MANAGEMENT OF PESTS AND DISEASES IN SNAP BEANS BY USE OF MICROBIAL ANTAGONISTS AND PLANT EXTRACTS

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FULANO ALEX MUREMI
BSc. Agriculture
University of Nairobi

DEPARTMENT OF PLANT SCIENCE AND CROP PROTECTION

FACULTY OF AGRICULTURE

UNIVERSITY OF NAIROBI

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DECLARATION

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

Fulano Alex Muremi Signature…… Date……

This thesis is submitted with our approval as University supervisors

Prof. James W. Muthomi Signature……..... Date…….....

Department of Plant Science and Crop Protection

University of Nairobi

Dr. Maina J. Wagacha Signature……..... Date…….....

School of Biological Sciences

University of Nairobi

Prof. Agnes W. Mwang'ombe Signature…….. Date…….....

Department of Plant Science and Crop Protection

University of Nairobi

DEDICATION

This M.Sc. Thesis is dedicated to my parents for their encouragement in my education and for instilling in me traits of integrity, hard work, and excellence and to my best friends Dorcas, Hyden and Francine for always being there for moral support and refuelling me throughout the period of this study.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

ALEs	Aqueous Leaf Extracts
CAN	Calcium Ammonium Nitrate
CFU	Colony Forming Units
DAP	Di-Ammonium Phosphate, (18%N and 46%P_2O_5)
EU	European Union
HCB	Hexachlorobenzene
HCDA	Horticultural Crops Development Authority
IFSS	International Food Safety Standards
IPM	Integrated Pest Management
KEPHIS	Kenya Plant Health Inspectorate Service
LM4	Lower Midland Zone 4
LSF	Liquid State Fermentation
MRLs	Maximum Residue Levels
NA	Nutrient Agar
OPs	Organophosphates
PDA	Potato Dextrose agar
POFT	Participatory On-Farm Trials
PSBs	Pod-Sucking Bugs
SSF	Solid-State Fermentation
WAE	Weeks after emergence

GENERAL ABSTRACT

Kenya's snap bean export market share has been lost to other African and Central American countries over the last five years as a result of interceptions due to presence of chemical residues above acceptable levels. The objective of this study was to evaluate the effectiveness of local antagonistic microorganisms and crude plant extracts against major pests and diseases of snap bean as alternatives to synthetic pesticides. Laboratory *in vitro* studies were conducted to evaluate the antifungal activity of microbials isolated from local environments while phytopathogenic fungi were isolated from diseased plant tissues. Screening of antifungal activity of microbial isolates against *Fusarium solani, Colletotrichum lindemuthianum, Alternaria solani* and *Rhizoctonia solani* was carried out using the dual culture method. Diameters of the pathogens cultures were determined and percentage growth inhibition calculated compared to controls. The active microbial isolates were identified and data was collected at two day intervals.

Field studies were carried out to evaluate efficacy of the most effective antifungal antagonists from *in vitro* studies as well as selected plant extracts from another related study. The antagonists included *Trichoderma viride, T. harzanium, T. asperellum* and *Paecilomyces* sp. while crude plant extracts were from turmeric, garlic, ginger and lemon. Field experiments were set up in farmer's field in Mwea for two cropping seasons, short rains and long rains. The fungal antagonists were multiplied on sterile sorghum grains while the crude plant extracts were prepared by blending plant material in ethanol and concentrated by evaporation under vacuum. They were applied weekly as foliar sprays, commencing one week after emergence until podding. Their efficacy was compared to that of Dithane M-45®, Confidor 70 WG®, Trianum® (*Trichoderma*) and Achook® (neem**).**

A total of 42 microbial isolates were isolated from local environments of which 69%, 19% and 12% fungi, bacteria and actinomycetes respectively that inhibited the mycelia growth of phytopathogens. The 16 most promising antagonists were all fungi and the most efficacious were *Paecilomyces* spp., *Trichoderma viride, T. asperellum* and *T. harzanium*. *Trichoderma harzanium* was most active in reducing mycelial growth of the test pathogens by up to 66%. The 16 fungal antagonists varied in activity while the phytopathogens varied in sensitivity over time. In field experiments, antagonistic fungi and crude plant extracts significantly ($P \leq 0.05$) reduced the population of whitefly and thrips. The crude plant extracts reduced the population of whitefly and thrips by up to 58% and 41% with corresponding reduction by antagonistic fungi of up to 30% and 18%, respectively. These treatments significantly reduced the disease index of angular leaf spot, anthracnose and rust. Antagonistic fungi and crude plant extracts reduced disease index by up to 50.9% and 33.5%, respectively. In addition, they significantly increased pod yield and reduced the pest and disease damaged pods. The results demonstrated that local environment has a great potential as sources of biologically active antagonistic microorganisms and plant-based compounds that can be exploited for integrated management of insect pests and diseases in snap bean production. This would reduce chemical residues and therefore enable the local growers of snap beans access the more lucrative export markets.

Key words: Fungal antagonists, maximum residue limits, plant extracts, rejections, Snap beans

CHAPTER ONE: INTRODUCTION

1.1 Background

Snap Beans are harvested at podding stage when pods are still immature and green (Odero *et al*., 2013). The harvested pods are for direct consumption and for canning (Wahome *et al*., 2013). Snap beans in Kenya are for export market, majorly to the European Union and also locally for niche markets (Mulanya, 2014; Okello and Swinton, 2011; Wahome *et al*., 2013). Among Kenya's vegetable snap beans account for one quarter of the export market (USAID-KHCP, 2015). Snap bean production areas in Kenya include Murang'a, Kirinyaga and Meru Counties accounting for 43%, 25%, and 7% of the total production respectively (Goro, 2013).

Varieties of snap beans grown based on market preferences in these areas include Amy, Teresa, Samantha, Serengeti, Julia, Paulista, Gloria, Claudia, Morgan and Monal (Monda *et al*., 2003; Odero *et al*., 2013: Wahome *et al*., 2013). Snap bean is a very high valued vegetable that generates income to smallholder farmers improving household livelihood (Nderitu *et al*., 2008; Odero *et al*., 2013; Wahome *et al*., 2013). A recent study by USAID depicts that European Union (EU) imports 25% Kenyan snap beans valued at 152 million US Dollars just behind Morrocco. Furthermore, United States, Singapore and United Arab Emirates are also Kenya's snap bean destinations (USAID-KHCP, 2015). In 2012, snap beans alone earned Ksh. 4 billion, from export (Mwangi, 2013). Production of snap beans is dominated by smallholder farmers and currently it is estimated that about 50,000 smallholder farmers grow snap beans in Kenya **(**Wahome *et al*., 2011; Wahome *et al*., 2013; USAID-KHCP, 2015).

The inputs needed in production of snap beans include certified seeds, fertilizer for planting and top dressing, and agrochemicals (MFarm, 2014). Small holder growers invest heavily in production of snap beans due to the high cost of these inputs and labour in order to have maximum productivity. However, the synthetic chemicals used dictate the marketability of the harvestable product in relation to maximum residue levels (MRLs) (Global GAP 2014; Keikotlhaile and Spanoghe, 2011; Wahome *et al*., 2013).

Production of snap beans is majorly constrained by insect pests and diseases (Keikotlhaile and Spanoghe, 2011). Insect pests that damage snap beans are aphids, diamond back moth, bean flies and whiteflies as major pests (Nderitu *et al*., 2008). On the other hand soil-borne diseases caused by fungi continue to be a threat in production of snap beans (Monda *et al*., 2003). It is recommended that smallholder farmers practice field hygiene, use certified seeds, carry out crop rotation and apply recommended pesticides (Monda *et al*., 2003). Production of snap beans in Kenya is also severely constrained by foliar diseases namely angular leaf spot, anthracnose and rust (Mulanya, 2014). Owing to this undesirable status, farmers at times incur great loss when the pods are intercepted due to poor quality and presence of harmful organisms and chemical residues above the recommended levels.

Rejection of harvested green pods is due to residual and persistent activity of chemicals (Business Daily Africa, 2012; USAID-KHCP, 2015). Solution to this is having integrated management approach where judicious use of chemicals is undertaken in the event of pest infestation and disease infection exceed economic threshold (Monda *et al*., 2003). Biopesticides use comes in handy so as to reduce the risk of pesticide residues on marketed snap beans that

leads to building consumer confidence and satisfaction at the same time increasing market access opportunities (Chandler *et al*., 2011).

Two classes of biopesticides are of economic importance in the management of pests and diseases in snap beans. These classes are microbials and botanicals which are commercially available but expensive. According to a survey conducted in Meru County, farmers in Kenya are aware of some biopesticides for management of insect pests and diseases but they lack information on their effectiveness for safe plant pest and disease management (Monda *et al*., 2003).

1.2 Problem Statement

Eighty percent of snap beans producers in Kenya are smallholder farmers who overly rely on the use of synthetic pesticides in managing pests and diseases. About 50,000 smallholder growers of snap beans target export market and the growing domestic market mainly elite supermarkets. Foliar fungal diseases namely angular leaf spot, anthracnose, powdery mildew and rust damage market preferred pod quality characteristics and pod yield of snap beans (Mulanya, 2014; UmassAmherst, 2015; Wahome *et al*., 2011). Fungal diseases make snap beans have a repulsive appearance and lower their marketability and thus the income arising from their sale. Field losses caused by anthracnose are up to 90% under climatic conditions favourable to the disease (Mohamed, 2013). The bean rust fungus (*Uromyces appendiculatus*) is of worldwide importance as a yield-reducing disease. According to Schwartz *et al.* (2004) bean rust causes 25 - 100% yield loss depending on the stage of infection and the prevailing weather conditions (Nyasetia, 2011).

Arthropod pests is another setback faced by smallholder farmers and include thrips, bean stem maggots, whiteflies, aphids, thrips, cut worms, pod borers, foliage beetles and red spider mites (Infonet-Biovision, 2015; Nderitu, 2008; UmassAmherst, 2015). Thrips infestation has been reported to cause yield losses as high as 60% (Ouma *et al*., 2014). This proportion includes direct losses through feeding and indirect losses such as being found in the harvested produce.

Chemical pesticides use on snap beans in Kenya pose threats of interception due to pesticides residue on produce (Fening *et al.*, 2014). There are also notable cases of use of banned pesticides resulting in interception and rejection of significant amounts of export snap beans to the European market. The strict maximum residue level (MRLs) requirements could result in low volumes of snap beans designated for export market because smallholder farmers may stop production due to non-compliances, high costs, limited access to analytical laboratories and delays in clearing of consignments (Ouma *et al.*, 2014). The impacts of misuse and overuse of the classes of pesticides pointed out earlier to the environment is undesirable (Fening *et al.*, 2014; Ouma *et al.*, 2014). The presence of pesticides in the ecosystem affects non-target organisms while pests develop resistance against particular insecticides or fungicides and they pollute the environment (Fening *et al.*, 2014; Nyasetia, 2011).

The stringent regulations by European Union (EU) especially on MRLs for fresh vegetables like snap beans can be addressed through the use of new technologies that do not result in chemical residues in marketable pods. It is therefore the use of biopesticides which are developed locally that such kind of smallholder farmers of snap beans can adopt as an ideal and cheaper option in an integrated approach towards management of insect pests and diseases (Srinivasan, 2012).

1.3 Justification

Use of biopesticides has gained popularity all over the world and formulations are available in the market as one of the alternatives for insect pest and disease management (Chandler *et al*., 2011; Ouma *et al.*, 2014; Srinivasan, 2012). This paradigm shift from chemical use to biopesticides squarely lies on change in attitude towards the use of chemical pesticide (Krishan, 2014). The quest to reduce pesticide application as a prerequisite to counter undesirable effects of chemical pesticides have led to application of biopesticides (Ouma *et al.*, 2014). The attributes that make biopesticides to be best alternatives to synthetic pesticides include having no toxic residues, are harmless to beneficial organisms and pose minimal risk to the environment and humans, are effective as the synthetic chemicals in managing pests and diseases and have high compatibility with other pest management techniques like use of biological agents after going through pest risk analysis (Chandler *et al*., 2011; Ouma *et al*., 2014; Sola *et al*., 2014).

Biopesticides can be locally sourced and therefore, as a new technology will be easily adopted by small holder farmers (Okello and Swinton, 2011; Sola *et al*., 2014). Another plus is that the use of biopesticides results in no development of resistance by pests and pathogens to the activities of antagonistic microorganisms and their products (Kimani, 2014). According to Ouma *et al* (2014) most biopesticides are slow acting and give better results when approached in an integrated pest management (IPM) programme.

The stringent requirements during snap beans marketing can be addressed by use of biopesticides that present premium value snap beans on niche markets at the same time enabling the smallholder farmers' find worth in investing in production (Kimani, 2014; Ouma *et al*., 2014). In

this context of integrating biopesticides in pest and disease management regimes, smallholder farmers will be able to overcome the constraints of a stringent regulatory framework while on the other hand coping with the market power of supermarkets (Chandler *et al*., 2011; Sola *et al*., 2014).

1.4 Objectives of the study

The broad objective of this study was to contribute to reduced synthetic pesticide application in snap bean production by use of locally sourced antagonistic microorganisms and plant extracts in the management of pests and diseases for improved pod quality and access to niche markets.

The specific objectives were:

i. To evaluate the activity of selected antagonistic microorganisms isolated from local environments in reducing the growth of phytopathogenic fungi *in vitro*.
ii. To determine the effectiveness of selected local antagonistic microorganisms and plant extracts in managing insect pests and diseases on snap beans in the field.

1.5 Hypotheses

i. Local environments harbour antagonistic microorganisms that interact with economically important phytopathogenic fungi.
ii. Antagonistic microorganisms and plant extracts from local environments can be used to manage insect pests and diseases in snap beans as alternatives to synthetic pesticides.

CHAPTER TWO: LITERATURE REVIEW

2.1 Economic importance of snap beans in Kenya

Production of snap beans in Kenya is by large scale commercial farmers and smallholder farmers, of which smallholder farmers in the rural areas form the biggest percentage of producers (Kinyuru *et al*., 2011; Odero *et al*., 2013; USAID-KHCP, 2015). This has led to improving incomes and alleviating poverty of such farmers who own between half and five acres of land (Nderitu *et al*., 2008; Odero *et al*., 2013). Snap bean is ranked first among the export horticultural crops in Kenya that earns foreign exchange (Nderitu *et al*., 2008). It is exported to European Union (Okello and Swinton, 2011; Horticultural Crops Development Authority **(**HCDA), 2014; USAID-KHCP, 2015) and in the recent past local consumption of snap beans has also been increasing hence most smallholder farmers supply the fresh produce to niche markets (HCDA, 2014; Odero *et al.*, 2013).

Snap beans are grown for fresh consumption, canning or freezing and this shows how pods are packaged at supermarkets in major urban centres where elite consumers are found. Various studies have been conducted on snap bean production and almost all show that trade in snap beans is highly profitable (Odero *et al*., 2013). Snap beans as a horticultural crop is regarded as an important crop that has a great potential for addressing food insecurity due to high nutritive value in both energy and amino acids content (Menge *et al*., 2014). Planting of snap beans is based on targeted market and mostly to have production all year round then they are planted at intervals of 2-3 weeks.

2.2 Snap bean market situation for smallholder farmers

The major problem in production of snap beans is marketing which almost all smallholder farmers face in the event that their produce is rejected during marketing (Keikotlhaile and Spanoghe, 2011). Rejection of harvested pods is because of disease and pest symptoms on these pods and poor grades. Rejection is also as a result of harmful chemical residues which emanates from application of pesticide regimes for example twice a week. This is done to meet buyers demand for specific grades of fresh pods of snap beans (GLOBALGAP, 2014). Such practice leads to overuse of these chemical pesticides that result to high MRLs in the produce (Business Daily Africa, 2012; Nyakundi *et al*., 2012; Ouma *et al*., 2014). In addition, some farmers have contracts with companies that do not permit sale of their produce outside the contract (Ndegwa *et al*., 2013; Okello and Swinton, 2011).

2.3 Stringent market conditions for Kenyan snap beans

Pre-export conditions are usually observed before marketing Kenyan snap beans in terms of postharvest activities and processing of beans meant for niche markets in Western countries hence need for upgrading of standards along the value chain (Keikotlhaile and Spanoghe, 2011; Odero *et al*., 2013). The importers of Kenyan snap beans are dictated by consumer demand for aesthetic quality attributes which necessitates meeting such requirements (Okello and Swinton, 2011). The snap beans are packed in facilities that are approved and registered with the Kenya Plant Health Inspectorate Services (KEPHIS) (Goro, 2013). Snap beans consignment is accompanied by a phytosanitary certificate which attests that inspection was conducted and was found free of quarantine pests.

In the event that residues of chemicals like dimethoate are found in snap beans entering supermarkets in European Union a heavy fine has to be paid by for the contaminated produce (Business Daily News, 2013). Due to this stringent requirement, inspection has to be conducted and the farmer pays for the cost of inspection that deprives them access to export market (Keikotlhaile and Spanoghe, 2011; Wahome *et al*., 2011). Meeting the standards implies that farmers in Kenya switching to new safer but more costly pesticides, investing in costly storage, packing and cooling facilities and keeping detailed technical information related to pesticide usage and produce handling practices both in the farm and in the grading and holding facilities in production of snap beans (Okello, 2011).

