



**Comparative analysis of fungal biocontrol agents on
Meloidogyne species infestation on potato cultivars
under different rhizosphere amendments**

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DECLARATION

I, Mukondeleli Ndivhuwo Ramatsitsi declare that this dissertation with the research title “Comparative analysis of indigenous fungal bio-control agents on *Meloidogyne enterolobii* and *Meloidogyne javanica* infestation on potato” submitted to North-West University is my original work. I have not previously in any form or part submitted to any other institution. All sources used in this work have been acknowledged through references.

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DEDICATION

To my nieces; Rilwele, Lethabo and Lesedi as well as my nephew; Thendokhae

The Lord said to Abraham, “*Look as far as you can see in every direction...*” **Genesis 13:14**

And God was challenging Abraham to dream bigger than what he could physically see.

I say to you, your dreams are valid and as big as you see them.

If my education, skills and expertise do not change the world;

it is useless.

And if it changes the world, but does not benefit my family;

it is not worth it.

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LIST OF ABBREVIATIONS

ANOVA:	Analysis of variance
ARC–ITSC:	Agricultural Research Council-Institute of Tropical and Subtropical Crops
BCA:	Bio-control agents
C:N:	Carbon to Nitrogen
CRD:	Completely randomised design
DNA:	Deoxyribonucleic acid
E:	Equilibrium
EC:	Electrical conductivity
HSD:	Turkeys Honestly significant difference
IAA:	Indole-3-acetic acid
ITS:	Internal transcribed spacer
J1:	First-stage juvenile
J2s:	Second-stage juvenile
J3:	Third-stage juvenile
J4:	Fourth-stage juvenile
LSU:	Large-sub unit
MEA:	Malt extract agar
NA:	Nutrient agar
NaOCl:	Sodium hypochlorite
NCBI:	National Center for Biotechnology Information
NCDS:	North Carolina Differential Scale
NCN:	National Collection of Nematodes
OC:	Organic compost

PCR:	Polymerase Chain Reaction
PDA:	Potato dextrose agar
Pf:	Final population density
Pi:	Initial population density
PPN:	Plant-parasitic nematodes
RCBD:	Randomised complete block design
RF:	Reproductive factor
RKN:	Root-knot nematodes
SAPPNS:	South African plant-parasitic nematodes survey
SSU:	Small-sub unit
v/v:	Volume/volume
VI:	Vigour index
VOC:	volatile organic compounds
WA:	Water agar

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THESIS SUMMARY

Potato (*Solanum tuberosum* L.) is a well-known food crop that is regarded as an important component in the worldwide battle against hunger and malnutrition. Potato has significant potential to help contribute to Africa's rising food demands since it can produce more food per hectare than most crops. However, among many other pests and diseases, root-knot nematodes (RKN), *Meloidogyne* species, are serious limitations in the potato industry. Yield reduction of potatoes is mainly due to tuber quality and quantity. Given the significant economic impact caused by RKN, a variety of nematode-control techniques, including the use of synthetic chemical nematicides, have been developed. The discontinuation of several pesticides as a result of the United Nations Framework Convention on Climate Change multilateral agreements has nonetheless increased the need to implement effective ecologically safe nematode management practices. Using bio-control strategies has been suggested as a considerably safer and more feasible option for managing RKN. Currently, a diverse array of species, including nematophagous fungi are known to serve as bio-control agents (BCA) against RKN. These eukaryotes have a selective adaptation to different soil and climatic conditions such as temperature, pH and salinity levels, which further influences their interaction with RKN and their host. Considering these factors, it may be inferred that indigenous fungal BCA would stand a better chance at establishment in the rhizosphere and managing target pathogens as compared to exotic species. The study focused on eight major areas, (1) Screen for *M. enterolobii* and *M. javanica* nematodes susceptibility of five potato cultivars, viz., 'Buffelspoort P1', 'Hertha', 'Larnoma', 'Mnandi' and 'Up-to-date'; (2) Isolate and identify indigenous fungal species as possible candidates for bio-control investigation; (3) Investigate parasitism of the indigenous fungal species on *M. enterolobii* and *M. javanica* eggs and juveniles *in vitro*; (4) Investigate pathogenesis of nematode-parasitic fungi on selected

seeds, viz., tomato, beetroot, swiss chard, dry bean, maize, sorghum and pumpkin; (5) Determine growth rate and geotropism of fungal species on different grain substrates, viz., red sorghum, white sorghum, barley, wheat and wheat bran; (6) Carry out *in vitro* screening of fungal species tolerance to stress and (7) Determine the effect of fungal species on *M. enterolobii* and *M. javanica* population densities on susceptible potato cultivar with and without compost incorporation under net house and field conditions. Reliability of measured variables was attained by using statistical levels of significance ($P \leq 0.05$) and coefficient of determination (R^2), with validity being ensured by conducting experiments at the same location over two seasons. Results of the first objective consistently demonstrated that potato cultivars, i.e., ‘Buffelspoort 1’, ‘Hertha’, ‘Larnoma’, ‘Mnandi’ and ‘Up-to-date’ were hosts to *M. enterolobii* and *M. javanica*, with the test nematodes inflicting damage to plants. Quadratic relationships between nematode reproductive factor (RF) values and initial population (P_i), in addition to confirming the density-dependent growth patterns of plant-parasitic nematodes (PPN), confirmed that the five test potato cultivars were susceptible to the two *Meloidogyne* species. Using serial dilution, a total of 25 fungal biotypes were isolated from soils collected from the rhizosphere of various fruit trees and vegetable plots. Soil samples were collected from plots with no previous record of fungal inoculations at the Agricultural Research Farm, North-West University (NWU), Molelwane, Mmabatho, North-West Province (25°48’36”S, 25°37’ 47.999”E). The biotypes were morphologically identified to genus level using colony characteristics, i.e., conidia, hyphal form and conidiophores. Using the molecular identification polymerase chain reaction (PCR) method, eight fungal biotypes were confirmed as follows: *A. flavus* (MT645322.1), *A. terreus* (MT316343.1), *Penicillium* sp. AL-38 IRH-2012b (KC341982.1), *Talaromyces minioluteus* (MN788118.1), *Tal. purpureogenus* (AB872818.1), *Tal. sayulitensis* (MZ014549.1), *Tri. ghanense* (MF078652.1) and *Tri. viride* (MW456070.1). *In vitro* fungal nematophagous assessment of the eight fungal species was carried out by

assessing the efficacy of indigenous fungal species on *Meloidogyne* species' eggs + second-stage juveniles (J2s) at 24, 48, 72 and 96 hours after exposure. *Talaromyces sayulitensis* had the highest *M. enterolobii* eggs parasitism (100%), in comparison to *A. terreus* which had the lowest parasitism (28%). *Aspergillus flavus*, *Tal. purpureogenus* and *Tal. sayulitensis* had the highest parasitism on *M. javanica* eggs (100%). On the other hand, *Penicillium* sp. had the lowest parasitic effects (55%). *Talaromyces sayulitensis* had the highest parasitic effect on *M. enterolobii* J2s (95%) and *A. terreus* had the lowest parasitism (60%). *Aspergillus flavus* and *Tal. sayulitensis* had the highest parasitic effects on *M. javanica* J2s (95%), whereas *Tri. ghanense* had the lowest parasitism (61%). The trend of the results showed that parasitism of the test fungi on *Meloidogyne* eggs and J2s increased with increasing time of exposure. The *in vitro* results exhibited that the test fungal species have the potential to be applied as BCA against *Meloidogyne* nematodes. Given that certain fungal species are parasitic and therefore with the potential of destroying many crops; their pathogenic effects were further assessed on different crops' seeds of economic importance, viz., tomato, dry bean, swiss chard, beetroot, maize, sorghum and pumpkin. It was observed that species within the same genus could affect crops in both favourable and unfavourable conditions. For instance, whilst *A. terreus* improved seed germination and seedling growth, *A. flavus* caused seed rot and reduced seed germination. It was demonstrated that the screened indigenous nematophagous fungal species, except for *A. flavus*, have exhibited seed germination enhancement and seedling enhancement. For the fungal isolates to be commercially developed for use as bio-control agents for problematic pathogens they should easily be produced and bulked. To commercially produce these fungal inoculants, they need to be cultured and bulked in the laboratory. Different grain substrates, viz., red sorghum, white sorghum, barley, wheat and wheat bran were screened for their performance as growth media for these respective fungal species. The fastest growth was observed on red sorghum followed by barley, white sorghum and wheat; whereas wheat bran

showed the least growth. On red sorghum, all fungal species grew towards both positive (+ve) and negative (-ve) geotropism, i.e., downwards and upwards, respectively. *Trichoderma viride* recorded the highest +ve growth at an average of 9.3 cm. On the other hand, *Tal. minioluteus* showed the highest negative (-ve) tropism growth at 8.8 cm. Although at varying degrees, all species exhibited the most rapid -ve geotropism growth on substrates, except for *Tal. purpureogenus*, which failed to grow at all on wheat bran. The grain substrate, especially the red and white sorghum, technique was effective in mass-producing the studied fungal species. Ideally, to successfully dominate and protect plants from stressors, BCA should tolerate varying environmental conditions when they are introduced. However, BCA are living micro-organisms and their survival and efficacy can be impeded by extreme conditions. Accordingly, the study further evaluated whether indigenous nematophagous fungal species could tolerate different levels of salinity, pH, nutrients and temperature. The results showed that, although being indigenous, *Aspergillus*, *Tal. sayulitensis* and *Tri. ghanense* failed to thrive in saline conditions and high pH. Compared to other species, *Penicillium* sp. failed to thrive under reduced nutrient levels. All fungal species exhibited minimal to no growth at 10-20 °C. Five nematophagous fungal species that showed the highest *in vitro* parasitism on *Meloidogyne* eggs + J2s were further assessed for their ability to reduce *M. enterolobii* and *M. javanica* population density on potato cultivar ‘Hertha’ with and without the incorporation of organic compost (OC) *in vivo*. The *in vivo* experiments were under a net house and repeated under field conditions. Results showed, though inoculated with *Meloidogyne* species; plant height, chlorophyll content, fresh shoot weight, dry shoot weight and bulb weight were significantly increased when administered with nematophagous fungal species ($P \leq 0.05$). On the other hand, gall rating, final J2s population density (Pf) and RF were significantly decreased ($P \leq 0.05$). These findings imply that the efficacy of indigenous nematophagous fungal species can be improved

by the application of OC to control *Meloidogyne* nematode infection and can be used as an environmentally safe alternative method to synthetic nematicides.

Keywords: Bio-control agents; geotropism; indigenous; interactions; *Meloidogyne*; nematophagous; potato; reproductive factor; root galls; parasitism; substrate; susceptible; stress responses.

CHAPTER 1: INTRODUCTION

1.1 Background

Nematodes are a diverse group of small, multicellular organisms that range from parasitic to beneficial and free-living species. Plant-parasitic nematodes (PPN) are the most common type of nematode that may affect living plants and are capable of penetrating roots or above-ground plant parts for feeding and reproduction (Ansari & Khan, 2012; Gullino *et al.*, 2020; Pulavarty *et al.*, 2021). Accordingly, they develop an obligate parasitic relationship with their hosts (Fosu-Nyarko & Jones, 2016). To date, over 4300 species of PPN have been reported, accounting for 7% of the Nematoda phylum (Decraemer & Hunt, 2006). They parasitise a broad variety of plant species, monocots and dicots, including weeds (Ntidi *et al.*, 2012; Onkendi *et al.*, 2014; Shapiro-Ilan *et al.*, 2015; Talwana *et al.*, 2016; Sikora, 2018).

Plant-parasitic nematodes pose a great threat to the global economy and are accountable for heavy losses in food production (Treonis *et al.*, 2018). Vegetable yields may occasionally decrease by up to 29% and even 100% for genotypes that are susceptible to PPN (Sabeh *et al.*, 2019). An estimated global \$173 billion worth of agricultural loss occurs each year due to PPN alone (Elling, 2013). Plant-parasitic nematode classification is mostly centred on feeding habits and dietary source materials (Palomares-Rius *et al.*, 2017; Hodda, 2022). Sedentary groups are the most damaging species among all the significant PPN because they establish a permanent feeding site. Owing to a fascinating and intricate process of host cell transformation, sedentary nematodes have a natural advantage over their migratory counterparts (Jones *et al.*, 2013; Escobar *et al.*, 2015; Palomares-Rius *et al.*, 2017; Smant *et al.*, 2018).

The three most commercially significant categories are root-knot (*Meloidogyne* species), cyst (*Heterodera* and *Globodera* species) and lesion nematodes (*Pratylenchus* species) (Sikora *et al.*, 2018). Comparatively, no other nematode species comes close to the huge economic relevance of *Meloidogyne* species (Trudgill & Blok, 2001; Moens *et al.*, 2009; Talwana *et al.*, 2016; Sikora *et al.*, 2018; Almohithet *et al.*, 2020). This is because of their wide host range, complex relationship with the host, level of damage caused by infestation and serious yield losses (Gregory *et al.*, 2017; Coyne *et al.*, 2018). *Meloidogyne* species were reported to cause approximately 15-25% reduction in global food production, with estimated economic losses of approximately 80 billion US dollars per year (Jiang *et al.*, 2018). The majority, if not all, of cultivated crops are affected by at least one or more of this ubiquitous group of nematodes (Moens *et al.*, 2009; Sikora *et al.*, 2018).

The most prevalent and commercially significant PPN that may parasitise potatoes are the root-knot nematodes (RKN), which belong to a group of around 24 genera (Saucet *et al.*, 2016; Jones *et al.*, 2017; Mhatre *et al.*, 2019). Potato production is limited by RKN because they cause lower yield, physical and chemical alterations in potato tubers, resulting in poor tuber quality and deformities, all of which render them unmarketable (Vovlas *et al.*, 2005b; Medina *et al.*, 2017). Potato yield losses brought on by nematode parasitism depend on many variables, such as cultivar, soil structure, nematode population density and time of planting and they may cause a more drastic reduction in yield at specific cropping systems (Vovlas *et al.*, 2005b; Wesemael *et al.*, 2011; Jones *et al.*, 2013; Medina *et al.*, 2017). Given that there are no commercially available RKN-resistant cultivars, controlling RKN in potatoes is challenging. Soil fumigants and synthetic nematicides are the most widely used nematode control strategies (Giannakou & Panopoulou, 2019; Ilieva *et al.*, 2021; Castellano-Hinojosa *et al.*, 2022). However, they may be expensive and hazardous to the environment and humans (Ebone *et al.*,

2019). Additionally, there is the possibility that nematodes might develop resistance as a result of excessive usage of particular nematicides (Wram & Zasada, 2019), resulting in reduced efficacy over time. Additional current approaches to reduce RKN include crop rotation with non-hosts or the use of biological controls and soil additives (Ntalli & Caboni, 2012; Forghani & Hajihassani, 2020), although such methods may not completely eradicate the nematode problem.

The need for effective ecologically sound management programs for RKN has become an urgent task globally. Hence, there have been global initiatives for developing sustainable eco-friendly nematode management approaches (Fourie *et al.*, 2016; Finch *et al.*, 2018; Kamalnath *et al.*, 2019; Khan *et al.*, 2020). One of these approaches includes the use of nematode-parasitic fungi, also referred to as nematophagous fungi, with the incorporation of organic soil amendments (Rizvi *et al.*, 2018). The introduction of organic compost (OC) into the soil provides multiple benefits including providing a carbon source of the antagonistic fungi and allowing the application of the fungi in any amount (Bonanomi *et al.*, 2011). The efficacy and survival of bio-control agents (BCA) that are associated with RKN depend on several parameters that include environmental factors such as pH and carbon dioxide (Coventry *et al.*, 2006; Smolińska & Kowalczyk, 2014).

1.2 Problem statement

Most commercial crops, including potatoes have been reported to have limited resistance to *Meloidogyne* species (Marais *et al.*, 2015). In such crops, yield losses escalate to as high as 50% to complete crop failure due to infestation by RKN, *M. incognita* (Pofu *et al.*, 2012). Management and control of nematodes have previously been extensively dependent on the use of synthetic nematicides (Ntalli & Caboni, 2012). However, following the global withdrawal

of several synthetic nematicides from agro-chemical markets because of their harmful effects, there has been a serious void of eco-friendly effective nematode management and control programs (Caboni *et al.*, 2015).

1.3 Justification of the study

Current programs of RKN management are limited largely to generic approaches such as crop rotation, intercropping, soil sterilisation and synthetic nematicides. These methods, are however rather ineffective and cause numerous unexpected drawbacks (Pimentel, 2009; Mitiku, 2018). However, the use of fungal BCA has been reported to have the potential for use in the management and control of nematode infestation and has attracted increasing attention from nematologists worldwide (Aminuzzaman *et al.*, 2013; Ferreira *et al.*, 2018; Bansal *et al.*, 2021). Introduced or exotic species may not survive in environments they are introduced into and may have unknown negative impacts on other soil microbes and pose ecological ramifications. On the other hand, indigenous species would have a substantial competitive advantage over introduced ones since they may be more adapted to local conditions. This study therefore sought to investigate the use of indigenous nematophagous fungi with the incorporation of organic amendments on the most economically important nematode species on potato cultivars.

1.4 Purpose of the study

1.4.1 Aim

This study aimed to develop an ecologically stable *Meloidogyne* bio-control management system.

1.4.2 Objectives

The objectives of this study were to:

- (1) Screen for *M. enterolobii* and *M. javanica* nematodes susceptibility levels of five potato cultivars, viz., 'Buffelspoort P1', 'Hertha', 'Larnoma', 'Mnandi' and 'Up-to-date'.
- (2) Isolate and identify indigenous fungal species as possible candidates for bio-control investigation.
- (3) Investigate parasitism of the indigenous fungal species on *M. enterolobii* and *M. javanica* eggs and juveniles.
- (4) Investigate the pathogenesis of nematode-parasitic fungi on selected seeds, viz., tomato, beetroot, swiss chard, dry bean, maize, sorghum and pumpkin.
- (5) Determine growth rate and geotropism of fungal species on different grain substrates, viz., red sorghum (*Sorghum bicolor* (L.) Moench subsp. *drummondii*), white sorghum (*S. bicolor* (L.) Moench), barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.) and wheat bran.
- (6) Carry out *in vitro* screening of fungal species tolerance to abiotic stress.
- (7) Determine effects of fungal species on *M. enterolobii* and *M. javanica* population densities on susceptible potato cultivar with and without compost incorporation under net house and field conditions.

1.4.3 Research questions

- (1) Would five potato cultivars, viz., 'Buffelspoort P1', 'Hertha', 'Larnoma', 'Mnandi' and 'Up-to-date' have different susceptibility levels?
- (2) Would the soil used harbour potential indigenous fungal bio-control agents that vary morphologically and molecularly?

- (3) Would indigenous fungal species parasitise *M. enterolobii* and *M. javanica* eggs and juveniles?
- (4) Would nematophagous fungi be pathogenic to selected seeds, viz., tomato, beetroot, swiss chard, dry bean, maize, sorghum and pumpkin?
- (5) Would fungal species vary in growth rate and geotropism on different grain substrates, viz., red sorghum, white sorghum, barley wheat and wheat bran?
- (6) Would fungal species tolerate different abiotic stress *in vitro*?
- (7) Would fungal species reduce *M. enterolobii* and *M. javanica* population densities on susceptible potato cultivar with and without compost incorporation under net house and field conditions?

1.4.4 Hypotheses

- (1) Five potato cultivars, viz., ‘Buffelspoort P1’, ‘Hertha’, ‘Larnoma’, ‘Mnandi’ and ‘Up-to-date’ will have different susceptibility levels.
- (2) The soil used would harbour potential indigenous fungal biocontrol agents that vary morphologically and molecularly.
- (3) Indigenous fungal species will parasitise *M. enterolobii* and *M. javanica* eggs and juveniles.
- (4) Nematode-parasitic fungi will not be pathogenic to selected seeds, viz., tomato, beetroot, swiss chard, dry bean, maize, sorghum and pumpkin.
- (5) Fungal species will vary in growth rate and geotropism on different grain substrates, viz., red sorghum, white sorghum, barley, wheat and wheat bran.
- (6) Fungal species will tolerate different abiotic stresses *in vitro*.

(7) Fungal species will reduce *M. enterolobii* and *M. javanica* population densities on susceptible potato cultivar with and without compost incorporation under net house and field conditions.

CHAPTER 2: LITERATURE REVIEW

2.1 Potato (*Solanum tuberosum* L.)

2.1.1 Potato growth requirements

Potato grows under a variety of climatological zones including temperate, tropical and sub-tropical regions, under diverse agro-ecological conditions including lowlands and highlands (Askew, 2001). For optimum yield, potatoes thrive at a temperature range of 15-20 °C (Nyawade *et al.*, 2019c). Potatoes can endure dryness, but if it is within six weeks after planting and during the tuber initiation period, their output will be significantly diminished (Nyawade *et al.*, 2019c). Potatoes are among the most drought-sensitive crops due to their shallow root system (Zarzynska *et al.*, 2017). Important physiological and biochemical processes are severely inhibited by drought, which results in poor plant performance and decreased tuber yield. However, the length and intensity of drought events, together with the cultivar and stage of plant growth, all have a major influence on the amount of this loss (Plich *et al.*, 2020).

Ideally, potatoes require a minimum of 750 mm and a maximum of 1200 mm rainfall as long as it is during the cold season (Gitari *et al.*, 2018). FAO (2012) stated that an altitude of 1500-4200 metres is best for potato productivity in tropical areas. The crop grows best on well-drained soils with a pH range of 4.8 to 5.8 and a salt threshold of up to 1.7ds/m (Haverkort & Verhagen, 2008). Potato cultivation requires thorough land preparation. Loam and sandy-loam soils are very suitable for potato cultivation (FAO, 2008). Agronomic practices such as weeding should be carried out as soon as the seedling emerges and when crops are 20 cm tall to ensure good crop development and yield. To prevent stolon from turning into aerials, shallow ridging should be practised. Potatoes require a range of micro- and macro-nutrients, such as nitrogen, phosphorus, potassium, calcium, magnesium and sulphur (Nyawade *et al.*, 2019a;

Nyawade *et al.*, 2019b; Gitari *et al.*, 2020), for strong growth performance and quality (FAO, 2019).

2.1.2 Importance of potatoes

2.1.2.1 Dietary importance

In a world where hunger is still widespread due to both lack of access to food and continuous inflations on food prices, potatoes have the potential to contribute to both food security and nutrition security. According to Devaux *et al.* (2020), potatoes are the most popular non-grain food in the world and the third most extensively eaten crop worldwide after rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.). More than one billion people throughout the world eat potatoes as their primary source of carbohydrates (Muthoni & Shimelis, 2023). Potatoes are grown for sustenance because they have the highest potential for yield growth and have a large amount of starch (16.1/100 g), protein (2.1/100 g), vitamin C (17.1 mg/100 g), potassium (443 mg/100 g), and necessary amino acids such as thiamin and niacin (DADO, 2015).

Prior to the agricultural revolution, people first made their potato consumption decisions based on characteristics including tuber colour, shape, size and skin elegance in the market (André *et al.*, 2007). However, human nutritional health-related studies emphasise the use of nutrient-rich potatoes and their key role in diet (in mineral malnutrition) as part of lifestyle change in consumers due to the sharp increase in chronic degenerative diseases like cancers, diabetes, asthma, cardiovascular disorders and tuberculosis, among many others (André *et al.*, 2007).

2.1.2.2 Economic importance

Potato is one of the pivotal income crops for addressing food insecurity and reducing poverty among smallholder and large-scale farmers in developing countries. Potatoes are

predominantly used as human food all around the world in various forms such as boiling, French fries, flakes, chips and powder (Araujo *et al.*, 2016). The South African potato industry is essentially self-sufficient, and a large portion of its fresh and processed product is exported. The primary potato industry is worth R8 billion, whereas the secondary potato industry is worth R25 billion in South Africa (PSA, 2021a).

2.1.3 Global potato production

In the northern hemisphere's temperate zone, where the crop is cultivated in the summer season, i.e., with the absence of frost, the cropping intensity is at its maximum Muthoni *et al.*, 2010). Potato is a significant source of revenue in these areas since it is primarily farmed as a cash crop. In tropical areas, the crop is important in the Andean highlands, the African highlands and the Rift Valley, as well as the volcanic mountains of West Africa and Southeast Asia, where production is for both staple foods and money (Muthoni *et al.*, 2010). In the subtropics, the crop is grown as a winter crop during the cool season, as is the case in the Mediterranean area, North India and southern China. The only regions where potatoes are not a primary staple food are the tropical lowlands, mostly because the temperatures are too high for potatoes to thrive (Haverkort *et al.*, 2013).

Around 375 million tons of potatoes were produced worldwide in 2022 on an estimated 17.80 million hectares of land (FAO, 2024). The worldwide potato output decreased by around 1% (368.25 million tons) since 2017. A decrease in production space was also seen, which could have affected worldwide outputs. China and India were the top two potato producers in 2022. According to estimates, China produced 95.50 million tons and India produced 56 million tons of potatoes in 2022 (FAO, 2024). Only Africa had a rise in potato production area in 2018, increasing by 1.90 million hectares (an increase of 3.3%) (WPC, 2020).

2.1.4 African potato production

Africa has a broad range of potato yields, averaging 12-15 tons per hectare. This is less than the 20 tons per hectare average yield for the entire world. From 2 million tons in 1960 to over 30 million tons in 2013, Africa produced more potatoes. The output increased from 8 to 24 million tons between 1994 and 2011, mostly as a result of an expansion of cultivated area (Harahagazwe *et al.*, 2018). African potato yields have fluctuated between 12 and 15 tons per hectare over the past 11 years, while the continent's overall producing area has ranged from 1.5 to 2 million hectares. In 2021, the top African potato-producing countries were Algeria, Egypt, Malawi, South Africa, Rwanda and Kenya, respectively (NPCK, 2022) (Figure 2.1).

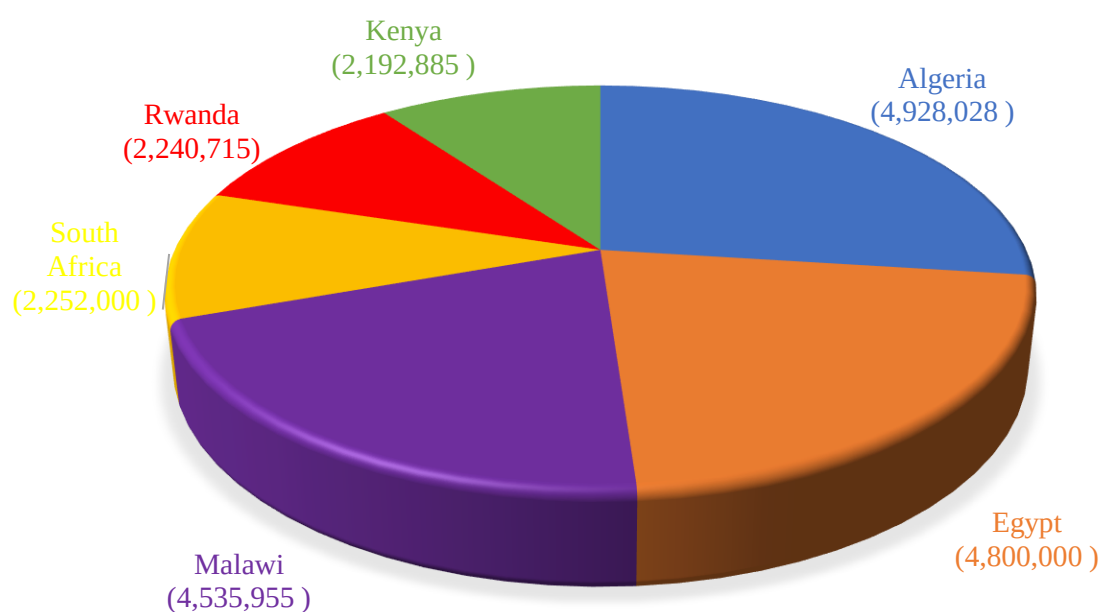


Figure 2.1 Top potato-producing African countries for 2021 (in tons). Source <https://npck.org/potato-production-growth-in-africa/>

Malawi was the top potato producer in Southern Africa (NPCK, 2022). In South Africa, potato constitutes the key vegetable crop. Potato is grown in sixteen geographically dispersed climatic zones and Provinces, viz., Limpopo, Mpumalanga, Free State, Eastern Cape, Western Cape,

Mpumalanga, KwaZulu-Natal, North-West and Gauteng. Limpopo is the top potato producer (DAFF, 2017). The potato industry in South Africa is highly advanced and makes use of cutting-edge tools, techniques and technologies across the value chain of the crop. In South Africa, potato is produced all year round. This is feasible since the nation has a wide range of geographical and climatic settings that are favourable for the growth of potatoes; as a result, fresh potato is available all year round (PSA, 2021b). In its immediate neighbouring countries, South Africa exports potatoes and potato seeds to nations such as Zimbabwe, Zambia, Angola and Mozambique. Following maize (*Zea mays* L.), sweet potato (*Ipomoea batatas* L.) and cassava (*Manihot esculenta* Crantz), potato is the fourth-most significant food crop in Malawi (Demo *et al.*, 2015). Despite Zambia's modest consumption, potato output has more than quadrupled over the past ten years (PotatoPro, 2020).

2.2 *Meloidogyne* genus

2.2.1 Distribution of *Meloidogyne* species in potato-producing regions

Root-knot nematodes (RKN), *Meloidogyne*, have approximately 105 different species (Ghaderi & Karssen, 2020). *Meloidogyne* species are found across a variety of plant-growing regions of the world, with temperate and tropical/subtropical areas having the largest populations of hosts (Santos *et al.*, 2019; Ghaderi & Karssen, 2020; Eisenback, 2022). The genus also contains the biggest variety of plant hosts and biological races, which are visually identical nematode species but may be distinguished by differential hosts and molecular methods (Rashidifard *et al.*, 2018; Wendimu, 2021).

Meloidogyne species represent a serious threat to agricultural productivity in Africa. This is due to the damage they inflict on a variety of agricultural products, particularly vegetables. These species inflict both direct and indirect damage, which lowers yield and quality while

increasing production expenses and resulting in revenue loss (Onkendi *et al.*, 2014; Coyne *et al.*, 2018). Owing to the generalised character of the above-ground symptoms, they are frequently disregarded or ignored and frequently misdiagnosed (Talwana *et al.*, 2016). With over 105 identified *Meloidogyne* species, 23 have been found in Africa (Moens *et al.*, 2009). *Meloidogyne* nematodes are polyphagous, obligatory pests that infest nearly all higher plant species, causing significant economic losses (Jones *et al.*, 2013). They are present across the world and are a major problem. More noteworthy, *M. enterolobii* can overcome resistance genes developed by some plants that are efficient against its thermophilic counterparts *M. incognita*, *M. javanica* and *M. arenaria* (Jones *et al.*, 2013).

Meloidogyne species are increasingly being detected in potato crops in the tropics and subtropics, generating significant economic effects owing to crop losses that are mostly dependent on cultivar, favourable environment and nematode density present during planting (Moens *et al.*, 2009; Jones *et al.*, 2013; Onkendi & Moleleki, 2013b). *Meloidogyne chitwoodi*, *M. fallax*, *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* have been described as common species parasitising potatoes in different climatic regions (Brodie *et al.*, 1993; Molendijk & Mulder, 1996; Vovlas *et al.*, 2005b; Onkendi & Moleleki, 2013b). *Meloidogyne chitwoodi* is a common and significant RKN species that affects potatoes in temperate countries, such as North America, Europe and Australia, where reports of severe damage, low tuber quality and economic losses have been made (Jones *et al.*, 2017). *Meloidogyne fallax* is also acknowledged as a severe potato pest in Europe, New Zealand and Australia (Jones *et al.*, 2017).

Meloidogyne javanica was reported to be the most widely distributed species (90%) in potato fields in Southern Brazil (Medina *et al.*, 2017). The researchers established that potato cultivars

have varying susceptibility levels to *M. javanica*, which is vital information for selecting promising offspring to create resistant materials. A similar survey found that several *Meloidogyne* species were parasitising potatoes in South Africa, including *M. incognita* (23%), *M. javanica* (23%), *M. arenaria* (17%), *M. enterolobii* (14%), *M. chitwoodi* (3%), *M. hapla* (1%) and unidentified *Meloidogyne* species (19%) (Onkendi & Moleleki, 2013b). This was done using multiple loci sequencing approaches, including the intergenic region. As a result, these studies reveal a global pattern of rising RKN infection issues in potato fields that are linked to climate change and the development of potato cultivars suited for planting in warmer climates (Onkendi & Moleleki, 2013b).

According to the South African plant-parasitic nematodes survey (SAPPNS) and the National Collection of Nematodes (NCN) databases, 453 PPN species were previously reported in South Africa (Marais *et al.*, 2015). Of these, 21% are associated with potato (Marais *et al.*, 2015), with *Meloidogyne* species being the most economically important genus that damage the crop. The highest RKN population density i.e., approximately 1 million per 50 g roots recorded was documented from potato during a survey in 2002 in Tshetshe village (North-West Province) (Mtshali *et al.*, 2002). *Meloidogyne incognita*, *M. javanica* and *M. arenaria* are the predominant tropical nematode species infecting potatoes in South Africa but also impact adversely on the growth and yield of the majority of other crops grown in the country (De Waele & Elsen, 2007).

Three more *Meloidogyne* species, viz., the cryophiles *M. chitwoodi*, *M. hapla* and the thermophile *M. enterolobii* have also been identified to damage potato crops in South African production areas (Onkendi & Moleleki, 2013b; Onkendi *et al.*, 2014; Agenbag, 2016). Onkendi & Moleleki (2013a) were the first to identify *M. enterolobii* from potatoes grown in KwaZulu-

Natal. The species was later also identified by Agenbag (2016) from potatoes in Mpumalanga. When Onkendi & Moleleki (2013b) investigated the distribution of PPN across potato-producing areas in South Africa, 81% of 78 composite potato samples obtained from fresh produce markets examined were infested with *Meloidogyne* species.

2.2.2 Economic significance of *Meloidogyne* nematodes on potato crops

Meloidogyne nematodes are the most prevalent and commercially significant of the approximately 24 genera of PPN that have been shown to parasitise potatoes (Esteves *et al.*, 2015). The six RKN species that cause the most harm to potato production globally are *M. enterolobii*, *M. chitwoodi*, *M. fallax*, *M. javanica*, *M. hapla*, *M. incognita* and *M. arenaria* (Fábia *et al.*, 2018). *Meloidogyne* infestations can cause yield loss of up to 50% and complete failure, i.e., 100% crop loss in potato fields (Jones *et al.*, 2017; Fábia *et al.*, 2018). It is challenging to precisely estimate yield loss due to *Meloidogyne* damages since other pests, diseases and abiotic variables collectively affect potato crop under field conditions (Jones *et al.*, 2017; Fábia *et al.*, 2018).

2.2.3 Signs and symptoms of *Meloidogyne* nematodes on potato crops

Meloidogyne species are characterised as ‘root-knot’ because of the structures they form on the roots after infection, resembling a knot or gall. Plant damage caused by *Meloidogyne* species is mostly due to the reduced availability of water and nutrients because of nematode feeding and disturbance of root anatomy/morphology (Mesa-Valle *et al.*, 2020). *Meloidogyne* species cause shape deformations, blistering or necrotic patches on the tuber surface as well as discolouration of tubers and visible infestation inside the tubers, with decreased tuber size, making potato tubers unfit for the fresh market or processing (Jones *et al.*, 2017; Fábia *et al.*, 2018). *Meloidogyne* nematodes are often difficult to detect and diagnose in the field because

of their minute size and obscure symptoms on crops which often resemble symptoms of abiotic stresses including nutrient and water stress (Palomares-Rius *et al.*, 2017). However, infection of potatoes by RKN exhibits both above and below-ground symptoms.

Above-ground symptoms of *Meloidogyne*-infested potato crops may exhibit stunting and nutritional deficiencies such as chlorosis, lack of vigour, early senescence and wilting (Jones *et al.*, 2017; Niere & Karuri, 2018). Thus, above-ground symptoms inflicted by RKN are generally similar to those exhibited by any plant having a damaged root system that is under abiotic stress as well as other soil-borne pathogen infections. On-farm sorting of tubers in the packing house and the rejection of RKN-damaged tubers by seed inspectors based on low/unacceptable seed quality and/or by processing plants as well as by fresh produce markets ultimately results in economic losses incurred by potato producers (Vermeulen, 2015).

The RKN below-ground symptoms are generally more specific than above-ground symptoms, characterised by the infamous galls [Figure 2.2 (a)]. Root-knot nematode damage tubers and leave them with blemishes [Figure 2.2 (b)], rendering them unsuitable for fresh markets and lowering processing quality. The area between the epidermis and vascular ring may also have internal necrosis in addition to external abnormalities (Vermeulen, 2015). The RKN-induced galls in the roots and tubers eventually obstruct normal water and nutrient uptake.

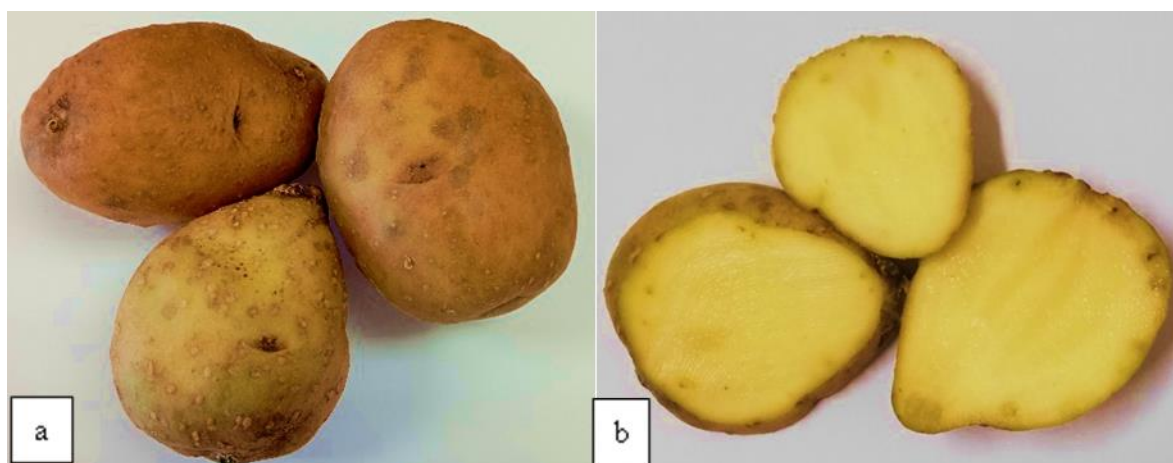


Figure 2.2 Potato tubers infected with *Meloidogyne enterolobii* with symptoms of (a) galling and (b) blemishes and root-knot females visible. Samples from North-West University (NWU) agricultural research field. Pictures taken by Ndivhuwo Ramatsitsi in Oct. 2021.

Typically, the infection of the potato root system does not cause damage, but as soon as the tubers start to form, the infective second-stage juvenile (J2s) invades them, its population increases and disseminates. Therefore, if the disease spreads quickly and infects potato roots and tubers, numerous nematode generations will develop before harvest, which usually happens 110 days after planting (Jones *et al.*, 2017). A combination of favourable temperatures, soil structure and moisture status might significantly worsen RKN infections (Jones *et al.*, 2017). In severe circumstances, RKN infection losses in potato crops might reach 100%. Additionally, depending on the planting season and the degree of soil infestation, losses might vary (Gomes & Souza, 2003).

2.2.4 Factors that aggravate *Meloidogyne* nematode damage

The RKN produce high numbers of offspring, particularly in warmer areas where they go through several life cycles in one season (Agrios, 2005). Temperature is a major contributing factor to high crop losses i.e., quality and yield, caused by RKN. Santos (2001) for example, indicated that up to 12 generations of *M. incognita* could occur within one growing season in Brazil, an indication of the increased quality losses that can be experienced by potato

producers. *Meloidogyne* species are particularly harmful when the potato crop is grown under the following conditions: (i) large initial population density (Pi) present at planting (Fourie *et al.*, 2010), (ii) susceptible cultivars are used (Mukhtar & Kayani, 2020) and (iii) infected tubers are planted (Mutala'liah *et al.*, 2019).

Meloidogyne infection sites also serve as entry points for other phytopathogens (Shi *et al.*, 2018). These pathogens penetrate the plant vascular system and alter water transport, causing widespread crop damage (Bird & Kaloshian, 2003). For example, wilt caused by pathogenic bacteria and fungi such as *Lecanicillium* species, *Ralstonia solanacearum*, *Fusarium* species and *Thanatephorus cucumeris* have been shown to occur as secondary infections to RKN (Brodie *et al.*, 1993; Scurrah *et al.*, 2005; Manzanilla-Lopez, 2009). Niere & Karuri (2018) identified further *Meloidogyne* interactions with *Rhizoctonia* and *Verticillium* fungal species, which exacerbate *Meloidogyne*-caused quantitative and qualitative potato yield loss.

2.2.5 The life cycle of *Meloidogyne* nematodes

Sexual reproduction occurs in some *Meloidogyne* species but species such as *M. incognita*, *M. javanica* and *M. arenaria* reproduce via mitotic parthenogenesis (Abad & Williamson, 2010). A *Meloidogyne* nematode life cycle usually starts with the hatching of fully developed J2s stimulated by soil conditions such as CO₂, soil pH, soil texture and soil moisture. The J2s, referred to as the infective stage of RKN, use sense organs, amphids, to locate host roots as soon as they hatch (Escobar *et al.*, 2015). The exudation of metabolites from the host root attracts and directs *Meloidogyne* J2s (Williamson & Čepulytė, 2017). When a suitable root is located, J2s migrate downward towards the meristem to enter the vascular cylinder. This is because root endodermis contains a Casparian strip which a nematode stylet cannot penetrate (Holbein *et al.*, 2019). The nematodes then secrete a mixture of cell wall-degrading enzymes,

such as cellulases, pectate lyases and polygalacturonase to push against the softened mid-lamella using their stylet and head to gain access to plant cells (Ali *et al.*, 2017a; Da Rocha *et al.*, 2021).

Under favourable conditions such as a temperature of about 27 °C and sufficient nutrient supply from the host plant, J2 moult through third-stage (J3), fourth-stage (J4) and adult female stage. Interestingly, *Meloidogyne* J2s and adult females are the only two stages that feed; with J3 and J4 lacking a functional stylet (Moens *et al.*, 2009; Velloso *et al.*, 2022). Such a peculiar observation is supported by *Meloidogyne* behaviour where in cases of J2s failing to establish a feeding site, they moult to males that are also not known to feed. Males are rare but present when conditions are unfavourable for female development, such conditions include high population density and scarcity of nutrients from stressed, dying or dead host plants (Sorribas *et al.*, 2020).

After the final moult, the adult female develops into a pear-like shape and enlarges 500 times more than the J2s size. In *Meloidogyne* species, the enlarged bodies of adult females can even protrude outside the gall (Niu *et al.*, 2022). The enlarged bodies and development of adult females are important for providing space during the secretion of a gelatinous matrix with a glycoproteinaceous nature in which hundreds of eggs containing larvae are deposited (Calderón-Urrea *et al.*, 2016). Orion *et al.* (2001) described the antimicrobial activity of the gelatinous matrix, including, protecting the developing larvae from external pathogens like fungi, furthermore, it serves as a barrier to water loss. The transition from first-stage juvenile (J1) to J2 occurs inside the egg.

2.2.6 Feeding habits of *Meloidogyne* nematodes

Feeding begins after J2s have invaded the root and moved within the root to a permanent feeding site. Inside a feeding site, J2s become sedentary and select certain cells that become their source of nutrients. The selected cells undergo remarkable morphological and metabolic changes termed giant cells, the infamous feeding sites (Cabrera *et al.*, 2015; Escobar *et al.*, 2015; Cabrera *et al.*, 2018; Niu *et al.*, 2022). Continued division and expansion of giant cells result in a root gall (Figure 2.3), a primary RKN symptom (Favery *et al.*, 2016; Al-Idrus *et al.*, 2017; Danish *et al.*, 2021). This specific cell deformation is attributed to the cell and those around it responding by repeatedly going through mitosis without cytokinesis (Davis & Mitchum, 2005; Mashela *et al.*, 2016). Inside a gall, the immobile J2s only move the head alternating feeding from one giant cell to another (Miyashita *et al.*, 2014; Favery *et al.*, 2016).

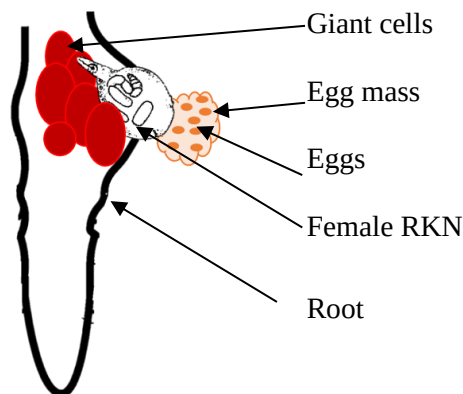


Figure 2.3 Egg mass protruding from the root with female *Meloidogyne* feeding on giant cells. Diagram created by Ndivhuwo Ramatsitsi.

The cytoplasm of the gigantic nematode cell is dense and metabolically active and its cell wall is abnormally thick. The surrounding root cells enlarge to produce a distinctive root gall as J2s develops through J3 and J4 to pear-shaped adult females (Castillo & Castagnone-Sereno, 2022; Niu *et al.*, 2022). Cabral *et al.* (2021) investigated the involvement of the Armadillo BTB Protein ABAP1 in the deoxyribonucleic acid (DNA) replication and transcription of

Meloidogyne species-induced galls. According to this research, ABAP1 is crucial for controlling the cell cycle during gall formation and is necessary for maintaining the homeostasis of the feeding site.

2.2.7 Plant and *Meloidogyne* nematodes interactions

Many nematode-plant interactions are obligatory for the success of nematode infection, which includes stimulation for J2s hatching, attraction, penetration of suitable tissues, J2s migration to feeding site, induction of feeding site and modification of cells (Davis & Mitchum, 2005; Mashela *et al.*, 2016). As soon as they locate a host, *Meloidogyne* J2s penetrate the plant in the root elongation zone using their protrusible oral stylet. After penetration, they migrate through different tissue layers to reach the vascular cylinder, where they induce the formation of five to seven root parenchyma cells that transform into specialised nematode-feeding sites, giant cells (Palomares-Rius *et al.*, 2017).

Meloidogyne nematodes attack plants using physical and biochemical infectivity, whereas plants generate defence responses that include both physical and biochemical strategies (Escobar & Fenoll, 2021). The ability of newly hatched J2s to locate their host is an important characteristic of plant-nematode interaction. This is because, unlike the free-living nematodes, RKN are biotrophic micro-organisms, and must locate their host in a relatively short time after hatching to survive. When they reach the root system, J2s congregate near the root elongation zone's surface, and continue using the same route for vascular migration (Bird *et al.*, 2009). This behaviour suggests that some form of biochemical communication between the juveniles and their respective hosts may occur prior to root invasion. This is generally referred to as chemotaxis.

Chemotaxis, the migration of RKN along a gradient of increasing concentrations of chemical signals, has been suggested to guide the nematode to distant hosts (Curtis, 2008; Reynolds *et al.*, 2011). Chemical signals and bioactive substances produced by the host can operate as short- and long-range attractants, directing the RKN to the root zone of the host. The migratory patterns of *M. incognita* and *M. graminicola* were analysed using Y-chamber olfactometers, which give the nematodes two routes to find their hosts (Reynolds *et al.*, 2011). The two *Meloidogyne* species were drawn to their preferred hosts by taking the shortest route, while they avoided the non-preferred hosts by taking the long route. Plant molecules from root tip and border cells influence the attraction of RKN *M. incognita* to host plants (Zhao *et al.*, 2000).

In plant-nematode interactions, Seinhorst (1966) introduced two concepts to describe nematode response: (i) host status and (ii) host sensitivity. Host status is described according to nematode reproductive function which estimates the maximum reproduction rate using the initial nematode population before planting and the final nematode population before harvesting, P_f/P_i ratio, as well as equilibrium density (E). Equilibrium is when the plant maintains the population density from planting to the end of the cropping cycle, making $P_f = P_i$ or $P_f/P_i = 1$ (Seinhorst, 1966; Schomaker & Been, 2006). A reproductive value of greater than one indicates that the plant was a susceptible host. On the other hand, a reproductive value of less than one would be an indication that the plant was not a nematode host, i.e., it was resistant.

Host sensitivity, instead, refers to plant growth and development in response to nematode infection, which could either be inhibition, neutral or stimulation (Seinhorst, 1966). A better understanding of plant-nematode interactions led to classifying plants as being either susceptible, tolerant or resistant according to interaction compatibility. Through intensive nematology research, several plants have been identified and characterised as either

susceptible, tolerant or resistant (Daneel *et al.*, 2018; Audil *et al.*, 2019; Verdejo-Lucas & Talavera, 2019).

2.3 Fungal bio-control agents

2.3.1 Plant and nematophagous fungi interactions

Nematode-parasitic fungi also referred to as nematophagous fungi, include a group of soil-living fungi that are natural enemies of RKN (Moosavi & Askary, 2015; Soares *et al.*, 2020). Nematophagous fungi are an extremely diverse group with the ability to transition from saprophytic to parasitic phases under a poor nutrition environment. These fungi have evolved sophisticated methods of parasitic effect on RKN (Degenkolb & Vilcinskis, 2016). This group of fungi attack RKN by secreting diffusible toxins, immobilising the nematodes before hyphal penetration through the nematodes' cuticles (Lopez-Llorca *et al.*, 2008).

The saprophytic/parasitic capabilities of the nematophagous fungi vary. While many fungi that produce traps and spores may survive in soil by themselves, endoparasites are typically more reliant on nematodes for nutrition (obligate parasites) (Moosavi & Zare, 2020). The capacity to trap nematodes is associated with a particular stage of fungal mycelial growth. The trapping (predatory) fungus has complex hyphal structures, such as hyphal nets, knobs, branches, or rings, in which nematodes are mechanically or adhesion-based caught. On the other hand, endoparasites use their spores to attack nematodes, which either adhere to their surface, germinate and invade the cuticle by producing cuticle-degrading enzymes, such as chitinases or swallow them in whole (Liang *et al.*, 2019; Karakaş, 2020).

Nematophagous fungi can directly attack, kill, immobilise, repel and confuse nematodes when finding their host, interfere with their giant cell development, compete for resources, or employ

a combination of these options (Schouten, 2016; Bogner *et al.*, 2017). For example, it has been observed how *Acremonium implicatum* can colonise the xylem of tomato roots and, in the soil, parasitise and destroy the eggs of *M. incognita* (Yao *et al.*, 2015). More examples of successful use of nematophagous fungi in the control of different RKN in different crops include; *Arythrobotrys oligospora*, *Candelabrella musiformis*, *Dactylaria eudermata* against *M. incognita* in tobacco (*Nicotiana tabacum* L.) plants (Hastuti & Nicklin, 2018) and *A. oligospora* against *M. incognita* in tomato plants (Singh *et al.*, 2013).

2.3.2 *Meloidogyne* nematodes and nematophagous interactions

2.3.2.1 Antibiosis

The nematophagous fungi antibiosis mechanism involves the production of *Meloidogyne*-toxic compounds by fungal biocontrol agents (Poveda *et al.*, 2020). These toxic compounds primarily include metabolites and enzymes, such as xylanase, pectinase and glucanase (Vos *et al.*, 2012; Girardi *et al.*, 2022). The fungal BCA produces various antimicrobial compounds that suppress or reduce the growth and/or proliferation of the parasitic nematodes (Lahlali *et al.*, 2022). Throughout the process of antibiosis, hydrolytic enzymes such as chitinase and β -1,3-glucanase are activated to degrade the RKN cuticle (Gortari & Hours, 2008; Al-Ani, 2018). An *in vitro* study by Molinari & Leonetti (2019) showed that such enzymes can degrade nematode eggshells and cuticles, and then subsequently absorb nematode nutrients. *Trichoderma* species produce a series of compounds with *Meloidogyne*-antagonistic properties that include cell wall degrading enzymes such as cellulase, xylanase, pectinase, glucanase, lipase, amylase, arabinase, protease and volatile metabolites such as 6-n-pentyl-2H-pyran-2-one (Aminuzzaman *et al.*, 2013; Ferreira *et al.*, 2018; Bansal *et al.*, 2021).

2.3.2.2 Parasitism

Meloidogyne parasitism by nematophagous fungi involves the fungal BCA making physical contact with eggs or J2s (Poveda *et al.*, 2020). Generally, parasitism of the host RKN by nematophagous fungi is referred to as mycoparasitism, where the mycoparasite gets some of its nutrients from the host. Physical modes of parasitism include RKN egg parasitism and J2s paralysis or disintegration leading to death either by predation or endoparasitism (Askary, 2015). Successful mycoparasitism is facilitated by physical penetration of the nematophagous fungi into the body of the host via the development of peculiar organs such as haustoria and appressoria, and secretion of enzymes. Parasitism by fungal BCA has been classified into three foremost groups; (i) nematode-trapping/predators, (ii) opportunistic or ovicidal and (iii) endoparasites (Liu *et al.*, 2009; Soares *et al.*, 2018). Nematode-trapping fungi have sophisticated hyphal structures such as nets, knobs, branches or rings that trap nematodes.

2.3.2.3 Systemic resistance

Plants have an inherent ability to avert pathogens, also referred to as resistance. This is formed by genes that detect and initiate immune responses against the invading pathogen (Birkenbihl *et al.*, 2017). Whilst some plants have evolved to efficiently utilise this ability and even amplify their resistance, other plants lack these genes against particular pathogens and therefore display susceptibility (Lorang *et al.*, 2007). Apart from direct mechanisms of attack on RKN, fungal BCA have plant rhizosphere-modifying effects that indirectly affect RKN. Colonisation of plant roots by beneficial endophytic and mycorrhizal fungi can protect plants against a wide range of RKN by inducing host resistance against pathogens. Induced resistance in plants can be defined as an enhancement of the plant's defensive capacity against pathogens that is acquired after appropriate stimulation (Mengistu, 2020).

Generally, when a plant is exposed to plant-pathogens, defense mechanisms that can alleviate or prevent adverse impact of plant-pathogens are activated. These mechanisms comprise progression of specific pattern-recognition receptors for recognition of microbe-based signals referred to as pathogen- or microbe-associated molecular patterns, or plant-based signals generated upon invasion (damage-associated molecular patterns) (Abdul Malik *et al.*, 2020). Two different types of systemic resistance can be conferred to host plants by beneficial fungal species namely induced systemic resistance and systemic acquired resistance (Birkenbihl *et al.*, 2017). The two are distinguished according to the biochemical pathways involved. Induced systemic resistance is salicylic acid-independent, whereas systemic acquired resistance is a salicylic acid-dependent pathway. For example, *Tri. harzianum* fungus can induce jasmonic acid- and salicylic acid-regulated defense pathways in tomato (*Solanum lycopersicum* L.) plant, causing resistance to the RKN *M. incognita*. Previous studies (Leonetti *et al.*, 2014; Mendoza-Mendoza *et al.*, 2018) have shown that, by colonizing the plant roots, *Trichoderma* stimulates their defense mechanisms against numerous plant-pathogenic micro-organisms, including *Meloidogyne*.

2.3.2.4 Chemoreception disruption that disturbs nematode chemotaxis

The ability of *Meloidogyne* species to detect the presence of a host and locate it depends on their sensory organs positioned around the mouth area of the head (Eisenback, 2009). These enable J2s to hatch from the eggs after perceiving environmental cues such as soil temperature, moisture, pH and carbon dioxide (Lu *et al.*, 2022). In the same manner that olfactory organs allow other organisms to have direction, PPN would not be able to navigate a host without these organs. Chemoreception takes place when messenger signals from the host bind to PPN sensory organs. However, these receptors can be blocked by binding to other substances which

have greater binding affinities, such as those produced by nematophagous fungi (Jansson & Lopez-Llorca, 2004).

Conidia from the nematophagous fungus *Drechmeria coniospora* have been shown to adhere to these sensory organs (Jansson, 1993; Jansson, 1994). Proteins on the conidia sticky buds bind to the glycoproteins of PPN sensory organs which block reception, ultimately decreasing PPN chemotaxis (Jansson, 1993). A dual mechanism of this fungus is shown in its ability to not only block reception but also parasitise the nematode (Jansson & Lopez-Llorca, 2004). Moreover, a study by O'Halloran *et al.* (2006) reported that host exudates are more stimulating to female J2s than males; this could be attributed to the fact that in *Meloidogyne*, it is only the females that feed. This mechanism of nematode confusion is yet to be tested on *Meloidogyne* species (Philbrick *et al.*, 2020).

2.3.3 Significance of indigenous fungal biotypes

The virulence, capacity to colonise the root surface and chlamydospore production of fungal biotypes vary and these characteristics are diverse enough to be significant for the fungus's employment as a biological control agent (Kerry, 2000). Indigenous biotypes would have substantial benefits over introduced ones since they may be more virulent against local populations of the nematode, compete more successfully with indigenous microflora, and are more suited to environmental circumstances (Stirling, 2018; Swibawa *et al.*, 2020). Furthermore, introduced/exotic species, on the other hand may have unknown negative impacts on other soil microbes and pose ecological ramifications.

2.3.4 Factors that improve the efficacy of nematophagous fungi

Fungal BCA are utilised in procedures and methods of integrated biological control programs, but sustainability is of major concern. Accordingly, altering agricultural methods or the environment to conserve and boost antagonistic species to lessen RKN damage should also be encouraged. Fungal BCA are subjected to a variety of biotic and abiotic conditions that might impact their capacity to survive, infect, spread and kill the target host (Abd-Elgawad & Askary, 2020; Palmieri *et al.*, 2022). Both biotic and abiotic variables may support or inhibit the activity and capacity to survive, infect, spread and kill the target host of BCA in the soil. It is thus of considerable interest to learn more about the physiological traits of fungi and how they can survive and adapt to different environmental situations.

Biotic factors, such as naturally existing soil microflora compete with the introduced BCA for scarce energy sources and can considerably impair the performance of the BCA (Abd-Elgawad & Askary, 2020). Fungal BCA engage with their target hosts in a variety of ways, including parasitism, predation, antagonism and competition. Nonetheless, they too have natural enemies that may parasitise them (Davies & Spiegel, 2011). In several ways, BCA in the soil can affect or be affected by other species, including non-target hosts or possible competitors, particularly indigenous organisms (Helmberger *et al.*, 2017). Temperature, humidity, soil moisture, soil texture, soil structure and sun ultraviolet radiation are all essential environmental parameters that can influence BCA survival and effectiveness (Abd-Elgawad & Askary, 2020). For the production of field crops outside of protected structures, environmental factors affect crop production directly and indirectly. As a result, controlling these factors in the field can be challenging, but adding adjuvants such as OC to increase BCA efficacy offers one approach (Helmberger *et al.*, 2017).

