MAKERERE UNIVERSITY

COLLEGE OF NATURAL SCIENCES

Department of Plant Sciences, Microbiology and Biotechnology

UTILIZATION OF FUNGAL AND BACTERIAL ENDOPHYTES FOR THE MANAGEMENT OF THE BANANA NEMATODE *RADOPHOLUS SIMILIS* IN EAST AFRICAN HIGHLAND BANANAS

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DECLARATION

I Kinalwa Nasser declare that this thesis is my original work and has not been submitted to Makerere University or any other University for the award of a degree.

Kinalwa Nasser

Signature ..... Date 20/January/2023

Supervisors' Approval

We confirm that the work presented in this thesis was carried out by the candidate and has been submitted after our approval as University supervisors.

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DEDICATION

This work is dedicated to my family for their love, support and encouragement during the period of study.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

East African Highland bananas (EAHB) (*Musa* spp., AAA-EA) are a staple food to many smallholder farmers in Uganda. Production is constrained by plant parasitic nematodes (PPN) with the burrowing nematode, *Radopholus similis* being the major PPN. This damages the root system and results into toppling of banana plants especially on rainy days. Management of *R. similis* is difficult especially in bananas because these nematodes reside inside the roots. Use of biocontrol agents (BCAs) in the management of *R. similis* in bananas is encouraged however the age of bananas to be enhanced with BCAs and how many times of enhancement with BCAs (inoculation regime) needs to be established. In this study, effect of cultural filtrates of the BCAs against *R. similis* was tested *in vitro* as an initial step in testing the BCAs against *R. similis.* In the screen house, effect of BCAs and their inoculation regimes on *R. similis* densities in banana plants was also tested. The effect of BCAs on plant growth was tested in the screen house. Five BCAs i.e., three bacteria endophytes; *Coccobacillus* spp (1DRB1), *Bacillus* spp (1HRB3) and *Bacillus* spp (1HRB4) and two fungal endophytes i.e., *Trichoderma asperellum* (Asperello T34) and *Trichoderma asperellum* (Real Trichoderma TRC900) were tested against *R. similis.* Effect of culture filtrates of BCAs on *R. similis* was determined as percentage paralysis and mortality of nematodes after *in-vitro* exposure of *R. similis* to culture filtrates of BCAs in petri dishes. Percentage (%) paralysis was determined after 3, 6 and 12 hours while percentage mortality was determined after 24 hours. All filtrates of fungal and bacterial endophytes paralyzed *R. similis*. Filtrates of all fungal and bacteria endophytes killed *R. similis* with 1HRB4 resulting in highest mortality (75.64%). Effect of BCAs, their inoculation regime on *R. similis* densities was determined as the total number of *R. similis* extracted from 5g of root at experiment termination. Effect of BCAs and their inoculation regime on growth of plants was determined by measuring height, fresh shoot weight, dry shoot weight, and fresh root weight. There were significant ($P < 0.001$) effect on reduction of *R. similis* densities in plants inoculated with fungal and bacterial isolates compared to the control plants that were not inoculated with BCAs. Inoculation regime with biocontrol agents had a significant ($P < 0.05$) effect on reduction of *R. similis* densities in banana plants. Biocontrol agents had significant ($P < 0.05$) effects on increasing plant growth of banana plants. Inoculation regime however had no significant ($P > 0.05$) effect on increasing plant growth of banana plants. Results from this study indicate that these isolates have the potential to be used as biocontrol agents against *R. similis* in bananas.

CHAPTER ONE

INTRODUCTION

1.1 Banana production and its constraints

The East African Highland Banana (EAHB) (*Musa* spp., AAA-EA) is a major food and cash crop for rural farmers in Uganda (Tushemereirwe *et al.,* 2004). Most farmers find the EAHB ideal for food and income than other banana cultivars due to its good sensory and cooking traits such as colour, texture, aroma, taste, and mouth feel etc. and can be harvested throughout the year (Kubiriba *et al.,* 2014). Despite its importance, production of the crop is constrained by both biotic and abiotic factors. Biotic factors include banana diseases such as Black Sigatoka disease also known as black leaf streak caused by *Mycosphaerella fijiensis* and results into 37% yield loss on East African Highland bananas (Tushemereirwe, 2003). In Uganda, black sigatoka alone can reduce yield by 30- 50%; hence it is considered as a major threat to the country's food security (Gale, 2012). Banana bacterial wilt (BBW) is caused by a bacterium *Xanthomonas campestris* pv. *Musacearum.* and can result in 100% yield loss (Tushemereirwe *et al*., 2003). Fusarium wilt also known as Panama disease is caused by the soil fungus *Fusarium oxysporum* f.sp. *cubense.* Fusarium wilt of banana can cause banana bunch weight reduction of up to 78% in severely affected plants due to poor development of banana fingers (Kangire, 1998).

The major banana pests include; the banana weevil, *Cosmopolites sordidus* Germar. Damage is caused by the larvae which feed and create tunnels into the banana corms resulting into snapping of plants, prolonged maturation rates and reduced yields. Severe infestations by this damaging insect pest can lead to total crop failure resulting into 100% yield loss (Sengooba, 1986). East African Highland Bananas are highly susceptible to banana weevil (Kiggundu *et al*., 2007).

1.2. Parasitic nematodes of bananas and their management

Other pests that threaten production of bananas in Uganda and in Africa are the plant-parasitic nematodes (PPNs) i.e., burrowing nematode *Radopholus similis* [Cobb] Thorne, root-lesion nematode *Pratylenchus. goodeyi* [Sher] et Allen, and spiral nematode *Helicotylenchus. multicinctus* [Cobb] Golden (Speijer & Fogain 1998). The burrowing nematode *Radopholus similis* is the most important nematode parasite in EAHB (Dochez *et al*., 2005). The nematodes infect root tissues and impair absorption and transportation of water and nutrients, in addition to

weakening plant anchorage in soil. Damage to the banana plant is associated with a reduction in bunch weight, loss of bunches due to plant toppling especially at the fruit-filling stage, increase in crop cycle duration and decrease in plantation longevity with yield losses ranging from 30% to 50% (Kashaija *et al.*, 1994). Nematode control has mainly relied on the use of nematicides and cultural control methods (Chabrier & Quénéhervé 2008) but nematicides are being discouraged in most countries due to their deleterious effect on human health and the environment for example they can be washed from soil into drinking water and lead to death. The nematicides also kill useful microorganisms in soil. Consequently, this has resulted into increase in nematode infestation levels.

1.3. Management of *Radopholus similis* using biocontrol agents

Use of the environmentally friendly biocontrol agents (fungal and bacterial endophytes) in various crops has been studied and several studies have demonstrated the potential of using endophytes in the management of a wide range of PPNs (Jatala, 1986, Dong *et al*., 2006, Elsen *et al.*, 2008, Akhtar & Malik, 2000, Schouteden, 2015).

The age of the plant to be inoculated and how many times of inoculation (Inoculation regime) with BCAs to achieve effective control of *R. similis* in crops has not been widely explored and hence it was explored in this study. Three bacterial endophytes *Bacillus* sp. (1HRB4), *Bacillus* sp. (1HRB3) and *Coccobacillus* sp. (1DRB1) isolated from banana corms and roots at the National Agriculture Research Organization (NARO) plant pathology laboratory in Kawanda (Uganda) were tested for management of Fusarium wilt disease in bananas at IITA- Sendusu and the same isolates were tested for management of *R. similis* in bananas in this study. Fungus *Trichoderma* spp. has been shown to have potential to kill PPNs and thus it was tested for management of *R. similis* (Stirling, 2018). Spores of two commercial fungal isolates, *Trichoderma asperellum* (Real Trichoderma TRC900) and *Trichoderma asperellum* (Asperello T34) were provided by Real IPM, Nairobi, Kenya and Biobest, Belgium respectively.

This study explored the effect of culture filtrates of BCAs on paralysis and mortality of *R. similis* in the laboratory. In the screen house, the best performing BCAs against *R. similis* was tested. The age at which banana plantlets were enhanced with BCAs and how many times the BCAs were inoculated into banana plants (inoculation regime) and its effect against *R. similis* and also was also tested.

1.4 Problem statement

In Uganda, 75% of small holder farmers grow bananas and 85% of banana produced is of the group EAHB (Tushemereirwe *et al.,* 2004). Banana production is constrained by the nematode *Radopholus similis* because these cultivars are very susceptible to *R. similis*. The nematodes infect root tissues and impair absorption and transportation of water and nutrients, in addition to weakening plant anchorage in soil. Damage to the banana plant is associated with a reduction in bunch weight, loss of bunches due to plant toppling especially at the fruit-filling stage and decrease in plantation longevity with yield losses ranging from 30% to 50% (Kashaija *et al.,* 1994). Tissue culture (TC) derived banana plantlets provide clean planting materials free from *R. similis* for the farmers compared to field grown suckers (Chabrier & Quénéhervé 2008). However, these plantlets are not only free of pests but are also free of the beneficial, naturally occurring microbial organisms. The effect of bacterial and fungal endophytes as BCAs against several plant parasitic nematodes (PPNs) has been widely studied. (Paparu et al., 2009, Vetrivelkalai et al., 2010, Stirling, 2018, Schouteden, 2015). However, little research on the effect of bacterial and fungal endophytes on *Radopholus similis* in EAHBs has been done and hence needs to be established. The age of the banana plant to be enhanced with BCAs and how many times the plant needs to be enhanced with BCAs (inoculation regime) is also not well understood and hence needs to be established

1.5 Objectives

The main objective of the study was to identify potential fungal and bacterial endophytes for management of the banana nematode *Radopholus similis* in East African Highland Bananas. The specific objectives were to;

i. Evaluate the *in vitro* antagonistic activity of culture filtrates of bacterial and fungal isolates against the infective stages of *R. similis*.

ii. Evaluate the effect of fungal and bacterial endophytes and inoculation regime on *R. similis* densities and growth of TC banana plants

1.6 Hypotheses

i. Bacterial and fungal filtrates do not paralyze or kill infective stages (Adult females and juveniles) of *R. similis*.

ii. Bacterial, fungal endophytes and their inoculation regimes do not reduce *R. similis* densities and do not promote growth of banana plants.