2.4 Major pests of snap beans

Insect pests are a very important constraint in snap bean production as they cause direct and indirect damage (Goro, 2013; Nderitu *et al*., 2008; Nyasani *et al*., 2013). Direct damage is through feeding and indirect damage is through transmission of viruses and contamination leading to low productivity (Infonet-Biovision, 2015). Bean stem maggots (*Ophiomyia* spp.), whiteflies (*Bemisia tabaci* and *Trialeurodes vaporariorum*) and aphids (*Frankliniella* spp. and *Megalurothrips sjostedti*) are the major insect pests in the production of beans in tropical climate and are more prevalent in drought conditions (Beebe *et al*., 2012). The main pests in snap bean production systems include thrips, bean stem maggots, whiteflies, aphids, thrips, cut worms, pod borers, foliage beetles and red spider mites (Infonet-Biovision, 2015; Nyasani *et al*., 2013).

Bean thrips (*Megalothrips* spp.) have piercing and sucking mouthparts and together with insects with the similar mouthparts cause the highest damage of up to 40% due to bud abscission and

flower abortion and up to 20% loss due to pod damage (Nderitu *et al*., 2008; Ouma *et al*., 2014). Such insects cause scars and blemishes on leaves and pods (Infonet-Biovision, 2015; National Farmers Information Service (NAFIS)**,** 2015). Other examples of insect pests with sucking and piercing mouthparts that affect snap beans are the whiteflies (*Bemisia tabaci* and *Trialeurodes vaporariorum*) that are of economic importance at larval stage and adult stage. Both the adult and larvae cause reduced plant growth, yellowing of leaves, and wilting of the plant when present in large numbers (Muvea, 2011). They produce honeydew, which may lead to growth of sooty mould on leaves and pods.

Heavy growth of sooty mould reduces photosynthesis affecting plant growth. Snap bean pods contaminated with sooty mould are unmarketable. Control measures on beans are justified if large whitefly numbers attack the plants during the early stages of the crop. Whitefly infestations after the onset of flowering are of no economic importance as they do not affect yield (Infonet-Biovision, 2015). Diagnostically adults are small (1-3 mm long), with two pairs of wings that are held roof-like over the body. The production of sooty mould reduces photosynthesis affecting plant growth that has a direct influence on crop yield (Infonet-Biovision, 2015).

Aphids directly cause damage by clustering on plant stems, leaves, and bean pods while sucking sap and cause the plants to stunt (NAFIS, 2015). Indirectly, aphids damage snap beans when they act as vectors of Bean Common Mosaic Virus. The species that transmit *Bean common mosaic virus* are *Aphis fabae* and *Aphis craccivora* (Beebe *et al*., 2012; Infonet-Biovision, 2015). Pod borers include African bollworm (*Helicoverpa armigera*) and the legume pod borer (*Maruca testulalis*) which feed on leaves, flowers, pods and seeds. Diagnostically African bollworm

caterpillars are 3 to 4 cm long and make clean circular holes in the pods while caterpillars of the legume pod borer attack pods at the point of contact with other pods (Infonet-Biovision, 2015). Pod borers are quarantine pests, and are particularly important in snap beans grown for export, for instance only one caterpillar found in a consignment sent to Europe, the whole consignment may be rejected (Infonet-Biovision, 2015).

The (*Ophiomyia* spp.) female bean fly pierces the young leaves to lay eggs and sucks sap that leaves yellow blotches on the leaves. Feeding of larva destroy the tissue causing the stem to swell and split and reduce formation of lateral roots (Infonet-Biovision, 2015). Spider mites (*Tetranychus* spp.) are phytophagous pests that inflict damage on snap bean leaves making them turn to bronze or a rusty, purple or yellow brown colour and webbing (Infonet-Biovision, 2015).

2.5 Major diseases of snap beans

Production of snap beans in Kenya is severely constrained by diseases according to Mulanya (2014) and the major foliar diseases are angular leaf spot (*Phaeoisariopsis griseola*), anthracnose (*Colletotrichum lindemuthianum*) and rust (*Uromyces appendiculatus* var. *appendiculatus*). These fungal diseases damage market preferred pod quality characteristics and pod yield of snap beans (Mulanya, 2014; Infonet-Biovision, 2015; Wahome *et al*., 2011; Beebe *et al*., 2012). Root rot is a fungal disease that affects seedlings of snap beans (Beebe *et al*., 2012; Infonet-Biovision, 2015). Common bacterial blight and halo blight are bacterial diseases that are known to reduce growth vigour and yield.

Bean rust (*Uromyces appendiculatus* var. *appendiculatus*) has been a major disease in snap bean production in Kenya which has made plant breeders spent sleepless nights to come up with cultivars that are resistant to rust due to its effect of lowering pod quality (Beebe *et al*., 2012; Ndegwa *et al*., 2013; Wagacha *et al*., 2007; Wahome *et al*., 2011). When environmental conditions favour rust fungi infestation and growth then farmers experience crop yield loss as there is increased severity of the disease (Beebe *et al*., 2012) which results in defoliation, stunted growth and subsequent reduced yields while infected pods may be rejected in the market due to the development of disfiguring lesions as earlier stated (Wagacha *et al*., 2007).

Another fungal foliar disease devastating snap beans is the angular leaf spot (*Phaeoisariopsis griseola*). Angular leaf spot can be diagnosed by looking at symptoms such as small dark brown spots with angular edges and are often numerous to give the foliage a checker-board appearance (Infonet-Biovision, 2014). The spots most often increase in size and then coalesce and cause yellowing which eventually turns necrotic causing premature defoliation (Infonet-Biovision, 2014; NAFIS, 2015). Humid weather favours the fungus to produce grey mould on the lower surface of the spots. Infected pods in the field have brown blotches (Infonet-Biovision, 2015).

Bean anthracnose (*Colletotrichum lindemuthianum*) is a seed-borne disease identified in snap bean fields even during seedling stage as pale brown sunken spots. Anthracnose symptoms can be observed in almost all above ground tissues for example dark brown lesions on leaves that are restricted to the veins on lower leaf surface, elongated and sunken lesion on stems and black sunken lesions on pods. These lesions lower the quality of marketable pods as the pods appear shrivelled (Amin *et al*, 2014; Mohammed, 2013).

The prevalent foliar bacterial disease of snap beans is the common bacterial blight caused by *Xanthomonas campestris* pv. *phaseoli*. The symptoms include lesions on leaves that first appear as small, watersoaked, light green areas. Leaf spots become dry and brown with a narrow yellow halo. Disease progress leads to spots expanding, eventually killing leaves. Pods also develop water soaked spots form and can develop into broad irregular blotches. When the attack is severe, pods may shrivel and seeds may not develop (Karavina *et al*., 2011).

Apart from foliar diseases, root rots are economically important in snap bean production. *Rhizoctonia* root rot is caused by *Rhizoctonia solani*, soil-borne fungus that attacks snap beans of almost any age (El-Mohamedy *et al*., 2015). The symptoms include seed rot and damping-off of seedling, stunting, yellowing, and death of older plants. Another type of root rot is *Fusarium* root tot that is caused by *Fusarium solani* f. sp. *phaseoli* that attacks snap beans later in the growing season (Muthomi *et al.*, 2014). Snap beans infected are stunted or yellowed but not usually killed, taproot and lower stem show reddish lesions, which later turn brown to black. Snap beans also suffer from Pythium root rot caused by *Pythium* spp. The later is characterised by pre-emergence damping-off and seedling wilt of snap beans (Muthomi *et al.*, 2014).

2.6 Management of insect pests in snap beans

Physical pest control measures employed include handpicking and destroying infested pods and pod borers which helps when the numbers of bean pod borers are low and in small fields and washing plants with a strong jet of water to knock off pest and to destroy their webs by also ensuring to spray the underneath of the leaves. However, this should be done early in the day to

allow the foliage to dry due to the fact that wetness of the foliage for an extended period is conducive to development of fungal diseases (Infonet-Biovision, 2015).

The cultural measures used in pest management in snap bean production involve crop rotation with non-related crops and weed control to remove alternate hosts that harbours the insect pests in the absence of the crop (Muvea, 2011; Richardson, 2012). Related to this is avoiding to plant snap beans near other leguminous crops that may be the source of pests. Planting early in the season helps in controlling bean fly as their numbers tend to be low during the early stages of the growing season and increase with time (Infonet-Biovision, 2015).

The provision of favourable growing conditions to improve plant vigour and to enhance tolerance to insect attack and damage is a very important cultural practice in pest management (Infonet-Biovision, 2015). For instance, soil fertility can be improved by adding organic fertilizer and manure that makes the crop plant not to be weakened at early stages of growth by pest infestation and lastly field sanitation is important.

Biological control has been advocated for use so as to reduce the MRLs in vegetables (Fening *et al.*, 2014; Nyasani *et al*., 2015). Formulated products of neem and pyrethrum are currently used whereas a mixture of garlic and pepper has been recommended to manage insect pests (Infonet-Biovision, 2015; Ogala, 2013). The use of chemical pesticide (Table 2.1) is the most common pest management strategy employed in snap bean production (Fening *et al*., 2014; Nderitu *et al*., 2007; Nyakundi *et al*., 2012). However, use of chemical pesticides in judicious way leads to reduced chemical applications in the production of snap bean according Nderitu *et al* (2009).

Table 2.1: Chemical pesticides used in controlling insect pests in snap beans and their application rates

Chemical pesticide	Pest	Application rate and interval
Cabarl,	Cutworms, Beetles and Caterpillars	50gm /20lts, at 15 to 21 days intervals
Chlopyrifos	Bean Fly, Aphids	150ml/100lts at 7 days intervals
Cypermethrine	Bean Fly, Aphids	100ml/20lt at 7 days intervals
Deltamethrin,	Cutworms, Beetles and Caterpillars	30-50 ml/20lts, at 7-10 days
Fenvalerate	Bean Fly, Aphid	100ml/20lts at 7 days intervals
Imidacloprid	Bean Fly, Aphids, whiteflies	570gm/100kg of seeds (also spray)
Triazopho,	Bean Fly, Aphids	30-60 ml/100lt, at 10-14 days intervals

Source: NAFIS, 2015; PCPB, 2015. gm-Grams, ml- Millitres and Lt- Litres

2.7 Management of diseases in snap beans

Management of fungal diseases in snap beans is always achieved by application of several disease management measures like cultural practices, cultivation of resistant varieties, and the use of protectant and systemic fungicides (Nyasetia, 2011). Using multiple resistant cultivars of snap beans is a good basis for foliar disease management which is much pragmatic in smallholder farming (Wahome *et al*., 2011). It is also the foundation of integrated disease management. Resistant varieties have been developed against various diseases, for example Theresa and Super Monet varieties are resistant to rust (Infonet- Biovision, 2015; Richardson, 2012) while Paulista are resistant to anthracnose.

Preventive strategies involve site selection were the farmer should know the cropping history of the field in which to establish snap bean crop. The use of certified seeds helps in controlling seed borne diseases such as anthracnose and periodical application of foliar fungicides are key in disease management (Nyasetia, 2011; University of Massachusetts, Amherst, 2015). Closely

associated with preventive measures are the cultural strategies in management of foliar diseases (rust, anthracnose and angular leaf spot) which include avoiding continuous cropping of beans in the same field to avoid inoculum build up, crop rotation with unrelated crops for three to four seasons and sprinkler irrigation should not be used as wetting the leaves provides suitable condition for foliar diseases spread (Infonet-Biovision, 2015). It is also required that there should be no walking in wet fields under snap beans (NAFIS, 2015).

On addition to these options, sanitation as a cultural method is through destroying snap bean residues after harvesting (Infonet-Biovision, 2015; NAFIS, 2015). In integrated disease management programs, antibiotics are incorporated to control foliar diseases in snap beans. These antibiotics are produced by antagonistic *Bacillus* and *Streptomyces* species that possess systemic activity against rust fungi (Amin *et al*, 2014; Wagacha *et al*., 2007). This has resulted in the increasing need to use biopesticides which are already formulated as described later in this chapter.

Fungicides for bean diseases management are most effective when used in the very early stages of the epidemic and preventatively (Amin *et al*, 2014). Effective fungicides include protectants such as chlorothalonil and dithiocarbamates, and systemic chemicals such as triazoles and carboxins (Nyasetia, 2011). In the last century, pesticides were largely adopted to counteract the action of pests and diseases and to increase plant health and yield. Efficacy levels of commercial fungicides in terms of reducing rust disease severity reach over 90% according to Stump *et al*. (2000) and Gent *et al*., 2001 (Nyasetia, 2011). However, continuous use of chemical fungicides for plant defence causes great environmental impact, the onset of resistance phenomena within

some populations of fungal pathogens as well as acute and general toxicity on humans and non-target organisms (Amin *et al,* 2014). Examples of chemical sprays used to control rust are Baycor® 30% EC, Bitertanol®, Anvil®, Alto® 100 SL, or Dithane M45® should be applied after every two weeks (NAFIS, 2014).

2.8 Synthetic agrochemicals used to manage pests and diseases in vegetables

Chemical pesticides used to manage insect pests and diseases include chlorinated hydrocarbons, organophosphates and carbamates (Nderitu *et al*., 2008; Nyakundi *et al*., 2012). These have been a major success in application over the years (Keikotlhaile and Spanoghe, 2011) but have been known to pose risks that have had a substantial impact on the environment; compounded further by indiscriminate and excessive use of the products (Keikotlhaile and Spanoghe, 2011; Nyakundi *et al*., 2012). Consequently, beneficial species have been lost and residual problems have increased, with subsequent impact on the food chain, groundwater contamination and resistance in pests (Keikotlhaile and Spanoghe, 2011).

In Kenya, insect pests are managed by dimethoate, imidacloprid (Confidor®), imidacloprid 100g/L + betacyfluthrin 45g/L (Thunder®), dimethoate, and lambda cyhalothrin 25g/Kg (Karate®), (Monda *et al*., 2013; Nderitu *et al.*, 2008; Ndung'u, 2013). Currently, most farmers still use the chemical pesticides on calendar spray regimes. Diseases in vegetables on the other hand are managed by fungicides like Dithane M45®and Anvil®, (Monda *et al*., 2013). These fungicides are mostly applied twice a week an aspect that leads to indiscriminate use of resulting to resistance, resurgence and residues (Prithusayak, 2011).

Despite the success of pesticide application in high value vegetable production like snap beans, these chemical pesticides possess toxins that endanger the health of farmers, consumers and the environment and as a result it is a new challenge to Kenyan snap bean farmers (Keikotlhaile and Spanoghe, 2011; Nyakundi *et al*., 2012). All over the world now, any agricultural produce should be pesticide residue free hence the emergent of stringent market conditions (Nyakundi *et al*., 2012) leading to ban of some chemicals. There are about 30 pesticides banned in Kenya between the 1986 and 2011 (Pest Control Products Board, 2014). The banned insecticides range from insecticides, herbicides, fungicides and soil fumigants.

2.9 Use of biopesticides in pest and disease management

Biopesticides provide a satisfactory alternative to chemical pesticides in the new era of integrated management strategies and increased enforcement of stringent regulations on agricultural products (Ouma *et al*., 2014). This comes about because of not posing residual problems (William, 2013; Opender, 2011; Krishan, 2014). They are also pest specific, have no negative effect on beneficial organisms and are environmentally benign (Kimani, 2014; William, 2013; Raudales and Gardener, 2008). Biopesticides have already pragmatically proven to be effective and sustainable in management of pests and diseases in other vegetables (Killani *et al*., 2011; Srinivasan, 2012). Biopesticides used in agriculture production majorly include microorganisms and botanicals (Kimani, 2014).

Microorganisms form various associations ranging from antibiosis, commensalism, parasitism, and symbiosis in nature. The exploitation of these interactions has resulted to biological control of plant pathogen. Some fungal antagonists are found in association with roots for, example in Brazil *Ulocladium atrum* was isolated from barley field and *Trichoderma viride* was isolated

from sugarcane field (Figueirêdo *et al*., 2010). It has been also been found that different species of the same genus exist in the soil freely or around the root zone like *T. harzianum* and *T. aureoviride* (Figueirêdo *et al*., 2010).

Most of the studies show that rhizosphere harbours most of the potential microbial antagonists for instance 85 rhizo-bacteria were isolated from the rhizosphere of plants growing in various desert areas and were screened for their antagonistic activity against different *Fusarium* isolates (Omar and Ahmed, 2014). Also microbials can be sourced from germplasm units according to Killani *et al* (2011). In Kenya, a number of formulated microbials (Table 2.2) have been recommended to manage pests and diseases in the production of vegetables and according Wafula (2014) they are effective when incorporated into IPM systems.

Formulated microbial biopesticides are available for treatment soil infected by soil borne pathogens such as *Fusarium* spp., *Pythium* spp. and *Rhizoctonia* spp. (Raudales and Gardener, 2008; Srinivasan, 2012; Ouma *et al*., 2014; Infonet-Biovision, 2015). Locally available microorganisms have been shown to prevent many diseases and a few pests in various crops when sprayed on a regular basis hence their use in snap beans deems most appropriate (Ouma *et al*., 2014; Infonet-Biovision, 2015; Raudales and Gardener, 2008).

Use of microbial based pesticides effectively reduce insect pest population and suppress disease development on crops. However, there are issues that need to be addressed. First, there is need to have focused studies on mode of action and pathogenicity of microbial antagonists. Contamination of microbial antagonists during mass production has been reported to reduce their

potency and shelf-life. The other challenges are that these microbial antagonists are slow in action and they are subjected to regulatory framework due to persistence, resistance and dispersal potential (Nawaz *et al*., 2016).

