Fort Hook was used to classify isolates into groups namely; highly aggressive, moderately aggressive and less aggressive. The method of inoculation and the toxin production affect the virulence and the aggressiveness of *Alternaria* sp. High levels of host-specific and non-host specific toxins; tenuazonic acid (prevents synthesis of proteins), pectin methyl lyase (break down the waxy cuticles) and tentoxin (inhibits photophosphorylation) does not induce disease development but do increase disease symptoms and facilitate colonization by killing cells prior to fungal invasion (Rotem 1994; Thymon *et al.,* 2016). Lyudmila *et al.,* (2015) reported that there exist intraspecific variations in the aggressiveness of *Alternaria alternata* towards the leaves of the host crop cultivars.

The pathogenicity and virulence of Alternaria species depends on host susceptibility or resistance as well as quantitative production of host-specific toxins and non-host specific toxins (Meena *et al.,* 2017). Licinio *et al.,* (2018) from their study on the pathogenicity of *A. alternata* on host crops reported that strong pathogenicity of *Alternaria* spp. is dependent on the expression of different enzymes which serve as virulence and aggressiveness factors.

5.3 Cultural and Morphological Characteristics of *Alternaria alternata*

Single spore isolates of *Alternaria alternata* from Uasin Gishu and Nandi counties demonstrated variation in cultural and morphological characteristics. In the present study, aerial mycelium growth, mycelial texture, mycelial colour, substrate colour, growth rate, conidia size and, shape and sporulation were used to group the 23 isolates of *Alternaria alternata* on potato dextrose medium (PDA) incubated at 25^{o}C for 12 days collaborated Marraiki *et al.,* (2012) who had isolated *Alternaria alternata* from the spinach leaves in Saudi Arabia and reported pure cultures on PDA at 25±1^{o}C had grayish white colony which later became black.

The conidia had 3-8 transverse septations and 1-2 longitudinal septation. Conidia were solitary or in short chains, mostly ovoid and smooth walled. The hyphae were branched, septate, brownish with olive brown septate conidiophore. These findings were in tandem with findings of Gilardi *et al.,* (2019) and Czjaka *et al.,* (2015) who reported that pure cultures of *Alternaria alternata* had dark gray to black colonies, the septa ranged from 30.95 µm and 43.69 µm long, 11.00 and 12.81 µm in the widest area and 3.00 µm to 3.82 µm in the narrowest area. The same results were similar with reports of Aslam *et al.,* (2019) from *Alternaria alternata* isolates from spinach in Pakistan. Pervaize *et al.,* (2018) also reported similar findings on *Alternaria alternata* from alfa alfa.

Bhaat *et al.,* (2000) isolated *Alternaria alternata* from spinach in Anusandham Samithan, India and reported that the isolates on PDA at 25±1^{o}C had circular and olivaceous black colonies; branched flexeous conidiophores arising from scars; conidia in long chains, obyriform with short cylindrical beaks, golden brown, smooth with 6-8 transverse and many longitudinal septa. Their results are in tandem with our present findings. Further, Sanjeev *et al.,* (2017) reported growth rate of 5.6 mm/day in

A. alternata isolated from brinjals which slightly deviates from the present study (3.69 mm/day), the mycelial growth was dull white with fluffy growth at the centre with excellent sporulation. Similar results had earlier been reported by Singh and Majumdar (2001).

Chethana *et al.,* (2018) studied 6 isolates of *A. alternata* causing purple blotch disease of onion and reported a significant variation in mycelial colour of the isolates, growth rate, sporulation, conidial length and width. The mycelial colour ranged from raised ashy white to flat ashy green to raised blackish green with all having black reverse. Isolates varied in sporulation ranging from 0.5×10^5 conidia/ml for low sporulating isolate to 4.5×10^5 conidia/ml for highly sporulating isolate which were similar though with slight variations with results of the current study. The conidial shape was obclavate with colour being golden brown or light brown. The conidial length ranged between 26.89 µm for the shortest to 76.15 µm for the longest and the width ranging from 9.50 to 23.82 µm. Their findings were in tandem to the findings from the present study.

Alternaria alternata isolated from pigeon pea produced abundant branched, septate, brownish mycelia. Conidiophores were simple, olive-brown, septate, and variable in length with terminal conidia which were either solitary or in short chains. Mature conidia measured from 10-30×5-12 µm, short conical beak or beakless. Conidia had 3-7 transverse septa, 1-5 longitudinal septa and in chains of 5-15 conidia (Mamta *et al.,* 2013). Their results are agreed with the findings from the current study and those of Raja and Reddy (2007) and Meena *et al.,* (2014). Devappa and Thejakumar (2016) reported a variation in mycelial colour, substrate colour and mycelial texture of *Alternaria alternata* on PDA medium incubated at 30^oC for 10 days. They reported a growth rate of 8.5 mm/day which differed to the present average of 3.69 mm/day.

Dipak *et al.,* (2013) while studying *Alternaria alternata* (Fr.) Keissler from *Gerbera jamesonii* reported similar findings. However, they recorded a varying growth rate from the present study (10.08 mm/day to 3.69 mm/day). Further, they reported a maximum sporulation (0.61×10^4 spores mm^{-2}) in PDA incubated at 27 ± 1^oC for 10 days. The sporulation was recorded within a period of 48 hours.

Waghunde and Patil (2010) and Guo *et al.,* (2011) noted that the maximum sporulation was obtained at 25^oC which is within the temperature at which the present study was done. Barry and Themis (2001) studied 70 isolates of *A. alternata* and noted that there was a great variation in mycelial colour ranging from lettuce green to olive green colonies with white margins, growth rate of about 7 mm/day which deviates slightly from our findings. The conidia were formed in chains of 6 to 14 in length having ovate shape with apical extensions. Their findings were in agreement with the present study and those of Hashem *et al.,* (2014).

The slight variations observed in growth rate and sporulation could be due to a number of factors such as; difference in incubation conditions, composition and availability of nutrients in the medium as reported by Dipak *et al.,* (2013) and Devappa and Thejakumar (2016). Dipak *et al.,* (2013); Varma *et al.,* (2007) and Singh *et al.,* (2007) noted variability due to different geographical locations of isolates within Alternaria species. Cultural variability in Alternaria species exists and the characteristics significantly serve the identification of races of the isolates being tested (Rotem, 1994).

