ACTIVITY OF EXTRACTS FROM SELECTED TANZANIAN SPICES ON MAJOR FUNGAL PATHOGENS AND BLIGHT DISEASES OF TOMATO

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) is affected by many fungal diseases and farmers rely on synthetic pesticides for management. Detrimental effects associated with injudicious use of chemical pesticides have caused a demand for alternative crop protection products. The objective of this study was to evaluate the antifungal activity of selected spices against major fungal pathogens and blight diseases of tomato. An ethnobotanical survey for ginger and turmeric and evaluation of awareness of botanical pesticides use and collection of commonly consumed spices was conducted in Tanzania. Ethanolic extracts from spices were prepared by maceration and tested for antifungal activity in posisoned food bioassay against *Phytophthora infestans*, *Alternaria solani*, *Fusarium oxysporum* f.sp. *lycopersici* and *Pythium* spp *in vitro,* their effect on seed germination and efficacy on severity of early and late blight diseases of tomato in. The most active extracts were fractionated in solvents with varied polarity, analyzed for biochemical composition and tested for fungicidal activity. Results indicated that among the tested spices, clove extract was the most active, inhibiting all the fungal pathogens (100%). Combined effect of the most active extracts showed that clove combined with either ginger, black pepper or turmeric inhibited growth of *P. infestans* (100%). The activity of solvent fractions was lower compared to the crude ethanolic extracts, except for clove fractioned in n-hexane which completely inhibited the growth of *P. infestans*. Therefore, the most active compounds were better extracted in ethanol. Gas chromatography- mass spectrometry analysis of the clove's n- hexane fraction showed high abundance of eugenol (74%) which is likely to be responsible for the high antifungal activity. High concentrations of the spice extracts deterred and slowed germination but low concentrations stimulated seed germination of up to 98%. Under field conditions black pepper extract reduced severity of late blight by 40% while clove extract reduced severity of early blight by 35% compared to the untreated control. The findings herein are proof of activity of spice extracts under *in vitro* and field conditions. This study recommends that the most active extracts be considered for development of a broad botanical fungicide for management of fungal diseases of tomato.

DECLARATION

I, Geraldin M. W. Lengai, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this thesis is my own original work and it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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CERTIFICATION

This is to certify that the undersigned have read this thesis titled “Activity of extracts from selected tanzanian spices on major fungal pathogens and blight diseases of tomato” and hereby recommend it to the NM-AIST Senate for award of the degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

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DEDICATION

To Myself

You can achieve anything you set your mind to.

To Gabriel “SweeBo” Lengai

We did this together. I mostly wrote while you napped and your support was in kicks, smiles and gentle glances. When it will be your turn, I will support you unreservedly.

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LIST OF ABBREVIATIONS AND SYMBOLS

AUDPC	Area under the disease progress curve
BCEU	Behavioual and Chemical Ecology Unit
CAVS	College of Agriculture and Veterinary Sciences
DAD	Diode-array detection
DAI	Days after incubation
DCM	dichloromethane
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FT-IR	Fourier-transform infrared spectrometry
FYM	Farmyard manure
GC-MS	Gas chromatography mass spectrometry
HPLC	High pressure liquid chromatography
HRMS	High resolution mass spectrometers
ICIPE	International Centre of Insect Physiology and Ecology
IPM	Integrated pest management
ISTA	International Seed Testing Association
LC-MS	Liquid chromatography mass spectrometry
MS	Mass spectrometry
NIST	National Institute of Standards and Technology
NM-AIST	Nelson Mandela African Institution of Science and Technology
NMR	Nuclear magnetic resonance
PCPB	Pest Control Products Board
PDA	Potato dextrose agar
Q-TOF	Quadroupole-time of flight
TLC	Thin layer chromatography
α	Alpha
β	Beta
γ	Gamma
Δ	Delta

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Tomato (*Solanum lycopersicum* L.) is an essentail source of vitamins to consumers and income to the farming communities worldwide. The tomato plant is grown for its fruit for the fresh market and processing into pastes, pulps, juices, powders and ketchup (Rao *et al*., 2020). In Tanzania, tomato is majorly grown for its commercial benefits as a source of livelihood to the farmers and those involved in its value chain (Mutayoba & Ngaruko, 2018; Mwatawala *et al*., 2019). The high yearly tonnage in tomato production of about 627 788 tonnes (Food and Agriculture Organization Corporate Statistical Database [FAOSTAT], 2020) makes the crop quite important and its health paramount. Throughout its growth span, tomato is attacked by different insect pests and diseases while in the field among them, fungal pathogens (Chidege *et al*., 2016; Bais *et al*., 2019; Shenge *et al*., 2020; Verma *et al*., 2020). Phytopathogenic fungi infect all parts of the tomato plants *viz.* seed, seedlings, leaves, stems, fruits and roots with reports of up to 100% crop loss under severe conditions (Bhalerao *et al*., 2019). Some of the fungal pathogens affecting tomato include species of *Alternaria*, *Phytophthora*, *Colletotrichum*, *Fusarium*, *Rhizoctonia*, *Pythium*, *Botrytis*, *Septoria*, *Ascochyta*, among others (Wonkaew & Sinsiri, 2014; Rahmatzai *et al*., 2017; Ghazal *et al*., 2019; Rao *et al*., 2020).

Diseases caused by pathogens, fungi included, reduce the quality and quantity of tomato fruits which further affects the marketability of the produce. For decades, farmers have relied on chemical pesticides for pest and disease management due to their effectiveness, quick knock down effects, applicability, availability and spread of information from one farming community to another (Sen *et al*., 2020). A wide range of chemical pesticides have been developed to manage different crop pests such as insects, bacteria, viruses, fungi and nematodes. Despite the worthy reasons for using synthetic pesticides, these chemicals have undesirable effects on humans, environment and other beneficial living organisms (Kariathi *et al*., 2017; Li *et al*., 2020). Misuse and overuse of chemical pesticides has also been cited as a barrier to international trade due to presence of toxic chemical residues in fresh agricultural produce intended for export (Dinham, 2003; Kussaga *et al*., 2014). The cry for safe food, a healthy population and a safe environment has attracted diverse views on the corrective

measures that need to be adopted in order to achieve agricultural and environmental sustainability (Yanar *et al*., 2011; Nain *et al*., 2020).

Alternatives to chemical crop protection products are one of the proposed items towards achieving healthy and safe food for human consumption and a habitable environment (Clerck *et al*., 2020). Research scientists have responded to these uproars by providing solutions such as breeding for resistance, biopesticides, improved cultural techniques, greenhouse farming and integrated pest and crop management (Guchi, 2015; Ridzuan *et al*., 2018; Verma *et al*., 2020). Biopesticides have particularly gained attention over the last few decades due to their effectiveness, biodegradability, availability of their resource materials and their ability to fit within any crop protection regime (Yanar *et al*., 2011; Arzoo & Biswas, 2013; Anjum *et al*., 2019). Biopesticides comprise a wide range of natural plant protection products derived from plants, microorganisms, minerals, semiochemicals or animals (Lengai & Muthomi, 2018).

Botanical pesticides are derivatives of plants, either as extracts, essential oils or minerals used to manage crop pests (Mizubuti *et al*., 2007; Gašić & Tanović, 2013). Use of plants as pesticides is historic with species such as pyrethrum, tobacco, sabadilla and rye having been used as insecticides in early days (Dubey *et al*., 2010). Other plants have since been identified, tested and reported to contain pesticidal properties with some such as neem, garlic, pyrethrum, thyme, rosemary, jojoba, cottonseed, dill, caraway seed, giant knotweed among others being commercialized as plant protection products (Zaker, 2016). Most of the pesticidal plants have been utilized as insecticides with diminutive focus towards fungal pathogens despite having fungicidal and fungistatic properties (Isman, 2015; Stevenson *et al*., 2017; Isman, 2020). This study focused on selected spices namely turmeric (*Curcuma longa*) clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum* spp), black pepper (*Piper nigrum*), lemongrass (*Cymbopogon citratus*), cardamom (*Elletaria cardamomum*) and ginger (*Zingiber officinale*) as plants with antifungal properties that could be used to make a broad-spectrum product usable on tomato against important fungal pathogens. Each of the plants selected for this study is used for culinary and medicinal purposes. The medicinal uses of some of the listed plants is well known amongst the farming communities where they are grown. Numerous reports detail the medicinal benefits associated with the spices aforementioned (Shahbazi *et al*., 2019, Mahfouz, 2020). The medicinal properties of each of the seven spices is what was tapped into during this study and applied towards important fungal pathogens of tomato (Nikolić *et al*., 2015). The

antifungal properties of these spices have also been reported elsewhere against a myriad of pathogens affecting various crops.

The properties that make the aforementioned plants candidates for consideration is due to their biochemical composition. The secondary metabolites present in those plants are responsible for the antifungal properties exhibited against important pathogens (Vidyasagar & Tabassum, 2013). Several studies have reported the biochemical profiles of some of the study plants herein and others have reported their antifungal activity (Kapoor *et al*., 2008; Bhuiyan *et al*., 2010; Hameed *et al*., 2016; Wang *et al*., 2019). This study seeks to link the antifungal activity of the study plants with the presence of biochemical compounds to point out their possible mode of action.

Botanical preparations have been used to treat seeds against seedborne pathogens. This is an important utilization of plant extracts because despite the effort taken by governments and private seed companies to ensure the presence of a formal seed system, some farmers still utilize informal seed sources (Muthoni & Nyamongo, 2008). With the concerns surrounding use of chemicals in agricultural systems, having alternatives to seed treatment is imperative. Botanical preparations have been used as seed treatments on a number crops such as tomato (Mbega *et al*., 2012), wheat (Mahal & Akter, 2019), sorghum (Masum *et al*., 2009), cabbage (Wolf *et al*., 2008), pea (Chandel & Kumar, 2017), cauliflower (Findura *et al*., 2020), cotton, barley (Ahmad *et al*., 2016) and cowpea (Akinbude & Ikotun, 2008) among others. Examples of plants used as seed dressers include garlic, ginger, black pepper, lemongrass, moringa, neem, clove, garden quinine, cumin, cedar, onions, bel leaf, turmeric and cinnamon. However, there has been reports that that despite the remarkable effect of plant extracts in seed dressing, some plants have toxic effects on the seeds and may interfere with germination and overall seed transition into seedlings. This is because of the presence of secondary metabolites which, at varied concentrations, may either stimulate or hinder germination of seed (Siddiqui, 2007; Han *et al*., 2008; Wolf *et al*., 2008; Shabana *et al*., 2017). The current study sought to investigate the effects of plant extracts from spices on the germination of tomato seeds.

Plant extracts have also been used as foliar sprays against a wide range of diseases caused by different phytopathogens (Zaker, 2016). In tomato production, plant extracts have been used against foliar diseases such as blights, spots and rots (Ganie *et al*., 2013; Rizwana, 2016; Ngegba *et al*., 2018). Some of the plants used as foliar sprays against fungal diseases of plants include garlic, clove, thyme, tea tree, rosemary, cinnamon, neem, moringa, lemon, turmeric

and pepper (Mohsan *et al*., 2017; Anjum *et al*., 2019; Khan *et al*., 2019; Wang *et al*., 2019; Najdabbasi *et al*., 2020). In the management of soil borne diseases affecting tomato, plant extracts have also been reported effective. Most soil borne fungal pathogens cause damping off, wilts and root rots in tomato and they include species of *Pythium, Fusarium, Rhizoctonia, Alternaria* and *Verticillium* (Yeole *et al*., 2016; Testen & Miller, 2017). Some spice plants have shown fungistatic and fungicidal activities against all these and other soilborne fungal pathogens. Examples of such plants include clove, cinnamon and black pepper (Aziz *et al*., 2012; Shukla & Dwivedi, 2012; Yoon *et al*., 2013; Estrada-Cano *et al*., 2017). Tomato is affected by selected fungal pathogens postharvest for instance species of *Alternaria, Phytophthora, Geotrichum* and *Colletotrichum* (Devi *et al*., 2016; Ahmed *et al*., 2017). There are plenty of plant extracts that have been reported effective against postharvest fungal pathogens of tomato. Antifungal extracts effective against postharvest fungi affecting tomato include those from cinnamon, clove, black pepper, turmeric and cardamom (Oros & Kállai, 2019).

Despite the vast number of publications emanating from research related to plant extracts (Isman & Grieneisen, 2014), there remains a shortage of commercial plant-based fungicides in the market of biopesticides. Review reports have attributed this gap to absence of enough data on chemical composition, efficacy and toxicity of plant extracts and positive checks especially for field experiments (Isman & Grieneisen, 2014). Information on the exact compounds responsible for antifungal activity in selected plants or their mode of action against pathogens is another challenge in commercializing plant-based fungicides. Despite these challenges, several botanical fungicides have been successfully registered for use in developed countries (Zaker, 2016). The commercially available botanical fungicides do not contain one active ingredient, but several compounds or even plant extracts or essential oils from several plants. The combination aspect of plant bioactive compounds into one product is likely to offer higher efficacy of the resultant botanical fungicides due to the synergistic effect (Eloff *et al*., 2017). Moreover, the product is likely to have diverse modes of action and the target pathogens will never develop resistance against the product because the compound combinations do not exist in nature (Oliveira *et al*., 2018). This study focused on the independent antifungal activity of plant extracts from spices and their combinations, their biochemical profiles and efficacy against important fungal diseases of tomato under *in vitro* and field conditions.

1.2 Statement of the problem

Tomato is affected by a wide range of fungal diseases with blights being of utmost importance. Blights of tomato continue to be a challenge for every tomato farmer and the diseases are mostly managed by use of chemical fungicides. However, use of chemical pesticides for disease management has detrimental ripple effects such as killing non-target organisms. Synthetic pesticides also pollute the environment due to accumulation of constituent compounds which take lengthy time to degrade. Accumulation of chemical compounds in the food produce is a health hazard and causes chronic poisoning to the consumer. A number of effective fungicides is required for one planting season of tomato for management of diseases, which further increases the cost of production. There is a concern surrounding food safety, especially vegetables and fruits, regarding presence of chemical residues which calls for alternative but effective crop protection strategies. Botanical extracts have the potential to fill that gap but they have to fulfil the requirements of efficacy and stability especially under field conditions. There exist botanical fungicides in the market, though minimal, and all of them can only be imported from the developed countries and are therefore expensive.

1.3 Rationale of the study

One of the alternative crop protection strategies to synthetic chemicals is use of natural products either from plants or microflora. Plants selected for this study are used as spices and as medicine to treat diverse human ailments. The property that makes the spices medicinal is what was being explored in the current study against important fungal pathogens of tomato. Extracts derived from these plants and used as pesticides have been proven effective against plant pathogens with notable success. A botanical fungicide will benefit a lot of small holder tomato farmers since it will reduce the excessive use of chemicals and the challenges associated with them. Extracts prepared from spices have little to no toxicity effects to the user or end consumer because they are already used as food additives and their biodegradability is also swift. The spices used in this study are also reliably produced in Tanzania which guarantees availability of resource materials for development of a botanical fungicide further creating an extra market for spice farmers.

1.4 Research objectives

1.4.1 Broad objective

To develop a broad-spectrum botanical fungicide from selected spices against major fungal diseases of tomato.

1.4.2 Specific objectives

(i) To establish medicinal and pesticidal value of ginger and turmeric in major growing regions in Northern and Eastern Tanzania.

(ii) To evaluate the antifungal effect of extracts from spices on tomato pathogens *in vitro.*

(iii) To identify bioactive compounds in spice extracts.

(iv) To evaluate efficacy of the botanical extracts on blight diseases of tomato under field conditions.

1.5 Hypotheses

(i) Medicinal and pesticidal value of turmeric and ginger is well known in Northern and Eastern Tanzania.

(ii) Spice extracts have antifungal properties against tomato pathogens.

(iii) Spices contain bioactive compounds with antifungal effect.

(iv) Spice extracts are efficacious against blight diseases of tomato.

1.6 Significance of the study

The study established that farmers in Tanzania are aware of botanical pesticides and they may be open to adopting them upon proof of efficacy. Some farmers have actually used ginger powder as a postharvest treatment for stored grains. This is a baseline for introducing botanical pesticides into the Tanzanian market, especially because the study plants are well known and already in use as spices and medicine. The plants selected for the study are all grown in Tanzania and have high antifungal activity on four of the most important fungal pathogens of tomato.

1.7 Delineanation of the study

Following are delineations of the current study:

(i) An ethnobotanical survey was carried out in selected Districts in Northern and Eastern Tanzania to establish the medicinal and pesticidal value of ginger and turmeric and also with an aim to evaluate the awareness of farmers on the use of botanical pesticides especially in Same District. During the survey, more spices were collected intended for antifungal evaluation against important phytopathogenic fungi. The level of awareness found among farmers producing ginger is enough to introduce a botanical fungicide developed from the spice they are readily producing and using for medicinal purposes. This activity was however carried out in two regions and would have been more representative of Tanzania as a whole if more regions were involved in the study.

(ii) Extracts prepared from the collected spices were tested for antifungal activity against important fungal pathogens *in vitro* and blight diseases of tomato under field conditions. Some spices inhibited growth of all the fungal pathogens 100% due to presence of bioactive compounds with high antifungal activity. Under field conditions the antifungal activity was reduced due to harsh environmental and disease factors, a circumstance that can be improved by formulating the highly inhibitory extracts in order to increase their stability and persistence.

CHAPTER TWO

LITERATURE REVIEW

2.1 Importance of tomatoes

Tomato is a fruit belonging to the solanaceous family of crops, utilized as a vegetable for its nutritional and economic benefits. The fruits of tomato are used fresh in salads, cuisines and gravy or processed into paste, creams, juices, ketchup, powder and pulp (Rao *et al*., 2020; Verma *et al*., 2020). In Tanzania, tomato is grown extensively throughout the country due to the favourable climatic and topographic conditions that favour its production (Mtui *et al*., 2010). Tomato farming is a source of income and supports the livelihoods of those participating in its value chain (Mwangi *et al*., 2020). However, production of tomatoes in many African countries is still below potential due to major challenges such as insect pests and diseases.

2.2 Plant health challenges in tomato production

Tomato is affected by a number of insect pests and diseases while in the field as well as postharvest. Among the diseases that affect tomato under field conditions, those caused by fungi are the majority leading to severe losses (Sanoubar & Barbanti, 2017). Species of fungi belonging to different genera such as *Phytophthora, Pythium, Fusarium, Alternaria, Rhizoctonia, Colletotrichum* cause various diseases on tomato leaves, stems, fruits and roots (Bais *et al*., 2019; Bhalerao *et al*., 2019). Each genus of fungi consists of various species, all of which cause diseases with similar symptoms in plants. Species of *Fusarium* that affect tomato are majorly *F. oxysporum* and *F. solani*. *Fusarium oxysporum* f. sp. *lycopersici* is the pathogen responsible for fusarium wilt in tomatoes contributing to about 80% of crop loss under favourable climatic conditions (Chougule & Andoji, 2016; Nefzi *et al*., 2016; Rao *et al*., 2020). The fusarium wilt pathogen is soilborne and it attacks tomatoes planted in the field as well as protected environments such as greenhouses (Timofte *et al*., 2018). In the absence of a suitable host, *Fusarium oxysporum* f. sp. *lycopersici* survives in the soil and on plant debris until a favourable host is available. The wilt pathogen enters the plant through the root and invades the vascular system hindering uptake of water and the brown vascular tissue is visible upon cutting the stem at the base (Timofte *et al*., 2018). The fusarium wilt symptoms in tomato are distinguished by yellowing on one side of the plant starting from the older leaves which wilt, dry and then defoliate. This wilt phytopathogen majorly reproduces by means of spores,

microconidia, macroconidia and chlamydospores. The macroconidia are a distinctive characteristic of the genus *Fusarium* as well as white to pink mycelia (Agrios, 2005).

The management strategies for fusarium wilt in tomato range from use of resistance varieties, soil sterilization, drenching the soil with fungicides and biological control. In Kenya, *Trichoderma asperullum* and *T. harzianum* are available as commercial antagonists for management of fusarium wilt in carnations caused by *Fusarium oxysporum* f. sp. *Dianthi* (Pest Control Products Board [PCPB], 2018). Despite availability of management methods, many smallholder farmers still struggle with wilt in tomatoes due to the high costs associated with successful management options. The synthetic chemicals may serve the purpose of fusarium wilt management, though they have negative effects such as environmental pollution and health hazard to humans (Yeole *et al*., 2016). Therefore, there is need for alternatives to fusarium wilt management and botanical preparations may serve that need given their degradability, antifungal activity and availability of resource materials (Arzoo & Biswas, 2013). Despite abundance of evidence of plant extracts inhibiting growth of *Fusarium in vitro* (Prasad *et al*., 2018; Clerck *et al*., 2020), there lacks a commercial product in the region designed for management of fusarium wilt of tomato. Efficacy tests involving botanical preparations are needed to prove efficiency against fusarium wilt under field conditions.

Blight diseases in tomato are majorly caused by fungal species of *Phytophthora* and *Alternaria*. *Phytophthora infestans* is an oomycete that reproduces asexually by means of zoospores and causes late blight of tomato (Agrios, 2005). Under temperatures of between 15° – 20° C and about 100% relative humidity, the sporangia *of P. infestans* germinate into a germ tube and infect the leaves of a host, such as tomato, especially under moist conditions, thus initiating infection (Agrios, 2005). Late blight infection is manifested as water-soaked spots on the bottom side of the leaves which enlarge over time and become grey to brown in colour. In a matter of days, the blight infected leaves wilt and *P. infestans* advances to the other leaves and eventually the entire plant. If no management action is taken, the entire tomato crop is infected with late blight and wilts to dryness within a week leading to crop and monetary losses (Peerzada *et al*., 2020). Under dry conditions, the late blight pathogen overwinters in the soil, volunteer crops and plant debris as oospores until the temperature and moisture conditions are favourable again for it to produce sporangiophores to start germination all over again. In the soil, the resting spores of *P. infestans* may be present for close to four years which serves as sources of inoculum once host crops such as tomatoes are planted. The mature sporangia are

disseminated by wind, water or attachments and once they land on appropriate hosts, germination and infection of late bight begins (Agrios, 2005). Late blight of tomato is majorly managed by frequent applications of synthetic contact and systemic fungicides. Fungal species of *Trichoderma* and *Aureobasidium* have the potential to inhibit growth of *P. infestans* (Difrancesco *et al.*, 2017; García-Núñez *et al*., 2017). Bacterial species of *Bacillus* and *Pseudomonas* also have antagonistic effect towards the late blight pathogen (Caulier *et al*., 2018). Plant extracts have also shown activity against *P. infestans* but commercial products in the region are still missing in the market (Han *et al*., 2018; Najdabbasi *et al*., 2020).

The genus *Alternaria* has a wide host range and therefore causes massive damage on various crops including tomato (Agrios, 2005). In tomato, *Alternaria* causes a number of diseases but the major concern is early blight caused by *Alternaria solani* distinguished by its dark to black mycelia, septate hyphae with pear-shaped conidiophores (Mihaescu *et al*., 2021). Early blight is noticeable on leaves, stems and fruits of tomato and symptoms are visible at early stages of growth through fruiting. On foliage, early blight is characterized by dark spots surrounded by chlorotic regions which become necrotic lesions as the infection progresses (Bais *et al*., 2019) while on fruits it is exhibited as dark velvety sunken regions on the stem end which begin as spots and enlarge as the infection advances (Agrios, 2005). Early blight is first manifested on the older leaves of tomato and as the disease intensifies, the young emerging leaves get infected. The disease is better managed by cultural practices, planting resistant varieties and frequent application of targeted fungicides (Verma *et al*., 2020). There are a number of plant extracts and biocontrol agents with antifungal activity against *A. solani*. Bacteria species of *Bacillus, Staphylococcus* and *Pseudomonas* have inhibited the growth of the early blight pathogen (Joseph *et al*., 2017; Chanthini *et al*., 2018). Fungal antagonists of genus *Trichoderma* and *Penicillium* have also shown antagonistic activity against *A. solani* (Devi *et al.*, 2017; Ragupathi *et al.*, 2020). Several plants contain bioactive compounds that slow or completely deter the growth of *A. solani* and consequently, development of early blight (Yeole *et al*., 2004; Castro *et al*., 2017; Amsaraj & Prasad, 2020). However, formulated products of plant origin designated for management of early blight disease in tomato are yet to be commercialized especially in sub-Saharan Africa.

2.3 Conventional approaches in tomato protection

Farmers rely on synthetic pesticides to manage the different insect pests and diseases that affect tomato throughout the production period (Majeed *et al*., 2011). This reliance on chemical

pesticides is out of the believe that high quality of a produce cannot be achieved without application of several pesticides. Farmers therefore apply pesticides indiscriminately as long as the chemicals will eradicate insect pests and diseases from their fields. Synthetic pesticides are relied upon since they are readily available, have varied modes of action, are easy to apply and have a quick knock down effect (Sumitra *et al*., 2012; Engindeniz *et al*., 2013). However, pesticides are also non-biodegradable, retain residues in the produce, pollute the environment and are toxic to humans, natural environment, and beneficial organisms in addition to being very expensive (Mizubuti *et al*., 2007; Mishra *et al*., 2015; Srijita, 2015). Despite being aware of the negative effects associated with chemical pesticides farmers sometimes apply them for pest management and production of aesthetically acceptable produce (Karianthi *et al*., 2017). In some instances, farmers do not necessarily follow guidelines on safe use of pesticides regarding dilution ratios, pre-harvest intervals, and use of appropriate gear during application or disposal of chemical containers (Damalas & Koutroubas, 2015).

2.4 New developments and consumer requirements in horticultural production

There has been a rising awareness regarding safety of food produce from production, postharvest handling, processing, transportation and consumption (Kussaga *et al*., 2014; Nain *et al*., 2020). Much of this is majorly associated with fruits and vegetables due to the fact they are mostly consumed fresh. Produce contamination by harmful microorganisms and presence of high residues of chemicals used to manage pests and diseases are the major causes of concerns surrounding food safety and by far, international trade of fruits and vegetables (Martinez & Poole, 2004). Farmers from developing states have had to deal with stringent requirements of sanitary and phytosanitary measures in order to access the lucrative markets of fresh fruits and vegetables offered by the developed nations. With most retailer organizations in the European countries demanding zero pesticide residues from the suppliers of fruits and vegetables, some farmers have had to opt out of the export market. Other farmers have been forced to subscribe to traceability systems and to private standards developed by major fruit and vegetable retailers as well as adopt organic farming (Martinez & Poole, 2004). Most of the fruits and vegetables are grown in open fields where an assortment of insect pests and diseases occur.

Farmers have mostly relied on pesticides to manage the pests and diseases and sometimes due to ignorance, lack of training or even misled advise by pesticide dealers, end up applying the chemicals injudiciously leading to presence of high residues in the produce (Dinham, 2003;

Karianthi *et al*., 2017). Presence of chemical residues above the permitted limits and traces of banned pesticides on the vegetables and fruits are grounds for interception, rejection and barring of access to international markets (Mwangi, 2013). Subscription to integrated crop management strategies, adoption of good agricultural practices, compliance to sanitary and phytosanitary requirements, and adoption of indigenous pest and disease management strategies are among the many combinations of options that the fruits and vegetable farmers have in order to access and thrive in the lucrative import markets (Kussaga *et al*., 2014; Zahid *et al*., 2016; Nain *et al*., 2020). In order to motivate the suppliers, consumers in the lucrative markets have offered to pay more for organically produced food (Nain *et al*., 2020). There is therefore hope for farmers who are willing to adopt safer methods of fruit and vegetable production targeted for international lucrative markets (Srijita, 2015; Nain *et al*., 2020).

2.5 Alternative pest management approaches in tomato crop protection

Given the high number of insect pests and diseases found in tomato fields, one pest management strategy is definitely not sufficient to offer protection against the biotic factors. A number of pest and crop management strategies is required to raise a healthy crop in a natural environment. The concept of multifaceted pest and disease management strategies seeks to interfere with pest activities, improve resilience of plants against pests or manipulate the environment to disfavour thriving of pests (Mizubuti *et al*., 2007). The plant genetic constitution may also be manipulated in order for the plant to withstand or deter pest attack (Bruggen *et al*., 2015). Planting seeds that are free of or resistant to diseases is one of the pests and disease management strategy. Scientists have explored breeding as a way of improving resistance of crops towards diseases through genetic manipulation (Gutierrez *et al*., 2016; Hickey *et al*., 2017). Notable success has been reported in plants such as wheat, barley, chilli, cocoa, tomato and potato (Ridzuan *et al*., 2018; Armstrong *et al*., 2019; Bais *et al*., 2019).

Some pests are quite sophisticated in morphology and physiology and therefore more complicated to handle compared to others thus calling for increased effort in their management. For instance, bacterial wilt caused by *Ralstonia solanacearum* is a disease that affects a wide range of crops especially in family Solanaceae where tomato belongs. Due to the devastating nature of the disease, bacterial wilt is only managed using a number of strategies. In Ethiopia, for example, combined strategies such as field sanitation, crop rotation, destruction of volunteer plants, intercropping, use of resistant varieties, planting disease free materials and use of biocontrol agents are used to manage bacterial wilt in tomato fields (Guchi, 2015).

Cultural practices have also been adopted to contribute in the management of crop pests and diseases and improvement of crop yield. Intercropping leeks (*Allium ampeloprasum*) with clover (*Trifolium* spp) reduced the severity of leak rust (*Puccinia allii*) and infestation by onion thrips (*Thrips tabaci*). There has been reported a significant reduction in blast of rice in fields where rice was intercropped with other crops as opposed to monocultured plots (Han *et al*., 2016). According to Gomez-Rodriguez *et al*. (2003) intercropping tomato plants with marigold (*Tagetes erecta*) reduced early blight (*Alternaria solani*) through allelopathic effects of marigold on the pathogen, alteration of microclimate at the canopy level and provision of a physical barrier deterring the spread of conidia. Zhang *et al*. (2019) reported reduced disease incidence in wheat when intercropped with faba bean (*Vicia faba*) and recommended that the practise is not sufficient on its own but better off as an IPM component.