2.3.5 Identification of fungal species

With an estimated 5.1 million species, fungi are the second largest class of eukaryotic organisms on the planet (O'Brien *et al.*, 2005; Kopytko *et al.*, 2017). In addition to having the potential to inhabit a broad range of natural and man-made niches, members of the fungi kingdom play important roles in human existence (Stajich *et al.*, 2009). Both fundamental (ecology, taxonomy) and applied (genomics, bioprospecting) uses of fungi in scientific study place a premium on species-level identification. This is particularly true for researchers studying natural products that use fungi as a source of bioactive secondary metabolites. Scientific names are essential for conveying knowledge about fungi because they help researchers identify other closely related species to better predict the evolution of chemical gene clusters (Schmitt & Barker, 2009) or to prioritise taxonomically related strains when a productive strain may attenuate the production of important bioactive compounds.

Most notably, whether industrial, agrochemical or medicinal products are to be made from a fungal strain, taxonomic identification of fungi is necessary. Identifying fungi with accuracy and precision is complex. Fungi have simpler body designs than other multicellular eukaryotes and the only characteristics that can be used to diagnose them are their bodies that produce spores, both sexually and asexually (Beakes & Thines, 2016; Nagy *et al.*, 2017; Lücking, 2019). It is practically hard to classify some fungi using conventional methods since they are solely known from vegetative structures (Koch *et al.*, 2017; Koch *et al.*, 2018). Thus, removal from their natural environment and in-depth laboratory analysis are needed for accurate identification.

2.3.5.1 Morphological identification

Morphology of eucaryotic species, such as fungi, is a fundamental source of data for taxonomic determinations, the evaluation of biodiversity and the comprehension of ecological and evolutionary processes. Information about morphology is easily available and may be processed at a reasonable price. Morphological data serves as both a starting point for molecular investigations and reference information to verify the outcomes of such analyses (Fodor & Hâruța, 2020).

The size and form of reproductive structures, i.e., spores, are the two main elements covered by morphology. The former is simpler to get, for example, as linear measurements. However, even when taken in a variety of projections, they offer little insight into the object's geometric structure (Zelditch *et al.*, 2004). In terms of data collection and processing, the latter, i.e., the form, presents greater challenges. However, it collects quantitative information about the geometric structure and enables the reconstruction of the biotypes' original appearance (Zelditch *et al.*, 2004; Fodor & Hâruța, 2020). It is a branch of geometric morphometrics that quantifies shapes and defines them as geometric details about the biotype (Kendall, 1977). The linear length and breadth measurements of the spore size are frequently utilised for species delimitations (Anikster *et al.*, 2005). Numerous words are employed depending on the shape of the fungi, i.e., “globose” or “cylindric”, or whether it resembles a certain natural or cultural entity, i.e., “ovoid” or “filiform” (Kušan *et al.*, 2014).

2.3.5.2 Molecular identification

The ground-breaking work by White *et al.* (1990) describing fungal nuclear ribosomal operon primers marked the beginning of the use of molecular data for the identification of fungi a little over 20 years ago. A new era of molecular phylogenetic sequence identification in the fungal

kingdom was inaugurated by the fungal DNA sequences produced with these primers for the large sub-unit (LSU-26S or 28S), small sub-unit (SSU-18S) and the entire internal transcribed spacer region (ITS1, 5.8S, ITS2; ca. 0.45-0.80 kb) (Seifert *et al.*, 1995; Cadez *et al.*, 2002). These three distinct areas have been shown to develop at different rates, leading to varied degrees of genetic variety. The SSU evolves the slowest and has the least amount of variation among taxa, whereas, ITS evolves the quickest and has the most variation (Cadez *et al.*, 2002; Mitchell & Zuccaro, 2006).

The SSU can be amplified and sequenced using the primer pair NS1 and NS4 for the phylogenetic location of a fungus at higher taxonomic levels (family, order, class and phylum) (White *et al.*, 1990). Alternately, the LSU can be amplified and sequenced using the primer pair LROR and LR6 if the identification needs to be done at the intermediate taxonomic levels (family, genera) (Vilgalys & Hester, 1990; Rehner & Samuels, 1995). To identify different species of fungi, the LSU region, which comprises the D1 and D2 hypervariable domains, can be useful on its own (Liu *et al.*, 2012) or in conjunction with the ITS area (Schoch *et al.*, 2012). Because it is the part of the rRNA cistron that evolves the most quickly, the ITS is the most helpful for species-level identification.

According to Schoch *et al.* (2012), the ITS region is one of the markers with the highest likelihood of accurate identifications. The ITS region has been validated as an appropriate fungal barcode by further fungi research (Seena *et al.*, 2010; Dentinger *et al.*, 2011; Kelly *et al.*, 2011). There are now many more ITS sequences accessible in GenBank as a result of this sequencing strategy (Engelmann *et al.*, 2009; Begerow *et al.*, 2010). Approximately 172,000 full-length fungal ITS sequences have been deposited in GenBank, with 56% of those having

a Latin binomial attached, representing roughly 2500 genera and 15,500 species (Schoch *et al.*, 2012).

2.6 Mass production of fungal bio-control agents

Bio-control fungal-based formulations have been explored against a variety of plant pathogens, *albeit*, with variable results (Radhakrishnan *et al.*, 2021; Lahlali *et al.*, 2022). An appropriate fungal agent, culture medium and formulation need to be developed in order to produce an effective bio-pesticides. An ideal bio-control fungus should be able to generate a high volume of inoculum at a reasonable cost, have a desired level of virulence and work with current application technologies (Jenkins *et al.*, 1998; Osman *et al.*, 2020). The procedure of formulating BCA is a multi-step that commences upon selection of the most effective BCA on the target pathogen. In essence, it entails mixing viable bio-agent culture with carriers and adjuvants to produce a high-quality formulation with a demonstrated efficacy and stable shelf life (Martinez *et al.*, 2023).

Scientifically, this calls for a complete comprehension of the pathogen, environment, application niche, ecology and biology of the BCA, as well as irrigation system types that are a part of the agrifood system. The BCA should be compatible with all carriers and adjuvants included in the formulation process and not negatively impacted by them (Teixidó *et al.*, 2022). Ultimately, each BCA would require carriers and adjuvants it is empirically compatible with. For BCA, a wide range of liquid and solid formulations have been manufactured. Generally, it is relatively more affordable and less complex to prepare liquid formulation than solid formulation; yet, solid formulation may enhance BCA stability (Maciag *et al.*, 2020; Vassilev *et al.*, 2020). A formulated product, biocide, consists primarily of the following components: (1) the active ingredient, bio-agent and/or secondary metabolites; (2) carriers, inert materials

that support and deliver the active ingredient in the target site; and (3) adjuvants, which include stabilizers, adherents, surfactants, additional nutrients. Adjuvants may also include substances that support and disperse the product in the intended environment while protecting the active ingredient from high temperatures, desiccation and UV radiation (Płaza & Achal, 2020)

2.7 *In vivo* application of fungal bio-control agents

2.7.1 Pre-application considerations

Bio-safety and efficiency are paramount to the success of BCA after they are applied (Müller-Schärer & Schaffner, 2008). The former is governed by testing for negative synergies or where the BCA impairs the ecological harmony. This can be in the form of establishing how the BCA interacts with animal, plant, insect or microbe species where they are intended to be applied. This vital step was defined as a risk assessment by Butt *et al.* (2001). Efficacy is determined in terms of the BCA's ability to survive in a range of abiotic extremes in their tested area of application. This includes ranges in soil salinity, pH, temperature, moisture, texture and nutrient levels in case of soil-applied BCA (Kessler *et al.*, 2003; Abd-Elgawad, 2020). This is because the BCA must be able to withstand these fluctuations as this will affect their survival which is determined by the BCA's ability to reproduce and multiply.

Similar to *Meloidogyne* species, several species of BCA are obligate parasites, while others are facultative (Lopez-Llorca *et al.*, 2008). The organic matter content is related to the life stages of nematophagous fungi. These are: (i) The saprophytic phase, where soil organic matter is used for carbon (energy) by nematophagous fungi and (ii) The parasitic phase, when a fungus enters the nematophagous phase, during which it will feed on nematode host (Persson & Jansson, 1999). Soil with low carbon to nitrogen (C:N) ratios tend to encourage the

development of conidia, whereas, soils with a relatively high C:N ratio appear to favour fungal BCA parasitism (Mo *et al.*, 2005).

2.8 Application methods

2.8.1 Seed inoculation

The practice of seed coating, which involves coating seeds with small quantities of fungal BCA inoculum or soaking seeds in inoculum, is gaining popularity as a reliable method of delivering fungal BCA (Meena *et al.*, 2017; Pedrini *et al.*, 2017). Utilising mixing tools such as a cement mixer or fluidised bed, microbial seed coating involves using a binder, sometimes a filler, combined with inoculum (Pedrini *et al.*, 2017). This guarantees that BCA are easily accessible as soon as germination commences at the early stages of plant growth, promoting healthy, quick establishment and ultimately increasing crop yield (Philippot *et al.*, 2013; Colla *et al.*, 2015). Winter wheat's development, nutrient absorption, grain production and quality were improved when fungal inoculants, *Tri. atroviride*, *Glomus intraradices* (previously *Rhizophagus irregularis*) and *G. mosseae* were inoculated simultaneously as seed coatings (Colla *et al.*, 2015).

2.8.2 Soil inoculation

Direct soil inoculation with liquid formulations is typically employed to deliver a significant volume of microbial inoculant into the soil. This method is intended to prevent damaging delicate seeds or to shield the inoculant from inhibitory substances applied to or generated by the seed, such as fungicides and antimicrobial compounds. When seeding, it can be done using either solid, liquid or encapsulated formulations (Malusá *et al.*, 2012). When fungal BCA are applied to the soil rhizosphere areas of a field in liquid formulations, they remain in the latent state and become active. Their shelf life in liquid formulations is aided by this feature. It has

been shown that *Purpureocillium lilacinum* and *Tri. viride* have a prolonged storage life of more than a year (Kolombet *et al.*, 2008; Manikandan *et al.*, 2010; Song *et al.*, 2016).

2.8.3 Plant inoculation

Plant inoculation with BCA includes spraying bio-control formulations on the aerial plant parts or dipping seedling roots in formulations before transplanting. Foliar spray application of *P. chlamydosporia* effectively reduced *M. chitwoodi* parasitism on potatoes (dos Santos *et al.*, 2014). The reproduction of *Meloidogyne* species, juvenile hatching and root galling were all significantly reduced by pre-planting root dipping in *Aspergillus allahabadii*, *P. chlamydosporia*, *Trichoderma asperellum* and *Kuklospora kentinensis* (Affokpon *et al.*, 2018). One hour of fungal root-dipping treatments resulted in considerably lower *Meloidogyne* populations 11 weeks after transplanting (Affokpon *et al.*, 2018).

CHAPTER 3: ASSESSING FIVE POTATO CULTIVARS' RESPONSE TO *MELOIDOGYNE* SPECIES

3.1 Introduction

The South African potato industry is essentially self-sufficient and a large portion of its fresh and processed product is exported to countries including Zimbabwe, Zambia, Angola and Mozambique (PSA, 2021a). However, potato yield and quality are hampered as a result of galls and blemishes on the tubers brought on by *Meloidogyne* nematodes infection and feeding. This is because *Meloidogyne* species deform roots by producing galls, which prevent roots from sufficiently absorbing water and nutrients (Fábia *et al.*, 2018). Furthermore, tuber deformation caused by the RKN renders potatoes unmarketable (Anyalewechi *et al.*, 2010; Fábia *et al.*, 2018; Bali *et al.*, 2021; Teklu *et al.*, 2022). The majority of crops produced in South Africa, including potatoes, are affected by tropical *Meloidogyne* species, such as *M. enterolobii*, *M. incognita*, *M. javanica* and *M. arenaria* (De Waele & Elsen, 2007; Onkendi & Moleleki, 2013a).

In South Africa, tropical species including *M. enterolobii*, *M. incognita*, *M. javanica* and *M. arenaria* are the most prevalent pests that affect a majority of crops, including potatoes (De Waele & Elsen, 2007; Onkendi & Moleleki, 2013a). Additionally, these parasites interact with other plant pathogens to create disease complexes and suppress defences against other pathogenic infections (Begum *et al.*, 2012). The severe losses brought on by RKN can be reduced by many different management strategies. Nematode management generally involves utilising synthetic nematicides, BCA, resistant hosts and cultural techniques including crop rotation (Stirling, 2018; Poveda *et al.*, 2020; Sivasubramaniam *et al.*, 2020; Sasanelli *et al.*, 2021). Even though using synthetic nematicides to control nematodes is a common and widely

used practice, it can have negative impacts on input costs, cause pollution and pose a risk to human health and other beneficial soil microbes (Oka, 2020; Abd-Elgawad, 2021). But prior to developing a nematode management strategy, it is essential to understand how the crop and RKN interact, i.e., whether the crop is susceptible, tolerant or resistant. Subsequently, the objective was to assess the response of five potato cultivars viz., ‘Buffelspoort P1’, ‘Hertha’, ‘Larnoma’, ‘Mnandi’ and ‘Up-to-date’ to *M. enterolobii* and *M. javanica*.

3.2 Study site

The experiments were carried out under laboratory and net house conditions at the Agricultural Research Farm, Plant Pathology Research laboratory, NWU, Mmabatho, Molelwane, North-West University, South Africa (25°48′36″S, 25°37′ 47.999″E). Experiments were conducted twice, Feb.-Apr. 2020 and Aug.-Oct. 2020.

3.3 Description of the experiments

3.3.1 Treatments and experimental design

A split-plot design with the two *Meloidogyne* species, viz., *M. enterolobii* and *M. javanica* as the main factors and potato cultivars, viz., ‘Buffelspoort P1’, ‘Hertha’, ‘Larnoma’, ‘Mnandi’ and ‘Up-to-date’ as the sub-plots were conducted under a net house. The cultivars were chosen on the basis that they had not been assessed for *Meloidogyne* response. In each experiment, five treatments of *M. enterolobii* and *M. javanica* as eggs and J2s; 0, 500, 1500, 2500 and 3500 (counted using a counter), were laid out in a randomised complete block design (RCBD) with five replications. Potato seeds, G0, and isolates of *Meloidogyne* species were received from Agricultural Research Council-Tropical and Subtropical Crops (ARC-TSC), Mbombela, South Africa. Nematodes were raised on susceptible tomatoes [(*S. lycopersicum* ‘Hotstuff’) MayFord

Seeds (Pty) Ltd, Pretoria, South Africa]. The inoculum was prepared by extracting eggs and J2s from tomato roots in 1% sodium hypochlorite (NaOCl) (Coyne *et al.*, 2007).

3.3.2 Experimental procedures

River sand and Hutton series red loam field soil was steam pasteurised at 121 °C for an hour. A 1:1 volume/volume (v/v) ratio mixture of river sand:field soil (72% sand, 20% silt, 8% clay) with a pH (H₂O) 6.25 was prepared. Thirty-centimetre (30-cm) diameter plastic pots were filled with the mixed soil and placed in the net house at 0.5 m inter-row and 0.5 m intra-row spacing. Potato seeds, one per pot, of ‘Buffelspoort P1’, ‘Hertha’, ‘Larnoma’, ‘Mnandi’ and ‘Up-to-date’ were sown in plastic pots. These pots were inoculated with different nematode populations seven days after emergence. Seedlings were inoculated by dispensing either 0, 500, 1500, 2500 or 3500 of *M. enterolobii* or *M. javanica* eggs and J2s using a 20 mL plastic syringe by placing into 5 cm holes on cardinal points of the stems and covered with growth medium. For each experiment, three sets of moisture meters and thermometers were inserted to 20 cm depths in randomly selected pots to monitor soil moisture content and soil temperature, respectively. Irrigation was applied by pouring 500 mL of tap water every second day into the tray of each pot using a watering can. LAN (28) was applied twice, 15 and 45 days after seedling emergence during the growing season at a rate of 0.6 g/plant in 300 mL water per pot. Plants were sprayed with mercaptothion (Malasol® /Malathion®) and tetradifon (Redspidercide®) alternatively every two weeks as preventative control of aphid and red spider mites, respectively.

3.3.3 Data collection

Plant variables: At 56 days after inoculation with nematodes, plant length was measured from the soil level to the tip of the flag leaf. Chlorophyll content was measured using a chlorophyll

meter (MINOLTA, SPAD-502, Konica Minolta) and stem diameter was measured at soil level using a digital vernier calliper. Shoots were cut at the soil level and were oven-dried for 72 hours at 52 °C for dry matter determination. Root systems for each seedling were removed from the pots and immersed in water to remove soil particles. Fully developed root galls were assessed using the North Carolina differential scale (NCDS) at 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls and 5 $\geq$ 100 galls per root system (Taylor & Sasser, 1978).

Nematodes: Nematodes were extracted from the total root system per plant by maceration and blending for 30 seconds in 1% NaOCl (Coyne *et al.*, 2007). The aliquot was passed through top-down nested 63 and 25 μ m screen open sieves. Contents of the 25 μ m screen sieve were poured into 100 mL plastic containers for counting under a stereomicroscope at 60 x 3 magnification. Soil per pot was thoroughly mixed and a 200 mL soil sample was collected. Nematodes were extracted from the soil samples using the modified sugar-floatation and centrifugation method (Jenkins, 1964). The soil was poured into a 4 L bucket with 2 L tap water and stirred, once the swirl had stopped, the aliquot was poured through top-down nested 150, 63 and 25- μ m screen open sieves. The contents of the 25- μ m screen sieve were poured into 50 mL plastic centrifuge tubes. Approximately, 3 g kaolin was added to each tube and the contents were centrifuged at 1750 rpm for 5 minutes. The kaolin solution was then decanted with nematodes having settled at the bottom of the tubes with soil particles. A 480 g of sugar per 1 L of tap water was poured into the centrifuge tubes and vigorously shaken to mix the settled soil with sugar solution before centrifuging for 60 seconds at 1750 rpm. The aliquot was decanted again onto a 25- μ m sieve with sugar being rinsed off the nematodes, which were then collected from the sieve into 100 mL plastic containers for counting under a stereomicroscope. Nematode numbers were converted to 5 L soil per pot to estimate the final nematode population

density (Pf). Reproductive factor ($RF = Pf/Pi$), which is a proportion of Pf and Pi, was computed for each inoculum level.

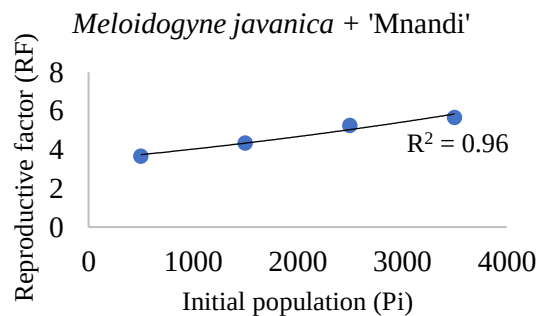
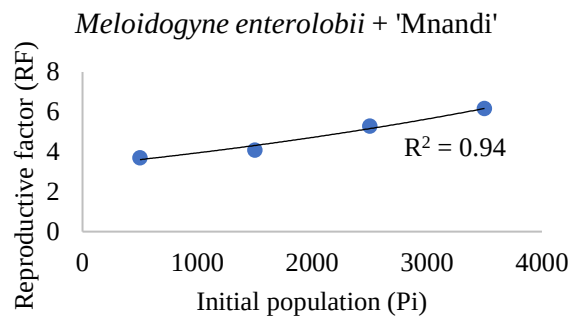
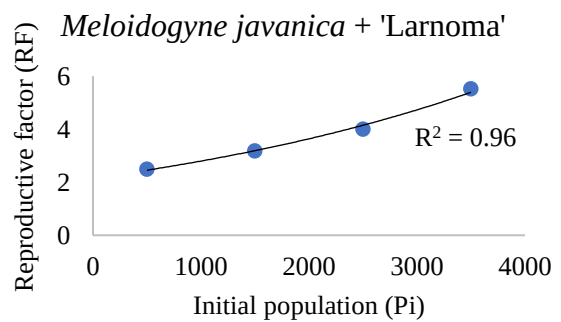
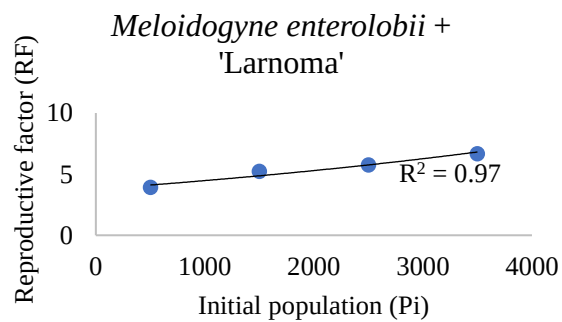
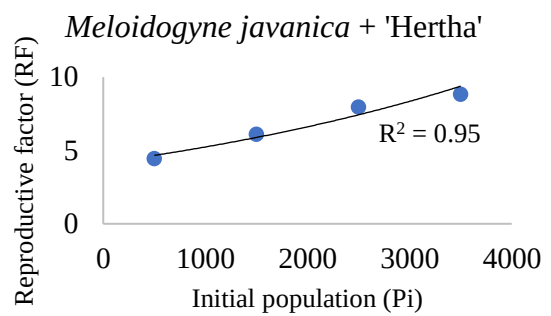
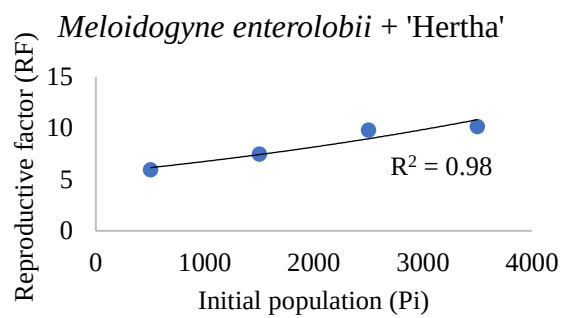
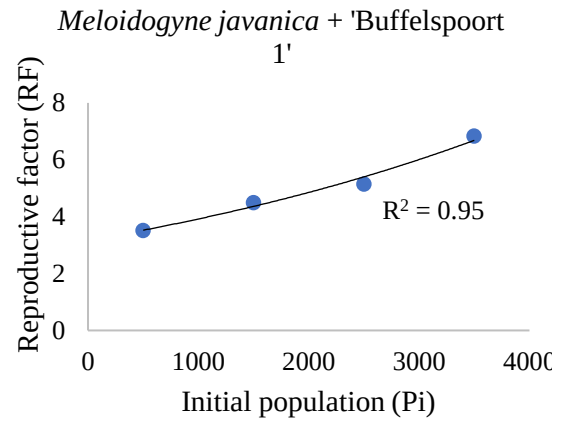
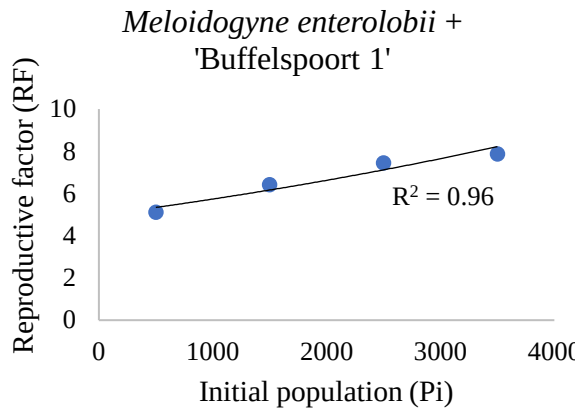
3.3.4 Data analysis

Nematode data was transformed through transformed through $\log_{10}(x + 1)$. Data were subjected to analysis of variance (ANOVA) using SAS software version 9.4 (SAS Institute, Inc., Cary, NC., USA, 2008). Nematode variables were subjected to stepwise regression analysis to determine the relation among different nematode Pi levels. Also, yield variables were subjected to stepwise regression analysis in order to determine the effect of different nematode species and nematode Pi on yield components (Gomez & Gomez, 1984). Mean separation for significant ($P \leq 0.05$) treatments was achieved through Tukey's Honestly Significant Difference (HSD). Unless otherwise stated, only predictive models which were significant at the probability level of 5% were discussed. Data analysis of interactions between the two growing seasons for variables in each potato cultivar were pooled by season.

3.4 Results

3.4.1 Reproductive potential of *Meloidogyne* on potato cultivars

There were no significant differences ($P \leq 0.05$) between data from the two growing seasons. The RF values at different levels of inoculation differed, allowing for the fitting of the best lines, which resulted in quadratic relationships (Figure 3.1).



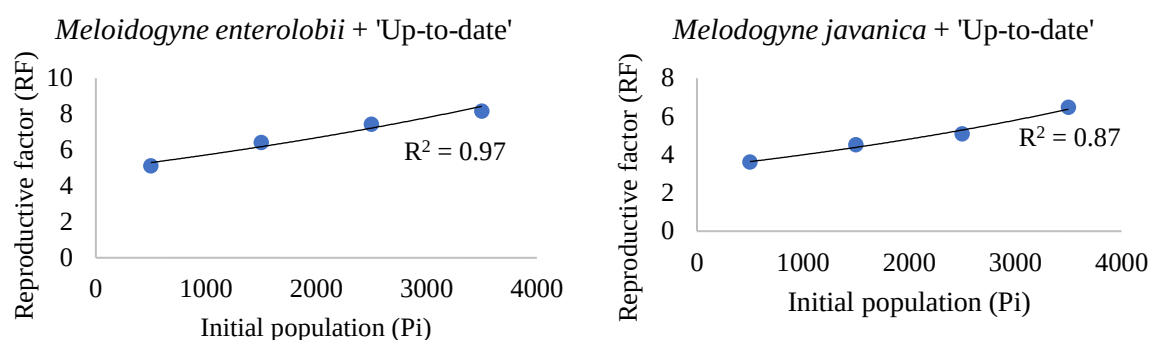


Figure 3.1 Relationship between reproductive factor (RF) and initial population (Pi) of *Meloidogyne enterolobii* and *Meloidogyne javanica* on five potato cultivars at 56 days after inoculation under net house conditions.

3.4.2 Potato-*Meloidogyne enterolobii* relations

Based on pooled data, *M. enterolobii* Pi had highly significant effects on Pf of ‘Hertha’ and ‘Larnoma’ ($P \leq 0.01$) and significant effects on ‘Buffelspoort P1’, ‘Mnandi’ and ‘Up-to-date’ ($P \leq 0.05$). Initial population densities were highly significant on the gall number of ‘Buffelspoort P1’ and ‘Hertha’ ($P \leq 0.01$) and significant on ‘Larnoma’ ($P \leq 0.04$). There was no significant difference in the gall number of ‘Mnandi’ and ‘Up-to-date’. *Meloidogyne enterolobii* Pi was highly significant on RF of ‘Buffelspoort P1’, ‘Hertha’ and ‘Mnandi’ ($P \leq 0.01$), and significant on ‘Up-to-date’ ($P \leq 0.03$) and ‘Larnoma’ ($P \leq 0.04$) RF. Cultivar ‘Hertha’ supported the highest *M. enterolobii* Pf and a subsequent higher RF (Table 3.1).

3.4.3 Potato-*Meloidogyne javanica* relations

Meloidogyne javanica Pi had significant effects ($P \leq 0.05$) on Pf of all cultivars. There were no significant differences in gall numbers across all cultivars. Initial population densities had highly significant effects ($P \leq 0.01$) on RF of ‘Buffelspoort P1’, ‘Hertha’, ‘Larnoma’ and ‘Mnandi’. But there were no significant differences in ‘Up-to-date’ RF. Cultivar ‘Hertha’ supported the highest *M. javanica* Pf and a subsequent higher RF (Table 3.1).

Table 3.1 Mean final population (Pf), gall numbers and reproductive factor (RF) of *Meloidogyne enterolobii* and *Meloidogyne javanica* on five potato cultivars at 56 days after inoculation under net house conditions.

Cultivar	Pi	<i>Meloidogyne enterolobii</i>			<i>Meloidogyne javanica</i>		
		Pf	Galls	RF	Pf	Galls	RF
Buffelspoort P1	500	2562.00d ^z ± 5.12	4.40a ± 0.89	5.12d ± 0.01	1757.00d ± 3.51	3.80a ± 1.30	3.51d ± 2.48
	1500	9624.00c ± 6.41	3.00b ± 1.87	6.42c ± 4.96	6731.00c ± 4.48	3.20a ± 0.83	4.49c ± 0.01
	2500	18617.00b ± 7.44	4.20ab ± 0.44	7.45b ± 4.18	12873.00b ± 5.15	3.60a ± 1.15	5.15b ± 0.02
	3500	27574.00a ± 7.88	4.60a ± 0.55	7.89a ± 4.97	23917.00a ± 6.83	3.80a ± 0.83	6.83a ± 0.00
HSD _{0.05}		20.11	1.16	1.01	20.01	n.s ²	1.00
P		0.05	< 0.01	< 0.01	0.04	0.51	< 0.01
F		2.99	17.45	18.53	0.16	18.53	17.45
Hertha	500	2831.00d ± 3.13	4.00ab ± 0.00	5.66d ± 0.63	2216.20d ± 3.96	4.40a ± 1.51	4.44d ± 0.79
	1500	11037.00c ± 4.47	4.80a ± 1.78	7.36c ± 0.29	8635.20c ± 8.45	4.20a ± 1.09	5.75c ± 0.56
	2500	24372.00b ± 4.47	4.00b ± 0.70	9.75b ± 0.17	19038.80b ± 3.72	4.20a ± 1.09	7.62b ± 0.46
	3500	35196.00a ± 8.94	4.00a ± 0.70	10.06a ± 0.25	30823.80a ± 2.46	4.00a ± 1.22	8.81a ± 0.70
HSD _{0.05}		21.12	0.50	2.12	16.02	n.s	0.66
P		< 0.01	< 0.01	< 0.01	0.02	0.34	< 0.01
F		1.52	14.40	7.07	3.60	17.23	17.23

Cultivar	Pi	<i>Meloidogyne enterolobii</i>			<i>Meloidogyne javanica</i>		
		Pf	Galls	RF	Pf	Galls	RF
Larnoma	500	1960.00d ± 3.02	4.40a ± 0.54	3.92d ± 0.14	1245.00d ± 2.63	3.40a ± 1.34	2.49d ± 3.12
	1500	7839.00c ± 4.78	3.00ab ± 1.73	5.23c ± 3.22	4772.00c ± 4.31	3.80a ± 0.83	3.18c ± 1.11
	2500	14374.00b ± 5.33	3.20a ± 2.16	5.75b ± 1.12	10001.00b ± 2.28	3.80a ± 0.44	4.00b ± 0.05
	3500	23313.00a ± 5.88	4.00a ± 2.23	6.66a ± 3.45	19349.00a ± 3.57	3.40a ± 0.54	5.53a ± 0.14
HSD _{0.05}		18.00	2.38	0.22	20.00	n.s	0.00
P		< 0.01	0.04	0.04	0.01	0.68	< 0.01
F		2.73	3.09	3.09	4.43	12.05	12.05
Mnandi	500	1853.00d ± 3.47	3.60a ± 0.89	3.71d ± 0.26	1839.00d ± 1.28	2.75a ± 0.83	3.68d ± 1.53
	1500	6136.00c ± 5.11	4.00a ± 0.71	4.09d ± 3.18	6521.00c ± 4.36	2.40a ± 0.89	4.35c ± 0.17
	2500	13242.00b ± 5.67	4.20a ± 0.44	5.29b ± 1.18	13141.00b ± 5.03	2.60a ± 1.14	5.26b ± 0.22
	3500	21648.00a ± 6.74	4.20a ± 0.44	6.19a ± 3.67	19856.00a ± 3.65	3.20a ± 0.44	5.67a ± 0.36
HSD _{0.05}		21.00	n.s	0.65	22.01	n.s	1.00
P		0.05	0.90	< 0.01	0.02	0.14	< 0.01
F		0.86	40.75	40.75	1.44	16.00	16.00
	500	2568.00d ± 5.21	2.20a ± 0.83	5.14ab ± 1.45	2398.00d ± 3.43	2.60a ± 0.89	4.79a ± 0.45

Cultivar	Pi	<i>Meloidogyne enterolobii</i>			<i>Meloidogyne javanica</i>		
		Pf	Galls	RF	Pf	Galls	RF
Up-to-date	1500	9598.00c ± 9.22	2.00a ± 1.00	6.39a ± 3.77	7506.00c ± 4.86	2.60a ± 0.56	5.00a ± 0.74
	2500	19102.00b ± 4.59	2.40a ± 0.54	7.64a ± 4.23	15479.00b ± 5.59	3.00a ± 0.71	6.19a ± 2.41
	3500	29082.00a ± 11.08	2.60a ± 0.54	8.31a ± 5.12	24464.00a ± 8.97	3.20a ± 0.44	6.98a ± 1.40
HSD _{0.05}		20.00	n.s	0.55	21.02	n.s	n.s
P		0.04	0.12	0.03	0.03	0.22	0.11
F		1.99	10.76	10.76	1.24	2.17	2.17

²Column means followed by the same letter were not significantly different ($P \leq 0.05$) according to Tukey's HSD, average ± standard deviation error.

²n.s. = not significant.

3.4.4 Response of potato cultivars to *Meloidogyne enterolobii*

Inoculation of 'Buffelspoort P1' with *M. enterolobii* significantly ($P \leq 0.01$) influenced chlorophyll content, but there were no significant differences in all other growth parameters. *Meloidogyne enterolobii* Pi had highly significant differences ($P \leq 0.01$) in stem diameter and tuber weight of 'Hertha', significant effects ($P \leq 0.05$) on plant height, fresh weight and tuber number, but there were no significant differences in chlorophyll content and dry weight. There were significant differences in chlorophyll content, fresh weight, tuber number and tuber weight but no significant differences in plant height, stem diameter and dry weight of 'Larnoma' across all Pi levels. *Meloidogyne enterolobii* Pi had highly significant effects ($P \leq 0.01$) in chlorophyll content, but no significant differences in all growth parameters of 'Mnandi'. *Meloidogyne enterolobii* had highly significant differences ($P \leq 0.02$) in stem diameter and dry weight, significant effects ($P \leq 0.05$) in fresh weight and tuber number, but no significant differences in plant height, chlorophyll content and tuber number of 'Up-to-date' (Table 3.2).

3.4.5 Response of potato cultivars to *Meloidogyne javanica*

Inoculation of 'Buffelspoort P1' with *M. javanica* significantly ($P \leq 0.05$) influenced plant height, tuber number and tuber weight but no significant influence in other growth parameters. There were highly significant differences on all 'Hertha' growth parameters, except stem diameter. *Meloidogyne javanica* Pi had high significant differences ($P \leq 0.01$) in all growth parameters of 'Larnoma'. *Meloidogyne javanica* Pi had significant influences ($P \leq 0.01$) in tuber number and tuber weight and significant differences in plant height, but there were no significant differences in other growth parameters of 'Mnandi'. There were highly significant differences ($P \leq 0.02$) in plant height and dry weight, significant differences ($P \leq 0.05$) in fresh

weight and tuber number but no significant differences in stem diameter, chlorophyll content and tuber weight of 'Up-to-date'.

Table 3.2 Effects of *Meloidogyne enterolobii* and *Meloidogyne javanica* initial population density (Pi) on potato cultivars' plant height (PH), stem diameter (SD), chlorophyll content (CC), fresh shoot weight (FW), dry shoot weight (DW), tuber number (TN) and tuber weight (TW) at 56 days after inoculation under net house conditions.

<i>Meloidogyne enterolobii</i>								
Cultivar	Pi	PH (cm)	SD (mm)	CC ($\mu\text{mol m}^{-2}$)	FW (g)	DW (g)	TN	TW (g)
Buffelspoort P1	0	16.84a ^z $\pm$ 4.90	9.57a $\pm$ 2.90	22.48a $\pm$ 3.12	26.98a $\pm$ 7.22	3.72a $\pm$ 1.66	5.50a $\pm$ 1.14	105.82a $\pm$ 42.69
	500	16.20a $\pm$ 5.27	8.26a $\pm$ 2.59	14.98bc $\pm$ 6.98	23.11a $\pm$ 13.67	2.45a $\pm$ 0.88	4.60a $\pm$ 1.67	97.93a $\pm$ 61.17
	1500	18.50a $\pm$ 8.14	8.04a $\pm$ 2.08	20.96ab $\pm$ 9.21	32.14a $\pm$ 21.63	4.27a $\pm$ 3.38	4.60a $\pm$ 1.14	88.75a $\pm$ 28.84
	2500	19.26a $\pm$ 7.09	8.21a $\pm$ 1.25	11.48c $\pm$ 3.84	26.00a $\pm$ 10.98	2.99a $\pm$ 1.76	5.10a $\pm$ 0.54	105.82a $\pm$ 41.39
	3500	17.22a $\pm$ 1.61	7.12a $\pm$ 1.01	23.62a $\pm$ 7.96	24.31a $\pm$ 15.79	3.53a $\pm$ 1.93	4.30a $\pm$ 0.89	83.20a $\pm$ 46.20
HSD _{0.05}		n.s ²	n.s	7.27	n.s	n.s	n.s	n.s
P		0.17	0.41	0.01	0.91	0.29	0.63	0.52
F		1.84	1.05	4.66	0.23	1.34	0.65	2.99
Hertha	0	11.98a $\pm$ 3.65	7.12a $\pm$ 1.78	16.26a $\pm$ 10.92	10.67a $\pm$ 5.77	1.16a $\pm$ 0.74	4.60a $\pm$ 1.34	135.82a $\pm$ 56.47
	500	10.22ab $\pm$ 1.77	6.72a $\pm$ 1.21	14.36a $\pm$ 4.86	6.78ab $\pm$ 3.59	0.83a $\pm$ 0.38	3.80ab $\pm$ 1.09	91.80ab $\pm$ 46.65
	1500	5.09b $\pm$ 4.76	3.70b $\pm$ 3.43	7.72a $\pm$ 7.05	7.13ab $\pm$ 0.80	0.80a $\pm$ 0.57	2.80b $\pm$ 0.83	48.85b $\pm$ 68.20
	2500	5.28b $\pm$ 4.98	3.27b $\pm$ 2.15	6.95a $\pm$ 6.73	4.48ab $\pm$ 4.35	0.76a $\pm$ 0.49	3.80ab $\pm$ 0.83	67.80ab $\pm$ 79.12
	3500	7.92ab $\pm$ 5.74	2.68c $\pm$ 2.57	13.94a $\pm$ 12.94	1.69b $\pm$ 1.75	0.46a $\pm$ 0.45	4.00ab $\pm$ 1.00	105.81ab $\pm$ 41.76

<i>Meloidogyne enterolobii</i>								
Cultivar	Pi	PH (cm)	SD (mm)	CC ($\mu\text{mol m}^{-2}$)	FW (g)	DW (g)	TN	TW (g)
HSD _{0.05}		4.22	3.50	n.s	2.45	n.s	0.60	28.11
P		0.05	< 0.01	0.33	0.05	0.38	0.04	0.02
F		2.87	6.41	1.24	2.06	1.11	2.05	1.52
Larnoma	0	27.06a $\pm$ 10.74	6.91a $\pm$ 3.31	32.91a $\pm$ 17.89	14.90a $\pm$ 14.15	2.15a $\pm$ 2.08	5.80a $\pm$ 0.83	141.33a $\pm$ 56.26
	500	17.46a $\pm$ 15.89	5.63a $\pm$ 3.19	13.94b $\pm$ 14.18	13.72a $\pm$ 1.86	1.56a $\pm$ 1.13	5.00a $\pm$ 1.41	157.45a $\pm$ 88.77
	1500	19.66a $\pm$ 11.28	5.08a $\pm$ 1.58	16.82b $\pm$ 16.82	6.52b $\pm$ 4.55	1.13a $\pm$ 0.86	3.40b $\pm$ 1.14	60.27b $\pm$ 45.87
	2500	25.38a $\pm$ 10.62	5.04a $\pm$ 1.85	27.22ab $\pm$ 14.55	6.23b $\pm$ 5.07	1.06a $\pm$ 0.59	5.20a $\pm$ 0.83	127.90a $\pm$ 63.53
	3500	25.98a $\pm$ 10.84	6.56a $\pm$ 3.67	22.18ab $\pm$ 11.46	13.72a $\pm$ 20.35	2.35a $\pm$ 3.05	4.40ab $\pm$ 1.51	116.15ab $\pm$ 106.87
HSD _{0.05}		n.s	n.s	14.28	16.48	n.s	1.59	67.23
P		0.39	0.77	0.05	0.03	0.72	0.05	0.02
F		1.09	0.44	2.60	0.54	0.52	2.93	2.73
Mnandi	0	20.68a $\pm$ 7.05	5.19a $\pm$ 1.99	18.00a $\pm$ 6.48	13.07a $\pm$ 6.80	1.24a $\pm$ 0.54	6.60ab $\pm$ 0.55	149.76a $\pm$ 57.21
	500	14.54a $\pm$ 5.61	3.67a $\pm$ 1.18	24.15a $\pm$ 19.9	12.87a $\pm$ 11.01	1.61a $\pm$ 1.04	7.00a $\pm$ 0.71	159.91a $\pm$ 56.02
	1500	15.32a $\pm$ 10.77	4.23a $\pm$ 2.53	8.96b $\pm$ 6.36	9.27a $\pm$ 8.10	0.96a $\pm$ 0.80	5.40bc $\pm$ 1.34	105.70a $\pm$ 95.90
	2500	16.30a $\pm$ 7.48	8.61a $\pm$ 7.63	9.51b $\pm$ 2.90	14.52a $\pm$ 12.31	3.03a $\pm$ 5.28	3.75c $\pm$ 0.90	105.66a $\pm$ 68.45

<i>Meloidogyne enterolobii</i>								
Cultivar	Pi	PH (cm)	SD (mm)	CC ($\mu\text{mol m}^{-2}$)	FW (g)	DW (g)	TN	TW (g)
	3500	13.85a $\pm$ 8.67	3.89a $\pm$ 2.48	7.30b $\pm$ 4.65	7.11a $\pm$ 4.79	0.68a $\pm$ 0.66	5.40bc $\pm$ 1.34	116.64a $\pm$ 55.35
HSD _{0.05}		n.s	n.s	13.88	n.s	n.s	1.26	n.s
P		0.51	0.36	0.05	0.71	0.62	< 0.01	0.50
F		0.86	1.15	2.43	0.54	0.67	4.91	0.86
Up-to-date	0	33.24a $\pm$ 1.61	12.50a $\pm$ 1.08	28.35a $\pm$ 4.67	44.55a $\pm$ 9.76	4.56ab $\pm$ 1.50	8.60a $\pm$ 2.16	114.20a $\pm$ 17.13
	500	33.38a $\pm$ 5.40	10.01ab $\pm$ 3.41	20.93a $\pm$ 6.14	34.49b $\pm$ 8.86	4.21ab $\pm$ 0.83	8.60a $\pm$ 1.81	94.09ab $\pm$ 27.64
	1500	30.56a $\pm$ 1.99	10.09ab $\pm$ 2.85	25.67a $\pm$ 5.07	33.85b $\pm$ 4.96	3.74b $\pm$ 0.51	6.80a $\pm$ 1.30	103.33ab $\pm$ 32.40
	2500	28.35a $\pm$ 7.04	8.84b $\pm$ 0.95	21.82a $\pm$ 6.70	33.12b $\pm$ 6.75	3.71b $\pm$ 0.56	7.20a $\pm$ 2.04	88.56b $\pm$ 12.36
	3500	31.91a $\pm$ 3.01	11.12ab $\pm$ 1.51	24.77a $\pm$ 4.71	41.37ab $\pm$ 5.94	5.15a $\pm$ 1.20	8.20a $\pm$ 1.48	111.11ab $\pm$ 24.1
HSD _{0.05}		n.s	2.21	n.s	9.55	1.34	n.s	23.21
P		0.36	0.02	0.28	0.05	0.02	0.24	0.04
F		1.17	1.65	1.37	2.62	1.79	1.50	1.99
<i>Meloidogyne javanica</i>								
Cultivar	Pi	PH (cm)	SD (mm)	CC ($\mu\text{mol m}^{-2}$)	FW (g)	DW (g)	TN	TW (g)
	0	25.36a $\pm$ 2.63	10.51a $\pm$ 2.21	33.98a $\pm$ 26.37	38.45a $\pm$ 8.51	5.71a $\pm$ 1.28	7.20a $\pm$ 0.83	156.79a $\pm$ 90.06

<i>Meloidogyne javanica</i>								
Cultivar	Pi	PH (cm)	SD (mm)	CC ($\mu\text{mol m}^{-2}$)	FW (g)	DW (g)	TN	TW (g)
Buffelspoort P1	500	22.28a $\pm$ 5.99	9.66a $\pm$ 1.64	27.12a $\pm$ 8.71	37.28a $\pm$ 14.33	5.41a $\pm$ 1.85	5.20b $\pm$ 0.83	127.30ab $\pm$ 63.94
	1500	17.10b $\pm$ 6.16	7.98a $\pm$ 1.18	28.02a $\pm$ 14.44	30.40a $\pm$ 17.72	3.14a $\pm$ 2.14	4.20b $\pm$ 1.93	63.94b $\pm$ 38.49
	2500	20.38a $\pm$ 4.77	9.02a $\pm$ 1.49	20.28a $\pm$ 7.96	37.55a $\pm$ 17.66	5.47a $\pm$ 2.61	4.40b $\pm$ 0.89	116.26ab $\pm$ 28.13
	3500	18.58ab $\pm$ 6.79	9.64a $\pm$ 2.46	31.28a $\pm$ 19.83	37.06a $\pm$ 15.39	5.51a $\pm$ 2.13	4.40b $\pm$ 1.14	75.32b $\pm$ 16.89
HSD _{0.05}		7.14	n.s	n.s	n.s	n.s	1.59	66.18
P		0.04	0.75	0.73	0.90	0.76	0.05	0.05
F		0.74	1.05	0.51	0.26	0.46	5.50	0.16
Hertha	0	24.40a $\pm$ 10.39	5.51a $\pm$ 1.01	43.48a $\pm$ 9.34	27.25a $\pm$ 23.95	4.60a $\pm$ 2.45	5.60a $\pm$ 1.94	241.00a $\pm$ 197.13
	500	16.60ab $\pm$ 8.10	5.18a $\pm$ 0.16	19.97b $\pm$ 13.79	13.56b $\pm$ 6.78	2.37b $\pm$ 1.39	3.40b $\pm$ 1.14	109.81b $\pm$ 49.00
	1500	12.70b $\pm$ 1.80	5.42a $\pm$ 0.53	22.48b $\pm$ 8.99	10.41c $\pm$ 3.29	1.05cb $\pm$ 0.46	3.40b $\pm$ 0.54	91.27b $\pm$ 44.46
	2500	11.92b $\pm$ 1.34	5.97a $\pm$ 1.17	14.78b $\pm$ 10.54	8.25c $\pm$ 2.61	0.52c $\pm$ 0.29	3.40b $\pm$ 0.89	83.58b $\pm$ 36.65
	3500	16.36ab $\pm$ 9.51	4.98a $\pm$ 0.91	19.62b $\pm$ 6.88	9.36c $\pm$ 8.21	1.13cb $\pm$ 0.94	2.20b $\pm$ 0.83	116.50b $\pm$ 57.7
HSD _{0.05}		9.33	n.s	14.19	16.83	1.83	1.60	109.76
P		0.03	0.46	< 0.01	0.04	< 0.01	< 0.01	0.02
F		2.11	0.95	6.08	2.01	7.14	4.92	3.60
	0	31.08a $\pm$ 11.28	6.59a $\pm$ 1.11	46.90a $\pm$ 12.54	53.54a $\pm$ 35.13	6.83a $\pm$ 3.58	8.40a $\pm$ 1.34	510.47a $\pm$ 116.45

<i>Meloidogyne javanica</i>								
Cultivar	Pi	PH (cm)	SD (mm)	CC ($\mu\text{mol m}^{-2}$)	FW (g)	DW (g)	TN	TW (g)
Larnoma	500	22.84b $\pm$ 8.24	4.84b $\pm$ 0.49	28.42b $\pm$ 12.01	43.06ab $\pm$ 27.75	4.97ab $\pm$ 2.33	6.20b $\pm$ 1.30	408.75ab $\pm$ 71.34
	1500	21.46b $\pm$ 8.52	4.82b $\pm$ 0.81	26.55b $\pm$ 3.39	38.29bc $\pm$ 26.26	4.06bc $\pm$ 1.96	5.60bc $\pm$ 0.89	300.03bc $\pm$ 151.61
	2500	18.40b $\pm$ 7.15	4.21b $\pm$ 0.89	24.37b $\pm$ 13.59	38.11bc $\pm$ 24.56	3.23bc $\pm$ 1.38	4.40c $\pm$ 1.14	229.58c $\pm$ 131.18
	3500	20.74b $\pm$ 1.56	5.15b $\pm$ 0.20	24.21b $\pm$ 7.30	29.20c $\pm$ 15.17	2.97c $\pm$ 0.43	4.80c $\pm$ 1.64	258.33bc $\pm$ 107.35
HSD _{0.05}		6.91	0.99	12.82	12.34	1.88	1.64	165.79
P		0.01	< 0.01	< 0.01	0.01	< 0.01	< 0.01	0.01
F		4.41	7.21	4.99	4.65	6.16	8.19	4.43
Mnandi	0	21.58a $\pm$ 9.72	5.34a $\pm$ 1.73	17.94a $\pm$ 15.83	18.40a $\pm$ 14.02	1.86a $\pm$ 1.21	6.80a $\pm$ 0.83	202.74a $\pm$ 49.34
	500	14.82b $\pm$ 3.94	6.00a $\pm$ 1.46	14.46a $\pm$ 10.75	12.87a $\pm$ 11.64	1.47a $\pm$ 1.53	5.30b $\pm$ 0.44	109.71ab $\pm$ 62.96
	1500	18.36ab $\pm$ 5.40	7.01a $\pm$ 2.19	11.60a $\pm$ 4.03	18.58a $\pm$ 24.84	1.65a $\pm$ 2.14	5.30b $\pm$ 0.84	104.99ab $\pm$ 67.73
	2500	18.08ab $\pm$ 3.13	5.53a $\pm$ 1.37	9.36a $\pm$ 5.92	17.36a $\pm$ 20.86	1.13a $\pm$ 1.19	4.40c $\pm$ 0.54	77.08b $\pm$ 21.72
	3500	17.58ab $\pm$ 3.98	5.49a $\pm$ 1.68	17.50a $\pm$ 8.66	15.78a $\pm$ 8.01	1.45a $\pm$ 0.68	4.20c $\pm$ 0.70	138.41ab $\pm$ 73.39
HSD _{0.05}		6.50	n.s	n.s	n.s	n.s	0.83	119.31
P		0.05	0.53	0.63	0.97	0.93	< 0.01	0.02
F		1.23	0.81	0.65	0.12	0.19	16.77	1.44
	0	34.58a $\pm$ 3.46	12.50a $\pm$ 1.08	28.35a $\pm$ 4.67	44.55a $\pm$ 9.76	4.56ab $\pm$ 1.50	8.20a $\pm$ 0.54	114.20a $\pm$ 17.13

<i>Meloidogyne javanica</i>								
Cultivar	Pi	PH (cm)	SD (mm)	CC ($\mu\text{mol m}^{-2}$)	FW (g)	DW (g)	TN	TW (g)
Up-to-date	500	24.78b $\pm$ 6.96	10.01a $\pm$ 3.41	20.93a $\pm$ 6.14	34.49b $\pm$ 8.86	4.21ab $\pm$ 0.83	6.40b $\pm$ 1.94	94.09a $\pm$ 27.64
	1500	26.86b $\pm$ 5.06	10.09a $\pm$ 2.85	25.67a $\pm$ 5.07	33.85b $\pm$ 4.95	3.74b $\pm$ 0.51	7.40ab $\pm$ 2.96	103.33a $\pm$ 32.40
	2500	25.50b $\pm$ 5.77	9.86a $\pm$ 2.06	24.59a $\pm$ 6.13	33.74b $\pm$ 6.90	3.82ab $\pm$ 0.69	6.60b $\pm$ 1.81	94.63a $\pm$ 11.52
	3500	29.26ba $\pm$ 1.65	11.12a $\pm$ 1.51	24.77a $\pm$ 4.71	41.37ab $\pm$ 5.94	5.15a $\pm$ 1.20	9.00a $\pm$ 1.00	111.11a $\pm$ 24.19
HSD _{0.05}		6.29	n.s	n.s	9.62	1.36	1.91	n.s
P		0.02	0.45	0.39	0.05	0.02	0.05	0.33
F		3.55	0.97	1.09	2.45	1.62	2.93	1.24
² Column means followed by the same letter were not significantly different ($P \leq 0.05$) according to Tukey's HSD, average $\pm$ standard deviation error.								
² n.s = not significant.								

3.5 Discussion

3.5.1 Host-status of *Meloidogyne* on potato cultivars

Following nematode extraction, a higher number of nematodes persisted inside the roots of all five cultivars. In both planting seasons, *M. enterolobii* and *M. javanica* Pi were not significantly associated with tuber yield and tuber number, but significantly associated with Pf and galling severity. According to the NCDS, galling severity scores for *M. enterolobii* were relatively high compared to *M. javanica*, with an average of 5 (> 100 galls) and 4-5 (31-100 and > 100), respectively. Accordingly, even if *Meloidogyne* species Pi did not reduce tuber yield or size; they still showed potential to substantially impede tuber quality.

A host's susceptibility is determined not only by its genotype but also by the number of nematodes that are affecting it, i.e., Pi as well as genotype. As such different initial population densities were administered. It has been demonstrated that plants suffer a significant decline in growth at high population densities (Kayani *et al.*, 2017), however, plant susceptibility is also affected by environmental factors (Starr & Mercer, 2009). This is not to say, lower Pi does not affect plant growth or yield; to the contrary. There were considerable differences between crop growth to different nematode Pi. In essence; because potato cultivars proved susceptibility to *Meloidogyne* nematodes, all inoculated nematode levels had the potential to cause quality damage to tubers. Even though results from this study demonstrated that there were no significant differences between tuber number and tuber mass at different inoculation levels within the same cultivar, tuber quality damage is generally not correlated with Pi. Be that as it may, a susceptible host supports *Meloidogyne* populations that would increase to have high Pi for the following cropping season. In any case, Pi ends up playing a pivotal role in crop yield.

At the lowest Pi, i.e., 500 eggs + J2s per pot, the highest nematode RF was 5.66 on cultivar ‘Hertha’ and at the highest Pi, i.e., 3500 eggs + J2s per pot, the highest nematode RF was 10.06 on cultivar ‘Hertha’. In general, as Pi increased, so did the RF for all cultivars. Although at varying degrees, all of the potato cultivars that were evaluated exhibited root knots, the infamous symptom that is typical of crops that have been infected by *Meloidogyne* nematodes. The response of various potato cultivars to RKN has been observed to vary significantly, with moderately susceptible cultivars harbouring fewer developing nematodes than highly susceptible cultivars (Getu *et al.*, 2021). According to Montasser *et al.* (2019), none of the 16 potato cultivars evaluated were completely resistant to nematode infection. Instead, the cultivars were classified as being either highly resistant, moderately susceptible, or highly susceptible to nematode infection. The RF, a factor directly proportional to galling severity, for *M. incognita* was lower on *C. pepo* ‘Amalthee’ than *C. sativus* ‘Dasher II’ (López-Gómez *et al.*, 2015; Verdejo-Lucas & Talavera, 2019).

During the current experiments, the mean soil temperature at 20 cm (representing the root zone) was around 25-29 °C in February-April 2020 and 25-29 °C in August-October 2020 (WU, 2021). These conditions, thus, represent a sufficient time for approximately two generations during the experiments, likely reflected in the galling severities and nematode Pf observed. The average generation time for many *Meloidogyne* species is 28–35 days at optimal base temperatures of approximately 24-28 °C (Bridge & Starr, 2007). At 25-30 °C, *Meloidogyne* species had an average of 15–33% infection rates on *S. lycopersicum* ‘BHN 589’, with J2s development relatively higher at 25 °C when compared to 30 °C. The same nematode species completed one generation cycle in 29 days at 30 °C (Velloso *et al.*, 2022). Potato crop optimum growing temperatures range between 17 and 26 °C (Kim & Lee, 2016; Rudić *et al.*, 2022),

which are the same temperatures that support *Meloidogyne* species growth and reproduction (Velloso *et al.*, 2022).

The soil sampled for planting in this study was a sandy loam texture, with clay contents ranging from 65 to 75% which may have facilitated the migration of *M. enterolobii* and *M. javanica* through the soil profile. Soil texture has also been correlated with nematode movement in soil, with coarse-textured soils facilitating increased migration of *Meloidogyne* species and crop damage (Koenning *et al.*, 1996; Gade & Hiware, 2017; Al-Ghamdi, 2021). *Meloidogyne incognita* showed an increased gall severity and egg-mass formation on carrots (*Daucus carota* Hoffm. ‘Shinheukjeon-5-chon’) in sandy soil. This behaviour was attributed to the nematodes’ increased penetration rates, which offer enough aeration for the nematodes owing to coarse soil characteristics, resulting in improved mobility (Kim *et al.*, 2017).

The cultivars and level of nematode inoculum had substantial impacts on the growth and reproduction of *M. enterolobii* and *M. javanica*. This demonstrated that the genetic diversity in potato cultivars varied depending on how they responded to the treatments. Based on Pf, galling severity and RF; potato cultivars’ response to the two nematodes varied: cultivar ‘Hertha’ was highly susceptible to *M. enterolobii* and *M. javanica*, while ‘Buffelspoort P1’ and ‘Up-to-date’ were moderately susceptible and ‘Larnoma’, ‘Mnandi’ and were susceptible. Crops within the same genera can also have different susceptibility levels to the same RKN. For example, there was a lower Pf/Pi ratio for *M. charantia* on pumpkins (*Cucurbita moschata* Duch.), followed by cucumber (*Cucumis sativus* L.) and bottle gourd (*Lagenaria siceraria* Molina) (Chandra *et al.*, 2010).

3.5.2 Host-sensitivity of potato cultivars under *Meloidogyne*

At all nematode inoculum levels, *M. enterolobii* showed severe symptoms as compared to *M. javanica*. This is most likely a result of *Meloidogyne* species' widely varied genetic makeup. Kim *et al.* (2017) establishment that *M. enterolobii* capacity to evade resistance on cotton (*Gossypium hirsutum* L.), is a testament to the species' remarkable virulence severity. The cultivars of inoculated vs non-inoculated plants produced the same quantity and weight of tubers in this study; however, because tuber growth was constrained by the size of the pot, different findings may have been obtained in the field. *Meloidogyne* damage to potatoes might vary depending on Pi, edaphic conditions and cultivar susceptibility (Brodie *et al.*, 1993; Castillo & Vovlas, 2007; Wassu *et al.*, 2023).

