CHARACTERIZATION AND FUNGICIDE SENSITIVITY OF TOMATO ISOLATES OF *ALTERNARIA SOLANI* SORAUER IN KAJIADO, KIAMBU AND KIRINYAGA COUNTIES, KENYA

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DECLARATION

I Andrew Nuwamanya declare that this thesis is my original work and has not been presented for the award of a degree in any other university or for any other award.

Signature…… Date……

Andrew Nuwamanya

Supervisors' approval:

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

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DEDICATION

I dedicate this thesis to all my teachers along this journey of formal education. This thesis is a testimony to the good work you have done in me. May God bless you!

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LIST OF ABBREVIATIONS AND ACRONYMS

ABC	ATP-binding cassette
a.i.	Active ingredient
ACZ	Agro-climatic zones
ANOVA	Analysis of Variance
AST	Agricultural Science and Technology
ATP	Adenosine triphosphate
CABI	Centre for Agriculture and Bioscience International
Cyt b	Cytochrome b gene
DMIs	Demethylation Inhibitors
DNA	Deoxyribonucleic acid
EAC	East African Community
EB	Early blight
EC_{50}	Effective concentration of a fungicide that reduces mycelial growth by 50 %
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FRAC	Fungicide Resistance Action Committee
GPS	Global Positioning System
HCDA	Horticultural Crop Development Authority, Kenya
HSD	Honestly Significant Difference
IUCEA	Inter University Council for East Africa
KNBS	Kenya National Bureau of Statistics

KU	Kenyatta University
MFS	Major Facilitator Superfamily
%MGI	Percent Mycelial Growth Inhibition
MoALF	Ministry of Agriculture, Livestock and Fisheries (Kenya)
NACOSTI	National Council for Science, Technology, and Innovation
NCBI	National Centre for Biotechnology Information
PCPB	Pest Control Products Board
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
RNA	Ribonucleic acid
rpm	rotations per minute
QoIs	Quinone Outside Inhibitors
SDHIs	Succinate dehydrogenase inhibitors
Sdh	Succinate dehydrogenase gene
SHAM	Salicylhydroxamic acid
SPSS	Statistical Package for the Social Sciences
SSA	sub-Saharan Africa
TAE	Triacetate EDTA
µm	Micrometer
µL	Microliter

ABSTRACT

Early blight (EB) caused by *Alternaria solani* is ranked as one of the most important tomato diseases in Kenya and farmers predominantly rely on synthetic fungicides to control it. However, there have been reports about the declining efficacy of some fungicides against EB control. This study was carried out to (i) determine the occurrence, importance and current management practices for tomato EB in Kirinyaga, Kajiado and Kiambu counties, Kenya; (ii) characterize *Alternaria solani* tomato isolates from the selected counties by morphological features and sensitivity to two commonly used fungicide groups and (iii) determine occurrence and spatial distribution of resistance-associated mutations in *A. solani* isolates from the three counties. A baseline survey was carried out in 175 tomato fields in Kirinyaga (n=58), Kajiado (77) and Kiambu (40) counties, data was collected using semi-structured questionnaires and field observation. Tomato shoots showing typical EB symptoms were collected from surveyed fields (one per field) and carried to Kenyatta University Pathology Laboratory, where a total of 96 *A. solani* isolates were isolated. Results indicate that EB was highly prevalent (75-91%) in all regions and all farmers were controlling it by fungicide application. A total of 40 fungicide products, representing 20 active compounds, with varying resistance risk levels, were in use against EB. Most farmers (83%) were applying the fungicides at higher than the recommended doses. Most farmers (81%) reported declines in effectiveness of fungicides, especially strobilurins and triazoles. The *Alternaria solani* isolates were characterized based on cultural features, conidial morphology and sensitivity to two fungicides; azoxystrobin (a strobilurin) and difenoconazole (a triazole) *in vitro* by poisoned food technique. One way analysis of variance revealed that colony and conidial parameters of isolates did not differ significantly (at α=0.05) across the study counties. Isolates were considered resistant to the fungicide whenever their % Mycelial Growth Inhibition (%MGI) at manufacturer's recommended dosage was below 50% and sensitive when above 50%. While all isolates were susceptible to Difenoconazole, majority of them (64%) were resistant to Azoxystrobin. Locations significantly differed in regard to sensitivity of isolates to fungicides with Kajiado and Kirinyaga isolates being least sensitive to Azoxystrobin and Difenoconazole, respectively. To determine the genetic basis of Azoxystrobin resistance, the cytochrome b gene (in all isolates) was PCR amplified, sequenced and analyzed for resistance-associated mutations at amino acid positions 129, 137 and 143. The F129L mutation was present in all Azoxystrobin resistant isolates plus 10 susceptible ones with MGI values close to the 50% threshold. Kajiado county had the highest percentage of mutated isolates (96.8%), followed by Kirinyaga (70%) and lastly Kiambu (40%). These findings indicate that Kenya's *A. solani* populations have developed resistance to some fungicides by mutation. The study recommends that anti-fungicide resistance strategies should be applied, for more effective management of tomato early blight.

CHAPTER ONE: INTRODUCTION

1.1 Background

Tomato (*Solanum lycopersicum* L.), is among the world's most important crops in terms of production, consumption, and trade. It belongs to family Solanaceae with origins in South America (Bai and Lindhout, 2007). In sub-Saharan Africa (SSA), tomato is extensively grown as a food and cash crop and contributes significantly to nutrition, employment, and income generation (Malherebe and Marais, 2015). According to FAOSTAT (2020), Kenya is among the leading tomato-producing countries in SSA, with an annual production of 599,458 tonnes. The crop accounts for about 7% of horticulture and 14% of vegetable production in the country (Mwangi *et al.,* 2015).

Despite its importance, tomato yields in Kenya have been declining due to many constraints. Early blight (EB) caused by *Alternaria solani* (Ellis and Martin) Sorauer is among the most significant tomato diseases in Kenya (Mugao *et al.,* 2020; Matumwabirhi, 2020; Mwangi *et al.,* 2015). Together, early blight and late blight (*Phytophthora infestans* (Mont.) de Bary) are estimated to cause 95.6% of all pre-harvest yield losses in Kenya's tomato yields (Waiganjo *et al.,* 2006). Brownish black lesions form on aerial parts of EB-affected plants and as these expand, plants lose more and more of their photosynthetic surface area (Foolad *et al.,* 2008), ultimately producing smaller, often lesioned fruits that fetch a low market value.

Management of early blight has remained a challenge especially among smallholder farmers in Kenya (Matumwabirhi, 2020). There has been an increasing tendency by farmers to rely on fungicides as the main method of control, mostly for its high efficacy at early blight control. According to Waiganjo *et al.* (2006), the highest pesticide use during tomato production in Kenya is for control of Early and Late blights with up to 40 applications per cropping season. The registered fungicides against EB in Kenya include multisite actors, Quinone outside inhibitors (QoIs), Demethylation inhibitors (DHIs) and Succinate dehydrogenase inhibitor (SDHI) fungicides. However, there has been a growing concern from farmers about the declining efficacy of some of the fungicides at controlling early blight (PCPB, 2005; 2019), and this is complicating EB management.

1.2 Statement of the problem

Control of tomato early blight in Kenya, is currently affected by declines in efficacy of some fungicides. According to farmers, this challenge is making tomato production expensive since they have to apply fungicides at doses higher than those recommended by manufacturers (PCPB, 2019). Also, the intensive use of fungicides is harmful to human and environmental health, and impacts negatively on the quality of harvested fruits for human consumption (Jørgensen *et al.,* 2017; Ishii *et al.*, 2009; Hardwick *et al.*, 2001).

In other tomato growing countries where such fungicide efficacy declines have been faced, research usually confirms development of resistance in *A. solani* isolates to available fungicides (Metz *et al.,* 2019; Nottensteiner *et al.,* 2019;

Leiminger *et al.,* 2016). Resistance development in *A. solani* especially to single-site fungicides has emerged as a major challenge in many tomato growing areas globally. Many authors have posited that as *A. solani* interacts with fungicides over time, its genome undergoes certain mutations in the genes targeted by fungicides (Samen *et al.*, 2016; Gudmestad *et al.*, 2013; Rosenzweig *et al.*, 2008; Pasche and Gudmestad, 2008). As a result, successive *A. solani* generations are becoming increasingly less sensitive to many fungicides being used globally (FRAC 2021; Rossi *et al.,* 2020; Weber and Hahn 2019; Ishii and Hollomon, 2015; Ktiller and Scheinpflug, 1987).

Examples of resistance-associated mutations that have been reported in *Alternaria solani* include G143A, F129L, G137R in *cyt b* gene that confer resistance to strobilurins (Fernández-Ortuño *et al.*, 2008; Grasso *et al.,* 2006; Pasche *et al.,* 2005) and SdhC-H134Q, SdhB-H278Y, and SdhC-H134R in *Sdh* genes for SDHI fungicides (Mostafanezhad *et al.*, 2021; Metz *et al.,* 2019; Mallik *et al.*, 2014).

A closer look at Kenya's tomato production systems reveals presence of many factors that would enable faster establishment and spread of mutant *Alternaria solani* biotypes if they emerged. For instance, most available fungicides in the country have been in continuous use for more than 20 years (PCPB, 2019) and the warm humid conditions in most tomato growing areas create favorable conditions for *A. solani* to complete many infection cycles in single cropping seasons (C. Kinyanjui (PCPB), personal communication, March 29, 2021). This

is complexed by lack of resistant tomato varieties and small land portions which make it difficult for farmers to have sufficient fallows or rotations between cropping seasons (Mwangi *et al.*, 2015).

1.3 Justification of the study

Despite farmers' complaints about declines in efficacy of some early blight fungicides (PCPB, 2005, 2019), little is known about the sensitivity of Kenya's *Alternaria solani* populations to fungicides being used in the country. Consequently, there is no empirical evidence to underpin regulations or recommendations for managing fungicide resistance. Hence, the declining efficacy of fungicides has largely been attributed to inappropriate use by farmers.

With *Alternaria*'s proven ability to develop resistance across many fungicide groups (Avenot *et al.*, 2016; Chowdhary *et al.*, 2013, Karaoglanidis and Thanassoulopoulos, 2003), efficacy declines could spread to many classes of fungicides, making tomato production very difficult.

It is therefore important to determine the sensitivity of *Alternaria solani* isolates on tomato to the commonly used fungicides so that fungicide resistant strains are detected early and management options adjusted at the earliest opportunity (Lucas, 2017; Hobbelen *et al.*, 2014), before they enter more difficult selection phases.

1.4 Objectives

1.4.1 General objective

To enhance sustainable production of tomato *Solanum lycopersicum* L. in Kenya through effective management of early blight caused by *Alternaria solani*.

1.4.2 Specific objectives

i. To determine the occurrence, importance and current management practices for tomato early blight in Kajiado, Kiambu and Kirinyaga counties, Kenya

ii. To characterize *Alternaria solani* isolates from the selected counties by cultural characteristics, morphological features and sensitivity to two commonly used fungicide groups

iii. To determine occurrence and spatial distribution of mutations associated with fungicide resistance among *A. solani* isolates in the selected counties

1.5 Research hypotheses

i. Early blight is among the most important tomato diseases in Kajiado, Kiambu and Kirinyaga counties and is managed by varying practices

ii. *Alternaria solani* tomato isolates from Kajiado, Kiambu and Kirinyaga counties vary significantly in terms of cultural features, morphological features and sensitivity to commonly used fungicide groups

iii. Mutations associated with fungicide resistance are present in *Alternaria solani* populations from Kajiado, Kiambu and Kirinyaga counties but are distributed unevenly across the three counties

1.6 Conceptual framework

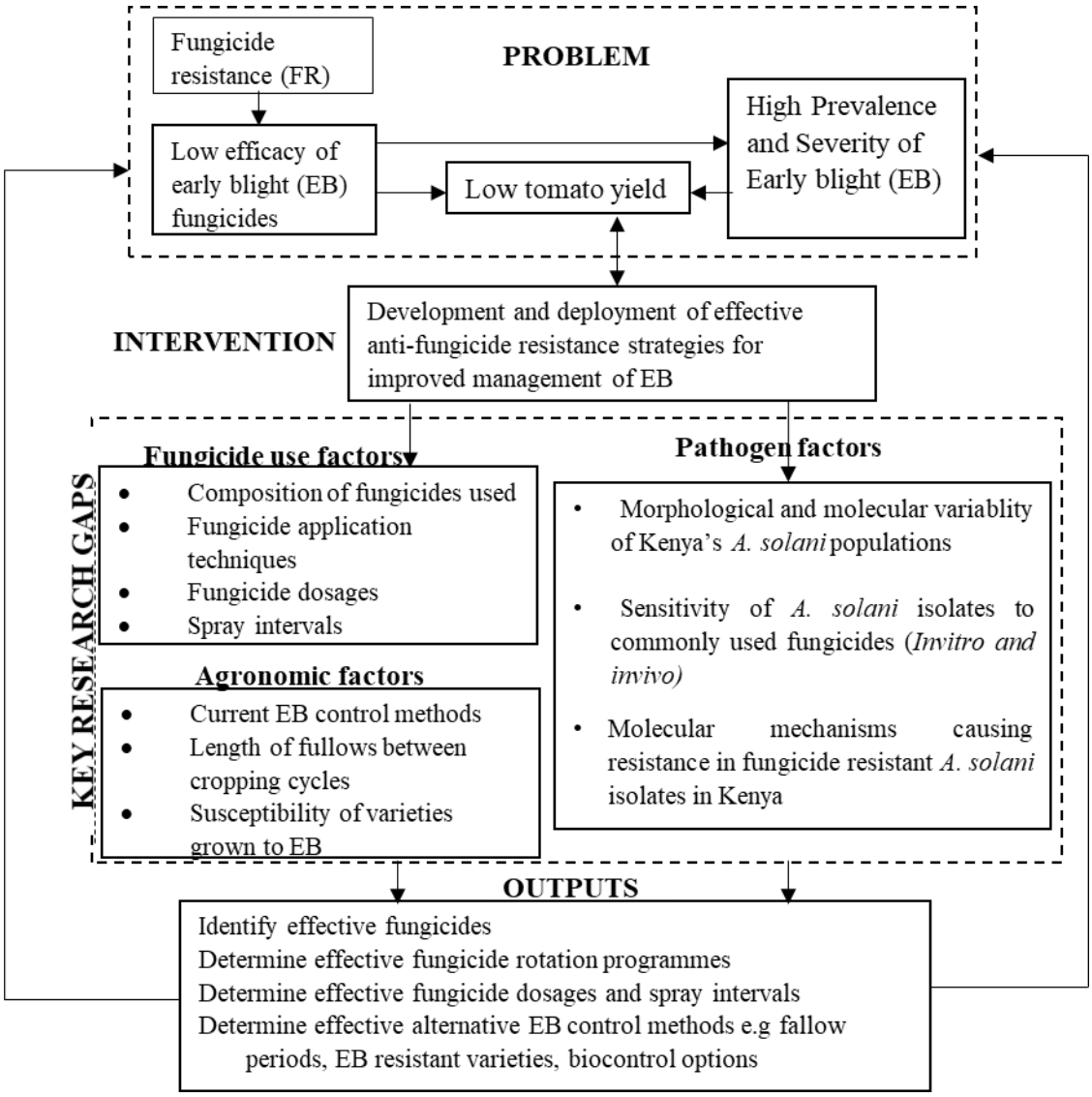

Figure 1.1: Conceptual framework

1.7 Significance of the study

This study has generated data on the current status and management of tomato early blight in Kirinyaga, Kiambu and Kajiado Counties of Kenya. Isolates of its causal agent, *Alternaria solani* have been characterized morphologically and by sensitivity to two fungicide groups (strobilurins and triazoles), that were reported to be least effective by farmers. The *Cytochrome b* gene which encodes for

Azoxystrobin target protein (cytochrome bc1 complex) has also been sequenced in all isolates and studied to identify mutations associated with Azoxystrobin resistance. The recommendations from this study will provide an informed basis for practitioners in crop protection for example fungicide manufacturers, farmers, pesticide regulators and scientists in formulating effective EB control options while countering the development of resistance.

CHAPTER TWO: LITERATURE REVIEW

2.1 Origin and nomenclature of tomato

Tomato, *Solanum lycopersicum* L., is a vegetable crop, with origins in western South America (Ecuador, Peru, and Chile) (Kimura and Sinha, 2008; Bai and Lindhout, 2007). It is believed that conquistadors from Europe were the first to domesticate tomato in Central America in the 16th century (Kimura and Sinha, 2008). Since then, Europeans distributed tomato in Europe and their colonies in the Middle East, Asia and Africa, including Kenya in 1933 (Atherton and Rudich, 1986). Today, tomato is among the most cultivated vegetables globally, with an annual production of over 120 million tons (FAOSTAT, 2020).

Tomato was initially placed in genus the *Solanum* as *Solanum lycopersicum* (lyco - "wolf," and persicum - "peach") by Linnaeus (1753). At that time, many people still thought that tomato was poisonous. This however changed when Miller (1754) formed a new genus, *Lycopersicon*, in which he assigned tomato and other edible species. The new name for tomato hence became *Lycopersicon esculentum* Mill. (esculentum meaning "edible"). However, this name was later found to breach the International Code of Botanical Nomenclature hence the original name, *Solanum lycopersicum* per Linneus has been retained (Darwin *et al.,* 2003; Spooner *et al.,* 2005).

The family where tomato belongs (Solanaceae) is among the largest in Kingdom Plantae (Kimura and Sinha, 2008). Many species in this family are important commercially, for example potato *Solanum tuberosum* L., eggplant *Solanum*

melongena L., chili pepper *Capsicum annum* L., *Capsicum frutescens* L., *Capsicum chinense* L.), tobacco *Nicotiana tabacum* L., night shade *Atropa belladonna L.*, mandrake *Mandragora officinarum* L., and ornamentals such as petunia *Petunia hybrida* L. (Sharma *et al.,* 2019; Kimura and Sinha, 2008). The presence of many commercially important plants in the family Solanaceae, makes tomato important as a model plant species (Kimura and Sinha, 2008).

2.2 Importance of tomato

2.3 Tomato production in Kenya

Tomato growing is believed to have started in Kenya in 1933 (Atherton and Rudich 1986). Today, the crop is one of the most cultivated vegetables, grown by both smallholder and medium-scale farmers (Infonet-biovision, 2021). According to FAOSTAT (2020), tomato accounts for 38% of the total vegetable production and 7% of horticultural production in Kenya. The major tomato growing counties in Kenya are Kirinyaga, Migori, Narok, Kajiado, Meru, Kiambu, Nakuru, Taita Taveta, Bungoma, Trans Nzoia (Table 2.1). Continuous tomato production in these counties has been enabled by availability of the optimal agro-ecological conditions required by the crop (Table 2.2).

Table 2.1: Tomato production data for the top ten tomato producing counties in Kenya

County	Harvested Area /Ha	Production /MT	Value (Ksh)
Kirinyaga	3,128 (14.3%)	54,185 (13.2%)	2,323,140,000
Migori	2,123 (9.7%)	32,568 (7.9%)	192,994,000
Narok	1,561 (7.1%)	20,744 (5.1%)	596,402,394
Kajiado	1,452 (6.6%)	42,789 (10.4%)	1,612,592,000
Meru	1,050 (4.8%)	9,951 (2.4%)	322,565,018
Kiambu	965 (4.4%)	9,132 (2.2%)	327,305,000
Nakuru	946 (4.3%)	15,179 (3.7%)	491,697,047
Taita Taveta	830 (3.8%)	38,026 (9.3%)	1,157,692,000
Bungoma	811 (3.7%)	21,305 (5.2%)	951,330,000
Trans Nzoia	733 (3.3%)	18,660 (4.6%)	638,237,500
National total	21,921	410,033	14,101,322,811

The figures in parentheses are percentages of national harvested area and production

(Source: MoALF data, 2020)

Table 2.2: Agro-ecological requirements for tomato production

Altitude	0 – 2,000 metres above sea level
Temperature	The optimum temperature range is 20 – 25 ºC (day) 15 – 17ºC (night). Warm humid conditions are favorable for development of many tomato pests and diseases (Tran, 2005).
Rainfall	Over 600 mm of rainfall annually. In dry areas, this may be supplemented with irrigation to maintain field moisture at around 60% (Tran, 2005). However, water logging should be avoided as it favours bacterial wilt (Nuruddin, 2001) and fruit rot (Jones, 1999)
Soils	Well drained sandy, loam, and clay loam soils, pH range 6.0 – 7.5. If the pH is less than 5.5, plant disorders such as blossom-end-rot can occur (Hanson *et al.*, 2001)
Nutrients	Due to their rapid growth and a long production period, tomatoes have high requirements of nutrients. For instance, to produce 1 ton of fruits the crop requires 1.36 - 3.63 kg N, 0.23 - 1.36 kg P, 2.27 - 5.45 kg K (Peet, 2008)

Spacing	Green house: 2 rows per bed (1 m wide); 40 cm between plants Open field: One row per bed (1 m wide); 40 cm between plants (Hanson *et al.*, 2001)

2.4 Tomato production systems in Kenya

Tomatoes are grown either in open fields or greenhouses. Open field cultivation is the most popular tomato production system in Kenya and accounts for 95% of tomato produced in the country (Sigei *et al.,* 2014). The varieties grown in open fields (determinate varieties) include Rio grande, Eden and Cal J among others.

In contrast, greenhouse farming is a relatively new production system in Kenya and accounts for only 5% of tomato produced (Sigei *et al.,* 2014). The varieties grown in green houses (indeterminate) include Anna F1, Prostar F1and Chonto F1 are grown under greenhouse conditions (Kanyua, 2018; Monsanto, 2017). According to Makunike (2007), one greenhouse tomato plant has a potential of giving up to 60 kg in its full cycle. Hence, greenhouse production is a promising technology for increasing tomato production in Kenya, if the adoption levels can increase.

2.5 Constraints to tomato production in Kenya

Tomato production in Kenya is constrained by many biotic and abiotic factors.

2.5.1 Abiotic constraints

These can be categorized as production-related and institutional-related constraints. Production-related constraints include inadequate capital and land, unreliability of rainfall in production areas, insufficiency of knowledge on

tomato production and declines in soil fertility among others (Sigei *et al.*, 2014). On the other hand, institutional related constraints include poor post-harvest technologies that hasten perishability and price fluctuations (Mwangi *et al.*, 2015, Sigei *et al.*, 2014).

2.5.2 Biotic constraints

Arthropod pests and diseases are considered the main challenges for tomato farming in Kenya (Ochilo *et al.*, 2019; Singh *et al.*, 2014; Waiganjo *et al.*, 2006). The most devastating tomato pests in Kenya include leaf miner moth *Tuta absoluta* Meyrick (Lepidoptera : Gelechiidae), whiteflies *Bemisia tabaci* Gennadius (Hemiptera : Aleyrodidae), African bollworm *Helicoverpa armigera* Hübner (Lepidoptera : Noctuidae), western flower thrips *Frankliniella occidentalis* Pergande (Thysanoptera : Thripidae), Red spider mites *Tetranychus* spp, Cutworms *Agrotis* spp, and Vegetable leaf miner, *Liriomyza sativae* Blanchard (Diptera : Agromyzidae) (Wakil *et al*., 2017; Gacheri, 2016).

On the other hand, the most significant tomato diseases in terms of yield loss caused include early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), powdery mildew (*Oidium lycopersici* Cooke and Massee), fusarium wilt (*Fusarium oxysporum* f.sp *lycopersici* (Sacc.) Snyder and Hans), bacterial wilt (*Ralstonia solanacearum* E.F. Smith), root-knot nematodes (*Meloidogyne* spp.), tomato yellow leaf curl virus and tomato spotted wilt virus (Infonet-biovision, 2021; Mwangi *et al.*, 2015; Singh *et al*., 2014; Kariuki *et al*., 2010). Such diseases reduce tomato yield quality and quantity resulting in loss of income (Goufo *et al*., 2008; Mizubuti *et al*., 2007). Early and late bllights are considered the most critical tomato diseases in Kenya and have been estimated

to cause 95.6% pre-harvest yield losses (HCDA, 2017; Waiganjo *et al.*, 2006).

2.6 Description of tomato early blight

Early blight (EB) is a major foliar disease of tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum*) (Rotem, 1994). Early blight symptoms in tomato include leaf blight, collar rots and fruit rot. Leaf blight is the most destructive stage of early blight infection (Rotem, 1994). Small dark patches appear first, then grow into brown-black lesions with concentric rings encircled by yellow halos (Fig. 2.1). Spores may then appear at the center of lesions, giving them a dark fuzzy appearance (Neils *et al.*, 2015).

As EB progresses, the rate of photosynthesis declines, the size, and quality of fruits reduce, leading to significant yield losses (Foolad *et al.*, 2008). In the fields where farmers delay to control the disease, complete defoliation of plants can occur leading to yield losses as high as 79%. According to Yadav and Dabbas (2012), a 1% increase in EB severity reduces tomato yields by up to 1.4%.

(a) **(b)**

Figure 2.1: (a) Tomato shoot affected by Early blight in a Kenyan field (b) Conidia of Alternaria solani which are the pathogen's infectious agents

(Source: Andrew Nuwamanya)

2.7 Aetiology of early blight causal agent, *Alternaria solani*

When early blight was first reported on tomatoes in 1892, there was great controversy about its causal agent (Chester, 1892). Galloway (1891) had associated the pathogenic fungus, *Macrosporium solani* (originally described by Ellis and Martin (1882)) with the disease but later an *Alternaria* species was also isolated from the lesions (Van der waals *et al.*, 2001). This *Alternaria* species bore spores that closely resembled those of *M. solani*, the only difference was that *Alternaria* spores were borne in chains while those of *M. solani* were borne singly (Van der waals *et al.*, 2001). This controversy was however resolved by Jones and Grout (1897) who isolated the two species from early blight lesions. One species was found to be pathogenic and was re-named *Alternaria solani* while the other, a saprophytic one was named *Alternaria fasciculata* (Cook and Ellis) Jones and Grout and later, *Alternaria alternata* (Fr: Fr.) Keissl).

2.8 Classification and morphological features for *Alternaria solani*

Alternaria solani is classified under Kingdom fungi, Eukaryota domain, Class dothideomycetes, order Hyphales and Family pleosporaceae (Ghafri *et al.,* 2019; Lawrence *et al.*, 2016). *Alternaria solani* hyphae are branched, septate, light brown turning darker at maturity. Its conidiophores, are borne individually but seldom in small groups, septated, flexuous or straight, dark in color, 50-90 µm in diameter (Ganie *et al.*, 2013).

