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Detection and management of phytophthora infestans on tomato in Iringa and Arusha regions, Tanzania

Ndala, Rachel I.

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DETECTION AND MANAGEMENT OF *Phytophthora infestans* ON TOMATO IN IRINGA AND ARUSHA REGIONS, TANZANIA

Rachel I. Ndala

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

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ABSTRACT

A study was conducted in Iringa and Arusha regions of Tanzania to determine the status of the of Late blight disease (LBD) on tomato and effect of synthetic fungicides on the disease. Leaf samples with symptoms of the LBD were collected from three farms per village in each region, and transported to the Nelson Mandela African Institution of Science and Technology (NM-AIST) laboratory for detection of the LBD-causing pathogen(s). In addition, field trials were set in each region to find out the efficacy of chemical fungicides on LBD control. Moreover, extracts from *Plectranthus barbatus*, *Sphaeranthus suaveolens*, *Lantana camara* and *Tephrosia vogelii* were tested *in-vitro* for their effects on the LBD-causing Phytophthora. The results showed that, the LBD was present in the study locations. In addition, despite the application of different synthetic fungicides on tomato, the percentage LBD incidences and severities were higher ranging from 42.1, 52.60 and 49.56, 39.39 in Iringa and Arusha regions, respectively. Such results implied that synthetic fungicides used by farmers in the study area do not fully control the LBD. The results also showed that there was significant difference ($p \leq 0.001$) between the effects of different plant extracts against the LBD. The crude extracts from *P. barbatus*, *S. suaveolens* and *L. camara* exhibited growth inhibition of *P. infestans* (*in-vitro*) almost similar with synthetic fungicide used as positive control. This study therefore, proposes *P. barbatus*, *S. suaveolens* and *L. camara* as candidates for further studies in developing alternative bio-fungicides against *P. infestans* in Tanzania.

DECLARATION

I, Rachel I. Ndala do here declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concomitantly submitted for degree award in any other institution.

Signature ……. Date ……..

Rachel I. Ndala

The above declaration is confirmed by

Signature …….. Date ……

Dr. Ernest R. Mbega (Principal supervisor)

Signature …….. Date ……

Prof. Patrick A. Ndakidemi (Co-supervisor)

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CERTIFICATION

The undersigned certify that, they have read the dissertation titled, "Detection and Management of *Phytophthora infestans* on tomato in Iringa and Arusha regions, Tanzania" and recommend for examination in fulfilment for the requirements for the degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

Signature …….. Date ……

Dr. Ernest R. Mbega (Principal supervisor)

Signature …….. Date ……

Prof. Patrick A. Ndakidemi (Co-supervisor)

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DEDICATION

This dissertation is dedicated to my beloved husband, Mr. Willy Mshambo

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LIST OF ABBREVEATION AND SYMBOLS

<	Less than
%	Percentage
^{O}C	Degree centigrade
µl	microliter(s)
ANOVA	Analysis of variance
cm	Centimetre
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetraacetic acid
g	Gram
HCl	Hydrogen Chloride
LSD	Least Significant Difference
Ml	Milligram
Masl	Metres above sea level
NaOCl	Sodium hypochlorite
PCR	Polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
SC	Suspension Concentrate

CHAPTER ONE

INTRODUCTION

1.1 Background information

Tomato (*Lycopersicon esculentum* L) is one of major vegetable crops grown for both nutritional and economical values worldwide (Wachira *et al.*, 2014). Nutritionally, tomatoes are a major source of Lycopene, a powerful anti-oxidant, and vitamins C, beta-carotene (vitamin A), biotin (Vitamin B), vitamin K, vitamin B6 (Pyridoxine), niacin (vitamin B3), vitamin E and mineral nutrients mainly potassium, copper, manganese and phosphorus (Bhowmik *et al.*, 2012).

In tropical Africa, tomato is a major source of income due to its growth cycles that is most varieties of tomato have a shorter maturity period and can be harvested more than four times a year (Lima *et al.*, 2004). Despite its economic importance, tomato is susceptible to a wide range of diseases such as bacterial wilt (*Ralstonia solanacearum*) (Aloyce *et al.*, 2017), verticillium wilt (*Verticillium dahliae*), early blight (*Altanaria solanii*), and late blight (*Phytophthora infestans*) among others (Foolad *et al.*, 2008; Kamoun, 2007). Of these diseases, the late blight disease caused by *Phytophthora infestans* (Mont.) de Bary is the most significant and economically important disease causing over 50% loss in the tropical Africa (Fontem *et al.*, 2005).

The late blight-causing pathogen also causes massive destruction in others crops in the solanacea family such as potato, eggplant, pear melon and tree tomato (Nowicki *et al.*, 2013). The disease causes loses in terms of reduced yield, poor quality of fruits and diminished storability (Nowicki *et al.*, 2012). The late blight disease-causing-pathogen is seriously deadly to tomato due to its biology (sexual and asexual reproduction), host range (more than 20 hosts), dispersal mechanism and persistence for a long period of time in the soil (Agrios, 2005). Also, late blight disease is constantly re-emerging in every growing season and where it occurs, the pathogen spreads very rapidly within the leaves and fruits of the infected plants in the field, and consequently, spreading out to the entire field causing economic loss and often total destruction of the crops in the field (Agrios, 2005). Furthermore, the pathogen has ability to produce both asexually and sexually (Fig. 1) and has different kinds of strains which allow faster development in the field (Nowicki *et al.*, 2012). The sexual and asexual

life cycles make the infection mechanisms of *P. infestans* very successful in the tropical environments and wide host range of plants (Nowicki *et al.*, 2012).

In Tanzania, the average tomato yield is 17.5 tons/ha which is far below the global average yield of 33.6 tons/ha (Aloyce *et al.*, 2017). One of the difficult challenges, in tomato production in the country has been the detection of late blight disease. Use of conventional methods for detection of the pathogens associated with the disease from complex disease symptoms are time consuming and expensive and take a long time to correctly identify them. With advancement in molecular technologies in studying pathogen systems, it appears obviously and convincingly that Polymerase chain reaction (PCR)- based method are more suitable and accurate in the detection of plant pathogens (Aloyce *et al.*, 2017).

Disease management involving cultural, biological and chemical control have been recommended but the disease is still challenging due to re-emergence of *P. infestans* with its enhanced ability to develop more virulent isolates through sexual recombination (Agrios, 2005). In addition, some of commonly used approaches such chemical control including using synthetic fungicides such as metalaxyl spray are ineffective to new strains of the oomycete produced as recombinants of fertilization (Axel *et al.*, 2012). The fungicides are expensive for most poor farmers to afford and they are challenged by development of resistance by the different *P. infestans* strains and also they have detrimental effects to human health and the environment. In addition, variation in pathogen strains further complicates not only management options but also how to correctly identify them (Abawi and Widmer, 2000). Therefore, options to use for alternative botanical extracts with pesticidal properties are currently promoted. For instance, plants such as *Vetiveria zizanoides, Cupressus bethamii* have been reported to inhibit sporangial germination and disease progression of *P. infestans* (Goufo *et al.*, 2010).

Therefore, this study in addition to using Polymerase Chain Reaction (PCR) - based approach for accurate detection of the pathogen, aimed at developing a sustainable management strategy for managing late blight disease-causing pathogen in Arusha, Tanzania.

1.2 Problem Statement and Justification

Late blight is a devastating disease of tomato worldwide including Tanzania. The disease is very destructive to tomato as it causes yield loss between 46% and 100% (Meya *et al.*, 2014). Management of the pathogen is complicated due to its biology, wide host range and infection mechanism of the pathogen (Nowicki *et al.*, 2012). Nevertheless, various management options such as the use of resistant cultivars, chemical, biological and cultural strategies have been used against late blight disease of tomato. Of these methods, synthetic chemical fungicide application is the most preferred approach used by farmers for the management of this disease due to direct effects on the pathogens and other pests. However, these chemicals are expensive, cause pathogens to developing resistance and are not environmentally friendly. As an alternative to synthetic chemicals use of the botanical plants in managing plant diseases pathogens are currently being promoted because of their effectiveness in sustainable disease management (Goufo *et al.*, 2008; Hubert *et al.*, 2013). To explore on their usefulness and efficacy this research aimed at testing the effects and developing a late blight disease management strategy based on botanical plants with potential fungicidal effect for application by tomato growers in Tanzania.

1.3 Objectives

1.3.1 Overall objective

The general objective of this study was to assess the current disease status and determine the effectiveness of leaf extracts of selected tomato plants for potential development of bio-fungicides against the late blight-causing pathogen in Tanzania.

1.3.2 Specific objectives

(i) To assess the incidence and severity of late blight disease (LBD) in tomato plants growing in Iringa and Arusha regions.

(ii) To identify the *P. infestans* using polymerase chain reactions (PCR).

(iii) To identify different tomato varieties grown by farmers and common synthetic fungicides used to manage late blight disease in Iringa and Arusha regions.

(iv) To evaluate effects of plant extracts against *P. infestans in-vitro.*

1.4 Hypothesis

Null hypothesis (Ho): The light-blight causing pathogen can effectively be controlled with plant extract from *Tephrosia vogelii, Plectranthus barbatus, Sphaeranthus suaveolens* and *Lantana camara.*

Alternative hypothesis (Hi): The light-blight causing pathogen cannot effectively be controlled with plant extract from *Plectranthus barbatus, Tephrosia vogelii, Sphaeranthus suaveolens* and *Lantana camara.*

1.5 Significance of the study

The outcome of the current study has provided updated information to the tomato stakeholders on the status of the late blight disease in Tanzania, particuraly in Iringa and Arusha (the study area) and the proposed sustainable diseases management option has included the use of plant extracts with potentials for developing bio-fungicides, the application of this finding by tomato growers is likely to improve tomato production in Tanzania.

CHAPTER TWO

LITERATURE REVIEW

2.1 Status of late blight disease of tomato in Tropical Africa

In the tropical Africa, late blight disease is constantly re-emerging in every growing season possibly due to favourable disease conditions including host susceptibility, existence of virulence races of the fungal organism and favourable environment (Agrios, 2005). The LBD-causing pathogen has ability to produce both asexually and sexually (Fig. 1). The sexual and asexual life cycles makes the infection of *P. infestans* successful in the warm and humid environments including Tanzania (Foolad *et al.*, 2008; Fry *et al.*, 2013; Nowicki *et al.*, 2012).

Disease management is mainly by chemical fungicides and the main chemicals groups being in the metalaxyl spray category. Nevertheless, these fungicides have been reported to be ineffective to new strains of the oomycete produced as recombinants of fertilization (Axel *et al.*, 2012). Some known strains of the LBD-causing pathogen are as shown in Table 1.

2.2 Pathogen description

The name *Phytophthora infestans* literally means, "Plant destroyer". It is a fungus-like organism classified under Oomycetes in the kingdom Chromista and order Peronosporales (Nelson *et al.*, 2001). It causes late blight disease in solanaceae plants especially during cool and moist period worldwide (Fry *et al.*, 2013). There are many different types of pathogen strains that genetically vary between their virulence and mating types (Table 1) (Kamoun *et al.*, 2015). The pathogen is commonly described as coenocytic oomycete with rare cross walls (Kamoun *et al.*, 2015). Sporangia are 29-36 x 19-22 µm and are ellipsoid to lemon shaped with a small pedicel. In culture, the pathogen mycelium is white and fluffy (Rumpf *et al.*, 2010). The primary sources of *P. infestans* inoculum originates from infected seed, weed infestation and or plant remain via asexual population (Kamoun and Smart, 2005).

Table 1: Some of different strains with different mating type of *P. infestans* in tropical Africa (Tomura *et al.*, 2017).

Pathogen type	Strain	Mating type
P. infestans	9173, 9174, PI0-1, PI1234-1	A1e, A1, A2e, A1e and A2e
Phytophthora sp	32716	A1e
P. vignae	30473, 30613	Homothallic
P. nicotianae	ATCC 38606 and ATCC 38607	(A2 mating type and A1 mating type)

2.3 Host range of the pathogen

There are over 120 host species of the *P. infestans* pathogen which have been identified including the common ones indicated in Table 2. On those hosts, the pathogen is capable to cause late blight disease at different growth stages and parts of the plants such as tissues, roots, tubers, herbaceous stems, woody trunks, foliage and fruit (Erwin and Ribeiro, 1996). Of the host, tomatoes and potatoes have been described to be the main hosts of the *P. infestans* while others are described as alternative hosts as shown in Table 2 below.

Table 2: Common hosts of *P. infestans* (Erwin and Ribeiro, 1996; Hooker, 1981).

Host	Type of host
Tomato (*L. esculentus*)	Main
Potato (*S. tuberosum*)	Main
Tree tomato (*S. betaceum*)	Alternative
Pear melon (*S. muricatum*)	Alternative
Nolana species	Alternative
Eggplant (*S. melongena*)	Alternative
Wild *Solanum* species	Alternative
Naranjilla (*S. quitoense*)	Alternative
Datura species	Alternative

The existence of this wide host range for the *P. infestans* complicates designing of appropriate management options in tomato. There is need to clearly identify the remaining hosts of the pathogens and their associated strains so that appropriate options may be designed and applied managing the late blight disease in tropical Africa.

2.4 Reproduction cycles of the *P. infestans*

The pathogen has two types of reproduction cycles (sexual and asexual), both of which have been described in three stages A-C (Fig. 1). In stage A, a fully effective organism is on the host and the pathogen follows either of the two modes of reproduction depending on the environmental conditions (Foolad *et al.*, 2008). If it goes through asexual reproduction, the pathogen sporangia germinates and releases zoospores which can then move chemo-tactically (in C) within the environment or directly through sporangia by wind or water. If it follows sexual reproduction in B, the pathogen produces two mating hormones α1 and α2, example for mating type A1 and A2 respectively which aid in the production of oospores (Tomura *et al.*, 2017). In stage C, mating of A1 and A2 take place and secreted mating hormones α2 (from A2) and α1(from A1) induce the sexual reproduction of the counter mating types A1 and A2, respectively hence production of oospore.

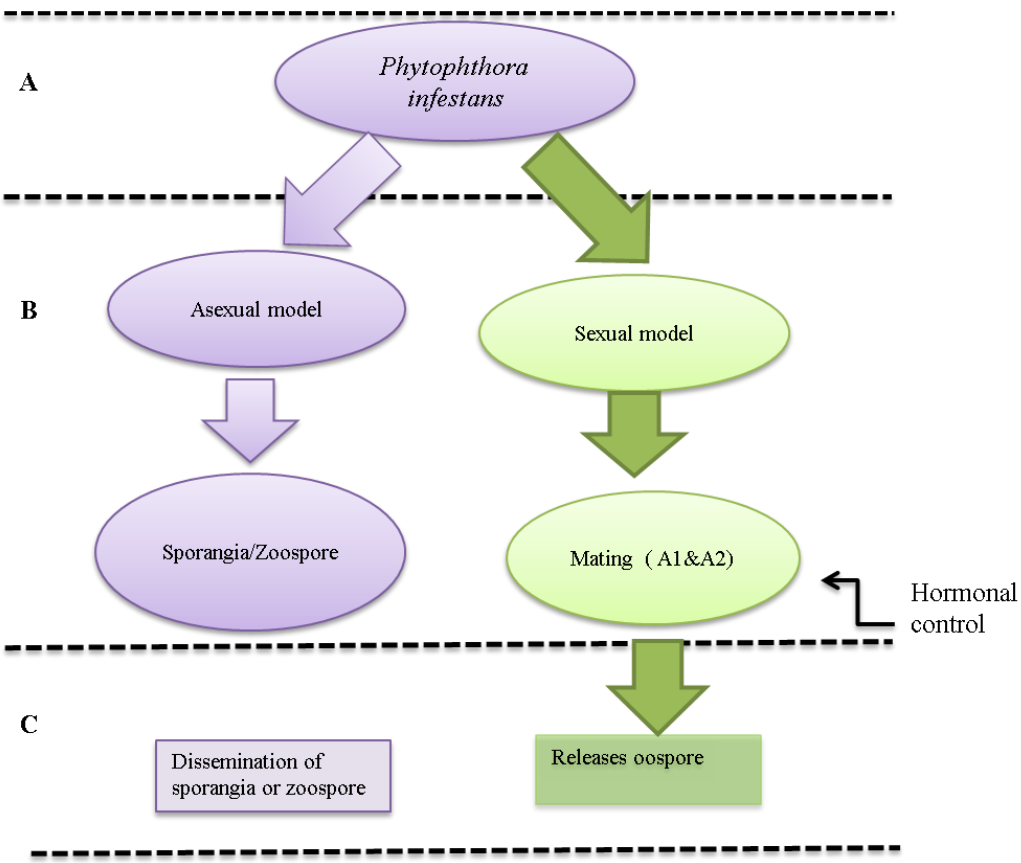

Figure 1: Reproduction of *P. infestans*

2.5 Infection mechanism of the pathogen and late blight disease development

For the pathogen (*P. infestans*) to effectively infect and colonize its host, a sequence of pathogenic processes is necessary (Fig. 2). In series, the pathogen sporangium or zoospore

has to be formed followed by cyst formation, then germination of the cyst/spore to form appressorium which will enhance penetration unto host and infection vesicle, then intercellular hyphal growth and haustorium formation and initiation of sporulation (Huitema *et al.*, 2004; Nowicki *et al.*, 2013). If these stages are successful, then colonisation on plant can be considered successful otherwise not based on some form of host resistance or interference by some environmental factors such as application of chemicals to control the pathogen or other nature based climatic conditions (Whisson *et al.*, 2016).

For successful colonisation, the pathogens' infection vesicle is supported by different virulence (AVR) and resistant (R) genes (Al-Babili and Beyer, 2005; Huitema *et al.*, 2004; Jones and Dangl, 2006).