The environment is filled with organisms belonging to different levels of the food chain and their interrelationships have been tapped for crop protection. Some organisms are predators and the concept of predation has been explored as a crop protection strategy with notable success. Classical biological control has been utilized especially against insect pests using parasitoids and predators and in other cases pathogens (Kenis *et al*., 2017). A predatory mite, *Neoseiulus longispinosus*, was reported to effectively reduce mite infestation by two-spotted spider mite (*Tetranychus urticae*) under glass house and laboratory conditions on okra plants (Rao *et al*., 2017). Powell and Pickett (2003) explored the response of aphids to sex pheromones and aphid plant-induced plant volatiles and reported that female aphid parasitoids responded to sex pheromones acting as kairomones and thus could be used for parasitization of aphid populations. The study concluded that pheromones could be used to induce plant defence by deterring colonization by aphids and attracting parasitoids and predators (Powell & Pickett, 2003). Semiochemicals have been effectively employed in management of insect pests in crop fields. Chermiti and Abbes (2012) reported population dependent reduction of tomato leaf miner, *Tuta absoluta,* using an IPM based sex pheromone dispenser. Sarles *et al*. (2015) reckoned that semiochemicals are important for host fruit location and reproduction of fruit flies. Therefore, use of kairomones, sex and mating pheromones as well as host-marking pheromones could be used to attract and kill emerging individuals and reduce oviposition especially in fruit flies (*Tephritidae* spp).

Natural environment also harbours microorganisms that exhibit antagonism towards other species through predation, parasitism and competition. Antagonism is important in microbial

interactions and has been used in development of biocontrol agents for management of important plant pathogens (Aw & Hue, 2017). Endophytic bacteria comprising *Stenotrophomonas maltophilia, Bacillus subtilis* and *Pseudomonas aeruginosa* have shown antagonism against *Rhizoctonia solani*, *Fusarium oxysporum*, *Erwinia carotovora* and *Pythium ultimum* under laboratory conditions. Effectiveness in antagonism is attributed to presence of bioactive compounds in the endophytic bacteria which are an important factor in biological control (Selim *et al*., 2016). *Metarhizium* spp has been widely utilized as an entomopathogenic fungi in management of insect pest with tabled success (Aw & Hue, 2017). Selected *Trichoderma* spp have also shown antagonistic activity towards many bacterial and fungal plant pathogens and has been formulated and commercialized.

Extracts from compost contain bioactive compounds and microorganisms which if tapped could improve plant growth and yield as well as plant health. Foliar sprays and soil drenching with compost teas increased commercial yield of kohlrabi (*Brassica oleracea*) and lettuce (*Lactuca sativa*) significantly (Pane *et al*., 2014). Compost tea used as a foliar spray and poultry litter extracts as a soil drench, reduced the incidence of late blight of tomato and potato under field conditions with a good economic feasibility (Islam *et al*., 2013). Compost tea and poultry litter also reduced the incidence of bacterial wilt (*Ralstonia solanacearum*) of brinjals (*Solanum melongena*) when used as a soil drench (Islam *et al*., 2014).

Plants also contain bioactive compounds which have been extensively explored for crop protection purposes. Plants such as neem (*Azadirachta indica*) and garlic (*Allium sativum*) have been commercialized as insecticides, effective against a wide range of insect pests (Infonet Biovision, 2018). Some plants are utilized as either extracts or essential oils which contain a variety of compounds with anti-pest properties. Garlic and ginger are utilised for their essential oils against a range of pests and pathogens (Koundal *et al*., 2018). Neem (*Azadirachta indica*), moringa (*Moringa oleifera*) and tephrosia (*Tephrosia vogelii*) have been reported to have insecticidal properties and used to manage a number of insects attacking different crops with notable success (Alao *et al*., 2018; Mikami *et al*., 2018; Seifi *et al*., 2018). The bioactive compounds in some plants may also have repellence effects without necessarily killing the insects (Babu *et al*., 2018). Anti-pest compounds in plants may also interfere with physiological activity of insects thereby reducing their infestation ability (Hamada *et al*., 2018). Plant compounds are also effective against nematodes and affect their infestation capability by inhibiting hatchability of second stage juveniles and eggs eventually reducing nematode

populations (Khan *et al*., 2008; Kepenekçi *et al*., 2016). In fungi, some plant compounds inhibit spore germination, interferes with mycelia development, delay sporulation and degrades important enzymes of those pathogens thereby reducing their pathogenicity (Martinez, 2012; Hadi *et al*., 2013). In bacteria, plant compounds cause leakage of cell content, interfere with cellular processes rendering the bacteria incapacitated for infection (Khan *et al*., 2009; Djeussi *et al*., 2013). Plant compounds also inhibit virulence capacity in viruses through inhibiting their enzymatic activity, hindering virus attachment to host cells and amplification (Bhanuprakash *et al*., 2008; Rajasekaran *et al*., 2013). Despite the extensive research dedicated to plant extracts and essential oils, there has not been much attention towards commercialization of plant-based fungicides especially in sub-Saharan Africa.

2.6 Role of biopesticides in plant health management

Biopesticides are products of natural components including plants, microorganisms and insects and they manage pests with less toxicity to the non-target organisms (Mizubuti *et al*., 2007; Kumar & Singh, 2015; Mishra *et al*., 2015). These products are important because unlike the synthetic pesticides they are easily degradable, less toxic to humans and the environment and do not have residual effects on food produce (Kimani, 2014; Kumar & Singh, 2015; Srijita, 2015). Biopesticides offer solutions to pest resistance due to synergistic effect by compound combintions which don't exist in nature, environmental and water pollution, public concerns about food safety and could greatly contribute towards sustainable agricultural productivity (Mishra *et al*., 2015). Biopesticides used in agriculture include microorganisms such as bacteria, fungi, viruses and protozoa and botanicals such as neem, garlic, pyrethrum and turmeric among others (Bautista-Banos *et al*., 2003; Goufo *et al*., 2008; Kimani, 2014). Bacteria species such as *Bacillus*, fungal species such as *Trichoderma* and plant species such as neem (*Azadirachta indica*) and turmeric (*Curcuma longa*) have been used in management of plant pests and diseases (Mishra *et al*., 2015).

Different plants contain different compounds which make them potential sources of biopesticides (Vidyassagar & Tabassum, 2012). In a report by Majeed *et al*. (2011), *Podophyllum hexandrum*, *Withania somnifera* and *Xanthium strumarium* were evaluated for activity and reported significant reduction of incidence of late blight of tomato (*Phytophthora infestans*) under field conditions. They also reported a better yield and recommended foliar applications of the tested plants in management of late blight of tomato. Mother tinctures of *Allium sativum, Glycyrrhiza glabra, Myroxylon balsamum* and *Aloe vera* were tested against

Fusarium guttiforme and were as effective as the fungicide used as positive control in reducing pineapple fusariosis *in situ* (Sales *et al*., 2016). According to an *in vitro* study by Mahajan *et al*. (2015), extracts of turmeric (*Curcuma longa*) led to morphological changes and interruption of the cytoplasmic membrane of *Staphylococcus aureus* which makes the plant a great source of antimicrobial compounds for pathogen management. Botanical pesticides are incorporable in integrated pest, disease and crop management programs since their effectiveness has been reported against insects, nematodes, bacteria, fungi and viruses that affect crops (Lengai *et al*., 2020).

2.7 Plants with antifungal properties

Plants belong to different families, a classification which dictates the types and amounts of bioactive compounds present in those plants. Plants within the same family may contain different types of bioactive compounds and in varying quantities (Vidyasagar & Tabassum, 2013). This translates to difference in activity against a variance of microorganisms in human, plant and animal species. Plants belonging to the same family may also have related compounds and with slightly difference in concentrations thus delivering similar antimicrobial activity (Karim *et al*., 2017). Some groups of compounds present in plants that exhibit antimicrobial properties include ketones, aldehydes, phenolics, tannins, terpenes, terpenoids, quinones, alkaloids, saponins, steroids, alcohols, flavonoids and quinones (Mizubuti *et al*., 2007).

Studies have shown potential of some plants to manage plant pathogenic fungi through different modes of action. Clove (*Syzygium aromaticum*) belonging to family Myrtaceae, repressed growth of *Penicillium digitatum*, causal agent of citrus green mould, by suppressing spore germination and germ tube elongation of the fungus. The study evaluated encapsulation of clove essential oil in order to improve its antifungal activity (Wang *et al*., 2017). When used in combination with mustard (*Brassica nigra*) belonging to family Brassicaceae, a synergistic effect was reported in both essential oils towards reducing the effects of *Botrytis cinerea*, causing grey mould in strawberry (Aguilar-Gonz´alez *et al*., 2015). Microencapsulation of essential oils from clove (*Eugenia caryophyllata*) and Mexican oregano (*Lippia berlandien*), of family Lamiaceae, proved effective against *Fusarium oxysporum* (Estrada-Cano *et al*., 2017).

Plants belonging to family mealiaceae have shown antifungal potential against important plant pathogenic fungi. When combined with synthetic fungicides and biocontrol agents, neem

(*Azadirachta indica*) was reported effective in managing *Alternaria carthami*, causal agent of leaf spot of safflower. In that study, neem oil was also combined with essential oil of castor (*Ricinus communis*) of family Euphorbiaceae (Gayanthri & Rao, 2017). Neem was also used as a seed coating agent in a study involving moringa (*Moringa olifera*) of family Moringaceae, and was reported effective in improving pathological and horticultural aspects of rough lemon seeds (Jaskani *et al*., 2018).

Essential oil of ginger (*Zingiber officinale*) of family Zingiberaceae, was reported effective against a number of pathogens causing rot diseases of ginseng (*Panax ginseng*). Effectiveness of ginger essential oil was tested against *Alternaria panax, Botrytis cinereal, Cylindrocarpon destructuns, Fusarium oxysporum, Sclerotinia sclerotiorum* and *Sclerotinia nivaurus* (Hussein & Joo 2018). In a study involving basil (*Ocimum gratissimum*), family Lamiaceae and lemon grass (*Cymbopogon citratus*), family Poaceae, ginger was also reported to suppress rot causing fungi of soursop fruit. The rotting fungi included *Rhizoctonia* spp, *Penicillium* spp and *Botrydiplopodia theobromae* (Okigbo *et al*., 2018). Chen *et al*. (2018) also reported effectiveness of ginger in managing rot diseases of harvested olive fruits caused by *Pestalotiopsis microspora*. The effects of ginger oleoresin on the pathogen included damaged plasma membrane and mycelia, distorted and shrivelled spores with degradation of unsaturated fatty acids. According to Choudhury *et al*. (2017), ginger in combination with *Clerodendrum infortunatum*, family Lamiaceae and *Polyalthia longifolia*, family Annonaceae, inhibited radial growth of *Rhizoctoia solani* and *Colletotrichum musae* causing sheath blight of rice and anthracnose of bean respectively.

Turmeric (*Curcuma* spp) belongs to family Zingiberaceae, like ginger, but with different bioactive constituents. Fu *et al*. (2018) reported curative effects of turmeric extracts on cucumber against *Podosphaera xanthii* which causes powdery mildew in cucurbits. Turmeric has also been reported effective against *Fusarium* spp (Akter *et al*., 2018). Synergistic effects were reported by Ghosh (2018) between turmeric and *Ixora coccinea* of family Rubiaceae against *Botrytis cinerea*.

Cinnamon belongs to family Luaraceae whose secondary metabolites include aldehydes, mono and sesquiterpenes, aromatics, esters, alcohols and ketones (Yu *et al*., 2020). Sohrabi *et al.* (2017) reported that the major compounds reported in cinnamon essential oil were *trans*-cinnamaldehyde and cinnamaldehyde diethyl acetal. Methanolic extracts of cinnamon bark was reported to contain a major compound cinnamaldehyde which is responsible for the

antimicrobial effect of the plant (Wang *et al*., 2005; Hameed *et al*., 2016). One of the derivatives of cinnamaldehyde has been reported active against *Phytophthora infestans* by Mackrill *et al*. (2020) who suggested that it could be used as a fungicide due to its activity and limited toxicity. The differences observed in the activity of cinnamon against various fungal pathogens may be attributed to geographical locations, varieties and environmental factors (Li *et al*., 2013). Some of the reviewed plants or their parts, have been commercialized into botanical fungicides especially in developed countries (Zaker, 2016).

2.8 Mode of action of botanical pesticides

Plants in different families contain varied bioactive compounds which affect wide ranged microorganisms in diverse ways. Plant compounds with antifungal properties may inhibit the growth of the entire fungus, hinder spore germination and sporulation, disrupt the cell membrane and also distort the morphology of the fungal pathogen. Understanding the mode of action of botanical pesticides is essential in developing a crop protection product since it spells effectiveness. *Juniperus procera* of family Cupressaceae and *Avicennia lunata* of family Acantheceae reduced the nucleic acid of *Curvularia lunata*, causal agent of leaf blight of rice. The compounds found in the two plants blocked synthesis of curvulalic acid and lunatin (Abdel-Ghany *et al*., 2015).

Cumin (*Cuminum cyminum*) seed extracts belonging to family Apiaceae act on fungal plant pathogen by increasing permeability of the cell membrane, reducing and swelling the branches of mycelia. These effects are as a result of the cuminic acid which also improve defence mechanisms of plants against fungal infections (Wang *et al*., 2017). Mexican marigold (*Tagetes erecta*) of family Asteraceae, kills cells of fungal pathogens an activity attributed to the presence of flavonoids which also distort the mycelia structure and cell physiology of the fungus. In addition, the compounds in the marigold improve the resistance of plants against fungal pathogens (Du *et al*., 2017). Ethanolic extracts of tola (*Parastrephia lepidophylla*) induced swelling of conidia and hindered conidial germination in a research that concluded the most likely mode of action is disrupting the plasma membrane (Ruiz *et al*., 2015). Terpenes found in a number of plants causes deformation of fungal mycelia as well as increased permeability of the fungal cell membrane (Gao *et al*., 2018).

Ginger oleoresin derived from ginger (*Zingiber officinale*) of family Zingiberaceae inhibits growth of fungal pathogens, spore germination, increases permeability of the fungal cell and

plasma membrane. Other effects include distortion and sunken mycelia as well as shrivelled spores (Chen *et al*., 2018). Ginger also inhibits conidial germination, synthesis of ergosterol as well as production of mycotoxins such as aflatoxins produced by *Aspergillus flavus* (Nerilo *et al*., 2015). The ability of ginger essential oils to inhibit production of mycotoxins in *A. flavus* is attributed to compounds such as γ-terpinene and citral and their ability to reduce expression of the genes responsible for aflatoxin production (Moon *et al*., 2018).

Turmeric (*Curcuma longa*) also belongs to family Zingiberaceae and the rhizomes have compounds with antifungal properties (Fu *et al*., 2018). Secondary metabolites such as sesquiterpenes isolated from the rhizomes of turmeric have supressed fungal plant pathogens (Han *et al*., 2017). Some of the effects of turmeric compounds on fungal pathogens include decreasing the growth rate of the hypha and mycelial growth (Akter *et al*., 2018; Mamarabadi *et al*., 2018). Aqueous extracts of turmeric have been reported to inhibit sporulation of *Alternaria alternata*, the causal agent of leafspot diseases of brinjals (*Solanum melongena*) (Chaudhari & Rajput, 2018).

Aloe vera of family Asphodelaceae also has antifungal activity against important plant fungal pathogens. It has been reported to destroy the cell membrane, inhibit conidial germination and general growth of hypha of *Aspergillus* and *Rhizoctonia* species (Medda *et al*., 2015; Saniasiaya *et al*., 2017). The gel found in the leaves of *A. vera* was recommended as a pre-harvest treatment in grapes due to its ability to inhibit production of ochratoxin in *Aspergillus carbonarius* (Dammak *et al*., 2018).

2.9 Importance of botanical fungicides

Tremendous accomplishments have been made in commercializing botanical pesticides. However, most of those products are insecticides with little success in products for management of fungi, bacteria, nematodes and viruses which also affect crops (Hikal *et al*., 2017). Plants such as neem have extensively been researched and formulated into products that manage different insect pests (Infonet-Biovision, 2018). Garlic has also been formulated into an insecticide in Kenya by Duduetech. Eucalyptus is successfully being used as a mosquito repellent (Navayan *et al*., 2017; Vivekanandhan *et al*., 2018). Intensive research has and is being conducted still focusing on plants with insecticidal properties (Rizvi *et al*. 2016), on various crops (Ingle *et al*., 2017), targeting different pests (Nia *et al*., 2015) and in different

parts of the world (Ntalli & Menkissoglu-Spiroudi, 2011; Oyedokun *et al*., 2011, Barbosa *et al*., 2013).

Fungal pathogens have caused great losses in crops since time immemorial. Fungal diseases are usually managed by use of synthetic chemical pesticides with notable success. Some pests such as *Phytophthora infestans* affect a wide range of crops and is mainly managed by use of a number of fungicides for successful containment (Mizubuti *et al*., 2007). Little has been achieved towards commercializing botanical fungicides especially in Sub-Saharan Africa. The available products made from plant essential oils are available in developed countries and they have been made from plants such as giant knotweed, thyme, rosemary, neem, cottonseed, dill, caraway seeds and garlic (Zaker, 2016).

2.10 Approaches to development of pesticides from plants

2.10.1 Sample collection and preparation

Whole plants or parts such as leaves, roots, fruits, barks, stems, nodes, rhizomes, cloves, seeds, peels, buds, husks and flowers are usually sought after and can be obtained from the growing regions or bought at specific markets (Gul & Bakht, 2013; Azwanida, 2015: Hadi *et al*., 2016; Akter *et al*., 2018). The plants and plant parts are then carried in appropriate packaging and gathered at a location that is suitable for further processing such as research laboratories. Samples are cleaned in running water and rinsed to remove foreign matter and dirt. They are then dried, preferably under shade to avoid photodegradation of bioactive compounds, or in ovens at regulated temperatures (Golshani & Sharifzadeh, 2013). In experiments involving solvents, the samples are ground into powder to increase the surface area for extraction and kept in opaque containers or khaki papers and free of moisture (Massiha *et al*., 2012).

2.10.2 Sample extraction

According to Silva *et al*. (2016), an idyllic extraction method should yield quality extracts, should be reliably fast and the extracts and the solvent should be easily separable. While choosing an extraction method, the motivating factor should be among others, targeted yield and phytochemical composition of the plant used (Azwanida, 2015). There are conventional and modern extraction methods and all of them depend on operating principles such as choice of solvent, solvent-particle ratio, use of co-solvents, environmental safety, temperature, pressure, plant matrix, polarity of solvents, human toxicity, extraction process and financial

feasibility (Azmir *et al*., 2013). Traditional extraction methods include Soxhlet and maceration while modern methods include but not limited to microwave assisted extraction, ultra-sound assisted extraction and supercritical fluid extraction (Azwanida, 2015). Each of these methods has advantages that make them suitable and demerits that have led to development of new techniques. Soxhlet for example, is one of the oldest methods of extraction and it works better with reduced solvent volumes. An extraction involving huge volumes of solvents may therefore not rely on Soxhlet method. Moreover, when not monitored, it could lead to flammable explosions since it depends on heating the solvent in use (Azwanida, 2015). Maceration operates on a principle similar to Soxhlet but can accommodate large volumes of solvent and plant samples in litres and kilograms respectively. Powdered plant samples are soaked in the solvent for a given period of time with constant stirring. This is to enhance extraction by maximizing the solvent solute interaction (Rwizana, 2016; Hadi *et al*., 2016) and for maceration choice of solvent, temperature and pH are vital. The disadvantage associated with maceration is the large amounts of organic wastes that need to be disposed after extraction (Azwanida, 2015).

Modern methods of extraction are improvements of the traditional methods and they seek to maximize yield components of the extraction within a short time, minimal solvent volumes while still maintaining human and environmental safety and guaranteed sustainability (Silva *et al*., 2016). For instance, supercritical fluid extraction relies on concepts of using safe solvents, solvents with different physiochemical properties, non-injurious aspect of carbon(iv) oxide and delivery of a solvent free extract (Silva *et al*., 2016). This method also prevents the extracts from atmospheric oxidation. While these techniques prove to be effective, they are also associated with costs that may not be met by small and medium enterprises (Awouafack *et al*., 2013).

2.10.3 Evaluation of the efficacy of botanical pesticides *in vitro and in vivo*

There are different methods of testing for efficacy of botanical pesticides under laboratory conditions. Each of those techniques aim at achieving the highest level of activity and with precision. Such techniques include poisoned food, disc diffusion and agar well diffusion. While using the agar well diffusion method the growth medium is allowed to molten on plates and the test pathogen plated on it. Wells are made of a certain diameter onto the medium and a known amount of extract are placed into the wells. After incubation, the extracts diffuse into the medium and their effectiveness is determined by the growth inhibition zones established

compared to the control treatments. This method is used for both bacteria and fungi (Golshani & Sharifzadeh, 2013; Muthii *et al*., 2014).

Disk diffusion assay involves asceptically preparing discs, usually from filter papers, to a uniform size and a known amount of the extracts poured onto each disc and are allowed to dry off. The plates are inoculated with the intended pathogen for 18 to 24 h before the discs are introduced while working with bacteria. The discs are placed on top of the media in equidistant positions depending on the number of treatments being evaluated together with the controls. When working with fungi, a known size of a mycelia growth of 8 to14 days is placed at the centre of the plate while the disks are placed at four equidistance locations. The control for fungi is either plates of fungi without discs or the discs are soaked in solvent used for the extraction. Effectiveness of the extracts is determined by measuring the inhibition zones around the discs compared to those of the control (Thiruppathi *et al*., 2010). This method is also commonly used in clinical trials for pharmaceutical products (Balouiri *et al*., 2016).

Poisoned food assay involves incorporating the extracts into the growth medium. A known weight of extracts is added into a known amount of growth medium and swirled to mix thoroughly before being poured into a plate. The mixture is allowed to set and then the test pathogen is inoculated at the centre of the plate. Agar plugs of bacteria cultures are used from a 24 to 48 h old culture while mycelia growth of a fungal pathogen used is usually 8 to14 days old. Control plates are usually pathogens grown on media with no extracts or with solvents only. Effectiveness is determined by calculating the percentage inhibition of growth by the treatments compared to the control plates (Al-Samarrai *et al*., 2013).

Field experiments involving plant extracts are done in comparison to already commercialized botanical pesticides and synthetic chemical pesticides in order to evaluate the efficacy of the proposed product. The efficacy trials are conducted in one or several regions and over a number of seasons, involving one or several crops depending on the target pathogen(s). The product concentrations that are reportedly effecitve are then formulated and further trials are done till an optimum product is achieved (Zhao *et al*., 2015; Lengai *et al*., 2017). The final product is presented to the relevant body for registration accompanied by efficacy data.

2.10.4 Identification of bioactive compounds in spice extracts

Bioactive compounds in plants may be isolated, purified and identified for the purposes of understanding modes of action or formulaton. Among the many techniques available for

isolation and purification of plant bioactive compounds, chromatography is the most common (Altemimi *et al*., 2017). Some of the most common chromatographic techniques include column chromatography, thin layer chromatography (TLC), high pressure liquid chromatography (HPLC), bioaffinity chromatography and cellular membrane chromatography (Cieśla & Moaddel, 2016). Identification of plant bioactive compounds has been possible when chromatographic techniques are coupled with mass spectrometry (MS). For instance, HPLC-MS is one technique that allows proper identification of compounds in plants since the MS aspect can specify the exact molecular weight of a compound. The performance of HPLC is also improved by coupling it with diode-array detection (HPLC-DAD) which aids in analysis and identification of complex plant compounds (Cieśla & Moaddel, 2016). Other techniques for identification of plant bioactive compounds include fourier-transform infrared spectrometry (FT-IR), nuclear magnetic resonance (NMR), gas chromatography mass spectrometry (GC-MS), hybrid quadroupole-time of flight (Q-TOF), high resolution mass spectrometers (HRMS) and liquid chromatography-mass spectrometry (LC-MS) (Cieśla & Moaddel, 2016; Ingle *et al*., 2017). The mass spectrometry inclusion in identification of bioactive compounds is important and thorough because it explicates the chemical and structural components of a compound (Ingle *et al*., 2017).

2.10.5 Formulation of botanical pesticides

The biodegradable nature of plant bioactive compounds that make them suitable candidates for crop protection also makes them a liability especially under natural conditions. In order to ensure stability, persistence and efficacy of plant compounds in natural conditions such as in open fields, it is imperative to formulate them. Formulation further improves applicability of botanical preparations as well as aids in their commercialization since the active compounds are standardized and stabilized (Kumar *et al*., 2019). Basic formulations may be aqueous or oil based and are made by adding a carrier material, stabilizer and a surfactant to the active compounds (Saravanakumar *et al*., 2015). The recent techniques in formulation of plant compounds include encapsulation of the bioactive compounds with materials such as chitosan, starch, cellulose acetate, alginate and polycarprolactone (Borges *et al*., 2018). Formulation of plant bioactive compounds has been reported to improve activity of botanical preparations as well as allow combination of several compounds belonging to one or more plants (Kumar *et al*., 2019).

2.11 Factors limiting adoption of botanical pesticides

Despite the proof of efficacy of botanical preparations under *in vitro* and field conditions there still remains a gap for their development and adoption by farmers. Some of the limiting factors include the need for high dosages under field conditions, reduced shelf-life especially in absence of formulation, strict registration processes, competition from synthetic pesticides, insufficient capital and facilities for development (Lengai & Muthomi, 2018). Huge amounts of plant materials are also needed for development of small quantities of plant extracts and storage and processing facilities of such may not be readily available. Some of the effective botanical preparations have also been reported toxic towards non target organisms (Grdiša & Gršić, 2013; Ekpo *et al*., 2017). Efficiacious botanical preparations are also from plants used as food as medicine and this is seen as competition for food products and further on land designated for agricultural production. There is not yet a ready market for many botanical pesticides especially in sub-Saharan Africa due to low awareness on the effectiveness and benefits associated with use of natural crop protection products (Lengai *et al*., 2020).

2.12 Opportunities for botanical pesticides

There is already a high demand for safe and healthy food, especially fruits and vegetables that has been produced organically and consumers are willing to pay a little more for that (Nain *et al*., 2020). Use of botanical pesticides is one way of naturally protecting crops from pest infestation and disease infection. In order to navigate through some of the challenges facing adoption of botanical pesticides, growing of plants with bioactive compounds of crop protection interest may be done in marginal areas where the communities may also benefit from a new source of income. Forested areas may also be used to grow short herbs, roots and rhizomes purposely for development of botanical pesticides. Non-food plants with bioactive compounds suitable for development of botanical pesticides may be domesticated for supply of enough source materials. Plants with highly effective bioactive compounds may be subjected to breeding in order to increase production of the compounds for development of botanical pesticides. Additionally, highly effective bioactive compounds identified in plants may be synthesized by chemical companies and formulated into pesticides (Lengai *et al*., 2020). In order to manage the high degradability of botanical preparations sespecially under field conditions, bioactive compounds may be encapsulated using nano particles which will increase efficacy of the formulated compound (Kumar *et al*., 2019).

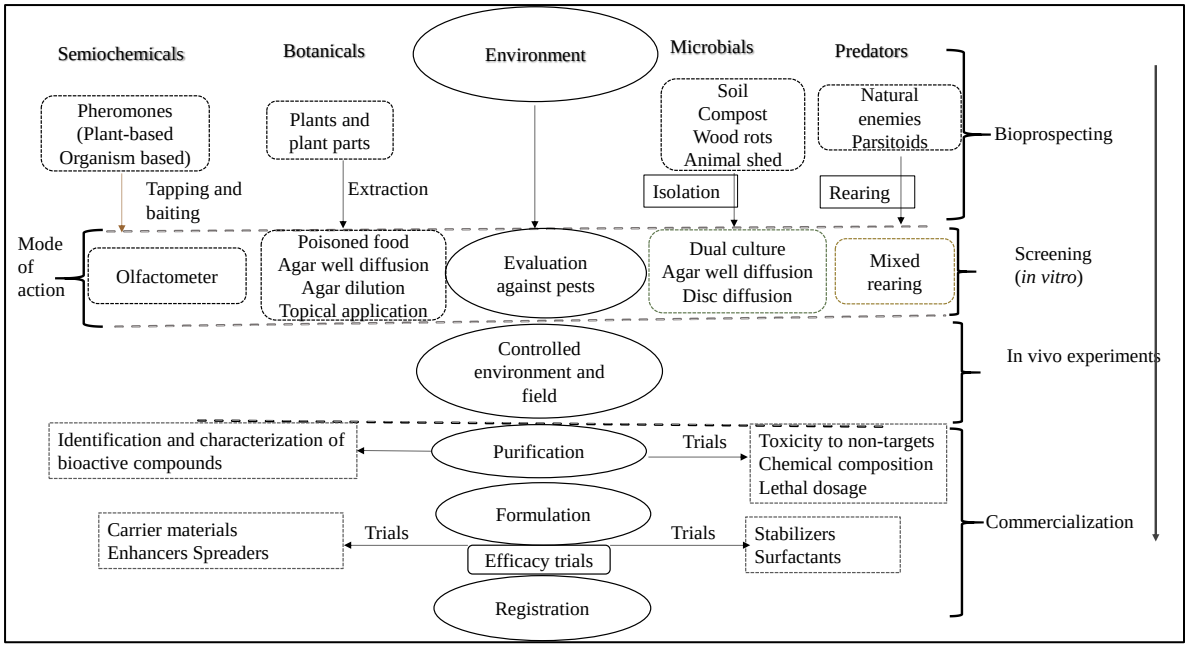

Figure 1: Steps in production and evaluation of different types of biopesticides (Lengai & Muthomi, 2018)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Identification of the ethnomedicinal value of ginger and turmeric in northern and eastern Tanzania

3.1.1 Ethnobotanical survey and collection of spice samples

A survey was conducted in Northern and Eastern regions of Tanzania, covering a total of six districts namely, Lushoto, Muheza, Korogwe, Same, Tanga and Moshi constituting trading markets and growing regions. Non-random sampling was adopted for the study removing the need to establish the population size since only a targeted group of people was needed. A total of 167 participants were purposively selected for homogeneity and were later categorized as farmers, traders and users of ginger and or turmeric. The study population comprised old and young, educated and less educated, working and non-working, male and female respodents.

A semi-structured questionnaire was used to gather information about production, sale and utilization of ginger and turmeric among the participants and in the region. Information was also sought regarding the participants' knowledge on medicinal and pesticidal value of ginger and turmeric. The questionnaires were written in English, but the questions were administered to the participants in Kiswahili, a language they were all conversant with. Access to the participants in different districts and their interiors was accorded by Agricultural Officers as well as village elders and organizational leaders. Consent to participate in the study was sought verbally with each respondent. The survey was undertaken in September through October 2018 during which samples of ginger, lemongrass, cardamom, black pepper, clove, cinnamon and turmeric were collected and gathered to the Life Sciences Laboratory at the Nelson Mandela African Institution of Science and Technology for further processing.

