EFFICACY OF PLANT EXTRACTS AND ANTAGONISTIC FUNGI AS ALTERNATIVES TO SYNTHETIC PESTICIDES IN MANAGEMENT OF TOMATO PESTS AND DISEASES

LENGAI W MARY GERALDIN

BSc Agriculture (Crop Protection)
(University of Nairobi)

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DECLARATION

This thesis is my original work and has not been submitted for the award of a degree in any other University.

Lengai W. Mary Geraldin

Signature ______________________Date__________________

This thesis is submitted with our approval as University supervisors:

Prof. James W. Muthomi

Department of Plant Science and Crop Protection

University of Nairobi

Signature ______________________________Date_______________

Dr. Maina Wagacha

School of Biological Sciences

University of Nairobi

Signature ______________________________Date_______________

Prof. Rama D. Narla

Department of Plant Science and Crop Protection

University of Nairobi

Signature ______________________________Date_______________

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Registration Number ____

College ____

Faculty/School/Institute____

Department ____

Course Name ____

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DEDICATION

This work is heartily dedicated to my beloved mum Margaret Kandithi and sister Lilian Lingai

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ABBREVIATIONS AND ACRONYMS

GRINFOBANK	Agricultural information bank
Bt	*Bacillus thuringiensis*
CAST	Council of Agricultural Sciences and Technology
EC	Emulsifiable concentrate
EPPO	European and Mediterranean Plant Protection Organisation
EU	European Union
FPEAK	Fresh Produce and Exporters Association of Kenya
GAP	Good agricultural practices
HCDA	Horticultural Crops Development Authority
ICIPE	International Centre for Insect Physiology and Ecology
IP/DM	Integrated Pest/ Disease Management
IPM	Integrated Pest Management
KALRO	Kenya Agricultural and Livestock Research Organisation
KEPHIS	Kenya Plant Health Inspectorate Service
LM	Lower Midland
LTD	Limited
MOA	Ministry of Agriculture
MRLs	Maximum Residual Levels
NAFIS	National Farmers Information Service
NICRA	National Initiative on Climate Resilient Agriculture
PAN	Pesticide Action Network
PCPB	Pest Control Products Board
PDA	Potato Dextrose Agar
PPPs	Plant Protection Products
SC	Soluble concentrate

TMV	Tobacco Mosaic Virus
TSWV	Tomato Spotted Wilt Virus
TYLCV	Tomato Yellow Leaf Curl Virus
USAID	Unite States Agency for International Development
WP	Wettable powder

GENERAL ABSTRACT

In the last few decades, farmers have relied on synthetic pesticides to manage crop pests and diseases. This is because synthetic pesticides are easily available, have quick knock down effect, have varied modes of action and are reliable. However, synthetic pesticides are not easily degraded, they leave residues in crop products, are expensive, are harmful to the user and are an environmental hazard. In addition, some pests and disease pathogens have developed resistance to synthetic pesticides and farmers have had to increase frequency of pesticide application since target markets demand aesthetically presentable produce. Presence of residues in fresh vegetables has led to increased interceptions and the produce has been denied access to lucrative markets. This has led to reduced export volumes, loss of market reputation, loss of employment and loss of income. The objective of this study was to evaluate the efficacy of plant extracts and antagonistic fungi compared to the synthetic pesticides in the management of pests and diseases of tomato under *in vitro* and field conditions.

Extracts from different plants were screened for activity against economically important fungal pathogens of tomato. Plant samples were extracted in ethanol and concentrated using a rotary evaporator. The extracts were tested for activity by poisoned food technique, which involved incorporating the extract into the culture media and sensitivity of the fungal pathogen was determined by measuring the pathogen colony radial growth. The most active extracts were further evaluated together with antagonistic fungi for efficacy in managing tomato pests and diseases under field conditions. Their efficacy was compared to synthetic pesticides, a commercial botanical and a commercial antagonist applied weekly. Data was collected on incidence and severity of early and late blight, population of white fly, damage by *Tuta absoluta* leaf miner, fruit yield and quality.

Out of the ten plant extracts evaluated Turmeric (*Curcuma longa*), lemon (*Citrus limon*), garlic (*Allium sativum*) and ginger (*Zingiber officinale*) showed significant activity against test pathogens. However, the test fungal pathogens varied in sensitivity to the different plant extracts with *Alternaria solani* being the most sensitive while *Fusarium oxysporum* f.sp. *lycopersici* was the least inhibited. Turmeric (*Curcuma longa*) extract was the most active and reduced mycelia growth of all the tested plant pathogens by up to 45% *in vitro*. It reduced mycelia growth of *Alternaria solani* by up to 70% while mint (*Mentha piperita*) was the least active. Under field conditions, plant extracts were effective in reducing populations of whiteflies and *Tuta absoluta* by up to 50% while the antagonists reduced the same pests by up to 30% compared to the negative control. Plant extracts and antagonistic fungi significantly reduced early and late blight diseases of tomato. Turmeric extract reduced early blight by up to 30% and late blight by up to 50% compared to the treated control. Majority of yield under grade 1 and 2 was from plants treated with a commercial botanical with neem as the source of extract while garlic (*Allium sativum*) extract had the highest yield under grade 3. Plant extracts and antagonistic fungi reduced pest and disease damage of fruit yield by up to 40% and 60%, respectively compared to the untreated control.

The comparative effectiveness of plant extracts and isolated antagonistic fungi with the synthetic pesticides and the commercialized antagonists and botanicals is proof that the crude products have significant potential. Therefore, there is need for comprehensive explorations into the local environment and more plants and organisms be identified and screened for antimicrobial properties and thereafter tapped and made available to farmers. This will help the average farmer reduce the production costs, have higher income and at the same time have clean, safe and quality produce for high value markets.

Key words: Plant extracts, antagonistic fungi, tomato, plant pathogenic fungi

CHAPTER ONE: INTRODUCTION

1.1 Background information

Tomatoes are grown almost by every household in the world either in a pot, kitchen garden, small scale or in a large scale for commercial production. It is one of the most important vegetables (Monte *et al*., 2013) in Kenya (Mutitu *et al*., 2003; Wachira *et al*., 2014). There are several improved varieties for pest and disease resistance, taste preferences and climate adaptability (Engindeniz *et al*., 2013). Tomato farming is an economic activity and offers a reliable income especially to small scale farmers, and are widely grown and consumed as vegetables (Wachira *et al*., 2014). In 2012, area under tomato production was 18,612 ha with a production of 397,000 MT and an income of 12.8 billion (HCDA, 2012-2013). Majority of the production is done in Kirinyaga (24%) and Taita Taveta Counties (7%). In Kirinyaga County and Loitoktok (in Kajiando County) production is under irrigation in Mwea and Namelock schemes, respectively. These areas are about 1159 m above sea level, have well drained clay or sandy loam soils, experience temperatures of between 20- 27^0C and receive rainfalls of up to 600 mm (USAID, 2013; Nafis, 2015).

Tomatoes are affected by insect pests and pathogens which reduce their quality, quantity and profitability (Engindeniz *et al*., 2013; Islam *et al*., 2013). Insect pests such as *Tuta absoluta* (Taha *et al*., 2012) flea beetles, aphids and leaf miners for instance affect the foliage while fruit borers affect the tomato fruits. Severe damage also occurs since some of these pests are disease vectors (Sumitra *et al*., 2012). Tomatoes are also affected by disease causing pathogens including bacteria (Sutanu and Chakrabartty, 2014), fungi (Goufo *et al*., 2008*)*, viruses (Joshua *et al*., 2003) and nematodes (Noling, 2013; Jacquet *et al*., 2005*)*. These pests and disease pathogens reduce the quality and quantity of the tomato yield and the losses can go up to 100% (Joshua *et al*., 2003; Goufo *et al*., 2008).

Some of the pathogens that affect tomatoes have a sophisticated morphology which makes them complicated to manage in the field (Mizubuti *et al*., 2007). For instance, the causal agent for late blight (*Phytophthora infestans* Mont.de Bary) is a highly developed oomycete and has only been managed by using protective synthetic fungicides and its losses are devastating (Mizubuti *et al*., 2007; Goufo *et al*., 2010). The complexity of pathogens therefore calls for a multi-faced approach in managing them and ideally an integrated approach suits best. With yield losses amounting to 100% due to these pests and diseases (Goufo *et al*., 2008), diverse and safe management options are essential.

In an effort to meet the demands of tomato, farmers resort to use of synthetic pesticides (Nashwa and Abo-Elyousr, 2012). However, concerns on the toxicity of the products used and their retention potential in the food products have been raised (Stangarlin *et al*., 2011; Business Daily, 2015). Apart from toxicity on the products, synthetic fungicides have other disadvantages such as environmental pollution, food contamination, health hazards to the farmers as well as possible elimination of natural enemies from the ecosystems. Indeed, use of synthetic pesticides poses problems within the markets due to maximum residue levels (MRLs) in tomato fruits and their requirements in the products (Pal and McSpadden, 2006; Campos, 2014; European Commision, 2014; Wagnitz, 2014; Wafula *et al*., 2014).

1.2 Problem statement

Tomato is infected by a large number of insect pests that include *Tuta absoluta*, flea beetles, fruit borers, aphids and whiteflies, from the time of emergence to harvesting (Sumitra *et al*., 2012; Engindeniz *et al*., 2013). Diseases include blights, mildews, cankers and wilts with collective losses of up to 100% (Goufo *et al*., 2008: Noling, 2013; KALRO, 2014). The farmers involved in horticultural export business find using synthetic pesticides the most convenient way of managing these pests and diseases (Birech *et al*., 2006).

While synthetic pesticides partially solve the menace, they also lead to more problems since synthetic pesticides are non-biodegradable, pollute the environment and leave residues in the produce (Bhattacharjee and Dey, 2014). They are harmful to applicants and continuous application lead to resistance build-up among the pests as well as pathogens (Stangarlin *et al*., 2011; Engindeniz *et al*., 2013; Wagnitz, 2014). Target markets have set strict quality requirements and require the food produce to be safe, clean and healthy for consumption (Business Daily, 2013; 2014; The East African, 2015).

Lack of awareness by farmers has seen them apply the synthetic pesticides intensely with to reduce pests and diseases (Goufo *et al*., 2008). Farmers do not observe the required pre-harvest intervals, handling requirements and application rates and make frequent applications to attain pest free fields which lead to presence of chemical residues in the produce leading to increased interceptions especially in the EU markets (The East African, 2015; People Daily, 2016). Several suppliers have been de-listed from the markets and a 90% reduction in the permitted levels of MRLs has been up to 0.02 ppm for some chemicals (Mwangi, 2013). In addition, sample size for verification of compliance has been increased to 10% and this has led to more losses including income, market reputation and support of livelihoods (Mwangi, 2013; Business Daily, 2016).

1.3 Justification

Satisfying consumer needs and taking care of human health and environmental safety can be achieved by either reducing the usage of synthetic pesticides or by supplementing with biological pesticides. Synthetic pesticides could be supplemented with biopesticides as alternative pest and disease management products (Mizubuti *et al*., 2007; Engindeniz *et al*., 2013). Biopesticides have been receiving much practical attention (Srijita, 2015) as substitutes to synthetic chemical plant protection products due to biodegradability, effectiveness in the long term, multiple modes of action on pests, target specificity, lack of toxic residues and can be cheaper than the synthetic pesticides especially if locally produced (Nashwa, 2011; Gupta *et al*., 2014). The fact that some of the plants used in developing biopesticides could be consumed makes them more beneficial and safe (Skaria, 2007; Raja, 2014; Srijita, 2015). Biopesticides have successfully worked to manage pests and diseases in other crops and losses have hence been minimized (Al-Samarrai *et al*., 2012; DejanMarciic *et al*., 2012 Naing *et al*., 2013; Degri *et al*., 2013; Raja, 2014).

Majority of the biopesticides available in Kenya are imported and some of the plants used to make them are exported from Kenya such as pyrethrum and chrysanthemums (Infonet-Biovision, 2015). Our local environment has flora and fauna which upon extensive explorations could make safe products for pest and disease management. In our environment there are also microbes which are found in the imported formulations of the biocontrols and if they could be tapped, there would be reduced risks of importing organisms which could be of phytosanitary harm to our environment (Chethana *et al*., 2012). Therefore, the local natural environment can be exploited fully to find safe alternatives to the synthetic pesticides which will reduce the presence of residues in vegetables, and reduce the costs of crop production systems under safe environments.

1.4 Objectives

The general objective of this study was to contribute to sustainable horticultural production through use of plant extracts and antagonistic fungi as alternatives to synthetic pesticides in managing pests and diseases.

The specific objectives of the study were:

i. To determine effectiveness of different plant extracts on selected fungal pathogens of tomato *in vitro.*

ii. To evaluate the efficacy of plant extracts and antagonistic fungi in managing pests and diseases of tomato under field conditions.

1.5 Hypotheses

i. The local environment has flora and fauna with antimicrobial activity and can effectively manage fungal pathogens of tomato.

ii. Biopesticides are as effective as synthetic pesticides in the management of tomato pests and diseases.

CHAPTER TWO: LITERATURE REVIEW

2.1 Tomato production in Kenya

Tomatoes are widely grown and consumed as vegetables (Wachira *et al*., 2014). The area under tomato production was 18,612 ha with a production of 397,000 MT and an income of 12.8 billion (HCDA, 2014). Majority of the production is done in Kirinyaga (24%) and Taita Taveta Counties (7%). In Kirinyaga and Loitoktok in Kajiando County, production is under irrigation in Mwea and Namelock schemes respectively. These areas are at least 1159 m above sea level, have well drained clay or sandy loam soils, experience temperatures of between 20-27^{0}C and receive rainfall of up to 600 mm (USAID, 2013; Nafis, 2015). Tomato in Kenya is grown for different markets which dictate the different varieties that are grown. The processing varieties produced include Cal-J, Riogrande, Roma VF, Parma VF, Rubino, Nema 1400 among others while fresh market varieties include Anna F1, Mavuno F1, Money maker, Marglobe, Capitan, Kentom 1 and beauty among others (MOA, 2012). Some of these are produced for the international markets, but most of the produce ends up in the local prime markets such as supermarkets and hotels while the remainder end up in the open air markets (Mungai *et al*., 2000).

2.2 Insect pests affecting tomatoes

Output of crops grown for human consumption is at jeopardy due to the occurrence of pests (Oerke, 2006). Tomatoes are subject to several insect pests from the time of emergence to harvesting. These pests can be classified according to the parts they affect in a plant such as foliage, leaves, and fruits or even stems. Flea beetles, aphids and leaf miners for instance affect the foliage while fruit borers affect the tomato fruits (Sumitra *et al*., 2012). Severe damage may also occur since some pests are disease vectors.

Some pests have less damage on the tomato plants while others can cause 100% yield loss in the field (Pascual *et al*., 2003; Mayfield *et al*., 2003).

Tuta absoluta is a moth and its larva (caterpillar) is the most destructive stage. It mines into the leaf tissue, feeds extensively (Santos *et al*., 2011) and also bores into fruits leaving symptomatic tiny holes. It also bores into stems causing breakage and can cause losses of between 50-100% in an infested field (Larry, 2013). Insecticides and predators have been used to manage it in countries like USA. Leaving plant debris in the field and continuous cropping of tomato are some of the conditions that lead to proliferation of leaf miners in a field (EPPO, 2005; KALRO, 2014). *Tuta absoluta* is better managed through IPM and aided by use of traps especially using pheromones (Santos *et al*., 2011; Cherif *et al*., 2013).

Thrips (*Thrips tabaci, Frankliniella occidentalis, F. schultzeli*) feed on the lower surface of the leaf and suck up the sap that exudes from the leaves (Ssemwogerere *et al*., 2013). They also attack buds, flowers and fruits and the attacked leaves show a silvery sheen and small black spots, thrips excreta. Collapse of plant cells can result in formation of deformed flowers, leaves, stems, shoots and fruits. Under heavy infestation, buds and flowers may fall off and the fruits may be deformed leading to a reduction in quality. Thrips are also virus carriers of tomato spotted wilt virus (TSWV) which can cause a 100% yield loss in a field (Mayfield *et al*., 2003). They can be managed through crop hygiene, weed control and using seeds for planting since they do not harbour TSWV. Some organophosphate and carbamate insecticides have some level of efficacy against thrips (Infonet-Biovision; Sonya *et al*., 2007; Funderburk *et al*., 2013).