In addition, the infection process involves secretion of different protein molecules which enable attachment of *P. infestans* to the host and breaking down physical defence barrier of the host plants (Huitema *et al.*, 2004).

On and in the host tissue, *P. infestans* suppresses the plant immunity through secretion of the effector proteins commonly known as apoplastic effectors (those formed on the outside) and cytoplasmic effectors (those formed inside) (Lo Presti *et al.*, 2015). Figure 2 successful colonisation by the pathogen leads unto symptom development and effects as described in Table 3.

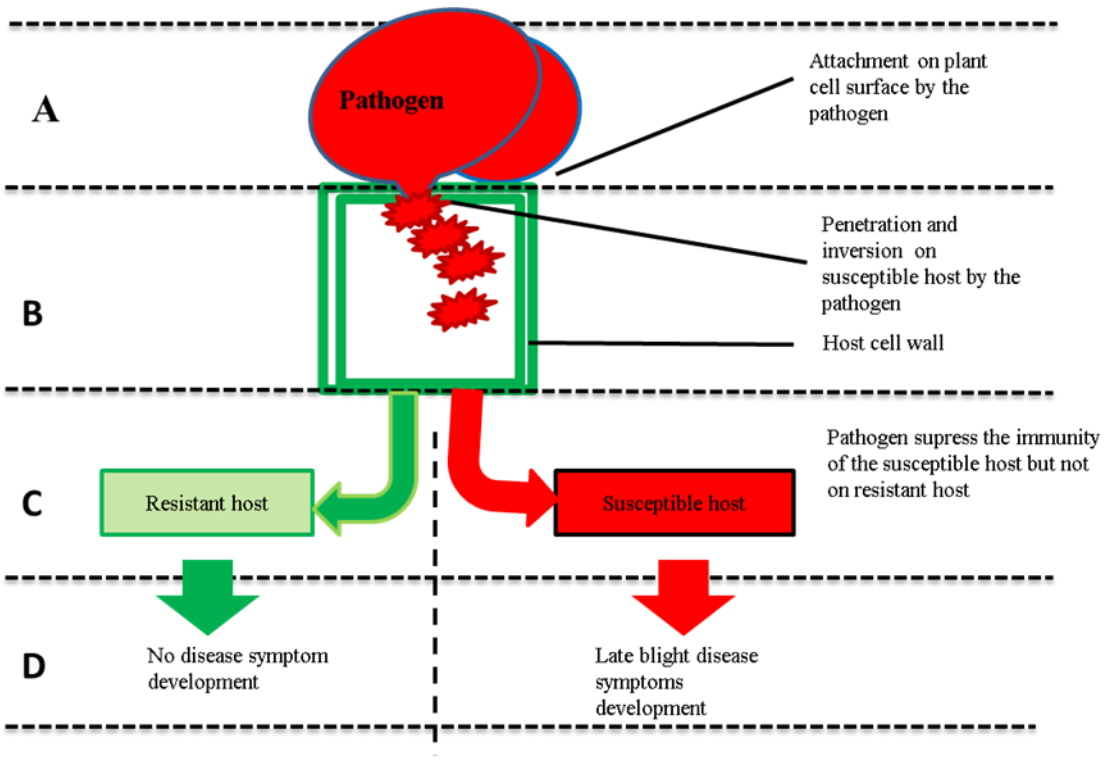

Figure 2: Stages of late blight disease development. In this model, two possibilities can occur upon pathogen contact to host as described in stages A-D. In stage A: The pathogen from different sources attaches the host, B: Invasion stage, C: Molecule exchange between pathogen and host where the pathogen supress the host immunity, D: There is a successful in susceptible variety and fail to colonize a resistant host. In susceptible host cell wall apposition is absent, the pathogen colonizes and supress then develops the Late Blight symptoms.

Table 3: Symptoms and or signs incited by *P. infestans* and their effects on different parts of the host tomato plant (Agrios, 2005).

Part of plant	Symptoms and Signs	Effect
Leaves	Necrosis, shrivelling, brown colouration, powdery and whitish	Reduced total number of photosynthetic cells rings
Petiole &stem	Elongated, blackened Water-soaked spots. Lesions covere with a grey	Reduced growth, infected fruitlets
Fruits	Circular greasy lesions A thin layer of white mycelium	Total plant failure to reach maturity

2.6 Management of late blight disease

There exist a number of management options that are used against late blight disease (Table 4). The most important ones are as discussed in the sections below.

2.6.1 Cultural control

Cultural practices such as timely weeding, crop rotation, elimination of volunteer tomato plants and planting clean seedling are major components of growers' strategy in disease management; and these methods can sometimes be able to limit the disease development to economical threshold levels. The cultural methods can prove useful in preventing introduction of inoculum to healthy transplants and prevent development or spread of inoculum between and within the field (Agrios, 2005). The principle behind the cultural practises is field sanitation i.e. keeping the field clean (Mpumi *et al.*, 2016). However, in the tropical Africa, there exist challenges is using this approach making it ineffective (Mpumi *et al.*, 2016). The challenges raise due to: (a) farmers' poor understanding of different factors that could lead to disease build up such use of unimproved susceptible tomato varieties, improper destruction of infected plant debris and continuous mono-cropping (b) existence of wide-host range for the pathogen within farms proximity (c) small-scale farming with mixed cropping options and (d) existence of different pathogen strains near or within farmer's field. Therefore, there is need to educate farmers and design specific management recommendation which will be suitable for managing late blight in the complex tropical African environment.

2.6.2 Fungicide application

This is the main approach used at global level in management of late blight in tomato and potato (Kamoun and Smart, 2005). For the chemical application to be effective, it must be applied before infection (Beckerman, 2008). In the tropical Africa, chemical application is the only most common and preferred approach though not always possible to small scale farmers due to high cost and those who afford it either fail to follow recommended rates of application or lack knowledge of handling the chemicals. Common fungicides used in the tropical Africa are chlorothalonil, which is usually applied before or upon disease development and metalaxyl which inhibit or reduce disease progress once symptoms are apparent it also inhibits ribosomal RNA (rRNA) polymerases in fungi by reducing incorporation of uridine which is the major cause of disease occurrence. Pre-treatment of tomato plants with the chemical such as dl-3-amino-butyric acid induce systemic-acquired resistance in the tomatoes, protecting them from late blight infection through inhibit

haustoria formation and growth of hyphae against *P. infestans* (Binyam, 2014). However, these applications increase production cost and the potential for human health and environmental risks associated with fungicide residual. Major chemical fungicide includes dithiocarbamates, such as mancozeb, which break down into carcinogens causing liver and thyroid tumours, and testicular effects (Novikova *et al.*, 2003). This involves that, fungicide residual leads to long-term risks for cancer development among tomato producers and consumers (Meya *et al.*, 2014). Also chemical applications associated with several challenges mainly, development of resistance by pathogens and also are associated with negative impacts to non-target organisms and pollution to the environment (Cohen, 2002; Kamoun *et al.*, 2015). Therefore, alternative method which is economical feasible, health safety and environmental friendly is required in sustainable management of the *P. infestans* pathogen in tomato.

2.6.3 Host-plant resistance

Since the outbreak of the late blight (LB) disease to potato in 1840s, the concern of developing LB resistant potato and tomato cultivars it was of great interest (Nowicki *et al.*, 2012). Since then, research evidence shows that resistance to *P. infestans* both race-specific resistance (i.e specific, vertical, or "gene-for-gene" interaction) and race-non-specific resistance (i.e, horizontal, or partial resistance) has developed (Nowicki *et al.*, 2012). Tomato with disease resistance ability by vertical resistance have been effective (at least initially) in avoiding growth and development of pathogen to the crop.

The problem of the pathogen to crop is due to its tendency of re-emergence with more virulent strain and sexual reproduction of *P. infestans* leading to more aggressive lineages, which make the resistance gene to be effective in only one or a limited number of pathogenic races (Fry, 2008). In comparison to vertical resistance, race-nonspecific resistance is often controlled by several genes or quantitative trait loci (QTL), which is partial resistance against multiple races of the pathogen. This is always slows, and not stop progress of the disease since LB disease spread quickly (Fry, 2008).

The key stone of disease management is the use of resistant crop cultivars but, durable resistance to late blight has not been available to growers, particularly in varieties that are in high demand by consumers' (Kamoun and Smart, 2005). However, most of different susceptible cultivars are still being grown due to cultural and economic values such as variety

popularity, factors which limit adoption of new or resistant varieties such as palatability, colour and shape of fruits.

Therefore, during screening for host resistance, it is better to consider farmer preference, high demand varieties, and economical factor so that the developed varieties improve the farmer's challenges faced in LB disease.

2.6.4 Biological control

Researcher has investigated the use of biological control that increases the potential as a solution to the late blight problem (García-Martínez *et al.*, 2012). Biological control includes the use of different microorganisms such as bacteria, fungi, nematodes and viruses (Shuping and Eloff, 2017). The great potential use of microorganism in management of late blight disease is based on the antagonistic nature of microbes to *P. infestans* (Shuping and Eloff, 2017). Also the use of the biological control is environmental friendly, the microorganism are available to the surrounding and its safety in human health.

Nevertheless in tropical Africa, limited information is available on use of biological control in managing late blight disease. The identification of the strain of the *P. infestans* is needed in order to increase the accuracy for the specific microbe or organism to the specific strain of the *P. infestans* rather than applying to general pathogen with no identification, as the pathogen reproduce so quickly with different strain continuously.

2.6.5 Plant extracts

Plant extracts are increasingly being explored for managing plant diseases due to the fact that they are portrayed as environmentally friendly and safe to humans contrast to synthetic fungicide (Goufo *et al.*, 2008). They contain active natural chemical that are effective in crop protection against the pathogen (Hubert *et al.*, 2013; Makoi and Ndakidemi, 2007; Ndakidemi and Dakora, 2003). Plant extracts are known to produce secondary metabolites such as phenolic/flavonoids and terpenes/monoterpenes which inhibit fungi, bacteria, and insects under laboratory, screen house and field tests (Hubert *et al.*, 2013; Makoi and Ndakidemi, 2007; Ndakidemi and Dakora, 2003).

Studies have shown that the use of synthetic fungicides in managing Late Blight disease levels were comparable with that of plant extracts this in Cameroon and Kenya (Piebiep Goufo *et al.*, 2010; Lengai *et al.*, 2016). Extracts from different plant like *Tephrosia vogelli*

and *Ageratum houstuianum* among others have been reported to be effective in reducing late blight severity (Goufo *et al*., 2010). Also the extract from *Ocimum gratissimum, Cupressus benthamii* and *Vetiveria zizanioides* are reported to have inhibitory capacity against the late fungal pathogens causing diseases in plants (Goufo *et al*., 2008; Goufo *et al*., 2010). A study in human model showed that plant extracts from *Sphaeranthus suaveolens*, exhibited remarkable strong antifungal activities. This prompted an idea of their possible action in controlling fungal pathogens such as *P. infestans* in Tomato. Although the reported plants were effective against fungal pathogens in plants, screening them against P. *infestans* will reveal their potential in combatting this disease.

The ability and effectiveness of different of plant extracts in reducing disease levels in plants is related to the mode of action of the plant extracts (Nashwa and Abo-ElyouSr, 2013). Some plant extract act directly on the pathogens while others induce systemic resistance in host plants to reduce disease occurrence and development. Also the modes of action of the extracts are comparable with those of the synthetic fungicides and then the effects are similar (Lengai *et al.*, 2016). As far as tropical Africa is concerned, use of plant extract is based on indigenous knowledge but very little has been documented (Olanya *et al*., 2012). This thus calls for research to quantify use and their potential in managing late blight disease in tomato in tropical Africa.

2.6.6 Integrated Disease Management (IDM)

Integration disease management is the better method in late blight disease management options in tropical regions where fungal inocula are abundant in most months of the year (Olanya *et al.*, 2012). These include variation of frequency of application based on host resistance of tomato varieties (reduced fungicide use), early planting and the use of improved crop variety (early and mid-maturity, tolerant variety). Other control measures include: use of disease-free seed; eliminating diseased tomato of off season and planting resistant cultivars. Also combined approaches consisting of cultural practices, biological control and among others (Makoi and Ndakidemi, 2007) have been reported to help farmers reduce the use of chemical fungicides. Although this approach is always encouraged, proper understanding by small scale farmers on how to create a balance of the procedures especially on which to start and when is always associated with some technical flow that is not always easily adopted in tropical Africa. For effective control of late blight, farmer must adopt on how this method must be used, knowing that only one method such as fungicides cannot be used alone.

However, it must be used as one tool in an integrated management strategy for effective control of late blight. For instance, using resistant cultivar in the field as the first line of defence, weeding, mulching, fungicide application as a last resort if necessary; that is integrated disease management which is cheap and appropriate in managing the disease.

Table 4: Management option of late blight disease

Method(s)	**Strengths**	**Weakness**
Cultural	Conserve soil nutrients and recycling through crop rotation and intercropping.	They are effective when used in combination with other methods.
Chemical	Fast effect on pathogens leading to increased yield production.	It is temporary They are not able to cure existing symptoms.
Integrated disease management (IDM)	They are cheap options to late blight management.	It depends on a crop production system.
Biological	Sustainable and environmental friendly, pathogen specific.	Developing the methodology takes a long time.
Resistant varieties	The constitutive defense makes the plant to thicken its cuticle and constitutively produce secondary metabolites which prevent the pathogen from attacking the plant.	Breeding for late blight resistance is a slow process.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Tomato varieties, fungicides and material or tools used in this study

The Tomato varieties, chemical fungicide and materials used in this study are as shown in Tables 5, 6 and 7.

Table 5: Tomato varieties used in this study.

No	Variety	Location
1.	Tanya	Iringa & Arusha
2.	Mkulima	Iringa & Arusha
3.	Eden F1(Eden F)	Iringa & Arusha
4.	Riogrande Safar (Riogrande S)	Iringa & Arusha
5.	Hybrid Imara F1 (H. Imara F1)	Iringa & Arusha

Table 6: Chemical fungicides used in this study

Fungicide trade name	Chemical formula	Nature of action
Ridomil Gold® Plus	Wettable formulation	Systemic Fungicide
Victory72 WP™ (Metalaxyl 80g/kg +. Mancozeb 640g/kg)	Wettable formulation	Systemic with curative and protective action
Ebony 72	Wettable formulation	Systemic and protectant
Milthane super	Contact fungicide	Protectant action
Banco	Wettable formulation	Systemic fungicide
Odeon	Wettable formulation	Curative and protectant

Table 7: Laboratory tools, chemicals and reagents used in this experiments

No	Materials and tools	Uses
1.	Petri dishes	Media preparation
2.	Sodium hypo chloride (NaOCl)	For sterilization
3.	Alcohol and Ethanol	For sterilization purposes
4.	Distilled water	Safe water to use in the laboratory
5.	Autoclave machine	Sterilization equipment at 121°C for 15 minutes
6.	V8 juice and rye agar	Media for fungal growing.
7.	Laminar floor	Safety working condition
8.	Incubator machine	For incubation
9.	Eppendorf tubes	For sample taking
10.	Pippete and pippete tips	For measuring volume
11.	Vortex machine	Mixing the sample solution
12.	Water bath heater	For heating purposes
13.	Centrifugal machine	For separation purposes
14.	Methanol/Acetyl acetate	For botanical plants extraction purposes
15.	Freezer/Refrigerator	For storing samples at different temperature
16.	Agarose sugar	For gel preparation
17.	Ethidium Bromide	For DNA vision
18.	TAE Buffer	For DNA extraction/gel running
19.	Master Mix	For DNA mixing for PCR preparation
20.	Microwave	For heating
21.	Loading dye	Colour mixing with DNA
22.	Loading gel	Gel used in the machine
23.	Ladder	Showing scale
24.	UV Translator	Machine for reading DNA bands
25.	Primers	For fungal isolation
26.	Gel electrophoresis	For loading DNA samples
27.	Polymerase chain reaction (PCR)	For DNA amplification

3.2 Study location

The study was conducted Kilolo District in Iringa region, which is located at latitudes 7°46'5.01"S and Longitudes 35°41'9.86"E and Arumeru District in Arusha region which is located at Latitudes 3° 23' 12.9300" S and Longitudes 36° 40' 58.7820" (Fig. 3). These districts were selected since they are the main tomato growing areas in the country and also due to high incidences and severity of the LBD. Survey studies were conducted these areas and Laboratory experiments were done at the NM-AIST laboratory in Arusha Region.

Figure 3: Map of Tanzania indicating the tomato growing regions (indicated by green colour) covered in this study

3.3 Assessment of disease in the study locations

Disease incidence refers to the proportion or percentage of diseased plants in an area. The disease incidence was determined by developing triplicate quadrats of 10m x 10m on the tomato fields each farm. The disease incidence was established by counting the number of plants with disease symptoms over the tomato number plants in the quadrat multiplied by 100%. Disease severity refers to the relative or absolute area of plant tissue affected by the disease. The disease severity was determined by using a scale rating of 1-4 (Table 8) as per protocol developed by Maerere *et al*. (2010a). Where score 1 representing no disease, 2 = low severity, 3 = moderate severity and 4 = high severity. The final scores of the disease incidence were obtained based on the formula below; and the score for the disease severity explained in table 5 below:

Table 8: The disease scoring scale of late blight disease showing levels and intervals

Level	**1**	**2**	**3**	**4**
Interval	1-20	21-50	51-80	81-100
Grade	Resistance	Less susceptible	Susceptible	Very susceptible

3.4 Sample collection, growth media preparation and culturing

A total of fifty four tomato leave samples which were infested by the late blight disease were randomly collected from different fields in northen (Arusha) and southern (Iringa) zones of Tanzania. In each zone, twenty seven samples were collected from three tomato fields per region. The collected samples were well labelled and transferred to the Nelson Mandela African Instutute of Science and Technology (NM-AIST) laboratory for further study (Plate 1). Eight vegetables (V8) media (V8 juice 200 mL, agar 20 g, and $CaCO_3$ 2 g in 800 mL of distilled water was autoclaved at 121°C and 15 psi for 15 minutes, allowed to cool to about 45°C) then amended with rye agar (2.5% Pimaricin aqueous solution and 20 mg/L Rifampicin SV sodium salt. Then the pH of the isolation medium was maintained at 5.76 and the media was poured into sterile petri dishes, covered and allowed to cool for 1-2 h under the laminar air flow cabinet, then the petri dishes were maintained at -4 degrees until used.