3.2 Evaluation of activity of plant extracts against fungal pathogens of tomato *in vitro*

3.2.1 Preparation of ethanolic extracts from spices

Maceration was adopted for this activity following modified procedures described by Rizwana (2016). A total of 40 kg of ginger and 40 kg of turmeric fresh rhizomes were washed under running water. The rhizomes were then rinsed with distilled water and spread out on warm concrete floors to dry out the surface water for two hours. Separately, the rhizome samples

were chopped into chips and spread out on khaki bags to dry under shade over two weeks (Golshani & Sharifzadeh, 2013). After drying, the dried ginger and turmeric chips were ground into powder and packed into khaki bags. Powder of each sample was soaked in 97% ethanol at the rate of 0.5 kg: 1.5 L amber glass bottles with constant shaking. The ethanol powder mixture was filtered through two layers of cheese cloth followed by Whatman No.1 filter paper. The ethanol in the filtrate was evaporated using a rotary evaporator (Heidolph) at 40°C. Extraction was done three times with evaporation of ethanol after every 72 h. The resultant ethanolic extracts were divided into three portions, one part for antifungal tests, one for fractionation and biochemical analysis and the other for field experiments. Dried samples of black pepper, clove, cinnamon, cardamom and lemongrass obtained during the survey were ground into powder and extracted in ethanol as aforementioned.

3.2.2 Isolation of fungal pathogens

Plant pathogenic fungi of interest were *Alternaria solani*, *Phytophthora infestans, Fusarium oxysporum* and *Pythium ultimum*, all affecting tomato plants on foliage, fruit or roots. Pure cultures of *Alternaria*, *Pythium* and *Fusarium* were obtained from the Plant Pathology Laboratory, University of Nairobi, while *P. infestans* was isolated from infected tomato samples. Cultures of *Fusarium*, *Alternaria* and *Pythium* were maintained on Potato Dextrose Agar (PDA) (Nashwa & Abo-Elyousr, 2012) while *Phytophthora* was maintained on decoction media. Procedures by Trione (1974) were modified to make decoction media by blending 200 g of young tomato seedlings in 500 mL of sterile distilled water. The tomato blend was filtered through cheese cloth and the filtrate topped to one litre with sterile distilled water followed by adding 39 g of PDA. The tomato blend and PDA solution was sterilized by autoclaving for 15 minutes at 121°C then allowed to cool to about 45°C before isolation and sub-culturing of *P. infestans*.

3.2.3 Bioassay of ethanolic extracts against fungal pathogens of tomato *in vitro*

Poisoned food technique was adopted for these bioassays and was carried out following modified procedures described by Muthomi *et al*. (2017). Preliminary experiments were conducted to arrive to an appropriate concentration of the extracts and also to rule out the antifungal activity of ethanol against the pathogens. A stock solution was prepared by adding 10 g of the extracts into 10 mL of ethanol. One hundred microlitres of the stock solution was further added to nine hundred microlitres of ethanol. Five hundred microlitres of that solution

was added to 15 mL of growth media intended to grow the fungal pathogens in 9 cm Petri dishes. The extracts were added into the media while it was at 45˚C, a temperature that allowed evaporation of the ethanol used to dulite the extracts. Positive control plates contained media amended with a commercial fungicide Ridomil Gold® (metalaxyl-M 40 g/kg and mancozeb 640 g/kg) at the rate of 2.5 g/L while negative control plates contained media with no amendments. A three-millimetre cork borer was used to scoop mycelia from the actively growing edges of seven-day old cultures of each of the intended pathogens and placed centrally on the plates. Each treatment was replicated four times and the experiment was repeated once. The plates were sealed and incubated at room temperature (25 – 27˚C). The diameter of the mycelia growth was recorded every two days after incubation (DAI) for 20 days. The effect of the treatments on the pathogens was measured by comparing the colony growth among treatments with that of the negative control and calculating the growth inhibition percentage. The following formula was adopted from Muthomi *et al*. (2017) for calculating percent growth inhibition.

$$\%Inhibition = \frac{Colony\ diameter\ (Control) - Colony\ diameter\ (Treatment)}{Colony\ diameter\ (Control)} \times 100$$

3.2.4 Bioassay of combinations of ethanolic extracts against *Phytophthora infestans in vitro*

The most inhibitory extracts from activity 3.2.3 *viz.* clove, black pepper, ginger and turmeric were selected for this activity. In the previous *in vitro* experiment involving individual extracts, 500 µL was incorporated into growth media. For this activity 500 µL was divided among all extracts involved in the combination. For instance, 250 µL was required for each extract in a combination involving two extracts. For a bioassay involving combination of three extracts, 167 µL was required from each extract while that with four extracts required 125 µLfrom each extract. The respective aliquots were incorporated into decoction media and a mycelium plug of seven-day old *P. infestans* was plated centrally. The media for positive control was amended with a fungicide, Ridomil Gold® (metalaxyl-M 40 g/kg and mancozeb 640 g/kg) while the media for negative control contained media with no amendment. Radial growth of *P. infestans* was recorded after every two days until 20 DAI. This data was used to compute the growth inhibition capacity of the extract combinations against *P. infestans* using the aforementioned formula.

3.3 Determination of the bioactive compounds in the crude extracts

3.3.1 Solvent fractionation of ethanolic spice extracts

Based on activity from the first stage of bioassays, in activity 3.2, four spice extracts were identified as most active against the tested fungal pathogens of tomato. These four spices, clove, black pepper, ginger and turmeric were considered for solvent fractionation. Sequential fractionation was adopted using three solvents as described by Fentahun *et al*. (2017) with suitable modifications. Five grams of each of the extracts were dissolved in 30 mL of distilled water in a separating funnel and shook to allow mixing. To the aqueous mixture, 30 mL of n-hexane were added and shook to allow dissolving of compounds, then allowed to settle for separation based on polarity and tapped out. This was done three times and was repeated using dichloromethane (DCM) and ethyl acetate. The solvents in the different fractions were evaporated under vacuum using a rotary evaporator. Four ethanolic extracts portioned in three solvents yielded 12 fractions.

3.3.2 Bioassay of solvent fractions of ethanolic spice extracts against *Phytophthora infestans*

For each of the 12 extracts fractions, 50 µl was amended with 15ml of decoction media and swirled to allow proper incorporation. The media was then poured into Petri plates in four replicates and allowed to set. Using a 3 mm cork borer, a mycelium plug of *P. infestans* was scooped from seven-day old cultures and placed centrally onto the Petri plates. The media for positive control was incorporated with a fungicide, Ridomil Gold® (metalaxyl-M 40 g/kg and mancozeb 640 g/kg) while the media for negative control had no amendment. The plates were labelled and sealed and maintained at room temperature (25 - 27°C). Radial growth of the pathogen was recorded after every two days until 20 days after incubation. This data was used to compute the inhibition capacity of the fractions as aforementioned in activity 3.2.

3.3.3 Gas Chromatography-Mass Spectrometry (GC-MS) analysis of solvent fractions

One milligram of each of the 12 fractions was weighed and put into Eppendorf tubes. One millilitre of the respective solvents was added into the different tubes, vortexed for 10 seconds and filtered through a plug fitted with a filter paper followed by laboratory grade anhydrous sodium sulfate (Fisher Scientific) to keep the samples water free. The formula $C_1V_1=C_2V_2$ was used to determine the concentration of the final solutions for analysis. The starting

concentration (C_1) of the solution multiplied by the initial volume (V_1) intended for processing equals to the final concentration (C_2) of the sample multiplied by the final volume (V_2) intended for analysis. The final volumes of the extracts were topped to one millilitre with respective solvents in glass vials and analyzed by GC-MS (Agilent Technologies Inc., Santa Clara, CA, USA) fitted with a low bleed capillary column with helium as the carrier gas flowing at the rate of 1.25 ml per minute. The initial oven temperature was 35˚C while the final reading was 285˚C. The mass selective detector was maintained at ion source temperature of 230˚C and a quadrupole temperature of 180˚C while the electron impact mass spectra were obtained at the acceleration energy of 70 eV (Cheseto *et al*., 2020).

3.4 Evaluation of effect of the ethanolic extracts on seed germination

Untreated seeds of Money Maker® tomato variety were obtained from a nursery farm in Mwea, Kirinyaga County, that supplies tomato farmers with seedlings established from seeds obtained from ripened tomato fruits. The seeds were carried in khaki bags to the Botany Laboratory of University of Nairobi for further experimentation. Phytotoxicity tests of the seven crude plant extracts were carried out following modified procedures by Islam and Kato-Noguchi (2014). A ten-dilution series was adopted where one millilitre of each stock extract was added to nine millilitres of sterile distilled water. Dilutions of up to 10^4 were used to make four cocnentrations of 10, 20, 30 and 40% (w/v). Tomato seeds were surface sterilized in 2% sodium hypochlorite, rinsed in three changes of sterile distilled water and then soaked overnight in the separate extract concentrations of each of the extracts, water and a fungicide Ridomil Gold® (metalaxyl-M 40 g/kg and mancozeb 640 g/kg). After 12 h of soaking, 25 seeds were incubated in a 4-layer blotter moist chamber and left for germination (International Seed Testing Association [ISTA], 2015). Germination data was collected from 7 days after incubation up to 20 days after germination because initial germination is expected within the first week after planting and expected to increase over time. Germination was considered if the radicle was completely out of the seedcoat by at least two millimeters. The experiment was laid out in a completely randomized design with three replicates and was repeated once. Data collected included number of roots and shoots and was used to compute germination percentage using the following formula adopted from ISTA (2015).

$$\% \text{ germination} = \frac{Number\ of\ seeds\ germinated}{Number\ of\ seeds\ planted} * 100$$

3.5 Evaluation of the efficacy of the plant extracts against blight diseases of tomato under field conditions

3.5.1 Experimental site

The experiments were conducted in the Field Station of College of Agriculture and Veterinary Science, University of Nairobi, Kenya. The location of the field station is in agroecological zone III which is characterized by an altitude of 900-1 860 m above sea level, a bimodal rainfall distribution, a mean annual minimum temperature of 13°C and a maximum of 23°C. The area has humic nitisols with good drainage and receives a 1 000 mm of rainfall annually, ideal for tomato production (Jaetzold *et al*., 2006). The station has a history of tomato production and therefore disease inoculum for early and late blight was readily available.

3.5.2 Raising of the experimental crop

Tomato seedlings of certified Money Maker® variety were grown in spindling trays over three weeks at the Botany Laboratory at CAVS, University of Nairobi. They were later transplanted onto plots of 3 m x 3 m into priorly prepared holes with soils containing diammonium phosphate applied at the rate of 200 kg/ha at a spacing of 60 cm x 60 cm. This specific spacing was adopted to allow propoer monitoring of the disease development and application of the treatments. The transplanted seedlings were watered every day for a week until they were successfully established. Other agronomic practices such as irrigation, gapping, weeding, topdressing, pruning and staking were done accordingly and uniformly for all plants in all plots. Five plants were selected within each plot and tagged for application of the treatment and monitoring throughout the experimental period.

3.5.3 Experimental design and treatment application

Following deductions made from the *in vitro* experiments, ethanolic extracts of clove, black pepper, turmeric and ginger were selected based on their activity to be tested for efficacy under field conditions. A stock solution was prepared at a concentration of 1g of extract per millilitre of ethanol. In hand sprayers of one litre capacity, five millilitres of each of the four ethanolic spice extracts' stock solution were added to one litre of water, followed by three drops of Tween 80, acting as a surfactant. Ridomil Gold® (metalaxyl-M 40 g/kg and mancozeb 640 g/kg) and Confidor® (Imidacloprid (chloro-nicotinyl) 700 g/kg) were respectively used as chemical fungicide and insecticide for the positive control. A negative control included no foliar application. These made a total of six treatments which were laid out in triplicate in

randomized complete block design and the experiments were carried out over two cropping cycles. Exactly two weeks after transplanting, the plants were sprayed with the treatments till fully drenched and thereafter once every week. In the second cropping cycle, there was heavy rainfall which favoured the development of late blight. Following this observation, the concentration of the extracts was doubled to 10 mL per litre of water. Data collection on distribution, incidence and severity of early and late blight diseases began one week after the first treatment application.

3.5.4 Assessment of blight intensity

Early blight and late blight were assessed using disease scales. Distribution was assessed on a scale of 0-2 where 0 = no disease in the entire plot, 1 = some spots in the plots showing diseases and 2 = disease distributed over the whole plot. Incidence, the number of plants showing infection out of the plant population in a plot in percentage, was assessed on a scale of 0 – 100% where 0 = no disease in the plot and 100 = disease symptoms in the entire plot. Severity was measured using modified scales by Horsefall and Barret (1945) and Henfling (1987) where 0 = no disease, 1 = <20% leaf area infection, 2 = 21-40% leaf area infected, 3 = 41-60% leaf area infected, 4 = 61-80% leaf area infected, 5 = 81-100% leaf area infected. There was no yield data due to high severity of late blight. Area under the disease progress curve (AUDPC) was used to compute the disease load present during the two cropping cycles as per the following formula.

$$\text{AUDPC} = \sum_{i=1}^{Ni-1} \frac{(yi+yi+1)}{2}(ti + t1 - ti) \text{ (Shaner \& Finney, 1977)}$$

3.6 Statistical data analysis

The survey data was coded and subjected to descriptive analysis using IBM SPSS Statistical Package Version 22 to obtain frequencies and valid percentages. The *in vitro* bioassay data of radial growth of the pathogen's mycelia was converted to percentage using the aforementioned formula. One-way analysis of variance was adopted for the different growth results as affected by various treatments. A split plot analysis was done on data involving various crude ethanolic extracts partitioned in different extracts where extracts were main plots and solvents were sub-plots. One way analysis of variance was also adopted for the field experiments. Means were separated using Tukey's test at 5%. Genstat® 15th Edition, VSN International was used for this analysis. Gas chromatography-mass spectrometry data was analysed using a Hewlett-Packard workstation equipped with ChemStation B.02.02 and the resultant compounds were compared with reference spectra published by National Institute of Standards and Technology (NIST) 05, 08, and 11 libraries for mass spectra and retention time.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Ethnomedicinal value of ginger and turmeric in northern and eastern Tanzania

4.1.1 Social characteristics of study participants

Out of the interviewed participants, traders were the majority (68.9%) followed by farmers (27.5%). About 58% of the respondents were male with most of the respondents aged between 20 – 39 years representing over 70% of youth. There was a small representation (13.8%) of participants belonging to the ages between 40 - 49 years and those above 60 years. About 70 % of the participants had received primary education and about 6.6 % of the participants had received tertiary education. There was a small representation of the civil servants and students. (Fig. 2).

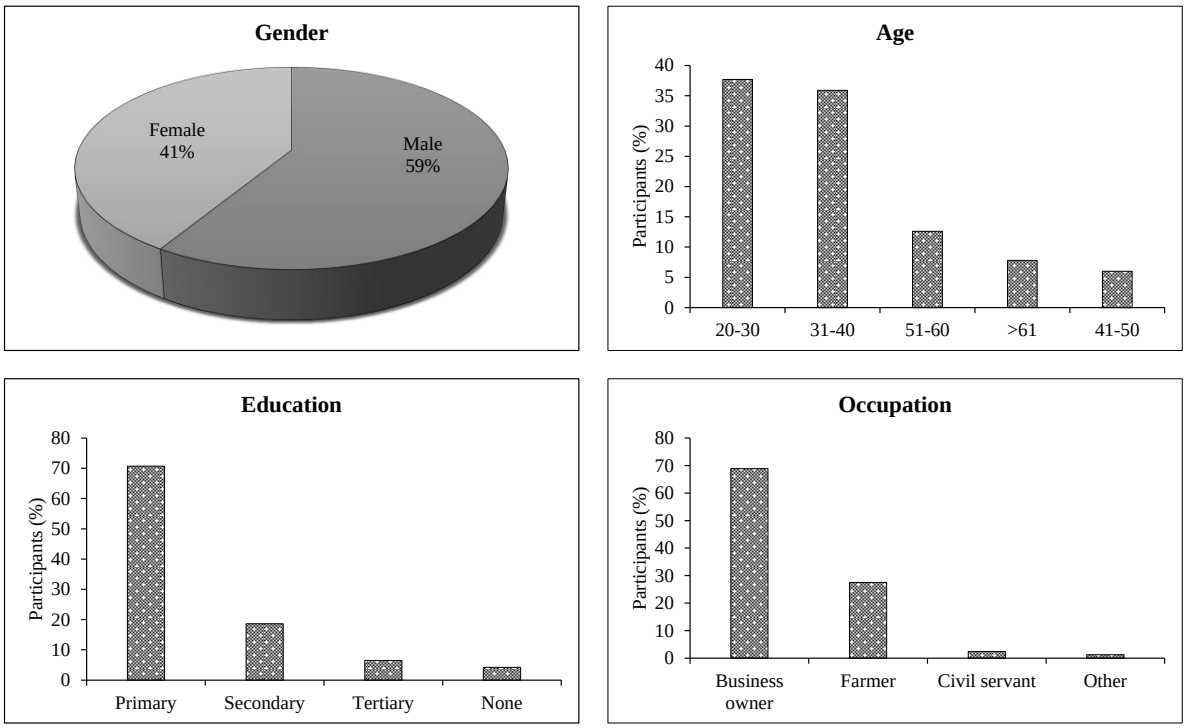

Figure 2: Gender, age, level of education and occupation of participants in selected districts in Northern and Eastern Tanzania

These results are in concurrence with the study by Mmasa and Mhagama (2017) that young people are involved in production and sale of agricultural produce. This also gives the youth an alternative source of income other than formal employment. The youth also have the strength to provide the necessary workforce needed in the agricultural sector (Kwenye &

Sichone, 2016). The observation that men were more involved in sale and growing of ginger and turmeric is supported by the role of the male as the heads of households and as owners of most of the family property along with providing for and protecting families (Silberschimdt, 2001). Therefore, they direct the activities carried out on the farms they own (Hewlett, 2000). However, the medicinal uses of ginger and turmeric were better elucidated by people above 40 years and this might be because of the number of years of experience an individual has about their society. Similar observations were made by Silva *et al*. (2011) who reckoned that the number of medicinal plants named by participants in their study was related to the age of the informant. However, Vanderbroek and Balick (2012) opined differently attributing such knowledge to migration habits which contribute to additional information. A higher level of education also exposes individuals to additional information and if well utilized, that information could lead to personal development. Kalirajan and Shand (1985) however, reported that there was no significant relationship between education of the farmers and attainment of higher rice yield according to the findings in their study. In the current study, respondents who had received tertiary education were aware of botanical pesticides and understood their benefits. This finding is supported by reports by Adebiyi and Okunlola (2013) who accounted that the level of education of an individual influences their understanding and hence adoption of new technologies. Adoption and utilization of new technologies would lead to increased agricultural productivity as well as improved quality of the produce.

4.1.2 Farmers' experiences in production of turmeric and ginger in Same District

Both turmeric and ginger were grown in Same district with the latter being widely grown by about 97% of the farmers. There were few farmers growing turmeric and it was observed that more farmers were venturing into turmeric farming at the time of the study. Ginger was majorly grown for sale and for home consumption with about 75% of the farmers growing it exclusively for sale. Ginger was grown on varying acreage ranging from a half to over five acres. About 55% of the farmers grew ginger on one to three acres of land. A small percent (10.8%) had over five acres of land under ginger production and 67% of farmers had grown ginger for over five years. Ginger was intercropped with other crops such as cassava, maize and arrowroots (Table 1).

Table 1: Farmers' experiences in production of turmeric and ginger in Same District

Questions	Category	Percent participants	Observations
Plants grown	Turmeric	2.7	Only a single farmer had turmeric at the time of data collection.
	Ginger	97.3	
Purpose of growing plants	Sale	75.7	Home uses include spicing tea and vegetables and marinating meat
	Home consumption	5.4	
	Both	18.9	
Acreage under ginger and turmeric production	<0.5	5.4	Ginger is intergrown with other crops like cassava, maize and arrow roots
	0.5 – 1	27.0	
	1 – 3	56.8	
	>5	10.8	
Number of years in ginger and turmeric farming	1 – 2	10.8	The number of years in ginger and turmeric production is relative to the age of the farmer
	2 – 5	21.6	
	>5	67.6	

All farmers interviewed in Same District used farmyard manure (FYM) for improvement of soil fertility in production of ginger and turmeric (Fig. 1). About 39 % of the farmers also used fertilizer while others used a combination of fertilizer, FYM and pesticides. Less than 2% of the respondent farmers used a combination of FYM and compost. The farmers using FYM and fertilizers were the majority representing about 58.7% of the respondents (Fig. 3). Ginger was mainly produced in Same District where the farmers owned up to five acres of land and most farmers had been in ginger production for more than ten years. This is attributed to land ownership culture and labour-intensive nature of land preparation (Ezra *et al*., 2017). In the current study, ginger was found to be intercropped with crops such as cassava, maize and arrow roots. Intercropping provides the farmers with alternative sources of food and income since ginger takes 7-10 months before it is harvested (Reddy *et al*., 2016). When intercropped with legumes crops such as cowpea, soy bean, mung bean and lablab the quality of the tillering and rhizomes of ginger also improves.

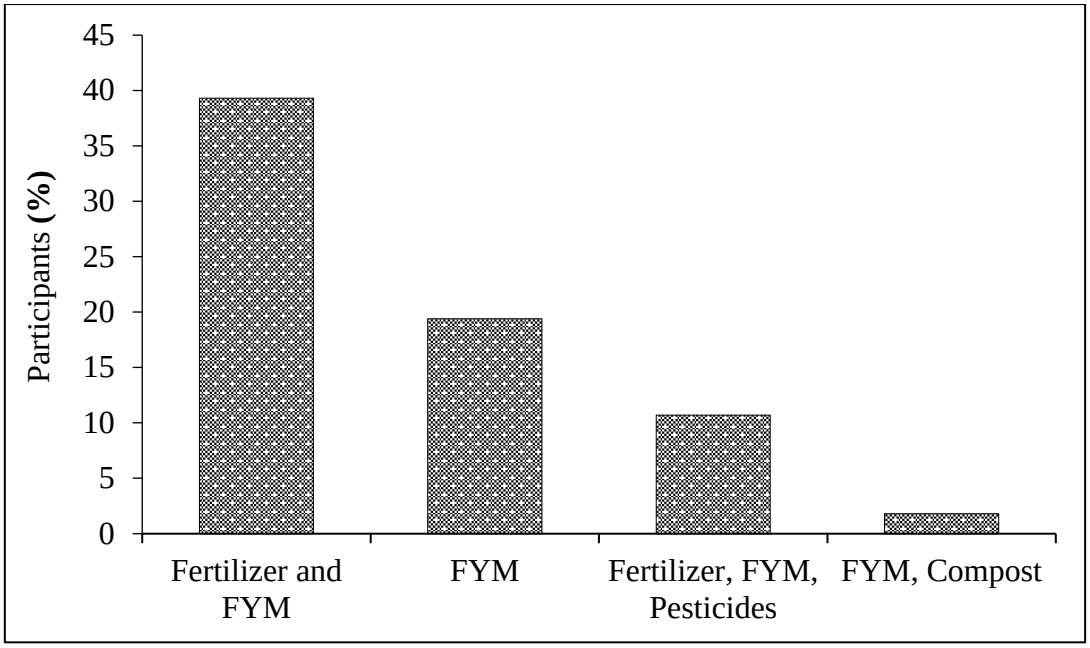

Figure 3: Percent of farmers using different types of inputs to grow turmeric and ginger in Same District at the time of the study

Intercropping one row of ginger with two rows of the legume crops also improves the nutrition status of the soil system where ginger was grown (Nwaogu & Muogbo, 2015). Ginger intercropped with maize and legume crops also has high economic returns as reported by Chapagain *et al*. (2018). Under agroforestry system, ginger was also reported to yield more and better rhizomes when intercropped with jatropha and sapota (Pandey *et al*., 2017). In the current study turmeric was rarely grown in the study districts. This explains the findings by Maerere (2014) who reported that less than one tonne of turmeric is exported from Tanzania per year. The respondents reported that turmeric used to be widely grown before the introduction of ginger, which has multiple uses and higher market demand. It was nevertheless observed that few farmers were again considering planting turmeric.

4.1.3 Trade of ginger and turmeric

Ginger was sold in all the sampled markets and it was the most common rhizome in the study districts with 100% availability in Lushoto, Moshi and Same. Turmeric was only available in some markets and in few districts. Less than 17% of the traders in Muheza, Tanga and Korogwe Districts sold a combination of ginger and turmeric. About 5.5% of traders in Muheza District exclusively sold turmeric (Fig. 4). Between 55 and 90% of the traders in Korogwe District sold fresh ginger rhizomes at the time of the survey. Processed ginger and turmeric were sold as either dried chips or powder. Chipping and drying of the ginger rhizomes were done at the farm while grinding was done in a processing factory. The high sale of fresh rhizomes is related to

their uses such as spices and medicine (Fig. 5). There was a distribution of both retailers and wholesalers in the sampled markets. Most of the customers in all districts, except Same, were retailers. Traders in Moshi, Korogwe and Lushoto had 100% retail customers. Muheza, Tanga and Same had a mixture of both retailers and wholesalers. Same District had the highest number of wholesalers and very few of retailers. This is because Same is a region of massive production of ginger. The small number of retailers in Same District could be explained by availability of ginger in almost every household in the district (Fig. 6). In all districts except Korogwe, ginger and turmeric were sold in company of other spices such as clove, cardamom, black pepper, cinnamon and garlic. Across all districts, the spices, vegetables and fruits were sold in the same market place with only 17.6% of traders in Muheza selling turmeric and or ginger only. In Lushoto and Same Districts, there were no fruits sold and in Korogwe only 11.2% sold ginger at the time of the study (Fig. 7).

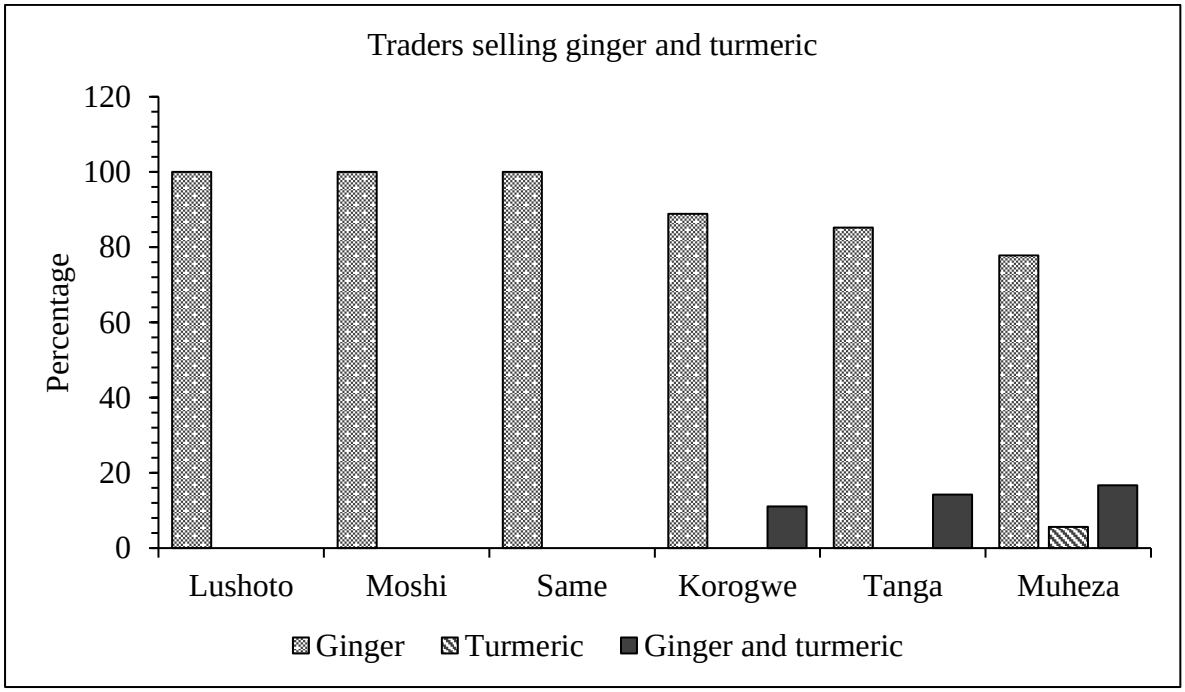

Figure 4: Percentage of traders selling turmeric and ginger in six districts in Northern and Eastern Tanzania

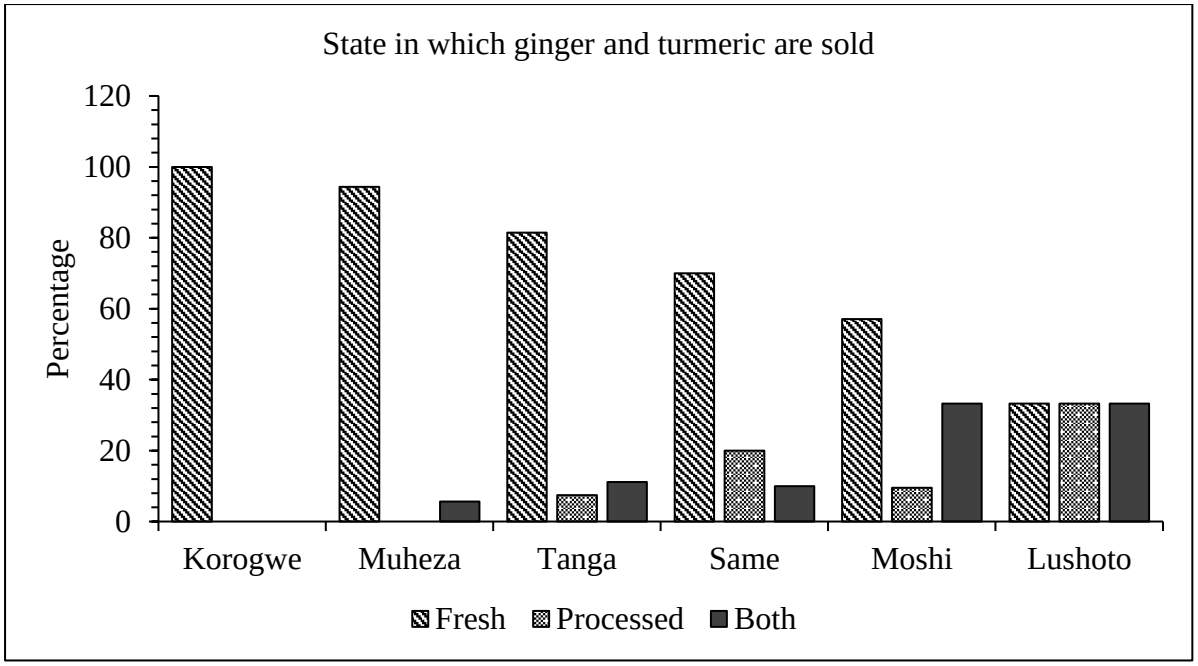

Figure 5: Percentage of traders selling fresh and processed turmeric and ginger in six districts in Northern and Eastern Tanzania