According to the findings of this study, symptoms may develop inside the tubers and only become obvious externally after harvest or storage. As a result, while nematode extraction from tubers enhances detection and verifies infection, visual inspection of tubers is not always sufficient for infection diagnosis. Evidenced by the growth of different genotypes when compared to the control, the potato's nutrients and water were sequestered by giant cells and tissue alterations brought on by nematode infections. These modifications obstruct water and nutrient transfer from infected roots to above-ground plant tissues, which inhibits plant development.

The present study's observations of the growth and parasitic behaviour of *M. javanica* in potato tubers and feeder roots imply that *M. javanica* infection has the potential to cause significant potato damage. The severity of root galling on feeder roots of cultivars 'Hertha', 'Up-to-date', 'Buffelspoort 1', 'Mnandi' and 'Larnoma', respectively as well as the significant impairment of plant growth caused by an increase in Pi in the soil proved that *Meloidogyne* has a high

potential for reproduction and damage on potato. A similar trend of declined potato growth response to *Meloidogyne* species was observed by Vovlas *et al.* (2005a).

3.6 Conclusion

The results show that the tested commercial potato cultivars have a variety of susceptibility levels to the two *Meloidogyne* species, given that none of the cultivars were able to suppress nematode penetration, feeding and/or reproduction. The cultivars ‘Hertha’, ‘Buffelspoort P1’ and ‘Up-to-date’ sustained higher nematode densities. On the other hand, in cultivars ‘Larnoma’ and ‘Mnandi’ the total nematode extracted from the total root system was relatively lower than that of the other cultivars, following the same pattern of results during both planting seasons. Based on these results, cultivar ‘Hertha’ was highly susceptible to the two *Meloidogyne* species, hence it was selected for further tests, i.e., **Chapter 9**. Overall, the screened cultivars are grown around South Africa, which may facilitate the spread of nematodes into un-infested areas while also allowing the preservation and growth of Pi already existing there. This study further demonstrates that comparatively, *M. javanica* is less aggressive to the potato than *M. enterolobii*.

CHAPTER 4: ISOLATION AND IDENTIFICATION OF FUNGAL BIOTYPES FROM SOILS AT DIFFERENT PLANT RHIZOSPHERES

4.1 Introduction

Micro-organisms are essential for managing plant diseases as well as for the growth and development of plants in the soil (Zarb *et al.*, 2005). Fungi are a group of micro-organisms that are an essential aspect of the soil ecological system, supporting the breakdown of organic matter and the recycling of soil nutrients to make them accessible to plants (Val-Moraes *et al.*, 2013). By applying these microbes' inherent defence mechanisms against a variety of soil-borne diseases, they may be used as bio-control agents. Bio-control refers to the application of effective organisms and their by-products to alleviate abiotic and biotic diseases and support overall productivity gains. The BCA help prevent diseases with minimal to no risk to the environment (Stirling, 2018).

There are numerous, i.e., more than 440, species of BCA on the market (van Lenteren *et al.*, 2018). Several different strategies are employed by BCA to suppress plant diseases in crops (Esmaeel *et al.*, 2020). A comprehensive list of globally registered BCA was given by van Lenteren *et al.* (2018) and comprises bacteria, fungi, mycoviruses and bacteriophages. Many agricultural plants' rhizospheres include specific antagonistic bacteria that can prevent various plant diseases and lessen the need for agrochemical pesticides. The application of these specific BCA in the management of soil-borne diseases has been the subject of research worldwide (Collinge *et al.*, 2019; Khan *et al.*, 2021; Collinge *et al.*, 2022; Pandit *et al.*, 2022).

Bio-control agents are readily present in the rhizosphere and can be exploited to improve crop production. This is because of the strong relationship between probiotic microbes that colonise

plant roots and their host cells, or rhizosphere and pests/pathogens (Anderson & Kim, 2018). The potential for the use of fungal BCA against plant pathogenic organisms has substantially increased because fungi have a very high reproduction rate (both sexually and asexually), a rapid generation period and are target-specific. In addition, they may transition from parasitism to saprotrophic in the absence of the host, which enables them to continue to exist sustainably (Thambugala *et al.*, 2020).

The use of nematophagous fungi BCA against nematodes is one of the biotechnological initiatives that is showing potential to replace the environmentally destructive nematicides. Effective integrated pest control strategies are required to construct the characterisation of entomopathogenic fungal strains as well as knowledge of the biological factors influencing how these strains behave when attacking pests that are vulnerable to them (Figueroa *et al.*, 2007). The traditional method of morphologically identifying fungi relies on the analysis of both macroscopic and microscopic features (Shenoy *et al.*, 2007). The form and size of the conidia are the characteristics that are most frequently utilised to distinguish between different species (Humber, 2012; Samson *et al.*, 2013).

Recent advances in molecular techniques, particularly those based on the analysis of DNA by the polymerase chain reaction (PCR), have made it possible to establish immediate, precise and widely applicable methodologies for the identification of various fungi (Schoch *et al.*, 2012). The ITS and ribosomal DNA genes enable the investigation of the evolutionary connections among several entomopathogenic fungal groupings (Rehner & Samuels, 1995; Driver *et al.*, 2000). In order to identify fungal biotypes with better accuracy and to assess their genetic diversity, it is important to compare the outcomes of morphological characterisation and molecular approaches (Hibbett *et al.*, 2011). The objective was to isolate indigenous fungal

biotypes as possible candidates for bio-control investigation, as well as identify the indigenous fungal biotypes at morphological and molecular level.

4.2 Study site

The experiments were carried out at the Agricultural Research Farm, Plant Pathology Research laboratory, NWU, Mmabatho, Molelwane, North-West Province, South Africa (25°48'36"S, 25°37'47.999"E). The climate of the area is classified as semi-arid, characterised by average summer temperatures ranging from 17 °C - 40 °C and winter temperatures ranging from 3 °C - 21 °C and rainfall during the year range between 1100 and 1600 mm/year (Figure 4.1). Soil samples around plant roots were collected at NWU fields from different crops and examined for the presence of fungi. Morphological identification of fungal biotypes was done at the Agricultural Research Farm, Plant Pathology Research laboratory, NWU. Molecular identification was done at Inqaba laboratory, Tshwane, Gauteng, South Africa. Soil samples were collected throughout the year of 2020.

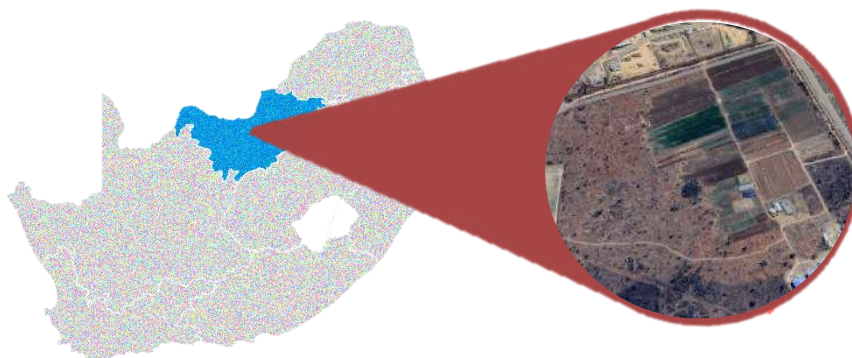


Figure 4.1 Geographical location of collected soil samples in planted areas at NWU campus and experimental farm, South Africa.

4.3 Description of the experiments

4.3.1 Treatments and experimental design

Twenty-five soil samples, i.e., treatments were replicated five times. Six tubes were lined up on a stand and labelled as follows: T₁, T₂, T₃, T₄, T₅ and T₆. Each tube was filled with 9 mL sterilised distilled water. Ten 90-mm Petri dishes were half-filled with potato dextrose agar (PDA) and laid out in a completely randomised design (CRD). In each Petri dish, 1 mL of the solution from tube T₅ was placed on the surface of PDA. Twenty-five pure biotypes were examined for growth structure, i.e., the colour and shape of the mycelia and spores.

4.3.2 Experimental procedures

Using a hand trowel, soil samples were collected from the rhizospheres of different fruit trees, i.e., peach (*Prunus persica* (L.) Batsch), guava (*Psidium guajava* L.) and fig (*Ficus carica* L.) and vegetable plots (at 10 cm depth) with no previous record of fungal inoculations at the Agricultural Research Farm. The soil samples were taken to the laboratory in sterile polythene zip-lock bags for further tests that took place within 24-48 hours after collection. Ten (10) g of rhizosphere soil sample was added to 10 mL of sterilised water and thoroughly mixed to obtain a soil suspension. The soil suspension was serially diluted to the appropriate concentration (10^{-2} g mL⁻¹ and 10^{-3} g mL⁻¹) and plated on PDA media (Biolab, Lawrenceville, Georgia, United States of America) (39 g L⁻¹) treated with Streptomycin (0.5 g L⁻¹) (Mast Diagnostics, United Kingdom). Petri dishes were incubated at 25 ± 2 °C and examined every two days for fungal colony growth. Individual colonies of fungi which vary in shape and colour were picked with a flame-sterilised inoculation needle and cultured separately on freshly prepared PDA. The fungal biotypes were sub-cultured every other day until pure distinct strains were observed (McLean, 1983). Pure biotypes were grown on different growth media, viz., PDA, malt extract agar (MEA), nutrient agar (NA) and water agar (WA) for growth rate comparison.

4.3.3 Morphological identification

Each fungal biotype was examined under a compound microscope (ZEISS, Research Microscopy Solutions, United Kingdom) at 63 μm magnification for structural characteristics after 48-72 hours. The biotypes were identified using Gaddeyya *et al.* (2012) approach, which took into account the growth pattern and colouration of the conidia and the texture of the mycelia. The microscopic determination was carried out by recognising features, such as phialides hyphae, conidial form and quantity of conidiophores. After the fungal structures were observed, microcultures were performed on slides with 2% WA and incubated at 25 ± 2 °C.

4.3.4 Molecular identification

The morphological identification was confirmed by molecular identification using sequencing of ITS gene region, with ITS1 and ITS2 sub-regions and their identities were confirmed by taxonomist, Inqaba Biotech Laboratory, Pretoria, South Africa. Genomic DNA was extracted on 14 days old cultures by aseptically scraping mycelia of the different biotypes from the respective PDA Petri dishes. Mycelial DNA was extracted using the Quick-DNA™ fungal/bacterial Miniprep Kit (Zymo Research, Catalogue No. D6005) (White *et al.*, 1990). The sequences were aligned using Clustal W and the resultant matrix comprising 600 bp was utilised for distance analysis in PAUP* 4.0-10 (Swofford, 1993), utilising the neighbour union technique (Saitou & Nei, 1987) and Bootstrap (Felsenstein, 1985) with 1000 cycles.

Amplification of the target genes was carried out by use of OneTaq® Quick-load® 2×Master Mix (NEB, Catalogue No. M0486) with primers presented in Table 4.1. Each Eppendorf tube comprised of 10 μl of NEB OneTaq 2X MasterMix with Standard Buffer (Catalogue No. M0482S), 1 μl of genomic DNA (10–30ng/ μL), 1 μl forward primer (10 μM), 1 μl reverse primer

(10µM) and 7µl nuclease-free water (Catalogue No. E476). Amplification cycles had an initial denaturation at 94 °C for 10 minute, 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 50 °C, elongation at 68 °C for 1 minute and final elongation at 72 °C for 10 minutes. The PCR amplicons were stored at 4 °C until electrophoresis.

Table 4.1 Internal transcribed spacer (ITS) primers sequences that were used for amplification of the ITS regions.

Name of primer	Target	Sequence (5' to 3')	Cycling conditions
ITS 1	Small-sub unit	TCCGTAGGTGAACCTGCGG	$\left. \begin{array}{l} 94^{\circ}\text{C for 30 sec} \\ 50^{\circ}\text{C for 30 sec} \end{array} \right\} 35 \text{ cycles}$
ITS 4	Large-sub unit	TCCTCCGCTTATTGATATGC	$\left. \begin{array}{l} 68^{\circ}\text{C for 1 min} \\ 68^{\circ}\text{C for 10 min} \end{array} \right\} 35 \text{ cycles}$

The integrity of the PCR amplicons was visualised on a 1% agarose gel (CSL-AG500, Cleaver Scientific Ltd) stained with EZ-vision® Bluelight DNA Dye. The extracted fragments were sequenced in the forward and reverse direction (Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit v3.1, BRD3-100/1000) and were purified using a Zymo kit (Zymo Research, ZR-96 DNA Sequencing clean-up Kit™, Catalogue No. D4050). The purified fragments were analyzed on the ABI 3500xl Genetic analyser (Applied Biosystems, ThermoFisher Scientific, United States of America) for each reaction and every sample. CLC Bio Main Workbench v7.6 was used to analyse the ab1 files generated by the ABI 3500xl Genetic analyser and the results were obtained by conducting a basic local alignment search tool (BLAST) search at the national center for biotechnology information (NCBI) (Altschul *et al.*, 1997).

4.3.4 Data collection

Soil characteristics: In the laboratory soil samples were air dried and sieved through a 2 mm sieve and pH, electrical conductivity (EC), soil texture and organic carbon (OC) were determined. Soil pH was tested using the Bouyoucos hydrometer technique (Bouyoucos, 1951) and EC was measured following Richards (1954) procedure. Soil texture was determined using standard glass/calomel electrodes in a 1:2.5 w/v soil-water suspension (McLean, 1983). Soil OC was tested following a method by Walkley (1947). Soil serial dilution method was used to carry out fungal spore isolations on PDA (Pradhan & Sukla, 2005).

Fungal biotypes: The observed pure biotypes' growth on different media was measured using a clear transparent ruler at 21 days after incubation. For identification purposes, seven-day-old mycelia of respective fungal species grown on PDA were collected from the colony edges and microscopically examined. The hyphae and spore colour, size and shape were used to identify the different species. The observed pure biotypes were assigned working numbers i.e., F-SP.1, F-SP.2, F-SP.3, ...F-SP.25). Following molecular identification, the fungal biodiversity index was calculated according to Gams & Bissett (1998).

4.3.5 Data analysis

Data for fungal biotypes' growth were subjected to ANOVA using SAS software version 9.4 (SAS Institute, Inc., Cary, NC., USA, 2008) (Gomez & Gomez, 1984). Mean separation for significant ($P \leq 0.05$) treatments was achieved through Tukey's Honestly Significant Difference (HSD). Unless otherwise stated, only predictive models which were significant at the probability level of 5% were discussed.

4.4 Results

4.4.1 Soil characteristics

The results on soil physical properties indicated in table 4.2 below:

Table 4.2 Physical properties of soil collected from North-West University agricultural research farm.

Bulk density	1 420 kg/m
Field capacity	320 mm/m
Wilting point	120 mm/m
Porosity	46%
PH	5.85-6.25
EC	132.12-151.80 μ s
OC	22.50-26.10%

4.4.2 Indigenous fungal biotypes growth on different media

Twenty-five fungal biotypes were isolated from field soil samples and were grouped according to their varying morphological characters. Such variations facilitate classifying and categorising species into their appropriate genera. Based on these traits, the isolated fungal biotypes were morphologically identified. Different macroscopic characteristics of various biotypes, such as colony colour and shape are displayed in Figure 4.2.

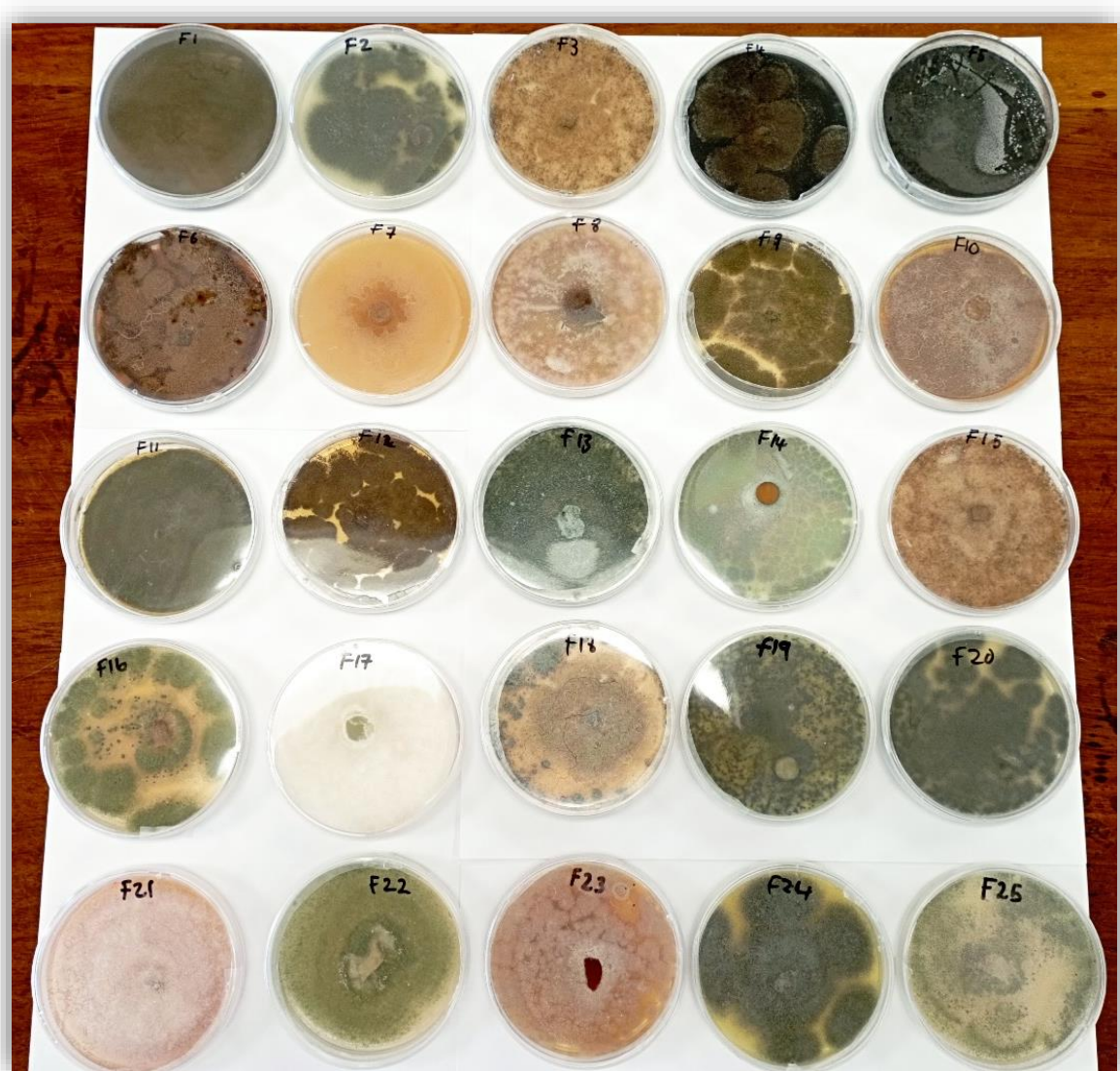


Figure 4.2 Pure fungal biotypes at 21 days after incubation. Pictures taken by Ndivhuwo Ramatsitsi.
Key: From the top left to bottom right, biotypes were labelled as F-SP.1, F-SP.2, ...F-SP.25.

Noteworthy, fungal biotypes showed slightly different morphological colours when grown on different media and had different growth rates. Biotype F-SP.12 had a dark brownish mycelial growth on PDA, whilst it showed light yellow mycelia growth on MA and WA. On the other hand, F-SP.21 showed a light brownish mycelial colour when grown on PDA, whilst it showed a white mycelial colour on both MA and WA (Figure 4.3).



(a) F-SP.12

(b) F-SP.13

(c) F-SP.20

(d) F-SP.21

Figure 4.3 Pure fungal biotypes grown on different media; potato dextrose agar (PDA), malt agar (MA) and water agar (WA) at 21 days after incubation. Pictures taken by Tuelo Motloba.

Furthermore, F-SP.12 grew relatively faster ($P \leq 0.05$) on PDA than on MA and WA; with 82 mm diameter growth at 21 days of incubation. Growth of biotype F-SP.13 also showed different morphological characters on different growth media. On PDA, F-SP.13 showed a green conidia colour and a brownish colour on MA; granted the growth rates were relatively comparable at 80 mm diameter at 21 days of incubation. Biotype F-SP.20 showed varied growth morphological characteristics on different growth media. On PDA, F-SP.20 showed a darker green mycelial colour that grew 90 mm in diameter within 21 days. Conversely, F.SP.20 showed a brownish mycelial colour on both MEA and WA with 60 mm and 78 mm diameter growth, respectively (Figure 4.4).

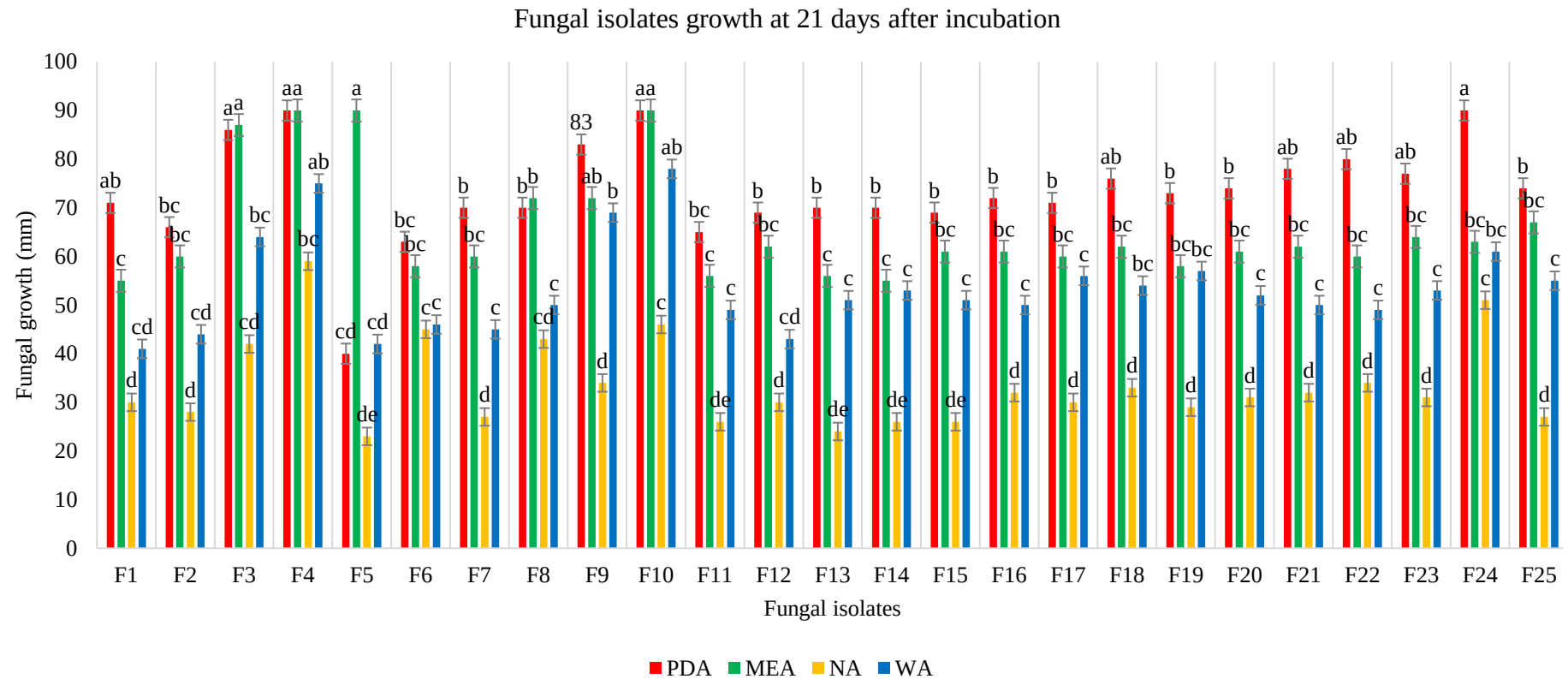


Figure 4.4 Mean radii growth of indigenous fungal biotypes at 21 days after inoculation on eight different agar media, potato dextrose agar (PDA), malt extract agar (MEA), nutrient agar (NA) and water agar (WA). Bars with different letters were significantly different ($P \leq 0.05$).

4.4.3 Indigenous fungal biotypes' morphology

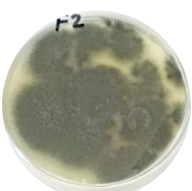
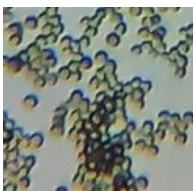
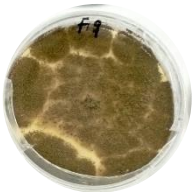
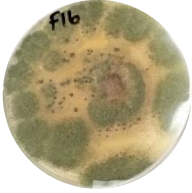
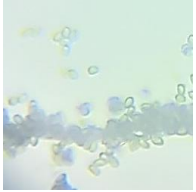
Twenty-five fungal biotypes were isolated from field soil samples and were grouped according to their varying morphological characters. Such variations facilitate classifying and categorising species into their appropriate genera. Based on these characteristics, the isolated biotypes were morphologically identified. In terms of colour, *Aspergillus* was distinguished by colonies that ranged from parrot green to deep green. Cultures showing conidial heads initially yellow when young, turning dark yellow-green as they age, and in older cultures, were deep grey-green; were marked also as *Aspergillus*, in accordance with Klich (2002). *Aspergillus*-group fungi were visually distinguished from other groups based on their yellow-green conidia, globose to sub-globose vesicles and biseriate seriations.

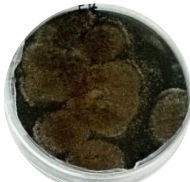
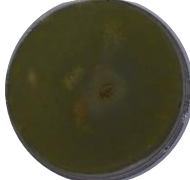
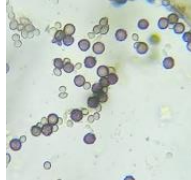
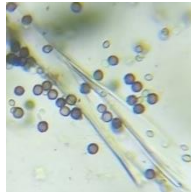
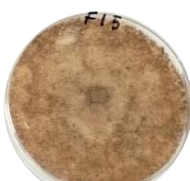
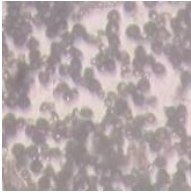
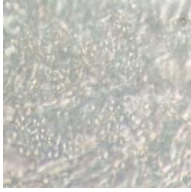
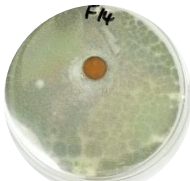
Penicillium was identified as a bluish-green colony colour without white borders and greyish green with white edges. Further, biotypes that secreted regularly occurring yellowish exudates were identified as *Penicillium*. Macroscopic observation of biotypes with multinucleate, septate and often colourless hyphae that make up the mycelium's typical heavily branching network were also grouped as *Penicillium*. *Talaromyces* were identified as biotypes with a yellowish-brown tint and a white reverse, i.e, background. Other biotypes that were dark green with white edges and light brown reverse and sparse spores were also identified as *Talaromyces*.

Pure biotypes displaying two to three concentric rings of yellow-green mycelium that were whitish in colour and spongy in substance, were identified as a *Trichoderma* species. The biotypes' microscopic features were comparable to those noted for the species *Tri. asperellum*. These comprised branching conidiophores, oval-round conidia with greenish-yellow pigmentation and the presence of ampulliform phialides (Samuels *et al.*, 1999). García-Núñez

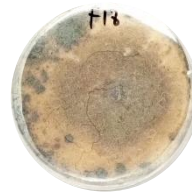
et al. (2017) identified *Trichoderma* biotypes that produced copious amounts of fluffy mycelium and two to three precisely defined rings of conidia and mycelia.

Because these morphological characteristics may be shared by multiple species within the same genera, it was challenging to identify the biotypes solely based on morphological data, let alone at a species level. Fungal biotypes that were different in colour but showed the same microscopic features were grouped into one genus. Microscopical examination of the isolated *Trichoderma* species revealed dense conidia, branching conidiophores, ampulliform phialides and slightly globose conidia with yellow-green pigmentation (Figure 4.5).

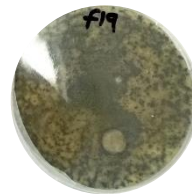
Biotype	General pigmentation appearances of isolated biotype	Macroscopic	Microscopic	Genus
F-SP.1	Initial yellow conidial heads that turn dark army green.			<i>Aspergillus</i>
F-SP.2	Growth initially yellow, turning dark green as they age.			
F-SP.9	Army green as mycelia.			
F-SP.16	Dark green mycelia.			

F-SP.3	Blackish growth colour.			<i>Penicillium</i>
F-SP.4	Initially white mycelia that turns grey green.			
F-SP.5	Dark green mycelia.			
F-SP.10	Light brown mycelia.			
F-SP.17	Light brown mycelia,			
F-SP.6	Dark green mycelia.			<i>Talaromyces</i>
F-SP.12	Brownish, with dark army green,			
F-SP.14	Yellowish-green mycelia.			

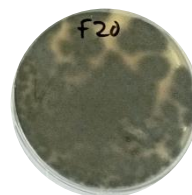
F-SP.18 Dark green mycelia.



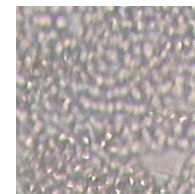
F-SP.19 Green mycelia.



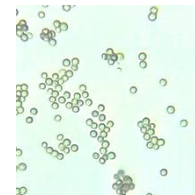
F-SP.20 Powder, army dark green.



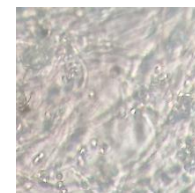
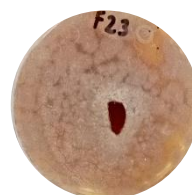
F-SP.21 Whitish mycelia.



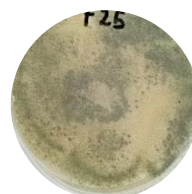
F-SP.22 Copious green fluffy mycelium and precisely defined rings.



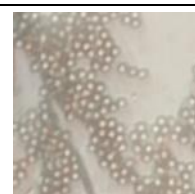
F-SP.23 Whitish mycelia.



F-SP.25 Green fluffy mycelia, with whitish heads.

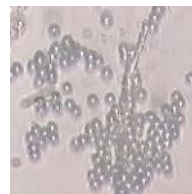


F-SP.7 Light brown.



Trichoderma

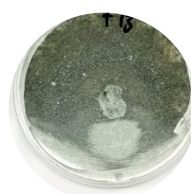
F-SP.8 Whitish mycelia.



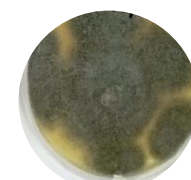
F-SP.11 Fluffy, dark green.



F-SP.13 Copious fluffy green mycelia.



F-SP.15 Fast growth in culture media and the emergence of conidia with a green hue; with a colourless reverse.



F-SP.24 Dark green mycelia.

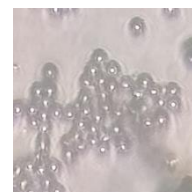


Figure 4.5 Morphological identification of fungal biotypes under compound microscope at 63 µm. Pictures taken by Ndivhuwo Ramatsitsi.

4.4.4 Indigenous fungal biotypes molecular identification

Generated DNA sequences of the respective fungal biotypes were queried by assessing their similarity against the biological sequence within the NCBI database. The BLAST nucleotide sequencing results and the use of the GENBANK (www.ncbi.nlm.nih.gov) gave different fungal species. After analysis, it became apparent that the acquired ITS rDNA sequences had strong similarities to species in Table 4.3.

Table 4.3 Molecular identification of indigenous fungal biotypes through DNA sequences analysis results by BLAST on NCBI.

Assigned #	Initial morphology Identity	GenBank Accession	Nearest BLAST match	Max. identity (%)	HSP length (bp)
F-SP.1	<i>Aspergillus</i> sp.1	MZ014549.1	<i>Talaromyces sayulitensis</i>	100	584
F-SP.2	<i>Aspergillus</i> sp.2	MZ014549.1	<i>Talaromyces sayulitensis</i>	100	577
F-SP.3	<i>Penicillium</i> sp.1	MT645322.1	<i>Aspergillus flavus</i>	100	596
F-SP.4	<i>Penicillium</i> sp.2	MW456070.1	<i>Trichoderma viride</i>	100	600
F-SP.5	<i>Talaromyces</i> sp.1	AB872818.1	<i>Talaromyces purpureogenus</i>	100	599
F-SP.6	<i>Talaromyces</i> sp.2	MZ014549.1	<i>Talaromyces sayulitensis</i>	100	579
F-SP.7	<i>Trichoderma</i> sp.1	MZ014549.1	<i>Talaromyces sayulitensis</i>	100	578
F-SP.8	<i>Trichoderma</i> sp.2	MN788118.1	<i>Talaromyces minioluteus</i>	97.69	562
F-SP.9	<i>Aspergillus</i> sp.3	KC341982.1	<i>Penicillium</i> sp. AL-38 IRH-2012b	94.32	545
F-SP.10	<i>Penicillium</i> sp.3	MT316343.1	<i>Aspergillus terreus</i>	99.83	602
F-SP.11	<i>Trichoderma</i> sp.3	MZ014549.1	<i>Talaromyces sayulitensis</i>	100	579
F-SP.12	<i>Talaromyces</i> sp.3	MZ014549.1	<i>Talaromyces sayulitensis</i>	100	580
F-SP.13	<i>Trichoderma</i> sp.4	MZ014549.1	<i>Talaromyces sayulitensis</i>	100	579
F-SP.14	<i>Talaromyces</i> sp.4	MZ014549.1	<i>Talaromyces sayulitensis</i>	100	577
F-SP.15	<i>Trichoderma</i> sp.5	MZ014549.1	<i>Talaromyces sayulitensis</i>	100	578

F-SP.16	<i>Aspergillus</i> sp.4	MZ014549.1	<i>Talaromyces sayulitensis</i>	100	579
F-SP.17	<i>Penicillium</i> sp. 4	MZ014549.1	<i>Talaromyces sayulitensis</i>	100	578
F-SP.18	<i>Talaromyces</i> sp.5	MZ014549.1	<i>Talaromyces sayulitensis</i>	100	579
F-SP.19	<i>Talaromyces</i> sp.6	MZ014549.1	<i>Talaromyces sayulitensis</i>	100	578
F-SP.20	<i>Talaromyces</i> sp.7	MZ014549.1	<i>Talaromyces sayulitensis</i>	100	579
F-SP.21	<i>Talaromyces</i> sp.1	MN788118.1	<i>Talaromyces minioluteus</i>	97.69	562
F-SP.22	<i>Talaromyces</i> sp.1	MZ014549.1	<i>Talaromyces sayulitensis</i>	95.45	545
F-SP.23	<i>Talaromyces</i> sp.1	MZ014549.1	<i>Talaromyces sayulitensis</i>	100	579
F-SP.24	<i>Trichoderma</i> sp.1	MF078652.1	<i>Trichoderma ghanense</i>	100	632
F-SP.25	<i>Talaromyces</i> sp.2	AB872818.1	<i>Talaromyces purpureogenus</i>	95.45	547

BLAST: Basic local alignment search tool.

DNA: Deoxyribonucleic acid.

NCBI: National center for biotechnology information.

4.4.5 Fungi biodiversity index

According to relative abundance, there was a higher occurrence of *Tal. sayulitensis*, followed by *Tal. purpureogenus*, *Tal. minioluteus* and *A. flavus*, *A. terreus*, *Penicillium* sp., *Tri. ghanense* and *Tri. viride* with the least abundance (Table 4.4).

$$Relative\ Abundance = \frac{n_i}{N} \times 100$$

where N is the total number of species and n_i is the number of individuals in species.

Table 4.4 Relative abundance of indigenous fungal species isolated from North-West University fields.

Species	Relative abundance
<i>Aspergillus flavus</i>	4
<i>Aspergillus terreus</i>	4
<i>Talaromyces purpureogenus</i>	8
<i>Talaromyces minioluteus</i>	8
<i>Talaromyces sayulitensis</i>	64
<i>Penicillium</i> sp.	4
<i>Trichoderma ghanense</i>	4
<i>Trichoderma viride</i>	4

4.5 Discussion

4.5.1 Soil characteristics influence on fungal biodiversity

Morphologically diverse fungal biotypes, a total of 25, illustrate the high biodiversity of indigenous fungal species. Soils rich in organic matter comprised a diverse range of fungal species with varying morphological traits. Based on the observations from the current study, diverse fungal biotypes were found in soil with high organic content; even though it has been previously established that fungi are adapted to a wide range of soil environments (Naranjo-Ortiz & Gabaldón, 2019). According to Ali (2021), organic matter in the soil influences the soil's physical, chemical and biological properties, as it serves as a supportive substrate for

biological activities and provides nourishment in the soil environment. Many biotic (plants and other living things) and abiotic (soil pH, moisture, salinity, structure and temperature) factors influence the variety and activity of fungi (López-Bucio *et al.*, 2015; Rouphael *et al.*, 2015).

4.5.2 Fungal biotypes growth on different media

All fungal biotypes grew faster in PDA as compared to NA, MEA and WA; subsequently, further culturing was done on PDA (Figure 4.4). Data on net radial growth were modified using a logarithmic formula to satisfy the normal distribution requirement. Significant variations in radial development among the media were recorded after 21 DAI ($P \leq 0.05$). Throughout most ecosystems, microbes may develop spontaneously. For continued development and reproduction, they require necessary additional nutritional supplies. After 21 days, the PDA medium produced fungal mycelia with the highest mean radial growth (Figure 4.4).

In comparison to all other media, biotypes grown on WA medium took more than five times longer to sporulate. According to the findings, different media may be used to either increase fungal biomass, trigger early sporulation, or initially recover and isolate unknown fungal biotypes. Understanding culture media conditions for optimum fungal growth is fundamental for maximising the potential of fungal biotypes. Moura *et al.* (2020) examined the impact of four culture media on the sporulation and growth of *Fusarium* and established that mycelial development and sporulation of biotypes varied significantly amongst the medium. The fungal growth rate is a function of both mycelia and sporulation. All of the fungal biotypes took noticeably longer to sporulate on WA than on any of the other media (Figure 4.4). This would appear to suggest that in order to sporulate early, the test biotypes may need an energy source that is not present in NA and WA. Slow growth on NA and WA may also be attributed to media pH. Mustafa *et al.* (2023) indicated that fungal growth and sporulation are influenced by pH.

With a PDA pH of 5.6, and MEA pH of 4.7; the two media supported relatively faster fungal growth compared to NA and WA which have pH of 6.8 and 6.3, respectively.

In general, fungi require sources of energy, including carbon, nitrogen, phosphorus, sulfur and different minerals (Harvey *et al.*, 2007). Because its nutritional needs mirror its natural environment, understanding fungi's endemic habitat may help choose a suitable culture medium. In addition, laboratory culture media, such as those used in industrial microbiology, water and food analysis, testing for antibiotic sensitivity and other procedures, are vital in the isolation and identification of particular fungi (Rämä & Quandt, 2021). A culture medium is frequently employed to choose, cultivate, or identify a particular type of microbe. In such circumstances, the composition of the medium will be determined by its purpose.

Individual biotypes' growth differed significantly ($P \leq 0.05$) between media. The growth medium PDA, followed by MEA are the only two media containing maltose as the carbon source and exhibited the maximum radial development. Because maltose is generated from glucose, it may have provided a more readily available and useful carbon supply for these biotypes. The fungal growth rate is a function of environmental factors, such as temperature, moisture and nutrient source. All of the fungal biotypes took noticeably longer to sporulate on WA than on any of the other media. This would appear to suggest that the biotypes may need other environmental signals, such as increased food availability than is present in plant tissue, in order to sporulate early. The majority of fungal species do not sporulate within plant tissue, hence the presence of plant extract in the medium may have had a sporulation-inhibiting impact (Murphy *et al.*, 2015).

4.5.3 Morphological identification of indigenous fungal biotypes

There was some extensive distribution of mainly four genera, i.e., *Aspergillus*, *Penicillium*, *Talaromyces* and *Trichoderma*; with relatively higher incidence rates of *Talaromyces* in all sample collection sites. These species' macroscopic traits, such as colony pigment and mycelial morphology, were found to be distinct. This is most likely explained by environmental factors such as the temperature during collection, the slight pH variations of the soil from which they were isolated, and the crops from where the soil was collected. Several studies (Shrestha *et al.*, 2006; Méndez *et al.*, 2011; Afshari *et al.*, 2015; De Ligne *et al.*, 2019) investigated and established the influence of light, temperature, pH and moisture on the pigmentation, texture, and mycelial density of fungal cultures.

The morphological colony characteristics of the various biotypes, when cultivated on PDA, may, therefore, have been modified by the environmental factors at the source where they were cultured and where they were collected. Microscopical examination of the isolated *Aspergillus*, *Penicillium*, *Talaromyces*, and *Trichoderma* exhibited varied spore structures from sparse to dense, differently branching conidiophores, ampulliform phialides and slightly to pronounced globose conidia. Similarly, Matas-Baca *et al.* (2022) identified unknown fungal biotypes through observation of conidia, conidiophores and ampulliform phialides.

These species' macroscopic traits, such as colony pigment and mycelial morphology, were found to be distinct. This may suggest that the species were of distinct strains, or that differences in environmental factors, during soil collection, such as temperature, moisture, pH and light from various soils produced the differences in their morphological traits. Shrestha *et al.* (2006) investigated the influence of light conditions, temperature, pH and moisture on the pigmentation, texture, and mycelial density of fungal cultures. As a result, the changes in

morphological characteristics, i.e., colour of the fungal biotypes can be attributed to both genetic and environmental factors. Accordingly, molecular confirmation was a necessary step following the morphologically identified species.

4.5.4 Molecular identification of indigenous fungal biotypes

Based on the precision they offer, molecular technologies for fungal identification are frequently utilised in conjunction with morphological characterisation. The identification of fungi isolated from soil based on visual characteristics alone is prone to misinterpretation. Accordingly, molecular confirmation would be a necessary step following morphologically identified biotypes. From the initially isolated 25 pure biotypes, a total of eight fungal species belonging to four genera; *Aspergillus*, *Penicillium*, *Talaromyces* and *Trichoderma* were identified. The DNA sequencing analysis of these fungal biotypes concluded that from 25, 16 *Talaromyces* species belong to 1 of the 4 genera. All cultured biotypes showed variations in mycelial colour and growth shapes. These are most likely explained by underlying genetic variations and/or environmental factors such as the temperature during collection, slight pH variations of the soil from which they were extracted and crops from where the soil was collected. Afshari *et al.* (2015) reported on the influence of environmental parameters such as temperature and pH on the mycelial pigmentation of *Penicillium* species. Similarly, Méndez *et al.* (2011) found that *Tal. purpurogenus* generates colourants in both solid and liquid growth media. They further stipulated that colour variances were caused by the influence of varied fungal growth temperatures.

The use of molecular identification in the current study amended the initial morphologically inaccurate identification of *Aspergillus* as *Penicillium* species and *Penicillium* as *Talaromyces*. The DNA bandwidths and nucleotide sequences in test samples were used to identify the fungal

species. The DNA fragments formed on a 1% agarose gel were all the same size. Biotype F-SP.1 produced the weakest and smallest DNA band, whilst biotype F-SP.21 produced higher and thicker matching DNA bands of the same size. According to previous studies, (Lee *et al.*, 2012; Heinsohn *et al.*, 2022) agarose electrophoresis is the most feasible and successful approach for separating DNA molecules depending on their sizes. Ortiz-García *et al.* (2015) demonstrated the value of ITS primers in accurately identifying fungal biotypes. Based on molecular analysis, the amplified ITS region of the *Trichoderma* biotypes produced a PCR product of about 600 bp in the current investigation. With the same primers, ITS1 and ITS4, 10 fungal species were examined, yielding amplified DNA fragment sizes of 500-600 bp (García-Núñez *et al.*, 2017). Using the primers ITS1 and ITS4, Hermosa *et al.* (2000) identified *Trichoderma* species that were around 560-600 bp in size.

A sample whose preliminary morphological identification was *Penicillium*, F-SP.3 biotype was ultimately identified as *Tal. sayulitensis* (MZ01549.1) and *Aspergillus* as *Tal. sayulitensis* (MZ01549.1) using molecular analysis. This emphasised the morphological authentication procedure's shortcomings. Furthermore, the procedure's limitations in identifying these biotypes at the species level indicate a gap that hampers an in-depth comprehension of fungal biology and the ecological relationships of specific species. The establishment of morphological inaccuracy identification of fungal biotypes is significant. This is especially imperative when it comes to distinguishing species that have the potential to be developed as ecologically stable bio-control agents for soil-borne plant diseases from those that are harmful to both humans and the environment. For instance, whilst *A. terreus* has been established as an endophytic species that improves crop growth and can be used as a bio-control agent (Abdelaziz *et al.*, 2023); *A. flavus* strain in the current study is a pathogenic species that has been shown to cause considerable economic losses in crops (Majumdar *et al.*, 2018). Despite

being morphologically distinct, F-SP.11 to F-SP.20 were later determined by molecular analysis to contain the same DNA nucleotide sequences and were all identified as *Tal. sayulitensis*. Neither of the species displayed any character variability at the gene level, resulting in them being assigned the same GenBank Accession number (MZ014549.1).

4.6 Conclusion

The growth rate bars offer a valuable tool for learning about the growth characteristics of filamentous fungi in various nutritional media and may aid in streamlining the procedure for assessing the susceptibility of fungi to various antifungals. Key biological characteristics of fungi include vegetative growth and sporulation on artificial culture conditions. In fungi, the minimal dietary needs may have a significant impact on the culture mycelial development, sporulation and shape. Given that PDA showed optimum and supported fast fungal growth, it was chosen as the sole growth medium throughout the study. Identification of fungal species based solely on morphological characteristics has never been sufficient and/or reliable. This is mainly supported by different fungal growth colours on different growth media. Accordingly, the identification of unknown fungal biotypes should be confirmed by molecular identification. From the soil samples, eight indigenous fungal biotypes were identified.

CHAPTER 5: *IN VITRO* ASSESSMENT OF FUNGI PARASITISM ON *MELOIDOGYNE ENTEROLOBII* AND *MELOIDOGYNE JAVANICA*

5.1 Introduction

Synthetic nematicides remain the most common short-term management strategy against RKN but have some drawbacks, particularly concerning their effects on human and environmental well-being (Chen *et al.*, 2020). For these reasons, several chemicals such as methyl bromide and aldicarb have been removed from agro-markets (Oka, 2020). Despite the availability of chemicals, such as 1,3-D-chloropicrin, chloropicrin-proprietary solvent, and 1,3-D-metam sodium, these have not been fully successful in achieving the same levels of effectiveness (Desaeger & Watson, 2019).

In search of effective RKN management interventions, understanding the relationship between primordial soil microbial communities and the balance of soil ecosystems has been considered the key to their success. Research efforts have been devoted to developing environmentally benign management strategies for RKN worldwide (Forghani & Hajihassani, 2020; Abd-Elgawad, 2022). Bio-control with microbial antagonists has been widely regarded as a promising tool for managing several kinds of plant diseases, including RKN. It is without a doubt that using fungal BCA to manage RKN provides an environmentally friendly and alternative method of controlling them. Fungal BCA are predominantly of interest and have demonstrated considerable potential among micro-organisms that parasitise or feed on RKN (Jang *et al.*, 2016; Abd-Elgawad & Askary, 2018; Forghani & Hajihassani, 2020; Abd-Elgawad, 2022; Hajji-Hedfi *et al.*, 2023).

However, non-indigenous or imported fungal BCA often experience poor adaptation to local climates/conditions and are generally not very effective against the indigenous target pathogen species (Fernandes *et al.*, 2012; Thambugala *et al.*, 2020). As such, it is important to isolate and identify indigenous biotypes of antagonistic micro-organisms with bio-control activity. The objective of this study was to assess the parasitism of the eight indigenous fungal species against *M. enterolobii* and *M. javanica*.

5.2 Study site

The experiments were carried out at the Agricultural Research Farm, Plant Pathology Research laboratory, NWU, Mmabatho, Molelwane, North-West Province, South Africa (25°48'36"S, 25°37'47.999"E). The experiments were carried out March 2021.

5.3 Description of the experiments

5.3.1 Treatments and experimental design

Eight treatments of eight fungal species, i.e., *A. flavus*, *A. terreus*, *Tal. Purpureogenus*, *Tal. Minioluteus*, *Tal. Sayulitensis*, *Penicillium sp.*, *Tri. Ghanense* and *Tri. viride*, identified from **Chapter 4** were laid out in CRD with five replications for each *Meloidogyne* species under laboratory conditions. The eight fungal species were screened for the potential nematocidal action against *M. enterolobii* and *M. javanica* eggs and J2s. For each treatment, 1 mL suspension of 200 ± 20 eggs/J2s was added to 5 mL culture filtrates of each fungal strain in the Petri dish. Eggs/J2s in Petri dishes were assessed for egg parasitism and J2s mortality every 24 h for four days.

5.3.2 Experimental procedures

Eggs of the two *Meloidogyne* species were extracted from galled tomato cv. 'Hotstuff' (MayFord Seeds (Pty) Ltd, Pretoria, South Africa) roots through the root maceration method as described by Coyne *et al.* (2007). When needed, dark brown egg masses of *Meloidogyne* were obtained from tomato plants. The roots of these plants were rinsed in 1% NaOCl solution with egg masses dislodged using a toothpick and placed in a Petri dish containing distilled water. Eggs displaced in water were counted and used in egg hatch bioassay. To assess the impact of indigenous fungi on *Meloidogyne* J2s mortality, the nematode egg masses were placed in distilled water in an incubator set at 25 ± 2 °C. The number of J2s mL⁻¹ of suspension was determined using a counting dish under a compound microscope (ZEISS, Research Microscopy Solutions, United Kingdom). For each treatment, 1 mL suspension of 200 ± 20 eggs and J2s was added to 5 mL culture filtrates of each fungal strain in the Petri dish. Eggs and J2s in Petri dishes were assessed for egg parasitism and J2s mortality at the following intervals: 24, 48, 72, and 96 h exposure. The bioassays were conducted by separately exposing *M. enterolobii* or *M. javanica* eggs and J2s to the identified fungal species; *A. flavus*, *A. terreus*, *Penicillium* sp., *Tal. minioluteus*, *Tal. purpureogenus*, *Tal. sayulitensis*, *Tri. ghanense* and *Tri. viride*. The fungi were grown on PDA for 21 days and spores were obtained by scraping the mycelial growth using a fibreglass hockey stick placed into sterile Petri dishes containing 10 mL of distilled water. The period of incubation was established through trial and error, wherein after 21 days all fungal species had grown to cover their respective Petri dishes. Using a haemocytometer, the conidia suspensions were standardised to 1×10^5 spores mL⁻¹ for all fungal species. Distilled water was used as an untreated control, with each experiment replicated five times.

5.3.3 Data collection

Eggs and J2s with hyphae growing from them and those surrounded by germinated spores were considered parasitised (Sun *et al.*, 2006) and counted on a nematode slide. Every 24, 48, 72 and 96 h, fungal species' ovicidal and nematicidal activities on *M. enterolobii* and *M. javanica* were assessed. This was achieved by examining and counting eggs/J2s that showed direct mycelial penetration and disintegration per replicate per treatment (Singh & Mathur, 2010). Observations were made under a compound microscope (ZEISS, Research Microscopy Solutions, United Kingdom) at 63 μ m magnification.

Egg and J2s parasitism % were calculated according to Singh & Mathur (2010).

$$\text{Egg parasitism (\%)} = [(m-n)/(100-n)] \times 100$$

Where: m indicates parasitism (%) and n indicates the total number of eggs.

5.3.4 Data analysis

Prior to analysis, data was transformed through $\log_{10}(x + 1)$. Parasitised *Meloidogyne* eggs and J2s data were subjected to ANOVA using SAS software (9.4 version). Means were separated using Tukey's HSD at a probability level of 5% (Gomez & Gomez, 1984).

5.4 Results

5.4.1 Effects of indigenous fungal species on *Meloidogyne* eggs

Fungal species had highly significant differences in their ability to parasitise ($P \leq 0.01$) in *M. enterolobii* and *M. javanica* eggs parasitism. For *M. enterolobii* eggs, the highest parasitism percentages were by *A. terreus* and *Tri. ghanense* at 99% and 86% after 96 hours, respectively. Whilst the lowest *M. enterolobii* egg parasitism was by *Penicillium* sp. at 50% after 96 h. For *M. javanica* eggs, the highest parasitism percentages were by *A. terreus*, *Tal. minioluteus* and

Tal. sayulitensis all at 99% after 96 hours; followed by *Tri. ghanense* and *Tri. viride* at 95% and 93% after 96 hours, respectively. On the other hand, *M. javanica* eggs lowest parasitism percentages were by *Penicillium* sp. at 75%, after 96 hours (Table 5.1).

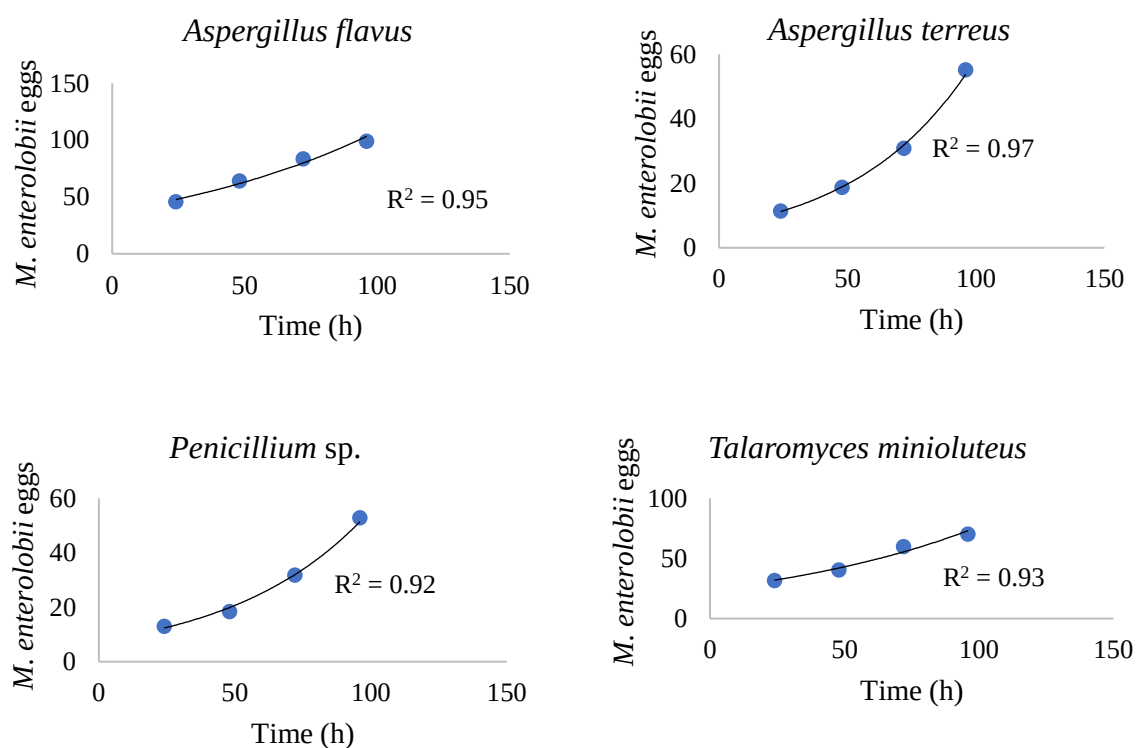
Table 5.1 Percentage eggs from *Meloidogyne enterolobii* and *Meloidogyne javanica* parasitised by eight indigenous fungal species after 24, 48, 72 and 96 hours of treatment.

<i>Meloidogyne enterolobii</i>				
Fungal species	24 hours	48 hours	72 hours	96 hours
<i>A. flavus</i>	12.00f ^z ± 1.22	18.60e ± 3.36	31.40g ± 1.52	55.20d ± 4.44
<i>A. terreus</i>	45.80a ± 5.49	64.20a ± 4.97	83.80a ± 5.32	99.00a ± 1.41
<i>Penicillium</i> sp.	13.80ef ± 4.58	18.80e ± 4.33	25.20f ± 1.92	50.40e ± 7.96
<i>Tal. minioluteus</i>	31.80b ± 2.39	40.60b ± 3.21	59.80c ± 2.86	72.20c ± 2.41
<i>Tal. purpureogenus</i>	25.40c ± 2.19	31.00d ± 2.00	40.80e ± 1.92	70.40c ± 2.55
<i>Tal. sayulitensis</i>	17.20de ± 3.35	34.60cd ± 2.89	49.80d ± 4.92	72.80b ± 4.55
<i>Tri. ghanense</i>	9.80b ± 1.48	15.80e ± 1.48	31.80f ± 1.52	51.20de ± 1.30
<i>Tri. viride</i>	21.40cd ± 6.06	38.20bc ± 3.35	65.80b ± 1.92	86.00b ± 1.87
HSD _{0.05}	5.11	4.44	4.35	4.64
P	< 0.01	< 0.01	< 0.01	< 0.01
F	46.99	108.21	177.96	114.93
<i>Meloidogyne javanica</i>				
<i>A. flavus</i>	23.00e ± 6.58	34.40g ± 1.30	46.00f ± 2.70	86.00d ± 2.30
<i>A. terreus</i>	63.40a ± 2.12	77.80a ± 1.95	91.80a ± 2.59	99.60a ± 0.45
<i>Penicillium</i> sp.	17.20f ± 1.92	42.60f ± 2.07	55.40e ± 1.67	75.60e ± 1.95
<i>Tal. minioluteus</i>	46.60b ± 2.41	54.60c ± 2.51	78.60ab ± 2.07	99.50a ± 1.09
<i>Tal. purpureogenus</i>	37.20c ± 3.19	63.80b ± 1.92	83.00b ± 2.55	95.40b ± 3.21
<i>Tal. sayulitensis</i>	32.00d ± 1.22	49.40d ± 1.52	79.80c ± 1.48	99.00a ± 1.41
<i>Tri. ghanense</i>	21.60e ± 1.14	44.80e ± 0.84	70.00d ± 1.87	89.00c ± 1.58

<i>Tri. Viride</i>	40.00c ± 3.39	56.20c ± 1.30	77.60c ± 2.07	93.60b ± 2.41
HSD _{0.05}	3.10	1.75	2.65	2.36
P	< 0.01	< 0.01	< 0.01	< 0.01
F	197.97	482.32	266.17	103.67

^aColumn means followed by the same letter were not significantly different ($P \leq 0.05$) according to Tukey's HSD, average ± standard deviation error.

Parasitism values at different times of exposure differed, allowing for the fitting of the best lines, which resulted in quadratic relationships. The regression model showed that the fungal species effect of exposure to *M. javanica* J2s exponentially increased over time (Figure 5.1).



