ANTIFUNGAL POTENTIAL OF PLANT BIOACTIVE COMPOUNDS, SULPHUR AND COPPER FORMULATIONS AGAINST *Alternaria brassicicola* (Schwein.) INFECTING KALES IN KIAMBU COUNTY, KENYA

BY

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DECLARATION

I, Victor Vedasto, affirm that this thesis is my original work and has not been presented for the award of a degree in any other university or any other award.

Signature Date 29/06/2025

Supervisors' approval

We confirm that the work reported in this thesis was carried out by the candidate under our supervision as university supervisors.

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DEDICATION

I dedicate this thesis to my mother and teachers in my academic background. This work is a product of your tireless material and non-material support to me. May the Almighty bless you abundantly.

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LIST OF ABBREVIATIONS AND ACRONYMS

ALS	Alternaria Leaf Spot
B.C	Before Christ
BLAST	Basic Local Alignment Search Tool
CRBD	Completely Randomized Block Design
DAP	Di Ammonium Phosphate
DHN	dihydroxy naphthalene
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine Tetra-Acetic Acid
G	grams
Ha	hectare
HCl	Hydrochloric acid
IDM	Integrated Diseases Management
ITS	Internal Transcribed Spacer
Kg	kilograms
Ksh	Kenyan shillings
KU	Kenyatta University
Lab	Laboratory
MEGA	Molecular Evolutionary Genetics Analysis
ml	Milliliters
MS	Mass spectrometry
NaCl	Sodium chloride
NCBI	National Centre for Biotechnology Information

NMR	Nuclear Magnetic Resonance
^{o}C	Centigrade or Celcius
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
pH	Measure of hydrogen concentration or power of hydrogen
WG	Wettable granule
WHO	World Health Organization

ABSTRACT

Kale is the most popular and majorly consumed green vegetable in both rural and urban settings in Kenya. It is cultivated by more than 90% of smallholder farmers in the country. One of the major biotic constraints hampering kale production in Kenya is Alternaria leaf spot (ALS) caused by *Alternaria brassicicola*. Most farmers manage the disease with the use of synthetic fungicides, which is not a sustainable approach. This study was conducted in Kiambu County to (i) determine the occurrence, prevalence, farmers' knowledge, and current management practices for ALS in kales; (ii) evaluate the cultural, morphological, and molecular features of *A. brassicicola* isolates infecting kales and (iii) evaluate efficacy of bioactive compounds present in *Jatropha curcas*, *Tephrosia vogelli, Persea americana, Cymbopogon citratus, Ocimum gratissimum,* and *Carica papaya* extracts and selected Sulphur commercial formulation (WETSULF® 80% w/w, THIOVIT® JET 80% w/w) and Copper (VIFRA® 40 WG, ISOCAP® 50WP) against *A. brassicicola* invitro. A survey was conducted in 180 kale fields, ninety (n=90) fields in Githunguri and (n=90) fields in the Lari sub-county, using a semi-structured questionnaire. The fresh kale leaves depicting the typical symptoms of ALS were collected, and 3-5 leaves from each plant were collected and transported to the Kenyatta University agriculture laboratory for analysis, where a total of 120 *A. brassicicola* isolates were obtained. The result revealed that ALS is prevalent in most kale fields (80-85%) in both sub-counties, and 57.2% of respondents manage the disease using fungicide; 45% of farmers reported the fungicide to be either less effective or ineffective. The other method used to manage the disease was the removal (pruning) of the infected leaves or plants by 33% of the total farmers. A test on farmers' knowledge of ALS and its management revealed that most respondents had knowledge ranging from low to medium. The cultural and morphological study of isolates revealed that most isolates were not significantly different from one another. When isolates were characterized at the molecular level, their sequences exhibited a low genetic diversity, and they fell into two Clades. The overall genetic distance (d) was 21%, while among Githunguri isolates it was 28% and 8% for Lari isolates. Other isolates were found to belong to other species of Alternaria, e.g. *A. alternata*, *A. altra*, and *A. aborescens,* suggesting that ALS in the study area is inflicted by not only *A. brassicicola* but also other Alternaria species. Among six plant species tested, the bioactive compounds from *J. curcas* (62.2% at 2000mg/ml, 69.9% at 4000mg/ml) followed by *T. vogelli* (60.3% at 2000mg/ml, 64.4% at 4000mg/ml) showed mycelial inhibition of $\geq$ 50 % at the 10thday after inoculation of *A. brassicicola.* The degree of mycelial inhibition at 4000mg/ml was significantly higher than at 2000mg/ml. Two sulphur formulations, THIOVIT JET 80W/W, which achieved pathogen growth inhibition by 68%, and WETSULF 80W/W by 64.5%, and only one Copper formulation, VIFRA 40WG (66%), were effective ($\geq$50%) against the pathogen at their manufacturer's recommended dosage levels. These findings suggest that bioactive compounds from *J. curcas* and *T. vogelli* are good candidates that can be considered in the commercial manufacture of control products for ALS. Most of the Copper and Sulphur formulations are effective against ALS and thus can be used by farmers as part of the Integrated Disease management strategy. For effective management of ALS, product manufacturers should take into account the genetic variability of *A. brassicicola* but also other Alternaria species that cause similar symptoms. Furthermore, there is a need to capacitate kale farmers on precise identification, early and sustainable management of ALS

CHAPTER ONE: INTRODUCTION

1.1 Background information

Kale, commonly known as Sukumawiki or *Brassica oleraceae var. acephala* D.C., is a cool-season crop in the Brassicaceae family (Swegarden, 2020). It is a hardy biennial with glaucous green or blue-green, frequently with finely split leaves, and an open growth style. The plant neither forms a head nor makes edible blooms; instead, the leaves grow upward from the rootstock. Kale cultivars come in a wide range of sizes and heights, as well as in the shape and colour of their leaves (Onyango and Onyango, 2002). Although there are many varieties of kale, only those with curly leaves are produced for human use. The majority of kale varieties bear a rosette of leaves at the top of the stem and are closely linked to wild cabbage (*Brassica oleracea var. capitata*). Kale can withstand both low temperatures of -15°C and high temperatures as well. Calcium, provitamin A, and vitamin C are all abundant in kale (Dikmetas et al., 2022; Zandstra *et al*., 2007). Kumar (2022) reported that the Asia Minor region (present-day Turkey) and the Eastern Mediterranean are places where kale, a leafy vegetable, originated. However, in other studies, the cultivation of the plant has been documented for generations, and it has been shown that people started to use it as food in 2000 B.C.

The cultivation of kale is easy and requires minimal effort. The ability to produce copious amounts of leaves that may be regularly harvested is another advantage of growing kale. However, this productivity is achieved when kale is properly cared for, such as by giving it enough water and nutrients. Due to lower production costs, which enable lower market

selling prices, it is affordable even for those with lower incomes. In a nutshell, kale is available and reasonably priced (Maina and Mwangi, 2008).

Over 90% of smallholder farmers cultivate kale and other brassica vegetables on plots of land that range from 1 to 2.5 ha. These contribute much to Kenya's internal economy (Rop et al., 2009). The majority of Kenya's kale output occurs in high elevations with consistent rainfall. They are significant local vegetable crops that are valued for their contribution to both health and nutrition as well as financial gain. It has been shown that kale has an anti-cancer effect and is responsible for good health. They are nutrient-rich in beta-carotene, vitamin C and fibre (Sanlier and Guler, 2018).

Kale production in Kenya is, however, constrained by diseases and pests. Several frequent diseases infect kale, including damping-off caused by *Pythium spp, Fusarium spp*, *Rhizoctonia solani,* and leaf spots due to *A. brassicicola* (Pscheidt and Ocamb, 2022; Rimmer et al., 2007; Iacomi et al., 2004). Thus, pests and diseases constrain kale production in Kenya. The principal disease among these is Alternaria leaf spot, which has a significant negative impact on both the quality and productivity of kale (Sabry et al. 2015; Komhormet et al., 2021; Rop et al., 2009).

1.2 Statement of the problem

Alternaria leaf spot is one of the most pervasive and damaging diseases that infects kale worldwide. Forty-five per cent of output can be lost as a result of the disease, particularly during the rainy season (Punyanobpharat et al., 2018). The disease results in significant losses of produce quantity and quality (AI-Lami et al., 2019). The losses in yield are usually attributed to reduced ability to synthesize carbohydrates, the quickening of senescence,

early pod shattering, and shrivelled seed (Ren et al., 2022). Although plant infection may mostly be cosmetic, crops with Alternaria leaf spots may be rejected by distributors and consumers. Even minor diseases can result in unmarketable crops. A drop in yield might occur as a result of leaf loss or weight loss from severe foliar disease. Unfortunately, synthetic fungicides are used to manage the disease; however, they pose risks to people and the environment and are frequently expensive to purchase by farmers (Komhorm, 2021). For instance WHO revealed that 0.75 million people become ill from pesticide poisoning each year (Gunnell et al., 2007). Furthermore, new physiological races of Alternaria species have been emerging as a result of the ineffectiveness of some fungicides due to pathogen resistance (Ferreira et al., 2006). Therefore, this shows that relying on synthetic fungicides by farmers in the management of ALS causes more negative effects on humans and the environment.

1.3 Justification of the study

Although the use of synthetic fungicides in plant disease control has been successful in improving agricultural output, several of these have been found to exhibit side effects in the form of carcinogenicity, detrimental effects and other residual toxicities (*Yazdani et al., 2011).* Several sustainable approaches for the management of Alternaria leaf spot have been proposed. One of the possible options, therefore, could be the use of plant bioactive compounds or botanicals, which are found to be largely nonphytotoxic, systemic and easily biodegradable. Botanical pesticides have been used for a long time in crop protection against insect pests and pathogenic microorganisms (Saxena et al., 2014; Lengai et al., 2016).

According to Choudhury (2018), plants have the natural ability to defend themselves against different enemies that surround them because they can secrete several metabolites such as phenols, terpenes, sulphur, and nitrogen compounds. The use of plant extracts has enabled new methods for pathogen management that secure the production of high-quality food. In several studies, it has been documented that pesticidal plants contain numerous bioactive components that can control fungal growth. Yazdani et al. (2011) reported that plant-derived compounds are regarded as a substantial source for novel lead structures to develop medicines and biocides, natural products.

Also, Sulphur and copper formulations have been reported to be safer fungicide formulations for humans and the environment and are even permitted for use in organic farming. However, there is a lack of information on the potential of bioactive compounds found in *J. curcas*, *Cymbopogon citratus*, *T. vogelli*, *P. americana*, *C. Papaya* and *Ocimum gratissium* to control the disease. Also, the effectiveness of Sulphur and Copper formulations commercialized in Kenya against ALS has not been investigated in the management of *A. brassicicola*. Therefore, the current study aimed to examine the antifungal potential of plant bioactive compounds and some selected Sulphur and copper formulations registered in Kenya against *A. brassicicola* as a sustainable way to manage Alternaria leaf spot disease.

1.4 Objectives

1.4.1 General objective

To contribute to sustainable kale production in Kenya through the utilization of less toxic, environment-friendly options to manage Alternaria leaf spot disease.

1.4.2 Specific objectives

i. To determine the occurrence, farmers' knowledge and current management practices for Alternaria leaf spot in kales in Kiambu County.
ii. To isolate, purify and characterize *A. brassicicola* isolates causing Alternaria leaf spot disease and determine genetic diversity (physiological races) of the pathogen infecting kales in Kiambu county.
iii. In vitro evaluation of bioactive compounds from selected plant species and sulphur-containing fungicides against *A. brassicicola* infecting kales in Kiambu county.

1.5 Hypotheses of the study

i. ALS is not prevalent in the study area, and there is no difference in farmers' knowledge level on ALS disease and its management

ii. The *A. brassicicola* isolates in Kiambu County do not differ significantly in cultural, morphological and molecular characteristics.

iii. The selected plant species, Cu and S formulations, are not effective against A. brassicicola infecting kales in Kiambu county

1.6 Conceptual framework

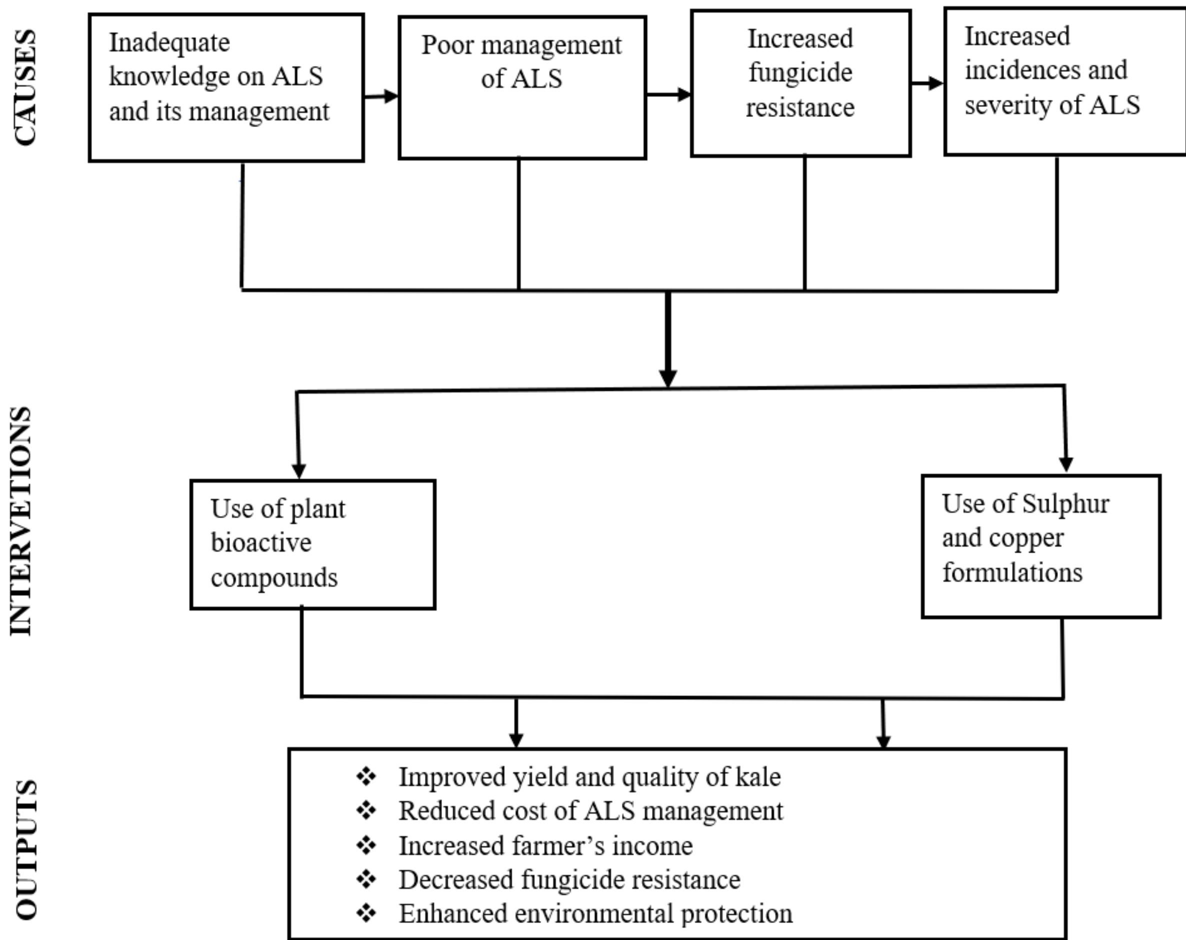


Figure 1.1: Conceptual framework

1.7 Significance of the Study

The current study has established the kale farmers' knowledge level, occurrence, prevalence, importance and current management practices of ALS. The study has shed light on the genetic diversity of the pathogen causing ALS in kale. The study has also established the botanicals with effective bioactive compounds against *A. brassicicola*. The identified effective plant and its associated bioactive compounds can be utilized to formulate products

at a large scale for the effective management of ALS. The chemical structures of the bioactive compounds can be studied and used to synthesize less toxic products for ALS control. Moreover, the study has established the efficacy of Sulphur and Copper-containing pesticides registered in Kenya against ALS as a more environmentally friendly chemical for crop production when compared to conventional fungicides

CHAPTER TWO: LITERATURE REVIEW

2.1 General information about Kale

2.1.1 Kale botany and varieties

Kale belongs to the Cruciferae family, which is currently known as the Brassicaceae family. The family is made up of several different vegetables, such as turnips, cauliflower, and broccoli. The term "kale" is Scottish and comes from the Latin and Greek words "coles" and "caulis." All plants that had a cabbage-like appearance were referred to by this term. Kale refers to several *Brassica oleracea* species, including sabellica, medullosa, costata, and Viridis. E, in both classic and contemporary literature, *Brassica oleracea ssp*. Kale has occasionally been referred to as acephala (Sharma et al., 2004; Dixon, 2007; Yamaguchi, 2012).

The term "kale" is applied to plants that do not produce a head. It includes various varieties of this leafy, green vegetable. The leaves of kale plants develop along a stalk. The plants have a wide range of appearances, including flat or crinkled leaves that are green, red, or tinted with purple (Šamec et al., 2019; Singh and Devi, 2015).

Studies on kale have reported that three kale cultivars can be distinguished; these include curly kales, Italian kales, Russian kales and collards (Figure 1). The most popular known cultivar in Europe, especially in Northern Europe, is the curly cultivar (*Brassica oleracea* convar. *acephala* var. *sabellica)* also known as the Scotch type. The second variety is Italian kale (*Brassica oleracea* convar. *acephala* var. *palmifolia*), also known as

Lacinato type. This variety is mainly cultivated in Tuscany, but was traditionally cultivated in Italian cuisine. Collards (*Brassica oleracea* convar. *acephala* var. *viridis*) are mainly cultivated in the United States, mainly in the Southern part of the country. Collards are characterized by a high resemblance to wild and feral cabbages, also known as "herbs of big leaves". The leaves of the collards are larger but also flat-roundish in shape. Another variety is Russian kale (Siberian type, *B. napus* var. *pabularia*, This variety is believed to have originated from the domestication of different species which are closely related (Christensen *et al*., 2011; Branca *et al*., 2012; Pelc *et al*., 2015; Hahn et al, 2016).

Figure 2.1: Different cultivars/varieties of kale

Source: (Hahn *et al*., 2022)

Cultivars (a) Curly variety/Scotch type (b) Italian/Lacinato kale type (c) Collards (d)Russian /Siberian kale type

2.1.2 History and origin of Kale

Kale originated in the eastern Mediterranean and Anatolia, where it was grown for food beginning in 2000 B.C. (Perry, 2018). Whereas, Curly-leaved varieties of cabbage were present together with flat-leaved varieties in Greece in the 4th century BC. Therefore, these varieties were named Sabellian kale by the Romans and also are considered to be the origin of modern kale (Perry, 2018). According to Kumar et al. (2022), the *Brassica overeager* family is thought to have descended from progenitors related to wild kale varieties.

Molecular analyses indicated that kale was supposed to be the first member of this group to be domesticated, and it eventually gave rise to other varieties like cabbage and broccoli.

Kale is shown as one of the "older" cabbages, and it is considered to originate from the wild cabbages. In America, kale arrived in the 1600s after Canada (Dixon, 2007). Due to its ease of cultivation, low cost, and ability to remove salt from the soil, kale was already a common crop in Croatia by that time. In the United States, kale was primarily utilized for decorations for the majority of the 20th century; but, in the 1990s, its nutritional content and concentration caused it to become a more well-known vegetable (Luczaj, 2012). The campaign known as Dig for Victory encouraged people to grow kale and other vegetables during the Second World War. This was because the vegetable was able to provide the missing nutrients in a normal diet due to rationing (Ginn, 2012). Kale in Europe is also known as a European vegetable and is still being grown and used widely in most European nations, including Scotland, Ireland, Germany, Netherlands, Italy and Sweden. While in France, it is a decorative element on the sides of roadways (Dixon, 2007).

In Kenya, kale was first brought by Europeans as a fodder crop when they arrived in the Kenya Highlands, and it has since become a significant vegetable with high demand. They have become staple foods amongst all categories of income groups in Kenya. Kale leaves are used to make stews of all kinds and are consumed as green vegetables together with other dishes, particularly grains. Kale and collard leaves have significant nutritional content compared to other vegetables in the Brassica family. The leaf yields produced in Kenya are still incredibly low, averaging 15 tons per hectare (Onyango and Onyango, 2002; Omiti et al., 2009).

2.1.3 Kales production in Kenya

In Kenya, kale is commonly known as Sukuma wiki, and several varieties are being produced (Onyango and Onyango, 2002; Wiersinga and de Jager, 2007). The most popular kale variety in Kenya right now is the thousand-headed kind. It is more productive than any other variety of kale in Kenya, yielding up to 37,500 kg/ ha and thus is highly preferred by farmers (Yegon, 2019). Other varieties being grown in Kenya are Tausi F1, Ethiopian kale kanzira, and Mfalme F1. Marrow stems and Moss Curled Kale are other varieties not extensively grown. When compared to other vegetables in Kenya and Uganda, kale is the most widely consumed vegetable. Most people prefer to serve it with ugali more than once a week. Smallholder farmers are the ones who widely grow kale (Owuor, 2006). Kale and collards are mostly grown in Kenya's Central Highlands for domestic use. Commercial farming is practised in Kiambu, which is close to Nairobi, and Nyandarua, which is close to Aberdare.

Woomer and Imbumi (2003) have further reported that kale is a vegetable that is produced in Kenya in tiny garden plots on practically all rural farms, along the sides of public roads, and in peri-urban and metropolitan areas. It can even be found in Nairobi, the largest metropolitan area in East Africa, with a population of around 3.75 million, along major thoroughfares and in a few isolated areas of open space. Even when they have no land, low-income families frequently grow and eat kale as their primary vegetable. When the product is for the export market, multinational grocery chains in the US, UK, and Netherlands control the major markets for kale from Kenya. Maintaining minimal residual levels is the driving force behind this direct involvement, particularly in restrictive countries like the US. These retailers make direct investments with farmers, primarily in

Central Kenya, to procure kale. For example, Sainsbury from England has a program for the supply of kale in the country's central regions. The authorities in Nyeri, which is located between Mount Kenya and the Aberdare Mountains, signed a contract with UK-based trading partners in 2016 to provide Mount Kenya with kale and collard greens (Krishnan, 2018; Matsaba et al., 2021).

2.1.4 Ecological conditions for Kale production

Kale performs well at low temperatures between 5-21°C, according to Qizilbash, (2015), it is the most well-known cool-season crop. It thrives in a variety of ecological situations and can endure frost and slightly alkaline soil, but it performs best in fertile soil with good drainage, a high organic matter content, and an ideal pH range of 6.0 to 7.5. 6 hours or more per day must be spent in direct sunshine. It does well in high and consistently moist soils however few varieties of kales do tolerate drought. In terms of rainfall requirements, the kale plant needs sufficient levels of moisture all year round. For the best crop yield, a rainfall of about 30-500mm annually is sufficient.

However, the crop will need to be irrigated once the rainfall becomes inadequate (Owino, 2019). On the other hand, Rodríguez et al. (2015), reported that temperatures between 4 to 21°C are ideal for kale plant growth. Kale plants this is the crop to be cultivated in both spring and autumn. It was added that kale is termed a hardy plant due to its ability to tolerate frost. Between 12 and 24°C is the ideal soil temperature for germination. After the threat of a hard frost has passed, kale seedlings need to be planted outdoors or in a cold frame for transplanting in the future. Planning the seeding process will ensure that the plants mature in cold conditions in time for the autumn harvest. The best time for seeding kale in warmer Southern regions is July. Soil for planting can be prepared by adding nitrogen sourced from

composted manure or bone meal. Kale seeds need to be planted at a spacing of 3.5 cm between each planting depth should be 12mm, and the final interrow spacing of between 45 and 60 cm is recommended (Singh and Devi, 2015; Hanna, 2015).

2.1.5 Agronomic requirements of Kale

According to Jagadeesh et al. (2022), kale is managed in the same ways as any other brassica crop. It is advised to alternate them with crops other than those from the Brassicaceae family and to transplant them on the same soil every three years. Nitrogen is assimilated quite considerably by Brassica plants. Therefore, the fundamental method of soil preparation entails deep ploughing and the heavy application of organic manure. Nitrogen fertilizer treatment improves vegetative development in kale crops and delays the onset of untimely senescence. In most cases, kale plants are propagated from seed. The seedlings initially form strong taproots and are followed by numerous lateral roots.

Planting seedlings at the end of summer or the beginning of autumn is typical planting time in warmer climates. Early bolting, which is triggered by low temperatures that encourage vernalization, leads to the production of palatable leaves throughout the winter. Some varieties may also be planted in the spring for a summer or autumn harvest (Haghighi et al., 2025). In Europe, Asia, and the United States, kale is widely consumed, and its popularity has recently increased. This could be because it is affordable, easy to cultivate, and tolerant of unfavourable weather conditions (increased salinity, drought, extreme temperature swings, etc.) Harvesting usually begins when the leaves reach the ideal size and level of softness.

2.2 Importance of Kales

2.2.1 Economic importance of Kales

Kale production plays an important role in improving both a country's and an individual farmer's economy (Lenné et al., 2005). According to Gallaher et al. (2013), kale has become the most demanded leaf vegetable in Kenya, and it is now regarded as a staple vegetable crop by almost all people of different groups. People eat kale leaves as green vegetables or use them to prepare stews of varied types again they are also used with other foods, such as grain foods. Nutritionally, the kale leaves and collards tend to have a higher composition of antioxidants compared to other plants from the genus Brassica (Wanjiku and Kimenye, 2007).

According to Beatrice and Kees (2010), kale production is a key source of income for many Kenyan communities. For instance, kale was found to be the crop that contributed the most to household income in a survey of households in the Kiambu area. Also, it was reported by Vilar et al. (2008) that kale is an all-year crop; therefore, it plays a very significant role in minimizing poverty levels, especially for most small-scale farmers.