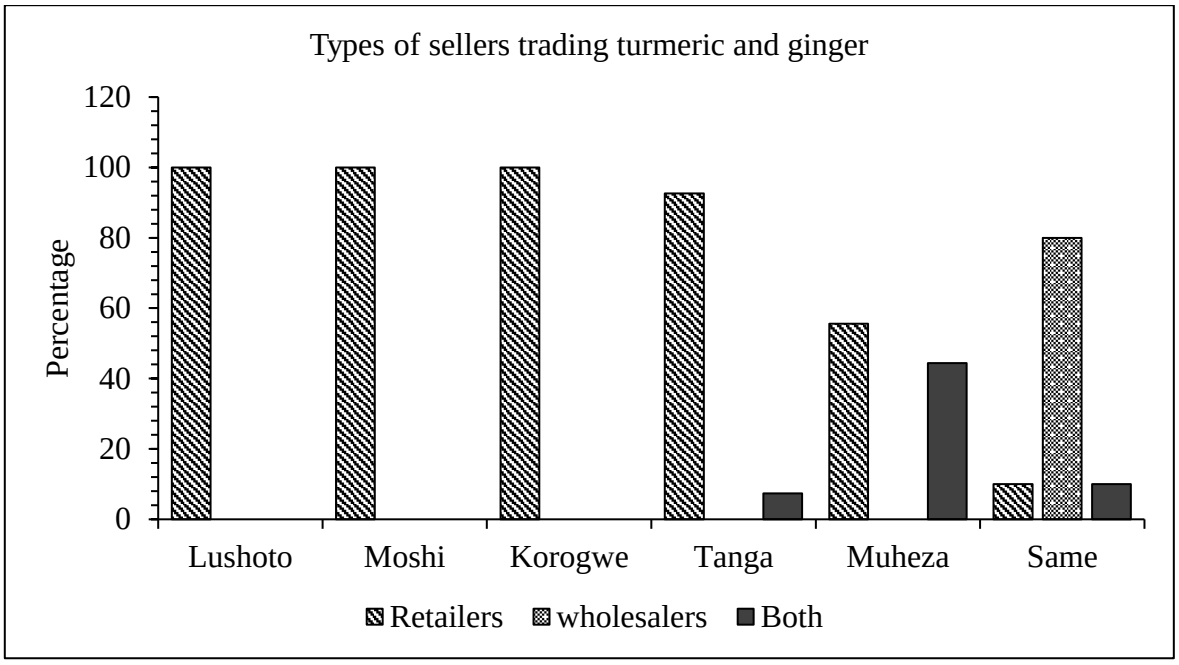

Figure 6: Percentage of retailers and wholesalers involved in purchase of turmeric and ginger in six districts in Northern and Eastern Tanzania

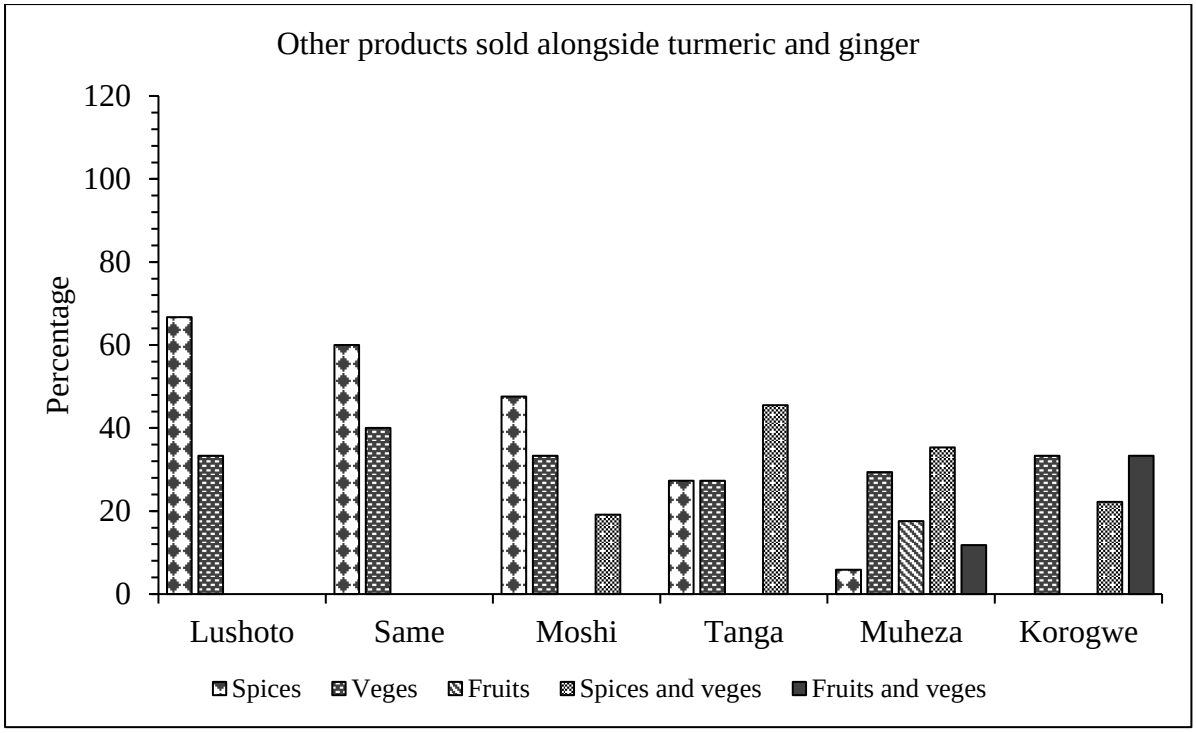

Figure 7: Percentage of traders selling other plant products alongside ginger and turmeric in six districts in Northern and Eastern Tanzania

It was observed that ginger and turmeric are always available in the market throughout the year. The prices however change according to time of the year and season (Mmasa, 2017). During harvesting, prices fall to about 0.3 dollars and could go up to 3 dollars per kilo of fresh rhizomes during the planting season. The prices at the harvesting season are usually determined by middlemen who buy the produce in large quantities and sell later at higher prices. Therefore, farmers do not benefit fully and have no say in the prices of their produce (Khanal, 2018). Augustino (2017) attributed the involvement of middlemen to failure of operations of the ginger factory in Same District. According to the interviewed ginger farmers, when the factory was operational, they sold their produce at better prices. The consideration of ginger and turmeric as sources of pesticides would hence create an alternative market for farmers and therefore a better income. There would be no conflict in the uses of these plants since they are always available in the markets. A recommendation by Maerere (2014) to enhance marketing of spices in Tanzania would be met by this alternative market of botanical pesticides. This would not only widen the market for such plants but also increase the income for the farmer. Other plants with essential oils have been considered for non-food uses with notable success and effectiveness (Isman *et al.*, 2011; Upadhyay, 2017). Therefore, spice plants in the current study may also be considered for pesticidal use for sustainable crop protection.

4.1.4 Utilization of ginger and turmeric

According to the interviewed sellers of turmeric and ginger, all the customers used the plants as spices with a certainty of 65-88%. Muheza District registered the highest usage of the study plants as spices. They accounted that the plants were used to spice tea, meats, vegetables and cereal meals. Turmeric and ginger were also used for medicinal purposes such as treating coughs, flu and skin wounds. Those using turmeric exclusively (3.7%) also had a beauty option added to their uses and this was common in areas where turmeric is grown. About 11% users in Muheza, Tanga and Korogwe Districts utilized the plants as spices, medicine and for beauty. High recognition of the plants as both spice and medicine, especially ginger, was registered in Lushoto District. Tanga District registered spice, medicinal and beauty uses of turmeric and ginger. This is explained by the fact that these and other plants are majorly produced in those regions (Table 2). All participating traders of ginger and turmeric used the plants as spices. Other participants used turmeric and ginger as a combination of spice and medicine. In all districts except Lushoto, between 70-94% of the participants used ginger as a spice. The rest of the respondents used the plants both as spices and medicine. The medicinal use of the plants was higher in Lushoto with over 65% adding the spice aspect (Table 2). All the users who participated in the study used the plants as spices. Between 66-88% of the user participants across all districts used ginger and turmeric as spices with Korogwe and Same Districts registering 100%. A few of the participants also used the plants as medicine especially in Moshi and Tanga Districts. Those using turmeric added the beauty aspect to their uses as registered among participants in Moshi and Tanga Districts. There was no registration on medicinal use of the study plants in Korogwe and Same Districts among users (Table 2).

These uses of spices reported in the current study have been reported elsewhere especially in Asia, where these plants originated from (Dubey, 2017). Other reported benefits of ginger include improving digestion, blood circulation, lowering cholesterol and also contain antimicrobial properties (Al-Awwadi, 2017; Zadeh & Kor, 2014). Some respondents claimed that ginger was used to improve libido as substantiated by Yadav *et al*. (2016) who reported that intake of about 15 g of ginger daily could lead to increased testosterone levels. Turmeric is widely used as a beauty product (Krup *et al*. 2013) and in the study region it was applied on brides on their wedding days.

Table 2: Percentage of participants utilizing ginger and turmeric as spice, medicine and beauty in selected districts in Northern and Eastern Tanzania

District	Spice	Spice and medicine	Spice, medicine, beauty	Total
Customers				
Muheza	88.9	0.0	11.1	100.0
Lushoto	66.7	33.3	0.0	100.0
Moshi	85.7	14.3	0.0	100.0
Tanga	70.4	18.5	11.1	100.0
Korogwe	77.8	11.1	11.1	100.0
Same	80.0	20.0	0.0	100.0
Trader participants				
Muheza	94.1	5.9	0.0	100.0
Lushoto	33.3	66.7	0.0	100.0
Moshi	89.5	10.5	0.0	100.0
Tanga	88.9	11.1	0.0	100.0
Korogwe	77.8	22.2	0.0	100.0
Same	70.0	30.0	0.0	100.0
User participants				
Moshi	88.8	5.6	5.6	100.0
Korogwe	100.0	0.0	0.0	100.0
Same	100.0	0.0	0.0	100.0
Tanga	66.7	22.2	11.1	100.0

4.1.5 Awareness on medicinal and pesticidal value of ginger and turmeric

About 59% of the interviewed farmers in Same District registered awareness on the medicinal value of ginger and turmeric at the time of the study. Ginger was majorly used to manage respiratory infections such as flu and coughs and it was used in combination with other plants such as lemon and garlic. Turmeric was used for healing skin related infection, wounds, and ulcers. About 13.5% of the interviewed farmers were aware of botanical pesticides and have used farm preparations from plants such as neem, moringa, Mexican sunflower and tephrosia to manage pests and diseases that affect vegetables on their farms. A negligible percent of the farmers had used ginger as an insecticide to manage postharvest insect pests of maize and beans

with notable success (Table 3). There was a correlation of 0.429 between respondents who were aware of botanical pesticides and those who had used ginger as a protective insecticide.

Trader participants across all sampled markets had a level of awareness regarding the medicinal value of ginger and turmeric. Participants from Lushoto and Same District registered the highest level of awareness at 66.7 and 60% respectively. Participants from Muheza District had the lowest level of awareness on medicinal value of ginger and turmeric and over 60% said they didn't know about the claim. Between 30-50% of participants from Lushoto and Tanga Districts claimed not to know any medicinal value of the study plants. Except in Lushoto, 11-57% of participants from the other districts were sure that there was no medicinal value in ginger and turmeric. Moshi and Korogwe Districts had the highest number of participants with knowledge of the medicinal value of ginger and turmeric (Table 4). Over 60% of the user participants in the sampled districts were aware of medicinal value of ginger and turmeric. Korogwe District registered 100% of awareness on medicinal value of the study plants. Between 11 and 38% of the participants had no knowledge on medicinal value of ginger and turmeric (Table 4).

Selected pharmaceutical shops sold products containing ginger either as tablets for soothing coughs, flu and other respiratory infections. They also sold slimming tea containing ginger and other plants such as lemon grass, lemon and fenugreek. Other natural products sold in the pharmaceutical shops included soaps containing neem, aloe and turmeric. The agro-shop operators that were interviewed were aware of botanical pesticides but sold none. The reasons behind agroshops not selling botanical pesticides were, not being common and less awareness among farmers. Some operators however said they would be willing to sell so long as the products are authentic, effective and farmers are aware of them and their effectiveness.

The study found that the respondents were totally unaware of the pesticidal value of turmeric. However, a slight percent of the interviewed farmers was aware that ginger has insecticidal properties. They had used ginger powder to protect maize and bean grains against storage pests with notable success. A section of farmers in Same District were aware of botanical pesticides and had used farm preparations from *Azadirachta indica*, *Tithonia diversifolia*, *Tephrosia vogelii* and *Moringa oleifera* to manage pests on vegetables and maize. The pesticidal properties and the availability of commercial formulations of these plants has been documented (Alao *et al*., 2018; Dimetry & El-Behery, 2018; Mikami *et al*., 2018; Seifi *et al*., 2018; Ngom *et al*., 2018).

Table 3: Farmers awareness on the medicinal and pesticidal value of ginger and turmeric and botanical pesticides in Same District

Question	Category	Percent of participants	Observations
Awareness on medicinal value	Yes No	59.5 40.5	Ginger used to treat flu, cough and chest infections. Turmeric was used for skin related infections, wounds and ulcers.
Awareness on pesticidal value	Yes No	2.7 97.3	Ginger was used as an insecticide to manage post-harvest pests of beans and maize
Awareness on botanical pesticides	Yes No	13.5 86.5	Farmers used farm preparations from plants such as neem, Mexican sunflower, moringa and tephrosia as insecticides

Table 4: Percentage of traders and consumers aware of the medicinal value of turmeric and ginger in Northern and Eastern Tanzania

District	Yes	No	Unsure	Total
Traders				
Muheza	23.5	11.8	64.7	100.0
Lushoto	66.7	0.0	33.3	100.0
Moshi	42.9	57.1	0.0	100.0
Tanga	31.8	18.2	50.0	100.0
Korogwe	42.9	57.1	0.0	100.0
Same	60.0	40.0	0.0	100.0
Users				
Moshi	61.1	38.9	0.0	100.0
Korogwe	100.0	0.0	0.0	100.0
Same	80.0	20.0	0.0	100.0
Tanga	88.9	11.1	0.0	100.0

4.2 Antifungal activity of spice extracts against fungal pathogens of tomato *in vitro*

4.2.1 Overview of antifungal activity of spice extracts

All the crude spice extracts tested for antifungal activity had a significant ($P \leq 0.05$) inhibition effect on growth of the pathogenic fungi of tomato. Clove extract was the most effective with an inhibition activity of about 85.6% against all the tested pathogen. Black pepper, turmeric and ginger extracts were also highly active against the pathogens with inhibitions of 81, 80 and 74% respectively. Clove, black pepper, turmeric and ginger were superior than Ridomil Gold® (metalaxyl-M 40 g/kg and mancozeb 640 g/kg) in inhibiting the growth of all the tested pathogenic fungi of tomato. Lemon grass and cinnamon were the least active with inhibitions of 49.6 and 48.4% respectively (Fig. 8). Susceptibility of the test pathogens differed among the tested pathogens. *Phytophthora infestans* was the most susceptible (85%), followed by *Pythium* (83%), both which belong to class oomycetes. *Alternaria solani* and *Fusarium oxysporum* f.sp. l*ycopersici* were averagely susceptible at about 55% (Fig. 9).

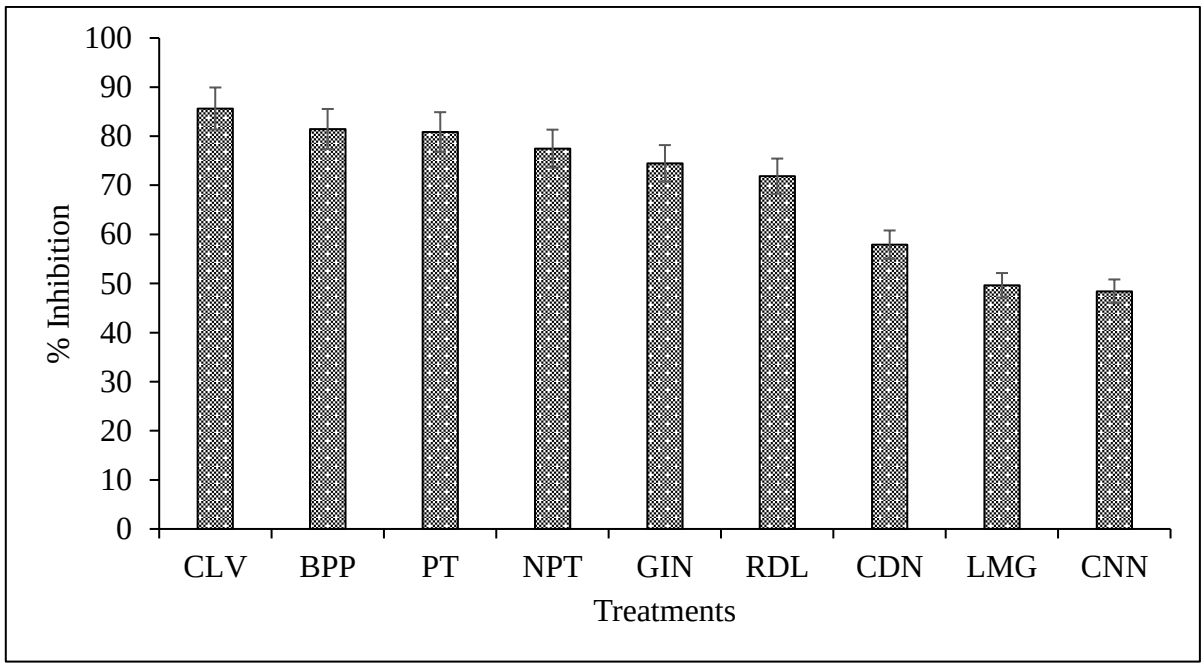

Figure 8: Inhibition percentage of crude spice extracts and a commercial fungicide against plant pathogenic fungi of tomato at 10 days after incubation. (CLV- clove, BPP- black pepper, PT- peeled turmeric, NPT- non peeled turmeric, GIN- ginger, RDL- Ridomil Gold® (metalaxyl-M and mancozeb), CDN- cardamom, LMG- lemongrass, CNN- cinnamon)

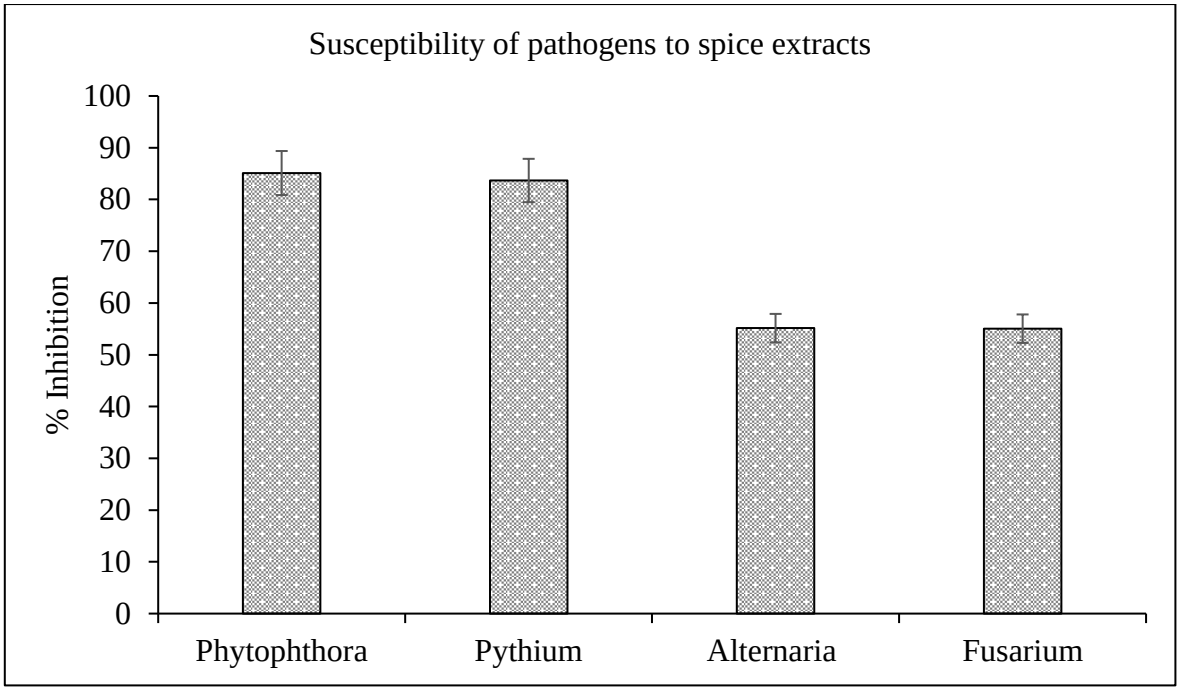

Figure 9: Susceptibility of fungal pathogens of tomato to various crude spice extracts and a commercial fungicide at 10 days after incubation

4.2.2 Activity of spice extracts against *Alternaria solani*

All the tested ethanolic spice extracts had an inhibition activity against the early blight pathogen, *Alternaria solani* throughout the experimental period. Five ethanolic extracts had an over 50% growth inhibition from 2 DAI. Clove, black pepper and turmeric extracts were the most effective with inhibitions and their activity was similar to that of the chemical fungicide, Ridomil Gold® (metalaxyl-M 40 g/kg and mancozeb 640 g/kg). Among the tested extracts, those from cinnamon and lemongrass were the least effective. Extracts from cardamom and ginger were averagely active against the early blight pathogen. The activity of the crude extracts increased between 4 to 8 DAI and then decreased from 10 to 20 DAI at a steady rate. Clove extracts maintained a high level of activity throughout the experiment period followed by black pepper and turmeric (Table 5). In the repeat experiment, the trend remained similar with clove extract leading in activity throughout the experimental period. However, black pepper and turmeric inhibited the growth of *A. solani* with an average activity of about 50%. Cinnamon and ginger remained less active with growth inhibitions of below 20% compared to the positive and negative controls. At some point between 10 and 20 DAI, the pathogen outgrew the extracts leading to negative inhibitions (Fig. 10, Table 6).

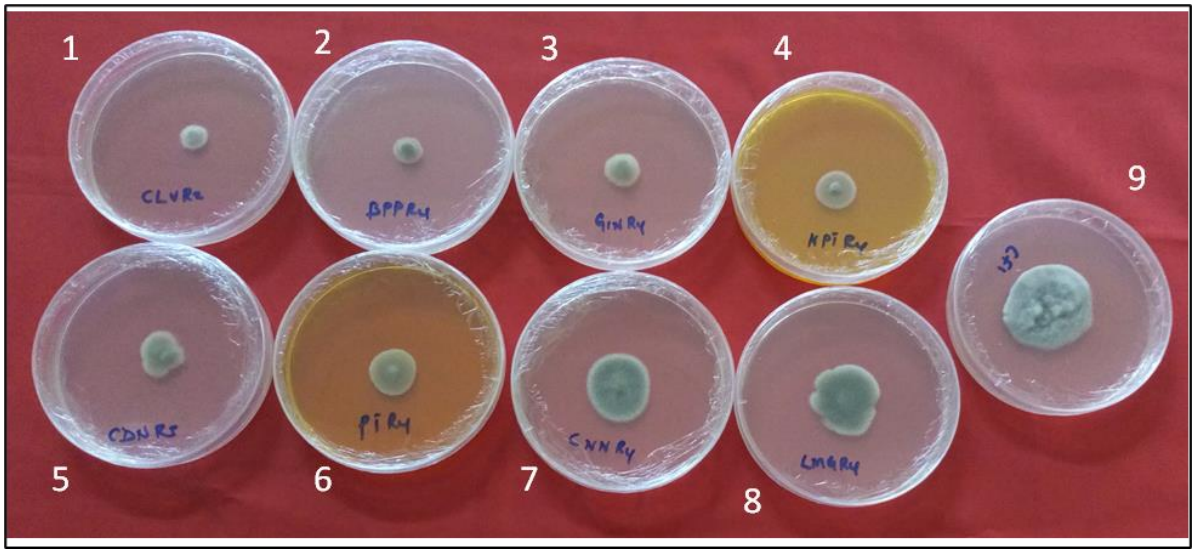

Figure 10: ***In vitro*** **activity of crude spice extracts against** ***Alternaria solani*** **at eight days after incubation. (1- clove, 2- black pepper, 3- ginger, 4- non peeled turmeric, 5- cardamom, 6- peeled turmeric, 7- cinnamon), 8- lemongrass), 9-negative control**

Table 5: Percentage inhibition of colony diameter of *A. solani* cultured on media amended with crude spice extracts, experiment 1

Treatments	Days after incubation										Mean	
	2	4	6	8	10	12	14	16	18	20		
Clove	65.4 a	73.9 a	78.3 a	75.7 a	76.9 a	73.3 a	71.9 a	68.9 a	63.6 a	59.1 a	71	a
Black pepper	53.9 abc	66.3 abc	66.7 ab	63.2 ab	64.4 ab	61.7 ab	59.2 ab	57.1 ab	55.5 a	52.2 a	60	ab
Ginger	39.5 cde	58.7 bc	60.0 b	58.1 ab	57.5 b	55.6 ab	53.1 ab	51.4 ab	48.6 a	47.0 ab	53	ab
Turmeric 1	55.8 abc	66.3 abc	62.5 ab	61.0 ab	60.6 ab	58.3 ab	57.1 ab	56.1 ab	52.7 a	52.2 a	58	ab
Turmeric 2	53.9 abc	64.1 abc	62.5 ab	59.6 ab	60.0 ab	58.9 ab	57.7 ab	55.7 ab	52.3 a	51.7 a	58	ab
Cardamon	44.2 bcd	55.4 c	58.3 b	53.7 bc	53.8 b	50.0 bc	45.4 bc	44.3 bc	39.1 ab	49.1 a	49	b
Cinammon	26.9 e	35.9 d	31.7 c	27.9 d	26.3 c	24.4 d	21.4 d	19.3 d	14.1 b	11.6 c	24	c
Lemongrass	30.8 de	41.3 d	38.3 c	35.3 cd	33.1 c	30.6 cd	27.0 cd	24.1 cd	17.7 b	16.8 bc	30	c
Ridomil	53.9 abc	69.6 ab	69.2 a	64.0 ab	63.8 ab	62.2 ab	61.2 ab	60.4 ab	57.3 a	34.1 abc	60	ab
SEM	3.4	2.8	3.5	4.1	4.0	4.8	4.7	5.0	5.4	6.8	3.9	
SED	4.9	3.9	4.9	5.8	5.6	6.7	6.6	7.1	7.6	9.6	5.5	
LSD (5% Level)	10.0	8.0	10.1	11.9	11.6	13.8	13.6	14.6	15.6	19.7	11	
CV (%)	14.6	9.4	11.9	14.8	14.4	18.1	18.6	20.7	24.1	32.7	15	

Means followed by the same letter(s) within each column do not differ significantly at $P \leq 0.05$

Table 6: Percentage inhibition of colony diameter of *A. solani* cultured on media amended with crude spice extracts, experiment 2

Treatments	Days after incubation										Mean
	2	4	6	8	10	12	14	16	18	20	
Clove	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Black pepper	51.7 bc	54.8 bc	49.1 cd	48.5 cd	40.0 c	41.3 c	41.1 cd	39.8 cd	40.9 cd	58.8 c	44.6 cd
Ginger	45.0 bc	40.5 c	37.0 d	39.4 d	32.9 c	35.6 c	31.7 d	32.1 d	36.8 d	37.1 c	36.8 d
Turmeric 1	60.0 b	56.0 bc	53.7 bc	53.0 bcd	44.3 bc	45.0 bc	42.2 bc	45.4 c	47.3 bcd	46.6 bc	49.8 bc
Turmeric 2	56.7 b	56.0 bc	57.4 bc	54.6 bc	47.9 bc	48.8 bc	51.1 bc	48.5 bc	50.5 bc	48.7 bc	52.0 bc
Cardamon	31.7 cd	22.6 d	17.6 e	12.1 e	-1.4 d	3.1 d	3.9 e	5.6 e	12.3 e	12.1 d	12.0 e
Cinammon	16.7 d	13.1 d	5.6 e	6.1 e	-5.7 d	2.5 d	4.4 e	4.6 e	8.2 ef	8.2 d	6.4 e
Lemongrass	18.3 d	20.2 d	15.7 e	3.0 e	-12.1 d	-5.6 d	-3.3 e	-6.1 e	0.0 f	-1.7 d	2.8 e
Ridomil	60.0 b	67.9 b	65.7 b	64.4 b	58.6 b	58.8 b	57.8 bc	58.2 b	58.6 b	54.7 b	60.5 b
SEM	4.7	3.7	3.4	3.1	3.9	3.4	2.6	2.6	2.4	2.9	2.5
SED	6.6	5.2	4.8	4.4	5.5	4.8	3.6	3.6	3.4	4.1	3.6
LSD (5%)	13.6	10.7	9.9	8.9	11.2	9.9	7.4	7.5	7.1	8.5	7.3
CV (%)	19.1	15.4	15.3	14.6	22.9	18.6	13.7	14.1	12.3	15.3	12.4

Means followed by the same letter(s) within each column do not differ significantly at $P \leq 0.05$

In the current study, clove was superiorly active in inhibiting the growth of *Alternaria solani* by up to 100% as at 20 DAI. This observation is consistent with findings by Sarfraz *et al*. (2018) who reported complete inhibition of *A. solani* by methanolic extracts of clove. The findings in this study however differ with those reported by Rahmatzai *et al*. (2017) who reported moderate activity of clove oil against *A. solani*. The differences may be due to susceptibility of the pathogen, quality of the extracts or concentration of the bioactive compouinds in clove subject to cultivar and geographical location where it was grown. Clove has been widely reported to inhibit growth of other species of *Alternaria*. Castro *et al*. (2017) reported superior activity of clove against *Alternaria alternata* that causes postharvest infections in the dragon fruit (*Hylocereus undatus*). According to Gadhi *et al*. (2020), clove essential oil was effective in inhibiting the growth of *A. alternata* causing alternaria leaf blight in chickpea (*Cicer arietinum*) by about 70%. Complete inhibition of growth in *Alternaria* spp has further been reported by Sanit (2016) who reported 100% effectiveness of clove extracts against species that cause dirty panicle disease in rice. Other species of *Alternaria* reported to be susceptible to clove, its oil or subsequent compounds include *A. porri* and *A. citrii* (Pawar & Thaker, 2007; Combrinck *et al*., 2011).

Black pepper extracts inhibited the growth of *Alternaria solani* by between 39 and 66% in the present study. Such activity has been reported by Yadav *et al*. (2019) where black pepper extracts reduced the growth of *Alternaria brassicae* causal agent of alternaria blight. Pattnaik *et al*. (2012) reported a reduced activity of about 29% against *A. solani* and a reduced incidence of alternaria canker by about 41% compared to the untreated control. Black pepper was interestingly reported to promote the growth of *A. ochroleuca*, an activity attributed to ability of the pathogen to detoxify the allelochemicals present in the extracts, allowing the pathogen to flourish (Shafique *et al*., 2018).