5.4 *In vitro* antifungal potential of ginger and turmeric against *Alternaria alternata*

Single spore isolates of *Alternaria alternata* was subjected to methanolic, ethanolic, acqueous and ethyl acetate extract of *Zingiber officinale* and *Curcuma longa* at

different levels of concentrations. Extracts of *Z. officinale* and *C. longa* showed varying antifungal potential against the *Alternaria alternata* depending on the solvent used for extraction and the concentration of the extract at which the fungi was subjected to. Methanolic extract of both *Zingiber officinale* and *Curcuma longa* stood out as the best solvent for extraction as it had the highest inhibition at all levels of concentrations (12.50 mg/ml, 25.00 mg/ml and 50.00 mg/ml). Methanolic extract of *Z. officinale* and *C. longa* demonstrated the highest antifungal activity which was significant ($P \leq 0.05$) against *Alternaria alternata* compared to ethanolic, aqueous and ethyl acetate extracts with percentage inhibition of 57.37% and 64.56% respectively at concentration of 50 mg/ml. At higher concentration (25.00 mg/ml and 50 mg/ml) the percent inhibition on the fungal growth was not significant ($P \leq 0.05$) from day 8^{th} in all the solvents of the extraction for turmeric and ginger.

These results are in conformity with the earlier findings of Vasudha *et al.,* (2018) who reported that extracts from *Z. officinale* and *Curcuma longa* had significantly inhibited mycelial growth of *Alternaria alternata* infecting different crops. They reported 73.64% by *Zingiber officinale* and 60.19% by *Curcuma longa* where the mycelial radial growth of *Alternaria alternata* decreased drastically with increase in concentrations of the test bioagents from 10 to 20 percent. Sajad and Abid (2017) studied the phytochemical contents of *Zingiber officinale* and reported that it contains several hydrocarbons. On subjection to *Alternaria alternata* at different concentrations, they noted a percentage growth inhibition of 45%, 66% and 73% at the concentrations of 5%, 10% and 20% respectively. Similar findings had earlier been documented by Bansod and Rai (2008). Rahmah *et al.,* (2013) and Olubunmi *et al.,* (2018) attributed the activity of ginger to the presence of phenolic amalgams and

hydrophobic essential oils (Mamarabadi *et al.,* 2018) which could be the active ingredients causing the present results.

The present results agreed with those of Wongkaew and Sinsiri (2014) who noted that ethanolic extracts of *Curcuma longa* were effective against *A. alternata* and *Pythium* sp. similar findings were earlier documented by Chethana *et al.,* (2012) who observed that garlic, turmeric and neem extracts inhibited the growth of *Alternaria pori*. From the study of Madhu *et al.,* (2017), ethanolic extracts of *Momordica charantia* showed 44% inhibition against the mycelial growth of *Alternaria alternata* and 100% on spore germination at the concentration of 60 mg/ml. In our study, methanolic, ethanolic, aqueous and ethyl acetate extracts of both *Curcuma longa* and *Zingiber officinale* demonstrated that 25 mg/ml minimum inhibitory concentration against *A. alternata* was obtained.

Muthomi *et al.,* (2017) reported efficacy of a number of plant extracts against important pathogens of tomato. Among the tested plant extracts, *Curcuma longa, Allium sativum, Citrus limon* and *Zingiber officinale* were the most effective extracts. They reported that *Curcuma longa* was the most active on all the tested fungal plant pathogens reducing their radial growth by 72% on *Alternaria solani*. *Curcuma longa* was sensitive against both young and old cultures of fungal pathogens. Extracts from *Zingiber officinale* and *Rosmarimus officinalis* showed high antifungal activity on young cultures of the fungal pathogens. There was variation in the antifungal activity of the plant extracts at different stages of growth of the fungal pathogens similar to the findings from the present study where variation in radial growth of *Alternaria alternata* was observed from day 1 to day 8 but henceforth the percent inhibition was not significantly different.

Similarly, Mudyiwa *et al.,* (2016) reported that ethanolic extracts of *Zingiber officinale* (ginger), *Allium cepa* (onion) and *Allium sativum* (garlic) at concentrations of 50%, 75% and 100% showed high inhibition activity against the mycelial growth of *Alternaria solani* whereas the plant extract concentration level increased, it resulted to direct decrease in mycelial growth. Humaira (2015) compared the ethanolic fractions of ginger (*Zingiber officinale*) viz; ethyl acetate, chloroform, petroleum ether, n-butanol and aqueous extracts against the *Alternaria alternata* causing leaf spot of spinach. The findings showed that ethyl acetate fraction displayed maximum antifungal inhibition on the *Alternaria alternata* followed by chloroform and petroleum ether while n-butanol and aqueous extract showed no inhibition effect, which differed with present study where methanolic extracts of both ginger and turmeric at higher concentrations demonstrated higher antifungal activity. Similar to the present findings, Neeta *et al.,* (2013) observed that increasing concentrations of ginger oil extract results to more suppression of the fungi. Researchers attributed the higher antifungal activity of *Zingiber officinale* to the high level of phytoconstituents of both volatile and non-volatile chemicals (Grzanna *et al.,* 2005; Rahmah *et al.,* 2013).

Variation in the activity of the evaluated plant extracts could be due to method of extraction, extraction solvent, nature and origin of the plants (Odhiambo *et al.,* 2009; Mahlo *et al.,* 2013). Further, the efficacy of the plant is influenced by sensitivity of the test pathogen strain and concentration of the extract (Bandor *et al.,* 2013; Brussoti *et al.,* 2013). Sumitra *et al.,* (2012) noted that the type and amount of active phytochemicals in a plant depends on the origin and nature of the plant. Also Mizubuti *et al.,* (2007) earlier noted that physiology and the growth rate of the test fungal pathogens affect the activity of the extracts since microorganism are fast

growers and some have highly improved morphologies. Javid and Rehman (2011) observed from their study that for effective efficacy from different plant decoctions against target pathogens then different level of concentrations are applied.

5.5 *In vivo* evaluation of plant extracts against Alternaria leaf spot of Spinach

Spinach plants were treated with methanolic extracts of both tumeric and ginger at the concentrations of 50 mg/ml and it was observed that tumeric and ginger reduced the disease index by 57.70% and 53.84% over control respectively. The results are in agreement with the findings of Biswas and Ghosh (2018) who reported a reduction in PDI over control in *in vivo* studies of Alternaria leaf blight of Mustard in the field with *L. camera*, *Z. officinale* and *A. sativum* recording a percent reduction in PDI of 71%, 22% and 55% at a concentration of 10%. Neeta *et al.,* (2103) noted that essential oil from Z. officinale drastically reduced the colony forming units per gram of *Alternaria* sp., *Apergillus* spp., *Fusarium* sp., *Penicillium* sp., and *Cladosporium* sp. in stored nuts, pulse and, spices at 200ppm, 500ppm, 1000ppm and 2000ppm.