1.7 Significance of the study

The study was conducted to identify the best performing BCAs, the age of banana plants to be enhanced with bacterial and fungal endophytes as BCAs and how many times of inoculations with endophytes into banana plants (inoculation regime) against *R. similis.* Results obtained from this study showed that bacterial and fungal isolates used in the study have the potential to be used as BCAs against *R. similis* and as promoters of banana plant growth. Also the results showed that inoculating several times with BCAs can reduce *R. similis* densities in banana plants. This information is very important for tissue culture laboratories and can be used in the production of Bio-enhanced and clean banana plantlets that protected against *R. similis* for farmers in Uganda. This can ultimately result into reduced *R. similis* infestation in field hence improvement in the banana production.

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CHAPTER TWO

LITERATURE REVIEW

2.1 *Radopholus similis* and their management in Bananas

The burrowing nematode *R. similis* belongs to the family Pratylenchidae, order Tylenchida and class Secernentea (Mendoza & Sikora 2009). *Radopholus similis* is wormlike, about 0.65mm long, 25µm wide and colourless (Agrios, 2005). Males have a degenerate stylet and counted as non-parasitic while adult females and juvenile are the active mobile forms and are infective (Sarah *et al.*, 1996). It is an endoparasitic migratory nematode, meaning it completes its life cycle within root tissue. All motile juvenile stages and females can infect root tissue and after root penetration, these life stages mainly feed and migrate into the cortical parenchyma and also into the stele. As the mature females migrate through root tissue, they lay eggs that are produced through either sexual reproduction with males or by hermaphroditistim (Sher, 1968, Kaplan & Opperman 2000). Once an egg hatches, the emergent second stage juvenile can migrate within the root and complete its entire life cycle within the root system, or it can leave the roots in search of another healthy host root.

Radopholus similis is found worldwide in tropical and subtropical regions of Africa, Asia, Australia, North and South America, and many island regions (O'Bannon, 1977). The widespread range of this nematode is due to its dissemination with propagative plant material, especially infected banana corms (Gowen *et al.*, 2005). Symptoms of burrowing nematode are most readily observable as dark and necrotic lesions on the root system. In banana, lesions may be present in both the roots and outer layer of the rhizome. Other symptoms include; yellowing, stunting, dieback, reduced fruit size, and thinning of the plant. Banana toppling occurs during heavy rain or wind due to severely reduced and damaged root system, which is unable to anchor the plant into the ground.

Damage caused by *R. similis* to the root tissue, leaves plants with little or no support or ability to take up water and translocate nutrients. Because of the damage that it causes to bananas, citrus, ornamentals, and other agricultural crops worldwide, *R. similis* damage has resulted in crop losses ranging 30%–80% (Gowen *et al.*, 2005) and this has made *R. similis* one of the most regulated nematode plant pests (Nicol *et al.*, 2011).

Figure 2. 1*:Radopholus similis*; full body and head region (Photo; J. Kisaakye)

Figure 2. 2: Toppling of banana plants caused by plant parasitic nematode damage to the root system (Photo; N. Kinalwa)

2.2 Management of *Radopholus similis*

Management of *R. similis* is an integrated approach involving use of physical measures such as staking of pseudo stems, preventive means such as use of clean Tissue Culture (TC) plantlets, cultural methods such as manure application, breeding for resistant varieties and chemical control involving the use of nematicides (Seenivasan, 2017). Nematicidal treatments to the soil in fields before planting of the bananas can greatly reduce populations of *R. similis* (Chabrier & Quénéhervé 2003). However, nematicides have environmental and human concerns including; the contamination of soil, plants, and groundwater and health risks to animals, farmers, and consumers hence their use is discouraged in many countries (Quénéhervé, 2009).

Studies on the use of BCAs in the management of *R. similis* have been conducted in pots under controlled screen house conditions, however, with less success in the field (Athman *et al.*, 2006). Studies on utilizing fungal and bacterial endophytes that live within plants have shown some promise in reducing populations of burrowing nematodes by inducing resistance to burrowing nematode in banana (Fotso *et al*., 2022, Kisaakye *et al* 2022, Kumar *et al*., 2021). Consequently, as new BCAs are isolated, it's necessary that these are tested for their potential in the management of *R. similis* and their performance evaluated.

2.3 Use of fungal and bacterial endophytes to control *Radopholus similis* in EAHBs

All microorganisms that inhabit, at least for one period of their life cycle, the interior of a plant, may be considered as endophytes. Endophytic microorganisms inhabit the interior of plants, especially leaves, branches and stems, showing no apparent harm to the host. By the systemic colonization of roots, they have the potential to develop into biological control agents (BCAs) of PPNs since they both inhabit the same plant tissue. Several studies have shown that the interaction between plants and some endophytic bacteria and fungi is associated with beneficial effects such as plant growth promotion and biocontrol potential against plant pathogens (Hallmann *et al.*, 2009*).* The use of endophytic bacteria and fungi for the management of plant-parasitic nematodes has been studied in EAHBs (Kisaakye *et al* 2022, Ochieno, 2022, Brader *et al*., 2014, Paparu *et al*., 2013, Hoang *et al*., 2020, Harish *et al*., 2009, Ngamau *et al*., 2012). Bacteria and fungi have been isolated from soil, host-plant tissues, nematodes and nematode eggs and cysts (Kumar & Dara, 2021).

Bacteria and fungi affect nematodes by a variety of modes for example, parasitizing, producing toxins, antibiotics, or enzymes, interfering with nematode plant host recognition, competing for nutrients, inducing systemic resistance of plants, and promoting plant health (Siddiqui & Mahmood, 1999). Bacteria and fungi are abundant organisms in soil and plant tissues, and some of them, for example bacteria of the genera *Pasteuria, Pseudomonas* and *Bacillus* have shown great potential for the biological control of nematodes (Ponpandian *et al*., 2019). *Trichoderma* species have long been recognized as potential BCAs of plant-parasitic nematodes (Meyer, 2003, Sharon *et al*., 2001, Hinterdobler *et al*., 2021, Sahebani & Hadavi, 2008, Yang *et al.*, 2012)

2.3.1 *Trichoderma* spp

Trichoderma spp. are fungi that are present in nearly all soils and other diverse habitats. In soil, they frequently are the most prevalent culturable fungi. They are favoured by the presence of high levels of plant roots, which they colonize readily (Kumar & Arthurs, 2021). Some strains are highly rhizosphere competent, i.e., able to colonize and grow on roots as they develop. The most strongly rhizosphere competent strains can be added to soil or seeds by any method. Once they come into contact with roots, they colonize the root surface or cortex, depending on the strain. Thus, if added as a seed treatment, the best strains will colonize root surfaces even when roots are a meter or more below the soil surface and they can persist at useful numbers for several months after application. In addition to colonizing roots, *Trichoderma* spp. attack, parasitize and gain nutrition from other microorganisms including fungi. Since *Trichoderma* spp. grow and proliferate best when there are abundant healthy roots, they have evolved numerous mechanisms for both attack of other microorganisms and fungi and for enhancing plant and root growth (Ibrahim, *et al.*, 2020). These mechanisms include; mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, solubilization and sequestration of inorganic nutrients, induced resistance, inactivation of the pathogen's enzymes (Mukhtar, 2018).

2.3.2 *Trichoderma asperellum* (Asperello T34)

Trichoderma asperellum as a BCA has been studied and used in the management of plant parasitic nematodes (PPNs) in several plants including Tomato and *Celosia argentea* Linn (Chowdhury *et al*., 2020). Although, the fungus *Trichoderma* is soilborne, it has been shown to colonize plant tissues and grow endophytically enabling plants to fight pathogens and also promote growth in several ways (Schuster & Schmoll, 2010).

Asperello T34 used in this study is a biological fungicide containing dry conidia of the beneficial fungus *Trichoderma asperellum* strain T34 which is used preventatively to protect greenhouse ornamentals, selected greenhouse vegetables and berries, and medicinal cannabis from soilborne diseases like *Pythium* spp., *Fusarium oxysporum* and *Phytophthora* spp (Trillas *et al*., 2006). If preventively applied, it protects plants from diseases due to its capacity to colonize soil and the plant roots (Trillas *et al*., 2006). It germinates and grows in a wide range of soils and substrates, and around the crop roots. It has a suppressive effect on the soil-borne pathogens *F. oxysporum*, *Pythium aphanidermatum* and *Phytophthora capsici* since it directly competes for space and nutrients and can parasitize the pathogenic fungus. Asperello near plant roots builds a physical barrier against pathogens and induces systemic resistance (ISR). It can be applied by spraying, drenching, dipping or via chemigation or irrigation. Asperello develops in a variety of pHs (4-9) and soil temperatures 15-35ºC (59-95°F), but its optimal growth temperature is 20-30ºC (68-86°F) and becomes active when the soil temperature is at least 10°C (50°F). The product is applied repetitively, at regular intervals (of 2 to 3 months), throughout the life of the crop in order to maintain the appropriate level of *Trichoderma* in soil.

2.3.3 *Trichoderma asperellum* (Real Trichoderma TRC900)

Real *Trichoderma asperellum* (TRC900) is an isolate of the naturally occurring *T. asperellum* found in soil. It is a bio-nematicide, bio-fungicide and biofertilizer promoting growth and branching of crop roots (Segarra *et al*., 2010). It works by competing directly with pathogens for biological space on the plant, through parasitism of competing microbes and by enhancing plant immune systems. Real *T. asperellum* can be used as part of a spray programme from propagation nursery to field trees against *Phytophora*, *Pythium*, nematode complex and both crown rot and collar rot in apples. It can also be used in several ways including; preparing planting holes with compost pre-treated with *Trichoderma*, dipping tree roots before planting followed by application to roots via drip irrigation every month (Sant *et al.,* 2010). For scab, to

reduce spores in the orchard, Real *T. asperellum* is sprayed on infected crop debris and branch structure. For control of root disease and nematodes in strawberries and raspberries, the plants are completely immersed in Real *T. asperellum* solution before planting and thereafter a monthly dose is applied to roots through drip irrigation.

2.4 *Bacillus* and *Coccobacillus* Gram negative bacteria

Bacillus bacteria have a rod shape and *Coccobacillus* bacterium have a shape which is an intermediate between coccus and bacillus bacteria. The shapes may range from round to short rods (Sarwar, 2021). *Bacillus* and *Coccobacillus* bacteria have been widely studied as endophytic bacteria (Forchetti *et al*., 2007, Pereira *et al*., 2012, Miguel *et al*., 2013, Kavamura *et al*. 2013, Agbobatinkpo *et al*., 2013, Suhandono *et al*., 2016, Khare & Arora 2010, Djafar *et al*., 2010, Chanway 1998). They have been isolated from a number of plants including; maize, sunflower, rice, papaya, coffee, bananas where they have shown to play a role as biocontrol agents and stimulants of plant growth (Verma *et al*., 2017). Several mechanisms used by these bacteria to fight plant pathogens and induce plant growth have been suggested including; production of auxins and gibberellins which improve plant growth (Forchetti *et al.,* 2007). Auxins have also shown to inhibit the growth of pathogens (Khare & Arora 2010). These bacteria have demonstrated ability to fight plant pathogens through production of lytic enzymes that degrade and kill plant pathogens (Kavamura *et al.,* 2013).