2.2.2 Nutritional importance of Kale

According to Swegarden et al. (2020), kale is a lower-calorie vegetable that is rich in vitamins, minerals, and phytochemicals. Due to the higher antioxidant content in kale than in other vegetables, kale has attracted more public attention in recent decades. Many phytochemicals from plants that are present in kale tissue can reduce the risk of chronic conditions such as diabetes, arthritis, cancer, and heart issues (Kaur and F., 2001; Houston and Harper, 2008; Zhang et al., 2012; Zhang et al., 2015; Kwak et al., 2016; Waterland et al., 2017; Walczyk et al., 2018; Makhaik et al., 2021).

In the worldwide superfood markets, organic kale leaves are economically necessary for making the powder that is employed to prepare nutritional supplements for many applications, including the food, beverage, pharmaceutical, and nutraceutical industries. To boost the nutritional value of processed food and beverages, such as smoothies and juices, kale powder is added. Furthermore, because of its excellent functional qualities, it is projected that the superfood industry will continue to see an enormous need for organic kale powder (Khalid *et al*., 2023; Subedi, 2023; Riar and Panesar, 2024).

Because of its many health benefits, top producers tend to include organic kale powder in their nutraceutical and nutritional supplement products. Several trace elements, including Ca^2+, K+, $Mg^{2+,}$ Fe^{2+}, Na^+, and Zn^{2+}, are also present in organic kale (Khalid *et al*., 2021; Khalid et al., 2022). It is well known that these compounds have antibacterial and anticarcinogenic properties. Moreover, plant lipids—among many other bioactive phytochemicals—have become prevalent and used as practical components in the creation of functional foods. These include medium-chain triglycerides, diglycerides, oleic acid, carotenoids, phytosterols, fatty alcohols, and polyunsaturated fatty acids (Szutowska et al., 2020; Das et al., 2022; Khalid et al., 2023).

Kale juice contains lutein, a xanthophyll compound that binds bile acids in the digestive system and prevents them from being reabsorbed. This reduces the buildup of cholesterol in the body, thus minimizing the risk of heart disease. Studies have indicated that kale juice helps to protect the heart disease, and one of the major heart disease risk factors is high cholesterol. Blood vessels may develop more fat deposits as a result, which will make it more difficult for blood to flow through arteries (Khalid et al., 2021). Also, kale juice was found to contribute to the reduction of body weight. For example, Boone (2013) showed

that juice made from kale is a great way to encourage weight loss. It reduces the appetite and can essentially eliminate the high need for food. The juice's high Sulphur content functions as a detoxifier and promotes fat burning. For this reason, health nuts favour kale juice as a post-workout beverage (Draelos, 2010).

Furthermore, due to the high calcium content present in kale, it helps in the formation of strong human bones. Also, Magnesium, which plays a role in the protection of the body against type 2 diabetes and heart disease, is a vital element contained in kale. The potassium contained in kale is crucial for keeping cells functioning properly. Kale has an anti-cancer activity, which is likely to be a result of glucosinolate breakdown products, which are also present in kale (Houston et al., 2008; Khalid et al., 2021).

2.3 Kale production constraints

2.3.1 Biotic constraints

The cabbage worm and cabbage looper are two caterpillars that are particularly drawn to the plant. Both like eating the plant's leaves and, if not eliminated, will quickly leave a plant bald. Aphids are another pest that attacks kale plants. The pests make kale plants deformed, stunted, and yellowed by sucking the juice out of the leaves (Berenbaum 1989, Nyabuga, 2004). Kale is also vulnerable to bacterial and fungal diseases, which can kill it (Damicone and Roberts, 2009). According to Singh Saharan et al. (2016), fungal infection is more frequent when conditions are wet. Alternaria leaf spot is one of the fungal infections on kale, which is characterized by the development of brown and grey spots which are surrounded by a chlorotic yellow halo on leaves.

According to Adhikari et al. (2024), reported that damping off disease mostly occurs in nursery soils and affects kale seeds and seedlings. *Fusarium spp, Pythium spp, and* Rhizoctonia *spp* are responsible for causing this disease. It prevents damaged seeds from germinating, but once a seedling becomes infected, it will rot and eventually die. On the roots of the diseased seedlings, white cottony growth is frequently observed. Root-knot nematodes also infect kales diminishing plant vigour, which results in crop stunting and eventual death (Tariq-Khan, 2017).

2.3.2 Abiotic constraints

Kale is a cool-season crop that thrives at temperatures between 18 and 24°C during the day, and between 4 and 7 °C during the night. High temperatures are unsuitable for kale. Therefore, early-season planting is recommended to avoid excessive temperatures during the seedling stage. Combinations of abiotic stressors sometimes result in outcomes that are more detrimental to productivity, growth, and consequently yield while also making plant management more difficult (Yadav et al., 2020).

Elevated CO_2 concentrations in crops have been reported to have a substantial direct impact on plant development and crop productivity. There have been a few direct effects observed on kale plants. Whereas in C3 plants, it has been found that an increase in CO_2 content results in a considerable rise in yield. However, insufficient research has been done to accurately anticipate future crop yield, much less the interplay of increasing CO_2, temperature, and UV-B. (Allen and Boote, 2000; Reddy and Hodges, 2000).

2.4 Alternaria Leaf Spot disease in Kales

Alternaria leaf spot in brassicas is reported to be caused by various Alternaria species. Kale, being among the brassica crops, is also affected by ALS. This disease is reported to be caused by *A. brassicicola, a* necrotrophic plant fungus that belongs to the order Dothideomycetes, class Pleosporales and family Pleosporaceae. The pathogen survives best in cool seasons since it prefers the extended period of leaf wetness. For new infections to occur, the temperature of between 13-24 ^{0}C. But also, the spores are released by the pathogen when the temperature is between 20-30 °C and the relative humidity of ~87%. These spores are then released in warm periods immediately after it rains. The disease is characterized by tiny black spots that develop on leaves, stems, and heads, which enlarge into huge lesions with distinctive concentric rings (Scheufele, 2013). Poursafar (2020) reported that tiny, dark spots on leaves change from brown to grey; Dark brown elongated lesions may occur on stems and petioles. Lesions may form concentric rings, have a purple-black edge, and crack in the centre. The leaf spots formed always appear round, grey to black, a little too large as a quarter, and frequently with a target pattern are leaf spots (Figure 2.2)

Figure 2.2: Kale foliage affected by Alternaria leaf spot

(Source: Victor Ngaiza)

When the pathogen releases a lot of its dark spores, the spots may appear velvety. Due to their smaller size compared to the spores produced by the pathogens that cause powdery and downy mildew, these spores are carried by the wind, but they normally do not travel very far. Spread is therefore uncommon among farms that are near but not neighbouring. Leaf spots typically become papery, dry, and brittle as they get older (Saharan et al., 2012; Kalayanamitra et al., 2023).

Furthermore, Sharma (2012) reported that it is crucial to destroy crops as soon as possible after harvesting, since this disease might live in crop waste. Crop waste's breakdown will be accelerated by breaking it into small pieces and adding it to the soil. An optimal rotation is three years. According to Javidan et al. (2023), leaf spot diseases cause a significant reduction in yield quantity and quality. The losses include diminished photosynthetic potential, rapid senescence, early pod shatter, and shrivelled seed as reported by Mohamed

(2015). Jagadeesh et al. (2022) reported that Alternaria leaf spot disease has significant economic consequences on a global scale and can occasionally cause yield reductions of 20–50% in crops like kale. Moreover, Saharan et al. (2016) reported that Alternaria leaf spots or black spots inflicted by Alternaria species affect leaves by decaying lesions, commonly characterized as being sooty and black, having chlorotic yellow haloes encircling the lesion sites.

The black necrotic lesions on seedlings, stems, leaves, and siliquae with encircling chlorotic areas are typical symptoms detected in the diseased kale plant, which can be afflicted at any stage of growth (Rop et al., 2012). Additionally, Alternaria black spot disease was cited by Spence et al. (2005) as one of the primary causes of Kenya's low yields of brassicas, particularly kale. An example of various ALS incidence levels recorded at different agroecological zones in Kenya is shown in Table 2.1.

Table 2.1: The mean ALS disease incidences at different altitudes, temperatures, and rainfall, agro-ecological zone for the disease survey conducted in Kenya

Province	Division	Altitude (m) a.s.l	Agroecological zone (AEZ)	Rainfall (mm)	Temperature (^{0}C)	Kale cultivar	Alternaria species	Disease incidence
Central	Lari	2387	UH1	1200-1600	13.5-15.2	LPS	*A. japonica*	30.3
Central	Lari	2396	UH1	1200-1600	13.5- 15.2	LPS	*A. brassicicola*	23.0
Central	Njabini	2676	UH1	1150-1600	10.0-14.6	LPS	*A. japonica*	9.3
Central	Njabini	2682	UH1	1150-1600	10.0-14.6	LPS	*A. brassicicola*	25.0
Central	Njabini	2704	UH1	1150-1600	10.0 -14.6	LPS	*A. japonica*	11.5
Central	Kinangop	2666	UH1	1150-1600	15.2-18.0	LPS	*A. japonica*	19.5
Eastern	Kibirichia	2277	LH1	1300-1500	21.4-22.3	THK	*A. japonica*	18.8
Nyanza	Central	1805	UM1	1400-2100	21.4-22.3	LPU	*A. japonica*	20.0
Nyanza	Kanemo	1282	LM2	1450-1600	21.4-22.3	LPU	*A. japonica*	16.3
Nyanza	Ugunja	1238	LM2	1450-1600	21.4-22.3	LPU	*A. japonica*	19.6
Nyanza	Ugunja	1241	LM2	1450-1600	21.4-22.3	LPU	*A. japonica*	18.3
Nyanza	Ugunja	1244	LM2	1450-1600	21.4-22.3	LPU	*A. japonica*	19.3
Nyanza	Ukwala	1207	LM2	1450-1600	21.4-22.3	LPU	*A. japonica*	14.8
Nyanza	Wagu	1374	LM1	1500-1900	21.4- 22.3	LPU	*A. japonica*	22.3

Nyanza	Ugunja	1313	LM2	1450-1600	21.4-22.3	LPU	*A. japonica*	19.5
Nyanza	Wagai	1312	LM2	1450-1600	21.4- 22.3	LPU	*A. japonica*	12.0
Western	Soy	2143	LH3	900-1100	15.1-17.9	CLRD	*A. brassicicola*	10.0
Rift Valley	Kapsakwoky	1961	LH2	1300-1700	16.4-18.8	LPU	*A. brassicicola*	22.0
Mean								17.9

Key: Agroecological zones: UH1= Sheep dairy zone, LH1= Tea dairy zone, UM1 =Coffee-tea zone, LM1 = Maize-sugarcane zone, LM2 =Marginal sugarcane zone, LH2 = Wheat-maize-pyrethrum LH3= Wheat-barley zone Kale varieties: LPU- Local kale propagated from suckers, LPS-Local kale propagated from seeds THK-Thousand headed kale, CLRD-Collards Source: Modified from Rop *et al*. (2009)

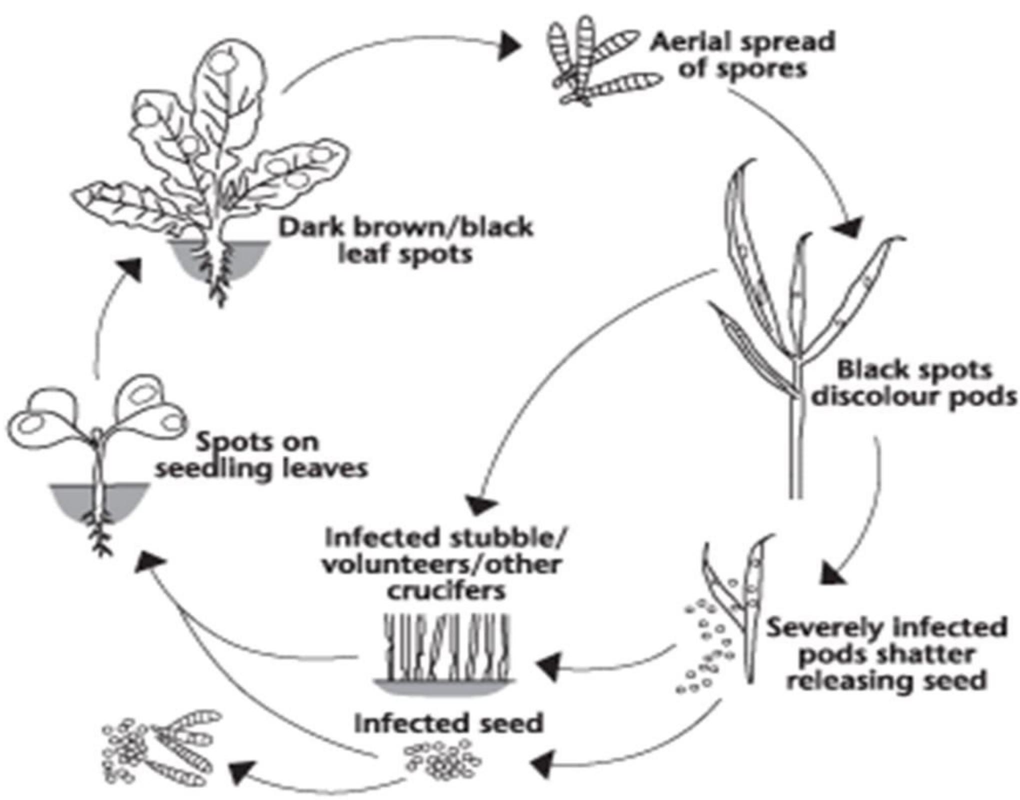


Figure 2.3: Life cycle of *Alternaria brassicicola*

(Source: Macioszek *et al*., 2018)

2.5 Pathogenesis and infection of *A. brassicicola*

According to Nowicki (2012), the four main sources of the infection are locally contaminated seeds, spores from plant debris in the topsoil, brassica weeds, and spores carried further by wind and air. Spores from infected plants can be spread to a maximum diameter of 1800 meters. Additionally, there are three main entryways into the host cell: penetration via the epidermis, through the stoma, and by an insect. The spores induce the release of several cell wall-degrading enzymes when they come into contact with the host cell, allowing the fungus to establish itself in the plant and begin destroying plant cells.

Amein (2011) and Nowicki (2012), added that the suggested mode of attack involves host-specific toxins, mainly AB toxins, which result in cell death by apoptosis. As a result, the host plant develops what appear to be dents and lesions. These are usually between 0.5 and 2.5 cm in diameter, brown, concentric circles with a yellow tinge around the edge. Necrosis usually appears 48 hours after infection. The mycelium of infected seeds can pierce the seed covering, and then it can remain viable for several years, in addition to living on the external seed coat where the spores can stay also reported that in some cases, it can even enter the embryo tissue of a seed (Cho, 2015, Macioszek et al., 2018).

However, all plant components, including pods, seeds, and stems, are susceptible to infection by *A. brassicicola*, which is particularly important as a post-harvest disease. It is not just confined to infecting leaves (Cramer, 2004). According to research by Kim (2009), *A. brassicicola,* like other Alternaria species, is a necrotrophic (death-causing) plant pathogenic fungus that secretes a wide range of poisonous secondary metabolites and proteins that kill cells either by directly harming cells or by inducing apoptosis in plants.

Spores are primarily transmitted through infected seeds (Nallathambi et al., 2020). The infection can spread widely and can induce seedling damping down at a rather early stage. It is not restricted to any particular region of the host plant (Macioszek et al., 2018; Nowicki, 2012). Additionally, it influences the host species at different phases of development. As was already indicated, seedlings have dark stem lesions that are followed by damping off. Older plants may have velvety, black blotches that resemble soot (Meena et al., 2016). Temperature, humidity, pH, reactive oxygen species, and host defence molecules all have an impact on the pathogenesis of *A. brassicicola (*Nowicki, 2012).

2.6 Cultural and morphological characteristics of *A. brassicicola*

According to Simmons (2007), the conidia are dark brown. They have smooth walls and can measure up to 60 x 14 µm. They produce muriform conidia that can be oblong or cylinder-shaped and are generated in chains of 8 to 10 spores (Meena et al., 2016). They are securely linked to conidiophores that can range in length from 100 to 200 µm and are olive-brown, septate, and growing.

Conidia normally develop in a continuous, chainlike structure, although it has also been o bserved that can branch at the base (Simmons, 2007). The mycelium has a variety of colours on the macroscopic level. When it is young, it is unpigmented, changing as it ages to an olive-grey, then a grey-black colour. The colonies of *A. brassicicola* are frequently black or dark brown (Köhl et al., 2010; Saha et al., 2016). Oliver (2024) also revealed that one factor contributing to the pathogen's lesions' dark appearance is the fungus's prodigious synthesis of chains of its dark spores. The pathogen transmits the disease to the kale present in the field, but also infects crop seeds, which ensures the pathogen's survival between crop cycles. A study by Hazowary et al. (2023) revealed that the isolates of A. brassicicola did not exhibit significant differences in pathogenic behaviour based on the assessment of the cultural and morphological variants.

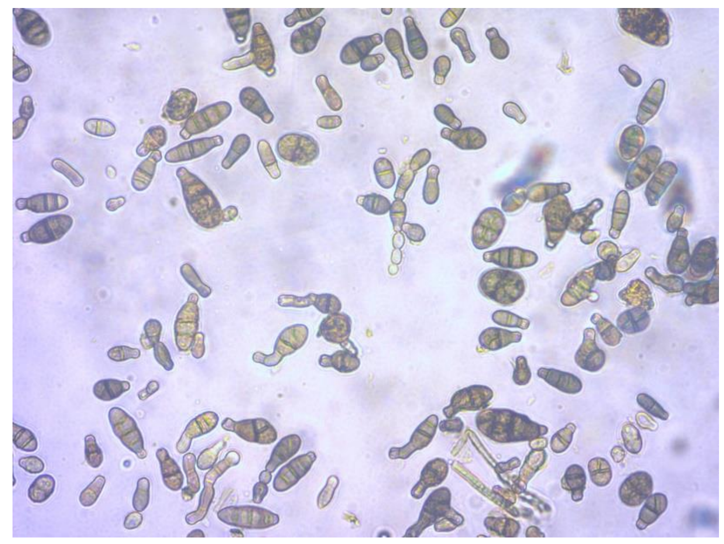


Figure 2.4: Conidia of Alternaria brassicicola

(Source: Victor Ngaiza)

2.7 Molecular characteristics and variability of *A. brassicicola*

Alternaria brassicicola species exhibit high levels of genetic variations (Van der Waals et al., 2004; Linde et al., 2010). Bock et al. (2002) analysed 18 samples from different locations and revealed that the pathogen had modest levels of genetic diversity along the New South Wales coast. Forty-three (43) out of 66 markers, 16.7–27.9% detected for each primer combination, were polymorphic, and the majority of isolates were identified as unique genotypes. Nei's measure of genetic distance and physical distance had a strong positive correlation ($r = 0.5486$, $p = 0.001$), and many isolates from particular areas tended to cluster together, indicating the possibility of population structure. *Alternaria brassicicola* isolates were subjected to a molecular study in Kenya by Reuben (2021), which uncovered a significant link between the various strains of *A. brassicicola* and *A. brassicae* and *A. alternata.* However, there is no research yet in East Africa on the genetic diversity of the *A. brassicicola* pathogen.

2.8 Management of Alternaria Leaf Spot disease in Kales

Alternaria Leaf spots in kale are mainly managed using fungicides (Jagadeesh et al., 2022; Ngaiza et al., 2024). These fungicides are sprayed directly on infected plants; they include active ingredients like chlorothalonil, fludioxonil, imazalil, iprodione, maneb, mancozeb, and thiram. The integrated management of the disease also includes improvement in sanitation and agricultural rotation, which are utilized to stop further outbreaks (Abbas et al., 2022).

Also, fungicides are used to treat seeds before planting. This has been reported by Nowicki *et al*. (2012) reported that many farmers pre-treat their seeds with fungicides to safeguard their crops. Iprodione and strobilurins, however, are the most frequently used active components in these fungicides. Iprodione most likely has two histidine residues in the enzyme target site that are affected. It ultimately prevents the formation of the germ tube. However, the fungus has become more resilient as a result of the widespread use of fungicides (Macioszek et al., 2018).

When applied soon after planting, mulch can aid in slowing down the spread of Alternaria spores already present in the soil (Altieri and Koohafkan, 2008; Gupta and Devi, 2014). Scheufele (2013) showed that black plastic or biodegradable plastic mulches were shown to be significantly less effective at controlling ALS than straw mulches, and mulched kale crops reported fewer and less severe problems with the disease than the control plants. Additionally, compared to other plants in the trial, the plants with straw mulch grew substantially taller.

Additionally, Fernandes et al. (2023) reported that although many Alternaria fungal diseases have similar symptoms, the fungus that causes them is typically very specialized in the types of plants that it may infect; therefore, crop rotation is crucial to avoiding Alternaria fungal spores from developing in the soil. Gardens that rotate their crops every four years can avoid Alternaria accumulating in the soil. The count of spores in the soil will also be minimized by quickly clearing away fallen leaves and dead plants. The damage caused by Alternaria is typically less severe in healthy, well-spaced plants than in their too-stressed relatives.

2.9 Use of plant bioactive compounds in the management of plant diseases

Plants tend to secrete secondary metabolites (SMs), also known as bioactive compounds such as phenols, terpenes, sulphur, and nitrogen compounds, that they can use to defend themselves against different enemies, including pathogens and insect pests (Parveez et al., 2009; Tapwal et al., 2011; Gahukar, 2016; Shuping and Eloff, 2017; Choudhury, 2018; Deshmukh et al., 2020). The presence of these compounds in plants has triggered extensive research on studying the bioactive compounds contained in various plant species and their efficacy once extracted and artificially utilized to manage pests and diseases in the laboratory, greenhouses and open field conditions (Arzoo et al., 2013; Anan and Athinuwat, 2016; Chohan et al., 2019). The use of plant bioactive compounds has enabled the implementation of a new method for pathogen management that secures the production of high-quality food (Agale et al., 2020). In several studies, it has been documented that pesticidal plants contain sundry bioactive components that can control fungal growth. Due to this ability, various plant species have been able to commercially synthesize products used in plant protection. For instance, Yazdani et al. (2011) reported that plant-derived

compounds are regarded as a substantial source for novel lead structures to develop medicines and biocides, natural products.

According to Wink (2006), more than 50,000 structures have been identified in plants by NMR, MS and X-ray analysis. However, as only less than 20% of all plants have been studied, likely that the actual number of secondary metabolites (SMs) or bioactive compounds in the plant kingdom would exceed 100,000 structures. SMs are produced in specific pathways, and the sites of synthesis can differ between types of compounds and between plant species

Several studies have reported the efficacy of plant bioactive compounds against plant pathogenic fungi. For instance, Tzortzakis and Economakis (2007) investigated the antifungal activity of lemon grass (*Cymbopogon citratus*) oil against *Colletotrichum coccodes*, *Botrytis cinerea*, *Cladosporium herbarium* and *Rhizopus stolonifera* and found that fungal spore production was inhibited up to 70 to 100% at 25 to 500 ppm of lemongrass oil concentration. A study by Ranasinghe et al. (2002) reported that essential oils of *Cinnamomum zeylanicum* and *Syzygium aromaticum* at concentrations of 0.03 to 0.11% (v/v) exhibited strong antifungal activity against *F. proliferatum*, *Lasiodiplodia theobromae* and *Colletotrichum musae*, the causal agents responsible for crown rot and anthracnose of banana.

Another study investigated the inhibitory effect of essential oil from *Satureja hortensis* against *Aspergillus parasiticus* as an aflatoxin producer. The compounds, carvacrol and thymol, were able to significantly inhibit fungal growth and AFB1 and AFG1 production at concentrations from 0.041 to 1.32 mM (Razzaghi-Abyaneh et al. 2008).

Another study found that *A. vera* leaf pulp at 1 to 105 µl/l had a good inhibitory effect on four postharvest fruit pathogens *Penicillium digitatum*, *Penicillium expansum*, *Botrytis cinerea* and *Alternaria alternata* (Shahbaz et al., 2022).

2.10 Use of sulphur and copper fungicides in the management of leaf spot disease

The oldest pesticide still in use is probably sulphur. Homer, a Greek poet who lived 3,000 years ago, wrote about the advantages of "pest-averting sulphur (Mathews, 2018). Either elemental sulfur or a lime-sulfur combination is utilized as inorganic Sulphur. Elemental sulphur can be found as dust or as a wettable powder; the latter is more frequently employed. However, chemical substances known as dithiocarbamates are the most widely used fungicides containing sulphur. Sulphur-containing fungicides function by inhibiting the fungal spores from germinating; hence, they must ideally be used before the development of disease for it to be most effective (Elagamey et al., 2023).

Generally, sulphur is regarded as a safe element. It is not known to pose any substantial dangers to people, animals, birds, or the environment, and it has low toxicity (Ataei, 2023).

It has been very challenging to find a pesticide that is safe to use because many of them are toxic to wildlife and helpful pollinators. Fortunately, fish, birds, and honeybees are unaffected by Sulphur (Anju et al., 2010). Since Sulphur occurs naturally in the environment, once in the soil will decompose and reintegrate into the earth's regular sulphur cycle. Again, Sulphur does not dissolve in water, so water runoff may not be a problem (Rickard and Luther, 2007).

In Kenya, several sulphur-based fungi have been registered by the Pesticide Control Products Board (PCPB) to be used by farmers. Among those pesticides, COSAVET® DF

– (Sulphur 80% w/w), BRAND SULPHUR® 99% w/w and SULGRO® 80 WDG are meant to manage the leaf spots and powdery mildew.