Maria and Maria (2019) reported that ginger and tumeric essential oils have high antibacterial, antifungal and herbicidal activity against and a wide range of bacteria, fungi and weeds. Extracts from ginger, tumeric, garlic and lemon reduced the population of Tuta absoluta and white flies by 55% and 63% respectively. Further, the same plant extracts reduced the intensity of early blight of tomato by 34% and late blight levels by 53% (Lengai *et al.,* 2017). John et al., (2018) found that extracts from tumeric rhizomes and moringa leaf had antifungal activity against Penicillium sp., *Fusarium solani*, *Aspergillus niger* and *A. flavus* at 25, 50 and 75 ml concentrations.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The study established the prevalence and incidences of Alternaria leaf spot on spinach caused by *Alternaria alternata* in Nandi and Uasin Gishu Counties.
2. The *Alternaria alternata* isolates from the different spinach growing areas showed significant difference in cultural and morphological characteristics such as mycelial growth, mycelial colour, substrate colour, conidial size and growth rate hence geographical variation of isolates.
3. Tumeric and ginger plant extracts evaluated at *in vitro* and *in vivo* were found to have antifungal potential against *A. alternata.* Methanolic extracts of both ginger and turmeric demonstrated the highest antifungal activity than ethanolic, acqueous and ethyl acetate extracts. However, turmeric extracts showed higher antifungal than ginger crude extracts against *A. alternata*.
4. The inhibition levels observed at *in vitro* and *in vivo* revealed that they have potential to manage plant pathogens and hence could be used instead of synthetic pesticides upon purification and formulation thereby minimizing risks and hazardous effects of the synthetic fungicides.

6.2 Recommendations

1. Farmers to be sensitized on the best control measures and the importance of adopting the control measures as soon as disease symptoms are observed.
2. Methanolic extracts of ginger and tumeric extracts can be recommended for use as bio-fungicide upon purification and formulation.

3. Further studies to be done to establishing genetic variation using molecular based techniques to compare with the cultural and morphological variations established in the study.
4. Further studies to be done on the plant extracts so as to profile the active phytochemicals which are present in the extracts that are responsible for this fungicidal activity.

REFERENCES

Abd El-Ghany, T. M., Roushdy, M. M. & Mohamed, A. A. (2015). Efficacy of Certain Plant Extracts as Safe Fungicides Against Phytopathogenic and Mycotoxigenic Fungi. *Agricultural and Biological Sciences Journal*, *1* (3), 71-75.

Abo-Elyousr, A. M. K. & Nashwa, S. M. A. (2012). Evaluation of various plants extracts against the early blight disease of tomato plants under green house and field conditions. *Plant Protection Science 48*, 74-79.

Agrios, G. N. (2005). Plant Pathology (5th edition). Elsevier- Academic Press, Newyork. p. 633.

Ahmed, K. S., Waqas, R., Muhammad, U. G., Yasir, I., Naveed, H. & Muhammad, H. R. (2016). Management of early blight of tomato through the use of plant extracts. *International Journal of Zoology Studies*, *1* (5), 1-4

Akrami, M. & Yousefi, Z. (2015). Biological control of fusarium wilts of tomato *Solanum lycopersicum* by *Trichoderma* spp. as antagonist fungi, Biological Forum-*An International Journal 7*: 887-892.

Al-Samarrai, G., Singh, H. & Syarhabil, M. (2012). Evaluating eco-friendly botanicals as alternatives to synthetic fungicides. *Annals of Agriculture and Environmental Medicine, 9*, 673-676.

Anjum, M. A., Ahmed, N., Babita, C. H. & Gupta, P. (2016). Plant Extracts in Post-Harvest Disease Management of Fruits and Vegetables. *Journal of Food Process Technology 7* (6), 592-600. http://doi:10.4172/2157-7110.1000592.

Anonymous (2014) Standard newspaper Kenya (7 0ct 2014)

Anonymous (2017). Daily Nation newspaper Kenya (28 July 2017).

Anuj, M., Rajib, R. & Jagatpati, T. (2014). *Alternaria* pathogenicity and its strategic controls. *Research Journal of Biology, 1,* 01-09.

Aslam, S, H., Aslam, M U., Abbas, A., Ali, M, A., Alam, M, W., Amrao, L. & Gleason, M, L. (2019). First Report of Leaf Spot of Spinach Caused by *Alternaria alternata* in Pakistan**.** *Plant Disease*, 103 (6), 1430-1442. https://doi.org/10.1094/PDIS-12-18-2211PDN.

Babu, S., Seetharaman, K., Nandakumar, R. & Johanson, I. (2000). Efficacy of fungal antagonists against leaf blight of tomato caused by *Alternaria solani* (Ell and Mart) Jones and Grout. *Journal Biological Control, 14* (2),79-81.

Ball, G. F. M. (2006) Vitamins in foods: Analysis, bioavailability, and stability. CRC Press.

Bandor, H., Hijazi, A., Ramma, H., Hachem, A., Saad, Z. & Badran, B. (2013). Techniques for the extraction of bioactive compounds from labanese *Urticadiotica*. *Journal of Phytomedicine Clinical Therapeutics, 1* (6), 507-513.

Bansod, S. & Rai, M. (2008) Antifungal activeity of essential oils from Indian medicinal plants against human pathogenic *Aspergillus fumigatus* and *Aspergillus niger*. *World Journal of Medical Sciences, 3* (2):81–88.

Barry, M. P. & Themis, J. M. (2001). Morphological, pathogenic, and molecular characterization of *Alternaria* isolates associated with Alternaria late blight of pistachio. *Phytopathology, 92,*406-416.

Bazzi, C., Gozzi, R., Stead, D. & Sellwood, J. (1988). A bacterial leaf spot of Spinach (Spinaciae oleracea L.) caused by a non-fluorescent Pseudomonas syringae van Hall. *Phytopathology Mediterranean Journal*, 27, 103-107.

Bdliya, B. S. & Gwio-Kura, K. K. (2007). Efficacy of some fungicides in the management of Cercospora leaf spot of groundnut in the Sudan savannah of Nigeria. *Journal of Plant Protection Research, 47* (3), 243 – 252.

Bhaat, J. C., Gahlain, A. & Pant, S. K. (2000). Record of *Alternaria alternata* on tomato, capsicum and spinach in Kumaon hills. *Indian Phytopathology, 53* (4), 495-498.

Biswas, M. K. & Ghosh, T. (2018). Evaluation of Phyto-extracts, Biological Agents and Chemicals against the Development of *Alternaria brassicae in vitro* and *in vivo European Journal of Medicinal Plants, 22* (3*)*, 1-9. http//:doi: 10.9734/EJMP/2018/40412.

Bourguet, D. & Guillemaud, T. (2016). The hidden and external costs of pesticide use. *Sustainable Agriculture Reviews: Springer*. Pp 35-120.

Bradley, G. A., Sistrunk, W. A., Baker, E. C. Cash, J. N. (1975). Effect of plant spacing, nitrogen and cultivar on spinach (*Spinacia oleracea* L.) yield and quality. *Journal 0f American Society for Horticultural Science, 10* (0), 45 – 48.

Brusotti, G., Cesain, I., Dentamaro, A., Caccialanza, G. & Massolin, G. (2013). Isolation and characterization of bioactive compounds from plant resources: the role of analysis in the ethno pharmacological approach. *Journal of Pharmaceutical and Biomedical Analysis, 87*, 218-228.