Botanicals are biochemicals that are derived from plants (plant extracts) (Kimani, 2014). The plants that botanicals are derived from can be collected in forests, farm fields or purchased (Obongoya *et al*., 2010; Al-Samarrai *et al*., 2012). Neem (*Azadirachta indica*) and pyrethrum (*Chrysanthenum* spp.) are widely used and thus formulated as products under pesticides (Table 2.3). According to studies carried out on the efficacy of botanicals, crude plant extracts are applied on crops with a hand sprayer to control pests (Degri *et al*., 2013) and diseases (Al-Samarrai *et al*., 2012). Some examples on the efficacy studies of botanicals in vegetable pests in the field clearly show how they are applied. For example, Degri *et al.* (2013) found out that the application of the aqueous leaf extracts (ALEs) of *Azadirachta indica* and *Chromolaena odorata* from the onset of podding and at fortnightly intervals, greatly reduced pod-sucking bugs (PSBs) infestation of cowpea crops.

In another study, neem (*Azadirachta indica*), tephrosia (*Tephrosia vogelii*), and tobacco (*Nicotiana tobacum*) applicatons had significant effect in reducing the population of whiteflies, aphids and thrips (Night *et al*., 2011). Efficacy of botanicals on vegetable diseases is exemplified in a study on phytotoxic effect of selected crude plant extracts on *Fusarium* yellows disease of common bean (Obongoya *et al*., 2010). In this study, it was found out that *Azadirachta indica* performed better than *Tagetes minuta*, *Nicotiana tobacum* and *Vinca rosea* in a participatory on-farm trials (POFT) conducted on 30 farms.

Table 1.2: Trade names, active substances of products, target pest and agent or distributor of biopesticides registered in Kenya

Trade name	Active substance	Target pest	Agent/manufacturer /distributor
BioCatch®	*Verticillium lecanii*	Aphids, whiteflies	Osho Chemical Industries Ltd
Biolep®	*Bacillus thuringiensis*	African bollworms	Insect (K) Ltd
Bio-Nematon®	*Paecilomyces lilacinus*	Root-knot nematodes	Osho Chemical Industries Ltd
Botanigard®	*Beauveria bassiana*	Aphids, thrips	Amiran (K) Ltd
Trianum-P®	*Trichoderma harzianum*	Soil-borne pathogens	Koppert B.S (K) Ltd
Trichotech®	*Trichoderma asperullum*	Soil-borne pathogens	Dudutech (K) Ltd

Source: PCPB, 2015

Table 2.2: Trade Names, active substances, target pest and agent or distributor of botanical pesticides available in Kenya

Trade Name	Active substance	Target pest	Agent / distributor
Flower DS EC®	Pyrthrins	Aphids, whiteflies	KAPI Ltd
Neemark EC®	Azadirachtin	Aphids, thrips, nematodes	Osho Chem. Industries Ltd
Neemraj Super®	Azadirachtin	Aphids, thrips, whiteflies	Amiran (K) Ltd
Nimbecidine EC®	Azadirachtin	Aphids, thrips, whiteflies	Osho Chem. Industries Ltd
Pyerin EC®	Pyrethrin	Aphids and whiteflies	Juanco SPS Ltd
Trilogy 70 EC®	Neem oil	Fungal Pathogens	Farmchem (K) ltd

Source: PCPB, 2015

A study conducted by Okunlola and Ainrirnola (2014) in Ondo State, Nigeria shows that indigenous knowledge of using botanicals is a success story in pest management in vegetables. Neem is the most used botanical biopesticide worldwide due to its insecticidal property (Chandler *et al*., 2011). Studies by Waiganjo *et al.* (2011) showed that formulated neem (Nimbecidine® and Achook®) controlled aphids and diamond back moth in cabbage. However, botanical biopesticides are not only insecticidal but they have microbial activity against plant

pathogenic fungi (Al-Samarrai *et al*., 2012). When botanicals are utilized optimally their effectiveness is registered in managing pests as in the case of pyrethrum in some economies according to Sola *et al.* (2014). Botanical biopesticides when used in agriculture provide sustainable pest management as in the case of microbial biopesticides (Sola *et al*., 2014).

2.10 Recent trends in marketing Kenyan snap bean pods

Export market share of Kenyan snap beans has been lost to other African and Central American countries over the last five years due to failure to meet phytosanitary and quality requirements that results in interception of fresh pods (CUTS, 2009; Ibui, 2015). The increased interceptions at ports of entry are overly termed as non-compliance with EU market requirements (Hortfresh, 2013). It is expected that fresh snap bean pods should be free from harmful organisms such bollworms, whiteflies, leaf miners, spider mites, thrips and microbial contaminants. Growers overly use synthetic agrochemicals to manage the pests leading to high levels of pesticide residues. In addition, contamination of pods with harmful organisms has affected access to the European markets.

The critical precondition is strict compliance with MRLs that dictates that the produce should not contain banned or higher amounts of agrochemical pesticide residues (CBI, 2015). Consequently, sampling and intensified inspections for residue analysis have been increased by 10% resulting in delivery delays (Andae, 2016; Hortfresh, 2016). Other requirements include traceability of products and certification (CBI, 2015). All the stated export requirements for vegetables are covered under GLOBAL GAP regulations. The other issues that growers should observe include

good agricultural practices (GAP), record keeping of all farm operations, environmental standards and welfare of farm workers enable them access export market (CUTS, 2009).

It is very expensive for smallholder growers to get GLOBAL GAP certificate due to need to invest in grading, cooling , safe handling of chemical pesticides facilities (Global GAP, 2014). This has made production cost to go up and as a result individual smallholder growers cannot keep up with the demand of GLOBAL GAP compliance. Non-compliance deprives the country valuable foreign exchange, loss of income by growers and further creates unemployment.

CHAPTER THREE: ANTIFUNGAL ACTIVITY OF MICROBIAL ISOLATES FROM LOCAL ENVIRONMENTS IN *IN VITRO* BIOASSAY

3.1 Abstract

Synthetic agrochemicals used to manage pest in snap beans lead to contamination of produce and environment hence the need of other options. *In vitro* studies were conducted to evaluate the antifungal activity of microorganisms isolated from local environment. Organic substrates were direct plated and pour plate was used to isolate microbials from soil while the phytopathogens were isolated from diseased tissues. Screening of antifungal activity of microbial isolates against *Fusarium solani, Colletotrichum lindemuthianum* and *Rhizoctonia solani* was carried out using the dual culture method. Growth inhibition was determined as reduction in pathogen colony diameter. The active microbial isolates were identified and their efficacy further evaluated against *Alternaria solani, Fusarium solani, Colletotrichum lindemuthianum* and *Rhizoctonia solani*. A total of 42 microbial isolates inhibited the mycelia growth of phytopathogens out of which sixteen were most promising antagonists. The most efficacious antagonists were *Trichoderma viride, T. harzanium, T. asperellum* and *Paecilomyces* spp. with *T. harzianum* that inhibited the test pathogens by up to 66%. Among the test phytopathogenic fungi, *Rhizoctonia solani* was the most sensitive to the antifungal activity of antagonistic fungi. The results demonstrated that local soils and dead organic substrates have a great potential as sources of biologically active antagonistic microorganisms that can be exploited as alternatives in management of diseases in French bean production.

Key words: Antifungal activity, biopesticides, microbial antagonists, phytopathogens

3.2 Introduction

The advocacy for ecofriendly options to manage diseases and as key components of integrated management in the present times becomes the preferred alternative of synthetic pesticides. The use of antagonistic microorganisms to manage diseases is seen as an alternative to synthetic pesticides in addressing the problem of harmful chemical residues on produce and pollution of the environment (Belete *et al*., 2015). Local environments such as rhizosphere of crop fields and pasture land, compost and organic substrates are rich in multiple species of antagonistic microorganisms and studies conducted reveal that they are effective in inhibiting mycelia growth of phytopathogens in *in vitro* bioassays (Belete *et al*., 2015; Figueirêdo *et al*., 2010; Omar and Ahmed, 2014).

Various studies have reported the effectiveness of antagonistic microorganisms in managing plant diseases (Belete *et al.,* 2015). For example, *Trichoderma* spp. was found to inhibit growth of *Fusarium oxysporum*, *Rhizoctonia solani*, and *Alternaria solani* (Patale and Mukadam, 2011). *Trichoderma aureoviride* suppressed mycelial growth of *Curvularia clavata* and *Fusarium solani* in dual culture bioassay (Sneha and Prasad, 2014). This implies that with relevant technology of mass multiplication and formulation, the adoption of local bioproducts in managing diseases is economically sound for small holder farmers and at the same time being safer to producers, consumers and environment. This study focused on evaluation of the activity of antagonistic microorganisms isolated from local environments in reducing the growth of phytopathogenic fungi *in vitro*.

3.3 Materials and Methods

3.3.1 Sources of antagonistic microorganisms

Potential microbial antagonists were collected from rhizosphere of plants in cultivated fields and pasture land, roots, cattle shed, decaying wood and decomposing organic matter. Soil samples were scooped at a depth of 10 cm after removing 2 cm topsoil and or litter. For every site, three soil samples were collected and mixed to make 50 grams composite sample that was packed in polythene bag. Each soil sample was air-dried by spreading on surface sterilized bench for one week. The samples were then placed in steam sterilized Khaki bags, labelled accordingly and stored at 4°C in the refrigerator for subsequent use.

3.3.2 Preparation of culture media

Nutrient Agar (NA) was prepared by dissolving 28 g of NA powder in 1000 ml of sterile distilled water, stirred vigorously and autoclaved for 20 minutes at 121^{o}C and 15 psi (Killani *et al.*, 2011). The NA media was cooled in a water bath set at 45 ^{o}C. Potato dextrose agar (PDA) was prepared by dissolving 39 g in 1000 ml sterile distilled water. The autoclaved PDA media was allowed to cool to 45^{o}C in a water bath and thereafter antibiotics added under sterile conditions using micropipettes. Tetracycline and streptomycin sulfate at a rate of 100 mg/L each (Rioux *et al*., 2014) were added to the molten PDA and stirred to mix thoroughly. Twenty ml of media was dispensed into sterile plastic Petri dishes in a laminar flow to ensure sterile conditions. Potato dextrose agar slants were also prepared by pouring 20 ml autoclaved PDA media in liquefied state into universal bottles. The universal bottles containing molten media were placed in a slanted position and media allowed to harden at a slope. They were used for storage of microorganisms used later in screening for antagonistic activity.

3.3.3 Isolation of antagonistic microorganisms

Microorganisms from soil and compost were isolated by serial dilution (Belete *et al*., 2015; Killani *et al*., 2011; Shobha and Kumudini, 2012). Ten grams of each composite soil sample was suspended in 100 ml sterile distilled water to obtain a suspension. The suspensions were homogenized by agitation using a magnetic shaker (Heidolph Unimax 1010) set at 200 revolutions per minute and further serial dilutions of 10^{-2}, 10^{-3} and 10^{-4} were prepared. One milliliter aliquot of serially diluted suspension from each dilution was pipetted onto plates in triplicate, ensuring that all microorganisms are isolated as distinct colonies. Into each plate, 20 ml of molten NA was poured. The suspension and molten media in the plates were thoroughly mixed by swirling in a gentle manner to uniformly spread the suspension. Incubation of bacterial cultures took 48 hours with Petri plates in inverted position under room temperature (23 ± 2 ºC) to prevent water droplets splashing the colonies.

The same procedure was repeated in isolating actinomycetes and fungi on 20 ml sterile molten PDA (45^{o}C) amended with antibiotics. Fungal and actinomycetes cultures were incubated for seven days under room temperature (23 ± 2 ºC). Roots and decaying woods were direct plating to isolate microorganisms. The decaying pieces of wood were washed with 70% alcohol for five minutes and then cut into approximately 3 mm blocks. Three pieces of wood were direct plated in molten PDA and NA and incubated at room temperature as others. After incubation, microorganisms showing clear zones of inhibition against other microorganisms were sub-cultured and purified on PDA for fungi and actinomycetes, and bacteria on NA. Purified cultures of bacteria were inoculated on sucrose-peptone agar slants while the fungi and actinomycetes were maintained on PDA slants.

3.3.4 Isolation of phytopathogenic fungi

Phytopathogens were isolated from diseased tissues of snap beans. The infected plant tissues were carried in Kraft bags and stored in the laboratory at 4^{o}C before isolation. Fungal phytopathogens were isolated from diseased tissues by direct plating. The diseased tissues were first washed in running water to remove surface soil, dust and other contaminants. Small pieces (3 mm in length) of plant tissues were cut using sterile surgical blade and then surface sterilized in 1.3 % sodium hypochlorite for two minutes. The plant tissues were rinsed three times in sterile distilled water and then blot dried using sterile absorbent paper. Four pieces were placed aseptically on PDA medium and incubated for seven days. Sub-culturing was done by aseptically cutting small pieces of mycelia at the growing edges of colonies and transferred to new PDA media to make pure cultures (Siameto *et al*., 2010).

3.3.5 Purification and storage of microorganisms

After incubation, microorganisms showing clear zones of inhibition against other microorganisms on agar were sub-cultured to obtain pure cultures. Sub-culturing was done by aseptically cutting small pieces of mycelia at edges of colonies and transferred to new PDA media to make pure cultures for fungi and actinomycetes. The pure cultures of bacteria were obtained using streak-plate technique on NA and were streaked on NA slants. The pure cultures of candidate fungal and actinomycetous isolates were inoculated on PDA slants. All the slant cultures were stored at 4°C in the refrigerator until needed for bioassays (Killani *et al*., 2011; Shobha and Kumudini, 2012).

3.3.6 Preliminary screening of microbial antagonists

Fusarium solani, Colletotrichum lindemuthianum and *Rhizoctonia solani* were used to evaluate the antagonistic effects of microorganism isolates using the dual culture method. Potato dextrose agar was prepared and dispensed into sterilized Petri dishes and allowed to solidify in the laminar flow. Each potential antagonistic microorganism was cut with 5 mm sterile cork borer to obtain agar discs. The agar discs having the antagonist microorganism were inoculated at four equidistant positions where each was 30 mm from the centre of the Petri dish.

Discs having young actively growing cultures, six days old of each phytopathogenic fungus were cut separately with sterilized 5-mm cork borers and inoculated at the centre of the cultured plates. The control plates included test pathogens inoculated alone at the centre and the experiment was replicated three times. The plates were incubated at room temperature (23 ± 2 ºC) for 10 days in the Plant Pathology Laboratory to allow adequate antagonist-pathogen interaction to take place. The plates were arranged in a completely randomized design on sterilized bench.

Colony diameters of the fungal pathogens in the test and control plates were measured and recorded. The colony diameter was given as the mean of two perpendicular diameters. Data was collected 9 days after inoculation (DAI) on PDA media by recording the growth diameters of the pathogens in millimetres. The data was used to obtain the inhibition in the mycelia growth of three test pathogens, *Fusarium solani, Colletotrichum lindemuthianum* and *Rhizoctonia solani* by the potential microorganism isolates. *Colletotrichum lindemuthianum* was isolated from snap bean leaves while *Fusarium solani* and *Rhizoctonia solani* were isolated from the roots. The

percentage growth inhibition was calculated according to Odebode *et al.* (2004) mathematical formula:

$$\text{Percentage growth inhibition} = \frac{\text{Dc} - \text{Dt}}{\text{Dc}} \times 100$$

Where: Dc; Mean diameter of pathogen in the control plates, and Dt; Mean diameter of the pathogen in the treatment plates (Killani *et al*., 2011).

The percentage of growth inhibition of each test pathogen by potential microorganism isolates was calculated and average percentage growth inhibition was used in rating effectiveness of isolates. Potential antagonistic microorganism isolates were categorized as effective in inhibiting radial growth of test pathogens by giving them a score as per modified Bogumił *et al.* (2013). Where; 1 – Low antagonistic activity (I < 51%), 2 – Moderate antagonistic activity (I = 51-59%), 3 – High antagonistic activity (I = 60-75%), and 4 – Very high antagonistic activity (I > 75%).

3.3.7 Identification of antagonistic fungi

After preliminary screening, active antagonistic fungi that were rated as having very high antagonistic activity and high antagonistic activity were identified (Watanabe, 2010) and characterized. Each isolate was cultured on PDA and identified based on their colony appearance, shape of conidia and conidiophores and branching pattern of phialides. They were also examined under microscope where slide preparations were stained with lactophenol-cotton blue. The morphological and microscopic authenticity of the antagonistic fungi was confirmed using different identification keys (Killani *et al*., 2011).

3.3.8 Determination of antagonistic activity of microorganisms against plant pathogens

Pure cultures of the most active antagonists based on preliminary screening were used to determine antagonism against the pure cultures of phytopathogens of economic importance in snap bean production. A volume of 20 ml of the media was poured aseptically into 9 cm sterile disposable Petri dishes and allowed to solidify at room temperature inside the laminar flow. The activity of the active antagonistic fungi was determined by the paired culture method. Mycelia agar disc, 5mm in diameter was cut from each culture of fungal plant pathogen and placed at the centre of PDA plate. Five millimetre plugs containing the antagonist were cut from pure cultures using a cork borer. The plugs were spot inoculated at four equidistant points on PDA medium, 30 mm from mycelial plug of the test fungus. Control plates were only inoculated with plugs of the fungal pathogens placed at the centre of PDA medium. For each test pathogen, the test was replicated 3 times. All the plates were incubated at room temperature (23 ± 2 ºC) for 10 days (Killani *et al*., 2011) and arranged in a completely randomized design.

Data was collected on 2^{nd}, 4^{th}, 6^{th}, 8^{th} and 10^{th}days by recording the colony diameters of the pathogens in millimetres using a pair callipers and ruler. The colony diameter was given as the mean of two perpendicular diameters. The degree of antagonism was determined by measuring the pathogen colony diameters and percentage inhibition calculated according to Silliman *et al* (2015):

$$\text{Percentage inhibition} = \frac{\text{A} - \text{B}}{\text{A}} \times 100$$

Where A is the diameter of mycelia growth of pathogenic fungus in control and B is the diameter of mycelia growth of pathogenic fungus with antagonist.