Whiteflies (*Bemisia tabaci*) attack tomatoes at all stages of growth and they suck up plant sap thus weakening the plant as well as yellowing. Their nymphs produce honey dew which reduces plant growth as well as the fruit quality. They affect tomatoes directly through feeding and indirectly as disease vectors (McCullan *et al*., 2003) such as tomato yellow leaf curl virus (TYLCV) (Pascual *et al*., 2003). Natural enemies such as parasitoid wasps should be conserved to help manage them. Plant barriers such as coriander can be used to repel whiteflies among other cultural activities. Neem based insecticides have been used to reduce egg-laying and also inhibit the growth and development of their nymphs (Lapidot *et al*., 2001: Infonet-Biovision, 2015).

Fruit borers (*Helicoverpa* spp and *Spodoptera* spp) attack the developing and mature fruits and are one of the most destructive pests of the tomato plants (Ghosh *et al*., 2011). They lay eggs on the lower side of the leaflets (Degri and Samaila, 2014) and when they feed on the leaves, they appear distorted since they feed on tips into the developing buds. The larva enters the fruit through the stem end and feed on the inner parts of the fruit. Through boring the fruits, they cause up to 70% yield loss (Infonet-biovision, 2015) since the fruit boring leads to decay. They can be controlled through use of biopesticides such as neem extracts, pyrethrin, rotenone or *Bacillus* sp (Ghosh *et al*., 2011). Conservation of natural enemies and use of cultural practices such as crop rotation and field sanitation also helps to manage the pest. Caterpillars can also be picked manually from the leaves (Chakraborty *et al*., 2011: Infonet-Biovision, 2015).

Spider mites (*Tetranychus* spp) suck up plant sap with their stylet-like mouth parts and can be found on both sides of the leaf but highly prefer the underside near the veins (Muzemu *et al*., 2011). They produce webbing on the foliage and on high infestation can mummify the fruits (Bauernfeind, 2005). Increased infestation can lead to defoliation and the affected plants produce small fruits which have low content of ascorbic acid. Tobacco spider mite (*Tetranychus evansi*) is the most destructive and can cause up to 90% yield loss (Jayasinghe and Mallik, 2013). There are no miticides registered yet but mites can be managed through farm hygiene especially removal of alternative hosts such as nightshade plants (Meck, 2010). Propagation materials should also be sourced from healthy and certified sources to avoid introduction of the mites in pest free fields (Azandeme-Hounmalon *et al*., 2014; Infonet–biovision, 2015).

2.3 Fungal diseases of tomato

Late blight (*Phytophthora infestans*) is identified by black or brown lesions on leaves and stems that may be small at first and are water soaked in appearance or have chlorotic borders, but soon expand rapidly and become necrotic (Schumann and Arcy, 2000). During wet weather, the lesions are covered with a grey to white mouldy growth. Affected stems and petioles may eventually collapse at the point of infection leading to death of all distal parts of a plant. Infected tomato fruits turn greasy, decay and can shrivel up and fall off the plant and those that remain attached never ripen (Alexandrov, 2011). Effects on the plant include extensive defoliation, reduced photosynthetic leaf area, loss of plant vigour, plant death, loss of fruits, and reproductive capacity and loss of seed. The pathogen's mycelia are spread by wind or water droplets from plant debris, volunteer tomato plants and perennial weeds of the nightshade family to susceptible hosts (Goufo *et al*., 2008). Disease development is favoured by cool moist weather, temperatures of 15-21^{0}C during the day and a relative humidity of 100% (Scot, 2008). The disease is manageable through IPM approach through strategies such as use of resistant varieties, crop rotation, and crop and field sanitation, protective fungicides among other practices (Mizubuti *et al*., 2007).

Effects of powdery mildew (*Oidium neolycopersici*) include yellowing, drying, necrosis and defoliation and tomatoes are affected at any stage under favourable environmental conditions for the disease to occur (Kubienova *et al*., 2013). Losses in plants can reach up to 50% in commercial productions where the disease is severe (Yonghao, 2013). The disease is considered a greenhouse disease but it is also found in open fields. Symptoms appear as light green and yellow blotches on the upper surface of the leaves. The fungus enlarges and chlorotic lesions turn purple with necrotic centres and the whole leaf could be covered by white fungal growth. The fungus survives on mycelia in living or dormant volunteer host plants (Updhyaya, 2013). The spores are easily dislodged from the infected leaves and carried long distances by wind or air currents. The infection could be polycyclic and is hence manageable by use of resistant varieties, cultural practices and fungicide application (Segarra *et al*., 2009).

Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*) is caused by a soil borne pathogen and it enters the plant through the roots to the vascular tissues (Akrami and Yousefi, 2015) which lead to famishment of the branches. The symptom is usually a yellowing of the lower leaves which gradually wilt and die. This form of wilt is favoured by warm temperatures, dry weather, acidic soils and presence of root knot nematodes. It can be introduced into a field through wind, water, wildlife or equipment being used in the field (Anitha and Rabeeth, 2009). Wilting kills the plant thus reducing its total productivity. *Fusarium* wilt is more severe where plants are infested with root knot nematodes (Mark, 2013). Crop rotation and growing resistant varieties is the most effective means to control the pathogen (Bonanomi *et al*., 2007).

Early blight (*Alternaria solani*) is a foliage and fruit disease and the fungus attacks the fruit at the stem end causing large sunken areas with concentric rings and a black velvety appearance (Junior *et al*., 2011). Other symptoms include irregular black / brown spots on older leaves and these spots enlarge forming lesions and can cause leaf fall. Ultimately, it leads to reduced fruit yields and also leaves the fruit open to sunscald (Ashour, 2009). The pathogen can attack the plant at any growing stage during the growing season but usually progresses most rapidly after fruits have set which increases fruit rot (Sallam *et al*., 2012). The pathogen can be controlled through crop rotation, use of drip irrigation, adequate soil fertility and a spray program using a recommended fungicide especially at fruit set (Gleason and Edmunds, 2006*;* Yazici *et al*., 2011).

Anthracnose fruit rot is caused by several members of the genus *Colletotrichum* (Janna, 2015) and the symptoms include black sunken lesions on the ripening fruit (Wani, 2011). As the lesions mature, the centre turns tan and small black fruiting bodies appear (Peter and Andy, 2005). The effect of this pathogen is noticeable on the ripe fruits, but it also affects the green fruits (Helene, 2015). The water soaked sunken spots can increase up to ½ inch in diameter. They enlarge and produce microsclerotia at the centre of the lesion beneath the skin surface and this exposes the fruits to secondary infections (Bautista-Banus *et al*., 2003). The fungus survives the winter on diseased tomatoes, in the soils and in seeds (Mark, 2006). Tomatoes become increasingly susceptible as they approach maturity (Helene, 2015). The disease can be controlled through harvesting at frequent intervals, picking all ripe fruits after each harvest, use of resistant varieties, crop rotation, field hygiene and use of disease free seeds or transplants (Infonet-biovision, 2015).

2.4 Losses caused by pests and diseases in tomato

Incidences of several insect pests like bugs, aphids, moths, miners and thrips among others have increasingly caused yield losses (Dhaliwal *et al*., 2010). Tomato is attacked by a number of arthropods, plant diseases and nematodes which significantly reduce fruit yield and quality (Engindeniz *et al*., 2013). Pest losses are estimated to cause 34.4% of attainable tomato yield with crop protection measures. Without management measures, the losses amount to almost 77.7% of the attainable yield (Zalom, 2003). Root note nematode species of *Meloidogyne* including *M. incognita* and *M. javanica* cause 24-38% loss in tomato (Hassan *et al*., 2010; Infonet-biovision, 2015).

Any pest or disease that affects any part of a tomato plant can lead to losses, either in quality or quantity. Some pests can cause 100% yield loss such as american leaf miner (*Tuta absoluta*) (KALRO, 2014) while others cause 70% yield loss such as fruit borers (Infonet-biovision, 2015; Sumitra *et al*., 2012). Other insect pests lead to production of deformed or injured fruits such as fruit borers and some insect pests also transmit dangerous viral diseases which can clear a whole tomato field (Sumitra *et al*., 2012).

Fruit borers such as bollworms (*Helicoverpa armigera*) can cause up to 95% damage of the tomato fruits (Sumitra *et al*., 2012) while nematodes (*Meloidogyne* spp) have been reported to cause up to 60% losses in tomatoes (Hassan *et al*., 2010). When nematode juveniles penetrate the root tips of plants, they initiate development of giant cells in the root tissues and galling of the roots occur impairing normal root functioning (Hassan et al., 2010; Escudereo *et al*., 2012).

Leaf miner *(Tuta absoluta)* and thrips (*Frankliniella and Thrips* spp) have been reported to cause tomato losses of up to 100 and 20%, respectively (Ssemwogerere *et al*., 2013; Taha *et al*., 2013). While most of the pests cause direct damage and loss, others cause damages indirectly such as disease transmission (Mayfield *et al*., 2003). Tomato spotted wilt virus is an insect transmitted virus and can cause up to 100% losses in tomato fields (Infonet-Biovision, 2015). Tomato diseases such as early blight, late blight, bacterial canker and anthracnose affect the quality and quantity of tomato produce which leads to loss of income (Mizubuti *et al*., 2007; Goufo *et al*., 2008; KARLO, 2014). Plant pests and diseases cause quality and quantity losses; as well as monetary losses and farmers are sometimes forced to plant varieties or species of plants that are resistant to diseases. In many cases these varieties are less productive, more costly and or commercially less profitable than other varieties (Engindeniz *et al*., 2013).

2.5 Management of tomato pests and diseases

Management of tomato pests and diseases is more effective through integrated approach due to reasons such as environmental concerns, cost effectiveness and attainment of the ultimate production goals (Mizubuti *et al*., 2007). An amalgam of cultural and mechanical approaches is effective in the management of pests and disease in any field (Massawe, 2010). Use of certified and disease free planting materials is highly advisable and this ensures that the farmer does not introduce a pathogen or pest in a pest-free area (infonet-biovision, 2015).

Field sanitation helps to remove possible hosts for pests and inoculum sources for diseases and it also leads to starvation of the larvae and disease causing microorganisms and eventual death (Infonet-Biovision, 2015). Pathogens survive on the plant debris until favourable hosts and establishment conditions are available as in the case of *Phytophthora infestans* and *Alternaria solani* the causative agents of late and early blight of tomato, respectively (Mizubuti *et al*., 2007; Goufo *et al*., 2008; 2010). Weeding is vital since some weeds are alternate hosts of pests and diseases. For instance, nightshades are important alternate hosts for most tomato pests such as spider mites (Azandeme-Hounmalon, 2014) and weeding is significant in management of all plant diseases (Hassan *et al*., 2010). Crop rotation reduces inoculum build up and helps kill the already present sources of inoculum in a particular field.

This is effective against major pests and all pathogenic diseases (Sumitra *et al*., 2012). Adequate nutrition dictates the health of plants since too little leads to deficiencies and can predispose them to pest and disease attack (Akrami and Yousefi, 2015). Using nitrogenous fertilizers is advisable as they reduce disease severity especially fungal diseases such as *Fusarium* wilts (Akrami and Yousefi, 2015). The system of watering should be appropriate to ensure it does not favour disease development. Furrow or drip irrigation can be done to manage foliar diseases of tomato such as early and late blight. Overhead irrigation could be used as it reduces mites, thrips and powdery mildew (ICIPE, 2014). However, furrow irrigation should be avoided in areas where soil borne diseases are prevalent such as bacterial and *Fusarium* wilts and the root knot nematodes (ICIPE and Infonet-Biovision, 2014). Notably, prolonged leaf wetness predisposes the plants to fungal and bacterial attacks and it is therefore advisable not to work within the fields when it is wet (Alexandrov, 2011; Shallam *et al*., 2012).

Plant spacing and populations needs to be observed well since overcrowding leads to creation of micro climates that favour thriving of pathogens (Mizubuti *et al*., 2007). Mulching at the surface of any crop is advised to avoid splashing of soil and spores to the leaves near the ground (Carrera *et al*., 2007) and it also reduces early and late blight diseases as well as bacterial cankers especially for the determinate tomato varieties (Infonet-Biovision, 2014). This helps to avoid soil borne pests and pathogens getting to the foliage, especially fungal diseases (Sherf *et al*., 1986).

2.6 Use of synthetic pesticides in small holder vegetable production systems

The sophisticated nature of some pests and disease pathogens has rendered use of synthetic pesticides as the only viable management option (Mizubuti *et al*., 2007). Synthetic pesticides 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, these pesticides are also non-biodegradable, retain residues in the produce, pollute the environment and are toxic to humans, natural environment, and beneficial organisms and are often expensive (Mizubuti *et al*., 2007; Mishra *et al*., 2015; Srijita, 2015). Farmers are not aware of these negative effects and hence they apply chemicals as long as their fields remain pest free and the produce is aesthetically presentable (KEPHIS, 2016). Farmers do not always follow guidelines on safe use of pesticides such as the appropriate dilutions, pre-harvest intervals and even use their bare hands to mix the chemicals and dispose the containers inappropriately. (Business Daily, 2013; 2014). This application of pesticides has led to many interceptions and rejections of the Kenyan fresh produce in EU markets (The East African, 2015).

Presence of traces of chemicals, use of banned chemicals, higher levels of chemical residues, unhealthy products, presence of contaminants and compromised food safety and quality are some of the reasons for bans against Kenyan produce (Mwangi, 2013). There have been strict controls at the market entry points for fresh produce and increased sample sizes for verification of compliance which add costs since they are paid for by the exporters (Mwangi, 2013; The East African, 2015). Farmers have had to deal with more stringent measures and some have opted out of the markets due to cost reasons (People Daily, 2016).

In addition, importers of fresh vegetables have opted for alternative suppliers from other competing countries and this has led to loss of markets and market reputation (Mwangi, 2013). Alteration of production processes, changes in the processes of pesticide assessment and inadequate pest management procedures are some of the reasons that harmonisation of MRLs by the EU has been done in efforts to enhance traceability (World Bank, 2003). New preferences have been made by the EU consumers who are willing to pay more for products that are organically grown (Michel, 2015; Srijita, 2015). In an effort to meet these requirements, Kenya is making efforts to close the gap between farmers' practices and consumer needs. Alternatives to synthetic pesticides are being sought, laboratories have been accredited to ensure effective pesticide monitoring programs and ensure that the produce leaving the country has met the set international standards (Daily Nation, 2015; KEPHIS, 2016; Business Daily, 2016). Advocacy on minimized chemical usage, use of alternative pest management options, training and creation of awareness are some of the options to enable the country regain market access and redemption of the consumer confidence (Daily Nation, 2015).

2.7 Use of biopesticides in crop production

Biopesticides are derivatives of natural products including plants, microorganisms and animals and they manage pests in a non-toxic manner (Mizubuti *et al*., 2007; Kumar, 2015; Mishra *et al*., 2015). These products are important because unlike the synthetic pesticides they are easily degradable, they are non-toxic to humans and the environment, they are target specific, are easily available and do not have residual effects on produce (Kimani, 2014; Kumar, 2015; Srijita, 2015). In addition, biopesticides offer solutions to pest resistance, environmental and water body pollution, public concerns about food safety and improves agricultural productivity (Mishra *et al*., 2015). Farmers have used crushed leaves of African marigold to control nematodes (PAN, 2005) while other damaging diseases have been controlled by use of biological agents from micro-organisms and plant origin. Late blight of potato and *Fusarium* wilt of different legumes, have been successfully controlled by microbial pesticides (Karimi *et al*., 2012; Islam *et al*., 2012). Chemical companies have come up with different formulations of the biologicals and are available for purchase by farmers (Dudutech, 2012).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 *Beauveria,* 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; Dunham, 2016).

Bacillus thuringiensis has been used in management of diamond back moth, *Beauveria* in control of mango hoppers and mealy bugs, neem in management of whiteflies and *Trichoderma* in management of rots and wilts in various crops (Dunham, 2016). Microbial antagonists are microorganisms that inhibit the growth of other organisms in the same ecosystem and are used in management of pests and disease pathogens (Mizubuti *et al*., 2007; Bautusta-Banos *et al*., 2003). They are found in the composts, rhizospheres of plants, cow sheds and generally in the habited and uninhabited environments. Some microbials have been registered by several companies as biopesticides including *Trichoderma harzianum* traded by Koppert® as Trianum®, Bt products by Monsanto, neem plant extracts by Organix Ltd and Amiran Kenya Ltd among others (Dunham, 2016; Infonet-Biovision, 2015). Genes of some of these microbes are inserted into plants and are used to enhance defence mechanisms of the plants against diseases or could help the plant to produce substances that are harmful to the pests or pathogens (Srijita, 2015).