Chethana, B. S., Ganeshan, G., Rao, A. S. & Bellishree, K. (2012). *In-vitro* evaluation of plant extracts, bioagents and fungicides against *Alternaria porri* causing purple blotch diseases of onions, Pest Management in Horticultural Ecosystems, *18* (2), 194-198.

Chethana, B.S., Girija, G., Archana, S. Rao. & Bellishree, K. (2018). Morphological and Molecular Characterization of *Alternaria* Isolates Causing Purple Blotch Disease of Onion. *International Journal of Current Microbiology and Applied Science,7*(4),3478-3493.https://doi.org/10.2056/ijcmas.2018.704.394 (04):3478-3493.

Ciqiong, C., Long, L., Zhang, F., Chen, Q., Chen, C. & Yu, X. (2018). Antifungal activity, main active components and mechanism of *Curcuma longa* extract against *Fusarium graminearum*. *13* (3), 194-204. https://doi.org/10.1371/journal. pone.0194284.

Correll, J. C., Teddy, E. M., Mark, C. B., Steven, T. K., Lynn, P. B. & Frank, J. D. (1994). Economically Important Diseases of Spinach. *The American Phytopathological Society, 78* (7) 653-690.

Czajka, A., Czubatka, A., Sobolewski, J. & Robak, J. (2015). First Report of Alternaria Leaf Spot Caused by *Alternaria alternata* on Spinach in Poland, *The American Phytophathological Society* (APS), *99* (5), 729-738. https://doi.org/10.1094/PDIS-10-14-1090-PDN.

Devappa, V. & Thejakumar, M. B. (2016). Morphological and Physiological Studies of *Alternaria alternata* causing leaf spot disease of Chilli, *Capsicum annuum* L. *International Journal of Apllied and Pure Science and Agriculture, 2* (5), 762-773.

Di, M., Xiang, Z. X., Hua, W. Y. & Wen, Z. X. (2007). Potential of bacterial secondary metabolites as bio-pesticides. *Journal of Shenyang Agriculture University, 38* (6), 811-815.

Dipak, T. N., Anil, P. G. & Lalan, S. (2013). Morphological and cultural characterization of *Alternaria alternata* (Fr.) Keissler blight of gerbera (*Gerbera jamesonii* H. Bolus ex J.D. Hook). *Journal of Applied and Natural Science, 5* (1), 171-178.

Elliott, J. A., (1917). Taxonomic characters of the genera *Alternaria* and *Macrosporium. American Journal of Botany, 4*: 439-476.

Ellis, M. B. (1968.) *Alternaria brassicae.* In: Descriptions of pathogenic fungi and bacteria (No. 162), Commonwealth Mycological Institute (CMI), Kew, Surrey, England.

Ellis, M. B. (1971). Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England. 464-497.

FAOSTAT. (2010). Production. Food and Agricultural Organization of the United Nations. http://fao-stat.fao.org/site/ 339/default.a spx (accessed 16 Jun. 2019).

FAOSTAT. (2016). Crops/ Regions/ World list of Production Quality of Spinach. The Food and Agricultural Organization of the United Nations. Rome, Italy.

Francisco, D. H. (2010). *Lippia graveolens* and *Carya illinoensis* Organic Extracts and there *in vitro* Effect against *Rhizoctonia Solani* Kuhn. *American Journal of Agricultural and Biological Sciences, 5* (3), 380-384.

Fries, E. M. (1832). Officiana Berlingianalund, Greifswald. *Systema Mycologicum*, 3, 520-521.

Gairola, S., Noresah, M. S., Bhatt, A., Chandra, P. & Kala, C. P. (2010). Influence of climate change on production of secondary chemicals in high altitude medicinal plants: Issues needs immediate attention. *Journal of Medicinal Plants Research.18*, 1825-1829.

Ginoya, C. M. & Gohel, N. M. (2015). Evaluation of newer fungicides against *Alternaria alternata* (Fr.) Keissler causing fruit rot disease of chilli. *International journal of Plant Protection, 8* (1), 169-173.

Goufo, P., Fontem, A. & Ngnokam, D. (2010). Evaluation of plant extracts for tomato late blight in Cameroon. *New Zealand Journal of Crop and Horticultural Science. 38,* 171-176.

Grover, R. K. & Moore, J. D. (1962). A toximetric study of fungicides against brown rot organisms *Sclerotinia fructicola* and *S. laxa*. *Phytopathology.52*, 876-880.

Grzanna, R., Lindmark, L. & Frondoza, C. G. (2005). Ginger an herbal medicinal product with broad anti-inflammatory actions. *Journal of Medicinal Food,* 8, 125-132.

Guo, R. J., Li, W. J., Yun, Y. M. & Feng, Z. J. (2011). Comparative study of the biological characteristics between *Alternaria alternata* and *Alternaria tabacina*. *Journal of Hunan Agricultural Univ*ersity, *37*(4), 411-414.

Harbant, S., Ghassan, F. & Mohds, S. (2011). Antifungal activity of *Capsicum frutescence* and *Zingiber officinale* against Key post-harvest pathogens in citrus, Int. Conference on Biomedical Engineering Technology.

Hasan, M. M., Hasan, M. S., Shamima, N., Nazia, B. I. & Kishowar, E. M. (2016). Bio-control of Root-Knot (*Meloidogyne incognita*) of Indian Spinach (*Basella alba* L.). *Universal Journal of Agricultural Research* 4 (6): 247-253, http://www.doi: 10.13189/ujar.2016.040604.

Hashem, A., Allah, A. B., Al-huqail, A. A. & Alqarawi, A. A. (2014). Report and characterization of *Alternaria alternata* (fr.) Keissler on avicennia marina (forsk.) Vierh forests of industrial yanb'a city, Saudi Arabia. *Pakistan Journal 0f Botany, 46* (2), 725-734.

Hassan, A. M., Chindo, P. S., Marley P. S. & Alegbejo, M. D. (2010). Management of root knot nematodes, *Meloidogyne* spp. on tomato, *Lycopersicon lycopersicum*, using organic wastes in Zaria, Nigeria. *Plant Protection Science, 46*, 34-38

Hassanein, N. M., Abou, Z. M. A., Youssef, K. A. & Mahmood, D. A. (2008). Efficacy of leaf extracts of Neem (*Azadirachta indica*) and Chinaberry (*Melia azedarach*) against early blight and wilt diseases of tomato. *Australian Journal of Basic and Applied Sciences, 2* (3), 763-777.

Herivony, O. A., Rado, R., Rahanira, R., Rigobert, A., Marson, R. & Fidele, R. (2016). Biological Potentials of Ginger Associated *Streptomyces* Compared with Ginger Essential Oil. *American Journal of Life Sciences, 4*, 152-163. https://doi: 10.11648/j.ajls.20160406.13.

Hong, S. G., Cramer, R. A., Lawrene, C. B., &Pryor, B. M. (2005). Alt a1 allergen homologs from *Alternaria* and related taxa: analysis of phylogenetic content and secondary structure. *Fungal Genetics and Biology, 42,* 119–129.