3.3.9 Data processing and analysis

Preliminary data summaries were generated using Microsoft Excel Spreadsheet. Percentage growth inhibition used to determine the level of antagonistic activity was carried out on MS Excel spreadsheet. Analysis of variance (ANOVA) was carried out on Percentage colony diameter inhibition.

3.4 Results

3.4.1 Antagonistic activity of microorganism isolates against phytopathogens

A total of 42 microorganism isolates were isolated from the rhizosphere of plants in cultivated fields, pasture land, roots, cattle shed, decaying wood and decomposing organic matter. The isolates showing clear zones of inhibition against other microorganisms comprised of fungi, actinomycetes and bacteria of which fungi isolates comprised the majority of the antagonists. Fungi isolates were 29, bacteria isolates were 8 while actinomycete isolates were 5 (Figure 1). The level of antagonistic activity of microbial isolates was based on mean percentage mycelia growth inhibition on three test pathogens. The mean colony diameter used to determine the level of antagonism was that of ninth day after inoculation (Tables 3.1; 3.2).

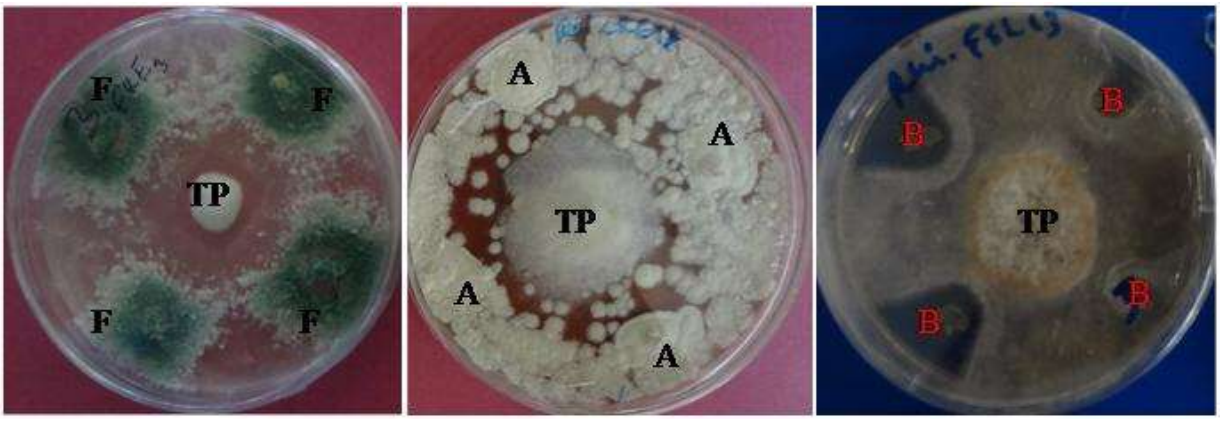


Figure 1: Fungal, actinomycetous and bacterial isolates screened for antifungal activity; F-fungal isolate, A-actinomycetous isolate, B- bacterial isolate and TP-test pathogen

Four fungal isolates had very high degree of antagonistic activity. The four isolates were from diverse sources including: milking shed mud, nappier grass rhizosphere, compost soil and decomposing wood. The mean percentage growth inhibitions exhibited by the four fungi were 80.4%, 79.7%, 77.7% and 77.2% (Table 3.1). Isolates that were rated to have high degree of antagonistic activity were also fungi. A total of 12 isolates had high degree of antagonistic activity. The 16 antagonist microorganism isolates with very high and high activity belonged to different genera, were *Trichoderma* was predominant. Other genus included *Paecilomyces, Epicoccum, Rhizoctonia*, *Sepedonium* and *Gloeosporium* (Figure 2).

Figure 2: Species of antagonistic fungi isolated from local environment

Table 3.1 Percentage colony diameter inhibition of three test pathogens by microbial isolates from diverse sources in dual culture test (Bogumil ranking)

Isolate designation	Source of collection	Group	% GI at 9th DAI			Mean	Bogumil ranking
			FO	CO	RO		
FKE3	Milking shade mud, UK	Fungi	76.4	92.1	72.6	80.4	4
FKF1	Nappier rhizosphere, UK	Fungi	76.4	92.5	70.1	79.7	4
FS4	Field Station compost, UK	Fungi	71.8	94.4	67.0	77.7	4
WOOD	Decomposing wood, UK	Fungi	67.4	83.7	80.5	77.2	4
B	Busia soil, PPL	Fungi	73.3	76.4	75.0	74.9	3
WRC(P)	Busia soil, PPL	Fungi	74.0	81.3	67.4	74.2	3
LH1K8D23	Culture, PPL	Fungi	67.5	79.0	70.9	72.5	3
FKE1	Milking shed mud, UK	Fungi	67.4	80.2	67.1	71.6	3
FKE2	Milking shed mud, UK	Fungi	66.3	72.6	72.4	70.4	3
DRC(M)	Busia soil, PPL	Fungi	64.1	71.0	65.3	66.8	3
LRC(M)	Busia soil, PPL	Fungi	63.5	78.2	67.0	69.6	3
DRC(C)	Busia soil, PPL	Fungi	61.9	83.3	63.2	69.5	3
A	Busia soil, PPL	Fungi	60.8	74.0	72.3	69.0	3
FKA2	Animal shed, UK	Fungi	59.2	65.8	56.7	60.6	3
DD2	Culture, PPL	Fungi	61.3	67.2	51.7	60.1	3
LH1K1N11	Culture, PPL	Fungi	62.5	63.5	54.2	60.1	3

UK; Upper Kabete Campus, PPL; Plant Pathology Laboratory, CDI; Colony diameter inhibition, DAI; Days after inoculation, FO; *Fusarium solani,* CO; *Colletotrichum lindemuthianum* and RO; *Rhizoctonia solani*.>75%- Very high and 60-75%- High degree of antagonistic activity.

Table 3.2: Percentage colony diameter inhibition of three test pathogens by microbial isolates from diverse sources in dual culture test (Bogumil ranking)

Isolate designation	Source of collection	Group	% GI at 9th DAI			Mean	Bogumil ranking
			FO	CO	RO		
FKA3	Animal shed, UK	Fungi	62.8	60.4	54.2	59.1	2
MAIZE	Maize roots	Fungi	52.3	69.6	50.0	57.3	2
D82	Culture, PPL	Fungi	54.6	66.7	48.8	56.7	2
BEANFS	Bean roots, UK	Fungi	55.7	60.4	52.7	56.3	2
FS1	Potato rhizosphere, UK	Bacteria	55.0	67.1	45.1	55.7	2
FKB1	Bean rhizosphere, UK	Fungi	53.9	57.9	50.2	54.0	2
WRC(C)	Busia soil, PPL	Fungi	43.8	63.5	48.7	52.0	2
CN	Compost, Ndumbuini	Fungi	49.4	58.7	46.9	51.7	2
C	Lantana rhizosphere, UK	Bacteria	61.4	65.6	28.2	51.7	2
PO	Culture, PPL	Fungi	50.0	55.2	46.3	50.5	2
LH19N1	Culture, PPL	Fungi	48.0	58.3	41.2	49.2	1
FKB	Bean rhizosphere, UK	Fungi	42.4	59.1	45.8	49.1	1
FKD	Compost, UK	Fungi	42.4	42.5	58.7	47.9	1
LK69	Busia soil, PPL	Actinomycete	47.5	56.3	40.0	47.9	1
ID3	Culture, PPL	Fungi	45.4	59.1	35.8	46.8	1
FKB2	Bean rhizosphere, UK	Bacteria	36.6	57.5	39.1	44.4	1
FSL13	Silt soil, UK	Bacteria	43.0	63.1	26.6	44.2	1
AS6	Busia soil, PPL	Actinomycete	38.5	56.3	37.3	44.0	1
DRC(M1)	Busia soil, PPL	Bacteria	33.9	54.0	34.2	40.7	1
LHIK7D22	Culture, PPL	Fungi	41.5	46.4	31.3	39.7	1
WRC(M)	Busia soil, PPL	Actinomycete	39.2	42.9	28.1	36.7	1
KSC14(W)	Culture, PPL	Bacteria	29.4	44.4	32.8	35.5	1
KSC14	Culture, PPL	Actinomycete	20.6	0.0	26.9	15.8	1
AS 7	Potato rhizosphere, UK	Bacteria	10.4	0.0	20.7	10.4	1
BN	Bean rhizosphere, UK	Fungi	5.1	0.4	17.0	7.5	1
KSC14	Field Station compost, UK	Bacteria	11.0	0.0	2.3	4.4	1

UK; Upper Kabete Campus, PPL; Plant Pathology Laboratory, CDI; Colony diameter inhibition, DAI; Days after inoculation, FO; *Fusarium solani*, CO; *Colletotrichum lindemuthianum* and RO; *Rhizoctonia solani*. 51-60%- Moderate and <51%- low antagonistic activity.

Table 3.3: Cultural and morphological characteristics of antagonistic fungi on PDA medium

Antagonist	Cultural and morphological characteristics
T. harzianum	Two concentric rings of mycelia, green conidia denser at the centre, globose conidia and slender phialides
T. asperellum	Conidiophores terminates into 2 or more philides and primary branching arise close to 90degrees to the main axis
T. viride	Granular mycelia, green conidia evenly distributed, globose conidia and slender phialides
T. reseei	Colony low on plate and white mycelium , ovoid green conidia , laterally branched conidiophores, singularly produced phialides
T. atroviride	Granular on PDA with green conidia unevenly distributed
T. pseudokonongii	White to green mycelia, branched conidiophores bearing elongated phialides with green oblong conidia and elongated phialides
T. konongii	Cylindrical green conidia
Trichoderma 1	White mycelia, globose green conidia and singularly produced phialides
Trichoderma 2	White mycelia, globose green conidia
Trichoderma 3	White mycelia, subglobose to ellipsoidal green conidia
Trichoderma 4	White mycelia, subglobose to ellipsoidal green conidia
Paecilomyces	Pink mycelia, multicellular and spherical conidia produced in sporodochia surface of the mycelium having densely clustered phialides
Epicoccum	Orange-brown colony with diffusible pigment, numerous black sporodochia, globose multicelluar conidia are globose
Rhizoctonia	Dark brown sclerotia and hypal strands branching at right angles
Sepedonium	Yellow mycelium, erect conidiophores, ellipsoid hyaline conidia produced singly on phliades
Gloeosporium	Separate conidiophores, unicell hyaline conidia

3.4.2 Activity of antagonistic fungi against plant pathogenic fungi

The antagonistic fungi significantly ($P \leq 0.05$) reduced colony diameters of the four test plant pathogens by up to 66% (Table 3.4). *Trichoderma harzianum* was the most efficacious in reducing mycelia growth of test plant pathogens by up to 65.8% followed by *T. viride* (65.3%). The least growth inhibition was observed in *Sepedonium* spp. (37.4%). The plant pathogens varied in their sensitivity to the different antagonistic fungi. *Rhizoctonia solani* was the most sensitive while *Alternaria solani* was the least to the activity of antagonistic fungi. *Trichoderma atroviride, Paecilomyces, Trichoderma viride* and *Trichoderma* isolate 3 had the highest biocontrol potency against *Alternaria solani, Rhizoctonia solani, Fusarium solani* f.sp. *phaseoli* and *Colletotrichum lindemuthianum*, respectively.

In experiment two, antagonistic fungi also significantly ($P \leq 0.05$) reduced the colony diameters of *A. solani, C. lindemuthianum, F. solani* f.sp. *phaseoli* and *R. solani* (Table 3.5). However, the inhibition of mycelia growth was less (up to 49%) compared to experiment one. *Trichoderma* isolate 1 and *T. harzianum* were the most effective and showed significant superiority amongst all the antagonistic fungi. *Rhizoctonia* was least in effectiveness in inhibiting growth of phytopathogens in experiment two compared to experiment one (Tables 3.4; 3.5).

Table 3.4: Percentage colony diameter inhibition of mycelial growth of plant pathogenic fungi by antagonistic fungi, *in vitro* experiment 1

Antagonist	*Alternaria*	*Rhizoctonia*	*Fusarium*	*Colletotrichum*	Mean
T. harzianum	56.4 ab	69.6 ab	68.9 b	68.5 abc	65.8 a
Trichoderma 1*	52.0 bc	44.5 g	55.4 def	59.8 ef	52.9 f
T. asperellum	52.1 bc	68.6 b	68.3 b	67.1 abcd	64.0 abc
Trichoderma 2*	48.3 cd	65.1 bcd	57.0 de	58.0 f	57.1 e
Paecilomyces	56.7 ab	74.1 a	57.5 d	64.0 cde	63.1 c
T. pseudokononggii	43.7 de	54.3 ef	55.4 def	43.3 h	49.2 g
T. konongi	52.8 abc	69.3 ab	67.4 bc	63.4 he	63.2 bc
T. reseei	52.8 abc	56.5 e	54.8 def	66.1 bcd	57.5 e
T. atroviride	58.3 a	67.7 bc	53.4 ef	61.2 ef	60.2 d
T.viride	48.2 cd	70.0 ab	73.0 a	70.1 ab	65.3 ab
Trichoderma 3*	55.7 ab	63.5 cd	64.6 c	71.5 a	63.8 abc
Trichoderma 4*	43.7 de	50.0 f	43.3 h	52.4 g	47.4 gh
Epicoccum	38.9 e	42.9 g	37.4 i	36.7 i	39.0 i
Rhizoctonia	33.5 f	61.3 d	47.7 g	43.3 h	46.4 h
Sepedonium	10.9 g	53.8 ef	42.0 h	42.8 h	37.4 i
Gloeosporium	45.0 d	67.2 bc	53.0 f	68.2 abc	57.6 de
Control	0.0 h	0.0 h	0.0 j	0.0 j	0.0 j
Mean	44.0	57.6	52.9	55.1	52.4
LSD ($P \leq 0.05$)	5.3	6.0	3.6	4.2	4.8
CV (%)	7.2	6.2	4.1	4.6	4.7

Means accompanied by different letter(s) in each column are significantly different (Duncan's multiple range test, $P \leq 0.05$).

Table 3.5: Percentage colony diameter inhibition of mycelial growth of plant pathogenic fungi by antagonistic fungi, *in vitro* experiment 2

Antagonist	*Alternaria*	*Rhizoctonia*	*Fusarium*	*Colletotrichum*	Mean
T. harzianum	48.1 ab	38.8 bc	51.5 b	53.3 a	47.9 ab
Trichoderma 1	49.5 a	38.6 bcd	58.0 a	49.2 ab	48.8 a
T .asperellum	46.9 ab	38.7 bc	43.2 c	53.4 a	45.6 b
Trichoderma 2	48.3 ab	35.5 bcde	52.3 b	52.0 a	47.0 ab
Paecilomyces	51.5 a	51.2 a	43.8 c	46.7 b	48.3 a
T. pseudokononGii	37.7 d	35.5 bcde	42.7 cd	38.1 cde	38.5 de
T. konongii	39.0 cd	31.8 ef	42.2 cd	36.6 cdef	37.4 de
T. reseei	38.9 cd	36.3 bcde	39.2 cde	32.2 f	36.6 e
T. atroviride	38.6 cd	32.8 cdef	37.0 de	39.7 cd	37.0 de
T.viride	34.6 d	40.2 b	40.8 cde	38.3 cde	38.5 de
Trichoderma 3	37.5 d	40.4 b	35.4 e	39.1 cd	38.1 de
Trichoderma 4	43.7 bc	39.7 b	44.7 c	40.6 c	42.2 c
Epicoccum	28.6 e	28.2 f	29.7 f	32.8 ef	29.8 f
Rhizoctonia	16.9 f	7.4 h	16.0 g	10.8 h	12.7 h
Sepedonium	17.6 f	21.0 g	30.1 f	16.6 g	21.3 g
Gloeosporium	38.8 cd	32.7 def	52.2 b	34.6 def	39.6 d
Control	0.0 g	0.0 i	0.0 h	0.0 i	0.0 i
Mean	36.2	32.3	38.7	36.1	35.9
LSD ($P \leq 0.05$)	5.6	5.7	5.9	5.0	5.6
CV (%)	9.4	10.6	9.3	8.4	8.7

Means accompanied by different letter(s) in each column are significantly different (Duncan's multiple range test, $P \leq 0.05$).

All antagonistic fungi that were screened significantly ($P \leq 0.05$) inhibited growth of the four test pathogens (Figure 3.1). At tenth day after inoculation (DAI) all the antagonistic fungi exhibited the highest inhibition activity on the mycelial growth of phytopathogenic fungi apart from *Gloeosporium* sp. against *C. lindemuthianum* and *R. solani.* Wholesomely, the biocontrol potency increased at a higher rate between the second and sixth DAI. There was variability in sensitivity by test pathogens and also notable variation in antifungal activity over time. Among the test phytopathogenic fungi, *R. solani* was the most sensitive to the antifungal activity of antagonistic fungi during screening in two *in vitro* experiments (Figure 3.2).

Paecilomyces sp. was the most effective and compared well with *T. harzianum* while the least was *Sepedonium* sp. in inhibiting mycelia growth of *A. solani. T. harzianum* was the most effective in inhibiting mycelial growth *C. lindemuthianum, F. solani* f. sp. *phaseoli* and *R. Solani*. *Rhizoctonia* isolate was the least in activity *a*gainst *C. lindemuthianum* and *Sepedonium* sp. was least against *F. solani* f. sp. *phaseoli* and *R. solani* (Figure 3.1). *T. harzianum* was the most active but the plant pathogens varied in their sensitivity to the different antagonistic fungi. *Alternaria* sp. was the least sensitive.