According to Simmons (2007), *A. solani* conidia are usually pale to olivaceous-brown, borne singly or in short chains. Conidia shapes vary from ellipsoidal to obclavate, 75-350 long and 20-30 µm wide at the broadest part and usually have 6–19 transverse and 0–8 longitudinal septa. Beaks are usually present in most conidia and measure about one-half to double the length of the conidium, septate, hyaline to pale brown and 5–9 µm in diameter (Meena *et al.,* 2017; Simmons, 2007).

According to Fry (2007), the genus *Alternaria* contains about 299 species of which *A. solani* is the most destructive to tomato globally. Based on the observation that Early blight was more prevalent in early maturing cultivars, Jones *et al.,* (1993) suggested the name ‘early blight’. This has enabled scientists to distinguish it from late blight which apparently, is more severe in late-maturing cultivars.

2.9 Infection and disease cycle of tomato early blight

The infection cycle begins when *Alternaria solani* spores land on a susceptible plant surface (Figure 2.2). Under moist warm conditions, the spores germinate to form a germ tube, which then develops an appresorium, that later penetrates the epidermis. Rotem (1994) reported that a temperature of 10-35^{o}C is required for germination of spores. *Alternaria solani* can also gain entry into plant tissues through stomata, wounds on the stem, and then cause disease (Kemmitt, 2002).

It has been reported that *A. solani* invades tissues of tomatoplants by producing enzymes that degrade cell walls. The pathogen also produces toxins to kill host cells and make their content available (Gulzar *et al*., 2018). *Alternaria solani* also secretes two enzymes extracellularly; a serine protease and metalloprotease that may be involved in its pathogenicity (Chandrasekaran *et al*., 2016; Chandrasekaran *et al*., 2014). Depending on cultivar susceptibility, leaf age and environmental conditions, symptoms may appear within a week after infection (Kemmitt, 2002).

Little is known about the molecular basis of *A. solani* infection. However, *A. solani* has been reported to secrete some phytotoxic compounds such as alternaric acid, alternariol, altersolanol A, solanapyrone A, B, C among others (Anderson *et al.,* 2008; Montemurro *et al.,* 1992). Alternaric acid and solanapyrones are known to induce necrosis and chlorotic symptoms (Adhikari *et al.,* 2017) but the contribution of other metabolites in disease development is not well documented.

Alternaria solani is a polycyclic pathogen as many infection cycles are possible in a single cropping season (Shuman, 1995). Primary infections on new tomato crops are caused by overwintering inoculum which can remain infective in uncultivated soil for 5–8 months (Pscheidt, 1985). The pathogen overwinters as mycelia, chlamydospores or conidia in soil (Pelletier, 1988; Shuman, 1995). *Alternaria solani* has also been reported to overwinter in other crops of family Solanaceae (Patterson, 1991; Basu, 1971).

On infected plants, sporulation occurs at temperatures between 5-30 °C, (Pscheidt, 1985) with the heaviest sporulation occurring after rain or dew. Spore production is initiated by daylight and accumulate over a 7–14-day period (Bashi and Rotem, 1975). Conidia are dispersed by rain splash and/or wind to the lower leaves of the plant where they germinate and infect (Rotem, 1994).

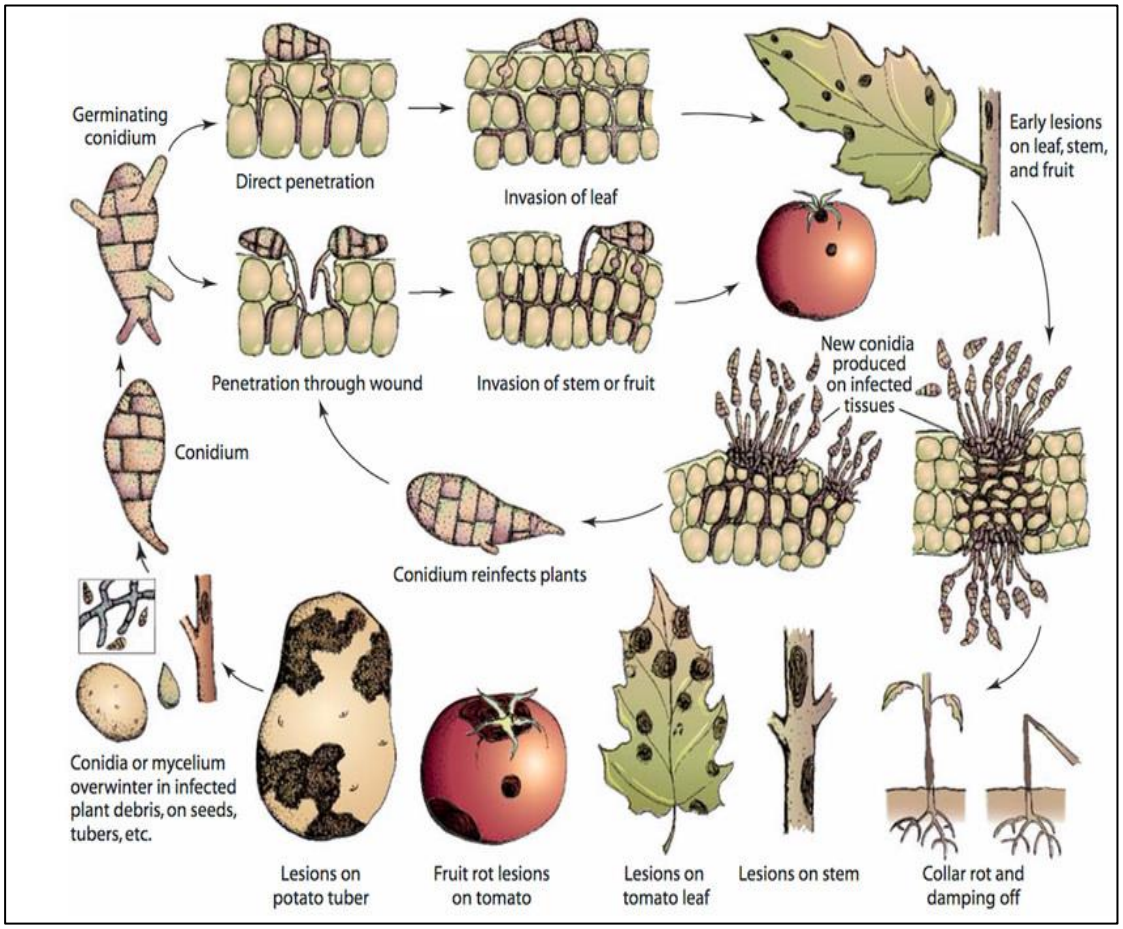

Figure 2.2. Disease cycle of early blight in tomato and potato (Adhikari et al., 2017)

2.10 Cultural, morphological and genetic variability of *Alternaria solani*

Many studies have reported high degrees of variability in *Alternaria solani* isolates basing on cultural, morphological, and genetic features. *A. solani* Isolates are usually highly variable even when they are collected from a single lesion (Kumar *et al.,* 2017) or field (Leiminger *et al.,* 2016).

The cultural characteristics that have been used to characterize *A. solani* include colony color, diameter, nature of margin and colony zonation (Nikam *et al.,* 2015; Marak *et al.,* 2014; Woudenberg *et al.,* 2014; Naik *et al.,* 2010). Various colors (ranging from creamy yellow, brown-black to olivaceous brown) have

been described among *Alternaria solani* isolates (Chohan *et al.,* 2015; Nikam *et al.,* 2015; Marak *et al.,* 2014). *Alternaria solani* cultures have been described to have concentric zonations or lack them while margins could either be regular (roughly circular) or irregular (Nikam *et al.,* 2015; Marak *et al.,* 2014). Kaul and Saxena (1988) grouped *A. solani* isolates into four discrete classes based on colony characteristics on Potato Dextrose Agar (PDA).

For morphological characterization, most studies use features of the conidia (*A. solani*'s infectious agent). Such features include conidia shape, length, and width, number of septa, presence or absence of beaks, the structure of conidiophores among others (Meena *et al.,* 2017; Loganathan *et al.,* 2016; Nikam *et al.,* 2015; Perez and Martinez, 2015; Marak *et al.,* 2014; Woudenberg *et al.,* 2014; Naik *et al.,* 2010). According to Loganathan *et al.,* (2016), conidia and beak lengths in *Alternaria solani* are significantly variable and can be used for characterization of the pathogen.

Genetically, most studies have characterized *Alternaria solani* using genes like ITS (Kumar *et al.,* 2017; Lourenço *et al.,* 2009), 18SrRNA (Al Husnain and AlKahtani, 2019; Ismail *et al.,* 2016; Loganathan *et al.,* 2016). Usually, high genetic dissimilarity among isolates is obtained even when isolates are collected from the same field or lesion (Kumar *et al.,* 2017; Leiminger *et al.,* 2016).

2.11 Management practices for Early blight

Management of tomato early blight is challenging because of its causal agent, *Alternaria solani* has a polycyclic life cycle and can form a large number of infective strains (Adhikari *et al.*, 2017). Nevertheless, there are three methods used for early blight control globally, namely; cultural methods, host resistance and fungicide application.

The most common cultural practices used for managing tomato early blight include field sanitation, rotating tomatoes with non-host crops and planting pathogen-free seeds. Furthermore, maintenance of plant vigor through adequate application of nitrogen and phosphorus has been reported to significantly reduce early blight severity (Li, 2012; Chaerani and Voorrips, 2006). However, cultural practices have not been effective in managing early blight because of the pathogen's soil-borne nature and ability to evolve into many infective strains (Foolad *et al*., 2008).

Use of host plant resistance has not been exploited satisfactorily, since only a few tomato varieties ("Plum Dandy", "Mountain Magic", "Mountain Merit" and "Mountain Supreme") are tolerant to early blight (Adhikari *et al.,* 2017). This is even complicated by the fact that EB resistant accessions do not perform well in terms of yield and consumers' preference (Yadav and Dabbas, 2012). Therefore, the tomato breeding for early blight resistance has largely stayed at trial level, with the developed accessions not available in most tomato growing countries,

Kenya inclusive. Consequently, early blight control has largely relied on regular application of synthetic fungicides (Foolad *et al.,* 2008).

Globally, the most commonly used fungicide groups against EB include multi-site enzyme inhibitors (such as Mancozeb, Zineb Propineb and copper salts), strobilurins (such as Azoxystrobin, pyraclostrobin and trifloxystrobin), Demethylation inhibitors (such as tebuconazole, difenoconazole, propiconazole) and Succinate dehydrogenase inhibitors for example boscalid (Mishra, 2012). These are manufactured by different companies and so they come under different trade names. Some formulations contain single active ingredients while others are mixed. Most globally available fungicides are registered for use in Kenya (PCPB, 2019).

2.12 Fungicide sensitivity in *Alternaria solani*

Many studies have reported variability of sensitivity to fungicides among *Alternaria solani* isolates. Samen *et al.* (2016) reported that 46.7 % of *A. solani* tomato isolates in Jordan valley, Israel had low sensitivity to Mancozeb, while 53.3% were less sensitive to Chlorothalonil. Shi *et al.* (2015) reported that 54 % of the *A. solani* tomato isolates from Shanxi province China showed high resistance to Boscalid. Mphahlele *et al.* (2018) reported high variability in among *A. solani* isolates from Limpopo province, South Africa in sensitivity to chlorothalonil, copper oxychloride and mancozeb with variations affected significantly by pathogen isolate, area of collection and fungicide tested.

It is postulated that consistent exposure to similar or closely related fungitoxic compounds drives a selection process in *A. solani* populations, giving rise to higher frequencies of resistant strains (Gudmestad *et al.,* 2013; Karaoglanidis *et al.,* 2011; Rosenzweig *et al.,* 2008). The resistance mechanisms proposed include mutations in genes encoding target proteins, over expression of target genes and fungicide effluxes (Table 2.3).

In particular, resistance develops faster in *Alternaria solani* against single-site fungicides since they target only one gene/stage in the fungal biochemical pathway (FRAC, 2021). This means that mutation of even a single nucleotide could modify the target site, making it difficult for the fungicide to effectively control the target disease.

Table 2.3: Confirmed fungal resistance mechanisms to commonly used fungicide groups

Fungicide group	**Example of active compounds**	**Mode of action**	**Possible resistance mechanisms**
Multisite enzyme inhibitors	Mancozeb, Zineb, Cu^{2+}salts Chlorothalonil, Cymoxanil, pyrimethanil, Carbendazim, cyprodinil	Inhibits DNA and RNA synthesis, affecting cell division and cellular metabolism.	-Fungicide efflux and detoxification (Yang *et al.,* 2019)
Strobilurins	Azoxystrobin, trifloxystrobin, pyraclostrobin	Inhibits mitochondrial respiration at the Qo site of cytochrome b, part of the cytochrome bc1 complex (Complex III), preventing spore	-Mutations in *cyt b* gene (Fernández-Ortuño *et al.,* 2008) -Induction of alternative, respiratory pathway sustained by alternative oxidase (Wood and Hollomon, 2003)

		germination and mycelial growth	-Efflux of fungicides by ABC or MFS transporters (Andrade *et al.,* 2000; Roohparvar *et al.,* 2007)
Demethylation inhibitors	Tebuconazole, Difenoconazole, Propiconazole	Inhibits C14-demethylation during ergosterol biosynthesis	- Mutations of the *Cyp51* gene leading to decrease in affinity of DMIs for their target site - Overexpression of the *Cyp51* gene leading to raised levels of sterol 14α-demethylase -Up-regulation of ABC or MFS transporters to increase efflux (Leroux and Walker, 2013)
Succinate dehydrogenase inhibitors	Boscalid, Fluopyram, Penthiopyrad, Fluxapyroxad	Inhibits the activity of mitochondrial Complex II(succinate dehydrogenase) and thus respiration in fungal cells	Mutations in *Sdh* genes (Avenot *et al.,* 2008; Ishii *et al.,* 2008)

2.13 Effect of mutations on sensitivity of pathogenic fungi to fungicides

As a mode of action, fungicides usually bind to active sites of one or a few proteins in the fungal biochemical pathway inhibiting key physiological or biochemical processes (Table 2.3). For example, strobilurins bind to the Qo site of cytochrome bc1 protein in the mitochondria, inhibiting fungal respiration (Fernández-Ortuño *et al.,* 2008), while demethylation inhibitors bind to sterol 14α-demethylase protein, inhibiting sterol biosynthesis (Leroux and Walker, 2013).

When mutations occur in fungal DNA, the nucleotide sequences in certain loci change which often results into modification of fungicide target sites in encoded proteins (Fernández-Ortuño *et al.,* 2008). For example, for strobilurin fungicides, a change from sequence GGT to GCT at position 143 in the fungal

Cyt b gene is known to result into G143A amino acid substitution (Alanine substituting Glycine at position 143) (Banno *et al.,* 2009; Ishii, 2009). The G143A-mutated-cytochrome-bc1-proteins have altered Qo sites and strobilurin fungicides can no longer bind on them. Therefore, fungal biotypes with such a mutation lose sensitivity to the fungicide (Figure 2.4).

Fungal biotypes with resistance mutations tend to be selected for whenever farmers apply the fungicide that they are resistant to, and so they continue accumulating in the fungal populations over time in case of continuous application of that fungicide (FRAC, 2021; Brent and Hollomon, 2007). A time then reaches when the fungicide can no longer suppress the fungus and manage the disease effectively. The time it takes from emergence of resistant pathogen strains to noticeable fungicide efficacy declines depends on various factors, in particular fungicide doses, spray frequency and rotations (Brent and Hollomon, 2007; Genet *et al.,* 2006; Kable and Jeffery, 1980), and whether alternative disease control methods are applied, e.g., host plant resistance and cultural methods (Fry, 2007).

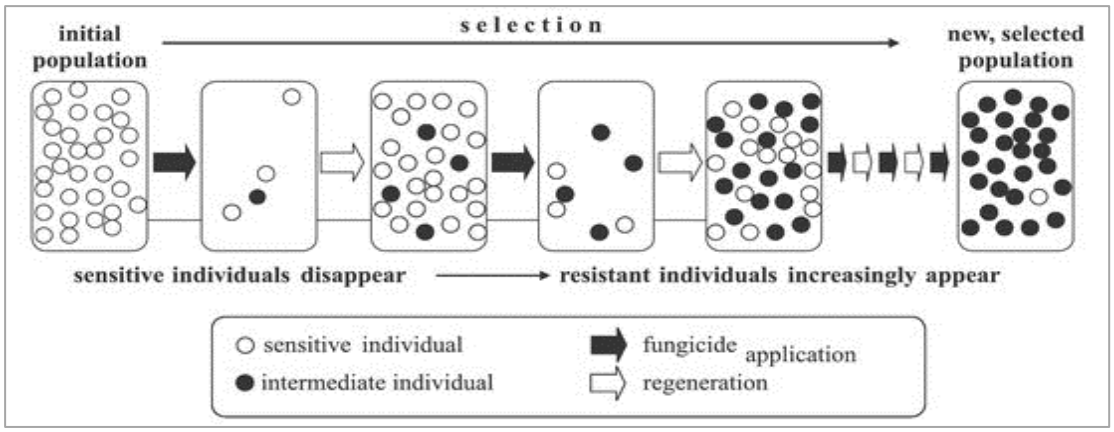

Figure 2.33. Illustration of fungicide resistance emergence and development (Adapted from Deising et al., 2008)

Examples of mutations reported to cause fungicide resistance in *Alternaria* diseases in some tomato-growing areas of the world are summarized in Table 2.4. Some fungicide sensitivity assays with *Alternaria solani* in Africa such as Mphahlele *et al.* (2018) (Limpopo, South Africa) have confirmed existence of resistant strains, but none have carried out molecular assays to detect any associated mutations. This is one of the knowledge gaps that this study intended to fill.

Table 2.4: Examples of resistance-associated mutations reported in *Alternaria* spp.

Fungicide group	Mutations	Pathogen (host crop)	Countries	Authors
Strobilurins	G143A, G137R, G143S, F129L in *Cyt b* gene	*Alternaria solani* (Potato, tomato) *A. alternata* (Potato, tomato), A. *tenuissima* (Pistachio) and *A. arborescens* (Pistachio),	USA, Germany Belgium, Sweden, Greece, Poland, South Africa	Nottensteiner *et al.,* 2019; Malandrakis *et al.,* 2018; Landschoot *et al.,* 2017; Duba *et al.,* 2017; Odilbekov *et al.,* 2016; Dube, 2014; Fairchild *et al.,* 2013; Pasche *et al.,* 2005
Demethylation inhibitors	F120L,Y131H K715R, Y781C, D1140G. T1628A in *cyp51* gene	*Alternaria alternata* (Paris root)	China	Sun *et al.*, 2021
Succinate dehydrogenase inhibitors	H278R, H278Y, H134R, H133R in *Sdh* genes	*Alternaria solani* (Potato)	Netherlands, Belgium, Germany and Great Britain	Mostafanezhad, *et al.*, 2021; Derpmann and Mehl, 2019; Metz *et al.*, 2019; Mallik *et al.*, 2014

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

This study was conducted in three major tomato growing areas in Kenya, representing three counties in Central and Rift valley regions (Figure 3. 1). These were: Mwea East in Kirinyaga County, Kabete in Kiambu County, and Loitokitok in Kajiado County.

Mwea East is classified among humid agroclimatic zones of Kenya (Braun, 1982). Located at the foothills of Mt. Kenya in central region, the area receives bimodal rainfall, ranging between 1212 – 2146 mm annually, while temperature varies between 8 -30^{o}C (Jaetzold and Schmidt, 1983). Coupled with fertile vertisols in most areas, such conditions enable production of a wide variety of crops all year round, which has made Mwea a food basket of Kenya (Nguetti *et al.,* 2018). The challenge, however, is that Mwea's warm humid conditions are also conducive for the infection process by many fungal pathogens, thus many diseases including Early blight are usually common and severe in such areas (Upadhyay *et al.,* 2019; Runno-Paurson *et al.,* 2015; Kemmitt, 2002).

Kabete is another significant tomato-growing area in Central Kenya. Over the years, many greenhouses have been erected in the area (Karume, 2015) that produce a variety of short maturing horticultural crops for the attractive market in the neighboring Nairobi metropolitan. This part of Kiambu County has been classified as semi-humid agro-climatic zone (Sombroek *et al.,* 1982). This area

receives bimodal rainfall ranging between 600-2000mm annually while the temperature is between 18-22^{o}C.

Loitokitok sub-county in Kajiado county lies on the foothills of Mt. Kilimanjaro in the southern region of Kenya, bordering Tanzania. Average annual rainfall ranges between 475 – 750mm while temperatures are between 12-27^{o}C (Jaetzold and Schmidt, 1983). Classified as a semi-arid ACZ, the sub-county consists of few areas with water availability surrounded by expansive dry grasslands. Tomato production there is dominated by smallholder farmers who must irrigate their fields all year round. A combination of warm conditions and intense irrigation in Loitokitok favors a wide range of tomato diseases including Early blight (Mantecón 2007).

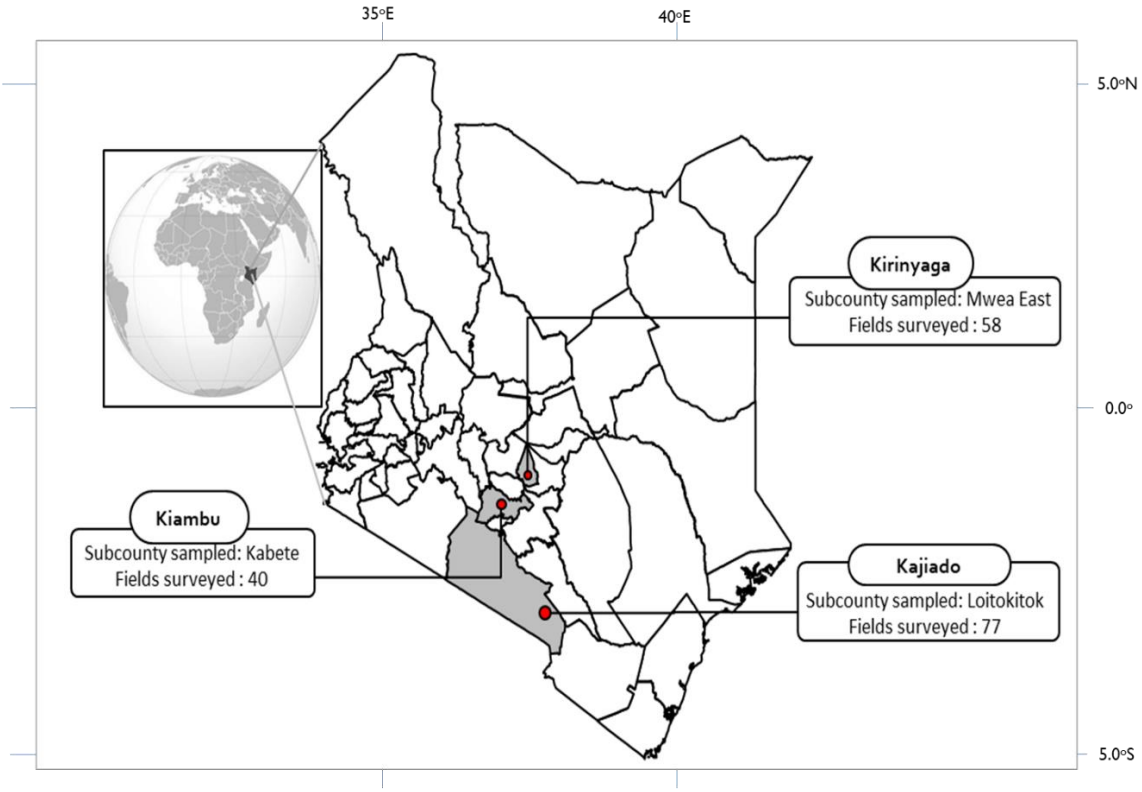

Figure 3.1: Map of Kenya showing location of study sites
(Developed using the .mapdata *package in R)*

3.2 Sampling technique

The number of tomato fields to survey in each study site was determined using the formula by Yamane (1967) (Equation 1).

$$n = \frac{N}{1 + N(e)^2} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where,

n= sample size,

N = Number of tomato fields in a selected sub-county according to the County Agriculture Office (Mwea east 70, Kiambu 50 and Kajiado 100)

e = level of precision or sampling error (0.05).

After determining the number of fields required in each area, fields were selected systematically along predetermined routes at 1 km intervals. In exceptional cases where there was no field available, the nearest tomato field was sampled. In total, 175 tomato fields were sampled; 58 in Mwea east, 77 in Loitokitok and 40 in Kabete.

3.3 Occurrence, importance, and management practices for early blight

3.3.1 Farmer's interviews and field visits

The questionnaire used (Appendix 3) had been pre-tested among ten tomato farmers in Thika, Kiambu County and validated. The questions (both open-ended and semi-structured) were programmed in Open Data Kit (ODK) software for electronic recording and automated transmission of data (Hartung *et al.,* 2010). The ODK Collect v1.16.0 app was downloaded from Google play store

(https://play.google.com/store/apps/details?id=com.lexi.android, Accessed 31st January 2021).

Approval to conduct this research was obtained from Kenyatta University Graduate School (Appendix 1), and National Council of Science, Technology and Innovation, Kenya (Appendix 2). Verbal consent was obtained from participants before interview

Data recorded included age, gender, and experience of the respondent in tomato production, area under tomato, number of cropping cycles per year, varieties grown, irrigation methods, prevalent pests and diseases and EB management practices including fungicide application procedures. Global Positioning System (GPS) coordinates were also taken from the central-most point of each field visited using the ODK Collect v1.16.0 app.

In assessing EB importance, respondents were asked to rate prevalent pests and diseases in terms of yield loss caused, on a 1-4 scale (developed for this study) where 1 represented low yield loss (<10%), 2-moderate (11-20%), 3-high (21%-30%) and 4 -very high yield loss (>40%). Respondents were also asked to state the EB control strategies used on their farms. Fungicide users were asked to identify each product by its trade name, dosage and frequency of sprays, and to rank their effectiveness on a three-level scale, i.e., low (1), moderate (2) or high (3). Resistance risk classes for active compounds in fungicide products were

obtained from Fungicide Resistance Action Committee (FRAC) website; *https://www.frac.info.*

3.4 Isolation and characterization of *Alternaria solani*

3.4.1 Collection of diseased tomato samples

In fields where early blight was prevalent, one tomato shoot having typical EB symptoms was collected. Samples were placed in labeled zip-lock bags and kept in a cool box for transport to the Pathology laboratory at Kenyatta University. In total, 144 samples were collected, 53 from Mwea east (Kirinyaga county), Kabete (Kiambu county n=30) and Loitokitok (Kajiado county, 61).