The findings in this study indicate that turmeric extract inhibited growth of *Alternaria solani* by between 42 and 66%. Similar level of activity has been reported by Amsaraj and Prasad (2020) who stated that methanolic extracts of turmeric inhibited growth of *A. solani* by about 73% in a poisoned food technique experiment. Rex *et al*. (2019) reported a growth inhibition of about 89% and deterred conidial germination of about 91% in *A. solani* by water extracts of turmeric. Antifungal activity of turmeric has been reported widely on various crops affected by different species of *Alternaria*. Pipliwal *et al*. (2017) reported an inhibition of about 70% in *A. burnsii*, the causal agent of blight in cumin (*Cuminum cyminum*) by water extracts of

turmeric. Pun *et al*. (2020) reported a growth inhibition of about 63% in *A. alternata* the causal agent of leaf spot of cabbage (*Brassica oleraceae* var. *capitata*) by a 10% concentration of fresh turmeric extracted in water. Effect of turmeric on the growth of *A. alternata* was also reported in niger (*Gizotia abyssinica*), brinjals (*Solanum melongena*), and tomatoes (*Solanum lycopersicum*) (Wongkaew & Sinsiri, 2014; Nagaraju *et al*., 2020; Shingne *et al*., 2020). Other species of *Alternaria* reported to show susceptibility to turmeric are *A. porri* and *A. brassicicola* (Suwitchayanon & Kunasakdakul, 2009; Chethana *et al*., 2012). In contrast to the high inhibition activity reported in this and other studies, Nagaraju *et al*. (2020) and Shingne *et al*. (2020) reported a reduced activity of turmeric extracts of 44 and 54% against *A. alternata* respectively.

Ethanolic extract of cinnamon was not as effective in reducing the growth of *Alternaria solani*, registering an inhibition capacity of 35.9% at four days after incubation. This finding is highly contrasted by Yeole *et al*. (2014) who reported 100% inhibition of *A. solani* by hexane and methanolic extracts of *Cinnamomum zeylanicum*. Such superior activity is also supported by Mishra *et al*. (2018) who reported complete inhibition of *A. solani* by cinnamon in a hanging drop technique experiment. Bahraminejad *et al*. (2016) attributed the superior activity of cinnamon against *Alternaria solani* to presence of major compounds found in the bark of the plant such as cinnamaldehyde and linalool. Superior activity of cinnamon in inhibiting growth of *Alternaria* spp was further reported by Castro *et al*. (2017). A study report by Rahmatzai *et al*. (2017) indicated similar findings as those in the present study of cinnamon only inhibiting growth of *A. solani* by up to 21.6%.

Cardamom extracts inhibited the growth of *Alternaria solani* by between 3 and 55% in the current study. Sarfraz *et al*. (2018) however, reported complete growth inhibition of *A. solani* by cardamom extracts. Shafique *et al*. (2017) reported an antifungal activity of about 80% by cardamom extracts against *Alternaria ochroleuca* the causal agent of alternaria leaf spot disease in money plant (*Epipremnum aureum*). In the present study cardamom also stimulated growth of *A. solani* by about 1.4%. This could be attributed to the ability of the pathogen to neutralize the allelochemicals in the cardamom extract thus allowing the pathogen to grow as explained by Shafique *et al*. (2017). Cardamom belongs to family Zingiberaceae and the bioactive compounds present in cardamom seeds include 1,8, cineole and constituents of terpineol, germacrene, *trans*-sabinene, bicyclo-germacrene, *delta*-carene, linalool, nerolidol, ocimenyl among others (Joshi *et al.,* 2017; Ashokkumar *et al.,* 2019).

Lemongrass was least active in suppressing the growth of *A. solani* in this study and in some cases, it was seen to promote growth. This activity of growth promotion was previously reported by Tzortzakis and Economakis (2007). However, Sen *et al* (2020) reported a higher activity of about 48% of lemongrass against *A. solani* in poisoned food technique experiment involving water extracts of *Cymbopogon citratus*. Lemongrass essential oil was reported to inhibit growth of *Alternaria alternata* by about 54% in a dose dependent poisoned food technique experiment (Moh *et al*., 2020). The activity of lemongrass is majorly attributed to presence of major compound, citral and according to Wang *et al*. (2019), this compound inhibits growth of fungi, hinders mycotoxin production, disrupts cell integrity of the fungus and destroys the spores. Lemongrass belongs to family Poaceae and studies have listed compounds such as citral, geranyl, geranial, geraniol and myrcene to be consistently present in cardamoms (Ali *et al*., 2017; Fokom *et al*., 2019).

In the present study, ginger extracts inhibited growth of *Alternaria solani* by between 31 and 60% over 20 DAI. Naik *et al*. (2020) similarly reported a growth inhibition of about 54% in *Alternaria solani* by ginger acetone extracts in a poisoned food technique experiment. The range of activity reported in this study also agrees with findings reported by Verma *et al*. (2020) and Rao *et al*. (2020). Mugao *et al*. (2020) reported an activity of about 47% while Muthomi *et al* (2017) reported an activity of about 37% on *A. solani* by ginger extracts in poisoned food technique experiments. Bhalerao *et al*. (2019) however, reported a higher inhibition of about 79% on *A. solani* by ginger extracts. According to Rex *et al*. (2019) ginger extracts inhibited the growth of *Alternaria solani* by up to 68.5% and germination of conidia by up to 75.5%. Activity of ginger has further been reported on other species of *Alternaria*. Rathore *et al*. (2018) reported a growth inhibition activity of about 49% in *Alternaria tenuissima*, causing alternaria leaf blight in pigeon pea, by ginger extracts. At a concentration of 10%, ginger extracts inhibited the growth of *Alternaria burnsii*, causal agent of blight in cumin (*Cuminum cyminum*) by up to 62.7% (Pipliwal *et al*., 2017). In a poisoned food technique experiment, Pun *et al*. (2020) also reported a growth inhibition of about 63.7% by ginger extracts against *Alternaria brassicicola*, causal agent for alternaria leaf spot of cabbage (*Brassica oleraceae*).

Alternaria species have been reported to withstand effect of chemicals and plant extracts by producing mycotoxins that hinder their antifungal activity (Pero *et al*., 1973; Thomma *et al*., 2003). According to Shafique *et al*. (2018) species of *Alternaria* produce chemicals that neutralize alleopathic effects of the plant extracts which increases the chances for the pathogen

to continue thriving. Therefore, the activity of plant extracts reported in the current study of plant extracts to completely inhibit the growth of *A. solani* is an indication of its ability to manage the pathogen effectively.

4.2.3 Activity of spice extracts against *Fusarium oxysporum* f.sp. *lycopersici*

All the tested spice extracts had a growth inhibition effect on the wilt pathogen. The activity seemed to decrease over time with reduced effect towards 30 DAI. Both turmeric extracts were highly effective against the pathogen with over 70% inhibition for the first 12 DAI and up to 40% at the end of the experiment. Extracts from clove and black pepper followed in activity with a similar trend of decreasing activity over time. Cinnamon, cardamom and lemongrass extracts were averagely effective starting at about 50% inhibition at 2 DAI and zero percent at 30 DAI. The activity of Ridomil Gold® (metalaxyl-M 40 g/kg and mancozeb 640 g/kg), the positive control, was above average, starting at 60% at 2 DAI and showing no growth inhibition activity at all at 20 DAI. Clove, black pepper, turmeric and ginger extracts were more effective than the positive control in inhibiting the growth of *Fusarium oxysporum* f. sp *lycopersici* (Table 7, Fig. 11). In the repeat experiment, clove extract completely inhibited the growth of the phytopathogen until 16 DAI and then by up to 90% until 20 DAI. Extracts from turmeric and ginger remained averagely active while Ridomil Gold® (metalaxyl-M 40 g/kg and mancozeb 640 g/kg) remained less active in inhibiting the growth of *Fusarium oxysporum* f. sp *lycopersici.* Cinnamon and lemon grass extracts were least active inhibiting the growth of the wilt pathogen by about 20% between 12 and 20 DAI. Effects of cinnamon on the growth of the pathogen ceased at 18 DAI (Table 8). These comparisons were drawn from the negative control. Turmeric, black pepper and clove visibly inhibited the characteristic pink pigmentation in *Fusarium* while ginger, lemon grass, cinnamon and cardamon allowed for higher pigmentation. These observations were made in comparison with the negative control (Fig. 12).

Figure 11: ***In vitro*** **activity of crude spice extracts against** ***Fusarium oxysporum*** **f. sp.** ***lycopersici*** **at eight days after incubation. (1- peeled turmeric, 2- non peeled turmeric, 3- black pepper, 4- clove, 5- ginger, 6- lemongrass, 7- cardamom, 8- cinnamon, 9-negative control)**

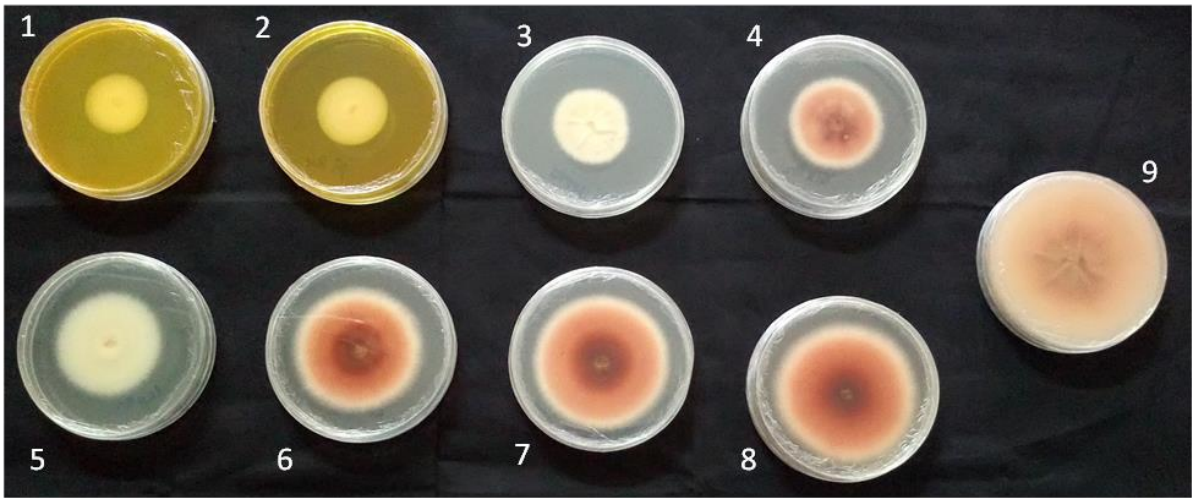

Figure 12: Activity of crude spice extracts on pigmentation of ***Fusarium oxysporum*** **f. sp.** ***lycopersici*** **at eight days after incubation. (1-non-peeled turmeric, 2- peeled turmeric, 3- black pepper, 4- ginger, 5- clove, 6-lemongrass, 7- cardamom, 8- cinnamon, 9- negative control)**

Table 7: Percentage inhibition of colony diameter of *Fusarium oxysporum* f. sp *lycopersici* cultured on media amended with crude spice extracts, experiment 1

Treatments	Days after incubation																					
	2		4		6		8		10		12		14		16		18		20		Mean	
Clove	72.62	a	72.3	ab	69.61	ab	67.86	bc	65.67	b	65	cd	58.33	cd	52.78	bc	47.5	cd	41.7	cd	61.33	cd
Black pepper	69.05	ab	70.27	ab	70.1	ab	69.05	abc	67	ab	67.5	bc	61.94	bc	57.78	ab	51.67	bc	48.3	bc	63.27	bc
Ginger	63.1	bc	63.51	bc	61.76	bc	62.3	c	57.33	c	60	d	53.33	d	47.22	c	39.72	d	33.3	d	54.16	d
Turmeric 1	70.24	ab	75	a	75.49	a	75.4	a	74.67	a	75	a	71.11	a	67.5	a	63.89	a	58.6	a	70.69	a
Turmeric 2	72.62	a	75.68	a	74.02	ab	74.6	ab	74	a	73.61	ab	69.17	ab	65	a	60.28	ab	55.6	ab	69.45	ab
Cardamon	63.1	bc	54.05	de	50	cd	49.21	d	45	de	45.83	e	37.5	e	30.28	d	23.61	e	16.9	e	41.55	e
Cinammon	55.95	c	49.32	e	44.61	d	44.05	d	39.67	e	41.67	e	33.33	e	26.39	d	20.56	e	133.3	e	36.89	e
Lemongrass	59.52	c	54.73	cde	51.96	cd	51.19	d	48.33	d	48.89	e	41.39	e	33.89	d	28.61	e	21.7	e	44.02	e
Ridomil	60.71	c	60.81	cd	42.16	d	28.17	e	23.67	f	23.89	f	13.61	f	5.28	e	2.78	f	0.0	f	26.11	f
SEM	1.7		2.0		2.6		1.5		1.7		1.6		1.7		2.1		2.2		2.1		1.6	
SED	2.4		2.8		3.7		2.1		2.4		2.2		2.4		2.9		3.2		3.0		2.2	
LSD (5%)	4.8		5.7		7.5		4.4		5.0		4.5		4.9		6.0		6.5		6.1		4.5	
CV (%)	5.1		6.1		8.6		5.2		6.2		5.6		7.0		9.6		11.9		13.0		6.0	

Means followed by the same letter(s) within each column do not differ significantly at $P \leq 0.05$

Table 8: Percentage inhibition of colony diameter of *Fusarium oxysporum* f. sp *lycopersici* cultured on media amended with crude spice extracts, experiment 2

Treatments	Days after incubation										Mean
	2	4	6	8	10	12	14	16	18	20	
Clove	100.0 a	100.0 a	1000.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	93.3 a	90.8 a	98.4 a
Black pepper	37.5 b	52.4 b	52.2 b	58.1 b	56.2 b	55.6 b	56.1 c	50.6 b	45.0 c	40.8 c	50.5 bc
Ginger	42.9 b	58.1 b	60.6 b	6.9 b	62.7 b	62.2 b	62.2 bc	59.2 b	54.7 bc	53.6 b	58.1 b
Turmeric 1	46.4 b	56.5 b	60.0 b	62.9 b	61.0 b	60.9 b	61.7 bc	57.2 b	53.3 bc	50.6 bc	57.1 b
Turmeric 2	35.7 b	52.4 b	56.7 b	60.1 b	60.3 b	61.3 b	63.6 b	59.7 b	57.2 b	54.7 b	56.2 b
Cardamon	5.4 c	23.4 c	22.8 d	30.2 d	27.4 d	25.3 d	25.8 e	23.6 d	18.1 de	14.2 de	21.6 d
Cinammon	3.6 c	20.2 c	17.2 d	24.2 de	19.2 de	15.6 e	13.1 f	5.8 e	0.0 f	0.0 f	11.9 e
Lemongrass	-25.0 d	0.0 d	5.0 e	17.3 e	15.8 e	10.6 e	10.8 f	5.6 e	12.5 e	8.9 ef	6.2 e
Ridomil	58.9 b	48.4 b	42.2 c	45.2 c	42.5 c	42.8 c	40.6 d	38.6 c	26.9 d	23.3 d	40.9 c
SEM	5.7	3.5	2.3	1.9	2.1	1.9	1.5	2.1	2.4	2.5	2.0
SED	8.1	5.0	3.3	2.7	2.9	2.6	2.1	3.0	3.5	3.5	2.9
LSD (5%)	16.6	10.2	6.7	5.6	6.0	5.4	4.3	6.1	7.1	7.2	5.9
CV (%)	33.7	15.4	10.0	7.5	8.4	7.8	6.2	9.5	12.2	13.2	9.1

Means followed by the same letter(s) within each column do not differ significantly at $P \leq 0.05$

Clove extract was highly effective in inhibiting the growth of *Fusarium oxysporum* f. sp. *lycopersici* in the current study. Ethanolic extracts of clove reduced the growth of this wilt pathogen by up to 100% in the repeat experiment up to 16 DAI. These findings are in agreement with Sharma *et al.* (2017) who reported complete inhibition of growth and sporulation of the fusarium wilt pathogen by clove essential oil in a dose dependent experiment. Extracts of clove containing 70% clove essential oil was also reported active against *Fusarium oxysporum* f. sp *chrysanthemi*, the causal agent of fusarium wilt in chrysanthemums by up to 97.5%. The same formulation also presented a healthy population of muskmelons grown in soils containing *Fusarium oxysporum* f. sp. *melonis*, but drenched with the clove extract formulation (Bowers & Locke, 2000). Effectiveness of clove is further reported by Sharma *et al*. (2018) who combined clove and lemongrass oil in water, a formulation that reduced the incidence of fusarium wilt of tomato by up to 70.6% compared to untreated control. The ability of clove extracts or essential oils has been observed and reported in other species of *Fusarium* affecting other crops other than tomato (Velluti *et al*., 2003: El-Samawaty *et al*., 2013; Hamad *et al*., 2015). Grata (2016) however reported reduced activity of clove against *Fusarium solani* causing fusarium wilt in white asparagus (*Asparagus officinalis*). However, that study also reported a superior activity of clove oil in inhibiting germination of conidia of *F. solani*.

Growth inhibition of *Fusarium oxysporum* f. sp *lycopersici* by black pepper extracts in the current study was between 27 and 70%. A similar range of activity was reported by Castellanos *et al*. (2020) who reported a 40% growth inhibition against the wilt pathogen by black pepper essential oil. A reduced activity of black pepper extracts and oils has been reported against fungal species of *Aspergillus, Rhizopus* and *Mucor* (Erturk, 2006; Pundir & Jain, 2010).

The susceptibility of *Fusarium oxysporum* f. sp. *lycopersici* to turmeric extracts in this study was 30 to 72%. In support of this finding, Wongkaew and Sinsiri (2014) reported that hot water extracts of turmeric inhibited the growth of *Fusarium oxysporum* f. sp. *lycopersici* by about 64%. Similar results have been reported in studies involving other species of *Fusarium*. In agreement with this finding, Yerukala *et al*. (2018) reported a growth inhibition of between 68 to 75% in *Fusarium oxysporum* f. sp. *ricini* the causal agent of wilt in castor (*Ricinus communis*) by turmeric. Shukla and Dwivedi (2012) reported that turmeric inhibited the growth of *Fusarium oxysporum* f. sp. *ciceris*, the causal agent for fusarium wilt in chickpea by about 87%. In the same study warm water extracts of turmeric inhibited the growth of *Fusarium udum*, causal agent for fusarium wilt in pigeon pea (*Cajanus cajan*) by 89%. However, Rao *et*

al. (2020) reported a comparatively low growth inhibition of about 35% in *Fusarium oxysporum* f. sp *lycopersici* by water extracts of turmeric. Akter *et al*. (2018) reported that different varieties and strains of turmeric contained different compounds thus having varied antifungal activity against pathogens. The study showed that major compounds present in the rhizomes were curcuminoids which were responsible for reducing the growth of *Fusarium solani sensu lato* isolated from *Tricheus manatus*. Turmeric oil has been reported to rapture cell membranes of *Phytophthora capsici*, the causal agent of phytophthora blight of pepper. This activity was attributed to presence of compounds in the oils such as *beta*-elemene, curcumenol, curcumol and curdione (Wang *et al*., 2019).

In the present study, susceptibility of *Fusarium oxysporum* f. sp. *lycopersici* to cinnamon extracts was low, with a growth inhibition of about 55% at 2 DAI which decreased to zero towards the end of the experimental period. These findings are contrasted by Clerck *et al*. (2020) who reported complete inhibition of *Fusarium culmorum* and *F. graminearum* by essential oils of *Cinnamomum cassia* and *C. zeylanicum*. In another study, Sarkosh *et al*. (2018) reported complete growth inhibition of *Fusarium solani* by cinnamon essential oil. El-Samawaty *et al.* (2013) reported a growth inhibition of between 53 – 61% on *F. anthophilium, F. fusarioids, F. solani, F oxysporum* and *F sporotrichioides* by cinnamon extracts in a poisoned food technique experiment. Pawar and Thaker (2007) also reported a remarkable antifungal activity of cinnamon essential oil against the growth of *Fusarium oxysporum* f. sp. *cicer* causing wilt in chick pea. Park *et al*. (2017) reported a growth inhibition of about 89% on *Fusarium oxysporum* f. sp. *fragariae* causing wilt in strawberry by cinnamon oil.

Against *Fusarium oxysporum* f. sp *lycopersici*, lemongrass showed an activity of between 5 and 59%. The extract also supported growth of the pathogen by about 25% at the onset of the experiment. An activity of between 66 and 100% has been reported in lemongrass against *Fusarium oxysporum, F. moniliformae, F. solani* and *Rhizoctonia solani* (Moh *et al*., 2020; Sarhan, 2020). In other studies, lemongrass has completely inhibited growth of *Fusarium oxysporum* f. sp *ciceris*, hindered its sporulation as well spore germination and reduced wilt incidence in chickpea by 88% (Moutassem *et al*., 2019). This high activity of lemongrass against *Fusarium* may have been attributed to abundance of bioactive compounds present in the plant (Raymond *et al*., 2020). According to Sharma *et al*. (2018) the mode of action exhibited by lemongrass oil is physical damage of the pathogen, lysis of cell content and disrupting the integrity of the cell membrane.

The activity of ginger against *Fusarium oxysporum* f. sp *lycopersici* in the current study ranged between 25 and 63%. A similar finding was reported in a study by Rao *et al*. (2020) of ginger extracts inhibiting growth of *Fusarium oxysporum* f. sp. *lycopersici* by up to 61%. Contrary to these findings, Azman *et al*. (2020) reported an inhibition of about 13.6% against *Fusarium oxysporum*. Yerukala *et al*. (2018) however, reported a reduced activity of about 49% by ginger against the tomato wilt pathogen. Prasad *et al*. (2018) reported a high activity of ginger of about 71% against *Fusarium solani* causing wilt and rot disease in pearl millet (*Pennisetum glaucum*). The activity of ginger against *Fusarium oxysporum* causing rot disease in ginseng (*Panax ginseng*) was as low as 13%.

The average susceptibility of *Fusarium* towards plant extracts and chemical control was average in the current study. This may be attributed to the nature of the cellular and toxic components of the wilt pathogen. The cell wall of *Fusarium* is made made of chitin and glucan (Schoffelmeer *et al*., 1999) which forms a protective layer of the pathogen. The ability of plant extracts such as turmeric and clove to inhibit growth of the wilt pathogen means their bioactive compounds have the ability to interfere with the protective nature of the chitinous cell wall of *Fusarium* and should therefore be considred for management of the wilt pathogen.

4.2.4 Activity of spice extracts against *Phytophthora infestans*

The spice extracts tested against this phytopathogen were significantly ($P \leq 0.05$) effective in inhibiting its growth (Fig. 13). Extracts from clove, turmeric and black pepper were significantly ($P \leq 0.05$) active with high inhibitions of 100% and lows of 53, 67 and 50% at 30 DAI in the first experiment. Extracts from ginger, cardamom and cinnamon were moderately active while lemon grass was least active with a high inhibition of 45% and a low of 20% at 30 DAI. Effects of clove, black pepper, ginger and turmeric remained above 50 % until end of the experiment. Clove extracts inhibited the growth of the pathogen by 100% in the first 4 DAI (Table 9). Under clove treatment, there was no growth in the first 2 DAI. In the repeat experiment, clove completely inhibited the growth of the pathogen throughout the experimental period. The activity of clove was similar to that of the positive control containing a fungicide, Ridomil Gold® (metalaxyl-M 40 g/kg and mancozeb 640 g/kg). At 20 DAI, turmeric extract was still effective inhibiting the growth by up to between 80 – 100%. Cinnamon and lemongrass extracts remained moderately active (Table 10). The reduced performance of Ridomil Gold® in the first experiment was because the concentration used was similar to that used for the spice extracts (Table 9). When the concentration was adjusted to 2.5 g/L as

recommended by manufacturer, the activity went higher as exhibited in the second experiment (Table 10).

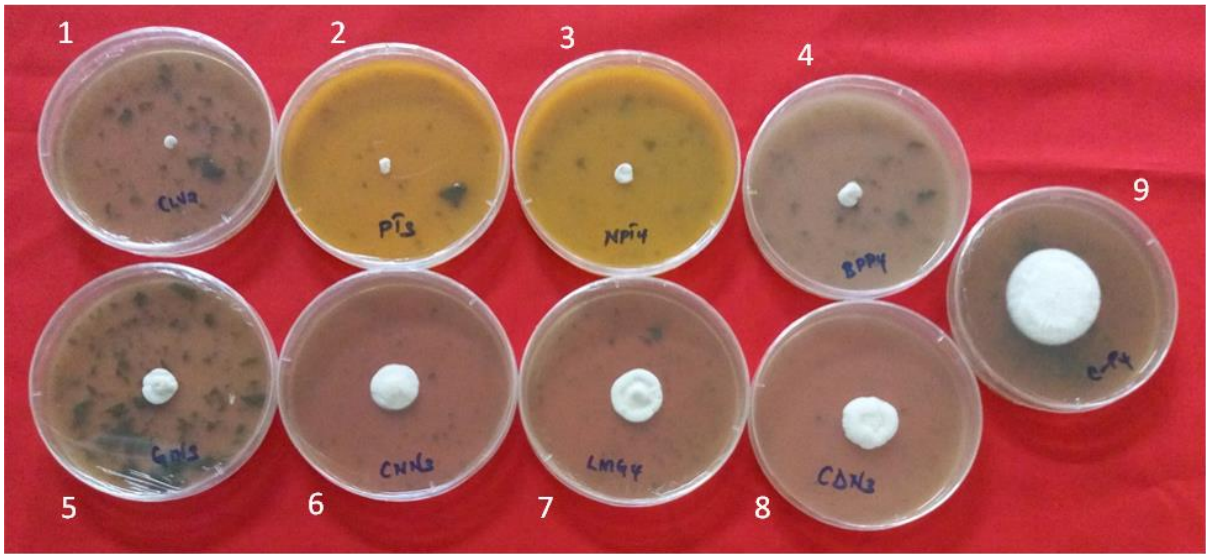

Figure 13: *In vitro* activity of crude spice extracts against *Phytophthora infestans* at eight days after incubation. (1- peeled turmeric, 2- non peeled turmeric, 3- black pepper, 4- clove, 5- ginger, 6- lemongrass, 7- cardamom, 8- cinnamon, 9- negative control)

Table 9: Percentage inhibition of colony diameter of *P. infestans* cultured on media amended with crude spice extracts, experiment 1

Treatments	Days after incubation																	
	2		4		6		8		10		12		14		16		18		20		30	
Clove	100.0	a	100.0	a	90.0	a	87.8	a	88.3	a	86.2	a	82.3	a	78.1	a	77.8	ab	67.8	ab	53.9	a
Black pepper	100.0	a	69.1	c	72.5	cd	69.9	b	69.9	b	68.5	b	68.5	b	66.4	bc	68.6	bc	58.6	bc	50.3	ab
Ginger	58.3	b	67.9	c	70.0	d	68.0	b	65.3	b	66.0	b	64.2	b	63.7	c	66.7	c	56.7	c	55.3	a
Turmeric 1	100.0	a	82.1	b	80.0	bc	80.8	a	81.1	a	79.7	a	78.9	a	77.7	a	79.2	a	69.2	a	67.8	a
Turmeric 2	100.0	a	84.5	b	85.0	ab	82.1	a	82.7	a	80.2	a	78.9	a	76.4	ab	78.9	a	68.9	a	64.4	a
Cardamon	58.3	b	51.2	d	50.0	e	44.9	c	46.9	c	47.8	c	45.0	c	45.9	d	51.1	d	41.1	d	34.2	bc
Cinammon	54.2	b	51.2	d	50.8	e	46.2	c	45.4	c	43.5	c	42.3	c	41.1	d	47.2	d	37.2	d	26.1	c
Lemongrass	41.7	c	44.1	d	45.0	e	43.6	c	43.4	c	42.7	c	41.5	c	40.4	d	44.2	d	34.2	d	20.8	c
Ridomil	8.3	d	3.6	e	5.8	f	2.6	d	2.0	d	5.6	d	5.4	d	5.5	e	15.6	e	5.6	e	0.0	d
s. e. m	2.1		2.0		1.8		1.6		1.7		2.0		1.9		2.3		2.1		2.1		3.9	
s. e. d	3.0		2.8		2.6		2.3		2.4		2.8		2.8		3.2		3.0		3.0		5.6	
L.S. D (5%)	6.2		5.7		5.3		4.8		4.9		5.7		5.7		6.6		6.2		6.2		11.4	
CV (%)	6.2		6.4		6.0		5.6		5.8		6.8		6.9		8.2		7.3		8.8		19.0	

Means followed by the same letter(s) within each column do not differ significantly at $P \leq 0.05$

Table 10: Percentage inhibition of colony diameter of *P. infestans* cultured on media amended with crude spice extracts, experiment 2

Treatments	Days after incubation								Mean
	4	6	8	10	12	14	16	18	20	
Clove	100 a	100.0 a	100 a	100.0 a	100.0 a	100 a	100.0 a	100.0 a	100.0 a	100.0 a
Black pepper	100 a	100.0 a	100 a	94.5 ab	91.1 ab	88 ab	86.0 ab	83.4 b	82.2 b	92.5 ab
Ginger	100 a	100.0 a	86 a	83.0 bcd	80.9 bc	79 bc	75.7 bc	71.9 bc	70.0 bc	84.7 bc
Turmeric 1	100 a	84.2 bc	85 ab	81.5 bcd	80.5 bc	78 bc	76.7 bc	74.4 bc	73.9 b	83.5 c
Turmeric 2	100 a	100.0 a	100 a	93.0 abc	92.4 ab	91 ab	88.3 ab	85.6 ab	81.9 b	93.4 ab
Cardamon	100 a	85.8 bc	85 ab	77.0 cd	72.9 cd	69 cd	65.0 cd	60.9 cd	59.4 cd	77.5 cd
Cinammon	84 b	75.0 c	73 b	68.5 d	63.1 d	59 d	54.3 d	50.0 d	49.7 d	67.7 d
Lemongrass	78 b	75.8 bc	73 b	68.5 d	64.0 cd	61 d	58.7 d	55.0 d	53.1 d	68.7 d
Ridomil	100 a	100.0 a	100 a	100.0 a	100.0 a	100 a	100.0 a	100.0 a	100.0 a	100.0 a
SEM	2	2.2	3	3.4	3.6	3	3.3	3.4	2.9	2.4
SED	3	3.1	4	4.8	5.0	5	4.6	4.8	4.1	3.5
LSD (5%)	5	6.4	9	9.8	10.3	10	9.5	9.8	8.4	7.1
CV (%)	4	4.8	7	8.0	8.6	9	8.3	8.9	7.8	5.7

Means followed by the same letter(s) within each column do not differ significantly at $P \leq 0.05$

Ethanolic extract from clove was the most active in inhibiting the growth of *P. infestans* followed by extracts of black pepper, turmeric and ginger. Clove extracts were the most active, completely inhibiting growth of the late blight pathogen. The results in this study are consistent with findings by Najdabbasi *et al.* (2020) who reported complete inhibition of mycelia growth of *P. infestans* by clove essential oil in a poisoned food technique experiment. Complete inhibition of *Phytophthora* spp has been reported before by Mohsan *et al*. (2017) who used aqueous extracts of *Parthenium hysterophorus* that completely inhibited growth of *P. capsici* the causal agent of blight and fruit rot of pepper. According to Badillo *et al*. (2010), aerial parts of *Artemisia ludoviciana* in a methanol-chloroform fraction completely inhibited growth of *Phytophthora* spp among them, *P infestans* in a paper disk agar diffusion experiment. Other extracts reported to completely inhibit the growth of *P infestans* include but not limited to *Terminalia belerica* and *Psoralea corylifolia* (Rani *et al*., 2015).