Botanicals include essential oils and plant extracts. While essential oils are volatile aromatic hydrophobic liquids from plant parts and mainly include terpenoids, plant extracts are dried plant parts obtained by filtration and evaporation and mainly consist of phenols, alkaloids, tannins and saponins and these give them the antifungal characteristics (Mizubuti *et al*., 2007; Vidyasagar and Tabassum, 2013). Different plant families have different bioactive compounds and thus exhibit varied modes of action. Neem from the *Meliaceae* family for instance, affects the reproductive and digestive system of the pests, garlic from the *Liliaceae* family has compounds that affect the neurosystem of insects while turmeric and ginger from the *Zingibereaceae* family has aromatic compounds that affect the morphology of the hyphae and mycelia structure of the fungal pathogen (Jahromi *et al*., 2012; Vidyasagar and Tabassum, 2013).

Plant extracts have been used in management of pests and disease both under controlled and field conditions and have been reported to be as effective as the synthetic counter parts (Goufo *et al*.,2008; Nashwa and Abo-Elyousr, 2012; Al-Samarrai *et al*., 2012; 2013). Reports on efficacy of biopesticides in pest and disease management are an indication that they have potential to replace the synthetics and can be incorporated in the crop management systems (Cao and Forrer, 2001; PAN, 2005; Nashwa, 2011; Chethana *et al*., 2012; Islam *et al*., 2012; Karimi *et al*., 2012; Fountain and Warren, 2013; Gonzalez, 2013; RAJA, 2014; Wafula *et al*., 2014).

Biopesticides have the capacity to balance between environmental safety and enhanced agricultural productivity (Kumar, 2015). From the recent concerns about food safety and food quality, increased demand for residue-free crop produce, increased organic food markets and for easier market registration and access, farmers ought to be trained on the necessity to embrace biocontrol of pests and diseases (Michel, 2015; KEPHIS, 2016). This will help them to overcome the issues of pest resistance, genetic variations in plant populations, reduction of beneficial species, environmental and water pollution and food poisoning which will improve the quality and safety of their produce (Mishra *et al*., 2015; Srijita, 2015). In turn, they will reduce the rate of interception and product rejection in the lucrative markets which will attract even more buyers for their produce (Daily Nation, 2015; The East African, 2015; People Daily, 2016). This boosts the agricultural productivity and economic level of the producing country.

In Kenya, biological agents have helped flower farms reduce the use of conventional pesticides by at least 50% (Casswell, 2015). Different companies have manufactured and or distributed biopesticides from natural sources and traded them to farmers (Table 2.1), and they have registered their products with the Pest Control Products Board (PCPB) in Kenya (Ngaruiya, 2003). Kenya is one of the leading producers of the natural pesticide, pyrethrin, which is a broad spectrum insecticide (Infonet- Biovision, 2015). The product is exported to developed countries: USA (60%), Europe (35%) and 5 % is used in Africa (Birech *et al*., 2006). Kenya has the potential to utilize the botanicals from neem (*Azadirachta indica*), pyrethrum (*Chrysanthemum cinerariaeofolium*) and other plants to manage pests and diseases in horticulture (Infonet-Biovision, 2015).

Table 2.1: Some botanical and microbial pesticides used in management of pests and diseases in Kenya

Product/trade name	Active ingredient	Target pest/ disease	Agent/Distributor
Achook 0.15 EC	Azadirachtin (0.15)%	Insect pests of horticultural crops	Organix Ltd
Flower DS EC	Pyrethrins (4%)	Aphids and whiteflies on vegetables	KAPI Ltd
Neemraj super 3000	Azadirachtin (0.03%)	Aphids, thrips, whiteflies, DBM, bollworms in vegetables	Amiran (K) Ltd
Nimbecidine EC	Azadirachtin (0.03%)	Aphids, thrips, whiteflies, leafminers, beetles and mites	Osho chemicals Ltd
Pyerin	Pyrethrin (75 g/l)	Aphids and whiteflies on flowers and vegetables	Juanco SPS ltd
Baticide WP	*Bacillus thuringiensis* var *Israelensis*	Mosquitoes in breeding sites	Insect (K) Ltd
Bio-Nematon 1.15 WP	*Paecilomyces lilacinus* (Fungus)	Root knot nematodes	Osho Chemical industries Ltd
Botaniguard ES	*Beauveria bassiana* strain GNA (Fungus)	Aphids, thrips, whiteflies in French beans and snow peas	Amiran (K) Ltd
Eco-T WP	*Trichoderma harzianum* strain k.d. (Fungus)	Soil borne diseases (*Fusarium*, *Pythium* and *Rhizoctonia*	Lachlan (K) Ltd

Source: Infonet- Biovison, 2015

Plant parts used for extraction of oils and extracts include seeds, roots, barks, flowers, rhizomes, leaves, peels and cloves among others and at times the whole plant could be used (Mizubuti *et al*., 2007; Yuliana *et al*.,2008; Iram *et al*., 2013). Plant parts with the required compounds are washed to remove dirt and other impurities. They are then dried using appropriate methods such as freezing, under sun or in ovens. They are then ground to obtain a homogenous sample as well as increase surface contact with the solvent system (Sasidharan *et al*., 2011). Extraction, depending on the type of bioactive compound involved, is aided by use of solvents which could be aqueous, non-aqueous and usually volatile and the solvent is then evaporated after filtration (Skaria, 2007). The method and solvent system used for extraction dictates the quality of the plant extracts acquired (Wongkaew and Sinsiri, 2014) and the nature of materials used in the extraction also matters (Agbenin and Marley, 2006).

The solvent system used and the temperatures involved are determined by the nature of the bioactive compound targeted (Platonov *et al*., 2014). Solvents such as methanol and ethanol are used to extract hydrophilic compounds while dichloromethane is used for lipophilic compounds (Sasidharan *et al*., 2011). These are however traditionally improved methods while modern techniques such as solid-phase micro-extraction and micro-wave assisted extraction have advantages such as reduction in organic solvents consumption and minimized sample degradation which compromises the quality of extracts and essential oils (Sasidharan *et al*., 2011). The solutions are filtered and the solvent evaporated under partial vacuum to obtain free oil. Powders are concentrated in certain amounts of the solvents at the time of application (Maragathavalli *et al*., 2012). The solvents used in the extraction process dictate the quality of oils, powders and extracts obtained (Platonov *et al*., 2014).

2.8 Quality requirements relating to chemical residues in fresh vegetables

Consumer demands, tastes and preferences of the fresh produce by the importing countries such as the EU have been changing (Steven, 2003; Michel, 2015). The consumers insist on safe and quality food produce, free from chemical residues, free from contaminants and those that meet a certain international criteria (Srijita, 2015; Michel, 2015). These lucrative markets have further offered to pay more for organically produced foods which is meant to attract farmers into adopting non-chemical production systems (Michel, 2015). Exporter of fresh vegetables must meet certain requirements for their produce to be accepted in the markets. Use of banned chemicals in the production systems such as Dimethoate, presence of pesticides higher than the allowed amounts in the produce and failure to conform to the stated sanitary and phytosanitary requirements will lead to more interceptions at the point of entry (Daily Nation, 2016; People Daily, 2016). The farmers using Dimethoate for instance, should not exceed 0.02ppm and for any other general pesticide, the general MRL is 0.01mg/kg (European Commission, 2016).

Small scale farmers are most hit by these conditions since they have relied on synthetic pesticides for pest and disease management for a long time and the harmful effects of pesticides are denying them access to market (Goufo *et al.*, 2008). Increased sampling also leads to delays in the delivery system which causes more losses due to the perishable nature of the fresh produce (Mwangi, 2013). Loss of shelf life of the produce leads to more economic losses since they could be re-shipped or destroyed at the exporter's cost. This in turn destroys the marketing reputation of the exporting country (The East African, 2015).

The stringent conditions for fresh vegetable produce has seen some farmers opt out of market which has negative effect on the economy of the country and the volume of fresh produce supply in the export markets (The East African, 2015). Small scale farmers have been forced to sell their produce in the local markets and they do not fetch as much income as the target markets which reduces the income and livelihoods of the employees dependent on the production and in the value chain (People Daily, 2016). The high interceptions and loss of markets have called for mitigation aspects to close the gap between market access and safe food produce (KEPHIS, 2016). Standards of practise in the agricultural sector have been raised to ensure food safety, sustainable production and improvement of market access which is implemented under GLOBALGAP (Wario, 2012).

Organisations such as the Fresh Produce Exporters Association of Kenya (FPEAK) have taken up the initiative to monitor small holder farmers and their production activities (Wario, 2012). Compliance to the standards of fresh produce production is being enhanced through training and certification of producers based on the KenyaGAP code (Wario, 2012). Introduction of traceability systems ensures the farmers adhere to safe agricultural practices which lead to production of safe food (World Bank, 2003; Wario, 2012). Use of biopesticides, accreditation of laboratories to monitor pesticide analysis programs, trainings and awareness creation on pesticide usage, provision of funds by the EU to small scale farmers and offers of attractive prices for food produced using natural methods are some of the prospects into sustainable agriculture and reliable market access and maintenance (Daily Nation, 2015; Michel, 2015; Business Daily, 2016; KEPHIS, 2016).

CHAPTER THREE
ANTIFUNGAL PROPERTIES OF PLANT EXTRACTS ON FUNGAL PATHOGENS OF TOMATO

3.1 Abstract

Indiscriminate use of synthetic pesticides has resulted in farmers losing access to niche and prime markets due to presence of residues on fresh vegetable produce. The markets require the produce to be aesthetically presentable, good quality, have no traces of banned pesticides and have the required limits of chemical residues. The objective of this study was to evaluate the effectiveness of locally available plant extracts in management of disease causing fungal pathogens of tomato *in vitro*. Plant samples from turmeric, garlic, ginger, lemon, pepper, mint, *Aloe*, neem, rosemary and marigold were extracted in ethanol and concentrated using a rotary evaporator. The extracts were screened *in vitro* for antifungal activity against tomato pathogens *Alternaria solani*, *Fusarium oxysporum* f.sp. *lycopersici*, *Pythium ultimum* and *Rhizoctona solani*. The crude extracts were incorporated into media and agar discs of the test pathogens inoculated at the centre of the plate. Antifungal activity was measured as reduction of the fungal colony radial growth after incubation. Turmeric extract was the most active and reduced colony radial growth by up to 40% while mint was the least active. *Alternaria solani* was the most susceptible with a reduction of colony radial growth by up to 70% while *Fusarium oxysporum* f.sp. *lycopersici* was the least susceptible. The study showed that plant extracts have the potential to inhibit growth of plant pathogens. The varied activity of plant extracts is dependent on the nature and parts of the plants used for extraction, the solvent system and susceptibility of the test pathogens. Therefore, further explorations into our natural environment would identify more plants with potential to manage pathogens and could replace the synthetic pesticides and further reduce residues in the tomato fruits thereby allowing tomato farmers to redeem and maintain access to prime markets.

Key words: Antimicrobial activity, Chemical residues, Synthetic pesticides, Tomato

3.2 Introduction

Plants have compounds which make them useful as sources of remedy to pests and diseases (Vidyasagar and Tabassum, 2013). These compounds inhibit the establishment, growth and development of disease causing pathogens including bacteria (Suthar *et al*., 2016) and fungi (Sesan *et al*., 2015) of different plants ranging from field crops (Jantasorn *et al*., 2016; Kekuda *et al*., 2016) as well as vegetables (Nefzi *et al*., 2016). Different plant families have varied compounds among the constituent plants which translate to different modes of action and target specificity (Vidyasagar and Tabassum, 2013). Presence of the antimicrobial compounds in these plants makes them useful as sources of pesticides which, if tapped, could be used for pests and disease management (Chougule and Andoji, 2016). Embracing use of products from natural sources to manage pests and diseases is environmental friendly and should be encouraged (Sesan *et al*., 2016) due to their biodegradability.

Farmers perceive use of synthetic pesticides as the most convenient way of controlling pests and diseases (Birech *et al*., 2006). Synthetic pesticides are non-biodegradable, expensive, cause environmental pollution, leave high levels of residues in the produce (Bhattacharjee and Dey, 2014). Chemicals are harmful to applicants (Engindeniz *et al*., 2013) and lead to resistance among pests and pathogens (Srijita, 2015) among other hazards (Stangarlin *et al*., 2011; Dudutech, 2012; Wagnitz, 2014). Markets have also raised alarms due to the pesticide levels in the products sold to them (East African Standard, 2011; Business Daily, 2013, 2014). There have been interceptions in the EU markets for produce found with traces of banned chemicals and higher levels of certain pesticides (The East African, 2015).

In regard to the harmful effects associated with use of synthetic pesticides, efforts to explore our natural environment for safer alternatives are being made (Gupta *et al*., 2014). Success has been reported from use of natural products and is hence gaining popularity due to their safety in use, easy availability and effectiveness in managing the pests and disease compared to synthetic products (Rodino *et al*., 2014). Their success is being attributed to their degradability, availability, nontoxicity and some of them are edible thus safe for human use, as well as the environment (PAN, 2005; Charlie, 2014; Wagnitz, 2014; Mohammed, 2014). Their demand is constrained to their mode of action and bio-degradability (Al-Samarrai *et al*., 2012) and the fact that they are non-phytotoxic which makes them a better alternative for pest and disease management (Nashwa, 2011). The interest in biopesticides is also based on the disadvantages associated with chemical pesticides especially their non-biodegradability and residue retention in the produce. In addition some of the synthetic pesticides are not readily available and are also expensive which increases the costs of production (Gupta and Dikshit, 2010).

Natural products have been successfully used in research studies and there is evidence that they can ultimately replace the synthetic pesticides (Bautista-Bosan *et al*., 2003; Mizubuti *et al*., 2007; Al-Samarai *et al*., 2012; Naing *et al*., 2013; Raja, 2014). They have been tested and found to reduce populations of insect pests (Sumitra *et al*., 2012; Singh *et al*., 2013; Nwachukwu and Asawalam, 2014) and pathogens alike (Mizubuti *et al*., 2007; Goufo *et al*., 2010; Yanar *et al*., 2011; Nashwa and Abo-Elyousr, 2012; Rodino *et al*., 2014). Most biopesticides that are used in Kenya are imported but some of their major constituents are grown in Kenya such as pyrethrum. Other natural components are available in Kenya's natural environment including microbial species like *Trichoderma, Paecilomyces* and *Bacillus* among others (Ngaruiya, 2003; Kimani, 2014; Infonet-Biovision, 2015).

The advantage of tapping our own environment for natural products is due to their availability which makes their adaptation inexpensive and also eliminates the risk of importing harmful organisms together with the foreign products (Kimani, 2014). In addition there will be surety of reliable products since markets are also contending with counterfeits which are less effective in pests and disease management (Ngaruiya, 2003). Natural products do not leave residues in the products and are hence advocated for incorporation in our cropping systems (Kimani, 2014).

The objective of this study was to evaluate the effectiveness of plant extracts on fungal pathogens of tomato *in vitro*.

3.3 Materials and Methods

3.3.1 Collection of plant samples

Plant samples were collected from the Field Station, Faculty of Agriculture, University of Nairobi and others purchased from a local market in Nairobi. The guiding principle in selection of the plant was the antimicrobial history from published reports by different researchers worldwide (Goufo *et al*., 2010; Al-Samarrai *et al*., 2012). The plants collected included turmeric (*Curcuma longa*), garlic (*Allium sativum*), ginger (*Zingiber officinale*), rosemary (*Rosmarinus officinalis*), pepper (chilli pepper), lemon (*Citrus limon*), mint (*Mentha piperita*), neem (*Azadirachta indica*), aloe (*Aloe vera*) and Mexican marigold (*Tagetes minuta*). The parts of the plant collected included leaves, roots, flowers, fruits, rhizomes and cloves (Figure 3.1).