3.4.2 Preparation of culture media (PDA)

Culture media was prepared according to Ainsworth (1961) under sterile conditions in a laminar flow cabinet. Thirty-nine grams of Potato Dextrose Agar (PDA) were dissolved in 1,000 ml of distilled water and autoclaved at 121^{o}C for 15 min. Autoclaved PDA media was allowed to cool to 45^{o}C and amended with tetracycline to inhibit bacterial growth (Rioux *et al.,* 2014). Approximately 20 milliliters of amended PDA was dispensed in 9 mm diameter petri plates and left to set overnight.

3.4.3 Isolation of *Alternaria* spp

Isolation of *Alternaria* spp from diseased tomato samples was carried out following a modified version of the protocol by Schulz *et al.* (1993). Infected leaves were surface sterilized in 1% Sodium hypochlorite for 3 minutes and

rinsed three times in sterile distilled water. Using a sterilized scalpel, three squares of ~ 5 mm^2 were cut from advancing edges of lesions and blotted dry using sterile filter paper. The sections were then plated on Potato dextrose agar (PDA) media, the petri plates sealed, and kept at 25°C in an electronic incubator for 5 days.

Only colonies that had creamy yellow, brown-black to olivaceous brown mycelia, which are characteristic of *A. solani* were subcultured (Chohan *et al.*, 2015). Using a sterilized inoculating needle, small sections of mycelial growth were cut from margins of such cultures and plated onto freshly prepared PDA, and incubated at 25^{o}C. Sub culturing continued until when pure cultures were obtained.

3.4.4 Preparation of single spore isolates

This was done on three-week old isolates using the single spore isolation method (Choi *et al.*, 1999). Using a fine inoculating needle, one conidium per isolate was picked from each isolate (under a research microscope) and transferred onto freshly prepared PDA medium amended with 0.2% streptomycin sulfate. Inoculated petri plates were incubated at 25^{o}C for 5 days. Cultures from such petri plates were kept in PDA slants at 4^{o}C and maintained by routine sub-culturing. All other characterization work was done using the single spore cultures.

3.4.5 Cultural characterization of isolates

Potato Dextrose Agar (PDA) media was prepared according to Ainsworth (1961) and amended with 0.1 g/l tetracyclin (antibiotic) at 50^0C. Twenty-milliliter volumes of PDA was dispensed into sterile 90 mm diameter petri plates. After setting, 5mm mycelial discs were cut from 7-day old *Alternaria solani* cultures and inoculated at the center of each petri plate. Three replicate plates were prepared for each isolate and incubated at 25ºC for 9 days, after which the cultural characteristics (colony diameter, color, nature of margin and colony zonation) were observed and recorded (Marak *et al.,* 2014).

3.4.6 Morphological characterization of the fungi

Morphological characterization of *Alternaria solani* was done based on previously reported methods (Nikam *et al.,* 2015; Marak *et al.,* 2014, Kumar *et al.,* 2017). Using a sterile scalpel blade, small sections of hyphal tips in 14-day-old cultures were 'brushed' onto a 2000 µL drop of sterile distilled water, on a microscope slide. Such slides were examined under a Zeiss - Primo Star microscope fitted with an AxioCam ERC 5s camera at magnification X40. The features studied included conidial parameters (such as shape, length (µm), and width (µm)) and number of septa. For isolates with beaked conidia, the beak length (µm) and number of beak septa were recorded.

All length and width measurements were done on five randomly selected conidia, for each isolate using an ocular micrometer. Morphological identification was later confirmed by pathogenicity tests (Section 3.4.7) and PCR

based methods (Section 3.5.3) using *Alternaria solani Cyt b* specific primers (Edin, 2012).

3.4.7 Pathogenicity tests on *Alternaria solani* isolates

To determine if the *Alternaria solani* isolates were pathogenic to tomato, a total of 20 randomly selected isolates were tested for pathogenicity on seedlings (Cultivar Riogrande). These tests were conducted under greenhouse conditions in pot experiments. Conidial suspensions were prepared from isolates by "flooding" four-week-old cultures with distilled water. The harvested conidial suspensions were visualized under a light microscope (magnification X40) and re-constituted to a density of ~10^5 spores per milliliter (Stammler *et al.,* 2014) using a hemocytometer slide.

Certified Riogrande seeds were originally planted in seedling trays containing autoclaved peat moss media at 25^{o}C and relative humidity 60% in a seed germinator. After germination, the seedlings were transferred to natural sunlight for 2 weeks (for hardening), after which they were transplanted into 4000 m^3 pots containing sterilized vermicompost media (Fig. 3.2). Using a hand sprayer, the conidial suspension from each isolate was inoculated onto leaves of three-week-old seedlings (3 replicates per isolate). For the control experiment, seedlings were sprayed with sterile distilled water.

For optimal growth, Diammonium phosphate fertilizer was applied as per the manufacturer's instructions (15g per pot weekly). The plants were regularly

monitored after every 2–3 days for EB symptoms and severity. Leaves showing typical early blight symptoms were collected from infected plants, two weeks after inoculation and the pathogen re-isolated from them to complete Koch's postulates (as described in Section 4.2.3).

Figure 3.2: Two-week old tomato plants at the start of the pathogenicity experiment

3.4.8 Evaluation of sensitivity of *Alternaria solani* isolates to commonly used fungicides

3.4.8.1 Selection of fungicides for evaluation

Two commercially formulated fungicides, *Score*® and *Ortiva*® were chosen to represent the fungicide groups reported by farmers as being the least effective at controlling tomato early blight (Section 4.1.9.2**).** Both are registered in Kenya the by Pesticides Control Products Board (PCPB).

Ortiva® is a contact and systemic fungicide registered for control of a broad range of fungal pathogens in various crops in Kenya. It is formulated as a soluble concentrate with a composition of 250g/l Azoxystrobin. The active ingredient Azoxystrobin belongs to Quinone outside inhibitor/ strobilurin group of fungicides and is known to inhibit respiration of fungi by binding to the Qo site

of cytochrome bc1 complex in the mitochondria (Fernández-Ortuño *et al.*, 2008). According to the FRAC (2021), Azoxystrobin is ranked as 11 meaning that the risk of pathogens developing resistance to it is high.

Score® is a broad-spectrum systemic fungicide registered for preventive and curative control of many foliar diseases in vegetables and ornamentals. The available commercial formulation in Kenya is an emulsifiable concentrate containing 250g/l Difenoconazole, a demethylation inhibitor (DMI) fungicide that has been available in the world market since 1996. Difenoconazole functions by inhibiting the biosynthesis of sterol, a key component in fungal cell membranes (Leroux and Walker, 2013). Its FRAC resistance risk rank is 3/medium.

3.4.8.2 Determination of fungicide concentrations

Manufacturers' recommended doses indicated on the fungicide labels were used to determine the concentrations evaluated in this study. For each of the fungicides, the concentrations were prepared as follows; the recommended rate (1ml/l for both Score® *and* Ortiva®), the recommended rate x1.5 (1.5ml for both) and twice the recommended rate (2ml/l for both). Dosages were converted from milliliters of fungicide per liter to milligrams of active ingredient per liter of PDA media (Fig. 3.3) to determine the working concentrations of active ingredients.

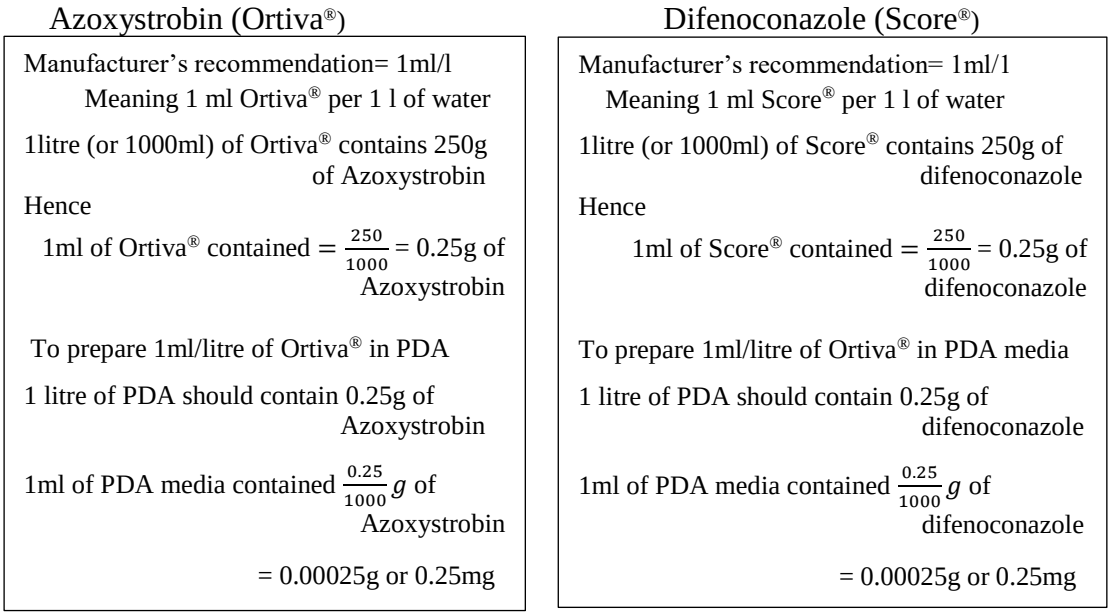

Figure 3.3: Calculations for conversion of manufacturer's dosage from milliliters of fungicide per liter to milligrams of active ingredient per liter of PDA media for the two tested fungicides

3.4.8.3 Evaluation of fungicide sensitivity among *A. solani* isolates

The poisoned food technique (Dhingra and Sinclair, 1985) was used for assaying fungicide sensitivity of *Alternaria solani* isolates. Potato Dextrose Agar media was prepared as described in section 3.4.2 and left to cool to 50^{o}C after which it was amended with fungicides, appropriately to achieve the required concentrations (0.25mg a.i/ml, 0.375mg a.i /ml and 0.5mg a.i/ml). This was followed by addition of 0.1mg of Salicylhydroxamic acid (SHAM) per liter of amended PDA to prevent the alternative oxidase pathway in *A. solani* (Rosenzweig *et al.*, 2008). The amended PDA was then placed on a rotary shaker to mix thoroughly and cool to room temperature. Three replicate plates were prepared for each isolate per fungicide concentration and each plate received twenty-milliliters of volumes of amended media. In control plates, PDA media (with no fungicide) was dispensed.

Using a cork borer, 5.0-mm-diameter mycelial plugs were cut from margins of 9-day old *A. solani* cultures and transferred to the center of media in each petri plate with the mycelial side facing down. Three replicate plates per isolate were prepared for each fungicide concentration and arranged in a completely randomized design in an incubator at 27°C. After 7 days, the diameter of *A. solani* colony in each plate (in millimeters) was measured in two perpendicular planes (Samen *et al.,* 2016) and the average taken.

The percentage mycelial growth inhibition (%MGI) for each isolate at each fungicide concentration was then determined using the formula below (Equation 2).

$$\%MGI = \left(1 - \left(\frac{Diameter\ of\ colony\ on\ fungicide\ ammended\ plate}{Diameter\ of\ colony\ on\ control\ plate}\right)\right) X\ 100 \ldots\ldots\ldots \quad (2)$$

(Shi *et al.*, 2015)

This experiment was performed twice.

The manufacturer's recommended dosage (MRD) was taken as a discriminatory dose for determining the sensitivity status of isolates. Isolates were considered resistant to the fungicide whenever their % MGI at manufacturer's recommended dosage was below 50% and sensitive when above 50% (Ishii *et al.,* 2009).

3.5 Detection of mutations associated with Azoxystrobin resistance

3.5.1 DNA extraction from *A. solani* isolates

DNA was extracted from pure mycelial cultures following a modified version of the protocol by Löffler *et al.* (1997). Using a sterile toothpick, a small lump of mycelia was transferred into a 2000 µL microcentrifuge tube followed by 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]. The tube was then kept in a freezer until the contents froze. Using a sterile toothpick, the mycelia was crushed to a fine paste, the tubes incubated in a water bath at 65^{o}C for 20 minutes after which an equal volume of chloroform: Isoamyl alcohol (24:1) was added. The tubes were then gently inverted 20 times for thorough mixing followed by centrifuging (13,200 rpm) for 5 minutes at 4^{o}C. The resulting supernatant was transferred to a new tube followed by adding equal amounts of ice-cold 100% ethanol and gentle mixing. The tube was then span at 13,200 rpm for 10 minutes and the supernatant discarded. The resultant DNA pellet was washed with 300 µl of 70% ethanol after which it was span at 10,000 rpm for 1 minute and the supernatant discarded. The DNA pellet was air-dried and dissolved in 50 µl of 1 x Tris-EDTA, pH 8.0.

3.5.2 Determination of DNA quality

The quality of DNA was determined by agarose gel electrophoresis. Agarose gel (1%) was prepared by adding 1g of agarose powder into 100ml of 1x TAE buffer and boiling the mixture (in an oven) to dissolve well. The mixture was then poured into a casting tray fitted with a comb and left to solidify. Using a micropipette, 1.5µl volumes of 1KB DNA marker (size standard) and DNA

isolates were separately mixed with a gel loading buffer (2 µL SYBR Green and 2 µL Ethidium bromide) and loaded into wells of the solidified gel. An electric voltage of 100 volts was connected to the gel for 30 minutes to facilitate the migration of the DNA through the gel. The gels were then visualized under a UV transilluminator (Innis *et al.*, 2012).

3.5.3 PCR and sequencing of the *Cyt b* gene

From *in vitro* tests, it was evident that while all isolates were sensitive to Difenoconazole, majority of them (64%) were resistant to Azoxystrobin (Section 4.2.4.1). Hence, to establish the cause of resistance to Azoxystrobin, the *cyt b* gene that codes for synthesis of the cytochrome bc1 complex (the Azoxystrobin target protein) (Musso *et al.*, 2020) was amplified and studied in all isolates.

Alternaria solani specific *Cyt b* primers (5'-GCTGCTTTAGCACTAATGCAC-3'(forward) and 5'-CAGAAGGTATCATTCTGGCAC-3' (reverse), designed using Eurofins design tool (https://www.eurofins.com/genomic-services/our-services/dna-rna-oligonucleotides/) and sourced from Macrogen Inc, Netherlands, were used to amplify the Cytochrome b region of rDNA in the isolates (Edin, 2012).

PCR reaction volumes of 30 µL contained 15 µL of Taq DNA polymerase, 1.5 µL of 10 µm/µL forward primer, 1.5 µL of 10 µm/µL reverse primer, 2.0 µL template DNA and 10 µL nuclease-free water. Polymerase Chain Reaction (PCR) was conducted in a gradient thermal cycler (Applied Biosystems), and

involved four stages; an initial denaturation at 94^{o}C for 5 min, 25 cycles of extension at 94^{o}C for 1 min, annealing at 58^{o}C for 1 min, and lastly stabilization at 72^{o}C for 5min.

3.5.4 Gel electrophoresis of PCR products

Agarose gel was prepared as described in section 3.5.2. Two microliter (µL) volumes of each PCR product and 1KB DNA marker (size standard) were separately mixed with gel loading buffer (2 µL SYBR Green and 2 µL Ethidium bromide). Stained mixtures were loaded into wells of the solidified gel suspended in 1x TAE buffer. An electric voltage of 100 volts was then connected to the gel for 30 minutes to facilitate the migration of the amplified PCR products. Formed DNA bands were visualized under UV light (Innis *et al.*, 2012) upon which clear bands confirmed both the amplification and *A. solani* identification (Edin, 2012).

3.5.5 Cleaning of PCR products

PCR products were cleaned by ethanol precipitation method (Green and Sambrook *et al.*, 2016). The products were transferred to fresh 1500 µL tubes followed by adding 5 µL of 3M sodium acetate and 150 µL of 100% ethanol. Tube contents were vortexed to mix thoroughly and left to precipitate at -20^{o}C overnight. This was followed by centrifuging the tube contents at 13000rpm at 4^{o}C for 30 minutes. Resultant DNA pellets were washed twice with 500 µL ice-cold 75% ethanol, spinning at 4^{o}C for 10 min each time. Ethanol was then discarded and the pellet span at top speed for 10 seconds. The pellet was then

left to air dry after which it was re-suspended in 20 µL nuclease-free water and sent to Macrogen Inc. (Amsterdam, Netherlands) for Sanger sequencing.

3.5.6 Bioinformatics analysis

The sequencing quality of reads was assessed using Bioedit® software. Raw sequences were trimmed to remove overlapping sections (noise) from the chromatograms. To support morphological and PCR identification of isolates, trimmed DNA sequences were blasted using *blastn* tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastnandPAGE_TYPE=BlastSearchandLINK_LOC=blasthome) in the GenBank database for similarity with published *Alternaria solani* sequences. At this stage, phylogenetic analysis was conducted on sequences to establish if there was genetic relationship between Azoxystrobin resistant and susceptible isolates. The phylogenetic tree was constructed in MEGA7 package (Tamura *et al.*, 2021), using neighbor joining method based on Tamura-3- model (Tamura and Nei, 1993). The bootstrap consensus tree was inferred from 1000 replicates.

The DNA sequences were then translated into amino acid sequences using NCBI's *blastx* tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastxandPAGE_TYPE=BlastSearchandBLAST_SPEC=andLINK_LOC=blasttabandLAST_PAGE=blastn).

To identify resistance-associated mutations, the obtained amino acid sequences were aligned using NCBI's constraint based alignment tool at https://www.ncbi.nlm.nih.gov/tools/cobalt/re_cobalt.cgi. Amino acid positions 129, 137 and 143 where mutations associated with Azoxystrobin resistance have been reported (Table 2.4) were analyzed.

3.5.7 Data analysis

Survey data was downloaded as a Microsoft Excel spreadsheet (from ODK) and exported into Statistical Package for Social Sciences (SPSS) version 23.0. Data on categorical variables such as gender and education level was expressed by frequencies and percentages while One way analysis of variance was carried out to compare study sites on selected quantitative variables. To determine relationships between some variables Spearman's correlation test was used.

Data on cultural and morphological features was originally entered into a Microsoft Excel spreadsheet and imported into Genstat® statistical software version 21. Descriptive data such as colony colors and nature of margins was summarized in form of frequencies and percentages of all isolates characterized while quantitative variables (such as colony diameters and conidia lengths) were subjected to analysis by one-way ANOVA.

Data on *in-vitro* sensitivity to fungicides was analyzed in R statistical software. A generalized linear model function involving Tukey's Honestly Significant Difference (HSD) tests (at α=0.05) was used to statistically compare mean

colony diameter and % Mycelial Growth inhibition (MGI) with four factors (county, isolates, fungicide and fungicide concentrations).

To analyze the effect of observed mutations on azoxystrobin sensitivity, one-way ANOVA was carried out between means of % MGI values in mutated and wild (un-mutated) isolates. This analysis was also done between colony diameters to determine the effect of mutations on physiological fitness of the isolates. A map of Kenya, showing the spatial distribution of Azoxystrobin resistant isolates and resistance-associated mutations in the surveyed counties was constructed using the .mapdata package in R.

CHAPTER FOUR: RESULTS

4.1 Occurrence, importance and management practices against tomato early blight

4.1.1 Demographic characteristics of farmers

Males constituted most farmers interviewed in the three study sites (Mwea east 84%, Kabete 65% and Loitokitok 93%). Majority of farmers were aged 31 - 50 years (Mwea east 81%, Kabete 92% and Loitokitok 87%). All farmers had attained some formal education i.e. Primary (39%), secondary (51%) and tertiary (18%). Farmers' experience in tomato production varied from 1 to 40 years, the overall average being 9.4 years (Table 4.1)

Table 4.1. Selected characteristics of tomato farmers interviewed in Mwea east, Kabete and Loitokitok subcounties, Kenya, January- April 2021

Characteristics	Mwea east (n=58)	Kabete (n=40)	Loitokitok (n=77)	Overall (n=175)
Gender (% respondents)				
Male	84.5	65.0	93.5	84.0
Female	15.5	35.0	6.5	16.0
Age (% respondents)				
20-30 years	6.9	2.5	9.1	6.8
31-40 years	44.8	37.5	58.4	49.1
41-50 years	36.2	55.0	28.6	37.1
>50 years	12.1	5.0	3.9	9
Formal education (% respondents)				
Primary	37.9	25.0	46.8	38.8
Secondary	44.8	72.5	44.1	50.9
Tertiary	17.2	2.5	9.1	10.3
Av. tomato growing experience (years± SD*)	10.4±5.2[a]	7.4±2.3[b]	9.6±4.8[c]	9.4±4.1

**Standard deviation. Means with different letters across rows are significantly different. Tukey's Honestly Significant Difference test at P ≤ 0.05*

4.1.2 Characteristics of the tomato fields

The studied farm characteristics varied significantly (P values < 0.05) across counties (Table 4.2). Loitokitok had the highest average tomato acreage (1.9 ha) followed by Mwea east (1.5ha) and lastly, Kabete (0.6 ha). On majority of the fields (81%), tomatoes were grown under open field conditions. Greenhouses were only common in Kabete accounting for 80% of all tomato farms surveyed there. The average estimated tomato yield was highest in Kabete (11.7 tons/ha) and lowest in Loitokitok (6.9tons/ha). Farmers' estimated yield was significantly higher (P-value 0.041) under greenhouse production than in open fields.

Table 4.2. Characteristics of tomato fields surveyed in Mwea east, Kabete and Loitokitok subcounties, January-April 2021

Characteristics	**Mwea east** (n=58)	**Kabete** (n=40)	**Loitokitok** (n=77)	**Overall** n=175
Av. farm size/ha ±SD	2.0±3.2b	1.0±0.4ab	2.3±1.9a	1.75±2.5
Av. tomato acreage /ha±SD	1.5±2.2ab	0.6±0.2b	1.9±1.1a	1.33±1.7
Estimated tomato yield* (ton/ha±SD)	7.1±1.6ab	11.7±2.7a	6.9±4.5b	8.57±2.8
Production system (% fields)				
Green house	1.7	80.0	0.0	18.2
Open field	98.3	20.0	100.0	81.1
Cropping pattern (% fields)				
Monocrop	63.7	77.5	75.3	72
Intercrop	36.2	22.5	24.7	28
Irrigation method (% fields)				
Drip	1.7	75.0	3.9	19.4
Furrow	93.1	12.5	84.4	70.8
Sprinkler	0.0	12.5	6.5	5.8
Others (watering can, diversion channels)	5.2	0.0	5.2	4.0

***Farmers estimated yield in terms of number of 'crates' or 'Forwards' harvested. Crates are wooden square containers with a capacity of 60-80Kg. 'Forwards' are trucks used to transport tomatoes to the market and each could carry an estimated 2 tonnes of tomatoes. SD- Standard deviation. Means with similar letters across rows are not significantly different. Tukey's Honestly Significant Difference test at P ≤ 0.05*

Tomatoes were grown as a monocrop on majority (72%) of the fields surveyed. Intercropping was practiced most in Mwea east and Loitokitok (21% and 19% of fields respectively) and least in Kabete (9% of fields). The commonest intercrop crops included maize, beans and green pepper. All farms visited practiced some form of irrigation. Majority of farmers (71%) used furrow, 19% drip and 9% sprinkler irrigation. In Mwea east and Loitokitok, other forms of irrigation were used for example watering cans and diversion channels on fields neighboring streams. These were observed on only 4% of the surveyed fields.

4.1.3 Farmers' knowledge and perception of early blight

A total of five major tomato diseases and four insect pests were present on at least 20% of the surveyed fields (Table 4.3). Early blight (85% of fields) was the most prevalent disease, followed by late blight (83%). Most farmers could identify EB as *"Baridi"* (Swahili word for cold), an indication that they associated it with cold weather. Blights (early and late) were also the highest ranked diseases in terms of yield loss caused. Early blight prevalence and overall yield loss rank were significantly highest in Mwea east and lowest in Kabete (P value 0.03).

Other major diseases identified in the fields included Bacterial wilt (48%), Fungal wilts (21%), and viral diseases (37%). The observed viral disease symptoms resembled those of Tomato Common Mosaic Virus (TCMV), Tomato Spotted Wilt Virus (TSWV) and Tomato Yellow Leaf Curl Virus (TYLCV). The major pests in the fields were tomato leaf miner (90%), thrips (53%), spider

mites (54%) and whiteflies (56%). Blights and tomato leaf miner were the highest ranked biotic constraints (average overall ranks above 3).

Table 4.3: Prevalence and farmer's ranking of major biotic constraints to tomato production in Mwea east, Kabete and Loitokitok subcounties, Kenya, January - April 2021

		Mwea east		Kabete		Loitokitok		Overall	
Diseases[b]	Scientific name	% fields	Rank[a] (x±SEM)	% fields	Rank[a] (x±SEM)	% fields	Rank[a] (x±SEM)	% fields	Rank[a] (x±SEM)
Early blight	*Alternaria solani*	91.4	3.5± 0.09	75.0	2.4 ±0.16	90.9	3.1±0.09	85.2	3.1±0.08
Late blight	*Phytophthora infestans*	87.9	3.3± 0.08	68.5	2.2± 0.14	80.5	3.5±0.06	80.6	3.2±0.06
Bacterial wilt	*Ralstonia solanacearum*	60.3	1.2± 0.06	94.3	2.1± 0.15	22.1	1.5±0.20	48.0	1.3±0.06
Fungal wilts	*Fusarium spp.,Verticillum spp,*	19.0	1.2± 0.12	25.7	1.1± 0.07	19.5	1.4±0.13	20.6	1.5±0.13
Viral diseases	TSWV, TCMV, TYLCV	34.5	1.2± 0.12	34.2	1.3± 0.09	40.3	1.8±0.07	37.1	1.4±0.06
Pests[b]									
Leaf miners	*Tuta absoluta*	93.1	2.5± 0.16	94.3	3.1 ±0.12	93.5	3.9±0.04	89.8	3.1±0.07
Thrips	*Thrips tabaci*	87.9	2.2± 0.12	60.0	1.8 ±0.11	28.6	1.2±0.17	53.1	1.8±0.08
Red spider mites	*Tetranychus evansi*	58.6	2.4± 0.15	40.0	1.1 ±0.06	62.3	1.4±0.11	54.2	1.4±0.12
Whiteflies	*Bemisia tabaci*	51.7	2.4± 0.07	80.0	1.8± 0.09	53.2	2.2±0.12	55.9	1.5±0.07

[a] Farmer's ranking in terms of yield loss caused (1-4) where 1 low (<10%), 2 Moderate (20-29%), 3 High (30-40%), 4 Very high (>40%). [b] Multiple answers possible
TSWV – Tomato Spotted Wilt Virus, TCMV – Tomato Chlorotic Mottle Virus, TYLCV – Tomato Yellow Leaf Curl Virus

4.1.4 Tomato varieties cultivated

A total of 19 tomato varieties were being grown on surveyed fields. Forty five percent of farmers grew one variety while the rest grew more than one. Anna F1 and Zara F1 were popular in Kabete while Big rock F1 and DRD F1 were dominant in Loitokitok (Fig. 4.1a). The most popular varieties in Mwea east were Terminator F1, Big rock F1 and Ansal F1. Whereas all these are improved varieties, their resistance/susceptibility to early blight could not be ascertained as this information was not found on their seed packs and neither was it available in any literature.