Hubert, J., Mabagala, R. B. & Mamiro, D. P. (2015). Efficacy of Selected Plant Extracts against *Pyricularia grisea*, Causal Agent of Rice Blast Disease. *American Journal of Plant Sciences,* 6, 602- 610.

Humaria, R. (2015). Exploiting antifungal potential of ginger for the management of *Alternaria alternata*, the cause of leaf spot disease of spinach. *Mycopathology, 13* (2), 97-104.

Ikpeama, A., Onwuka, G. I. & Nwankwo, C. (2014). Nutritional Composition of Tumeric (*Curcuma longa*) and its Antimicrobial Properties. *International Journal of Scientific and Engineering Research. 10*, 1085-1089.

Infonet-biovision(2015). Tomatoes, Retrieved from, http://www.infonetbiovision. (Retrieved on 22, March 2019).

Ivan, S., Ryan, J. H., Beiquan, M. & James. M. (2014). Lettuce and spinach cultivation. American Society of Agronomy. *Crop Science Society of America*, pp 53-86. http://doi:10.2135/cssaspecpub33.c4

Javaid, A. & Rehman, A. H. (2011). Antifungal activity of leaf extracts of some medicinal trees against *Macrophomina phaseolina*. *Journal of Medicinal Plants Research*, *5* (13), 2858-2872.

Jeff, W. & Janet, M. (2012). Automated Spore Measurements Using Microscopy Image Analysis and Peak Recognition of Near Monodisperse Aerosols. *Aerosol Science Technology*, *46* (8), 862-873. http://doi:10.1080/02786826.2012.674232 (Retrieved on 16th Jan, 2019).

John, W. C., Ihum, T. A., Olusolape, O. & Janfa, N. (2018). Efficacy of Turmeric Rhizome (*Curcuma longa*) and Moringa Leaf (*Moringa oleifera*) Extract in Treatment against Fungi Associated with Maize Seeds *Asian Plant Research Journal. 1(2) 1-8.*

Joly, P. (1959). Le Genre Alternaria. Paul Lechevalier Bulletin trimestiel de la Societe Mycologique de France, *75*, 149-158.

Keissler, K. (1912). Zur kenntnis der pilzflora krains. *Beihefte zum Botanischen Zentralblatt, 29*, 395–440.

Kimani, V. (2014). Bio-Pesticides development, use and regulation in Kenya, Regional Experts Workshop on Development, Regulation and Use of Bio-Pesticides in East Africa, Nairobi, Kenya.

Kohla, J., Tongerena, C. A. M., Groenenboom-de-Haasa, B. H., R., Hoofa, R. A., Driessenb, R. & Heijdenc, J. (2010). Epidemiology of dark leaf spot caused by *Alternaria brassicicola* and *Alternaria brassicae* in organic seed production of cauliflower. *Journal of Plant Pathology*, 59, 358–36. http://doi:10.1111/j.1365-3059.2009.02216.x7.

Koike, S. T., M, Cahn, M., Cantwell, S., Fennimore, M., Lestrange, E., Natwick, R., Smith, F. & Takele, E. (2011). Spinach production in California. Publication 7212. University of California, Agriculture & Natural Reources. http://anrcatalog.ucdavis.edu/vegetableropProduct-tioninCalifornia/7212.spex (retrieved 16 Jun. 2019).

Koren, G. (2007). Medication safety in pregnancy and breastfeeding. McGraw-Hill Professional. P. 279.

Kumar M., Bhadauria V., Singh K., Singh C. & Yadav A. K. (2013). Evaluation of fungicide efficacy for the management of Alternaria leaf spot disease of chilli. *Plant Pathology, 12*, 32-35.

Kumar, S. (2015). Biopesticide, an environment friendly pest management strategy, *Journal of Biofertilizers and Biopesticides*, *6*, 1-3.

Kuna, P., Kaczmarek, J. & Kupczyk, M. (2011). Efficacy and safety of immunotherapy for allergies to *Alternaria alternata* in children. *Journal of Allergy and Clinical Immunology*, *127*, 502-508.

Lengai, G. M. W., Muthomi, J. W. & Rama, D. N. (2017). Efficacy of Plant Extracts and Antagonistic Fungi in Managing Tomato Pests and Diseases under Field Conditions. *Journal of Agriculture and Life Sciences, 4* (2) 20-27.

Licinio, D., Jose, A. D. R. & Ana, O. (2018). Mechanism of the *Alternaria alternata* Pathogenicity in 'Fortune' Mandarin. *Horticulturae*, *4*:54-62. http://doi:10.3390/horticulturae4040054.

Lyudmila, Y., Natalya, N. K., Marina, A. P., Natalia, V. S. & Boris, T. Z., (2015). Virulence of *Alternaria* strains toward potato and tomato cultivars.*17*, 121-132.

Madhu, G., Sushil, S. & Rekha, B. (2017). Phytotoxicity of *Momordica Charantia e*xtracts against *Alternaria Alternata. Journal of Pharmaceutical Sciences & Researc,. 9* (1), 28-34.

Madhusankha, G. D. M., Thilakarathna, R. C. N., Liyanage, T. & N S. B. (2018).Comparison of phytochemical properties of Indian and Sri Lankan turmeric rhizomes (*Curcuma longa*).*Journal of Pharmacognosy and Phytochemistry, 3*, 1995-1998.

Mahlo, S. M., Chauk, H. R., McGaw, L. J. & Eloff, J. N. (2013). Antioxidant and antifungal activity of selected plant species used in traditional medicine. *Journal of Medicinal Plants Research, 7* (33), 2444-2450.

Malkhan, S. G. Shahid, A., Masood, A. & Kangabam, S. S. (2012). Efficacy of plant extracts in plant disease management. *Journal of Agricultural Sciences, 3* (3), 425-433.http://doi:10.4236/as.2012.33050.

Mamarabadi, M., Abbas, T. & Younes, R. (2018).Antifungal activity of recombinant Thanatin in Comparison with two plant extracts and a chemical mixture to control fungal plant pathogens. Http://dol.org/10.1186/s13568-018-0710-4.

Mamta, S., Raju, G. & Suresh, P. (2013). Occurrence of *Alternaria alternata* causing Alternaria blight in pigeon pea in India. *Advances in Bioscience and Biotechnology, 4*, 702-705. http://doi:10.4236/abb.2013.46092.

Mamunur, M. R., Humayun, M. K., Mokbul, M. H., Rejwan, B. & Mohammad, A. I. K. (2015). Eco-Friendly Management of Chilli Anthracnose (*Colletotrichum capsici*). *International Journal of Plant Pathology, 6* (1), 1-11. Http://doi: 10.3923/ijpp.2015.1.11.

Manoharan, K. P., Asmawi, M. Z., Choon, T., Sasidharan, S., Lachumy S. J. & Ismail, R. (2010). Inhibitory effect of ethanolic and water extracts of two varieties of ginger on selected bacterial and fungal isolates. *Pharmacology 3*, 951-958.