Use of fungal and bacterial endophytes as biocontrol agents against plant parasitic nematodes in most crops including bananas has been studied. However, there is little information on the timing of inoculation and in what quantities (inoculation regime). This study focused on the use of fungal and bacterial endophytes and their inoculation regime in the management of *Radopholus similis* in bananas and also establishing the effect of these endophytes on banana plant growth.

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CHAPTER THREE

IN-VITRO SCREENING OF CULTURE FILTRATES OF BACTERIAL AND FUNGAL ENDOPHYTES AGAINST *RADOPHOLUS SIMILIS*

Abstract

Despite banana being an important food and cash crop to rural famers in Uganda, its production is constrained by diseases such as banana bacterial wilt, Fusarium wilt and black sigatoka and pests such as banana weevil and plant parasitic nematodes. Banana burrowing nematode *Radopholus similis* is one of the major constraints in banana production in Uganda. Biological control through the use of bacterial and fungal endophytes is considered as an alternative in the integrated control of *R. similis.* This study was conducted to test the effect of culture filtrates of three bacteria endophytes i.e., *Coccobacillus* spp (1DRB1), *Bacillus* spp (1HRB3) and *Bacillus* spp (1HRB4) and two fungal endophytes i.e., *Trichoderma asperellum* (Asperello T34) and *Trichoderma asperellum* (Real Trichoderma TRC900) on *R. similis.* Bacterial and fungal endophytes were cultured on solid media and then transferred to liquid media. Cultures in liquid media were centrifuged and the filtered to obtain culture filtrates fee from cells and spores. Effect of culture filtrates on *R. similis* was evaluated as a percentage paralysis and mortality after *in-vitro* exposure of *R. similis* to culture filtrates. Percentage paralysis was determined after 3, 6 and 12 hours of exposure of *R. similis* to culture filtrates while percentage mortality was determined after 24 hours of exposure. All filtrates of fungal and bacterial endophytes caused paralysis of *R. similis* with 1HRB4 resulting in the highest percentage paralysis i.e., 22.49% at 12 hours, 16.67% at 6 hours and 6.9% at 3 hours of exposure to the filtrate. Filtrates of all the fungal and bacterial endophytes killed *R. similis* with 1HRB4 showing the highest percentage mortality (75.64%) after 24 hours of exposure to filtrates. Percentage paralysis increased with increased time of exposure to culture filtrates in all fungal and bacterial endophytes. There were significant ($P < 0.001$) differences in percentage paralysis and percentage mortality between the biocontrol agents (BCAs) and the control at 3, 6 and 12 hours of exposure to culture filtrates for percentage paralysis and at 24 hours of exposure for percentage mortality. These results show that these bacterial and fungal endophytes have potential to be used as BCAs in management of *R. similis*.

3.1 Introduction

Radopholus similis is the most economically important parasitic nematode of banana in the world (Sekora & Crow, 2012, Coyne *et al*., 2018). Infection by this burrowing nematode causes toppling of banana plants leading to about 60% yield loss (Tushemereirwe *et al*., 2015). Management of *R. similis* in banana by small holder farmers involves the use of clean planting material such as tissue culture derived banana plantlets and hot water treated suckers, cultural control methods such as mulching, use of chemical nematicides, breeding for resistance and biocontrol methods involving use of fungal and bacterial endophytes (Kumar *et al.,* 2021). Use of clean planting material is expensive and cultural control is labour intensive to smallholder farmers. Breeding for resistance is an efficient method for the management of *R. similis* but resistant varieties of banana are yet to be developed, a process which takes over 20 years (Batte *et al.*, 2020). Use of chemical nematicides is an effective approach in the management of *R. similis* but their use has been discouraged owing to the harmful health and environmental such as being poisonous to humans and useful microorganism, their persistence in food chains causing their accumulation in tissues of organisms resulting into harmful effects such as diseases to animals because they are non-biodegradable (Mendoza *et al*., 2008).

Biocontrol involving use of eco-friendly microbes such as bacteria and fungi against *R. similis* is encouraged in the integrated management of *R. similis*. Several studies on use of bacterial and fungal endophytes in the management of plant parasitic nematodes have been conducted and have proved that they have potential to manage plant parasitic nematodes (Mendoza *et al*., 2008, Latz *et al*., 2018). In order to ensure effective management of *R. similis* in banana using Bio Control Agents (BCAs), the best performing BCAs and their mode of action against *R. similis* need to be identified. This study aimed at the use of culture filtrates from five BCAs i.e., 3 bacterial and 2 fungal against *R. similis.* The three bacterial isolates were sourced from IITA laboratory in Kawanda were being tested for their action against Fusarium wilt of bananas hence the need to be tested against *R. similis* in bananas. At Kawanda, the bacterial isolates were maintained on broth media in eppendorf tubes, stored at -20^{o}c in a freezer and were activated before use by thawing. The two fungal isolates are commercial products of spores of fungus *Trichoderma asperellum* that has been considered a biopesticide which has been widely used against several plant parasitic nematodes (PPN) but has not been tested against *R. similis* in bananas

3.2 Materials and methods

3.2.1 Isolation and multiplication of *Radopholus similis* cultures

Radopholus similis nematodes were extracted from 5 g of macerated root tissue of *R. similis* infested banana plants over a period of 48 h using the modified Baermann technique (Coyne *et al*., 2014). The pure culture of *R. similis* was maintained at the IITA Nematology laboratory at Namulonge, Sendusu - Uganda on carrot discs. *R. similis* was cultured on carrot (*Daucus carota*) discs according to the technique described by (Coyne *et al.*, 2014). The nematode populations were sub-cultured every 5 to 7 weeks. To sub-culture, the nematodes were collected in a test tube by rinsing the petri dishes containing the carrot discs with sterile distilled water. The nematodes were surface sterilized with streptomycin sulphate (2,000 ppm) for 4 hours followed by three rinses with sterile distilled water. Carrots were surface sterilized with 96% ethanol and peeled two times. The carrots were then cut into discs of about 5 mm diameter and placed in sterile 35 mm diameter petri dishes. About 50 nematodes were placed on each carrot disc. The dishes were sealed with parafilm and incubated at 28°C in the dark in an incubator for one month.

3.2.2 Culturing of bacterial isolates and production of bacterial filtrates

The bacterial isolates *Bacillus* gram negative (1HRB3), *Coccobacillus* gram negative (1DRB1) and *Bacillus* gram negative (1HRB4) were cultured on Yeast Peptone Glucose Agar (YPGA) media, (yeast extract 5 g, peptone 5 g, glucose 10 g, and agar 15 g) (Tripathi *et al.*, 2009). Pure cultures from a single colony of the bacteria were obtained by streaking on YPGA and incubating for 72 hours at 27-30^{o}C (Figure. 3.1).

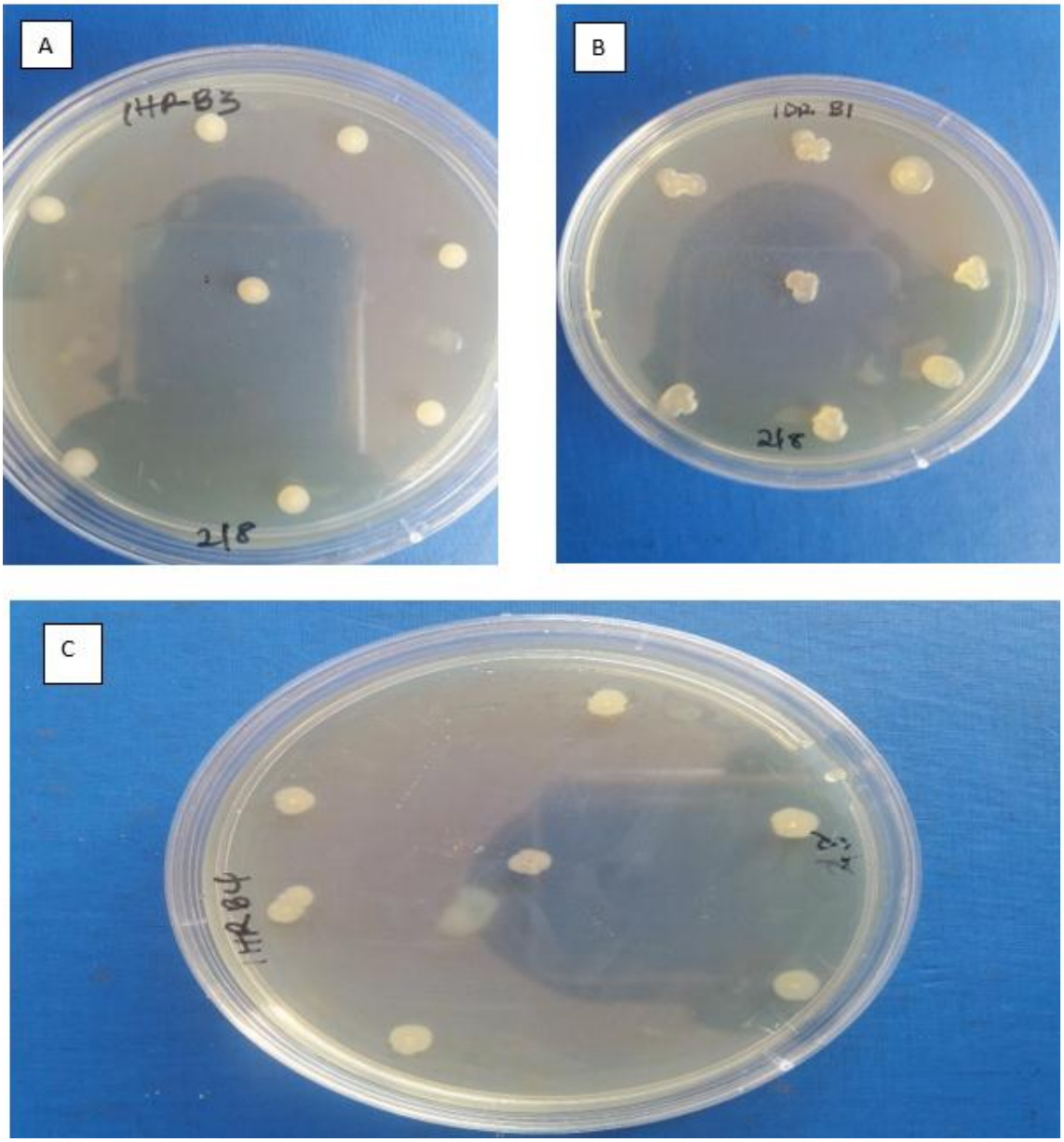

Figure 3. 1: Pure Cultures of bacterial isolates on YPGA media. A: Colonies of 1HRB3 B: Colonies of 1DRB1 C: Colonies of 1HRB4

To obtain culture filtrates, the bacteria cells were washed off a single YPGA plate with sterile distilled water (SDW) and 1ml of the cell suspension was added into a 250ml conical flask containing 100ml of sterile YPG Broth (YPGB) media (Figure 3.2). The bacterial cultures were incubated for 72 h at 25 ± 2^{o}C on a rotary shaker at 300 rpm. After growth, the culture media was centrifuged at 10,000 rpm for 15 min, the supernatants filtered through 0.22 µm membrane

filters and the filtrate collected in sterile 50ml falcon tubes (Mwaura *et al*., 2009). The collected filtrate was maintained in the freezer at -20^{0}C until required.