Black pepper extract was also active in reducing the mycelia growth of *P infestans in vitro* by between 82 and 100%. Antimicrobial properties of black pepper have been widely investigated on various pathogens enlisting bacteria, fungi and viruses (Mahfouz *et al*., 2020). A study by Nikolić *et al*. (2015) reported growth inhibition of *Streptococcus aureus* and *Aspergillus niger* by essential oils of black pepper and attributed the activity to presence of bioactive compounds present in those oils. To further support the antifungal effects of black pepper, Mahfouz *et al*. (2020) reported complete growth inhibition of *Penicillium citrinum* while growth of *Aspergillus niger* and *A. flavus* was inhibited to some extent after using aqueous seed extracts. The antifungal properties of black pepper are attributed to presence of major compounds such as *beta* - caryophellene, sabinene, *delta* - bisabolene, copaene among others (Singh *et al*., 2004; Aziz *et al*., 2012). Acetone fractions completely inhibited growth of *Fusarium graminearum* and to some extent *Aspergillus ochraceus* and *Penicillium viridcatum*. The major compounds present in that acetone fraction were piperine and its constituents (Singh *et al*., 2004).

Turmeric showed antifungal activity against *P. infestans* by 64 and 100 %. In agreement with these findings, ethanolic extracts of turmeric inhibited the growth of *Phytophthora infestans* by up to 50% in a poisoned food technique experiment compared to a complete inhibition by the commercial fungicide, copperoxychloride and mancozeb, used as positive control in the experiment (Wongkaew & Sinsiri, 2014). Han *et al*. (2017), also reported antifungal activity of a sesquiterpene isolated from turmeric (*Curcuma zedoaria*) rhizomes against important

pathogens of rice, tomato and wheat as *Rhizoctonia solani, P. infestans* and *Puccinia tricina* respectively in a greenhouse experiment.

In this study, the activity of cinnamon extracts was above average, inhibiting the mycelial growth of *P. infestans* by between 84 and 49% at two and 20 DAI respectively. This finding is in contrast with other findings that reported strong activity of cinnamon extracts and oil against phytopathogens. Wang *et al*. (2005) reported 100% growth inhibition of *Curiolus versicolor* and *Laetiporus sulphureus* both which cause rots in wood, by essential oils from leaves of *Cinnamomum osmophloeum*. Essential oils of cinnamon were also reported to be 100% active in inhibiting mycelial growth and spore germination of *Aspergillus niger, A. oryzae* and *A. ochraceus* (Hu *et al*., 2019). Cinnamon extracts extracted in carbon (iv) oxide completely inhibited growth of *Botrytis cinerea*, the causal agent of grey mold disease in strawberry in a food poisoned technique experiment. This study by Šernaitė *et al*. (2019) reported that no growth of the pathogen was detected 7 DAI. Clerck *et al*. (2020) also confirmed efficiency of cinnamon essential oils in inhibiting growth of species of *Botrytis* and *Fusarium*. These findings agree with the present study that despite the activity of cinnamon against other pathogens, it was less effective against *Phytophthora*. However, according to Sarkhosh *et al*. (2018) cinnamon inhibited growth of *Fusarium solani*, *Phytophthora palmivora* and *Colletotrichum gleosporioides*. According to Clerck *et al*. (2020), the essential oil of cinnamon majorly contained phenols, phenylpropanoids, aldehydes and organosulfurs.

In the present study, extracts of cardamom were averagely active against *P infestans* starting at an inhibition of 100% at 2 DAI and ending with 59% at 20 DAI. In support of this finding, a study by Ansary *et al*. (2016), reported ineffective activity of water extracts of cardamom (*Elettaria cardamomum*) in reducing motility of zoospores of *Phytophthora capsici*. Contrary to the findings in this study, Kapoor *et al*. (2008) reported 100% effectiveness of cardamom essential oil against *Aspergillus flavus* in an inverted plate experiment. Methanolic extracts of cardamom have also been reported to completely inhibit *Alternaria solani* at 8 DAI (Sarfraz *et al*., 2018). Similarly, the essential oils extracted in methanol and carbon tetrachloride were also highly effective in inhibiting the growth of *Aspergillus niger, Alternaria solani, Aspergillus oryzae* by about 40%. Kapoor *et al*. (2008) attributed this activity to 1,8 cineole and alpha – terpineol which were reportedly the major compounds in the cardamom essential oils as per a GC-MS analysis conducted during the study. These compounds were also confirmed present

in cardamon essential oils by Mutlu-Ingok and Karbancioglu-Guler (2017) who further reported the oil's effectiveness against bacterial species of *Campylobacter*.

According to the findings in this study, lemongrass was the least active extract against *Phytophthora infestans*. Contrary to this finding, Nyamath and Karthikeyan (2018) reported effectiveness of lemon grass extracted in cold and warm water as well as methanol against *Aspergillus niger* and *Colletotrichum musae*. Raymond *et al*. (2020) also reported activity of lemongrass in inhibiting mycelial growth of *Fusarium oxysporum, Helminthosporium oryzae* and *Colletotrichum falcatum* in a dose dependent experiment. Essential oil derived from lemon grass was reported effective in inhibiting germination of spores and germ tube elongation of *Botrytis cinerea, Colletotrichum coccides, Cladosporium herbarum* and *Rhizopus stolonifer*. This study by Tzortzakis and Economakis (2007) also reported an increased rate of sporulation in *Aspergillus niger* supported by lemongrass oil. The antimicrobial activity of lemongrass has been attributed to the major compound citral and its constituents (Nyamath & Karthikeyan, 2018; Hartatie *et al*., 2019; Sattary *et al*., 2020).

Ginger extracts inhibited growth of *P. infestans* by between 55 and 100% throughout the experimental period. Such activity has been reported previously by Okigbo *et al*. (2018) who described effect of ethanolic and cold-water extract of ginger against species of *Rhizoctonia, Botryodiplopodia, Corticinum* and *Penicillium* in a dose dependent experiment. In concurrence with the present study, ethanolic extract of ginger inhibited mycelial growth of *Alternaria alternata* by up to 90% and completely inhibited spore germination of the pathogen causing leaf spot of cabbage. Ethyl acetate fraction of the ethanolic ginger extracts further reduced the biomass of the pathogen by up to 100% in a dose dependent experiment (Rizwana, 2016). Ginger oleoresin has been reported effective against important fungal pathogens in agriculture. In a study by Chen *et al*. (2018), ginger oleoresin showed high antifungal activity against *Pestalotiopsis microspore*, the pathogen responsible for rotten disease of Chinese olives (*Canacium album*). The oleoresin also inhibited spore germination, interfered with the cell membrane, damaged the plasma membrane and morphology of *P. microspore*. Antifungal activity of ginger was further explored by Nerilo *et al*. (2015) who reported its ability to deter production of the mycotoxin, aflatoxin, produced by *Aspergillus niger* in addition to inhibiting conidial germination of the same pathogen. The antimicrobial activity of ginger is attributed to presence of bioactive compounds as reported by various studies with citral and gingerol being

the major compounds (Manasa *et al*., 2013; Shareef *et al*., 2016; Moon *et al*., 2018; Hussein & Joo, 2018).

The cell wall of *Phytophthora infestans* is maily made up of cellulose and glucan both of which are apparently important in the infection process of the late blight pathogen (Grenville-Briggs *et al*., 2008). Presence of appressorial structures and proteins is also important for pathogenicity of *Phytophtora infestans* as they majorly contain enzymes (Resjo *et al*., 2017). Therefore, a suitable management option of the pathogen may be targeted towards manipulation of the cellular components and infection process of *P. infestans*. Mancozeb and metalaxyl designed for management of late blight act majorly as protectants and by disrupting the biochemical and enzymatic processes of the pathogen (Wong & Wilcox, 2001; Gullino *et al*., 2010). Metalaxyl has been reported to interfere with synthesis of nuceliec acid in *Phytophtora infestans* (Fisher & Hayes, 1984). The susceptibility of *Phytophtora infestans* towards extracts of say clove in the current study is an indication that bioactive compounds in the plant have disruption properties towards the development of the pathogen. Since the cellwall of *P. infestans* is important for its infection process (Grenville-briggs *et al*., 2010), plant bioactive compounds interfering with the integrity of its membrane stands a chance for effective management of the pathogen.

4.2.5 Activity of spice extracts against *Pythium*

Pythium species tested was highly susceptible to the different crude extracts (Fig. 14). The most active spice extracts against the pathogen were those from clove, black pepper, ginger, turmeric and positive control with inhibitions of between 85 and 100%. Clove and ginger extract completely inhibited the growth of the pathogen throughout the experimental period. Extracts from cardamom, cinnamon and lemon grass were moderately active in growth inhibition. Black pepper, turmeric and Ridomil Gold® (metalaxyl-M 40 g/kg and mancozeb 640 g/kg) had completely inhibited growth of the pathogen in the initial days after incubation (Table 11). In the repeat experiment, the trend was similar with high activity observed from extracts of clove, black pepper, ginger and turmeric recording an inhibition of between 65 and 100%. Clove extract remained the most effective while cinnamon and lemon grass were least active (Table 13).

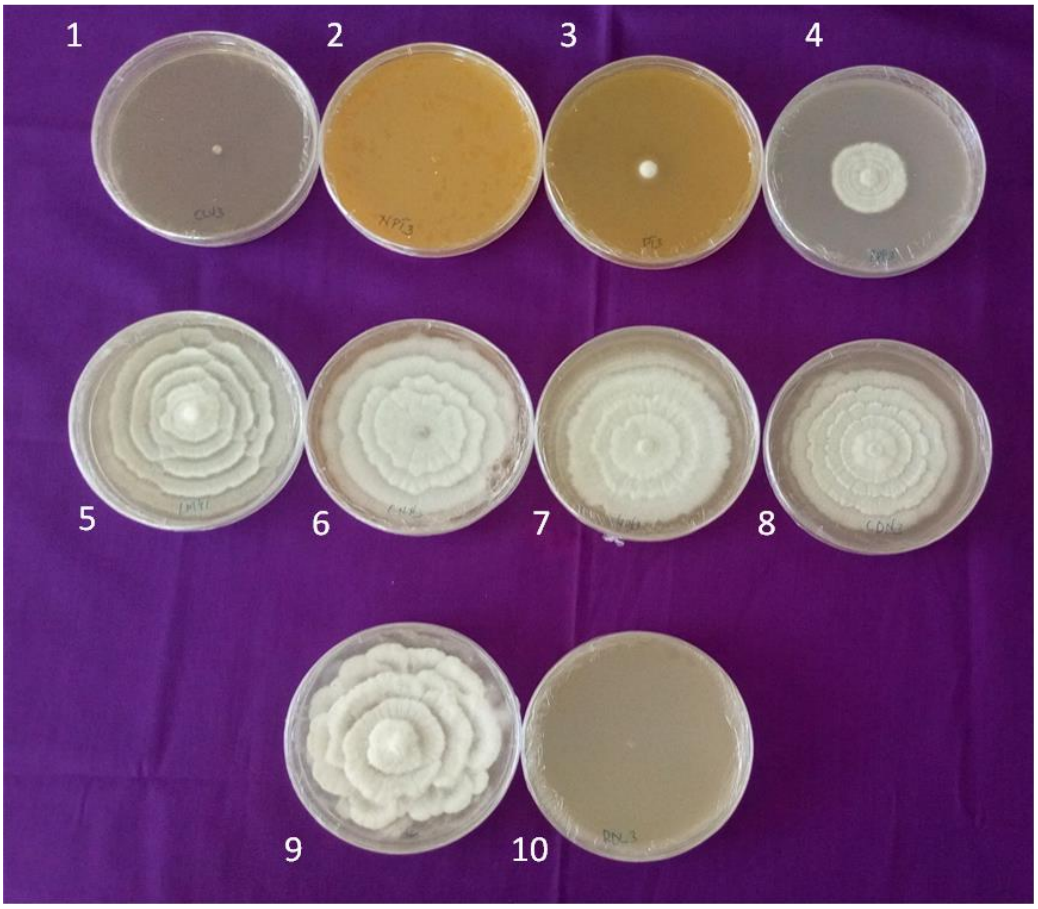

Figure 14: *In vitro* activity of crude spice extracts against *Pythium* at eight days after incubation. (1-clove, 2-non-peeled turmeric, 3-peeled turmeric, 4-black pepper, 5-lemongrass, 6- cinnamon, 7-ginger, 8-cardamom, 9-negative control, 10- positive control)

Table 11: Percentage inhibition of colony diameter of *Pythium* cultured on media amended with crude spice extracts, experiment 1

Treatments	Days after incubation										Mean	
	2	4	6	8	10	12	14	16	18	20		
Clove	100.0 a	100 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100 a	100.0 a	100	a
Black pepper	100.0 a	100 a	100.0 a	100.0 a	100.0 a	100.0 a	96.7 a	95.6 a	94 a	87.5 a	97.42	a
Ginger	100.0 a	100 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100 a	100.0 a	100	a
Turmeric 1	83.3 ab	91 ab	92.0 a	93.1 a	93.1 a	92.5 a	91.9 a	91.7 a	92 a	91.1 a	91.09	a
Turmeric 2	100.0 a	100 a	96.3 a	96.4 a	96.4 a	96.1 a	96.4 a	96.4 a	96 a	96.4 a	97.08	a
Cardamon	68.3 b	70 b	65.7 b	63.9 b	55.8 b	52.2 b	49.4 b	43.9 b	40 b	35.3 b	54.51	b
Cinammon	69.2 b	69 bc	64.7 b	63.6 b	59.2 b	55.3 b	52.8 b	49.2 b	49 b	47.2 b	57.88	b
Lemongrass	12.5 c	41 c	49.3 b	52.5 b	48.6 b	47.8 b	46.7 b	44.4 b	43 b	42.2 b	42.83	b
Ridomil	100.0 a	100 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	98.6 a	98 a	97.2 a	99.39	a
SEM	6.1	6	4.6	4.8	5.7	6.2	6.7	7.5	8	7.7	5.96	
SED	8.7	8	4.5	6.8	8.1	8.7	9.5	10.6	11	10.9	8.43	
LSD (5%)	17.8	17	13.3	14.0	16.6	17.9	19.5	21.8	22	22.4	17.29	
CV (%)	15.1	14	10.7	11.3	13.6	14.9	16.5	18.8	19	19.9	14.5	

Means followed by the same letter(s) within each column do not differ significantly at $P \leq 0.05$

Table 12: Percentage inhibition of colony diameter of *Pythium* cultured on media amended with crude spice extracts, experiment 2

Treatments	Days after incubation									
	2	4	6	8	10	12	14	16	18	20
Clove	86.2 a	91.7 a	93.9 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Black pepper	85.3 a	91.7 a	93.9 a	100.0 a	100.0 a	100.0 a	95.3 a	94.2 a	89.2 a	86.9 a
Ginger	81.9 a	83.3 ab	83.5 ab	83.5 a	78.9 ab	79.4 a	76.4 a	72.5 a	68.6 a	64.7 a
Turmeric 1	85.3 a	91.2 a	93.1 a	98.1 a	98.4 a	96.8 a	100.0 a	96.9 a	98.3 a	98.3 a
Turmeric 2	86.2 a	91.2 a	93.1 a	95.8 a	96.5 a	96.8 a	96.4 a	96.1 a	96.1 a	95.7 a
Cardamon	79.3 a	71.9 b	69.6 b	59.6 b	55.1 bc	49.1 b	41.1 b	33.6 b	25.8 b	20.6 b
Cinammon	65.5 b	56.8 c	51.9 c	36.5 bc	44.2 c	30.6 bc	20.3 b	16.7 b	8.3 b	7.8 b
Lemongrass	62.1 b	50.5 c	45.0 c	28.5 c	24.0 c	18.8 c	11.1 b	6.7	5.6 b	5.6 b
Ridomil	86.2 a	91.2 a	93.5 a	97.7 a	97.1 a	96.8 a	96.4 a	95.8 a	95.3 a	94.7 a
s. e. m	2.7	2.9	3.3	5.0	7.0	5.6	6.4	7.0	7.7	8.5
s. e. d	3.8	4.0	4.7	7.0	9.8	8.0	9.0	9.9	10.9	12.0
L.S. D (5%)	7.8	8.3	9.6	14.4	20.2	16.3	18.5	20.3	22.4	24.6
CV (%)	6.8	7.1	8.3	12.8	18.0	15.2	18.0	20.6	23.6	26.6

Means followed by the same letter(s) within each column do not differ significantly at $P \leq 0.05$

In this study, *Pythium* was completely susceptible to clove extracts. In agreement with this finding, Kareem *et al*. (2009) reported complete inhibition of *Pythium aphanidermatum*, the causal agent of damping off in cucumber (*Cucumis sativus*). The study further reported effectiveness of clove oil, extracted by steam distillation, in reducing severity of damping off *in vivo*. Clove oil and its pure compounds was reported to show biocidal activity against *Pythium* in a study report by McMaster *et al*. (2013). Black pepper extracts inhibited the growth of *Pythium* by between 85 and 100%. Pattnaik *et al*. (2012) however reported an activity of about 37% when *Pythium debaryanum* was treated with black pepper water extract. In a greenhouse experiment, black pepper extracts increased resistance of tomato plants against gray mold disease caused by *Botrytis cinerea* (Ghazal *et al*., 2019).

Turmeric extract inhibited the growth of *Pythium* spp by between 83 and 100% in the current study. High inhibition activity by turmeric of about 82% on *Pythium ultimum*, the causal agent of damping off in chilli (*Capsicum annum*) was also reported by Zagade *et al*. (2012). An average activity of about 56% was however reported by Wongkaew and Sinsiri (2014) on *Pythium* by hot water turmeric extracts. Muthomi *et al*. (2017) also reported an activity of about 55.6% on *Pythium* in a poisoned food technique involving ethanolic extracts of fresh rhizomes of turmeric. To further contrast the findings in this study, Gholve *et al*. (2014) reported a growth inhibition of about 24% on *Pythium ultimum* the causal agent of damping off in brinjals (*Solanum melongena*) by turmeric extracts.

Cinnamon extracts inhibited the growth of *Pythium* by between 7 and 69% in the current study. However, aqueous and water cinnamon extracts were reported to completely inhibit the growth of *P. aphanidermatum* and *P. sulcatum* (Mvuemba *et al*., 2009; Al-Shemmary *et al*., 2018). Ginger extracts inhibited the growth of *Pythium* by between 64 and 100% in the current study. Suleiman and Emua (2009) similarity reported complete inhibition of *Pythium aphanidermatum*, causing root rot in cowpea (*Vigna unguiculata*) by ginger. Gholve *et al*. (2014) reported an average activity of about 53% against *P. ultimum* causing damping off in brinjals (*Solanum melongena*). Ravi *et al*. (2017) reported a complete lysis of hyphal structures of *Pythium myriotylum*, causing soft rot in ginger (*Zingiber officinale*) by water extracts of wild ginger (*Zingiber zerumbet*) in a well diffusion experiment. Zagade *et al*. (2012) similarly reported a high growth inhibition activity of about 63% by water extracts of ginger against *Pythium ultimum* causing damping off of chili.

According to Cooper and Aranson (1967) the cell wall of *Pythium* is mainly made up of glucan and glucose which may be easily dissolved by the extracts found to completely inhibit growth of the pathogen in the current study. Eugenol for instance, a major compound in clove, has been reported to act on pathogens by disrupting their cell mebrane thereby affecting its permeability (Olea *et al*., 2019). Most of the extracts studied herein also interfered with the rosette appearance of the *Pythium* mycelial colony. This activity is possibly due to intereference with the nucleic acid of the fungus, a mode of action similar to that of metalaxyl as reported in *P. splendens* by Kerkenaar (1981).

4.2.6 Activity of combinations of various ethanolic spice extracts against *P. infestans*

The activity of combinations of spice different extracts was evaluated on *P. infestans* only due to its high susceptibility and importance in tomato. All combinations were highly effective against the pathogen (Fig. 15). Total growth inhibition was observed from combinations of clove + ginger, clove + turmeric and clove + black pepper, all completely inhibiting growth of *P. infestans*. Clove extract was a common denominator in the most active combinations. No growth was observed in the treatments until at 8 days after incubation. The combinations inhibited growth of the pathogen by between 65 to 100% up until 20 days after incubation. However, combination of all the spice extracts did not guarantee total growth inhibition. Least activity was observed from combinations of extracts of ginger + turmeric + black pepper, ginger + clove + black pepper, ginger + black pepper + turmeric. Black pepper was a common denominator in the less active combinations (Table 13). In the repeat experiment, there was growth throughout the experiment period. High growth inhibition was recorded from the combinations of turmeric + clove, clove + black pepper, turmeric + clove + black pepper and ginger + clove extract with records of 91.9%, 85%, 76.9% and 73% respectively. There was no significant ($P \leq 0.05$) difference the combinations and the positive control (Table 14).

Figure 15: ***In vitro*** **activity of combinations of various crude spice extracts against** ***Phytophthora infestans*** **at eight days after incubation. (1- turmeric + clove, 2- ginger + clove, 3- clove + black pepper, 4- turmeric + clove + black pepper, 5-ginger + turmeric + clove, 6- ginger + turmeric + clove + black pepper, 7- ginger + black pepper, 8- ginger + clove + black pepper, 9- ginger + turmeric + black pepper, 10- ginger + turmeric, 11- turmeric + black pepper, 12- negative control, 13- positive control)**

Table 13: Percentage inhibition of colony diameter of *Phytophthora infestans* cultured on media amended with combinations of crude spice extracts, experiment 1

Treatments	Days after incubation													
	8		10		12		14		16		18		20	
Ginger +turmeric	89.1	b	89.3	c	88.7	bcd	87.2	bc	83.8	bcd	83.5	bcd	81.4	bc
Ginger +clove	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a
Ginger +black pepper	84.8	b	84.8	c	83.6	d	82.1	c	79.7	cd	76.5	cde	71.4	cd
Turmeric + clove	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a
Turmeric + black pepper	86.4	b	86.6	c	87.5	cd	86.5	bc	82.5	bcd	84.4	bc	82.5	bc
Clove + black pepper	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a
Ginger + turmeric + clove	100.0	a	97.8	ab	97.7	ab	94.6	ab	93.8	abc	89.4	ab	86.4	bc
Ginger + turmeric + black pepper	85.3	b	82.1	c	78.9	d	76.0	c	73.1	d	70.0	e	65.3	d
Ginger + clove + black pepper	87.5	b	85.7	c	82.0	d	78.0	c	75.6	d	71.5	de	67.2	d
Turmeric+ clove+ black pepper	100.0	a	97.3	ab	97.3	abc	96.6	ab	95.6	ab	92.9	ab	91.1	ab
Ginger + turmeric + clove +black pepper	100.0	a	97.3	ab	97.3	abc	93.9	ab	90.9	abc	86.8	bc	84.2	bc
Ridomil	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a
s. e. m	1.0		1.8		2.0		2.4		2.9		2.6		2.8	
s. e. d	1.5		2.6		2.9		3.3		4.1		3.7		3.9	
L.S. D (5%)	3.0		5.2		5.8		6.8		8.3		7.5		7.9	
CV (%)	2.2		3.9		4.4		5.2		6.5		5.9		6.4	

Means followed by the same letter(s) within each column do not differ significantly at $P \leq 0.05$

Table 14: Percentage inhibition of colony diameter of *Phytophthora infestans* cultured on media amended with combinations of crude spice extracts, experiment 2

Treatments	Days after incubation																	
	2		4		6		8		10		12		14		16		18		20	
Ginger +turmeric	47.5	bc	56.5	c	50.7	c	47.9	e	46.4	de	45.3	de	43.9	efg	44.3	ef	46.4	f	42.8	g
Ginger +clove	60.0	b	100.0	a	100.0	a	93.6	ab	89.6	ab	86.7	ab	83.9	abc	80.4	bc	77.5	abcd	73.6	bcde
Ginger +black pepper	22.5	d	37.0	d	31.8	d	33.0	f	33.2	e	35.2	e	35.7	g	38.0	f	42.2	f	38.9	g
Turmeric + clove	100.0	a	100.0	a	100.0	a	100.0	a	100.0	ab	97.7	ab	97.1	abc	95.6	ab	95.0	abcd	91.9	ab
Turmeric + black pepper	47.5	bc	63.9	c	58.1	c	55.9	e	54.6	de	66.8	bc	53.6	def	54.4	def	56.1	ef	52.5	fg
Clove + black pepper	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a	95.7	a	94.6	abc	91.8	ab	90.3	abcd	85.0	abc
Ginger + turmeric + clove	100.0	a	100.0	a	87.2	b	79.3	cd	76.4	bc	78.8	bc	71.1	cd	69.0	cd	69.4	cde	65.8	def
Ginger + turmeric + black pepper	42.5	c	58.3	c	50.7	c	47.3	e	43.6	de	42.2	e	39.6	fg	39.6	f	41.7	f	37.8	g
Ginger + clove + black pepper	100.0	a	100.0	a	78.4	b	75.0	d	70.0	c	64.8	cd	61.1	de	59.2	de	60.0	def	56.1	efg
Turmeric+ clove+ black pepper	100.0	a	100.0	a	85.8	b	85.1	bc	80.5	bc	73.8	bc	70.0	cd	67.7	cd	75.3	bcde	62.8	def
Ginger + turmeric + clove + black pepper	100.0	a	100.0	a	87.2	b	84.0	cd	85.0	b	83.2	abc	81.1	bc	79.8	bc	79.7	abcd	76.9	bcde
Ridomil	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a	100.0	a
s. e. m	3.0		1.8		2.1		1.7		2.7		4.3		3.6		4		4.2		4.0	
s. e. d	4.2		2.6		3.0		2.5		3.8		6.1		5.1		6		6.0		5.7	
L.S. D (5%)	8.6		5.3		6.0		5.0		7.8		12.4		10.4		11		1.2		11.6	
CV (%)	7.8		4.4		5.4		4.6		7.4		12.0		10.4		11		12.2		12.3	

Means followed by the same letter(s) within each column do not differ significantly at $P \leq 0.05$

Different combinations of ethanolic clove, black pepper, turmeric and ginger extracts tested for antifungal activity against *P. infestans* inhibited its growth by between 50 and 100%. The most active combinations were between clove with black pepper, ginger and turmeric. The activity of the three combinations was equal and similar to that of the fungicide, Ridomil Gold®, completely inhibiting the growth of *P. infestans*. The high antifungal activity observed under the effect of extract combination is attributed to the synergistic effect among the many compounds present in the extracts belonging to different families. The aspect of synergy in botanical pesticides is important because it presents a combination of compounds that do not exist naturally (Oliveira *et al*., 2018). This compound combination is important because once exposed to the fungal pathogens, it would be impossible for them to develop resistance due to the many modes of actions that the compounds may exhibit. The concept of synergy in botanical pesticides has been explored widely on insects, fungi, animalcules and ants, with positive observations (Huang *et al*., 2014; Melo *et al*., 2020). A combination of extracts of *Mansoa alliacea* and *Allamanda cathartica* was more effective in inhibiting the growth and spore germination of *Athelia rolfsii,* the causal agent of stem rot disease in peanut than the individual extracts (Parwanayoni *et al*., 2018). Species of pepper when combined with *Acarus calamus* and *Coscinion fenestratus* were reported quite effective against *Alternaria brassicicola, Colletotrichum capsici* and *Fusarium oxysporum* f. sp *cubense* in different ratios and concentrations (Rueangrit *et al*., 2019).

Pure compounds, cinnamaldehyde and citronellal were recommended for consideration as preservatives for citrus after their combined effect was effective in hindering growth of *Penicillium digitatum* responsible for green mold disease in *Satsuma mandarin* fruits (Ouyang *et al*., 2020). Though some bioactive compounds are active against pathogens even on their own, majority of them do not show any effect on growth or development of the pathogens. Individual constituents of thyme oil showed no activity against carmine spider mite, *Tetranychus cinnabarinus* but the oil itself exhibited strong acaricidal effects (Wu *et al*., 2017). Major bioactive compounds of lemongrass, lominene and citral, exhibited cytotoxic effects in larvae of cabbage looper, *Trichoplusia ni* (Tak *et al*., 2017). Some studies have further recommended use of botanical extracts with the recommended fungicides but at lower concentrations. A combination of extracts from *Polyalthia longifolia* with 25% of the recommended fungicide, mancozeb, for controlling purple blotch reduced the growth of *Alternaria porri* by about 82% (Kalsoom *et al*., 2019). This combination of botanical extracts with fungicides is to support reduced usage and reliance of synthetic pesticides (Deepa *et al*.,

2013). The concept of synergy is also employed in developing synthetic pesticides. The fungicide used as a positive control in this study, Ridomil Gold® is also a combination of mancozeb and metalaxyl.

4.3 Bioactive compounds present in the ethanolic spice extracts

4.3.1 Activity of ethanolic extracts fractionated in various solvents against *P. infestans*

All the solvents extracted various compounds from various crude spice extracts which variably affected the growth of *P. infestans*. The resultant fractions inhibited growth of *Phytophthora infestans* to different extents. From the black pepper extracts, all fractions were active against the pathogen. The activity was about 20% and below in all fractions. There was no consistency in the inhibition from start to the end of experiment. Despite the low activity in all the fractions, the ethyl acetate fraction was more active compared to the hexane and dichloromethane (DCM) fractions (Table 15). In the repeat experiment, ethyl acetate fraction was not tested. Inhibition effect from hexane and DCM fractions was noticeable. Highest inhibition level by hexane fraction was 62% while the least was at 19%. The highest inhibition registered by the DCM fraction was 44% and the overall activity was moderate (Table 16). The most active compounds in black pepper were better extracted by ethyl acetate and DCM.