On triplicate petri dishes, three small pieces of diseased tomato leaves (taken from the margins of a lesion) were of about 1 cm square were placed equidistantly on each of three Petri dishes (each with a diameter of 9 cm) the media. The inoculated plates were incubated at 16-18 ºC and observations were made after three days to five days. Growth of cotton-like mycelia characterized by water-like droplets was recorded as positive for LBD-causing fungi. Pure culture was obtained by transferring small portion of mycelia from white mycelia to sterilized fresh amended-rye agar medium and incubated at 20 ºC for 3-5 days in darkness. Fungal mycelia were scooped using a sterile needle into sterile 1.5 mL Eppendorf tubes for DNA extraction and the remaining pure culture was maintained on rye agar for further study and references at -4 ºC. Using the same pure culture, isolates were inoculated on cultivar Tanya prior identification of virulent strains of *P. infestans*. Those which induced LBD symptoms were considered for PCR analysis.

Plate 1: Tomato leaf sampes collected from Iringa and Arusha farmers' field.

3.5 Identification of *P. infestans* with pathogenicity testing and PCR

Tomatoes (Tanya seeds) were grown in the screen house and inoculation was done at 5-6 weeks after sowing. The inoculum was prepared using procedures described by Goufo *et al.* (2008). It was collected with a fine brush into 10 mL Single distilled water (SDW) containing 0.01% Tween 20. The suspensions was shaken in a vortex to dislodge sporangia, and then filtered through a double layer of cheesecloth to remove mycelia fragments. Its sporangial suspension was determined and adjusted using haemocytometer to the concentration of 2×10^4 sporangia/mL. The suspension was incubated at 8 ◦C for 1 hour to promote zoospore release prior to use. One drop of 2×104 spores/mL of sporangial suspension of selected *P. infestans* isolates and water as control was applied on the abaxial side of each leaves using a Pasteur pipette. After inoculation, plants were allowed to dry, and then covered with a polyethylene (Plate 2) bag for 24 hours to maintain high humidity around the leaves. Irrigation and weeding were also done as described by Fontem *et al*. (2004).

Plate 2: Inoculation activities of the *P. infestans* to Tomato (Tanya variety) grown in the screen house.

3.6 Deoxyribonucleic acid extraction

Deoxyribonucleic acid extraction was performed using a Quick-DNA™ Miniprep Kit Catalog No's. D4068 and 4069. Fungal mycelia of 50 mg (wet weight) were suspended into 200 µl of isotonic buffer. The mycelia were centrifuged in a microcentrifuge at 10 000 x *g* for 1 minute. The sample were then transferred up to 400 µl supernatant to a Zymo-Spin™ III-F Filter in a Collection Tube and centrifuged at 8 000 x *g* for 1 minute. Then 200 µl of Genomic Lysis Buffer was added to the sample and filtrated into the collection tube. Then 800 µl of the mixture to a Zymo-Spin™ IIC Column3 in a collection tube were centrifuged at 10 000 x *g* for 1 minute. After the flow through from the collection tube, 200 µl of pre-Wash Buffer was added to the Zymo-Spin™ IIC Column in a new collection tube and was centrifuged at 10 000 x *g* for 1 minute. This was followed by adding 500 µl g-DNA Wash Buffer to the Zymo-Spin™ IIC Column and centrifuge at 10 000 x *g* for 1 minute.

The last step was to transfer the Zymo-Spin™ IIC Column to a clean 1.5 mL micro centrifuge tube and 100 µl (35 µl minimum) DNA Elution Buffer was added directly to the column matrix. The content was centrifuged at 10 000 x *g* for 30 seconds to elute the DNA. The DNA pellets were re-suspended with 50 µl of TE buffer (10 mM Tris HCL and 1 mM EDTA). The DNA obtained was stored at -20 ^{o}C for then, PCR analysis.

3.7 Polymerase Chain Reaction (PCR)

The primers that target the Internal Transcribed Spacer (ITS) with 600 bp were used. PINF (CTCGCTACAATAGGAGGGTC) and ITS5b (GGAAGTAAAAGTCGTAACAAGG) Forward and reverse primer respectively were used for PCR-based identification of fungal isolates based on procedure by Judelson and Tooley (2000). The PCR premix was made up of 8.5 µl water, 0.5 µl PINF forward, 0.5 µl of ITS5b reverse and 3µl of DNA and, was added mixed in 1.5 mL eppendorf tube.

The PCR conditions were as follows: Denaturation temperature at 94 ^{o}C for 30 seconds followed by 35 cycles at 94 ^{o}C for 30 seconds, annealing temperature at 55 ^{o}C for 30 seconds, extension temperature at 72 ^{o}C for 1 minute. Then the temperature was held at 4 ^{o}C for infinity.

3.8 Gel electrophoresis

Polymerase chain reaction products were obtained through electrophoresis generally containing 1% agarose gel in 100 mL volume (1 g of agarose was mixed with 100 mL of 1x TBE in a 250 mL conical flask). The mixture was boiled in a microwave oven for about 1-2 minutes to completely dissolve the agarose. The solution was then cooled at 60 °C seconds by keeping it into cold running water. Two drops of easy dye vision was added to the solution and was mixed well. The gel was poured into a gel tray with comb giving required number of wells. Bubbles were removed by using disposable pipette tip. The gel matrix was ready to use after 45 minutes.

Polymerase chain reaction product with loading dye and ladder was loaded onto a solidified gel matrix using a sterile pipette and the electrophoresis was set at 100 v for 30 min, and was visualized afterwards with the aid of UV light in a Trans illuminator to see the absence or the presence amplified fragments. The amplification of the DNA product was indicated by a band with a product size of 600 bp. The band images obtained from the UV light Trans illuminator were taken by the gel documentation system. The gel matrix was then disposed on specialized trash containers set for such purposes at the NM-AIST laboratory.

3.9 Identification of different tomato varieties grown by farmers and common synthetic fungicides

In the surveyed villages, common tomato varieties grown by farmers and common fungicides used for managing the LBD were identified. Then, a research-farmer managed trial was established, one field at Ilula village, Kilolo District, Iringa and another one at Ngarenanyuki, Arumeru District in Arusha, region. Split-plot design with three replications was adopted in each field. Tomato varieties were considered as the main-plot and the chemical fungicides as the sub-plot. These varieties include Tanya, hybrid imara, Rio grande, EdenF1 and Mkulima while chemical fungicides were Victory72, Ridomil, Ibony72, Milthane, Tancopper, Banco and Odeon. Tomato varieties without management were used as control. The experiment was carried out in February to March 2018 and March to May 2018 in Iringa and Arusha, respectively, to determine the incidence and severity of LBD on tomato. Field preparation, sowing of seeds, weeding and application of fungicides were done according to farmers' routine.

3.10 Collection of selected plant samples

Four medicinal plants were selected from different fields in Arusha region, Tanzania due to their antimicrobial and antifungal activities as per Goufo *et al*. (2010) and Al-Samarrai *et al*. (2012). The plants collected included *Plectranthus barbatu*, *Tephrosia vogelii*, *Sphaeranthus suaveolens* and *Lantana camara* (Table 9 and plate 3). The collected plants were dried under shade at 25–27 ◦C for 10 days.

P. barbatus *L. camara* *S. suaveolens* *T. vogelii*

Plate 3: Pictures of selected plants whose extracts were evaluated for *P. infestans* mycelial inhibition

Table 9: Uses of selected medicinal plants.

Name of plants	Family	Local name	Uses
Tephrosia vogelii	Fabaceae	Vogeli tephrosia/ Tchieuc	It controls larval stages of mosquitoes and soft-bodied insects and mites. Dried leaves protect stored legume seeds from bruchids.
Plectranthus barbatus	Lamiaceae	Coleous, makandi	Leaves used for medicinal or antimicrobial activities (Musila *et al.*, 2017).
Sphaeranthus suaveolens	Asteraceae	Sphaeranthus Indicus(General name)	Leaves used as traditional medicine as anti-malaria, antibacterial and antifungal agents (Mahajan *et al.*, 2015). Whole plant is drunk as a cough relaxant (Mahajan *et al.*, 2015).
Lantana camara	Verbenaceae	Lantana	Inhibitory and stimulatory biochemical interactions between plants. Essential oil of *Lantana camara* has antibacterial activity against bacteria (Saikia and Sahoo, 2011).

3.11 Bioassay preparation of plant extracts

In this experiment, 10 g of plant extract (powder) was macerated using a mortar and pestle containing 100 mL of distilled water. The exudation of biochemical compounds was allowed overnight and biomass was filtered by using of standard Whatman No. 1 filter paper. The filtered extracts were sterilized by autoclaving at 121 °C and 15 psi for 15 minutes. Inhibition of different extracts to the pathogen (Poison food technique) was used *in-vitro*. Three mL of plant extract of the stock solution was added in V8 juice media and poured in sterilized Petri plates containing the V8 media (V8 juice 200 mL, agar 20 g, and CaCO3 2 g in 800 mL of distilled water was autoclaved at 121 °C and 15 psi for 15 minutes, allowed to cool to about 45 °C). The disc of 5 mm diameter of test fungi taken from a 3 day old culture were placed at the centre of triplicate Petri dishes and incubated at room temperature. After three days, the radial growth of mycelium were measured and compared with the results of control. The

following formula was used to calculate inhibition percentage for each fungus in treatment (Goufo *et al*., 2008).

$$\%\ of\ inhibition = \frac{\text{Colony diameter without extract} - \text{Colony diameter with extract}}{\text{Colony diameter without extarct}} \times 100\%$$

3.12 Extraction and formulation of crude extracts from plant samples

Crude extracts from collected botanical plant samples were extracted by aqueous methanol and acetyl acetate solvent using Soxhlet extraction method using the modified method by Al-Samarrai *et al*. (2012, 2013). Plant sample materials were blended and for each sample 1 Kg of dried extract was dissolved in 1 litre of methanol and acetyl acetate in the ratio of 1:1 (w/v) of dry powder/solvent, respectively, using procedure described by Al-Samarrai *et al*. (2012; 2013 with modifications followed by soaking for 48 hours. The beakers containing the content was sealed properly with aluminium foil and left for 72 hours. The extract was filtered through Whatman No. 1 filter paper on a plug of glass wool in a glass column and the solvents was evaporated through the vacuum using a rotary evaporator. Extracts of each plant was weighed separately and dissolved in Dimethyl sulfoxide (DMSO) solvent to give a stock solution. Then, the concentrate was transferred from round flask to the universal bottle and passed through nitrogen to remove the extraction solvent and the extract were kept to cool.

3.13 Evaluation of antimicrobial activity of crude plant extracts

Screening of the crude plant extracts for antimicrobial properties was done following modified procedures described by Al-Samarrai *et al*. (2012, 2013). The V8 juice medium was prepared and cooled. Then plant extracts were incorporated at a ratio of 1 mL plant extract: 30 mL of V8 medium and the mixture was dispensed into Petri dishes. After the media had set, 5 mm agar discs was cut from 14 day old fungal pathogen cultures and placed at the centre of the plate and incubated at room temperature. The V8 Petri dishes without plant extracts were used as negative control and V8 mixed with synthetic fungicide were included as positive controls. Observations were made at 48 to 72 hours after plating and antifungal activity was determined by measuring the fungal colony radial growth using the same formula as previously described under section 3.11.

3.14 Statistical analysis

The data collected from farmers' and experimental fields were tomato variety, chemical fungicide sprayed and disease incidence and severity during the main cropping season, February to May 2018 in Arusha and Iringa region. Late blight disease severity and incidence were analysed using analysis of variance (ANOVA) facilitated by statistical analysis package Genstat, 16th Edition. Duncan's Multiple Range Test was used to separate the means of disease incidence and severity while Least Significant Difference (LSD) test was used for mean separation of means between tomato varieties grown and chemical fungicides used at $p < 0.05$.

The PCR amplification was performed in an Eppendorf thermocycler gradient PCR machine (BIO-RAD, C1000 Touch ^{TM}) under PCR condition(s) with Reverse and Forward primer(s). Analysis of the PCR product was performed by electrophoresis on 1% agarose gel, pre-stained with10 µl of easy dye vision at and visualized under UV light.

Data collected on inhibition of mycelia growth were analysed using 3-way analysis of variance (ANOVA) STATISTICA software. The Fisher's least Significance Difference (LSD) was used to compare treatment at $p = 0.05$ level of significance.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Status of the late blight disease in the study locations

The results indicated relatively same disease incidence and severity trend in all the study locations. The disease incidences on tomato were relatively high (50%) and disease severities were about 15% in Iringa and Arusha (Fig. 4).

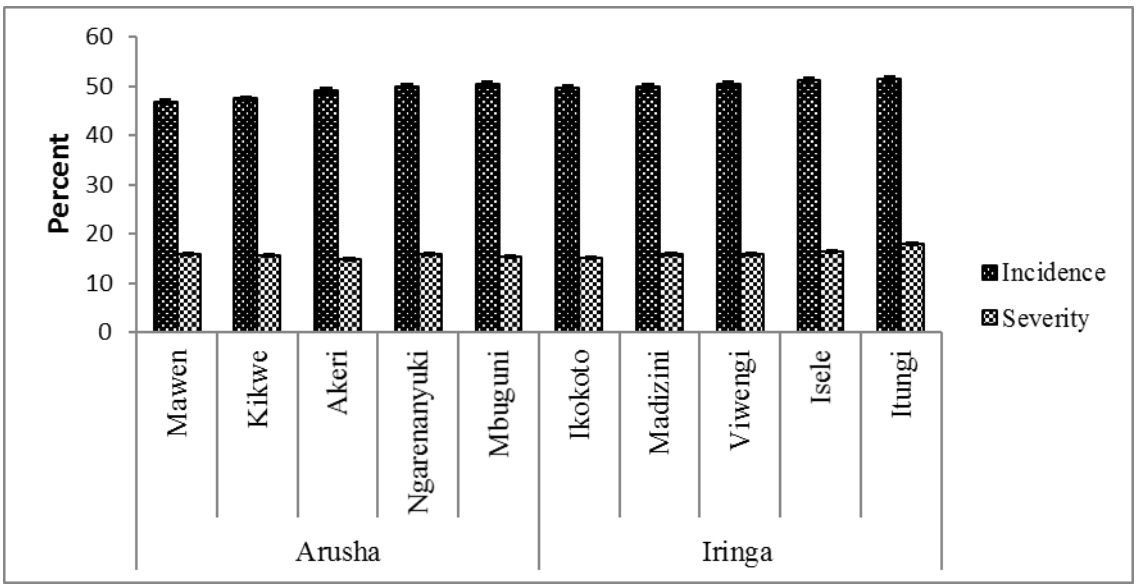

Figure 4: Average late blight disease incidences and severity on tomato in different villages covered in this study**.**

4.1.2 *Phytophthora infestans* identification

A total of 54 isolates were obtained from disease plant samples. Out of these only 15 indicated pathogenic symptoms on tomato variety (Tanya) (Fig. 5). Deoxyribonucleic acid (Makule *et al.*, 2017) from those isolates was extracted and results are presented on Plate 4.