Among the reasons for choice of cultivars, yield (78%) was the most frequently mentioned (Figure 4.1 b). Other factors included longevity of harvesting period (40%), size of fruits (23%), shelf life of fruits (22%), water stress tolerance (16%) and price of seedlings (15%). Resistance to pests and diseases was only considered by 19% of the farmers interviewed. Only 4 farmers (representing 2% of total) had their own nurseries so on most fields, the seedlings had been purchased from commercial nurseries.

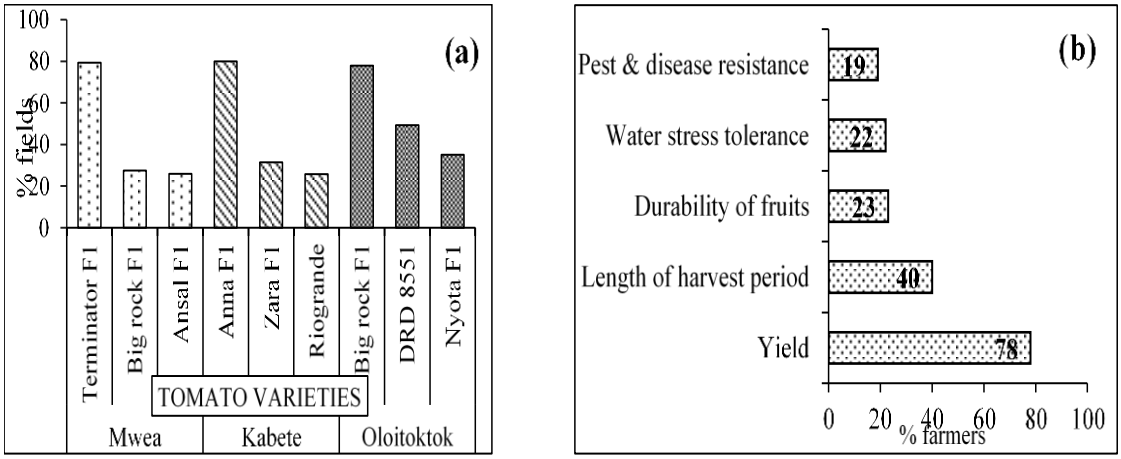

Figure 4.1: (a) Main tomato varieties grown in the surveyed sub-counties (b) Factors influencing farmers' preference of varieties

Majority of farmers (66%) could grow tomatoes for more than two cropping cycles on the same fields per year. Considering that the average tomato season in Kenya is 4-5 months, this means that many farmers in surveyed areas could plant a new crop in the same field immediately after harvesting the old one. Some fields had been under continuous tomato production for more than 10 years without any fallow periods or rotation with any other crop. Since all farmers could practice some form of irrigation, tomato production on most fields could be undertaken all year round without defined periods for planting or harvest.

4.1.5 Control methods used against early blight

All farmers used synthetic fungicides for management of early blight. Although common cultural practices that can supplement EB control (such as weeding, pruning, and staking) were observed on most farms, only 10% of the farmers could associate these with disease management. Biological control methods

involving use of fungal antagonists (still on trial) were observed on only two farms, one in Kabete and another in Mwea east. Only 7% of the farmers interviewed had knowledge on integrated disease management.

4.1.6 Composition and resistance risk of fungicides used

A total of 40 fungicide products representing 20 active compounds were in use against early blight (Table 4.4). Of these, 24 contained single active compounds while 16 were mixtures. Active compounds represented 6 chemical groups/ modes of action. Mancozeb (present in 38% of the fungicides) was the most common active compound. Other common active compounds were propineb, cymoxanil, chlorothalonil, azoles, carbendazim and Azoxystrobin. Most active compounds (70%) fell in FRAC resistance risk categories above ‘Low’.

Table 4.4: Fungicide products used in the surveyed subcounties, their active compounds and FRAC Resistance risk codes

Fungicide product	Active compound(s)	FRAC Resistance risk* (Code/meaning)
Ridomil Gold®	Metalaxyl + Mancozeb	(4/High) + (M3/Low)
Milraz®	Propineb + cymoxanil	(M3/Low) + (27/Low to medium)
Oshothane®	Mancozeb	M3/Low
Mistress®	Cymoxanil+ Mancozeb	(27/Low to Medium) + (M3/Low)
Agromax®	Cymoxanil+ Mancozeb	(27/Low to Medium) + (M3/Low)
Ortiva®	Azoxystrobin	11 (High)
Milthane Super®	Mancozeb	M3/Low)
Antracol®	Propineb	M3/Low)
Victory®	Metalaxyl+ Mancozeb	(4/High) + (M3/Low)
Score®	Difenoconazole	3 (Medium)
Linkmil®	Mancozeb+ Metalaxyl	(4/High) + (M3/Low)
Bayfidan®	Triadimenol	3/Medium
Classic®	Tebuconazole	3 /Medium
Daconil®	Chlorothalonil	M5 /Low
Funguran®	Copper hydroxide	M1/Low
Wetsulf®	Sulfur	M2/Low
Goldazim®	Carbendazim	1/High
Cover®	Azoxystrobin+ Propiconazole	(11/High) + (3/Medium)
Isacop®	Copper oxychloride	M1/Low
Greencop®	Copper oxychloride	M1/Low
Blue Shield®	Copper hydroxide	M1/Low
Ivory®	Mancozeb	M3/Low
Penncozeb®	Mancozeb	M3/Low
Equation Pro®	Cymoxanil	M3/Low
Bayleton®	Femoxadone	Unclassified
Komesha®	Cymoxanil+ Propineb	(27/Low to medium) + (M3/Low)

Absolute®	Azoxystrobin + Difenoconazole + Hexaconazole	(11/High) + (3/Medium) + (3/Medium)
Rodazim®	Carbendazim	1/High
Trinity Gold®	Copper oxychloride+ Cymoxanil+ Mancozeb	(M1/Low) + (27/Low) + (M3/Low)
Nordox®	Copper	M1/Low
Volar MZ®	Dimethomorph + Mancozeb	(40/Low to medium) + (M3/Low)
Azoxystop®	Azoxystrobin + Difenoconazole	(11/High) + (3/Medium)
Nativo®	Trifloxystrobin + Tebuconazole	(11/High) + (3/Medium)
Ranson®	Carbendazim+ Triadimefon	(1/High) + (3/Medium)
Mixanil®	Cymoxanil+ Chlorothalonil	(27/Low to Medium) + (M5/Low)
Farmerzeb®	Mancozeb	M3/Low
Z-Force®	Mancozeb	M3/Low
Stargem®	Mancozeb	M3/Low
Tajiri®	Mancozeb+ Cymoxanil	(M3/Low) + (27/ Low to Medium)
Top Guard®	Thiophanate methyl	1/High

**FRAC=Fungicide Resistance Action Committee. The risk codes were obtained from their website https://www.frac.info*

4.1.7 Farmers' preference for fungicide products

Farmers' preference for fungicide products differed significantly across study sites (Table 4.5). The most commonly used brand names were Ridomil Gold® (Metalaxyl+Mancozeb) (66% of fields), Milraz® (Propineb + cymoxanil) (29%) and Oshothane® (Mancozeb) (21% of fields) (Table 5). Price of the fungicide (72%), prevailing weather (70%) and perception on efficacy (67%) were the

major factors influencing the choice of fungicides. With exception of Ridomil Gold®, the effectiveness ranking of fungicide products did not differ significantly (at α=0.05) across study sites. Ridomil Gold® (overall rank 2.1) and Milraz® (2.2) were ranked as the most effective but also the most expensive.

Most farmers believed that severity of Early blight could get higher during cold weeks so during such times, more farmers would apply the products perceived to be most effective (Ridomil Gold® and Milraz®). During warmer periods, the brands perceived to be less effective were applied more. In Loitokitok, which is at the border between Kenya and Tanzania, there was a general perception that fungicides purchased from Tanzania were more effective than those sold in Kenya even when the active compounds and/or brand names were similar. Most farmers interviewed (81%) viewed chemical control of Early blight using available fungicides as moderately effective, only 19% ranked it as highly effective.

Table 4.5: Major fungicide products used by tomato farmers in Mwea east, Kabete and Loitokitok subcounties, Kenya in January -April, 2021

	Mwea east		Kabete		Loitokitok		Overall			
Brand name[a]	% fields	Rank[b] (x±SEM)	% fields	Rank[b] (x±SEM)	%fields	Rank[b] (x±SEM)	% fields	Rank[b] (x ±SEM)	P value[c]	P value[d]
Ridomil Gold®	81.0	2.5± 0.09	94.3	2.4 ±0.16	41.6	2.2± 0.14	66.2	2.1±0.08	0.044	0.048
Milraz®	55.2	2.3± 0.08	42.9	2.2± 0.14	3.9	2.1± 0.15	28.5	2.2±0.06	0.039	0.052
Oshothane®	31.0	2.2± 0.06	40.0	2.1± 0.15	5.2	1.1± 0.07	20.5	1.8±0.06	0.043	0.061
Mistress®	32.8	1.2± 0.12	31.4	1.1± 0.07	3.9	1.3± 0.09	20.0	1.5±0.13	0.048	0.068
Agromax®	10.3	1.2± 0.12	5.7	1.3± 0.09	29.9	1.5± 0.16	17.7	1.4±0.06	0.043	0.087
Milthane Super®	8.6	1.1± 0.16	20.0	1.2± 0.09	6.5	2.2± 0.12	10.3	1.1±0.13	0.047	0.075
Antracol®	6.9	1.2± 0.12	15.0	1.3± 0.09	18.8	1.5± 0.16	14.3	1.3±0.06	0.043	0.087
Ortiva®	8.6	1.0± 0.09	5.7	1.1± 0.19	25.0	1.0± 0.05	15.4	1.0±0.06	0.043	0.087
Score®	6.9	1.0± 0.13	20.0	1.2± 0.07	12.5	1.3± 0.17	12.0	1.1±0.13	0.047	0.075

[a]Multiple answers allowed. [b]Farmers' ranking on effectiveness of the fungicide where 1=low, 2=Moderate, 3=High [c]P value for fungicide products at α=0.05, [d]P value for Farmer's ranking at α=0.05

4.1.8 Fungicide dosages and spray interval

On majority of the fields (83%), farmers reported that they were not following the manufacturers' recommendations on fungicide dosages and spray intervals. Most applied higher than recommended dosages of especially the cheaper fungicides, in attempt to increase their effectiveness.

Price (90%), weather (74%) and perceived effectiveness (67%) were the major factors influencing spray dosages and intervals. Higher dosages were applied during colder weeks than on warmer ones. Overall, the average dosages for the more costly fungicides (i.e Ridomil Gold® (Metalaxyl+Mancozeb) and Milraz® (Propineb+Cymoxanil) were lower than those recommended on labels while those for less expensive ones were much higher. An example is Mistress® (Cymoxanil+ Mancozeb), a low-cost locally manufactured fungicide whose average dosage was double the manufacturer's recommendation. Similarly, the spray intervals were shorter for low-cost fungicides than for the more expensive ones. Comparatively, the fungicide dosages were lowest and spray intervals longest in Kabete. Loitokitok had the highest fungicide dosages and shortest spray intervals (Figure 4.2).

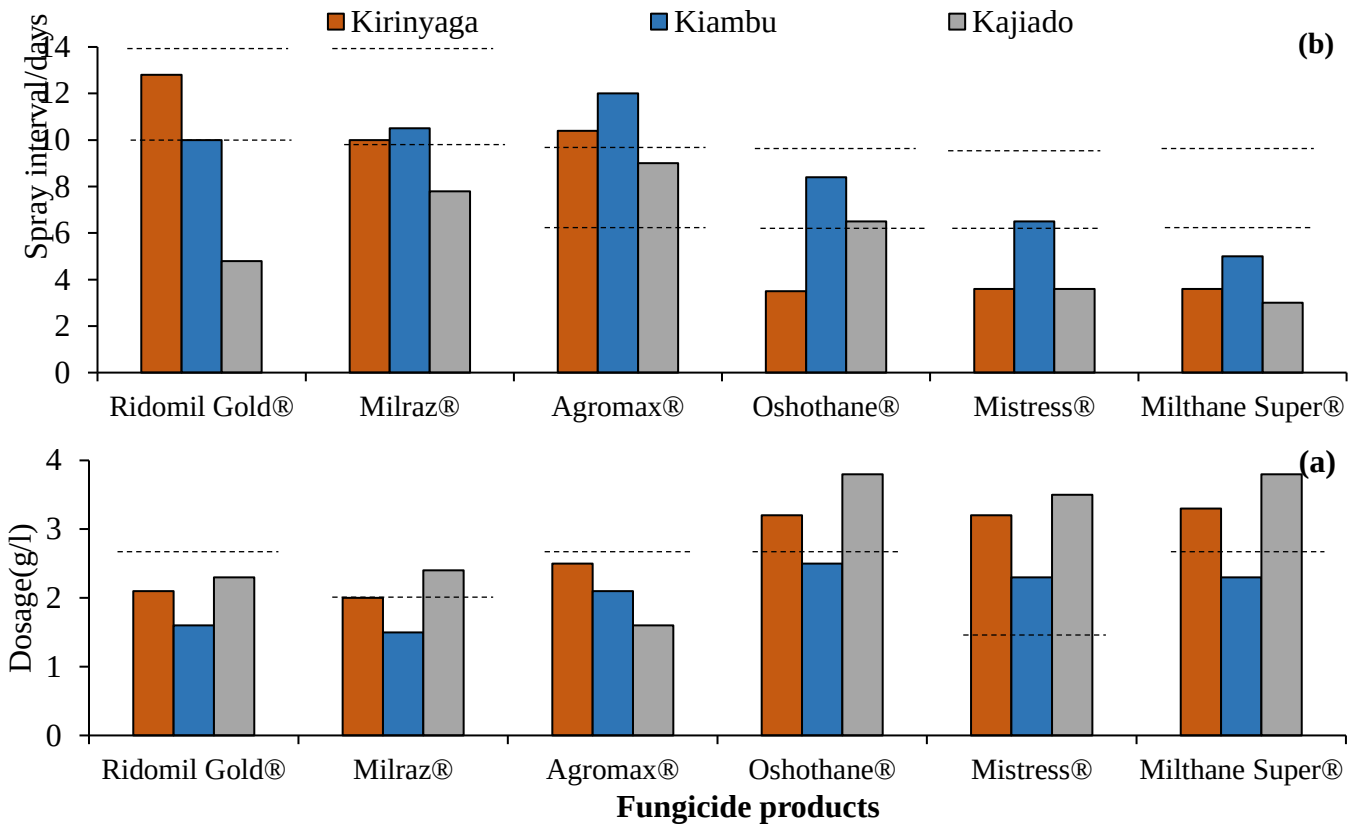

Figure 4.2: Farmers' application rates for 6 most commonly used fungicide products against Early blight relative to manufacturer's recommended levels (indicated by dotted lines)

(a) *Average farmers' spray dosages* ***(b)*** *Average farmers' spray intervals.* Farmers' dosages were calculated from responses on volume of fungicide per Knapsack pump(16-20l) or mixing drum (320-800l). [c]Farmers' spray intervals were calculated from responses on number of sprays per week or month.

Advice on choice of fungicides, spray dosage and intervals, timing of application was sought mostly from fellow farmers (72%), agrochemical shops (49%), visiting agronomists (28%) or other sources (11%).

4.1.9 Timing of fungicide application

On most fields in Mwea east (91%) and Loitokitok (78%), fungicide application typically started from the first week after transplanting (Table 4.6). In Kabete, the first fungicide application would occur much later, especially in greenhouses. Most farmers (74%) applied fungicides as a preventive measure for early blight, only few (22%) waited until appearance of the first symptoms. In some greenhouses in Kabete (n=8; 5% of total), farmers reported to only apply fungicides whenever the weather turned cold.

Table 4.6: Decision factors on fungicide application among interviewed tomato farmers in Mwea east, Kabete and Loitokitok subcounties, Kenya, January – April 2021

	Mwea east	Kabete	Loitokitok	Overall
1.What informs decision to start applying fungicides? %				
Cold weather	-	20	-	4.6
First symptoms	17.2	50.0	10.4	21.7
Prevention	82.8	30.0	89.6	73.7
2.When after planting does the first fungicide application occur? (% farmers)				
In the first week	91.3	12.5	77.9	67.4
In the first 2 weeks	8.6	25.0	22.1	18.2
In the first one month	-	62.5	-	14.3
3.Factors for choice of fungicide* (% farmers)				
Price	72.4	65.7	75.3	72.4
Weather	86.2	54.2	64.9	70.0
Effectiveness	63.7	71.4	67.5	67.0

*Multiple answers allowed

4.1.9.2 Farmers perception on declining efficacy of fungicides

Most farmers (142 out of 175 or 81%) had experienced declines in efficacy observed of at least one fungicide product during early blight management (Table 4.7). Loitokitok had the highest proportion of such farmers (92%), followed by Mwea east (88%) and lastly Kabete (50%). To a typical farmer, declining efficacy meant that early blight disease had gained resistance to the fungicides (Figure 4.3)

A total of 25 fungicide products were reported to have declined in efficacy against early blight. Such products contained mostly single-site active compounds for example Azoxystrobin (60% of the mentioned fungicide products), difenoconazole (20%) and tebuconazole (20%). This declining efficacy of fungicides was attributed most to development of resistance in early blight disease (71% of the farmers), counterfeit fungicides (31%), and climate change (19%).

Table 4.7: Perceptions of tomato farmers on declining efficacy of some fungicides in Mwea east, Kabete and Loitokitok subcounties, Kenya

Farmers who had observed declining efficacy of at least one fungicide (% farmers)	Loitokitok	92.2
	Mwea east	87.9
	Kabete	50.0
Major a.i s in fungicide products reported to have declined efficacy against EB[a b] (% products)	Azoxystrobin	60
	Tebunoconazole	20
	Difenoconazole	24
	Trifloxystrobin	20
Reasons for declining efficacy of fungicides[b] (% farmers)	Resistance	71.4
	Counterfeit fungicides	31.4
	Climate change	18.8
	Didn't know	24.0

a.i –Active ingredient. a-Some mentioned fungicides contained more than one active ingredient. b-Multiple answers possible. Figures in parentheses indicate frequencies

Figure 4.3. Typical farmer's understanding of fungicide resistance.
Even when the farmer had applied the fungicide (as visible in a), early blight lesions kept expanding leading to rejection of such fruits by buyers. Approximately 30% of all harvested fruits were being discarded at this grading site in Mwea east, Kirinyaga County, Kenya (b).

4.2 Characterization of *Alternaria solani* isolates

4.2.1 Cultural characterization of *Alternaria solani* isolates

On the 5th day after culturing, a total of 122 isolates had creamy white to green colors characteristic of *Alternaria*. These were sub-cultured and characterized but only 96 of these were later confirmed as *Alternaria solani* by PCR (Section 3.5.4). Thirty-five confirmed isolates were from Kirinyaga, Kiambu 30 and Kajiado 31.

In culture, the fungus grew as profuse mycelia on PDA. At first, the mycelia was hyaline but later turned gray to brown, septate and branching irregularly, as it grew. Isolates did not differ significantly (at α=0.05) in studied cultural characteristics across the selected counties (Table 4.8 and Appendix 4). Isolate

KYG24 from Kirinyaga had the highest recorded colony diameter at 85mm while KJD18 from Kajiado had the lowest colony diameter at 65.5mm.

Regarding color, most colonies (45 of 96 or 45%) were greenish-white, 22 were creamish-white, 10 green and 1 grey. On reverse plate, most colonies were pigmented creamish white (49 out of 96), greenish-brown (42) and brown (5). About half (55.2%) of the isolates had irregular margins and majority (62.5%) of isolates had concentric zonation (Figure 4.4).

Table 4.8: Summary of cultural characteristics of *Alternaria solani* isolates from Kirinyaga, Kiambu and Kajiado counties, Kenya

Characteristic	Kirinyaga n=35	Kiambu n=30	Kajiado n=31	Overall n=96
Colony diameter (mm ± SD)	78.60 ± 8.81a	75.09 ± 7.62ab	76.68± 8.58b	74.77 ± 7.59
Range/mm	66.0-85.0	66.5-83.5	65.5-84.0	
Colony colour (Top) %				
Green	17.1	26.7	45.2	29.2
Creamish white	45.7	6.7	12.9	22.9
Greenish white	37.1	63.3	41.9	46.9
Grey	00	3.3	00	1.0
Pigmentation (down) %				
Brown	2.8	66.7	6.4	5.2
Creamish white	60.0	33.3	58.1	51.0
Greenish brown	37.1	60	35.5	43.8
Nature of margin (%)				
Irregular	85.7	33.3	74.2	55.2
Regular	14.3	66.7	25.8	44.8
Colony zonation (%)				
Concentric zonation	40.0	86.7	64.5	62.5
No zonation	60.0	13.3	35.5	37.5

SD- Standard deviation. Means with similar letters in rows are not significantly different. Tukey's Honestly Significant Difference test at $P \leq 0.05$

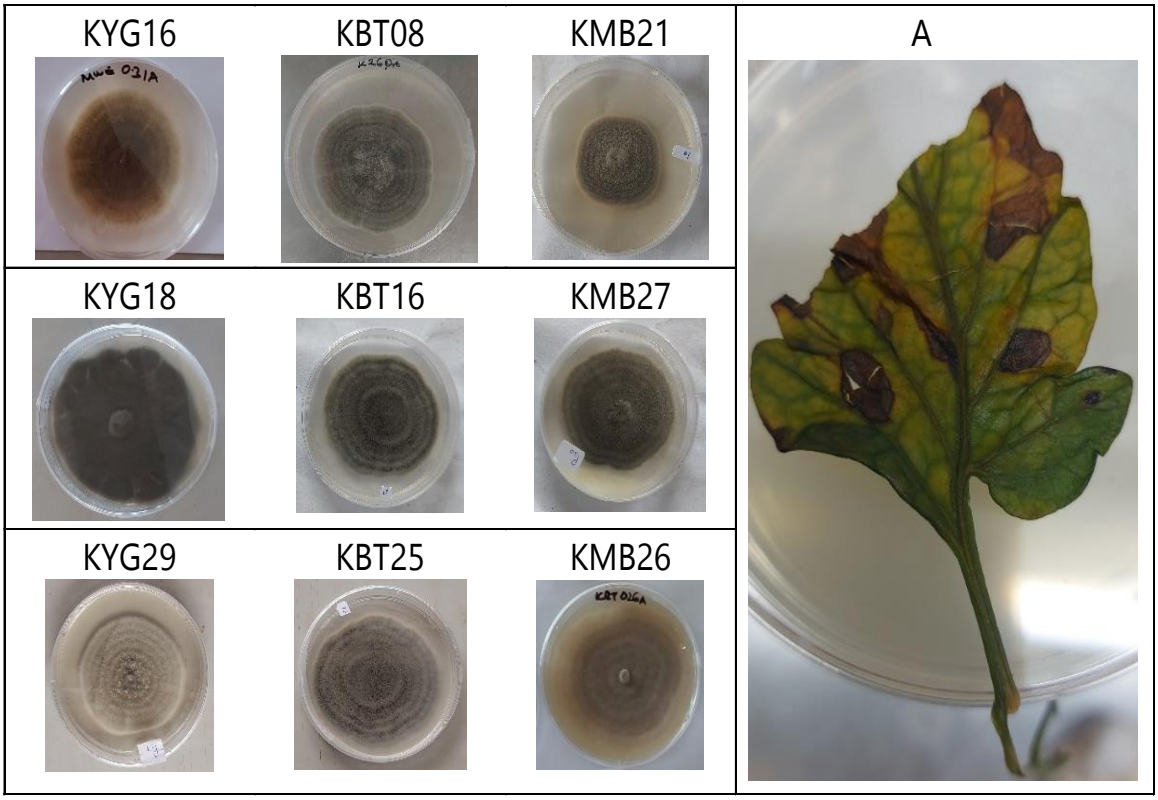

Figure 4.4: Nine-day cultures for some isolates. Picture A represents one of the infected tomato leaves from which Alternaria solani was isolated

4.2.2 Morphological characteristics of isolates

A total of 117 isolates had morphological features that matched those of *Alternaria solani* described by Simmons (2007). However, only 96 of these were confirmed as *A. solani* by molecular methods. In the early stages, hyphae were thin (diameter 2.5-2.8µm), hyaline but thickened slightly (4.41-4.44µm diameter) with age.

Conidiophores were 200-230 µm long, flexuous or straight, and were either solitary in many isolates or in small groups in a few others. At apices, conidiophores enlarged slightly with scars indicating points of conidia attachment. Conidia were pale to olivaceous-brown, borne singly or in short

chains, on conidiophores. They were straight, or slightly flexuous tapering to a beak in some isolates or flat-ended in some others.