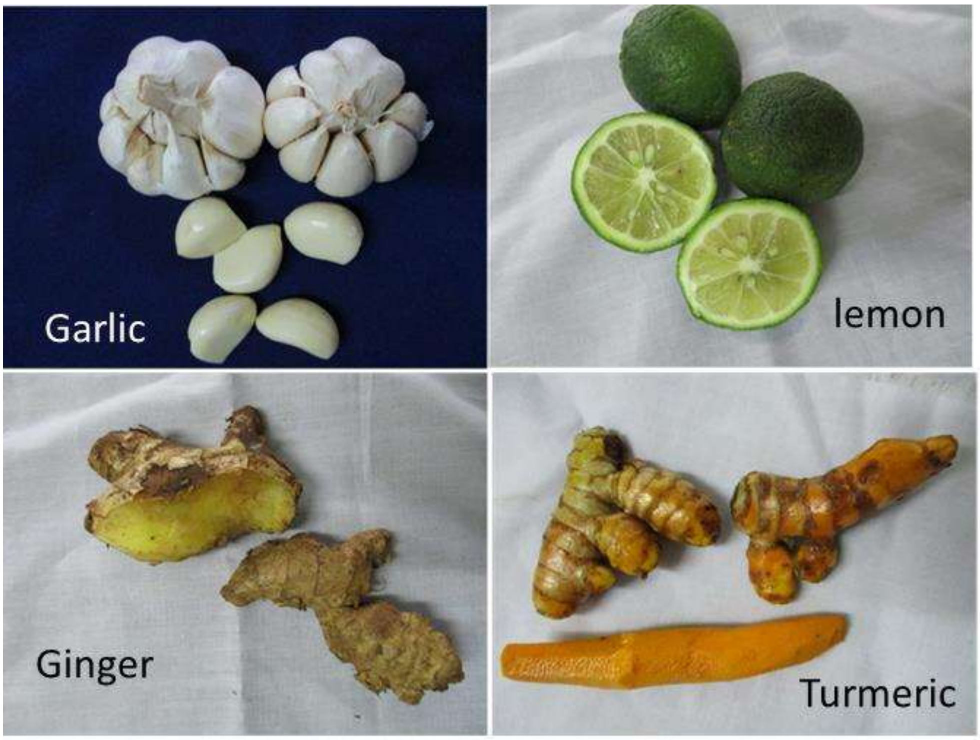

Figure 3.1: Some of the plant parts used as sources of crude plant extracts in the study

3.3.2 Extraction of crude extracts from plant samples

Crude extracts from plant samples were extracted using the modified method by Al-Samarrai *et al* (2012; 2013). The plant samples were washed under running tap water and rinsed in distilled water. Sample materials, they were blended to paste, except for lemon, in sterile distilled water and 100 g of the material weighed and put in a conical flask. Five hundred millilitres of 95% ethanol was added to the materials and constantly stirred for 30 minutes. The mixture was then filtered through two layers of cheese cloth followed by Whatman No. 2 filter paper. The alcohol in the filtrate was evaporated under vacuum at 60^0C and 10 ml of the concentrated solution was retained. The concentrated stock solution was put in a screw capped universal bottle and stored in a refrigerator at 4^0C.

3.3.3 Isolation and maintenance of fungal pathogens of tomato

Plant pathogens were isolated from diseased tomato plants which were washed under running tap water, cut into 3 mm pieces, sterilized in 1.3% sodium hypochlorite and rinsed in three changes of sterile distilled water. These were blot-dried, plated on Potato Dextrose Agar (PDA) amended with streptomycin (4mg/litre of media) and incubated for growth at room temperature (23±2^{0}C). The isolated fungi, *Alternaria solani*, *Pythium ultimum*, *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *lycopersici,* were purified by sub-culturing them onto molten PDA media and incubated at room temperature.

3.3.4 Evaluation of antimicrobial activity of crude plant extracts

Screening of the crude plant extracts for antimicrobial properties was done following modified procedures described by Al-Samarrai *et al.* (2012; 2013). Potato dextrose agar medium was prepared and cooled to 45^{0}C. Plant extracts were then incorporated at a ratio of 1ml extract: 50ml medium and the mixture was dispensed into Petri dishes. After the media had set, 5mm agar discs cut from 14 day old fungal pathogen cultures were placed at the centre of the plate and incubated at room temperature. Control plates had media not amended with plant extracts. Observations were made at 2, 4, 6 and 8 days after plating and antifungal activity was determined after measuring the fungal colony radial growth using the following formula:

$$\%\text{inhibition} = \frac{(\text{Colony diameter without extract} - \text{Colony diamter with extract})}{\text{Colony diameter without extract}} * 100$$

3.3.5 Data analysis

Data collected was subjected to analysis of variance using Genstat® 15th Edition and means separated using Fischer's Protected LSD. The overall mean was derived by a split plot design analysis.

3.4 Results

The evaluated crude plant extracts inhibited the radial growth of the test pathogens (Figure 3.2). There were varied levels of inhibition with turmeric (*Curcuma longa*) having the highest radial growth inhibiting capacity against all the tested pathogens and it significantly ($p \leq 0.05$) reduced the growth of *Alternaria solani* by up to 70%. Turmeric was followed in activity by lemon (*Citrus limon*) and garlic (*Allium sativum*) while mint (*Mentha piperita*) had the least overall inhibitory effect (Table 3.1). Radial growth of the tested pathogens was reduced by all the crude plant extracts and *Alternaria solani* was the most susceptible while *Fusarium oxysporum* f.sp. *lycopersici* was the least susceptible (Table 3.1).

The trends were the same for the repeat experiments (Table 3.3). The test pathogens exhibited different levels of susceptibility to the extracts on different days after incubation. The effectiveness of plant extracts in inhibiting colony growth reduced after incubation among all the extracts (Table 3.3, Table 3.4, Table 3.5, Table 3.6). Turmeric maintained a high level of inhibition and especially against *Alternaria solani* throughout the experimental period (Table 3.3). When tested against *Fusarium oxysporum* f.sp. *lycopersic*i, the effectiveness of the extracts reduced over time within the monitoring days (Table 3.5). *Rhizoctonia solani* exhibited low susceptibility to the crude extracts from day two of incubation and towards the end of the monitoring time the growth rate of the treated plates was equal to that of the controls (Table 3.6).

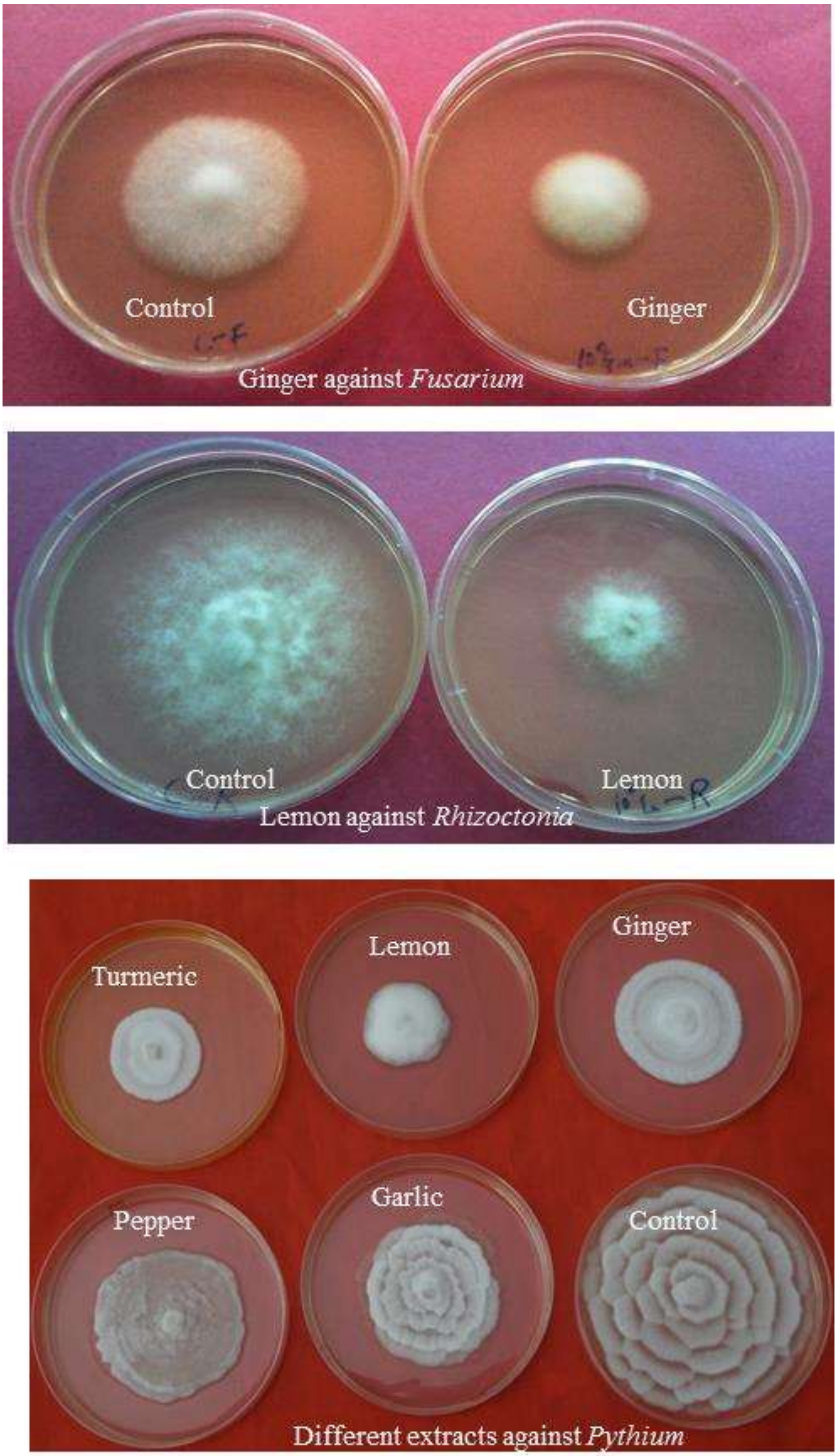

Figure 3. 2: *In vitro* activity of different extracts against test fungal pathogens at eight days after inoculation

Table 3. 1: Percentage inhibition of colony diameter of different tomato plant pathogens cultured on media amended with crude plant extracts at eight days-experiment 1

Source of extracts	*Pythium*	*Alternaria*	*Rhizoctonia*	*Fusarium*	Mean
Turmeric	55.6a	72.9a	33.5a	36.7a	49.4a
Garlic	20.5de	43.5b	24.7b	8.7d	22.6c
Lemon	49.3a	35.0c	14.4c	4.9f	31.6b
Pepper	29.3cd	15.9de	35.6a	27.5b	19.2cd
Ginger	31.5bc	37.9bc	0.0c	4.8ef	17.8d
Rosemary	44.7ab	22.4d	0.0c	4.7f	15.9d
Neem	14.0e	8.0f	0.0c	7.0de	4.1ef
Aloe	17.3de	9.3f	0.0c	2.3g	6.5ef
Mint	20.9de	9.8ef	0.0c	8.5d	1.6fg
Marigold	45.5ab	11.6ef	0.0c	11.0c	7.4ef
Control	0.0f	0.0g	0.0c	0.0h	0.0g
LSD ($p \leq 0.05$)	11.6	6.8	5.5	2.1	3.8
CV (%)	26.7	19.3	38.9	13.8	33.7

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

Table 3. 2: Percentage inhibition of colony diameter of different tomato plant pathogens cultured on media amended with crude plant extracts at eight days-experiment 2

Source of extracts	*Pythium*	*Alternaria*	*Rhizoctonia*	*Fusarium*	Mean
Turmeric	55.3a	72.6a	33.2a	8.2d	44.1a
Garlic	20.2de	43.2b	24.4b	8.4d	24.9b
Lemon	49.0a	34.7c	14.2c	2.0g	21.1c
Pepper	29.0d	15.6de	35.3a	6.7de	14.3d
Ginger	34.8cd	37.6bc	0.0d	36.4a	24.2bc
Rosemary	44.4bc	22.1de	0.0d	10.7c	14.1d
Neem	13.7ab	7.8f	0.0d	4.4f	4.4g
Aloe	20.0e	9.0ef	0.0d	27.2b	9.6e
Mint	20.6de	9.5ef	0.0d	4.5ef	5.5fg
Marigold	45.2de	11.3ef	0.0d	4.6ef	8.8ef
Control	0.0f	0.0g	0.0d	0.0g	0.0h
LSD ($p \leq 0.05$)	11.6	6.7	5.5	2.1	3.4
CV (%)	26.9	19.5	38.9	14.2	30.4

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

Table 3. 3: Percentage inhibition of colony diameter of *Alternaria solani* cultured on media amended with crude plant extracts

Source of extracts	Days after incubation			
	2	4	6	8
Turmeric	100.0a	80.4a	78.6a	73.6a
Garlic	100.0a	70.7a	61.9b	45.0b
Lemon	75.8b	43.7b	37.5d	36.8c
Pepper	59.0d	32.1bc	26.8e	18.2e
Ginger	62.5c	37.8bc	49.4c	39.6c
Rosemary	53.7e	32.1bc	27.4e	24.6d
Neem	36.4g	20.5c	15.7f	10.3g
Aloe	39.2f	20.6c	17.3f	11.8g
Mint	57.0d	25.0c	15.6f	14.8ef
Marigold	51.3e	44.6b	18.6f	14.1f
Control	0.0h	0.0d	0.0g	0.0h
LSD (P ≤ 0.05)	2.9	19.1	7.7	3.6
C V (%)	3.4	35.7	16.7	9.5

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

Table 3. 4: Percentage inhibition of colony diameter of *Pythium ultimum* cultured on media amended with crude plant extracts

Source of extracts	Days after incubation			
	2	4	6	8
Turmeric	71.9a	70.5a	69.2a	67.4a
Garlic	45.5e	18.6f	24.7c	28.7c
Lemon	66.7b	45.3b	36.5b	33.0b
Pepper	12.5h	2.5i	1.6g	5.9g
Ginger	59.7c	23.9e	25.6c	27.6c
Rosemary	49.2d	31.9c	35.8b	37.4c
Neem	12.5h	4.5h	3.1f	8.5f
Aloe	31.2f	12.2g	9.2e	5.9f
Mint	17.7g	2.3i	17.5d	11.4e
Marigold	46.6e	27.2d	24.4c	23.0d
Control	0.0i	0.0j	0.0g	0.0h
LSD P ≤ 0.05)	2.6	1.7	1.7	2.5
C V (%)	4.8	5.4	5.3	8

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

Table 3. 5: Percentage inhibition of colony diameter of *Fusarium oxysporum* f.sp. *lycopersici* cultured on media amended with crude plant extracts

Source of extracts	Days after incubation			
	2	4	6	8
Turmeric	48.5a	46.7a	48.6a	39.1a
Garlic	36.8b	16.7c	9.0e	6.6d
Lemon	32.4b	39.2b	40.3b	31.5b
Pepper	17.8c	12.5d	2.7dg	3.1def
Ginger	30.9b	3.3fg	5.6fg	5.4def
Rosemary	14.7d	8.3e	3.1fg	3.3def
Neem	8.7e	10.8e	2.8fg	2.7ef
Aloe	5.8f	14.6cd	6.3ef	4.9def
Mint	5.7f	4.2fg	16.0d	19.9c
Marigold	5.7f	12.4d	28.9c	23.2c
Control	0.0f	0.0g	0.0g	0.0f
LSD P ≤ 0.05)	6	3.7	4.6	3.3
C V (%)	22.2	16.5	21.3	18.1

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

Table 3. 6: Percentage inhibition of colony diameter of *Rhizoctonia solani* cultured on media amended with crude plant extracts

Source of extracts	Days after incubation			
	2	4	6	8
Turmeric	82.3a	62.7a	43.8a	36.0a
Garlic	72.3b	55.6b	37.1b	24.7b
Lemon	56.4c	45.9c	32.1c	20.0c
Pepper	57.3c	55.9b	45.6a	35.6a
Ginger	35.9e	18.6e	5.3e	0.0d
Rosemary	43.0d	47.4c	14.1d	0.0d
Neem	42.7d	22.1e	13.5d	0.0d
Aloe	55.9c	28.5d	13.5d	0.0d
Mint	10.9f	0.0f	0.0f	0.0d
Marigold	9.8f	0.0f	0.0f	0.0d
Control	0.0g	0.0f	0.0f	0.0d
LSD P ≤ 0.05)	4.1	4.2	4.1	2.1
C V (%)	6.8	9.6	15.2	13.5

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

3.5 Discussion

The evaluated crude plant extracts inhibited the mycelial growth of the tested fungal pathogens of tomato. Turmeric (*Curcuma longa*) was the most effective among the extracts and it inhibited all the pathogens. It was particularly superior in inhibiting the colony growth of *Alternaria solani* and *Pythium ultimum*. Lemon (*Citrus limon*) and Garlic (*Allium sativum*) were also effective. These findings were in agreement with those reported by Wongkaew and Sinsiri (2014) who found out that ethanolic extracts of turmeric were effective against *Alternaria alternata*, *Pythium* sp and *Fusarium oxysporum* f.sp. *lycopersici.* The results of the study were also in concurrence with the report by Chethana *et al*., (2012) who worked with garlic, neem and turmeric extracts and found that they inhibited growth of *Alternaria porri*, the causal agent of purple blotch in onions. However, our results differ with those reported by Chethana *et al*. (2012) who reported that garlic extracts were superior to those from turmeric against *Alternaria porri*. These differences could be due to the nature of the plant materials used. Fresh materials increase the extraction efficiency since they swell the plant tissues which enhance solvent- material contact (Bandor *et al*., 2013).