One of the first copper-based fungicides ever used was Bordeaux, a solution created by mixing copper sulfate and calcium hydroxide with water (Pscheidt and Ocamb, 2022). Cu-containing fungicides are still useful in pest management programs because of their wide range of applications, resilience to recurring wet weather events, and affordability. Cu-containing fungicides function by killing harmful organisms in plant leaves by "denaturing enzymes and other critical proteins, which kill the pathogen cells. According to Zambolim (2016), a wide range of activity, durability in the face of repeated wet weather events, and affordability make the Cu-containing fungicides still useful in pest management programs. Copper fungicides such as the Bordeaux mixture have been accepted and used in organic farming in several countries (van Bruggen et al., 2016).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

The survey was conducted in Kiambu County, Lari and Githunguri sub-counties. According to Wawira (2016), the Lari sub-county is largely mountainous, located between 0°50’ and 1°40’ S and 36°35’ and 36°43’ E. The landscape is divided into two agroecological zones, the lower and the upper highland zones, with altitudes varying from 1760m above sea level in the lower zone to 2610m a.s.l. in the upper zone. At an altitude of 1,400m above sea level, rainfall varies from 700mm in the low altitude zone to 1400mm in the upper zone (Wawira, 2016).

Also, Wawira (2016) reports that rainfall is bimodal. The long rains typically occur between mid-March and May, which is followed by a cold period with light rain falling in extremely fine drops and frost from June to August. The short-term rains typically occur between mid-October and November and average 1600mm annually. The temperature range is 13.8-25.8ºC. Soils in the landscape are highly fertile, very deep, well-drained, dark reddish brown, strongly calcareous and saline in many places. The soils have high organic carbon content (3-4%), which reflects the high level of applied organic matter, and low nitrogen, while phosphorus levels remain average (Makokha et al., 2011). Githunguri is an agricultural sub-county in Kiambu county, Kenya, which is geographically located at 1° 3’ 31.08” S 36° 46’ 40.48” E with an elevation of 1979.37 meters (6494.0 feet) above sea level. Githunguri is characterized by a Marine west coast (Classification: Cfb). The sub-county’s yearly average temperature is 20.4ºC. Githunguri typically receives about 127.13 millimetres of precipitation and has 209.83 rainy days (57.49% of the time) annually (Gathaku, 2023).

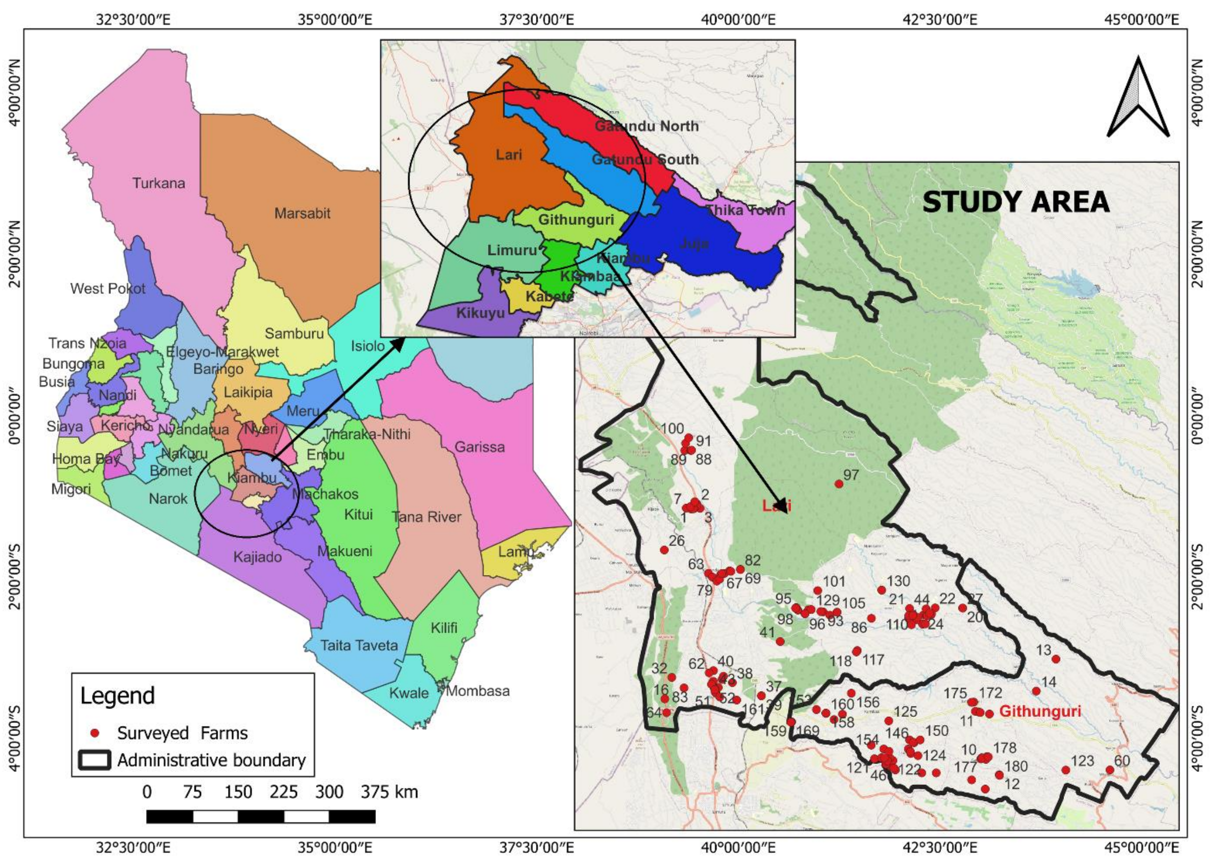


Figure 3.1: Map of Kenya showing the study sub-counties in Kiambu County and the surveyed farms

3.2 Occurrence, Prevalence, Farmers' Knowledge and Current Management Strategies for Alternaria Leaf Spot in Kiambu County

Kiambu County was selected for this study due to its agroecological conditions that favour kale production and high incidences of ALS. The County Agriculture Office provided data on the number of kale farmers, which made up the sampling frame for a sample size of 90 farms in Lari and 90 farms in Githunguri. The formula, as presented by Kara (2015) (Eq. 1), was used to determine this sample size.

Formula: $$S = \frac{Z^2 * (P) * (1-P)}{C^2} \quad \text{(Eq. 1)}$$

P = % of picking a choice, expressed as a decimal (0.5). Sample size, Z = Z value (e.g., 1.96 for 95% confidence), C confidence interval, expressed as decimal (0.098).

Regarding this study, p=0.65. This value was determined after collecting the data from the Sub-County office on the number of kale farmers in each sub-county. A cross-sectional survey study design was employed for this study, and a stratified sampling technique was adopted where each ward constituted a stratum, and from each stratum, 90 kale farms were randomly selected, considering the farm size ≥0.25 acres and the distance of 2 km between the farms. Thirty-six (36) respondents, equivalent to 20% of the study sample, were used to pretest the tool, and these respondents were not included in the main survey. To determine farmers' knowledge of ALS disease, a test of 12 items was included in the questionnaire. The items covered the identification of disease symptoms in the field, knowledge of disease transmission mechanisms, management approaches and their sustainability. The items were categorized based on binary choice (1 = yes, 2 = no).

The data collected were farmers' demographic variables, farm characteristics and farmers' knowledge level. Kale yield was considered for two weeks, this was because the majority of kale farmers in the study sub-counties harvest the kale leaves every two weeks, and thus each farmer reported the average per two weeks. Disease incidence was determined by selecting 3-5 sampling units, depending on the farm size, for each farm. In each sampling unit, a quadrant was made, and the number of diseased plants was counted out of the total number of plants in the quadrant. Then, the formula (Eq. 2) developed by Cheshkova (2022), was used to calculate the disease incidence;

$$Disesase\ incidence = \frac{Number\ diseased\ plants}{Total\ number\ of\ plants} \times 100 \qquad \text{(Eq. 2)}$$

To get the disease incidence for a given farm, the average disease incidence for sampling units was calculated. Farmers' knowledge level was determined by administering a test with twelve items where the farmers had to respond ‘Yes’ or ‘No’ based on their understanding of each item. For categorization, a 0-4 score was regarded as low, a 5-8 score was medium, and a 9-12 score was considered as high knowledge level.

To explain the relationship between the dependent variable (the kale farmer’s knowledge level of ALS and its management and the independent variables (socio-economic factors i.e. interval or ratio scale), the multinomial logistic regression model was used in SPSS version 22 (Eq. 3).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \ldots\ldots\ldots\ldots \beta_n X_n + \epsilon -$$
(Eq. 3)

Where X_1...Xn represents a vector of socio-economic predictors influencing ALS disease knowledge levels. ϵ is a random error with a mean of zero and a constant standard deviation σ (Table 1).

Table 3.1: Definition of multinomial regression variables

Variables	Definition
Dependent variables	
ALS knowledge levels	Low, Medium, High
Independent variables	
Age HH (years)	Continuous variable
Education of HH	1 = None, 2 = Primary, 3 = Secondary, 4 = Tertiary
Gender of HH	0 = Male, 1 = Female
Kale farming experience (years)	Continuous
Household members in farming	Continuous
Total farm size (acres)	Continuous
Kale farm size (acres)	Continuous variable
Training on ALS	1 = yes, 2= no
Credit access	1 = yes, 2 = no
Yield in t ha^{-1}	Continuous
Household monthly income (Kshs)	Continuous
Number of trainings per year	1 = One. 2 = Two. 3 = Three. 4 = More than three
Number of kale planting seasons per year (seasons)	1 = one, 2 = Two, 3 = Three, 4 = More than three seasons
How long has ALS been experienced (years)	1 = <One, 2 = Two-three, 3 = four-six, 4 = > more than seven
Extension service	1 = yes, 2 = no
Disease incidence	Continuous

3.3 Isolation and characterization of *Alternaria brassicicola* from infected kale plants

3.3.1 Collection of diseased kale samples

In each kale field considered for this study, the number of sampling units in the field was determined based on farm size and disease distribution, and then in each unit, the leaves with typical symptoms of Alternaria leaf spot were collected. A maximum of ten samples

in each field were collected. The collected samples were packed in labelled khaki bags and placed in a cool box then transported to Kenyatta University for isolation.

3.3.2 Isolation of *Alternaria spp*

A modified version of the Van der Walls (2004), procedure was used to isolate Alternaria species from diseased kale samples. Using a sterile razor blade, the lesion's margins were sliced into five-millimetre pieces, which were surface sterilized in 1% NaOCl for 3 minutes and rinsed three 3 times in sterile distilled water. The washed slices were blot-dried and then plated on modified Potato Dextrose Agar (PDA) at room temperature, 25± 2^0C for nine (9) days with treatment of 1 g/L streptomycin sulfate as an antibiotic. The cultures were monitored and sub-cultured regularly to prolong the life span and maintain purity.

3.3.3 Purification of Alternaria isolates

Routine subculturing was done to obtain a pure culture, whereby a fungus colony that is 10 days old was sliced into mycelial blocks along the periphery using a 5 mm diameter, sterile corn borer. These blocks were transferred to fresh PDA under a laminar flow hood using a sterile inoculating needle and spread over plates.

For microscopic characterization, the slides were prepared from the individual cultures of each *A. brassicicola* culture that were nine (9) days old and were stained with Lactophenol blue solution. The shape, size, and width of the conidiogenous hyphae were examined using a digital microscope (Kumar *et al*., 2007). This procedure was done to enable the identification of the pathogen at the genus level; isolates were categorized based on the observed cultural and morphological features. At the molecular level, only individuals identified to be Alternaria species were selected (Akwa et al., 2020; Bessadat *et al*., 2020).

3.3.4 Cultural characterization

PDA media was prepared according to the procedure by Acharya and Hare (2022). In each sterile plate, a twenty-millilitre volume of PDA was poured. This was followed by placing at the centre a 5mm mycelial disc cut from the seven-day-old cultures. The four replicates were incubated at 250 °C for 9 days. The cultures were then characterized in terms of colony diameter, colour, nature of margin and colony zonation (Marak *et al*., 2014).

3.3.5 Morphological characterization of the fungi

Five-millimetre (5mm) parts of hyphal tips were cut using a sterile scalpel blade and then brushed into 2000 µl of sterile distilled water on a microscope slide. The slides were observed under a robust light microscope (Zeiss-Primo Star) fitted with an AxioCam ERC 5s at a magnification of ×40. Data on the conidia (such as shape, length, diameter, width and number of septa) were determined. The beak length (µm) and number of beaked septa were recorded. Four randomly selected conidia for every isolate were considered for measurement of the width and length of conidia and beak conidia. These characteristics were found to match the published morphological characteristics of the pathogen. This was further supported by pathogenicity tests and PCR-based reactions.

3.3.6 Pathogenicity test on *Alternaria brassicicola* isolates

The experiment was performed to confirm if the isolates were pathogenic to kale. The "COLLARDS" variety, which is grown by most respondents in the study area and is susceptible to ALS, was used in the study. The experiment was conducted in the greenhouse, and the optimal conditions (16- 23^{0}C and 80% relative humidity) are optimal greenhouse conditions for the growth of kale. Kale seedlings were placed into germination trays containing a well-sterilised soil mixture (2 sand:1 soil). The seedlings were well

managed up to 2 weeks after sowing, and at two leaves, they were transplanted into the prepared pots.

Twenty (20) isolates were randomly sampled for the study. Four-week-old cultures were used to prepare the pathogen inoculum, done by flooding each culture plate with sterile distilled water. To achieve the density of -10^5 spores per millilitre (Stammler et al., 2014), a hemocytometer with conidial suspension was used, and spore density was adjusted accordingly. All agronomic practices, including fertilization, weeding and irrigation, were applied as recommended. Two (2) grams of Diammonium phosphate (DAP) were applied to the plants during transplanting, and the plants were irrigated every two days.

The three-week-old seedlings were inoculated by hand spraying each isolate in three replicate plants. Inoculation was repeated twice after two (2) days. To check if the isolates were able to cause infection, the plants were monitored every 2-3 days. The leaves that developed two weeks after inoculation were collected and taken to the laboratory for pathogen re-isolation as described by Koch's postulates.

Figure 3.2: Kale seedlings fourteen days after transplanting, at the start of pathogenicity

(Source: Victor Ngaiza)

3.4 Determination of genetic diversity among the *A. brassicicola* isolates

3.4.1 DNA extraction

In 20 mL of potato dextrose agar, fungi were cultured for 9 days at 25^0 C. Pure mycelial cultures of representative *A. brassicicola* isolates were used to extract DNA using the standard procedure as outlined by Zhang *et al*. (2010). With the use of a sterile toothpick, a 20mm piece was harvested from the pure culture and put into a 1.5 ml Eppendorf tube with lysis buffer (400 mM Tris-HCl (pH 8), 60 mM EDTA- pH 8.0, 150 mM NaCl, and 1% sodium dodecyl sulphate).

A loopful of mycelia was transferred using a sterile toothpick, to a 2000µL microcentrifuge tube, a volume of 400 µl of lysis buffer [400mM Tris-HCl, pH 8.0, 60mM EDTA, pH 8.0, 150mM NaCl, 1% sodium dodecyl sulfate, 2%Polyvinyl pyrrolidone, 1% β-

mercaptoethanol] was added to the tube contents and placed in a freezer. The frozen contents and the mycelium were crushed with a sterile toothpick, then the tubes were placed in a water bath at 65 °C for 20 minutes. An equal volume of chloroform (400 µl): Isoamyl alcohol (24:1) was added, and the tubes were inverted using hands gently 20 times, followed by centrifugation at 10,000 rpm for 5 minutes at 4˚C. Then the supernatant was transferred to a new Eppendorf tube, and the volume was recorded.

An equal amount of ice-cold 100% ethanol was added and gently mixed by inverting the tube about 20 times, then the tube was spun again at 10,000 rpm for 10 minutes, and the supernatant was discarded. The pellet that had pigments and lysis buffer was re-added, and the extraction procedure was repeated. The resultant DNA pellet was then washed with 300 µl of 70% ethanol, followed by spinning the pellet at 10,000 rpm for 1 minute; the supernatant was discarded. The DNA pellet was finally air dried and dissolved in 20 µl of 1 x Tris-EDTA, pH 8.0 and 3 µl of the purified DNA was used in 50µl of PCR mixture (Liu et al., 2000).

3.4.2 Polymerase chain reaction of the extracted DNA

The *Amk1* DNA gene and intergenic transcribed nucleotide sequences were amplified using eukaryotic primers in a gradient thermal cycler (Applied Biosystems, Waltham, USA). The Amk1 gene was amplified using the primers 5′-GTCTGCGATTTCGGTCTTGC-3′ and 5′-GGTATGGGTGCTTGAGTGCT-3′ (Loganathan *et al*., 2016). For 18s amplification, the PCR conditions were changed to include a first denaturation at 94°C for 3 min, then 35 cycles of 72°C for 30s, 61.5°C for 30s, and 72°C for 5 minutes.

3.4.3 Gel electrophoresis of the PCR products

A 2% agarose gel was used to electrophorese five (5) µl of the PCR product, and a 500 base pair (bp) DNA marker was separately mixed with loading dye and then stained with two microliters (2µl) of SYBER VIEW. The stained PCR product and loading dye were loaded using a 10 µl micropipette into the wells of the solidified agarose gel submerged in 1×TAE buffer. To enable the migration of PCR products, a gel tank was connected to a 100-volt current and allowed to run for 20 minutes. The gel tank was switched off, the lid was removed, and the gel was allowed to cool for 10 minutes. The gel was removed from the tank and visualised and viewed with a trans illuminator (Okoro et al., 2009; Tsuji et al., 2000). The appearance of the clear band confirmed that DNA was amplified and *Alternaria brassicicola* was present. Following that, the amplicons' relative molecular sizes were determined.

3.4.4 Cleaning of the PCR products

Cleaning of the confirmed PCR products was done using the precipitation method described by Green and Sambrook *et al*. (2016). A total of 50µl of the PCR product was transferred to a 1500 µl Eppendorf tube, and then 5 µl of 3M sodium acetate was added. This was followed by the addition of 100µl of 100% ethanol. The tube contents were chilled overnight at -20 ^{0}C to allow precipitation. Centrifugation of the tube contents at 13,000 rpm at 40 °C for 10 minutes was done. The resultant DNA pellets were washed twice with 700µl of ice-cold cold, followed by spinning at 10,000 rpm at 4°C for 5 minutes each time. Ethanol was discarded, and the DNA pellet was air-dried overnight. The air-dried DNA was then re-dissolved into 20µl TE buffer and finally sent to Macrogen Inc., Netherlands for Sanger sequencing.

3.4.5 Sequencing of PCR products

The purified PCR products were delivered to Macrogen Inc., Netherlands, where they were sequenced according to a modified version of the procedures found in the manual for the Applied Biosystems ABI PRISMTM DyeTerminator. The Cycle Sequencing Ready Reaction Kit was utilized. This yielded partial nucleotide sequences for the 18S rDNA gene in the *A. brassicicola* isolates.

3.4.6 Bioinformatics analysis

The nucleotide sequences were trimmed to remove the low-quality reads and adapter sequences, and alignment was done using the BioEdit 7.2 software (https://bioedit.software.informer.com/7.2/). Using the BLASTn program, each cleaned sequence was blasted for similarities with already published reference sequences in the GenBank Database. Multiple sequence alignment for the sequences was performed using MEGA 11 software (https://www.megasoftware.net/). Then Clusta*W* in Mega 11 software was used to determine the genetic diversity among the *A. brassicicola.* The software was used to determine the genetic distance within the isolates from one sub-county, between sub-counties and the overall genetic distance for all isolates. Then, the Kimura-2-parameter with 1000 bootstraps and Gamma distribution model in Mega 11 software was used to construct the Maximum Likelihood (ML) phylogenetic tree, which enabled to establishment and showing of the relationships between each isolate as described by Jafar et al. (2024). This made it possible to distinguish between the pathogen's several physiological races at the molecular level.

3.5 In vitro evaluation of the efficacy of plant bioactive compounds against *A. brassicicola*

3.5.1 Collection of plant materials

Six plants from different families reported to have medicinal values in traditional medicine were selected for this study (Table 3.2). Fresh and disease-free plant parts, including leaves, flowers, bulbs, and seeds, were collected from the study area. The collected samples were washed thoroughly, 2-3 times with running water, followed by one washing in distilled water and used for extraction.

Table 3.2: Ethnobotanical data of the plant species selected for the study

Botanical name	Common name	Part to be used	Bioactive compound (s)
Jatropha curcas L.	Purging nut, Barbados nut or physic nut	Leaves, seeds, seeds bark	Essential oil
Cymbopogon citratus DC.	Lemon grass	Leaves	Phenols, flavonoids, and flavones
Carica papaya L.	Pawpaw	Leaves/seeds	Alkaloids, flavonoids and terpenes.
Tephrosia vogelli	Fish bean	Leaves	Deguelin, tyrosine and rotenone
Ocimum gratissium L.	Love basil, African basil, wild basil	Leaves	Flavonoids, tannins and eugenol
Persea americana Mill.	Avocado	Leaves and seed	Flavonoids, tannins, phenolic, and alkaloids.

3.5.2 Isolation of bioactive compounds from selected plant species

The thoroughly washed plant parts (leaves, seeds, barks, stems) were shade-dried for fifteen days (15) then ground into a blender to make a powder. The serial exhaustive extraction method as described by Ngouana et al., (2021) and Azmir et al. (2013), was employed where 25g of dry powder of plant material was put into 250 ml of petroleum ether (10 ml: 1 g solvent to dry weight) and placed in a shaking apparatus. Before decanting, the solvents were shaken for an hour to allow extraction. The same amount of solvent was added to the marc (the residues), and it was shaken for an additional hour. The process was carried out six times. After allowing the marc to dry, the extraction procedure was carried out once more using benzene, chloroform, methanol, and ultimately ethanol.

The extract was passed through Whatman (no. 2) filter paper using a Büchner funnel, and the solvent was eliminated using vacuum distillation in a rotary evaporator set at 65 ^{0}C. The extracts were reduced to a minimal amount, put in pre-weighed beakers, and allowed to completely dry while being exposed to a cool air stream. Each solvent's extracted mass was measured. Upon the complete evaporation of the solvent, each of these solvent extracts was quantified and kept in a dark, tightly sealed bottle. The percentage yield of bioactive compounds for each solvent was determined by dividing the weight of the extract by the total of dry powder dissolved and then multiplying by 100% as shown in equation 4 below.

$$\%\,Yield = \frac{Weight\ of\ the\ solvent\ extract}{weight\ of\ dry\ powder\ dissolved\ \ solvent} x100 \qquad \text{(Eq. 4)}$$

3.5.3 Screening of plant bioactive compounds, Sulphur and Copper against *A. brassicicola*

The poisoned food technique was used to test the antifungal activity of five solvent extracts obtained from plant materials, including Petroleum ether, Benzene, Chloroform, Methanol, and Ethanol. Two (2) g of each solvent extract were diluted with 2 ml of methanol before being placed into a 1000 ml PDA medium to make a concentration of 2000µg. For the concentration of 4000µg, 4g of each solvent extract was diluted with 4 ml of methanol and then mixed in the autoclaved media. The mixture was poured into 20 ml Petri plates and left to cool. A 5 mm disc of the test fungus culture, which was 7 days old, was placed in the middle of the Petri plates once the media had solidified. The Sulphur-containing fungicides (WETSULF® – Sulphur 80% WDG at 1.5g/litre and THIOVIT® JET 80% w/w at 5g/litre), copper (VITRA® 40 WG at 2g/litre and ISOCAP® 50WP at 2.5g/litre) and MISSTRESS® 72WP (Cymoxanil 8%+Mancozeb 64%) were tested. For each concentration, five replicates were prepared. The control was 1000 ml of medium with 2 ml of methanol. As specified by Singh and Tripathi (1999), the plates were incubated at 25 °C. The pathogen's radial mycelial growth in the test plates was measured to assess the efficacy of the bioactive compounds in the extract. This was compared to the control to determine the percentage of the pathogen's mycelia growth inhibition (Manmohan and Govindaiah, 2012 (Eq. 5)

$$PI(\%) = \frac{Mc-Mt}{Mc} x100 \qquad \text{(Eq. 5)}$$

Where: Mc=Mycelial growth in control

Mt=Mycelial growth in treatment

PI (%) = Pathogen's mycelia inhibition (%).

Data were collected on the 5th, 8th, and 10th days after inoculation and the experiment was conducted twice.

3.6 Data analysis

Statistical Software for Social Sciences (SPSS version 24) was used to encode and analyze the survey data. The frequencies and means were summarized by descriptive statistics. Multinomial logistic regression was used to assess how socio-demographic factors and farm characteristics affected the knowledge levels of farmers regarding ALS. Because ALS knowledge categories (response variables) were categorized at three levels (low, moderate, and high) based on the farmer's score on each of the twelve items, the regression model was acceptable. For each ALS variable, the relationship between sociodemographic traits and ALS knowledge levels was examined using cross-tabulations and descriptive statistics. ANOVA was used to analyze the mean differences (numerical variables).

The original raw data on physical and cultural characteristics were tabulated into an Excel spreadsheet and then imported into the statistical program GenStat version 21. Quantitative characteristics like conidia and colony lengths were analyzed using one-way ANOVA, while descriptive data like colony colours and margin type were condensed into frequencies and percentages of all isolates characterized. Data on percentage mycelial growth inhibition for each treatment were subjected to statistical analysis using the R-software (Ri386 2.15.3) and their means were separated employing Tukey’s test at the 0.05% level of probability.

CHAPTER FOUR: RESULTS

4.1 Occurrence, prevalence, farmers' knowledge and current ALS management practices

4.1.1 Demographic characteristics of respondents

The results showed that most of the interviewed respondents were from male-headed households (Githunguri 61.1%, Lari 62.2%). The majority of the respondents (Githunguri 73.3%, Lari 64.4%) had attained up to the secondary level of education, which implies that farmers can obtain and comprehend information on disease management approaches. On age, the majority of the interviewed respondents were aged between 51 and 60 years (Githunguri 37.8%, Lari 32.2%), which was followed by the age group of 41 to 50 years (Githunguri 22.2%, Lari 25.6%)

The study found that the majority of kale farmers (Githunguri 97.80%, Lari 96.70%) own the land where they grow kale, while a minority (Githunguri 3.30%, Lari 2.20%) have either hired or are managing the farms on behalf of the owners. On farmers' occupations, most farmers (Githunguri 57.80%, Lari 54.40%) were solely kale farmers, while in Githunguri, 42.20% and in Lari, 45.60% were doing both kale farming and formal occupations. Kale farming experience for the interviewed farmers was from 1 year to more than 40 years; however, the experience of between 11-20 years (Githunguri 41.1%, Lari 32.2%) dominated the study.