The fractions from clove extracts were also active against *P. infestans*. The hexane fraction was most active inhibiting the pathogen's growth completely at 100% from 4 to 20 DAI (Fig. 16). Ethyl acetate and DCM fractions of clove were the least active registering high inhibitions at 20 and 12% and lower inhibitions of -1 and 3% respectively. The growth inhibition trend from start to end of the experiment was inconsistent (Table 15). In the repeat experiment, hexane fraction completely inhibited growth of the pathogen from start to the end. The other two fractions remained least active with high inhibitions of 23% and lows of -10%. The trend was however inconsistent (Table 16). The most active compounds in clove were better extracted in hexane.

Fractions from turmeric extracts were averagely active registering high inhibitions of 48% and lows of 6%. Activity of hexane and DCM fractions were almost similar and the former had a steady decline from start to the end of the experiment. In the first experiment, activity of DCM fraction was fluctuating up to the 8th DAI then thereafter decreased steadily (Table 15). In the repeat experiment, the activity of hexane, DCM and ethyl acetate fractions was almost similar. Apart from the second day after incubation, there was a steady decline in the activity towards

the end of the experiment. The activity of hexane fractions had high inhibitions of 59% and low inhibitions of about 37%. The ethyl acetate fraction had a low inhibition at some point of about 10% and the activity fluctuated throughout the experiment. The activity of DCM fractions was above average registering a growth inhibition of 58%. Hexane and DCM fractions were more active in inhibiting the growth of *P. infestans* (Table 16). Active compounds in turmeric were better extracted in hexane and DCM.

Ginger fractions extracted with hexane and DCM were more active in inhibiting the growth of *Phytophthora infestans* than those of ethyl acetate. The DCM fractions were more active than that of hexane and the activity decreased with fluctuations. The activity of hexane fractions decreased steadily until 18 DAI (Table 15). In the repeat experiment, hexane and DCM fractions remained more active in inhibiting growth of the phytopathogen than that of ethyl acetate with inhibitions of up to 60%. Activity of ethyl acetate fraction was below average with the highest inhibition being about 30%. Dichloromethane fractions of turmeric were active registering a growth inhibition of about 53%. The most active compounds in ginger were extracted in hexane and DCM (Table 16).

The high coefficients of variation indicated in Tables 15 and 16 were due to the differences in concentration of bioactive compounds which were responsible for the varied antifungal activity recorded against *P. infestans*.

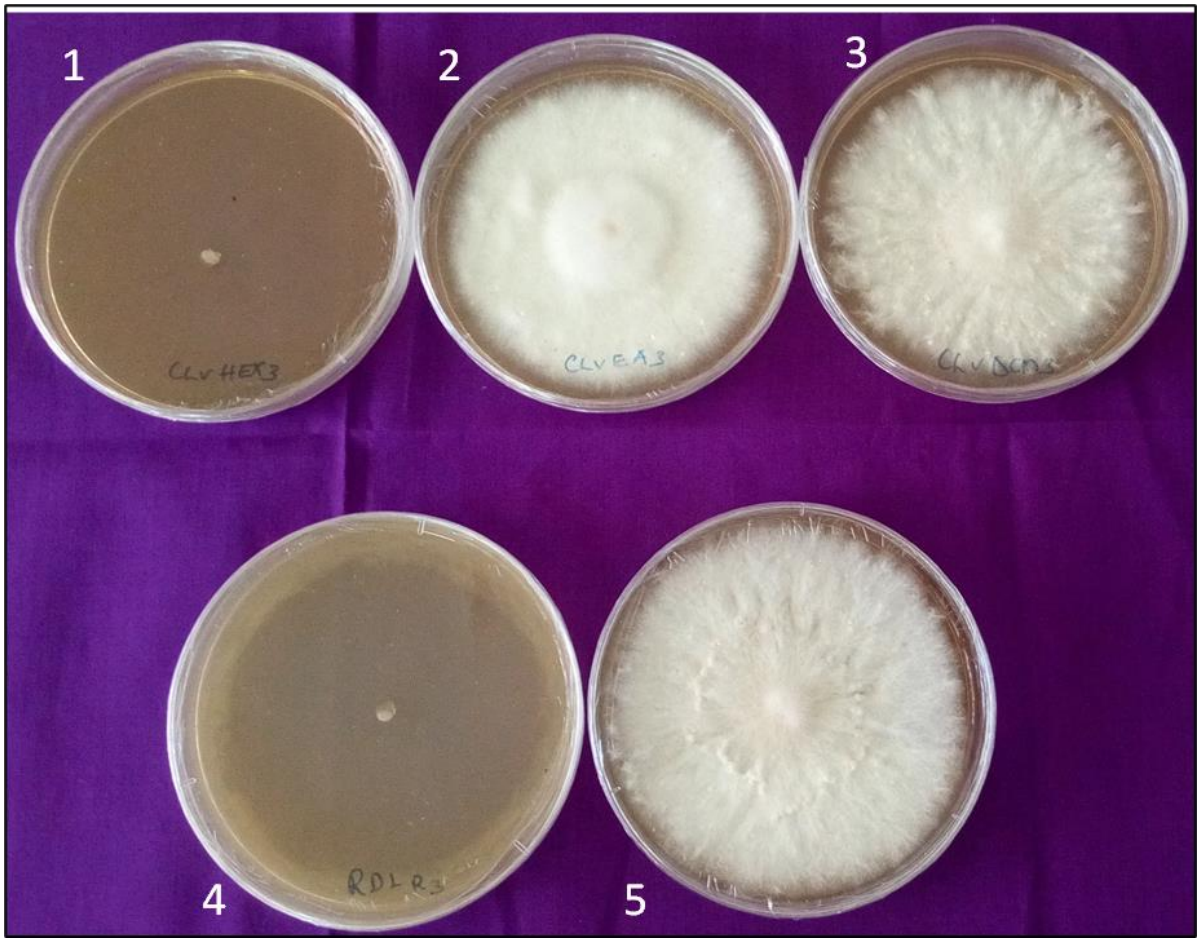

Figure 16: ***In vitro*** **activity of clove extract partitioned in 1-n-hexane, 2-ethyl acetate, 3-dichloromethane compared to 4- positive (Ridomil Gold® (metalaxyl-M and mancozeb)) and 5- negative control against** ***Phytophthora infestans*** **at 10 days after incubation**

Table 15: Percentage inhibition of colony diameter of *Phytophthora infestans* cultured on media amended with solvent fractions of ethanolic spice extracts, experiment 1

Extracts	Black pepper		Clove			Turmeric			Ginger			Statistics			
Solvents/DAI	H	DCM	H	EA	DCM	H	EA	DCM	H	EA	DCM	s.e.m	s.e.d	LSD (5%)	CV (%)
2	2.5	17.5	100.0	-15.0	-10.0	37.5	10.0	30.0	20.0	10.0	7.5	3.2	4.6	9.3	34.1
4	62.0	37.0	100.0	23.2	21.3	59.3	43.5	53.7	39.8	31.5	53.7	2.0	2.8	5.8	8.7
6	35.8	29.1	100.0	16.9	16.2	53.4	39.9	52.0	32.4	25.7	52.0	5.2	7.3	14.9	27.8
8	30.3	44.1	100.0	17.0	16.0	52.1	38.8	49.5	51.6	24.5	47.9	8.1	11.5	23.5	46.7
10	26.4	25.5	100.0	12.7	13.6	49.5	35.0	46.4	51.8	23.2	43.2	7.3	10.4	21.2	46.9
12	23.8	26.6	100.0	10.9	13.7	48.8	32.0	46.5	52.0	21.1	42.6	7.5	10.7	21.8	14.6
14	21.4	25.4	100.0	6.1	11.1	46.4	29.3	43.2	52.1	19.6	41.4	7.6	10.8	22.1	53.6
16	22.5	26.9	100.0	4.4	9.8	46.5	29.1	42.1	53.5	20.9	42.9	7.6	10.7	21.9	53.6
18	24.7	30.8	100.0	0.0	13.6	48.1	31.1	58.1	55.3	24.7	45.3	8.2	11.6	23.6	50.4
20	19.4	25.6	100.0	0.0	0.0	43.3	23.6	53.9	60.6	20.3	39.4	9.5	13.5	27.5	66.1

(H- hexane, EA- ethyl acetate, DCM- dichloromethane)

Table 16: Percentage inhibition of colony diameter of *Phytophthora infestans* cultured on media amended with solvent fractions of ethanolic spice extracts, experiment 2

Extracts	Black pepper		Clove			Turmeric			Ginger			Statistics			
Solvents/ DAI	H	DCM	H	EA	DCM	H	EA	DCM	H	EA	DCM	s.e.m	s.e.d	LSD (5%)	CV (%)
2	2.5	17.5	100.0	-15.0	-10.0	37.5	10.0	30.0	20.0	10.0	7.5	3.2	4.6	9.3	34.1
4	62.0	37.0	100.0	23.2	21.3	59.3	43.5	53.7	39.8	31.5	53.7	2.0	2.8	5.8	8.7
6	35.8	29.1	100.0	16.9	16.2	53.4	39.9	52.0	32.4	25.7	52.0	5.2	7.3	14.9	27.8
8	30.3	44.1	100.0	17.0	16.0	52.1	38.8	49.5	51.6	24.5	47.9	8.1	11.5	23.5	46.7
10	26.4	25.5	100.0	12.7	13.6	49.5	35.0	46.4	51.8	23.2	43.2	7.3	10.4	21.2	46.9
12	23.8	26.6	100.0	10.9	13.7	48.8	32.0	46.5	52.0	21.1	42.6	7.5	10.7	21.8	14.6
14	21.4	25.4	100.0	6.1	11.1	46.4	29.3	43.2	52.1	19.6	41.4	7.6	10.8	22.1	53.6
16	22.5	26.9	100.0	4.4	9.8	46.5	29.1	42.1	53.5	20.9	42.9	7.6	10.7	21.9	53.6
18	24.7	30.8	100.0	0.0	13.6	48.1	31.1	58.1	55.3	24.7	45.3	8.2	11.6	23.6	50.4
20	19.4	25.6	100.0	0.0	0.0	43.3	23.6	53.9	60.6	20.3	39.4	9.5	13.5	27.5	66.1

(H- hexane, EA- ethyl acetate, DCM- dichloromethane)

All the fractions derived from different solvents influenced the growth of *P. infestans* by a significant ($P \leq 0.05$) degree. The fraction observed to completely inhibit the growth of the late blight pathogen was from clove extracted in hexane. This clove hexane fraction inhibited the growth of the pathogen 100% throughout the experiment. The activity of the clove hexane fraction was also similar to that of the ethanolic extract as well as that of the fungicide containing mancozeb and metalaxyl. Similar findings were reported in a study involving leaf extracts of *Melianthus comosus* where acetone extracts were more active in inhibiting the growth of a wide range of fungal pathogens of agricultural importance (Eloff *et al*., 2017). The study further indicated that the activity of independent compound, oleanolic acid, isolated from *M. comosus* was not as high as that of the crude extracts. The antifungal activity of the methanol fractions and crude extracts especially against *Colletotrichum gleosporioides* and *Penicillium expansum* was similar to that of the comparative fungicide containing dicarboximide (Eloff *et al*., 2017). According to Awouafack *et al*. (2013) fractions of *Eriosema robustum* were highly active against *Aspergillus fumigatus* and *Cryptococcus neoformans* than isolated compounds. The study also confirmed that highest antifungal activity was observed in crude extracts and recommended their usage given the economic feasibility and synergy of the constitutive compounds. Moreover, acetone, DCM and ethyl acetate fractions of *Combretum erythrophyllum* leaves showed high antifungal activity against *Candida albicans* and *Aspergillus fumigatus* (Mtunzi *et al*., 2017).

4.3.2 Gas chromatography- mass spectrometry analysis of fractionated spice extracts

Different solvents were effective in extracting various compounds from the fractions of extracts in the present study. There was similarity in presence of some major compounds but their abundance differed among the spices and among the solvents. The major compounds detected in black pepper were E-caryophyllene, piperine, copaene, *alpha*-cubebene, caryophyllene oxide, *delta*-elemene, toluene, *ar*-turmerone, *alpha*-humulene, *ar*-curcumene, *delta*-cadinene, ethyl oleate, piperine, stigmasterol, *gamma*- sitesterol, *alpha*-copaene and terpinolene. The abundance of specific compounds was different as extracted by various solvents. Piperine was a major and common compound found in black pepper and its abundance differed when extracted in dichloromethane (86%), ethyl acetate (17%) and hexane (5.8%). E-caryophyllene also commonly detected in black pepper yielded differently in DCM (0.2%), ethyl acetate (0.3%) and hexane (5.75). This difference in abundance among compounds extracted in different solvents was observed in all black pepper extracts (Table 17- 19).

In clove, major compounds detected were toluene, eugenol, methyl salicylate, thiazole, E-caryophyllene, *delta*- cadinene, *gamma*- sitesterol, *alpha*- humulene, *ar*-curcumene, caryophyllene oxide, methyl linoleate, methyl palmitate and limonene. There were no significant compounds extracted in dichloromethane. The major compound in clove was eugenol and was abundantly extracted in hexane (73%) followed by E-caryophyllene (10.25%) (Table 20, 21).

In ginger, major compounds detected were *ar*- curcumene, *beta*-sesquiphellandrene, *alpha*-copaene, E- caryophyllene, cadinene, *gamma*- elemene, bisabolene, turmerone and t*rans*-sesquisabinene hydrate, linoleic acid, toluene, *alpha*- zingiberene, premnaspirodiene, caryophyllene, *ar*- turmerone, nerolidol, *delta*- elemene, *alpha*- copaene, *beta*- elemene, E-caryophyllene, *ar*- curcumene, *beta*- sesquiphellandrene, *gamma*- elemene, eucalyptol and borneol. The abundance of major compounds also differed with solvents. *Alpha*-zingiberene was abundantly extracted in hexane (24.4%) followed by DCM (9.7%) while beta-sesquiphellandrene was better extracted in hexane (12%) followed by DCM at 6% (Table 22-24).

In turmeric, major compounds detected were E-caryophyllene, *ar*-turmerone, *beta*-sesquiphellandrene, *beta*- bisabolene, curlone, *alpha*- zingiberene, *ar*- curcumene, turmerone, *alpha*- himachalane and E- atlantone, caryophyllene, toluene, eucalyptol, sesquithujene, *alpha*-*trans*- bergamotene and *alpha*- humulene. *Ar*- turmerone was abundantly extracted in DCM (30%) followed by hexane (25%). Another major compound was turmerone and was abundantly extracted in hexane (27.7%) followed by DCM (18.5%) (Table 25-27).

Table 17: Bioactive compounds present in black pepper extracted in dichloromethane

Peak No.	Retention time (min)	Compound identity	Abundance (%)
1	17.83	E - Caryophyllene	0.26
2	19.91	Caryophyllene oxide	0.04
3	20.44	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.α.,4a.α.,7.β7a.β.,7b.α.)]-	0.05
4	20.58	α-Cubebene	0.14
5	20.58	α - Copaene	0.13
6	20.60	Copaene	0.10
7	22.29	4,4'-methylenebis Benzenamine	0.01
8	22.39	3-methyl, Dibenzothiophene	0.01
9	29.55	4-Methyl-2-(phenylacetyl)phenol	1.77
10	29.56	Benzamide, 2-methoxy-N-(2,4-dimethoxyphenethyl)-	1.61
11	30.56	Piperine	8.88
12	31.69	Piperine	0.95
13	32.19	Piperine	86.05
14	34.30	2(3H)-Furanone, 3,4-bis(1,3-benzodioxol-5-ylmethyl)dihydro-, (3R-trans)-	0.02

Table 18: Bioactive compounds present in black pepper extracted in ethyl acetate

Peak no.	Retention time (min)	Compound identity	Abundance (%)
1	3.27	Thiazole, tetrahydro-	0.91
2	3.81	2-Pentanol, formate	3.03
3	3.95	Benzenesulfonylhydrazide, N2-(3-nitrobenzylideno)-	2.38
4	4.27	Heptaethylene glycol monododecyl ether	2.69
5	4.41	Sulfur tetrafluoride	3.92
6	4.44	Thiopropionamide	2.43
7	4.77	1,6-Dideoxy-l-mannitol	9.38
8	4.83	10-Oxatetracyclo[5.5.2.0(1,5).0(8,12)]tetradecane-9,11,14-trione, 4-[(2-methoxyethoxy)methoxy]-5-methyl-	7.73
9	5.19	6-Nitro-o-tolunitrile	6.61
10	5.22	10-Oxatetracyclo[5.5.2.0(1,5).0(8,12)]tetradecane-9,11,14-trione, 4-[(2-methoxyethoxy)methoxy]-5-methyl-	3.08
11	5.34	Benzeneacetonitrile, 4-nitro-	5.78
12	5.42	Thiazole, tetrahydro-	6.22
13	5.63	Benzenesulfonylhydrazide, N2-(3-nitrobenzylideno)-	10.72
14	6.34	2.34 Ethyl propanoate	6.23
15	6.46	Formic acid, butyl ester	0.86
16	16.92	Eugenol	0.43
17	29.52	Benzamide, 2-methoxy-N-(2,4-dimethoxyphenethyl)-	1.31
18	29.68	2,7-Naphthiridin-1(2H)-one, 4,5-ethano-8-methylamino-	0.41
19	30.54	Piperine	1.43
20	32.17	Piperine	17.42

Table 19: Bioactive compounds present in black pepper extracted in hexane

Peak no.	Retention time (min)	Compound identity	Abundance (%)
1	3.61	3-[N-[2-Diethylaminoethyl]-1-cyclohexenylamino]propylnitrile	8.14
2	3.84	1-Pentene, 2-methyl-	5.48
3	16.73	Terpinolene	3.19
4	17.77	E - Caryophyllene	5.75
5	18.21	α- Humulene	1.53
6	18.49	Ar- Curcumene	1.80
7	18.63	1S,2S,5R-1,4,4-Trimethyltricyclo[6.3.1.0(2,5)]dodec-8(9)-ene	2.05
8	18.73	α- Selinene	2.34
9	19.82	Caryophyllene oxide	2.29
10	20.51	α- Copaene	1.78
11	20.67	Ar- Turmerone	1.88
12	23.74	Hexadecanoic acid	4.65
13	23.98	Ethyl hexadecanoate	1.77
14	25.40	9,12-Octadecadienoic acid (Z,Z)-	6.14
15	25.45	Oleic Acid	3.83
16	25.57	Methyl linoleate	2.98
17	25.62	Ethyl Oleate	2.34
18	29.53	o-Anisic acid, 2-methyloct-5-yn-4-yl ester	1.77
19	30.49	Bicyclo[4.1.0]heptan-3-one, 4,7,7-trimethyl-, [1R-(1.alpha.,4.alpha.,6.alpha.)]-	2.08
20	32.06	Piperine	5.83

Table 20: Bioactive compounds present in clove extracted in ethyl acetate

Peak no.	Retention time (min)	Compound identity	Abundance (%)
1	3.70	1,4,7,10,13,16-Hexaoxacyclooctadecane	1.21
2	3.71	Bis(n-propylthio)methane	2.58
3	3.75	1,4,7,10,13,16-Hexaoxacyclooctadecane	4.10
4	3.94	Thiopropionamide	1.77
5	3.96	Isobutyl 2,5,8,11-tetraoxatridecan-13-yl carbonate	1.64
6	4.09	2-Pentanethiol	1.95
7	4.10	2,5-Anhydrogluconic acid	8.14
8	4.39	Butanamide, 2-hydroxy-N,3,3-trimethyl-	2.75
9	4.42	Thiopropionamide	3.41
10	4.47	1,6-Dideoxy-l-mannitol	3.27
11	4.55	Germacyclopent-3-ene, 1,1,3,4-tetramethyl-	4.04
12	4.59	Formic acid, 3-methylbut-2-yl ester	4.98
13	4.68	Butanamide, 2-hydroxy-N,3,3-trimethyl-	9.17
14	4.91	Formic acid, 3-methylbut-2-yl ester	4.87
15	5.16	10-Oxatetracyclo[5.5.2.0(1,5).0(8,12)]tetradecane-9,11,14-trione, 4-[(2-methoxyethoxy)methoxy]-5-methyl-	8.59
16	5.24	1,2,4-Triazole, 4-(4-dimethylamino-3-nitrobenzylidenamino)-	8.05
17	5.26	Thiazole, tetrahydro-	8.87
18	5.39	Germacyclopent-3-ene, 1,1,3,4-tetramethyl-	9.16
19	5.63	Thiopropionamide	3.10
20	6.32	Ethyl propanoate	5.06

Table 21: Bioactive compounds present in clove extracted in hexane

Peak no.	Retention time (min)	Compound identity	Abundance (%)
1	3.84	Methyl-cyclopentane,	5.88
2	16.69	α - Terpinene	0.24
3	16.75	α -Terpinyl acetate	0.55
4	16.93	Eugenol	73.94
5	17.16	α - Copaene	1.01
6	17.77	E - Caryophyllene	10.25
7	18.21	α - Humulene	1.39
8	18.51	ar - Curcumene	0.31
9	18.64	α - Zingiberene	0.34
10	18.75	β - Panasinsene	0.19
11	19.03	β - Sesquiphellandrene	0.38
12	19.03	Δ - Cadinene	0.47
13	19.82	Caryophyllene oxide	1.25
14	20.44	10,10-Dimethyl-2,6-dimethylenebicyclo [7.2.0] undecan-5. beta. -ol	0.18
15	20.46	Tetracyclo [6.3.2.0(2,5).0(1,8)] tridecan-9-ol, 4,4-dimethyl-	0.31
16	20.85	N-(2-Cyclopropylphenyl) cyclopropanecarboxamide	0.24
17	24.04	Ethyl hexadecanoate	0.32
18	25.62	Linoleic acid ethyl ester	0.24
19	38.72	γ - Sitosterol	0.53
20	40.56	β - Humulene	0.17

Table 22: Bioactive compounds present in ginger extracted in dichloromethane

Peak no.	Retention time (min)	Compound identity	Abundance (%)
1	6.65	Hexanal	4.38
2	14.72	n -Decanal	3.13
3	18.50	ar - Curcumene	3.50
4	18.65	α- Zingiberene	9.74
5	18.78	(+)-g- Cadinene	3.35
6	18.78	γ- Muurolene	1.44
7	18.82	β -Bisabolene	2.46
8	19.02	β- Sesquiphellandrene	5.58
9	19.74	Pyrazine, 2-methoxy-3-(1-methylethyl)-	1.13
10	20.29	7-epi-cis-sesquisabinene hydrate	1.25
11	20.50	Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)-	19.00
12	20.61	b-Eudesmol	4.07
13	20.62	Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)-	4.24
14	20.67	Ar-tumerone	1.93
15	20.72	Tumerone	3.67
16	20.93	7-epi-trans-sesquisabinene hydrate	2.20
17	21.40	o-Cymene	1.34
18	21.63	7-Oxabicyclo[4.1.0]heptane, 1-(1,3-dimethyl-1,3-butadienyl)-2,2,6-trimethyl-, (E)-	3.44
19	22.27	6-Methyl-6,7-dihydro-9H-5-oxa-9-azabenzocyclohepten-8-one	2.95
20	23.99	Ethyl hexadecanoate	2.89

Table 23: Bioactive compounds present in ginger extracted in ethyl acetate

Peak no.	Retention time (min)	Compound identity	Abundance (%)
1	4.00	Butane, 1-(1-ethoxyethoxy)-	2.31
2	4.20	Germacyclopent-3-ene, 1,1,3,4-tetramethyl-	3.31
3	4.37	2,3-Dihydrothiophene 1,1-dioxide	4.45
4	4.41	Thiazole, tetrahydro-	2.41
5	4.51	Methanethioamide, N,N-dimethyl-	6.86
6	4.56	N-(2-Sulfanylethyl)-2-oxopropanamide	3.05
7	4.56	3-Methylseleno-2-benzo[b]thiophenecarboxaldehyde	3.50
8	4.58	Ether, bis[2-(ethylthio)ethyl]	2.23
9	4.77	Thiazole, tetrahydro-	2.18
10	4.91	Ether, bis[2-(ethylthio)ethyl]	4.81
11	4.92	Thiazole, tetrahydro-	2.70
12	5.03	Cyclopropane, 1,1-dichloro-2,3-diethyl-, trans-	7.67
13	5.10	Thiopropionamide	7.30
14	5.15	Methanethioamide, N,N-dimethyl-	8.57
15	5.15	3,4-Methylenedioxy-.beta.-nitrostyrene	2.21
16	5.23	Benzenesulfonylhydrazide, N2-(3-nitrobenzylideno)-	10.52
17	5.65	1-(1-Propen-1-yl)-2-(2-thiopent-3-yl)disulfide	2.57
18	5.71	Borane, chlorodipropyl-	2.21
19	5.82	Methanethioamide, N,N-dimethyl-	6.00
20	6.34	2.34 Ethyl propanoate	3.24

Table 24: Bioactive compounds present in ginger extracted in hexane

Peak no.	Retention time (min)	Compound identity	Abundance (%)
1	3.13	3,5,5-Trimethylhexyl S-2-(dimethylamino)ethyl propylphosphonothiolate	0.64
2	3.49	1-Pentene, 2-methyl-	2.40
3	3.83	Cyclopentane, methyl-	3.63
4	3.83	1-Pentene, 2-methyl-	3.54
5	17.76	E- Caryophyllene	0.62
6	18.11	E-β- Farnesene	0.63
7	18.51	Ar-Curcumene	7.54
8	18.68	α Zingiberene	24.43
9	18.76	α- Copaene	4.34
10	18.77	α -Farnesene	9.82
11	18.82	β- Bisabolene	3.45
12	19.02	β- Sesquiphellandrene	12.03
13	20.27	Cis - α- Bergamotene	0.80
14	20.60	a-Eudesmol	0.97
15	20.60	β- Eudesmol	1.31
16	20.67	Ar-tumerone	0.68
17	20.71	Tumerone	0.92
18	23.70	geranyl-p-cymene	0.68
19	23.99	Hexadecanoic acid, ethyl ester	0.98
20	25.56	Methyl linoleate	0.81

Table 25: Bioactive compounds present in turmeric extracted in dichloromethane

Peak no.	Retention time (min)	Compound identity	Abundance (%)
1	17.78	E- Caryophyllene	1.46
2	18.50	Ar- Curcumene	2.61
3	18.65	α- Zingiberene	3.83
4	18.82	β- Bisabolene	1.29
5	19.01	β- Sesquiphellandrene	4.87
6	19.46	E- Nerolidol	0.51
7	19.82	Cis- Sesquisabinene hydrate	0.74
8	19.82	7-epi-cis-sesquisabinene hydrate	0.66
9	20.71	Ar- Turmerone	30.84
10	20.75	Tumerone	18.52
11	21.12	Curlone	18.78
12	21.24	Curcuphenol	0.55
13	21.60	6S, 7R- Bisabolone	1.97
14	21.81	Furazan	0.88
15	21.89	E- α- Atlantone	3.12
16	22.08	Tumerone	0.69
17	24.16	2,6-Nonadien-4-one, 9-(3-furanyl)-2,6-dimethyl-, (E)-	1.76
18	24.17	3-Methylbut-2-enoic acid, 3-methylphenyl ester	1.06
19	24.37	3-Methylbut-2-enoic acid, 3-methylphenyl ester	1.38
20	24.37	Hexane, 1,6-dibromo-	1.04

Table 26: Bioactive compounds present in turmeric extracted in ethyl acetate

Peak no.	Retention time (min)	Compound identity	Abundance (%)
1	5.62	Methyl 2-(methylthio)butyrate	10.29
2	5.57	Propane, 1,2-bis(ethylthio)-	9.20
3	4.83	Acetic acid, (butylthio)-, methyl ester	7.93
4	5.62	Thiazole, tetrahydro-	7.32
5	5.41	1,2-Propanediol, 3-(butylthio)-	7.30
6	5.69	Thiazole, tetrahydro-	5.84
7	4.34	Thiopropionamide	5.39
8	6.32	Ethyl propanoate	5.17
9	4.75	Thiopropionamide	4.69
10	5.72	3,3'-Methylenebis(1,5,8,11-tetraoxacyclotridecane)	4.53
11	4.29	Thiazole, tetrahydro-	4.48
12	4.02	Thiazole, tetrahydro-	3.50
13	5.41	Thiazole, tetrahydro-	3.42
14	4.06	10-Oxatetracyclo[5.5.2.0(1,5).0(8,12)]tetradecane-9,11,14-trione, 4-[(2-methoxyethoxy)methoxy]-5-methyl-	2.93
15	4.63	Thiazole, tetrahydro-	2.81
16	4.49	Bis(n-propylthio)methane	2.28
17	3.81	Methanethioamide, N,N-dimethyl-	1.91
18	3.98	D-Glucose, 2,3,5,6-tetra-O-methyl-	1.84
19	20.73	Tumerone	1.22
20	20.68	Ar- Turmerone	1.16

Table 27: Bioactive compounds present in turmeric extracted in hexane

Peak no.	Retention time (min)	Compound identity	Abundance (%)
1	17.77	E- Caryophyllene	2.01
2	18.50	Ar- Curcumene	3.11
3	18.65	α- Zingiberene	7.63
4	18.81	β- Bisabolene	1.69
5	19.02	β- Sesquiphellandrene	6.63
6	19.44	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	0.37
7	19.65	Benzene, 1-methyl-3-(1-methylethyl)-	0.68
8	19.82	1-Isopropenyl-3,3-dimethyl-5-(3-methyl-1-oxo-2-butenyl)cyclopentane	0.41
9	19.98	Benzene, 1-ethyl-2,4-dimethyl-	1.12
10	19.99	Benzene, 1-(3-cyclopentylpropyl)-2,4-dimethyl-	1.32
11	20.23	Ether, 3-hydroxy-2-butyl 1-(p-tolyl)ethyl	0.40
12	20.28	Pyridine, 2,4,6-trimethyl-, 1-oxide	0.44
13	20.37	Curlone	0.35
14	20.37	Benzenamine, N-ethyl-3-methyl-	0.35
15	20.72	Ar-tumerone	25.09
16	20.72	Tumerone	27.72
17	21.12	Curlone	12.78
18	21.30	5-Isopropyl-2-methylphenyl 2-methylbut-2-enoate	0.36
19	21.60	6S,7R- Bisabolone	0.99
20	21.89	E- α- Atlantone	2.40

The present study used ethanol for extracting compounds from the various spices for the first round of *in vitro* experiments. The ethanolic extracts were further partitioned with solvents of varied polarity. Specifically, clove ethanolic extracts partitioned in hexane completely inhibited the mycelia growth or development of *P. infestans*. This activity was similar to the crude clove ethanolic extracts. Therefore, ethanol was a suitable solvent for maximum extraction of antimicrobial compounds in the spice plants. Use of ethanol as an extracting solvent was supported by Dirar *et al*. (2018) who reported that use of 50 and 70% ethanol yielded high levels of phenolic content extracted from *Guiera senegalensis*. Similar claims were made by Rodriguez-Rojo *et al*. (2012) who reported that there was high phenolic content extracted from *Rosmarinus officinalis* after using ethanol as a solvent. Use of alcohols and especially ethanol has been widely researched and advocated for due to claims of delivering high yield of important compounds (Lapornik *et al*., 2005; Markom *et al*., 2006; Hayouni *et al*., 2007: Manasa *et al*., 2013; Dhawan *et al*., 2017). Factors such as temperature, extraction time, energy and extraction method, are to be considered since they also influence the yield of the targeted compounds (Rodriguez-Rojo *et al*., 2012). The features of ethanol that made it

suitable for use as an extractant are polarity, preservative nature and safety for human consumption.