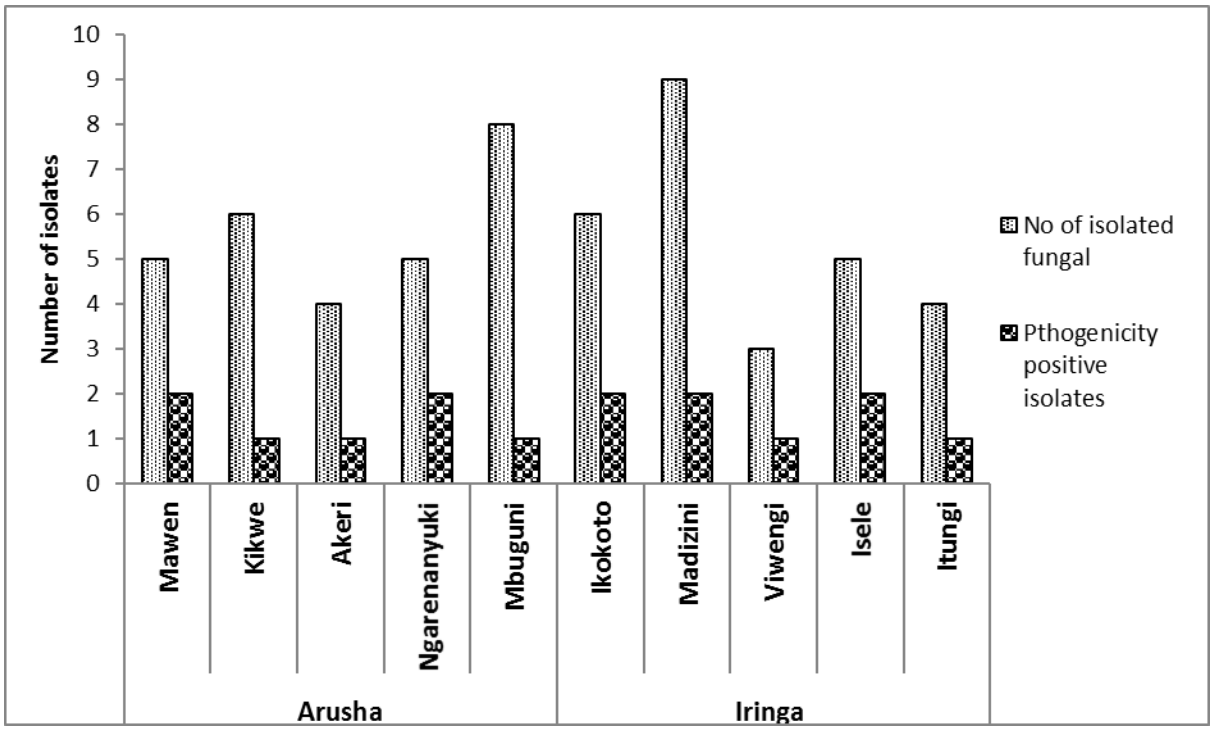

Figure 5: Pathogenicity positive isolate from Iringa and Arusha region

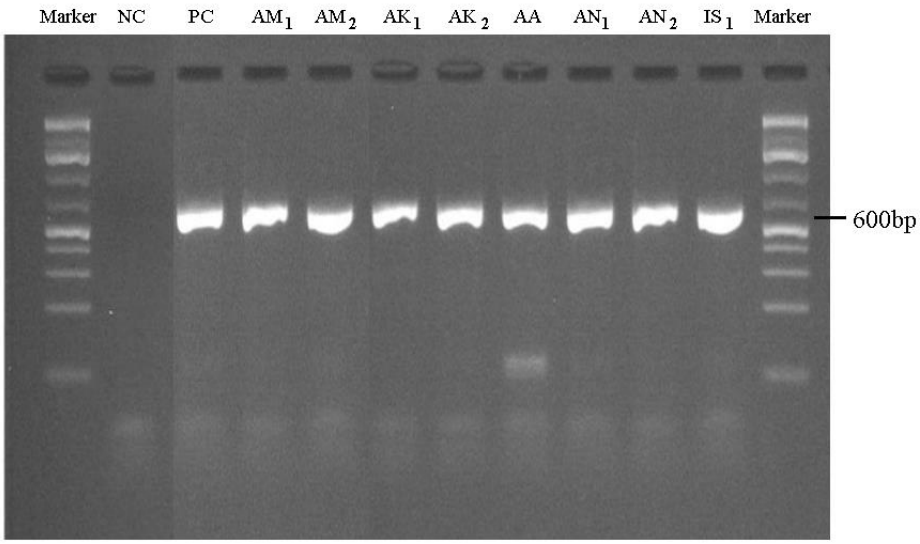

Plate 4: Amplification of DNA sample of *P. infestans* pathogen. Where by PC = Positive control, NC = Negative control (master mix) and AM1, AM2, AK1, AK2, AA, AN1, AN2, IS1. Mawen, Kikwe, Akeri and Ngarenanyuki in Arusha region and IS1 in Iringa region.

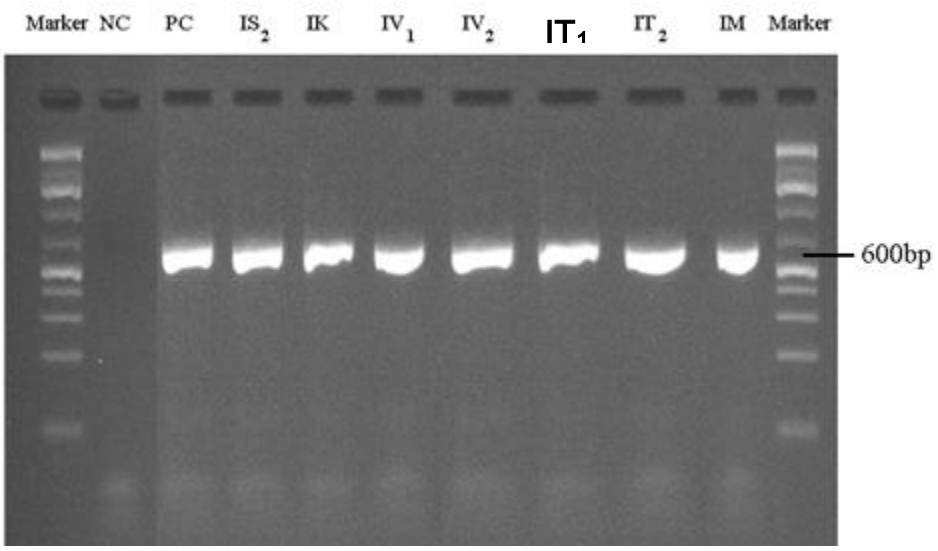

Plate 5: Amplification of DNA sample of *P. infestans* pathogen.

Where by PC = Positive control, NC = Negative control (master mix) and IS2, IK, IV1, IV2, IT1, IT2, IM are (Mawen, Kikwe, Akeri andNgarenanyuki in Arusha region, Isele, Ikokoto, Viwengi, Itungi and Madizini in Iringa region: 1= first sample and 2 = Second sample from the same village).

4.1.3 Common tomato varieties, chemical fungicides and LBD incidence and severity in research-farmer managed trials in Iringa and Arusha regions.

Results showed that in all regions, common tomato varieties were Tanya, Mkulima, Eden F1, Rio grande safar, Hybrid Imara F1. Chemical fungicides were Ridomil Gold® Plus, Victory72 WP™ (Metalaxyl 80g/kg + Mancozeb 640g/kg), Ebony 72, Milthane super, Banco and Odeon. Of these, Ridomil Gold® Plus, Victory72 WP™ (Metalaxyl 80g/kg + Mancozeb 640g/kg) and Ebony 72 (Fig. 6). Tanya was the most popular tomato variety in the study area. These varieties and fungicides used for research-farmer trials and results are shown in Table 10 and 11.

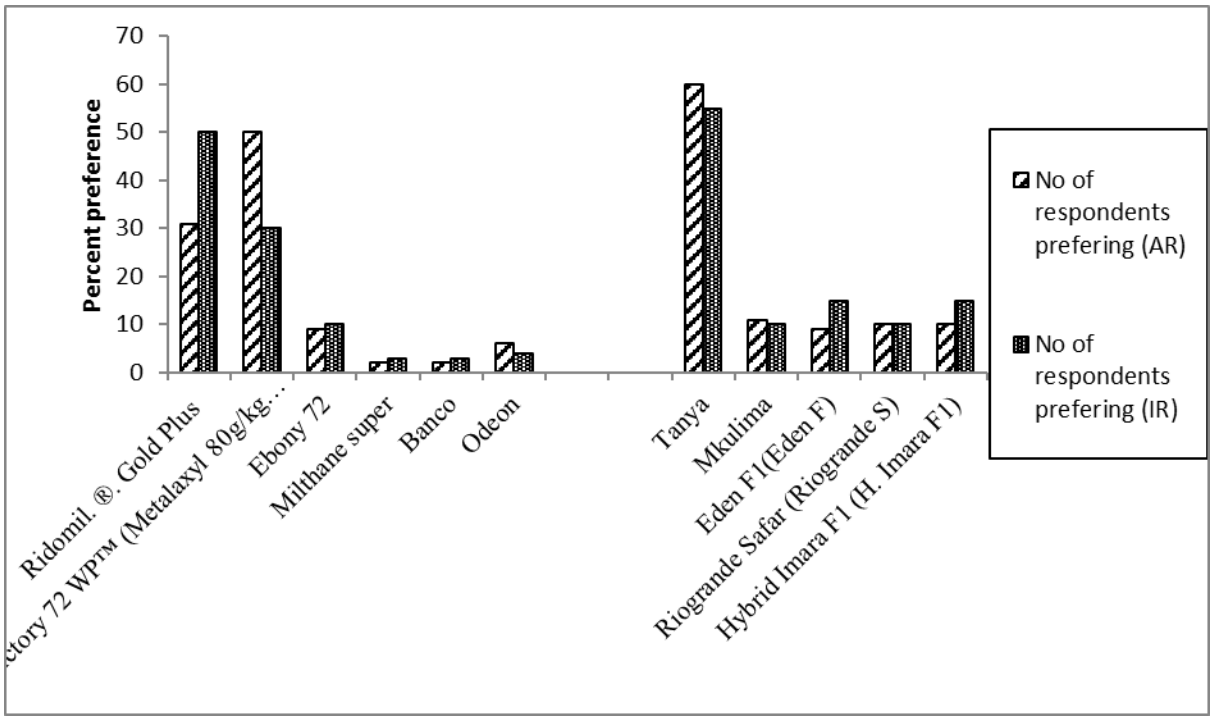

Figure 6: Common Tomato varieties, chemical fungicides and LBD incidence and severity in research-farmer managed trials in Iringa and Arusha regions.

The results for research-farmer managed trial showed that there was significant different (p=0.01) between disease incidence in different tomato varieties in Iringa region (Table 10). The highest disease incidence was recorded on variety Tanya (57.57%) and the lowest (48.43%) even though was not significant with other tomato varieties Eden F1, Mkulima and Rio grande was recorded for tomato variety Hybrid Imara (Table 10).

The results also showed that there was significant different (p=0.001) between disease severity in different tomato varieties in Iringa region (Table 6). The highest disease severity was observed on the tomato variety Tanya (20.28%), Rio grande (19.95%) and Eden F1 (15.99%) inoculated with LB followed by tomato variety Mkulima (14.94%) (Table 10). However, statistically, this study indicated that tomato varieties Eden F1, Mkulima, Hybrid Imara and Rio grande had no significant levels of susceptibility/pathogenicity to late blight disease (Table 10).

The results also showed that there was significant different (p=0.01) between disease incidence in different tomato varieties in Arusha region (Table 10). The highest disease incidence (62.7%) was recorded for variety Tanya and the lowest (49.2%) even though was

not significant with other tomato varieties Eden F1, Mkulima, Hybrid Imara and Rio grande was recorded for tomato variety (Table 10).

Table 10: Late blight disease incidence and severity of five tomato variety in Iringa and Arusha

		Mean				Mean	
Region	**Variety**	**Incidence**	**Severity**	**Region**	**Variety**	**Incidence**	**Severity**
Iringa	Hybrid imara	48.43a	15.28a	Arusha	H.imara	44.59a	15.92a
Iringa	Eden F1	48.52a	15.99a	Arusha	Eden F1	46.00a	15.69a
Iringa	Mkulima	48.94a	14.94a	Arusha	Mkulima	47.5a	13.77a
Iringa	Rio grande	48.12a	19.95a	Arusha	Rio grande	49.2a	13.73a
Iringa	Tanya	57.57b	20.28b	Arusha	Tanya	62.7b	18.58b
Grand mean		50.51	16.29		Grand mean	48.8	15.5
LSD		2.6	1.4		LSD	2.98	1.3
Ftest		**	***		F-test	**	***

Values presented are means. **, *** = significant at $P \leq 0.05$, $P \leq 0.001$ respectively. Dissimilar letter(s) in a column have significant difference at ($P = 0.05$) from each other using Fishers Least significance Difference (LSD) test

Results showed that, there was significant difference ($p < 0.001$) between disease incidence and severity on tomato sprayed with different synthetic chemical fungicides. The highest disease incidences (78.70% in Iringa and 78.85% in Arusha) and severity (27.56% in Iringa and 26.92% in arusha) were recorded on tomatoes that were not sprayed with any chemical fungicides (Table 11). Of the fungicides sprayed, fungicides Victory72, Odeon and Banco resulted in the lowest disease incidence and disease severities in all regions (Table 11).

Table 11: Effect of different chemicals fungicide on LBD incidence and severity in Iringa and Arusha regions

		Mean				Mean	
Region	**Variety**	**Incidence**	**Severity**	**Region**	**Variety**	**Incidence**	**Severity**
Iringa	Victory72	42.10a	13.63a	Arusha	Victory72	39.39a	12.48ab
Iringa	Odeon	43.57ab	14.46a	Arusha	Odeon	41.26a	13.66ab
Iringa	Banco	44.41abc	14.20a	Arusha	Banco	42.64ab	14.60b
Iringa	Milthane	46.77bcd	14.15a	Arusha	Milthane	44.98bc	13.77ab
Iringa	Ridomil	47.58cd	13.01a	Arusha	Ridomil	45.91bc	11.87a
Iringa	Ebony 72	48.38d	14.92a	Arusha	Ebony 72	47.59cd	13.78ab
Iringa	Tan Copper	52.60e	18.38b	Arusha	Tan Copper	49.56d	17.2c
Iringa	No Mgt	78.70f	27.56c	Arusha	No Mgt	78.85e	26.92d
Grand mean		50.51	16.29		Grand mean	48.8	15.5
LSD		1.5	1.3		LSD	1.7	1.2
Ftest		***	***		F-test	***	***

Values presented are means*** = significant at P ≤ 0.001. Dissimilar letter(s) in a column have significant difference at (P = 0.05) from each other using Fishers Least significance Difference (LSD) test.

4.1.4 Evaluation of *in-vitro* efficacy of different plant extracts against *Phytophthora infestans*

Using different extracting solvents, the results indicated that there were significant differences ($p \leq 0.001$) between extracting solvents on percentage inhibition of *P. infestans* (Table 12). Methanol was superior in inhibiting mycelia growth of *P. infestans* as compared with ethyl acetate. The result from Table 12 also showed that there was a significance different ($p \leq 0.001$) among the plant extracts on the growth of *P. infestans*. However, all plants showed antifungal activity against *Phytophthora infestans*. From tests of plant extracts, *Plectranthus barbatus* (94±1.0) and *Lantana camara* (91±2.4) were the strongest of all treatment and these were statistical similar to the positive control (97±0.0) (Table 12. In this study, *Tephrosia vogelii* extract showed poor results in inhibiting the mycelia growth of *P. infestans* (16±3.5) as compared with other plant extracts. The negative control did not inhibit the mycelia growth of *P. infestans*.

Interactive effectiveness of the solvent and the plant extracts on inhibition of mycelia growth of the *P. infestans* was observed to be significant different ($p \leq 0.001$). The results revealed that, the combination of solvent and plant extracts strongly inhibited the growth of mycelia of *P. infestans* (Fig. 7). Methanolic extracts from *Plectranthus barbatus*, *L. camara* and *S. suaveolens* inhibited the mycelia growth of the pathogen from 80% and above except *T. vogelii* which inhibited the growth of the pathogen to below 18% as shown in Fig. 7. In ethyl acetate plant extraction, the inhibition percentage was observed to be statistically similar with that of methanolic extract except that the inhibition by *S. suaveolens* was lowered to 70%.

The interactive effect of incubation time and plant extracts was observed and it was found to be significantly different at $p \leq 0.001$. The combination of the incubation time and plant extracts strongly inhibited the growth of mycelia of the *P. infestans*. These results revealed that, the three plant extracts: *Plectranthus barbatus*, *Lantana camara* and *Sphaeranthus suaveolens* were the best on inhibiting the mycelia growth of *P. infestans* and statistically similar between 48 hours and 72 hours. However, the performance of *T. vogelii* in inhibiting the growth of the pathogen significantly decreased at 72 hours as compared with 48 hours as shown in Fig. 8.

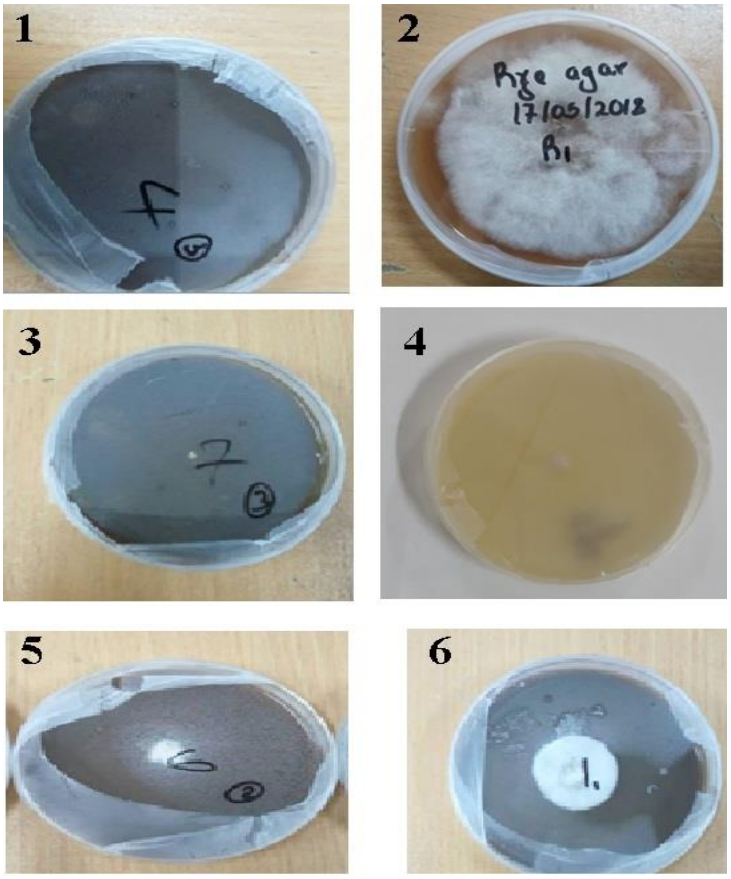

Plate 6: Assessment of the activity of plant extracts in the inhibition of *P. infestans* mycelia growth using Food Poisoning Technique.

Where 1 = Positive control (Otiva chemical fungicide), 2 = Negative control (untreated), 3 = *Plectranthus barbatus*, 4 = *Lantana camara*, 5 = *Sphaeranthus suaveolens*, 6 = *Tephrosia vogelii*.