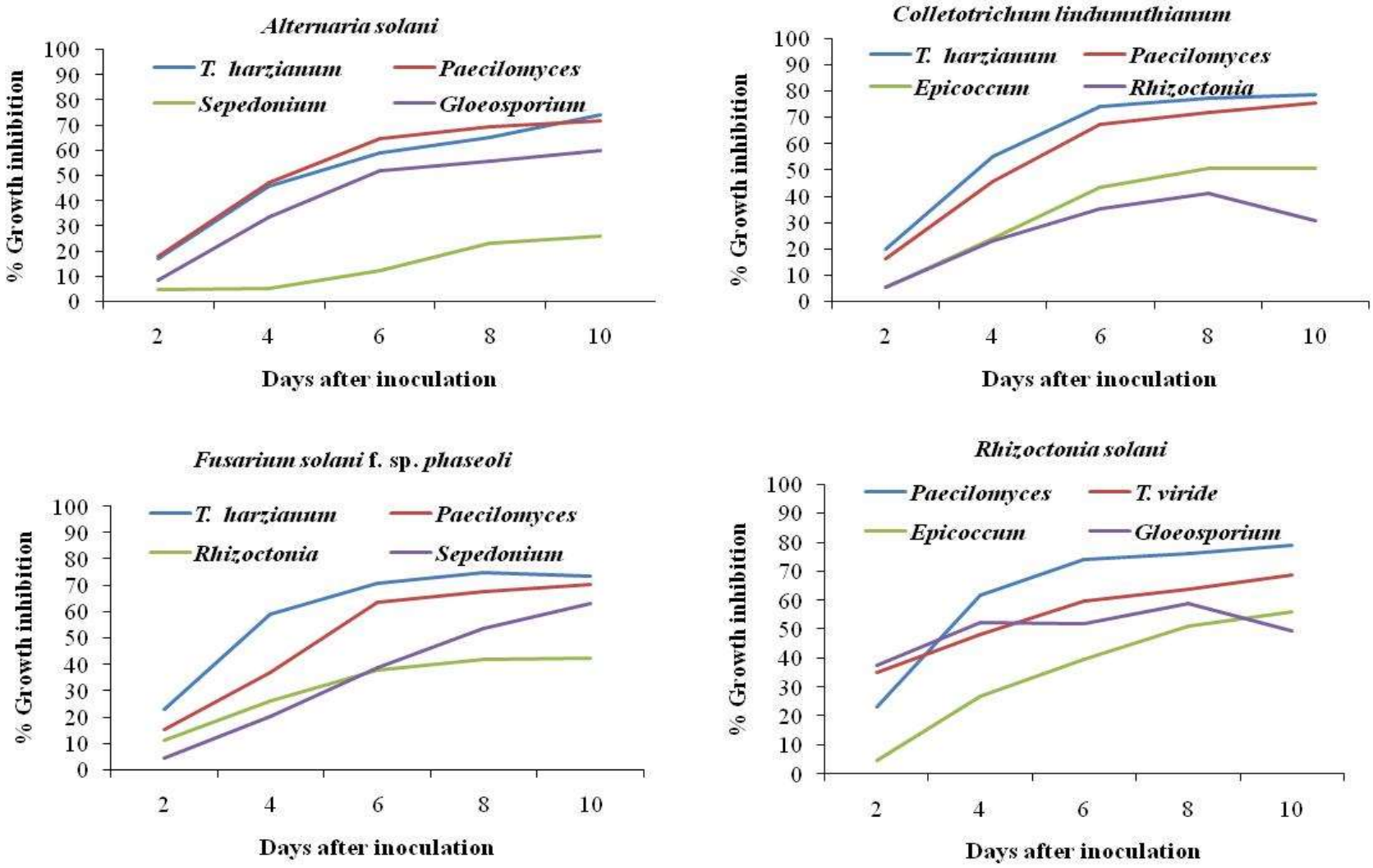


Figure 3: Percentage colony diameter inhibition of mycelial growth of phytopathogenic fungi by antagonistic fungi *in vitro*

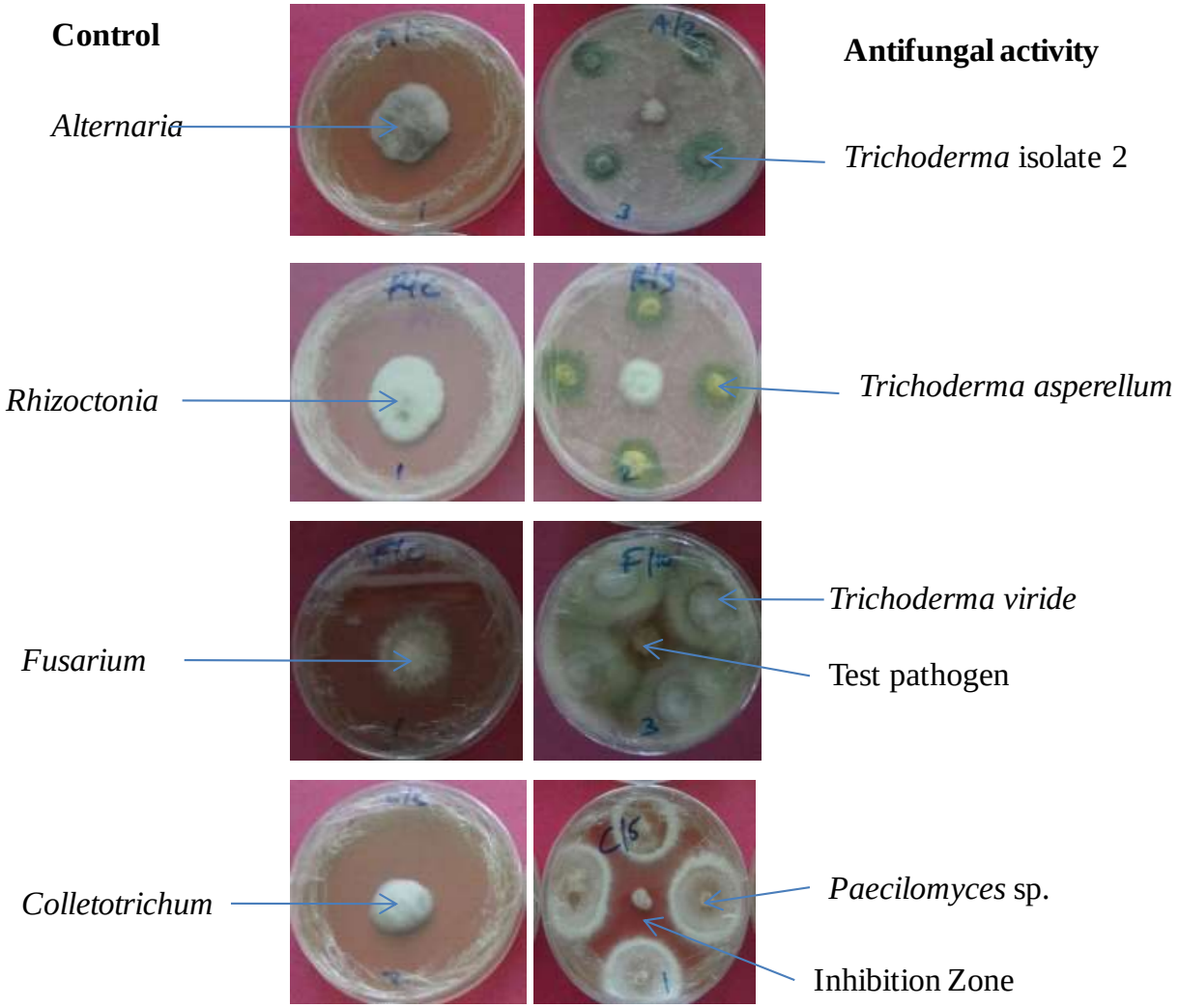


Figure 4: Antagonistic activity of some active fungal isolates against phytopathogens

3.5 Discussion

Evaluation of antimicrobial efficacy of microorganisms in *in vitro* bioassays is a sound step in developing products for crop protection. Microorganisms isolated from local sources included fungi, bacteria and actinomycetes had antagonistic activity against plant fungal pathogens namely *Fusarium solani*, *Colletotrichum lindemuthianum* and *Rhizoctonia solani*. A considerable variation was observed among, as well as within the fungal, bacterial and actinomycetous antagonists with regard to the colony diameter inhibition of the three test

pathogens at the ninth DAI. The dual culture assay by Živković *et al.* (2010) showed similar findings to those observed in this study as all antagonistic microorganisms inhibited the mycelial growth of *C. acutatum* and *C. gloeosporioides*, with varying efficiencies.

Similar findings were reported by Lahlali and Hijri (2010) that antagonistic fungi had significant antagonistic activity against *Rhizoctonia solani* when tested in an *in vitro* dual culture. In another study, similar findings were reported on microbial antagonists exhibiting varying degree of antagonistic effect against all the test pathogenic fungi (*Botryodiplodia theobromae, Alternaria porri, Fusarium oxysporum* and *Sclerotium rolfsii*) on PDA medium (Sivanantham *et al.,* 2013). The number of actinomycetes was 12% of the total microbial isolates and their antifungal activity was found to be low in this study`. This could be attributed to the type of media used as it could not fully support growth of actinomycetes.

In line with the findings, Prapagade *et al*. (2008) reported that only a few isolates of actinomycetes were antagonistic to fungi. Findings in the current study were contrary to findings by Muiru (2000) and Ara *et al.* (2012) who reported that actinomycetes isolates exhibited varying degree of antifungal activity. The antifungal activity of actinomycetes is through production of inhibitory substances, antibiotics and enzymes that degrade cell walls of fungi through lysis Kaur *et al*., 2015. The antagonistic activity of bacteria to suppress fungal pathogens is attributed to the antibiotic metabolites that may penetrate the phytopathogen cell and inhibit its activity by chemical toxicity (Devi *et al.,* 2012*;* Sivanantham *et al.,* 2013*;* Živković *et al*., 2010).

The lumping together of all groups of microorganisms did not yield satisfactory comparative results in dual culture bioassay as only fungal isolates were the most active in inhibiting fungal pathogens. Some bacteria had pronounced inhibition zones though the diameters of mycelia of test plant pathogens were bigger relative to most fungal isolates hence regarded to have lower antifungal activity. According to these results dual culture method where microbial antagonist is multiply inoculated equidistantly from the centre where the test pathogen is inoculated can only be used to compare activity of one group of microbial antagonist. The slight inconsistency of results across the two *in vitro* bioassays could be attributed to sub-culturing, storage time in refrigerator of microorganism isolates and further compounded by room temperature fluctuations. The same variables could have been the cause of shifts in degree of activity among the microorganism isolates. The difference in response to antagonistic activity by the four test pathogens was the result of variation in composition of the cell wall exhibited by these pathogens.

In dual culture screening, different antagonistic fungi isolates differed in their ability to suppress the growth of the four test pathogens, *A. solani, C. lindemuthianum, F. solani* f. sp *phaseoli* and *R. solani* over time. Variation in biocontrol potency of *Trichoderma* spp. against phytopathogens has been reported (Prasad and Rageswaram, 1999; Sarker and Sharma, 2001; Pan and Bhagat, 2007; Reddy *et al*., 2014). The different *Trichoderma* spp. also varied in antagonistic activity against different fungi an aspect reported in many studies according to findings by Muthukumar *et al.* (2011) Ramzan *et al.* (2014), Reddy *et al*. (2014), Belete *et al.* (2015) and El-Naggar *et al.* (2016).

Other species that had antagonistic activity against the test pathogens included species of *Epicoccum, Rhizoctonia, Sepedonium* and *Gloeosporium.* Kortekamp (1997) reported that *Epicoccum* had antagonistic activity against *Plasmopara viticola* that cause downy mildew fungus of grapes. The efficacy of *Epicoccum* in inhibiting mycelial growth of phytopathogens may be due to its production of numerous antifungal compounds such as flavipin (Madrigal and Melgarejo, 1994).

Paecilomyces sp. in this study showed profound effect of suppressing the four test pathogens and corroborates well with the results reported by Ramzan *et al.* (2014). In 2015, Perveen *et al* reported similar findings that *Paecilomyces* sp. was highly efficacious against *Sclerotium rolfsii* and *Pythium aphanidermatum* in dual culture bioassays. Biocontrol potency of *Paecilomyces* can be attributed to production of active metabolites that inhibit growth of other microorganisms. Another attribute of *Paecilomyces* is the capability to colonise the agar surface much faster compared to the pathogen (Muhammad and Amusa, 2003).

This status of microorganism antagonist–phytopathogen specificity may be as a result of differences in levels of hydrolytic enzymes produced by each species or isolate when they attack the mycelia of the pathogens (Reddy *et al* ., 2014). This study advocates that hyphal interaction and parasitism are subsets of mycoparasitism that were exhibited by antagonistic fungi. Mycoparasitism is a vital mechanism of antagonism of the fungal antagonist to give protection to plants against pathogen attack (Hermosa *et al.*, 2000; Howell, 2003; Reddy *et al*., 2014; Belete *et al*., 2015). The microorganism isolates used in this study variably inhibited the growth of the four phytopathogens. This corroborated findings by Riungi *et al*. (2007) who reported that

Alternaria sp., *Epicoccum* sp. and *Trichoderma* sp. reduced colony diameters of *Fusarium graminearum in vitro*. Mallikarjuna and Gowdu (2015) reported that *Gloeosporium* and *Rhizoctonia* alongside other isolates from soil exhibited antagonistic potential against *M. phaseolina* by inhibiting its mycelia growth.

All *Trichoderma* isolates in the current study were effective in suppressing the growth of test pathogens by more than 50%. *Trichoderma harzianum* had the highest antagonistic activity among all the antagonistic microorganisms relative to the control. This was in line with the findings by Soliman *et al.* (2015) who reported that among the antagonists tested, *T. harzianum* highly retarded the growth of *Botrytis cinerea* in culture. Findings in the current study were consistent with findings by Muthukumar *et al.* (2011) in regard to *Trichoderma* spp. antagonism.

Antagonistic activity of *Trichoderma* is attributed to various mechanisms key among them being antibiosis and competition. In addition studies by Muthukumar *et al* (2011) and Hermosa *et al.* (2000), indicate that *Trichoderma* spp. produce enzymes, volatile and non-volatile metabolites that degrade cell wall of pathogens and inhibit mycelia growth. The results herein were also in line with those by Reddy *et al* (2014) who reported that all the isolates of *Trichoderma* spp. were highly efficacious on the growth of *Fusarium oxysporum* f. sp. *lycopersici*, *Alternaria solani*, *Aspergillus niger* and *Macrophomina phaseolina* in *in vitro* bioassay. Muthukumar *et al*. (2011) also reported the inhibitory activity of *Trichoderma* spp. isolated from chilli rhizosphere against *Phythium aphanidermatum.*

CHAPTER FOUR: EFFICACY OF ANTAGONISTIC FUNGI AND CRUDE PLANT EXTRACTS IN MANAGEMENT OF SNAP BEAN INSECT PESTS AND DISEASES

4.1 Abstract

The use of synthetic pesticides reduces the competitiveness of Kenyan snap bean pods due to stringent regulations by importers as a result of presence of chemical residues. This study was conducted to determine the effectiveness of selected local antagonistic fungi and plant extracts in managing insect pests and diseases of snap beans. Field experiments were set up in farmer's field for two cropping seasons. *Trichoderma viride, T. harzanium, T. asperellum, Paecilomyces* sp., turmeric, garlic, ginger and lemon were evaluated. These treatments were applied weekly as foliar sprays and their efficacy was compared to that of commercial formulations of Dithane M-45®, Confidor 70 WG®, Trianum® (*Trichoderma*) and Achook® (neem**).** Crude plant extracts had higher efficacy in reducing the population of insect pests than antagonistic fungi. The crude plant extracts reduced the population of whiteflies and thrips by up to 58% and 41% while antagonistic fungi had a corresponding 30% and 18% reduction, respectively. *Trichoderma* spp. reduced severity of angular leaf spot, rust and anthracnose by up to 37.5%, 67% and 20.7%, respectively. The crude plant extracts and antagonistic fungi increased marketable pod yield by 25.6% and 17.3%, respectively. The results demonstrated that local local microbial antagonists and plant-based compounds are effective in managing pests and diseases and their use will reduce chemical residues enabling the local snap beans producers' access the export markets.

Key words: Biopesticides, maximum residue limits (MRLs), snap beans, synthetic pesticides

4.2 Introduction

Antagonistic microorganisms and plant extracts have been found effective and sustainable in management of pests and diseases in other vegetables (Killani *et al*., 2011; Srinivasan, 2012). Among the antagonistic fungi utilized as bioagents include *Trichoderma* spp., and *Paecilomyces* spp. *Trichoderma* spp. produce diffusible and volatile antibiotics plus hydrolytic enzymes which are the crucial mechanisms behind their biocontrol activity (Navaneetha *et al.*, 2015). *Paecilomyces* as entomopathogenic fungi is reported to suppress several insects as a foliar spray though with varying efficacies (Archana and Ramaswamy, 2012).

Plant extracts are reputed to constitute various bioactive compounds and secondary metabolites like alkaloids, flavonoids and phenolic compounds. These substances possess synergistic effects on retarding growth of pests and suppression of pathogens hence act as insecticidal and antifungal agents (Ahmed *et al*., 2012). Plant extracts have been successfully applied against insect pests and diseases (Shahid *et al.*, 2015). In Kenya formulated products of neem and pyrethrum are currently used whereas a mixture of garlic and pepper has been recommended to manage insect pests (Infonet-Biovision, 2015; Ogala, 2013). In a study by Waiganjo *et al*., (2011), neem oil extracts, Achook® and Nimbecidine® suppressed the population of aphids and diamondback moth in cabbage.