Neem (*Azadirachta indica*) was among the least active extracts and this differs with reports by Agbenin and Marley (2006) who worked with neem and garlic and found them effective in reducing the mycelial growth of *Fusarium oxysporum* f.sp *lycopersici*. Among the tested pathogens, *Rhizoctonia solani* and *Fusarium oxysporum* f.sp *lycopersici* were the least susceptible to the extracts which differs with the findings by Rodino *et al*., (2014) who worked with marigold extracts and reported their superior effect in reducing the mycelial growth of *Rhizoctonia solani*. Findings in the current study further differed with those of Javaid and Rehman (2011) who reported ethyl extracts from neem to be effective in reducing fungal growth *in vitro*. These differences could be as a result of the different solvents and concentrations used since various neem extract concentrations gave different efficacy results. The time taken during the extraction could also be a factor to consider since some methods of extraction need more time to yield better extracts than others (Bandor *et al*., 2013)

The varied activity exhibited by the plant extracts against the pathogens throughout the monitoring period could be due to the susceptibility, tolerance or resistant levels of the pathogens, degradation of the extracts with time or growth rates of the pathogens. Similar observations were made by Wongkaew and Sinsiri (2014) who underscored that the origin of the plant has effect on the effectiveness of the resultant extracts. The physiology of some pathogens is sophisticated which makes them hard to manage (Mizubuti *et al*., 2007) while others are fast growers which makes them flourish before the effect of the extracts is established (Rodino *et al*., 2014).

The antimicrobial activity of turmeric could be attributed to presence of active constituents in the rhizome including flavonoids, alkaloids, saponins (Reddy *et al*., 2012). The major compounds are aromatic oils, turmerones and curcuminoids which possess the antimicrobial effect (Wongkaew and Sinsiri, 2014). The same authors have reported that when ethanol is used as the extraction solvent, a higher level of effectiveness is observed from the plant extracts. This is because ethanol being a polar solvent, it produces high yield of phenolic concentration and gives even better quality extracts when diluted with water (Bandor *et al*., 2013). The method of extraction and the solvent system used dictate the quality of extracts yielded (Odhiambo *et al*., 2009; Javaid and Rehman, 2011; Mahlo *et al*., 2013; Bandor *et al*., 2013). Dabur *et al*. (2007) however reported water extracts to be more effective than organic extracts while Bandor *et al*. (2013) reported that use of water in extraction increases the amount of impurities which could impair the quality of extracts. The evaluated plants belong to different families and each plant family contain different compounds that have been reported to have antimicrobial effects on several pathogens. Plants from *Zingiberaceae* family such as ginger and turmeric have curcuminoids and turmerones which are responsible for their exhibited antifungal activity and especially inhibition of spore germination (Damalas, 2011). Plants under the *Rutaceae* family such as lemon contain α and β-phellandrene and limonene which have antimicrobial properties while plants from *Asteraceae* family including *Tagetes* spp. have compounds such as piperitone and piperitonone which modify the structure of mycelia of fungal pathogens (Vidyasagar and Tabassum, 2013). The method of extraction, sensitivity of test strains, concentration of the extracts, origin of plants, solvent extraction systems and the type of active compounds present in the plants affect the effectiveness of the plant extracts against the test pathogens (Vidyasagar and Tabassum, 2013; Wongkaew and Sinsiri, 2014).

The activity of crude extracts is variable depending on the nature and origin of the plants used (Nashwa and Abo-Elyousr, 2012), the method of extraction and the solvent system used (Bandor *et al*., 2013; Brussoti *et al*., 2013; Mahlo *et al*., 2013), the physiology and growth rate of the test fungal pathogens (Mizubuti *et al*., 2007). Different concentrations are needed for efficacy from different plant extracts and against different pathogens (Javaid and Rehman, 2011). The study showed that locally available plants have antifungal properties which if tapped using the right methods and the right concentrations identified, they could be formulated and availed to farmers as pesticides. The inhibition levels reported show that they have potential to manage disease pathogens and hence could replace pesticides when being formulated.

CHAPTER FOUR
EFFICACY OF PLANT EXTRACTS AND ANTAGONISTIC FUNGI IN MANAGING TOMATO PESTS AND DISEASES

4.1 Abstract

Synthetic pesticides are expensive, have adverse effects on environment, and leave harmful residues on food products. This has led to a shift to use of biological pesticides since they are effective in production of safe and healthy food that is aesthetically valuable. The objective of this study was to evaluate the effectiveness of plant extracts and fungal antagonists in managing pests and diseases of tomato under field conditions. Crude extracts were from turmeric, garlic, ginger and lemon while the antagonistic fungi were two isolates of *Trichoderma,*commercial formulations Ridomil Gold®, Isacop 80 WP® and Confidor SC 200®, Achook 0.15 EC® and Trianum® were used as checks. Each product was applied weekly, commencing two weeks after transplanting. Their effectiveness was determined as reduction in population of pests and disease intensities, pest and disease damage and improvement in fruit yield and quality compared to untreated controls. Plant extracts reduced the population of whiteflies and *Tuta absoluta* by 63% and 55%, respectively and compared favourably with the standard synthetic pesticides. Plant extracts and antagonistic fungi reduced the intensity of early blight by 34% and 23%, respectively; and late blight levels by 53% and 70%, respectively. The plant extracts reduced pest and disease damage on fruits by up to 40% and 65%, respectively. Plants treated with Achook®, a commercial botanical product, produced the highest yield under grade 1 and 2 while plants treated with garlic extracts had the highest yield under grade 3. The results showed that plant extracts and antagonistic fungi from the local environment can be incorporated in integrated pest and disease management in tomato and can help reduce overuse of synthetic pesticides. More plants should be identified and their active compounds formulated for use as biopesticides

Key words: Synthetic pesticides, Plant extracts, antagonistic fungi, Tomato pests and diseases

4.2 Introduction

Tomato is one the most important vegetables in the world and it is grown for its diverse use both for the fresh market and processing industries (Wachira *et al*., 2014). Fresh tomato is used in salads, sauces, stews and puree among others while the processed tomato is mainly for value addition such as pastes (Mungai *et al*., 2000). In Kenya, tomatoes are grown commercially mainly by small scale farmers as a source of income and livelihood (Mutitu *et al*., 2003). However, tomato is affected by insect pests and pathogens which adversely affect quality, quantity and profitability (Engindeniz *et al*., 2013; Islam *et al*., 2013). Insect pests affect plants directly by feeding and indirectly though transmission of diseases (Sumitra *et al*., 2012). Tomato diseases are caused by bacteria (Sutanu and Chakrabartty, 2014), fungi (Goufo *et al*., 2008*)*, viruses (Joshua *et al*., 2003) and nematodes (Noling, 2013; Jacquet *et al*., 2005*)*. They reduce the quality and quantity of the tomato yield and the losses can go up to 100% (Goufo *et al*., 2008).

Farmers have relied on synthetic pesticides to manage pests and diseases in tomato (Mizubuti *et al*., 2007). For example, late blight (*Phytophthora infestans* Mont.de Bary) is managed by a combination of protective and curative synthetic fungicides yet the losses in the field are devastating (Mizubuti *et al*., 2007; Goufo *et al*., 2010). Integrated crop management is therefore important in the cropping systems both for pest and disease management (Goufo *et al*., 2008). In an effort to meet market demand of tomato, farmers have resorted to continuous use of synthetic pesticides (Nashwa and Abo-Elyousr, 2012). However, there is growing concern on toxicity of the synthetic pesticides due to retention of their residues in the food products (Stangarlin *et al*., 2011). In addition, the synthetic pesticides have negative effect on environment such as pollution due to non-biodegradability, health hazards to the farmers, toxicity to non-target natural enemies and other beneficial organisms (Mizubuti *et al*., 2007; Engindeniz *et al*., 2013; Naing *et al*., 2013; Bhattacharjee and Dey, 2014).

The above concerns have led to consumer markets developing stringent quality requirements with regard to maximum residue levels (MRLs) of pesticides in fresh vegetables (Pal and McSpadden, 2006; Wagnitz, 2014; Wafula *et al*., 2014; Campos, 2014; European Commission, 2014). These requirements have become more stringent especially for amounts of chemical residues in fresh vegetables (People Daily, 2016). Due to non-compliance with the market requirements, fresh vegetable produce was recently denied access to lucrative markets and this has increased losses since the rejected produce has to be redirected to local markets which do not fetch good prices (The East African, 2015). However, some consumers for the produce sold at the local open air markets are not aware of the chemical residue concerns and the associated health risks (Srijita, 2015).

Therefore, introduction of biopesticides in vegetable production systems will help to reduce the risks associated with the use of synthetic chemicals (Goufo *et al*., 2008). Natural products are non-toxic, easily biodegradable, safe to non-targets and natural enemies and do not retain residues in the food products (Kimani, 2014). Edibility of some plants with antimicrobial properties make them even better alternatives to the synthetic pesticides for use in sustainable agriculture (Srijita, 2015). In addition, some of the biopesticides can be incorporated into soils which make them better in the management of soil-borne pathogens such as *Fusarium* (Ngaruiya, 2003). The objective of this study was to evaluate the efficacy of plant extracts and antagonistic fungi in management of tomato pests and diseases under field conditions.

4.3 Materials and Methods

4.3.1 Description of the experimental site

On farm field experiments were conducted in Mwea, Kirinyaga County, with a long history of tomato growing and Kabete Field Station. Mwea has ideal climatic conditions for tomato growth as it receives annual rainfall of 1100-1250 mm in two seasons with long rains in mid-March and short rains in mid-October. The temperatures in the region range between 15.7 and 27.9^{0}C which are ideal for tomato production. Mwea is in the agro ecological zone of Lower midland (LM4) (Jaetzold *et al*., 2006). Agro-ecological zone LM4 falls under an altitude of 1159 meters above sea level. Mwea has well drained soils and reliable source of water for irrigation which makes tomato production a year round practice (Jaetzold *et al*., 2006). Kabete Field Station is located in Nairobi which is in agro-ecological zone (AEZ) III and has a bimodal rainfall distribution. The area is at an altitude of 900-1860 m above sea level and receives about 1000mm of rainfall annually with mean annual maximum temperature being 23^{0}C and minimum going up to 13^{0}C. The soils are humic nitosols with kaolinite clay minerals. The soils are deep with good drainage and usually dark brown to brown ideal for tomato production (Jaetzold *et al*., 2006).

4.3.2 Description of experimental materials

Fungal antagonists from a parallel study and active plant extracts against fungal pathogens of tomato from *in vitro* experiments were selected for field experiments and evaluated for their pest and disease management against synthetic pesticides, a commercial botanical and a commercial antagonist. The plant extracts evaluated were turmeric (*Curcuma longa*), garlic (*Allium sativum*), ginger (*Zingiber officinale*) and lemon (*Citrus limon*) while the antagonistic fungi were two isolates of *Trichoderma* labelled as *Trichoderma* Sp1 and *Trichoderma* Sp2. Commercial synthetic fungicides Isacop 80 WP® (Copper oxychloride- 50% metallic copper) from Twiga Chemicals Ltd, Ridomil Gold® (4% Metalaxyl-M and 64% Mancozeb) from

Syngenta Ltd, and an insecticide; Confidor SC 200® (0.125g/l Imidacloprid) from Bayer Crop Science Ltd while commercial plant extract and antagonist were Achook 0.15 EC® (*Azadirachta indica*) from Organix Ltd and Trianum® (*Trichoderma harzianum*) from Koppert Biological Systems Ltd, respectively, which were used as standard checks.

4.3.3 Experimental design and layout

Field experiments were carried out in Mwea and Kabete over two cropping cycles between October 2015 and April 2016. Tomato seedlings were transplanted onto plots of 3m x 3m at a spacing of 60cm x 90cm. Plant extracts and antagonistic fungi selected based on their *in vitro* activity were evaluated together with commercial formulations and untreated control plots. A total of 10 treatments replicated thrice included four crude plant extracts from turmeric, garlic, ginger and lemon; two antagonistic microorganisms *Trichoderma* sp 1 and *Trichoderma* sp 2, a commercial botanical formulation (Achook 0.15 EC®- *Azadirachta indica*), a commercial microbial formulation (Trianum®- *Trichoderma harzianum*); a positive control (combination of a commercial insecticide (Confidor SC 200®), two synthetic fungicides (Isacop 80 WP® and Ridomil Gold®) and a negative control.

The extracts were applied at the rate of 10ml/litre; commercial products were applied according to the manufactures' guidelines and the antagonists had a spore concentration of $1x10^8$ in a litre of water which constituted the stock solution. The plants in the negative control plots were sprayed with water only. The experiment was laid out in a randomized complete block design. The treatment application was initiated two weeks after transplanting the tomato seedlings and thereafter the subsequent applications were done at seven day intervals. All the necessary agronomic practices such as fertiliser application, watering and weeding were carried out as per the requirement. Data was collected on population of pest, pest damage, disease distribution, incidence and severity, fruit yield and quality.

4.3.4 Assessment of disease intensity

Early blight (Figure 4.1) and late blight were the most prevalent diseases and they were assessed on a weekly basis commencing three weeks after transplanting until the end of harvesting. Distribution of each disease was assessed on a scale of 0-2, where 0 = no disease in the whole plot, 1 = disease present in spots within a plot, and 2 = disease distributed over the whole plot. Disease incidence was assessed as the number of plants showing infection out of the total number of plants per plot and converted to percent, where 0 = no disease and 100% = all plants showing infection. The percent values of disease incidence were then converted into proportion, where 0 = No disease and 1 = all plants infected. Disease severity was assessed on ten plants randomly selected from the central rows within each plot on a 0-5 scale modified from 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.

Figure 4. 1: Early blight symptoms on tomato foliage

The scores of disease distribution, incidence and severity were used to calculate percent disease index as follows:

$$\text{Disease index} = \frac{\text{Distribution score} + \text{Incidence score} + \text{Severity score}}{\text{Maximum disease score (8)}} * 100$$

Disease distribution had a maximum score of 2, incidence had a maximum score of 1 while disease severity had a maximum score 5, thus giving total cumulative maximum disease score of 8.

Area under disease progress curve was calculated using the following formula:

$$AUDPC = \Sigma\, [(X_{i+1} + X_i) / 2][t_{i+1} - t_i]$$

Where $\sum$ - Sum total of the disease, X_i- Disease measure on first assessment, X_{i+1} – disease measure on the subsequent assessment, t_i – time in days on the first assessment, t_{i+1}- time in days of the subsequent assessment

4.3.5 Assessment of pest population and damage

Pests assessed were whiteflies (*Bemisia tabaci*) and the American leaf miner (*Tuta absoluta,* Figure 4.2) and this commenced from the third week after transplanting until the end of harvesting. Pest assessment was done following methods modified from NICRA (2012). For both whiteflies and *Tuta absoluta*, ten plants were sampled from the central rows in each plot. The 10 plants were tagged and two leaflets were selected from each of the leaves on which the number of whitefly nymphs was counted on the underside and this was during the early morning. Damage by *Tuta absoluta* was assessed as the number of mines on all the leaves of the ten plants sampled. Assessment of pest population and damage was done on a weekly basis before the subsequent treatment application was done.

Figure 4. 2: *Tuta absoluta* damage on tomato foliage and fruit

4.3.6 Assessment of fruit yield and quality

Harvesting commenced when fruit maturity indices were observed and the ripe fruits were harvested after showing the pink tinge. Harvesting was done on a weekly basis for six continuous weeks and each plot was harvested separately. The weight of the harvested fruits were recorded and later categorized into different grades according to FAO (2015). Grading was done as follows: Grade 1, No decay, no foreign materials, no injury, fairly firm and not overripe, attractive and well-shaped, at least 50mm, fairly uniform in size and colour; Grade 2, same characteristics as class 1 but 40mm in size; Grade 3, same characteristics as class 1, but 30mm in size. The grades 1-3 were the marketable portion while those with pest, disease and any other form of damage were the unmarketable portion. The number and weight of fruits showing pest and disease damage was recorded.

4.3.7 Data collection and analysis

Data on populations of whitefly nymphs and *Tuta absoluta* damage on leaves was collected on weekly basis as well as distribution, incidence and severity of early and late blight diseases. Yield categories were also collected and all the data was subjected to analysis of variance using Genstat® 15th Edition. Means were separated using Fischer's Protected LSD.