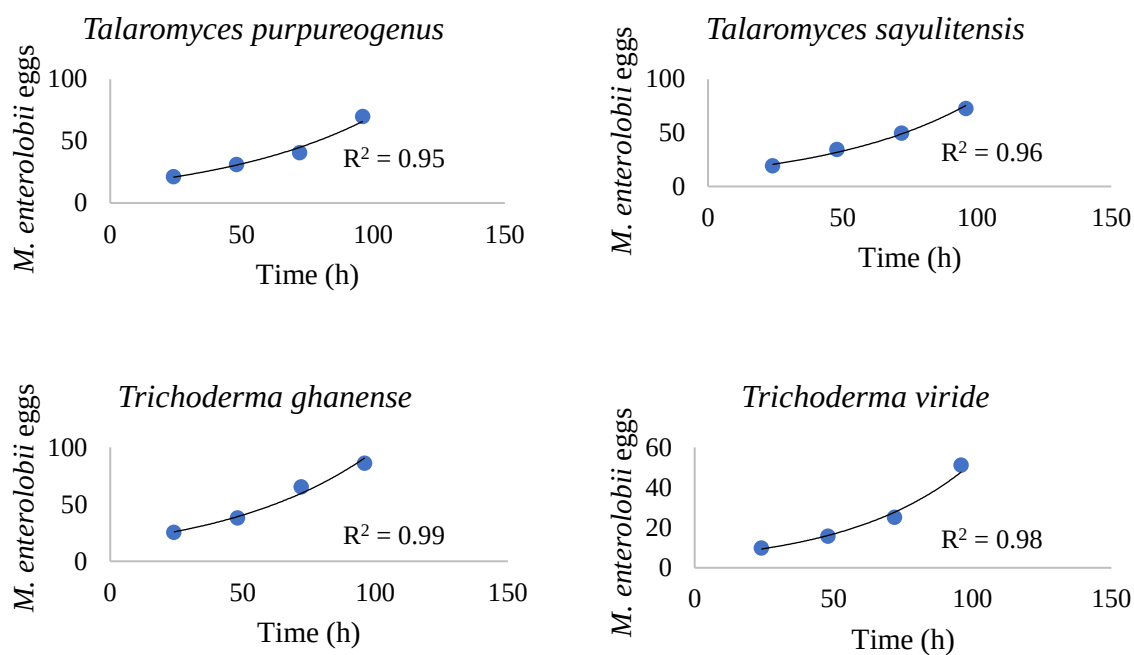
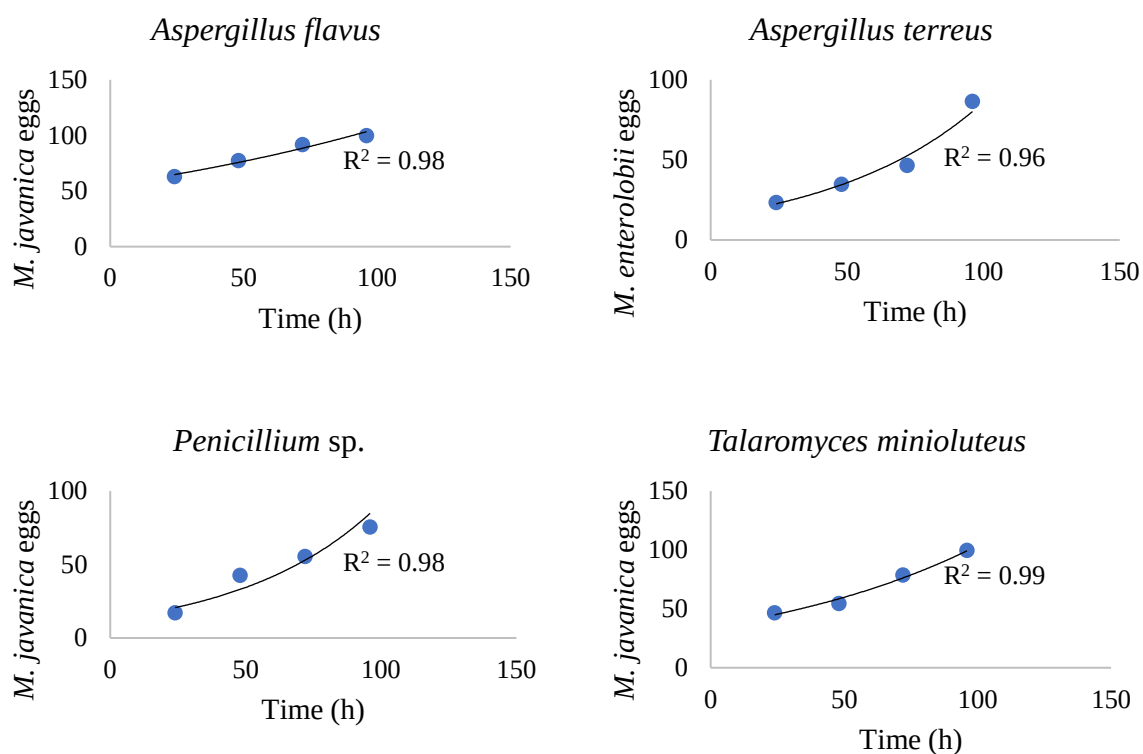


Figure 5.1 Effects of indigenous fungal species on *Meloidogyne enterolobii* eggs.

The regression model showed that exposure of *M. javanica* eggs to fungal species exponentially increased over time (Figure 5.2).



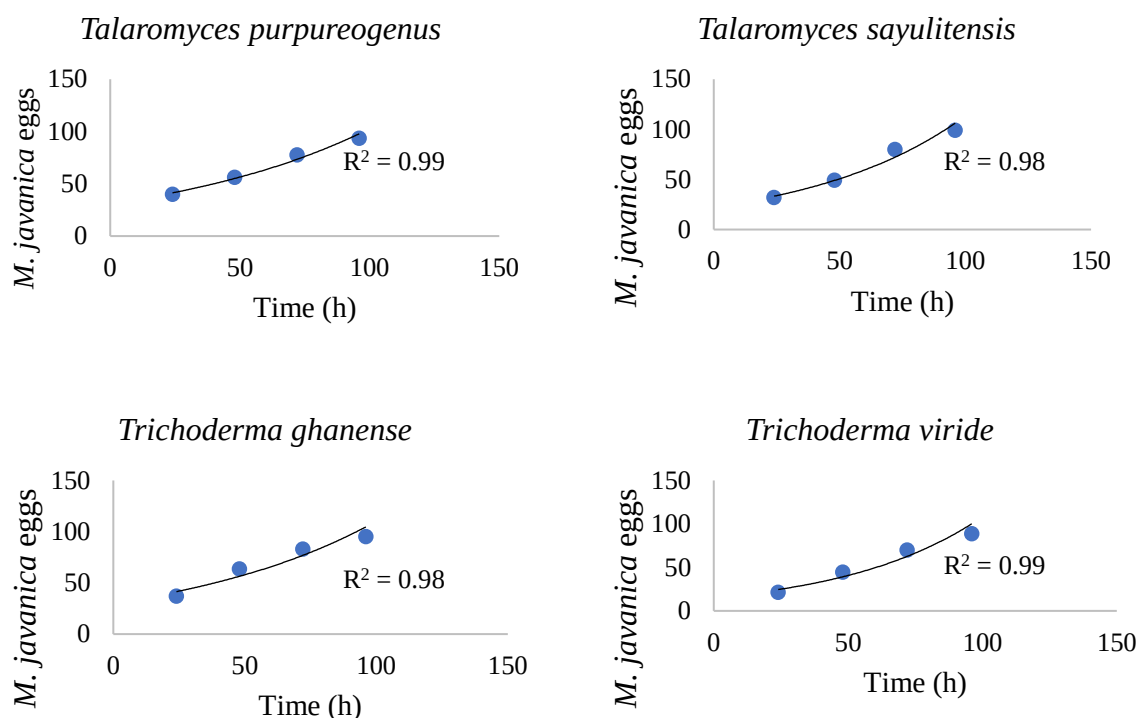


Figure 5.2 Effects of fungal species on *Meloidogyne javanica* eggs.

In vitro assessment exhibited different morphological characteristics of *M. enterolobii* and *M. javanica* eggs infected by conidia and spore suspension of fungal species (Figure 5.3).

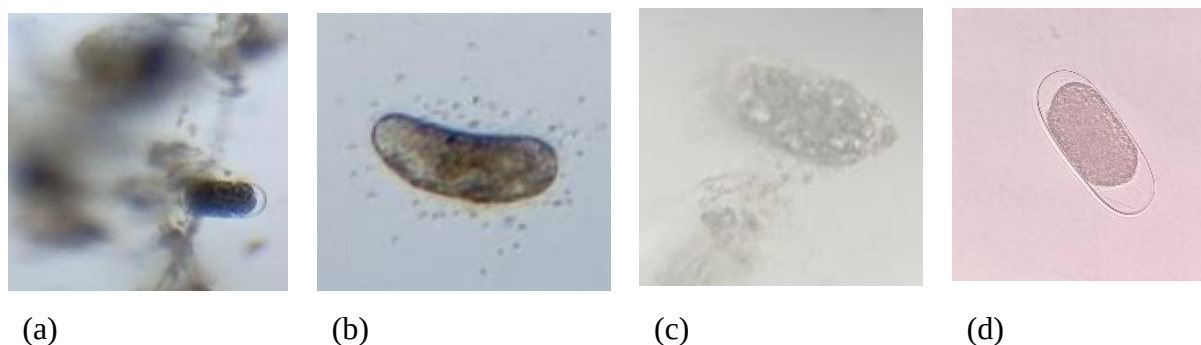


Figure 5.3 *Meloidogyne enterolobii* and *Meloidogyne javanica* eggs infected by conidia and spore suspension of fungal species *in vitro* with (a) *M. enterolobii* eggs parasitised by the mycelium of *Tal. minioluteus*; (b) *M. javanica* eggs surrounded by *A. terreus* spores; (c) the degraded egg by *Tal. sayulitensis* and (d) *M. enterolobii* intact eggs under control group. Observations were made under 63 μ m magnification. Pictures taken by Ndivhuwo Ramatsitsi.

Aspergillus terreus, *Penicillium* sp. and *Tal. minioluteus* hyphae networks had initiated *Meloidogyne* egg trapping as early as 24 h after exposure [Figure 5.3(a)]. Microscopic examinations showed that *M. enterolobii* eggs that were inoculated with *A. flavus* conidia suspension, were surrounded with germinated spores and the egg began to lose its shape and turgidity [Figure 5.3(b)]; whilst there were fewer eggs of *M. javanica* at 24 h. Hyphal structures of *Tal. purpurogenus* initiated egg entanglement at 24 h, while other eggs were surrounded by spores. The conidia suspension of *Tal. sayulitensis* had a strong lethal and parasitic effect on *M. javanica* wherein fewer eggs were noted as early as 24 h, demonstrating egg degradation [Figure 5.3(c)].

The majority of *M. enterolobii* eggs were surrounded by *Tal. sayulitensis* germinated spores, compared to others that were floating. *Meloidogyne enterolobii* and *M. javanica* eggs exposed to *Tri. viride* were disintegrated at 24 h. This was evidenced by fewer eggs that were observed. Eggs of the two *Meloidogyne* species were partly surrounded by *Tri. ghanense* spores while the others were trapped with mycelia. All observed responses became more pronounced with time over four days. In contrast, *M. enterolobii* and *M. javanica* eggs remained intact in the control group [Figure 5.3(d)].

5.4.2 Effects of indigenous fungal species on *Meloidogyne* J2s

Fungal species had highly significant differences ($P \leq 0.01$) in *M. enterolobii* and *M. javanica* J2s parasitism. For *M. enterolobii* J2s, the highest parasitism percentages were by *A. terreus* and *Tal. minioluteus* at 95% after 96 hours. On the other hand, the lowest *M. enterolobii* J2s parasitism was by *Tri. ghanense* at 61% after 96 hours. For *M. javanica* J2s, the highest parasitism percentages were by *Tri. viride* and *Tal. sayulitensis* at 94% and 91% after 96 hours,

respectively. On the other hand, *M. javanica* J2s' lowest parasitism percentages were by *Penicillium* sp. at 61%, after 96 hours (Table 5.2).

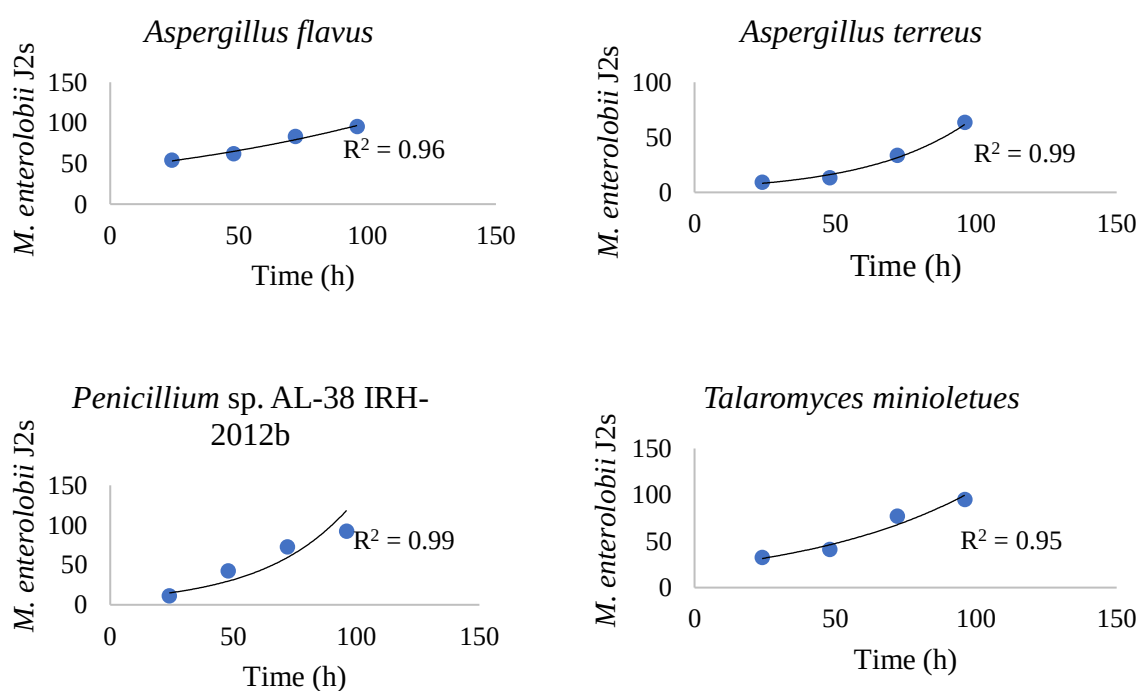
Table 5.2 Percentage eggs and J2s from *Meloidogyne enterolobii* and *Meloidogyne javanica* parasitised by the eight fungal species after 24, 48, 72 and 96 hours of treatment.

<i>Meloidogyne enterolobii</i>				
Fungal species	24 hours	48 hours	72 hours	96 hours
<i>A. flavus</i>	9.20e ^z ± 1.30	13.20e ± 2.17	33.20f ± 1.14	63.80d± 1.14
<i>A. terreus</i>	54.00a ± 2.00	62.20a ± 2.12	83.40a ± 2.17	95.40a± 2.95
<i>Penicillium</i> sp.	9.20e ± 1.30	31.20c ± 0.83	43.00e ± 1.00	63.40d± 1.82
<i>Tal. minioluteus</i>	32.40b ± 1.52	41.20b ± 1.30	77.00b ± 2.74	95.00a± 2.74
<i>Tal. purpureogenus</i>	11.40de ± 1.14	23.40d ± 3.91	40.80e ± 0.84	63.40d± 0.84
<i>Tal. sayulitensis</i>	21.40c ± 0.89	33.40c ± 1.52	53.40d ± 2.41	72.40c± 2.41
<i>Tri. ghanense</i>	12.40d ± 3.13	20.20d ± 1.48	41.60e ± 1.14	61.80d± 0.83
<i>Tri. viride</i>	11.00de ± 1.00	42.60b ± 1.34	72.40c ± 1.14	92.40b± 1.09
HSD _{0.05}	2.04	3.34	2.36	2.80
P	< 0.01	< 0.01	< 0.01	< 0.01
F	540.52	250.59	842.31	539.05
<i>Meloidogyne javanica</i>				
<i>A. flavus</i>	12.40c ± 2.14	41.60e ± 1.22	62.60b ± 1.64	82.20c± 1.92
<i>A. terreus</i>	32.40ab ± 1.14	41.20c ± 1.22	53.00c ± 2.12	73.20d± 1.09
<i>Penicillium</i> sp.	12.40e ± 1.14	32.60de ± 1.14	54.40c ± 4.44	61.60c± 1.14
<i>Tal. minioluteus</i>	27.00b ± 2.74	41.00c ± 1.00	64.00b ± 2.00	80.40c± 0.55
<i>Tal. purpureogenus</i>	20.80d ± 0.84	42.20bc ± 1.78	52.60c ± 1.14	71.80d± 1.30
<i>Tal. sayulitensis</i>	23.40e ± 1.41	43.00b ± 1.41	74.20a ± 2.86	91.80b± 1.92
<i>Tri. ghanense</i>	21.80cd ± 0.84	33.20d ± 0.83	54.20c ± 2.59	64.20e± 1.79
<i>Tri. viride</i>	35.40a ± 2.95	53.60a ± 1.82	74.60a ± 4.62	94.80a± 4.21

HSD _{0.05}	2.48	1.78	3.80	2.61
P	< 0.01	< 0.01	< 0.01	< 0.01
F	90.49	142.25	48.85	117.36

²Column means followed by the same letter were not different ($P \leq 0.005$) according to Tukey's HSD, average $\pm$ standard deviation error.

Juveniles' mortality was found to be directly proportional to the duration of exposure. The highest percentages of *M. enterolobii* J2s mortalities were from both *A. terreus* and *Tal. minioluteus* at 95%, followed by *Tri. viride* at 92% after 96 h. On the other hand, the lowest percentages of *M. enterolobii* J2s mortalities were from *Tal. purpureogenus* at 61%. On *M. javanica* J2s, the highest parasitism was from *Tri. viride* and *Tal. sayulitensis* at 94% and 91%, respectively. Whilst *Penicillium* sp. exhibited the lowest *M. javanica* J2s parasitism at 61%. Parasitism values at different times of exposure differed, allowing for the fitting of the best lines, which resulted in quadratic relationships. The regression model showed that exposure of *M. enterolobii* J2s to fungal species exponentially increased over time (Figure 5.4).



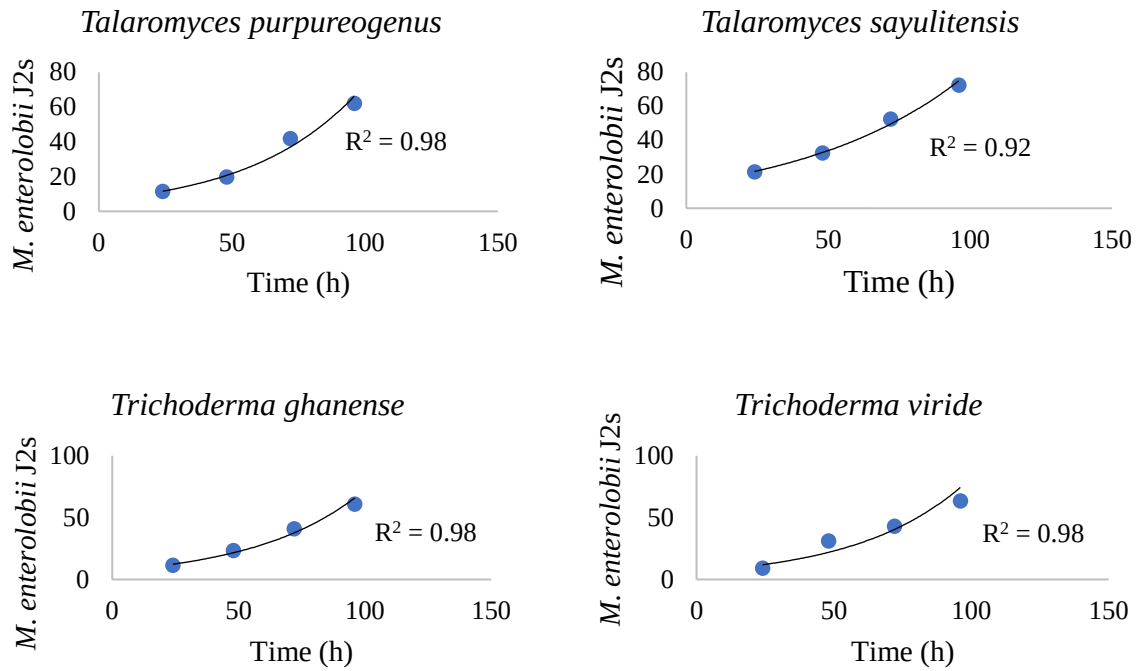
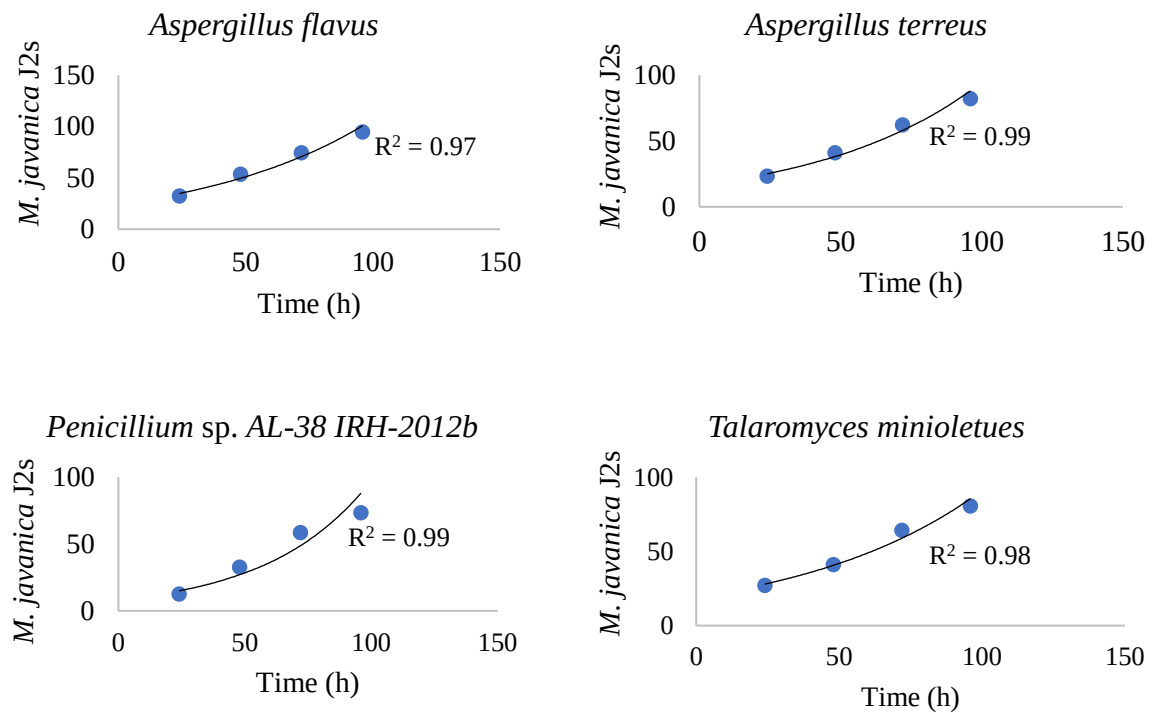


Figure 5.4 Effects of indigenous fungal species on *Meloidogyne enterolobii* J2s.

The regression model showed that exposure of *M. javanica* J2s to fungal species exponentially increased over time (Figure 5.5).



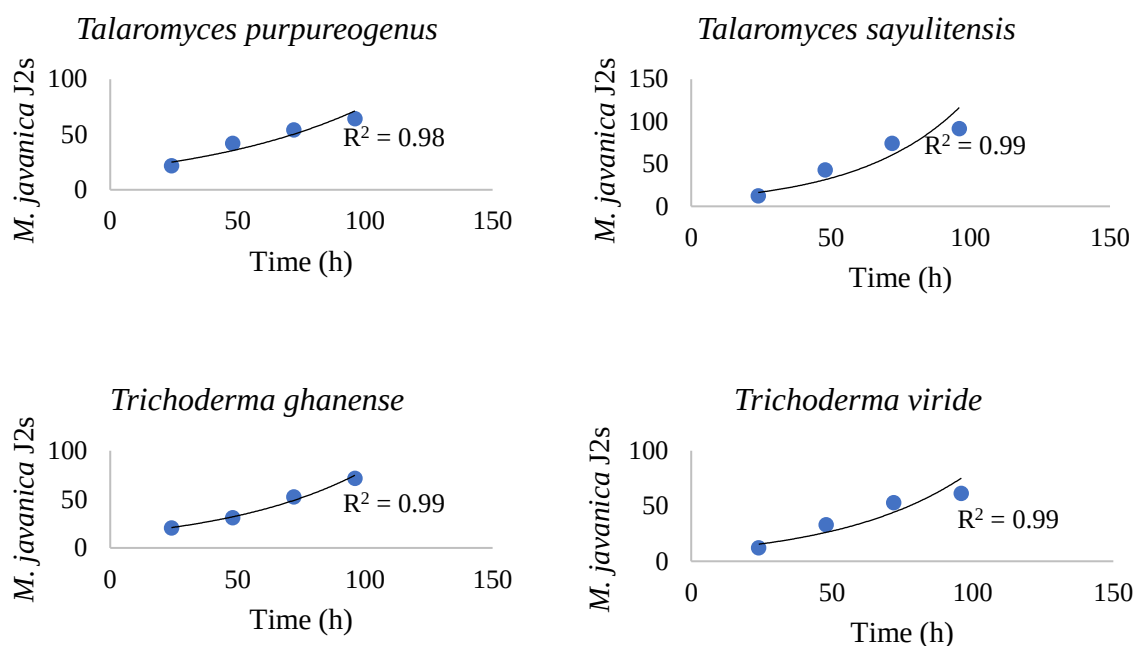


Figure 5.5 Effects of indigenous fungal species on *Meloidogyne javanica* J2s.

All eight fungal species were parasitic to J2s of both *Meloidogyne* species (Figure 5.6).

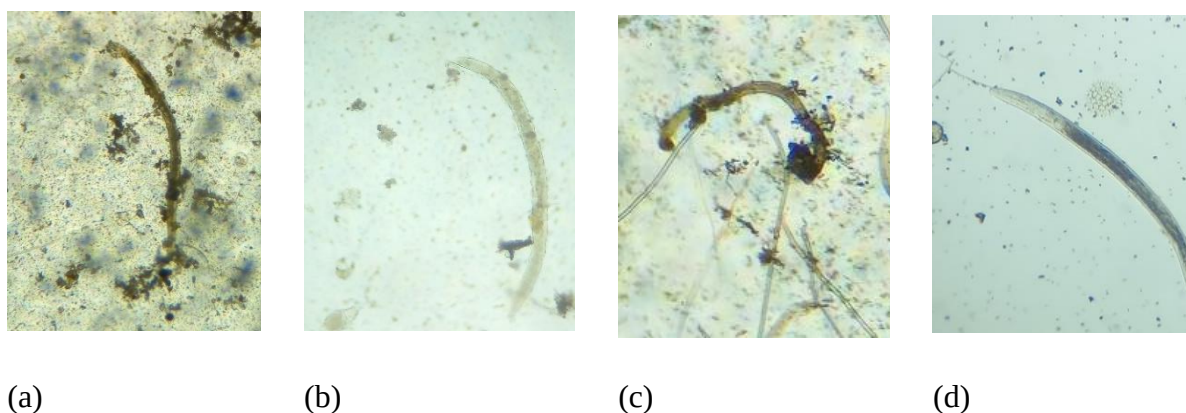


Figure 5.6 *Meloidogyne enterolobii* and *Meloidogyne javanica* J2s infected by the conidia suspension of indigenous fungi *in vitro*, with (a) dead *M. enterolobii* second-stage juvenile surrounded by *A. flavus* spores, (b) *M. javanica* second-stage juvenile husk parasitised by the conidia of *Tal. minioluteus* (c) *M. javanica* second-stage juvenile entangled and parasitised by hyphae of *Tri. viride* and (d) alive and healthy *M. enterolobii* second-stage juvenile in the control group. Observations were made under 63 μ m magnification. Pictures taken by Ndivhuwo Ramatsitsi.

Microscopic examinations demonstrated that the freshly hatched *M. enterolobii* J2s were rigid when poked with a toothpick and dull after being exposed to *A. flavus* 24 h [(Figure 5.6(a))]. These nematodes appeared to be in a state of paralysis with minimal movement. At 48 h through 72 h, most of the J2s were stiff and started showing the stylet of dead J2s. At 24 h, *M. javanica* J2s had turned into a lifeless husk after parasitism by *Tal. minioluteus* [Figure 5.6(b)].

Some J2s of both *M. enterolobii* and *M. javanica* became rigid and immobile, with germinated spores of *Tal. purpurogenus* adherent to or parasitising on the nematode's surface. *Trichoderma* hyphae entangled and paralysed *M. enterolobii* J2s at 24 h [Figure 5.6(c)]. *Trichoderma* conidia germinated with many hyphae and penetrated the integument. Even some *Meloidogyne* J2s began to degrade at 48 h. In contrast, the nematodes' bodies' colour and the shape of the J2s remained intact in the control group [Figure 5.6(d)].

5.5 Discussion

The culture filtrate investigations showed that all eight fungal species were successful in preventing *M. enterolobii* and *M. javanica* eggs from hatching and causing J2s mortality. The mechanisms of fungal BCA infection are essentially similar; egg/J2s cuticle penetration, egg/J2s digestion and J2s immobilisation (Ortiz-Urquiza & Keyhani, 2013; Zhang *et al.*, 2020). As such, three parameters were evaluated in the *in vitro* bioassay technique to screen fungal species candidates: (i) eggs/juveniles' entanglement/parasitism, (iii) egg hatch inhibition and (iv) juvenile mortality. In the pathogenicity test, some fungal species had lower J2s parasitic effects, but higher egg parasitism; while others had higher J2s parasitic effects, but lower egg parasitism. For instance, *Tal. minioluteus* parasitised 72% of *M. enterolobii* eggs but 95% of J2s, *A. terreus* parasitised 99% of *M. javanica* eggs and 73% of J2s. This indicates the involvement of different mechanisms amongst different species.

5.5.1 Fungal species parasitism on *Meloidogyne* eggs

Given that *Meloidogyne* egg shells serve as the primary defence against parasitic fungal invasion, spores and hyphae need to adhere to the egg's surface before penetrating it (Yang *et al.*, 2007). The main mode of action of the majority of fungal species on nematodes that was observed was spore parasitism. This mode of action destroyed *Meloidogyne* eggs by direct fungal penetration after the formation of appressoria around the eggs. *Talaromyces sayulitensis* and *Tal. purpurogenus* showed evidence of extracellular biochemical secretion that played an important role in the infection process of eggs as they enabled the respective fungus to degrade the *M. enterolobii* and *M. javanica* eggshell. *Talaromyces* is an endophytic genus with species known to produce toxic metabolites such as siderophore ferrirubin, herquiline B and 3-O-methylfunicone (Vinale *et al.*, 2017). These metabolites could have induced the observed *Meloidogyne* eggshell degradation.

The intricate, stratified nematode eggshell, which is mostly composed of proteins, i.e., chitin, collagen, and fibres, is important in protecting RKN against nematicidal fungal BCA invasions (Chen & Peng, 2019). According to Esteves *et al.* (2009), proteases such as chitinases can degrade *Meloidogyne* eggshells. These enzymes have been considered to be involved in the fungal infection process and serve as virulence factors (Esteves *et al.*, 2009). At the same time, the eggshell may also serve as a site of infection for fungi with potent enzymatic armaments (Al-Ani *et al.*, 2022). The primary defence against parasitic fungal infection may be nematode eggshells. Germinated spores and penetration structures must adhere to the surface before entering the eggs (Yang *et al.*, 2007).

Microscopic observations indicate that most J2s were deformed inside the eggs; suggesting that the test fungal species suppressed hatching of the two *Meloidogyne* species J2s. This interaction

could have been through the use of nemato-toxic metabolites, such as chitinase, and lytic enzymes such as proteases and lipases that break down nematode eggshells (Kalele *et al.*, 2010). Naz *et al.* (2021) recently reported on *M. incognita* egg hatch inhibition induced by exposure to *A. flavus*. Nguyen *et al.* (2021) isolated the nematicidal metabolite, cyclopiazonic acid, from *A. flavus* that degrades *Meloidogyne* eggshells. *Penicillium* species have also been reported to produce the same mycotoxin.

Trichoderma suppresses phytopathogen growth primarily through competition, antibiosis and mycoparasitism (Gajera *et al.*, 2016; Fan *et al.*, 2020). Sharon *et al.* (2008) demonstrated that eggs attached to *Trichoderma* conidia became non-viable, reducing the rate of egg hatch. The four isolated genera are among the most significant fungi in the world due to their remarkable bio-control capacity on plants and phytopathogens in the soil or plant root system (Jang *et al.*, 2016; Poveda *et al.*, 2020; Tazi *et al.*, 2021). The culture filtrates revealed that all tested fungal species were effective in suppressing egg hatching of *M. enterolobii* and *M. javanica*.

5.5.2 Fungal species parasitism on *Meloidogyne* J2s

Microscope examinations indicated that parasitism during the infection process were most likely the key modes of action that functioned as the first phase of attack *in vitro* against *Meloidogyne* J2s. Fungal mycelia attached or parasitised the surface of *M. enterolobii* and *M. javanica* J2s germinated a high number of hyphae and spores, pierced the integument and reproduced on J2s surface. Mobility of *M. incognita* J2s was progressively reduced over 48 h and was essentially immobile after 72 h exposure to *P. chrysogenum* Snef1216 (Sikandar *et al.*, 2020). Similarly, Rajinikanth *et al.* (2016) reported that the mortality of RKN increased exposure time under *Tri. viride*.

Results in the current study suggest that the fungal species may have secreted enzymes that causes J2s physiological abnormality and mortality. Furthermore, the parasitised J2s deformed and started to disintegrate, with some J2s completely dissolving/disintegrating. Sharon *et al.* (2001) showed direct parasitism of *Tri. harzianum* (T-203) on *M. javanica* *in vitro*, as a result of extracellular protease that was secreted by the fungus. Singh & Mathur (2010) also established the *in vitro* efficacy of indigenous fungi; where *A. terreus* was found to be highly toxic against *M. incognita* J2s, causing high mortality (68%). Similarly, Jang *et al.* (2016) reported that the culture filtrate of *A. niger* at various concentrations showed nematicidal activity against *M. incognita* J2s.

5.5.3 Host and stage-specific

Remarkably, the test fungal species displayed host specificity between the two *Meloidogyne* species and the growth stages. From the observation, it was evident that different fungi antagonise different stages, i.e., eggs/J2s, of the respective *Meloidogyne* species, and the same fungi can antagonise different *Meloidogyne* species at different rates. For instance, *Tal. purpurogenus* had the highest ovicidal activity (80%) after 48 hours, whilst the same species had the least larvicidal activity (9%) after 48 hours on *M. javanica*. *Aspergillus flavus* had the highest *M. javanica* ovicidal activity of 62%, but the lowest *M. enterolobii* ovicidal activity of 4% at 24 hours. This demonstrates that endoparasitic fungi have different host preferences.

Nonetheless, the differences in fungi parasitism between different *Meloidogyne* species and different growth stages can be attributed to both the parasitising fungi and the parasitised nematode. *Meloidogyne* egg is covered with a gelatinous matrix that protects them from pathogens (Escobar *et al.*, 2015). It is possible that the firmness of this gelatinous matrix is different across genera and species. This would explain why *A. flavus* had 62% and 4% ovicidal

activities between for *M. javanica* and *M. enterolobii*, respectively. Given that J2s stages can move around in their surroundings, makes it tricky for fungus to parasitise. Many fungi, nevertheless, such as *Trichoderma* have evolved in order to parasitise mobile stages of nematodes by using intricate and advanced predation structures (Su *et al.*, 2017; Sood *et al.*, 2020), such as trapping structures to immobilise nematodes.

5.6 Conclusion

All eight tested indigenous fungal species parasitised both *Meloidogyne* eggs and J2s, with *A. terreus*, *Tal. minioluteus*, *Tal. purpureogenus*, *Tal. sayulitensis* and *Tri. viride* exhibiting higher parasitism. Accordingly, these fungal species are potential *Meloidogyne* BCA.

CHAPTER 6: EFFECTS OF INDIGENOUS FUNGI ON CROP SEEDS

6.1 Introduction

Seed germination is one of the most important and complex periods of plant establishment and growth. During this period, plant physiological processes are controlled and altered by different elements such as moisture availability, oxygen, light and the presence or absence of plant pathogens (Carrera-Castaño *et al.*, 2020; Haj Sghaier *et al.*, 2022). The use of fungi as BCA has generated a great deal of attention and some have even been produced as commercial bio-control products (Askary, 2015). However, several species of fungi are parasitic and known to destroy unfathomable amounts of crops, forests, stored goods and structures while also harming both human and animal health (Fisher *et al.*, 2012; Peng *et al.*, 2021; Rokas, 2022). Accordingly, determining the unintended consequences of using fungi as BCA is necessary before one can develop and commercialise them.

Given that the fungal species used are indigenous and their effects on other crops has not been established yet, the objective was to address pathogenesis of nematode-parasitic fungi on selected seeds, *viz.*, tomato, beetroot, swiss chard, dry bean, maize, sorghum and pumpkin.

6.2 Study site

The experiments were carried out at the Agricultural Research Farm, Plant Pathology Research laboratory, NWU, Mmabatho, Molelwane, North-West Province, South Africa (25°48'36"S, 25°37'47.999"E). The experiments were carried out in April 2021.

6.3 Description of the experiments

6.3.1 Treatments and experimental design

Eight treatments of fungal species and control (untreated) were replicated five times. These were laid out in a CRD *in vitro* in a growth chamber at room temperature, 25 ± 2 °C. Seeds (MayFord Seeds (Pty) Ltd, Pretoria, South Africa) of tomato (*S. lycopersicum* ‘Hotstuff’), swiss chard (*Beta vulgaris* L. subsp. *vulgaris* ‘Fordhock giant’), beetroot (*B. vulgaris* subsp. *vulgaris* ‘Detroit’), dry bean (*Phaseolus vulgaris* L. ‘PAN 148’), pumpkin (*Cucurbita moschata* L.), maize (*Zea mays* L.) and sorghum were purchased at NWK, Mahikeng, South Africa and were tested for fungal pathogenicity.

6.3.2 Experimental procedures

Following a method by Coninck *et al.* (2020), seeds were disinfected with 10% NaOCl solution for 5 minutes and washed three times for 5 minutes with sterile distilled water. The seeds were put on a sterile paper towel and allowed to air dry for 24 hours inside a laminar flow cabinet (FILTA-MATIX) before being coated and tested. A suspension of 21 days old fungal species was prepared by adding each fungus’ spores and mycelia to water before being added to the chitosan mixture and agitated frequently. For each fungi:chitosan mixture, a ratio of 2:1 mL solution was adjusted for respective fungal species. Previously sterilised and dried seeds were submerged in their corresponding slurry mixture for 2-5 seconds before being allowed to air dry for 48 hours at room temperature (28 °C). The seeds were submerged in a sterilised glass beaker containing 200 mL of slurry for each treatment. Ten seeds/Petri dish/treatment placed on Whatman No. 1 filter paper were used to carry out the germination test. To maintain moist conditions, 5 mL of distilled water was infused into respective Petri dishes. When a radicle longer than 5 mm was noted, seeds were deemed to have germinated and inspected for necrosis

and rotting. The experiment was repeated twice for validation, data were compared and did not differ between the experiments.

6.3.3 Data collection

Germination rate was carried out at a two-day interval (number of germinated seeds per assessment). The germination percentage was calculated and the growth rate of the respective plumules and radicles of the germinated seeds was measured. Vigour indices (VI) were calculated using the mathematical formula as described and explained by Abdul-Baki & Anderson (1973). Vigour determines the potential for rapid and uniform germination/emergence under various conditions. The study ran for 14 days and was terminated soon after growth measurements.

$$\text{Vigour index (VI)} = \text{length of plumule (lp)} + \text{length of radicle (lr)} \times \text{germination}$$

6.3.4 Data analysis

All data were subjected to ANOVA using the SAS version 9.4 package (SAS Institute, Inc., Cary, NC., USA, 2008) and Tukey's HSD was used for mean comparisons at the probability level of 5% (Gomez & Gomez, 1984).

6.4 Results

6.4.1 Seeds pathogenesis

Based on seed germination rate, plumule growth, radicle growth and seedling vigour index, the two *Trichoderma* species showed the highest seedling growth, followed by *Talaromyces*, *A. terreus* and *Penicillium* sp., respectively. On the other hand, *A. flavus* inhibited seed germination and caused seed rot.

6.4.2 Seeds germination rate

Based on seed germination rate over 14 days, the two *Trichoderma* species showed the highest seedling growth, followed by *Talaromyces*, *A. terreus* and *Penicillium* sp., respectively. Whilst *A. flavus* inhibited seed germination and caused seed rot, *A. terreus* promoted seed germination and subsequently improved seedling growth. Figure 6.1 illustrates different vegetables' germination rate trends.

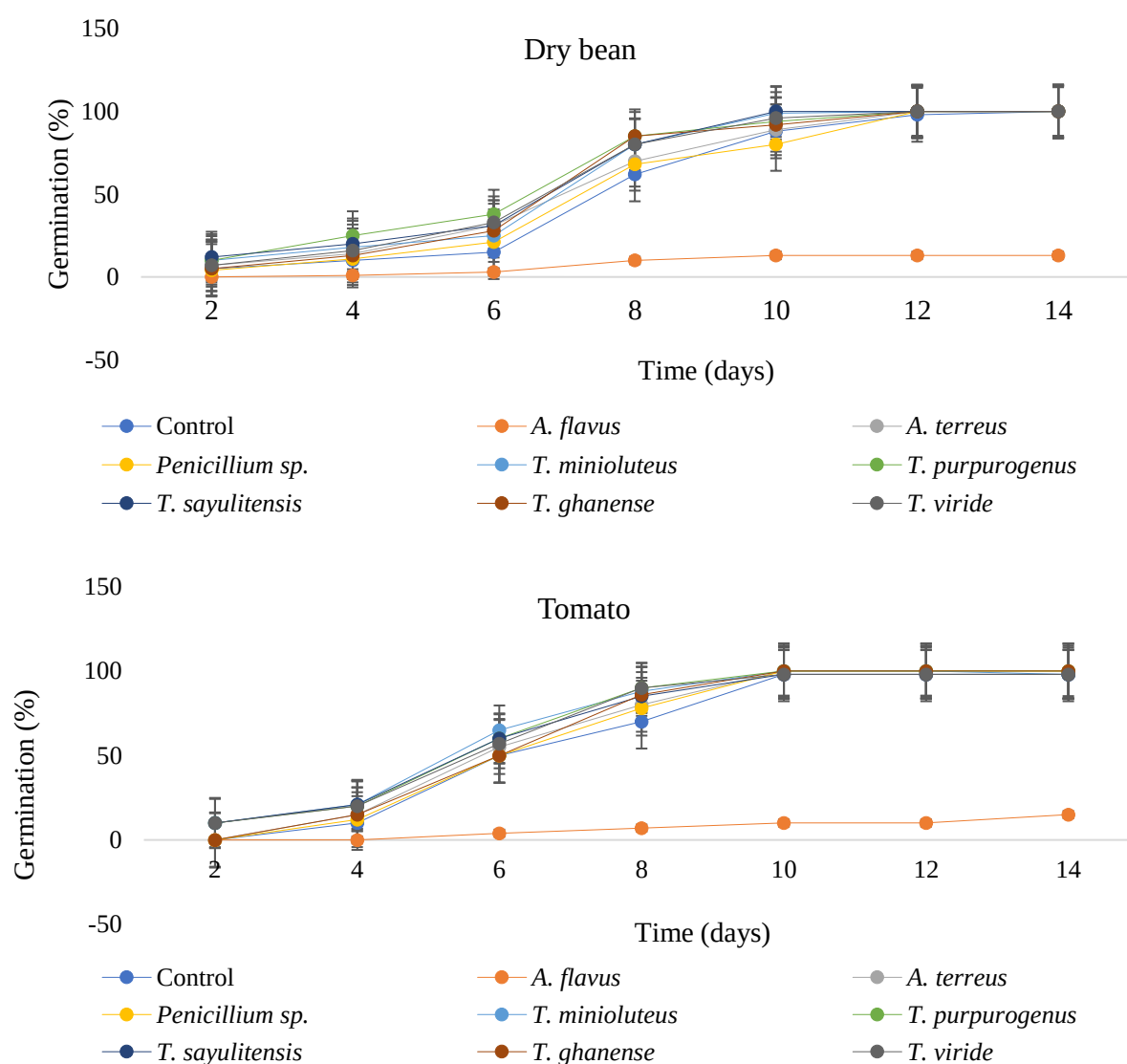


Figure 6.1a Effects of eight indigenous filamentous fungi on the germination of dry bean and tomato seeds 14 days after inoculation.

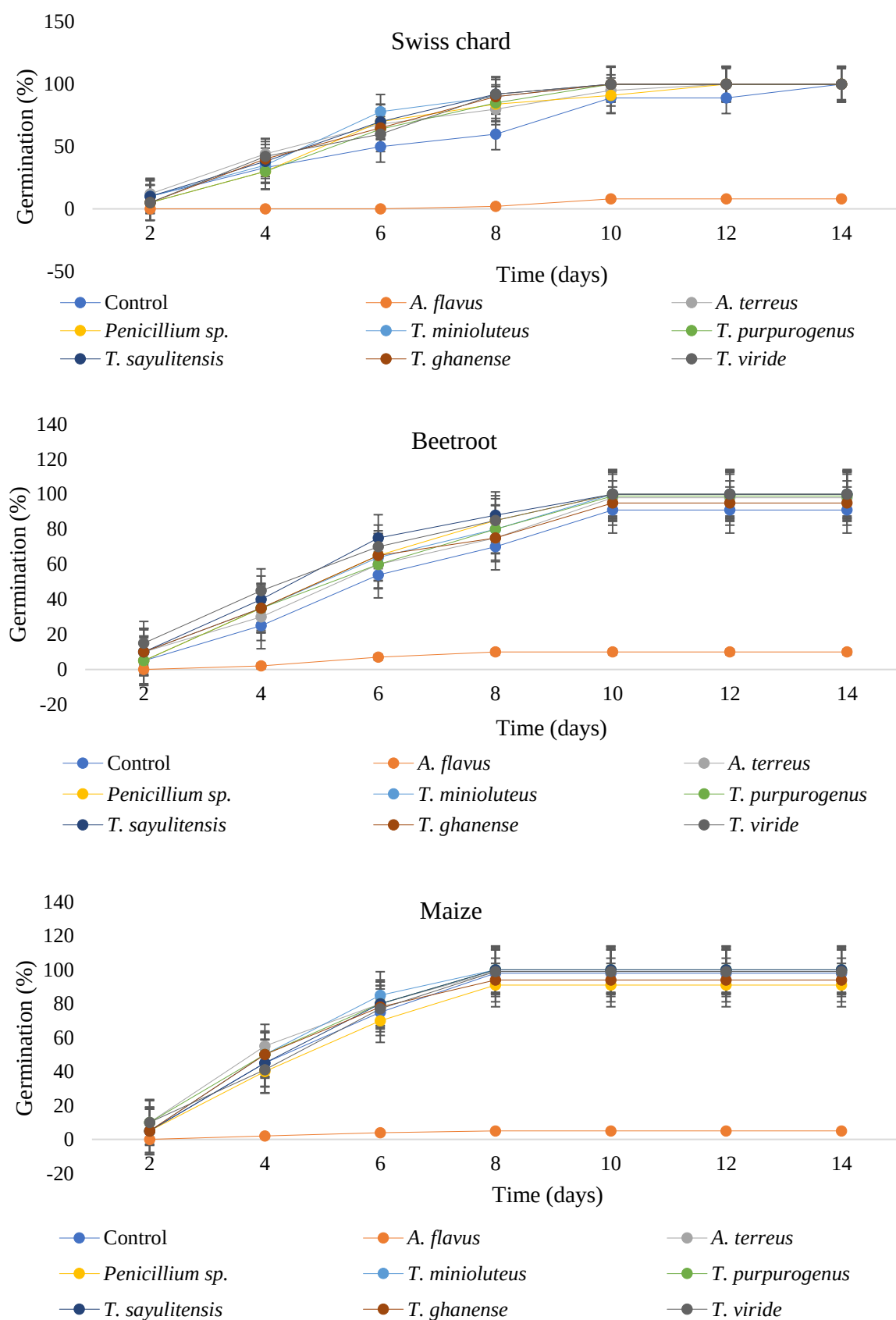


Figure 6.1b Effects of eight indigenous filamentous fungi on the germination of swiss chard, beetroot and maize seeds 14 days after inoculation.

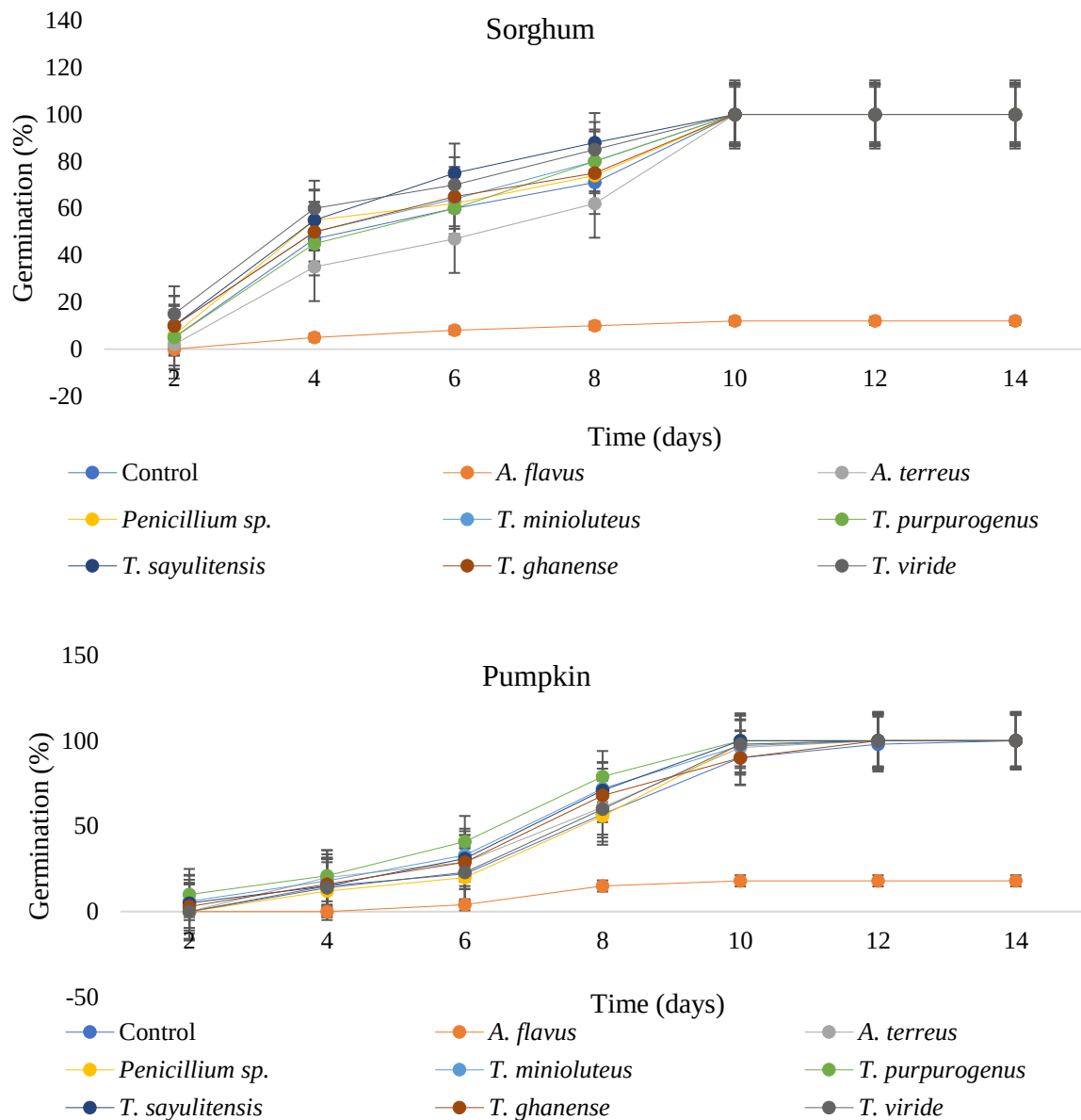


Figure 6.1c Effects of eight indigenous filamentous fungi on the germination of sorghum and pumpkin germination seeds 14 days after inoculation.

6.4.3 Seedling plumule growth

Fungal species had significantly different effects on seedling radicle growth ($P \leq 0.05$), with an evident increase compared to *A. flavus*, which exhibited the lowest radicle growth in all seedlings (Figure 6.2).

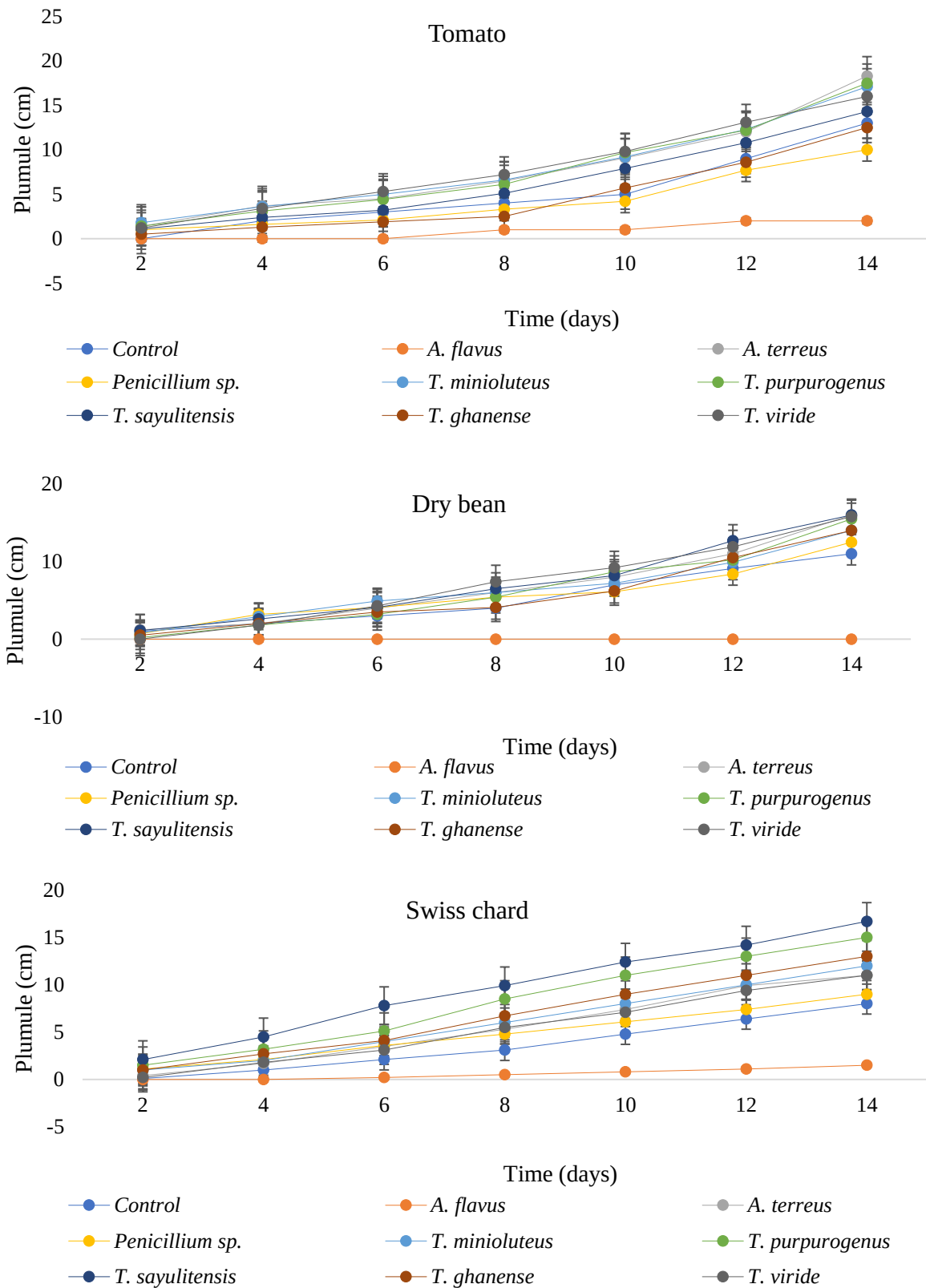


Figure 6.2a Effects of eight indigenous filamentous fungi on the plumule growth of dry bean, tomato and swiss chard 14 days after inoculation.