Conidia lengths ranged from 16.72 – 20.48 μm (Mean 18.46, St. dev. 3.83) while widths were between 11.87-12.13 μm (mean 11.44, St. dev.2.2) (Table 4.9 and Appendix 4). Three conidia shapes were identified; ellipsoidal (54%), obclavate (35%), obvoid (10%). All conidia had at least 2 transverse septations (range 2-5). Majority (69.8% or 67 of all isolates) had longitudinal septa in their conidia, ranging from 1 - 3. Only 56% (or 56 isolates) had beaked conidia. Beak lengths ranged from 19.7 – 6.8 μm (Mean 10.9, St. dev. 3.8) while the number of beak septa varied from 1-4 (Figure 4.5). Chlamydospores were formed in old cultures (older than 2 months) of *A. solani*. These were dark brown in color, thick walled and round to oval in shape.

Table 4.9: Morphological characteristics of *Alternaria solani* isolates collected from Kirinyaga, Kiambu and Kajiado counties, Kenya

Characteristic	Kirinyaga (n=35)	Kiambu (n=30)	Kajiado (n=31)	Overall (n=96)
Conidia shape (% isolates)				
Ellipsoidal	68.5	40	51.6	54.2
Obclavate	28.6	33.3	45.7	35.4
Obvoid	2.9	26.7	3.2	10.4
Beaks on conidia (% isolates)				
Isolates with beaked conidia	60	60	48.4	56.3
Isolates without beaked conidia	40	40	51.6	43.7
Conidia dimensions				
Av. length(µm ± SD)	20.48±3.81a	16.72 ±2.34b	18.00±2.38b	18.44±3.29
Av. width (µm ± SD)	11.87±1.89b	11.22±1.96a	12.13±1.77a	11.78±2.24
Av. beak length(µm± SD)	10.26±3.85b	13.99±3.62a	8.98±1.51c	10.97±2.76
Septations				
No. of transverse septa (range)	2-5	2-5	2-5	2-5
No. of longitudinal septa (range)	0-2	0-2	0-2	0-2
No. of beak septa (range)	0-2	0-2	0-2	0-2

**Means followed by similar letters in rows are not significantly different. Tukey's Honestly Significant Difference test at $P \leq 0.05$*

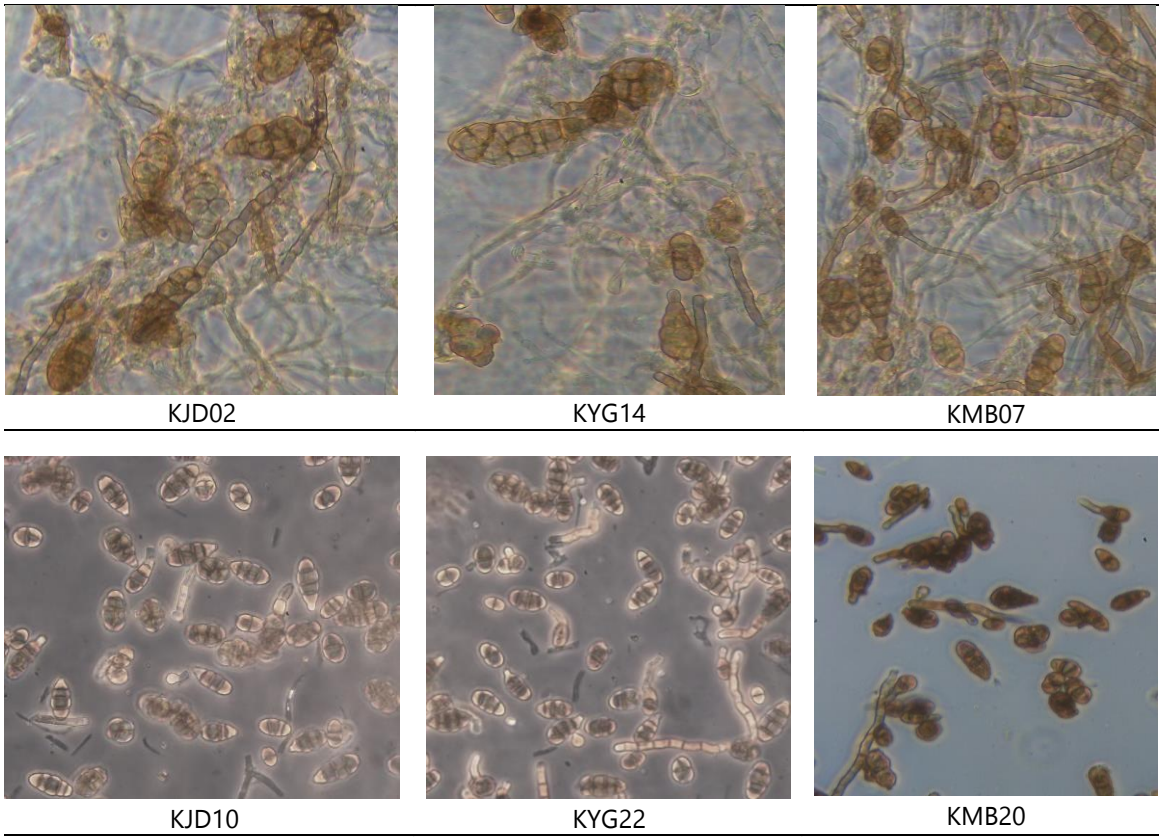

Figure 4.5: Conidial features of some isolates at medium power (X40)

4.2.3 Pathogenicity tests for *Alternaria solani* isolates

Symptoms started appearing 3 days after spraying the *A. solani* inoculations. Brown, irregular spots (2-4 mm in diameter) with concentric zonations at the center, appeared on leaves (Figure 4.6). In some cases, the spots enlarged in size reaching up to 10mm in diameter in the second week after inoculation

Re-isolated cultures from infected leaves had close similarity with inoculated isolates in terms of cultural and morphological features. This confirmed the pathogenicity of tested *Alternaria solani* isolates on tomato as per Koch's postulates.

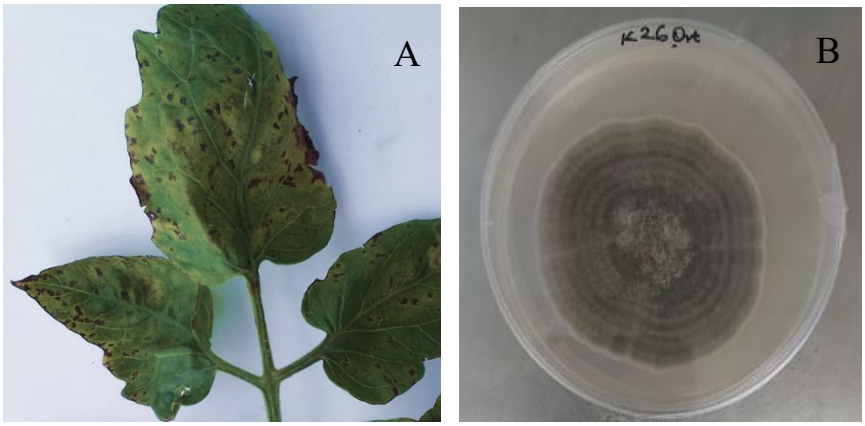

Figure 4.6: Results of the pathogenicity test. A-Early blight lesions on tomato leaves. B- Re-isolated Alternaria solani isolate

4.2.4 Sensitivity of *Alternaria solani* isolates to two commonly used fungicide groups

4.2.4.1 Sensitivity at Manufacturer's recommended dosage (MRD) (0.25mg a.i/L)

At this dosage, both azoxystrobin and difenoconazole significantly reduced colony diameter in all isolates ($p<0.05$) compared to the control (without fungicide). However, difenoconazole was more effective at inhibiting colony growth (lower %MGI values, at $\alpha=0.05$) than azoxystrobin for all isolates (Figure 4.7). All isolates were sensitive to difenoconazole at this dosage with %MGI values above the 50% threshold (range 54.44-96.5%). The most insensitive isolate to Difenoconazole (M24) was from a field in Kirinyaga where tomato had been grown continuously for 10 years. At this field, difenoconazole and other triazoles had been in use for only 5 years.

Most isolates (62 out of 96, or 64.6%) were resistant to Azoxystrobin at MRD. Isolate KJD6 from Kajiado demonstrated the highest resistance to Azoxystrobin (MG1 15.44%). This isolate was obtained from a field where the farmer had

abandoned Azoxystrobin usage, after experiencing great declines in effectiveness against early blight.

Site significantly influenced the sensitivity of isolates to the fungicides tested. Mean comparison tests between average %MGI values at MRD (α=0.05), revealed that Kirinyaga isolates were the least sensitive to difenoconazole while Kiambu isolates were the most sensitive to the fungicide. For Azoxystrobin, Kajiado isolates were the least sensitive, followed Kirinyaga ones and lastly, those from Kiambu (Figure 4.8). Kajiado county accounted for the majority of azoxystrobin-resistant isolates (47%), followed by Kirinyaga (35%) and lastly Kiambu.

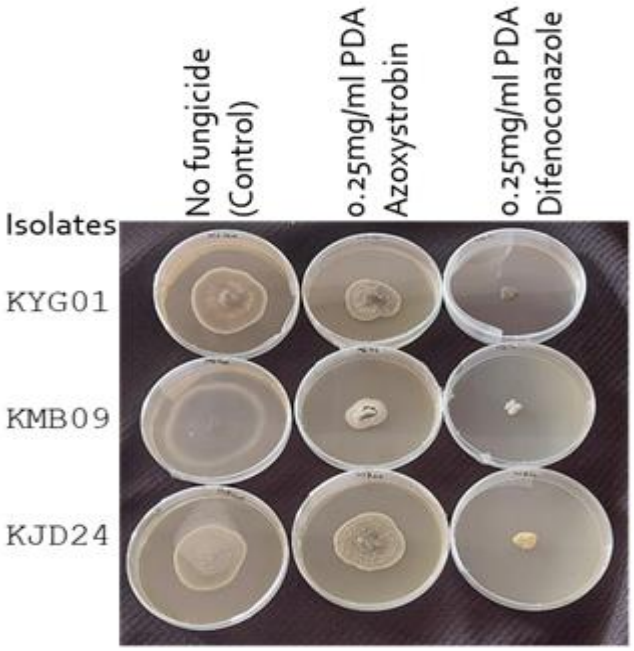

Figure 4.7: Seven day old cultures for three isolates at manufacturer's recommended dosage compared with the control for two fungicides, (Azoxystrobin and Difenoconazole)

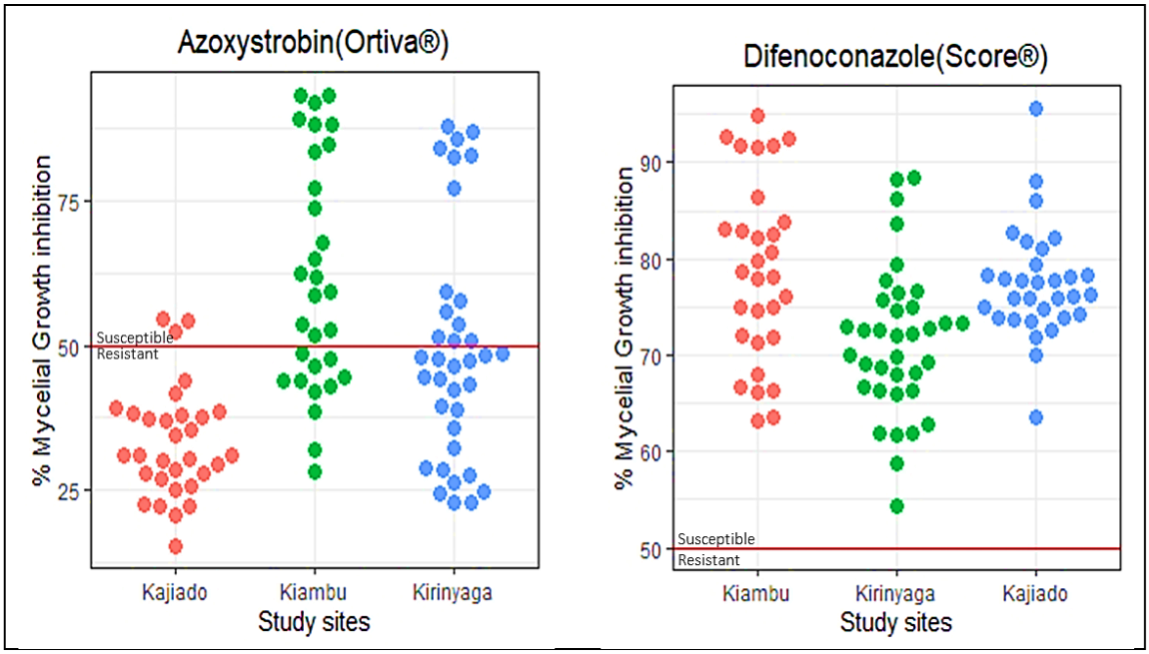

Figure 4.8: Sensitivity of Alternaria solani isolates from three study sites in Kenya to fungicides (Azoxystrobin and Difenoconazole) at Manufacturer's recommended dosage. Isolates with % MGI <50% at this dosage were considered resistant to the fungicide.

4.2.3.2 Sensitivity at other dosage levels

Increase in fungicide concentration significantly increased the sensitivity (by increasing % MGI) of all isolates to the fungicides tested. Mycelial growth inhibition was however site-dependent, with Kajiado isolates having the lowest sensitivity to Azoxystrobin (at α=0.05) (Figure 4.9). Kirinyaga and Kiambu isolates did not differ significantly in sensitivity to azoxystrobin at all dosages (α=0.05). At 0.5mg a.i ml^{-1} (double the manufacturer's recommendation), 12 isolates were still resistant to Azoxystrobin with, isolate KJD32 from Kajiado County (MGI 33.5%), being the most resistant.

Difenoconazole was more effective than azoxystrobin at inhibiting mycelial growth at all doses and as was the case for Azoxystrobin, site affected the

sensitivity of isolates to Difenoconazole. Kirinyaga isolates were the least sensitive at all difenoconazole concentrations followed by Kajiado and lastly Kiambu. Percent MGI for Kiambu and Kajiado isolates did not differ significantly at 0.25 mg/ml and 0.375 mg/ml difenoconazole concentrations (α=0.05).

At double the manufacturer's recommended dosage, isolates did not significantly differ by site in sensitivity to difenoconazole. Isolate KYG19 (% MGI 62.5) had was the least sensitive at this dosage.

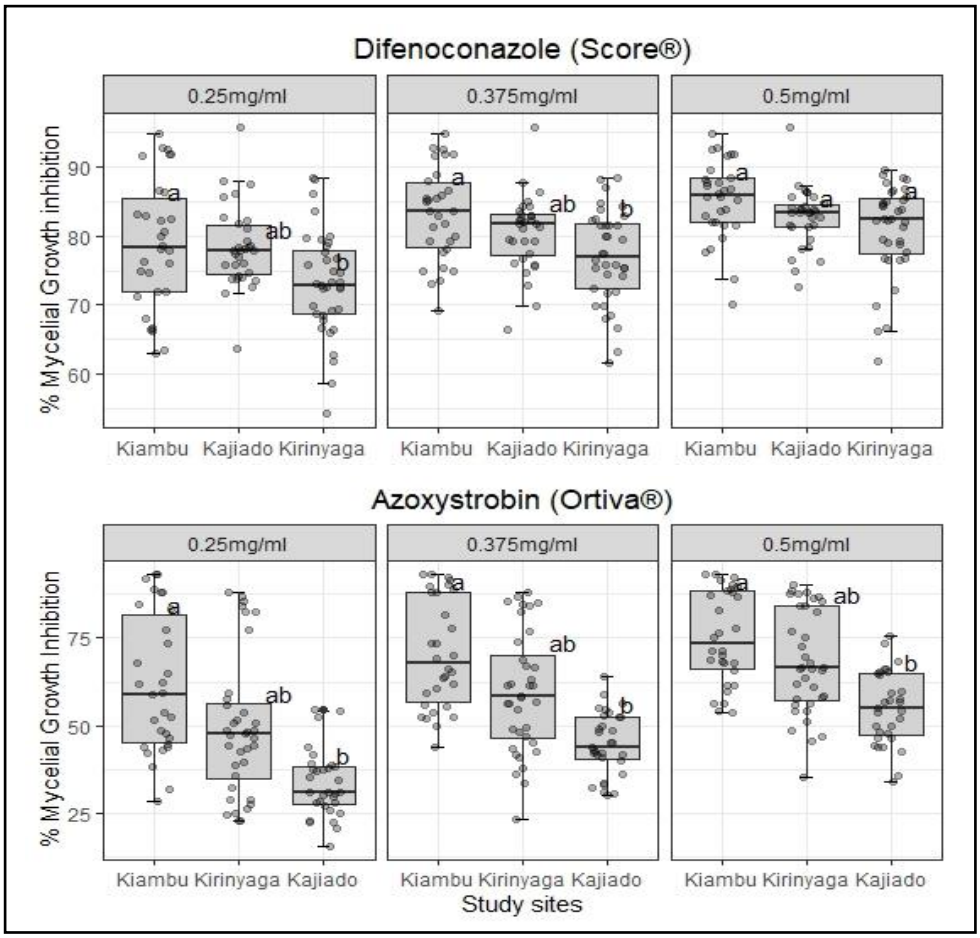

Figure 4.9: Sensitivity of Alternaria solani isolates to Azoxystrobin and Difenoconazole at different concentrations

4.3 Detection of resistance-associated mutations in the *cyt b* gene

4.3.1 PCR results and confirmation of *Alternaria solani* identity

The two primer pairs amplified an ~ 450 bp fragment of *cyt b* gene in each *A. solani* isolate (Figure 4.10). Ninety-six trimmed DNA sequences, ~ 210 bp long (Appendix 7), were obtained (Kirinyaga (n=35), Kiambu (30) and Kajiado (31)). In the NCBI database, sequences showed high percent similarities (98-99.5%) with *Alternaria solani* accession numbers DQ209285.1 and DQ209284.1. This served as the final confirmatory step for *Alternaria solani*.

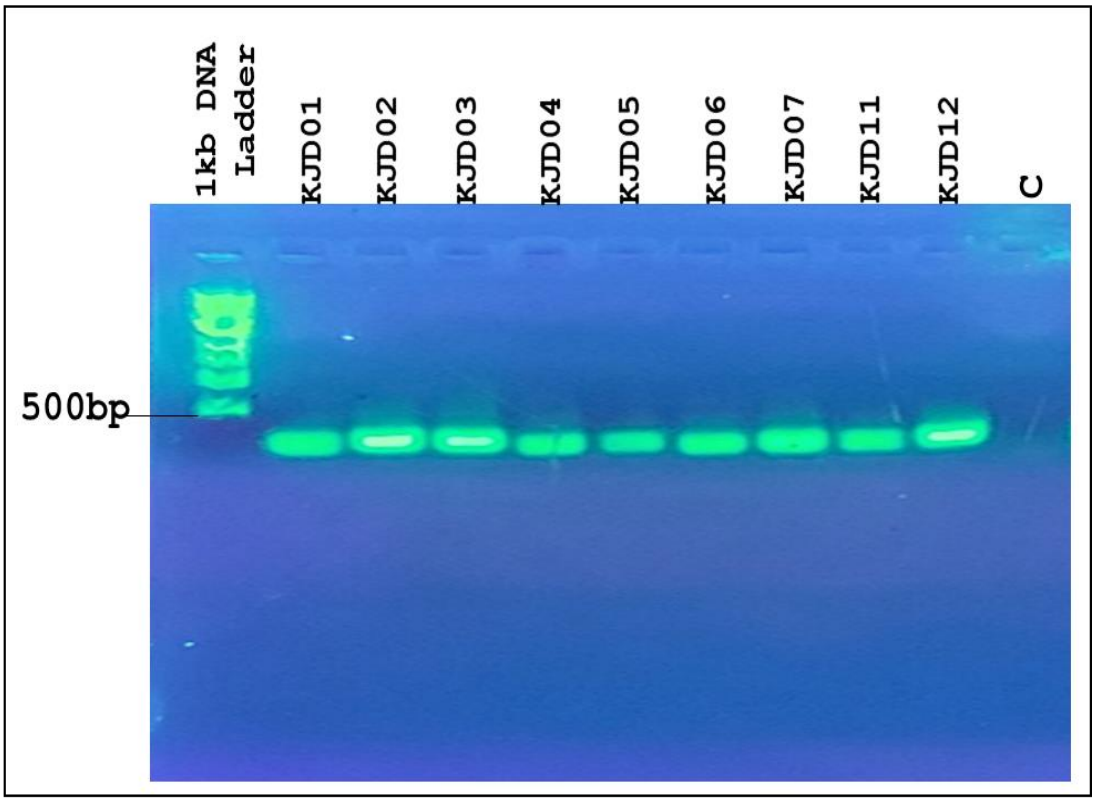

Figure 4.10: PCR products of cyt b region of A. solani isolates KJD01-12
C, Negative control

4.3.2 Phylogenetic analysis

During phylogenetic analysis, it was expected that isolates would cluster according to sensitivity levels to Azoxystrobin. However, this did not happen as many isolates clustered randomly (Fig. 4.11). This observation was attributed to random nucleotide substitutions in the DNA sequences which were not necessarily associated with sensitivity to azoxystrobin.

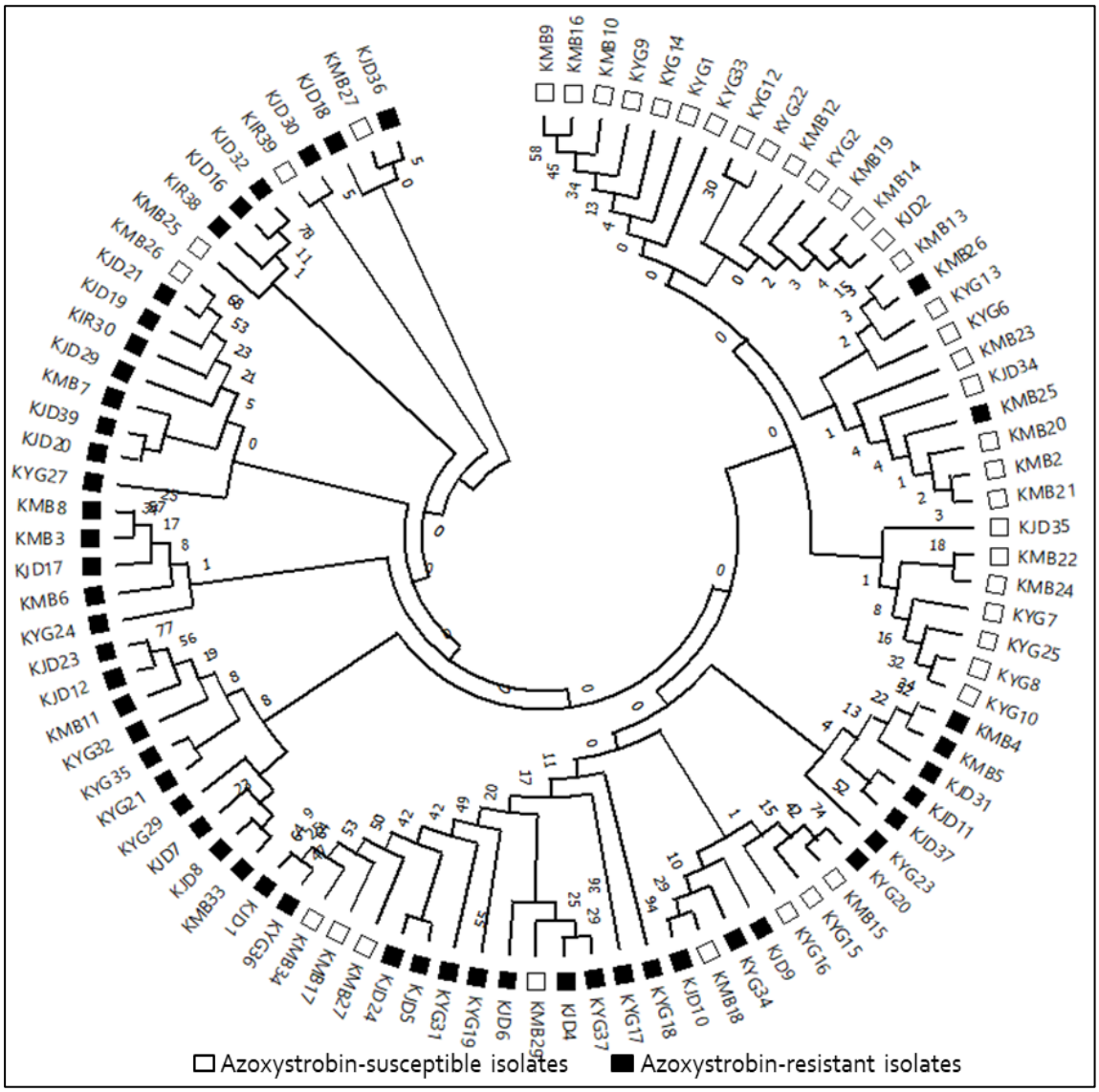

Figure 4.11: Phylogenetic analysis of Alternaria solani isolates based on alignment of their Cyt b sequences. The consensus tree was constructed by Neighbour joining method based on the Tamura 3 model in MEGA7. Bootstrap values calculated on 1000 replicates are indicated in the branches.

4.3.4 Multiple sequence alignment (MSA) of sequences

From *Blastx*, the obtained sequences were ~50 amino acids long, covering positions 110 -170 of the *Alternaria solani Cyt b* gene in most of the sequences. Positions where Azoxystrobin resistance mutations have been reported (129, 137 and 143) were present in all sequences hence they were sufficient for MSA. Therefore, the sequences were aligned against the wild *cyt b gene* sequence (Accession number ABB5714.1) obtained from the NCBI database. MSA revealed that F129L mutation (Leucine replacing Phenylalanine at amino position 129) was present in majority (75%) of isolates. F129L was detected in all azoxystrobin resistant isolates and 10 isolates with % MGI values slightly above 50% threshold (Fig. 4.12).

At positions 137 and 143, some isolates had certain random amino acid substitutions (for example Serine replacing Glycine) but these were somewhat random and their presence did not correlate significantly with Azoxystrobin sensitivity. Other mutations for example G137R (Arginine substituting Glycine at amino acid position 137) and G143A (Alanine substituting Glycine at position 143) that have been associated with Azoxystrobin resistance in other tomato growing areas were not present in the analyzed sequences.