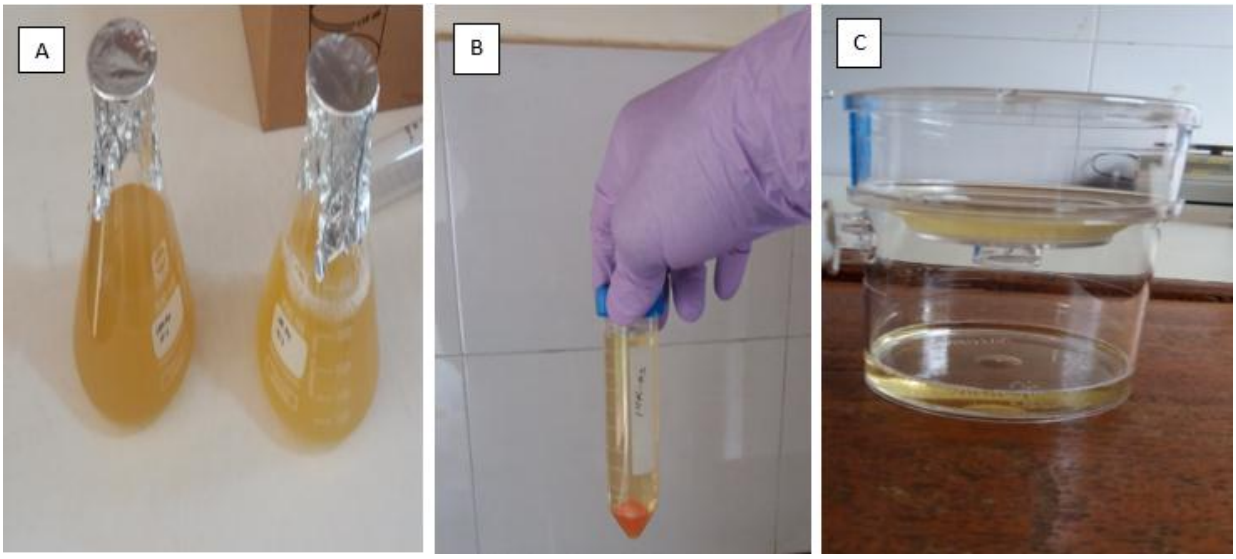

Figure 3. 2: Preparation of bacterial filtrates. A: culturing bacterial isolates in YPG Broth media. B: collection of supernatants after centrifuging. C: Collection of filtrates through membrane filter

3.2.3 Culturing of fungal isolates and production of fungal filtrates

Spores of two commercial fungi, *T. asperellum* (Real Trichoderma TRC900) and *T. asperellum* (Asperello T34) were provided by Real IPM, Nairobi, Kenya and Biobest, Belgium respectively. The fungal isolates were cultured on half strength Potato Dextrose Agar (PDA) medium (19.5 g/l PDA and 6.6 g/l agar) supplemented with antibiotics (streptomycin sulphate (0.2 g/l), penicillin G (0.1 g/l) and chlortetracycline (0.05 g/l) in 90mm diameter petri dishes for 14 days. Mycelial disks (7 mm diameter) of each fungal isolate were obtained from a single colony and each isolate was grown in a conical flask containing 100 ml Potato Dextrose Broth (PDB) media and incubated at 25 ± 2^{o}C for 14 days. After incubation, the cultures were filtered into pre-sterilized conical flasks using 0.22 µm membrane filters. The filtrates were then stored in a freezer at -20^{o}C until required.

3.2.4 In vitro screening of fungal and bacterial filtrates against *R. similis*

Mixed stages of *R. similis* including females, males and juveniles were obtained from carrot disc cultures and used for testing the inhibitory activity of fungal and bacterial filtrates against *R. similis*. One milliliter (1ml) of the respective microbial filtrate was transferred to a sterile glass petri dish (30 mm diameter) containing 100 mixed stages of *R. similis* in 100 µl of sterile distilled water (SDW). One milliliter of sterile distilled water was used in the control treatment (Athman *et al*., 2006). Each treatment was replicated three times. The experiment was set up on a laboratory bench in a completely randomized design at 25 ± 2^{o}C. The bioassay was repeated twice. The effect of bacterial and fungal filtrates on nematode paralysis was assessed after 3, 6, 12 hours of exposure to culture filtrate, while nematode mortality was assessed only after 24 hours of exposure. To check for nematode paralysis, nematodes were probed with a fine needle and were considered alive if they moved and considered paralyzed if they did not move. To check for nematode mortality, the nematodes were first rinsed with SDW on a 28μm sieve then and transferred back into the petri dishes containing SDW. The nematodes were then observed under a stereo microscope and nematodes that remained paralyzed after probing with a fine needle were considered dead. The nematodes that were exposed to SDW only without filtrates were used as a control for both paralysis and mortality assays.

3.3 Data analysis

Both percentage paralysis and mortality were tested for normality and equality of variances. For nematode paralysis and mortality data, the generalized linear model (GLM) was used for analysis. All statistical analysis was done using R software v4.0.2 (R Core Team, 2020) and graphs drawn using Microsoft excel.

3.4 Results

3.4.1 Effect of culture filtrates of BCAs on paralysis of *Radopholus similis*

Table 3.1 shows results of the percentage paralysis of *R. similis* after exposure to BCA filtrates for 3, 6 and 12 hours. Among the BCAs, there was a significant (P<0.001) effect of their filtrates on percentage paralysis of *R. similis* across time of exposure with the highest percentages of paralysis observed after 12 hours. Exposure to filtrates of 1HRB4 resulted in the highest percentage of paralysis (22.49%) at 12 hours of exposure. There was a significant (P < 0.001) difference in percentage paralysis of *R. similis* between BCAs and the control at 3, 6 and 12 hours of exposure

Table 3. 1: Paralysis of *Radopholus similis* after 3, 6 and 12 hours of exposure to culture filtrates of bacterial endophytes 1HRB3, 1HRB4, 1DRB1 and fungal endophytesT34 and TRC900.

Treatment	Paralysis of *R. similis* (%)		
	3 h	6 h	12 h
1HRB3 (Bacteria)	6.62 ± 1.5 a	15.93 ± 2.3 a	18.14 ± 1.2 ab
1DRB1 (Bacteria)	4.94 ± 1.6 a	9.64 ± 0.9 b	12.89 ± 1.9 b
1HRB4 (Bacteria)	6.9 ± 1.8 a	16.67 ± 1.8 a	22.49 ± 2.5 a
T34 (Fungal)	4.27 ± 1.7 a	9.71 ± 1.8 b	17.52 ± 0.7 ab
Trc900 (Fungal)	7.15 ± 1.8 a	9.52 ± 0.7 b	17.53 ± 1.4 ab
Control	0.46 ± 0.2 b	0.50 ± 0.2 c	3.08 ± 1.4 c
P value	P < 0.001	P < 0.001	P < 0.001

Data are Means ± SE of 3 replications. Values in same columns followed by different letters are significantly different at P < 0.05 based on Tukey's HSD test.

3.4.2 Effect of filtrates of BCAs on mortality of *Radopholus similis*

Table 3.2 shows results of percentage mortality of *R. similis* after exposure to BCA filtrates for 24 hours. There was a significant (P < 0.001) difference in percentage mortality between BCAs and the control in the experiments. Bacterial isolate 1HRB4 resulted in the highest percentage mortality compared to all BCAs in the experiments.

Table 3. 2: Percentage mortality of *Radopholus similis* after 24 hours exposure to culture filtrates of bacterial endophytes 1HRB3, 1HRB4, 1DRB1 and fungal isolatesT34 and TRC900.

Treatment	Mortality of *R. similis* (%)	
	Experiment 1	Experiment 2
1DRB1 (Bacteria)	12.85 ± 2.4 d	18.89 ± 2.2 c
1HRB3 (Bacteria)	48.04 ± 6.7 b	30.57 ± 3.3 b
1HRB4 (Bacterial)	75.64 ± 5.8 a	47.77 ± 3.8 a
T34	45.39 ± 2.2 bc	26.18 ± 0.1 b
TRC900	36.62 ± 3.7 c	21.49 ± 1.9 b
Control	0.49 ± 0.3 e	1.29 ± 0.7 d
P value	P<0.001	P<0.001

Data are Means ± SE of 3 replications. Values along columns followed by different letters are significantly different at $P < 0.05$ based on Tukey's HSD test.