Marcin, N., Marzena, N., Anna, N. & Elzbieta, U. K. (2012) Alternaria black spot of crucifers: Symptoms, importance of disease, and perspectives of resistance breeding. *76*, 5-19 http://doi: 10.2478/v10032-012-0001-6.

Maria, D. I. & Maria A. B. (2019). Ginger and Turmeric Essential Oils for Weed Control and Food Crop Protection. *Plants 8*, (59) 1-14,

Marraiki, N. N., Siddiqui, I., Rizwana, H. & Javaid, J. (2012). First report of *Alternaria alternata* leaf spots on spinach in Saudi Arabia. *The Journal of Animal & Plant Sciences,22* (1), 247-248.

Massawe, R. S. C. (2010). Impact of nematode pest management strategies on nematode communities in tomato productions systems in Zimbabwe, Doctor of Philosophy, University of Zimbabwe

McKinney, H. H. (1923). A new system of grading plant diseases. *Agricultural Research, 26*, 95-98.

Meena, M., Gupta, S. K., Prashant, S., Andleeb, Z., Manish, K. D. & Ram, S. V. (2017). Alternaria toxins: Potential Virulence Factors and Genes Related to Pathogenesis. *Frotiers in Microbiology*, 8, 1451-1465. http:// doi.10.3389/fmicb. 2017.0151.

Meena, R. K., Sharma, S. S. & Singh, S. (2014). Studies on variability in *Alternaria alternata* (Kessler) causing Leaf blight of Isabgol, *Plantago ovata. Journal of Agriculture Research, 12* (2), 63-70.

Mehdi, S. R., Elena, M. V., Bahare, S., Javad, S. R., Karl, R. M., …& Seyed, A. A. (2017).Plants of the Genus *Zingiber* as a Source of Bioactive Phytochemicals: From Tradition to Pharmacy. Review. Molecules.

Ministry of Agriculture. (2000). Local and Export Vegetables: Growing Manual. Ministry of Agriculture, Rural Development, Kenya &Japan International Cooperation Agency, Printed by Agricultural Information Resource Centre, Nairobi, Kenya.

Ministry of Agriculture. (2016). External Evaluation of Horticulture and Food Security Program. Ministry of Agriculture, Kenya.

Mishra, J., Tewan, S. & Arora, K. N. (2015). Biopesticides, where we stand, springer. 37-75.

Mizubuti, G. S. E., Junior, V. L. & Forbes, G. A. (2007). Management of late blight with alternative products. *Pest Technology,. 1*(2), 106-116.

Mohammed, N., Al-qumboz, A. & Samy, S. A. (2019). Spinach Expert System: Diseases and Symptoms. *International Journal of Academic Information Systems Research, 3* (3), 16-22.

Morelock, T. E. & Correll, J. C. (2008). Vegetables: *Asteraceae, Brassicaceae, Chenopodiaceae,* Cucurbitaceae & Spinach. Springer, New York, P. 189-218.

Mudyiwa, R. M., Chiwaramakanda, S., Manenji, B. T. & Takawira, T. (2016).Anti-*Alternaria solani* Activity of Onion (*Allium cepa*), Ginger (*Zingiber officinale*) and Garlic (*Allium sativum*) *in vitro. International Journal of Plant & Soil Science, 10*(4), 1-8. http://doi: 10.9734/IJPSS/2016/24488.

Mustapha, F. A. J., Abdelhafiz, A. D. & Mohammed, S. A. (2017). Environmental and Human Health Impacts of Pesticide Use in Agriculture. Environment and Life Sciences Research Center, Kuwait Institute for Scientific Research, Kuwait.

Muthomi, J. W., Geraldin, M. W. L., Maina, J. W. & Rama, D. N.(2017). *In vitro* activity of plant extracts against some important plant pathogenic fungi of tomato**.** *Australian Journal of Crop Science, 11* (06), 683-689. http://doi: 10.21475/ajcs.17.11.06.p399.

Nadia, J., Javaid, A., Ahmed, E. & Sharif, A. (2014). Managment of causal organism of collar rot of bell pepper (*Sclerotum rolfsii*) by organic solvents extracts of *Datura metel* fruit. *Pakistan Journal of Phytopathology, 26,* 15-20.

NCScAO (2018). Nandi Central Sub-county Agricultural Offices, Nandi county, Kenya.

Neergaard, P. (1945). Danish species of Alternaria and Stemphylium taxonomy, parasitism and economic significance. Oxford University Press, London, Oxford: 559.

Nees, V. E. G. (1817). Das System der Plize Urid Schwamme, Wurzburg, 234.

Neeta, S., Richa, T. & Madhu, P. S. (2013). *Zingiber officinale* Roscoe. Oil: A preservative of stored commodities against storage mycoflora *International. Journal of Current Microbiology and Applied Science,* 2(7), 123-134.

Noman, E., Al-Gheethi, A. A., Rahman, N. K., Talip, B., Mohamed, R. and Kadir, O. A. (2018). Single Spore Isolation as a Simple and Efficient Technique to obtain fungal pure culture. IOP Conf. Series: *Earth and Environmental Science*, 140, 012-055. http://doi:10.1088/1755-1315/140/011055

Odhiambo, A. J., Siboe, G. M., Lukhoba, C. W. & Dossaji, F. S. (2009). Antifungal activity of crude extracts of selected medicinal plants used in combination in Lake Victoria basin, Kenya. *Plant Product Research Journal. 13*, 35-43.

Ojiambo, P. S. (1997). Cultural studies and epidemiology of *Alternaria sesami* and significance of sesame (*Sesamum indicum* L.) seed transmission and plant age on *Alternaria* leaf spot severity. M. Sc. thesis. University of Nairobi, Nairobi, Kenya. Pp 111.

Olubunmi, A. A., Felix, A. A., Daniels, J. A., Oloswol, O. O. & Ayodele, B. O. (2018). Phytochemical screening and antifungal activities of *Zingiber officinale* on mycotoxygenic fungi associated with the deterioration of *Pennisetum glaucume* grains. *Journal of Advances in Microbiology*, *13* (1), 1-11.

Pankaj, S. & Richa, S. (2017). Antifungal screening of *Curcuma longa* and *Zingiber officinale* against dermatophytes causing superficial Mycosis. *International Journal of Pharmaceutical Research and Health Science, 5*, 1980-1983.

Patel, D. K. (2015). *Curcuma longa* Linn. Cultivation: The process for its Medicinal use and Conservation. *The Pharmaceutical Innovation Journal, 1*, 99-101.

Pervaize, A. A., Willy, R., Shawkat, A. & Hamid, A. N. (2018). First report of *Alternaria alternata* causing leaf spot and blight symptoms on alfalfa in Canada. *Canadian Journal of Plant Pathology, 40* (3), 1-5. http://doi:10.1080/07060661.2018.1470111.