4.4 Results

4.4.1 Effectiveness of crude plant extracts and antagonistic fungi in reducing populations of tomato pests

Plant extracts and antagonistic fungi significantly ($p \leq 0.05$) reduced the populations of whiteflies and *Tuta absoluta* damage compared to the negative control (Figure 4.3). The plant extracts were more effective in reduction of the populations of insects compared to the antagonists with extracts having reductions of up to 63% of whiteflies and 55% of *Tuta absoluta* while the antagonists reduced the populations by up to 28% and 23% in whiteflies and *Tuta absoluta* respectively (Figure 4.3). Achook 0.15 EC®, a commercial botanical, significantly ($p \leq 0.05$) reduced the populations of whiteflies and *Tuta absoluta* compared to all the other treatments and to the negative control. There was varied activity among the treatments throughout the monitoring period with changes in populations of the whitefly nymphs (Table 4.1; Table 4.2) in the two cropping cycles. The trend effect of the treatments on the populations of whiteflies and *Tuta absoluta* was similar for both seasons (Figure 4.3).

There were variations in the effectiveness of the treatments in reducing the populations of pests and disease levels over the monitoring period. The differences among the treatments were not significant ($p \geq 0.05$) especially in management of whiteflies (Table 4.1; Table 4.2) in both seasons. However, from 7 weeks after transplanting, the differences among treatments were significant in managing the populations of *Tuta absoluta* in season one and this trend was observed until the end of the season (Table 4.3). These significant differences were also observed in the beginning of season two until week 7 (Table 4.4).

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Table 4. 1*:* Mean number of whitefly nymphs on tomato crop sprayed with crude plant extracts and fungal antagonists during cropping cycle one in Mwea, Kirinyaga County

Treatments	Weeks after transplanting									Total
	3	4	5	6	7	8	9	10	11	
Turmeric extract	0.0d	0.3d	3.0cde	3.3bc	2.3cd	2.7cd	2.7d	1.7c	1.3c	21.0cd
Garlic extract	0.0d	1.3cd	3.3bcd	2.3cd	0.7e	1.7d	2.7d	2.3bc	2.3abc	20.7cd
Ginger extract	0.0d	1.7cd	2.3de	3.3bc	2.7cd	3.3c	2.3d	3.0bc	2.3abc	25.0c
Lemon extract	1.0ab	1.7cd	1.7e	1.3d	2.3cd	2.3cd	2.7d	1.3c	1.7bc	21.0cd
Trichoderma Sp 1	0.3cd	5.7a	4.3bc	2.7cd	5.3a	3.3c	3.3cd	4.0b	2.7abc	37.3b
Trichoderma Sp 2	1.3a	4.0ab	4.7ab	2.7cd	3.7bc	5.3ab	5.3bc	2.3bc	3.0ab	36.3b
Synthetics*	1.0ab	4.3ab	4.3bc	4.3b	4.7ab	6.3a	6.3ab	7.0a	2.3abc	45.3a
Achook 0.15%EC®	0.7bc	1.3cd	1.7e	1.3d	1.7de	2.3cd	1.7d	1.7c	1.3c	17.7d
Trianum T-22®	1.0ab	2.7bc	4.0bc	3.3bc	3.3bc	4.7b	3.7cd	3.3bc	3.3a	37.7b
Control	1.3aa	4.3ab	6.0a	6.3a	2.7cd	2.7cd	8.3a	1.7c	3.3a	48.7a
LSD ($p \leq 0.05$)	0.6	1.7	1.4	1.5	1.3	1.1	2.1	1.9	1.3	5.1
CV (%)	54.8	36.5	23.7	28.3	25.9	18	30.9	38.8	33.2	9.5

Means followed by the same letter(s) within each column do not differ significantly at $p \leq 0.05$. Synthetics* was a combination of Ridomil Gold®, Isacop 80 WP® and Confidor SC 200®. Total disease = 8 (Distribution = 0-2; Incidence = 0-1; Severity = 0-5)

Table 4. 2*:* Mean number of whitefly nymphs on tomato crop sprayed with crude plant extracts and fungal antagonists during cropping cycle two in Mwea, Kirinyaga County

Treatments	Weeks after transplanting									Totals
	3	4	5	6	7	8	9	10	11	
Turmeric extract	3.3bc	1.7bc	2.3b	2.3b	2.3abc	2.3bc	1.7bc	0.3c	0.7c	21.7d
Garlic extract	0.7e	1.7bc	1.3bc	1.3c	1.3c	1.3c	1.7bc	2.3b	1.7bc	16.7de
Ginger extract	2.3cde	1.7bc	2.3	1.7c	2.3abc	2.3bc	1.7bc	1.3bc	1.3bc	22.3d
Lemon extract	2.7bcd	1.7bc	0.7bc	1.3c	1.3c	1.3c	0.7c	1.7bc	2.3b	18.0de
Trichoderma Sp 1	4.3b	2.3abc	1.7bc	2.3b	2.3abc	2.7bc	2.3ab	1.7bc	3.7a	30.3bc
Trichoderma Sp 2	2.3cde	3.0ab	2.3b	2.3b	3.7a	2.3bc	2.3ab	1.7bc	2.3b	29.7c
Synthetics*	2.7bcd	3.3ab	4.7a	2.3b	4.0a	3.3bc	3.3ab	3.7a	2.3b	36.7b
Achook 0.15%EC®	1.0de	0.7c	1.3bc	1.3c	1.7bc	1.7c	1.3bc	1.3bc	0.3c	13.7de
Trianum T-22®	4.3b	2.3abc	4.7a	1.7c	3.3ab	2.7bc	2.3ab	2.3b	0.7c	31.3bc
Control	7.7a	4.3a	5.3a	3.7a	2.7abc	5.3a	2.3ab	2.3b	2.3b	46.7a
LSD ($P \leq 0.05$)	1.8	2	1.2	0.5	1.6	1.3	1.4	1.3	1.3	6.5
CV (%)	32.9	50.7	25.6	15.6	37.5	28.8	42.5	40.7	43	14.1

Means followed by the same letter(s) within each column do not differ significantly at ($p \leq 0.05$). Synthetics* was a combination of Ridomil Gold®, Isacop 80 WP® and Confidor SC 200®. Total disease = 8 (Distribution = 0-2; Incidence = 0-1; Severity = 0-5)

Table 4. 3*:* Mean number of *Tuta absoluta* mines on tomato crop sprayed with crude plant extracts and fungal antagonists during cropping cycle one in Mwea, Kirinyaga County

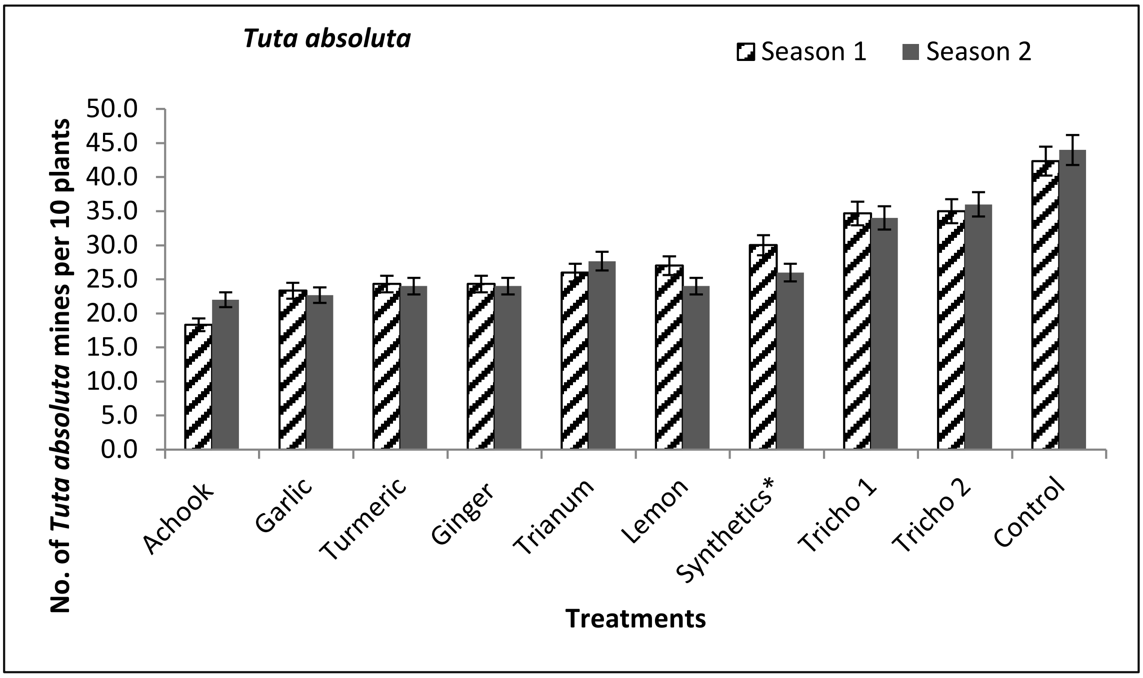
Treatments	Weeks after transplanting									Totals
	2	3	4	5	6	7	8	9	10	
Turmeric extract	1.7bc	2.3ab	1.7b	1.3d	2.3d	3.0c	2.3c	1.7b	2.7ab	24.3d
Garlic extract	1.3c	2.7ab	2.3ab	2.3c	1.3e	2.3d	2.3c	1.3b	3.3a	23.3d
Ginger extract	1.3c	2.7ab	2.3ab	1.3d	3.3c	3.3c	2.3c	1.3b	2.3b	24.3d
Lemon extract	1.7bc	2.7ab	2.3ab	3.3b	1.3e	2.3d	3.3b	2.3ab	1.3c	27.0cd
Trichoderma Sp 1	2.3ab	3.3a	2.3ab	2.3c	2.3d	4.7b	2.3c	3.3a	2.3b	34.7b
Trichoderma Sp 2	2.7a	2.7ab	2.0ab	2.3c	3.7c	5.3b	5.3a	2.7ab	2.3b	35.0b
Synthetics*	1.7bc	3.0ab	3.3a	3.3b	2.3d	2.3d	3.3b	1.3d	2.3b	30.0c
Achook 0.15%EC®	1.3c	1.7b	1.3b	1.3d	2.3d	2.3d	1.3d	1.3d	1.3c	18.3e
Trianum T-22®	1.3c	2.3ab	2.7ab	2.3c	4.3b	2.3d	2.3c	3.0a	1.3c	26.0d
Control	2.3ab	3.7a	3.3a	4.3a	5.3a	7.0a	5.3a	3.0a	2.0bc	42.3a
LSD ($p \leq 0.05$)	0.8	1.4	1.2	0.9	0.6	0.6	0.9	0.9	0.9	3.8
CV (%)	28	30.8	29.8	22.5	12.2	9.4	16.7	23.4	23.5	7.7

Means followed by the same letter(s) within each column do not differ significantly at ($p \leq 0.05$). Synthetics* was a combination of Ridomil Gold®, Isacop 80 WP® and Confidor SC 200®. Total disease = 8 (Distribution = 0-2; Incidence = 0-1; Severity = 0-5)

Table 4. 4: Mean number of *Tuta absoluta* mines on tomato crop sprayed with crude plant extracts and fungal antagonists during cropping cycle two in Mwea, Kirinyaga County

Treatments	Weeks after transplanting									Totals
	3	4	5	6	7	8	9	10	11	
Turmeric extract	1.7d	3.3bc	1.3c	1.7b	3.7bc	2.7b	1.7bc	1.7cdef	2.3bcd	24.0cd
Garlic extract	4.3b	2.3cd	1.7c	1.7b	2.3d	1.7e	1.3bc	3.7ab	2.7bc	22.7cd
Ginger extract	3.3bc	3.3bc	2.3bc	1.3b	4.7ab	1.7e	0.3c	0.7df	1.3cd	24.0cd
Lemon extract	1.7d	5.7a	1.7bc	2.3b	2.3cd	2.7b	1.7bc	1.3cdef	2.3bcd	24.0cd
Trichoderma Sp 1	4.7d	2.3cd	3.3ab	2.3b	2.3cd	3.3ab	2.7b	2.3bcde	3.0b	34.0b
Trichoderma Sp 2	6.3a	4.7ab	2.3bc	1.3b	4.3ab	4.3a	2.7b	3.7ab	2.3bcd	36.0b
Synthetics*	2.3cd	1.7d	1.3c	3.7a	1.7d	4.7a	2.3b	2.7bc	1.3cd	26.0cd
Achook 0.15%EC®	2.7cd	1.3d	1.7bc	1.3b	1.3d	1.7e	4.3a	1.7cdef	1.3d	22.0d
Trianum T-22®	3.3bc	2.3cd	1.3c	2.3b	1.7d	3.3ab	2.7b	4.3a	1.7cd	27.7cd
Control	6.3a	4.0b	4.3ab	4.7a	5.7a	3.7ab	2.3b	2.3bcd	4.7a	44.0a
LSD ($p \leq 0.05$)	1.3	1.5	1.5	1.2	1.3	1.5	1.3	1.5	1.2	5
CV (%)	20.9	28	41.9	29.8	25.4	29.1	34	36	30.1	10.2

Means followed by the same letter(s) within each column do not differ significantly at ($p \leq 0.05$). Synthetics* was a combination of Ridomil Gold®, Isacop 80 WP® and Confidor SC 200®. Total disease = 8 (Distribution = 0-2; Incidence = 0-1; Severity = 0-5)



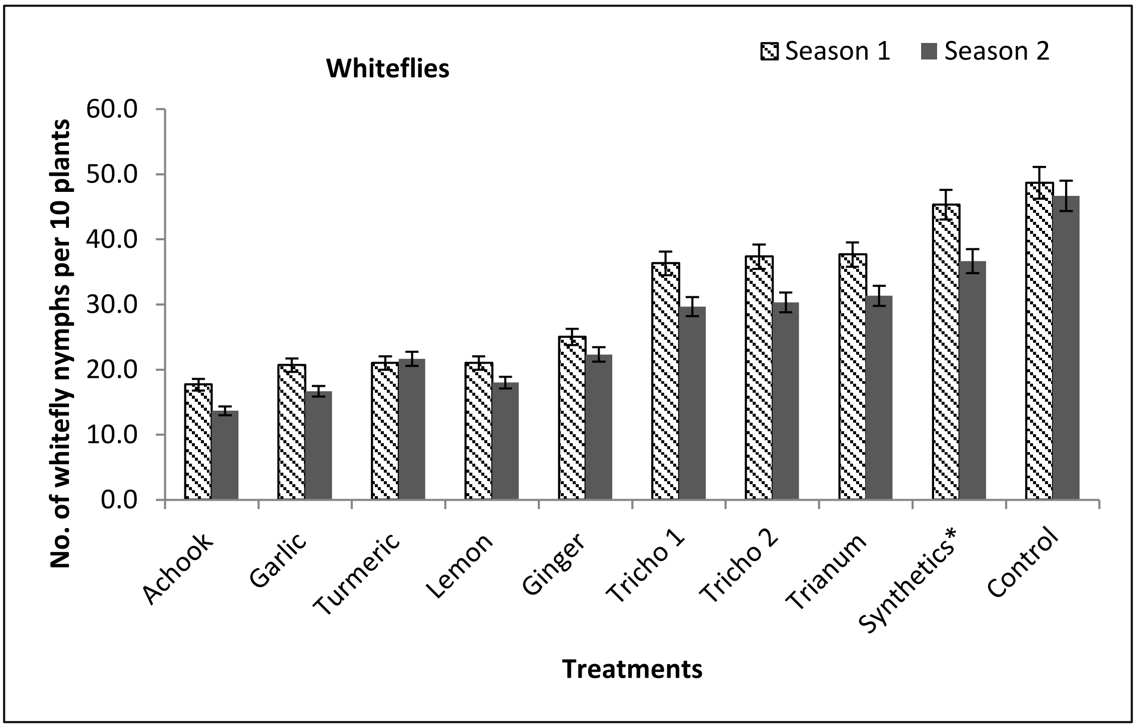


Figure 4. 3: Number of *Tuta absoluta* mines and whitefly nymphs on tomato crop sprayed with crude plant extracts and antagonistic fungi in Mwea, Kirinyaga County. Synthetics* was a combination of Ridomil Gold®, Isacop 80 WP® and Confidor SC 200®. Tricho 1- *Trichoderma* sp 1, Tricho 2- *Trichoderma* sp 2.

4.4.2 Effect of crude plant extracts and antagonistic fungi on early and late blight of tomato

Crude plant extracts and antagonistic fungi reduced intensity of early blight in Mwea. The disease levels increased with time and the effectiveness of the extracts and antagonists also varied with time. There were significant ($P \leq 0.05$) differences during some weeks of crop growth among the treatments while on other weeks, the effects were similar (Table 4.13; Table 4.14). There were no significant ($P \geq 0.05$) differences in season one while season two exhibited significant ($P \leq 0.05$) differences among the treatments in reducing early blight (Figure 4.4). Among the plant extracts, turmeric was significantly ($p \leq 0.05$) effective in reducing early blight in season two while among the antagonists, *Trichoderma* sp 1 was effective in reducing early blight in the field in season two (Figure 4.4) relative to the synthetic pesticides and non-treated controls.