Dead and paralyzed nematodes appeared straight, while live nematodes retained the characteristic sigmoid shape and exhibited movement. (Figure. 3.3)

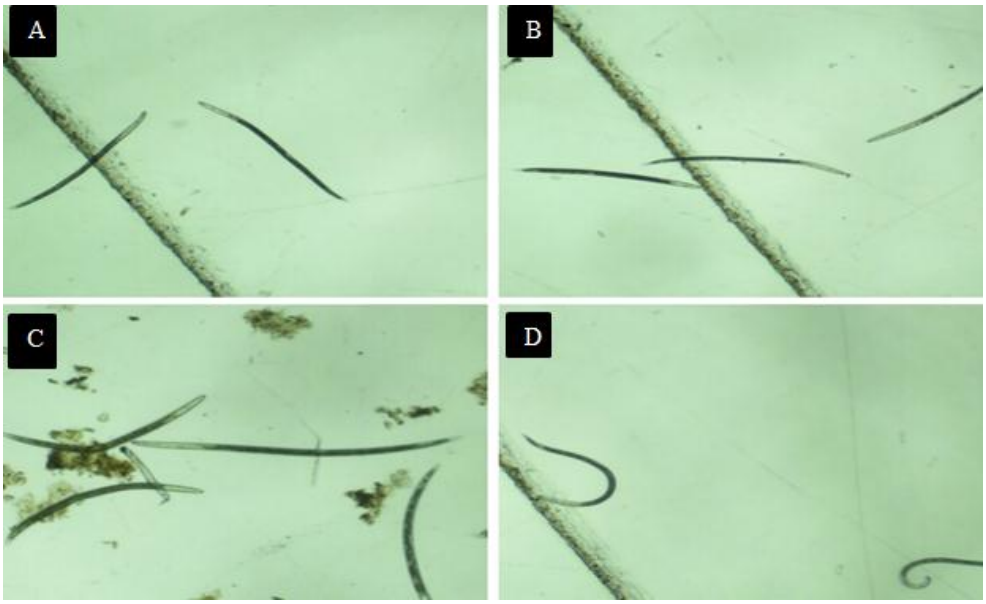

Figure 3. 3: *Radopholus similis* paralysed by isolate 1HRB4 (A), 1HRB3 (B),1DRB1 (C) No paralysis in Control (Sterile Distilled Water) (D)

3.5 Discussion

Management of *R. similis* in bananas by use of BCAs is encouraged because it is environmentally friendly to humans and other organisms. This study focused on effect of BCA filtrates on paralysis and mortality of *R. similis in-vitro.* Culture filtrates of isolates were used for this study to test whether they have nematicidal activity against of *R. similis* and if these could be used for further study in the screen house.

All BCA filtrates paralysed *R. similis* and percentage paralysis increased with time of exposure of *R. similis* to filtrates. Percentage paralysis of *R. similis* by all BCA filtrates differed from that of the control (Sterile Distilled Water with no filtrate of BCAs) and this implies that the filtrates contained compounds that were able to paralyze *R. similis*. Percentage paralysis increases with increase in exposure time of *R. similis* to BCA filtrates because of the increase in the time for the compounds to act on *R. similis*.

In related studies, Athman *et al*., (2006) demonstrated increase in percentage paralysis with increase in time of exposure of *R. similis* to culture filtrates of endophytic fungus *F. oxysporum*. Sharon *et al*., (2007) carrying out *in vitro* bioassays, verified that extracts of *T. asperellum* released in soil were capable of immobilizing juveniles of *Meloidogyne javanica* and reducing egg viability. Many fungi have been studied and are known to produce compounds that are toxic to nematodes (Hallmann & Sikora, 1996).

Filtrates of all BCAs killed *R. similis* indicating that these contain compounds that have ability to kill *R. similis* hence have nematicidal effects which are not reversible. Reversibility of nematicidal effects has however been demonstrated with culture filtrates of bacterial isolates against various nematode species when exposed for less than 48 hours (Soliman *et al*., 2019).

Numerous studies have reported that bacterial culture filtrates possess *in vitro* nematicidal activity (Mendoza *et al*., 2008, Hemeda & Deeb, 2019, Youssef *et al*., 2020). *Trichoderma* species have also been examined for their ability to kill plant-parasitic nematodes and mostly *T. asperellum* and *T. harzianum* have been tested, however, most of the studies have been performed on *Meloidogyne* species (Temitope *et al*., 2020). Also, Al Kader (2009) demonstrated that filtrates of *Trichoderma* spp. had nematicidal effects.

Differences in percentage mortality amongst BCAs was attributed to isolates being different with each producing compounds that act on *R. similis* differently and this could further be investigated.

In related studies by Cayrol *et al*., (1989), Hallmann & Sikora, (1996), Zareen *et al*., (2001), Meyer *et al*., (2004), Anke & Stadler, (1995), Chen *et al.,* (2000), it was demonstrated that culture filtrates can also differ in their activity according to the culture medium, culture conditions, and isolate, and they can determine the type of secondary metabolites produced.

This study has demonstrated that filtrates of the bacterial and fungal endophytes tested may contain compounds which may be harnessed and used as biopesticides for the management of *R. similis* and possibly other nematode species. Percentage mortality and paralysis from the study was not up to the expected percentage (100%) like for other chemical pesticides but none the less, the culture filtrates were able to paralyze and kill *R. similis* an indicator of their potential to be used in the future as biopesticides. Since culture filtrates caused a high percentage mortality of up to 75%, these results could be used to select isolates to test their effects on *R. similis* in the screen house.

3.6 Conclusions

The effect of bacterial and fungal filtrates on paralysis and mortality of the infective stages of *R. similis* was studied and based on the results the following conclusions can be drawn;

Culture filtrates of bacterial and fungal endophytes contain compounds that can paralyse and kill *Radopholus similis in-vitro*

Exposure of *R. similis* to culture filtrates of bacterial and fungal endophytes for a long time increases the paralysis and mortality of *R. similis*.

Culture filtrates of bacterial endophytes paralysed and killed *R. similis* more than culture filtrates of fungal endophytes

3.7 Recommendations

Since all culture filtrates caused paralysis and mortality of *R. similis*, the isolates from which these filtrates were made can be selected for testing in both screen house and field trials against *R. similis.* This is because BCAs may act against *R. similis* in laboratory but may fail to act against *R. similis* in banana plants in the screen house or field.

Since the culture filtrates were able to paralyse and kill *R. similis*, this indicates that they contain active compounds that can kill *R. similis* and these could be harnessed, investigated and maybe used as in future in *R. similis* management.

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CHAPTER FOUR

EFFECT OF BACTERIAL AND FUNGAL ENDOPHYTES ON *RADOPHOLUS SIMILIS* DENSITIES AND BANANA PLANT GROWTH IN THE SCREEN HOUSE

Abstract

The use of bacterial and fungal endophytes is a promising and safe alternative approach for controlling plant-parasitic nematodes. Understanding the timing of inoculation with these biological control agents (BCAs) is important in order to achieve reduced *Radopholus similis* densities in Tissue Culture (TC) derived banana plantlets. This study was conducted to test the effect of BCAs, their different inoculation regimes on *R. similis* densities in TC derived plantlets and their effect on banana plant growth. This study was carried out in a split plot design involving seven inoculation regimes with five BCAs. The BCAs included three bacterial endophytes i.e., *Coccobacillus* spp. gram negative (1DRB1), *Bacillus* spp. gram negative (1HRB3) and *Bacillus* spp gram negative (1HRB4) and two commercial fungal endophytes i.e., *Trichoderma asperellum* (Asperello T34) and *Trichoderma asperellum* (Real Trichoderma TRC900). Inoculation regimes involved inoculation of TC derived plantlets once, twice and thrice with BCAs by drenching the bacterial and fungal inoculum through soil in the potting bag. There were significant ($P < 0.001$) differences in *R. similis* densities in banana plants inoculated with the different isolates. Inoculation regime had no significant ($P > 0.05$) effect on *R. similis* densities in banana plants. At some inoculation regimes, plants inoculated with BCAs had more growth than control plants and at other inoculation regimes, control plants had mor growth than plants inoculated with BCAs. Only plants inoculated with bacterial isolate 1DRB1 had the more growth than control plants at all inoculation regimes. Inoculation regime had no significant ($P > 0.05$) effect on plant growth.

4.1 Introduction

Microbial endophytes are microorganisms that live in the tissues of a plant without causing any harm to their host plant. Endophytic microbes can enter and colonize plants from plant to plant or from the soil to the plants (Verma *et al.*, 2018 & Haung *et al*., 2016). They inhabit plants and produce biologically important compounds that could be used for promoting plant growth, potential biocontrol agents against pathogens and pests, immune defense and fitness, and enabling the plant to withstand stresses (Mehmood *et al*., 2019 & Soldan *et al.*, 2019).

The use of endophytic bacteria and fungi to improve agricultural productivity through efficient management of pests and diseases is a sustainable alternative that is encouraged. Endophytic bacteria and fungi have shown great potential in promoting plant growth, in the biological control of phytopathogens, destructive pests, and insects, in inducing tolerant traits in response to abiotic stresses, and in inducing greater immune fitness in different plants (Chhipa & Deshmukh 2019, Omomowo & Babalola 2019, Khare *et al*., 2018).

Biological control of PPNs including *R. similis* using endophytic microorganisms has been studied and is encouraged for sustainable pest management in banana production. Biocontrol agents such as fungi and bacteria have been tested in management of PPNs in various crops including bananas (Zum *et al*., 2006, Chaves et al., 2009, Mendoza & Sikora, 2009). In bananas, use of endophytic microorganisms as BCAs has been studied in management of banana pests such banana weevils and nematodes such as *Radopholus similis* (Akello *et al*., 2008, Paparu *et al.*, 2009 & Athman *et al*., 2006).

How these endophytic microorganisms enter, establish, and firmly colonize plant tissues and introduce functional traits that positively influence plant productivity however needs to be well understood. Also at what age of the plant should the endophytic microorganisms enter the plant to achieve more beneficial qualities and in what quantities needs to be well understood for effective use of BCAs as a pest management strategy. In this chapter, five BCAs that were tested for *in vitro* nematicidal potential in Chapter 3 were tested for *R. similis* management potential in the screen house using tissue culture (TC) derived banana plantlets.

4.2 Materials and methods

4.2.1 Preparation of fungal inoculum

Two commercial *Trichoderma* products; Real Trichoderma TRC900 and Asperello T34 were used in this study. TRC900 was provided as pure spores, while T34 was provided as a formulated product by Biobest, Belgium. To prepare inoculum, 10g of each of these products was dissolved in 50ml of sterile distilled water (SDW) and spores quantified using a hemocytometer under a light microscope. The suspension was adjusted to the required spore concentration of 1.5×10^7 spores/ml.