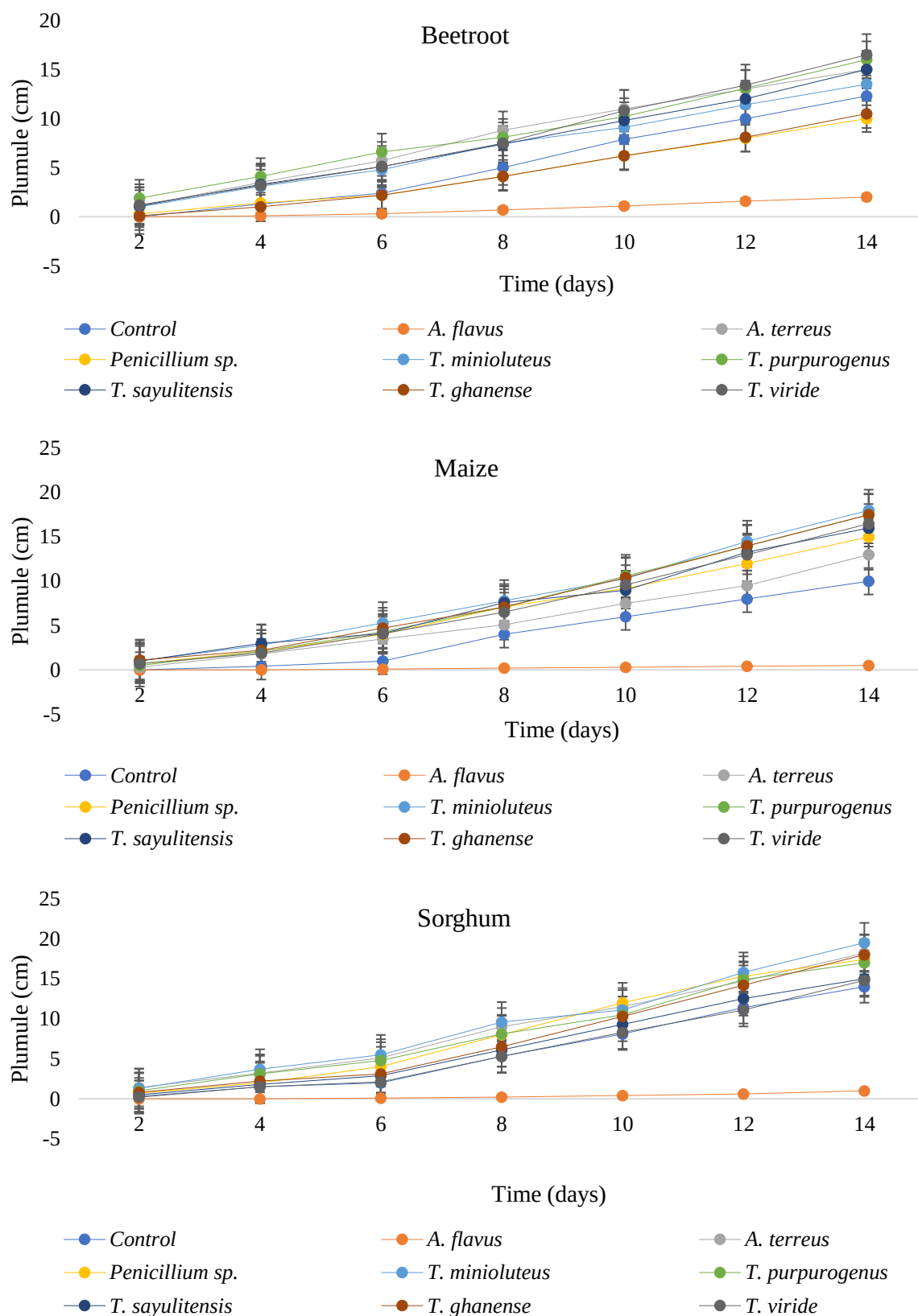


Figure 6.2b Effects of eight indigenous filamentous fungi on the plumule growth of beetroot, maize and sorghum 14 days after inoculation.

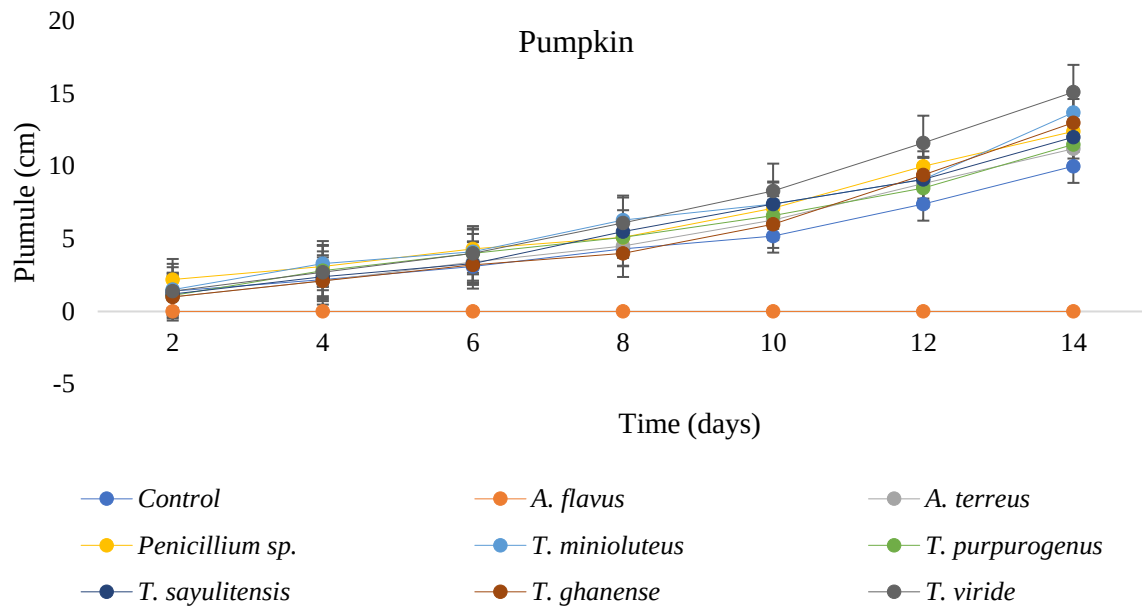


Figure 6.2c Effects of eight indigenous filamentous fungi on the plumule growth of pumpkin 14 days after inoculation.

6.4.4 Seedling radicle growth

Fungal species had significantly different effects on seedling radicle growth ($P \leq 0.05$), with an evident substantial increase compared to the control. However, *A. flavus* exhibited the lowest radicle growth in all seedlings (Figure 6.3).

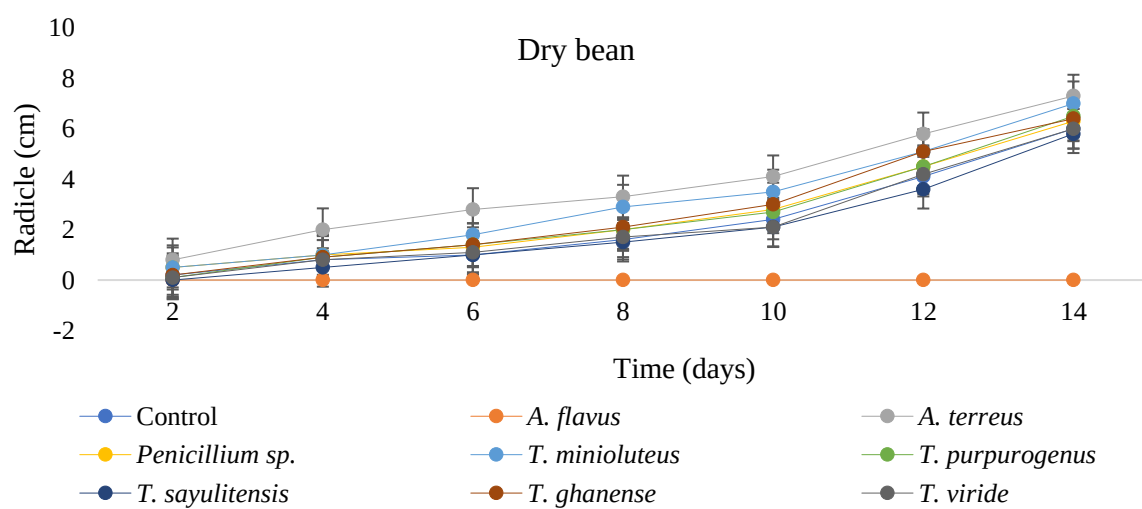


Figure 6.3a Effects of eight indigenous filamentous fungi on the radicle growth of dry bean 14 days after inoculation.

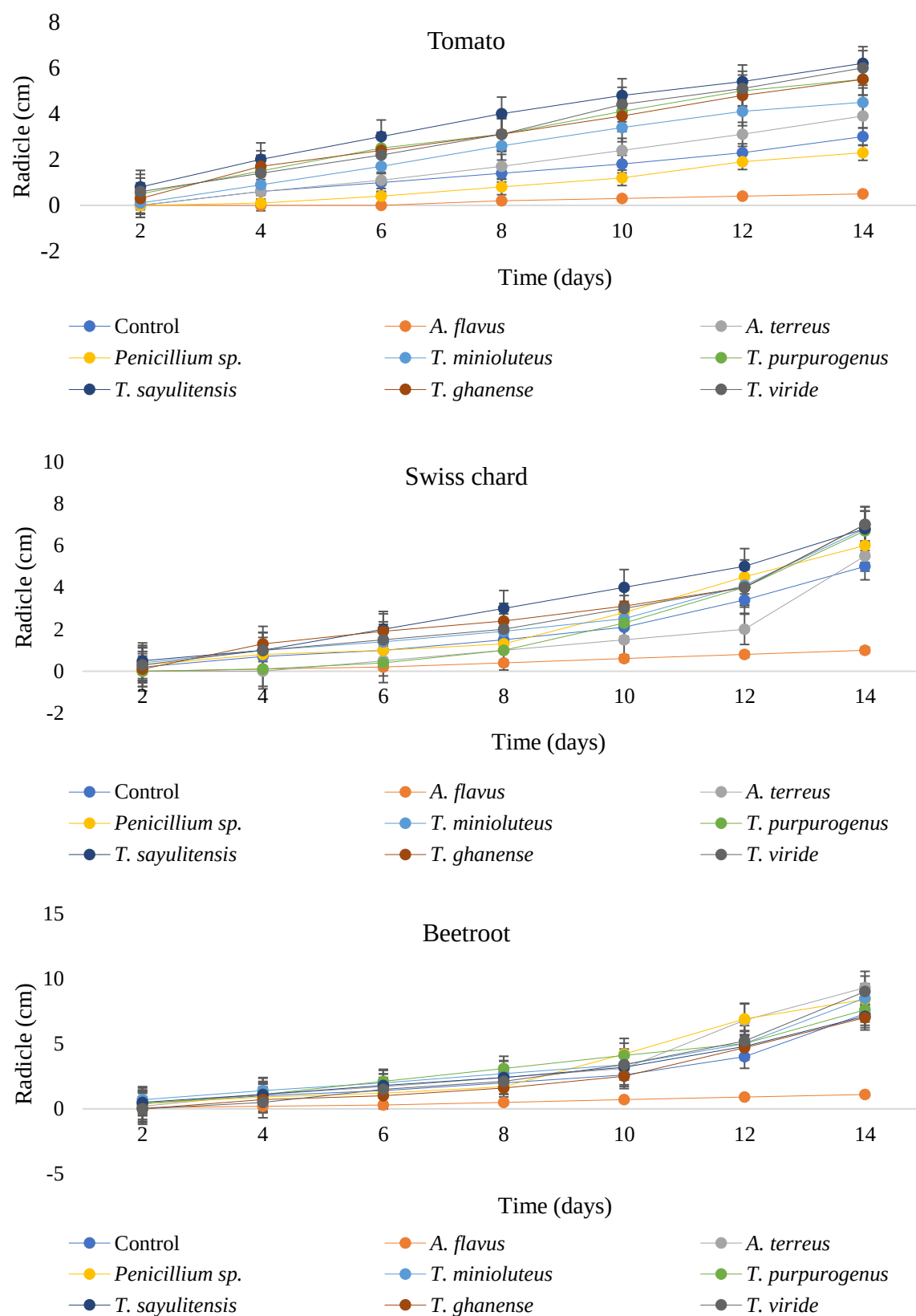


Figure 6.3b Effects of eight indigenous filamentous fungi on the radicle growth of tomato, swiss chard and beetroot 14 days after inoculation.

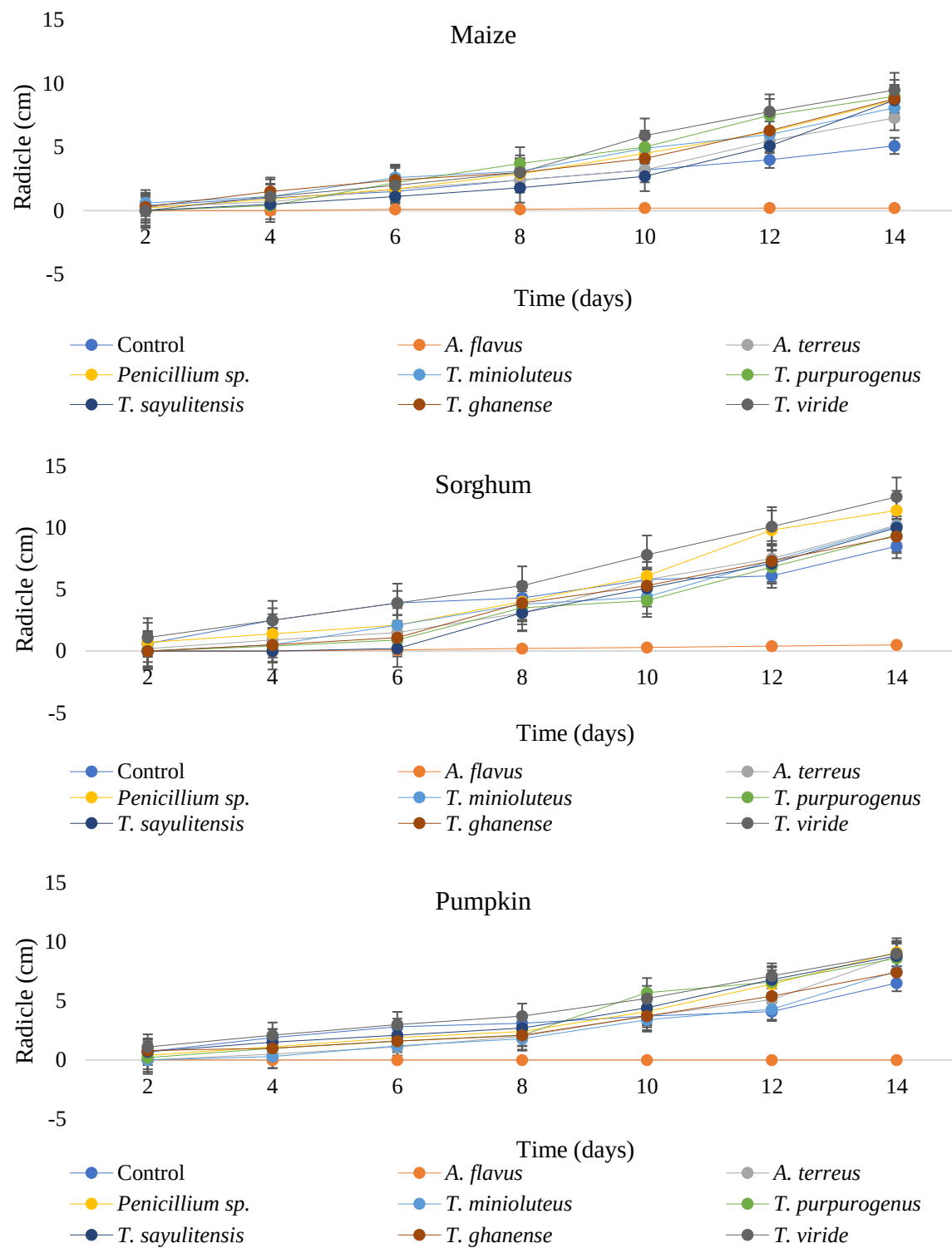


Figure 6.3c Effects of eight indigenous filamentous fungi on the radicle growth of maize, sorghum and pumpkin 14 days after inoculation.

6.4.5 Seedling vigour index

To further analyse the effect of different species on germination, plumule and radicle growth rate, the seedling vigour index was calculated (Figure 6.4).

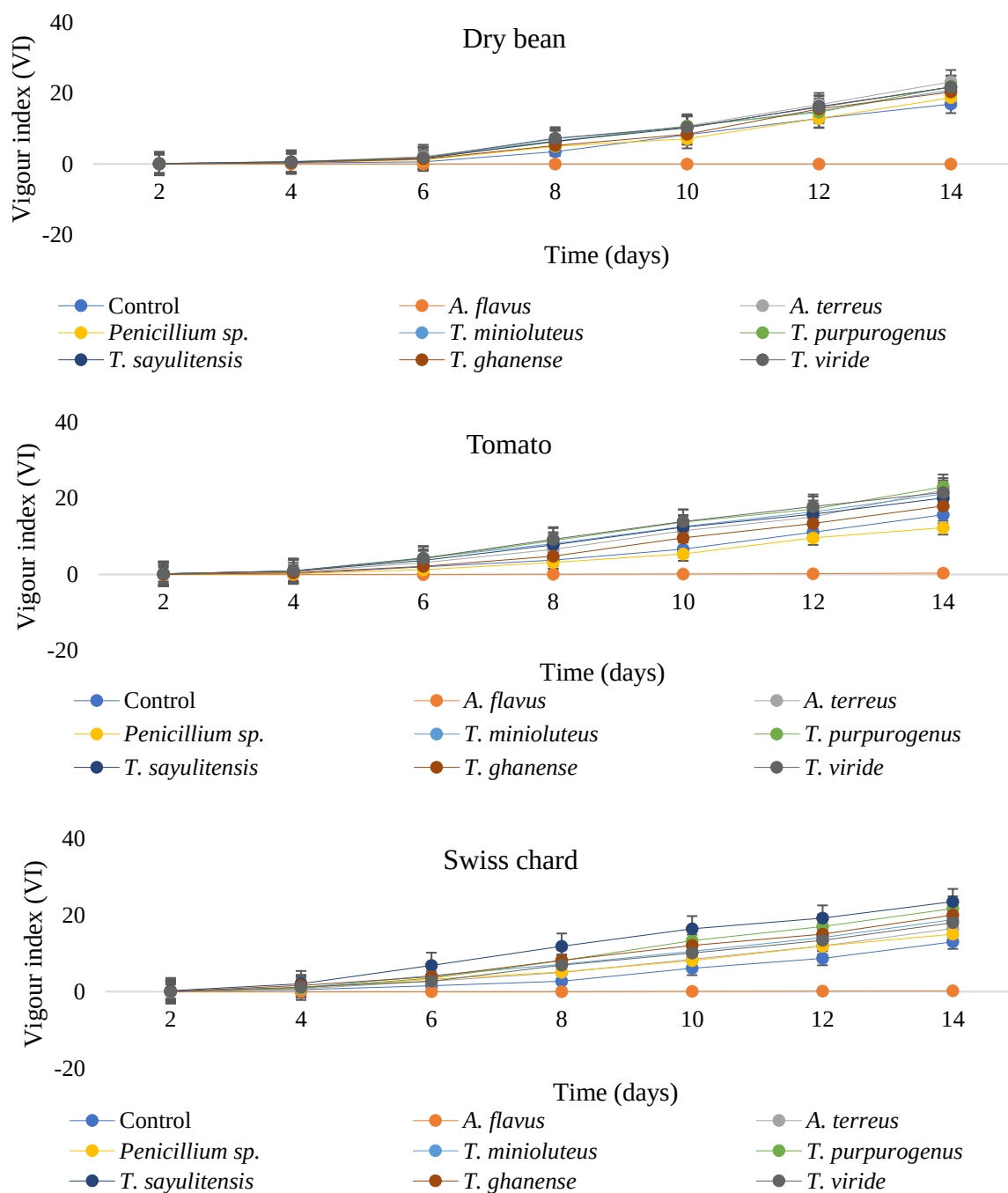


Figure 6.4a Effects of eight indigenous filamentous fungi on vigour index of dry bean, tomato and swiss chard 14 days after inoculation.

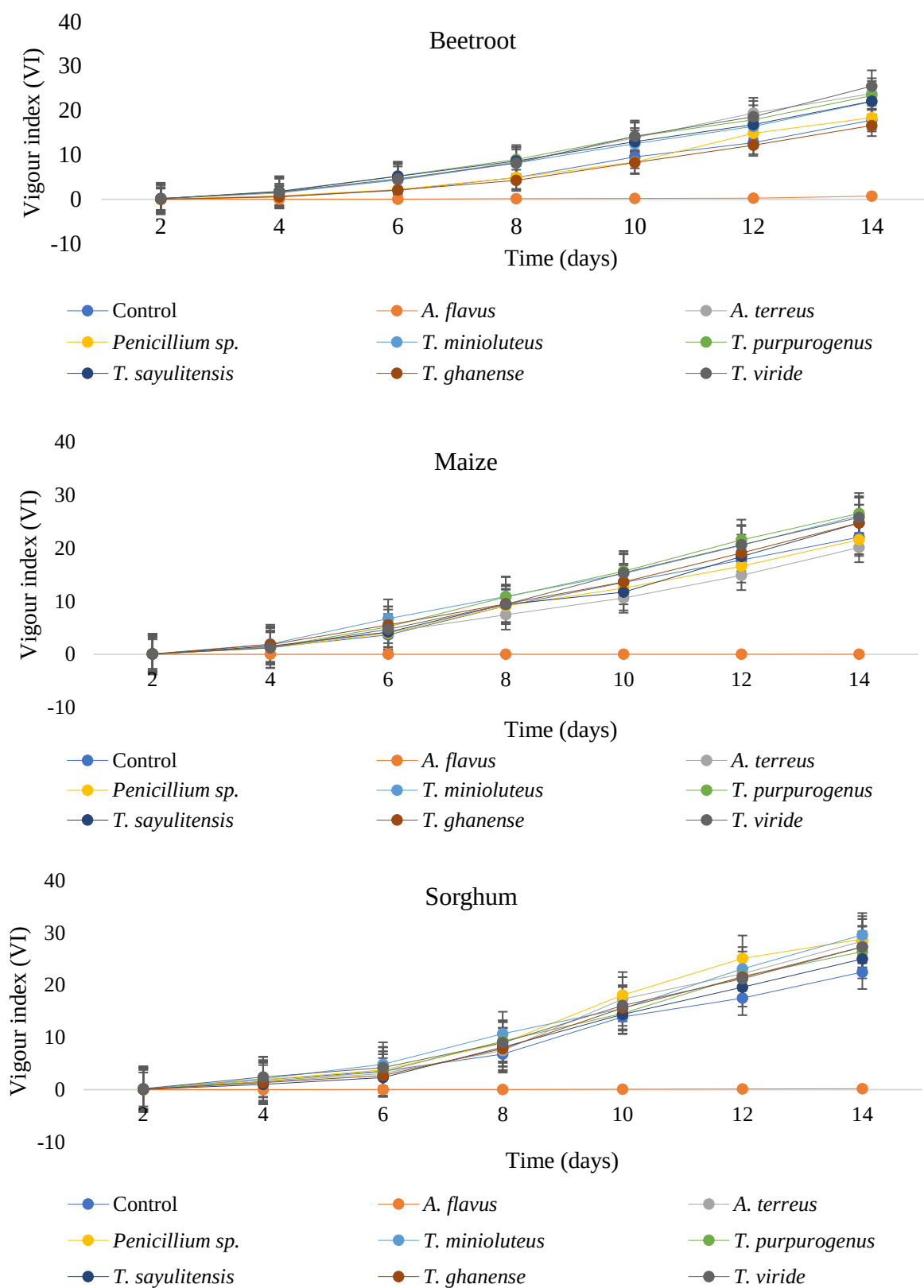


Figure 6.4b Effects of eight indigenous filamentous fungi on vigour index of beetroot, maize and sorghum 14 days after inoculation.

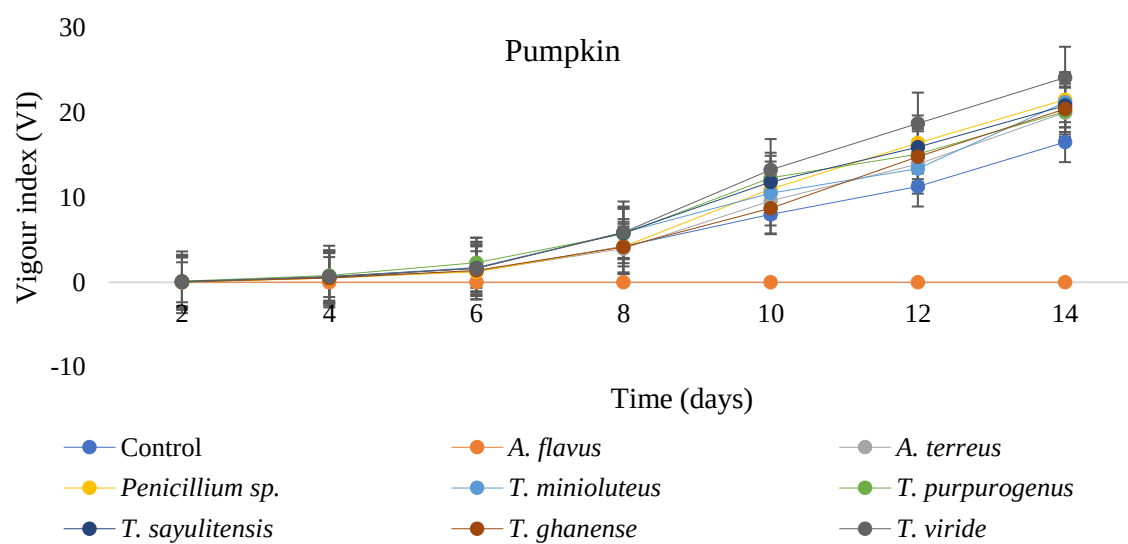
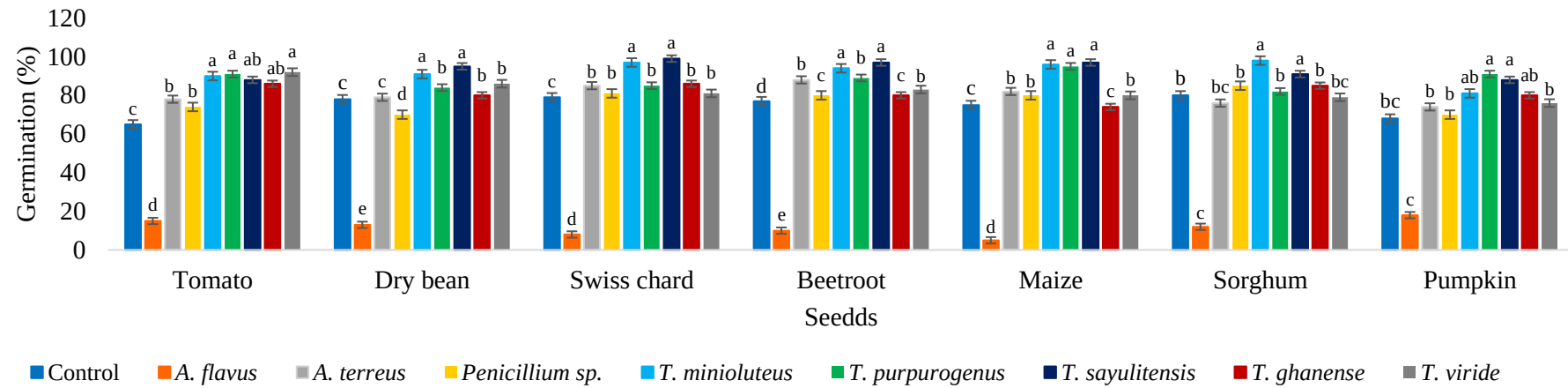
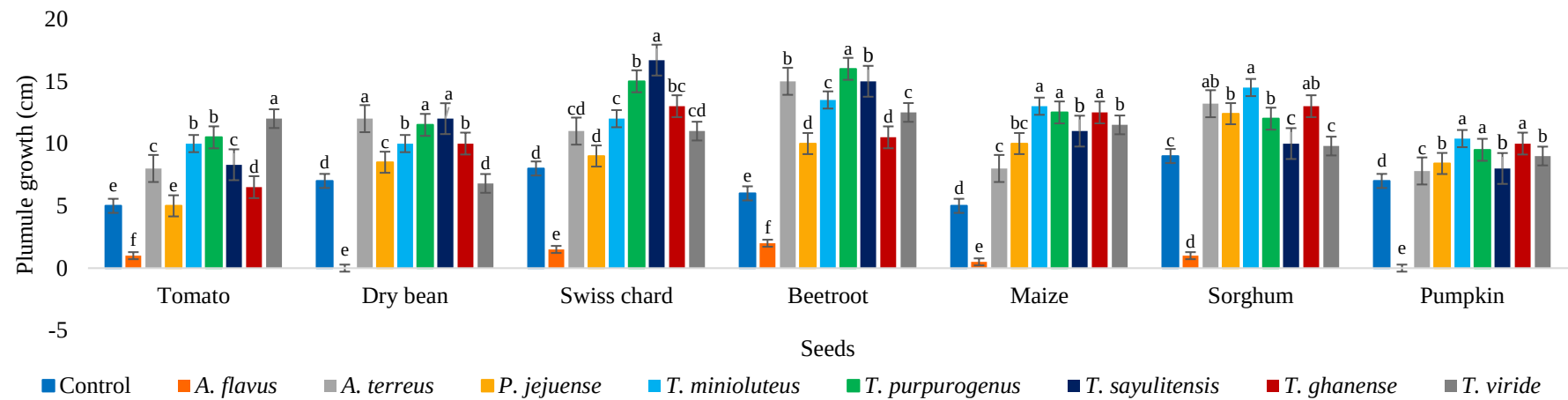


Figure 6.4c Effects of eight indigenous filamentous fungi on vigour index of pumpkin 14 days after inoculation.

(i) Seed germination under different fungal isolates



(ii) Plumule growth under different fungal isolates



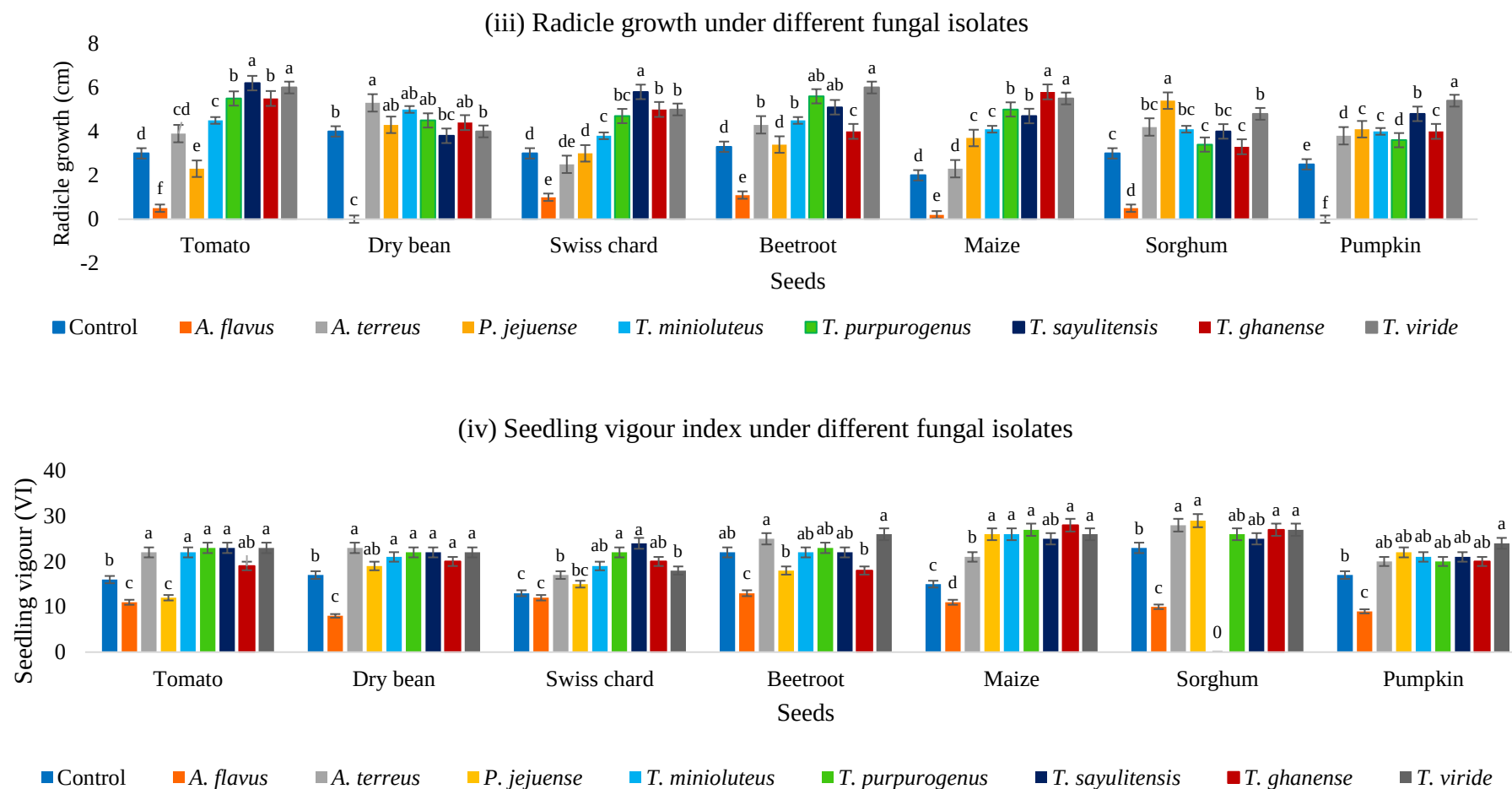


Figure 6.5 Response of (i) seed germination percentage, (ii) radicle growth, (iii) plumule growth and (iv) seedling vigour index of different seeds at 14 days exposure to eight indigenous filamentous fungi.

6.5 Discussion

6.5.1 Indigenous fungal species pathogenicity on seeds

Whilst *A. flavus* caused seed rot and reduced seedling growth, *A. terreus* improved seed germination and seedling growth of tested vegetables. This shows that species from the same genus can inhibit seed germination and seedling growth, whilst other species promote growth. *Aspergillus* is a genus of filamentous fungi that are typically opportunistic and prevalent in soil, air and decaying plant matter (Perrone *et al.*, 2007; Mousavi *et al.*, 2016; Abdel-Azeem *et al.*, 2019). Previous research has shown the inhibitory effects of Aflatoxin B1 from *A. flavus* on seed germination and seedling development in other agricultural plants (Avilala *et al.*, 2011; Yaqub-Bhat & Fazal, 2011; Divakara *et al.*, 2015). Yoo *et al.* (2018) previously reported that after four weeks of tomato seed treatment with *A. terreus* species, JF02, JF07, JF27 and JF44, the seedlings had significantly increased shoot length in comparison to the control group.

Some species from the same genus inhibit seed germination and seedling growth. A dimorphic fungus, i.e., parasitic and saprophytic, *A. flavus* is an opportunistic pathogen of both human and plants. In addition to causing disease conditions in significant food and feed crops including maize, peanut (*Arachis hypogaea* L.), cottonseed (*Gossypium herbaceum* L.) and tree nuts, this fungus also exudes aflatoxins, secondary metabolites which are poisonous and carcinogenic to humans (Majumdar *et al.*, 2018; Segura-Palacios *et al.*, 2021). Past research (Avilala *et al.*, 2011; Divakara *et al.*, 2015) has shown the inhibitory effects of Aflatoxin B1 from *A. flavus* on seed germination and seedling development in other agricultural plants. The administration of varying doses of aflatoxins significantly reduced sorghum (*Sorghum bicolor* L.) seed germination and seedling vigour (Divakara *et al.*, 2017). Similarly, Yaqub-Bhat & Fazal (2011) investigated the influence of *A. flavus* metabolites on wheat seed germination and

seedling development and discovered that greater concentrations of *A. flavus* culture filtrate inhibited seed germination, root and shoot lengths.

6.5.2 Fungal species effect on seed germination and seedling growth

In the current study, it was observed that *Penicillium* sp. promoted the growth of grain seeds, maize and sorghum, more than those of vegetable seeds. Increased root biomass could promote abiotic stress tolerance and aid the plants in the absorption of water and nutrients in far distances. The stimulation of seed germination by *Penicillium* sp. may have resulted from the synthesis of plant growth hormones such auxins, gibberellic acids, abscisic acid, ethylene and jasmonate. It has been shown in several investigations that *Penicillium* species generate gibberellic acid, which is necessary for the mitotic division of seeds (Leitão & Enguita, 2016; Ali *et al.*, 2017b; Altaf *et al.*, 2018). In a study by Mushatq *et al.* (2011), pre-soaking tomato seeds with *P. extractsa* considerably enhanced seed germination and subsequent seedling growth of up to 90% higher than untreated seeds.

The two *Trichoderma* species showed the fastest seed germination and seedling growth compared to other tested species. Furthermore, 100% seed germination was observed on *Trichoderma*-treated seeds. According to Shahid *et al.* (2011), chickpea (*Cicer arietinum* L.) germination and vigour were improved when seeds were treated with *Tri. viride*; whilst Lalitha & Arunalakshmi (2012) discovered improved seed germination and root length in mustard (*Brassica juncea* L.). The application of *Tri. viride* promoted wheat plant height, root length, leaf length, number of leaves and grain production (Mahato *et al.*, 2018). *Trichoderma ghanense* improved the shoot and root length of rye (*Secale cereale* L.) seedlings (Bridžiuvienė *et al.*, 2022). It has been reported (Martínez-Medina *et al.*, 2014) that inoculation of seeds by

Trichoderma species led to an increase in auxin, a decrease in cytokinin and abscisic acid content in melon (*C. melo* L.) and further stimulated melon root and shoot growth.

Compared to the control, *Tal. minioluteus* improved the germination rate of *S. lycopersicum* significantly by 99%. *Talaromyces* species promoted the formation of lateral roots on the test seeds, which would result in an improved nutrient uptake capacity and an increase in the biomass of the roots and shoots. *Talaromyces* species have been shown to increase crop growth through their abilities to produce siderophores, IAA and dissolving phosphorus (Zhao *et al.*, 2021). *Aspergillus* is a genus of filamentous fungi that are typically opportunistic, prevalent in soil, air and decaying plant matter and they generate a variety of secondary metabolites. Some *Aspergillus* species limit nematode activity, function as bio-controls for plant diseases and foster plant development (Waqas *et al.*, 2015).

6.6 Conclusion

The results of this study established that test species, except for *A. flavus*, could potentially be effective seed inoculants for stimulating the growth developmental process of seedlings. These test species may also have endophytic characteristics and their application may aid in achieving the development of robust seedling stands.

CHAPTER 7: GROWTH AND TROPISM OF FUNGI ON GRAIN SUBSTRATES

7.1 Introduction

Formulations based on fungal BCA have a wide range of applications as plant bio-pesticides against a variety of plant pathogens (Radhakrishnan *et al.*, 2021; Lahlali *et al.*, 2022). Developing a suitable fungal agent, growth medium and formulation is necessary to generate biopesticides that work. A pristine beneficial fungus should be able to produce a large amount of inoculum at a reasonable cost, possess the necessary virulence and be compatible with the technology used in modern applications (Jenkins *et al.*, 1998; Osman *et al.*, 2020). *Aspergillus*, *Penicillium*, *Talaromyces* and *Trichoderma* are facultative fungi that may be both endophytic and saprophytic (Vadlapudi *et al.*, 2017; Hyde *et al.*, 2019). It has been established that these fungal species do grow and sporulate easily in semi-synthetic media such as corn meal agar (CMA), MEA, NA, PDA and WA. These laboratory growth media are rich in carbon and nitrogen, important nutrients for fungal growth and sporulation (Campbell *et al.*, 1983; Wang *et al.*, 2019).

Bulk production of these fungal species by use of growth media is however relatively expensive and is therefore often only used for experimental purposes. According to Nozaki *et al.* (2004), the requirements for fungal development and sporulation of different species are not always the same. It is also recognised that some culture media are more conducive to fungal sporulation than others. It is therefore important that for commercial production of these endophytic fungi, an economically viable inoculum production technology should be developed. To provide fungal-based bio-pesticides at a reasonable cost, low-tech mass production procedures and inexpensive media must be established.

Biological control or growth promotion can be applied in various ways and these include seed treatment or direct soil inoculation. To establish the most appropriate application, the growth pattern of these respective fungi must be determined. The growth pattern would indicate whether it grows following the radicle or plume or grows simultaneously alongside both radicle and plume. In other words, it is important to determine the geotropic response of the potential biocontrol agents. Different fungi have been noted to vary in their geotropism response (Moore, 1996; Stočkus & Moore, 1996). Fungal species have been categorised into three geotropic responses: (i) Positive geotropic (ii) Negative geotropic (iii) Neutral geotropic (Moore, 1991). The geotropic response of the different fungal species would determine their method of application i.e., whether as seed treatment or above soil application. The objective was to determine growth rate and geotropism of fungal species on different grain substrates, viz., red sorghum, white sorghum, barley, wheat and wheat bran.

7.2 Study site

The experiments were carried out at the Agricultural Research Farm, Plant Pathology Research laboratory, NWU, Mmabatho, Molelwane, North-West Province, South Africa (25°48'36"S, 25°37'47.999"E). The experiments were conducted in May-June 2021.

7.3 Description of the experiments

7.3.1 Treatments and experimental design

Five parallel experiments were undertaken: one for each growth media, viz., whole grains of red sorghum, white sorghum, barley, wheat and wheat bran. These experiments were carried out under laboratory conditions. In each experiment, eight fungal species were taken as treatments and replicated five times, making a total of forty experimental units. The experimental units were laid out in an incubator at 25 ± 2 °C in a CRD with five replications.

Each growth substrate was inoculated with two 5 cm disks of appropriate fungal species. The eight treatments of fungal species were first cultured on PDA and incubated at 25 ± 2 °C for 21 days. No control was included for these experiments since no universal grain media supports growth of all fungal species the same. All experiments were conducted twice, but datasets presented herein is from a single trial.

7.3.2 Experimental procedures

Following a procedure by Bhadauria *et al.* (2012), 50 cm centrifuge tubes were sterilised with 70% ethanol by soaking for 12h, after which they were air-dried inside a laminar airflow. All grains were soaked in tap water overnight, except for wheat bran as this was in powder form. The excess water was drained by decanting using top-down open sieves of different sizes, following which all the media was autoclaved at 121 °C for 15 min. The grains were allowed to cool inside the laminar airflow and tubes were individually half-filled with respective grains. Two 5 cm fungal species disks were placed in the middle of the substrates facing opposite sides; thus, in support of both negative and positive geotropism (Figure 7.1). As the grains used have different granule size and weight, each tube was completely filled with each grain type. The tubes were then filled with the respective grains, closed and sealed with parafilm. All tubes were incubated at 25 ± 2 °C and growth rate and direction measured every 48 h for 22 days. After 22 days, 5 grams of colonised grain was mixed with 5 mL of distilled water using a vortex for 1 minute. The resultant suspension was inoculated and cultured on PDA. Using a haemocytometer, spore number was calculated after 48 hours.

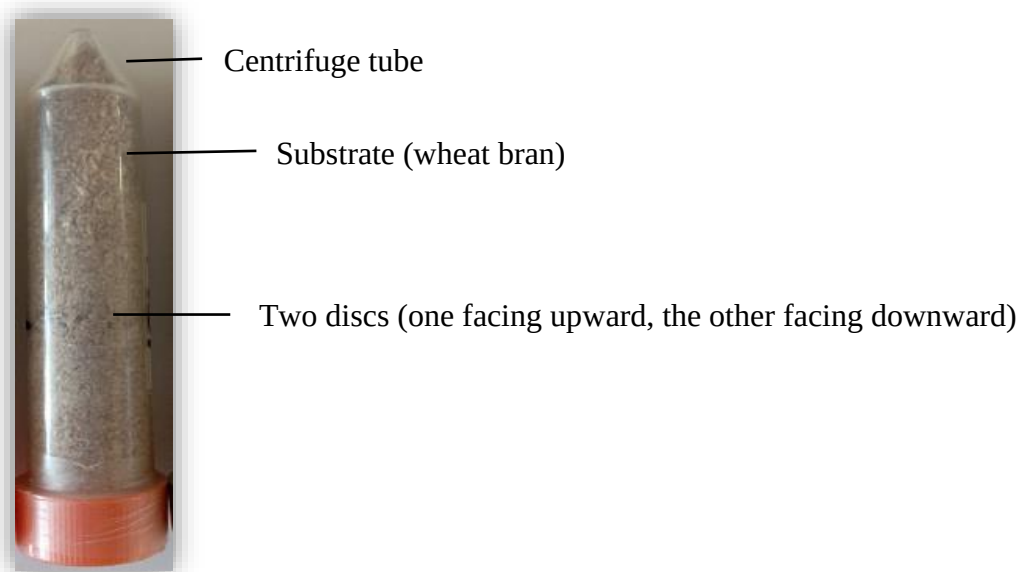


Figure 7.1 Centrifuge tube filled with wheat bran and inoculated with two 5 cm PDA discs with fungi; one disc facing upwards and another facing downwards. Picture taken by Ndivhuwo Ramatsitsi.

7.3.3 Data collection

At 48 h after inoculation, fungal growth was assessed for growth direction and growth rate. Using a clear transparent ruler, the growth was measured from the centre of the tube, inoculation point and recorded. The study ran for 21 days and was terminated soon after growth measurements.

7.3.4 Data analysis

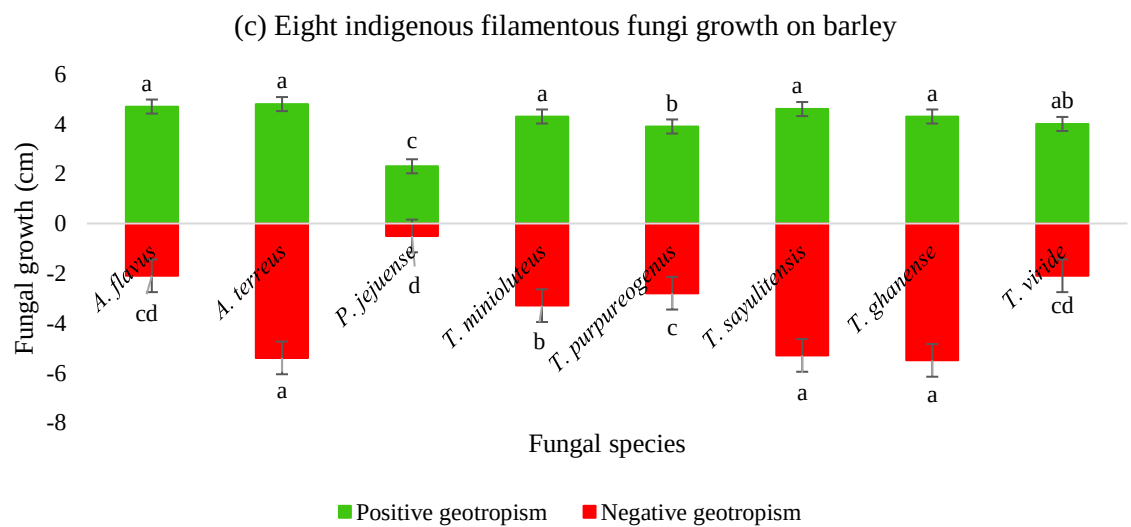
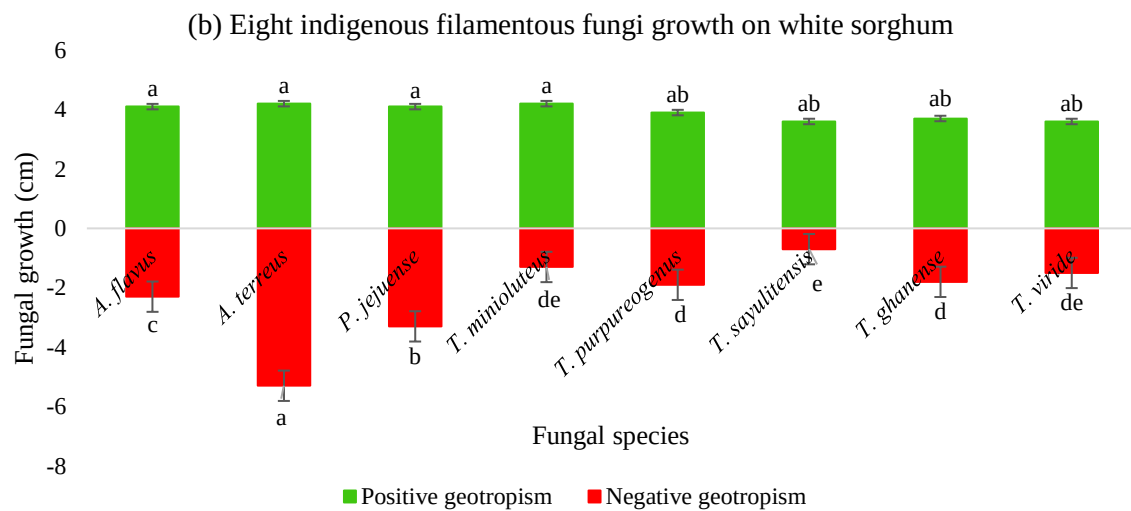
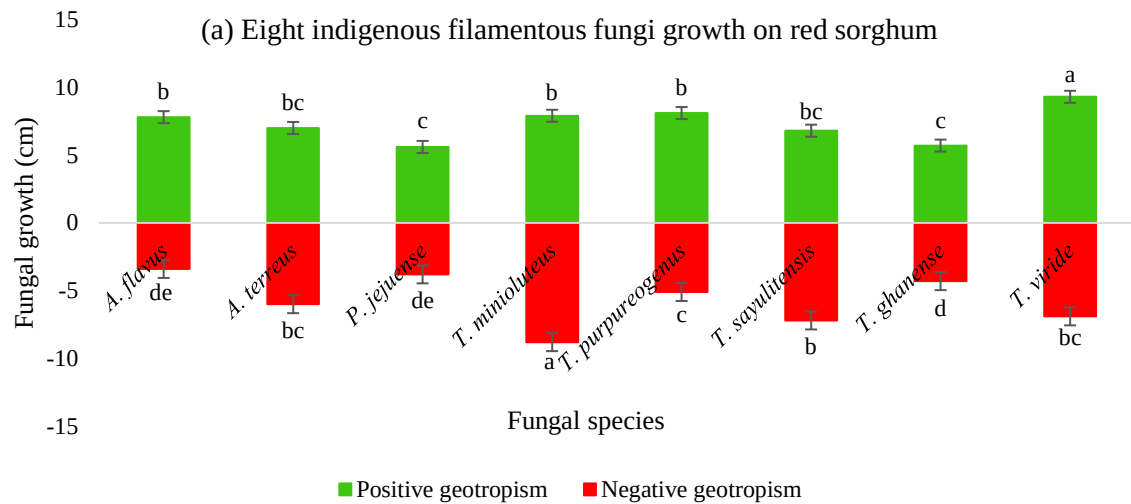
Fungal species growth was subjected to the ANOVA procedure using SAS software (9.4 version) to determine significant treatments at the probability level of 5%. Mean separation was achieved using Tukey's HSD (Gomez & Gomez, 1984).

7.4 Results

7.4.1 Indigenous fungi growth rate and geotropism on grain substrates

Figure 7.2 shows that among the substrates tested, the mean growth of all eight fungal species was significantly higher ($P \leq 0.05$) on red sorghum than on other growth media. On red sorghum, all fungal species grew towards both positive (+ve) and negative (-ve) geotropism. *Trichoderma viride* recorded the highest +ve growth at an average of 9.3 cm. On the other hand, *Tal. minioluteus* showed the highest -ve tropism growth at 8.8 cm [Figures 7.2 (a), (b), (c), (d) and (e)]. Even though white sorghum supported a relatively higher -ve geotropism than +ve, this was not the case for *A. terreus*; as it grew 5.3 cm +ve and 4.2 cm -ve tropism [Figure 7.2 (b)]. All the other species; *A. flavus*, *Penicillium* sp., *Tal. minioluteus*, *Tal. purpureogenus*, *Tal. sayulitensis*, *Tri. ghanense* and *Tri. viride*, each had an overall growth of over 4 cm -ve tropism and an overall growth less than 4 cm +ve tropism. Of all the test species, *Tal. sayulitensis* had the least overall +ve tropism growth of 0.7 cm.

Barley substrate supported both +ve and -ve tropisms, with the highest recorded growth of +ve 4.8 cm for *A. terreus* and 2.3 cm for *Penicillium* sp. The highest observed growth of -ve tropism was *Tri. ghanense* (5.5 cm) and the lowest was *Penicillium* sp. (0.5 cm). All fungal species had a relatively higher -ve tropism than +ve, except *Tal. purpureogenus* which had a higher +ve geotropism than -ve at +ve 4.2 cm and -ve 3.9 cm growth. The highest -ve tropism recorded was 5.2 cm for *A. flavus*. The least fungal growth rate for all species was observed on wheat bran, with the highest overall +ve tropism recorded on *A. terreus* at 3.1 cm and no -ve growth on *Tri. viride*; whilst *Tal. purpureogenus* did not grow at all in either tropism. The highest -ve tropism was recorded for *Tal. minioluteus* at 1.6 cm.



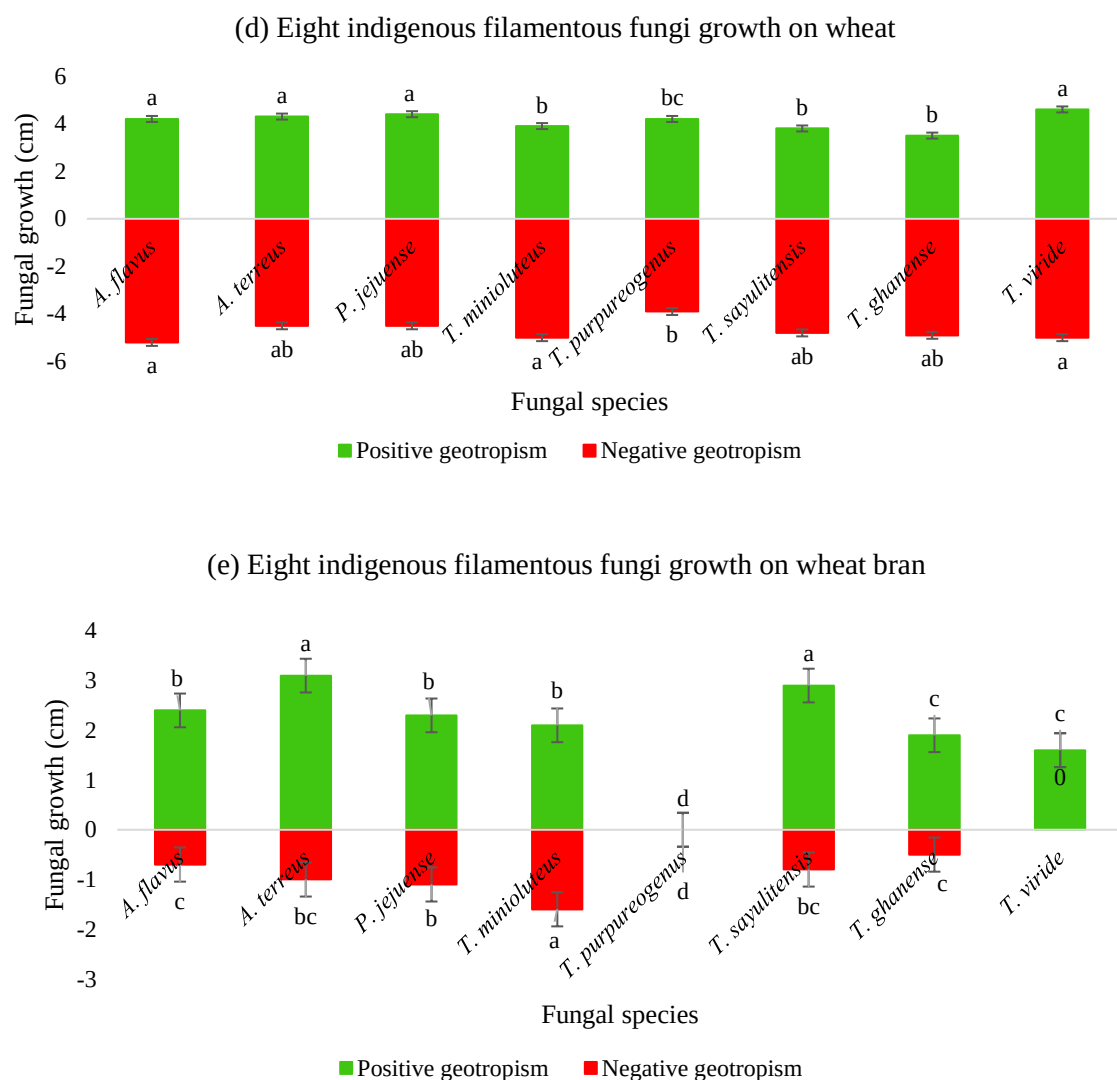


Figure 7.2 Growth of eight filamentous fungi cultured on grain substrates at 30 days after inoculation.
^aSame letters above the bars indicate no significant difference according to Tukey's HSD at $P \leq 0.05$.

7.4.2 Fungal spore count

Fungal species spore count was highly significant ($P \leq 0.01$) for fungi previously cultured on barley, wheat and wheat bran substrates (Table 7.1). The number of spores significantly varied ($P \leq 0.05$) amongst fungi previously cultured on red sorghum and white sorghum. The highest mean spore count was recorded on *Tal. minioluteus* (399.00 ± 1.30) and *Tri. viride* (241.00 ± 8.60) when cultured in red sorghum. On the other hand, *Penicillium* sp. (121.70 ± 3.40) yielded the least spore count (Table 7.1).

Table 7.1 Mean spore number of fungal species incubated from various substrates to PDA after 48 hours (per 5 mL water).

Treatments	Red sorghum	White sorghum	Barley	Wheat	Wheat bran
<i>A. flavus</i>	172.44d ^z ± 4.90	95.96c ± 2.90	70.60e ± 1.10	173.00b ± 2.70	53.20ef ± 4.10
<i>A. terreus</i>	178.94d ± 3.80	139.02a ± 0.70	101.64bc ± 3.10	137.80c ± 0.70	92.88b ± 1.10
<i>Penicillium</i> sp.	118.70d ± 3.40	84.16cd ± 9.10	71.00e ± 34.00	108.14de ± 1.40	60.92de ± 2.50
<i>Tal. minioluteus</i>	399.00a ± 1.30	56.30f ± 2.00	83.14d ± 0.80	107.94de ± 0.20	81.78bc ± 3.30
<i>Tal. purpureogenus</i>	188.30d ± 3.80	72.88de ± 11.80	97.54c ± 1.90	114.42d ± 6.30	53.40ef ± 0.060
<i>Tal. sayulitensis</i>	220.80c ± 2.60	135.60a ± 7.70	108.80b ± 1.60	115.62d ± 2.60	112.44a ± 0.90
<i>Tri. ghanense</i>	170.50d ± 3.80	61.88ef ± 1.80	123.34a ± 1.90	98.42e ± 6.30	70.10cd ± 0.60
<i>Tri. viride</i>	260.40b ± 8.60	108.64b ± 2.90	66.34e ± 0.50	665.30a ± 2.10	45.04f ± 2.70
HSD _{0.05}	22.00	12.27	9.64	12.14	12.45
P	0.05	0.05	0.01	< 0.01	0.01
F	2.23	3.78	7.00	79.40	6.36

^zColumn means followed by the same letter were not different ($P \leq 0.005$) according to Tukey's HSD, average ± standard deviation error.

7.5 Discussion

7.5.1 Fungi growth rate and geotropism sensitivity

Grains are relatively affordable, easily available and act as the best nutritive media for the mass production of many micro- and macro-organisms (Lakshmi & Alagammai, 2001; Agale *et al.*, 2018). Results exhibited a general trend of fungal species neutral geotropic response for most of the test fungal species cultured on wheat substrate [Figure 7.2 (d)]. Among the test fungi grown under wheat bran substrate, the *Tri. viride* fungal species was conspicuously different from the rest showing only -ve geotropism [Figure 7.2 (e)]. The general trend of the geotropic neutral response of the fungi response was also noted in the other growth media *albeit* with varying strengths of -ve and +ve geotropism [Figures 7.2 (a), (b) and (c)]. Fungal species have been reported to respond to influence by various tropisms i.e., phototropism, geotropism and barrier tropism (Moore, 1991; Moore, 1996). As this experiment was carried out in an incubator with no lighting, phototropism was not evaluated.