The formal education levels for the interviewed respondents ranged from those with no formal education (Githunguri 4.4%, Lari 2.2%) to tertiary level (Githunguri 6.70%, Lari

5.60%). However, the study was majorly dominated by secondary-level farmers (Githunguri 73.30%, Lari 64.4%) while the primary level was (Githunguri 15.60% and Lari 27.80%) (Table 4.1). Monthly household income in the Lari sub-county was significantly higher at 22,794 Ksh than in the Githunguri sub-county at 21,394 Ksh ($P \geq 0.05$) (Table 4.1).

Table 4.1. Selected social demographic variables of kale farmers interviewed in Githunguri and Lari sub-counties, Kenya, May 2023

Sociodemographic variables for the respondents	Category	Githunguri (n=90)	Lari (n=90)	Overall (n=180)
Gender (% of respondents)	male	61.1	62.2	61.7
	female	38.9	37.8	38.3
Education level (% of respondents)	none	4.4	2.2	3.3
	primary	15.6	27.8	21.7
	secondary	73.3	64.4	68.9
	tertiary	6.7	5.6	6.1
Age group (years) (% of respondents)	20-30	3.3	4.4	3.9
	31-40	18.9	15.6	17.2
	41-50	22.2	25.6	23.9
	51-60	37.8	32.2	35.0
	>60	17.8	22.2	20
Land ownership (% of respondents)	owner	97.8	96.7	97.2
	hired	2.2	2.2	2.2
	manager	0.0	1.1	0.6
Occupation (% of respondents)	farmer	57.8	54.4	56.1
	formal	42.2	45.6	43.9
Experience (% of respondents)	1-10	26.7	26.7	26.7
	11-20	41.1	32.2	36.7
	21-30	15.6	28.9	22.2
	>30	16.7	12.2	14.4
Monthly Household income (Ksh)		21394 ±1226.87b	22794±1371.09[a]	22094±918.86

SE-Standard Error, Means followed by the same letter across the rows indicate that there was no significant difference between the two sub-counties according to the Independent Samples T-Test at P≤ 0.05

4.1.2 Selected farm characteristics for the surveyed fields

The study revealed that 43.9% of respondents in both sub-counties grow kale for two seasons (Table 3) and 42.2% of respondents obtain seeds by harvesting from previous plants in their fields. Most of the interviewed respondents (59.4%) practice both rainfed and irrigation kale farming, which indicates the likelihood of respondents having kale on

the farm throughout the year. 38.9% practice only rainfed farming, while a minority, 1.7%, practice only irrigation farming, as indicated in Table 4.2

Besides, the study found that the majority of respondents practice intercropping (63.3%) in comparison to monocropping (15.6%), crop rotation (20.6%) and the least relay cropping (0.6%). Kale was found to be mostly intercropped with other vegetables and cereals (29.4%) such as Spinacia oleracea, *Brassica oleracea var. capitata*, *Solanum tuberosum* and *Zea mays*. The average kale yield for two weeks did not differ significantly, with Lari 1219.681kg/acre in Githunguri 1197.85kg/acre. The average kale farm size in Lari was significantly higher at 0.6 acres than in the Githunguri sub-county at 0.49 acres. The average total farm size in Lari did not vary significantly between the two sub-counties which was 1.78 and 1.55 for Githunguri and Lari respectively. Disease incidence was significantly higher at 28.34% in the Lari sub-county than in the Githunguri sub-county at 31.78%. Average ALS disease incidence in Lari was significantly higher (31.78%) than in Githunguri (28.34%). (Table 4.2)

Table 4.2: Selected farm characteristics for the surveyed fields in Githunguri and Lari Sub-counties, Kenya, May 2023

Selected Farm characteristic		Githunguri (n=90)	Lari (n=90)	Overall (n=180)
Seasons (% of fields)	One	23.3	24.4	23.9
	Two	36.7	51.1	43.9
	Three	22.2	16.7	19.4
	More than three seasons	17.8	7.8	12.8
Seed source (% of fields)	From a certified dealer	44.4	38.9	41.7
	Seedlings from the market	15.6	10.0	12.8
	From own harvest	34.4	50.0	42.2
Cropping system (% of fields)	Rainfed	37.8	40.0	38.9
	Irrigated	2.2	1.1	1.7
	Both	60.0	58.9	59.4
Cropping system (% of fields)	Monocropping	20.0	11.1	15.6
	Intercropping	56.7	70.	63.3
	Relay cropping	1.1	0.0	0.6
	Crop rotation	22.2	18.9	20.6
Intercrop (% of fields)	Cereal crops tree/tree crops	2.2	1.1	1.7
	None	43.3	35.5	37.8
	Cereal crops	4.4	1.1	2.8
	Legumes	0.0	1.1	0.6
	Tree/tree crops	0.0	1.1	0.6
	Vegetable+cereals	30.	28.9	29.4
	Vegetables+legumes+cerea	7.8	18.9	13.3
	Others	1.1	0.0	0.6
Yield (kg/acre in 2 weeks± (SD)		1197.859±27.85[a]	1219.681±40.81[a]	1208.770±24.76
Total farm size (Acre±S.E)		1.5519±0.18[a]	1.7850 ± 0.24[a]	1.6685±0.15
ALS incidence (%±S. E)		28.34±0.85[b]	31.78±0.78[a]	30.06±0.59

Kale farm (acres±SE)	0.49 ±.050[b]	0.63±0.65[a]	0.56±.04

SE-Standard Error, Means followed by the same letter across the arrows indicate that there was no significant difference between the two sub-counties according to the Independent Samples T-Test at P≤ 0.05

4.1.3 Occurrence, prevalence and current management practices for ALS

4.1.3.1 Occurrence of ALS

The study showed that ALS is abundant in farmers' fields where the majority of the interviewed respondents experience the disease (Githunguri 83.3%, Lari 80.5%).

4.1.3.2 Growth stage when kale farmers experience ALS

Most respondents (38.9% in Githunguri, 45.6% in Lari) experienced ALS throughout all crop growth stages from the seedling stage to the harvesting stage, (26.00% in Githunguri, 31.1% in Lari experience ALS at the harvesting stage), (20% in Githunguri, 6.7% in Lari experienced ALS during the vegetative stage only), (1.1% Githunguri, 2.2% in Lari experienced ALS during seedling crop stage only). This shows that the disease is a problem throughout the season when the crop is in the field. (Figure 4.1)

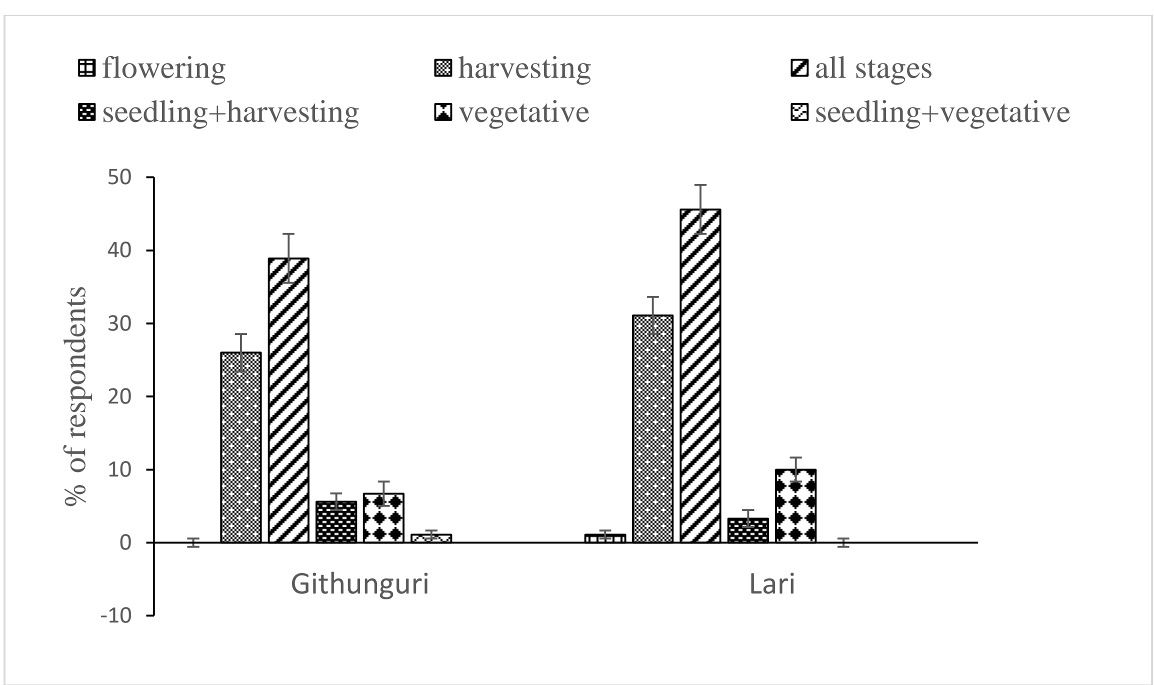


Figure 4.1: Kale growth stages when ALS is experienced by a farmer in Githunguri and Lari sub-county, Kiambu county, Kenya

4.1.3.3 Duration of ALS experienced by kale farmers

This study revealed that most of the respondents in both sub-counties (42.2% in Githunguri and 40% in Lari) had experienced ALS for a period of more than seven (>7) years, (27.8% in Githunguri, 32.2% Lari) had experienced between 4-6 years), (22.2% Githunguri, 22.2% Lari had experienced ALS 2-3 years), and the minority of respondents (7.8% Githunguri, 5.6% in Lari had experienced ALS for less than 1 year as shown in Figure 4.2

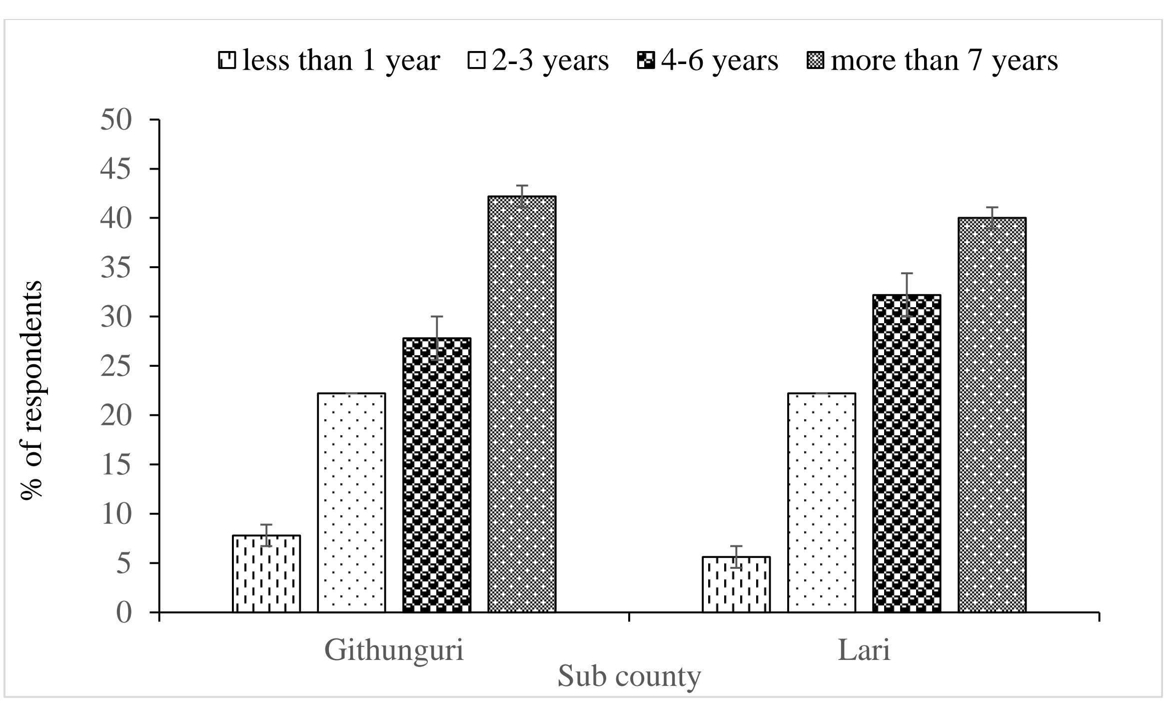


Figure 4.2: Kale growth stages when ALS is experienced by a farmer in Githunguri and Lari sub-county, Kiambu county, Kenya

4.1.3.4 Management approaches employed by kale farmers against ALS

The study found that a high proportion of kale respondents from both sub-counties (60% in Githunguri and 54.4% in Lari) were using chemical fungicides to manage ALS. Also, the removal of the infected leaves (pruning) and uprooting of the entire infected plants were techniques used by several respondents (13.9% in Githunguri and 16.1% in Lari) as shown in Figure 4.3.

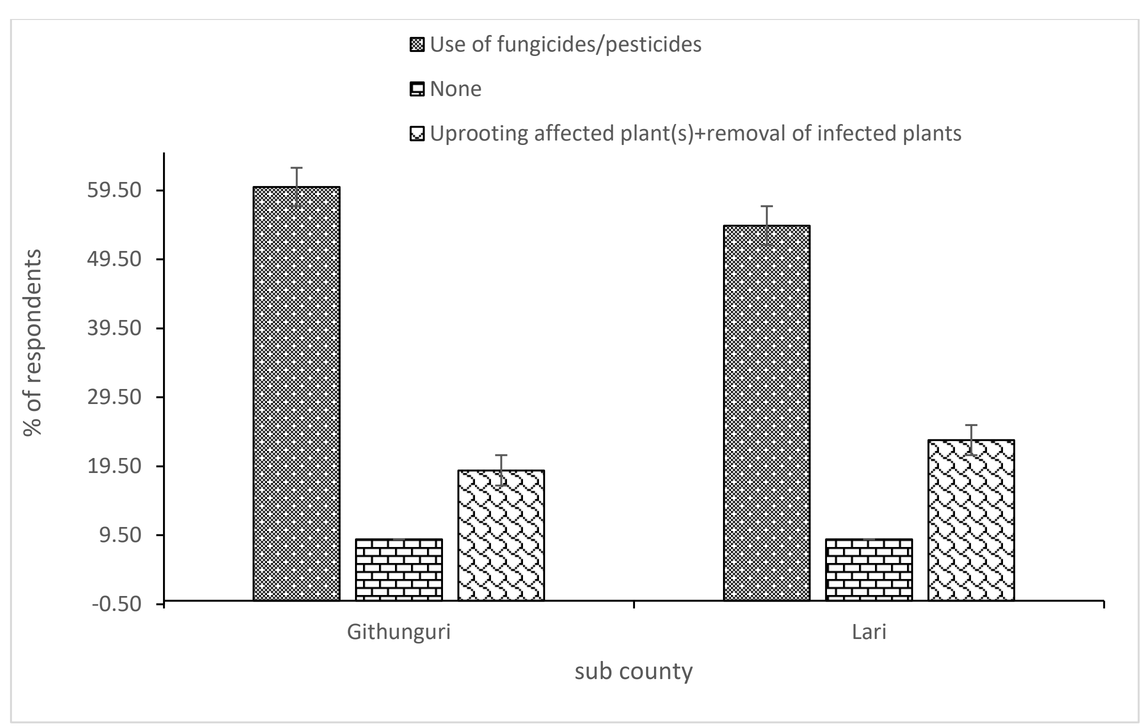


Figure 4.3: Alternaria leaf Spot disease management approaches used by kale farmers in Githunguri and Lari sub-counties, Kiambu county, Kenya

4.1.3.5 Kale Varieties Resistant to ALS

Open-pollinated (local) variety has been reported as a disease-resistant kale variety grown in both sub-counties (Githunguri 43.3%, Lari 51.1%). This was followed by Thousand Headed (Githunguri 17.8%, 8.9%), Mfalme 1 (Githunguri 20%, Lari 22.2%), Ethiopian kale variety (17.8%, 8.9%), and lastly Tausi F1 variety (Githunguri 0%, 1.1%) (Figure 4.4)

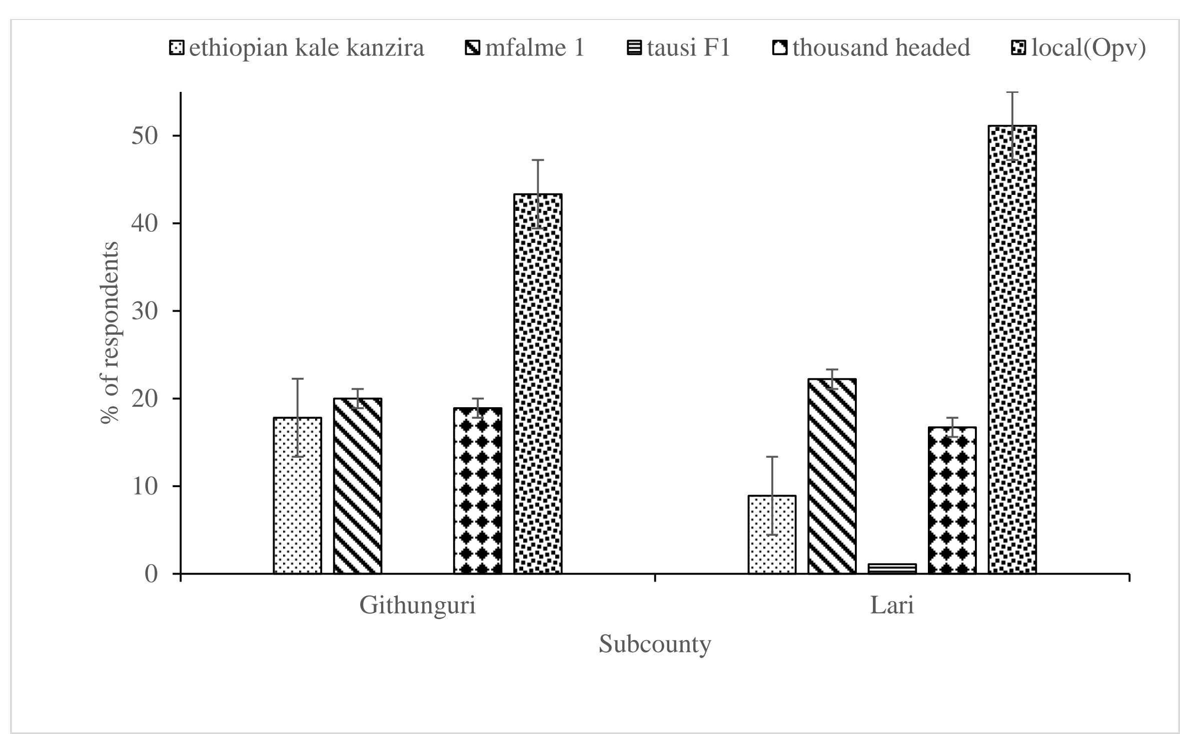


Figure 4.4: ALS-resistant variety among the cultivated kale varieties in Githunguri and Lari sub-county, Kiambu-Kenya

RIDOMIL GOLD® fungicide is used by a greater number of respondents from both sub-counties (Githunguri 31.1%, Lari 30%), MISTRESS® fungicide (4.4% in Githunguri, 7.8% in Lari), followed by ALPHATATA® insecticide (Githunguri 3.3%, Lari 7.8%), GREENCOP® fungicide (3.3% in Githunguri, 4.4% in Lari) and the least applied pesticide in both sub-counties was BESTOX® insecticide (Githunguri 3.3%, Lari 1.1%).

Table 4.3: Pesticide products used by kale farmers against ALS in Githunguri and Lari sub-counties, Kiambu county, Kenya

	% of kale fields	
Fungicide	Githunguri	Lari
RIDOMIL GOLD®	31.1	30
MISTRESS®	4.4	7.8
TATA ALPHA®	3.3	7.8
GREENCOP®	3.3	4.4
BESTOX®	3.3	1.1
Others	13.3	5.6
No fungicide	41.1	43.3

4.1.3.6 Effectiveness of pesticides used against ALS

The pesticides used by respondents of this study against ALS were reported to be less effective by the majority (Githunguri 35.6%, Lari 40%), while a small proportion of the respondents reported that the pesticides are very effective (3% in Githunguri and 0% in Lari). However, some respondents reported the pesticides were effective (15.6% in Githunguri and 6.7% in Lari) (Figure 4.5).

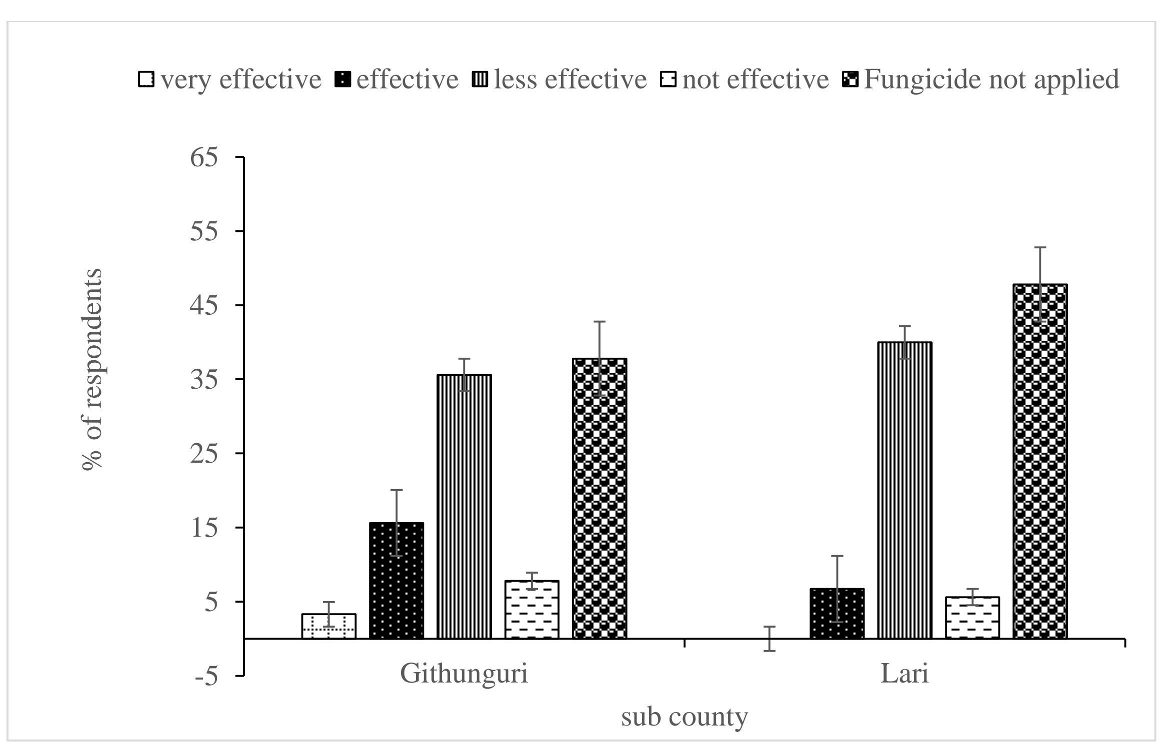


Figure 4.5: Effectiveness of the pesticides used by kale farmers in Githunguri and Lari sub-counties-Kiambu County, Kenya

4.1.4 Farmers' knowledge of ALS and its management

The results on the tested items for farmers' knowledge level are shown in Table 4.4 below

Table 4.4: Items in the test for farmers' knowledge on ALS and its management in Githunguri, Lari sub-counties-Kiambu county, Kenya

	Qn	Yes	No
1	Alternaria leaf spot in kale can cause up to 45% yield loss or more if not managed	99.4% (179)	0.0056% (1)
2	Alternaria leaf spots appear round, grey, to black concentric rings that look like a target	99.4% (179)	0.0056% (1)
3	During the rainy season, the Alternaria leaf spot is less severe than in the dry season	53.89% (97)	46.11% (83)
4	The source of kale seedlings has nothing to do with ALS	53.8% (97)	45(81)
5	Alternaria leaf spot disease is not a seed-borne disease	61.11% (110)	38.9% (70)
6	It is cheaper to use botanicals than chemical fungicides to manage Alternaria leaf spots	60% (108	40% (72)
7	There are some kale varieties resistant/tolerant of Alternaria leaf spots	55% (99)	45% (81)
8	Copper and sulphur-containing pesticides are safer than other synthetic fungicides when used to manage Alternaria leaf spots	47.7% (86)	52.2% (94)
9	Fungicides to control ALS in kale are usually sprayed directly on the plants	73.9% (133)	26.1% (47)
10	The fungicides to control leaf spot disease are usually applied at a frequency of 7-14 days	82.78% (149)	17.2% (31)
11	Management of Alternaria leaf spot disease using chemical pesticides causes more negative than positive effects on both humans and the environment	14.4% (26)	85.6% (154)
12	Management of ALS using chemical fungicides increases the quality of the kale produce	76.7% (138)	23.33% (42)

*(*percentage of respondents followed by its number of respondents)*

The significant predictors influencing farmer's knowledge level concerning ALS disease (Table 7) includes number of seasons per year (β = 0.848, Odds=7.855), number of years of experience in kale farming (β = 0.03, Odds = 0.019), whether a farmer receives training/not (β = 0.002, Odds = 0.01), number of trainings per year (β =0.472, Odds= 2.942) age (β =-1.324, Odds=1.665), credit access (β =-0.293, Odds=0.096), and number

of household members in farming (β = 0.471, Odds = 6.694) and total farm size (β = -0.051, Odds= 0.081). (Table 4.5)

Table 4.5: Socio-economic variables influencing farmers' knowledge of ALS and its management

	Low			Moderate		
	β	**Odds**	**Sig**	**β**	**Odds**	**Sig**
Intercept	6.688	1.966	.161	7.704	9.497	.002
Age of HH Head	-1.324	7.432	.005**	-1.182	15.163	.000**
Number of seasons per year	1.162	7.746	.005**	-.848	7.855	.005**
Occupation of a farmer	-1.162	1.655	.198	.448	.833	.361
Years of experience in kale farming	.077	3.643	.006**	.003	.019	.0090**
Credit access	-.293	.096	.757	1.237	.479	.010**
Number of trainings per year	.215	.179	.000	-.472	2.942	.0001**
How long has ALS been experienced	1.644	12.190	.000**	-.861	10.078	.002**
Whether the extension service was received	-.132	.052	.819	-.008	.001	.979
Kale farm size	.149	.025	.874	-.122	.095	.758
Whether a farmer receives training/not	.290	.490	.484	.002	.000	.001**
Income per month	.000	.048	.826	.000	.886	.347
No. of Household members in farming	.277	.685	.408	.471	6.694	.010**
Incidence of ALS	-.027	.235	.628	-.081	6.889	.009**
Gender	-0.079	0.007	0.934	0.169	0.123	0.726
Education	-0.127	0.002	2.204	-0.153	0.149	0.858
Total farm size	-0.766	2.815	0.003**	-0.051	0.081	0.004**

a. *The reference category is: high*

*** denotes a statistical significance at P≥0.05*

Age of household head (β =-1.324, Odds=1.665), disease incidence (β = -0.027, Odds = 0.235), credit access (β =-0.293, Odds=0.096), and total farm size (β = -0.766, 2.815) were significant negative predictors of farmers' knowledge on ALS disease. Regression analysis indicated that years of experience in kale farming (β = 0.77, Odds =3.643), how long the farmer has experienced ALS (β = 1.644, Odds = 12.190), number of seasons per

year (β = 1.162, Odds = 7.746), number of trainings per year (β =0.215, Odds= 0.00) and total farm size (β = -0.766 Odds= 2.815) were significant predictors for high knowledge on ALS disease and its management (Table 7)

4.2 Cultural, morphological characterization and determination of genetic diversity of *Alternaria brassicicola* isolates

4.2.1 Cultural characterization of *A. brassicicola* isolates

On the fifth day after subculturing, 120 of the single-spore isolates had grey to creamy colours, which are typical features of *A. brassicicola.* Sixty-four (64) of the confirmed isolates were from Lari, while fifty-six (56) were from the Githunguri sub-county. Initially, most of the cultures were creamish white, which grew as a profuse mycelium in the media. This gradually changed to grey and then brown; some isolates branched irregularly while others branched regularly. For the studied cultural characteristics, the isolates did not differ significantly across the sub-counties. An isolate from Githunguri recorded the highest colony diameter at 69.1mm, while from Lari, the highest diameter was 67.0mm. For the isolates' colony colour, it was observed that 36 out of 120 isolates or 30%, were olive grey, 18 out of 120 isolates or 15%) 15% were creamish grey, 19.2% were creamish white, 6.7% were grey black, and 29.2% were greenish grey. On pigmentation (colour of isolates upside down), 71 out of 120 or 59.2% were brown coloured, 37 out of 120 or 30.8% isolates were black, 6.7% were creamish grey and 4 out of 120 isolates or 3.3% were grey black (Table 4.6).