4.3 Testing viability of fungal spores

Viability of spores was tested by spreading 0.1ml of a suspension of spores of each isolate at a concentration of $3x10^6$ spores/ml on Potato Dextrose Agar (PDA) and then three sterile microscope coverslips placed randomly on the surface of each PDA plate. Three plates were used per isolate, sealed with parafilm and incubated at 25 ± 2^oC for 18 hours. One hundred conidia under each cover slip were selected and evaluated for germination under a light microscope. Germinated conidia were identified as those that produced a germ tube twice the diameter of a conidium while non germinated conidia were identified as those without a germ tube (Opisa *et al*., 2018). The spores of the isolates were considered viable at 90-99% conidia germination

4.4 Preparation of bacterial inoculum

The three bacterial isolates used in *in-vitro* study (Chapter 3) i.e., *Bacillus* gram negative (1HRB3), *Coccobacillus* gram negative (1DRB1) and *Bacillus* gram negative (1HRB4) were cultured on Yeast Peptone Glucose Agar (YPGA) (yeast extract 5 g, peptone 5 g, glucose 10 g, and agar 15 g) on 30mm diameter petri dishes (Tripathi *et al*., 2015) Pure cultures from a single colony of the bacteria were obtained by streaking on YPGA and incubating for 72 hours at 27 – 30^oC.

Bacterial cells were washed off the YPGA plates with sterile distilled water (SDW) and 1ml of cell suspension was added into 250 ml of sterile YPG Broth (YPGB) in 250ml conical flasks and incubated for 48 h at 27 - 30°C in a rotary shaker at 125 rpm. The cell suspension was adjusted to the required concentration (1.5 x 10^7 CFU/ml) using the standard optical density (OD) reading of 0.5 on the spectrophotometer (600nm) (Nakato *et al*., 2019).

4.5 Tissue culture banana plants

The TC banana plantlets, Mbwazirume variety of EAHBs were obtained from a commercial TC laboratory at the deflasking stage; the time when the plantlets are removed from the test tubes and transferred to the hardening phase. Deflasked tissue-cultured banana plants were washed off all the adhering media with tap water and sorted for uniformity in size. The plants were planted in weaning trays (60 cm x 30 cm) and allowed to grow for one month on soil, manure and saw dust medium. They were then transplanted into 3-litre potting bags (5×9×4 cm) where they were allowed to grow for one month before inoculation with *R. similis.*

Figure 4. 1. Tissue culture derived banana plantlets on weaning plates in a humidity chamber.

4.6 Experimental design and Biocontrol agent treatments

The experiment was conducted twice in a split plot design with BCAs as the main plot factor and inoculation regime as the sub plot factor. For each inoculation regime, ten (10) plants were inoculated with each of the five BCAs (3 bacterial and 2 fungal endophytes) individually. Inoculation regimes involved variation in the number of times and age the plants were inoculated

with BCAs as shown in Table 4.1. For each inoculation regime, ten plants not inoculated with any BCAs were used as control. The plants were inoculated with the BCAs by the drenching method. On weaning trays, each of the TC derived plants was drenched with 3mls of BCAs suspensions. In pots, 250ml of BCA suspension were drenched into each pot. All bacterial and fungal suspensions used for inoculation were at $1.5x10^7$ concentration.

Table 4. 1. Inoculation regimes with BCAs of Tissue culture derived banana plants. X represents inoculation with BCAs

Inoculation regime	Age of banana plants at inoculation with BCAs		
	Deflasking from test tubes	2 weeks	6 weeks
1	X		
2		X	
3			X
4	X	X	
5	X	X	X
6	X		X
7		X	X

4.7 Inoculation of plants with *Radopholus similis*

At eight (8) weeks post deflasking all the plants were inoculated with nematodes. A pure culture of *R. similis* obtained from carrot discs was suspended in sterile distilled water (SDW) in a beaker, and the number of juveniles and females in a 2 ml suspension estimated under a light microscope. To inoculate plants with nematodes, three holes (3-5 cm deep) were made in potting soil at the base of the plant at equal distance from one another (Paparu *et al*, 2009). A mixture of 1000 juveniles and female nematodes in a 2mL suspension were then pipetted into the three holes per plant. Plants were not watered for 24 h to ensure that the nematodes are not washed away.

Figure 4. 2. Inoculation of banana plants with *R. similis* (A) Holes made in the potting soil close to plant roots through which *R. similis* is introduced (B) Inoculation of *R. similis* through holes into roots of the plant.

4.9 Endophyte re-isolation from banana plants

At the end of each experiment, all plants were evaluated for endophyte colonisation by re-isolation of endophytes form the plants. For each plant, three roots and the entire rhizome were used. The roots and rhizomes of inoculated and control banana plants were first disinfected in 5% NaOCl for 1 min and in 75% ethanol for 1 min. They were then rinsed three times using

sterile distilled water and blotted on sterile tissue paper. For each root, two pieces of approximately 0.5 cm long were cut from the tip, middle and base (total of six root pieces per root). For the inner and outer rhizome, six pieces each were used for re-isolation. Surface-disinfected root and rhizome pieces were inserted halfway in PDA supplemented with antibiotics (0.1 g penicillin G, 0.2 g streptomycin sulfate and 0.05 g chlortetracycline L1) in 90 mm diameter petri dishes and incubated in the laboratory for 7 days for fungal isolates (Schuster & Schmoll, 2010). Fungi growing from plated root and rhizome pieces were viewed under a compound microscope (100X and 400X magnification) and identified as *T. asperellum.*

For bacterial isolates, root and rhizome pieces were inserted in YPGA in 90 mm diameter petri dishes and incubated in the laboratory for 3 days. For bacterial isolates, colony morphology and color were used to identify the bacterial isolates. 70 plants (35 treatments and 35 control) were sampled and 420 root pieces and 420 corm pieces were plated, i.e., 6 root pieces and 6 corm pieces per plant (Schuster & Schmoll, 2010). The presence of *T. asperellum* or bacterial isolate in at least one of the root or corm sections was considered an indication of successful colonization of a plant. The data was expressed as percentage colonization i.e., number of plant replicates colonized/number of plant replicates excised ×100.

4.8 Data collection and analysis

At experiment termination (8 weeks after nematode inoculation), the following plant growth parameters were measured; fresh shoot weight, fresh root weight, plant height, and dry shoot weight. The dry shoot weight was measured by weighing banana plant shoots that were previously dried in a dry air oven at 70°C for 7 days.

Fresh shoot and root weight were measured by weighing fresh banana shoots and roots respectively before drying. The plant height was measured by taking the linear dimensions from the corm to the tip of the youngest leaf using a ruler.

Nematodes were extracted from 5 g of macerated root tissue over a period of 48 h using the modified Baermann technique (Coyne *et al*., 2014). Samples were stored in a fridge (4°C) to keep nematodes alive until they were counted. Nematode suspension volume was reduced to 25ml and nematodes quantified from 3-2 ml aliquots under a light microscope. A generalized linear model (GLM) was used to analyze nematode density data and plant growth parameters data. Data was presented as tables of means and standard error and graphs where applicable. All

statistical analysis was done using R software v4.0.2 (R Core Team, 2020) and graphs were made using Microsoft excel.

4.9 Results

4.9.1 Re-isolation of fungal and bacterial endophytes from inoculated banana plants

At termination of each experiment, *T. asperellum* was successfully re-isolated from 56 of 84 root and rhizome pieces (67%), representing 9 of the 14 fungus-treated plants. No fungus was re-isolated from the control plants. Bacterial isolates were successfully re-isolated from 82 of 126 root and rhizome pieces (65%) representing 14 of the 21 bacterial treated plants. No bacterial isolate was re-isolated from the control plants.

4.9.2 Effect of BCAs and their inoculation regimes on *R. similis* densities in TC derived banana plants

Radopholus similis densities differed significantly ($P < 0.0001$) among plants inoculated with the different BCAs. Across inoculation regimes, plants inoculated with BCAs had reduced *R. similis* densities than the control plants not inoculated with BCAs (Table 4.2). There were no significant ($P = 0.611$) differences in *R. similis* densities across inoculation regimes.

Table 4. 2: *Radopholus similis* densities in Tissue culture derived banana roots following inoculation with BCAs and nematodes at different inoculation regimes in the screen house

Isolates	Nematode density (X1000) per 100g of root in different inoculation regimes						
	Deflasking	2wks	6wks	Deflasking+ 2wks	Deflasking+ 2wks+6wks	Deflasking+ 6wks	2wks+6wks
1DRB1	21.2 ± 6.5 bc	14.2 ± 3.6 c	20.6 ± 5.6 abc	27.5 ±10.5ab	30.7 ±6.2abc	16.3 ± 7.2 b	21.2 ± 4.4 c
1HRB3	17.2 ± 3.8 c	37.1 ± 9.9 ab	13.4 ± 4.1 c	28.1 ± 6.8 ab	19.8 ±5.3bcd	32.2 ± 7.1 ab	14.2 ± 3.2 c
1HRB4	16.2 ± 4.9 c	22.6 ± 6.1 bc	18.6 ± 6.1 bc	17.8 ± 4.0 b	17.6 ± 4.5 cd	35.4 ±12.7ab	16.9 ± 5.8 c
T34	40.6 ± 10.5 ab	25.1 ± 4.3 bc	35.1 ± 1.4 ab	16.5 ± 5.1 b	12.9 ± 3.5 d	18.3 ± 5.1 b	38.3 ± 8.4 ab
TRC900	26.9 ± 9.1 bc	22.3 ± 8.4 bc	30.2 ± 7.1 abc	24..1 ±4.7 ab	33.7 ± 5.9 ab	44.1 ± 6.7 a	28.8 ± 4.8 bc
CONTROL	45.5 ± 5.9 a	41.3 ± 5.4 a	40.9 ± 5.3 a	37.3 ± 4.9 a	46.3 ± 6.6 a	49.8 ± 13.6 a	49.2 ± 6.7 a
P value	P=0.013	P=0.046	P=0.096	P=0.117	P<0.0001	P=0.068	P<0.0001

Data are Means ±SE in the experiment. Values in columns followed by different letters are significantly different ($P < 0.05$) based on Tukey's HSD test.

4.9.3 Effect of BCAs and their inoculation regime on height of TC derived banana plants

There were significant ($P < 0.001$) differences in height of plants inoculated with the different BCAs. Across inoculation regimes, height of plants with height of plants inoculated with BCAs being higher than that of control plants at some inoculation regime while in other inoculation regimes the height of control plants being higher than that of plants inoculated with BCAs (Table 4.4). Only plants inoculated with bacterial isolate 1DRB1 had higher heights than control plants across all inoculation regimes. Height of plants was not significantly ($P = 0.097$) different across inoculation regimes.