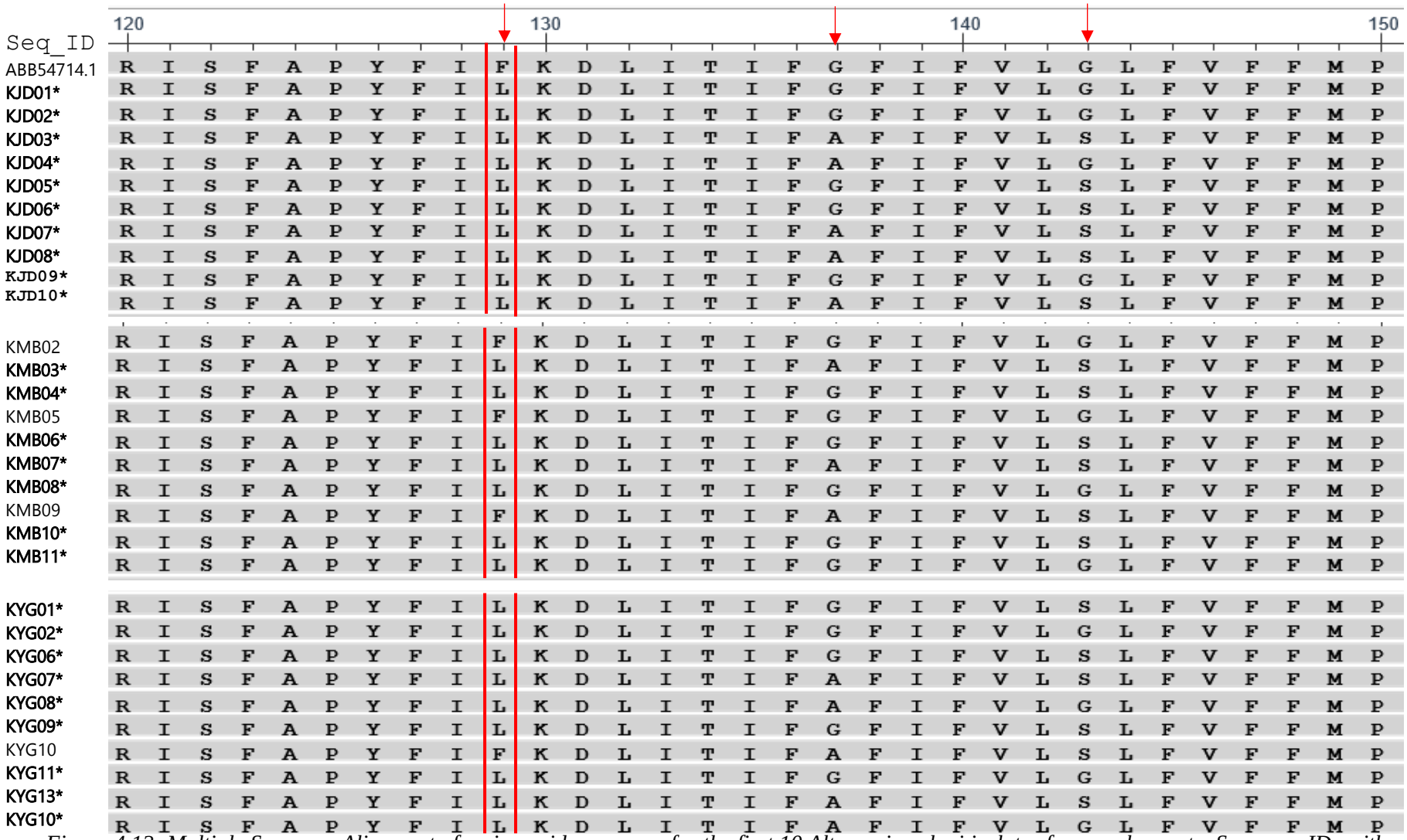

Figure 4.12: Multiple Sequence Alignment of amino acid sequences for the first 10 Alternaria solani isolates from each county. Sequence IDs with asterick (*) indicate isolates with F129L mutation. ABB5714.1 –Reference A. solani sequence from NCBI database. Isolate IDs KYG-Kirinyaga, KMB-Kiambu, KJD-Kajiado

4.3.3 Effect of F129L mutation on mycelial growth and sensitivity of isolates to Azoxystrobin

Isolates were sorted by colony diameter, % mycelial growth inhibition and amino acid present at position 129. Seventy-two isolates (or 75% of all) had F129L mutation while 24 lacked it. All Azoxystrobin resistant isolates and 10 others with near-threshold percent mycelial growth inhibition had the F129L mutation. One-way ANOVA revealed that F129L mutants were significantly less sensitive to Azoxystrobin (lower % MGI values) than wild isolates at all azoxystrobin concentrations (Table 4.10).

In control plates (no fungicide), F129L mutants grew significantly slower than the un-mutated isolates. However, this was not the case in Azoxystrobin amended plates, wherein the wild isolates grew significantly faster than F129L mutants (Table 4.10)

Table 4.10: Comparison of mean % mycelial growth inhibition and colony diameter for F129L mutants and non-mutated isolates at varying azoxystrobin concentrations

	Mean Percent Mycelial Growth inhibition		
Treatment*	F129L mutants (n=72)	Wild strains (n=24)	P value
0.25mg/ml	35.09 ± 1.14b	70.07±2.58a	0.011
0.375mg/ml	47.61 ± 1.29b	75.72 ±2.08a	0.014
0.5mg/ml	57.40± 1.38b	80.15±1.61a	0.047
	Mean colony diameter(mm)		
0mg/ml (Control)	44.48±1.99b	69.5 ±1.17a	0.039
0.25mg/ml	56.16 ± 0.86a	37.66±1.81b	0.011
0.375mg/ml	34.74±1.52a	28.37±1.14b	0.041
0.5mg/ml	27.39±1.74a	18.22±0.94b	0.033

**Means followed by similar letters in rows are not significantly different. Tukey's Honestly Significant Difference test at $P \leq 0.05$*

4.3.4 Spatial distribution of Azoxystrobin resistant isolates and F129L mutation by county

Kajiado had the highest number of isolates with F129L mutation (30 or 96.8%), followed by Kirinyaga (25 or 71%) and lastly Kiambu with 14 (47%) (Figure 4.13).

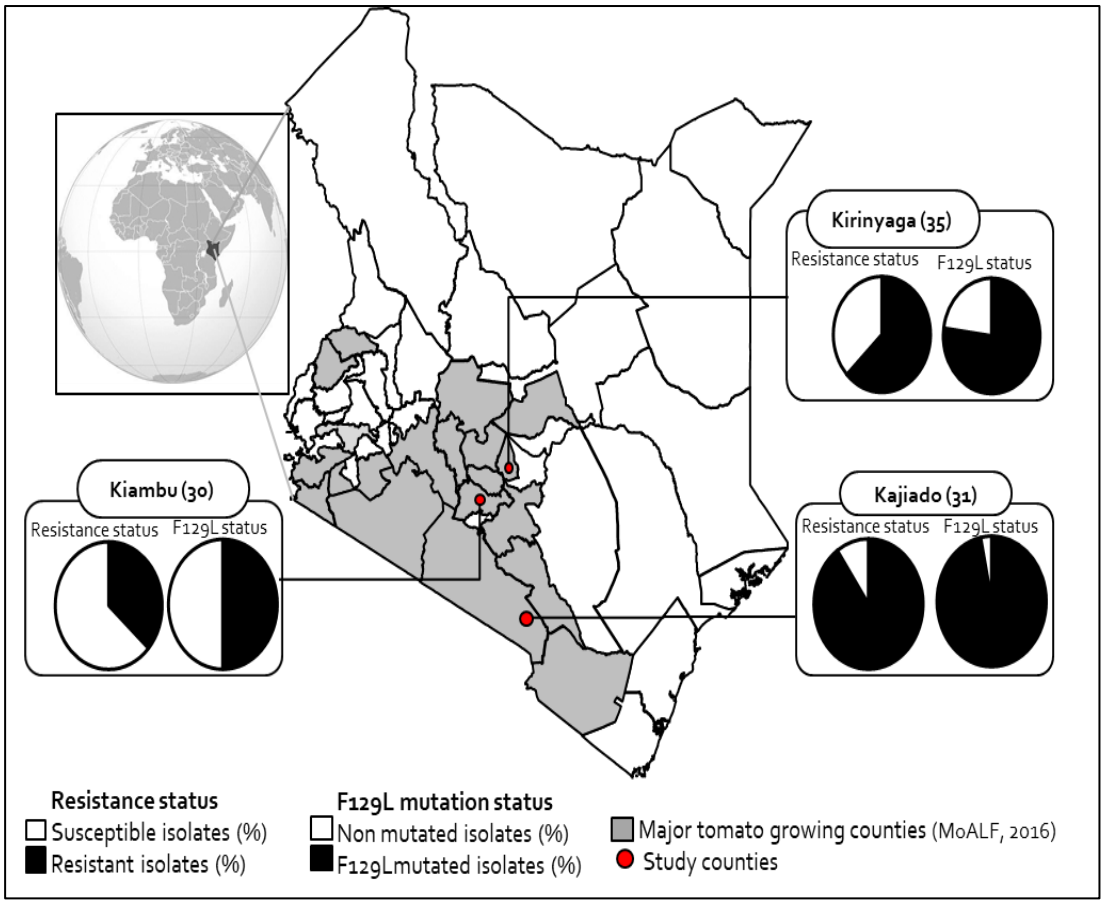

Figure 4.13: Map of Kenya showing distribution of Azoxystrobin resistant isolates and F129L mutants in the studied counties. The number of Alternaria solani isolates characterized per county is indicated in parentheses after county name.

➢

CHAPTER 5: DISCUSSION

5.1 Occurrence, importance and management practices for Early blight

5.1.1 Demographic characteristics of tomato farmers

The results revealed that males constituted the majority of tomato farmers across the three counties under study. This is consistent with findings from related studies done in Kenya (Ochilo, 2019; Nguetti *et al.,* 2018; Mwangi *et al.*, 2015), Tanzania (Angelina, 2014) and in Nigeria (Usman and Bakari, 2013). The male dominance has been attributed to the fact that more males than females tend to own and control the use of key production factors (such as land and capital) in most communities in Kenya (Ochilo, 2019; Barasa *et al.,* 2019; Mwangi *et al.,* 2015) and Africa (Anang *et al.,* 2013; Usman and Bakari, 2013). Therefore, since tomato production is a capital-intensive process, it was not surprising, that more males than females were involved in tomato growing in all the three studied counties.

Majority of the tomato farmers interviewed were aged between 31-50 years. This is consistent with findings from previous studies involving tomato farmers in Kenya (Barasa, *et al.,* 2019; Angelina, 2014; Nguetti *et al.,* 2018). The finding however contrasts with Mwangi *et al.* (2015) and Anang *et al.* (2013), who reported dominance of a younger age group (21-40 years). The age group 31-50 years has been described as one where people tend to have more access to important factors of production like land and capital. The fact that all of them had attained some level of formal education is important since this has been associated with better understanding of aspects of disease control at farm level

(Barasa *et al.,* 2019; Awan *et al.,* 2012). However, the findings from this study did not show significant correlation between the level of education and early blight control, for example, with regard to conformity to manufacturer's recommended spray dosages. This therefore underscores a need for regular training of farmers on up-to-date EB management strategies regardless of farmers' education level.

5.1.2 Characteristics of tomato fields in studied counties

The findings showed that respondents owned small farm sizes ranging from 1.0ha in Kiambu to 2.3 ha in Kajiado. These land ownership patterns can be attributed to the high population density in the surveyed counties (KNBS, 2019). According to Mwangi *et al.* (2015), land size limits application of important disease management practices for example crop rotation and fallowing. It was therefore not surprising that tomato was grown for up to 3 times per year (without rotations) on majority of the fields. Considering that an average tomato season takes ~ 4 months, it means that there was always a tomato crop in the fields and/or neighboring ones throughout the year. Once fungicide-resistant *A. solani* individuals evolve in such fields, they can multiply quickly from inocula transferred across successive seasons (Abuley *et al.,* 2019; Fry, 2007). This is further complicated by the fact that tomato was grown as a monocrop on most of the fields, hence there were no non-host barriers that would impede the spread of fungicide-resistant strains.

5.1.3 Occurrence and importance of early blight

The high yield loss associated with EB in Kirinyaga County can be attributed to the humid conditions experienced there in most times of the year that favour the disease (Runno-Paurson *et al.,* 2015, Upadhyay *et al.,* 2019; Kemmitt, 2002). In Kajiado (a warmer area), the fact that fields were irrigated all year round (in combination with the warm climate) could have contributed to a high EB severity (Mantecón, 2007) and consequently, high yield losses reported by farmers. In Kiambu, the low yield loss was attributed to dominance of greenhouse tomato production in the county, which has been linked with low severity of EB (Gullino *et al.,* 2020; Hanan *et al.,* 1978).

5.1.4 Management practices employed against Early blight

This study established that farmers in all the studied counties were relying on synthetic fungicides as the main method for EB control, confirming previous reports (Mwangi *et al.,* 2015; Nyankanga *et al.,* 2004). More to that, Early blight-resistant tomato varieties were not yet available in surveyed areas; regardless of the yield losses, EB was causing in the fields. It is therefore important for tomato breeders in Kenya, to consider incorporating EB resistance traits in the accessions being developed which will provide a viable control alternative, now that declines in fungicide efficacy are being experienced.

The fact that cultural practices with potential to reduce EB severity were evidenced on most of the fields (even if only a few farmers could associate these with disease control) provides a promising strategy to supplement control of fungicide-resistant strains in the fields. For example, majority of farmers had

planted certified, pathogen-free seed/seedlings, most fields had been weeded and fertilizers were being applied on most of the fields. According to FRAC (2021), such practices reduce the agronomic risk for resistance development and establishment. Hence these should be promoted in Kenya.

The active compounds in most commonly used fungicide products against EB represented only six chemical groups and six modes of action. This is a low number of chemical groups used when compared to those registered for EB control in the country (PCPB, 2019). This could be attributed to the fact that advice on fungicide use was obtained most from fellow farmers and agrovet shops, hence only the locally availed and/or well-known fungicides were the ones being applied. With *A. solani*'s proven ability to develop cross resistance across fungicide classes (Avenot *et al.,* 2016; Chowdhary *et al.,* 2013; Karaoglanidis and Thanassoulopoulos, 2003), the narrow diversity of modes of action among the fungicides used presented a high resistance risk for the pathogen and could complicate the management of resistant strains in future. Sensitizing farmers about the need to increase the diversity of fungicides applied and rotating them regularly may help to address this challenge.

Most farmers indicated that they were not adhering to manufacturer's recommendations on fungicide dosages and spray intervals. This may be attributed to the fact that most farmers had adopted a quantity driven approach (not quality driven) as evidenced by their choices on cultivars grown, number of cropping cycles per year among others. As reported by Udimal *et al.* (2022),

such farmers tend to hold the belief that reduction in pesticide application leads to yield reduction hence pay less attention to recommended pesticide use practices. As postulated in other studies (Jørgensen *et al.,* 2017; Ishii, 2006), this tendency among farmers becomes worse whenever they experience declines in efficacy of pesticides and increase in severity of the targeted pest/pathogen.

The higher spray dosages in Kajiado can be attributed to high EB prevalence in the county, favored in part by the warm climate and also the fact that farmers could access fungicides more cheaply in neighboring Tanzania. Over dosage of fungicides has been shown to favor establishment of fungicide-resistant individuals among pathogens (Brent and Hollomon, 2007; Genet *et al.,* 2006; Kable and Jeffery, 1980). In Kiambu, the long spray intervals are explained by the low disease intensity under greenhouse production.

Without alternative control options, there is a potential for fungicide resistant *A. solani* strains to multiply quickly in such fields and overwhelm tomato production. Coupled with wide deviations from the recommended spray frequencies for most fungicides, this presents a high risk for resistance development.

5.1.5 Farmers' perceptions on declining efficacy of fungicides

Majority of farmers interviewed had observed declines in performance of at least one fungicide, and could attribute this to resistance development by the pathogen. However, they were not employing any resistance management

strategies. This lack of attention by farmers on resistance management can be explained by their ignorance of anti-resistance strategies and increases the risk since they continue with the same practices that promote resistance. Strategies for example alternating available fungicide products, if incorporated in an integrated EB management programme can delay/slow down fungicide resistance (Hobbelen *et al.,* 2013; LaMondia, 2001), making EB control more efficient and sustainable.

Most fungicides reported to have declining efficacy contained active compounds with single site mode of action such as strobilurins and azoles, which is coherent with literature (Odilbekov *et al.*, 2019; He *et al.,* 2019; Rosenzweig *et al.,* 2008). Resistance develops faster for single-site fungicides since they target only one gene/stage in the fungal biochemical pathway (FRAC 2021). This means that even mutation of a single nucleotide is enough to modify the target site, making it difficult for the fungicide to effectively suppress pathogen populations.

5.2 Characterization of *Alternaria solani* isolates by cultural and morphological features, and fungicide sensitivity

5.2.1 Cultural and morphological features

The observed cultural features in isolated *Alternaria solani* matched with those reported in previous studies (Nikam *et al.,* 2015; Naik *et al.*, 2010). Isolates were highly diverse in terms of colony diameter, color and zonation patterns. However, although they are useful for initial screening, cultural features may be

affected by other factors for example culture media used and incubation temperature, and this limits their utility.

The recorded morphological features satisfactorily identified the isolates as *Alternaria solani* as has been described in previous studies (Nagrale *et al.*, 2013; Ramjegathesh and Ebenezar, 2012; Simmons 2007). However, in contrast with Loganathan *et al.* (2016), the finding in this study is that, the measured conidia and beak lengths did not vary significantly among the isolates and so it would be difficult to use them for characterization of *Alternaria solani* by counties of origin in Kenya.

5.2.3 Sensitivity of *A. solani* isolates to Azoxystrobin and Difenoconazole

5.2.3.1 Sensitivity of *A. solani* isolates to Difenoconazole (representing triazoles)

The results in the present study demonstrate that there was wide variation in sensitivity of *Alternaria solani* isolates from the three counties to Difenoconazole. Even though all isolates were susceptible to Difenoconazole, a significant number (10.4%) had low sensitivity (% MGI values between 50-60%) at discriminatory dosage. This suggests that resistance mechanisms against Difenoconazole (and/or other triazoles) could be already developing in the *Alternaria solani* populations. Regulation of triazole use through adherence to recommended doses and spray intervals would therefore be important at this early stage, to slow down the process of resistance development (FRAC, 2021; Jørgensen, 2015). Further studies should also be carried out specifically to analyze the *cyp51* gene (the triazole target gene) for occurrence and/or

prevalence of any mutations that have been associated with difenoconazole resistance in *Alternaria* populations in other areas like China (Sun *et al.*, 2021).

The fact that no resistant isolates were detected at the 50% MGI threshold, may be attributed to limitations of the methodology used. Therefore, I recommend that future sensitivity assays with azoles on *Alternaria solani* in Kenya should consider alternative techniques such as % germination inhibition (for spores) and regression of % MGI to determine EC50 values for isolates (requires at least 5 fungicide concentrations).

5.2.3.2 Sensitivity of *A. solani* isolates to Azoxystrobin (representing strobilurins)

The data in the present study indicates that resistance has developed in *Alternaria solani* populations from the three counties to Azoxystrobin with 75% of the isolates resistant at the manufacturer's recommended dosage. Isolates with MGI values as low as 33% could be identified at double the manufacturer's recommended dosage. This means that it would be difficult to achieve desired early blight control by spraying Azoxystrobin-containing fungicides even if farmers were to increase the dosage. According to PCPB (2021), Azoxystrobin fungicides have been continually used in Kenya for 23 years, which is much more time than it has taken to develop resistance in other countries. For example, in the USA, resistance developed just 2 years after Azoxystrobin introduction (Pasche *et al.,* 2005).

The finding that *A. solani* isolates have lost sensitivity to Azoxystrobin *in-vitro* is consistent with farmers' observation of declining efficacy of this class of

fungicides at EB control. There is, therefore, a need to strictly regulate the usage of Azoxystrobin (and other strobilurins) for EB control and if possible, suspend them especially in an area like Kajiado where 28 out of the sampled 31 isolates were resistant.

5.3. Occurrence and spatial distribution of mutations associated with in *Alternaria solani* resistance to fungicides

5.3.1 Occurance of resistance-associated mutations

Through sequence analysis of the *cyt b* gene in all isolates, it was confirmed that the F129L mutation (Leucine substituting Phenylalanine at amino acid position 129) was present in the *Alternaria solani* populations in the three counties showing resistance to Azoxystrobin. This is the first report of azoxystrobin resistance-associated mutations in any plant pathogen in Kenya. However, similar findings have been reported in other tomato growing countries e.g Germany (Leiminger *et al.,* 2016), Sweden (Odilbekov *et al.,* 2019) and USA (Pasche *et al.,* 2005).

Pasche *et al.* (2005) reported that F129L mutations cause meager losses in Azoxystrobin sensitivity. However, in contrast, in the current study this mutation was detected in highly resistant isolates with MGI values as low as 22.5%. This finding, when combined with the observation that some isolates with MGI values in the range of 50-65% had F129L mutation, while other lacked the mutation suggests that other resistance mechanisms could be contributing to Azoxystrobin resistance in Kenya's *A. solani* populations. Future studies may therefore explore other causes of fungicide resistance such as transporter-mediated

fungicide effluxes as reported by Andrade *et al.* (2000) and Roohparvar *et al.* (2007).

5.3.2 Fitness costs for F129L mutants

The results in this study indicate that wild *A. solani* isolates grew significantly faster in vitro than F129L mutated ones in absence of the fungicide. This indicates that F129L mutants are less fit than wild isolates in absence of Azoxystrobin. Hence, it is likely that if Azoxystrobin fungicides were suspended for some time, the wild Azoxystrobin susceptible isolates would re-establish faster than mutated ones, as has been demonstrated in post-harvest fungi by Bradshaw *et al.*, (2021). However, the length of such a waiting time for *Alternaria solani* remains to be determined and verified experimentally.

5.3.3 Spatial distribution of F129L mutations in study counties

Data in this study indicates that Kajiado county had the highest proportion of F129L mutated isolates at 96.8%. This finding is coherent with fungicide use practices in the area. the survey (Section 4.1.8), it was established that farmers in Kajiado were applying the highest doses of most fungicides (Azoxystrobin inclusive), which could be causing faster selection of F129L mutated isolates, causing them to dominate in the county's *Alternaria solani* populations.

The finding that Kiambu county had a relatively lower proportion (47%) of F129L mutated isolates may be attributed to the relatively lower Azoxystrobin

dosages applied, considering the low EB yield loss perception since tomato production in the county is majorly done in greenhouses.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Survey data indicated that Early blight (EB) was among the most prevalent and highest ranked (in terms of yield loss caused) biotic constraints to tomato production in the surveyed counties and was managed mainly by application of synthetic fungicides; however, the fungicide use practices did not take into account the risk of *Alternaria solani* developing resistance to the fungicides.

2. *Alternaria solani* isolates from the studied counties did not differ significantly by cultural and morphological features. However, they were highly variable in sensitivity to Azoxystrobin and Difenoconazole fungicides with majority of them resistant to Azoxystrobin.

3. The F129L mutation (Amino acid Leucine substituting Phenylalanine at position 129) in the *cytochrome b* gene, was detected in all azoxystrobin resistant *A. solani* isolates; this mutation was unevenly distributed across the study counties; Kajiado county had the highest proportion of F129L mutated isolates followed by Kirinyaga and lastly Kiambu.

6.2 Recommendations

1. Stakeholders in pesticide regulation should ensure that farmers are adequately trained on resistance management strategies for example fungicide rotations, adherence to manufacturers' recommendations on dosage and spray intervals and fallows between cropping cycles.

2. Use of azoxystrobin and other strobilurin based fungicides should be suspended in the main tomato production regions to allow re-establishment of susceptible individuals in *Alternaria solani* populations.

3. Future studies should investigate other possible fungicide resistance mechanisms in *Alternaria* for example fungicide effluxes and target gene overexpressions.

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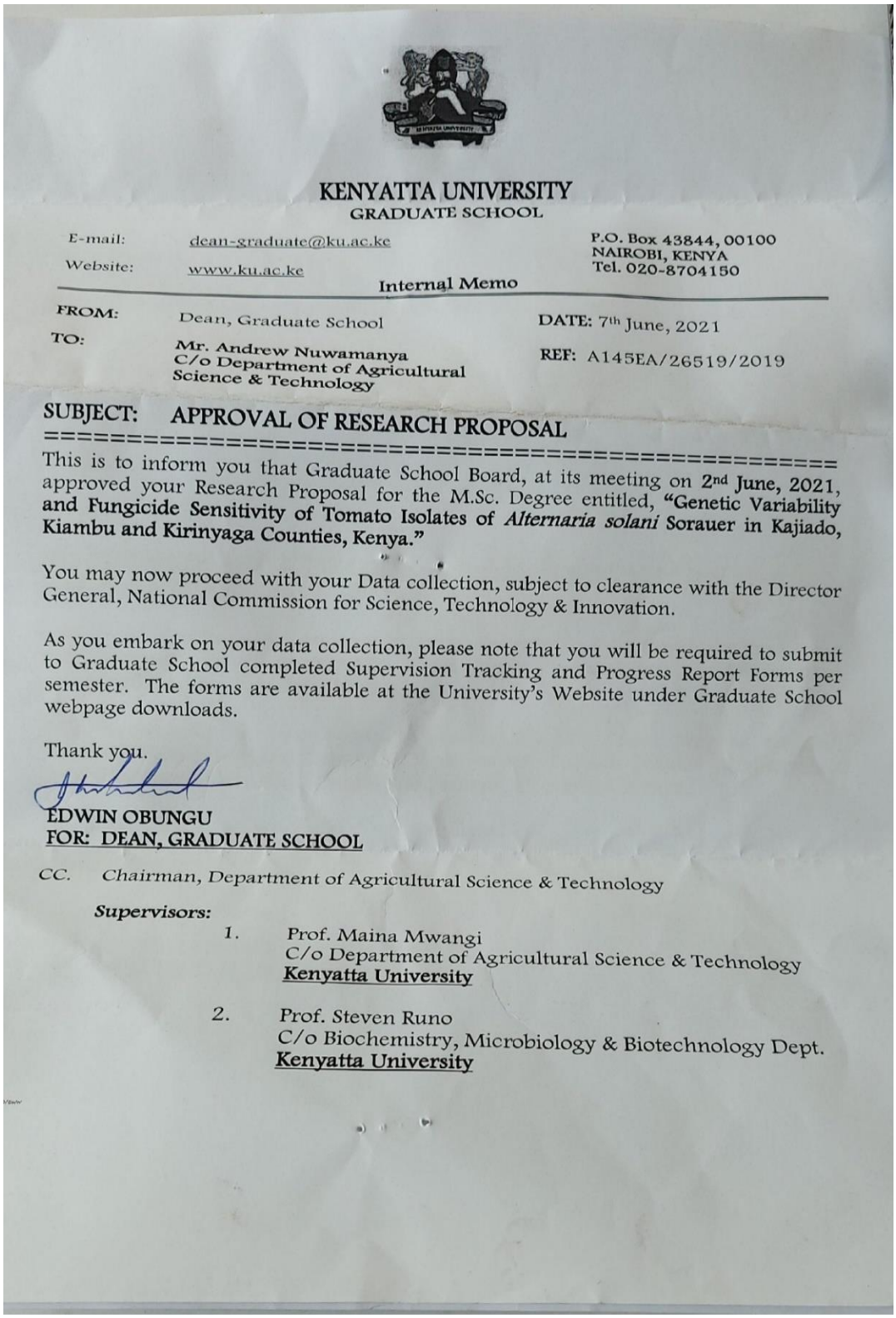
APPENDICES

Appendix 1: Graduate School Proposal Approval



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NAIROBI, KENYA
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Internal Memo

FROM: Dean, Graduate School
DATE: 7th June, 2021

TO: Mr. Andrew Nuwamanya
C/o Department of Agricultural
Science & Technology
REF: A145EA/26519/2019

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting on 2nd June, 2021, approved your Research Proposal for the M.Sc. Degree entitled, **"Genetic Variability and Fungicide Sensitivity of Tomato Isolates of *Alternaria solani* Sorauer in Kajiado, Kiambu and Kirinyaga Counties, Kenya."**

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.