Regarding the texture of the colony, 12 out of 120 isolates or 10% were flat, 32.5% were medium raised, while the majority of the isolates (69 out of 120 isolates or 57.5%) had raised colonies. On the nature of colony margins, 64 out of 120 isolates or 53.3% had

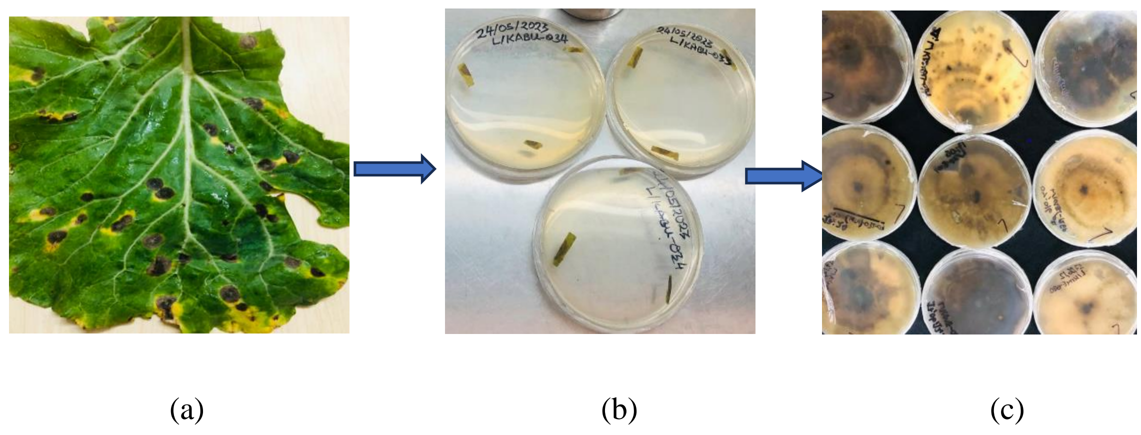
regular margins while 56 out of 120 isolates or 46.7% had irregular margins. On the density of the colony, the majority of the isolates, 53.3 %, were dense, while 32.5% were medium dense, and 14.2% of isolates were lense dense. On zonation of the colony, the majority of isolates (65 out of 120 or 54.2%) were non-zoned colonies, while 55 out of 120 or 45.8% were zoned colonies (Table 4.6)

Table 4.6: Cultural characteristics of *Alternaria brassicicola* isolates from Lari and Githunguri sub-county

Characteristic	**Lari n=64**	**Githunguri n=56**	**Overall n= 120**
Colony diameter (mm+Error)	51.79±1.187a	53.71±1.135a	52.64±0.832
Range (mm)	38.42-67	44-69.1	38.42-69.1
Colony colour (Top) %			
Olive grey	22.6	33.912.519.6	3015
Creamish grey	17.2	8.9	19.2
Creamish white	18.8	24	6.7
Grey black	4.7		29.2
Greenish grey	32.8		
Pigmentation (down)%			
Brown	60.9	57.1	59.2
Black	31.3	30.4	30.8
Creamish grey	6.3	7.1	6.7
Grey black	1.6	5.4	3.3
Texture (%)			
Flat	7.8	25	10
Medium-sized	12.5	41.1	32.5
Raised	67.2	46.4	57.5
Nature of margin (%)			
Regular	56.3	50	53.3
Irregular	43.8	50	46.7
Density of the colony (%)	14.1	14.3	14.2
Lense dense	25	32.5	32.5
Medium dense	60.9	44.6	53.3
Dense			

Zonation (%)			
Positive	48.4	42.9	45.8
Negative	51.6	57.1	54.2

Means followed by the same letter across the rows do not differ significantly according to the Independent Samples T-Test at P≤ 0.05



(a) (b) (c)

Figure 4.6: (a) Diseased kale leaf sample before isolation, (b) PDA media inoculated with diseased and sterilized leaf section ready for incubation, (c) Twelve-day cultures for some A. brassicicola isolates

4.2.2 Morphological characteristics of *Alternaria brassicicola* isolates

The conidia were olivaceous-brown, obclavate to obovoid in shape, and were born in the chain-like structure of 8-10 spores (Figure 4.7). This chain was sometimes branching at the base. The conidia were attached to septate conidiophores, which were olive brown, extending to between 100-200µm. Initially, hyphae were thin (diameter 2.2 -3.1µm) and hyaline, but with time hyphae became thickened to 4.7-4.4 in diameter (Table 4.7). Regarding the conidial shape, the majority of the isolates (75 out of 120 isolates or 62.5%) were obclavate shaped, 22.5% were ellipsoidal shaped, while a few (18 out of 120 or 15% isolates) were obovoid shaped (Figure 4.7). Isolates that had been isolated were the majority (79 out of 120 or 65.8%) (Table 4.7).

The conidia average length was not significantly different among isolates from the different sub-counties at $P \leq 0.05$, while for the conidia average width, Githunguri isolates (13.45±0.53) were significantly wider than Lari isolates (11.46±0.43). For the average beak length, there was no significant difference between Githunguri (5.13±0.61) and Lari isolates (5.1±1.09). All isolates had at least 2 transverse septations, and the range was between 2-5 septa. The number of longitudinal septations ranged from 0-2, where the majority of the isolates had no longitudinal septations on their conidia. The number of beak septa for all isolates ranged from 0 to 2, meaning that some isolates had some conidia with beaks that had no septations

Table 4.7: Morphological features of Alternaria isolates collected from Lari and Githunguri Sub-County

Characteristic	Lari n=64	Githunguri n=56	Overall N=120
Conidia shape (% isolates)			
Obclavate	62.9	62.1	62.5
Ellipsoidal	16.1	29.3	22.5
Obvoid	21	8.6	15
Beaks on conidia (%isolates)			
Isolates with beaked conidia	75.8	52.2	65.8
Isolates without beaked conidia	24.2	44.8	34.2
Conidia dimensions			
Av. Conidial length (µm)	23.27±0.69[a]	23.45±0.69[a]	23.36±0.48
Av. Conidial width (µm)	11.46±0.43[b]	13.45±0.53[a]	12.42±0.34
Av. Beak length (µm)	5.13±0.61[a]	5.1±1.09[a]	5.11±0.61
Septations			
No. of transverse septa (range)	2-4	2-4	2-4
No. of longitudinal septa (range)	0-2	0-2	0-2
No. of beak septa (range)	0-1	0-1	0-1

**Means followed by the same letter across the rows are not significantly different according to the Independent Samples T-Test at P≤ 0.05*

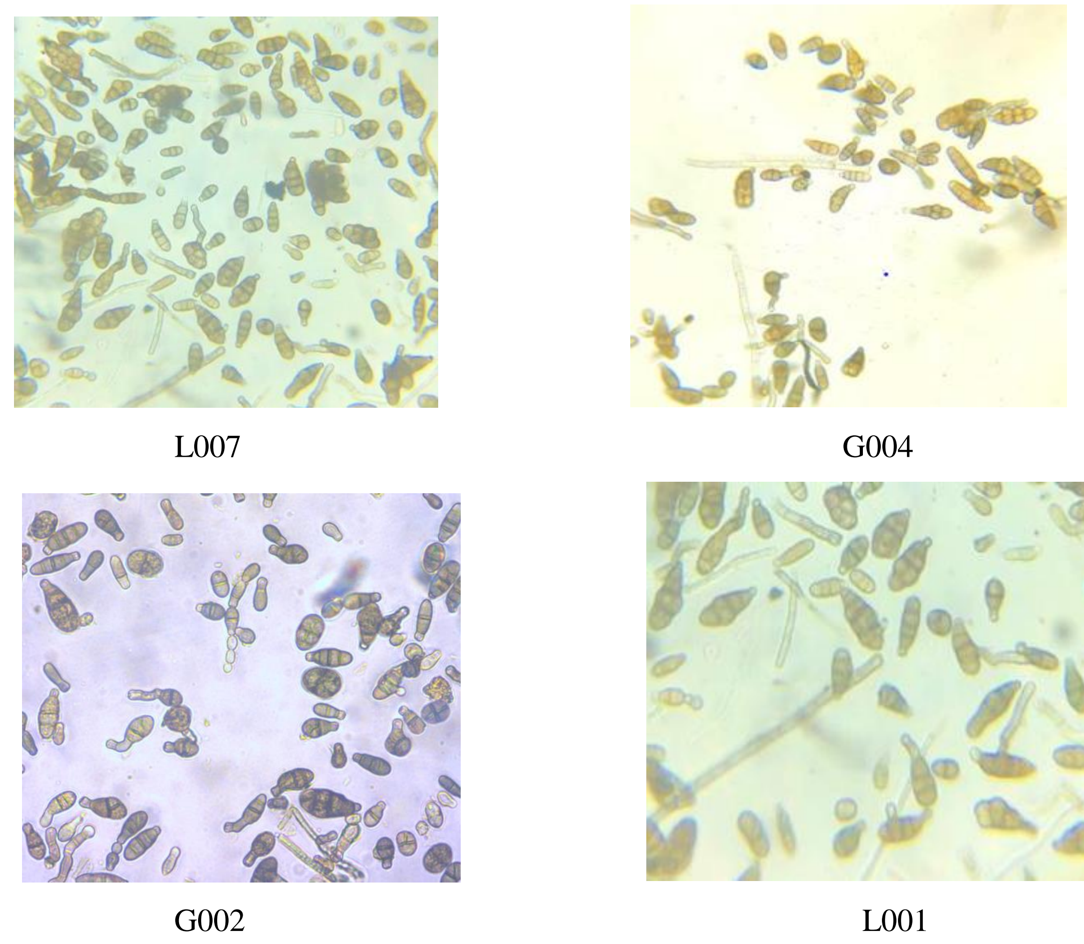


Figure 4.7: Conidial features of selected *A. brassicicola* isolates at medium power of (×40)

4.2.3 Pathogenicity tests for *Alternaria brassicicola* isolates

The symptoms of the disease were observed three days after inoculating the plants with *A. brassicicola.* The symptoms started to develop on the lower and older leaves, progressing to the young ones. The symptomatic leaves displayed round, brown spots with concentric rings. Gradually, the spots started developing a yellow halo encircling the spots, and the spots were cracking in the middle necrotic part. The necrotic areas continued to enlarge with time. After re-isolating the pathogen, the cultures appeared to be similar to inoculated isolates both culturally and morphologically. Based on Koch's postulates of pathogenicity, this similarity was used to confirm that the pathogen is *Alternaria brassicicola* on kale.

(a) (b)

Figure 4.8: (a) ALS symptomatic kale leaves, (b) Re-isolated Alternaria brassicicola isolate

4.2.4 Determination of genetic diversity among the isolates

4.2.4.1 PCR results and confirmation of *A. brassicicola*

The pair of forward and reverse primers amplified a 445bp fragment of the Amk1 gene in the extracted DNA for each isolate. Ninety-six (96) trimmed DNA sequences ~210bp long for the *A. brassicicola* isolates from the Lari and Githunguri sub-counties, were obtained. In the NCBI database, the sequences for the isolates showed 80-97% similarities with *A. brassicicola* accession AY515257. However, other isolates showed ≥80% similarity to other species of Alternaria, including *A. altenata, A. altra* and *A. aborescensis.*

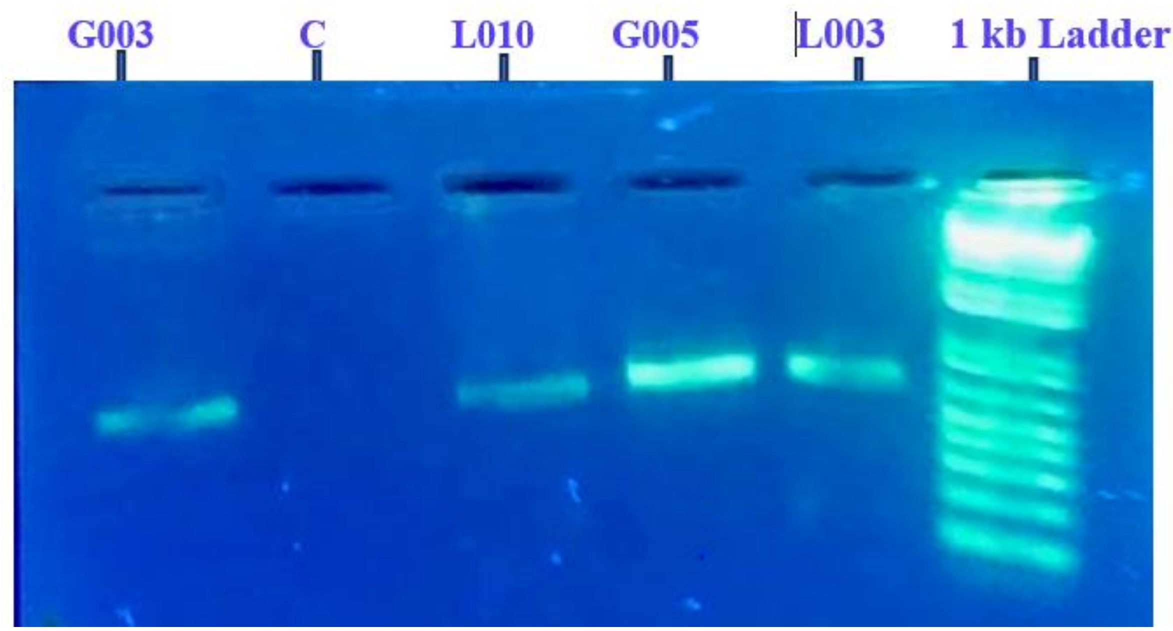


Figure 4.9: PCR products of Mitogen-activated region (MAP) of *A. brassicicola*, G003, L003, G005, L010 and C for control

4.2.4.2 Phylogenetic analysis

The phylogenetic tree was developed to show the diversity of the isolates. The sample clustered in two clades that were isolated from either the Githunguri or Lari sub-counties.

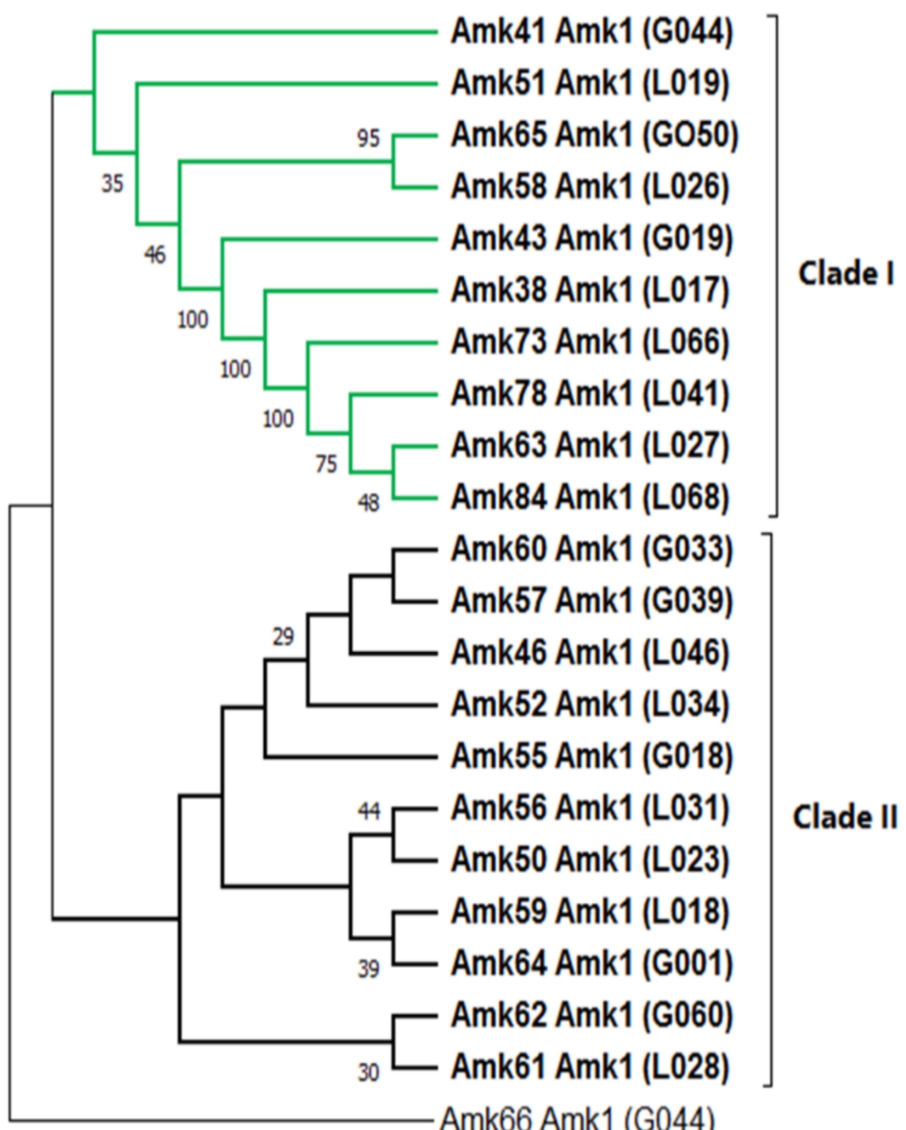


Figure 4.10: Maximum likelihood phylogenetic tree for Alternaria brassicicola isolates based on their genetic distance

The consensus tree was constructed by the Neighbour-joining method based on the Kimura-2-parameter model and the Gamma distribution model in Mega 11 software. Bootstrap values were calculated on 1000 replicates, indicated in the branches.

4.2.4.3 Genetic diversity of *Alternaria brassicicola* isolates

The genetic distance within Lari isolates was 0.28 (28%) greater than Githunguri isolates at 0.08 (8%). The genetic distance between Githunguri and Lari isolates was 0.21 (21%) greater than between Lari and Githunguri isolates, 0.02 (2%). The genetic distance within Clade I isolates was 0.34 (34%) greater than within Clade II isolates, 0.05 (5%). The genetic distance between Clade I and Clade II isolates was 0.28(28%). The overall mean genetic distance in the entire population was 0.21 (21%) (Table 4.7)

Table 4.8: Genetic diversity for the sequences of the isolates

		distance	%d	S. E
Within the sub-county	Lari	0.28	28	0.03
	Githunguri	0.08	8	0.01
Between sub-county	Lari*Githunguri	0.21	21	0.00
	Githunguri*Lari	0.02	2	0.00
Within Clades	Clade I	0.34	34	0.04
	Clade II	0.05	5	0.01
Between Clades	CladeI*Clade II	0.03	3	0.00
	Clade II*CladeI	0.28	28	0.00
Overall mean distance in the entire population		0.21	21	0.02

4.3 In vitro screening of plant bioactive compounds, Sulphur and Copper against *A. brassicicola*

4.3.1 Yield of plant bioactive compounds from the extraction process

The results show that the percentage yield of bioactive compounds varies depending on the plant species and the solvent used. The highest total yield was recorded from *P. americana* (16.72%), followed by *C. citratus* (11.15%), and the lowest yield was from *J. curcas* (7.67%) (Table 4.8).

Table 4.9: The percentage (%) yield of bioactive compounds under serial exhaustive extraction in 1:10 (plant material: solvent) for six (6) different plant species

	Petroleum ether	Benzene	Chloroform	Methanol	Ethanol	Total yield
C.papaya	1.80	1.55	1.58	2.18	1.88	8.99
J. curcas	1.49	0.76	1.38	2.02	2.02	7.67
C. citratus	1.68	1.49	1.14	3.92	2.92	11.15
P.americana	6.11	0.34	2.52	4.5	3.25	16.72
O.grattisium	0.80	1.98	1.18	2.3	2.13	8.39
T. vogelli	1.16	2.63	2.1	1.96	1.06	8.91

4.3.2 Efficacy of plant bioactive compounds against *Alternaria brassicicola*

The mycelium inhibition concentration of 4000mg/ml was significantly higher than that at 2000mg/ml at $p \leq 0.05$. Most of the bioactive compounds significantly reduced the mycelial growth, but *T. vogelli* and *J. curcas* were more effective. There was a significant difference in mycelial growth inhibition among bioactive compounds from different plant species. Two out of six plant species attained ≥50% mycelial inhibition; these were *J. Curcas* and

T. vogelli. Two sulphur formulations (THIOVIT® Jet 80%w/w, WETSULF® 80%w/w) and a Copper formulation (VITRA® 40WG) were able to attain ≥ 50% mycelial growth inhibition. The mycelial growth inhibition by Sulphur and Copper formulations was significantly higher than for most plant bioactive compounds, but lower than Chlorothalonil, a conventional synthetic fungicide.

At 2000mg/ml, *J. curcas* had the most effective bioactive compound, which reduced the growth of *A. brassicicola mycelia* by 62.7% on the 10th day and was followed by *T. vogelli* (60.3%). The bioactive compounds from other treatments did not attain 50% inhibition. *P.americana* reduced mycelial growth by 31.4%, *C. citratus* by 22.046%, *C. papaya* by 20.8%, and the least effective was *O. gratissium* (18.7%). For sulphur and Copper formulations, MISTRESS® (Sulphur) 68.4% was the most effective formulation followed by VITRA® 40WG (Copper) 67% while WETSULF® 80% w/w (Sulphur) also reduced the mycelia growth by 64.8%, the least effective formulation was ISOCAP® 50WP (Copper) which reduced mycelial inhibition by 30.5%, while Chlorothalonil which was used as a positive control reduced mycelial growth by 70.8%. However, on the 10th day, there was no significant difference in the level of mycelial growth inhibition between THIOVIT®80%w/w Jet, VITRA® 80%w/w and WETSULF® 80% w/w; the significant difference was only recorded between those three formulations and ISOCAP® 50WP.