Table 4. 3: Height of Tissue culture derived banana plants following inoculation with BCAs at different inoculation regimes in the screen house

Isolates	Height (cm) at different inoculation regimes						
	Deflasking	2wks	6wks	Deflasking+ 2wks	Deflasking+ 2wks+6wks	Deflasking+ 6wks	2wks+6wks
1DRB1	27.1 ± 0.6 a	21.9 ± 1.4 a	26.3 ± 1.2 a	23.6 ± 1.5 a	24.5 ± 1.2 ab	21.5 ± 1.4 a	25.4 ± 1.9 a
1HRB3	22.5 ± 1.1 b	22.3 ± 1.7 a	21.1 ± 1.2 c	23.4 ± 1.5 a	20.3 ± 1.7 cd	21.9 ± 1.0 a	21.8 ± 1.0 bc
1HRB4	23.5 ± 1.0 b	22.5 ± 1.1 a	25.1 ± 0.9 ab	17.7 ± 1.0 b	26.4 ± 0.8 a	17.7 ± 0.8 b	24.9 ± 0.9 ab
T34	20.9 ± 1.4 b	19.7 ± 1.2 a	20.1 ± 1.0 c	22.3 ± 0.5 a	21.5 ± 0.9 bc	22.2 ± 1.2 a	19.4 ± 1.1 c
TRC900	17.4 ± 0.8 c	21.6 ± 1.0 a	22.2 ± 1.3 bc	21.5 ± 1.5 a	17.2 ± 1.6 d	19.9 ± 1.2 ab	21.5 ± 0.6 c
CONTROL	21.4 ± 1.0 b	20.8 ± 1.0 a	22.1 ± 1.0 bc	20.9 ± 0.9 ab	21.3 ± 1.0 bc	20.1 ± 0.9 ab	21.8 ± 1.1 bc
P value	P<0.0001	P=0.572	P=0.002	P=0.007	P<0.0001	P=0.060	P=0.003

Data are Means ±SE in the experiment. Values in columns followed by different letters are significantly different ($P < 0.05$) based on Tukey's HSD test.

4.9.4 Effect of BCAs and their inoculation regime on Fresh Root Weight (FRW) of TC derived banana plants

Fresh root weight of plants inoculated with the different BCAs differed significantly ($P < 0.0001$). Only plants inoculated with bacterial isolate 1DRB1 had their fresh root weights higher than those of control plants across all inoculation regimes (Table 4.5). There were no significant ($P = 0.128$) differences in fresh root weight of plants across inoculation regimes.

Table 4. 4: Fresh root weight of Tissue culture derived banana plants following inoculation with BCAs at different inoculation regimes in the screen house

Isolates	Fresh root weight (grams) of bananas at different inoculation regimes						
	Deflasking	2wks	6wks	Deflasking+ 2wks	Deflasking+ 2wks+6wks	Deflasking+ 6wks	2wks+6wks
1DRB1	77.3 ± 7.8 a	74.5 ± 12.1 a	73.9 ± 6.4 ab	66.7 ± 9.3 a	65.9 ± 5.0 a	67.9 ± 8.8 a	83.4 ± 11.6 a
1HRB3	51.6 ± 7.8 bcd	64.8 ± 13.1 a	55.5 ± 5.9 b	51.1 ± 8.2 ab	43.5 ± 6.5 b	52.9 ± 5.1 ab	54.5 ± 5.8 bc
1HRB4	62.4 ± 5.9 ab	58.1 ± 6.1 ab	81.3 ± 7.8 a	45.8 ± 6.3 b	73.1 ± 5.2 a	40.3 ± 6.3 b	69.2 ± 5.4 ab
T34	42.9 ± 5.9 cd	39.8 ± 6.5 b	55.1 ± 5.6 b	49.4 ± 3.5 ab	63.1 ± 5.1 a	52.8 ± 5.6 ab	39.2 ± 4.9 c
TRC900	35.9 ± 5.6 d	57.8 ± 4.9 ab	59.1 ± 5.9 b	54.4 ± 6.4 ab	38.2 ± 6.8 b	48.6 ± 6.3 ab	53.6 ± 4.3 bc
CONTROL	57.1 ± 6.5 bc	59.1 ± 7.3 ab	61.6 ± 7.9 b	60.8 ± 7.6 ab	62.6 ± 7.6 a	61.6 ± 7.8 a	59.7 ± 7.2 b
P value	P=0.001	P=0.114	P=0.032	P=0.335	P<0.0001	P=0.098	P<0.0001

Data are Means ±SE in the experiment. Values in columns followed by different letters are significantly different ($P < 0.05$) based on Tukey's HSD test.

4.9.5 Effect of BCAs and their inoculation regime on Fresh Shoot Weight (FSW) of TC derived banana plants

There were significant ($P < 0.001$) differences in fresh shoot weight of banana plants inoculated with different BCAs. Only Plants inoculated with bacterial Isolate 1DRB1 had their fresh root weight higher that of control plants across all inoculation regimes (Table 4.6). Inoculation regime had a significant (P = 0.047) effect on the fresh shoot weight of the plants.

Table 4. 5: Fresh shoot weight of Tissue culture derived banana plants following inoculation with BCAs at different inoculation regimes in the screen house

Isolates	Fresh shoot weight (grams) of bananas at different inoculation regimes						
	Deflasking	2wks	6wks	Deflasking+ 2wks	Deflasking+ 2wks+6wks	Deflasking+ 6wks	2wks+6wks
1DRB1	106.5 ± 11.2 a	85.3 ± 11.0ab	95.9 ± 6.9 ab	84.3 ± 10.2 a	88.4 ± 6.2 ab	74.5 ± 9.1 a	115.6 ±14.8a
1HRB3	81.5 ± 8.8 ab	91.0 ± 21.4 a	70.0 ± 7.1 c	81.7 ± 18.0 a	62.6 ± 8.4 cd	63.7 ± 5.1 ab	66.9 ± 6.9 c
1HRB4	87.4 ± 9.7 ab	71.6 ± 6.7 ab	100.5 ± 5.6 a	54.4 ± 5.7 b	90.5 ± 3.9 a	49.2 ± 5.7 b	93.0 ± 7.0 ab
T34	65.9 ± 8.5 bc	58.2 ± 7.2 b	64.3 ± 6.3 c	71.4 ± 3.4 ab	72.6 ± 5.3 bc	74.2 ± 8.8 a	57.4 ± 8.1 c
TRC900	47.8 ± 6.7 c	76.7 ± 5.9 ab	78.9 ± 12.1 bc	66.6 ± 8.7 ab	47.0 ± 8.2 d	63.1 ± 6.7 ab	72.2 ± 4.5 bc
CONTROL	65.9 ± 4.9 c	70.2 ± 5.5 ab	75.9 ± 6.0 bc	69.8 ± 5.6 ab	66.6 ± 5.2 c	63.7 ± 4.9 ab	69.8 ± 5.4 bc
P value	P<0.0001	P=0.288	P=0.006	P=0.304	P<0.0001	P=0.140	P<0.0001

Data are Means ±SE in the experiment. Values in columns followed by different letters are significantly different (P < 0.05) based on Tukey's HSD test.

4.9.6 Effect of BCAs and their inoculation regime on Dry Shoot Weight (DSW) of TC derived banana plants

Dry shoot weight of plants differed significantly ($P < 0.0001$). At inoculation regimes, dry shoot weight of plants inoculated with BCAs was higher than that of control plants while in other inoculation regimes, dry shoot weight of plants inoculated with BCAs was lower than that of the control plants (Table 4.7). There were no significant ($P = 0.217$) differences in dry shoot weight of plants across inoculation regimes.

Table 4. 6: Dry shoot weight of Tissue culture derived banana plants following inoculation with BCAs at different inoculation regimes in the screen house

Isolates	Dry shoot weight (grams) of bananas at different inoculation regimes						
	Deflasking	2wks	6wks	Deflasking+ 2wks	Deflasking+ 2wks+6wks	Deflasking+ 6wks	2wks+6wks
1DRB1	9.5 ± 0.9 a	7.6 ± 1.2 a	8.4 ± 0.8 ab	8.6 ± 1.4 a	8.2 ± 0.6 ab	7.5 ± 1.2 a	9.5 ± 1.2 a
1HRB3	6.6 ± 0.7 bc	7.7 ± 1.6 a	6.9 ± 0.8 b	7.7 ± 1.7 a	6.2 ± 0.8 bc	6.4 ± 0.7 ab	6.2 ± 0.7 bc
1HRB4	8.3 ± 0.8 ab	6.3 ± 0.6 ab	10.5 ± 0.7 a	6.1 ± 0.8 a	7.7 ± 0.4 ab	4.9 ± 0.7 b	7.8 ± 0.8 ab
T34	5.6 ± 0.7 cd	4.9 ± 0.6 b	6.4 ± 0.7 b	6.4 ± 0.3 a	6.7 ± 0.6 abc	5.9 ± 0.6 ab	5.6 ± 0.8 c
TRC900	4.3 ± 0.6 d	6.9 ± 0.5 ab	6.8 ± 1.2 b	5.9 ± 0.9 a	5.1 ± 0.8 c	6.5 ± 0.9 ab	5.9 ± 0.4 bc
CONTROL	7.9 ± 0.8 ab	8.0 ± 0.8 a	8.3 ± 0.9 ab	7.9 ± 0.9 a	8.2 ± 0.8 a	7.6 ± 0.8 a	7.9 ± 0.8 ab
P value	P<0.0001	P=0.153	P=0.012	P=0.410	P=0.009	P=0.227	P=0.005

Data are Means ±SE in the experiment. Values in same columns followed by different letters are significantly different ($P < 0.05$) based on Tukey's HSD test.

4.10 Discussion

This study focused on two factors i.e., effect of BCAs and their inoculation regimes on *R. similis* densities in banana plants and their effect on growth of the banana plants in the screen house. All bacterial and fungal isolates were effective in reducing *R. similis* densities compared to the control. This implies that these isolates have the potential to be used as BCAs against *R. similis* in bananas. Reduction of *R. similis* densities in plants by bacteria and fungi happens through direct antagonistic activity against the nematodes for example through production of compounds by BCAs that have nematicidal effects. Indirectly, bacteria and fungi can reduce *R. similis* density through promotion of growth in the plant so that the plant establishes fast enough before the nematodes cause significant effects and induced systemic resistance that is developed by the plant due to presence of BCAs within the plant.