Crude extract of garlic bulb contain allicin (Wei *et al.*, 2011) while the rhizome of turmeric contain curcuminiods (60% curcumin) all responsible for insecticidal and antimicrobial properties (Ahmed *et al*., 2013). This study investigated the effectiveness of local antagonistic microorganisms and crude plant extracts against major pests and diseases of snap bean as

alternatives to synthetic pesticides for reduced chemical residues in the produce destined for niche markets.

4.3 Materials and Methods

4.3.1 Description of the experimental site

The field study was carried out in Mwea, Kirinyaga County, which is a major snap bean growing area in Kenya. Mwea Division is in Kirinyaga South District, Kirinyaga County. The experimental site is in lower midland zone 4 (LM4) and the types of soils are the nitosols. The soils have good water holding capacity, aeration and dark reddish brown colour. The average rainfall in Mwea is about 850 mm with a range of 500 - 1250 mm per annum divided into long rains (March – June with an average of 450 mm) and short rains (Mid-October to December with an average of 350 mm). The rainfall is characterized by uneven distribution in total amounts, time and space. Since production of snap beans is all year round, farmers depend on both rainfall and irrigation. The temperature ranges from 15.6º C to 28.6º C with a mean of about 22ºC which meets the ecological requirements for snap bean production (Kamanu *et al*., 2012).

4.3.2 Multiplication of microbial antagonists and preparation of inoculum

The selected antagonistic microorganisms in slants of PDA stored at four degrees were thawed before mass multiplication. Sorghum was used as a substrate for mass multiplication since it was locally available and has shown good performance as a substrate (Upadhyay and Mukhopadhyay, 1986; Singh *et al*., 2014; Kumar *et al.*, 2014). Two hundred grams of sorghum grains were placed in 1000 ml flasks containing 400 ml of distilled water and then half boiled for 20 minutes and cooled. The boiled sorghum was mixed thoroughly with the help of a glass rod until all the substrate particles were evenly moistened and no lumps were present.

The sorghum grains in flasks were autoclaved at 121^{o} C for 20 minutes and left to cool. Re-autoclaving was done after one day before inoculating with mycelia agar plugs. The cool autoclaved sorghum was aseptically transferred into strong polythene sleeves. Five mycelial agar plugs having a diameter of 10 mm of each of the four antagonistic fungi were inoculated in polythene sleeves with cooled autoclaved sorghum under sterile conditions in the laminar flow hood. The contents were then shaken well to evenly disperse the inocula before incubation at room temperature (23 ± 2 ºC) for 14 days. During the incubation, the contents of the polythene sleeves were shaken once after two days to prevent aggregation and to improve aeration.

Conidia of each isolate were harvested by flooding the substrate having fungal growth with sterile distilled water. Spore suspension was obtained by filtering the flooded substrate with antagonistic fungi through two layers of cheesecloth. The spore concentration recorded on this substrate was between 43.1 x 10^{8} CFU/ ml and 68.3 x 10 CFU/ ml. Tween 20 at 0.05 % was added to conidial suspension and standardized by serial dilution with the aid of a haemocytometer to 1×10^{8} conidia ml^{-1} for each antagonistic fungus. The conidia suspension for each antagonist was prepared in the same way as during standardization. One drop of 0.05% Tween 20 (polyoxyethylene sorbitol esteris) surfactant was added into conidia suspensions and was agitated to have even dispersion of conidia and used as foliar sprays.

4.3.3 Preparation of plant extracts

Fresh turmeric rhizome, garlic corm, ginger rhizome and lemon fruit each weighing 100 g were finely blended separately and extracted with 95% ethanol measuring 500 ml. The ethanol extracts was concentrated by vacuum evaporation and 10 ml of each crude plant extract was

transferred to a universal bottle. Ten ml of each of the extract was added into five litres of water having one drop of 0.05% Tween 20 and used as foliar sprays. The concentration used in this study was arrived at after conducting preliminary test on effectiveness and effect on plants.

4.3.4 Field experiment layout, design and agronomic practices

Field experiments were set up in farmer's field in Mwea, Kirinyaga County for two cropping seasons under irrigation. The first cropping season was between November, 2015 to January, 2016 while the second was between March to May, 2016. Samantha, snap bean variety that is only resistant to anthracnose (Race Lambda) and bean Common Mosaic Virus was planted. The field was divided into plots measuring 3 metres by 3 metres with 1 metre alleys between the plots and 1 M alleys between the blocks. The crop was planted in single rows with spacing of 15 cm x 30 cm in each plot. Di-ammonium phosphate (DAP) at a rate of 200Kg/ha was mixed well with soil before placement of seeds. First weeding was carried out two weeks after emergence (WAE) while second weeding was at fifth WAE. Calcium ammonium nitrate (CAN) was applied at a rate of 100Kg/ha at flowering stage. Furrow irrigation was applied twice per week in absence of rain during the two cropping seasons.

The treatments included antagonistic fungi and plant extracts which were compared to commercial formulated biopesticides and commonly used synthetic pesticides. These were as follows: i) *Trichoderma viride*, ii) *T. harzianum*, iii) *T. asperellum, iv) Paecilomyces, v)* Turmeric, vi) Garlic, vii) Ginger, viii) Lemon, ix) Dithane M-45® and Confidor 70 WG®, x) Trianum® (commercial *Trichoderma*), xii) Achook® (commercial neem**)**, and xii) Control (sprayed with water).

Antagonistic fungi and plant extracts were applied as foliar sprays as outlined under section 4.3.2 and 4.3.3 respectively. Commercial neem, Achook® was applied at a rate of 20 ml in 20 Litres of water. Commercial *Trichoderma* (Trianum®) was applied at a rate of 5g in 5 litres of water having a concentration of 1×10^8 spores/ml**.** However, synthetic fungicide, Dithane M-45® (Mancozeb 80% m/m) was applied at a rate of 50gm in 20 Litres of water. The synthetic insecticide, Confidor® WG 70 (imidacloprid 700g/Kg) was applied at a rate of 5g in 20 Litres of water and control plots were sprayed with water. Synthetic pesticides, Dithane M-45® and Confidor® 70 WG were alternatively applied. The treatments were replicated three times and the experiment was laid out in a randomized complete block design (RCBD). Treatment application was from one week after emergence at one week interval for seven weeks.

4.3.5 Assessment of pest infestation

The main pests assessed in the trials were whiteflies and thrips. The assessment of the pests was conducted at early hours of the day as reported by El-Shafie and Abdelraheem (2012). The population of whiteflies was determined by counting the number of nymphs on lower surface of the leaf. Nymphs on ten lower leaves were counted from ten plants that were tagged in a zigzag manner from inner rows of each plot (Wafula, 2014). This was carried out five times at third, fourth, fifth, sixth and seventh week after emergence (WAE).

The assessment of population of thrips was carried out three times fifth, sixth and seventh WAE. Ten flowers each from ten randomly tagged plants from inner rows per plot were harvested. The harvested flowers were kept in 70% ethanol. Each flower was placed in a Petri dish, dissected and washed with water making sure that no thrips was lost with the debris (Nderitu, *et al*., 2008).

Adult and nymph thrips were observed under dissecting microscope and counted with aid of a tally counter (Wafula, 2014).

4.3.6 Assessment of disease distribution, incidence and severity

Evaluation was done on the distribution, incidence and severity of angular leaf spot, rust and anthracnose from second to seven WAE. Distribution of disease was done by observing how each disease was spread in the whole field using a 0-2 distribution scale; where 0 = No disease, 1 = spots, 2 = whole plot (Muthomi, Personal communication). Assessment of incidence of disease involved counting diseased plants that showed symptoms of each disease and the percentage of disease incidence calculated according to the formula by Wheeler (1969):

$$\text{Percentage disease incidence} = \left[\frac{\text{Number of infected plants}}{\text{Total number of plants}}\right] 100$$

Assessment of severity was based on a scale of 0-5 (Stavely, 1985); where 0 = no disease, 1 = < 20%, 2 = 21-40%, 3 = 41-60%, 4 = 61-80%, 5 = 81-100% leaf area infected. Ten plants were randomly sampled and tagged from three inner rows in each plot by scoring three trifoliate leaves sampled at bottom, middle and top of each plant (Wahome *et al*., 2011).

Area under disease progress curve (AUDPC) was computed from the mean severity scores of data recorded at each assessment day at seven days interval as described by Shaner and Finney (1977) as follows;

$$\text{AUDPC} = \sum_{i=1}^{n-1} [(Y_{i+1} + Y_i)/2] (X_{i+1} - X_i)$$

Where, Yi = disease severity at ith assessment, Xi = number of days after inoculation at the ith assessment and n = total number of assessments.

Total disease indices were computed using scores of distribution, incidence and severity. Percentage disease index was computed using the formula modified from McKinney (1923):

$$\text{Percentage disease index} = \frac{\text{Distribution score} + \text{incidence score} + \text{severity score}}{2(\text{distribution}) + 1(\text{incidence}) + 5(\text{severity})} \times 100$$

4.3.7 Assessment of yield and yield components

Harvesting of fresh pods was done once a week for three weeks in whole plots. Fresh pods harvested per plot were graded into two major categories namely marketable and non-marketable. The category of marketable was graded into extra fine and fine where the attributes for extra fine were a width of 6 mm and the fine - width between 6 mm to 8 mm (Wahome *et al*., 2011). The fresh weight of each of the grades per plot was determined in grams at every harvest. Unmarketable pods were separated into thrips damage, disease damage, deformed, overgrown and other damages and their weight taken. The pod yield was converted to yield per hectare according to Wahome *et al.* (2013).

4.3.8 Data processing and analysis

Preliminary data summaries were generated using Microsoft Excel Spreadsheet. Data on insect pest population, disease incidence, disease severity, area under disease progress curve (AUDPC), disease index and yield parameters was subjected to analysis of variance using Genstat®, Release15.1. Mean separation of the treatments was accomplished using Fisher's protected Least Significant Difference (Steel *et al.*, 1999).

4.4 Results

4.4.1 Effect antagonistic fungi and crude plant extracts on insect pest population

The fungal antagonists and crude plant extracts significantly ($P \leq 0.05$) reduced the population of whiteflies on snap bean (Tables 4.1; 4.2) compared to the control during the two cropping seasons. Plant extracts however had a significantly ($P \leq 0.05$) higher efficacy in reducing the population of whitefly compared to the fungal antagonists. Crude plant extracts compared well with commercial neem formulation, Achook 0.15EC® and alternate application of synthetic pesticides, Dithane M-45® and Confidor® 70 WG. During the March-May 2016 cropping season, spray application of crude plant extracts were as effective as formulated neem (Achook 0.15EC®) and more efficacious than other treatments (Table 4.2). Population of whiteflies during the November 2015-January 2016 cropping season was higher than the March- May, 2016 cropping season.

Crude plant extracts had significantly ($P \leq 0.05$) higher efficacy in reducing population of thrips compared to the fungal antagonists. The crude plant extract sprays compared well with commercial neem formulation Achook 0.15EC® and alternate application of synthetic pesticides, Dithane M-45® and Confidor® 70 WG. The activity of antagonistic fungi in reducing population of thrips compared well with Trianum® (Commercial *Trichoderma*). The crude plant extracts and antagonistic fungi reduced the thrips infestation by up to 45.3% and 19.1% respectively during the November, 2015-January, 2016 cropping season.

Table 4.1: Number of whiteflies per leaf on snap beans sprayed with different antagonistic fungi and crude plant extracts during the November, 2015- January, 2016 cropping season

Treatment	Weeks after emergence					Mean	% Reduction
	3	4	5	6	7		
T. viride	63.7 abc	52.0 bc	64.3 b	43.3 abc	26.0 ab	44.6 b	22.4
T. harzianum	54.7 abc	52.3 bc	50.3 bcd	52.3 ab	31.0 a	44.6 b	22.4
T. asperellum	55.7 bcd	47.0 bcd	56.0 bc	48.0 ab	24.3 ab	42.8 b	25.5
Paecilomyces	67.3 ab	56.0 ab	49.0 bcd	49.3 ab	24.0 ab	40.8 b	29.0
Turmeric extract	52.7 cde	40.3 d	31.7 de	24.3 d	18.3 bcd	24.8 cde	56.9
Garlic extract	56.7 bcd	27.0 e	28.7 e	26.3 d	13.7 cd	22.9 def	60.1
Ginger extract	43.3 ef	30.0 e	23.7 e	23.3 d	13.3 cd	20.1 eg	65.0
Lemon extract	49.7 de	39.7 d	32.0 de	33.0 cd	13.0 d	26.0 cde	54.7
Dithane® + Confidor®	34.7 f	42.0 cd	40.3 cde	44.7 cd	10.3 d	31.8 cd	44.7
Trianum®	63.7 abc	51.0 bc	60.7 b	40.3 bc	22.7 abc	41.2 b	28.2
Achook®	56.0 bcd	45.0 cd	32.7 de	41.0 bc	17.3 bcd	30.3 c	47.2
Control	73.7 a	64.3 a	85.3 a	57.0 a	30.0 a	57.4 a	0.0
LSD ($P \leq 0.05$)	11.1	9.7	17.2	13.1	8.6	7.9	
CV (%)	11.8	12.5	22.0	19.3	24.9	13.1	

Means accompanied by different letter(s) in each column are significantly different (Fisher's protected Least Significant Difference, $P \leq 0.05$)

Table 4.2: Number of whiteflies per leaf on snap beans sprayed with different antagonistic fungi and crude plant extracts during the March-May, 2016 cropping season

Treatment	Weeks after emergence					Mean	% Reduction
	3	4	5	6	7		
T. viride	6.4 abc	5.2 b	6.7 bc	4.3 c	8.0 bc	6.1 bc	20.6
T. harzianum	5.5 cde	5.2 b	6.0 cd	5.2 ab	7.7 bc	5.9 cd	23.2
T. asperellum	5.6 bcd	4.7 bcd	5.6 cd	4.8 bc	6.7 cd	5.5 de	29.0
Paecilomyces	6.7 ab	4.3 bcd	4.9 de	4.3 c	6.0 de	5.2 e	31.9
Turmeric extract	5.3 cde	4.0 cd	2.9 fg	2.4 d	4.0 fg	3.7 fg	51.7
Garlic extract	5.7 bcd	2.7 f	2.9 fg	2.8 d	5.0 ef	3.8 fg	50.5
Ginger extract	4.3 ef	3.0 ef	2.6 fg	2.5 d	6.0 de	3.7 fg	51.9
Lemon extract	5.0 de	4.0 de	3.5 f	3.3 d	6.0 de	4.3 f	43.6
Dithane® + Confidor®	3.5 f	4.2 bcd	4.0 ef	4.5 bc	5.3 def	4.3 f	44.2
Trianum®	6.4 abc	5.1 bc	7.9 ab	4.9 bc	9.0 ab	6.6 b	13.7
Achook®	5.6 bcd	4.5 bcd	1.8 g	3.2 d	2.7 g	3.6 g	53.7
Control	7.4 a	6.4 a	8.5 a	5.7 a	10.3 a	7.7 a	0.0
LSD ($P \leq 0.05$)	1.1	1.0	1.3	0.4	1.4	0.6	
CV (%)	11.8	13.0	16.7	11.6	12.8	7.0	

Means accompanied by different letter(s) in each column are significantly different (Fisher's protected Least Significant Difference, $P \leq 0.05$)

Trichoderma spp. reduced the population of thrips by up to 10.5% while *Paecilomyces* sp. 19.9% during the March- May, 2016 cropping season and with up to 28.8% corresponding reduction by crude plant extracts. Synthetic pesticides, Dithane M-45® and Confidor® 70 WG had the highest reduction of thrips population of up to 35.7% followed by garlic extract (31.5%) and Achook® (31.1%) during the second season. However, the population of thrips was higher during the March- May, 2016 cropping season.

Table 4.3: Number of thrips in 10 flowers on snap beans sprayed with different antagonistic fungi and plant extracts for two seasons

Treatment	November, 2015-January, 2016 cropping season					March- May, 2016 cropping season				
	Weeks after emergence			Mean	% Reduction	Weeks after emergence			Mean	% Reduction
	5	6	7			5	6	7		
T. viride	18.0 abc	14.3 bc	70.3 ab	34.2 bcd	23.3	78.3 b	37.3 b	35.3 cd	50.3 c	12.8
T. harzianum	20.0 ab	18.3 bc	71.0 ab	36.4 b	18.4	84.3 b	38.0 b	37.3 bc	53.2 b	9.9
T. asperellum	17.3 abc	17.3 bc	70.0 ab	34.9 bc	21.7	91.3 a	36.0 bc	35.3 cd	54.2 b	8.9
Paecilomyces	19.7 ab	23.7 ab	67.7 bc	37.0 b	17.0	65.7 c	32.3 cd	31.7 de	43.2 d	19.9
Turmeric extract	19.7 ab	19.3 b	52.7 de	30.6 cde	31.4	53.7 d	24.7 e	24.7 f	34.3 f	28.8
Garlic extract	10.7 c	14.3 bc	42.3 e	22.4 f	49.8	47.0 d	22.7 e	25.0 f	31.6 f	31.5
Ginger extract	17.7 abc	15 bc	56.3 cd	29.7 de	33.4	53.3 d	29.0 d	29.7 ef	37.3 e	25.8
Lemon extract	12.7 bc	15.7 bc	58 bcd	28.8 e	35.4	51.7 d	22.3 e	28.7 ef	34.2 f	28.9
Synthetics	11.0 c	8.7 c	20.3 f	13.3 g	70.2	48.3 d	17.7 f	16.3 f	27.4 g	35.7
Trianum®	21.3 a	16.7 bc	71.3 ab	36.4 b	18.4	78.0 b	38.0 b	41.0 b	52.3 bc	10.8
Achook®	16.0 abc	14.7 bc	49.0 de	26.6 ef	40.4	53.3 d	23.3 e	19.3 g	32.0 f	31.1
Control	22.0 a	28.3 a	83.3 a	44.6 a	0.0	91.3 a	46.3 a	51.7 a	63.1 a	0.0
LSD ($P \leq 0.05$)	6.9	8.4	12.4	4.5		6.3	3.9	4.8	2.6	
CV (%)	23.8	28.8	12.4	8.5		5.6	7.6	9.0	3.6	

Means accompanied by different letter(s) in each column are significantly different (Fisher's protected Least Significant Difference, $P \leq 0.05$). (Synthetics: Dithane M-45® and Confidor® 70 WG).