For most of the treatments, there was a significant difference in mycelial growth inhibition at different days, i.e., 5th, 8th, and 10th days after inoculation. There was no significant difference in mycelial growth inhibition at the 10th day between *J. curcas* and *T. vogelli,* while there was a significant difference in mycelial inhibition between *P. americana, C. citratus, C. papaya,* and *O. gratissium.* Among Sulphur formulations (THIOVIT®

80%w/w Jet and WETSULF®80%w/w), there was no significant mycelial growth reduction on the 10th day after inoculation, while among copper formulations, VIFRA® 40WG and ISOCAP® 50WP, there was a significant difference in mycelial growth inhibition. Moreover, there was no significant mycelial growth inhibition between THIOVIT® 80%w/w Jet and WETSULF® 80% w/w, while ISOCAP® 50 WP inhibition was significantly different from others (Table 4.9)

Table 4.10: Percentage of mycelial growth inhibition under different treatments at 2000mg/ml for bioactive compounds, Copper and Sulphur formulations at their recommended rate

Treatments	5th day PI (%) ±S. E	8th day PI (%) ±S. E	10th day PI (%) ±S. E
Mistress 72WP (Chlorothalonil)	62.304±1.78[a]	66.82 ±0.69[a]	70.8±0.86[a]
Thiovit Jet 80w/w-S	58.22±4.51[a]	61.24 ±4.59[ab]	68.44±1.16[a]
Jatropha curcas	55.17±2.68[a]	57.516±3.45 [ab]	62.668±2.48[a]
Vifra 40WG- Cu	54.8±3.57[a]	62.12±1.74 [ab]	67.02±2.19[a]
Wetsulf 80% w/w-S	54.02±2.55[a]	62.148±1.87[ab]	64.84±2.13[a]
Tephrosia vogellii	50.53±3.93[a]	52.932±3.00 [ab]	60.3±3.46[a]
Persea americana	25.814±2.38[b]	27.902±2.82[c]	34.314±2.89[b]
Isocap 50 WP -Cu	23.15±3.09[bc]	27.78±2.43[c]	30.49±2.13[bc]
Cymbopogon citratus	12.92±1.55[bc]	18.374±1.86[c]	22.046±1.45[cd]
Carica papaya	12.14±1.94[bc]	15.61±1.36[c]	20.282±1.02[cd]
Ocimum gratissium	11.12±2.61[c]	16.02±2.58[c]	18.66±2.67[d]
Control	0.0[d]	0.0[d]	0.0[e]
P value	P<0.05	P<0.05	P<0.05

**Means followed by similar letters are not significantly different according to Tukey's Honestly Studentized Range (HSD) test at P≤0.05*

Figure 4.11: Percentage of mycelium growth inhibition under different treatments at 2000mg/ml on the 10th day of inoculation. Error bars represent Standard Errors (SE)

At 4000mg/ml, bioactive compounds from *J.curcas* recorded the highest level of mycelial growth inhibition 69.9%) on the 10th day after inoculation. This was followed by *T.vogelli* (64.4%). while *P.americana* reduced mycelial growth by 38.64%, *C.papaya* by 31.46%, *C. citratus* by 30.06%, and the least effective was *O. gratissium* (24.06%). However, there

was no significant difference in mycelial growth inhibition on the 10th day between *J. curcas* and *T.vogelli* while was significant difference was recorded among *C. citratus, C.papaya,* and *Ocimum gratissium.*

Table 4.11: Mycelial growth inhibition under different treatments at 4000mg/ml for bioactive compounds, Copper and Sulphur formulations at their manufacturer's recommended dosage

Treatment	5th day PI (%) ±S. E	8th day PI (%) ±S. E	10th day PI (%) ±S. E
Mistress 72WP (Chlorothalonil)	64.52±0.62[a]	67.44±0.53[a]	71.78±0.80[a]
Jatropha curcas	58.99±2.56[a]	65.236±2.92[a]	69.9±3.91[a]
Thiovit Jet 80w/w- **S**	56.55±3.54[a]	62.9±4.78[a]	68.358±2.11[a]
Vifra 40WG- Cu	54.66±3.08[a]	61.58±1.25[a]	65.06±1.52[a]
Tephrosia vogellii	53.72±2.14[a]	59.94±1.11[a]	64.44±2.43[a]
Wetsulf 80% w/w-**S**	51.68±2.57[ab]	58.14±2.51[a]	64.12±1.72[a]
Isocap 50 WP-Cu	36.22±6.86[bc]	42.03±5.94[b]	46.83±4.99[b]
Persea americana	27.2±3.30[cd]	30.914±3.24[bc]	38.636±1.83[bc]
Carica papaya	20.132±3.58[d]	29.838±2.50[bc]	31.46±3.60[cd]
Cymbopogon citratus	18.92±1.54[d]	22.674±1.57[c]	30.066±1.20[cd]
Ocimum gratisssium	14±2.80[d]	19.54±3.38[c]	24.06±2.32[d]
Control	0[e]	0[d]	0[e]
P value	P<0.05	P<=0.05	P<=0.05

Means followed by similar letters in rows are not significantly different according to Tukey's Honestly Studentized Range (HSD) test at P≤0.05

On the 10th day, a significant increase in the level of mycelial inhibition was recorded when the concentration of bioactive compounds was doubled, i.e. 4000mg/ml (Table 4:10)

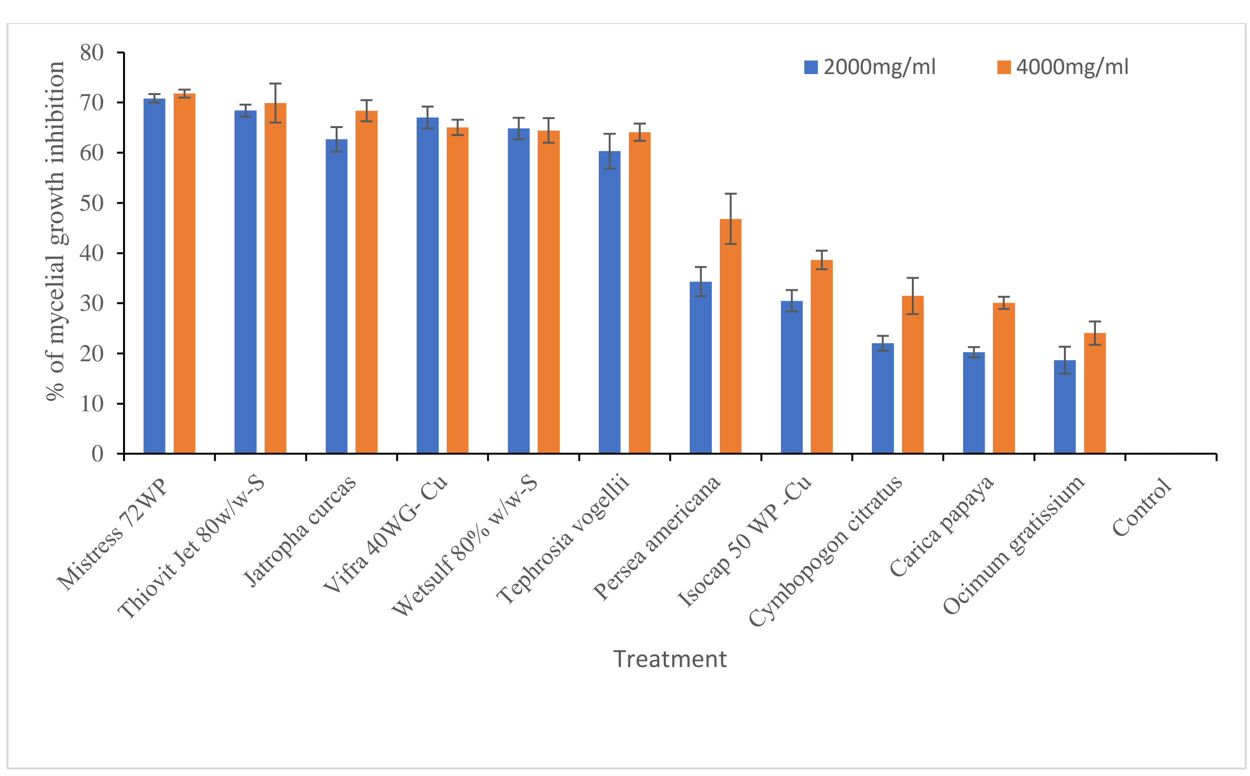


Figure 4.13: Comparisons of mycelial growth inhibition at 2000mg/ml and 4000mg/ml on the 10th day after inoculation. Error bars represent Standard Errors (SE)

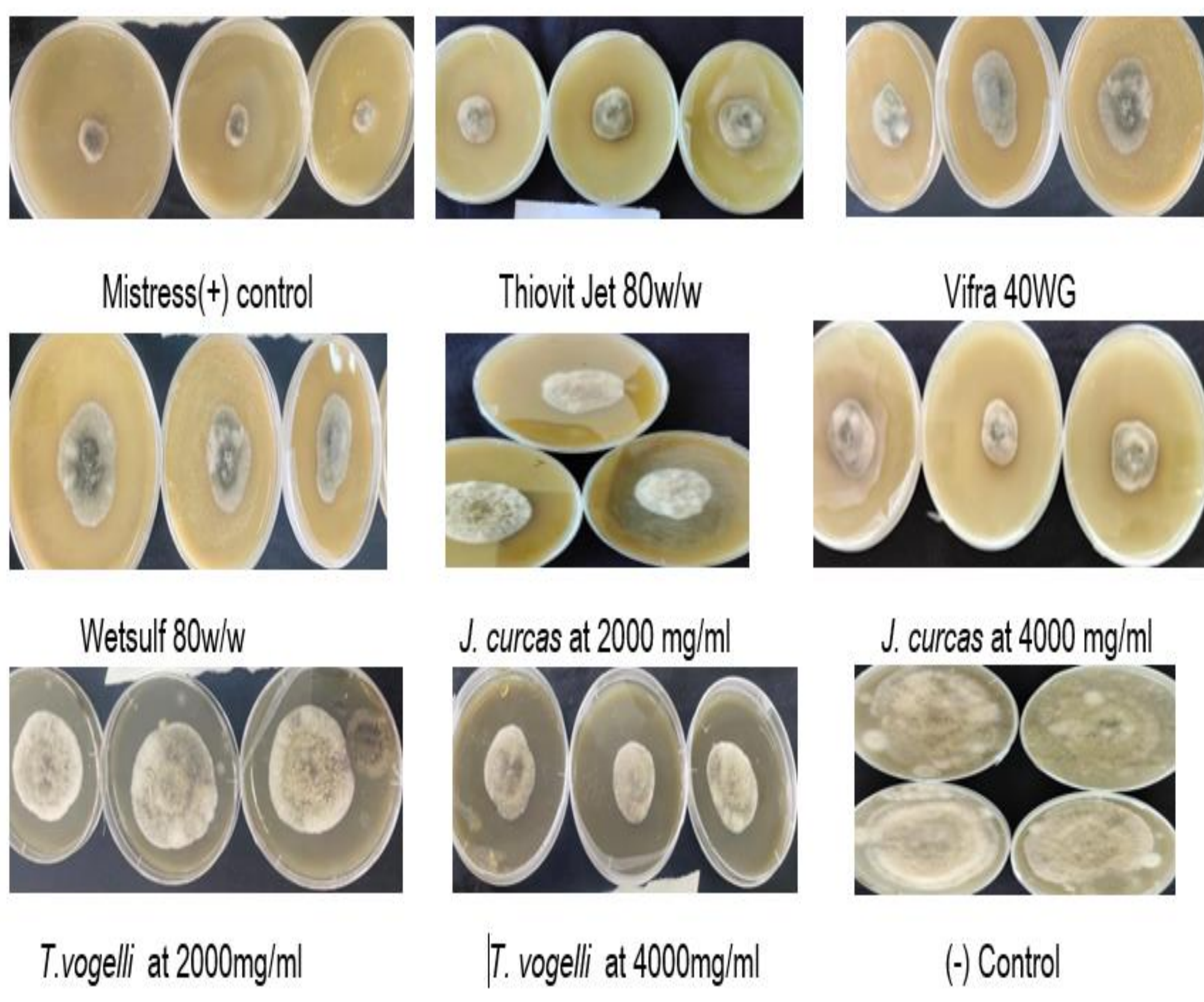


Figure 4.14: Alternaria brassicicola mycelial growth inhibition under treatments that attained ≥50% inhibition on the 10th day after inoculation. Sulphur and Copper formulations were at the manufacturer's recommended dosage levels

CHAPTER FIVE: DISCUSSIONS

5.1 Occurrence, farmers' knowledge and current management practices for ALS

5.1.1 Demographic characteristics of respondents

The majority of kale respondents from the study area were males, and this finding was inconsistent with the study conducted in Kabete, Kenya, by Achola et al. (2014), who found that the proportion of female kale farmers (67%) was almost double that of the male farmers. This was attributable to more women being empowered to start enterprises that improve their economic status through self-help groups and funding from government and nongovernmental programmes. However, for this study, the dominance of males in the study can be explained by the fact that men hold and control the means of production, including land and capital (Anang et al., 2013; Barasa et al., 2019).

A large number of the kale respondents interviewed were ≥50 years old. This result was consistent with that of Achola et al. (2014), who found that 61% of the kale farmers in Kabete, Kenya, were ≥ 50 years old. This can be explained by the fact that many youths tend to migrate from rural to urban areas in search of white colour occupations while leaving the adult population who have retired to undertake farming activities. Additionally, it was also reported by Mbambo (1998) reported that the majority of farmers in Kenya are aged above 45 years.

The fact that most respondents have attained formal education implies that farmers can obtain and comprehend information on disease management approaches. Awan *et al.* (2012) found that there is a strong association between the level of education and a better understanding of disease management strategies. Nevertheless, the study found that there

was no association between the level of education and the farmer's knowledge of ALS and its management.

The majority of farmers in the study area were found to be well-experienced in kale farming. Pratiwi and Suzuki (2017) found that the more the farmer is experienced, the better the understanding of the crop and the higher the ability to adopt knowledge from other farmers or experts.

5.1.2 Farmers' knowledge

In the farmer's training, the study revealed that the more trained farmers were likely to have moderate to high knowledge of ALS and its management. This confirms that farmer training helps to improve their skills in agricultural practices including pest and disease management (Pretty, 2008; Mushobozi, 2010; Sajeev et al., 2012; Emeana et al., 2019). In line with these results, Rasanjali et al. (2021) and Mwakidoshi et al. (2023) reported that the advancement of farmers' knowledge and skills depends on improved agricultural training programs. According to a different study carried out in China by Yang et al. (2008), farmers who attended Farmer Field School (FFS) considerably improved their knowledge of vegetable pests, insect and disease ecology, natural enemies, and pest management; conventionally trained farmers did not significantly improve their knowledge in any of these sections.

The study also revealed that farmers who grow kale in more seasons in a year are likely to have moderate to high knowledge of ALS. The pathogen is more favoured in the cool season; thus, ALS disease incidence and severity may be higher during the cool season (Rop et al., 2009; Jantasorn et al., 2017). Considering that kale can take between three to

six months in the field when a farmer does two or more seasons in a year, they will have kale in the field for almost a whole year. According to Damicone and Roberts (2009), the tendency of growing kales all year round increases ALS disease incidences, this is because *A. brassicicola* tends to persist in the soil and crop debris.

It was revealed that households with more members engaged in kale cultivation were probably more knowledgeable about the symptoms and treatment of ALS disease. According to a study by Zossou et al. (2020), there is a household-level exchange of agricultural information and expertise; therefore, the larger the household size, the higher the degree of knowledge about crop management. According to Whitehouse (2011), the majority of African societies share this solidarity of skills and knowledge exchange at the household level. The level of knowledge for the younger kale producers regarding ALS and its management was moderate relative to high. This is inconsistent with the findings of Macharia et al. (2014) and Mwaura et al. (2021). According to a different study by Ebewore and Isiorhovoja (2019), a farmer's age was statistically significant at $P<0.05$ for predicting their level of plant disease management knowledge.

5.1.3 Characteristics of kale farms in the studied Lari and Githunguri Subcounties

The average kale farm size reported in this study is consistent with that of Lefsrud and Kopsell (2005), being between 0.2 to 0.4 ha. According to Achola (2014), kale farm size can affect crop management practices, including disease management techniques and crop rotation. This can be related to the results of this study, where most farmers do not practice regular crop rotation; instead, they perform monocropping, relay cropping and intercropping. Handbook (2006) reported that crop rotation in kale farming minimizes the ALS disease severity.

This study found that most kale farmers obtain seeds and seedlings from their previous harvest; this practice increases the risk of ALS spread. Infected seeds or seedlings are a primary source of *A. brassicicola* inoculum (Scheufele, 2013). Köhl et al. (2010) found that ALS is not restricted to leaves but also damages the fruit-bearing branches and pods, which turn black when colonized. The pathogen's mycelium grows both on the external and internal parts of the kale seeds. The pathogen survives in seeds and results in lower germination rates of the infected seedlings. This happens mostly when a farmer uses non-certified seeds. This is supported by Scheufele (2013), who found that *A. brassicicola* colonized seeds and seedpods 14 days after flowering, and infection increased slowly until seeds began to develop, and then increased sharply during seed maturation. According to Bishaw et al. (2007), the quality of kale seeds for the plants inoculated with *A. brassicicola* was significantly lower than in untreated plants, as only 80% of seeds were viable. A study by Scheufele (2013) recommended the purchase of tested and certified kale seeds to minimize ALS infection from seeds. The intercropping of kale with other brassica vegetables such as *Brassica oleracea var. capitata*, *Brassica oleracea var. italica*, and *Brassica oleracea var. botrytis* increases the chance of spread, thus increasing ALS incidence (Carrillo-Reche et al., 2023). However. Lodha et al. (2018) found that lower disease severity was experienced in kales intercropped with cereals.

5.1.4 Occurrence of ALS

ALS development is favoured by cool and moist weather conditions that are important for the proper germination of the spores (French et al., 2019). The pathogen prefers cool weather and long periods of leaf wetness, making autumn weather and produces spores after exposure to high relative humidity (~87%) and temperatures between 20-300 (Carmody 2017; Kirarei, 2019). Thus, Lari and Githunguri sub-counties were ideal environments for the growth and reproduction of *A. brassicicola*. More than 95% of the surveyed farms were found to be affected by ALS. ALS is associated with a high loss of kale yield in the studied sub-counties due to the weather conditions and management practices used by farmers. Spores of the pathogen are also spread by winds and splashing water (Singh Saharan et al., 2016; Meena et al., 2022).

5.1.5 Management practices used by farmers against ALS

This study revealed that most of the kale farmers in the study areas employ pesticides as the principal management method for ALS. This result was also supported by Rop et al. (2009) and Kirarei (2009) studies. Another finding revealed that most of the farmers mistakenly regard ALS as being caused by insect pests and thus tend to manage the disease using insecticides such as Duduba® 400EC, Betafos® 263 EC and ALFAGOLD® 100 EC products, which compromises the actual ALS management. Other farmers think ALS is caused by cold conditions, and hence they leave the diseased plants unattended. The lack of familiarity with the disease makes the farmers use approaches which are not viable for ALS management. This result is similar to that of Mandiriza-Mukwirimba et al. (2016), who found that more than 30% of brassica farmers were not able to distinguish ALS and other leaf spots.

The farmers did not report knowledge of any kale varieties resistant to *A. brassicicola,* but there an OPV or local kale varieties that are tolerant. This result was consistent with Seif and Nyambo (2013), who found that resistance against ALS had not yet been reported in commercially available kale varieties marketed in Kenya. In other areas where kale is grown, there has been limited availability of commercial kale varieties which are highly tolerant or resistant to ALS (Scheufele et al., 2013; Hahn et al., 2016; Al-Lami, 2023).

In this study, other factors that determined the choice of a kale variety included the marketability of the leaves, the productivity of the variety, tolerance against pests and diseases and the life span of the variety in the field. Similar factors for kale variety selection were reported by Ordás and Cartea (2008). Most respondents reported either less effectiveness or ineffectiveness of the pesticides they use against ALS; this might probably be attributed to a lack of rotations on the pesticides and the use of insecticides instead of fungicides to manage ALS. Also, the ineffectiveness of the pesticide can be attributed to the stage of the disease at which farmers start using the pesticide.

On the other hand, close intervals and more frequent application of pesticides would, in the long run, render it less effective (Lucas et al., 2015). Patel et al. (2018) found that some ingredients, such as Azoxystrobin, were less effective against ALS. Most of the respondents from the study area have reported that they tend to apply the pesticides even less than fourteen days after the previous application. Higher levels of pesticides can be attributed to the high prevalence of ALS disease and a lack of knowledge on the frequency of pesticide application. Ntow et al. (2006) found that over 85% of farmers did not read or follow the pesticide label instructions, including the interval of chemical application. According to Scheufele (2013) and Roller (1999), the continued dependency on synthetic

chemicals to manage ALS may lead to increased resistance of *A. brassicicola* strains. Also, the lack of adherence to the manufacturer's recommendations, including the dosage and the frequency of application, may increase ALS prevalence, incidence and severity of the disease.

5.2 Characterization of *Alternaria brassicicola* isolates by cultural, morphological and genetic features

The colony features that were used to characterize *A. brassicicola* were similar to the features described by Kiran et al. (2018) and Punyanobpharat et al. (2018). There was a great diversity in the colony features such as colony diameter, colony length, margin, zonation, colony colour, pigmentation and beak length, width and septa number. The variation in the colony features may be attributed existence of different pathotypes within the population. These results are supported by the study by Hazowary et al. (2023), who recorded great variability in the colony features of the *A. brassicicola* isolates from diseased cabbage leaf samples collected from major areas growing cabbage in Aslam, India. These features include conidial length, breadth, and septal count. In comparison to the morphological features described by Sharma *et al.* (2021) and Pattanamahakul and Strange. (1999), the features characterized for the isolates were satisfactory to confirm the *A. brassicicola.* Additionally, the conidia with or without beaks were observed among the isolates, which is a typical characteristic of *A. brassicicola* as described by (Gao et al., 2014; and Singh et al., 2023).

5.2.1 Genetic diversity of *A. brassicicola* isolates

The study revealed that the population of *A. brassicicola* was genetically diverse between sub-counties and within each sub-county. Genetic diversity means the number and frequencies of alleles at each locus, the isolates clustered in two (2) genetic groups of ancestors (Clade I and Clade II). However, there was an intermix of isolates from either Githunguri or Lari sub-counties into Clade I and Clade II. This suggests the epidemiological history of *A. brassicicola* has moved from one of subcounty. This was the first study to determine the genetic diversity of *Alternaria brassicicola* causing leaf spots in kale in Kenya and sub-Saharan Africa. This study has revealed that there is low genotypic diversity of 0.21= 21% for *A. brassicicola* from the study area, which is lower than the level of genotypic diversity (0.53=53%) for *A. brassicicola* reported in New York State by a study conducted by Kreis et al. (2016). Similarly, Bock et al. (2005) and Linde et al. 2010), studies reported high genotypic diversity for the *A. brassicicola* population in Australia and suggested that recombination was occurring occasionally for *A. brassicicola*.

Similar results were reported by Bock *et al.* (2005), who studied five populations of *A. brassicicola* infecting *Cakile maritima* and found high levels of genetic diversity. Results of genetic studies using amplified fragment length polymorphisms indicated a randomly mating population; also, the populations studied were found to be in linkage disequilibrium, suggesting that some clonality exists among the populations. According to Sharma et al. (2021), this high level of genotypic diversity was thought to be the result of genetic recombination occurring in the population.

On the other hand, other Alternaria species, other than *A. brassicicola* were responsible for causing ALS. These were *A. alternata, A. altra* and *A. aborescensis*. This result is

supported by the study conducted by Rop et al. (2009), who reported that ALS was inflicted not only by *A. brassicicola* but also by other Alternaria species, including *A. japonica*. Reuben (2021) found that ALS in kale and cabbage in Kenya is associated with either *A. brassicicola, A. alternata* or *A. brassicae*. Al-Lami *et al*. (2019) reported ten species of Alternaria associated with ALS, these included *A. alternata, A. arborescens, A. brassicae, A. ethzedia, A. hordeicola, A. infectoria, A. japonica, A. malvae, A. metachromatica* and *A. tenuissima.*

5.3 Efficacy of plant bioactive compounds, Sulphur and copper formulations against *Alternaria brassiciciola*

This study revealed that two out of the six plant species tested were able to suppress the growth of *A. brassicicola* mycelium at ≥ 50%. These were *J.curcas* and *T.vogelli.* However, *J. curcas* was significantly more effective than *T. vogelli* at both concentrations of 2000mg/ml and 4000mg/ml of the bioactive compounds. The result of gas chromatography by Sama *et al*., 2012) for various samples of *J. curcas* indicated that it contains unsaturated oil and has a variable fatty acid composition depending on the oil. The most important ones were vaccenic (50.18%), nonadecanoic (26.95%) and palmitic (12.87%) acids and linolelaidic (49.04%), nonadecanoic (25.7%) and palmitic (15.11%) acids. Some of these fatty acids were known for their antifungal activities

Jatropha curcas is reported to contain essential oils that have proven to be effective against other plant pathogens. *Jatrpoha curcas* oil showed inhibition of mycelial growth ranging from 31.59 to 81.96% on some fungi including *Fusarium chlamydosporum*, *Aspergillus niger* and *Penicillium glabrum* (Sama et al., 2012). Furthermore, it was also found that

when *Jatropha curcas* oil concentration is increased, the level of mycelial growth inhibition increases (Cordova-Albores et al., 2014; Najjar et al., 2014; Sama et al., 2021).

Moreover, the presence of cumin aldehyde, carvacrol, indole-5 fatty acids, and others present in *J. curcas* extracts was characterized as the potential compounds for the management of plant pathogenic fungi, including *Alternaria spp.* (Abd-Elbaky and Gharib 2021). Different parts of the *J. curcas* plant, including the leaf and seed, contain compounds that have antimicrobial activity. These compounds include higher steroids, terpenoids (mainly phorbol ester), flavonoids and alkaloids (mainly jatrophines (Krishnan and Paramathma, 2009; Ramadan, 2022; Ramadan Hassanien, 2023)

The application of jatropha extract is a promising technique for management of phytopathogens in vegetable production because it is cost cost-effective and environmentally friendly approach (Gakuhar et al., 2016; Deresa et al., 2023). Furthermore, the jatropha seeds are used to extract biodiesel, which has also been reported to contain high antifungal properties. produce biodiesel, which can also be used against fungal pathogens (Gaikwad et al., 2012).

Several studies have reported the use of *T. vogelii* as a botanical insecticide under laboratory and field conditions (Belmian et al., 2012; Mkenda et al., 2015; Tembo et al., 2018). The antifungal activity of *T. vogelli* may be due to the presence of tephrosine and degueline, which are the major active compounds occurring in all plant parts along with the minor components of tephrosin and rotenone (Lin 2021). This was also supported by Stevenson et al. (2012), who revealed that *T. vogelli* is characterized by the presence of rotenoids, rotenone, deguelin and obovatin 3-*O*-methylether chromatograph results.

A study to demonstrate the chemical variation in *T. vogelii* across locations in three countries of East Africa revealed considerable variation in chemistry influencing the bioactivity of plant materials (Mkindi et al., 2019; Kerebba et al., 2019). Some studies have reported variability in the composition of *T. vogelli*, where the important chemotypes may be absent or in lower concentration. this variability may be attributed to the location where the plant is collected. Therefore, the phytochemical analysis is very important before the local use or commercialization of the *T. vogelli* extracts and products (Mkindi et al., 2019).