Thank you.

EDWIN OBUNGU
FOR: DEAN, GRADUATE SCHOOL

CC. Chairman, Department of Agricultural Science & Technology

Supervisors:

1. Prof. Maina Mwangi
C/o Department of Agricultural Science & Technology
Kenyatta University

2. Prof. Steven Runo
C/o Biochemistry, Microbiology & Biotechnology Dept.
Kenyatta University

Appendix 2: NACOSTI Research Authorisation

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Ref No: **839404**

Date of Issue: **28/October/2021**

RESEARCH LICENSE

This is to Certify that Mr.. Andrew M. Nuwamanya of Kenyatta University, has been licensed to conduct research in Kajiado, Kiambu, Kirinyaga on the topic: Genetic variability and fungicide sensitivity of tomato isolates of Alternaria solani Sorauer from Kajiado, Kiambu and Kirinyaga counties, Kenya for the period ending : 28/October/2022.

License No: **NACOSTI/P/21/13512**

839404

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix 3: Survey questionnaire

I am a student at Kenyatta University carrying out a survey to evaluate farmers' perceptions and knowledge on Early blight in tomato farms of Kajiado, Kiambu and Kirinyaga counties. You have been selected randomly as one of the tomato farmers in this area. Information collected will only be used for academic and research purposes and will be confidential. In case of any question(s) concerning the study, Contact Andrew Nuwamanya (0721381978) or email: *amnuwamanya@gmail.com*. I now request for your permission to begin the interview.

Starting time:……

End time: ……..

County	Subcounty	Location	Village	Farm ID.	GPS coordinates of the field

SECTION A: HOUSEHOLD CHARACTERISTICS

1 (a) Name of the respondent

……....

(b)Mobile No

……

(c) Gender of the respondent: Male ☐ Female ☐

(d) Age of the respondent (in years)

…….

(e) What is the highest educational level attained by the respondent? None ☐ Primary ☐ Secondary ☐ Tertiary ☐

SECTION B: TOMATO PRODUCTION PRACTICES

2 (a) How long have you been growing tomatoes?

.....

(b)What is your total farm size?

…….....

(c) What is the size of your farm under tomato production (acres)? ……

3 (a) Which varieties of tomato do you grow on your farm? Rank them in order of preference:

Rank	Variety	Area size	Why chosen over others

(b)(i) How many seasons do you grow tomato per year? …….

(ii) In which months of the year do you have tomato in your field?.....……..

(c) What is the approximate tomato yield on your farm ? ……..

(d) (i) Which tomato production system do you use? Greenhouse…… Open field ……. Both….

(ii) If open field, what do you rely on as a source of water for your crop? Rainfall ☐ Irrigation ☐ Both ☐

(iii) If you irrigate, which irrigation method do you use on your farm? Drip ☐ Furrow ☐ Sprinkler ☐ Others (Specify) ……

4. (a)(i) Which crops do you intercrop your tomatoes with?

…….. ……. ……

(ii) Which crops do you grow adjacent to your tomato field?

…… ……..

(b) How long do you wait to grow tomatoes again on your land after a season?

…….

SECTION C: FARMERS' KNOWLEDGE ON TOMATO PESTS AND DISEASES IN THEIR FARMS

5. Which diseases and pests have been affecting your tomatoes? Rank them in terms of magnitude of yield loss caused Very high (> 40%)…… High (21-39%)…….Medium (11-20%)…… Low (1-10%)

(a) Diseases

Rank	Disease	Estimated yield loss
1		
2		
3		
4		
5		

(b) Pests

Rank	Pest	Estimated yield loss
1		
2		
3		
4		
5		

SECTION C: MEASURES TAKEN BY TOMATO FARMERS TO MANAGE TOMATO DISEASES

6. Which management practices do you use against diseases on your tomato farm? Rate their effectiveness by ticking where applicable

Management practice (Grouped into the following categories)	**Effectiveness**			
	High	**Moderate**	**Low**	**Not effective**
Cultural, e.g. early planting, intercropping, weeding, certified seed, mulching, crop rotation				
Physical, e.g. hot water treatment of seeds,				

destruction of diseased crops				
Biological e.g use of antagonistic bacteria and fungi				
Chemical control through use of fungicides				
Integrating measures? specify				
Others (Specify)				

7. (a) For chemical control of early blights, when do you start applying the fungicide(s)?

……

(b) What informs your decision to start applying the fungicide(s)?

……

……

(c) For each of the fungicide(s) used, State the trade name, dosage, frequency of spray and effectiveness rating (*H= Highly effective M= Moderately effective L= Less effective)*

Fungicide trade name	Dosage	Frequency of spray: X1 /week; X1/ 2 weeks; X1 / month; X1/2months	Rate the Effectiveness of the fungicide H/ M/ L

8. (a) Do you strictly follow dosage recommendations according to fungicide labels?

Yes ☐ No ☐

(b) If No, State why not?

……

……

9. (a) Do you interchange between different fungicide chemical products in a tomato growing season?

Yes ☐ No ☐

(b) (i) If Yes, is it always or some times?

Always ☐ Sometimes ☐

(ii) State the reason(s) why you interchange between different chemical products in a growing season?
……
(c) (i) Have you observed any decrease in effectiveness of any fungicide over the years in your tomato growing? Yes ☐ No ☐
(ii) If yes, state the brand names of fungicides whose effectiveness has decreased over the year(s).
……
……
……
(iii) Which factors do you think are responsible for the declining efficacy of some Early blight fungicides?
……
……
……

10. What is your source of information regarding tomato production and pest management practices?
Radio…….. Tv…… Mobile phones…… Extension officer…….. Newspaper……..Others (Specify) ……..

END*

Appendix 4: Detailed Cultural characteristics of *Alternaria solani* isolates collected from Kajiado, Kiambu and Kirinyaga counties, January-April 2021

Isolate ID	Colony diameter	Colony colour	Pigmentation (Reverse plate)	Nature of Margin	Zonation
KYG01	74	Green	Greenish brown	Regular	-
KYG02	74.5	Creamish white	Greenish brown	Regular	-
KYG04	71.5	Creamish white	Greenish brown	Regular	+
KYG06	85	Creamish white	Creamish white	Irregular	+
KYG07	85	Greenish white	Creamish white	Irregular	-
KYG08	85	Creamish white	Creamish white	Irregular	-
KYG09	67.5	Creamish white	Creamish white	Irregular	+
KYG10	70.5	Green	Creamish white	Irregular	+
KYG11	78	Green	Creamish white	Irregular	+
KYG13	85	Greenish white	Creamish white	Irregular	+
KYG14	72	Green	Creamish white	Irregular	-
KYG15	85	Creamish white	Creamish white	Irregular	-
KYG16	66	Greenish white	Greenish brown	Regular	+
KYG17	68	Creamish white	Greenish brown	Irregular	+
KYG18	85	Greenish white	Greenish brown	Irregular	+
KYG19	73.5	Creamish white	Greenish brown	Irregular	+
KYG20	69.5	Green	Greenish brown	Irregular	-
KYG21	71.5	Greenish white	Creamish white	Irregular	+
KYG22	74	Creamish white	Greenish brown	Irregular	-
KYG23	67	Green	Creamish white	Irregular	-
KYG24	85	Greenish white	Creamish white	Irregular	+
KYG25	67	Greenish white	Creamish white	Irregular	-
KYG27	85	Greenish white	Creamish white	Irregular	-
KYG28	85	Greenish white	Creamish white	Irregular	-
KYG29	69	Greenish white	Greenish brown	Regular	-
KYG30	78	Greenish white	Greenish brown	Irregular	-
KYG31	72.5	Greenish white	Brown	Irregular	-
KYG32	75.5	Greenish white	Creamish white	Irregular	-
KYG33	85	Creamish white	Creamish white	Irregular	+
KYG34	85	Creamish white	Creamish white	Irregular	-
KYG35	68	Creamish white	Creamish white	Irregular	-
KYG36	73.5	Creamish white	Creamish white	Irregular	-
KYG37	72	Creamish white	Greenish brown	Irregular	-
KYG38	83	Creamish white	Creamish white	Irregular	+
KYG39	69	Creamish white	Greenish brown	Irregular	-
KMB02	74	Creamish white	Creamish white	Regular	+
KMB03	75.5	Creamish white	Creamish white	Regular	+

KMB04	86.5	Green	Greenish brown	Irregular	+
KMB05	77	Green	Greenish brown	Regular	+
KMB06	75	Greenish white	Greenish brown	Regular	-
KMB07	71.5	Greenish white	Greenish brown	Regular	-
KMB08	69	Greenish white	Brown	Regular	+
KMB09	68.5	Greenish white	Brown	Regular	-
KMB10	66	Greenish white	Creamish white	Regular	+
KMB11	66.5	Green	Creamish white	Regular	+
KMB12	67.5	Green	Creamish white	Regular	+
KMB13	85	Green	Greenish brown	Irregular	+
KMB14	85	Greenish white	Greenish brown	Irregular	+
KMB15	85	Greenish white	Greenish brown	Irregular	+
KMB16	83	Greenish white	Greenish brown	Irregular	+
KMB17	70.5	Greenish white	Greenish brown	Regular	+
KMB18	70	Greenish white	Greenish brown	Irregular	+
KMB19	71	Green	Greenish brown	Regular	-
KMB20	77	Green	Greenish brown	Irregular	+
KMB21	82	Green	Creamish white	Irregular	+
KMB22	74.5	Green	Creamish white	Regular	+
KMB23	72.5	Grey	Greenish brown	Regular	+
KMB24	66.5	Greenish white	Creamish white	Regular	+
KMB25	75	Green	Creamish white	Irregular	+
KMB26	76	Green	Creamish white	Regular	+
KMB27	82.5	Green	Greenish brown	Regular	+
KMB29	84	Green	Greenish brown	Irregular	+
KMB31	76	Green	Greenish brown	Regular	+
KMB33	76	Greenish white	Greenish brown	Regular	+
KMB34	76	Greenish white	Greenish brown	Regular	+
KJD01	73.5	Creamish white	Brown	Irregular	+
KJD02	74.5	Green	Greenish brown	Irregular	+
KJD04	70	Creamish white	Creamish white	Irregular	+
KJD05	69.5	Creamish white	Creamish white	Irregular	+
KJD06	72	Creamish white	Creamish white	Irregular	+
KJD07	74	Green	Creamish white	Irregular	+
KJD08	84	Greenish white	Creamish white	Irregular	+
KJD09	78.5	Green	Creamish white	Irregular	+
KJD10	76	Green	Creamish white	Regular	+
KJD11	68.5	Green	Creamish white	Irregular	-
KJD12	79.5	Green	Greenish brown	Irregular	-
KJD16	70	Green	Greenish brown	Irregular	+
KJD17	76	Greenish white	Brown	Irregular	-
KJD18	65.5	Green	Creamish white	Irregular	-

KJD19	75	Greenish white	Creamish white	Irregular	-
KJD20	73.5	Green	Creamish white	Irregular	-
KJD21	78.5	Green	Creamish white	Irregular	-
KJD23	72	Green	Greenish brown	Irregular	+
KJD24	79	Greenish white	Greenish brown	Irregular	+
KJD25	80	Greenish white	Greenish brown	Irregular	+
KJD26	85	Green	Creamish white	Regular	+
KJD27	82.5	Green	Creamish white	Regular	+
KJD29	74.5	Green	Greenish brown	Regular	+
KJD30	68	Greenish white	Greenish brown	Irregular	-
KJD31	69	Greenish white	Greenish brown	Irregular	+
KJD32	70	Greenish white	Greenish white	Regular	-
KJD33	82.5	Greenish white	Greenish brown	Irregular	+
KJD34	77	Greenish white	Creamish white	Regular	-
KJD35	75	Greenish white	Creamish white	Regular	-
KJD36	73	Greenish white	Creamish white	Irregular	+
KJD37	73.5	Greenish white	Greenish brown	Regular	+

Isolates KYG01-39-Kirinyaga, KBT02-34-Kiambu, KJD01-48-Kajiado. + Concentric zonation –No zonation. All features were recorded in three replicates of each isolate.

Appendix 5: Detailed table of Morphological characteristics of *Alternaria solani* isolates collected from Kajiado, Kiambu and Kirinyaga counties, January-April 2021

Isolate ID	Conidia shape	Conidia dimensions		No. of conidia septa		Beak length*/µm	Beak septa (No.
		L*/µm	W*/µm	Tra.	Long.		
KYG01	Ellipsoidal	18.5	8.3	3-4	0	-	-
KYG02	Obclavate	18.8	8.8	3-4	1-2	-	-
KYG04	Ellipsoidal	22.3	8.2	3-5	0-1	19.7	2-3
KYG06	Ellipsoidal	14.7	8.6	2-4	0	12.3	1-2
KYG07	Ellipsoidal	24.7	10.1	2-3	0-1	9.61	1-2
KYG08	Obclavate	17.4	8.9	2-3	0-1	9.4	1-2
KYG09	Obclavate	25.7	11.6	3-4	0-1	7.4	0-1
KYG10	Obvoid	27.1	13.4	3-4	1-2	-	-
KYG11	Obclavate	15.5	13.5	3-4	0-1	6.9	1-2
KYG13	Obclavate	21.5	8.8	2-4	0-1	6.7	0-1
KYG14	Ellipsoidal	26.6	12.9	3-4	0-1	9.0	1-2
KYG15	Obclavate	21.5	11.7	3-4	1-2	11.8	1-2
KYG16	Obclavate	19.7	10.7	2-3	1-2	-	-
KYG17	Obclavate	15.9	8.6	3-4	1-2	6.4	1-2
KYG18	Ellipsoidal	17.2	10.8	3-4	0	7.1	
KYG19	Obclavate	16.6	9.6	2-3	0	6.2	1-2
KYG20	Ellipsoidal	19.8	9.5	2-3	0	-	-
KYG21	Ellipsoidal	23.1	11.3	2-4	1-2	-	-
KYG22	Obvoid	21.5	8.0	3-4	0	8.9	1-2
KYG23	Ellipsoidal	17.4	13.2	2-3	0	11.9	1-2
KYG24	Ellipsoidal	19.4	12.8	3-4	1-2	15.4	2-3
KYG25	Ellipsoidal	22.5	13.9	2-3	0	14.7	2-3
KYG27	Ellipsoidal	18.9	11.8	3-5	0	11.2	2-3
KYG28	Obclavate	18.8	9.4	3-4	0	-	-
KYG29	Obclavate	15.4	12.3	2-5	1	5.1	1-2
KYG30	Ellipsoidal	25.7	8.8	2-4	0	-	-

KYG31	Obclavate	25.2	13.7	3-4	1-2	-	-
KYG32	Ellipsoidal	17.9	12.4	2-3	1-2	16.6	3-4
KYG33	Ellipsoidal	25.0	11.1	3-4	0	11.4	2-3
KYG34	Obclavate	15.1	13.5	3-4	0	-	-
KYG35	Obclavate	22.1	11.7	3-4	1-2	-	-
KYG36	Obclavate	17.2	10.3	3-5	1-2	-	-
KYG37	Obclavate	21.9	10.4	3-4	1-2	7.8	2-3
KYG38	Ellipsoidal	16.3	8.6	3-4	0	-	-
KYG39	Obclavate	17.8	13.4	2-4	1-2	8.7	2-3
KMB02	Ellipsoidal	19.2	10.5	2-4	1-2	19.6	1-3
KMB03	Ellipsoidal	18.0	13.1	3-4	0	-	-
KMB04	Ellipsoidal	22.2	9.1	3-5	1-2	12.8	3-4
KMB05	Obvoid	10.3	8.9	3-4	1-2	19.5	2-3
KMB06	Obvoid	13.1	12.3	3-5	1-2	13.0	2-4
KMB07	Ellipsoidal	18.1	10.6	2-4	2-3	11.1	1-2
KMB08	Ellipsoidal	17.2	13.1	3-4	0	-	-
KMB09	Obclavate	15.1	7.5	2-4	1-2	17.3	2-3
KMB10	Ellipsoidal	18.7	8.2	3-4	0	-	-
KMB11	Ellipsoidal	17.4	9.5	2-3	1-2	16.5	2-3
KMB12	Ellipsoidal	16.1	8.8	2-4	0-1	16.8	2-3
KMB13	Ellipsoidal	18.7	7.5	3-5	1-2	10.9	2-4
KMB14	Ellipsoidal	15.4	13.2	3-4	0	-	-
KMB15	Obclavate	16.2	12.6	3-4	0-1	10.2	1-2
KMB16	Obclavate	15.4	10.8	3-4	0	-	-
KMB17	Ellipsoidal	16.8	9.7	2-4	1-2	16.5	2-4
KMB18	Obvoid	18.9	11.3	3-5	0	-	-
KMB19	Ellipsoidal	18.1	11.5	2-4	0	13.2	2-4
KMB20	Ellipsoidal	18.1	7.4	3-5	0-1	13.8	2-4
KMB21	Ellipsoidal	15.2	7.8	3-5	0-1	-	-
KMB22	Ellipsoidal	17.8	9.3	2-4	0	-	-
KMB23	Obvoid	15.8	12.0	2-4	0-1	-	-
KMB24	Obclavate	18.8	11.1	3-4	1-2	10.1	1-2

KMB25	Ellipsoidal	18.7	12.1	3-5	0	-	-
KMB26	Obclavate	13.2	11.4	3-4	0-1	8.8	2-4
KMB27	Ellipsoidal	16.5	9.5	3-4	0-1	18.9	2-4
KMB29	Ellipsoidal	16.8	8.0	2-4	0-1	-	-
KMB31	Obvoid	16.7	7.7	2-4	1-2	14.3	2-4
KMB33	Ellipsoidal	15.9	11.5	3-5	1-3	10.0	2-4
KMB34	Obclavate	19.3	12.0	2-4	0-1	-	-
KJD01	Obclavate	17.5	12.8	2-4	0-1	11.6	3-5
KJD02	Ellipsoidal	15.4	11.3	3-5	1-2	-	-
KJD04	Obclavate	15.7	14.1	3-5	1-2	7.4	3-5
KJD05	Ellipsoidal	19.0	12.0	3-4	0-2	9.6	4-5
KJD06	Obvoid	19.7	14.4	3-4	0-1	-	-
KJD07	Obclavate	18.4	10.8	2-4	0-1	-	-
KJD08	Ellipsoidal	20.8	11.0	3-4	0-1	-	-
KJD09	Obclavate	16.0	11.1	2-4	1-2	6.8	2-5
KJD10	Ellipsoidal	15.4	12.9	3-5	0	9.4	3-4
KJD11	Obclavate	18.0	15.1	3-5	0	-	-
KJD12	Obvoid	21.4	13.6	4-5	2	11.7	2-5
KJD16	Ellipsoidal	14.4	13.0	3-5	2	7.2	3-4
KJD17	Obclavate	21.8	14.1	4-5	2	-	-
KJD18	Ellipsoidal	20.3	16.1	4-6	2	-	-
KJD19	Obclavate	16.9	16.7	4-6	1	7.8	2-3
KJD20	Ellipsoidal	19.7	16.0	4-5	1	9.7	2-3
KJD21	Obvoid	15.5	14.4	3-5	1	9.6	2-3
KJD23	Ellipsoidal	20.3	11.6	4-5	1	-	-
KJD24	Ellipsoidal	15.4	15.1	3-5	1	10.0	3-5
KJD25	Ellipsoidal	16.0	12.7	3-4	1	7.7	3-5
KJD26	Ellipsoidal	20.4	12.0	4-6	1	10.5	3-4
KJD27	Obclavate	17.3	12.6	3-6	1	-	-
KJD29	Obclavate	16.9	13.1	4-6	2	8.1	3-4
KJD30	Ellipsoidal	20.9	10.7	4-6	0	7.5	3-4
KJD31	Ellipsoidal	17.7	11.7	3-5	2	-	-

KJD32	Ellipsoidal	16.4	15.5	3-5	2	9.6	3-4
KJD33	Ellipsoidal	14.7	11.7	4-5	2	-	-
KJD34	Obclavate	14.3	10.4	3-5	1	-	-
KJD35	Ellipsoidal	21.3	12.8	2-4	2	10.1	2-4
KJD36	Obclavate	20.9	16.0	3-6	2	-	-
KJD37	Ellipsoidal	21.2	14.9	4-5	0	8.6	2-4

L-Length, W-Width Tran. –Transverse, Long.- Longitudinal

Isolates KYG01-39-Kirinyaga, KBT02-34-Kiambu, KJD01-48-Kajiado.*Means of three replicates

Appendix 6: Mean colony diameters for all isolates at different concentrations of Azoxystrobin (Azo) and Difenoconazole (Dif)

Isolate	Fungicide	0.25mg a.i /ml	0.375mg a.i/ml	0.5mg a.i/ml
KYG1	Azo	5 ± 0 bB	5 ± 0 bB	5 ± 0 bB
	Dif	14.3 ± 0.17 cA	11.7 ± 0.33 bA	8.2 ± 0.17 aA
KYG2	Azo	34 ± 0.29 cA	27.5 ± 0.29 bA	20.2 ± 0.44 aA
	Dif	14.2 ± 0.17 cB	11.2 ± 0.17 bB	9.3 ± 0.17 aB
KYG3	Azo	25.7 ± 0.44cA	19 ± 0.29bA	13.7 ± 0.17aA
	Dif	15.8 ± 0.44cB	13.3 ± 0.17bB	10.7 ± 0.17aB
KYG6	Azo	5 ± 0aA	5 ± 0aA	5 ± 0aA
	Dif	11.3 ± 0.17cB	10.2 ± 0.17bB	8.7 ± 0.17aB
KYG7	Azo	33.5 ± 0.29cA	17.3 ± 0.17bA	8.8 ± 0.17aA
	Dif	26 ± 0.29cB	11.8 ± 0.17bB	11.7 ± 0.6aB
KYG8	Azo	35.2 ± 0.6cB	22 ± 0.29bB	19.3 ± 0.6aB
	Dif	22.5 ± 0.29cA	12 ± 0.29bA	9.5 ± 0.29aA
KYG9	Azo	31 ± 0.58cB	24.8 ± 0.44bB	20 ± 0.29aB
	Dif	5 ± 0cA	5 ± 0bA	5 ± 0aA
KYG10	Azo	27.2 ± 0.6cB	25.3 ± 0.88bB	5 ± 0aB
	Dif	16.3 ± 0.17cA	13 ± 0.29bA	8.2 ± 0.17aA
KYG12	Azo	15.3 ± 0.44cB	10.2 ± 0.6bB	7.3 ± 0.44aB
	Dif	17.5 ± 0.76cA	16.7 ± 0.44bA	13.2 ± 0.44aA
KYG13	Azo	31.7 ± 0.88cA	31.5 ± 0.5bB	25.3 ± 0.88aB
	Dif	16.8 ± 0.17bB	13.8 ± 0.17aA	10.1 ± 6.21aA
KYG14	Azo	30.8 ± 0.17bA	23.3 ± 0.33aB	22.3 ± 0.33aB
	Dif	17.8 ± 0.17cB	15.5 ± 0.29bA	12.2 ± 0.17aA
KYG15	Azo	33.7 ± 0.33bA	25.5 ± 0.87bB	23.2 ± 0.44bB
	Dif	21 ± 0.29cB	13.5 ± 0.29bA	11.2 ± 0.17aA
KYG16	Azo	33.7 ± 0.6cA	28.8 ± 0.17bB	25.5 ± 0aB
	Dif	17.2 ± 0.17cB	14.2 ± 0.17bA	9.5 ± 0.29aA
KYG17	Azo	32.3 ± 0.44bA	32.7 ± 0.33bB	20.8 ± 0.17aB
	Dif	5.0 ± 0.0aB	5 ± 0.00aA	5.0 ± 0.00aA
KYG18	Azo	35.2 ± 0.44bA	34.2 ± 0.17bB	25.8 ± 0.44aB
	Dif	16.2 ± 0.17bB	12.2 ± 0.17aA	11.2 ± 0.17aA
KYG19	Azo	41 ± 0.5bA	34.7 ± 0.6aB	34.2 ± 0.44aB
	Dif	14.5 ± 0.29bB	10.8 ± 0.17aA	9.7 ± 0.17aA
KYG20	Azo	5 ± 0aA	5 ± 0aB	5 ± 0aB
	Dif	10.5 ± 0.29cB	9.3 ± 0.17bA	7.8 ± 0.17aA
KYG21	Azo	12.5 ± 0.29cA	10.2 ± 0.17bB	5 ± 0aB
	Dif	10.2 ± 0.17bB	9.3 ± 0.17bA	7.8 ± 0.17aA
KYG22	Azo	16.8 ± 0.17cA	10.2 ± 0.17bB	6.8 ± 0.17aB
	Dif	9.2 ± 0.17cB	7.7 ± 0.17bA	6.7 ± 0.17aA
KYG23	Azo	5 ± 0aA	5 ± 0aB	5 ± 0aB
	Dif	13.5 ± 0.29cB	10.3 ± 0.17bA	9.7 ± 0.17aA