In a related study, Poveda *et al*., (2020) reported that fungi in the genus *Trichoderma* have potential as biocontrol agents against plant parasitic nematodes by either direct parasitism, paralysis or production of nematicidal compounds. Further still, genus *Trichoderma* have the ability to stimulate plant defenses against plant parasitic nematodes through induced systemic resistance in plants. *Trichoderma asperellum* produced volatile compounds such as δ-lactone 6-*n*-pentyl-2*H*-pyran-2-one (6-PAP) (Jeleń *et al.,* 2014). More recently, organic extracts of the soil-borne fungus *Trichoderma* sp. YMF 1.00416 were also tested for nematicidal activity (Yang *et al*., 2012). Others include 6-(1'-pentenyl)-2*H*-pyran-2-one, massoialactone, δ-decalactone, and viridepyronone (Reino *et al.,* 2008) also have nematicidal effect and have shown to reduce plant parasitic nematode densities in lettuce plants (Wonglom, *et al.,* 2020).

Endophytic bacteria including *Bacillus* spp i.e., *B. pumilis, B. megaterium, B. subtilis* and *Pasteuria fluorescens* are able to reduce densities of plant parasitic nematodes directly through production of chitinolytic enzymes that degrade the egg shells or larvae inside the eggs. Indirectly bacteria colonize the host root surface, forming a physical barrier and degradation of root exudates disorienting nematodes movements so reducing root penetration (Migunova & Sasanelli, 2021).

Inoculation regime had no significant effect on reducing *R. similis* densities for all BCAs implying that as long as plants have been inoculated with BCAs and there is effective colonization by the BCAs, it does not matter how many times one enhances them with BCAs. These result differ from results of a study by Paparu *et al*., (2009) where several inoculations with fungal endophyte *Fusarium oxysporum* reduced *R. similis* densities in banana plants.

Plants inoculated with BCAs had increased growth differently at different inoculation regimes. Some plants inoculated with BCAs had higher growth than control plants while some plants had lower growth than control plants. This indicates that these BCAs have the potential to cause growth though the time of enhancement with BCA and quantity of BCAs needs to be observed. Consistency of the bacterial isolate *Coccobacillus* gram negative (1DRB1) in having higher growth than the control plants across all inoculation regimes except with dry shoot weight was an indication of its potential to promote of growth in banana plants compared to other BCAs. The mechanisms with which these isolates promote growth in these banana plants need to be further studied.

Since plants inoculated with the same bacterial isolate had lower *R. similis* densities than all the other isolates and the same plants have shown more growth, this makes bacterial isolate 1DRB1 a good BCAs capable of reducing *R. similis* densities and also promotion of growth. In related studies, *coccobacillus* gram negative bacteria have demonstrated ability to kill plant parasitic nematodes (Safni et al., 2018). In their study, such bacteria are capable of producing proteolytic enzymes gelatinase, protease, and chitinase that have nematicidal effects. Also, *coccobacillus* gram negative bacteria have been characterized as a plant growth promoting Rhizobacteria (PGPR) (Rajendran *et al*., 2012). Plant Growth Promoting Rhizobacteria use different mechanisms of promoting plant growth and also improving the health of plants. Such mechanisms include; phosphate solubilization, biological nitrogen fixation, improvement of other plant nutrients uptake, and phytohormone production (like indole-3-acetic acid) (Olanrewaju *et al*., 2017).

Inoculation regime had no significant effect on plant growth parameters of height, fresh root weight and fresh shoot weight. This implies that it does not matter the number of times inoculation with BCAs is done to enhance growth as long as there is effective colonization of plants with BCAs (Jaber *et al.,* 2018). Despite successful colonization of BCAs in banana plants, this however did not translate to increase in plant growth in some plants. Inconsistent effects of fungi and bacteria on plant growth parameters have been reported. Akello *et al*., (2008) did not observe a major difference in plant growth following inoculation of banana (*Musa* spp.) with endophytic *B. bassiana* strain compared to the control treatment.

4.11 Conclusions

The effect of BCAs and the inoculation regime on densities of *R. similis* and their effect on banana plant growth was studied and based on the results, the following conclusions can be made;

All fungal and bacterial isolates in this study reduced *R. similis* densities in banana plants

All fungal and bacterial isolates used in this study promoted growth of banana plants but at different inoculation regimes.

Inoculation of banana plants with BCAs several times does not reduce the density of *R. similis* in banana plants

Inoculation of banana plants with BCAs several times does not result into increased banana plant growth

4.12 Recommendations

This study provides information on the utilization of bacterial and fungal endophytes in management of *R. similis* in bananas. From the conclusions, the recommendations below can be made;

These bacterial and fungal isolates should be used as biocontrol agents against *R. similis* in bananas but along with other pest management strategies.

Tissue culture derived banana plants should be enhanced with these bacterial and fungal endophytes before given to farmers to keep them protected against *R. similis*

These bacterial and fungal endophytes should be further studied for their plant growth promotion abilities and then they can also be used as growth promoters.

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CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 General discussion

This study focused on the effect of culture filtrates of bacterial and fungal endophytes on *Radopholus similis* in the laboratory. Also the effect of bacterial and fungal endophytes on *R. similis* in banana plants in the screen house was studied. The effect of age at which the banana plants are enhanced with endophytes and how many times of inoculation with endophytes (inoculation regime) on *R. similis* in banana plants in the screen house was also studied.

All culture filtrates of bacterial and fungal isolates paralyzed *R. similis* highly after 3, 6 and 12 hours of exposure compared the control solution which was sterile distilled water. This showed that these filtrates contained compounds that had the ability to paralyze *R. similis*. Further still, since percentage paralysis of *R. similis* by culture filtrates of all isolates of fungi and bacteria differed from that of the control, it was evident that the filtrates contained compounds that were able to paralyze *R. similis*.

Mortality of *R. similis* was used as an indicator for the identification of the isolates that can be potential BCAs in the management of *R. similis*. All culture filtrates of bacterial and fungal isolates caused higher mortality of *R. similis* compared to the control. This was an indication that the BCA filtrates contained compounds that were toxic to *R. similis*. Presence of compounds that are toxic to *R. similis* by fungal and bacterial culture filtrates was also reported in related studies by Sharon *et al*., (2007) carrying out *in vitro* bioassays, and verified that extracts of *T. asperellum* released in soil had compounds that were capable of immobilizing juveniles of *Meloidogyne javanica* and reducing egg viability. Many fungi have been studied and are known to produce compounds that are toxic to nematodes (Hallmann & Sikora, 1996). Also studies by Youssef *et al*., (2020), Hemeda & Deeb, (2019) have reported that culture filtrates of bacteria (*Bacillus* sp.) contain compounds that are toxic to nematodes.

All banana plants enhanced with bacterial and fungal endophytes had reduced *R. similis* densities compared to the control plants that were not enhanced with endophytes. Reduction of *R. similis* densities in plants enhanced with bacteria and fungi endophytes was attributed to the direct antagonistic activity of the endophytes against the nematodes through production of compounds by BCAs that had nematicidal effects.

Poveda *et al*., (2020), working on a related study reported that fungi in the genus *Trichoderma* have potential as biocontrol agents against plant parasitic nematodes by either direct parasitism, paralysis or production of nematicidal metabolites. Migunova & Sasanelli, (2021) reported that endophytic bacteria including *Bacillus* spp i.e., *B. pumilis, B. megaterium, B. subtilis* and *Pasteuria fluorescens* are able to reduce densities of plant parasitic nematodes directly through production of chitinolytic enzymes that degrade the egg shells or larvae inside the eggs. Indirectly bacteria colonize the host root surface, forming a physical barrier and degradation of root exudates disorienting nematodes movements so reducing root penetration.

Inoculation regime i.e., the age at which banana plants were enhanced with endophytes and how many times the plants were enhanced with endophytes had no effect in reducing *R. similis* densities in the banana plants. This implies that it does not matter how many times you inoculate with BCAs as long as the is maximum colonisation with BCAs. These results however differ from results of a related study by Paparu *et al*., (2009) working on the dual inoculation of banana plants with endophyte *Fusarium oxysporum* and that dual inoculations with this endophyte reduced *R. similis* densities in banana plants.

There were differences in growth of plants inoculated with both fungal and bacterial endophytes at the different inoculation regimes. Some plants showed higher growth than control plants that were not enhanced with BCAs at some inoculation regimes while other plants inoculated with BCAs showed little growth than control plants at other inoculation regimes. This shows the potential of these endophytes to promote growth though the timing of inoculation into the plants has to be observed. Only plants inoculated with bacterial endophyte 1DRB1 had higher growth than control plants at all inoculation regimes and plants inoculated with the same endophyte had reduced R. similis densities than control plants. This makes 1DRB1 a good biocontrol agent with ability to promote growth in banana plants.

Inoculation regime had no significant effect on plant growth despite successful colonization of BCAs in banana plants. It does not matter how many times you inoculate banana plants with endophytes to enhance growth as long as there is successful colonization by the endophytes. Akello *et al*., (2008) working on a related study did not observe a major difference in plant growth following inoculation of banana (*Musa* spp.) with endophytic *B. bassiana* strain compared to the control treatment.

5.2 General conclusions

Culture filtrates of all bacterial and fungal isolates paralyzed and killed *R. similis*. This showed that these filtrates contained compounds that can kill *Radopholus similis*

Exposure of *R. similis* to culture filtrates of all bacterial and fungal endophytes for a long time increases the percentage mortality

Plant enhanced with fungal and bacterial endophytes had reduced *R. similis* densities compared to control plants that were not enhanced with endophytes. Also plants enhanced with bacterial and fungal endophytes showed relatively higher growth compared to control plants in the screen house at some inoculation regimes but in other inoculation regimes, control plants had higher growth.

Age at which banana plants are enhanced with endophytes and number of times of inoculation with endophytes (inoculation regime) has no effect on *R. similis* densities in banana plants. Inoculation regime also has no effect on growth of banana plants

5.3 General recommendations

This study provides information on the potential bacterial and fungal endophytes that can be used as BCAs in the management of *R. similis* in EAHBs which is one of the major pests of bananas. Proper management of *R. similis* is essential for the improvement of banana production amongst rural farmers in Uganda. The following are the recommendations from this study;

Tissue culture laboratories involved in the production of clean banana planting materials should integrate production of bio-enhanced TC derived banana plantlets in their production chain.

This will ensure production of banana plants that are enhanced with beneficial microbes and also banana plants that are protected from *R. similis.*

Since culture filtrates of bacterial and fungal isolates contained compounds that had the potential to paralyze and kill *R. similis* in the laboratory, the compounds in these filtrates should be further explored and could be used as biopesticides against *R. similis* in future.

The bacterial and fungal endophytes in this study should further be explored and maybe used as biofertilizer due to their ability to enhance growth of the banana plants.

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