Table 12: Descriptive statistics Results

Treatment	% Inhibition
Solvent	
Methanol	56±5.4a
Ethyl acetate	55±5.2b
Incubation time	
48 Hours	56±5.1a
72 Hours	56±5.5a
Plant extracts/Treatment	
Negative control	0±0.0d
Positive control	97±0.0a
Tephrosia vogelii	16±3.5c
Plectranthus barbatus	94±1.0a
Sphaerunthus suaveolens	80±3.4b
Lantana camara	91±2.4a
Three - Way ANOVA (F - value)	
Solvent	33.36***
Incubation Time	0.69 ns
Plant Extracts	5257.82***
Solvent*Incubation Time	1.17 ns
Solvent*Plant Extract	19.72***
Incubation Time* Plant Extract	28.95***
Solvent* Incubation Time* Plant Extract	1.32ns

Each value is a mean ± standard error of three replicates, *** = significant at P≤0.001 and ns = not significant. Means within the same column followed by the same letter (s) are not significant different at P = 0.05) based on the Fisher's Least Significant Different (LSD) test.

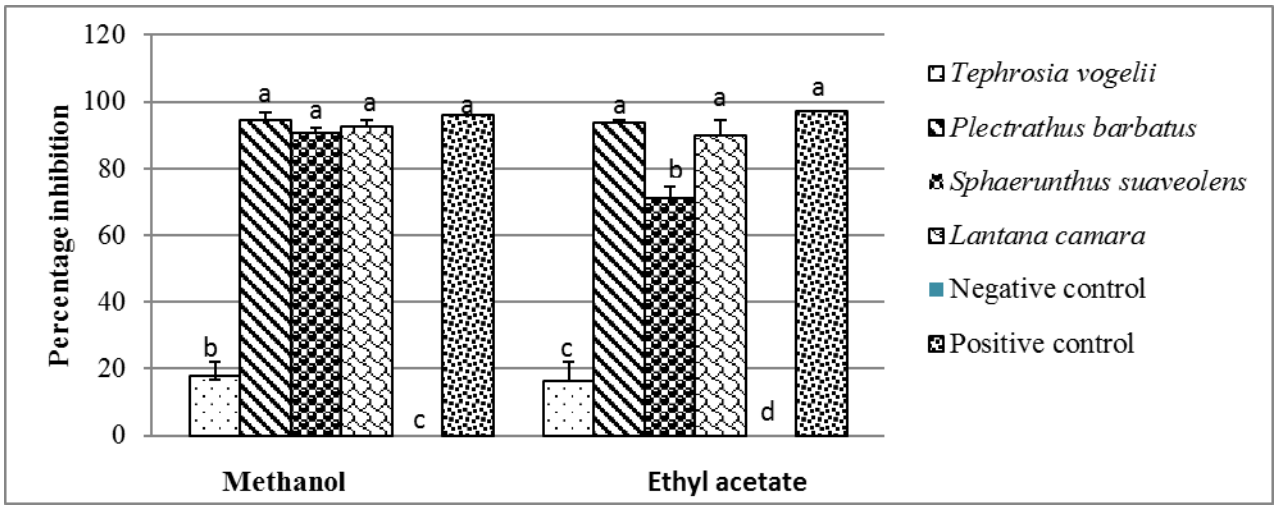

Figure 7: Interaction between the solvent and the plant extract in inhibiting the growth of *P. infestans*

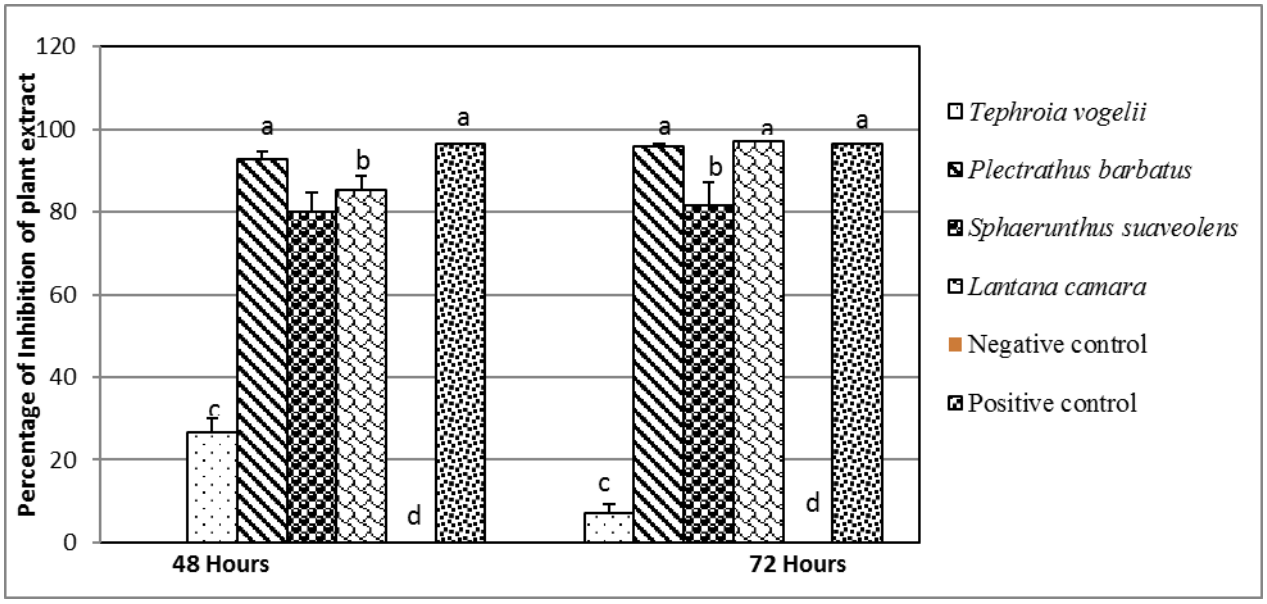

Figure 8: Interaction between the Incubation time and the plant extract in inhibiting the growth of *P.infestans*

4.2 Discussion

The results showed that most of the tomato varieties growing by farmers in the study locations succumbed to the pathogen and disease attack thus and the trend of disease incidence and severity were moderately higher. Of the varieties, Tanya which appeared to be very popular was also very susceptible to the LBD. Such observations open an opportunity for plant breeders to find ways of introgressing resistant traits to Tanya. If such efforts are not

fastened, there will be a possibility that there will be subsequent spread and development of the pathogens and the disease to levels that will affect tomato production (Meya *et al.*, 2014). Farmers in the surveyed areas spray their tomato plants with different chemical fungicides against LBD. The chemical fungicides used by the farmers in the surveyed areas were not able to give a substantial protection to tomato varieties against the development of the LBD in the study areas. Similar observations were reported by Maerere *et al.* (2010) who pointed a possibility that failure of chemical fungicides to protect the plants can be associated with development of resistance by the pathogens. The chemical fungicide used by the farmers against the disease might either be not effective or the farmers were mis-using the chemicals in respect to dosages, fungicide type and/or application methods. However, this situation is not friendly to health of farmers, consumers and the environment Maerere *et al.* (2010). As a follow up on finding reasons to why the LBD incidence was high in farmers' fields despite application of different fungicides, a trial was established with common tomato varies and fungicides and the results portrayed the same trend indicating slightly that the sprayed tomato plants had relatively lower disease incidences and severities compared with the unsprayed.

In this study, a number of fungal isolates were obtained and most those that were positive for pathogenicity testing implied beyond doubt that they organisms isolated from diseased leaves were indeed responsible for the LBD symptoms observed in the field. A proof on identity was made using PCR (Judelson and Tooley, 2000) and amplification of the DNA from the isolated organisms, producing a product size of 600 bp confirmed that the isolates were indeed *P. infestans.*

The nature of chemical solvent used in extraction of the active compounds from the plants has been reported to dictate the quality of extracts yielded (Javaid and Rehman, 2011; Mahlo *et al.*, 2013; Odhiambo *et al.*, 2009). In this study, methanol and ethyl acetate as polar and nonpolar solvent respectively were used in extracting and preparing the crude extracts from the tested plants. Results reveal that, the methanolic plant extract was the best and significantly inhibited the mycelial growth of *P. infestans*. In this study the methanol leaf extract of *L. camara* were effective on inhibiting the growth of *P. infestans*. Such findings are in line with Vashist and Jindal (2012) who reported the tree to have inhibition on fungal activities.

In this study, methanol crude extract was effective in controlling the activities of *P. infestans* as compared with ethyl acetate extracts. This means that methanol was effective in the

extraction of active polar compounds from the plant which was more effective than the nonpolar compounds extracted by ethyl acetate (Azwanida, 2015).

This study has also showed that all plant extracts used in the current study have active compounds with inhibitory effects to the growth of *P. infestans* as compared with the negative control. Such observations were not surprising since different plant parts are known to produce secondary metabolites such as phenolic/flavonoids and terpenes/monoterpenes which inhibit pathogen growth (Ndakidemi and Dakora, 2003; Makoi and Ndakidemi, 2007; Hubert *et al.*, 2013).

The current findings revealed that the *Plectranthus barbatus*, *Lantana camara*, and *S. suaveolens* crude extracts inhibited the mycelial growth of the *P. infestans* and these were statistically similar with the commercial chemical fungicide. Such results are very promising and the tested plants may be used as potential candidate for developing bio-pesticide in controlling *P. infestans* in tomato. Research evidence has shown the presence of different active compounds with antifungal and antibacterial properties in *Plectranthus barbatus* (Musila *et al.*, 2017), *Lantana camara* (Mpumi *et al.*, 2016) and *Sphaeranthus* species (Mahajan *et al.*, 2015).

Tephrosia vogelii extract from this study was poor in inhibiting the mycelial growth of *P. infestans.* Despite of the poor inhibition of the *T. vogelii* on *P. infestans* from this study, other studies have reported the great potential in controlling insect pests in the field and storage (Vashist and Jindal, 2012).

The interactive effects between the extraction solvents and plant crude extracts showed that ethyl acetate significantly lowered the inhibition ability of *S. suaveolens*. Furthermore, the interactive effects between incubation time and plant extracts revealed that, the performance of *T. vogelii* in inhibiting the growth of *P infestans* was significantly decreased at 72 hours of incubation. Further studies must be conducted to establish the mechanism of the interactive effects observed in the current findings.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study has revealed that most of the tomato varieties growing by farmers in the study locations succumbed to the pathogen and disease attack and the trend of disease incidence and severity were moderately higher in all location. Tomato variety Tanya appeared to be very popular; however it was very susceptible to LBD. Isolation of the LBD-causing organisms was made and confirmation by pathogenicity testing and PCR revealed beyond doubt that the LBD symptoms in the field were indeed caused by *P. infestans*. Chemical fungicide application appeared to be common in the study locations; however they did not give a total protection of tomato against LBD.

This study also identified *Plectranthus barbatus, Lantana Camara* and *Sphaeranthus suaveolens* plant extracts as potential candidates for developing bio-fungicides against *Phytophthora infestans* in Tanzania.

5.2 Recommendations

This study identified tomato variety (Tanya) to be very popular in the study location despite the fact that, it was very susceptible to the LBD as compared to other varieties. There is a need for plant breeders to find sources and ways of identifying sources of resistance to LBD in order to introgress those resistant traits to Tanya as step towards LBD management and tomato production in the study locations and Tanzania in general.

This study also showed that, there is a potential for developing natural fungicides based on plant-derived products namely *Plectranthus barbatus, Lantana camara* and *Sphaeranthus suaveolens* for late blight disease control. Such plants are recommended for further studies as candidates for identification of chemical ingredients and formulation of bio-fungicides for managing the LBD-causing pathogen in Tanzania.

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DIFFERENT PLANT EXTRACTS AGAINST *Phytophthora infestans* (MONT.) DE BARY IN TOMATO IN VITRO.

RESEARCH ARTICLE

Rachel .I. Ndala[1, 2], Ernest Rashid Mbega[2] and Patrick. A. Ndakidemi[1]

[1]School of Life Sciences and Bio-engineering the Nelson Mandela African Institution of Science and Technology (NM- AIST), P.O. Box 447, Arusha, Tanzania.

[2]Centre for Research, Agricultural Advancement, Teaching Excellence and Sustainability in Food and Nutrition Security (CREATES- FNS).

*Corresponding author:rachelndal41@gmail.com

Abstract

The objective of this study was to evaluate the efficacy of plant extracts in managing late blight disease in tomato, *in vitro*. Crude extracts were from *Plectranthus barbatus*, *Tephrosia vogelii*, *Sphaeranthus suaveolens* and *Lanatana camara*. These were compared with commercial formulations Otiva fungicide and untreated as negative control. Their effectiveness were determined by measuring the inhibition zone of the mycelial growth of the pathogen recorded in triplicate at 48 hours and 72 hours. The results showed significant differences ($p \leq 0.001$) among the extracting solvents on percentage inhibition of *Phytophthora infestans*. Methanol was superior in inhibiting the growth of mycelial growth of *P. infestans* as compared with ethyl acetate. Furthermore, all plants tested showed antifungal activity against *P. infestans*. The *P. barbatus*, *L. camara* and *S. suaveolens* were comparable with the commercial fungicide in inhibiting the growth of *P. infestans*. In this study, *T. vogelii* extract showed poor results in inhibiting the mycelial growth of *P. infestans* as compared with other plant extracts. Also, it was observed that, there was significant ($p < 0.05$) interactive effects between solvent and plant extracts and between incubation time and plant extracts.

Key words: Tomato, Late blight disease, Plant extracts, inhition percentage.

1. Introduction

Tomato is one of the most important vegetables in the world grown for its different use, fresh market and processing industries [1]. Nutritionally, the crop is a major source of lycopene a powerful anti-oxidant, vitamins such as vitamin A,B,C,D,E,K and mineral nutrients mainly potassium and phosphorus [2]. Tomato grown for fresh tomato is used in making different products such as salads, sauces, stews and puree among others while the processed tomato is only for value addition such as pastes. In tropical Africa, tomato is a major source of income due to its growth cycles , that is, most varieties of tomato have a shorter maturity period and can be harvested more than four times a year [3]. In Tanzania, tomatoes are grown commercially mainly by small scale farmers as a source of income and livelihood [4].

Despite its economic importance, tomato is susceptible to a wide range of diseases such as bacteria [5], fungi [6], viruses [7]and nematodes [8] among others which adversely affect quality, quantity and profitability. Of these diseases, the fungal disease like late blight disease caused by *Phytophthora infestans* (Mont.) de Bary is among the economically important diseases which reduce the quality and quantity of the tomato yield and the losses can go up to 100% [6][9].The disease causes loses in terms of reduced yield, poor quality of fruits and diminished storability [10]. The late blight disease-causing-pathogen is seriously deadly to tomato due to its biology (sexual and asexual reproduction), host range (more than 20 hosts), dispersal mechanism and persistence for a long period of time in the soil [11]. In Tanzania, the average tomato yield is 17.5 tons/ha which is far below the global average yield of 33.6 tons/ha.

Farmers rely on the use of synthetic fungicides to manage late blight diseases of tomato by a combination of protective and curative synthetic fungicides yet the problem is still the challenge [12][13]. In an effort to meet market demands of tomato, farmers have resorted to continuous use of synthetic pesticides. However, there is a growing concern about toxicity of synthetic pesticides due to retention of their residues in the food products [14]. Also synthetic fungicide have negative effects on the environment because of its poor biodegradability leading to pollution, health hazards to the farmers, toxicity to non-target natural enemies, and loss of biodiversity among others [12]. In addition, in the market there is the issue of maximum residue levels (MRLs) of pesticides to the vegetables which disqualify the product with respect to quality standards or requirements in the market [15]. Therefore, integrated crop management such as the use of cultural practices (rogueing), biological practices (fungus) and chemical

fungicides as a last option is important in the cropping systems for disease management [16]. Therefore, introduction of bio-pesticides in tomato production systems will reduce the risks associated with the use of synthetic chemicals [16].

Bio-pesticides are non-toxic, easily biodegradable, safe to non-targets and natural enemies and do not retain residues in the food products [17]. Moreover, some plants with fungicidal activity can inhibit *Phytophthora infestans* making them even better alternatives to synthetic pesticides for sustainable agriculture [15]. The objective of this study was to evaluate the *in vitro* activity and effectiveness of selected plant extracts on *P. infestans* infecting tomato.

2. **Materials and Methods**

2.1. *Isolation and maintenance of Phytophthora infestans of tomato*

Leaves of tomato with infected symptoms of late blight disease were collected from different fields in Iringa and Arusha regions of Tanzania, and used to isolate the pathogen causing late blight disease. In the laboratory the tissues of tomato leaves sample were washed with 2% sodium hypochlorite and 70% ethanol then rinsed with sterilized distilled water for 5 to 10 minutes, followed by blotting of excess moisture with a sterile blotting paper. The clarified V-8 agar (to make 1 litre, V8 juice 200 ml, agar 20 g, and $CaCO_3$ 2 g in 800 ml of dH_2O) amended with Rifampicin and rye agar was used as isolation medium. The pH of the isolation medium was 5.76. The medium was autoclaved at 121°C and 15 psi for 15 minutes, allowed to cool to about 45°C thereafter it was amended with antibiotics (2.5% Pimaricin aqueous solution and 20 mg/L Rifampicin SV sodium salt). Rifampicin was dissolved in small volume of ethanol then mixed with distilled water. Antibiotics were filtered by using ultrafilters-microfilters (Sartorius, Minisart® steril 600 kPa max.) before being added to the growth medium. After amendments, 25 - 30 mL of the medium were poured to each of the Petri dishes and allowed to solidify in the laminar hood. Each sample was divided into 1 cm segments whereby 4 segments were placed equidistantly on each of five Petri dishes (9 mm diameter) containing V8 agar amended with antibiotics. The inoculated plates were I ncubated at 20ºC and observations were made on 3rd day whereby white colonies (cotton-like mycelia) characterized by water-like droplets were observed. The isolated fungi, *Phytophthora infestans* was purified by sub-culturing them onto molten rye agar and incubated at room temperature. Polymerase Chain Reaction (PCR) method use in the identification of the *P. infestans* .The primers that target the Internal Transcribed Spacer (ITS) with 600 bp were used. PINF (CTCGCTACAATAGGAGGGTC) Forward and ITS5b

(GGAAGTAAAAGTCGTAACAAGG) reverse primer were used for PCR-based identification of fungal isolates based on procedure by [18]. The PCR premix was made up of 8.5 µl of water, 0.5 µl of PINF forward, 0.5 µl of ITS5b reverse and 3µl of DNA and, was added mixed in 1.5 ml eppendorf tube.