4.4.2 Effect antagonistic fungi and crude plant extracts on snap bean diseases

The antagonistic fungi and crude plant extracts had effect against incidence, severity and disease index of angular leaf spot, rust and anthracnose on snap beans (Figure 4.1). Both the fungal antagonists and plant extracts significantly ($P \leq 0.05$) reduced severity of angular leaf spot, rust and anthracnose in snap bean (Table 4.4). Antagonistic fungi were more effective in reducing incidence of the three foliar fungal diseases than crude plant extracts and compared well with formulated *Trichoderma* (Trianum®). *Trichoderma harzianum* was the second most effective after synthetic pesticides, Dithane M-45® and Confidor® 70 WG (36.4%) in reducing angular leaf spot in snap beans by up to 27.5% while turmeric extract was the most efficacious crude plant extract by up to 17%.

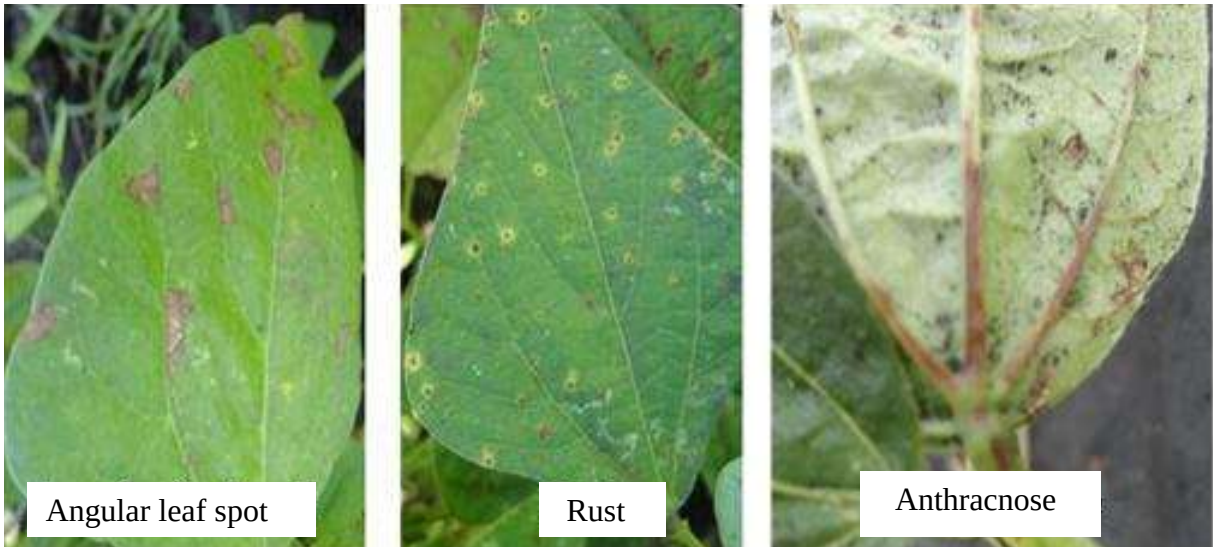


Figure 5: Symptoms of economically important diseases of snap beans that were assessed

Trichoderma viride was the most effective in reducing severity of angular leaf spot by up to 43.3% during the March- May, 2016 cropping season. The corresponding reduction in incidence of angular leaf spot was up to 22.4% and 50% by turmeric extract and alternate application of synthetic pesticides, Dithane M-45® and Confidor® 70 WG, respectively. Among the antagonistic fungi, *T. viride* was the most efficacious in reducing the incidence of rust by up to 77.5% and 75.1% during the two seasons, respectively (Figure 4.4). However, all the *Trichoderma* spp. performed better than alternate application of synthetic pesticides, Dithane M-45® and Confidor® 70 WG in reducing the incidence of rust. Similar results were recorded during the March-May, 2016 cropping season. Ginger extract was the most efficacious in reducing incidence of rust by up to 63.3% among the crude extracts during the first cropping season while turmeric (45.6%) and ginger (44.4%) during the second cropping season. The efficacy of lemon extract and Achook® were comparable in reducing incidence of rust.

The application of antagonistic fungi had a profound impact and compared well with alternate application of synthetic pesticides, Dithane M-45® and Confidor® 70 WG in the incidence of anthracnose in snap bean. Among the crude plant extracts, turmeric was the least in efficacy and comparatively similar to Achook® in reducing incidence of anthracnose *Trichoderma viride* and garlic extract reduced the incidence of anthracnose by up to 60.1% and 33.3%, respectively (Figure 4.4).

Table 4.4: Incidence (%) of angular leaf spot, rust and anthracnose on snap beans sprayed with different antagonistic fungi and plant extracts for two seasons

	November, 2015-January, 2016 cropping season			March-May, 2016 cropping season		
Treatment	ALS	Rust	Anthracnose	ALS	Rust	Anthracnose
T. viride	61.1 cd	5.8 f	15.8 g	37.8 h	4.2 f	14.2 g
T. harzianum	59.2 d	7.2 ef	18.6 ef	39.2 gh	5.3 ef	17.8 f
T. asperellum	60.3 d	7.2 ef	19.4 def	42.8 f	4.7 ef	18.6 f
Paecilomyces	66.7 b	8.3 ef	21.7 cde	49.4 e	7.2 de	21.9 e
Turmeric extract	67.8 b	11.4 d	26.7 b	51.7 de	9.2 cd	27.5 d
Garlic extract	69.6 b	14.2 c	22.2 cd	54.4 cd	10.0 c	25.8 d
Ginger extract	69.9 b	9.4 de	22.2 cd	56.7 c	9.4 cd	30.0 c
Lemon extract	80.1 a	16.4 bc	23.6 bc	55.8 c	13.9 b	33.1 b
Dithane® + Confidor®	51.9 e	8.1 ef	16.5 fg	33.3 i	5.8 ef	15.3 g
Trianum®	60.4 d	8.9 e	22.2 cd	41.7 fg	5.6 ef	18.6 f
Achook®	66.9 bc	16.9 b	26.0 b	60.8 b	13.6 b	30.0 c
Control	81.7 a	25.6 a	33.3 a	66.7 a	16.9 a	35.6 a
LSD ($P \leq 0.05$)	5.6	2.3	3.1	2.9	2.4	2.4
CV (%)	5.0	11.7	8.3	3.5	15.9	6.0

Means accompanied by different letter(s) in each column are significantly different (Fisher's protected Least Significant Difference, $P \leq 0.05$).

The antagonistic fungi and crude plant extracts significantly ($P \leq 0.05$) reduced the severity of angular leaf spot, rust and anthracnose when applied on snap beans under field conditions (Table 4.5). There was great variation in efficacy among the treatments with *Trichoderma* spp. being more efficacious in managing rust and anthracnose. However, alternate application of synthetic pesticides had the highest efficacy in managing angular leaf spot. Rust was more sensitive in response to foliar application of antagonistic fungi and crude plant extracts compared to other diseases.

Trichoderma spp. reduced severity of ALS by up to 35% and 37.5% during the two seasons, respectively (Table 4.5). *Trichoderma* spp. compared well with alternate application of synthetic pesticides that reduced severity of ALS by up to 38.8% during November, 2015-January, 2016 cropping season. Among crude plant extracts, ginger and lemon did not have significant ($P \geq 0.05$) effect on angular leaf spot when compared to the untreated plots during the November, 2015-January, 2016 cropping season. *Trichoderma* spp. significantly reduced rust development by 67% while crude extracts, Trianum® and Achook® by 39%, 45% and 27% respectively. Among the crude plant extracts, Turmeric was the most efficacious on rust with a reduction of 49%.

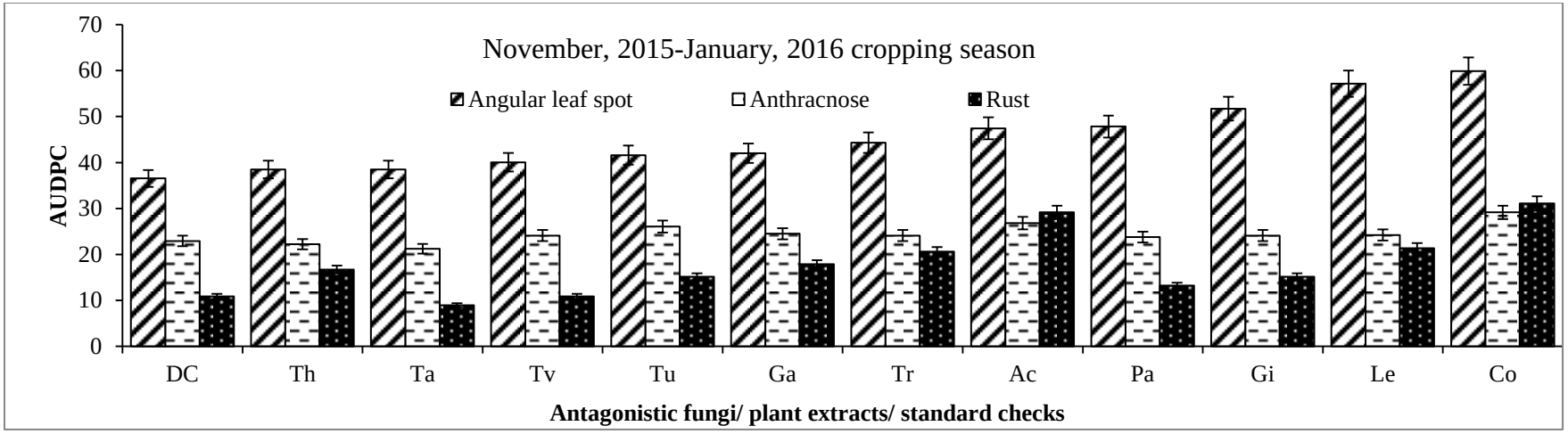
During the November, 2015-January, 2016 cropping season, the highest reduction (by up to 20.7%) in severity of anthracnose was by *T. asperellum* while the least was by Achook® (8.2%) (Table 4.5). There were no marked differences in severity of anthracnose on snap bean plots that received antagonistic fungi and alternate spray application of synthetic pesticides during the March-May cropping season from the preceding season though severity was higher than the first cropping season.

Table 4.5: Severity of angular leaf spot, rust and anthracnose on snap beans sprayed with different antagonistic fungi and crude plant extracts for two seasons

	November, 2015-January, 2016 cropping season			March-May, 2016 cropping season		
Treatment	ALS	Rust	Anthracnose	ALS	Rust	Anthracnose
T. viride	1.2 cde	0.3 f	0.9 bc	1.0 f	0.2 g	0.8 cd
T. harzianum	1.2 cde	0.4 f	0.8 c	1.0 f	0.3 fg	0.8 bcd
T. asperellum	1.1 de	0.4 f	0.8 c	1.0 f	0.4 f	0.9 bcd
Paecilomyces	1.4 cde	0.4 f	0.9 bc	1.2 de	0.5 e	1.0 b
Turmeric extract	1.2 cde	0.5 d	0.9 bc	1.2 de	0.5 e	1.0 b
Garlic extract	1.3 cde	0.8 b	0.9 bc	1.2 cd	0.6 cd	1.0 b
Ginger extract	1.3 bc	0.7 bc	0.9bc	1.3 bc	0.7 bc	1.3 a
Lemon extract	1.7 ab	0.7 bc	0.9 bc	1.3 b	0.7 bc	1.2 a
Dithane® + Confidor®	1.1 e	0.3 f	0.8 c	1.0 f	0.3 fg	0.8 d
Trianum®	1.3 cde	0.6 c	0.9 bc	1.1 e	0.5 e	0.9 bcd
Achook®	1.4 bcd	0.8 b	0.9 bc	1.4 b	0.9 a	1.2 a
Control	1.8 a	1.1 a	1.1 a	1.6 a	1.0 a	1.4 a
LSD ($P \leq 0.05$)	0.3	0.1	0.1	0.1	0.1	0.2
CV (%)	12.4	13.5	6.8	5.0	10.8	9.3

Means accompanied by different letter(s) in each column are significantly different (Fisher's protected Least Significant Difference, $P \leq 0.05$).

Area under disease development curve (AUDPC) values showed that angular leaf spot, anthracnose and rust development was significantly affected by the application of antagonistic fungi and plant extracts. The data on AUDPC showed significant differences ($P \leq 0.05$) among antagonistic fungi and plant extracts. During the November, 2015-January, 2016 cropping season *Trichoderma asperellum* reduced development of anthracnose and rust by 27.1% and 71.3% respectively while *Trichoderma harzianum* reduced angular leaf spot by35.7%. During the March-May, 2016 cropping season *Trichoderma viride* reduced development angular leaf spot and rust by 37.5% and 76.9% respectively while *Trichoderma asperellum* reduced anthracnose by 40.6%. Among the crude plant extracts, turmeric and garlic resulted in the least AUDPC value during both cropping seasons (Figure 4.2).



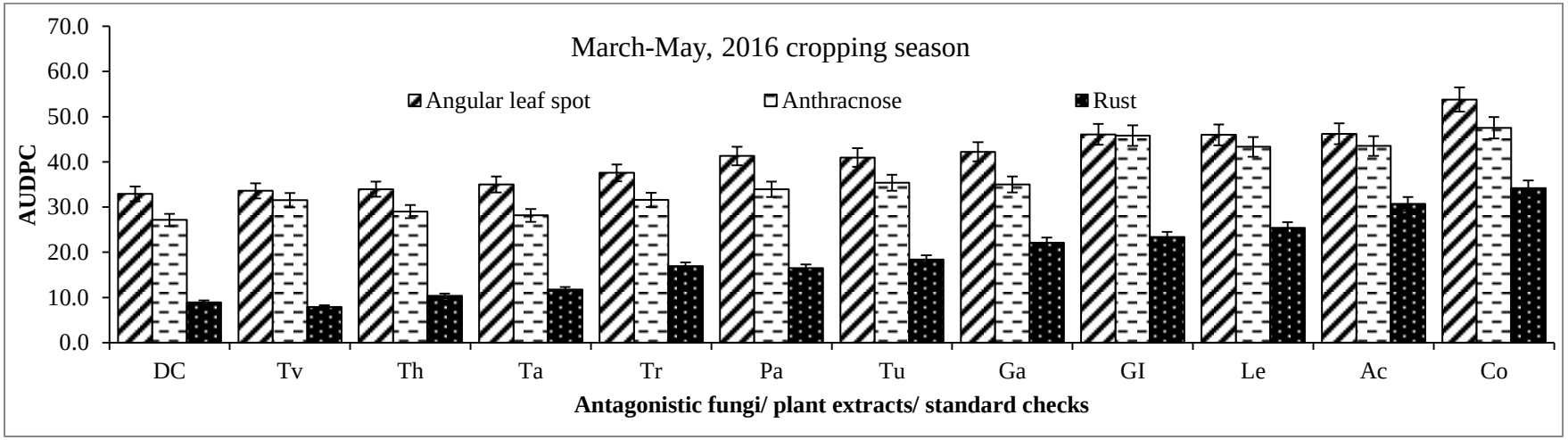


Figure 6; AUDPC of angular leaf spot, anthracnose and rust on snap beans sprayed with different antagonistic fungi and crude plant extracts. Tv-*T.viride*, Th-*T. harzianum*, Ta-*T. asperellum*, Ps-*Paecilomyces*, Tu- Turmeric, Ga-Garlic, Gi-Ginger, Le-Lemon, DC-Dithane® + Confidor®, Tr-Trianum®, Ac-Achook® and Co- Control).

Antagonistic fungi compared well with alternate application of synthetic pesticides, Dithane M-45® and Confidor® 70 WG and commercial *T. harzianum* (Trianum®) in reducing anthracnose (Table 4.6). *T. viride* had similar efficacy level with alternate application of synthetic pesticides, Dithane M-45® and Confidor® 70 WG in suppressing rust. *T. viride* compared well with commercial *T. harzianum* (Trianum®) in suppressing angular leaf spot. Among the plant extracts, turmeric and garlic were superior and had the least disease indices in both seasons and were more efficacious than Achook®.

Trichoderma viride reduced disease index of rust and anthracnose by up to 47.3% and 22.7%, respectively while *T. harzianum* reduced disease index of angular leaf spot by up to 22.2% during the November, 2015-January, 2016 cropping season (Table 4.6). Turmeric reduced the disease index of angular leaf spot and rust by up to 17.8% and 33.5%, respectively while ginger reduced disease index of anthracnose by up to 14.9% during the same season. During the March-May, 2016 cropping season, *T. viride* reduced disease index of angular leaf, rust and anthracnose by 36.7%, 50.9% and 33.3%, respectively while the corresponding reduction of angular leaf spot and rust by turmeric extract was up to 17%, and 24.2% with garlic reducing disease index of anthracnose by 23%.