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The survey data showed that ALS is prevalent in almost all kale farms in the study area. Most of the farmers manage the disease using fungicides, but some farmers unknowingly use insecticides. The knowledge of ALS and its management varies among kale farmers and is determined by various socio-economic factors and farm characteristics.

2. *Alternaria brassicicola* isolates from the study area did not vary significantly by cultural and morphological features, but the pathogen is genetically diverse, falling into two clades. ALS infecting kale in the study area is caused by *A. brassicicola* and other Alternaria species, including *A. alternata*, *A. altra* and *A. aborescens.*

3. The bioactive compounds from *J. curcas* and *T. vogellii* showed high antifungal potential against *A. brassicicola.* Sulphur formulation (Wetsulf 80% w/w and Thiovit 80% w/w) and Copper (Vifra 40 WG) were confirmed to be effective against *A. brassicicola* at their manufacturer's recommended rate

6.2 Recommendations

1. To boost the farmers' knowledge of ALS, there is a need to create more awareness on the identification of diseases in the field, varieties tolerant to the disease and sustainable disease management approaches. This role can be facilitated by the government and other practitioners, such as NGOs.

2. For the effective management of ALS, the products currently being marketed should be tested for effectiveness to account for the genotypic diversity of *A.*

brassicicola and effectiveness against other Alternaria species responsible for causing ALS in kale in the study area.

3. Further studies on genetic diversity and geographic dispersion of the different races are necessary.
4. Bioactive compounds from *J. curcas* and *T. vogellii* can be extracted for formulation and commercialization at an industrial scale to manage ALS.
5. Sulphur and Copper formulations can be used as a part of Integrated Pest Management against *A. brassicicola.*

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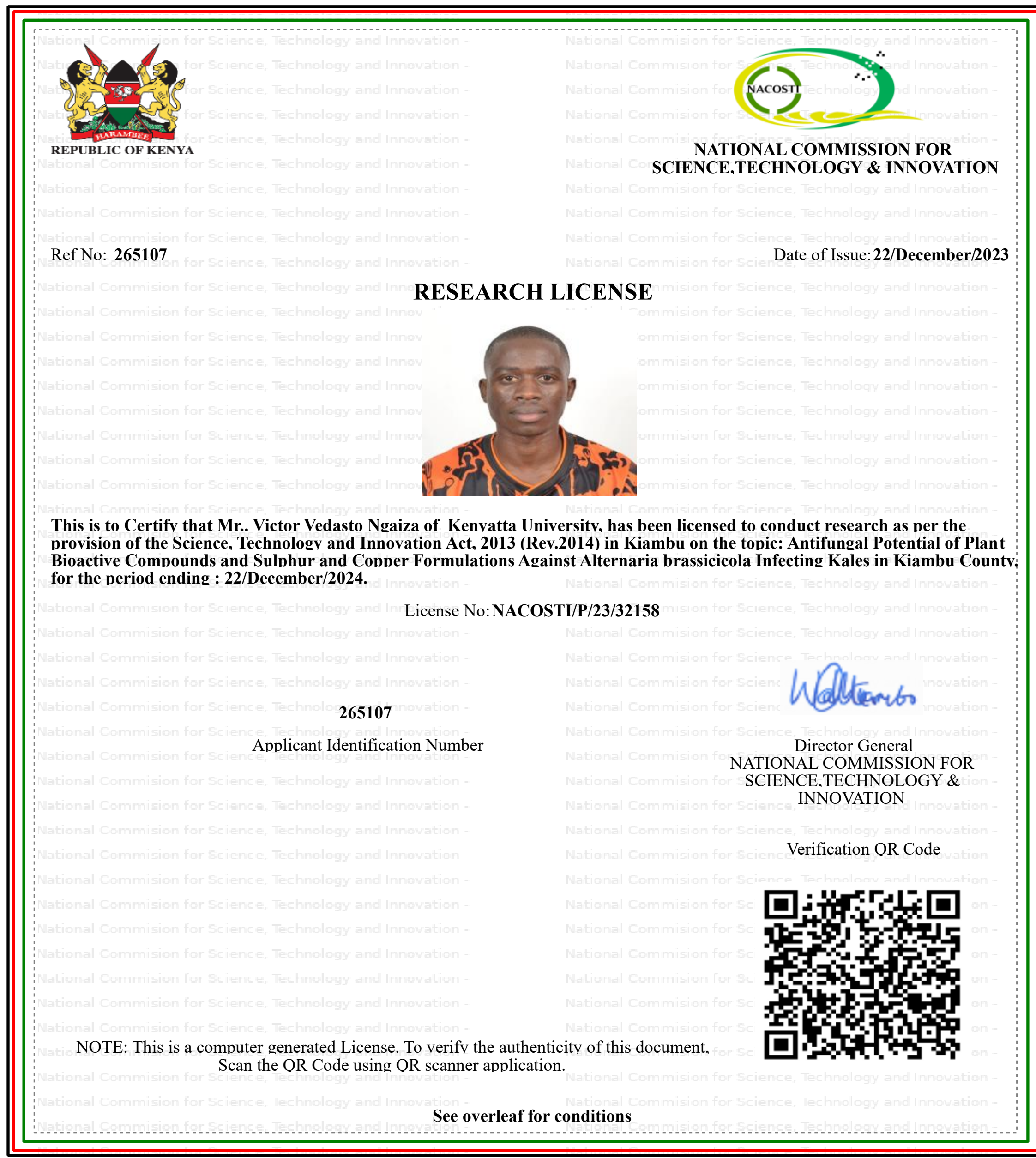
APPENDICES

Appendix 1: National Commission for Science, Technology, and Innovation (NACOSTI) Research Permit



REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **265107**

Date of Issue: **22/December/2023**

RESEARCH LICENSE

This is to Certify that Mr.. Victor Vedasto Ngaiza of Kenyatta University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Kiambu on the topic: Antifungal Potential of Plant Bioactive Compounds and Sulphur and Copper Formulations Against Alternaria brassicicola Infecting Kales in Kiambu County. for the period ending : 22/December/2024.

License No: **NACOSTI/P/23/32158**

265107

Applicant Identification Number

Director General

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

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See overleaf for conditions

Appendix 2: Research approval letter from the graduate school

KENYATTA UNIVERSITY
OFFICE OF THE EXECUTIVE DEAN, GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Internal Memo

FROM: Executive Dean, Graduate School **DATE:** 17th October 2023

TO: Mr. Victor Vedasto **REF:** A145EA/20937/2021
c/o Department of Agricultural Science and Technology

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting on **11th October 2023**, approved your Research Proposal for the M.Sc. Studies. Degree entitled ***"Antifungal Potential of Plant Bioactive Compounds, Sulphur and Copper Formulations Against Altenaria Brassicicola Infecting Kales in Kiambu County.***

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking and Progress Report Forms per semester. The Forms are available at the University's Website under Graduate School webpage downloads.

Also, please ensure that you publish article(s) from your thesis before submitting it to Graduate School for examination as per the Commission for University Education and Kenyatta University guidelines.

Thank you.

REUBEN MURIUKI
FOR: EXECUTIVE DEAN, GRADUATE SCHOOL

cc. Chairman, Department of Agricultural Science and Technology

Appendix 3: Detailed cultural characteristics of the isolates from Githunguri and Lari Sub-counties

Sample code	colour(front)	colour reverse	Texture	margin	Density	Media alteration	Zonation	diameter (mm)
G003	Grey	Creamish grey	flat	regular	medium dense	dark brown	+	59
G029	Creamish grey	Brown	raised	Irregular	less dense	no	+	60.25
G011	Grey	Brown	raised	regular	dense	dark brown	+	48.8
L021	Grey	Brown	raised	irregular	medium dense	dark brown	+	40.38
L053	Creamish white	Brown	raised	regular	medium dense	dark brown	+	52.26
L047	Greenish grey	Black	raised	regular	dense	grey black	+	44.6
G009	Grey	Black	medium raised	irregular	dense	grey black	+	44.92
G027	Grey	Black	flat	irregular	medium dense	dark brown	-	65
L055	Creamish white	Brown	raised	regular	medium dense	no	-	64.2
G017	Grey black	Brown	medium raised	irregular	dense	dark brown	+	39.5
G036	Grey	Brown	raised	irregular	dense	dark brown	-	46
G038	Grey	Brown	raised	irregular	medium dense	dark brown	-	61.55
L043	Greenish grey	Brown	raised	regular	dense	dark brown	-	44.84
G013	Greenish grey	Grey black	raised	irregular	dense	grey black	-	46.8
L050	Creamish white	Creamish grey	raised	regular	dense	no	-	48.66

G041	Creamish white	Brown	raised	regular	medium dense	no	-	62.282
L064	Creamish white	Brown	medium raised	irregular	medium dense	dark brown	+	53.8
G028	Greenish grey	Brown	medium raised	regular	medium dense	no	+	49.5
L034	Grey	Brown	raised	regular	dense	no	-	48.38
G007	Grey black	Black	raised	regular	dense	grey black	+	60.5
G008	Grey black	Black	medium raised	irregular	dense	grey black	+	54.8
L058	Creamish grey	Brown	raised	irregular	dense	no	-	58.8
L008	Creamish grey	Creamish grey	flat	irregular	dense	no	+	44.92
L005	Grey	Brown	medium raised	irregular	dense	grey black	+	47.8
G015	Greenish grey	Brown	raised	regular	dense	dark brown	+	54
L036	Greenish grey	Brown	raised	regular	dense	greenish brown	+	38.42
L003	Grey	Black	medium raised	irregular	medium dense	grey black	+	62.25
L012	Creamish white	Brown	raised	irregular	dense	dark brown	+	42.6
G048	Creamish grey	Brown	medium raised	regular	medium dense	no	-	53.8
L010	Grey	Black	raised	regular	dense	grey black	+	64.8
G004	Grey	Black	flat	regular	less dense	grey black	+	49
L014	Greenish grey	Black	flat	regular	less dense	green	+	54.6
G009	Creamish grey	Brown	raised	regular	dense	dark brown	+	48.55

L038	Greenish grey	Brown	raised	regular	dense	greenish brown	-	47.7
G054	Creamish white	Brown	medium raised	regular	dense	no	-	65.5
G026	Greenish grey	brown	medium raised	regular	less dense	no	-	58.6
G016	Grey	Creamish grey	raised	regular	dense	dark brown	+	64.5
L017	Grey	Brown	medium raised	regular	dense	dark brown	+	45.7
G021	Creamish white	Creamish grey	flat	irregular	less dense	no	+	38.22
L033	Greenish grey	Brown	raised	regular	less dense	no	-	59.5
G044	Greenish white	Brown	medium raised	regular	dense	no	-	64.2
G020	Grey	Brown	raised	irregular	dense	dark brown	-	37.64
G019	Grey	Brown	raised	irregular	dense	dark brown	+	50.78
G022	Grey	Brown	raised	irregular	dense	dark brown	+	53.66
G047	Grey	Brown	medium raised	regular	medium dense	no	-	60.4
L046	Greenish grey	Brown	raised	regular	dense	no	-	48.54
L058	Creamish white	Brown	medium raised	irregular	less dense	dark brown	-	66.8
L067	Greenish grey	Brown	medium raised	irregular	less dense	no	-	49.2
L024	Grey black	Brown	raised	regular	dense	grey black	+	52.58
L023	Creamish white	Creamish grey	flat	irregular	less dense	no	+	50
L019	Creamish grey	Black	raised	irregular	dense	greenish brown	+	12.34

L034	Creamish white	Brown	raised	regular	dense	dark brown	+	48.38
G012	Grey black	Grey black	raised	irregular	medium dense	black	-	65.4
L056	Greenish grey	Brown	medium raised	regular	dense	no	-	64.2
G018	Creamish grey	Brown	flat	regular	less dense	dark brown	+	54.8
L031	Greenish grey	Brown	raised	regular	dense	dark brown	+	64.77
L039	Greenish grey	Black	medium raised	regular	medium dense	grey black	-	38.65
L026	Greenish grey	Black	raised	regular	moderate dense	no	+	48.66
L018	Grey	Brown	raised	irregular	dense	no	+	67.52
G033	Grey	Black	raised	regular	dense	dark brown	+	48.4
L028	Grey black	Black	flat	irregular	medium dense	no	+	42.66
L060	Creamish green	Black	flat	irregular	dense	no	-	60.4
L027	Grey	Brown	raised	regular	dense	dark brown	-	40.66
L001	Grey	Black	medium raised	irregular	dense	grey black	-	46.5
G050	Greenish grey	Black	medium raised	irregular	medium dense	grey black	-	66
G014	Grey	Brown	raised	irregular	dense	dark brown	-	44.1
L035	Grey	Brown	raised	irregular	dense	dark brown	-	66.7
G005	Grey	Black	raised	regular	dense	grey black	-	47.8
L015	Creamish grey	Brown	medium raised	regular	dense	dark brown	-	62.6
G024	Creamish white	Brown	medium raised	regular	moderate dense	dark brown	+	40.66

G014	Grey	Brown	raised	irregular	dense	dark brown	-	44.1
L052	Greenish white	Brown	raised	irregular	dense	dark brown	-	56.55
L066	Creamish white	Brown	raised	irregular	medium dense	grey black	+	66
L020	Grey black	Black	raised	regular	dense	grey black	+	62.8
L061	Creamish white	Brown	medium raised	irregular	dense	no	-	53.5
G041	Creamish black	Brown	raised	regular	medium dense	no	-	62.282
G010	Grey grey	Black	medium raised	irregular	dense	grey black	+	56.4
L041	Greenish grey	Brown	raised	regular	dense	no	-	66.8
L040	Greenish grey	Greenish grey	medium raised	irregular	medium dense	no	+	49.5
L043	Greenish grey	Brown	raised	regular	dense	dark brown	+	48.44
G049	Creamish grey	Brown	raised	irregular	less dense	no	-	51
L035	Greenish grey	Brown	raised	regular	less dense	no	+	49
G030	Greenish grey	Black	medium raised	regular	medium dense	dark brown	+	44
L068	Greenish grey	Black	medium raised	irregular	less dense	no	-	45.8
L029	Grey	Black	raised	irregular	dense	no	+	39.48
L004	Grey	Black	medium raised	regular	dense	grey black	+	40.8
L016	Grey	Brown	raised	regular	dense	dark brown	+	63.8
G051	Greenish grey	Black	medium raised	irregular	medium dense	no	-	58.6

G052	Greenish grey	Black	medium raised	irregular	medium dense	no	-	56.4
G053	Greenish black	Black	medium raised	irregular	medium dense	no	-	61.6
L048	Creamish green	Brown	raised	regular	dense	dark brown	+	57.6
G046	Greenish grey	Brown	medium raised	irregular	less dense	no	-	55.6
G040	Greenish grey	Greenish black	flat	irregular	medium dense	no	+	50.67
G043	Greenish grey	Brown	raised	regular	dense	no	-	58.7
L007	Grey	Creamish grey	raised	regular	dense	no	-	48.5
L051	Creamish grey	Brown	raised	irregular	dense	no	-	56.55
G039	Grey	Brown	medium raised	irregular	dense	dark brown	-	41.28
G037	Creamish grey	Creamish grey	medium raised	regular	medium dense	dark brown	-	48.55
L063	Creamish grey	Creamish grey	medium raised	irregular	medium dense	no	-	59.6
G031	Greenish grey	Brown	medium raised	regular	dense	no	-	55.54
G035	Creamish white	Black	raised	regular	less dense	grey black	+	53.45
G006	Creamish black	Black	semi raised	irregular	medium dense	no	-	48.9
L042	Greenish grey	Brown	raised	irregular	medium dense	no	+	54.6
G001	Grey black	Brown	Flat	regular	medium dense	no	+	64.5
G025	Creamish white	Brown	raised	irregular	medium dense	no	-	40.33

G032	Greenish grey	Brown	raised	regular	dense	no	+	49
L054	Creamish white	Brown	raised	regular	medium dense	no	-	48.54
L006	Grey	Black	raised	regular	dense	grey black	+	38
L030	Greenish grey	Black	raised	regular	dense	dark brown	+	56.31
G042	Creamish grey	Brown	medium raised	irregular	medium dense	no	+	56.74
L044	Creamish white	Black	raised	irregular	less dense	no	+	52.2
L032	Greenish grey	Black	raised	irregular	medium dense	no	+	52.88
L052	Grey	Brown	raised	regular	dense	dark brown	+	40.66
G045	Creamish white	Brown	raised	irregular	dense	no	-	69.1
G002	Grey	Black	flat	regular	medium dense	no	+	64.5
G023	Greenish black	Black	medium raised	regular	medium dense	no	+	44.84
L065	Greenish grey	Brown	raised	regular	less dense	no	-	49.7
L013	Creamish grey	Brown	medium raised	irregular	dense	dark brown	+	50.6
L025	Grey	Brown	raised	irregular	medium dense	no	+	51.66
L037	Greenish grey	Black	raised	regular	dense	dark brown	-	60.34

Appendix 4: Detailed morphological characteristics of the isolates from Githunguri and Lari sub-counties

Sample code	Shape	Conidia dimensions		No. of conidia septa		beak length	Beak septa No.	Presence of conidia
		Length (µm	Width(µm)	Trans.	Long.			
G003	Obclavate	25.90	18.26	1-3	0-2	4.27	0	yes
G029	Obclavate	25.51	9.89	1-2	0	1.07	0	yes
G011	Obclavate	24.63	14.00	3-4	2-4	6.58	0	yes
L021	Obclavate	16.60	10.07	2-4	0-1	3.90	0	yes
L053	Obclavate	14.00	9.37	1-2	0	6.13	0	yes
L047	Obclavate	21.28	10.13	1-2	0	5.39	0-1	yes
G009	Obclavate	23.49	13.40	2-3	0-1	7.43	0	yes
G027	Ellipsoidal	17.15	12.27	2-4	2-4	0	0	no
L055	Obclavate	22.35	9.75	1-3	2-3	3.08	0	yes
G017	Obclavate	19.08	13.12	1-2	0	5.35	0-1	yes
G036	Obclavate	28.05	13.95	3	1-2	4.13	2-3	yes
G038	Ellipsoidal	22.10	12.23	3-4	2-4	0	0-1	no
L043	Obclavate	25.61	11.72	2-3	0-1	4.866	0	yes
G013	Obclavate	27.52	18.32	2-3	0-1	0	1-2	no
L050	Ellipsoidal	23.95	10.54	1-3	1-2	18.71	1-2	yes
G041	Ellipsoidal	31.58	10.37	1.00	0-1	0	0	no
L064	Obclavate	22.77	14.77	1-2	1-2	3.76	0	yes
G028	Obclavate	21.93	16.41	2-4	1-4	26.17	0	yes
L034	Obovoid	19.49	9.89	2-4	1-2	5.24	0-1	yes
G007	Ellipsoidal	18.30	18.75	2-3	0-1	0	0	no
G008	Obclavate	20.57	8.04	2-3	0-1	0	0	no

L058	Obclavate	42.27	12.24	1-3	0-1	14.15	0-1	yes
L008	Obvoid	19.79	15.06	1-2	0	15.87	0	yes
L005	Obvoid	24.97	10.81	2-4	2-3	0	0	no
G015	Ellipsoidal	20.74	13.48	3-4	1-2	0	0	no
L036	Obclavate	26.98	10.87	2-4	1-2	4.77	0-1	yes
L003	Obvoid	20.42	10.30	2-4	1-2	6.28	0-1	yes
L012	Obvoid	21.30	13.50	1-3	0	7.54	0-1	yes
G048	Obclavate	23.50	20.57	2-3	1-2	0	1-2	no
L010	Obclavate	27.36	7.36	2-3	0	0	0	no
G004	Obvoid	20.17	11.08	1.2	0-1	0	0	no
L014	Ellipsoidal	20.88	10.20	2-3	1-2	0	0	no
G009	Obclavate	23.49	13.40	2-3	0-1	7.43	0	yes
L038	Obclavate	20.26	11.06	2-3	0-1	9.23	0	yes
G054	Obclavate	16.66	9.03	0-1	0-1	0	0	no
G026	Obclavate	27.10	23.20	1-2	0-1	3.86	0	yes
G016	Obclavate	25.47	15.56	1-3	0	3.59	2	yes
L017	Obvoid	20.63	10.38	1-2	0-1	4.11	0-1	yes
G021	Ellipsoidal	16.62	11.59	1-3	0	0	0	no
L033	Obvoid	28.49	14.53	2-3	1-2	5.03	1-2	yes
G044	Obclavate	23.94	10.19	3-4	1-2	3.97	0	yes
G020	Ellipsoidal	14.76	9.53	1-3	0	0	0-1	no
G019	Obclavate	25.95	18.61	2-3	0-1	5.61	0-1	yes
G022	Obvoid	14.98	10.10	1-3	1-2	28.44	0-1	yes
G047	Obclavate	23.94	10.20	3-4	1-2	3.98	0	yes
L046	Obclavate	23.64	12.36	2-4	1-2	0	0	no
L058	Obclavate	22.35	9.75	1-3	2-3	3.08	0	yes
L067	Obclavate	22.35	9.75	1-3	2-3	3.08	0	yes

L024	Obclavate	23.83	14.21	1-2	1-2	0	0	no
L023	Obclavate	20.48	9.47	1-2	0	6.78	0	yes
L019	Obclavate	23.83	14.21	1-2	1-2	0	0	no
L034	Obovoid	19.49	9.89	2-4	1-2	5.24	0-1	yes
G012	Obclavate	20.57	8.04	2-3	0-1	0	0	no
L056	Obovoid	23.11	9.70	2-3	0	5.07	1-2	yes
G018	Ellipsoidal	30.51	16.71	3	1-2	0	0	no
L031	Obclavate	15.62	9.39	0-2	0-1	2.88	0-1	yes
L039	Ellipsoidal	16.17	9.63	1-3	0	0	0	no
L026	Obclavate	34.51	15.46	2-3	0-1	0	0	no
L018	Obclavate	20.07	14.67	1-2	1-2	12.80	0-1	yes
G033	Obclavate	45.52	18.43	3-4	1-2	10.71	0	yes
L028	Obclavate	20.58	8.06	2-3	1-2	4.36	1-2	yes
L060	Ellipsoidal	23.95	10.54	1-3	1-2	18.71	1-2	yes
L027	Obclavate	30.12	15.08	1-2	2-3	0	0	no
L001	Obclavate	21.81	14.49	2-3	0-1	6.07	0-1	yes
G050	Ellipsoidal	18.30	18.75	2-3	0-1	0	0	no
G014	Obclavate	29.56	19.66	2-4	0-1	13.93	0	yes
L035	Ellipsoidal	25.33	14.02	0	0	4.57	0	yes
G005	Ellipsoidal	25.22	17.26	0-1	0-1	26.13	0	yes
L015	Obclavate	24.13	3.55	2-3	0	4.50	0-2	yes
G024	Obclavate	16.66	9.03	0-1	0-1	0	0	no
G014	Obclavate	29.56	19.66	2-4	0-1	13.93	0	yes
L052	Obclavate	23.54	9.28	0-1	0-2	10.24	1-2	yes
L066	Obovoid	24.30	10.82	3	0-2	0	0	no
L020	Obovoid	19.08	3.40	2-3	1-2	4.65	0-1	yes
L061	Obclavate	22.77	14.77	1-2	1-2	3.76	0	yes

G041	Ellipsoidal	31.58	10.37	2-3	0-1	0	0	no
G010	Obclavate	27.59	15.29	1-2	0-1	3.32	0	yes
L041	Obclavate	22.77	14.77	1-2	1-2	3.76	0	yes
L040	Obclavate	23.94	10.20	3-4	1-2	3.98	0	yes
L043	Obclavate	25.61	11.72	2-3	0-1	4.86	0	yes
G049	Obclavate	23.94	10.20	3-4	1-2	3.98	0	yes
L035	Obovoid	22.19	15.10	1-2	0-1	6.60	0	yes
G030	Obclavate	24.13	3.56	2-3	0	4.5	0-2	yes
L068	Obclavate	14.00	9.37	1-2	0	6.13	0	yes
L029	Obclavate	26.89	22.16	2-4	2-3	3.74	0	yes
L004	Obovoid	22.82	10.11	1-2	0-1	5.54	0	yes
L016	Obclavate	13.72	8.47	1-2	0-1	6.76	1-2	yes
G051	Obclavate	23.64	12.36	2-4	1-2	0.00	0	no
G052	Obovoid	20.43	10.30	2-4	1-2	6.29	0-1	yes
G053	Obclavate	26.80	8.50	3-4	2-4	4.15	0	yes
L048	Obovoid	30.19	13.85	1-3	0-1	6.00	0-1	yes
G046	Ellipsoidal	18.93	11.26	1.00	0	0	0-1	no
G040	Ellipsoidal	22.11	12.23	3-4	2-4	0	0-1	no
G043	Obclavate	21.28	10.13	1-2	0	5.40	0-1	yes
L007	Obclavate	23.33	12.86	1-2	0-1	2.73	1	yes
L051	Obclavate	13.99	9.36	1-2	0	6.13	0	yes
G039	Ellipsoidal	16.17	9.63	1-3	0	0	0	no
G037	Obclavate	23.49	13.40	2-3	0-1	7.43	0	yes
L063	Obclavate	23.33	12.86	1-2	0-1	2.73	1	yes
G031	Ellipsoidal	17.15	12.27	2-4	2-4	0	0	no
G035	Obclavate	27.59	15.29	1-2	0-1	3.32	0	yes
G006	Obclavate	25.90	18.26	1-3	0-2	4.26	0	yes