2.2. *Collection of plant samples*

Five important medicinal plants were selected from different field in Tanzania which has antimicrobial and antifungal activity with guiding principle of different researchers worldwide [13]. The plants collected included *Tephrosia vogelii*, *Plectranthus barbatus*, *Sphaeranthus suaveolens* and *Lantana Camara* as described in Table 1. The parts of the plant collected are only leaves as shown in figure 1. Plant leaves, collected were air-dried (25–27 ◦C) for 10 days.

P. barbatus *L. camara* *S. suaveolens* *T. vogelii*

Figure 1: Pictures of selected plants whose extracts were evaluated for *P.infestans* mycelial inhibition

Table 1: Uses or ailments treated of selected medicinal plants.

Name of plants	Family	Local name	Uses
Tephrosia vogelii	Fabaceae	Vogeli tephrosia/ Tchieuc	-It controls larval stages of mosquitoes and soft-bodied insects and mites -Dried leaves protect stored legume seeds from bruchids
Plectranthus barbatus	Lamiaceae	Coleous, makandi	It is economically important with horticultural, medicinal and food uses and also is used in the antimicrobial activities [19]
Sphaeranthus suaveolens	Asteraceae	Sphaeranthus Indicus(General name)	-Leaves used as traditional medicine as as anti- malaria, antibacterial and antifungal agents [20] Whole plant is drunk as a cough relaxant[20]
Lantana camara	Verbenaceae	Lantana	-Inhibitory and stimulatory biochemical interactions between plants -Essential oil of *Lantana camara* has antibacterial activity against all the bacterial strains [21]

2.2. *Screening bioassay preparation of plant extract*:

Screening of plant extract was conducted in laboratory conditions. For this purpose, 10 g of different plant powder were macerated with 100 ml of distill water. The exudation of biochemicals was kept overnight and biomass was filtered by using of standard Whatman No. 1 filter paper. Inhibition of different extracts to the pathogen (Poison food technique) were also

determined in lab conditions. Plant extract at 3 mL of each stock solution was added in V8 juice media pour sterilized petri plates. The disc of 5 mm diameter of different fungi 3 days old culture were placed at the centre of Petri plates. Three replicates were kept for each treatment and incubated at room temperature. After the three days inoculation, radial growth of mycelium were measured and compared with the results of control. The following formula of inhibition percentage was applied for each fungus in treatment.

$$\text{Inhibition percentage} = \frac{\textit{Colony diameter without extract} - \textit{Colony diameter with extract}}{\textit{Colony diameter without extarct}} \times 100\%$$

2.3. *Extraction and formulation of crude extracts from plant samples*

Crude extracts from plant samples were extracted using the modified method [22][23]. Plant sample materials were blended and for each sample 1 kg of dried extract was dissolved in 1Litre of methanol and ethyl acetate respectively in the ratio of (1:1 wt/vol, dry powder/solvent) followed by soaking for 48 hrs. Then, the concentrate transferred from round flask to universal bottle and passed to nitrogen to remove the extraction solvent and the extract were kept to cool place.

2.4. *Evaluation of antimicrobial activity of crude plant extracts*

Screening of the crude plant extracts for antimicrobial properties was done following modified procedures described by [22][23]. V8 juice medium was prepared and cooled. Plant extracts were then incorporated at a ratio of (1mL extract: 30 mL medium) per petri dish that means 3 mL of the dissolved extract were added to of 90 mL of molten V8 juice media, mixed and then poured equally into three 10 cm^3 petri dishes. After the media had set, 5 mm agar discs cut from 14 day old fungal pathogen cultures were placed at the centre of the plate and incubated at room temperature. Positive control had meia amended with Otiva as chemical fungicide and negative control plates had media not amended with plant extracts. Observations were made at 48 and 72 hours after planting and antifungal activity was determined after measuring the fungal colony radial growth using the following formula shown at 2.2.

3. Data analysis

Growth inhibition (GI) was calculated as per the following formula:

$$GI = \frac{(A - B)}{A} \times 100\%$$

Where Ais the radial diameter of fungus growing on the control plate; andB is the radial diameter of fungus growing on the experimental plate.

All experiments were conducted in triplicates and the data collected on inhibition of mycelial growth of *Phytophthora infestans* were analysed using by 3-way ANOVA (Analysis of variance) using a statistical software STATISTICA. The Fisher's least Significance Difference (LSD) was used to compare treatment at P = 0.05 level of significance.

Results

Effects of Incubation time on percentage inhibition of *P. infestans*

Aftre 5 mm diameter of the pathogen being placed to the centre of the petri dishes containing media and plant extracts the observation as made for 48 hours and 72 hours. There was no significance difference in incubation time on inhibiting the growth of the pathogen (Table 3).

Effects of Plant extracts on percentage inhibition of *P. infestans*

The result from Table 3 showed that there was a significance different ($p \leq 0.001$) among the plant extracts on the growth of *P. infestans*. However, all plants showed antifungal activity against *Phytophthora infestans*. From the tests of plant extracts, *Plectranthus barbatus* (94±1.0) and *Lantana camara* (91±2.4) were the best of all plant treatments and these were statistical similar to the positive control (97±0.0) Table 3. This was followed by *Sphaeranthus suaveolens* (80±3.4). In this study, *Tephrosia vogelii* extract showed poor results in inhibiting the mycelial growth of *P. infestans* (16±3.5) as compared with other plant extracts. The negative control did not inhibit (0 ±0.0) the mycelial growth of *P. infestans*.

Interactive effects between solvent and plant extracts

Interactive effectiveness of the solvent and the plant extracts on inhibition of mycelial growth of the *P. infestans* was observed to be significantly different ($p \leq 0.001$). It was revealed that, the combination of solvent and plant extracts strongly inhibited the growth of mycelia of *P. infestans* (Figure 3). In methanolic plant extracts to all of the three plants *P. barbatus*, *L. camara* and *S. suaveolens* inhibited the mycelial growth of the pathogen from 80% and above except *T. vogelii* which inhibited the growth of the pathogen below 18% as shown in Figure 3. In ethyl acetate plant extraction, the inhibition percentage of was observed to be statistically similar with that of methanolic extract except that the inhibition by *S. suaveolens* was lowered to 70 % .

Interactive effects between incubation time and Plant Extracts

The interactive effect of incubation time and plant extracts was observed and it was found to be significantly different ($p \leq 0.001$). The combination of the incubation time and plant extracts strongly inhibited the growth of mycelia of the *P. infestans*. Our results revealed that, the three plant extracts, *Plectranthus barbatus, Lantana camara* and *Sphaeranthus suaveolens* were the best on inhibiting the mycelial growth of *P. infestans* and statistically similar between 48 hours and 72 hours. However, the performance of *T. vogelii* in inhibiting the growth of the *P. infestans* significantly decreased at 72 hours as compared with 48 hours as shown in Figure 4.

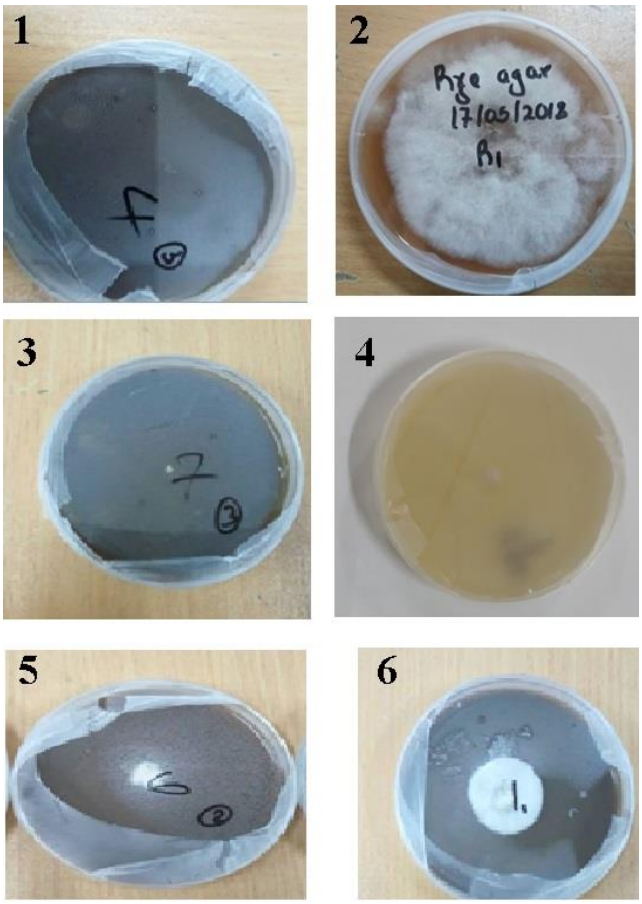

Figure 2: Assessment of the activity of plant extracts in the inhibition of *P.infestans* mycelia growth using Food Poison Technique. Where 1=Positive control, Otiva

chemical fungicide, 2= Negative contro, untreated, 3= *Plectranthus barbatus*, 4= *Lantana camara*, 5= *Sphaeranthus suaveolens*,6= *Tephrosia vogelii*.

Table 3: Descriptive statistics Results

TREATMENT	% INHIBITION
SOLVENT	
Methanol	56±5.4a
Ethyl acetate	55±5.2b
INCUBATION TIME	
48 Hours	56±5.1a
72 Hours	56±5.5a
PLANT EXTRACTS/TREATMENT	
Negative control	0±0.0d
Positive control	97±0.0a
Tephrosia vogelii	16±3.5c
Plectranthus barbatus	94±1.0a
Sphaeranthus suaveolens	80±3.4b
Lantana camara	91±2.4a
3 - WAY ANOVA (F VALUE)	
Solvent	33.36***
Incubation Time	0.69 ns
Plant Extracts	5257.82***
Solvent*Incubation Time	1.17 ns
Solvent*Plant Extract	19.72***
Incubation Time* Plant Extract	28.95***
Solvent* Incubation Time* Plant Extract	1.32ns

Each value is a mean ± standard error of three replicates, *** = significant at P≤0.001 and ns = not significant. Means within the same column followed by the same letter (s) are not not significantly different at (P=0.05) from each other using Fishers Least Significant Different (LSD) test.

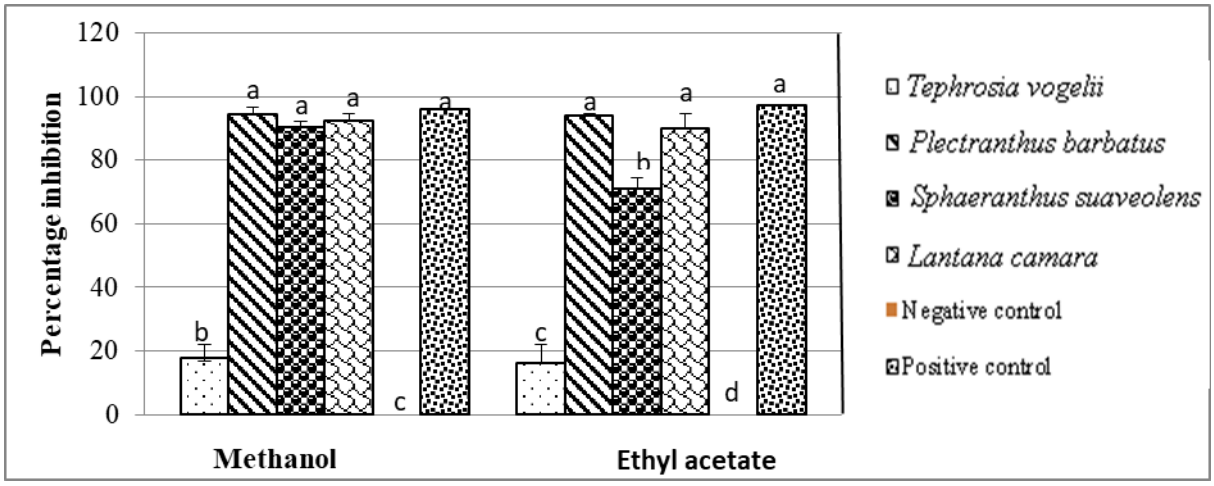

Figure 3: Interaction between the solvent and the plant extract in inhibiting the growth of *P. infestans*

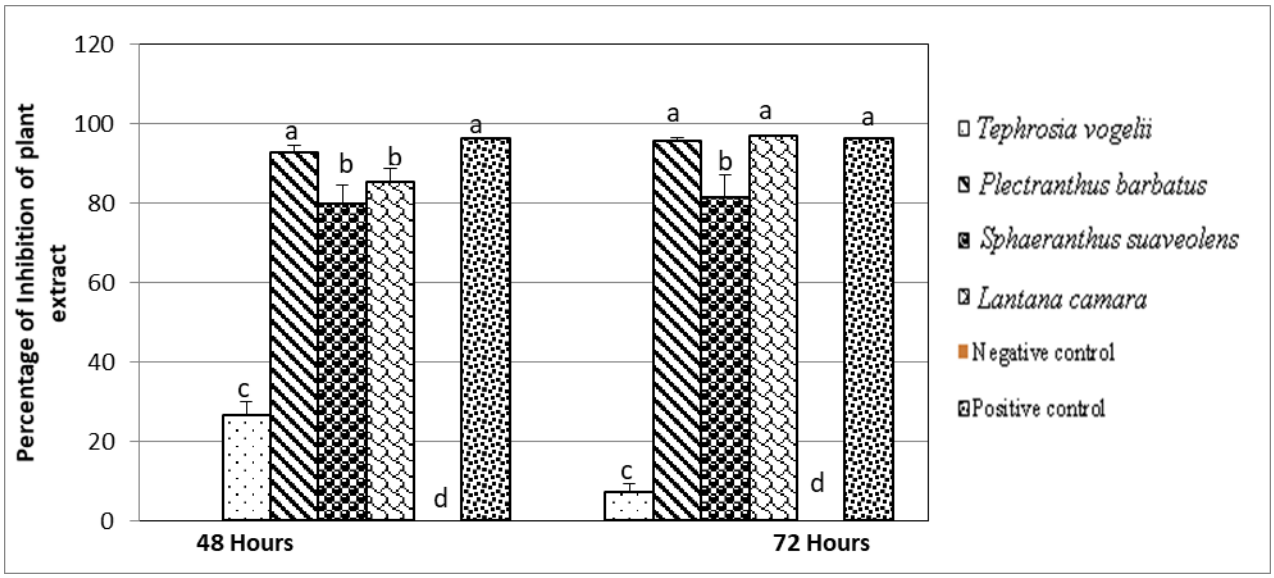

Figure 4: Interaction between the Incubation time and the plant extract in inhibiting the growth of *P. infestans*

Discussion

Effects of Methanol and Ethyl acetate extracting solvent on percentage inhibition of *P. infestans*

The nature of chemical solvent used in extracting the active compounds from the plants may dictate the quality of extracts yielded [24][25][26][27]. In this study, methanol and ethyl acetate as polar and nonpolar solvent were used in extracting and preparing the crude extracts from the tested plants. Results from Table 3 reveals that, the methanolic plant extract was the best and significantly inhibited the mycelial growth of *P. infestans*. Similar to our results, the methanol leaf extract of *L. camara* reported to have better inhibition to tested fungal activities [28]. Also, [19] also reported that crude extracts of *P. barbatus* had the lowest MIC values against the test microbes compared to the other this is because the methanol was not used as the extracting solvent. From our study, methanol crude extract was effective in controlling the activities of *P. infestans* as compared with ethyl acetate extracts. This means that methanol was effective in the extraction of active polar compounds from the plant which were more effective than the nonpolar compounds extracted by ethyl acetate [29].

Effects of Plant extract on percentage inhibition of *P. infestans*

The results of the study showed that all plant extract have active compounds with inhibitory effects to the growth of *P .infestans* as compared with the negative control. Different plant parts are known to produce secondary metabolites such as phenolic/flavonoids and terpenes/monoterpenes which inhibit pathogen growth [30][31][32].

Our findings revealed that the *Plectranthus barbatus*, *Lantana camara*, and *S. suaveolens* crude extracts inhibited the mycelial growth of the *P. infestans* and these were statistically similar with the commercial chemical fungicide. Such results are very promising and may be used in future to develope potential candidate pesticide in controlling *P. infestans* in tomato and potato. Reseach evidence has shown the presence of different active compounds with antifungal and antibacteril properties in *Plectranthus barbatus* [19], *Lantana camara* [33] and *Sphaeranthus* species [20].

T. vogelii extract from this study was poor in inhibiting the mycelial growth of *P. infestans.* Despite of the poor inhibition of the *T.vogelii* on *P.infestans* from this study, other studies have reported the great potential in controling insect pests in the field and storage [28].

The interactive effects between the extraction solvents and plant crude extracts showed that ethyl acetate significantly lowered the inhibition ability of *S. suaveolens.* Furthermore, the interactive effects between incubation time and plant extracts revealed that, the performance of *T. vogelii* in inhibiting the growth of *P infestans* was significantly decreased at 72 hours of incubation. Further studies must be conducted to establish the mechanism of the interactive effects observed in our findings.