Gopalakrishnan *et al.* (1999) reported that sorghum was the ideal cereal for the mass production of *Paecilomyces farinosus*. In the case of *Lecanicillium lecanii*, sorghum was found to be the ideal cereal for mass production (Lakshmi & Alagammai, 2001), which is in confirmation with the findings of the current study. In plants, phototropism is associated with geotropism expression through the effect of radiation on IAA which in turn plays a role in geotropism (Kumari & Panigrahi, 2019; Yun *et al.*, 2023). The role of IAA in fungal species has not been established. Fungal species have been reported to produce certain growth hormones, such as gibberellin and ethylene, that drive their physiological processes (Adeleke & Babalola, 2021; Alfiky & Weisskopf, 2021). This probably can explain the different -ve and +ve geotropism strengths between different fungal species observed in the figures. This variation is under hormone control and generation by fungal species although genetic dependence could also be

influenced by the growth media in which they were cultured. External influence of geotropism response in fungal species has been reported (Corrochano & Galland, 2016; Bielčik *et al.*, 2019; Yu & Fischer, 2019). This could explain the geotropism variation response in the current study.

Although it could be deduced that geotropism response and strength in fungal species are inherently genetically controlled, there is nevertheless an indication that external influence/factors could alter this response. This could explain the variation of geotropism strengths exhibited in Figures 7.2 (a), (b), (c), (d) and (e). The variation of geotropic response between fungal species could therefore be explained as possibly occurring as a result of their inherently genetic constitution in their growth hormone generative capacity. *Trichoderma viride* showed stronger -ve tropism and *Tal. minioluteus* showed stronger +ve geotropism which could be an indication different capacity of hormone production capabilities and therefore differing responses to different growth media. Past studies (Lin *et al.*, 2014; Begum *et al.*, 2019; Wahab *et al.*, 2022; Datta *et al.*, 2023; Hafeez *et al.*, 2023) established that biochemical molecules can alter physiological processes in plants or fungi and in turn influence geotropic response.

As these fungal species are being investigated for their possible use as biocontrol of soil-borne pathogens, their +ve or -ve geotropism characteristics are important for the determination of the application method of the fungal biocontrol agent. For instance, a geotropic neutral BCA would be appropriate to be applied as a seed treatment. During seed germination of bio-control treated seed the fungal growth would be both upwards and downwards i.e., alongside the plumule and radicle protecting against soilborne pathogens. On the other hand, a +ve geotropic biocontrol fungus would only grow downwards protecting the radicle. Similarly, a -ve geotropic bio-control fungus would grow upwards only protecting the plumule. It could

therefore be concluded that neutral geotropic biocontrol fungi would be the most appropriate biocontrol agent. It is important to note that the study did not determine/investigate the fungal horizontal response i.e., protecting the germinating seed on a horizontal plane. To come up with effective bio-control technology, further work needs to be undertaken particularly looking at analysing for synergistic effect of combining fungal species with different geotropism properties.

7.5.2 Fungal spore count post culture on grain substrates

Noteworthy, the lowest spore count of *Tal. purpureogenus* (188 ± 3.80) previously cultured on red sorghum, was still higher than the highest spore counts in species previously cultured in all the other substrates. The nutritional quality of red sorghum, which is abundant in tartaric acid, citric acid, vitamin C and sugars, accounts for the increased conidial production in sorghum grains. Moore-Landecker (1972) stimulated spore germination in the laboratory by adding inorganic ions, carbohydrates, amino acids, lipids and vitamins to the growth medium. A medium that encourages extensive sporulation may not be the greatest for ensuring spore quality. As a result, optimising spore production must take spore quality into account (Jackson & Bothast, 1990; Wyss *et al.*, 2001). The current study further validated this fact by determining which grains examined for mass production of fungal species.

7.6 Conclusion

Sorghum substrates, both red and white, supported faster fungal growth compared to other substrates. Therefore, the two provide suitable affordable media that is commercially viable for biotechnological products based on endophytes. These results may be relevant for the mass production of advantageous endophytes for agricultural application.

CHAPTER 8: *IN VITRO* ASSESSMENT OF INDIGENOUS FUNGAL SPECIES TOLERANCE TO ABIOTIC STRESS

8.1 Introduction

Soil abiotic stress is a worldwide problem and has become a major factor limiting agricultural and forestry production (Patil *et al.*, 2013). Soil characteristics influence the establishment and subsequent growth of micro-organisms in the rhizosphere. Due to their failure to colonise the host rhizosphere in unfavourable natural conditions, several fungal BCA that have promising potential when tested *in vitro* sometimes fail under field conditions. Because of this, researchers need to better understand the intricate web of interactions between biotic and abiotic elements that are in close contact with these BCA in order to optimise their benefits through safe, skilled application and cutting-edge technology. Tolerance of plants to abiotic and biotic stress can be improved by the application of BCA (Rawat *et al.*, 2013); however, the applied BCA strains must acclimatise to the abiotic stress well.

In South Africa, indigenous fungal species have demonstrated tremendous potential as BCA against important plant pathogens (Coombes *et al.*, 2016). However, abiotic conditions such as temperature have an impact on BCA effectiveness, much like with other microbial control agents (Jaronski, 2010). Abiotically stressed soils that are acidic, have high salinity levels and are nutrient-deficient were shown to benefit very slightly from the use of BCA; regardless of whether the fungi have bio-control or bio-fertiliser qualities (Begum *et al.*, 2019). Therefore, it is highly useful to determine abiotic stress tolerance in beneficial soil fungi. Fungal BCA that thrives in harsh conditions, such as high acidity, high salinity or relatively high temperatures, may in turn have an impact on their symbiotic hosts' ability to adapt to a comparable spectrum of situations. It is generally recognised that two key characteristics must be present or improved

when choosing acceptable fungal BCA for use as biocides: (i) high virulence against the intended pests and (ii) environmental stress tolerance (Ibarra-Cortés *et al.*, 2013; Kumar *et al.*, 2016). The objective was to assess abiotic stress tolerance of indigenous fungal species.

8.2 Study site

The experiments were carried out at the Agricultural Research Farm, Plant Pathology Research laboratory, NWU, Mmabatho, Molelwane, North-West Province, South Africa (25°48'36"S, 25°37'47.999"E). The experiments were conducted in August 2021.

8.3 Description of the experiments

8.3.1 Treatments and experimental design

To investigate the stress tolerance of the indigenous fungal species to different conditions, a concentration gradient culture was established following a modified procedure by Yu *et al.* (2021). To match saline-alkali soil, NaCl and NaHCO₃ were added at various concentrations to the PDA medium. To generate final salt stress, medium concentrations (w/v, g mL⁻¹) of 1, 4, 7 and 10% were added. Using a normal PDA pH of 5.77, pH stress media (w/v, g mL⁻¹) of 6.25, 6.50, 6.75 and 7.00 were obtained. To simulate a nutrient-deficient soil, the PDA medium was diluted to 25, 50, 75 and 100%. To test for extreme temperatures, fungal species were grown under 10, 20, 30 and 40 °C, by changing incubator temperature, and growth was measured. When media in the Petri dishes had solidified and cooled, a 5-mm diameter mycelial disc of each fungal species was placed in the middle of the agar plate and incubated at 25 ± 2 °C. The controls comprised all fungal species grown on PDA following recommended preparation, i.e., suspending 39 g of the agar in 1 L water.

8.3.2 Experimental procedures

Four parallel experiments, each for a tolerance test, i.e., salinity, pH, nutrient and temperature were laid out in a CRD, and replicated five times *in vitro* in an incubator. The eight fungal species, i.e., *A. flavus*, *A. terreus*, *Tal. Purpureogenus*, *Tal. Minioluteus*, *Tal. Sayulitensis*, *Penicillium sp.*, *Tri. Ghanense* and *Tri. viride* identified in **Chapter 4**, were cultured in modified PDA media. Temperature was changed to 10, 20, 30 and 40 °C during the temperature assessment experiment. Two separate incubators were used at a time, wherein one was set at 10 °C and the other at 20 °C. After eight days, growth was measured and the media plates discarded, after which the incubators were set at 30 and 40 °C. The experiments were carried out twice for each temperature set. For the controls, the inoculated media plates were incubated at 25 ± 2 °C and observed every two days for eight days.

8.3.3 Data collection

The radii of the species' growth were recorded using a ruler every 48 hours for eight days.

8.3.4 Data analysis

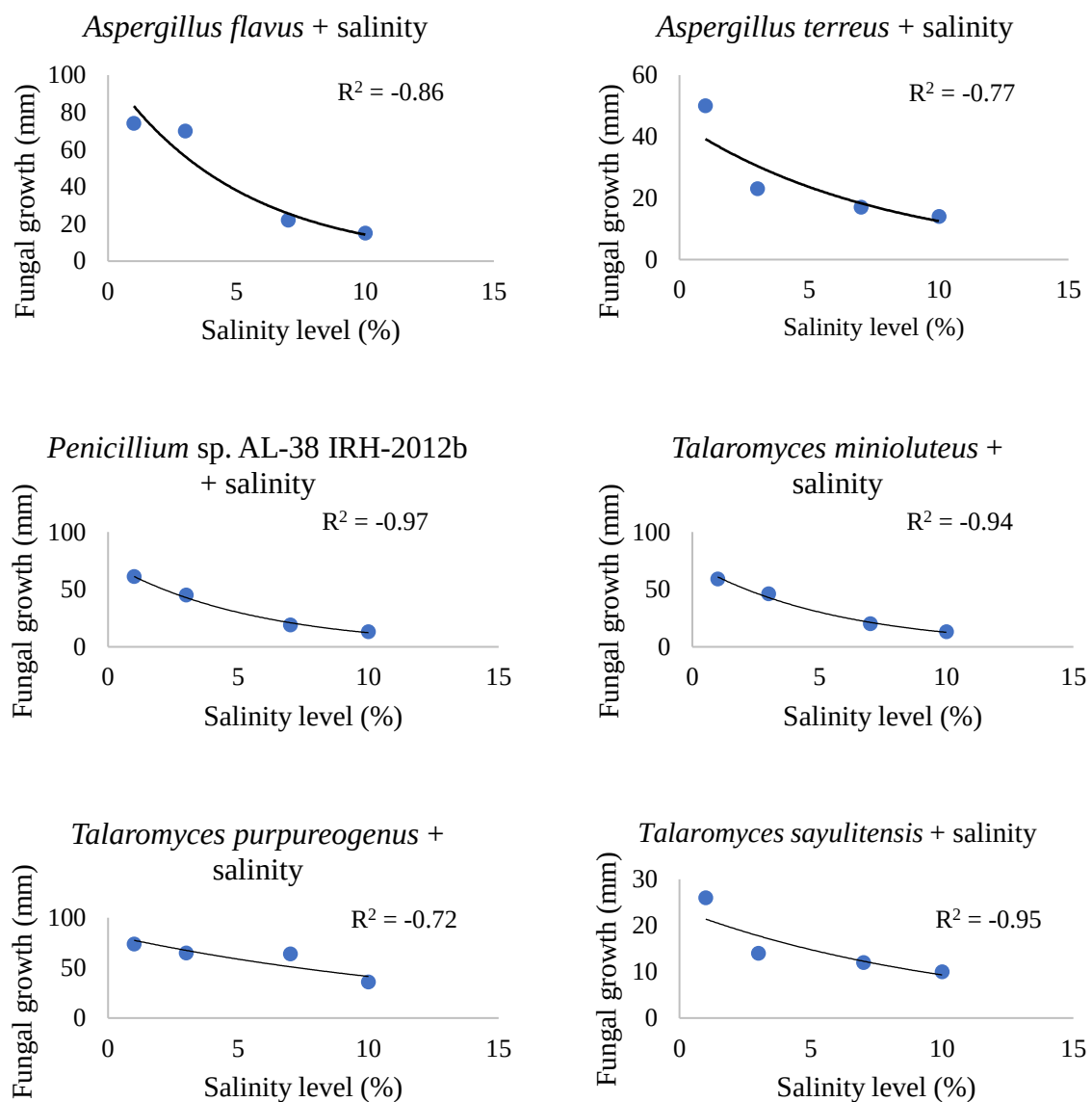
All data were subjected to ANOVA using the SAS version 9.4 package (SAS Institute, Inc., Cary, NC., USA, 2008) and the Student t-test used for mean comparisons (Gomez & Gomez, 1984).

8.4 Results

8.4.1 Response of fungal species to salinity

Every 48-h following incubation, the radius of each colony was measured. The growth responses of *A. flavus*, *A. terreus*, *Penicillium sp.*, *Tal. minioluteus*, *Tal. purpureogenus*, *Tal. sayulitensis*, *Tri. ghanense* and *Tri. viride* differed significantly ($P \leq 0.05$) to various levels of

salt, pH, nutrient deficiency and temperature [Figures (8.1), (8.2), (8.3) and (8.4)]. There was no significant difference ($P \leq 0.05$) between levels 1-4% and 7-10% salt concentrations amongst all species. The radii of *A. flavus* significantly decreased as salt concentration increased (Figure 8.1). Nonetheless, all eight isolates could grow at 10% salt concentration, with *Tal. minioluteus* tolerating salt stress better than the other species.



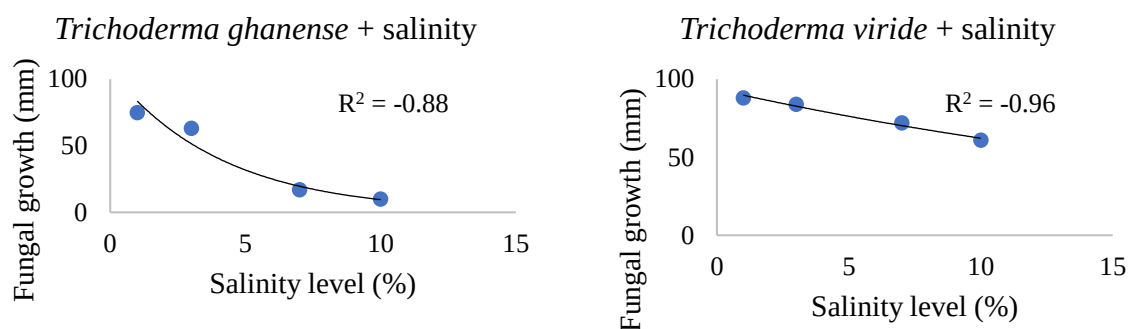


Figure 8.1 Effect of different levels of salinity on fungi radii growth (mm) at eight days incubation period.

Aspergillus flavus exhibited decreased radii as salt concentration increased (Figure 8.2)

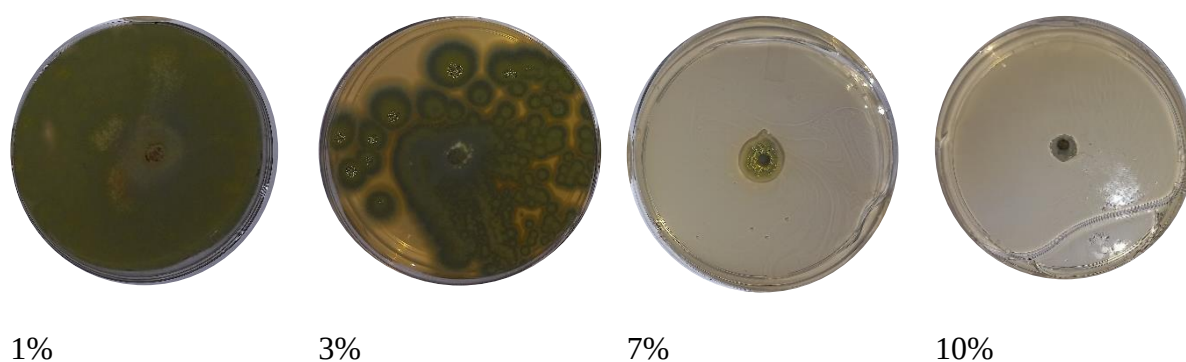


Figure 8.2 *Aspergillus flavus* radii growth under salt-amended PDA at 21 days after incubation.

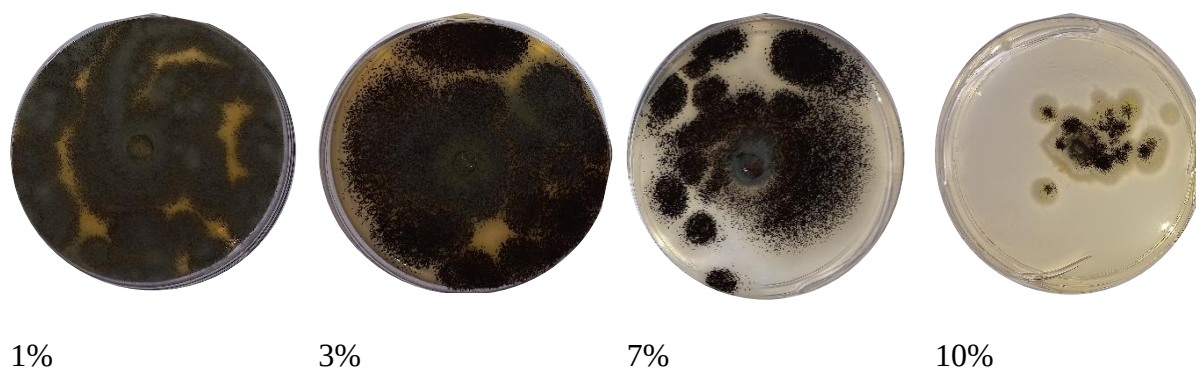


Figure 8.3 *Talaromyces minioluteus* radii growth under salt-amended PDA at 21 days after incubation.

There were highly significant differences ($P \leq 0.01$) on all salinity levels, but no significant differences in control. All eight fungal species thrived at 1% salt concentration, with *Tal. sayulitensis* showing the least tolerance to salt stress than the other species (Table 8.1).

Table 8.1 Fungal growth (mm) at different doses of salinity-amended PDA at 21 days after incubation.

Fungi	Control (0%)	1%	4%	7%	10%
<i>A. flavus</i>	88.20a ^z ± 1.78	74.80b ± 2.86	71.20b ± 1.64	21.80c ± 0.84	14.80c ± 0.44
<i>A. terreus</i>	89.80a ± 2.48	53.00e ± 2.44	49.40c ± 15.02	17.00de ± 0.70	12.80d ± 0.83
<i>Penicillium</i> sp.	88.20a ± 1.48	62.40c ± 2.41	43.40c ± 2.30	17.60de ± 1.67	12.20d ± 0.83
<i>Tal. Minioluteus</i>	88.20a ± 5.63	58.60d ± 1.14	48.20c ± 3.19	20.80c ± 1.48	13.00d ± 0.70
<i>Tal. Purpureogenus</i>	86.20a ± 3.89	74.60b ± 1.81	66.40b ± 1.14	63.80b ± 1.92	35.60b ± 1.14
<i>Tal. Sayulitensis</i>	82.20a ± 2.38	26.60f ± 1.34	14.00d ± 0.00	12.20f ± 0.44	10.00e ± 0.70
<i>Tri. Ghanense</i>	89.20a ± 1.09	75.40b ± 2.30	65.80b ± 1.92	15.80e ± 0.83	11.80d ± 1.64
<i>Tri. Viride</i>	89.60a ± 6.02	74.40b ± 0.89	84.80a ± 1.30	71.40a ± 1.67	63.60a ± 1.81
HSD _{0.05}	n.s. ²	2.54	7.29	1.63	1.45
P	0.16	< 0.01	< 0.01	< 0.01	< 0.01
F	81.57	472.42	74.04	1720.45	1395.20
^z Column means followed by the same letter were not different according to Tukey's HSD at the probability level of 5%, average ± standard deviation error.					
² n.s. = not significant.					

8.4.2 Response of fungal species to pH

From the *Talaromyces* genus, *Tal. purpureogenus* adapted to changing pH levels compared to *Tal. minioluteus* and *Tal. sayulitensis*. Both *Aspergillus* species halted their radial growth under pH 7.00 (Figure 8.4).

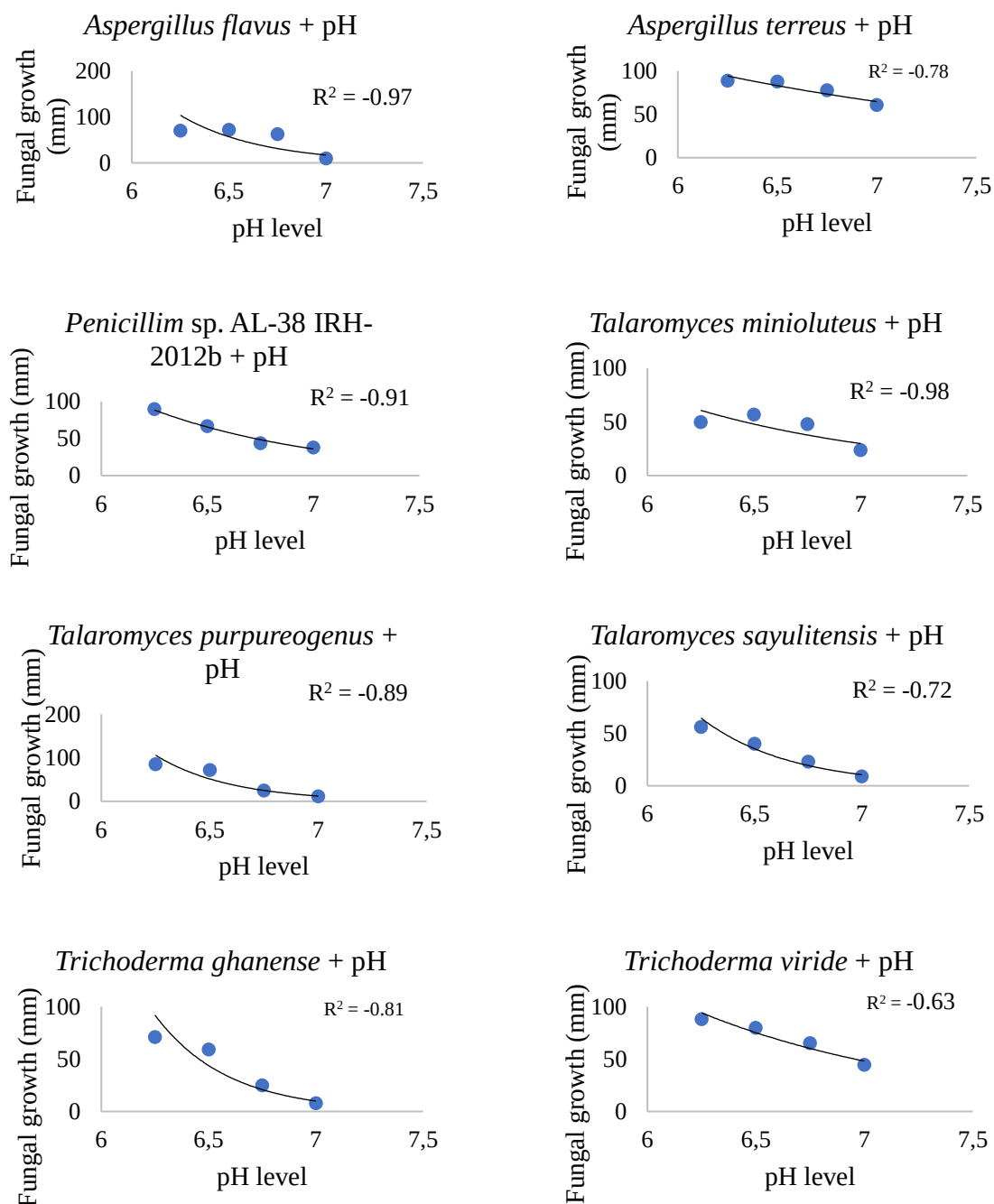


Figure 8.4 Effect of different levels of pH on fungi radii growth (mm) at eight days incubation period.

The radii of species decreased as pH level increased; but *Tal. purpureogenus* was able to grow at a pH of 6.75 (Figure 8.5), with *Tri. viride* demonstrating superior adaptation than the other species (Figure 8.6).

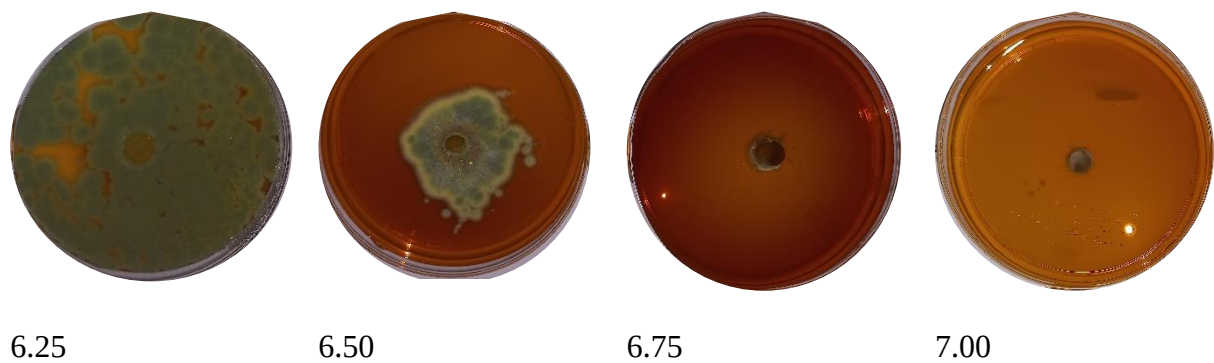


Figure 8.5 *Talaromyces purpureogenus* radii growth under pH-amended PDA at 21 days after incubation.

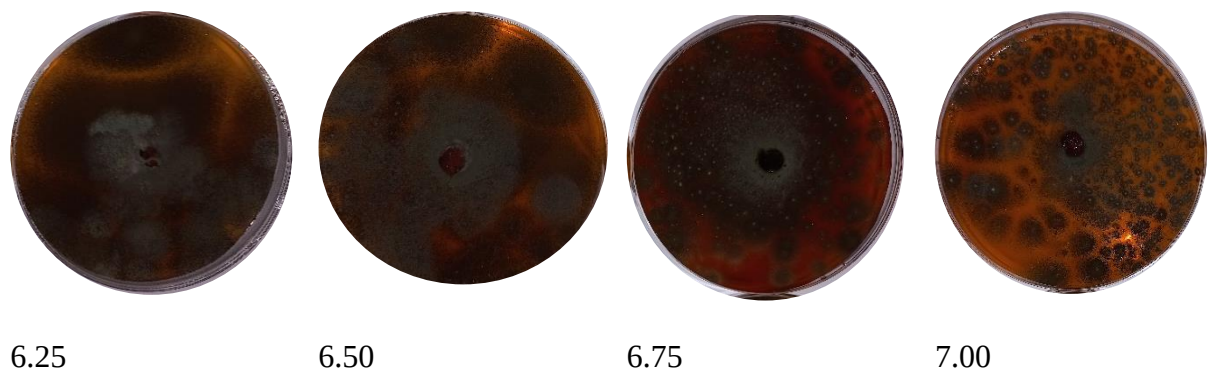


Figure 8.6 *Trichoderma viride* radii growth under pH-amended PDA at 21 days after incubation.

There were highly significant differences ($P \leq 0.01$) amongst all the eight fungal species at all pH levels. Even though *Tri. viride* showed better adaptation to pH levels, *Tri. ghanense* failed to grow from 6.75 pH amended PDA (Table 8.2).

Table 8.2 Fungal growth (mm) at different doses of pH-amended PDA at 21 days after incubation.

Fungi	Control (5.77)	6.25	6.50	6.75	7.00
<i>A. flavus</i>	88.20a ^z ± 1.78	70.40c ± 2.30	72.20c ± 1.30	62.40b ± 2.30	10.00e ± 0.00
<i>A. terreus</i>	89.90a ± 2.48	85.20b ± 3.11	71.80c ± 1.30	14.40e ± 1.14	8.40e ± 4.15
<i>Penicillium</i> sp.	90.00a ± 0.00	85.20b ± 1.48	69.20c ± 1.92	43.60d ± 2.60	36.60c ± 1.67
<i>Tal. Minioluteus</i>	48.20d ± 5.63	51.40d ± 1.14	56.60d ± 1.14	50.40d ± 1.81	25.20d ± 1.30
<i>Tal. Purpureogenus</i>	86.20ab ± 3.89	88.80a ± 1.30	83.60b ± 4.39	88.60a ± 2.19	62.20b ± 1.64
<i>Tal. Sayulitensis</i>	82.20b ± 2.38	37.00f ± 1.58	37.60e ± 1.94	53.00c ± 2.23	9.00e ± 0.70
<i>Tri. Ghanense</i>	89.20a ± 1.09	71.20c ± 1.30	70.60c ± 1.10	9.60f ± 0.54	10.00e ± 0.00
<i>Tri. Viride</i>	74.60c ± 6.02	88.60a ± 1.34	81.60b ± 1.14	61.20b ± 4.65	79.80a ± 178
HSD _{0.05}	4.52	2.53	2.41	3.02	2.42
P	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
F	81.57	557.64	401.70	659.48	1080.32

^zColumn means followed by the same letter were not different according to Tukey's HSD at $P \leq 0.05$, average ± standard deviation error.

8.4.3 Response of fungal species to nutrient status

The decrease in nutrient concentration significantly affected the radii of the colonies (Figure 8.7).

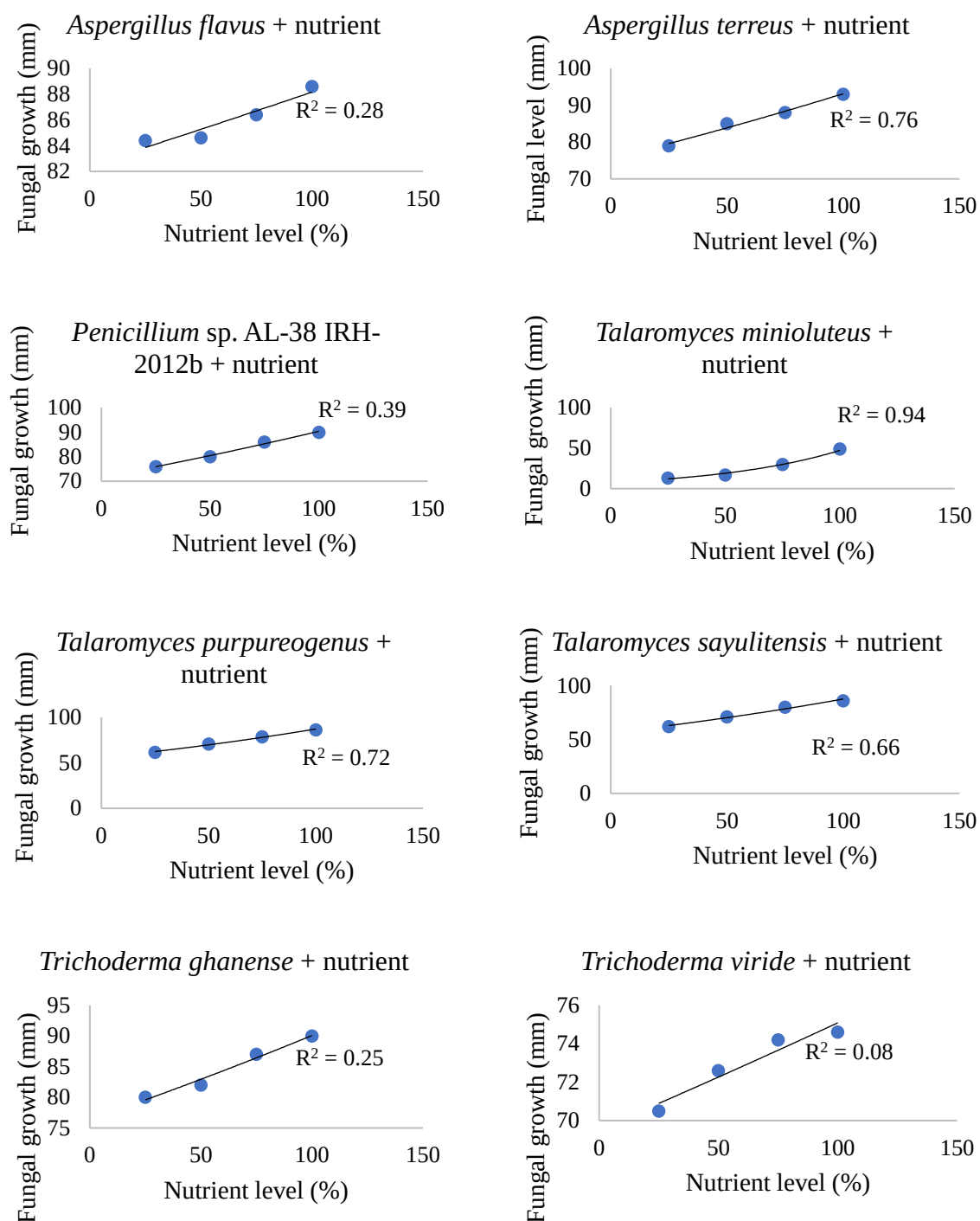


Figure 8.7 Effect of different levels of nutrient on fungi radii growth (mm) at eight days incubation period.

As the concentration of nutrients decreased, the radii of the colonies did not decrease, but their thickness was reduced, with *Tal. sayulitensis* tolerating the nutrient stress better than the other species. (Figure 8.8).

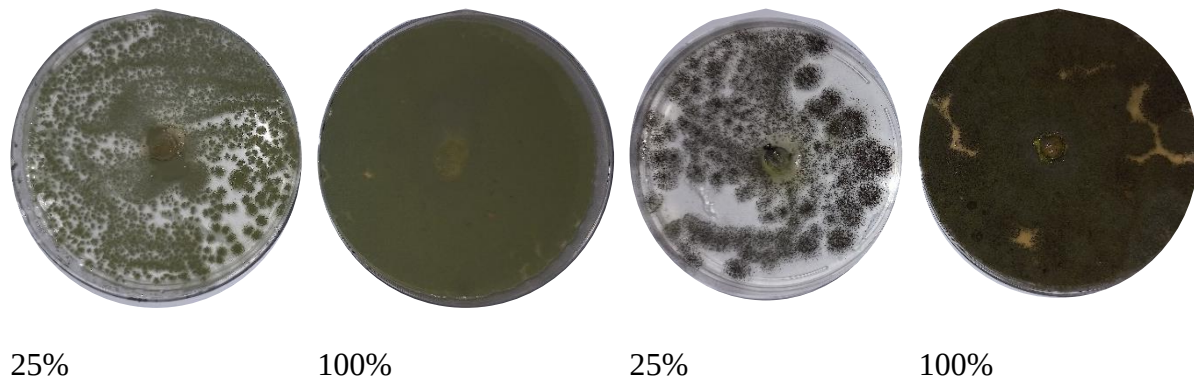


Figure 8.8 *Talaromyces sayulitensis* (left) and *Aspergillus terreus* (right) radii growth under nutrient-amended PDA at 21 days after incubation.

Changes in nutrient status had highly significant differences ($P \leq 0.01$) across all nutrient levels. Even at 100% nutrient status, *Tal. minioluteus* showed the lowest growth rate (Table 8.3).

Table 8.3 Fungal growth (mm) at different doses of nutrient-amended PDA at 21 days after incubation.

Fungi	Control (100%)	25%	50%	75%
<i>A. flavus</i>	88.20a ^z ± 1.78	84.40a ± 2.70	84.60a ± 3.20	86.40a ± 3.04
<i>A. terreus</i>	89.80a ± 2.48	68.80c ± 6.18	84.00a ± 8.21	89.60a ± 0.89
<i>Penicillium</i> sp.	88.20a ± 1.48	75.60b ± 4.27	82.20a ± 1.92	86.40a ± 3.36
<i>Tal. minioluteus</i>	78.20b ± 5.63	14.20e ± 1.30	14.60c ± 1.51	24.60d ± 4.61
<i>Tal. purpureogenus</i>	86.20a ± 3.89	61.60d ± 6.06	70.80b ± 9.73	78.80b ± 3.70
<i>Tal. sayulitensis</i>	82.20a ± 2.38	68.00c ± 6.44	73.20b ± 3.70	82.60a ± 3.74
<i>Tri. ghanense</i>	89.20a ± 1.09	85.40a ± 3.13	88.60a ± 1.94	88.00a ± 3.08
<i>Tri. viride</i>	89.60a ± 6.02	70.00bc ± 6.44	74.20b ± 4.43	72.60c ± 3.43
HSD _{0.05}	4.52	6.28	6.54	4.41
P	< 0.01	< 0.01	< 0.01	< 0.01
F	81.57	107.42	111.42	202.72
^z Column means followed by the same letter were not different according to Tukey's HSD at P ≤ 0.05, average ± standard deviation error.				
² n.s. = not significant.				

8.4.4 Response of fungal species to temperature

Results demonstrated that there was no growth observed on species at temperatures below 10 °C and above 40 °C. However, it was observed that it kept the fungal spores viable. Fungi growth rates increased when the temperature was raised from above 20 °C (Figure 8.9).

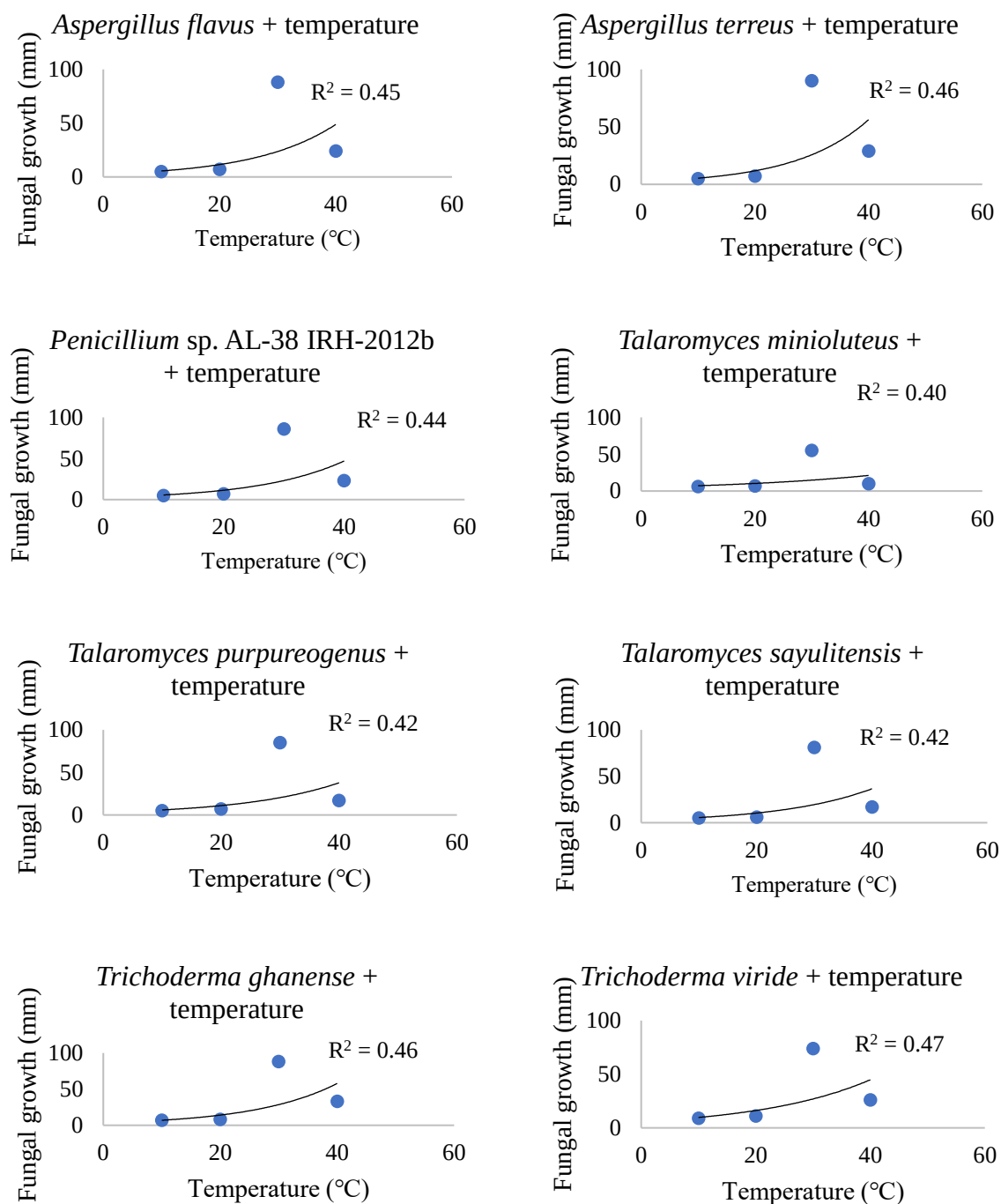


Figure 8.9 Effect of different levels of temperature on fungi radial growth (mm) at eight days incubation period.

Minimal growth was observed on fungal species at temperatures less than 20 °C. In addition, increasing the temperature above 40 °C inhibited mycelial growth. However, it was established that it keeps the fungal spores viable. Fungal growth rates increased when the temperature was raised from above 20 °C. The range between 27-30 °C was found to be ideal for mycelial development (Figure 8.10).

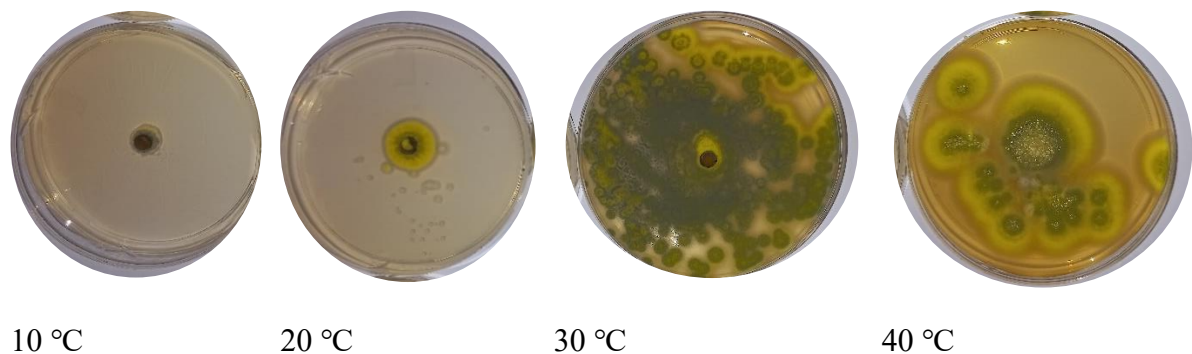


Figure 8.10 *Talaromyces purpurogenus* radii growth under nutrient-amended PDA at 21 days after incubation.

The temperature had highly significant differences ($P \leq 0.01$) across all fungal species, but there were no significant differences in the control. At 10 and 40 °C, none of the fungal species grew optimally. The optimal growth temperatures varied from 27 to 32 °C amongst the eight fungal species (Table 8.4).

Table 8.4 Fungal growth (mm) at different temperatures at 21 days after incubation.

Fungi	Control (27 °C)	10 °C	20 °C	30 °C	40 °C
<i>A. flavus</i>	88.20a ^z ± 1.78	5.00d ± 0.00	7.40cd ± 0.54	82.40a ± 0.85	23.80d ± 0.83
<i>A. terreus</i>	89.80a ± 2.48	5.00d ± 0.00	7.00d ± 0.00	80.60a ± 0.79	29.60b ± 1.51
<i>Penicillium</i> sp.	88.20a ± 1.48	5.00d ± 0.00	7.60c ± 0.54	80.10a ± 0.32	22.00e ± 0.70
<i>Tal. minioluteus</i>	88.20a ± 5.63	5.80c ± 0.44	6.20e ± 0.44	68.20c ± 0.71	9.40h ± 0.54
<i>Tal. purpureogenus</i>	86.20a ± 3.89	5.20d ± 0.44	6.20e ± 0.44	78.10b ± 0.87	47.20a ± 0.83
<i>Tal. sayulitensis</i>	82.20a ± 2.38	5.00d ± 0.00	6.20e ± 0.44	76.30b ± 0.46	15.80g ± 2.38
<i>Tri. ghanense</i>	89.20a ± 1.09	6.60b ± 0.54	8.20b ± 0.11	82.20a ± 0.54	32.00b ± 0.70
<i>Tri. viride</i>	89.60a ± 6.02	8.80a ± 0.72	12.00a ± 0.70	74.60b ± 1.05	26.60c ± 1.14
HSD _{0.05}	n.s. ²	0.40	0.58	4.52	1.34
P	0.16	< 0.01	< 0.01	< 0.01	< 0.01
F	81.57	93.04	90.43	81.57	265.16

^zColumn means followed by the same letter were not different according to Tukey's HSD at $P \leq 0.05$, average ± standard deviation error.

²n.s. = not significant.

8.5 Discussion

8.5.1 Fungal species' tolerance to salinity

Results from the current study showed that the growth of *Tal. purpurogenus* and *Tri. viride* can be promoted by 10% NaCl; making them tolerant and highly tolerant, respectively. However, as incubation time increased, the fungal growth gradually decreased for seven species, except for *Tri. viride*; which exhibiting remarkable NaCl tolerance. As previously described by Pirhadi *et al.* (2016), fungal species were classified as highly tolerant, tolerant, moderately tolerant, or sensitive based on how much radial growth was inhibited on salt-amended PDA media in comparison to pure PDA media.

The salt amendment media resulted in a gradual decrease in mycelial development and sporulation of *A. flavus*, *A. terreus*, *Penicillium* sp., *Tal. minioluteus*, *Tal. sayulitensis* and *Tri. ghanense*, making them sensitive. This observation shows that different fungal species from the same genus can have different tolerance capacities for the same salinity level, which could be attributed to different metabolites from individual species. For instance, Zhang *et al.* (2022) observed that two species from the same genus, *Tri. harzianum* SS and *Tri. harzianum* ST, responded differently to different salt stress levels, with *Tri. harzianum* ST responding the most consistently. This has further been corroborated in field studies where *Tri. asperellum* RM-28, which tolerates saline (4% NaCl) environments, improved the growth and resistance of sorghum-sudangrass seedlings (Anam *et al.*, 2019). Similarly, Zhang *et al.* (2022) observed that Cucumber (*C. sativus* 'Chuanlv 21') leaves' total chlorophyll content dropped from 1.29 mg g⁻¹ to 1.00 mg g⁻¹ when NaCl concentrations increased (0, 100 and 200 mM). Application of *Tri. atroviride* HN082102.1, however, caused total chlorophyll content to rise by 16.57, 13.93 and 19.68%. Accordingly, all the tested indigenous fungal species have the potential to

be applied for crop improvement and protection in soils with approximately 4% (700 mM) salinity.

Salinity is one of the most pervasive factors limiting crop production and slowing the development of BCA (Regragui *et al.*, 2003). High salt levels in the soil cause limited water and nutrient absorption, reducing germination and seedling development (Machado & Serralheiro, 2017). *Trichoderma viride* exhibited saline-stress tolerance, showing better performance in another previous study (Guo *et al.*, 2018). Given that the current findings show that the presence of NaCl in media had no detrimental effect on the tested *Tri. viride* and *Tal. purpurogenus* in terms of growth and sporulation, it follows that saline soils may not slow down their antagonistic effect on pathogens. This means that these fungal species could successfully thrive in high-saline soils, colonise the rhizosphere and subsequently could protect plants from both biotic and abiotic stressors. In an *in vitro* and *in vivo* study, a salt tolerant *Trichoderma* significantly lowered biotic (*F. oxysporum* f.sp. *ciceri*) and abiotic (salinity) stressors in chickpea (Rawat *et al.*, 2013).

8.5.2 Fungal species' tolerance to pH

Trichoderma ghanense and *Tri. viride* were more tolerant to a wide pH range followed by *Penicillium* sp. and *Tal. minioluteus*. This could be the attribution of the production of different enzymes and other metabolites such as alkaloids, terpenoids and esters. These metabolites are known to aid in adapting to a wide range of pH and promote greater mycelial development and biological activity (Kredics *et al.*, 2003). *Trichoderma*, *Penicillium* and *Talaromyces* species' potential as inoculant BCA in soils where changing pH is anticipated is supported by their improved tolerance and survival at increased pH levels.

Since only species with tolerance capabilities grow in alkaline environments, the mycelia produced by *Tri. ghanense* and *Tri. viride* make them viable as potential candidates for use in soils with diverse pH levels. Additionally, both *Tri. ghanense* and *Tri. viride* changed the pH of the medium to levels close to neutrality, i.e., from 7.50 to 6.10, which is an impressive development that sets them apart from the other examined species. Given that the influence of pH stress reduces the generation of the mycelium, as observed in the failure of *Talaromyces*' growth at 6.50-7.00 pH level. This provides evidence to support the use of *Tri. ghanense* and *Tri. viride* in crop production under alkaline soils. This is because not only did they prove adaptable to alkaline pH, but they also altered the pH to an extent that is more suitable for most crops (Altomare *et al.*, 1999).

Cabral-Miramontes *et al.* (2022) determined that, *Tri. harzianum* is adaptable to high pH, like *Tri. ghanense* and *Tri. viride* from the current study, it exhibited the ability to alter the pH of the medium. As a result, the effects of pH did not prevent mycelial development and biomass increase is beneficial in the formation of plant-microbe relationship, resulting in a mutualistic interaction. The interaction, longevity and proliferation of the plant or the microbe are influenced by the physicochemical characteristics of the rhizosphere. Neutral pH is one of the factors that controls the persistence of the mutualistic interaction (Husson, 2013; Weil & Brady, 2017).

Current results demonstrated that the two *Trichoderma* species exhibited hyphal growth within 48 h of incubation at a pH of 7.00. On the other hand, *Aspergillus* and *Talaromyces* species were growing at a minimal rate. This was true for all four pH levels. *Trichoderma* is one type of fungal genus with species that adapts to a variety of pH-related growth conditions (Miao *et al.*, 2020). The generation of hyphae and conidia is unaffected by neutral pH levels, as indicated

in Figure 8.6. Most *Trichoderma* strains prefer a slightly acidic pH with optimal growth and conidiospore generation in the range of 4.6 and 6.8 (Kumar, 2013; Kumari *et al.*, 2014; Singh *et al.*, 2014; Zehra *et al.*, 2017).

Measuring the growth of the biomass and variations in the pH are vital, to determine whether or not the fungal species in question has the potential to alter the pH of its growth media and in essence of its rhizosphere. According to research by Limón *et al.* (2004), fungal development is more favoured by an acidic pH than an alkaline one. Past studies have reported on several fungal species, including *Tri. viride* (Verdin *et al.*, 2004) and *A. niger* (Srivastava & Thakur, 2006), that have been successfully cultured in mineral salt media at pH 5.5. Under *in vitro* conditions, *Tri. harzianum* T22 Rifai acidified the media, allowing solubilisation as metallic Mn, Fe, Cu and Zn oxides and phosphate rock (Altomare *et al.*, 1999).

8.5.3 Fungal species' tolerance to nutrient deficiency

The fungal species morphology was examined under a compound microscope (ZEISS, Research Microscopy Solutions, United Kingdom) at 63 µm. It was observed that the hyphae in the 100% nutrient level had distinct and turgid hyphal structures. Of all the test species, *Tal. minioluteus* had the least growth of 14.20, 24.60 and 24.60 mm at 25, 50 and 75% nutrient status, respectively. On the other hand, *Tal. purpureogenus* and *Tal. sayulitensis* had a better growth response averaging between 61.60 and 82.60 mm under the same nutrient status. Species from the same genus may respond differently to nutrient source availability. This might be because, in addition to nutrient sources, fungal growth is governed by other factors such as media pH.

Taking into account the fact that some soils typically lack nutrients, and if a fungal BCA cannot grow quickly and well in a nutrient-poor environment, they might not be able to successfully establish in the growth site and outcompete other local microbes. Such BCA with slow growth may also not survive in the soil for a long period. Important bio-control techniques involve BCA competition for the growing site and colonising it (Contreras-Cornejo *et al.*, 2016). By functioning as the primary decomposers of plant biomass, storing enormous amounts of nutrients in their mycelium and assisting in the movement of those resources between the soil's organic and mineral fractions, fungi are important players in the soil nutrient cycles. The majority of the nutrients absorbed by plants are provided by soil fungi through their relationships with plant roots (Gadd, 2006).

8.5.4 Fungal species' tolerance to different temperatures

The findings revealed that *Tal. purpureogenus* had the largest radial growth (47 mm) at 40 °C. The ability of *Tal. purpureogenus* to grow in a variety of thermal ranges is evident, with greater growth occurring at relatively high temperatures. *Trichoderma* species showed the best growth at 27 and 30 °C; with the highest temperature for *Trichoderma* species growth observed at 32 °C when grown on PDA. The standard incubation temperature for fungal growth such as *Aspergillus*, *Trichoderma* and *Penicillium* is 30 °C (Santos & Linardi, 2004). Similarly, Affokpon *et al.* (2015) reported the same observations wherein *in vitro* sporulation and mycelial development were both greatest at 28 °C.

Most species produced the most spores when incubated at this temperature in the dark. Following three weeks of incubation, fungal species were able to develop normally and distinguished by strongly stimulated sporulation. One of the species' survival mechanisms observed in cold conditions, such as those between 4 and 5 °C, is the induction of

chlamydospores; which was also observed. *Trichoderma* species and *Tal. purpureogenus* spores demonstrated better heat resistance at 40 °C, in contrast with other test species. This finding suggested that *Trichoderma* species spores are heat-tolerant at temperatures between 45 and 75 °C. The presence of stability to temperature fluctuations in fungal BCA is an important criterion for the organism's survival in harsh conditions.

8.6 Conclusion

The tested fungal species might be employed to increase plant growth in sustainable agriculture. Selecting BCA that are effective in a wide range of conditions, such as soil texture, moisture, temperature extremes and competition, is essential to ensuring efficient bio-control. The tolerance of BCA to harsh and sudden fluctuating climatic conditions is a significant challenge to the transition from conventional to organic agriculture, even more so now due to climate change. The fact that *Tal. sayulitensis*, *Tal. purpureogenus*, *Tal. minioluteus*, *A. flavus*, *A. terreus*, *Tri. viride*, *Tri. ghanense* and *Penicillium* sp. live a multitrophic existence in nature; one that includes soil saprophytism, nematode egg parasitism and plant root endophytism indicates that they can adapt throughout time and different agro-ecological regions.

CHAPTER 9: EFFICACY OF INDIGENOUS FUNGI ON

MELOIDOGNYE UNDER POTATO

9.1 Introduction

The RKN of the *Meloidogyne* genus is one of the most significantly destructive PPN in the world, endangering the establishment and production of over 5500 plants, including vegetable crops and weeds. *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla* are the four most commonly reported RKN species. *Meloidogyne enterolobii* is an aggressive RKN species that is being studied for the characterisation and/or establishment of management practices (da Silva Campos *et al.*, 2017). In South Africa, RKN is among the relevant and damaging groups of PPN and is widely distributed throughout potato-producing regions (Marais *et al.*, 2015).

Given the significant economic effect of RKN, a variety of nematode-control techniques, including the use of chemical nematicides, have been developed. The loss of pesticides as a result of EU laws (EC No. 1107/2009) has nonetheless increased the need to implement effective nematode (biological) control (Zhang *et al.*, 2017). Pesticides are hazardous to human health and the environment. A considerably safer and more workable option for managing PPN is being explored, utilizing bio-control strategies. Currently, a diverse array of species, including fungi, bacteria, viruses, protists, nematode antagonists and other invertebrates, are known to serve as BCA against RKN (Abd-Elgawad & Askary, 2018); Zeilinger *et al.*, 2016).

Small peptide effectors and enzymes, among other bioactive compounds secreted by fungal companions, aid in colonisation and foster symbiotic and pathogenic partnerships by promoting both pathogen defence and symbiotic associations (Zeilinger *et al.*, 2016). Nematophagous fungi of the genera *Aspergillus*, *Talaromyces* and *Trichoderma* are promising BCA that have

demonstrated effective antagonistic effects against a wide range of PPN (Abd-Elgawad & Askary, 2018). Nevertheless, because the soil is a dynamic matrix, the level of RKN mitigation may be modified by many factors. Temperature, moisture, soil texture and structure, nematode density proliferation and fungal species of exotic BCA may all affect the efficiency of fungal BCA *in situ*. Under certain conditions, the product is rendered ineffective if it fails to adapt to drastically shifting environmental circumstances. Indigenous BCA, on the other hand, can easily adapt to such environments.

Soil amendments, to encourage the activity of indigenous soil microbes, are some forms of biological control by which a multitude of natural enemies suppress RKN. It has been demonstrated that adding organic material to the soil is advantageous for soil fertility, plant development and disease control (El-Nagdi & El Fattah, 2011; Abu-Shahba *et al.*, 2021). Different organic materials, including animal waste, organic fertiliser, litterfall and organic additions with nemato-toxic characteristics against nematodes are utilised to create OC (El-Nagdi & El Fattah, 2011). Through several processes, including (i) the formation of nematode-killing compounds during decomposition, (ii) better soil physico-chemical characteristics and (iii) increased microbial activity, these organic supplements compete with the RKN (Aoudia *et al.*, 2012).

The objective was to determine effects of fungal species on *M. enterolobii* and *M. javanica* population densities on susceptible potato cultivar with and without compost incorporation under net house and field conditions.

9.2 Study site

The study was conducted under net house conditions at the Agricultural Research Farm, Plant Pathology Research laboratory, NWU, Mmabatho, Molelwane, North-West Province, South

Africa (25°48'36"S, 25°37'47.999"E) in summer (February-April) 2022 and repeated during spring (September-November) 2022.

9.3 Description of the experiments

9.3.1 Treatments and experimental design

The experiments were conducted using potato cultivar 'Hertha', a cultivar that showed highest susceptibility to the two *Meloidogyne* species as reported in **Chapter 3**. Four parallel experiments, two of *M. enterolobii* on potato cultivar 'Hertha' with and without OC incorporation, and two of *M. javanica* on the same potato cultivar with and without OC incorporation were conducted under net house conditions. Five fungal species were selected based on efficacy to parasitise *Meloidogyne* eggs + J2s in **Chapter 5** and not pathogenic to seeds in **Chapter 6**.