Table 4.6: Percentage disease index on snap beans sprayed with different antagonistic fungi and crude plant extracts for two cropping seasons

Treatment	November, 2015-January, 2016 cropping season			March-May, 2016 cropping season		
	ALS	Rust	Anthracnose	ALS	Rust	Anthracnose
T. viride	42.1 def	12.9 f	25.1 f	32.1 fg	13.2 h	24.4 e
T. harzianum	40.7 fg	14.2 ef	25.0 f	33.9 f	15.5 gh	25.1 de
T. asperellum	40.9 efg	14.0 ef	25.1 f	37.3 e	16.2 fgh	26.2 cde
Paecilomyces	45.4 cde	15.1 ef	26.7 def	41.7 d	19.3 def	27.3 cd
Turmeric extract	43.0 def	16.3 de	29.7 b	42.0 d	20.4 cde	28.4 c
Garlic extract	43.9 cde	20.7 b	28.2 bcd	42.9 cd	21.7 cd	28.2 c
Ginger extract	47.9 bc	18.0 cd	27.4 cde	45.0 bc	22.2 bcd	34.0 b
Lemon extract	51.0 ab	19.7 bc	28.2 bcd	44.4 bc	23.5 bc	33.6 b
Dithane® + Confidor®	37.1 g	13.0 f	25.2 f	29.9 g	15.4 gh	23.9 e
Trianum®	42.7 def	16.6 de	25.7 ef	37.1 e	18.2 efg	26.0 cde
Achook®	46.1 cd	21.1 b	28.9 bc	45.9 b	25.2 ab	33.2 b
Control	52.3 a	24.5 a	32.2 a	50.6 a	26.9 a	36.6 a
Mean	44.4	17.2	27.3	40.0	19.8	28.9
LSD ($P \leq 0.05$)	4.1	2.4	1.7	2.2	3.1	2.5
CV (%)	5.4	8.4	3.7	3.2	9.4	5.2

Means accompanied by different letter(s) in each column are significantly different (Fisher's protected Least Significant Difference, $P \leq 0.05$).

4.4.3 Effect antagonistic fungi and crude plant extracts on yield of snap beans

The yield parameters on which the efficacy of antagonistic fungi and crude plant extracts was evaluated included the number of pods, grades of marketable pods and grades of nonmarketable pods (Figure 4.3). The crude plant extracts significantly ($P \leq 0.05$) increased the number of pods/ plant (Table 4.7) more than antagonistic fungi. Crude plant extracts effect on number pods /plant was comparable to alternate application of synthetic pesticides Dithane M-45® and Confidor® 70 WG and Achook®. *Paecilomyces* was the only treatment that resulted in a decrease in total pod

yield relative to control. Crude plant extracts and antagonistic fungi increased extra-fine pod yield by 25.6% and 17.3%, respectively compared to alternate application of synthetic pesticides Dithane M-45® and Confidor® 70 WG (101.0%), Achook® (78.3%) relative to control. Achook® application resulted in a remarkable increase in fine pods above all treatments.

Figure 7: Grades of marketable and non-marketable pods of snap beans

Table 4.7: Number of pods per plant, total pod yield and marketable pods (Kg/ha) of snap beans sprayed with different antagonistic fungi and plant extracts for both cropping seasons

Treament	November, 2015-January, 2016 cropping season				March-May, 2016 cropping season			
	No. pods/plant	Total pod yield	Marketable pods		No. pods/plant	Total pod yield	Marketable pods	
			Extra-fine	Fine			Extra-fine	Fine
T. viride	5.3 e	6070 abc	1523 cd	61.1 b	6.4 cd	5474 d	1787 f	86.7 ef
T. harzianum	6.5 bcd	7659 a	1556 cd	61.1 b	7.0 c	5639 d	2120 ef	100.4 de
T. asperellum	5.6 de	6333 abc	1541 cd	74.1 b	5.9 d	5278 d	2420 de	77.2 fg
Paecilomyces	5.8 cde	4989 c	1044 d	70.4 b	5.8 d	4511 e	1387 g	76.2 fg
Turmeric extract	6.0 cde	6611 abc	1730 bc	55.5 bc	8.5 b	7829 b	3313 b	130.4 ab
Garlic extract	7.6 abc	6996 ab	1559 cd	29.6 c	8.6 b	6354 c	2487 de	114.1 cd
Ginger extract	8.2 ab	7711 a	1400 cd	68.5 b	8.2 b	5821 cd	2587 d	105.9 cd
Lemon extract	7.2 abcd	7156 ab	1376 cd	66.5 bc	8.0 b	5620 d	2253 de	87.6 ef
Dithane® + Confidor®	8.5 a	8037 a	2426 a	59.3 bc	10.0 a	9077 a	3670 a	118.2 bc
Trianum®	6.0 cde	6274 abc	1167 d	59.2 bc	5.7 de	5356 d	2203 e	84.4 efg
Achook®	7.5 abc	7078 ab	2152 ab	104.0 a	9.5 a	7517 b	2937 c	138.5 a
Control	5.5 de	5333 bc	1207 cd	42.6 bc	5.0 e	4359 e	1153 g	69.6 g
Mean	6.6	6687.3	1556.7	62.6	7.4	6069.5	2360	99.1
LSD ($P \leq 0.05$)	1.6	1710.1	482.3	27.6	0.7	626.1	338.5	15.2
CV (%)	13.9	15.1	18.3	26.1	5.8	6.1	8.5	9.1

Means accompanied by different letter(s) in each column are significantly different (Fisher's protected Least Significant Difference, $P \leq 0.05$).

Antagonistic fungi reduced disease damaged pods more than crude extracts (Tables 4.8; 4.9). Garlic extract significantly reduced thrip-damaged pods by 45.5% followed by Achook® (40.6%). *Trichoderma* spp. significantly reduced disease-damaged pods by 48.4% while Trianum® by 38.9%. Dithane® and Confidor® (49.1%) had a slight reduction in disease-damaged pods over *Trichoderma* spp. The application of antagonistic fungi and crude plant extracts variably resulted in increase in deformed pods. Plant extract treated snap beans had higher yields of overgrown and others grades than antagonistic fungi.

Table 4.8: Weight of different categories of non-marketable pods (Kg/ha) of snap beans sprayed with different antagonistic fungi and plant extracts in cropping season 1

Treatment	Thrip damaged	Disease damaged	Deformed	Overgrown	Others
T. viride	1330 abc	815 d	2415 abc	740.7 d	451.9 c
T. harzianum	1452 ab	837 d	2715 a	755.6 d	451.8 c
T. asperellum	1326 abc	800 d	1889 cde	855.6 cd	588.9 bc
Paecilomyces	1222 abcd	867 d	1344 ef	796.3 d	733.3 ab
Turmeric extract	1341 abc	1548 ab	1281 f	918.5 cd	663.1 abc
Garlic extract	852 d	1267 bc	2456 abc	1148.1 b	622.2 bc
Ginger extract	1189 abcd	1674 a	2637 ab	1070.4 bc	751.9 ab
Lemon extract	1041 bcd	1107 cd	2130 abc	892.6 cd	607.4 bc
Synthetics	1059 bcd	807 d	1981 cd	1363.0 a	911.1 a
Trianum®	1344 abc	985 cd	1478 def	855.6 cd	751.9 ab
Achook®	926 cd	1600 ab	2096 bc	807.4 d	685.2 abc
Control	1563 a	1585 ab	1285 f	944.4 bcd	692.6 abc
Mean	1220.4	1157.7	1975.6	929.0	659.3
LSD ($P \leq 0.05$)	371.9	364.5	540.5	208.4	225.6
CV (%)	18.0	18.6	16.2	13.2	20.2

Means accompanied by different letter(s) in each column are significantly different (Fisher's protected Least Significant Difference, $P \leq 0.05$). (Synthetics=Dithane® and Confidor®).

Table 4.9: Weight of different categories of non-marketable pods (Kg/ha) of snap beans sprayed with different antagonistic fungi and plant extracts in cropping season 2

Treatment	Thrip damaged	Disease damaged	Deformed	Overgrown	Others
T. viride	581.5 a	407.4 c	2415 abc	741 c	451.9 c
T. harzianum	725.9 a	459.3 bc	2715 a	756 c	755.6 ab
T. asperellum	663.0 a	400.0 c	1889 cd	856 c	881.5 ab
Paecilomyces	611.1 a	433.3 c	1344 d	796 c	733.3 abc
Turmeric extract	670.4 a	774.1 a	1281 d	1530 a	1107.4 a
Garlic extract	548.1 a	633.3 abc	2456 abc	1148 abc	622.2 bc
Ginger extract	711.1 a	837.0 a	2637 ab	1070 abc	940.7 ab
Lemon extract	670.4 a	737.0 ab	2130 abc	1489 ab	607.4 bc
Synthetics	663.0 a	633.3 abc	1981 bcd	1363 ab	911.1 ab
Trianum®	781.5 a	603.7 abc	1989 bcd	1104 abc	751.9 abc
Achook®	463.0 a	666.7 abc	2096 abc	807 c	685.2 bc
Control	655.6 a	607.4 abc	1285 d	944 bc	692.6 bc
Mean	645.4	599.4	2018.2	1050	762.0
LSD ($P \leq 0.05$)	291.9	283.9	718.0	486.2	347.4
CV (%)	26.7	28.0	21.0	27.3	26.9

Means accompanied by different letter(s) in each column are significantly different (Fisher's protected Least Significant Difference, $P \leq 0.05$). (Synthetics=Dithane® and Confidor®).

4.5 Discussion

Application of antagonistic fungi and crude plant extracts as sprays reduced the population of whiteflies and thrips on snap bean. Plant extracts were more efficacious in reducing insect pest populations compared to the antagonistic fungi. All crude plant extracts significantly reduced the population of whiteflies while garlic extract significantly reduced the population of thrips. These findings are in agreement with those of Kiani *et al.* (2012) who reported that the garlic-onion-pepper extract controlled western flower thrips in the strawberry in greenhouse. Mohapatra *et al.* (2012) reported that garlic amidst other botanicals exhibited best antifeedant for vegetable pests. Similarly, a study done in Nigeria by Kalu *et al*. (2010) illustrated the larvicidal activities of the extract of garlic bulb against larvae of *Culex quinquefasciatus.* Ali *et al.* (2014) reported the efficacy of leaf extracts of garlic and turmeric in reducing the larval, pupal and adult *Tribolium castaneum.*

The repellency of garlic has been reported by Ahmad *et al*. (2013) to reduce population build of *Trogoderma granarium* by 32.2%. Garlic repellent nature of compounds in plant extracts has been reported by Nwachukwu *et al*. (2014) who attributed the high mortality of maize weevil of up to 90% due to high amounts of allicin in fresh garlic. In this present study garlic effectiveness to reduce the population of insect pests could be due to allicin which is a key component of volatile oils known to have insecticidal properties (Regnault-Roger, 1997).

Foliar sprays of antagonistic microorganisms and crude plant extracts showed antifungal activity against economically important fungal foliar disease of snap bean. These findings were in line with studies by Soliman *et al.* (2015) who reported that antagonistic fungi significantly reduced grey mold on cucumber. In 2014, Panwar *et al.* reported that foliar application of *T. harzianum* and *T. viride* significantly reduced head blight severity on wheat. Antagonistic microorganisms significantly suppressed rust which was in agreement with the findings of Chhetry and Mangang (2012). Similar findings were reported by Akrami (2015) that *Trichoderma spp*. significantly reduced the incidence of Fusarium wilt cucumber (*Cucumis sativus*).

Foliar spray of *Trichoderma harzianum* reduced intensity of angular leaf spot, rust and anthracnose less than 50% in this study. These results were not in agreement with findings by El-Mougy *et al*. (2016) who reported that *Trichoderma harzianum* totally reduced incidence of leaf spot (*Alternaria tenuissima*) on broad bean plants in the field in Egypt. The effectiveness of antagonistic fungi on snap bean foliar pathogens can be attributed to antimicrobial toxins and plant growth promoting substances (Sawant, 2014). The latter are said to induce host resistance against the pathogens.

Plant extracts reduced severity of angular leaf spot, anthracnose and rust with garlic being more efficacious than turmeric, ginger and lemon. This was in line with the results of Wei *et al.* (2011) who reported that garlic extract effectively suppressed development of leaf mold in tomato. Garlic extract was reported to reduce the incidence of leaf spot and rust on sunflower by 50-56% (Poorniammal and Sarathambal, 2009). The effectiveness of plant extracts of the present study was similar to what Hossain and Hossain (2013) reported that plant extracts effectively reduced the incidence of Tikka disease on groundnuts was by up to 63.6%.

The findings of this study were agreeable to what Onyeani and Osunlaja reported in 2012 that plant extracts were suppressed severity of disease. A study conducted by Kumar *et al.* (2012) illustrated that garlic, ginger and lemon were efficacious against angular leaf spot, anthracnose and rust. Garlic was found to have the least percentage disease index over all botanicals against spot of Palak (*Cercospora beticola*) (Yashoda, 2011). Gurjar *et al*. (2012) reported that extracts of garlic, turmeric and ginger had antimicrobial activity against fungi of agricultural significance. The antimicrobial activity of garlic and turmeric are attributed to allicin and curcumin, respectively Wei *et al.* (2011).

Plant extracts used as foliar application increased pod yield of snap bean over control. Poorniammal and Sarathambal (2009) reported similar findings of increase in yield of sunflower when sprays of garlic extract, neem oil, *Calotropis gigantea* leaf extract, *Prosopis juliflora* leaf extract were applied to manage leaf spot and rust. Such findings fully support deduction by Khan *et al*. (1996) that applications of plant extracts show significant increase in yield. Hossain and Hossain (2013) also reported increase in the number of pods on groundnut by plant extract foliar applications. However, the results of this study differed with the findings by Amin *et al.* (2014), Testaye (1997) and Sharma *et al*. (2008) who reported

that both *Trichoderma* spp. foliar application resulted in yield loss in common beans. The increase in yield was as a result of antagonistic fungi and plant extracts suppressing the population of pests and foliar diseases that led to minimal flower abortion resulting in increase in the number of pods per plant.

Antagonistic fungi and crude plant extracts are effective in management of insect pests and diseases in snap beans in fields. Crude plant extracts were effective in reducing population of whiteflies while *Trichoderma* spp. were effective in lowering development and spread of rust, anthracnose and angular leaf spot. By using biopesticides from local environment in managing pests and diseases in snap beans farmers will have increased marketable pod yields more than commercially formulated biopesticides that are of foreign origin as demonstrates in this study. Further, locally sourced biopesticides will be less costly compared to exotic formulated ones.

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The results obtained from the laboratory studies demonstrate how the exploitation of local environment in search of microbial antagonists against phytopathogenic fungi is promising. The use of antagonistic fungi as an alternative to synthetic pesticides for the management of *Alternaria solani, Colletotrichum lindemuthianum, Fusarium solani* and *Rhizoctonia solani* was effective under *in vitro* conditions. All the antagonistic fungi tested had antifungal effect on the mycelia growth of phytopathogens with *Trichoderma* spp. and *Paecilomyces* giving more promising results than other isolates. *Trichoderma* spp. and *Paecilomyces* could be used for management of fungal diseases in snap beans grown by farmers in Kenya.

Application of crude plant extracts as foliar sprays in snap beans reduced the population of whiteflies and thrips more than antagonistic fungi. The crude plant extracts sourced locally from fresh plants could successfully be used in place of synthetic insecticides in the management of insect pest in snap beans. Crude plant extracts were more efficacious in reducing population of whiteflies than alternate application of Dithane® and Confidor® 70 WG. Small holder growers will find it cheaper to use plant extracts namely turmeric, garlic and ginger as opposed to formulated botanical, Achook® as the results for the two cropping seasons indicated that they were more effective in reducing the population of whiteflies.

The effectiveness of these plant extracts against white flies translated to better pod yield with greater volumes of marketable pods. Though alternate application of Dithane® and Confidor® 70 WG was most effective in reducing population of thrips, garlic was more promising than all other plant extracts and formulated neem, Achook®. These plant extracts are effective in reducing whiteflies and thrips and are readily available, eco-friendly and most importantly

known not to having toxic residues. Local environments are rich sources of antagonistic fungi that are effective in suppressing foliar fungal diseases in snap beans. *Trichoderma harzianum* compared well with Dithane M-45® and Trianum® in suppressing development and spread of angular leaf spot. All the *Trichoderma* spp. were effective in lowering development and spread of rust and anthracnose across the two seasons better than alternate application of synthetics pesticides and much better than commercial *Trichoderma*.

Antagonistic fungi increased pod yield of snap bean and at the same time reduced anaesthetic attributes due to disease infection on pods hence contributing to more volumes of marketable pods. Small scale farmers of snap beans will find it economical in producing high quality produce that will make Kenyan snap bean pods to be highly competitive at niche markets. The livelihoods of small holder farmers will be improved when biopesticides use would be to adopted hence minimize produce interceptions due to harmful residues as higher incomes will be generated and employment created.

5.2 Recommendations

i. Further work on continued bio-prospecting to explore active microorganisms and botanicals from local environments should be carried out.
ii. Awareness creation and promotion of use of biopesticides by small scale vegetables farmers on adverse effects of synthetics pesticides and advantages of biopesticides should be conducted.
iii. Further research to determine active compounds and formulation of antagonistic fungi and botanicals will enhance ease of application in fields.
iv. A policy on exploitation, formulation, registration and adoption of local biopesticides to realize the benefits of sustainable agriculture should be developed.

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