Solvent fractionation was also performed on the ethanolic extracts and each solvent yielded different compounds. There were similar compounds in a particular plant extracts but their abundance differed depending on the solvent used in fractionation. Such remarks have been echoed by various studies regarding similar plants (Amelia *et al*., 2017, Tran *et al*., 2019; Koch *et al*., 2017; Sahoo *et al*., 2019). The differences in abundance of bioactive compounds in similar plants is subject to geographical locations, agronomic practices, environmental conditions, species and cultivars of the plants and extraction systems (Herath *et al*., 2017; Uddin *et al*., 2017; Munda *et al*., 2018; Liang *et al*., 2021). Some solvents are better extractants of selected bioactive compounds than others due to polarity of the solvents and the nature of the target compounds (Sing *et al*., 2004). The antifungal activity of ethanolic spice extracts was higher than that of many fractions due to ability of the solvents to extract certain bioactive compounds. This removes the need for fractionation and proves reliability of ethanol as an extracting solvent.

4.4 Effect of the ethanolic spice extracts on seed germination

All the extracts and their different dilutions had an effect on the germination percentage of tomato seeds (Table 28A, 28B). Extracts from black pepper showed significant effects on the germination percentage of the seeds under different dilutions. The highest initial germination percentage was recorded by the fourth (10^4) dilution and was up to 60%. Seeds treated with black pepper extracts took about 8 DAI to reach 50% germination. The first (10^1) dilution delayed germination of the seeds and only gave 60% germination by the end of 20 DAI. The second (10^2) and third (10^3) dilutions supported maximum germination till the end registering a percentage of above 90 by 20 DAI. The highest germination percentage recorded under black pepper extracts by the end of the experiment was under the third dilution giving about 97%.

Dilutions derived from cardamom extracts exhibited varied effects on the germination percentage of tomato seeds. The first dilution showed slowed germination of the seeds from beginning to the end of the experiment with a final germination percentage of about 50%. The fourth dilution supported maximum germination yielding up to 93%. The second and third dilutions also had relatively higher germination percentages. It took about 10 days for the seeds

treated with cardamom to attain a 50% germination percentage. The highest initial and final germination percentages were recorded under the fourth dilution.

The fourth dilution of clove extracts registered the highest germination percentage of above 90. The first dilution only allowed up to 20% of germination by the end of the experiment. Third and fourth dilutions supported maximum germination throughout the experiment. It took the seeds under clove treatment about eight to nine days to attain a 50% germination. High germination percentages were recorded at 10 days after incubation. The fourth dilution registered the highest initial and final germination percentages.

The first dilution of cinnamon supported average germination allowing up to about 66% at the end of the experiment. The highest initial and final germination percentage was recorded under the fourth dilution yielding about 49 and 98% respectively. By the 10^{th} day, seeds treated with extracts of cinnamon had achieved between 80 and 90% germination supported by the second and third dilution. Fifty percent germination was achieved between the eight and nineth day after incubation.

There was no germination recorded under the first dilution of ginger for the first 10 days after incubation. This dilution allowed up to 10% germination by the end of 20 DAI. The highest initial and final germination was about 18 and 80% respectively and was recorded under the third dilution of ginger. The highest maximum germination was also recorded by the third dilution and it attained 50% germination by 10 DAI.

The highest initial germination percentage (53%) recorded by seeds treated with lemongrass was recorded by the fourth dilution and attained 50% germination by 7 days after incubation. The fourth dilution also registered the highest final germination of up to 92%. All the dilutions recorded high germination percentages by the 20^{th} day after incubation. The first dilution allowed over 70% germination. This was the highest initial germination percentage for a first dilution compared to all other treatments. The second, third and fourth dilutions of lemongrass all allowed higher germination percentage.

From the dilutions derived from turmeric, the highest initial germination was recorded by the third dilution at 32% while the highest final germination was about 92%. The first dilution supported zero germination by 7 DAI but allowed a germination percentage of about 37% by 20^{th} day after incubation. The second and third dilutions allowed higher germination percentages.

The commercial fungicide used as a positive control registered an initial germination of 37% under the fourth dilution. The highest final germination was recorded by the fourth dilution at about 90%. The first dilution allowed a germination percentage of 48. The first dilution allowed a germination of 78%, higher all treatments. Distilled water registered the highest initial germination percentage of 56% and a final of 88%. All extracts performed better than water towards the end of the experiment.

All the treatments investigated in this study influenced the germination percentage of Money Maker® tomato seeds. Among the plant extracts, highest germination was recorded in seeds treated with cinnamon, black pepper, cardamom, lemongrass, turmeric, clove and ginger in that order with comparisons drawn from the negative and positive control. Only seeds treated with ginger and water had a germination percentage of below 90%. Different dilutions influenced the germination of the seeds. The more dilute treatments allowed a higher germination percentage especially with the plant extracts. The first dilution of ginger, for instance, did not allow any germination until 10 days after incubation. Apart from ginger and turmeric, all other plant extract treatments exhibited maximum germination at the fourth dilution.

The germination percentages reported in this study have been reported elsewhere, either involving tomato or other plant seeds. Nwachukwu and Umechuruba (2001) reported a germination percentage of 50 – 53% in the seeds of African yam bean (*Sphenostylis stenocarpa*) treated with lemon grass extracts. This was however a lower germination compared to 92% of tomato seeds treated with lemon grass as reported in the current study. Sorghum (*Sorghum bicolor*) seeds treated with lemongrass harmlessly transitioned into seedlings and the extract was recommended as a seed dresser by Somda *et al*. (2007). The effect of turmeric and clove reported in the current investigation mirrors that of Suwitchayanon and Kunasakdakul (2009) who reported a germination percentage of between 82 and 92% in Chinese kale seeds treated with clove and turmeric respectively. The concentrated treatments of ginger did not allow germination of seed for up to 10 days and only supported a 10% germination rate at the end of the experiment. This observation was also similar to the seeds treated with concentrated dilutions of turmeric. According to Han *et al*. (2008), seeds of soy bean (*Glycine max*) and chive (*Allium schoenoprasum*) treated with ginger extracts recorded reduced germination percentage and retarded seedling development due to interference with physiological processes such as water intake.

Table 28A: Germination percentage of tomato seed roots treated with different dilutions of various crude spice extracts

Treatments/	Days after incubation				
Dilutions	7	10	13	16	20
Black pepper					
10^1	0.0	10.7	37.3	52.0	60.0
10^2	38.7	82.7	88.0	90.7	90.7
10^3	44.0	90.7	94.7	97.3	97.3
10^4	62.7	89.3	92.0	93.3	93.3
Cardamom					
10^1	0.0	5.3	21.3	34.7	50.7
10^2	1.3	58.7	70.7	80.0	86.7
10^3	12.0	64.0	74.7	81.3	88.0
10^4	34.7	82.7	89.3	89.3	93.3
Clove					
10^1	0.0	1.3	2.7	9.3	20.0
10^2	13.3	60.0	80.0	80.0	88.0
10^3	12.0	81.3	84.0	86.7	90.7
10^4	33.3	74.7	86.7	88.0	90.7
Cinnamon					
10^1	0.0	1.3	18.7	32.0	66.7
10^2	24.0	82.7	88.0	89.3	93.3
10^3	30.7	73.3	77.3	78.7	81.3
10^4	49.3	93.3	97.3	98.7	98.7
s. e. m	7.7	7.0	5.9	5.6	5.2
s. e. d	10.9	9.9	8.4	7.9	7.4
L. S. D (5%)	21.8	19.7	16.8	15.8	14.8
CV (%)	62.8	21.0	15.1	13.3	11.6

Table 28B: Germination percentage of tomato seed roots treated with different dilutions of various crude spice extracts

Treatments/	Days after incubation				
Dilutions	7	10	13	16	20
Ginger					
10^1	0.0	0.0	2.7	5.3	10.7
10^2	0.0	21.3	41.3	53.3	65.3
10^3	18.7	56.0	62.7	69.3	82.7
10^4	16.0	74.7	80.0	81.3	81.3
Lemongrass					
10^1	0.0	30.7	53.3	64.0	74.7
10^2	8.0	66.7	84.0	89.3	93.3
10^3	36.0	81.3	90.7	90.7	90.7
10^4	53.0	84.0	92.0	92.0	92.0
Turmeric					
10^1	0.0	13.3	26.7	33.3	37.3
10^2	9.3	74.7	86.7	89.3	90.7
10^3	32.0	88.0	90.7	90.7	92.0
10^4	28.0	74.7	82.7	85.3	86.7
Ridomil Gold®					
10^1	4.0	21.3	34.7	40.0	48.0
10^2	16.0	56.0	69.3	74.7	81.3
10^3	32.0	68.0	86.7	86.7	89.3
10^4	37.3	65.0	84.0	86.7	90.7
Water	56.0	73.3	84.0	85.0	88.0
s. e. m	7.7	7.0	5.9	5.6	5.2
s. e. d	10.9	9.9	8.4	7.9	7.4
L. S. D (5%)	21.8	19.7	16.8	15.8	14.8
CV (%)	62.8	21	15.1	13.3	11.6

The authors attributed this finding to presence of allelochemicals that are soluble in water found in ginger plant, especially the stems. However, according to Karabuyuk and Aysan (2018), ginger extracts did not reduce germination percentage in tomato seeds treated against bacterial speck disease caused by *Pseudomonas syringae* pv. *tomato*. Similarly, Chandel and Kumar (2017) reported a germination percentage of 84 and 88% in garden pea (*Pisum sativum*) seeds treated with ginger and turmeric respectively. The study further reported increased dry weight in seeds and seedlings treated with turmeric extracts.

The commercial fungicide used in this study as a positive control recorded a maximum germination percentage of about 90%. Black pepper, cardamom, cinnamon and lemongrass extracts however exceeded this percentage by registering up to 98% germination. This finding

is contrary to that of Alam *et al*. (2014) who reported that the highest germination of about 95% was recorded in seeds of chili (*Capsicum annum*) treated with a fungicide as compared to plant extracts of neem, garlic, ginger and allamonda leaves.

In the current study, black pepper extracts supported a germination of about 97%. Being that Siddiqui (2007) reported black pepper leachates inhibited germination and retarded early development of black gram (*Vigna mungo*) seeds, the high germination rate in the current study can only be supported by the concentration of the extracts used to treat the tomato seeds. The authors concluded that high concentrations of the black pepper leachates were responsible for the reduced germination as well as impaired physiological aspects such as chlorophyll synthesis (Siddqui, 2007).

The most concentrated solution of cinnamon supported a germination percentage of about 66% while the least supported about 98% germination in the current study. Contrary to this finding, Wolf *et al*. (2008) reported that concentrated versions of cinnamon oil had negative effects on germination of cabbage (*Brassica oleracea*) seeds.

4.5 Efficacy of the spice extracts against blight diseases of tomato under field conditions

Throughout the first cropping cycle, severity of late blight was highest in the negative control plots except on the eighth week after application. At first week after treatment application, there was no significant ($P \leq 0.05$) differences among the treatments in reducing the severity of late blight. However, plots treated with black pepper extracts had exceptionally low severity (Table 29). At two, three and four weeks after treatment application, late blight disease remained low only in the positive control treated with Ridomil Gold®. Effect of all the crude spice extract treatments showed relatively similar amount of disease. The severity of late blight increased in the fourth week after transplanting across all treatments though the trend in reducing the disease remained the same, with commercial fungicide having the lowest disease. Severity of late blight started to decline across weeks five, six and seven but then surged again at week eight. There were no significant ($P \leq 0.05$) differences among the treatments at these weeks. The only significant ($P \leq 0.05$) difference noted among the treatments was at week three, where the disease load was highest in the negative control. The behaviour of treatments fluctuated throughout the weeks, in tandem with changes in the disease load (Table 29).

There was increased severity of late blight in cropping cycle two. During the first two weeks after application, the severity remained low among all treatments. However, as at the third

week after application the disease surged throughout week five where all plants wilted except for plots treated with the fungicide. There were no significant ($P \leq 0.05$) differences among treatments in the first two weeks after treatment application. Significant ($P \leq 0.05$) differences among treatments were only observed in week five. The activity of plant extracts in reducing severity of late blight was similar, though it was lower than that of the fungicide. The highest severity was recorded in the untreated plots. As at week five after application, only the positive control, treated with Ridomil Gold®, was able to reduce the severity of late blight (Table 30).

Area under the disease progress curve (AUDPC) indicated that the fungicide, Ridomil Gold® (metalaxyl-M and mancozeb), was most effective in reducing the severity of late blight in both cropping cycles while the negative control had the highest severity (Fig. 17). Among the plant extracts, black pepper was the most effective in reducing the severity of late blight by 40% followed by ginger, turmeric and clove in order of activity. Despite the disease developing rapidly in the second cropping cycle, the first cycle showed high disease load as indicated by the AUDPC values (Fig. 17)

Table 29: Severity of late blight disease assessed from tomatoes sprayed with crude spice extracts during cropping cycle one

Treatments	Weeks after application							
	1	2	3	4	5	6	7	8
Clove	2.5 a	2.5 a	1.7 b	2.0 a	1.6 a	1.4 a	1.0 a	1.7 a
Black pepper	1.9 a	2.2 a	1.6 b	2.0 a	1.2 a	0.9 a	0.3 a	2.7 a
Turmeric	2.6 a	2.3 a	1.7 b	2.1 a	1.9 a	1.1 a	0.6 a	1.9 a
Ginger	2.1 a	2.1 a	1.8 b	2.0 a	1.9 a	1.1 a	0.0 a	2.3 a
Fungicide	2.6 a	1.5 a	1.1 b	1.9 a	1.7 a	1.0 a	0.1 a	1.7 a
Control	3.1 a	2.8 a	2.9 a	2.7 a	2.5 a	2.3 a	1.6 a	2.4 a
s. e. m	0.8	0.6	0.4	0.4	0.5	0.6	1.0	0.8
s. e. d	0.7	0.5	0.3	0.3	0.4	0.5	0.8	0.6
L.S. D (5%)	1.5	1.0	0.6	0.7	0.9	1.1	1.8	1.4
CV (%)	33.0	25.2	19.4	18.2	26.8	44.7	53.8	37.7

Means followed by the same letter(s) within each column do not differ significantly at $P \leq 0.05$

Table 30: Severity of late blight disease assessed from tomatoes sprayed with crude spice extracts during cropping cycle two

Treatments	Weeks after transplanting			
	1	2	3	5
Clove	0.7 a	0.9 a	3.9 ab	5.0 a
Black pepper	1.3 a	1.5 a	3.9 ab	5.0 a
Turmeric	0.9 a	1.1 a	3.5 b	5.0 a
Ginger	0.5 a	1.1 a	3.7 b	5.0 a
Fungicide	0.5 a	0.5 a	1.7 c	3.1 b
Control	0.9 a	0.7 a	4.5 a	5.0 a
s. e. m	0.3	0.3	0.1	0.1
s. e. d	0.4	0.4	0.2	0.1
L.S. D (5%)	0.8	0.9	0.4	0.2
CV (%)	25.8	26.2	6.7	2.7

Means followed by the same letter(s) within each column do not differ significantly at $P \leq 0.05$

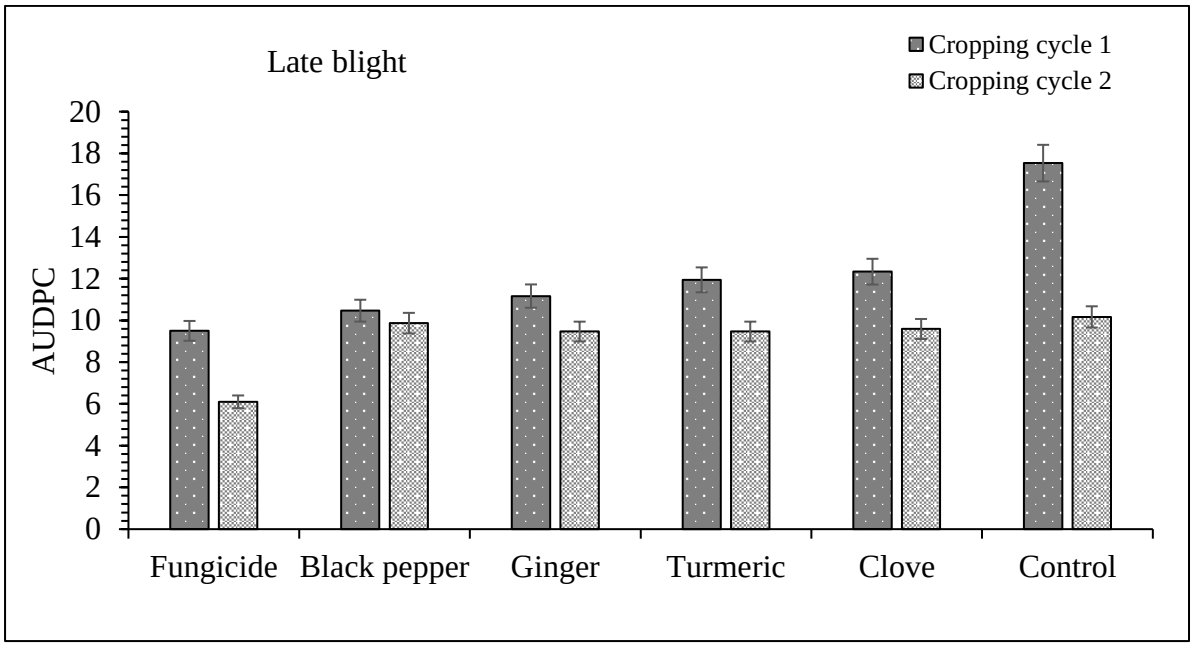

Figure 17: Area under disease progress curve of late blight disease on tomatoes sprayed with crude spice extracts for two cropping cycles

There was low severity of early blight disease in the first cropping cycle. Effects of the treatments on reducing the disease had no significant ($P \leq 0.05$) differences. At the first week after application, the lowest disease was observed in plots treated with clove and the negative control. In the second week after application no disease was observed among all the treatments. On the third week after application, symptoms of early blight reappeared on plots treated with ginger and the positive control. At the fourth week after application, there was disease in plots treated with black pepper, turmeric, ginger and fungicide, while all other plots had no

symptoms. After week six there was disease in all plots with no significant ($P \leq 0.05$) differences among the treatments. High severity was recorded in plots treated with black pepper, ginger and the positive control (Table 31). Activity of all the treatments in reducing the severity of early blight fluctuated throughout the cropping cycle.

During the second cropping cycle, severity of early blight remained low in all the treatments. The disease was however still high in the positive control. There were no significant ($P \leq 0.05$) differences among treatments in the first and second weeks after application (Table 32). AUDPC indicated that clove was very effective in reducing the disease load of early blight in both cropping cycles by about 35%. The activity of clove was followed by that of turmeric after which the fungicide followed. The highest disease load was recorded in plots treated with ginger followed by the negative control. The trend observed in cropping cycle one was relatively similar to that of the second cropping cycle (Fig. 18).

Table 31: Severity of early blight disease assessed from tomatoes sprayed with crude spice extracts during cropping cycle one

Treatments	Weeks after transplanting							
	1	2	3	4	5	6	7	8
Clove	0.0 a	0.0	0.0 a	0.0 a	0.0 a	0.5 a	2.2 a	2.1 a
Black pepper	0.3 a	0.0	0.0 a	0.1 a	0.9 a	1.1 a	2.3 a	2.5 a
Turmeric	0.4 a	0.0	0.0 a	0.1 a	0.5 a	0.5 a	2.4 a	2.3 a
Ginger	0.1 a	0.0	0.1 a	0.2 a	0.5 a	1.4 a	3.0 a	1.9 a
Fungicide	0.2 a	0.0	0.4 a	0.3 a	0.0 a	0.4 a	2.3 a	2.8 a
Control	0.0 a	0.0	0.0 a	0.0 a	0.0 a	1.6 a	3.3 a	1.9 a
s. e. d	0.3	0.0	0.2	0.2	0.4	0.5	0.4	0.6
L.S. D (5%)	0.6	0.0	0.4	0.5	0.8	1.1	0.9	1.4
CV (%)	56.8	0.0	7.1	88.2	76.0	58.1	10.0	9.0

Means followed by the same letter(s) within each column do not differ significantly at $P \leq 0.05$

Table 32: Severity of early blight disease assessed from tomatoes sprayed with crude spice extracts during cropping cycle two

Treatments	Weeks after transplanting			
	1	2	3	5
Clove	0.1 a	1.0 a	0.6 b	0.5 b
Black pepper	0.3 a	1.3 a	1.1 b	0.4 b
Turmeric	0.5 a	0.6 a	0.8 b	0.5 b
Ginger	0.3 a	1.0 a	1.0 b	0.5 b
Fungicide	0.4 a	0.9 a	2.2 a	1.8 a
Control	0.3 a	1.1 a	0.5 b	0.7 b
s. e. m	0.2	0.2	0.2	0.2
s. e. d	0.2	0.3	0.3	0.2
L.S. D (5%)	0.5	0.6	0.6	0.5
CV (%)	61.9	33.1	32.7	41.0

Means followed by the same letter(s) within each column do not differ significantly at $P \leq 0.05$

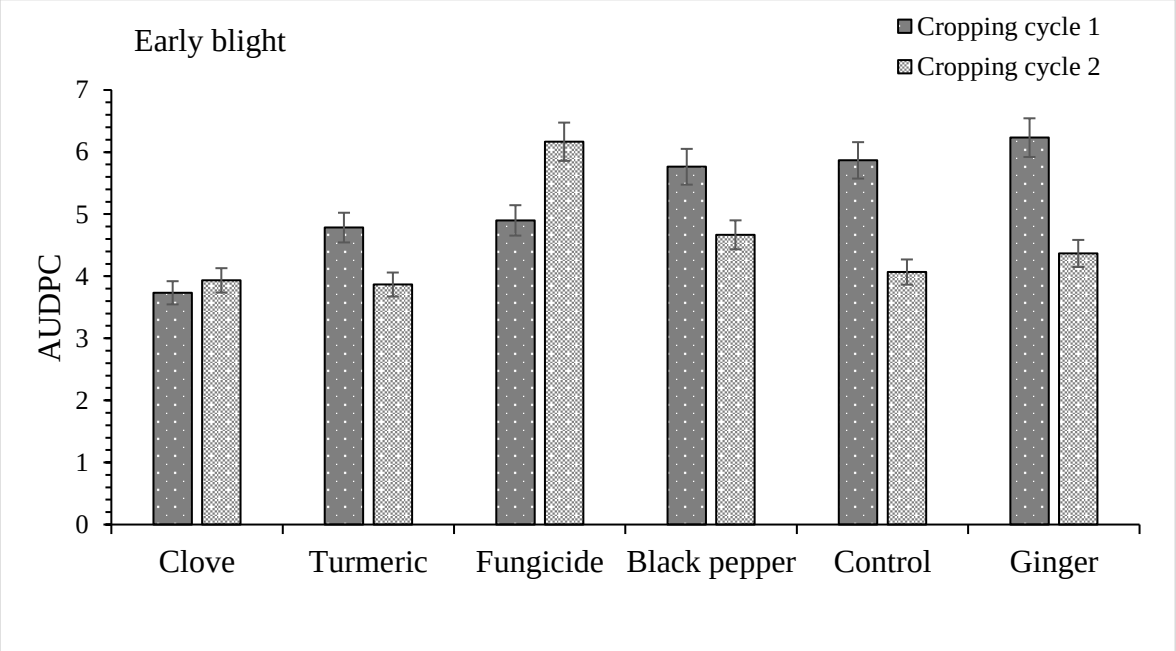

Figure 18: Area under disease progress curve of early blight disease on tomatoes sprayed with crude spice extracts for two cropping cycles

Plant extracts evaluated in this study reduced the severity of early blight under field conditions. Clove was the most effective in reducing the disease compared to the rest and even to the recommended fungicide. Activity of clove extract and oil against early blight has been

previously reported. Derbalah *et al*. (2012) reported that clove oil reduced severity of early blight by about 48% under greenhouse conditions. The oil also reduced severity of early blight by about 34% in field experiments and also increased yield and improved the vegetative parameters of tomato plants (Rahmatzai *et al*., 2017). While black pepper was not as effective in reducing the early blight disease in the current study, Pattnaik *et al*. (2012) reported that *Piper nigrum* reduced severity of early blight by about 36%. There are other plants that have been reported to reduce the severity of early blight by far and the authors attributed the activity to presence of antifungal compounds in the plant extracts and oils. Despite the reduced antifungal activity of lemongrass in the *in vitro* studies of the current study, Abd-El-Khair and Haggag (2007) reported that water extracts of lemongrass reduced severity of early blight by about 81%. Other plant extracts reported to reduce severity of early blight include basil, neem, *Pongamia pinnata, Ageratum conyzoides, Vitex negundo* and garlic (Nashwa & Abo-Elyousr, 2012: Pattnaik *et al*., 2012).

Ethanolic extracts of spices reduced severity of late blight by between 29 and 40% compared to the negative control. This level of activity by plant extracts has been reported by a study involving suspensions of *Frangula alnus, Rheum palmatum* and *Galla chinesis* against late blight of potato with a 10% higher activity than copper fungicides (Forrer *et al*., 2017). Gopi *et al*. (2020) however reported ineffectiveness of extracts of *Zingiber officinale* and *Curcuma longa* on late blight of tomato under field conditions and attributed the findings to epidemiology of the disease and lack of persistence of the extracts. Despite the positive results associated with several plant extracts reducing the severity of late blight under field conditions, the fungicides containing copper, metalaxyl or mancozeb are still the most effective (Shrestha & Ashley, 2007; Baka, 2014; Islam *et al*., 2019). In the current study, the activity of Ridomil Gold® containing metalaxyl and mancozeb was also highly effective in reducing the AUDPC of late blight of tomato and especially under the most favourable environmental conditions for disease development.

The different activity levels exhibited by plant extracts in this and other studies under field conditions is due to environmental and technical factors. Plant extracts in their basic form, without any form of formulation, are subject to rapid degradation due to environmental conditions such as temperature, light and precipitation (Oliveira *et al*., 2018). The bioactive constituents in the plant extracts are also highly volatile and are vulnerable to processes such as oxidation which renders them inactive (Pavela & Benelli, 2016).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Based on the specific objectives, the following conclusions were made:

(i) This study found high levels of awareness among farmers and traders on medicinal value of ginger and turmeric but negligible awareness on botanical pesticides. Though ginger was reliably produced in Same District, there are plenty of spices in various markets which provides a reliable source of material for production of plant-based fungicides. The high usage of spices for culinary and medicinal purposes creates an avenue for introduction of a spice-based fungicide being that intended users are already familiar with the plants.

(ii) Extracts from clove, black pepper, ginger and turmeric exhibited high antifungal activity against *A. solani, P. infestans, F. oxysporum* f. sp. *lycopersici* and *Pythium.* The antifungal activity which was similar to that of Ridomil Gold® is due to presence of bioactive compounds in spices which also exhibited synergistic effects on inhibiting growth of *P. infestans*. Reported findings provide a basis for development of a botanical fungicide for management of diseases of tomato caused by the tested pathogens.

(iii) Gas chromatography-mass spectrometry analysis of clove, black pepper, turmeric and ginger indicated presence of major and minor bioactive compounds which are attributed to the high antifungal acitivity reported in the study. The bioactive compounds were better extracted in ethanol majorly due to its polarity and its use is supported by being a preservative and its uses in food processing. The high abundance of bioactive compounds such as eugenol and piperine provides evidence that some spices can be relied upon as sources of active indredients for development of a plant-based fungicide.

(iv) Different concentrations of the spice extracts influenced germination of farmer saved tomato seeds. This provides insight into their use as seed treatment against seed borne pathogens by farmers and also for development of a botanical seed treatment product. Efficacy of ethanolic spice extracts against early and late blight diseases was satisfactory but could be improved by formulation. A higher concentration may be required to increase the efficacy in addition to formulation for longevity and stability under varying environemtnal conditions.

5.2 Recommendations

Following the findings in this study, the following recommendations are made:

(i) There are stringent requirements regarding food safety especially of fruits and vegetables particularly for farmers who wish to access the international markets. There is therefore a need to sensitize farmers on the use of safe yet effective crop protection products such as botanical pesticides.

(ii) For the farmers who rely on their own saved seeds as planting materials for the subsequent season, a seed treatment product needs to be developed from the plant extracts studied herein. This would help the farmers to overcome the challenges of seed borne diseases

(iii) Bioactive compounds present in spices can be extracted in ethanol and there is no need to isolate pure compounds due to cost and efficacy. The extracts should be used in their crude form for synergy and cost feasibility.

(iv) The high antifungal activity reported in the current study should support the quest for developing a broad-spectrum botanical fungicide for management of fungal diseases of tomato. In order to tap into the synergistic potential of the plants reported in this study, the fungicide should contain combined spice extracts. Efficacy of the spice extracts should be increased by higher concentrations and formulation for persistence and stability.

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RESEARCH OUTPUT

(i) Papers

Lengai, G. M., Muthomi, J. W., & Mbega, E. R. (2020). Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. *Scientific African*, *7*, 1-13. https://doi.org/10.1016/j.sciaf.2019.e00239

Lengai, G. M., Muthomi, J. W., & Mbega, E. R. (2021). Effect of plant extracts on important fungal pathogens and germination of tomato seed. *International Journal of Biosciences*, 18(4), 77-92. http://dx.doi.org/10.12692/ijb/18.4.77-92

Lengai, G. M., Mbega, E. R., &Muthomi, J. W., Activity of ethanolic extracts of spices grown in Tanzania against important fungal pathogens and early blight of tomato. Accepted at *Bulgarian Journal of Agricultural Science.*

(ii) Poster presentation

Antifungal activity of extracts from selected Tanzanian spices on major fungal pathogens and blight diseases of tomato