Fungi inoculum preparation and inoculum procedure: In each experiment, seven treatments; *A. terreus*, *Tal. minioluteus*, *Tal. purpureogenus*, *Tal. sayulitensis*, *Tri. viride* and negative controls (un-inoculated plants and inoculated with nematodes alone) were replicated five times and laid out in RCBD. Each potato was inoculated with 2500 ± 20 of *M. enterolobii* or *M. javanica* eggs + J2s. Isolates of *Meloidogyne* species were raised on nematode-susceptible tomato cultivar 'Hotstuff'. The inoculum was prepared by extracting eggs + J2s from tomato roots in a 1% NaOCl solution (Coyne *et al.*, 2007). In a similar way to net house experiments, four parallel experiments were laid out and the same nematode extraction and inoculation procedures were repeated, with the exception that the seeds were planted in the soil bed at 60 cm intra-row spacing and 1 m inter-row spacing. Red sorghum grains, which showed the fastest growing rate in **Chapter 7**, were used to mass-produce the five fungal species. Sorghum grains were steeped in water at a volume ratio of 2:1 (water: sorghum) for 12 hours.

Excess water was drained and the grains were sealed in polypropylene bags and autoclaved at 121 °C for 20 minutes at 1 atmosphere pressure. The materials were allowed to cool to room temperature being inoculated with the fungal colonies that had been growing for 14 days on red sorghum (Cumagun & Moosavi, 2015). To get inoculum powder, the substrate was incubated for 14 days, air dried, milled and put through top-down 80- and 50-mm mesh sieves at the same time. Talc was added to the fungal inoculum from the powdered sorghum grains in a 1:2 ratio, allowed to dry at room temperature and mixed to create the final product. Colony-forming units of the various fungal species were estimated by suspending 1 g of dry product that had been mass-produced on substrates and serially diluting the powder (Singh *et al.*, 2016).

Compost preparation: Culterra compost was bought from Game warehouse, Mmabatho, North-West Province, South Africa. The compost was first pasteurised at 121 °C for 90 minutes. This was done to ensure that the microbial content of the compost was the respective fungal species inoculated. After cooling down, 80 g of compost was incorporated in 30-cm diameter planting pots for net house experiments and in seed planting holes in field experiments and thoroughly mixed with the soil. Each pot was filled with a 1:1 ratio (v/v) mixture of 10 L steam pasteurised Hutton series with a red loam field + river sand (72% sand, 20% silt, 8% clay) with a pH (H₂O) 6.25.

9.3.2 Experimental procedures

The pots were placed in the net house at 0.5 m inter-row and 0.6 m intra-row spacing. Potato seeds ‘Hertha’G0, were sown in planting pots and each seedling was inoculated with 2500 ± 20 of *M. enterolobii* or *M. javanica* eggs + J2s using a 20 mL plastic syringe by placing into 5 cm holes on cardinal points of the stems and covered with growing medium seven days after emergence of leaves. Irrigation was applied in all trials by pouring 500 mL tap water every

second day into the tray of each pot from a watering can. Plants in all trials were sprayed with mercaptothion (Malasol[®] /Malathion[®]) and tetradifon (Redspidercide[®]) alternatively every two weeks as preventative control of aphids and red spider mites, respectively. The net house experiments were terminated 56 days after inoculation with nematodes. Treatments, water regime and insect control were the same as described in **Chapter 3** for the net house experiments. Four parallel experiments were laid out and the same nematode extraction and inoculation procedures were repeated, except that the seeds were sown in the ground at 10 cm intra-row spacing and 1 m inter-row spacing. The field experiments were terminated 110 days after planting, a period to allow for potato maturity.

9.3.3 Data collection

At 56 days after inoculation with nematodes, shoots were cut at the soil level and root systems were removed from the pots and then immersed in water to remove soil particles. Chlorophyll content was measured using a chlorophyll meter (MINOLTA, SPAD-502) and stem diameter was measured at soil level using a digital vernier caliper. Shoots were cut at the soil level and were oven-dried for 72 hours at 52 °C for dry matter determination. Fully developed root galls were assessed using the NCDS at 0=no galls, 1=1-2 galls, 2=3-10 galls, 3=11-30 galls, 4=31-100 galls and 5 > 100 galls per root system (Taylor & Sasser, 1978). Nematodes were extracted from the total root system per plant by maceration and blending for 30 seconds in a 1% water solution of NaOCl (Coyne *et al.*, 2007). The aliquot was passed through top-down nested 63 and 25 µm screen open sieves. Contents of the 25 µm screen sieve were poured into 100 mL plastic containers for counting under a stereomicroscope.

Soil per pot was thoroughly mixed and 200 mL of soil sample was collected for analysis. Nematodes were extracted from soil samples using the modified sugar-floatation and

centrifugation method (Jenkins, 1964). The soil was poured into a 4 L bucket with 2 L tap water and stirred, once the swirl had stopped, the aliquot was poured through top-down nested 63 and 25- μm screen open sieves. The contents of the 25 μm screen sieve were poured into 50 mL plastic centrifuge tubes. Approximately 3 g kaolin was added to each tube and the contents were centrifuged at 1750 RPM for 5 min. Kaolin solution was then decanted with nematodes having settled at the bottom of the tubes with soil particles. A 480 g sugar/l L tap water was poured into the centrifuge tubes and vigorously shaken to mix the settled soil with the sugar solution prior to centrifuging for 60 seconds at 1750 RPM. The aliquot was decanted again onto a 25- μm sieve with sugar being rinsed off the nematodes, which were then collected from the sieve into 100 mL plastic containers for counting under a stereomicroscope. Nematode numbers were converted to 5 L soil per pot to estimate Pf. *Meloidogyne* RF = Pf/Pi, which is a proportion of Pf and Pi, was computed for each inoculum level.

9.3.4 Data analysis

Data were subjected to using SAS software (SAS Institute, Inc., Cary, NC., USA, 2008). Nematode data was transformed through transformed through $\log_{10}(x + 1)$ and were subjected to stepwise regression analysis in order to determine the relation among different nematode Pi levels. Also, growth variables were subjected to stepwise regression analysis in order to determine the effect of the two nematode species and nematode Pi on potato growth (Gomez & Gomez, 1984). Mean separation for significant ($P \leq 0.05$) treatments was achieved through Tukey's HSD. Unless otherwise stated, only predictive models which were significant at the probability level of 5% were discussed.

9.4 Results

9.4.1 Effect of fungi on *Meloidogyne* species on potato ‘Hertha’ under net house conditions

Table 9.1 represents data on the effect of fungal species on nematode parameters. *Talaromyces sayulitensis* had significantly higher nematotoxic activity ($P \leq 0.01$); resulting in the lowest root gall values of 2.60 ± 1.14 and 2.20 ± 0.44 on *M. enterolobii* and *M. javanica*, respectively. *Talaromyces purpureogenus* demonstrated the least antagonistic activity toward *M. enterolobii* J2s, whilst *Tri. viride* demonstrated the least antagonistic activity toward *M. javanica* J2s. When compared to the negative controls, the Pf of the two *Meloidogyne* species was substantially reduced ($P \leq 0.05$) across all treatments; all resulting in RF of < 1 (Table 9.1).

Table 9.1 Effects of nematophagous fungi on *Meloidogyne enterolobii* and *Meloidogyne javanica* with and without organic compost (OC); (i) number of galls, (ii) final population (Pf) and (iii) reproductive factor (RF) at 56 days after inoculation under net house conditions.

<i>Meloidogyne enterolobii</i> and nematophagous fungi with OC			
Treatment	Pf	Gall	RF
+ Control (P)	$0.00e^z \pm 0.00$	$0.00d \pm 0.00$	$0.00d \pm 0.00$
– Control (P + N)	$8611.00a \pm 40.79$	$4.20ab \pm 1.30$	$5.61a \pm 0.04$
<i>A. terreus</i>	$364.00de \pm 100.10$	$3.20a \pm 0.82$	$0.36c \pm 0.10$
<i>Tal. minioluteus</i>	$480.20d \pm 143.01$	$3.00b \pm 1.41$	$0.48c \pm 1.14$
<i>Tal. purpureogenus</i>	$823.40b \pm 66.73$	$3.40a \pm 1.14$	$0.82b \pm 0.06$
<i>Tal. sayulitensis</i>	$328.20de \pm 16.93$	$2.20c \pm 0.44$	$0.32c \pm 0.02$
<i>Tri. viride</i>	$777.20c \pm 118.18$	$3.60a \pm 1.14$	$0.77b \pm 0.12$
HSD _{0.05}	32.47	1.10	2.12
P	0.03	0.01	0.03
F	4.65	6.56	4.66
+ Control (P)	$0.00d \pm 0.00$	$0.00c \pm 0.00$	$0.00d \pm 0.00$

<i>Meloidogyne enterolobii</i> and nematophagous fungi without OC			
Treatment	Pf	Gall	RF
– Control (P + N)	10660.80a ± 457.30	5.20a ± 2.86	6.66a ± 0.45
<i>A. terreus</i>	468.60c ± 31.54	3.40a ± 1.51	0.46c ± 0.03
<i>Tal. minioluteus</i>	529.00c ± 118.66	3.20ab ± 1.09	0.52 ± 0.11
<i>Tal. purpureogenus</i>	927.60b ± 36.55	3.60a ± 1.14	0.92b ± 0.03
<i>Tal. sayulitensis</i>	405.60cd ± 45.04	2.60b ± 1.14	0.40c ± 0.04
<i>Tri. viride</i>	814.40b ± 126.50	3.40a ± 0.89	0.81b ± 0.12
HSD _{0.05}	24.32	2.84	2.45
P	0.02	0.04	0.02
F	5.21	0.83	5.25
<i>Meloidogyne javanica</i> and nematophagous fungi with OC			
+ Control (P)	0.00e ± 0.00	0.00d ± 0.00	0.00c ± 0.00
– Control (P + N)	4541.00a ± 51.52	8.80a ± 1.3	4.44a ± 0.05
<i>A. terreus</i>	141.40d ± 21.50	1.60c ± 0.54	0.14bc ± 0.02
<i>Tal. minioluteus</i>	261.20c ± 52.96	2.20b ± 0.3	0.26bc ± 0.05
<i>Tal. purpureogenus</i>	339.40c ± 71.45	2.20b ± 0.84	0.33b ± 0.07
<i>Tal. sayulitensis</i>	418.62b ± 68.7	3.80b ± 1.37	0.71b ± 1.27
<i>Tri. viride</i>	333.00c ± 136.61	2.40b ± 0.89	0.33b ± 0.13
HSD _{0.05}	25.41	2.74	2.11
P	0.03	0.04	0.03
F	5.47	2.31	4.77
<i>Meloidogyne javanica</i> and nematophagous fungi without OC			
+ Control (P)	0.00e ± 0.00	0.00d ± 0.00	0.00c ± 0.00
– Control (P + N)	5524.00a ± 51.52	4.40a ± 2.91	5.54a ± 0.05
<i>A. terreus</i>	305.40cd ± 56.90	2.20c ± 0.44	0.30bc ± 0.05
<i>Tal. minioluteus</i>	401.20c ± 109.47	2.20c ± 0.83	0.40b ± 0.11

<i>Meloidogyne javanica</i> and nematophagous fungi without OC			
Treatment	Pf	Gall	RF
<i>Tal. purpureogenus</i>	479.40c ± 61.37	2.00c ± 0.70	0.47b ± 0.06
<i>Tal. sayulitensis</i>	249.00d ± 19.68	3.40b ± 0.54	0.24bc ± 0.01
<i>Tri. viride</i>	673.00b ± 102.77	2.00c ± 0.01	0.67b ± 0.13
HSD _{0.05}	27.14	0.88	1.37
P	0.03	0.05	0.03
F	5.11	0.36	5.12
The results are the mean and standard deviation of five replicates. ² Data with different letters indicates a significant difference at $P \leq 0.05$ according to Tukey's HSD, with average ± standard deviation error.			
P: Plant alone, P + N: Plant and nematodes.			

9.4.2 Response of potato 'Hertha' to *Meloidogyne* species under net house conditions

Meloidogyne enterolobii ± OC: With OC incorporation, fungal species had highly significant differences ($P \leq 0.01$) in plant height and tuber number, significant differences ($P \leq 0.05$) in stem diameter, chlorophyll content, dry weight and tuber number. However, there were no significant differences in fresh weight. Without OC, treatments had significant differences ($P \leq 0.05$) on all potato growth parameters, but no significant differences on fresh weight (Table 9.2).

Meloidogyne javanica ± OC: With the incorporation of OC, treatments had significant differences ($P \leq 0.05$) on all growth parameters, but no significant differences in stem diameter, fresh weight and tuber number. Without OC, fungal species had highly significant differences ($P \leq 0.01$) in plant height and chlorophyll content and significant differences ($P \leq 0.05$) in fresh weight, dry weight, tuber number and tuber weight. However, there were no significant differences in stem diameter.

Table 9.2 Effects of *Meloidogyne enterolobii* and *Meloidogyne javanica* and indigenous nematophagous fungi on potato ‘Hertha’ plant height (PH), stem diameter (SD), chlorophyll content (CC), shoot fresh weight (FW), shoot dry weight (DW), tuber number (TN) and tuber weight (TW) with and with and without organic compost (OC) at 56 days after inoculation under net house conditions.

<i>Meloidogyne enterolobii</i> and nematophagous fungi with OC							
Treatment	PH (cm)	SD (mm)	CC ($\mu\text{mol m}^{-2}$)	FW (g)	DW (g)	TN	TW (g)
+ Control (P)	9.82c ^z ± 8.07	1.86c ± 1.40	80.68c ± 28.08	27.09a ± 5.41	4.79a ± 5.20	2.20d ± 1.78	10.88d ± 10.66
– Control (P + N)	10.82bc ± 0.86	2.52c ± 0.44	65.48d ± 26.85	26.90a ± 2.75	2.34c ± 2.31	4.20b ± 3.03	47.89a ± 12.12
<i>A. terreus</i>	15.12b ± 3.38	4.38a ± 1.16	82.16c ± 31.85	29.55a ± 4.31	1.86d ± 1.47	2.60d ± 0.83	13.23d ± 17.17
<i>Tal. minioluteus</i>	11.28b ± 3.01	2.12c ± 0.59	143.64a ± 59.73	24.52a ± 1.46	2.01c ± 1.94	5.40a ± 1.81	50.37a ± 17.57
<i>Tal. purpureogenus</i>	17.00a ± 7.74	1.98c ± 1.92	81.74c ± 42.26	25.84a ± 5.32	2.28c ± 1.27	3.40c ± 1.94	42.93a ± 13.97
<i>Tal. sayulitensis</i>	20.50a ± 1.34	3.30b ± 0.01	109.80b ± 50.36	29.70a ± 0.12	2.40b ± 0.00	4.00b ± 0.07	34.18b ± 1.34
<i>Tri. viride</i>	10.58bc ± 8.11	1.72c ± 1.28	78.78c ± 37.10	25.89a ± 4.54	3.06b ± 2.11	2.20d ± 0.54	18.05c ± 18.41
HSD _{0.05}	5.25	0.88	10.33	n.s ²	0.56	1.10	7.28
P	< 0.01	0.05	0.04	0.71	0.05	0.02	< 0.01
F	25.65	4.12	0.16	0.14	0.10	1.99	8.35
<i>Meloidogyne enterolobii</i> and nematophagous fungi without OC							
+ Control (P)	9.37b ± 6.39	1.80c ± 1.25	67.44b ± 27.81	19.76a ± 13.22	2.37b ± 1.24	1.75cd ± 2.36	7.44c ± 9.24
– Control (P + N)	9.82b ± 6.11	1.86c ± 0.62	61.85b ± 60.80	28.09a ± 7.63	4.79a ± 1.67	2.20c ± 2.79	10.88c ± 11.98

<i>Meloidogyne enterolobii</i> and nematophagous fungi with OC							
Treatment	PH (cm)	SD (mm)	CC ($\mu\text{mol m}^{-2}$)	FW (g)	DW (g)	TN	TW (g)
<i>A. terreus</i>	19.82a $\pm$ 6.18	3.44a $\pm$ 1.45	73.34b $\pm$ 20.30	25.62a $\pm$ 3.07	2.77b $\pm$ 2.61	3.40b $\pm$ 0.83	42.93a $\pm$ 17.17
<i>Tal. minioluteus</i>	10.82b $\pm$ 2.13	2.52b $\pm$ 1.32	98.04a $\pm$ 23.40	26.06a $\pm$ 2.37	2.34b $\pm$ 1.57	2.20c $\pm$ 1.81	38.05b $\pm$ 17.57
<i>Tal. purpureogenus</i>	21.36a $\pm$ 5.31	3.70a $\pm$ 0.50	64.16b $\pm$ 36.73	27.05a $\pm$ 7.50	2.47b $\pm$ 1.60	4.00b $\pm$ 1.94	47.89a $\pm$ 13.97
<i>Tal. sayulitensis</i>	20.68a $\pm$ 0.30	3.16a $\pm$ 0.28	67.88b $\pm$ 3.29	20.10a $\pm$ 0.87	3.12b $\pm$ 0.37	5.40a $\pm$ 0.89	50.37a $\pm$ 2.80
<i>Tri. viride</i>	11.50b $\pm$ 5.07	2.64b $\pm$ 1.18	98.30a $\pm$ 57.42	28.59a $\pm$ 2.12	2.63b $\pm$ 3.36	2.60c $\pm$ 0.54	38.23b $\pm$ 18.41
HSD _{0.05}	9.87	0.64	12.73	n.s	1.33	0.34	4.64
P	0.04	0.05	0.05	0.63	0.05	0.03	0.03
F	0.12	0.10	0.15	0.23	0.28	1.07	0.45
<i>Meloidogyne javanica</i> and nematophagous fungi with OC							
+ Control (P)	19.82a $\pm$ 6.25	3.44a $\pm$ 1.10	97.02c $\pm$ 16.01	40.62a $\pm$ 9.04	2.77a $\pm$ 2.24	3.40a $\pm$ 0.89	42.93a $\pm$ 19.09
– Control (P + N)	16.52b $\pm$ 4.13	4.47a $\pm$ 1.18	104.18b $\pm$ 48.68	36.46a $\pm$ 8.24	2.05b $\pm$ 2.26	3.60a $\pm$ 0.54	34.18b $\pm$ 20.96
<i>A. terreus</i>	14.36c $\pm$ 8.77	2.68a $\pm$ 2.03	112.28b $\pm$ 62.67	35.79a $\pm$ 5.98	2.80b $\pm$ 2.56	3.80a $\pm$ 0.44	18.04c $\pm$ 9.31
<i>Tal. minioluteus</i>	18.72b $\pm$ 1.75	3.94a $\pm$ 1.81	130.50a $\pm$ 45.15	38.29a $\pm$ 2.21	1.56b $\pm$ 1.18	4.20a $\pm$ 2.17	48.93a $\pm$ 15.66
<i>Tal. purpureogenus</i>	16.26b $\pm$ 3.81	3.54a $\pm$ 1.20	131.04a $\pm$ 72.21	33.30a $\pm$ 5.27	2.03b $\pm$ 1.77	3.40a $\pm$ 0.89	40.03a $\pm$ 18.48
<i>Tal. sayulitensis</i>	26.10a $\pm$ 0.01	3.30a $\pm$ 0.11	112.20b $\pm$ 0.04	29.70a $\pm$ 0.00	2.40b $\pm$ 0.03	4.00a $\pm$ 0.00	31.10b $\pm$ 0.02

<i>Meloidogyne javanica</i> and nematophagous fungi with OC							
Treatment	PH (cm)	SD (mm)	CC ($\mu\text{mol m}^{-2}$)	FW (g)	DW (g)	TN	TW (g)
<i>Tri. viride</i>	16.66b $\pm$ 3.56	3.76a $\pm$ 1.11	96.00c $\pm$ 75.69	38.27a $\pm$ 11.02	3.19a $\pm$ 2.79	3.80a $\pm$ 1.48	32.98b $\pm$ 19.85
HSD _{0.05}	1.88	n.s	10.55	n.s	0.45	n.s	9.41
P	0.04	0.69	0.05	0.15	0.04	0.18	0.05
F	0.56	0.15	0.29	2.10	0.04	1.80	0.09
<i>Meloidogyne javanica</i> and nematophagous fungi without OC							
+ Control (P)	21.36a $\pm$ 2.64	3.70a $\pm$ 0.91	71.52b $\pm$ 28.44	23.05a $\pm$ 7.66	3.12b $\pm$ 3.19	5.40a $\pm$ 1.51	50.37a $\pm$ 20.43
– Control (P + N)	20.68a $\pm$ 2.33	3.16a $\pm$ 1.32	74.00b $\pm$ 13.45	24.95a $\pm$ 16.17	2.47c $\pm$ 2.26	4.00ab $\pm$ 3.46	47.89b $\pm$ 14.76
<i>A. terreus</i>	13.67c $\pm$ 9.01	3.94a $\pm$ 4.91	78.32b $\pm$ 51.33	10.09c $\pm$ 11.16	2.32c $\pm$ 2.64	3.80b $\pm$ 2.16	35.08c $\pm$ 30.36
<i>Tal. minioluteus</i>	13.06c $\pm$ 8.11	2.26a $\pm$ 1.27	89.33b $\pm$ 60.14	19.79b $\pm$ 19.52	4.15a $\pm$ 3.58	2.00c $\pm$ 1.02	40.93b $\pm$ 17.82
<i>Tal. purpureogenus</i>	18.82b $\pm$ 3.56	2.58a $\pm$ 0.88	127.87a $\pm$ 32.02	18.14b $\pm$ 14.48	3.01b $\pm$ 2.99	4.20ab $\pm$ 1.78	57.64a $\pm$ 27.60
<i>Tal. sayulitensis</i>	21.10a $\pm$ 0.01	3.10a $\pm$ 0.11	71.40b $\pm$ 0.06	21.20a $\pm$ 1.36	1.25d $\pm$ 1.28	4.00ab $\pm$ 0.34	48.10b $\pm$ 15.88
<i>Tri. viride</i>	15.14c $\pm$ 6.15	2.66a $\pm$ 1.72	60.28bc $\pm$ 23.89	23.58a $\pm$ 15.74	2.62c $\pm$ 2.09	4.40ab $\pm$ 1.81	20.13d $\pm$ 15.73
HSD _{0.05}	2.59	n.s	11.23	2.41	1.01	1.13	5.55
P	0.01	0.33	< 0.01	0.04	0.03	0.04	0.03
F	6.43	0.97	8.08	0.11	1.49	1.85	4.86

The results are mean and standard deviation of five replicates. ²Data with different letters indicates a significant difference at $P \leq 0.05$ according to Tukey's HSD, with average $\pm$ standard deviation error.

P: Plant alone, P + N: Plant and nematodes.

²n.s. = not significant.

9.4.3 Effect of fungi on *Meloidogyne* species on potato ‘Hertha’ under field conditions

Treatments had significant differences ($P \leq 0.05$) on both *M. enterolobii* and *M. javanica* Pf, gall number and RF with and without compost. Roots under all five fungal species retained considerably lower egg and J2s densities at 56 DAI for both *M. enterolobii* and *M. javanica* than the – control (P + N). Host-status analyses showed that all fungal species examined, except for *Trichoderma* (RF value > 1) for *M. enterolobii*, had lower efficacy in reducing *M. incognita* impacts on potato growth without OC (Table 9.3).

Table 9.3 Effects of nematophagous fungi on *Meloidogyne enterolobii* and *Meloidogyne javanica* with and without organic compost (OC); (i) number of galls, (ii) final population (Pf) and (iii) reproductive factor (RF) under field conditions.

<i>Meloidogyne enterolobii</i> and nematophagous fungi with OC			
Treatment	Pf	Gall	RF
+ Control (P)	0.00e ^z ± 0.00	0.00d ± 0.00	0.00d ± 0.00
– Control (P + N)	3200.80a ± 98.73	9.40a ± 0.89	3.20a ± 0.01
<i>A. terreus</i>	652.60c ± 26.58	6.40b ± 1.140	0.65b ± 0.01
<i>Tal. minioluteus</i>	564.80cd ± 40.03	3.40cd ± 0.54	0.56b ± 0.01
<i>Tal. purpureogenus</i>	764.00c ± 29.25	5.40c ± 0.54	0.76bc ± 0.03
<i>Tal. sayulitensis</i>	323.60d ± 34.35	4.80c ± 0.44	0.32b ± 0.01
<i>Tri. viride</i>	935.20b ± 29.34	5.80c ± 1.30	0.93b ± 0.02
HSD _{0.05}	55.78	1.32	1.27
P	0.02	0.05	0.02
F	5.32	0.17	5.32
<i>Meloidogyne enterolobii</i> and nematophagous fungi without OC			
+ Control (P)	0.00c ± 0.00	0.00c ± 0.00	0.00d ± 0.00
– Control (P + N)	3860.80a ± 26.97	11.60a ± 1.11	3.86a ± 0.02
<i>A. terreus</i>	968.60b ± 31.54	8.00b ± 1.00	0.96c ± 0.02
<i>Tal. minioluteus</i>	935.80b ± 39.30	4.20c ± 0.83	0.93c ± 0.01

<i>Meloidogyne enterolobii</i> and nematophagous fungi with OC			
Treatment	Pf	Gall	RF
<i>Tal. purpureogenus</i>	1287.00b ± 27.52	6.20b ± 0.83	1.28b ± 0.01
<i>Tal. sayulitensis</i>	639.60c ± 21.64	6.40b ± 0.89	0.63c ± 0.02
<i>Tri. viride</i>	1105.20b ± 28.76	7.40b ± 0.89	1.10b ± 0.03
HSD _{0.05}	62.21	2.02	0.21
P	0.02	0.05	0.02
F	5.33	0.45	5.34
<i>Meloidogyne javanica</i> and nematophagous fungi with OC			
+ Control (P)	0.00d ± 0.00	0.00c ± 0.00	0.00c ± 0.00
– Control (P + N)	2261.00a ± 15.31	3.20a ± 1.300	2.26a ± 0.01
<i>A. terreus</i>	145.20c ± 26.55	2.60ab ± 1.14	0.14b ± 0.02
<i>Tal. minioluteus</i>	134.00c ± 28.53	2.00b ± 1.00	0.13b ± 0.02
<i>Tal. purpureogenus</i>	219.80b ± 16.45	2.40ab ± 0.54	0.21b ± 0.02
<i>Tal. sayulitensis</i>	139.00c ± 27.97	2.40ab ± 1.12	0.13b ± 0.03
<i>Tri. viride</i>	359.00b ± 33.96	1.60b ± 0.54	0.35b ± 0.01
HSD _{0.05}	57.63	0.89	1.32
P	0.02	0.02	0.03
F	5.47	2.47	5.47
<i>Meloidogyne javanica</i> and nematophagous fungi without OC			
+ Control (P)	0.00d ± 0.00	0.00b ± 0.00	0.00c ± 0.00
– Control (P + N)	3611.00a ± 40.79	4.20a ± 1.92	3.61a ± 0.01
<i>A. terreus</i>	324.20c ± 16.55	3.40b ± 1.14	0.32b ± 0.04
<i>Tal. minioluteus</i>	246.00c ± 24.78	3.60a ± 1.14	0.24b ± 0.03
<i>Tal. purpureogenus</i>	950.80b ± 26.75	3.20b ± 1.300	0.95b ± 0.00
<i>Tal. sayulitensis</i>	220.20c ± 13.44	3.00b ± 1.00	0.22b ± 0.02
<i>Tri. viride</i>	844.00b ± 33.36	3.20b ± 0.83	0.84b ± 0.02
HSD _{0.05}	57.63	0.74	1.82

P	0.01	0.04	0.03
F	6.17	4.34	0.86

The results are the mean and standard deviation of five replicates. ²Data with different letters indicates a significant difference at $P \leq 0.05$ according to Tukey's HSD, with average $\pm$ standard deviation error.

P: Plant alone, P + N: Plant and nematodes.

²n.s. = not significant.

9.4.4 Response of potato 'Hertha' to *Meloidogyne* species under field conditions

***Meloidogyne enterolobii* $\pm$ OC:** With OC incorporation, fungal species had highly significant differences ($P \leq 0.01$) in plant height, chlorophyll content, fresh weight, dry weight, and significant differences ($P \leq 0.05$) in stem diameter and tuber weight. However, there were no significant differences in tuber number. Without OC, treatments had highly significant differences ($P \leq 0.01$) in plant height, chlorophyll content, fresh weight and dry weight, and significant differences ($P \leq 0.05$) in stem diameter and tuber weight. However, there were no significant differences in tuber number (Table 9.4).

***Meloidogyne javanica* $\pm$ OC:** With the incorporation of OC, treatments had highly significant differences ($P \leq 0.01$) in stem diameter, chlorophyll content, fresh weight, dry weight and tuber number, significant differences ($P \leq 0.05$) in plant height and no significant differences in tuber weight. Without OC, fungal species had highly significant differences ($P \leq 0.01$) in stem diameter, chlorophyll content, dry weight, tuber number and tuber weight. Treatments had significant differences ($P \leq 0.05$) on plant height and fresh weight (Table 9.4).

Table 9.4 Effects of *Meloidogyne enterolobii* and *Meloidogyne javanica* and indigenous nematophagous fungi on potato ‘Hertha’ plant height (PH), stem diameter (SD), chlorophyll content (CC), shoot fresh weight (FW), shoot dry weight (DW), tuber number (TN) and tuber weight (TW) with and with and without organic compost (OC) at 110 days after inoculation under field conditions.

<i>Meloidogyne enterolobii</i> and nematophagous fungi with OC							
Treatment	PH (cm)	SD (mm)	CC ($\mu\text{mol m}^{-2}$)	FW (g)	DW (g)	TN	TW (g)
+ Control (P)	10.33b ^z $\pm$ 0.98	3.46b $\pm$ 0.43	194.18a $\pm$ 4.02	25.52b $\pm$ 1.56	2.01b $\pm$ 0.02	8.40a $\pm$ 1.14	142.28b $\pm$ 21.30
– Control (P + N)	5.21d $\pm$ 0.63	1.56d $\pm$ 0.30	169.90a $\pm$ 3.67	19.90bc $\pm$ 1.23	1.21c $\pm$ 0.13	5.63a $\pm$ 0.54	122.99c $\pm$ 5.70
<i>A. terreus</i>	8.78c $\pm$ 1.06	2.88b $\pm$ 0.16	107.48c $\pm$ 3.81	26.26b $\pm$ 0.80	1.73c $\pm$ 0.24	6.86a $\pm$ 1.09	147.96b $\pm$ 38.92
<i>Tal. minioluteus</i>	18.68a $\pm$ 0.94	4.36a $\pm$ 0.20	150.16b $\pm$ 13.48	37.02a $\pm$ 0.96	3.89a $\pm$ 0.41	6.60a $\pm$ 0.54	153.51a $\pm$ 34.89
<i>Tal. purpureogenus</i>	11.30b $\pm$ 1.65	3.18b $\pm$ 0.10	134.20ab $\pm$ 4.96	32.02a $\pm$ 1.91	2.73b $\pm$ 0.15	6.64a $\pm$ 1.14	149.45b $\pm$ 38.11
<i>Tal. sayulitensis</i>	11.76b $\pm$ 1.06	3.12b $\pm$ 0.49	134.84ab $\pm$ 15.19	33.32a $\pm$ 1.57	2.91b $\pm$ 0.14	6.60a $\pm$ 1.14	109.40d $\pm$ 8.42
<i>Tri. viride</i>	8.58c $\pm$ 0.78	2.04c $\pm$ 0.19	88.06b $\pm$ 1.77	21.86b $\pm$ 0.90	1.37c $\pm$ 0.13	7.22a $\pm$ 0.83	162.47a $\pm$ 9.60
HSD _{0.05}	4.21	0.80	38.63	7.17	1.04	n.s ²	12.81
P	< 0.01	0.05	< 0.01	< 0.01	< 0.01	0.28	0.04
F	11.52	3.67	17.64	15.46	12.25	1.16	4.45
<i>Meloidogyne enterolobii</i> and nematophagous fungi without OC							
+ Control (P)	7.82b $\pm$ 0.94	3.34a $\pm$ 0.16	182.38a $\pm$ 6.04	22.8b $\pm$ 0.68	1.43c $\pm$ 0.27	6.60a $\pm$ 0.54	154.42a $\pm$ 32.38
– Control (P + N)	3.28d $\pm$ 0.31	1.20c $\pm$ 0.29	166.38a $\pm$ 3.66	16.78c $\pm$ 0.82	0.92d $\pm$ 0.29	5.20a $\pm$ 0.44	126.84b $\pm$ 25.83

<i>Meloidogyne enterolobii</i> and nematophagous fungi without OC							
Treatment	PH (cm)	SD (mm)	CC ($\mu\text{mol m}^{-2}$)	FW (g)	DW (g)	TN	TW (g)
<i>A. terreus</i>	6.76b $\pm$ 0.57	1.78c $\pm$ 0.19	94.40c $\pm$ 3.18	20.54b $\pm$ 0.82	1.44c $\pm$ 0.26	5.80a $\pm$ 0.83	131.60a $\pm$ 7.52
<i>Tal. minioluteus</i>	15.70a $\pm$ 0.72	3.90a $\pm$ 0.30	128.32b $\pm$ 3.99	31.76a $\pm$ 1.63	3.46a $\pm$ 0.39	5.60a $\pm$ 0.89	114.58b $\pm$ 7.74
<i>Tal. purpureogenus</i>	8.62b $\pm$ 0.50	2.28b $\pm$ 0.14	111.46a $\pm$ 2.28	23.32b $\pm$ 0.85	2.15b $\pm$ 0.11	5.40a $\pm$ 1.14	111.22b $\pm$ 15.34
<i>T.al sayulitensis</i>	9.38b $\pm$ 0.49	2.68b $\pm$ 0.32	117.24b $\pm$ 1.06	25.30b $\pm$ 1.23	2.41b $\pm$ 0.26	6.80a $\pm$ 0.44	124.55b $\pm$ 56.53
<i>Tri. viride</i>	5.36c $\pm$ 0.26	1.26c $\pm$ 0.38	77.90c $\pm$ 3.09	18.44c $\pm$ 0.73	1.01c $\pm$ 0.09	5.60a $\pm$ 0.54	120.95b $\pm$ 7.50
HSD _{0.05}	2.02	0.54	18.12	4.78	0.77	n.s	25.47
P	< 0.01	0.02	< 0.01	< 0.01	< 0.01	0.28	0.03
F	12.14	1.20	15.48	10.36	15.16	1.17	3.12
<i>Meloidogyne javanica</i> and nematophagous fungi with OC							
+ Control (P)	9.92b $\pm$ 0.87	2.66a $\pm$ 0.35	121.38c $\pm$ 6.58	40.33a $\pm$ 4.05	2.55a $\pm$ 0.37	7.00b $\pm$ 1.00	151.07a $\pm$ 19.27
– Control (P + N)	8.40b $\pm$ 1.38	2.48a $\pm$ 0.33	183.96a $\pm$ 5.08	32.21b $\pm$ 2.24	2.22a $\pm$ 0.30	5.83c $\pm$ 1.09	144.98a $\pm$ 13.96
<i>A. terreus</i>	8.28b $\pm$ 2.62	1.92b $\pm$ 0.52	80.17d $\pm$ 3.17	26.30b $\pm$ 3.88	1.27b $\pm$ 0.51	7.41b $\pm$ 0.89	130.49a $\pm$ 19.19
<i>Tal. minioluteus</i>	17.00a $\pm$ 1.91	3.22a $\pm$ 1.79	169.06b $\pm$ 4.19	31.20b $\pm$ 1.20	2.41a $\pm$ 0.28	7.22b $\pm$ 0.83	161.08a $\pm$ 4.50
<i>Tal. purpureogenus</i>	8.30b $\pm$ 0.68	2.74a $\pm$ 0.23	82.44d $\pm$ 4.59	23.65c $\pm$ 8.33	1.80b $\pm$ 0.54	6.48b $\pm$ 0.89	154.66a $\pm$ 15.25
<i>Tal. sayulitensis</i>	15.88a $\pm$ 3.05	2.86a $\pm$ 0.48	107.00d $\pm$ 3.46	24.12c $\pm$ 9.74	2.32a $\pm$ 0.92	8.67a $\pm$ 1.34	146.79a $\pm$ 8.11

<i>Meloidogyne javanica</i> and nematophagous fungi with OC							
Treatment	PH (cm)	SD (mm)	CC ($\mu\text{mol m}^{-2}$)	FW (g)	DW (g)	TN	TW (g)
<i>Tri. viride</i>	12.18a $\pm$ 0.79	2.64a $\pm$ 0.19	99.70d $\pm$ 7.99	29.38b $\pm$ 0.97	2.12a $\pm$ 0.12	6.61b $\pm$ 1.34	119.78a $\pm$ 10.45
HSD _{0.05}	3.22	0.55	15.98	3.33	0.40	1.25	n.s
P	0.05	< 0.01	< 0.01	0.01	0.01	< 0.01	0.65
F	3.61	18.52	85.36	0.99	7.34	10.45	0.20
<i>Meloidogyne javanica</i> and nematophagous fungi without OC							
– Control (P)	8.98c $\pm$ 0.60	2.36a $\pm$ 0.19	150.46a $\pm$ 20.08	33.26a $\pm$ 2.66	2.26a $\pm$ 0.22	6.66b $\pm$ 0.89	217.20a $\pm$ 68.76
– Control (P + N)	7.06c $\pm$ 0.59	1.74b $\pm$ 0.26	153.52a $\pm$ 5.44	27.06a $\pm$ 1.29	1.25b $\pm$ 0.08	5.82b $\pm$ 1.09	122.23c $\pm$ 18.55
<i>A. terreus</i>	5.48c $\pm$ 0.37	1.20b $\pm$ 0.14	60.62b $\pm$ 3.17	20.90b $\pm$ 1.14	0.82c $\pm$ 0.07	6.82b $\pm$ 0.44	114.62b $\pm$ 25.49
<i>Tal. minioluteus</i>	14.14b $\pm$ 2.34	2.68a $\pm$ 1.19	93.10b $\pm$ 3.59	21.38b $\pm$ 1.64	1.96a $\pm$ 0.22	6.00b $\pm$ 0.70	113.80c $\pm$ 23.98
<i>Tal. purpureogenus</i>	6.46c $\pm$ 0.28	2.12a $\pm$ 0.17	79.66c $\pm$ 6.25	21.79b $\pm$ 1.53	1.12b $\pm$ 0.18	6.44b $\pm$ 0.89	171.09b $\pm$ 68.66
<i>Tal. sayulitensis</i>	20.50a $\pm$ 2.27	2.92a $\pm$ 0.54	114.76b $\pm$ 3.50	28.88a $\pm$ 1.12	2.54a $\pm$ 0.19	9.63a $\pm$ 0.54	179.61b $\pm$ 28.37
<i>Tri. viride</i>	7.94c $\pm$ 0.35	1.47b $\pm$ 0.23	87.26c $\pm$ 3.98	25.06b $\pm$ 3.10	1.55b $\pm$ 0.33	6.87b $\pm$ 1.64	141.07b $\pm$ 39.77
HSD _{0.05}	4.26	1.78	21.83	7.22	0.52	2.87	35.47
P	0.05	< 0.01	< 0.01	0.04	0.01	< 0.01	< 0.01
F	3.61	18.52	85.36	0.99	7.34	12.39	9.08

The results are the mean and standard deviation of five replicates. ²Data with different letters indicates a significant difference at $P \leq 0.05$ according to Tukey's HSD, and average $\pm$ standard deviation error.

P: Plant alone, P + N: Plant and nematodes.

²n.s. = not significant.

9.5 Discussion

9.5.1 Potato response to *Meloidogyne* with and without organic compost

According to the findings from this study, *M. enterolobii* and *M. javanica* had a detrimental impact on potato plant growth by significantly reducing shoot weight and tuber mass (Table 9.4). These findings support the previously established studies on the effect of *Meloidogyne* species on crops such as carrots (Ahmad *et al.*, 2019), cucumbers (Mukhtar & Kayani, 2019) and tomatoes (Strajnar *et al.*, 2012). Plant growth parameters, plant height, chlorophyll, fresh shoot weight and dry weight, were all compromised by *Meloidogyne* infestation. However, there is evidence that the potato plants' growth was enhanced by the tested fungi (Table 9.4). Plant colonisation by certain fungal species can boost plant defense against RKN. This was especially seen with *Tal. minioluteus*, *Tal. purpureogenus*, *Tal. sayulitensis* and *Tri. viride*, that showed a significant increase in diverse plant growth parameters when compared to the positive and negative controls (Tables 9.2 and 9.4). The same was observed in **Chapter 6**, wherein, some of these tested fungal species increased seedling growth.

It has been established that fungal species achieve this physiological positive impact by using two mechanisms: synthesis of plant-like hormones such as cytokinin and gibberellin, as well as mobilising and increasing plant defence mechanisms (Rodriguez *et al.*, 2009; Alam *et al.*, 2021; Baron & Rigobelo, 2022). An increase in tuber mass was observed in potato seedlings inoculated with *Tal. sayulitensis* and *Tri. viride*. *Talaromyces* and *Trichoderma* are obligate root symbionts that help protect their host plants against biotic stresses including PPN infection. This is achieved via several mechanisms such as higher nutrient uptake, altered root morphology, competing for space and nutrition with PPN and inducing plant systemic resistance. The symbiosis between a plant and fungal BCA has also been reported to result in increased root growth stimulation (Poveda *et al.*, 2020).

Although all treatments had nematicidal action, the administration of *Talaromyces* species induced the greatest levels of J2s mortality and penetration inhibition. The species' direct parasitism (observed in **Chapter 5**) might be responsible for the nematicidal activity (Liu *et al.*, 2009; Poveda *et al.*, 2020; Soares *et al.*, 2018). In comparison to the positive control, all treatments were able to decrease *Meloidogyne* Pf densities to varied degrees in both the soil and root matrices. *Talaromyces* species showed higher efficacy in reducing nematode impact on potato seedlings, which could have been attributed to various mechanisms. The mechanisms involved have also been previously established within a range of other *Aspergillus* and *Talaromyces* species (Schouten, 2016). These mechanisms may include repellent activity reducing J2s attraction to roots, attenuated or delayed development of adult females and reduction of their fecundity (Forghani & Hajihassani, 2020; Le *et al.*, 2016; Schouten, 2016).

The fungal species showed significantly higher nematicidal activities with the incorporation of OC. It has been suggested that fungi emit microbial compounds into the environment in which they thrive (Hyde *et al.*, 2019; Thambugala *et al.*, 2020; Manzar *et al.*, 2022). A similar trend has been established by previous research when the incorporation of OC increased the efficacy of fungal species against *Meloidogyne* species on different crops (Rizvi *et al.*, 2018; d'Errico *et al.*, 2022). The incorporation of OC improved the efficacy of fungal BCA against the two *Meloidogyne* species. In a study by Rousk & Bååth (2007), barley straw resulted in fungal growth rates that peaked at roughly 12 times the control value. There was a noticeable effect of nitrogen addition, with the strongest addition increasing fungal growth by 35% when combined with barley straw and 15% when combined with alfalfa (Rousk & Bååth, 2007). Fungal growth promotion following N addition has previously been documented (Bardgett *et al.*, 1999). In the net house conditions, fungal species exhibited efficacy on *M. enterolobii* and *M. javanica* population densities. However, the nematophagous fungi efficacy was not

maintained under field conditions; *albeit* they still reduced nematode damage when compared to the negative controls. Some factors could have contributed to fungal species' failure to maintain a similar efficacy to that observed under net house conditions. It is possible that in the field, potato crops were exposed to other nematode species and/or other microbial infections that increased potato susceptibility and subsequently increased *M. incognita* penetration and populations.

In a study by Daneel *et al.* (2018), a similar trend was noted; where the glasshouse study showed a reduction in *M. incognita* population density and field experiments failed to reduce *Meloidogyne* population densities. Under field temperatures, exudates from stressed potato crops could have increased *Meloidogyne* attraction to the root zone. Further, uncontrolled temperatures in the field could have reduced the emission of potato volatile organic compounds (VOC). According to Daussy & Staudt (2020), temperature affects plant VOC emissions; whereas an increase in temperature reduces plant VOC emissions. Noteworthy, nematophagous fungal impact on the two *Meloidogyne* species was also improved with OC incorporation as did under net house trials.

9.6 Conclusion

The present investigation did show how the studied indigenous fungal species, *A. terreus*, *Tal. minioluteus*, *Tal. sayulitensis*, *Tal. purpureogenus* and *Tri. viride* could enhance potato plant development when they are infested with *Meloidogyne*. The applied fungal species reduced the gall index, gall number and nematode RF, as well as fecundity, demonstrating their potential effectiveness against RKN.

CHAPTER 10: SUMMARY, GAPS AND PROSPECTS

10.1 Summary

The study focused on eight major areas, i.e., (1) Screen for *M. enterolobii* and *M. javanica* nematodes susceptibility levels of five potato cultivars, viz., ‘Buffelspoort P1’, ‘Hertha’, ‘Larnoma’, ‘Mnandi’ and ‘Up-to-date’; (2) Isolate indigenous fungal biotypes as possible candidates for bio-control investigation, and morphologically and molecularly identify the indigenous fungal biotypes; (3) Investigate parasitism of indigenous fungal biotypes on *M. enterolobii* and *M. javanica* eggs and juveniles; (4) Investigate pathogenesis of nematode parasitic fungi on seven selected crop seeds, viz., tomato, beetroot, swiss chard, dry bean, maize, sorghum and pumpkin; (5) Determine growth rate and geotropism of fungal biotypes on different grain media, viz., red sorghum, white sorghum, barley, wheat and wheat bran; (6) Examine stress tolerance of fungal species *in vitro* and (7) Determine effects of indigenous fungal species on *M. enterolobii* and *M. javanica* population densities on a susceptible potato cultivar with and without compost incorporation under greenhouse and field conditions.

10.1.1 Potato-*Meloidogyne* interactions

The present study’s observations of the two *Meloidogyne* species parasitic behaviour on potato tubers and feeder roots indicate that *Meloidogyne* infection has the potential to cause significant potato damage. Current results showed that the tested commercial potato cultivars have different levels of susceptibility to *Meloidogyne* nematodes, given that none of the cultivars were able to suppress nematode reproduction. Cultivars ‘Hertha’, followed by ‘Up-to-date’ and ‘Buffelspoort 1’ sustained higher nematode densities. This study further demonstrated that comparatively, *M. enterolobii* was more virulent to the potato than *M. javanica*; although *M. javanica* still significantly impeded potato quality.

10.1.2 Indigenous fungal biotypes isolation and identification

The fungal growth rates offer a valuable tool for learning about the growth characteristics of filamentous fungi in various nutritional media. Research on growth characteristics has significance for the development of nematophagous fungi. Key biological characteristics of fungi include vegetative growth and sporulation under field conditions. In fungi, the minimal dietary needs may have a significant impact on the culture mycelial development, sporulation and shape. From the original 25 pure biotypes, eight different species were confirmed at the species level. This shows that while examining morphological traits to identify fungal biotypes has limited accuracy, it is still fairly reliable. All cultured species showed variations in mycelial colour. These were most likely explained by underlying genetic variations and/or environmental effects such as the temperature and pH of the soil from which they were isolated.

10.1.3 Fungi-*Meloidogyne* interactions

The genus *Talaromyces* exhibited the highest eggs + J2s parasitism on the two *Meloidogyne* species. Whilst *Tal. sayulitensis* had the highest parasitic effect on *M. enterolobii* egg, *Tal. sayulitensis* had the highest *M. javanica* egg parasitism. *Talaromyces minioluteus* had the highest parasitism on *M. enterolobii* J2s effects and *Tal. sayulitensis* had the highest parasitism on *M. javanica* J2s.

10.1.4 Fungal species pathogenesis

The results of this study established that test species, except for *A. flavus*, could potentially be effective seed inoculants for stimulating the germination and growth developmental process of seedlings. These test species may also have endophytic characteristics and their application may aid in achieving the development of robust seedling stands.

10.1.5 Fungal species growth and geotropism sensitivity

It was established that the grain substrate technique, especially on red sorghum, was effective at mass-producing the studied fungal species. This research provides alternative nutritional sources for the mass production of endophytic fungal species. This stands to reason, laboratory growth media are feasible for *in vitro* fungal studies, whilst grain substrates are feasible for economic mass production. These grains may be used to mass produce the fungi, perhaps increasing their efficacy as a bio-control agent that is also inexpensive and widely available.

10.1.6 Fungal species stress tolerance

The findings demonstrated that although being indigenous, *Aspergillus*, *Tal. sayulitensis* and *Tri. ghanense* did not thrive in conditions of high salinity and alkalinity; *Penicillium* sp. failed to grow at lower nutritional levels and all fungal species failed to grow at temperatures between 10-20 °C. The production of chlamydospores by *Trichoderma* was recognised as a means of surviving unfavourable conditions. Additionally, *Aspergillus* species from the same genus can have both favourable and unfavourable effects on crops. The adaptation of BCA to habitats with sudden fluctuating climatic conditions is a significant challenge to the transition from conventional to organic agriculture, even more currently due to climate change. This challenge could be alleviated by using BCA with relatively high abiotic stress tolerance.

10.1.7 Fungi-Meloidogyne-potato tri-trophic interactions

The current experiments exhibited the effect of indigenous fungal species on *Meloidogyne* indices to be significantly reduced compared to the negative control, confirming their bio-control potential. This study provides insights into the practical usage of indigenous nematophagous fungi as BCA against the RKN, *M. enterolobii* and *M. javanica* on potato plants. The results demonstrated that inoculation of *M. enterolobii* and *M. javanica* J2s with

Aspergillus, *Talaromyces* and *Trichoderma* led to significant levels of antagonistic activity. However, the test fungal species gave inconsistent results in terms of efficacy inside net house and field conditions. Further studies are required to evaluate the efficacy of these fungal species in commercial greenhouses and optimise their use on plants.

11.2 Conclusion

Five commercial potato cultivars were highly susceptible to the two *Meloidogyne* species that are prevalent in potato-producing regions of South Africa. The eight indigenous nematophagous fungi were effective in parasitising and subsequently reducing *Meloidogyne* population densities. The test nematophagous fungi further exhibited endophytic characteristics by improving potato root elongation as well as overall plant growth. Results also showed that incorporation with OC improved the efficacy of nematophagous fungi. Accordingly, the five indigenous nematophagous fungal species have the potential for use as registered commercial BCA in potato cultivars, including other *Meloidogyne*-susceptible crops.

11.3 Research gaps and prospects

Numerous studies, including the current one, demonstrated the relationship between *Meloidogyne* nematodes and potato crops, and the devastating impacts on potato production thereof. Nevertheless, this has not been matched by efforts towards research in ecologically safe nematode management practices. Furthermore, of those research outputs elucidating nematicidal alternative measures, even fewer are adopted by South African farmers. This is an indication that there is a gap between scientific research output and farmers' implementation of research recommendations. To date, South African farmers continue to spray nematicides as their primary line of defence to manage nematodes. Regardless of scientific research-based

data indicating the detrimental impacts of pesticides on the environment and people, several extremely hazardous pesticides, such as velum, paraquat, atrazine and 2.4D remain legal and in use in South Africa. This issue is particularly pressing such that even the United Nations recently urged South Africa to conform to the United Nations' recommendations and prohibit the use of highly hazardous pesticides (Wardle, 2013; Wagg *et al.*, 2014; Hannula *et al.*, 2017; Payi, 2023). This could be due to farmers' concerns and uncertainty about losing money, should they change procedures they are already familiar with and confident in regarding efficacy levels.

Meloidogyne nematodes continue to adversely impact the South African potato industry; the same is true for other crops, especially those used in rotation with potatoes. To achieve sustainable *Meloidogyne* management practice, it is necessary to provide future recommendations for growers whose expected crop yield returns are severely affected by RKN parasitism. Research must be carried out to identify mass culture strains of potential BCA for field adaptation. Although worldwide BCA products are available, emphasis should be placed on the need to employ indigenous material. The latter are less costly and more adaptable to the areas they are being applied, and therefore they justify recommending their widespread use.