KYG24	Azo	50.3 ± 0.31aA	45.6 ± 0.19aB	42.1 ± 0.32aB
	Dif	13.5 ± 0.29bB	10.7 ± 0.17aA	9.5 ± 0.29aA
KYG25	Azo	32.8 ± 0.6cA	22.2 ± 0.17bB	14.8 ± 0.17aB
	Dif	18.5 ± 0.29bB	11.7 ± 0.33aA	9.8 ± 0.44aA
KYG26	Azo	32.8 ± 0.17bA	30 ± 0.29bB	26.5 ± 0.29aB
	Dif	15.3 ± 0.17cB	11.8 ± 0.44bA	8.8 ± 0.17aA
KYG27	Azo	30.5 ± 0.5bA	28.2 ± 0.6bB	22.7 ± 0.88aB
	Dif	13.2 ± 0.44bB	11.3 ± 0.17abA	9.5 ± 0.5aA
KYG29	Azo	37.7 ± 0.88cA	31.5 ± 0.76bB	27.7 ± 0.6aB
	Dif	11.3 ± 0.17aB	9.3 ± 0.17aA	8.8 ± 0.17aA
KYG30	Azo	41.7 ± 0.6cA	28 ± 0.5bB	20.8 ± 0.44aB
	Dif	16.5 ± 0.29bB	10.5 ± 0.29aA	8.3 ± 0.17aA
KYG31	Azo	40 ± 0.58cA	31.3 ± 0.44bB	27.2 ± 0.44aB
	Dif	14.7 ± 0.17bB	10.5 ± 0.29aA	9.7 ± 0.17aA
KYG32	Azo	37.3 ± 0.33cA	33 ± 0.5bB	26.3 ± 0.33aB
	Dif	10.2 ± 0.17aB	9.3 ± 0.17aA	8.8 ± 0.17aA
KYG33	Azo	28.2 ± 0.44cA	25.2 ± 0.44bB	21.5 ± 0.29aB
	Dif	9.2 ± 0.17aB	8.3 ± 0.17aA	7.3 ± 0.33aA
KYG34	Azo	28.5 ± 0.29cA	14.8 ± 0.17bB	7.5 ± 0.29aB
	Dif	17 ± 0.29cB	13.2 ± 0.17bA	10.3 ± 0.33aA
KYG35	Azo	30.3 ± 0.33cA	19 ± 0.29bB	14.3 ± 0.33aB
	Dif	11.7 ± 0.17bB	9.2 ± 0.17aA	8.2 ± 0.17aA
KYG36	Azo	6.7 ± 0.17bA	6.2 ± 0.17aB	5.5 ± 0.29aB
	Dif	13.2 ± 0.17cB	9.3 ± 0.44abA	8.2 ± 0.17aA
KYG37	Azo	22.3 ± 0.44bA	20.2 ± 1.36abB	18 ± 0.29aB
	Dif	15.8 ± 0.17cB	12.7 ± 0.33bA	10 ± 0aA
KYG38	Azo	34 ± 0.58cA	30.3 ± 0.33bB	25 ± 0.29aB
	Dif	15.7 ± 0.33bB	12.2 ± 0.17aA	10.8 ± 0.17aA
KYG39	Azo	32.2 ± 0.44cA	23.7 ± 0.33bB	20.8 ± 0.44aB
	Dif	13.8 ± 0.44bB	10 ± 0.29aA	8.7 ± 0.33aA
KYG40	Azo	33.5 ± 0.5cA	27.5 ± 0.29bB	21.2 ± 0.17aB
	Dif	18.8 ± 0.17cB	15.2 ± 0.17bA	12.7 ± 0.33aA
KBT2	Azo	6.3 ± 0.17aA	6.3 ± 0.17aA	6.3 ± 0.17aA
	Dif	10 ± 0bB	7.8 ± 0.17aB	6.8 ± 0.17aB
KBT3	Azo	30 ± 0.29cA	26 ± 0.29bA	21.7 ± 0.17aA
	Dif	5 ± 0aB	5 ± 0aB	5 ± 0aB
KBT4	Azo	6.2 ± 0.17bA	7 ± 0bA	7.3 ± 0.17aA
	Dif	13 ± 0.29bB	11.2 ± 0.17bB	12.5 ± 0.29aB
KBT5	Azo	42.8 ± 0.44cA	28 ± 0.29bA	23.7 ± 0.17aA
	Dif	12.3 ± 0.17cB	11.3 ± 0.17bB	10.2 ± 0.17aB
KBT6	Azo	26.8 ± 0.17bA	20.7 ± 0.44aA	20.3 ± 0.17aA
	Dif	21.5 ± 0.29cB	20.3 ± 0.17bB	19 ± 0.29aB
KBT7	Azo	38.3 ± 0.33bA	30.3 ± 0.17aA	29.2 ± 0.44aA
	Dif	24.3 ± 0.33cB	20.5 ± 0.29bB	17.5 ± 0.29aB
KBT8	Azo	45.5 ± 0.29cA	38.2 ± 0.44bA	30.2 ± 0.44aA
	Dif	11.5 ± 0.29bB	10 ± 0.29abB	8.8 ± 0.44aB
KBT9	Azo	36.8 ± 0.44bA	31.5 ± 0.29bA	25.8 ± 0.6aA

	Dif	16.7 ± 0.17cB	14.3 ± 0.17bB	12.3 ± 0.17aB
KBT10	Azo	39.5 ± 0.29bA	23.8 ± 0.6aA	22.7 ± 1.45aA
	Dif	19.7 ± 0.33cB	13.8 ± 0.17bB	10 ± 0.29aB
KBT11	Azo	35.8 ± 1.36cA	27.8 ± 0.44bA	19.8 ± 0.44aA
	Dif	9.2 ± 0.17aB	7.7 ± 0.17aB	7 ± 0aB
KBT12	Azo	35.5 ± 0.29cA	25 ± 0.58bA	16.2 ± 0.44aA
	Dif	5 ± 0aB	5 ± 0aB	5 ± 0aB
KBT13	Azo	6.3 ± 0.44aA	6.5 ± 1.04aA	6.3 ± 0.73aA
	Dif	16.2 ± 0.17bB	13.7 ± 0.17abB	11.3 ± 0.33aB
KBT14	Azo	6.3 ± 0.73aA	5.3 ± 0.33aA	6.2 ± 0.6aA
	Dif	12.8 ± 0.17bB	10.8 ± 0.44aB	9.3 ± 0.17aB
KBT15	Azo	5.7 ± 0aA	5 ± 0aA	5 ± 0.33aA
	Dif	20.5 ± 0.29cB	15.2 ± 0.17bB	13.7 ± 0.17aB
KBT16	Azo	21.8 ± 0.44bA	5.5 ± 0.29aA	5 ± 0aA
	Dif	15 ± 0.29bB	9.2 ± 0.17aB	8 ± 0.29aB
KBT17	Azo	28.5 ± 0.29cA	22.3 ± 0.88bA	16 ± 1.15aA
	Dif	5 ± 0aB	5 ± 0aB	5 ± 0aB
KBT18	Azo	30.5 ± 0.76bA	25.5 ± 0.29aA	24.2 ± 0.44aA
	Dif	5 ± 0aB	5 ± 0aB	5 ± 0aB
KBT19	Azo	7.5 ± 0.29aA	5.7 ± 0.67aA	5.3 ± 0.33aA
	Dif	12 ± 0.29bB	10 ± 0.29abB	8.7 ± 0.17aB
KBT20	Azo	10.5 ± 0.76bA	10.3 ± 1.17bA	5.7 ± 0.67aA
	Dif	9 ± 0.29aB	9 ± 0.29aB	6.5 ± 0.29aB
KBT21	Azo	7.5 ± 0.29bA	5 ± 0aA	5 ± 0aA
	Dif	10.2 ± 0.17bB	7.8 ± 0.33aB	6.5 ± 0.29aB
KBT22	Azo	35.2 ± 0.44cA	30.7 ± 1.76bA	26.3 ± 0.88aA
	Dif	18.8 ± 0.17bB	16.2 ± 0.17bB	12 ± 0.29aB
KBT23	Azo	21.3 ± 0.88cA	14.7 ± 0.67bA	9.8 ± 0.44aA
	Dif	18.2 ± 0.17cB	14.2 ± 0.17bB	10.5 ± 0.29aB
KBT24	Azo	7.8 ± 0.44bA	5 ± 0aA	5 ± 0aA
	Dif	15.5 ± 0.29cB	10.2 ± 0.17bB	8.3 ± 0.17aB
KBT25	Azo	25 ± 0.58bA	20.7 ± 0.44aA	19.3 ± 0.17aA
	Dif	17.2 ± 0.17cB	12.3 ± 0.33bB	9 ± 0aB
KBT26	Azo	25.3 ± 0.33cA	18.2 ± 0.17bA	15.7 ± 0.88aA
	Dif	15.3 ± 0.17cB	12.7 ± 0.33bB	8.2 ± 0.17aB
KBT27	Azo	15 ± 0.58bA	13.7 ± 0.88bA	7.5 ± 0.29aA
	Dif	5 ± 0aB	5 ± 0aB	5 ± 0aB
KBT29	Azo	32.3 ± 0.44cA	28.3 ± 0.88bA	22.2 ± 0.17aA
	Dif	15.2 ± 0.17cB	13 ± 0.29bB	9 ± 0.29aB
KBT31	Azo	23.7 ± 0.44cA	20.2 ± 0.17bA	15.2 ± 0.17aA
	Dif	13.5 ± 0.29cB	11.2 ± 0.17bB	10.2 ± 0.17aB
KBT33	Azo	28.5 ± 0.29cA	26.5 ± 0.29bA	18.5 ± 0.29aA
	Dif	5 ± 0aB	5 ± 0aB	5 ± 0aB
KBT34	Azo	34.8 ± 0.17cA	25.8 ± 0.17bA	23.7 ± 0.33aA
	Dif	12.7 ± 0.33cB	10.8 ± 0.44bB	9.5 ± 0.29aB
KJD01	Azo	40.8 ± 0.44bA	30.2 ± 0.17cA	28.3 ± 0.17dA
	Dif	17.8 ± 0.17bB	15.5 ± 0.29bB	14.5 ± 0.29cB
KJD02	Azo	41.8 ± 0.17bA	30.5 ± 0.29bA	26.8 ± 0.17aA

	Dif	15 ± 0.76cB	12.3 ± 0.33bB	9.8 ± 0.17aB
KJD03	Azo	32 ± 0.29bA	23.2 ± 0.17aA	22.7 ± 0.33aA
	Dif	10.5 ± 0.29bB	9.3 ± 0.17abB	8 ± 0.29aB
KJD04	Azo	34 ± 0.29bA	33.7 ± 0.17bA	28.2 ± 0.17aA
	Dif	9.3 ± 0.17bB	8.2 ± 0.17aB	7.7 ± 0.17aB
KJD05	Azo	43.5 ± 0.29cA	40.3 ± 0.33bA	30.3 ± 0.17aA
	Dif	11.7 ± 0.17bB	10.7 ± 0.17bB	9 ± 0.29aB
KJD06	Azo	56.7 ± 0.44cA	31.3 ± 0.33bA	16 ± 0.29aA
	Dif	17.8 ± 0.17cB	13.5 ± 0.29bB	12.2 ± 0.17aB
KJD07	Azo	35.8 ± 0.17cA	30 ± 0.29bA	25.7 ± 0.17aA
	Dif	14.2 ± 0.17cB	12.3 ± 0.17bB	9.3 ± 0.17aB
KJD11	Azo	38.2 ± 0.44cA	28.8 ± 0.17bA	20.8 ± 0.17aA
	Dif	13.2 ± 0.17cB	11.7 ± 0.44bB	9.8 ± 0.17aB
KJD12	Azo	39 ± 0.29cA	34 ± 0.58bA	32.2 ± 0.17aA
	Dif	14.8 ± 0.17cB	10.2 ± 0.17bB	8.8 ± 0.17aB
KJD14	Azo	42 ± 0.29cA	40.2 ± 0.17bA	32.7 ± 0.17aA
	Dif	12.3 ± 0.33bB	11.5 ± 0.29bB	10.5 ± 0.29aB
KJD18	Azo	33.3 ± 0.17cA	27.8 ± 0.17bA	24.8 ± 0.17aA
	Dif	11.8 ± 0.17bB	9.2 ± 0.17aB	8.7 ± 0.17aB
KJD19	Azo	26 ± 0.58cA	23.7 ± 0.33bA	18.5 ± 0.29aA
	Dif	20.5 ± 0.29cB	17.8 ± 0.44bB	15.3 ± 0.17aB
KJD20	Azo	37.2 ± 0.17cA	34 ± 0.5bA	26.8 ± 0.44aA
	Dif	17.5 ± 0.29cB	12.8 ± 0.17bB	11 ± 0.29aB
KJD23	Azo	38.5 ± 0.58cA	35.5 ± 0.5bA	30.8 ± 0.44aA
	Dif	13.5 ± 0.29bB	9.7 ± 0.17aB	8.7 ± 0.17aB
KJD24	Azo	34 ± 0.29cA	27.8 ± 0.44bA	20.2 ± 0.17aA
	Dif	11 ± 0.29bB	10.7 ± 0.17bB	9.5 ± 0.29aB
KJD27	Azo	44.7 ± 0.88cA	32 ± 0.58bA	27.2 ± 0.44aA
	Dif	11.8 ± 0.17bB	8.2 ± 0.17aB	7.3 ± 0.33aB
KJD29	Azo	20 ± 0.58bA	18.2 ± 0.44bA	11.7 ± 0.17aA
	Dif	9.2 ± 0.17bB	7.3 ± 0.33abB	7.2 ± 0.17aB
KJD30	Azo	32.8 ± 0.6cA	28.2 ± 0.6bA	24.2 ± 0.44aA
	Dif	13.7 ± 0.33cB	12 ± 0.29bB	10.3 ± 0.33aB
KJD32	Azo	46 ± 0.29cA	30 ± 0.58bA	27.3 ± 0.33aA
	Dif	12.2 ± 0.44bB	10.8 ± 0.44bB	9.2 ± 0.17aB
KJD34	Azo	31.3 ± 0.33cA	28.3 ± 0.44bA	21.8 ± 0.44aA
	Dif	12.7 ± 0.33cB	10.7 ± 0.17bB	8.5 ± 0.29aB
KJD35	Azo	40.3 ± 0.33cA	36.3 ± 0.33bA	31.2 ± 0.44aA
	Dif	13.5 ± 0.29cB	10.8 ± 0.44bB	8.8 ± 0.44aB
KJD36	Azo	37.2 ± 0.17cA	30.8 ± 0.17bA	29.5 ± 0.29aA
	Dif	5 ± 0aB	5 ± 0aB	5 ± 0aB
KJD37	Azo	34.3 ± 0.44cA	30.5 ± 0.29bA	28 ± 0.29aA
	Dif	11.2 ± 0.17cB	9.2 ± 0.17bB	6.8 ± 0.17aB
KJD38	Azo	19.2 ± 0.17cA	15.2 ± 0.17bA	11.2 ± 0.17aA
	Dif	11.8 ± 0.17cB	9.7 ± 0.44bB	8.2 ± 0.17aB
KJD41	Azo	30.5 ± 0.29cA	25.7 ± 0.17bA	22.2 ± 0.17aA
	Dif	12.2 ± 0.17bB	11.3 ± 0.17abB	10.3 ± 0.33aB
KJD43	Azo	47.7 ± 0.17cA	37.2 ± 0.33bA	22.2 ± 0.17aA

	Dif	15.2 ± 0.17cB	13 ± 0.29bB	11.2 ± 0.17aB
KJD44	Azo	38.3 ± 0.17cA	31.8 ± 0.17bA	26.5 ± 0.29aA
	Dif	11.3 ± 0.33cB	8.8 ± 0.17bB	7.8 ± 0.17aB
KJD45	Azo	46.3 ± 0.17bA	42.3 ± 0.17aA	41.3 ± 0.17aA
	Dif	13.2 ± 0.17cB	10.8 ± 0.17bB	9.5 ± 0.29aB
KJD46	Azo	35 ± 0.29cA	26 ± 0.29bA	22.7 ± 0.17aA
	Dif	10.2 ± 0.17bB	8.2 ± 0.17aB	7.5 ± 0.29aB
KJD47	Azo	38.8 ± 0.17cA	30.5 ± 0.29bA	26.8 ± 0.17aA
	Dif	14 ± 0.29bB	11.7 ± 0.44aB	10.8 ± 0.17aB
KJD48	Azo	46.8 ± 0.17cA	41.8 ± 0.17bA	21.8 ± 0.17aA
	Dif	11.5 ± 0.29bB	9.2 ± 0.17aB	8.5 ± 0.29aB

Azo-Azoxystrobin, Dif-Difenoconazole, Isolates KYG01-39-Kirinyaga, KBT02-34-Kiambu, KJD01-48-Kajiado. All diameters are means of three replicates

Means with similar lower case letters across rows are not significantly different at p=0.05

Means with similar upper case letters across columns are not significantly different at p=0.05

Appendix 7: *Alternaria solani* DNA sequences generated from this study

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG1
caggaatcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatcttat
tacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaactat
gttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaatgataccttctg

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG2
ggatcaggaatcctttagtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatctt
attacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaacta
tgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaatgataacct

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG4
gctggatcaggaatcctttagtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagat
cttattacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaa
ctatgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaatgatac

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG6
gatcaggaatcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatctt
attacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaacta
tgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaatgaatacct

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG7
ggaatcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatcttattaca
atatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaactatgttgt
ggcaaaccctatgcaaactcctgcagctatcgtgccagaatgaataccttctgaca

> *Alternaria solani* (Mwea east, Kirinyaga County) KYG8
atcaggaatcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatcttat
tacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaactatg
ttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaatgataccttc

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG9
ggttataggaaatacgaaagagctcttttgtcctctttaatatcaaagagctacacaatatttgcatactattcg
tacctctttatttgtgcttttatgcctaatgtattaggagatagtgaaaactatgttgtggcaaaccctatgcaaa
ctcctgcagctatcgtgccagagaataccttctga

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG10
tgcttggatcaggaatccttataggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaa
agatcttattacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtga
aaactatgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaatgata

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG11

gctggatcaggaatcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaag
atcttattacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaa
actatgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaatgatacct

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG13
caggaatcctttaggggatcaggaaactacgaaagaaatcttttgccccttatttcatatttaaagatcttatta
caaaatttgcatttatatttgattatctttatttgtgtgcgttatgcctaatggattaggagatagtgaaaactatgt
tgtgggaaaccctatgcaaactcctgcagctatcgtgccagaatgataccttctggggg

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG14
ggatcaggaatcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatct
tattacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaact
atgttgtggcaaaccctatgcaaactcctgcagctat

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG15
gatcaggaatcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatctt
attacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaacta
tgttgtggcaaaccctatgcaaactcctgcagcta

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG16
atcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatcttattacaatat
ttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaactatgttgtggca
aaccctatgcaaactcctgcagctatcgtgcca

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG17
atcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatcttattacaatat
ttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaactatgttgtggca
aaccctatgcaaactcctgcagctatcgtgcca

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG18
gggaaatccttttaggtgtatcagggaaactacgaaagaatatcttttgctccttatttcatatttaaagatctta
ttacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaactat
gttgtggcaaaccctatgcaaactcctgcagctatcgt

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG19
tgctggatcaggaatcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaag
atcttattacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaa
actatgttgtggcaaaccctatgcaaactcctgcagcta

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG20
gacaggaatccttttcggtgtatcaggaaactacgaaagaatatctttatgctccttatttcatatttaaagatct
tattacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaact
atgttgtggcaaaccctatgcaaactcctgcagctatcgtg

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG21
ggaatcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatcttattaca
atatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaactatgttgt
ggcaaaccctatgcaaactcctgcagctatcgtgccagatgat

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG22
ggatcaggaatcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatct
tattacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaact
atgttgtggcaaaccctatgcaaactcctgcagctatcgtgcc

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG23
gatcaggaagcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatctt
attacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaacta
tgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaatgat

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG24
atcaggaatcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatcttat
tacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaactatg
ttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaagaatacctt

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG25
gatcaggaatcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatctt
attacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaacta
tgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaatga

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG27
aggaatccttttcgtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatcttattac
aatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaactatgttgt
ggcaaaccctatgcaaactcctgcagctatcgtgccagaagata

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG28
gatcaggaatcctttagtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatcttat
tacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaactatg
ttgtggcaaaccctatgcaaactcctgcagctatcgtgccagatga

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG29
cctttctaggtgtatcaggaaactacgaaagaatatacttatatcgctccttatttcatatttaaagatcttattac
aatattctgcattctatattctgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaactatg
ttgtggcaaaccctatgcaaactcctgcagctatcgtgccagattga

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG30
ggaatccttttcggtgtatcaggaaacttacgaaagaatatcttttgctcccttatttcatatttaaagatcttatt
acaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaactatgt
tgtggcaaaccctatgcaaactcctgcagctatcgtgccagaatgaa

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG31
atcaggaatcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatcttat
tacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaactatg
ttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaatga

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG32
gatcaggaatccttttaggtgtatcaggaaacttacgaaagaatatcttttgctcccttatttcatatttaaagat
cttattacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaa
ctatgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaaga

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG33
acaggaatccttttcggtgtatcaggaaactacgaaagaatatctttatgctccttatttcatatttaaagatctt
attacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaacta
tgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagatgaatac

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG34
gaggatcaagggaaatcctctctaggtgtatcaggaaactacgaaagaatatacttcttgctccttatttcata
tttaaagatcttattacaatattctgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagat
agtgaaaactatgttgtggcaaaccctatgcaaactcctgcagctatcgtgcc

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG35
ggatcaaggaaatcctttataggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaa
gatcttattacaatatttagcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtga
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> *Alternaria solani* (Mwea east, Kirinyaga County), KYG36
tagatcaggaatccttttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagat
cttattacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaa
ctatgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaagata

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG37
gctggatcaggaatccttttagtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagat
cttattacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaa
ctatgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaatga

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG38
gctggatcaggaatccttttagtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagat
cttattacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaa
ctatgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaatgataa

> *Alternaria solani* (Mwea east, Kirinyaga County), KYG39

gatcaggtaatcctttaggtgtatcaggtaactacgatagaatatcttttgctccttatttcatatttaaagatctt
attacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaacta
tgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagatgataccttctg

> *Alternaria solani* (Kabete, Kiambu County), KMB2
gctggatcaggaatcctttagtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagat
cttattacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaa
ctatgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaatactg

> *Alternaria solani* (Kabete, Kiambu County), KMB3
gctggatcaggaatcctttagtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagat
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ctatgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaata

> *Alternaria solani* (Kabete, Kiambu County), KMB4
tggatcaggaatcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatc
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atgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaatgataacc

> *Alternaria solani* (Kabete, Kiambu County), KMB5
tggatcaggaatcctttagtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatctt
attacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaacta
tgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagatgataacct

> *Alternaria solani* (Kabete, Kiambu County), KMB6
atcaggtaatcctttctaggtgtatcaggtaactacgatagatatcttttgctccttatttcatatttaaagatctta
ttacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaactat
gttgtggcaaaccctatgcaaactcctgcagctatcgtgccagatgataacctt

> *Alternaria solani* (Kabete, Kiambu County), KMB7
tggatcaggaatcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatc
ttattacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaact
atgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaatgatacct

> *Alternaria solani* (Kabete, Kiambu County), KMB8
tggatcaggaaatcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagat
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ctatgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaatgatacc

> *Alternaria solani* (Kabete, Kiambu County), KMB9
aggaatccttttcggtgtatcaggaaacttacgaaagaatatcttttgctcccttatttccatatttaaagatctt
attacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaacta
tgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagatgaataacct

> *Alternaria solani* (Kabete, Kiambu County), KMB10
ggatcaggaatcctttaggtgtatcaggaaactacgaaagaatatcttttgctccttatttcatatttaaagatct
tattacaatatttgcatttatatttgtattatctttatttgtgttctttatgcctaatgtattaggagatagtgaaaact
atgttgtggcaaaccctatgcaaactcctgcagctatcgtgccagaatgataacct

> *Alternaria solani* (Kabete, Kiambu County), KMB11
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> *Alternaria solani* (Kabete, Kiambu County), KMB12
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> *Alternaria solani* (Kabete, Kiambu County), KMB13
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> *Alternaria solani* (Kabete, Kiambu County), KMB14
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> *Alternaria solani* (Kabete, Kiambu County), KMB15
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> *Alternaria solani* (Kabete, Kiambu County), KMB16
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> *Alternaria solani* (Kabete, Kiambu County), KMB17
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> *Alternaria solani* (Kabete, Kiambu County), KMB18

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> *Alternaria solani* (Kabete, Kiambu County), KMB19
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> *Alternaria solani* (Kabete, Kiambu County), KMB20
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> *Alternaria solani* (Kabete, Kiambu County), KMB21
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> *Alternaria solani* (Kabete, Kiambu County), KMB22
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> *Alternaria solani* (Kabete, Kiambu County), KMB23
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> *Alternaria solani* (Kabete, Kiambu County), KMB24
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> *Alternaria solani* (Kabete, Kiambu County), KMB25
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> *Alternaria solani* (Kabete, Kiambu County), KMB26
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> *Alternaria solani* (Kabete, Kiambu County), KMB27
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(f1)

> *Alternaria solani* (Kabete, Kiambu County), KMB29
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> *Alternaria solani* (Kabete, Kiambu County), KMB31
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> *Alternaria solani* (Kabete, Kiambu County), KMB33
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> *Alternaria solani* (Kabete, Kiambu County), KMB34
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD1
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD2
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD4
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD5

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> *Alternaria solani* (Loitokitok, Kajiado County), KJD6
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD7
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD8
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD9
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD10
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD11
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD12
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD16
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD17
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD18
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD19
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD20
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD21
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD23
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD24
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD25
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD26
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD27
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD29
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD30
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD31
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD32
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD33
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD34
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD35
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD36
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> *Alternaria solani* (Loitokitok, Kajiado County), KJD37
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