Plant extracts and antagonistic fungi reduced late blight intensity in Mwea (Figure 4.4) compared to the untreated control. One isolate of *Trichoderma* was the most effective followed by extracts of turmeric, ginger and garlic compared to the untreated controls (Figure 4.4). There was no late blight in season two in Mwea since weather conditions were not favourable for its establishment. In Kabete, the plant extracts and antagonistic fungi reduced early blight levels compared to the treated control. There were differences among the plant extracts and antagonistic fungi but they were not significant in relation to the untreated control (Figure 4.5). Plant extracts and antagonistic fungi did not significantly reduce late blight in Kabete compared to both the standard and untreated control and the disease levels were high (Figure 4.5).

Table 4. 5: Percentage disease index for early blight assessed from tomatoes sprayed with plant extracts and antagonistic fungi during cropping cycle one in Mwea, Kirinyaga County

Treatments	Weeks after transplanting									Mean
	3	4	5	6	7	8	9	10	11	
Turmeric extract	24.9b	50.0a	50.0a	56.9a	68.1a	63.9b	97.2a	98.6a	100.0a	73.6a
Garlic extract	29.4ab	51.4a	51.4a	58.3a	63.9a	68.1ab	91.7a	94.4a	98.6a	73.1a
Ginger extract	36.0ab	50.0a	55.6a	58.3a	65.3a	69.4ab	84.7aa	94.4a	97.2a	73.5a
Lemon extract	31.8ab	50.0a	54.2a	59.7aa	69.4a	80.6ab	95.8a	98.6a	100.0a	75.2a
Trichoderma sp 1	34.2ab	50.0a	55.6a	62.5a	66.7a	70.8ab	88.9a	95.8a	98.6a	74.6a
Trichoderma sp 2	26.7b	51.4a	58.3a	68.1a	76.4a	77.8ab	100.0a	100.0a	100.0a	78.1a
Synthetics*	24.9b	50.0a	56.9a	59.7a	65.3a	69.4ab	94.4a	95.8a	100.0a	74.2a
Achook 0.15%EC®	26.7b	51.4a	62.5a	63.9a	75.0a	81.9a	100.0a	100.0a	100.0a	78.3a
Trianum T-22®	49.0a	50.0b	55.6a	61.1a	68.1a	69.4ab	95.8a	97.2a	100.0a	76.7a
Control	39.7ab	52.8a	61.1a	59.7a	77.8a	80.6ab	100.0a	100.0a	100.0a	79.2a
LSD ($P \leq 0.05$)	18	2.7	13.3	12.5	18.2	15	14.8	7.7	3.2	7.3
CV (%)	32.5	3.1	13.9	12	15.3	12	9.1	4.6	1.9	5.7

Means followed by the same letter(s) within each column do not differ significantly at ($p \leq 0.05$). Synthetics* was a combination of Ridomil Gold®, Isacop 80 WP® and Confidor SC 200®. Total disease = 8 (Distribution = 0-2; Incidence = 0-1; Severity = 0-5)

Table 4. 6: Percentage disease index for early blight assessed on tomatoes sprayed with plant extracts and antagonistic fungi during cropping cycle two in Mwea, Kirinyaga County

Treatments	Weeks after transplanting										Mean
	3	4	5	6	7	8	9	10	11	12	
Turmeric extract	50.0ab	50.0b	51.4a	51.4d	51.4a	54.2b	54.2b	56.9b	61.1b	65.3b	55.9e
Garlic extract	52.8ab	50.0b	50.0b	51.4d	51.4a	58.3ab	62.5ab	66.7ab	70.8ab	76.4ab	61.0de
Ginger extract	52.8ab	51.4ab	51.4b	55.6cd	54.2a	63.9ab	66.7ab	70.8ab	75.0ab	79.2ab	64.0bcd
Lemon extract	52.8ab	52.8ab	52.8ab	59.7abcd	54.2a	56.9ab	66.7ab	70.8ab	75.0ab	79.2ab	64.0bcd
Trichoderma sp 1	52.8ab	51.4ab	51.4b	59.7abcd	55.6a	63.9ab	70.8ab	76.4a	79.2a	83.3a	65.2bcd
Trichoderma sp 2	55.6ab	52.8ab	52.8ab	65.3ab	56.9a	63.9ab	76.4a	79.2a	80.6a	84.7a	68.2bc
Synthetics*	56.9a	54.2a	55.6a	68.1a	59.7a	61.1ab	73.6a	68.1ab	83.3a	87.5a	70.0b
Achook 0.15%EC®	52.8ab	50.0b	50.0b	55.6cd	54.2a	59.7ab	63.9ab	72.2ab	72.2ab	76.4ab	62.1cde
Trianum T-22®	51.4ab	50.0b	50.0b	58.3bcd	52.8a	59.7ab	68.1ab	72.2ab	76.4ab	80.6ab	64.0bcd
Control	51.4ab	51.4ab	50.0b	61.1abc	58.3a	66.7a	66.7ab	70.8ab	75.0ab	79.2ab	85.0a
LSD ($P \leq 0.05$)	5.8	3	3.3	8.1	7.5	9.8	15.5	13.3	15.8	15.6	6.6
CV (%)	6.4	3.3	3.8	8.1	8	9.4	13.5	16.2	12.3	11.5	5.8

Means followed by the same letter(s) within each column do not differ significantly at ($p \leq 0.05$). Synthetics* was a combination of Ridomil Gold®, Isacop 80 WP® and Confidor SC 200®. Total disease = 8 (Distribution = 0-2; Incidence = 0-1; Severity = 0-5)

Table 4. 7: Percentage disease index for early blight assessed from tomatoes sprayed with plant extracts and antagonistic fungi in Kabete Campus, Nairobi County

Treatments	Weeks after transplanting									Mean
	1	2	3	4	5	6	7	8	9	
Turmeric extract	24.9a	27.1b	35.7a	45.6a	43.3c	14.2d	32.2b	26.1cd	18.8d	25.7b
Lemon Extract	31.8a	30.8a	34.0a	40.3a	45.0bc	15.8d	22.2c	21.5d	21.5cd	26.8b
Trichoderma sp 1	28.1a	30.8a	36.7a	47.9a	37.2c	27.1c	31.1b	26.9c	21.5cd	30.4b
Trichoderma sp 2	30.4a	30.8a	34.6a	41.3a	52.5b	26.7c	22.8c	25.3cd	25.3bc	30.7b
Synthetics*	24.9a	27.5b	35.7a	45.3a	62.5a	68.1a	50.0a	62.5a	62.6a	48.1a
Control	24.9a	27.5b	38.5a	52.1a	53.1b	37.5b	29.9b	38.8b	29.9b	31.6b
LSD($P \leq 0.05$)	10.8	1.5	6.7	13.5	7.9	9.2	6.8	4.7	5.2	6.2
CV (%)	21.6	2.9	10.3	16.4	8.9	16	12	7.6	9.6	10.5

Means followed by the same letter(s) within each column do not differ significantly at ($p \leq 0.05$). Synthetics* was a combination of Ridomil Gold®, Isacop 80 WP® and Confidor SC 200®. Total disease = 8 (Distribution = 0-2; Incidence = 0-1; Severity = 0-5). In Kabete only one season was carried out and with fewer treatments.

Table 4. 8: Percentage disease index for late blight assessed from tomatoes sprayed with plant extracts and antagonistic fungi, Mwea, Kirinyaga County

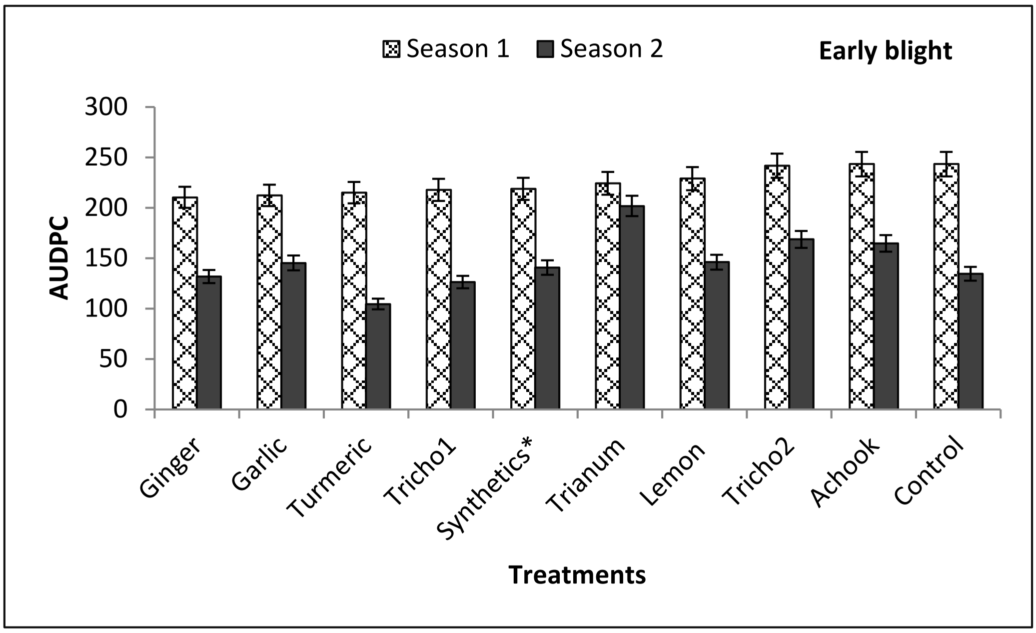
Treatments	Weeks after transplanting					Mean
	3	4	5	6	7	
Turmeric extract	0.0b	2.5b	5.1b	7.9a	9.7a	5.4abc
Garlic extract	0.0b	9.7a	6.1b	13.5a	0.0a	5.6abc
Ginger extract	0.0b	11.5a	12.9ab	12.9a	0.0a	7.7abc
Lemon extract	12.9a	3.3b	13.3ab	38.8a	9.3a	15.1a
Trichoderma sp 1	0.0b	3.3b	6.1b	9.3a	0.0a	3.5c
Trichoderma sp 2	0.0b	11.5a	17.9a	30.3a	0.0a	12.1abc
Synthetics*	0.0b	3.3b	17.5a	7.9a	0.0a	4.4bc
Achook 0.15%EC®	0.0b	5.1b	21.1a	35.3a	9.3a	14.6ab
Trianum T-22®	0.0b	5.1b	6.5b	7.9a	0.0a	5.3abc
Control	14.3a	11.5a	17.9a	22.5a	0.0a	11.7abc
LSD ($P \leq 0.05$)	3	4.3	7.6	31.7	10.3	9
CV (%)	65.1	37.5	35.4	98.3	211.8	61.2

Means followed by the same letter(s) within each column do not differ significantly at ($p \leq 0.05$). Synthetics* was a combination of Ridomil Gold®, Isacop 80 WP® and Confidor SC 200®. Total disease = 8 (Distribution = 0-2; Incidence = 0-1; Severity = 0-5)

Table 4. 9: Percentage disease index for late blight assessed from tomatoes sprayed with plant extracts and antagonistic fungi in Kabete, Nairobi County

Treatments	Weeks after transplanting										Mean
	3	4	5	6	7	8	9	10	11	12	
Turmeric extract	26.1a	28.8a	40.4a	68.1a	69.4a	91.7ab	54.2b	56.9ab	75.0a	100.0a	63.7a
Lemon Extract	13.3b	25.4a	39.0a	68.1a	76.4a	91.7ab	68.1a	54.2bc	79.2a	100.0a	64.4a
Trichoderma sp 1	13.9b	29.2a	35.8a	62.5a	76.4a	90.3ab	63.9ab	63.9a	76.4a	100.0a	63.3a
Trichoderma sp 2	13.1b	27.8a	37.2a	63.9a	75.0a	88.9ab	61.1ab	55.6bc	77.8a	100.0a	62.3a
Synthetics*	0.0c	26.8a	28.2b	41.1b	59.7b	84.7b	54.2b	48.6c	55.6b	73.6b	48.3b
Control	13.6b	28.1a	37.2a	56.9a	75.0a	97.2a	56.9ab	63.9a	80.6a	100.0a	63.0a
LSD($P \leq 0.05$)	2.4	3.5	5.2	13.6	8.9	10	12.3	7.5	11.6	1.8	5.8
CV (%)	9.8	6.9	7.9	12.5	6.8	6	11.3	7.2	8.6	1	5.2

Means followed by the same letter(s) within each column do not differ significantly at ($p \leq 0.05$). Synthetics* was a combination of Ridomil Gold®, Isacop 80 WP® and Confidor SC 200®. Total disease= 8 (Distribution = 0-2; Incidence = 0-1; Severity = 0-5)



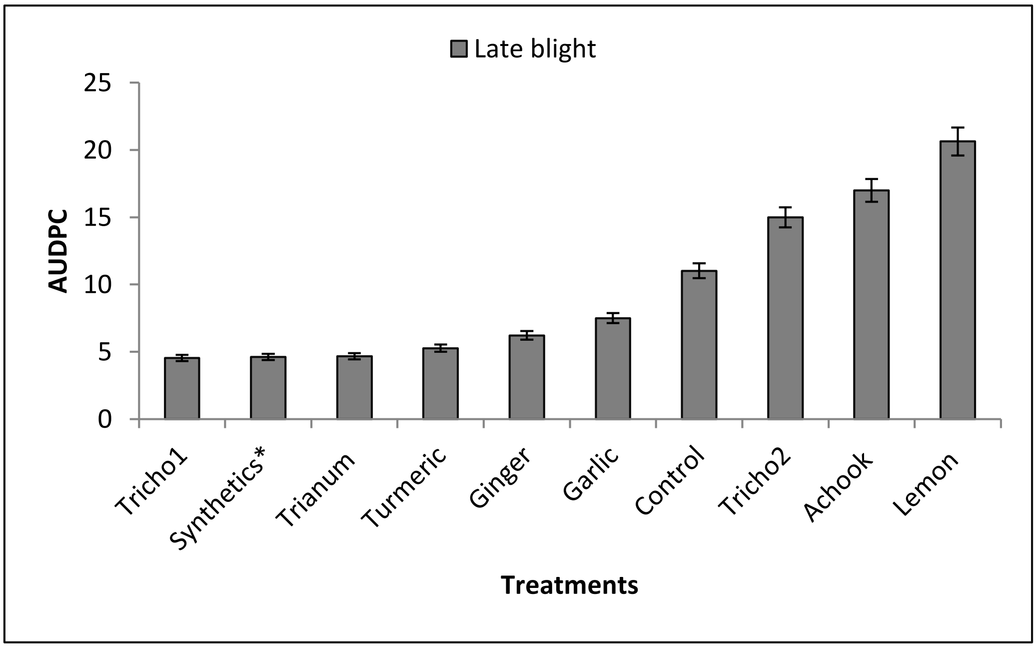


Figure 4. 4: Area under disease progress curve (AUDPC) of early and late blight of tomato on a tomato crop sprayed with crude plant extracts and antagonistic fungi in two cropping cycles in Mwea, Kirinyaga County. Synthetics* was a combination of Ridomil Gold®, Isacop 80 WP® and Confidor SC 200®. Total disease = 8 (Distribution = 0-2; Incidence = 0-1; Severity = 0-5). Tricho 1- *Trichoderma* sp 1, Tricho 2- *Trichoderma* sp 2 Late blight was only available in season 1.