CONCLUSION

This study shows that, there is a potential for developing natural fungicides based on plant-derived products for late blight control. The *Plectranthus barbatus, Lantana Camara* and *Sphaeranthus suaveolens* plant extracts were effective as commercial synthetic pesticide in reducing the growth of *Phytophthora infestans* and hence can be used alone as alternative to chemical fungicide. *T.vogelii* extract was not efficiency on inhiting the mycelial growth of the pathogen, and hence it is not advocated in this purpose.

Acknowledgments

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STATUS AND MANAGEMENT OPTIONS OF *PHYTOPHTHORA INFESTANS, A CAUSAL AGENT OF THE* LATE BLIGHT DISEASE OF TOMATO, IN TROPICAL AFRICA

R. I. Ndala[1, 2] P. A. Ndakidemi[1,] and E. R. Mbega[2].

[1]School of Life sciences and Engineering, [2]Centre for Research, Agricultural Advancement, Teaching Excellence and Sustainability in Food and Nutrition Security (CREATES- FNS), Nelson Mandela African Institution of Science and Technology (NM- AIST), P.O. Box 447, Arusha, Tanzania.

Corresponding Author E-mail addresses:rachelndal41@gmail.com

ABSTRACT

Late blight disease caused by *Phytophthora infestans* (Mont.) de Bary is a serious challenge in tomato (*Solanum lycopersicum* L or *Lycopersicon esculentum* L) production worldwide. In tropical Africa, the disease is increasing always due to its biology and different complex infection mechanism(s) used by its causative pathogen to survive, spread, and invade the tomato host plant and environment. Management options of the disease, such as integrated disease management (IPM), use of resistant tomato varieties, use of plant extracts and synthetic chemical pesticides have been recommended but are either poorly practised or limited in tropical Africa. Thus, this review discusses the status and commonly recommended management options against late blight disease-causing pathogen(s) including the pathogen strain complexes so that tomato growers and other stakeholders can understand specific strains and how they can design appropriate managerial approaches to halt challenges of the disease in tomato production in the tropical Africa.

Key words: Host range, Pathogen infection Mechanism, Sign and Symptoms and Plant extract.

INTRODUCTION

Tomato (*Solanum lycopersicum* L. or *Lycopersicon esculentum* L.) is one of major vegetable crops grown for both nutritional and economical values worldwide (FAOSTAT, 2011; Davis, 2006; Jones, 2007). Nutritionally, tomatoes are a major source of lycopene a powerful antioxidant, vitamins C, beta-carotene (vitamin A), biotin (Vitamin B), vitamin K, vitamin B6 (Pyridoxine), niacin (vitamin B3), vitamin E and mineral nutrients mainly potassium, copper, manganese and phosphorus(Mitchell *et al*., 2007; Bhowmik *et al*., 2012).

Despite its economic importance, tomato is susceptible to a wide range of diseases, such as bacterial wilt (*Ralstonia solanacearum*) (Aloyce *et al*., 2017), verticillium wilt (*Verticillium dahliae*), early blight (*Altanaria solanii*), and late blight (*Phytophthora infestans*) among others (Foolad, 2007; Kamoun, 2007). Of these diseases, the late blight disease caused by *Phytophthora infestans* (*P. infestans)*(Mont.) de Bary is the most significant and economically important disease causing over 50% loss in the tropical Africa (Fontem *et al*., 2005). The late blight-causing pathogen also causes massive destruction in others crops in the solanacea family such as potato, eggplant, pear melon and tree tomato (Agrios, 2005; Nowicki *et al*., 2013).

The late blight disease causes loses in terms of reduced crop yield, poor quality of fruits and diminished storability (Nowicki *et al*., 2012). The late blight disease-causing-pathogen is seriously deadly to tomato due to its biology, wide host range and difficulties in managing it. This article, therefore, reviews the status, biology of the tomato late blight pathogen, its host range, and management options under African tropical climatic conditions. The information that is discussed here will be vital for accurate understanding of the causes, host range and how the pathogen can be managed within the tropical African context.

Status of tomato late blight disease in tropical Africa: In the tropical Africa, late blight disease is constantly re-emerging in every growing season and where it occurs, it spreads very rapidly within the leaves and fruits of the infected plants in the field, and consequently, spreading out to the entire field causing economic loss and often total destruction of the crops in the field (Agrios, 2005). Furthermore, the pathogen has ability to reproduce both asexually and sexually (Fig 1.) and has different kinds of strains which allow faster development in the field (Nowicki *et al*., 2012). The sexual and asexual life cycles make the infection by *P. infestans* on tomato and other hosts to be very successful in the tropical environments (Nowicki *et al*., 2012). The asexual form of the *P. infestans* serves as a major vehicle driving epidemics during the growing seasons whereas the sexual form proliferate during the off-seasons (Foolad *et al*., 2008). After attack, the pathogen kills the leaves, stems and fruits of the affected host plant (Agrios, 2005).

Disease management involving cultural, biological and chemical control have been recommended but the disease is still challenging due

to re-emergence of *P. infestans* with its enhanced ability to develop more virulent isolates through sexual recombination (Agrios, 2005). Chemical control which include synthetic fungicides such as metalaxyl spray are among the most widely used methods, however, they are ineffective to new strains of the oomycete produced as recombinants of fertilization (Jackson, 2010). The fungicides are also costly to be afforded by most poor farmers, are challenged by development of resistance by the different *P. infestans* strains, and they have detrimental effects to human health and the environment. In addition, variation in pathogen strains (Table 1) further complicates not only management options but also how to correctly identify them. Therefore, there is a need to use improved methods such as Polymerase Chain Reaction (PCR) for accurate identification of pathogen strains involved in order to design strain-specific management options for tropical Africa.

Biology of *P. infestans*

Pathogen description: The name *Phytophthora infestans* literally means, "Plant destroyer". It is a fungus-like organism classified under Oomycetes in the kingdom Chromista and order Peronosporales (Nelson, 2008). It causes late blight disease in solanaceae plants especially during cool and moist period worldwide (Fry *et al*., 2013). There are many different types of pathogen strains that genetically vary between their virulence and mating types (Table 1) (Kamoun *et al*., 2015).

The pathogen is commonly described as coenocytic oomycete with rare cross walls (Kamoun *et al*., 2015). Sporangia are 29-36 x 19-22 µm and are ellipsoid to lemon shaped with a small pedicel. In culture, the pathogen mycelium is white and fluffy (Rumpf *et al*., 2010). The primary sources of *P. infestans* inoculum originate from infected seed; diseased weed hosts and or plant remain via asexual population (Kamoun and Smart, 2005).

Table1. Some of different strains with different mating type of *P. infestans* in tropical Africa (Tomura *et al*., 2017).

Pathogen type	Strain	Mating type
P. infestans	9173, 9174, PI0-1, PI1234-1	$A1^e$, A1,$A2^e$, $A1^e$ and $A2^e$
Phytophthora sp	32716	$A1^e$
P. vignae	30473, 30613	Homothallic
P. nicotianae	ATCC 38606 and ATCC 38607	(A2 mating type and (A1 mating type)

Host range of the *P. infestans*: There are over 120 host species of the *P. infestans* pathogen including tomato, eggplant, potato and others (Table 2). On those hosts, the pathogen is capable to cause late blight disease at different growth stages and parts of the plants such as tissues, roots, tubers, herbaceous stems, woody trunks, foliages and fruits (Erwin and Ribeiro 1996; Martin *et al*., 2014). Among these plants, tomatoes and potatoes have been described to be the main hosts of the *P. infestans* while others are described as alternative hosts as shown in Table 2 below.

The existence of this wide host range for the *P. infestans* complicates designing of appropriate management options in tomato. There is need to clearly identify the remaining hosts of the pathogens and their associated strains so that appropriate management options may be designed and applied for managing the late blight disease in tropical Africa

Reproduction cycles of the *P. infestans:* The pathogen has two types of reproduction cycles (sexual and asexual), both of which have been described in three stages A-C (Fig 1). In stage A, a fully effective organism is on the host and the pathogen follows either of the two modes of reproduction depending on the environmental conditions (Foolad *et al*., 2008). If it goes through asexual reproduction, the pathogen sporangia germinates and releases zoospores which can then move chemo-tactically (in C) within the environment or directly through sporangia by wind or water. If it follows sexual reproduction in B, the pathogen produces two mating hormones α1 and α2, example for mating type A1 and A2 respectively which aid in the production of oospores (Tomura *et al*., 2017). In stage C, mating of A1 andA2 take place and secreted mating hormones α2 (from A2) and α1(from A1) induce the sexual reproduction of the counter mating types A1 and A2, respectively hence production of oospore.

Infection mechanism of the pathogen and late blight disease development: For the pathogen (*P. infestans*) to effectively infect and colonize its host, a sequence of pathogenic processes is necessary (Fig. 2) (Whisson *et al*., 2016). In series, the pathogen sporangium or zoospore has to be formed followed by cyst formation, then germination of the cyst/spore to form appressorium which, enhances penetration unto host and infection vesicle, then intercellular hyphal growth and haustorium formation and initiation of sporulation (Huitema *et al*., 2004; Kamoun, 2001; Hardham, 2007; Nowicki *et al*., 2013). If these stages are successful, then colonisation on plant can be considered successful otherwise not based on some form of host resistance or interference by some environmental factors such as application of chemicals to control the pathogen or other nature based climatic conditions (Shimony and

Friend, 1975; Mendgen and Hahn, 2002; Whisson *et al*., 2016).

Table 2. Common hosts *P. infestans*.

Host	type of host	Reference
Tomato(*L.esculentus*)	Main	Erwin and Ribeiro, 1996
Potato(*Solanumtuberosum*)	Main	Erwin and Ribeiro,1996
Tree tomato (*S. betaceum*)	Alternative	Erselius *et al*., 2002
Pear melon(*S. muricatum*)	Alternative	Turkensteen 1978; Abad *et al*., 1995
Nolanaspecies	Alternative	Erselius *et al*., 2003
Eggplant(*S.melongena*)	Alternative	Hooker, 1981
Wild Solanum species	Alternative	Abad *et al*., 1995
Naranjilla(*S. quitoense*)	Alternative	Erselius *et al*., 2003
Datura species	Alternative	Sunita and Sen, 1997

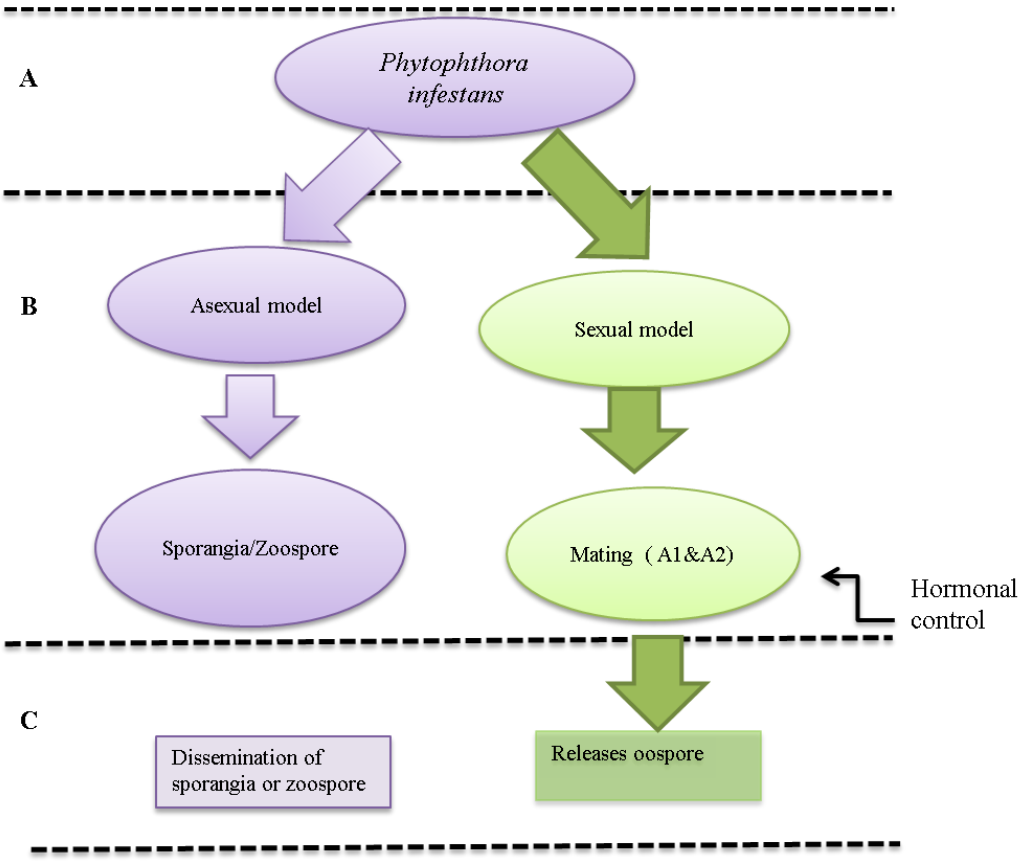

Figure 1. Reproduction of *P. infestans*.

For successful colonisation, the pathogens' infection vesicle is supported by different virulence (AVR) and resistant (R) genes (Huitema *et al*., 2004; Beyer *et al*., 2005: Jones and Dangl, 2006). In addition, the infection process involves secretion of different protein molecules which enable attachment of *P. infestans* to the host and breaking down physical defence barriers of the host plants (Huitema *et al*., 2004).

On and in the host tissue, *P.infestans* suppresses the plant immunity through secretion of the effector proteins commonly known as apoplastic effectors (those formed on the outside) and cytoplasmic effectors (those formed inside) (Lo Presti *et al.*, 2015). Successful colonisation by the pathogen leads unto symptom development and effects as described in Table 3.

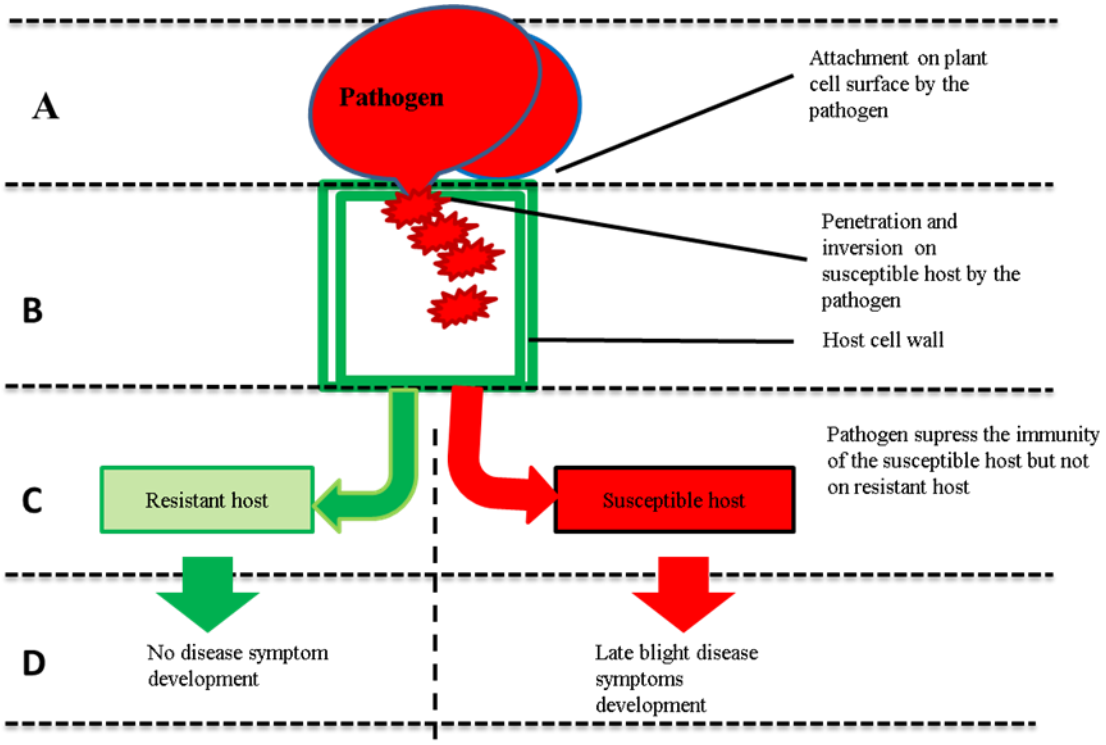

Figure 2. Stages of late blight disease development. In this model, two possibilities can occur upon pathogen contact to host as described in stages A-D. In stage A: The pathogen from different sources attaches the host, B: Invasion stage, C: Molecule exchange between pathogen and host where the pathogen supress the host immunity, D: There is a successful in susceptible variety and fail to colonize a resistant host. In susceptible host cell wall apposition is absent, the pathogen colonizes and supress then develops the late blight symptoms as showed in Table 3 below.

Table 3. Symptoms and or signs incited by *P. infestans* and their effects on different parts of the host tomato plant.

Part of plant	Symptoms and Signs	Effect	References
Leaves	Necrosis, shrivelling, brown colouration, powdery and whitish rings	Reduced total number of photosynthetic cells	(Wallance, 2014; Restrepo *et al*., 2015).
Petiole and stem	Elongated, blackened Water-soaked spots. Lesions covered with a grey	Reduced growth, infected fruitlets	Nelson, 2008).
Fruits	Circular greasy lesions A thin layer of white mycelium	Total plant failure to reach maturity	(Agrios, 2005)

Management of late blight disease: There exist a number of management options that are used against late blight disease (Table 4). The most important ones are as discussed in the sections below.