Prasad, Y, & Naik, M. K. (2003). Evaluation of genotypes, fungicides and plant extracts against early blight of tomato caused by *Alternaria solani*. *Indian Journal Plant Protection, 31* (2), 49-53.

Premachandra, D. W. T. S. & Gowen. S. R. (2015). Influence of pre-plant densities of Meloidogyne incognita on growth and root infestation of spinach (*Spinacia oleracea* L.) (Amaranthaceae) – an important dimension towards enhancing crop production, Future of Food: *Journal Food, Agriculture and Society, 3* (2), 18-26.

Rahmah, A. N., Mostafa, A. A., Abdel-Megeed, A., Yakout, S. M. & Hussein, S. A. (2013). Fungicidal activities of certain methanolic plant extracts against tomato phytopathogenic fungi. *African Journal of Microbiological Research, 7*, 517-524.

Raja, P. & Reddy, A.V. (2007). Morphological and biological variability of *Alternaria* spp. causing leaf spot and fruit rot of brinjal. *Journal of Mycology and Plant Pathology*, *37* (2), 336-338.

Rayner, R. W. (1970). A Mycological Colour Chart. Commonwealth Mycological Institute, Kew, UK.

Richa, S. & Meenakshi, S. (2011).Synergistic Antifungal Activity of *Curcuma longa* (Tumeric) and *Zingiber officinale* (Ginger) Essential oils Against Dermatophytes infections. *Journal of Essential Oil Bearing Plants, 14*, 38-47.

Rodino, S., Butu, A., Butu, M. & Cornea, C. P. (2014). *In vitro* activity of some plant extracts against damping off diseases of tomato. *Journal of International Scientific Publications, Agriculture and Food*, 240-244.

Rotem, J. (1994). The genus *Alternaria*: Biology, epidemiology and pathogenicity. The American Phytopathological Society Press, St. Paul, Minnesota.

Saha, D., Fetzner, R., Burkhardt, B., Podlech, J., Metzler, M. & Dang, H. (2012). Identification of a polyketide synthase required for alternariol (AOH) and alternariol-9-methyl ether (AME) formation in *Alternaria alternata*.*7*, 405-415.

Sajad, A. M., &Abid, H. Q. (2017). Antifungal activity of *Zingiber officinale* oil against plant pathogenic fungi isolated from solanaceous vegetable fruits. *Asian Journal of Pharmacy and Pharmacology*, *3* (4), 121-124.

Sami, J. M., Marissônia, A. N., Maria, S. X. F., Marcos, P. S. C. & Ailton, R. (2012). Survey and prevalence of species causing Alternaria leaf spots on *Brassica* species in Pernambuco. Scientific communication, 30 (2),73-78. http://doi.org/10.1590/S0102-05362012000200027.

Sanjeev, J. R. K., Mesta, I. B., Biradar, S., Mushrif, K. &Ajjappalavar, P.S. (2017). Studies on the cultural and growth characteristics of *Alternaria alternata*, *Colletotrichum melongenae* and Phomopsis vexans the causing fruit rot of brinjal. *International Journal of Current Microbiology and Applied Science*, 6 (6), 1062-1069. doi: https://doi.org/10.20546/ijcmas.2017.606.122

Sato, A., Takeda, H., Oyanagi, W., Nishihara, E. & Murakami, M. (2010). Reduction of cadmium up take in spinach, *Spinacia olerac*ea L. by soil amendment with animal waste compost. *Journal of Agricultural Research, 181*, 298-304.

Shabana, Y. M., Mohamed, E. A., Atef, A. S., El-Sawy, M, M., Ibrahim, S. D. & Ahmed, W. Y. (2017). Efficacy of plant extracts in controlling wheat leaf rust disease caused by *Puccinia triticina*. *Egyptian Journal of Basic and Applied Sciences*, 4 (1), 67-73. https://doi.org/10.1016/j.ejbas.2016.09.002.

Shafique, S., Bajwa, R., Akhtar, N. & Hanif, S. (2011). Fungitoxic activity of aqueous and organic solvent extracts of *Tagetes erectus* on phytopathogenic fungus-*Ascochytarabiei*. *Pakistan Journal of Botany, 43*, 59-64.

Sharma, P. K., Singh, V. & Ali, M. (2016). Chemical composition and antimicrobial activity of fresh rhizome essential oil of *Zingiber officinale* Roscoe. *Pharmacognosy Journal, 8*,185–190.

Sherf, A. F. & Macnab, A. A. (1986). Vegetable Diseases and Their Control. John Wiley & Sons, Inc. ISBN: 0-471-05860-2.

Simmons, E. G. (1965). Typification of Alternaria, Stemphylium and Ulocladium. Mycologia, 59, 67-92.

Simmons, E. G. (2007). Alternaria. An Identification Manual. In: Biodiversity No 6. CBS fungal diversity centre, Utrecht, The Netherlands.

Singh, H., Ghassan, F. & Mohd, S. (2011). Anti-fungal activity of *Capsicum frutescence* and *Zingiber officinale* against key post-harvest pathogens in citrus. *International Conference on Biomedical Engineering and Technology, 11*, 123-131.

Singh, J. & Majumdar, V. L. (2001). Efficacy of plant extract against *Alternaria alternata*. The incitant of fruit rot of pomegranate (*Punica granatum* L). *Journal of Mycology and Plant Pathology, 31* (3), 346-349.

Singh, P. C. & Singh, D. (2006). *In vitro* evaluation of fungicides against *Alternaria alternata*. *Annals of Plant Protection Science, 14* (2), 500-502.

Singh, V., Vhrivastava, A., Jadon, S., Wahi, N., Singh, A. & Sharma, N. (2015). Alternaria Diseases of Vegetable Crops and its Management Control to Reduce the Low Production. *International Journal of Agriculture Sciences, 13*, 834-840.

Sinha, P. P. & Prasad, R. K. (1989). *In vitro* evaluation of fungicides against *Alternaria* spp. *Indian Journal of Mycology and Plant Pathology, 19,* 204-205.

Slavov, S., Mayama, S. & Atanassov, A. (2014). Some aspects of epidemiology of *Alternaria alternata* of tobacco pathotype, *18* (3), 234-243. http://doi. 10.1080/13102818.2004.10817125.

Srinath, K. V. & Sarwar, M. (1965). Alternaria blight of pyrethrum. *Curr. Sci*. 34,295.

Stangarlin, J. R., Kuhn, O. J., Assi, L. & Schwan-Estrada, K. (2015).Control of plant diseases using extracts from medicinal plants and fungi. https://www.researchgate.net/publication/268204141 (Retrieved on 12 February 2019).

Sumitra, A., Kanojia, A. K., Kumar, A., Mogha, N.& Sahu, V. (2012). Biopesticide formulation to control tomato lepidopteran pest menace. *Current Science, 102* (7), 1051-1057.