L042	Obclavate	39.38	20.65	3-4	1-2	20.65	1-2	yes
G001	ellipsoidal	16.17	9.63	1-3	0	0	0	no
G025	Obclavate	24.33	13.54	2-3	1-2	8.12	0	yes
G032	Ellipsoidal	20.52	9.73	1-3	0-1	44.49	0	yes
L054	Obclavate	26.6	15.76	2-3	0-1	9.50	0-1	yes
L006	Obclavate	24.05	13.80	1-3	1-3	6.16	0	yes
L030	Ellipsoidal	18.46	10.19	1-2	1.00	0	0	no
G042	Obclavate	23.51	20.57	2-3	1-2	0	1-2	no
L044	Obovoid	17.50	13.15	1-2	0-1	12.07	0-1	yes
L032	Obclavate	24.13	3.55	2-3	0	4.50	0-2	yes
L052	Obclavate	23.54	9.28	0-1	0-2	10.24	1-2	yes
G045	Obovoid	23.71	12.39	3-4	0	0	0	no
G002	Obclavate	26.82	16.93	1-2	0	0	0	no
G023	Obclavate	26.82	16.93	1-2	0	0	0	no
L065	Obovoid	30.19	13.85	1-3	0-1	6.00	0-1	yes
L013	Ellipsoidal	31.58	10.37	2-3	0-1	0	0	no
L025	Ellipsoidal	31.58	10.37	2-3	0-1	0	0	no
L037	Obclavate	20.57	8.04	2-3	0-1	0	0	no

Appendix 5: DNA sequence of *Alternaria brassicicola* generated for this study

>*Alternaria brassicicola* **(Kimende-Lari sub county), L031**

AAGGCCATCGATGTATGGAGTGTAGGATGCATTCTGGCTGAGATGCTTAGCG
GAAAGCCTCTGTTCCCAGGAAAGGACTGTAAGTATTGCGCACATAGGATGTG
CCAGGAGCTCTTTGCTAACTGTAGTCAGACCACCACCAACTCACGCTGATTCT
CGATGTGCTTGGTACGCCTACCATGGAGGATTACTACGGCATCAAGTCCCGC
CGAGCTCGTGAATACATTCGGTCGCTGCCATTCAAGAAGAAGATTCCGTGGA
AGGCCATGTTCCCTAAGACCAACGATCTGGCGCTTGACCTGCTCGAGCGGTT
ACTCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCAAGCAC
CAATACCAG

>*Alternaria brassicicola* **(Githiga- Githughuri subcounty), (G050)**

AAGGCCATCGATGTGTGGAGTGTAGGATGCATTCTGGCTGAGATGCTTAGCG
GAAAGCCTCTGTTCCCAGGAAAGGACTGTAAGTATTGCGCACACAGGATGCG
CCAGGGGCTCTTTGCTAACTGTAGTCAGACCACCACCAACTCACGCTGATTCT
CGATGTGCTCGGTACGCCTACCATGGAGGATTACTACGGCATCAAGTCCCGC
CGAGCTCGTGAATACATTCGATCGTTGCCATTCAAGAAGAAGATTCCGTGGA
AGGCCATGTTCCCTAAAACCAACGATCTGGCGCTTGACCTGCTCGAGCGGTT
ACTCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCAAGCAC
CCATACCAG

>*Alternaria brassicicola* **(Nyambari -Lari subcounty), (L034)**

AAGGCCATCGATGTATGGAGTGTAGGATGCATTCTGGCTGAGATGCTTAGCG
GAAAGCCTCTGTTCCCAGGAAAGGACTGTAAGTATTGCGCACATAGGATGTG
CCAGGAGCTCTTTGCTAACTGTAGTCAGACCACCACCAACTCACGCTGATTCT
CGATGTGCTCGGTACGCCTACCATGGAGGATTACTACGGCATCAAGTCCCGC
CGAGCTCGTGAATACATTCGGTCGCTGCCATTCAAGAAGAAGATTCCGTGGA
AGGCCATGTTCCCTAAGACCAACGATCTGGCGCTTGACCTGCTCGAGCGGTT
ACTCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCAAGCAC
CCATACAAG

>*Alternaria brassicicola* **(Kijabe- Lari) (L017)**

AGAACTCAAGCACAATACCAGAGCTCAAGCACCAGTACCAGACCTCTAGCAC
CAATACCAGGCCCCAAGCACAATACCAGAGCTCTACCACCATTACCAGTGCC
CAAACACTCTTTGCAAACTGTAGTCAGACCTCCACCAACTCACGCTGATTCTC
GATGTGCTCGGTACGCCTACCATGGAGGACTACTACGGCATCAAGTCCCGCC
GAGCTCGCGAATACATTCGGTCGCTGCCATTCAAGAAGAAGATTCCGTGGAA
GGCCATGTTCCCTAAGACCAACGATCTGGCGCTTGACCTGCTCGAGCGGTTA
CTCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCAAGCACC
CATACCAG

>*Alternaria brassicicola* **(Nyambari- Lari) (L019)**

AAGGCCATCGATGTGTGGAGTGTAGGATGCATTCTGGCTGAGATGCTTAGCG
GAAAGCCTCTGTTCCCAGGAAAGGACTGTAAGTATTGCGCACACAGGATGCG
CCAGGAGCTCTTTGCTAACTGTAGTCAGACCACCACCAACTCACGCTGATTCT
CGATGTGCTCGGTACGCCTACCATGGAGGATTACTACGGCATCAAGTCCCGC
CGAGCTCGTGAATACATTCGATCGCTGCCATTCAAGAAGAAGATTCCGTGGA
AGGCCATGTTCCCTAAAACCAACGATCTGGCGCTTGACCTGCTCGAGCGGTT
ACTCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCACCCCC
CATACCAG

>*Alternaria brassicicola* **(Nyambari- Lari) (L046)**

AAGGCCATCGATGTATGGAGTGTAGGATGCATTCTGGCTGAGATGCTTAGCG
GAAAGCCTCTGTTCCCAGGAAAGGACTGTAAGTATTGCGCACATAGGATGTG
CCAGGAGCTCTTTGCTAACTGTAGTCAGACCACCACCAACTCACGCTGATTCT
CGATGTGCTCGGTACGCCTACCATGGAGGATTACTACGGCATCAAGTCCCGC
CGAGCTCGTGAATACATTCGGTCGCTGCCATTCAAGAAGAAGATTCCGTGGA
AGGCCATGTTCCCTAAGACCAACGATCTGGCGCTTGACCTGCTCGAGCGGTT
ACTCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCAAGCAC
C-ATACCAG

> *Alternaria brassicicola* **(Githiga-Githunguri) (G060)**

AGGCCATTAACCCAAGGAGAGTAGGATGCATTCTGGCTGAGATGCTTAGCGG
AAAGCCTCTGTTCCCAGGAAAGGACTGTAAGTATTGCGCACATAGGATGTGC
CAGGAGCTCTTTGCTAACTGTAGTCAGACCACCACCAACTCACGCTGATTCTC
GATGTGCTCGGTACGCCTACCATGGAGGATTACTACGGCATCAAGTCCCGCC
GAGCTCGTGAATACATTCGGTCGCTGCCATTCAAGAAGAAGATTCCGTGGAA
GGCCATGTTCCCTAAGACCAACGATCTGGCGCTTGACCTGCTCGAGCGGTTA
CTCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCAAGCACC
CATACCAG

>*Alternaria brassicicola* **(Kijabe- Lari subcounty) (L018)**

AAGGCCATTGATGTATGGAGTGTAGGATGCATTCTGGCTGAGATGCTTAGCG
GAAAGCCTCTGTTCCCAGGAAAGGACTGTAAGTATTGCGCACATAGGATGTG
CCAGGAGCTCTTTGCTAACTGTAGTCAGACCACCACCAACTCACGCTGATTCT
CGATGTGCTCGGTACGCCTACCATGGAGGATTACTACGGCATCAAGTCCCGC
CGAGCTCGCGAATACATTCGGTCGCTGCCATTCAAGAAGAAGATTCCGTGGA
AGGCCATGTTCCCTAAGACCAACGATCTGGCGCTTGACCTGCTCGAGCGGTT
ACTCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCAACACC
CATACCAG

> *Alternaria brassicicola* **(Ikinu-Githunguri sub county) (G019)**

AGACCTCCAGCACCATACCAGCACTCAAGCACCCGTCTGGGATCCCTAGCAC
CAATACTCTGTTCCCAGTAAAGGACTGTAAGTATTGCGCACACAGGATGCGC
CAGGGGCTCTTTGCTAACTGTAGTCAGACCACCACCAACTCACGCTGATTCTC
GATGTGCTCGGTACGCCTACCATGGAGGATTACTACGGCATCAAGTCCCGCC
GAGCTCGTGAATACATTCGATCGTTGCCATTCAAGAAGAAGATTCCGTGGAA

GGCCATGTTCCCTAAAACCAACGATCTGGCGCTTGACCTGCTCGAGCGGTTA
CTCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCAAGCACC
CATACCAG

>*Alternaria brassicicola* **(L023) (Kagwe Lari subcounty)**

AAGGCCATCGATGTATGGAGTGTAGGATGCATTCTGGCTGAGATGCTTAGCG
GAAAGCCTCTGTTCCCAGGAAAGGACTGTAAGTATTGCGCACATAGGATGTG
CCAGGAGCTCTTTGCTAACTGTAGTCAGACCACCACCAACTCACGCTGATTCT
CGATGTGCTTGGTACGCCTACCATGGAGGATTACTACGGCATCAAGTCCCGC
CGAGCTCGTGAATACATTCGGTCGCTGCCATTCAAGAAGAAGATTCCGTGGA
AGGCCATGTTCCCTAAGACCAACGATCTGGCGCTTGACCTGCTCGAGCGGTT
ACTCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCAACACC
C-ATACCAG

>*Alternaria brassicicola* **(Githiga- Githunguri subcounty) (G033)**

AAGGCCATCGATGTATGGAGTGTAGGATGCATTCTGGCTGAGATGCTTAGCG
GAAAGCCTCTGTTCCCAGGAAAGGACTGTAAGTATTGCGCACATAGGATGTG
CCAGGAGCTCTTTGCTAACTGTAGTCAGACCACCACCAACTCACGCTGATTCT
CGATGTGCTCGGTACGCCTACCATGGAGGATTACTACGGCATCAAGTCCCGC
CGAGCTCGTGAATACATTCGGTCGCTGCCATTCAAGAAGAAGATTCCGTGGA
AGGCCATGTTCCCTAAGACCAACGATCTGGCGCTTGACCTGCTCGAGCGGTT
ACTCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCAAGCAC
CCATACCAC

> *Alternaria brassicicola* **(Githiga-Githunguri sub county) (G018)**

AAGGCCATCGATGTATGGAGAGTAGGATGCATTCTGGCTGAGATGCTTAGCG
GAAAGCCTCTGTTCCCAGGAAAGGACTGTAAGTATTGCGCACATAGGATGTG
CCAGGAGCTCTTTGCTAACTGTAGTCAGACCACCACCAACTCACGCTGATTCT
CGATGTGCTCGGTACGCCTACCATGGAGGATTACTACGGCATCAAGTCCCGC
CGAGCTCGTGAATACATTCGGTCGCTGCCATTCAAGAAGAAGATTCCGTGGA
AGGCCATGTTCCCTAAGACCAACGATCTGGCGCTTGACCTGCTCGAGCGGTT
ACTCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCAAGCAC
CCATACCAG

> *Alternaria brassicicola* **(Nyambari -Lari sub county) (L026)**

AAGGCCATCGATGTGTGGAGTGTAGGATGCTTTCTGGCTGAGATGCTTAGCG
GAAAGCCTCTGTTCCCAGGAAAGGACTGTAAGTATTGCGCACACAGGATGCG
CCAGGGGCTCTTTGCTAACTGTAGTCAGACCACCACCAACTCACGCTGATTCT

CGATGTGCTCGGTACGCCTACCATGGAGGATTACTACGGCATCAAGTCCCGC
CGAGCTCGTGAATACATTCGATCGTTGCCATTCAAGAAGAAGATTCCGTGGA
AGGCCATGTTCCCTAAAACCAACGATCTGGCGCTTGACCTGCTCGAGCGGTT
ACTCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCAAGCAC
CCATACCA

>*Alternaria brassicicola* **(Gitiha- Githunguri sub county) (G044)**

AAGGCCATCGATACCTGGCGAGTAGGATGCATCTTGGCTGAGATGCTTAGTG
GAAAGCCTCTGTTCCCAGGAAAAGACTGTAAGTATTGCGCAAATATCATGTA
TCAGGACCTGGTTGCTAATTGTCATTAGACCACCACCAGCTTACGCTGATTCT
CGATGTGCTCGGCACACCCACCATGGAGGACTACTACGGAATTAAATCTCGC
CGAGCTCGCGAATACATTCGATCGCTGCCGTTCAAGAAGAAGATTCCGTGGA
AGGCCATGTTCCCCAAGACCAACGACCTGGCACTTGACCTGCTCGAGCGGTT
ACTCGCTTTCAACCCTGTCAAGCGCATCACAGTCGAGGAAGCACTCAAGACC
CCATACCAG

> *Alternaria brassicicola* **(Gitiha- Githunguri sub county) (G044)**

AGGGTCGAGAAGTGTGGATGGGAGGATGCATGCTGGCTGGAATGCTTAGCGG
AAAGCCTCTGTTCCCAGGAAAGGACTGTAAGTATTGCGCACATAGGATGTGC
CAGGAGCTCTTTGCTAACTGTAGTCACACCACCACCAACTCACGCTGATTCTC
GATGTGCTCGGTACGCCTACCATGGAGGATTACTACGGCATCAACTCCCGCC
GAGCTCGTGAATACATTCGGTCGCTGCCATTCAAGAAGAAGATTCCGTGGAA
GGCCATGTTCCCTAATACCAACGATCTGGCGCTTGACCTGCTCGAGCGGTTAC
TCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCACACCCCC
ATTACAA

>*Alternaria brassicicola* **(Kijabe -Lari subcounty) (L041)**

AGGCCTCAAGCACAATACCAGCACTCAAGCACCAATACCAGACCTCTAGCAC
CAATACCAGGACTCCAGCACCGTACGAGACCTCTACCACCATTACCGGAGCC
CAACCACCAATACCAGAACTCAAGCACCCATACCAGACCCCAAGCACATTCT
CGATGTGCTCGGTACGCCTACCATGGAGGATTACTACGGCATCAAGTCCCGC
CGAGCTCGTGAATACATTCGGTCGCTGCCATTCAAGAAGAAGATTCCGTGGA
AGGCCATGTTCCCTAAGACCAACGATCTGGCGCTTGACCTGCTCGAGCGGTT
ACTCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCAAGCAC
CCATACCAG

> *Alternaria brassicicola* **(Nyambari- Lari sub county) (L027)**

AGCCCTCAAGCACCATACCAGCGCTCAAGCACCCATACCAGCCCTCAAGCAC
CCATACCAGACCTCAAGCACCATACCGGCCCTCAAGCACCCATACCAGACCC
CAAGCACCCATACCAGACCTCAAGCACCAGTACCAGACCCCCAGCACCATTC
CGATGCGCTCGCACCACCTACGATGCTGGATTACTACTACAGAACCTCCCCA
CCAGTTCATGAATACATTCAGTCGCTGCCATTCAAGAAGAAGATTCCGTGGA
GGGCCATGTTCCCTAAGACCAACGATGTGGCGCTTGACCTGCTCGAGCGGTT
ACTCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCACACCG
CCATACCAG

> *Alternaria brassicicola* **(Kimende Lari sub county) (L068)**

AGCACTCTAGCACAATACCAGCACTCTAGCACCAATACCAGGGCTCTAGCAC
CCATACCAGGGCTCTAGCACAATACCAGAGCTCTAGCACCAATACCAGAACT
CAAACACCCATACCAGAGCTCAAACACCCATACCAGAGCTCAAGCACCCATT
CGAGGTGCCCAGCACCCCTACCAGGGCGGAATCCTCCGGACGCGAGTCCCGC
CAAGCTCGTGAATACGTTCGGTCGCTGCCGTTCAAGAAGAAGATTCCGTGGA
AGGCCATGTTCCCTAAGACCAACGATCTGGCGCTTGACCTGCTCGAGCGGTT
ACTCGCTTTCAACCCTGTCAAGCGAATGACAGTCGAGGAAGCACTCAAGCAC
CCATACCAG

>*Alternaria brassicicola* **(Kaburu- Lari sub county) (L066)**

AGGGCTCTAGCACAATACCAGCACTCAAGCACCAATACCAGACCTCTAGCAC
CAATACCAGGGCTCCAGCACAATACCAGAGCTCAACCACCCATACCAGTACT
CAAACAACAATAGCAGACGGAAGCACCAGTACAAAAACTCAGGTTGAATTA
CGATGTGCTCGGCACCCCTACCATGGAGGATTAATACGGCATCGAGTCCCGC
CGAGCTCGAAAATACATTCGATCGCTGCCATTCAAGAAGAAGATTCAGTGGA
AGGCCATGTTCCCTAAGACCAAAGATCTGGCGCTTGGCCTGCTCGAGTGGTT
ACTCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCGAGCAA
CCATACCA

>*Alternaria brassicicola* **(Kimende- Lari sub county) -(L028)**

AAGGCCCTCGCCGTCTGGAGAGTTGGATGCATTCTGGCTGAGATGCTTAGCG
GAAAGCCTCTGTTCCCAGGAAAGGACTGTAAGTATTGCGCACATAGGATGTG
CCAGGAGCTCTTTGCTAACTGTAGTCATACCACCACCAACTCACCCTGATTCT
CGATGTGCTCTGTACGCCTACCATGGAGGATTACTACGGCATCAAGTCCCGC
CGAGCTCGTGAATACATTCGGTCGCTGCCATTCAAGAAGAAGATTCCGTGAA
AGGCCATGTTCCCTAAGACCAACGATCTGGTGCTTGACCTGCTCAAGCGGTTA
CTCGCTTTCAACCCTGTTAAGCTAATCACAGTCGAGGAAGCACTCAAACACC
CGTACCAC

> *Alternaria brassicicola* **(Githiga- Githunguri sub county) (G039)**

AAGGCCATCGATGTATGGAGTGTAGGATGCATTCTGGCTGAGATGCTTAGCG
GAAAGCCTCTGTTCCCAGGAAAGGACTGTAAGTATTGCGCACATAGGATGTG
CCAGGAGCTCTTTGCTAACTGTAGTCAGACCACCACCAACTCACGCTGATTCT
CGATGTGCTCGGTACGCCTACCATGGAGGATTACTACGGCATCAAGTCCCGC
CGAGCTCGTGAATACATTCGGTCGCTGCCATTCAAGAAGAAGATTCCGTGGA
AGGCCATGTTCCCTAAGACCAACGATCTGGCGCTTGACCTGCTCGAGCGGTT
ACTCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCAAGCAC
CCATACCAG

> *Alternaria brassicicola* **(Githiga-Githunguri sub county) (G001)**

AAGGCCATCGATGTATGGAGTGTAGGATGCATTCTGGCTGAGATGCTTAGCG
GAAAGCCTCTGTTCCCAGGAAAGGACTGTAAGTATTGCGCACATAGGATGTG
CCAGGAGCTCTTTGCAACTGTAGTCAGACCACCACCAACTCACGCTGATTCTC

GATGTGCTCGGTACGCCTACCATGGAGGATTACTACGGCATCAAGTCCCGCC
GAGCTCGTGAATACATTCGGTCGCTGCCATTCAAGAAGAAGATTCCGTGGAA
GGCCATGTTCCCTAAGACCAACGATCTGGCGCTTGACCTGCTCGAGCGGTTA
CTCGCTTTCAACCCTGTCAAGCGAATCACAGTCGAGGAAGCACTCAACCATA
CACACCAG

Appendix 6: Survey questionnaire for this study

I am a student at Kenyatta University conducting a farm survey in this area. The survey aims to investigate the occurrence, knowledge levels, prevalence, and current management practices for the Alternaria leaf spot in Kales in Kiambu County.

The information provided by you in this survey will be used for research purposes and is completely confidential. It will not be used in a manner which would allow the identification of your responses. I ask for your consent to conduct the survey

Questionnaire number: ……………………. Date: ……………………

Section A: Details of the respondent's area

Sub County: …………………………

Location: ……………………

Village: ……………………… Nearest town: ……………………….

Section B: Socio-demographic characteristics of kale farmers

Name of the farmer: ………………………………. Contact: ………………

(a). Sex: (a) Male (b) Female

(b). Age of household head (Yrs.) ……………...

(c). Education level: (1) None (2) Primary (3) Secondary

(4) Tertiary

(d). Farm ownership(s): (1) Owner (2) Hired (3) Partnership

(4) Manager

(e). What is the size of your farm under kales (in acres)? ………………

(f) What is your total farm size (in acres) …………………...

(f). How many household members in farming?.........................

(g) What is the household monthly income (in Ksh)?............................

(h) Occupation of the head

(1) Farmer (2) Famer + other occupations (3) non-farmer (4) others

C: Farmer's Knowledge of management of Alternaria Leaf spot disease

Please answer the questions below in practical knowledge on Alternaria leaf spot disease in kale and its management (indicate if the statement is true or false)

Questions	True/false
1. Alternaria leaf spots in kale can cause yield loss of up to 45% if not managed.	
2. Alternaria leaf spots appear round, grey to black concentric rings that look like a target.	
3. During the rainy season, the Alternaria leaf spot is more severe than in the dry season.	
4. The source of kale seedlings has nothing to do with leaf spot disease spread.	
5. Alternaria leaf spot is not a seed-borne disease.	
6. It is cheaper to use botanicals than chemical fungicides to manage Alternaria leaf spots.	
7. There are some kale varieties resistant/tolerant of Alternaria leaf spots.	
8. Copper and Sulphur-containing pesticides are safer than other synthetic fungicides when used to manage Alternaria leaf spots.	
9. Fungicides to control Alternaria leaf spots in kale are usually sprayed directly on infected plants.	
10. The fungicides to control leaf spot disease are usually applied at a frequency of 7-14 days.	
11. Management of Alternaria leaf spots using chemical pesticides causes more negative than positive effects on both humans and the environment.	
12. Management of leaf spot disease using synthetic fungicides compromises the quality of kale produce.	

Section D: Kale Management and Production Practices

(a) How many planting seasons do you have kale per year?

(1) One (2) Two (3) Three (4) More than two seasons

(b) For how long have you been growing kale (in years)?.........................

(c) On a scale of 1-10, score your preference for these kale varieties.

(i) Ethiopian kale (Kanzira)

(ii) Mfalme F1

(iii) Marrow stem

(iv) Moss-curled kale

(v) Thousand headed

(d). Why do you prefer the above variety(s)? (Tick all that apply)

Varieties	High yield	Early maturity	Ready market	Readily available	Tolerance	Longer dormancy
Ethiopian (Kanzira)						
Mfalme 1						
Marrow stem						
Moss curled						
Thousand headed						

(e). Where do you get your kale seedlings/ seeds from?

(1) Purchase from a certified dealer (2) Buy seedlings from market

(3) Select from previous harvest (4) Borrow seedlings from a neighbour

(5) Others (Specify)………………

(f). What type of cropping systems do you practice?

(1) Mono-cropping (2) Intercropping (3) Relay cropping (4) Crop rotation.

(g). If you practice intercropping, which crops do you intercrop with kale? (Tick all that apply)

(1) other vegetables (2) cereal crops (3) legumes (4) tree/tree crops 7) Other(s) ……………………….

(h) What type of kale farming do you do?

(1) Rainfed (2) Irrigated (3) Both

Section E: Alternaria leaf spot disease and its management

(a). Do you experience dark leaf spot disease in kale farming?

(1) YES (2) NO

(b). At what stage of crop growth do you notice the diseases?

(1) Seedling (2) Vegetative (3) Flowering (4) Harvesting (5) All stages

(c). How long has the dark leaf spot problem on your farm?

(1) <1 yr. (2) 2-3yrs (3) 4-6yrs (4) >7yrs

(d). On what variety (s) is it most severe? (Rank in order of priority)

(1) Ethiopian Kanzira (2) Mfalme F1 (3) Marrow stem

(4) Moss curled kale (5) Thousand headed

(e). What control measures do you apply to manage this disease?

(1) Use of fungicides (2) Use of Resistant variety(s)

(3) Uprooting affected plants (4) Using certified kale seeds

(5) None

(6) Others (Specify)…………..……………………………

(f). If you use fungicides, which fungicide(s) do you apply?

Specify trade name………………………………………….

(g). How effective is the fungicide(s) applied?

(1) Very effective (2) Effective (3) Less effective

(4) Not effective

(h). At what frequency is the fungicide(s) applied?

Specify ……………………………………...

(i). If use resistant variety(s), which kale (s) do you use?

Specify ……………………………………….

(j). From where do you get the knowledge on dark leaf spot disease management practices?

(1) Government extension staff (2) Private extension staff

(3) Field days (4) Agrovet shops (5) Radio/TV (6) Newspapers

(7) Neighbors.

(k) If from extension how many times in a year has the extension visited? ……………………….

(l). Are you aware of botanicals, Sulphur and Copper fungicides strategies used in managing Alternaria diseases?

(1) Yes (2) No (skip to question "n")

(m). How did you know about the plant extract and Sulphur and copper fungicides (s)/strategies?

(1) Government extension staff (2) Private extension staff

(3) Field days (4) Agrovet shops (5) Radio/TV

(6) Newspapers

(7) Neighbors

(n). How do you determine when to initiate control of dark leaf spot disease on your kale? (Tick all that apply)

(1) When I see disease symptoms (2) Changing weather conditions

(3) At a particular crop growth stage (4) Follow extension recommendations

(5) Chemical company agronomist/salesmen recommendations

(6) My own experience (7) Specify Others ………………….

(o) Any training(s) attended by farmer about kale disease management ……………

(p) Number of pieces of training per year

(1) None (2) One (3) Two (4) Three (5) More than three

(m) Do you have access to credit

(1) Yes (2) No

Section F: Post-harvest handling and management

(a). What is your average yield of kale in bags per Acre (in kg)?......................

(b). Are you a member of any SACCO/Growers Association/Farmers group?

(1) Yes (2) No

(c). If yes in Q9, name and that you are registered with.

………………………………………………………………

Thank you very much for participating in this survey