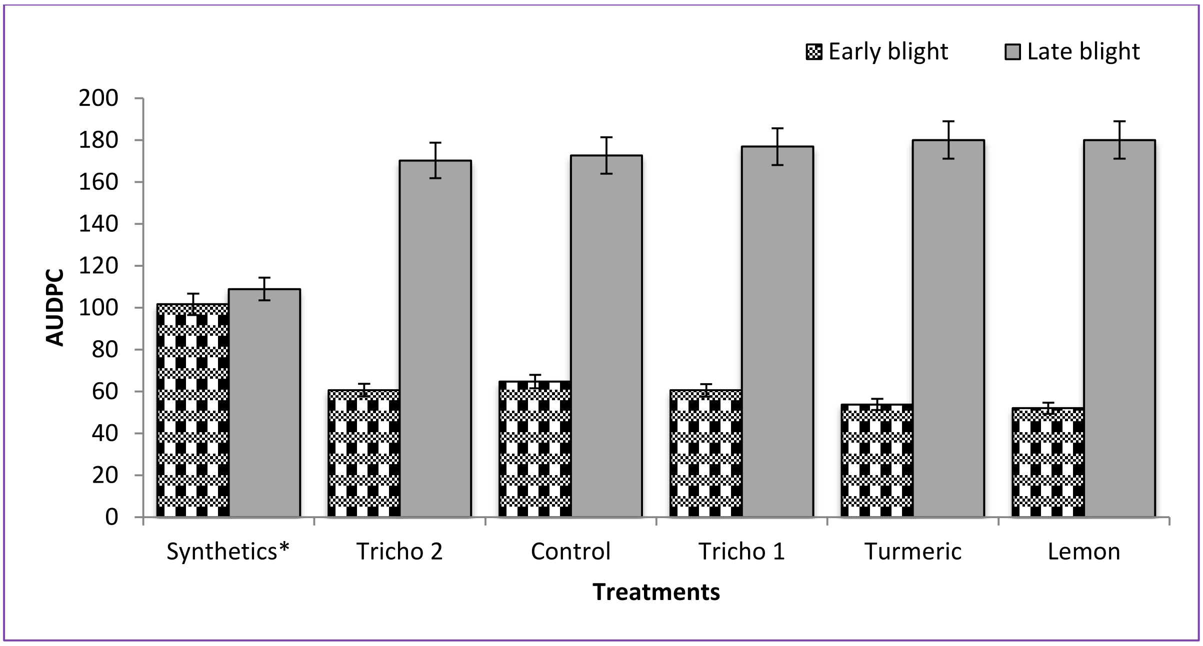


Figure 4. 5: Area under disease progress curve (AUDPC) of early and late blight of tomato on a tomato crop sprayed with crude plant extracts and antagonistic fungi Kabete, Nairobi County. Synthetics* was a combination of Ridomil Gold®, Isacop 80 WP® and Confidor SC 200®. Total disease= 8 (Distribution = 0-2; Incidence = 0-1; Severity = 0-5). Tricho 1- *Trichoderma* sp 1, Tricho 2- *Trichoderma* sp 2

4.4.3 Effectiveness of crude plant extracts and antagonistic fungi in improving tomato fruit yield and quality

There were significant differences ($p \leq 0.05$) in fruit yield of tomatoes treated with different crude plant extracts and antagonistic fungi in Mwea. Yield from plants sprayed with Achook 0.15 EC®, a commercial botanical formulation, had the highest yield of grade 1 and 2 of marketable fruits while yield from plants treated with garlic extract had a significantly ($p \leq 0.05$) higher yield of grade 3 fruits. Plant extracts significantly reduced yield with pest infestations by more than 35% and those with disease infection by more than 50% in season 1 in comparison to the untreated controls (Table 20). Majority of the yield fell under the grade 3 category for both season one and two with the highest being from the garlic extracts (Table 20; Table 21). In season two, yield trends for grades 1, 2 and 3 remained the same (Table 21). Plant extracts reduced pest infestations on fruit yield with more than 40% and disease infections by more than 65% which was a significant improvement from season 1 (Table 21).

There was negligible yield in Kabete due to the high disease levels.

Table 4. 10: Fruit yield (Kg/ha) for different grades harvested from tomato crop treated with crude plant extracts and antagonistic fungi during the first cropping cycle in Mwea, Kirinyaga County

Treatments	Grade 1	Grade 2	Grade 3	Non-marketable
Turmeric extract	180.0i	67.2i	574.0g	1466.0j
Garlic extract	406.3c	301.5c	2361.0a	1866.0h
Ginger extract	235.5g	129.6e	1244.0e	1710.0i
Lemon extract	207.4h	112.1ef	688.0f	2836.0c
Trichoderma Sp 1	472.0b	405.5b	1914.0c	2330.0e
Trichoderma Sp 2	301.1e	91.7fh	1917.0c	3652.0b
Synthetics*	345.8d	112.0efg	478.0g	2682.0d
Achook 0.15%EC®	512.0a	438.6a	1627.0d	2059.0h
Trianum T-22®	211.3h	89.3h	2164.0b	2179.0f
Control	261.1f	226.2d	1244.0e	3829.0a
LSD ($p \leq 0.05$)	17.6	19.6	109	118.9
CV (%)	3.3	5.8	4.5	2.9

Means followed by the same letter(s) within each column do not differ significantly at ($p \leq 0.05$). Synthetics* was a combination of Ridomil Gold®, Isacop 80 WP® and Confidor SC 200®

Table 4. 11: Fruit yield (Kg/ha) for different grades harvested from tomato crop treated with crude plant extracts and antagonistic fungi during the second cropping cycle in Mwea, Kirinyaga County

Treatments	Grade 1	Grade 2	Grade 3	Non-Marketable
Turmeric extract	0.0h	66.1h	573.0h	1106.0g
Garlic extract	211.7c	300.5c	2353.0a	1484.0f
Ginger extract	11.4g	128.3e	1243.0f	1336.0f
Lemon extract	14.0g	111.1f	682.0g	1695.0e
Trichoderma sp 1	261.7b	402.3b	1908.0e	1908.0d
Trichoderma sp 2	76.8e	91.2g	1915.0d	2636.0b
Synthetics*	135.8d	111.1f	474.0i	3246.0a
Achook 0.15%EC®	307.7a	434.3a	1955.0c	2443.0c
Trianum T-22®	11.6g	91.0g	2159.0b	1805.0de
Control	61.3f	223.4d	1242.0f	2320.0c
LSD ($p \leq 0.05$)	4.6	2.4	7.2	150.3
CV (%)	2.1	0.7	0.3	4.4

Means followed by the same letter(s) within each column do not differ significantly at ($p \leq 0.05$). Synthetics* was a combination of Ridomil Gold®, Isacop 80 WP® and Confidor SC 200®

4.5 Discussion

Crude plant extracts and antagonistic fungi reduced the populations of *Tuta absoluta* and whiteflies. Plant extracts were more effective in managing the insect pests compared to the antagonistic fungi. A commercial botanical, Achook 0.15 EC (neem) was however highly effective compared to other treatments. These findings agree with those of Nwachukwu and Asawalam (2014) who reported that fresh garlic juice reduced populations of maize weevils while Damals (2011) reported that turmeric has repellent property and it reduced populations of *Tribolium castaneum, Sitophilus granaries* and *Rhyzopertha dominica.* Report by Sumitra *et al*. (2012) also showed that neem and ginger (*Zingiber officinales*) effectively reduced populations of the leaf cutting beetle in mango. The antagonistic fungal sprays were not effective in pest management and this could be due to the source they were isolated from (Karimi *et al*., 2012), carrier material used (Slavica and Brankica, 2013) and the mode of action (Pal and McSpadden, 2006) since there is need for physical contact between the pest and the antagonist. There is also a difference in efficacy depending on whether the formulation used is liquid or dry which dictates the stability of the antagonist and its activity (Slavica and Brankica, 2013). Crude plant extracts and antagonistic fungi compared favourably to the commercial formulations of extracts and microbials as well as the synthetic pesticide.

The effectiveness of the plant extracts in reducing populations of insect pests in the field is attributed to the presence of volatile compounds which include saponins, alkaloids, tannins (Mizubuti *et al*., 2007), triterpenoids, sulphurous and polyacetate derivatives (Javaid and rehman, 2011). The activity of neem is attributed to the diterpenoids, triterpenoids, polyphenolics and polyacetate derivatives found in the plant (Javaid and Rehman, 2011). These compounds could lead to blockage of the tracheal system of the insects which eventually leads to death (Mathew *et al*, 2014). They also affect the growth and development of insects by impairing their normal functioning (Damals, 2011). Compounds from plants have been found to have deterrent effects towards the insects and turmeric for instance has bioactive constituents, turmerones and curcuminoids, which interfere with the insect's behaviour and growth (Damalas, 2011). Ginger and garlic have also been reported to have repellent properties (Ishii *et al*., 2010; Jahromi *et al*., 2012). Allicin is a compound in garlic and it is responsible for the repellent activity in garlic (Jahromi *et al*., 2012). The volatile active ingredients in garlic are sulphide compounds produced by rapid degradation of allicin. Its effects are persistent which explains the continuous reduction of pest populations in the field (Jahromi *et al*., 2012).

Effiom *et al*. (2012) reported that lemon has long lasting repellent effects on insects due to its volatile phytochemical extracts. Incorporation of biological control in cropping systems has been reported to reduce pest population by over 50% (Kasina *et al*., 2010; Muthomi *et al*., 2014) and also reduce production cost (Kasina *et al*., 2010). A single product is not effective enough to manage the pests thus the need to be incorporated into an integrated approach for additivity, synergy and antagonism (Odhiambo *et al*., 2009; Mahmoud *et al*., 2011; Mahlo *et al*., 2013). Several biopesticides have been formulated and commercialized including Neemraj Super 3000®, a neem based insecticide from Amiran (K) Ltd which controls aphids, thrips, whiteflies, diamond backmoth and bollworms; Halt 50 WP®, a bacterial base insecticide from Lachlan (K) Ltd used to manage diamond backmoth in brassicas and caterpillars in roses; Amblytech C®, a predator based insecticide from Dudutech (K) Ltd used to manage thrips and spider mites on flowers; Ditera DC®, a fungus based nematicide from Safina EA Ltd, used to manage nematodes in ornamentals; Eco-T WP®, (Trichoderma spp) used to manage soil borne pathogens (Infonet Biovision, 2015).

Both the extracts and the antagonistic fungi reduced the levels of early blight compared to the untreated control. The disease levels were however very high especially for early blight. The disease reduction potential was similar to that of the synthetic fungicides and these results agree with findings by Nashwa and Abo-Elyousr (2012) who evaluated neem, garlic, thorn apple and other plant extracts and found them effective in reducing the severity of early blight. Rodino *et al*., (2014) also reported that extracts from rosemary (*Rosmarius officinalis*) and jimson weed (*Datura stramonium*) reduced the disease levels in the field.

The variation in efficacy against plant diseases is as a result of several factors. The reduced efficacy of plant extracts efficacy in the field has been attributed to low concentrations of the bioactive compounds (Mizubuti *et al*., 2007). The extraction method and the solvent system used has also been cited as a major determinant of the quality and yield of plant extracts (Mizubuti *et al*., 2007; Odhiambo *et al*., 2009; Javaid and Rehman, 2011; Mahmoud *et al*., 2011; Bandor *et al*., 2013). However, Dabor *et al*., (2007) reported that water extracts were the most effective compared to organic extracts which is contrary to our results and those of Mahmoud *et al*. (2011) who worked with ethyl acetate and reported organic extracts as the most effective. This is further confirmed by Reddy *et al*. (2012) who also reported ethanolic extracts to be the most effective. Bandor *et al*. (2013) reported that polar solvents produce better yield of extracts than non-polar solvents.

The reduction of late blight by antagonistic fungi and extracts could be due to isolation source of the antagonists (Karimi *et al*., 2012) as well as the medium of growth (Naing *et al*., 2013). This however disagrees with Chethana *et al*. (2012) who reported average disease reduction by fungal antagonists and attributed it to prevailing environmental conditions. The antagonistic microbial species also differ in activity and mode of action. Hyperparasitism is a mode of action characteristic to *Trichoderma* species (Pal and McSpadden, 2006) and are also target specific (Srijita, 2015).

The finding that the effectiveness of synthetic fungicides in reducing disease levels is comparable to that of plant extracts was also echoed by Goufo *et al*. (2010) who reported plant extracts having similar results with synthetic fungicides against late blight in Cameroon. Nashwa (2011) reported that plant extracts from sweet basil, oleander, jimson weed and neem were effective in reducing early blight in the field but Ridomil Plus® was more effective and similar findings were reported by Ghorbani *et al*., (2005) who recounted copper oxychloride fungicide to be highly effective in managing late blight compared to the compost extracts. The latter further attributed the findings to the limited amounts of bioactive compounds in the extracts and their degradation with time.

Plant extracts and antagonistic fungi were effective in reducing late blight of tomatoes and compared well with the commercial fungicide formulations. These findings agree with studies by Islam *et al*. (2013) who worked with compost tea in an IPM program and reported that they reduced late blight severity effectively. Extracts from *Tephrosia vogelli, Clausena aniseta* and *Ageratum houstuianum* among others have been reported to be effective in reducing late blight severity by Goufo *et al*. (2010). Mizubuti *et al*, (2007) has also reviewed reports by several researchers that extracts from garlic, turmeric and pepper (*Piper longum*) have been found to reduce late blight severity in tomato plants. Antagonists from species of *Penicillium*, *Pseudomonas* and *Trichoderma* have also been reported to reduce late blight disease effectively (Mizubuti *et al*., 2007). The effectiveness of plant extracts in reducing disease levels on tomato plants in the field is related to the mode of action of the plant extracts (Nashwa and Abo-Elyousr, 2012). Some of them act directly on the pathogens while others induce systemic resistance in host plants resulting in reduced disease development (Nashwa, 2011). The modes of action of the extracts compare to those of the synthetic fungicides and thus the effects are similar.

In the present study, there was increase in yield and in quality of tomato fruits from the plant treated with extracts and antagonists. This differs with findings by Stangarlin *et al*., (2011) who reported that there were no yield differences in fruits harvested from plants sprayed with several plant extracts. The variation in activity of the plant extracts in reducing disease and enhancing fruit quality could be due to differences in the active chemical ingredients of the plants used, solvent extraction systems (Mizubuti *et al*., 2007; Bandor *et al*., 2013) and the fungal species evaluated (Nashwa and Abo-Elyousr, 2012). Some plant extracts and antagonistic microbes have some growth promotion effect which could increase the yield of the plants (Naing *et al*., 2013). Far from this, some biopesticides induce disease resistance systems of the plants which lead to healthy growth of the plants and thus better productivity (Naing *et al*., 2013).

Quality improvement and increase in yield is as a result of reduced pests and diseases during growth and fruit development. Authors that have reported reduced populations of pests (El Shafie and Abdelhareem, 2012; Rizvi and Jaffar, 2015) and diseases (Nashwa and Abo-Elyousr, 2012) have also reported remarkable increase in tomato yield. Other plant extracts have been reported to have a growth promotion effect (Culver *et al*., 2012) resulting to increased tomato yield. Growth promotion effect has also been reported upon using microbial pesticides in managing pests and diseases (Shah *et al*., 2013; Rahman *et al*., 2014; Singh *et al*., 2015).

The study showed that plant extracts and antagonistic fungi are efficacious in reducing pest populations, disease levels and increasing yield. Therefore, they can be incorporated in an integrated pest and disease management program, thereby reducing heavy application of synthetic pesticides which have negative effect on environment and leave harmful residues on the produce. This would help to meet the increasingly stringent quality requirements and hence improve access to prime markets, resulting in increased incomes for small scale tomato growers.

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CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Plant extracts inhibited growth of plant pathogens and exhibited varied effectiveness during the monitoring period. Extracts from the Zingibereaceae family were most active including Turmeric and ginger followed by extracts from plants in families Amaryllidaceae and Rutaceae. Mint belonging to family Lamiaceae was the least inhibiting. The varied activity among plant extracts against the test pathogens is an indication that there is a difference in the chemical composition of the different bioactive compounds. Susceptibility of the test pathogens was also varied and this could be due to rate of growth, morphology or the class to which the fungi belong. Plant extracts as well as antagonistic fungi reduced populations of assessed pests and diseases. The plant extracts were more effective in reducing the populations of insect pests compared to the fungal antagonists. Plant extracts also reduced the intensity of late blight more effectively compared to the fungal antagonists. Plant extracts as well as the antagonistic fungi compared favourably with the synthetic fungicides in reducing populations of pests and disease intensities. There was also notable yield difference among the plants treated with biologicals compared to those treated with synthetic fungicides.

Locally available plants and microorganisms have the potential to manage pests and diseases of tomato and hence have potential to replace the synthetic products since these natural products are environmentally friendly, non-toxic, target specific and do not retain residues in foods. Use of natural products will ensure clean, quality and healthy food produce with no chemical residues and therefore if used instead of the synthetic pesticides, there will be reduced interceptions in the lucrative markets. Farmers will hence redeem their access to these and gain more supply destinations since their produce will meet the required quality, safety and MRL requirements.

5.2 Recommendations

From the conclusions it is recommendable that:

i. Antagonistic microbes and plant extracts be incorporated into the integrated crop management programs to reduce accumulation of residues in fresh vegetables and production systems.

ii. Plants and microorganisms with antimicrobial activity need to be formulated into forms that are storable for longevity of shelf life and ease of application.

iii. More explorations should be made into the natural environment for plants and microorganisms with active compounds which could be used in the place of synthetic pesticides.

iv. The synergistic effects between and among the extracts and antagonistic microorganisms should also be investigated.

v. Chemical analyses should be done on the plant extracts to find the composition of their active compounds to establish their effects on host plants, target organisms as well as the non-target organisms.

vi. Awareness should be raised on use of biopesticides in management of pests and diseases especially to the small scale farmers and a policy on campaigns to promote use of biopesticides.

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