Suruchi, V. & Vikas, K. (2015). Pharmacological profile of Tumeric oil. A review.

Taiga, A. & Friday, E. (2009). Variations in Phytochemical Properties of Selected Fungicidal Aqueous Extracts of Some Plant Leaves in Kogi State, Nigeria. *American-Eurasian Journal of Sustainable Agriculture*, 3, 407-409. http://dx.doi.org/10.4236/ajps.2015.65065.

Tatsadjieu, N. L., Dongmo, P. M. J., Ngssoum, M. B., Etoa, F. X. & Mbofung, C. M. F. (2009). Investigation on the essential oil of *Lippi arugosa* from Cameroon for its potential use as antifungal agent against *Aspergillus flavus*. *Food Control Journal*, *20*,161-166.

The Ministry of Agriculture, Livestock and Fisheries (2017). Climate Risk Profile for Uasin Gishu County. Kenya County Climate Risk Profile Series. Nairobi, Kenya.

Thomma. B. (2003). *Alternaria* spp. from general saprophyte to specific parasite. *Molecular Plant Pathology, 4,* 226-236.

Thymon, L. S., Cummings, T. F. & Johnson, D. A. (2016). Pathogenicity and aggressiveness of three *Alternaria* spp. on potato foliage in the U.S North West Plant. 100, 797-801. http://doi. 10.1094/PDIS-08-15-0942-RE.

Varma, K. P., Sher, S. & Gandhi, S. K. (2007). Variability among *Alternaria solani* isolates causing early blight of tomato. *Indian Phytopathology 60* (2), 180-186.

Vasudha, A., Kadam, D. N., Dhutraj, D. V. P.& Patil, D. D. (2018). Bio Efficacy of Bio Agents and Botanicals against *Alternaria alternata* (Fr.) Keissler Causing Leaf Spot of Pomegranate. *International Journal of Current Microbiology and Applied Science, 7* (11), 1146-1155. https://doi.org/10.20546/ijcmas.2018.711.133.

Wachira, M. J., Mshenga, M. P. & Saidi, M. (2014). Comparison of profitability of small scale green house and open-field tomato production systems in Nakuru North District, Kenya. *Asian Journal of Agricultural Sciences, 6*, 54-61.

Waghmare, M. B. & Kamble, S. S. (2010). Efficacy of carbendazim against *Alternaria alternata* causing leaf spot of rose. *Bioinfonet* 7, 241-248.

Waghunde, R. R. & Patil, R. K. (2010). Physiological studies of the Alternaria fruit rot (*Alternaria alternata*) of watermelons. *Journal of Plant Disease Sciences, 5* (1), 73-75.

Wongkaew, P. & Sinsiri, W. (2014). Effectiveness of ringworm cassia and turmeric plant extracts on growth inhibition against some important plant pathogenic fungi. *American Journal of Plant Science*, 5, 616-626.

Yu, S. D., Peng, W. S., Qin, J. Z. & Jun, W. W. (2008). *In vitro* bio-control of *Alternaria* sp. *Journal of Northwest Asia*, 36, 173-178.

APPENDICES

Appendix I: Alternaria leaf spot (ALS) field survey questionnaire

Section I: Background information

Farmer ID: ---------- Name of farmer: ---------- Date: -----/-------/2018

Age: -------- Sex: (M) (F) Village: ---------- Agro-Ecological Zone: ----------

Latitude: ---------- Longitude: ---------- Altitude (m): ----------

Head of household (M/F): ---------- Highest level of education: ----------

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Section II: Information on production practices

I. How many years have you practiced spinach production? ----------

---

II. Area under spinach production (acres): ----------

III. Varieties of spinach grown: ----------

IV. Sources of seeds: a) Own ----------b) Neighbor ----------

c) Market---------- d) Agro-shop----------

V. Other crops grown on the farm ----------

VI. What method(s) of field preparation do you practice? ----------

---

VII. Pre-season practices ----------

VIII. Do you mix spinach crop with other crops? (Yes) (No)

IX. If yes, with which crops? ----------

X. Do you practice crop rotation in spinach production? (Yes) (No)

XI. If yes, with what crops? ----------

XII. Do you use any soil amendments in spinach production? (Yes) (No)

XIII. If yes, which ones? ----------

XIV. What are the most common diseases of spinach in your spinach field? ----------

---

XV. What methods of pest and disease control do you employ? ----------

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XVI. (a) Do you know this disease? (Show farmer a photo of spinach leaf showing symptoms of Alternaria leaf spot) a) (Yes) (No)

(b) Occurrence- (Presence) or (Absence) in the farm

…….

XVII. What method(s) do you use to control it? ----------

XVIII. How do you handle the crop residues after harvest? ----------

---

XIX. Yield per harvest (kg)? ----------

XX. Disease incidence (%) of Alternaria leaf spot ----------

Other observations ----------

	Q_1	Q_2	Q_3
Incidence			

Appendix II : ANOVA table for the incidence from the different sites

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	1.06	0.53	0.01	
Sites	39	42814.84	1097.82	24.67	<.001
Error	78	3471.33	44.5		
Total	119	46287.23			

Appendix III: ANOVA table for the incidence from different sub-counties

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	1.1	0.5	0	
Sub-county	6	18362.7	3060.4	12.17	<.001
Error	111	27923.5	251.6		
Total	119	46287.2			

Appendix IV: ANOVA table for the altitude of the different sites surveyed

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	42	8641.5	205.8	1.15	0.3
Altitude	2	3307.3	1653.7	9.21	< 0.001
Error	75	13468.5	179.6		
Total	119	25417.3	213.6		

Appendix V: ANOVA table for the growth rate of the different isolates

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	0.00403	0.00201	0.17	
Isolate	22	5.13579	0.23345	20.24	<.001
Error	44	0.50757	0.01154		
Total	68	5.64739			

Appendix VI: ANOVA table for the sporulation of the different isolates

Source of variation	d.f.	s.s.	m.s.	v.r.
Replication	2	5.12E+04	2.56E+04	0.03
Isolate	22	4.22E+09	1.92E+08	238.45
Error	44	3.54E+07	8.05E+05	
Total	68	4.26E+09		

Appendix VII: ANOVA table for the % inhibition by the different plant extracts on *Alternaria alternata*

Solvent	DF	Tumeric M	Ginger M	Tumeric E	Ginger E	Tumeric W	Ginger W	Tumeric E.A	GingerEA
Concentration	2	484.65	518.5	658.12	513.27	125.97	1331.96	859.41	1643.06
Days	5	1144.74***	1046.41***	872.44***	403.9***	1048.86***	617.36***	1041.38***	595.43**
Total error	10	28.48	28.24	37.72	24.41	7.457	60.76	37.07	74.17
Total	17								

(*, **, ***) and ns=significant at ((P≤0.05, P≤0.01, P≤0.001) and not significant at (P≤0.05).

Appendix VIII: Similarity Index/Anti-Plagiarism Report

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