Cultural control: Cultural practices such as timely weeding, crop rotation, elimination of volunteer tomato plants and planting clean seedling are major components of growers' strategy in disease management; and these methods can sometimes limit the disease development to economical threshold levels. The cultural methods can prove useful in preventing introduction of inoculum to healthy transplants and prevent development or spread of inoculum between and within the field (Garrett and Dendy, 2001; Agrios, 2005). The principle behind the cultural practises is field sanitation i.e. keeping the field clean (Kirk, 2009). A good example for applicability of this principle include three major practices (1) rogueing, to prevent the spread of diseases from one plant to another (Kirk, 2009) (2) Timely planting, to create unfavourable environment condition for the growth and development of the pathogen and avoiding the rainy season (Olanya *et al*., 2001) (3) mulching, this is very important method after weeding so as to prevent the spreading of the disease though rain splash (Olanya *et al*., 2001). However, in the tropical Africa, there exist

challenges is using cultural control options including this approach making it ineffective. The challenges raised include (a) farmers' poor understanding of different factors that could lead to disease build up such use of unimproved susceptible tomato varieties, improper destruction of infected plant debris and continuous mono-cropping, (b) existence of a wide-host range for the pathogen within farms proximity (c) small-scale farming with mixed cropping options and (d) existence of different pathogen strains near or within farmer's field. Therefore, there is need to educate farmers and design specific management recommendation which can be suitable for managing late blight in the complex tropical African environment such as manipulation of planting date, planting density and intercropping that are often used to reduce build-up of air-borne inoculum as well as an escape mechanisms for the late blight disease.

Fungicide application: This is the main approach used at global level in the management of late blight in tomato and potato (Kamoun and Smart, 2005). For the chemical application to be effective, it must be applied before infection (Beckerman, 2008). In the tropical Africa, chemical application is the only most common and preferred approach though not always possible to small scale farmers due to high cost and those who afford it either do not follow recommended rates of application or lack knowledge of handling the chemicals. Common fungicides used in the tropical Africa are chlorothalonil, which is usually applied before or upon disease development and metalaxyl, which inhibit or reduce disease progress once symptoms are apparent due to its ability to inhibit ribosomal RNA (rRNA) polymerases in fungi. The metalaxyl is capable of reducing incorporation of uridine which is the major cause of disease occurrence (Gisi*et al.* 2011). Pre-treatment of tomato plants with the chemical such as dl-3-amino-butyric acid induce systemic-acquired resistance in the tomatoes, protecting them from late blight infection through inhibition of haustoria formation and growth of the *P. infestans* hyphae (Tsedaley, 2014). However, these applications increase production cost and the potential for human health and environmental risks associated with fungicide residual. Some of examples of chemical fungicide reported includes dithiocarbamates, such as mancozeb, which break down into carcinogens causing liver and thyroid tumours, and testicular effects (Novikova *et al*., 2003; USA-EPA, 2004; Meya *et al*., 2014). Also chemical applications are associated with several challenges mainly, development of resistance by pathogens and also are associated with negative impacts to non-target organisms and pollution to the environment (Shtienberg *et al*., 1994; Cohen, 2002; Kamou *et al*., 2015). Therefore, alternative method which is economical feasible, health safety and environmental friendly is required in sustainable management of the *P. infestans* pathogen in tomato.

Host-plant resistance: Since the outbreak of the late blight (LB) disease to potato in 1840s, the concern of developing LB resistant potato and tomato cultivars has been of great interest (Nowicki, 2012). Since then, research evidence has shown that resistance to *P. infestans* both race-specific resistance (i.e specific, vertical, or "gene-for gene" interaction) and race-non-specific resistance (i.e, horizontal, or partial resistance) can be developed (Nowicki, 2012). Some reports show that, tomato with disease resistance ability by vertical resistance have been developed and are effective (at least initially) in avoiding growth and development of pathogen to the crop (Fry, 2008).

The pathogen has a tendency of re-emergence with more virulent strains leading to more aggressive lineages and this makes the resistance gene to be effective in only one or a limited number of pathogenic races (Fry, 2008). In comparison to vertical resistance, race-nonspecific resistance is often controlled by several genes or quantitative trait loci (QTLs), which is partial resistance against multiple races of the pathogen. This is always slow and cannot stop the progress of the disease since LB disease spread quickly (Fry, 2008).

The cornerstone of disease management is the use of resistant crop cultivars but, durable resistance to late blight has not been available to growers, particularly in varieties that are in high demand by consumers (Kamoun and Smart, 2005). As a result, most of different susceptible cultivars are still being grown due to cultural and economic values such as variety popularity, factors which limit adoption of new or resistant varieties such as palatability, colour and shape of fruits (Walker *et al*., 2003). Host resistance to late blight has been reported as an effective strategy in the management of late blight and have long-term economic benefits for small-scale farmers. It also reduces the ability of pathogen to resist fungicide by minimizing changes in the population structure of *P. infestans* (Sharma *et al*., 2015).

The use of host plants (cultivar) with very high resistance has proved to be helpful in reducing the amount of fungicides (Jones 1998). Late blight in tomato is controlled by dominant gene (Nowicki *et al*., 2012). For example, Ph-1, a completely dominant gene and is known for conferring resistance against tomato race-0 (T0), however, it was rapidly overcome by new races of the pathogen. The Ph-2 gene provides only partial resistance to tomato plants. A disadvantage is that instead of blocking the disease, Ph-2 only reduces its development rate and hence may not be effective when more aggressive isolates are present. Resistance provided by Ph-3, on the other hand, has been reported to be considerably effective against a wide range of *P.infestans* isolates. In terms of inheritance, both Ph-2 and Ph-3 display incomplete dominance (Nowicki *et al*., 2012). As reported elsewhere, race change in *P. infestans* in

common and has complicated the development of plants which are fully tolerant to this disease. However, tomato varieties reported to have a reasonable resistance to late blight resistance in Africa are not exceptional as they are also subjected to partial protection due to race changes. Therefore, breeding programs should rely on more durable resistance mechanisms such as the introgression of several resistance genes (quantitative resistance) (Elsayed *et al*., 2011).

Therefore, it is vital that during screening for host resistance, it is better to consider farmer preference, high demand varieties, and economical factor so that the developed varieties improve the farmer's challenges faced in LB disease.

Biological control: Researchers have investigated the use of biological control as a potential strategy and solution to the late blight problem (Martinez, 2012; Nega, 2014). Biological control includes the use of different microorganisms such as bacteria, fungi, nematodes and viruses (Shuping and Eloff, 2017). The great potential use of microorganism in management of late blight disease is based on the antagonistic nature of microbes to *P. infestans* (Berg and Hallmann, 2006; Shuping and Eloff, 2017). Also, the use of the biological control is environmental friendly, the microorganism are available to the surrounding and its safety in human health. One of the most cited microorganisms that have been used for biological antagonistic against the *P. infestans* include fungi known as *Trichoderma harzianum* (Fatima *et al.,* 2015). The *T. harzianum* has been reported to be effective in reducing incidence of *P. infestans* through secretion of different antifungal substances of various toxic substances and antibiotic metabolites which are involved in the inhibition and lysis of pathogenic fungi (Lorito *et al*., 1993). Other fungi inhibiting *P.infestans* include *Gliocladium spp* and *Penicillium funiculosum* (Fatima *et al*., 2015). Also, there is complementarity of bacteria with biocontrol activity from the genera Bacillus, Pseudomonas, Rahnella and Serratia (Daayf *et al*., 2003). For example, Stephan *et al*. (2005) reported that metabolites from *Bacillus subtilis* have bio control abilities and antagonistic activities against late blight in tomatoes (Júnior *et al*., 2006). In view of the reported successes on the use of biological control agents in other parts of the world, similar approach could significantly be deployed in Africa to control late blight disease in Tomato. This is due to the fact that the microbes can easily be isolated from local environments and used sustainably without harm to non-target organisms and environment.

Nevertheless, in tropical Africa, limited information is available on use of biological control in managing late blight disease. The identification of the strain of the *P. infestans* is needed so that to increase the accuracy for the specific microbe or organism to the specific strain of the *P.infestans* rather than general group of pathogen strains with no identification.

Plant extracts: Plant extracts are increasingly being explored for managing plant diseases due to the fact that they are portrayed as environmentally friendly and safe to humans contrast to synthetic fungicide (Varma and Dubey, 1999; Goufo *et al*., 2008). They contain active natural chemical that are effective in crop protection against the pathogen (Hubert *et al*., 2013; Ndakidemi and Dakora 2003; Makoi and Ndakidemi, 2007). Plant extracts are known to produce secondary metabolites such as phenolic/flavonoids and terpenes/monoterpenes which inhibit fungi, bacteria, and insects under laboratory, screen house and field tests (Wang *et al*., 2007; Hubert *et al*., 2013; Ndakidemi and Dakora, 2003).

Studies have shown that the use of synthetic fungicides in managing late blight disease levels were comparable with that of plant extracts in Cameroon and Kenya (Geraldin, 2016; Goufo *et al*., 2010). Extracts from different plant such as *Tephrosiavogelli* and *Ageratum houstuianum* among others have been reported to be effective in reducing late blight severity (Goufo *et al*., 2010). Also the extract from *Ocimum gratissimum, Cupressus benthamii* and *Vetiveria zizanioides* have been reported to have inhibitory capacity against the late blight fungal pathogens (Goufo *et al*., 2008; Goufo *et al*., 2010). A study in human model showed that plant extracts from *Sphaeranthus suaveolens*, exhibited remarkable strong antifungal activities. This prompted an idea of their possible action in controlling fungal pathogens such as *P. infestans* in tomato. Although the reported plants were effective against fungal pathogens in plants, screening them against P. *infestans* will reveal their potential in combatting this disease.

The ability and effectiveness of different plant extracts in reducing disease levels in plants has been reported to be related to the mode of action of the plant extracts (Nashwa and Abo-Elyousr, 2012). Some plant extract act directly on the pathogens while others induce systemic resistance in host plants to reduce disease occurrence and development (Nashwa, 2011). Also the modes of action of the plant extracts are comparable with those of the synthetic fungicides as previously described and also as per report by (Geraldin, 2016). As far as tropical Africa is concerned, use of plant extract is based on indigenous knowledge but very little has been documented (Olanya *et al*., 2012). This thus calls for research to quantify use and potential of plant extracts in managing late blight disease in tomato in tropical Africa.

Integrated Disease Management (IDM): Integrated disease management has been cited to be among the best method in late blight disease management in tropical regions where fungal inocula are abundant in

most months of the year (*Olanya et al*., 2004). A mode IDM package includes cultural practices (early planting and the use of improved crop variety (early and mid-maturity variety, good crop husbandry), tolerant variety and reduction of frequency of synthetic chemical application (Agrios, 2005). Other examples of the IDM package includes: sowing of disease-free seed; eliminating diseased tomato plants, maintaining weed free environments and minimised chemical sprays (Kirk *et al*., 2013). The IDM can also include other different combined approaches consisting of cultural practices; host plant resistance, biological control and others (Makoi and Ndakidemi 2007) have been reported to help farmers reduce the use of chemical fungicides. Although this approach is always encouraged, proper understanding by small scale farmers on how to create a balance of the procedures especially on which to start and when is always associated with some technical flow that is not always easily adopted in tropical Africa. Thus, there is need to train farmers on how to effectively adopt the IDM techniques, which however calls for research to develop cost effective farmers' based recommendations suitable for managing the late blight in different location in tropical Africa.

Table 04. Management option of late blight disease.

Method(s)	Strengths	Weakness
Cultural	They play a greater role in maintaining the soil health fertility, nutrient recycling through crop rotation and intercropping (Altieri, 1999; Abawi and Widmer, 2000; Huber and Haneklaus, 2007).	They are effective when used in combination with other methods (Fravel, 2005; Nowicki *et al*., 2012).
Chemical	Fast effect on pathogens leading to increased yield production such as the use of fungicide with mancozeb and metalaxyl	It is temporary (Lyr, 1995). They are not able to cure existing symptoms (Beckerman, 2008).
Integrated disease management(IDM)	They are cheap options to late blight management, IDM package include the use of cultural, biological and fungicide application(Martin *et al*., 1994; Kirk ,2009; Kirk *et al*., 2013)	It depends on a crop production system (Agrios, 2005)
Biological	Sustainable and environmental friendly, pathogen specific (Emmert and Handelsman, 1999)	Developing the methodology takes a long time
Resistant varieties	The constitutive defence makes the plant to thicken its cuticle and constitutively produce secondary metabolites which prevent the pathogen from attacking the plant (Niks and Rubiales,2002)	Breeding for late blight resistance is a slow process (Kim and Mutschler, 2005; Matlon and Minot, 2007).

Conclusion: Worldwide, tomato is considered as the most important vegetable food crop due to its beneficial nutritional and economical roles. However, tomato is highly susceptible to late blight disease causing low production of the crop. In tropical Africa, the disease is a serious challenge to production. Management of the disease is limited by biology, wide host range and environment conditions under which the pathogen works. Despite a number of management options that have been discussed in this review including cultural, resistant varieties, biological, chemical, integrated disease management and use of plant extracts, only one management option (synthetic chemical) is commonly used despite its harmful effect to target and non-target environment and cost involved. However, this review has also shown that use of plants extracts is increasingly important, thus there is need to conduct research on pathogen identification, selected plant in management late blight disease in tomato within the context of tropical Africa.

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Output 2: Poster Presentation

DETECTION AND MANAGEMENT OF *Phytophthora infestans* ON TOMATO IN IRINGA AND ARUSHA REGION, TANZANIA

Rachel I. Ndala , Patrick Ndakidemi and Ernest Mbega

Nelson Mandela African Institution of Science and Technology, School of Life Science and Bio-Engineering P.0.Box 447, Arusha, Tanzania.

Introduction

Tomato (*Lycopersicon esculentum* L) is grown for both nutritional and economical values worldwide. Late blight disease caused by *Phytophthora infestans* (Mont.) de Bary y important disease causing over 50% loss in the tropical Africa (Fontem *et al.*, 2005).

In Tanzania, the average tomato yield is 17.5 tons/ha which is far below the global average yield of 33.6 tons/ha (Aloyce *et al.*, 2017).

With advancement in molecular technologies in studying pathogen systems, it appears obviously and convincingly that Polymerase chain reaction (PCR)- based method are more suitable and accurate in the detection of plant pathogens (Aloyce *et al.*, 2017).

The extensive uses of synthetic insecticide for the past years have disrupted natural enemies as a result of bioaccumulation and bio-magnification effects.

These have necessitated the need to search for novel compounds with unique mechanism of action that would target physiological processes that are crucial to pathogen survival.

It is in this vein that *selected* leaf extracts which is used by local communities in Tanzania for management of *P. infestans* were evaluated against mycelia growth of *P. infestans*

Objective

To identify the *P. infestans* using polymerase chain reactions (PCR) and to evaluate effects of plant extracts against *P. infestans in-vitro*

Methodology

- **Diseased tomato leaves collection, culturing, DNA extraction and PCR reaction**

-The tomato leaves with symptomatic leaves were collected from the farmers field to the NM-AIST laboratory

-V8 media was used as culturing media

-DNA extraction was done by using Quick-DNA™ Miniprep Kit Catalog No's. D4068 and 4069.

-PCR The primers that target the Internal Transcribed Spacer (ITS) with 600 bp were used. PINF (CTCGCTACAATAGGAGGGTC) Forward and ITS5b (GGAAGTAAAAGTCGTAACAAGG) reverse primer were used for PCR-based identification of fungal isolates based on procedure by Judelson and Tooley (2000).

- **Medicinal plant collection**

-Laves of *Plectranthus barbatus, Lantana camara, Sphaeranthus suaveolens* and *Tephrosia vogelii* were collected from different field of Arusha .

P. Barbatus *L. camara* *S. suaveolens* *T.vogelii*

- **Preparation of extracts**

Pulverized leaves of selected medicinal plants were sequentially macerated using methanol and ethyl acetate for 48h and 72h. The respective extracts were filtered through Whatman filter paper number 1 in a glass column. Solvents were removed using a rotary evaporator .

Results

-A total of 54 isolates were obtained from disease plant samples. Out of these only 15 indicated pathogenic symptoms on tomato variety.

-All plants showed antifungal activity against *Phytophthora infestans*. *Plectranthus barbatus* (94±1.0) and *Lantana camara* (91±2.4) were the strongest of all treatment and these were statistical similar to the positive control (97±0.0). *Tephrosia vogelii* extract showed poor results in inhibiting the mycelia growth of *P. infestans* (16±3.5) as compared with other plant extracts. The negative control did not inhibit the mycelia growth of *P. infestans*.

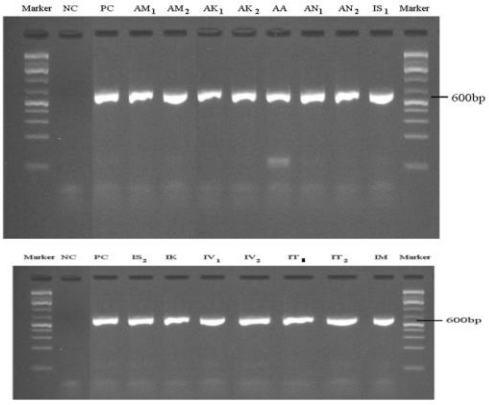

Figure1: Amplification of DNA sample of *P. infestans* pathogen

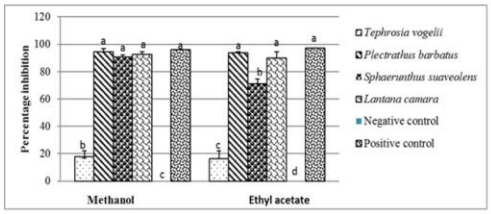

Figure 2: Interaction between the solvent and the plant extract in inhibiting the growth of *P. infestans*

Conclusion

- Polymerase chain reaction (PCR)- based method are more suitable and accurate in the detection of *P. infestans*
- The use of environmentally safe, biodegradable and cheaply available indigenous methods is most recommended method to control mosquitoes.
- Current study publicized the presence of active ingredients responsible for mycelia growth inhibition in leaf extracts from selected medicinal plants
- The traditional use of leaves from selected medicinal plants for the management of *P. infestans* in Tanzania is hereby justified in this study.

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