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APPENDICES

Appendix A: ANOVA table for Chapter 3

Appendix A.1 Analysis of variance for *Meloidogyne enterolobii* and *Meloidogyne javanica* final population on potato genotypes at 56 days after inoculation (n = 25) under net house conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
‘Buffelspoort P1’ + <i>Meloidogyne enterolobii</i>					
Rep	4	20036.96762	5009.24191		
Treatment	4	29148.26666	7287.06667	2.99	0.0508
Error	16	38994.01746	2437.12609		
Corrected Total	24	88179.25174			
‘Buffelspoort P1’ + <i>Meloidogyne javanica</i>					
Rep	4	1879.451784	469.862946		
Treatment	4	1555.845184	388.961296	0.16	0.0459
Error	16	39099.81102	2443.73819		
Corrected Total	24	42535.10798			
‘Hertha’ + <i>Meloidogyne enterolobii</i>					
Rep	4	12525.18292	3131.29573		
Treatment	4	22689.71012	5672.42753	1.52	0.0042
Error	16	59554.29196	3722.14325		
Corrected Total	24	94769.18500			
‘Hertha’ + <i>Meloidogyne javanica</i>					
Rep	4	70792.39678	17698.09920		
Treatment	4	96430.94858	24107.73714	3.60	0.0283
Error	16	107226.2589	6701.6412		
Corrected Total	24	274449.6043			
‘Larnoma’ + <i>Meloidogyne enterolobii</i>					
Rep	4	74148.10630	18537.02658		
Treatment	4	27481.93014	6870.48254	2.73	0.0060
Error	16	40233.8376	2514.6149		
Corrected Total	24	141863.8741			
‘Larnoma’ + <i>Meloidogyne javanica</i>					
Rep	4	71912.1051	17978.0263		
Treatment	4	271178.0651	67794.5163	4.43	0.0133
Error	16	244635.5324	15289.7208		
Corrected Total	24	587725.7026			

'Mnandi' + <i>Meloidogyne enterolobii</i>					
Rep	4	32624.56790	8156.14197		
Treatment	4	13065.39738	3266.34934	0.86	0.0590
Error	16	60809.0155	3800.5635		
Corrected Total	24	106498.9808			
'Mnandi' + <i>Meloidogyne javanica</i>					
Rep	4	20072.04936	5018.01234		
Treatment	4	45706.26812	11426.56703	1.44	0.0265
Error	16	126700.6873	7918.7930		
Corrected Total	24	192479.0048			
'Up-to-date' + <i>Meloidogyne enterolobii</i>					
Rep	4	6585.008456	1646.252114		
Treatment	4	2381.938296	595.484574	1.99	0.0454
Error	16	4798.77590	299.92349		
Corrected Total	24	13765.72266			
'Up-to-date' + <i>Meloidogyne javanica</i>					
Rep	4	5810.756936	1452.689234		
Treatment	4	1698.525496	424.631374	1.24	0.0347
Error	16	5492.74006	343.29625		
Corrected Total	24	13002.02250			

Appendix A.2 Analysis of variance for *Meloidogyne enterolobii* and *Meloidogyne javanica* gall number on potato cultivars at 56 days after inoculation (n = 25) under net house conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
‘Buffelspoort P1’ + <i>Meloidogyne enterolobii</i>					
Rep	4	3.36000000	0.84000000		
Treatment	4	73.36000000	18.34000000	18.53	<.0001
Error	16	15.84000000	0.99000000		
Corrected Total	24	92.56000000			
‘Buffelspoort P1’ + <i>Meloidogyne javanica</i>					
Rep	4	5.44000000	1.36000000		
Treatment	4	53.04000000	13.26000000	17.45	0.5147
Error	16	12.16000000	0.76000000		
Corrected Total	24	70.64000000			
‘Hertha’ + <i>Meloidogyne enterolobii</i>					
Rep	4	3.76000000	0.94000000		
Treatment	4	46.96000000	11.74000000	14.40	<.0001
Error	16	13.04000000	0.81500000		
Corrected Total	24	63.76000000			
‘Hertha’ + <i>Meloidogyne javanica</i>					
Rep	4	9.06000000	2.26500000		
Treatment	4	46.01785714	11.50446429	17.23	0.3424
Error	16	10.68214286	0.66763393		
Corrected Total	24	65.76000000			
‘Larnoma’ + <i>Meloidogyne enterolobii</i>					
Rep	4	14.24000000	3.56000000		
Treatment	4	39.04000000	9.76000000	3.09	0.0461
Error	16	50.56000000	3.16000000		
Corrected Total	24	103.84000000			
‘Larnoma’ + <i>Meloidogyne javanica</i>					
Rep	4	1.84000000	0.46000000		
Treatment	4	39.04000000	9.76000000	12.05	0.6831
Error	16	12.96000000	0.81000000		
Corrected Total	24	53.84000000			
‘Mnandi’ + <i>Meloidogyne enterolobii</i>					
Rep	4	0.40000000	0.10000000		
Treatment	4	65.20000000	16.30000000	40.75	0.9004

Error	16	6.40000000	0.40000000		
Corrected Total	24	72.00000000			
‘Mnandi’ + <i>Meloidogyne javanica</i>					
Rep	4	4.00000000	1.00000000		
Treatment	4	32.00000000	8.00000000	16.00	0.1446
Error	16	8.00000000	0.50000000		
Corrected Total	24	44.00000000			
‘Up-to-date’ + <i>Meloidogyne enterolobii</i>					
Rep	4	0.96000000	0.24000000		
Treatment	4	22.16000000	5.54000000	10.76	0.1247
Error	16	8.24000000	0.51500000		
Corrected Total	24	31.36000000			
‘Up-to-date’ + <i>Meloidogyne javanica</i>					
Rep	4	7.36000000	1.84000000		
Treatment	4	5.76000000	1.44000000	2.17	0.2232
Error	16	10.64000000	0.66500000		
Corrected Total	24	23.76000000			

Appendix A.3 Analysis of variance for *Meloidogyne enterolobii* and *Meloidogyne javanica* reproductive factor on potato cultivars at 56 days after inoculation (n = 25) under net house conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
‘Buffelspoort P1’ + <i>Meloidogyne enterolobii</i>					
Rep	4	3.36000000	0.84000000		
Treatment	4	73.36000000	18.34000000	18.53	<.0001
Error	16	15.84000000	0.99000000		
Corrected Total	24	92.56000000			
‘Buffelspoort P1’ + <i>Meloidogyne javanica</i>					
Rep	4	5.44000000	1.36000000		
Treatment	4	53.04000000	13.26000000	17.45	<.0001
Error	16	12.16000000	0.76000000		
Corrected Total	24	70.64000000			
‘Hertha’ + <i>Meloidogyne enterolobii</i>					
Rep	4	3.76000000	0.94000000		
Treatment	4	46.96000000	11.74000000	14.40	<.0001
Error	16	13.04000000	0.81500000		
Corrected Total	24	63.76000000			
‘Hertha’ + <i>Meloidogyne javanica</i>					
Rep	4	9.06000000	2.26500000		
Treatment	4	46.01785714	11.50446429	17.23	<.0001
Error	16	10.68214286	0.66763393		
Corrected Total	24	65.76000000			
‘Larnoma’ + <i>Meloidogyne enterolobii</i>					
Rep	4	14.24000000	3.56000000		
Treatment	4	39.04000000	9.76000000	3.09	0.0461
Error	16	50.56000000	3.16000000		
Corrected Total	24	103.84000000			
‘Larnoma’ + <i>Meloidogyne javanica</i>					
Rep	4	1.84000000	0.46000000		
Treatment	4	39.04000000	9.76000000	12.05	0.0001
Error	16	12.96000000	0.81000000		
Corrected Total	24	53.84000000			
‘Mnandi’ + <i>Meloidogyne enterolobii</i>					
Rep	4	0.40000000	0.10000000		

Treatment	4	65.20000000	16.30000000	40.75	<.0001
Error	16	6.40000000	0.40000000		
Corrected Total	24	72.00000000			
'Mnandi' + <i>Meloidogyne javanica</i>					
Rep	4	4.00000000	1.00000000		
Treatment	4	32.00000000	8.00000000	16.00	<.0001
Error	16	8.00000000	0.50000000		
Corrected Total	24	44.00000000			
'Up-to-date' + <i>Meloidogyne enterolobii</i>					
Rep	4	0.96000000	0.24000000		
Treatment	4	22.16000000	5.54000000	10.76	0.0302
Error	16	8.24000000	0.51500000		
Corrected Total	24	31.36000000			
'Up-to-date' + <i>Meloidogyne javanica</i>					
Rep	4	7.36000000	1.84000000		
Treatment	4	5.76000000	1.44000000	2.17	0.1196
Error	16	10.64000000	0.66500000		
Corrected Total	24	23.76000000			

Appendix A.4 Analysis of variance for potato cultivars' plant height under *Meloidogyne enterolobii* and *Meloidogyne javanica* at 56 days after inoculation (n = 25) under net house conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
'Buffelspoort P1' + <i>Meloidogyne enterolobii</i>					
Rep	4	144.5320000	36.1330000		
Treatment	4	208.8040000	52.2010000	1.84	0.1713
Error	16	454.9440000	28.4340000		
Corrected Total	24	808.2800000			
'Buffelspoort P1' + <i>Meloidogyne javanica</i>					
Rep	4	218.7696000	54.6924000		
Treatment	4	31.2376000	7.8094000	0.27	0.0499
Error	16	465.2024000	29.0751500		
Corrected Total	24	715.2096000			
'Hertha' + <i>Meloidogyne enterolobii</i>					
Rep	4	133.1344000	33.2836000		
Treatment	4	182.9684000	45.7421000	2.87	0.0575
Error	16	255.2496000	15.9531000		
Corrected Total	24	571.3524000			
'Hertha' + <i>Meloidogyne javanica</i>					
Rep	4	381.5702667	95.3925667		
Treatment	4	408.6597976	102.1649494	2.11	0.0373
Error	16	775.599536	48.474971		
Corrected Total	24	1565.829600			
'Larnoma' + <i>Meloidogyne enterolobii</i>					
Rep	4	1567.398400	391.849600		
Treatment	4	364.086400	91.021600	1.09	0.3941
Error	16	1334.633600	83.414600		
Corrected Total	24	3266.118400			
'Larnoma' + <i>Meloidogyne javanica</i>					
Rep	4	858.3256000	214.5814000		
Treatment	4	469.5256000	117.3814000	4.41	0.0136
Error	16	425.818400	26.613650		
Corrected Total	24	1753.669600			
'Mnandi' + <i>Meloidogyne enterolobii</i>					
Rep	4	633.4605440	158.3651360		
Treatment	4	145.6461440	36.4115360	0.86	0.5108

Error	16	680.474416	42.529651		
Corrected Total	24	1459.581104			
‘Mnandi’ + <i>Meloidogyne javanica</i>					
Rep	4	283.4656000	70.8664000		
Treatment	4	116.0296000	29.0074000	1.23	0.0571
Error	16	377.1184000	23.5699000		
Corrected Total	24	776.6136000			
‘Up-to-date’ + <i>Meloidogyne enterolobii</i>					
Rep	4	117.2632560	29.3158140		
Treatment	4	87.4605760	21.8651440	1.17	0.3601
Error	16	298.5645440	18.6602840		
Corrected Total	24	503.2883760			
‘Up-to-date’ + <i>Meloidogyne javanica</i>					
Rep	4	98.0496000	24.5124000		
Treatment	4	313.0496000	78.2624000	3.55	0.0297
Error	16	353.1904000	22.0744000		
Corrected Total	24	764.2896000			

Appendix A.5 Analysis of variance for potato cultivars' stem diameter under *Meloidogyne enterolobii* and *Meloidogyne javanica* at 56 days after inoculation (n = 25) under the net house.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
'Buffelspoort P1' + <i>Meloidogyne enterolobii</i>					
Rep	4	2.51693600	0.62923400		
Treatment	4	17.55345600	4.38836400	1.05	0.4115
Error	16	66.70998400	4.16937400		
Corrected Total	24	86.78037600			
'Buffelspoort P1' + <i>Meloidogyne javanica</i>					
Rep	4	5.61545600	1.40386400		
Treatment	4	15.26897600	3.81724400	0.74	0.7504
Error	16	82.7136240	5.1696015		
Corrected Total	24	103.5980560			
'Hertha' + <i>Meloidogyne enterolobii</i>					
Rep	4	57.71996000	14.42999000		
Treatment	4	85.17376000	21.29344000	6.41	0.0028
Error	16	53.1248800	3.3203050		
Corrected Total	24	196.0186000			
'Hertha' + <i>Meloidogyne javanica</i>					
Rep	4	2.56385767	0.64096442		
Treatment	4	2.77980012	0.69495003	0.95	0.4606
Error	16	11.69631821	0.73101989		
Corrected Total	24	17.03997600			
'Larnoma' + <i>Meloidogyne enterolobii</i>					
Rep	4	7.89373600	1.97343400		
Treatment	4	17.05561600	4.26390400	0.44	0.7767
Error	16	154.4052240	9.6503265		
Corrected Total	24	179.3545760			
'Larnoma' + <i>Meloidogyne javanica</i>					
Rep	4	3.20260000	0.80065000		
Treatment	4	15.80452000	3.95113000	7.21	0.0016
Error	16	8.77188000	0.54824250		
Corrected Total	24	27.77900000			
'Mnandi' + <i>Meloidogyne enterolobii</i>					
Rep	4	17.12489600	4.28122400		
Treatment	4	82.85473600	20.71368400	1.15	0.3686

Error	16	287.9532640	17.9970790		
Corrected Total	24	387.9328960			
‘Mnandi’ + <i>Meloidogyne javanica</i>					
Rep	4	12.70750400	3.17687600		
Treatment	4	9.28638400	2.32159600	0.81	0.5395
Error	16	46.12105600	2.88256600		
Corrected Total	24	68.11494400			
‘Up-to-date’ + <i>Meloidogyne enterolobii</i>					
Rep	4	5.03682400	1.25920600		
Treatment	4	37.74502400	9.43625600	1.65	0.0214
Error	16	91.7124560	5.7320285		
Corrected Total	24	134.4943040			
‘Up-to-date’ + <i>Meloidogyne javanica</i>					
Rep	4	7.17869600	1.79467400		
Treatment	4	24.86121600	6.21530400	0.97	0.4530
Error	16	102.9674640	6.4354665		
Corrected Total	24	135.0073760			

Appendix A.6 Analysis of variance for potato cultivars' chlorophyll content under *Meloidogyne enterolobii* and *Meloidogyne javanica* at 56 days after inoculation (n = 25) under net house conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
'Buffelspoort P1' + <i>Meloidogyne enterolobii</i>					
Rep	4	1540.781600	385.195400		
Treatment	4	533.997600	133.499400	4.66	0.0138
Error	16	4206.018400	262.876150		
Corrected Total	24	6280.797600			
'Buffelspoort P1' + <i>Meloidogyne javanica</i>					
Rep	4	415.4296000	103.8574000		
Treatment	4	547.8456000	136.9614000	0.51	0.7310
Error	16	470.654400	29.415900		
Corrected Total	24	1433.929600			
'Hertha' + <i>Meloidogyne enterolobii</i>					
Rep	4	470.3615360	117.5903840		
Treatment	4	356.0277760	89.0069440	1.24	0.3347
Error	16	1151.282704	71.955169		
Corrected Total	24	1977.672016			
'Hertha' + <i>Meloidogyne javanica</i>					
Rep	4	58.762793	14.690698		
Treatment	4	2727.992378	681.998094	6.08	0.0036
Error	16	1794.077205	112.129825		
Corrected Total	24	4580.832376			
'Larnoma' + <i>Meloidogyne enterolobii</i>					
Rep	4	2176.608224	544.152056		
Treatment	4	1180.687824	295.171956	2.60	0.0576
Error	16	1817.012496	113.563281		
Corrected Total	24	5174.308544			
'Larnoma' + <i>Meloidogyne javanica</i>					
Rep	4	1048.855720	262.213930		
Treatment	4	1826.360600	456.590150	4.99	0.0083
Error	16	1463.266280	91.454143		
Corrected Total	24	4338.482600			
'Mnandi' + <i>Meloidogyne enterolobii</i>					
Rep	4	334.385664	83.596416		

Treatment	4	1042.645744	260.661436	2.43	0.0592
Error	16	1715.692736	107.230796		
Corrected Total	24	3092.724144			
‘Mnandi’ + <i>Meloidogyne javanica</i>					
Rep	4	271.3681760	67.8420440		
Treatment	4	275.6401760	68.9100440	0.65	0.6359
Error	16	1699.580704	106.223794		
Corrected Total	24	2246.589056			
‘Up-to-date’ + <i>Meloidogyne enterolobii</i>					
Rep	4	83.2277040	20.8069260		
Treatment	4	180.2161840	45.0540460	1.37	0.2889
Error	16	526.9789760	32.9361860		
Corrected Total	24	790.4228640			
‘Up-to-date’ + <i>Meloidogyne javanica</i>					
Rep	4	61.7926560	15.4481640		
Treatment	4	141.9464560	35.4866140	1.09	0.3933
Error	16	519.4117040	32.4632315		
Corrected Total	24	723.1508160			

Appendix A.7 Analysis of variance for potato cultivars' fresh weight under *Meloidogyne enterolobii* and *Meloidogyne javanica* under the net house at 56 days after inoculation (n = 25).

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
'Buffelspoort P1' + <i>Meloidogyne enterolobii</i>					
Rep	4	899.9717360	224.9929340		
Treatment	4	212.0453360	53.0113340	0.23	0.9155
Error	16	3633.804624	227.112789		
Corrected Total	24	4745.821696			
'Buffelspoort P1' + <i>Meloidogyne javanica</i>					
Rep	4	525.7356240	131.4339060		
Treatment	4	243.0948640	60.7737160	0.26	0.9010
Error	16	3780.506296	236.281644		
Corrected Total	24	4549.336784			
'Hertha' + <i>Meloidogyne enterolobii</i>					
Rep	4	58.2663360	14.5665840		
Treatment	4	222.3106160	55.5776540	2.06	0.0530
Error	16	431.6463040	26.9778940		
Corrected Total	24	712.2232560			
'Hertha' + <i>Meloidogyne javanica</i>					
Rep	4	245.219346	61.304837		
Treatment	4	1267.429348	316.857337	2.01	0.0411
Error	16	2521.536362	157.596023		
Corrected Total	24	4034.185056			
'Larnoma' + <i>Meloidogyne enterolobii</i>					
Rep	4	406.3119760	101.5779940		
Treatment	4	325.1978160	81.2994540	0.54	0.0371
Error	16	2418.716224	151.169764		
Corrected Total	24	3150.226016			
'Larnoma' + <i>Meloidogyne javanica</i>					
Rep	4	12753.36464	3188.34116		
Treatment	4	1574.62564	393.65641	4.65	0.0111
Error	16	1355.82672	84.73917		
Corrected Total	24	15683.81700			
'Mnandi' + <i>Meloidogyne enterolobii</i>					
Rep	4	236.8186160	59.2046540		
Treatment	4	188.0666160	47.0166540	0.54	0.7104

Error	16	1399.720464	87.482529		
Corrected Total	24	1824.605696			
‘Mnandi’ + <i>Meloidogyne javanica</i>					
Rep	4	1975.733744	493.933436		
Treatment	4	111.718304	27.929576	0.12	0.9745
Error	16	3818.259936	238.641246		
Corrected Total	24	5905.711984			
‘Up-to-date’ + <i>Meloidogyne enterolobii</i>					
Rep	4	307.7202160	76.9300540		
Treatment	4	530.8666160	132.7166540	2.62	0.0571
Error	16	810.911464	50.681967		
Corrected Total	24	1649.498296			
‘Up-to-date’ + <i>Meloidogyne javanica</i>					
Rep	4	301.9486160	75.4871540		
Treatment	4	505.6189360	126.4047340	2.45	0.0581
Error	16	824.701904	51.543869		
Corrected Total	24	1632.269456			

Appendix A.8 Analysis of variance for potato cultivars' dry weight under *Meloidogyne enterolobii* and *Meloidogyne javanica* at 56 days after inoculation (n = 25) under net house conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
'Buffelspoort P1' + <i>Meloidogyne enterolobii</i>					
Rep	4	15.95898400	3.98974600		
Treatment	4	22.89410400	5.72352600	1.34	0.2966
Error	16	68.1124160	4.2570260		
Corrected Total	24	106.9655040			
'Buffelspoort P1' + <i>Meloidogyne javanica</i>					
Rep	4	2.56616000	0.64154000		
Treatment	4	9.75020000	2.43755000	0.46	0.7646
Error	16	84.95564000	5.30972750		
Corrected Total	24	97.27200000			
'Hertha' + <i>Meloidogyne enterolobii</i>					
Rep	4	1.39494400	0.34873600		
Treatment	4	1.25746400	0.31436600	1.11	0.3871
Error	16	4.54265600	0.28391600		
Corrected Total	24	7.19506400			
'Hertha' + <i>Meloidogyne javanica</i>					
Rep	4	6.93264600	1.73316150		
Treatment	4	53.42342464	13.35585616	7.14	0.0017
Error	16	29.94694536	1.87168408		
Corrected Total	24	90.30301600			
'Larnoma' + <i>Meloidogyne enterolobii</i>					
Rep	4	10.92602400	2.73150600		
Treatment	4	6.94426400	1.73606600	0.52	0.7205
Error	16	53.14569600	3.32160600		
Corrected Total	24	71.01598400			
'Larnoma' + <i>Meloidogyne javanica</i>					
Rep	4	65.30669600	16.32667400		
Treatment	4	48.83333600	12.20833400	6.16	0.0034
Error	16	31.7174240	1.9823390		
Corrected Total	24	145.8574560			
'Mnandi' + <i>Meloidogyne enterolobii</i>					
Rep	4	20.77372000	5.19343000		
Treatment	4	16.86596000	4.21649000	0.67	0.6233

Error	16	100.9543200	6.3096450		
Corrected Total	24	138.5940000			
‘Mnandi’ + <i>Meloidogyne javanica</i>					
Rep	4	11.06662400	2.76665600		
Treatment	4	1.46598400	0.36649600	0.19	0.9390
Error	16	30.52753600	1.90797100		
Corrected Total	24	43.06014400			
‘Up-to-date’ + <i>Meloidogyne enterolobii</i>					
Rep	4	3.76941600	0.94235400		
Treatment	4	7.27133600	1.81783400	1.79	0.0294
Error	16	16.21022400	1.01313900		
Corrected Total	24	27.25097600			
‘Up-to-date’ + <i>Meloidogyne javanica</i>					
Rep	4	4.02234400	1.00558600		
Treatment	4	6.72970400	1.68242600	1.62	0.0210
Error	16	16.58545600	1.03659100		
Corrected Total	24	27.33750400			

Appendix A.9 Analysis of variance for potato cultivars' tuber number under *Meloidogyne enterolobii* and *Meloidogyne javanica* at 56 days after inoculation (n = 25) under the net house.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
'Buffelspoort P1' + <i>Meloidogyne enterolobii</i>					
Rep	4	6.24000000	1.56000000		
Treatment	4	31.04000000	7.76000000	0.65	0.6374
Error	16	22.56000000	1.41000000		
Corrected Total	24	59.84000000			
'Buffelspoort P1' + <i>Meloidogyne javanica</i>					
Rep	4	10.16000000	2.54000000		
Treatment	4	2.56000000	0.64000000	5.50	0.0555
Error	16	15.84000000	0.99000000		
Corrected Total	24	28.56000000			
'Hertha' + <i>Meloidogyne enterolobii</i>					
Rep	4	5.20000000	1.30000000		
Treatment	4	8.40000000	2.10000000	2.05	0.0457
Error	16	16.40000000	1.02500000		
Corrected Total	24	30.00000000			
'Hertha' + <i>Meloidogyne javanica</i>					
Rep	4	7.10000000	1.77500000		
Treatment	4	28.07142857	7.01785714	4.92	0.0089
Error	16	22.82857143	1.42678571		
Corrected Total	24	58.00000000			
'Larnoma' + <i>Meloidogyne enterolobii</i>					
Rep	4	5.36000000	1.34000000		
Treatment	4	16.56000000	4.14000000	2.93	0.0542
Error	16	22.64000000	1.41500000		
Corrected Total	24	44.56000000			
'Larnoma' + <i>Meloidogyne javanica</i>					
Rep	4	9.04000000	2.26000000		
Treatment	4	49.44000000	12.36000000	8.19	0.0009
Error	16	24.16000000	1.51000000		
Corrected Total	24	82.64000000			
'Mnandi' + <i>Meloidogyne enterolobii</i>					
Rep	4	6.59000000	1.64750000		
Treatment	4	17.49000000	4.37250000	4.91	0.0090

Error	16	14.26000000	0.89125000		
Corrected Total	24	38.34000000			
‘Mnandi’ + <i>Meloidogyne javanica</i>					
Rep	4	3.36000000	0.84000000		
Treatment	4	26.16000000	6.54000000	16.77	<.0001
Error	16	6.24000000	0.39000000		
Corrected Total	24	35.76000000			
‘Up-to-date’ + <i>Meloidogyne enterolobii</i>					
Rep	4	9.84000000	2.46000000		
Treatment	4	13.84000000	3.46000000	1.50	0.2496
Error	16	36.96000000	2.31000000		
Corrected Total	24	60.64000000			
‘Up-to-date’ + <i>Meloidogyne javanica</i>					
Rep	4	53.84000000	13.46000000		
Treatment	4	23.84000000	5.96000000	2.93	0.0541
Error	16	32.56000000	2.03500000		
Corrected Total	24	110.24000000			

Appendix A.10 Analysis of variance for potato cultivars' tuber weight under *Meloidogyne enterolobii* and *Meloidogyne javanica* at 56 days after inoculation (n = 25) under the net house.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
'Buffelspoort P1' + <i>Meloidogyne enterolobii</i>					
Rep	4	20036.96762	5009.24191		
Treatment	4	29148.26666	7287.06667	2.99	0.5208
Error	16	38994.01746	2437.12609		
Corrected Total	24	88179.25174			
'Buffelspoort P1' + <i>Meloidogyne javanica</i>					
Rep	4	1879.451784	469.862946		
Treatment	4	1555.845184	388.961296	0.16	0.0559
Error	16	39099.81102	2443.73819		
Corrected Total	24	42535.10798			
'Hertha' + <i>Meloidogyne enterolobii</i>					
Rep	4	12525.18292	3131.29573		
Treatment	4	22689.71012	5672.42753	1.52	0.0244
Error	16	59554.29196	3722.14325		
Corrected Total	24	94769.18500			
'Hertha' + <i>Meloidogyne javanica</i>					
Rep	4	70792.39678	17698.09920		
Treatment	4	96430.94858	24107.73714	3.60	0.0283
Error	16	107226.2589	6701.6412		
Corrected Total	24	274449.6043			
'Larnoma' + <i>Meloidogyne enterolobii</i>					
Rep	4	74148.10630	18537.02658		
Treatment	4	27481.93014	6870.48254	2.73	0.0260
Error	16	40233.8376	2514.6149		
Corrected Total	24	141863.8741			
'Larnoma' + <i>Meloidogyne javanica</i>					
Rep	4	71912.1051	17978.0263		
Treatment	4	271178.0651	67794.5163	4.43	0.0133
Error	16	244635.5324	15289.7208		
Corrected Total	24	587725.7026			
'Mnandi' + <i>Meloidogyne enterolobii</i>					
Rep	4	32624.56790	8156.14197		
Treatment	4	13065.39738	3266.34934	0.86	0.5090

Error	16	60809.0155	3800.5635		
Corrected Total	24	106498.9808			
‘Mnandi’ + <i>Meloidogyne javanica</i>					
Rep	4	20072.04936	5018.01234		
Treatment	4	45706.26812	11426.56703	1.44	0.0265
Error	16	126700.6873	7918.7930		
Corrected Total	24	192479.0048			
‘Up-to-date’ + <i>Meloidogyne enterolobii</i>					
Rep	4	6585.008456	1646.252114		
Treatment	4	2381.938296	595.484574	1.99	0.0454
Error	16	4798.77590	299.92349		
Corrected Total	24	13765.72266			
‘Up-to-date’ + <i>Meloidogyne javanica</i>					
Rep	4	5810.756936	1452.689234		
Treatment	4	1698.525496	424.631374	1.24	0.3347
Error	16	5492.74006	343.29625		
Corrected Total	24	13002.02250			

Appendix B: ANOVA tables for Chapter 4

Appendix B.1 Analysis of variance for fungal growth at 21 days after incubation.					
Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
Potato dextrose agar					
Rep	4	1448.096000	1448.096000		
Treatment	1	633.895457	633.895457	6.59	0.0114
Error	122	11726.93654	96.12243		
Corrected Total	124	13808.92800			
Malt extract agar					
Rep	4	2155.392154	2155.392154		
Treatment	1	98.007765	98.007765	0.98	0.0352
Error	122	12256.08808	100.45974		
Corrected Total	124	14509.48800			
Nutrient agar					
Rep	4	2.40384615	2.40384615		
Treatment	1	1.49084163	1.49084163	0.01	0.0530
Error	122	12195.09731	99.95981		
Corrected Total	124	12198.99200			
Water agar					
Rep	4	77.3355385	77.3355385		
Treatment	1	100.7218281	100.7218281	0.89	0.0446
Error	122	13745.75063	112.67009		
Corrected Total	124	13923.80800			

Appendix C: BLAST consensus for Chapter 5

RID: KN6JTX4W013

Database: nr

Query= F1 Consensus

Length=584

Score E

Sequences producing significant alignments: (Bits) Value

gi|2029238279|gb|MZ014549.1| Talaromyces sayulitensis isolate... 1054 0E00
gi|365769131|gb|JN565299.1| Penicillium verruculosum strain 1... 1049 0E00
gi|323651436|gb|HQ832990.1| Penicillium sp. BMP2937 18S ribos... 1048 0E00
gi|323651435|gb|HQ832989.1| Penicillium sp. BMP2894 18S ribos... 1042 0E00
gi|323651434|gb|HQ832988.1| Penicillium sp. BMP2893 18S ribos... 1040 0E00
gi|1226044067|gb|MF574592.1| Talaromyces annesophieae isolate... 1037 0E00
gi|1916862438|ref|NR_170732.1| Talaromyces annesophieae CBS 1... 1037 0E00
gi|1725841687|gb|MN330064.1| Talaromyces sp. Strain MS Pai-PC... 1034 0E00
gi|1725841687|gb|MN330064.1| Talaromyces sp. Strain MS Pai-PC... 65 2E-05
gi|1216650020|gb|CP017345.1| Talaromyces pinophilus strain 1-... 1034 0E00
gi|1216650020|gb|CP017345.1| Talaromyces pinophilus strain 1-... 1026 0E00
gi|1216650020|gb|CP017345.1| Talaromyces pinophilus strain 1-... 1026 0E00
gi|1216650020|gb|CP017345.1| Talaromyces pinophilus strain 1-... 1026 0E00
gi|1216650020|gb|CP017345.1| Talaromyces pinophilus strain 1-... 1017 0E00
gi|1216650020|gb|CP017345.1| Talaromyces pinophilus strain 1-... 1009 0E00
gi|1216650020|gb|CP017345.1| Talaromyces pinophilus strain 1-... 105 2E-17
gi|1039673679|emb|LT558962.1| Talaromyces pinophilus genomic ... 1034 0E00
gi|430768584|dbj|AB474749.2| Acremonium cellulolyticus Y-94 g... 1034 0E00
gi|727366068|gb|KM458826.1| Talaromyces cellulolyticus strain... 1032 0E00
gi|727366028|gb|KM458786.1| Talaromyces cellulolyticus strain... 1032 0E00
gi|312434314|gb|HQ607835.1| Talaromyces verruculosus isolate ... 1032 0E00
gi|291498385|gb|GU566197.1| Penicillium pinophilum strain A26... 1032 0E00
gi|1473247740|gb|MH860684.1| Talaromyces funiculosus culture ... 1031 0E00
gi|1564358007|gb|MK461563.1| Penicillium sp. Isolate strain B... 1031 0E00
gi|1039673680|emb|LT558963.1| Talaromyces pinophilus genomic ... 1030 0E00
gi|2235063530|gb|ON427048.1| Talaromyces sp. Strain XM1-2 sma... 1030 0E00
gi|2235063523|gb|ON427041.1| Talaromyces sp. Strain XM1-2-1 s... 1030 0E00
gi|2235063514|gb|ON427032.1| Talaromyces sp. Strain JGT26S-10... 1030 0E00
gi|386685952|gb|JQ776546.1| Penicillium pinophilum strain SGE... 1030 0E00
gi|326579940|gb|HM469418.1| Penicillium pinophilum strain KUC... 1030 0E00
gi|1511083613|gb|MK139987.1| Talaromyces pinophilus strain 6H... 1029 0E00
gi|2172391645|gb|OM106404.1| Penicillium sp. Isolate GLBRC206... 1029 0E00
gi|189047028|dbj|AB369480.1| Penicillium pinophilum genes for... 1028 0E00

gi|2259521439|emb|OW985285.1| Talaromyces pinophilus genomic ... 1027 0E00
 gi|317383363|gb|HQ631042.1| Penicillium sp. 4 TMS-2011 vouche... 1027 0E00
 gi|291498433|gb|GU566245.1| Penicillium pinophilum strain F10... 1027 0E00
 gi|2306377000|gb|OP482313.1| Talaromyces annesophieae isolate... 1026 0E00
 gi|2222228392|gb|ON211310.1| Talaromyces pinophilus strain TP... 1026 0E00
 gi|2222228390|gb|ON211309.1| Talaromyces pinophilus strain TP... 1026 0E00
 gi|2222228388|gb|ON211308.1| Talaromyces pinophilus strain TP... 1026 0E00
 gi|2119982826|gb|OK584745.1| Talaromyces verruculosus isolate... 1026 0E00
 gi|2119982819|gb|OK584738.1| Talaromyces pinophilus isolate Y... 1026 0E00
 gi|312434503|gb|HQ608025.1| Talaromyces verruculosus isolate ... 1026 0E00
 gi|225355236|gb|FJ795356.1| Talaromyces pinophilus strain 1-9... 1026 0E00
 gi|1735343740|gb|MK450750.1| Talaromyces sp. CMV-2019d strain... 1024 0E00
 gi|1318461055|gb|MG745314.1| Talaromyces sp. Isolate XJ2 smal... 1024 0E00
 gi|1036125647|gb|KX067868.1| Penicillium sp. Isolate Liaoning... 1024 0E00
 gi|1030027887|gb|KU931362.1| Uncultured fungus clone WJ87 18S... 1024 0E00
 gi|571431108|gb|KF800214.1| Uncultured fungus clone CMH123 18... 1024 0E00
 gi|334690535|gb|JF817288.1| Talaromyces pinophilus strain Can... 1024 0E00
 gi|321531578|gb|HQ839772.1| Penicillium pinophilum strain F01... 1024 0E00
 gi|969986897|gb|KT336529.1| Talaromyces pinophilus isolate M2... 1023 0E00
 gi|1318461054|gb|MG745313.1| Talaromyces sp. Isolate XJ3 smal... 1022 0E00
 gi|76593945|gb|DQ200258.1| Trichoderma viride internal transc... 1022 0E00
 gi|1473244841|gb|MH857785.1| Talaromyces flavus var. flavus c... 1021 0E00
 gi|2259526189|emb|OW986071.1| Talaromyces muroii genomic DNA ... 1021 0E00
 gi|1736272964|gb|MN431394.1| Talaromyces muroii strain CBS _7... 1021 0E00

ALIGNMENTS

>gb|MZ014549.1| Talaromyces sayulitensis isolate DTO 439-E1 small subunit ribosomal RNA gene,
 partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed
 spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Length=860

Score = 1054.5 bits (1168), Expect = 0E00

Identities = 584/584 (100%), Gaps = 0/584 (0%)

Strand = Plus/Minus

Query 1 TTCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAACTCCTACCTGATCCGAGGTCAA 60

|||||

Sbjct 687 TTCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAACTCCTACCTGATCCGAGGTCAA

628

Query 61

CCGTGGTAAAACATGGTGGTGACCAACCCCGCAGGTCCTTCCCGAGCGAGTGACAGAGC 120

|||||

Sbjct 627
CCGTGGTAAAACATGGTGGTGACCAACCCCCGCAGGTCCTTCCCGAGCGAGTGACAGAGC 568

Query 121
CCCATACGCTCGAGGACCAGACGGACGTCGCCGCTGCCTTTTCGGGCAGGTCCCCGGGGGG 180
|||||

Sbjct 567
CCCATACGCTCGAGGACCAGACGGACGTCGCCGCTGCCTTTTCGGGCAGGTCCCCGGGGGG 508

Query 181
ACCACACCCAACACACAAGCCGTGCTTGAGGGCAGAAATGACGCTCGGACAGGCATGCCC 240
|||||

Sbjct 507
ACCACACCCAACACACAAGCCGTGCTTGAGGGCAGAAATGACGCTCGGACAGGCATGCCC 448

Query 241
CCCGGAATGCCAGGGGGCGCAATGTGCGTTCAAAGATTTCGATGATTCACGGAATTCTGCA 300
|||||

Sbjct 447
CCCGGAATGCCAGGGGGCGCAATGTGCGTTCAAAGATTTCGATGATTCACGGAATTCTGCA 388

Query 301 ATTCACATTACTTATCGCATTTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCA
360
|||||

Sbjct 387 ATTCACATTACTTATCGCATTTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCA
328

Query 361 TTGTTGAAAGTTTTGACAATTTTTCACAGTACTCAGACAGCCCATCTTCATCAGGGTTCA
420
|||||

Sbjct 327 TTGTTGAAAGTTTTGACAATTTTTCACAGTACTCAGACAGCCCATCTTCATCAGGGTTCA
268

Query 421
CAGAGCGCTTCGGCGGGCGCGGGCCCCGGGGACGTGCGTCCCCCGGCGACCAGGTGGCCCC 480
|||||

Sbjct 267
CAGAGCGCTTCGGCGGGCGCGGGCCCCGGGGACGTGCGTCCCCCGGCGACCAGGTGGCCCC 208

Query 481
GGTGGGCCCCGCCAAAGCAACAGGTGTATTAGAGACAAGGGTGGGAGGTTGGGCCGCGAGG 540
|||||

Sbjct 207
GGTGGGCCCCGCCAAAGCAACAGGTGTATTAGAGACAAGGGTGGGAGGTTGGGCCGCGAGG 148

Query 541 GCCCGCACTCGGTAATGATCCTTCCGCAGGTTACCTACGGAAA 584
|||||

Sbjct 147 GCCCGCACTCGGTAATGATCCTTCCGCAGGTTACCTACGGAAA 104

>gb|JN565299.1| *Penicillium verruculosum* strain 101119 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

gi|614788526|gb|KJ433640.1| *Talaromyces verruculosus* strain IARI-SF3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Length=581

Score = 1049.0 bits (1162), Expect = 0E00

Identities = 581/581 (100%), Gaps = 0/581 (0%)

Strand = Plus/Minus

Appendix D: ANOVA table for Chapter 6

Appendix 6.1 Analysis of variance for *Meloidogyne* parasitism fungal species at 96 hours after exposure *in vitro* (n = 40).

Source	Degree of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + eggs					
Rep	4	133.25000	33.31250		
Treatment	7	10330.57500	1475.79643	114.93	<.0001
Error	28	359.55000	12.84107		
Corrected Total	39	10823.37500			
<i>Meloidogyne javanica</i> + eggs					
Rep	4	31.350000	7.837500		
Treatment	7	2411.575000	344.510714	103.67	<.0001
Error	28	93.050000	3.323214		
Corrected Total	39	2535.975000			
<i>Meloidogyne enterolobii</i> + J2s					
Rep	4	389.00305	97.25076		
Treatment	7	13969.17500	1746.14687	539.05	<.0001
Error	28	90.70000	3.23929		
Corrected Total	39	14448.87805			
<i>Meloidogyne javanica</i> + J2s					
Rep	4	16.750000	4.187500		
Treatment	7	5074.800000	724.971429	177.36	<.0001
Error	28	114.450000	4.087500		
Corrected Total	39	5206.000000			

Appendix E: ANOVA table for Chapter 7

Given that seedling vigour, is a function of plumule, radicle and germination; ANOVA for seedling vigour only is presented herein.

Appendix 7.1 Analysis of variance for tomato seedlings' vigour on fungal species at 14 days after inoculation.					
Control					
Source	Degrees of freedom	Sum of squares	Mean Square	F	P
Rep	4	6.3554372	1.5888593		
Treatment	6	233.4132543	38.9022090	36.43	<.0001
Error	24	25.6301394	1.0679225		
Corrected Total	34	265.3988309			
<i>Aspergillus flavus</i>					
Rep	4	282.230101	70.557525		
Treatment	6	6305.049809	1050.841635	10.45	<.0001
Error	24	2413.188998	100.549542		
Corrected Total	34	9000.468908			
<i>Aspergillus terreus</i>					
Rep	4	2.7782174	0.6945543		
Treatment	6	365.5104305	60.9184051	107.47	<.0001
Error	24	13.6046973	0.5668624		
Corrected Total	34	381.8933452			
<i>Penicillium</i> sp. AL-38 IRH-2012b					
Rep	4	17.4532665	4.3633166		
Treatment	6	735.3043329	122.5507222	42.22	<.0001
Error	24	69.6641809	2.9026742		
Corrected Total	34	822.4217803			
<i>Talaromyces minioluteus</i>					
Rep	4	5.6091232	1.4022808		
Treatment	6	349.4550498	58.2425083	33.13	<.0001
Error	24	42.1898486	1.7579104		
Corrected Total	34	397.2540216			
<i>Talaromyces purpureogenus</i>					
Rep	4	3.9692576	0.9923144		
Treatment	6	170.0276752	28.3379459	24.49	<.0001

Error	24	27.7703622	1.1570984		
Corrected Total	34	201.7672950			
<i>Talaromyces sayulitensis</i>					
Rep	4	36.23780401	9.05945100		
Treatment	6	57.51447349	9.58574558	14.00	<.0001
Error	24	16.4318358	0.6846598		
Corrected Total	34	110.1841133			
<i>Trichoderma ghanense</i>					
Rep	4	2.2073809	0.5518452		
Treatment	6	366.2763589	61.0460598	55.72	<.0001
Error	24	26.2950887	1.0956287		
Corrected Total	34	394.7788285			
<i>Trichoderma viride</i>					
Rep	4	8.5916984	2.1479246		
Treatment	6	227.8067484	37.9677914	29.19	<.0001
Error	24	31.2137008	1.3005709		
Corrected Total	34	267.6121476			

Appendix F: ANOVA table for Chapter 8

Since red sorghum stimulated faster fungal growth and sporulation, ANOVA for growth on red sorghum only is presented herein.

Appendix F.1 Analysis of variance for *Aspergillus flavus* growth on red sorghum at 21 days after incubation.

Source	Degree of freedom	Sum of Squares	Mean Square	F	P
Positive geotropism					
Rep	4	0.91360000	0.22840000		
Treatment	4	72.90960000	18.22740000	60.18	<.0001
Error	16	4.84640000	0.30290000		
Corrected Total	24	78.66960000			
Negative geotropism					
Rep	4	0.48800000	0.12200000		
Treatment	4	46.48400000	11.62100000	77.86	<.0001
Error	16	2.38800000	0.14925000		
Corrected Total	24	49.36000000			

Appendix F.2 Analysis of variance for *Aspergillus terreus* growth on red sorghum at 21 days after incubation.

Source	Degree of freedom	Sum of Squares	Mean Square	F	P
Positive geotropism					
Rep	4	2.87600000	0.71900000		
Treatment	4	32.42000000	8.10500000	38.78	<.0001
Error	16	3.34400000	0.20900000		
Corrected Total	24	38.64000000			
Negative geotropism					
Rep	4	1.43840000	0.35960000		
Treatment	4	78.66240000	19.66560000	249.41	<.0001
Error	16	1.26160000	0.07885000		
Corrected Total	24	81.36240000			

Appendix F.3 Analysis of variance for *Penicillium* sp. AL-38 IRH-2012b growth on red sorghum at 21 days after incubation.

Source	Degree of freedom	Sum of Squares	Mean Square	F Value	P
Positive geotropism					
Rep	4	2.01040000	0.50260000		
Treatment	4	45.31040000	11.32760000	50.27	<.0001
Error	16	3.60560000	0.22535000		
Corrected Total	24	50.92640000			
Negative geotropism					
Rep	4	0.33360000	0.08340000		
Treatment	4	54.69760000	13.67440000	76.87	<.0001
Error	16	2.84640000	0.17790000		
Corrected Total	24	57.87760000			

Appendix F.4 Analysis of variance for *Talaromyces minioluteus* growth on red sorghum at 21 days after incubation.

Source	Degree of freedom	Sum of Squares	Mean Square	F Value	P
Positive geotropism					
Rep	4	1.59760000	0.39940000		
Treatment	4	80.65760000	20.16440000	71.21	<.0001
Error	16	4.53040000	0.28315000		
Corrected Total	24	86.78560000			
Negative geotropism					
Rep	4	0.9656000	0.2414000		
Treatment	4	199.9376000	49.9844000	196.87	<.0001
Error	16	4.0624000	0.2539000		
Corrected Total	24	204.9656000			

Appendix F.5 Analysis of variance for *Talaromyces purpureogenus* growth on red sorghum at 21 days after incubation.

Source	Degree of freedom	Sum of Squares	Mean Square	F Value	P
Positive geotropism					
Rep	4	8.4616000	2.1154000		
Treatment	4	111.2816000	27.8204000	119.97	<.0001
Error	16	3.7104000	0.2319000		
Corrected Total	24	123.4536000			
Negative geotropism					
Rep	4	1.61360000	0.40340000		
Treatment	4	77.50960000	19.37740000	156.40	<.0001
Error	16	1.98240000	0.12390000		
Corrected Total	24	81.10560000			

Appendix F.6 Analysis of variance for *Talaromyces sayulitensis* growth on red sorghum at 21 days after incubation.

Source	Degree of freedom	Sum of Squares	Mean Square	F Value	P
Positive geotropism					
Rep	4	5.62560000	1.40640000		
Treatment	4	60.40160000	15.10040000	59.12	<.0001
Error	16	4.08640000	0.25540000		
Corrected Total	24	70.11360000			
Negative geotropism					
Rep	4	0.4120000	0.1030000		
Treatment	4	159.4080000	39.8520000	207.02	<.0001
Error	16	3.0800000	0.1925000		
Corrected Total	24	162.9000000			

Appendix F.7 Analysis of variance for *Trichoderma ghanense* growth on red sorghum at 21 days after incubation.

Source	Degree of freedom	Sum of Squares	Mean Square	F	P
Positive geotropism					
Rep	4	5.55760000	1.38940000		
Treatment	4	46.43760000	11.60940000	143.06	<.0001
Error	16	1.29840000	0.08115000		
Corrected Total	24	53.29360000			
Negative geotropism					
Rep	4	0.61840000	0.15460000		
Treatment	4	78.25040000	19.56260000	48.99	<.0001
Error	16	6.38960000	0.39935000		
Corrected Total	24	85.25840000			

Appendix F.8 Analysis of variance for *Trichoderma viride* growth on red sorghum at 21 days after incubation.

Source	Degree of freedom	Sum of Squares	Mean Square	F	P
Positive geotropism					
Rep	4	3.7296000	0.9324000		
Treatment	4	141.8416000	35.4604000	117.85	<.0001
Error	16	4.8144000	0.3009000		
Corrected Total	24	150.3856000			
Negative geotropism					
Rep	4	0.7360000	0.1840000		
Rep	4	0.7360000	0.1840000	0.78	0.5521
Error	16	3.7560000	0.2347500		
Corrected Total	24	151.2000000			

Appendix F.9 Analysis of variance for fungal spore count post growth on grain substrates at 21 days after incubation.

Source	Degree of freedom	Sum of Squares	Mean Square	F	P
Red sorghum					
Rep	4	1489.02025	1489.02025		
Treatment	4	13827.31086	13827.31086	2.23	0.01
Error	16	223701.2417	6213.9234		
Corrected Total	24	247152.4031			
White sorghum					
Rep	4	100.352000	100.352000		
Treatment	4	3592.967538	3592.967538	3.78	0.05
Error	16	34195.32390	949.87011		
Corrected Total	24	37968.81900			
Barley					
Rep	4	253.472000	253.472000		
Treatment	4	2600.008754	2600.008754	7.00	0.01
Error	16	13372.74890	371.46525		
Corrected Total	24	16842.24400			
Wheat					
Rep	4	117.37013	117.37013		
Treatment	4	30531.33647	30531.33647	79.40	< 0.01
Error	16	13842.34978	384.50972		
Corrected Total	24	45854.72000			
Wheat bran					
Rep	4	483.144500	483.144500		
Treatment	4	3189.611779	3189.611779	6.36	0.01
Error	16	18049.67426	501.37984		
Corrected Total	24	22141.64400			
Control					
Rep	4	3.20000	3.20000		
Treatment	4	9140.10119	9140.10119	0.96	0.03
Error	16	342803.3083	9522.3141		
Corrected Total	24	364793.3877			

Appendix G: ANOVA table for Chapter 9

Appendix G.1 Analysis of variance for fungal growth on salinity-amended media at 21 days after incubation *in vitro* (n = 40).

Source	Degree of Freedom	Sum of Squares	Mean Square	F	P
1% salinity					
Rep	4	21.85000	5.46250		
Treatment	7	12773.10000	1824.72857	472.42	<.0001
Error	28	108.15000	3.86250		
Corrected Total	39	12903.10000			
4% salinity					
Rep	4	114.35000	28.58750		
Treatment	7	16444.80000	2349.25714	74.04	<.0001
Error	28	888.45000	31.73036		
Corrected Total	39	17447.60000			
7% salinity					
Rep	4	9.65000	2.41250		
Treatment	7	19247.50000	2749.64286	1720.45	<.0001
Error	28	44.75000	1.59821		
Corrected Total	39	19301.90000			
10% salinity					
Rep	4	4.10000	1.02500		
Treatment	7	12382.37500	1768.91071	1395.20	<.0001
Error	28	35.50000	1.26786		
Corrected Total	39	12421.97500			

Appendix G.2 Analysis of variance for fungal growth on pH-amended media at 21 days after incubation *in vitro* (n = 40).

Source	Degree of Freedom	Sum of Squares	Mean Square	F	P
6.25 pH					
Rep	4	15.85000	3.96250		
Treatment	7	14965.77500	2137.96786	557.64	<.0001
Error	28	107.35000	3.83393		
Corrected Total	39	15088.97500			
6.50 pH					
Rep	4	24.250000	6.062500		
Treatment	7	9776.400000	1396.628571	401.70	<.0001
Error	28	97.350000	3.476786		
Corrected Total	39	9898.000000			
6.75 pH					
Rep	4	28.85000	7.21250		
Treatment	7	25184.00000	3597.71429	659.48	<.0001
Error	28	152.75000	5.45536		
Corrected Total	39	25365.60000			
7.00 pH					
Rep	4	14.85000	3.71250		
Treatment	7	26454.30000	3779.18571	1080.32	<.0001
Error	28	97.95000	3.49821		
Corrected Total	39	26567.10000			

Appendix G.3 Analysis of variance for fungal growth on nutrient-amended media at 21 days after incubation *in vitro* (n = 40).

Source	Degree of Freedom	Sum of Squares	Mean Square	F	P
25% nutrient					
Rep	4	121.75000	30.43750		
Treatment	7	17687.60000	2526.80000	107.42	<.0001
Error	28	658.65000	23.52321		
Corrected Total	39	18468.00000			
50% nutrient					
Rep	4	147.85000	36.96250		
Treatment	7	19915.17500	2845.02500	111.42	<.0001
Error	28	714.95000	25.53393		
Corrected Total	39	20777.97500			
75% nutrient					
Rep	4	38.75000	9.68750		
Treatment	7	16484.00000	2354.85714	202.72	<.0001
Error	28	325.25000	11.61607		
Corrected Total	39	16848.00000			
100% nutrient					
Rep	4	65.150000	16.287500		
Treatment	7	6966.975000	995.282143	81.57	<.0001
Error	28	341.650000	12.201786		
Corrected Total	39	7373.775000			

Appendix G.4 Analysis of variance for fungal growth at different temperatures at 21 days after incubation *in vitro* (n = 40).

Source	Degrees freedom	of	Sum of Squares	Mean Square	F	P
10 °C						
Rep	4		0.90000000	0.22500000		
Treatment	7		62.80000000	8.97142857	93.04	<.0001
Error	28		2.70000000	0.09642857		
Corrected Total	39		66.40000000			
20 °C						
Rep	4		1.8500000	0.4625000		
Treatment	7		130.0000000	18.5714286	90.43	<.0001
Error	28		5.7500000	0.2053571		
Corrected Total	39		137.6000000			
30 °C						
Rep	4		65.150000	16.287500		
Treatment	7		6966.975000	995.282143	81.57	<.0001
Error	28		341.650000	12.201786		
Corrected Total	39		7373.775000			
40 °C						
Rep	4		1.650000	0.412500		
Treatment	7		2011.900000	287.414286	265.16	<.0001
Error	28		30.350000	1.083929		
Corrected Total	39		2043.900000			

Appendix H: ANOVA table for Chapter 10

Appendix H.1 Analysis of variance for *Meloidogyne enterolobii* and *Meloidogyne javanica* final population on potato ‘Hertha’ treated with nematophagous fungi with and without compost at 56 days after inoculation (n = 35) under net house conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	179832.91	179832.91		
Treatment	6	15796033.40	15796033.40	4.65	0.0387
Error	24	108711433.7	3397232.3		
Corrected Total	34	124687300.0			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	45926.41	45926.41		
Treatment	6	62091180.18	62091180.18	5.21	0.0292
Error	24	381263162.0	11914473.8		
Corrected Total	34	443400268.6			
<i>Meloidogyne javanica</i> + compost					
Rep	4	78325.11	32165057		
Treatment	6	1163611.26	384561.64	5.47	0.0347
Error	24	3216854.64	541632.32		
Corrected Total	34	5641321.51			
<i>Meloidogyne javanica</i> – compost					
Rep	4	87721.20	87721.20		
Treatment	6	40015879.31	40015879.31	5.11	0.0307
Error	24	250458534.1	7826829.2		
Corrected Total	34	290562134.6			

Appendix H.2 Analysis of variance for *Meloidogyne enterolobii* and *Meloidogyne javanica* gall number on potato ‘Hertha’ treated with nematophagous fungi with and without compost at 56 days after inoculation (n = 35) under net house conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	7.55714286	7.55714286		
Treatment	6	11.42857143	11.42857143	6.56	0.0154
Error	24	55.75714286	1.74241071		
Corrected Total	34	74.74285714			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	1.42857143	1.42857143		
Treatment	6	0.11428571	0.11428571	0.04	0.8398
Error	24	88.05714286	2.75178571		
Corrected Total	34	89.60000000			
<i>Meloidogyne javanica</i> + compost					
Rep	4	3.65714286	3.65714286		
Treatment	6	2.57857143	2.57857143	2.31	0.0480
Error	24	35.65000000	1.11406250		
Corrected Total	34	41.88571429			
<i>Meloidogyne javanica</i> – compost					
Rep	4	8.92857143	8.92857143		
Treatment	6	0.86428571	0.86428571	0.36	0.0519
Error	24	76.49285714	2.39040179		
Corrected Total	34	86.28571429			

Appendix H.3 Analysis of variance for *Meloidogyne enterolobii* and *Meloidogyne javanica* reproductive factor on potato ‘Hertha’ treated with nematophagous fungi with and without compost at 56 days after inoculation (n = 35) under net house conditions.

Source	Degrees freedom	of Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	0.08092000	0.08092000		
Treatment	6	7.03360286	7.03360286	4.66	0.0384
Error	24	48.28194571	1.50881080		
Corrected Total	34	55.39646857			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	0.02126286	0.02126286		
Treatment	6	27.79474571	27.79474571	5.25	0.0287
Error	24	169.4770200	5.2961569		
Corrected Total	34	197.2930286			
<i>Meloidogyne javanica</i> + compost					
Rep	4	1730.06	1730.06		
Treatment	6	15661959.11	15661959.11	4.77	0.0364
Error	24	105097725.1	3284303.9		
Corrected Total	34	120761414.3			
<i>Meloidogyne javanica</i> – compost					
Rep	4	0.03703000	0.03703000		
Treatment	6	17.82144643	17.82144643	5.12	0.0305
Error	24	111.3253521	3.4789173		
Corrected Total	34	129.1838286			

Appendix H.4 Analysis of variance for potato ‘Hertha’ plant height under *Meloidogyne enterolobii* and *Meloidogyne javanica* treated with nematophagous fungi with and without compost at 56 days after inoculation (n = 35) under net house conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	9.3549729	9.3549729		
Treatment	6	747.6085029	747.6085029	25.65	<.0001
Error	24	932.808313	29.150260		
Corrected Total	34	1689.771789			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	0.00357143	0.00357143		
Treatment	6	3.30178571	3.30178571	0.12	0.0478
Error	24	904.5563571	28.2673862		
Corrected Total	34	907.8617143			
<i>Meloidogyne javanica</i> + compost					
Rep	4	44.64014286	44.64014286		
Treatment	6	13.33028571	13.33028571	0.56	0.0459
Error	24	760.0284286	23.7508884		
Corrected Total	34	817.9988571			
<i>Meloidogyne javanica</i> – compost					
Rep	4	9.1441429	9.1441429		
Treatment	6	238.4235000	238.4235000	6.43	0.0163
Error	24	1186.296357	37.071761		
Corrected Total	34	1433.864000			

Appendix H.5 Analysis of variance for potato ‘Hertha’ stem diameter under *Meloidogyne enterolobii* and *Meloidogyne javanica* treated with nematophagous fungi with and without compost at 56 days after inoculation (n = 35) under net house conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	13.81728571	13.81728571		
Treatment	6	16.18400000	16.18400000	4.12	0.0508
Error	24	125.7301429	3.9290670		
Corrected Total	34	155.7314286			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	0.02414286	0.02414286		
Treatment	6	0.13828571	0.13828571	0.10	0.0576
Error	24	43.04328571	1.34510268		
Corrected Total	34	43.20571429			
<i>Meloidogyne javanica</i> + compost					
Rep	4	0.60357143	0.60357143		
Treatment	6	0.28350000	0.28350000	0.15	0.6987
Error	24	59.47192857	1.85849777		
Corrected Total	34	60.35900000			
<i>Meloidogyne javanica</i> – compost					
Rep	4	0.26414286	0.26414286		
Treatment	6	1.89778571	1.89778571	0.97	0.3318
Error	24	62.54492857	1.95452902		
Corrected Total	34	64.70685714			

Appendix H.6 Analysis of variance for potato ‘Hertha’ chlorophyll content under *Meloidogyne enterolobii* and *Meloidogyne javanica* treated with nematophagous fungi with and without compost at 56 days after inoculation (n = 35) under net house conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	669.6035714	669.6035714		
Treatment	6	248.2581779	248.2581779	0.16	0.04691
Error	24	49085.83372	1533.93230		
Corrected Total	34	50003.69547			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	1189.856571	1189.856571		
Treatment	6	229.120071	229.120071	0.15	0.0544
Error	24	50021.79479	1563.18109		
Corrected Total	34	51440.77143			
<i>Meloidogyne javanica</i> + compost					
Rep	4	5001.555571	5001.555571		
Treatment	6	711.903500	711.903500	0.29	0.0597
Error	24	77621.95636	2425.68614		
Corrected Total	34	83335.41543			
<i>Meloidogyne javanica</i> – compost					
Rep	4	340.12129	340.12129		
Treatment	6	11914.08750	11914.08750	8.08	0.0077
Error	24	47178.27693	1474.32115		
Corrected Total	34	59432.48571			

Appendix H.7 Analysis of variance for potato ‘Hertha’ fresh weight under *Meloidogyne enterolobii* and *Meloidogyne javanica* treated with nematophagous fungi with and without compost at 56 days after inoculation (n = 35) under net house conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	1380.928973	1380.928973		
Treatment	6	19.537786	19.537786	0.14	0.7113
Error	24	4483.008116	140.094004		
Corrected Total	34	5883.474874			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	23.89728571	23.89728571		
Treatment	6	10.17901786	10.17901786	0.23	0.6367
Error	24	1432.447165	44.763974		
Corrected Total	34	1466.523469			
<i>Meloidogyne javanica</i> + compost					
Rep	4	64.3968514	64.3968514		
Treatment	6	103.8021607	103.8021607	2.10	0.1575
Error	24	1585.318828	49.541213		
Corrected Total	34	1753.517840			
<i>Meloidogyne javanica</i> – compost					
Rep	4	14.15700571	14.15700571		
Treatment	6	1.75168286	1.75168286	0.11	0.0456
Error	24	523.3191800	16.3537244		
Corrected Total	34	539.2278686			

Appendix H.8 Analysis of variance for potato ‘Hertha’ dry mass under *Meloidogyne enterolobii* and *Meloidogyne javanica* treated with nematophagous fungi with and without compost at 56 days after inoculation (n = 35) under net house conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	0.00464143	0.00464143		
Treatment	6	0.70007143	0.70007143	0.10	0.0570
Error	24	216.8486414	6.7765200		
Corrected Total	34	217.5533543			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	0.01515571	0.01515571		
Treatment	6	1.07888643	1.07888643	0.28	0.0561
Error	24	123.7964150	3.8686380		
Corrected Total	34	124.8904571			
<i>Meloidogyne javanica</i> + compost					
Rep	4	2.15426286	2.15426286		
Treatment	6	0.16116071	0.16116071	0.04	0.0494
Error	24	123.4765207	3.8596416		
Corrected Total	34	125.7919543			
<i>Meloidogyne javanica</i> – compost					
Rep	4	7.60980571	7.60980571		
Treatment	6	8.94103143	8.94103143	1.49	0.0310
Error	24	191.9262600	5.9976956		
Corrected Total	34	208.4770971			

Appendix H.9 Analysis of variance for potato ‘Hertha’ tuber number under *Meloidogyne enterolobii* and *Meloidogyne javanica* treated with nematophagous fungi with and without compost at 56 days after inoculation (n = 35) under net house conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	4.12857143	4.12857143		
Treatment	6	7.77857143	7.77857143	1.99	0.0280
Error	24	125.0642857	3.9082589		
Corrected Total	34	136.9714286			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	0.35714286	0.35714286		
Treatment	6	3.15000000	3.15000000	1.07	0.0391
Error	24	94.37857143	2.94933036		
Corrected Total	34	97.88571429			
<i>Meloidogyne javanica</i> + compost					
Rep	4	0.255464651	0.374568652		
Treatment	6	2.06428571	2.06428571	1.80	0.1887
Error	24	36.62142857	1.14441964		
Corrected Total	34	38.68571429			
<i>Meloidogyne javanica</i> – compost					
Rep	4	0.35714286	0.35714286		
Treatment	6	5.60000000	5.60000000	1.85	0.0427
Error	24	96.6142857	3.0191964		
Corrected Total	34	102.5714286			

Appendix H.10 Analysis of variance for potato ‘Hertha’ tuber weight under *Meloidogyne enterolobii* and *Meloidogyne javanica* treated with nematophagous fungi with and without compost at 56 days after inoculation (n = 35) under net house conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	33.534321	33.534321		
Treatment	6	3794.445161	3794.445161	8.35	0.0069
Error	24	14545.57099	454.54909		
Corrected Total	34	18373.55047			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	46.62624143	46.62624143		
Treatment	6	81.80914571	81.80914571	0.45	0.0371
Error	24	5815.836601	181.744894		
Corrected Total	34	5944.271989			
<i>Meloidogyne javanica</i> + compost					
Rep	4	47.82542286	47.82542286		
Treatment	6	30.38320286	30.38320286	0.09	0.0563
Error	24	10353.58681	323.54959		
Corrected Total	34	10431.79544			
<i>Meloidogyne javanica</i> – compost					
Rep	4	84.7660129	84.7660129		
Treatment	6	948.4811429	948.4811429	4.86	0.0347
Error	24	6240.859233	195.026851		
Corrected Total	34	7274.106389			

Appendix H.11 Analysis of variance for *Meloidogyne enterolobii* and *Meloidogyne javanica* final population on potato ‘Hertha’ treated with nematophagous fungi with compost and without compost at 110 days after inoculation (n = 35) under field conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	91224.70	91224.70		
Treatment	6	83831944.46	83831944.46	5.32	0.0278
Error	24	504697945.1	15771810.8		
Corrected Total	34	588621114.3			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	20812.1	20812.1		
Treatment	6	207486351.6	207486351.6	5.33	0.0275
Error	24	1244884591	38902643		
Corrected Total	34	1452391754			
<i>Meloidogyne javanica</i> + compost					
Rep	4	907.20	907.20		
Treatment	6	71180216.26	71180216.26	5.47	0.0258
Error	24	416439805.1	13013743.9		
Corrected Total	34	487620928.6			
<i>Meloidogyne javanica</i> – compost					
Rep	4	21542.6	21542.6		
Treatment	8	161074487.3	161074487.3	6.17	0.0185
Error	32	836017230.3	26125538.4		
Corrected Total	34	997113260.3			

Appendix H.12 Analysis of variance for *Meloidogyne enterolobii* and *Meloidogyne javanica* gall number on potato ‘Hertha’ treated with nematophagous fungi with compost and without compost at 110 days after inoculation (n = 35) under field conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	1.15714286	1.15714286		
Treatment	6	1.40000000	1.40000000	0.17	0.0568
Error	24	264.4142857	8.2629464		
Corrected Total	34	266.9714286			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	1.42857143	1.42857143		
Treatment	6	5.60000000	5.60000000	0.45	0.0558
Error	24	395.6571429	12.3642857		
Corrected Total	34	402.6857143			
<i>Meloidogyne javanica</i> + compost					
Rep	4	3.21428571	3.21428571		
Treatment	6	3.77857143	3.77857143	2.47	0.0256
Error	24	48.89285714	1.52790179		
Corrected Total	34	55.88571429			
<i>Meloidogyne javanica</i> – compost					
Rep	4	6.91428571	6.91428571		
Treatment	8	10.86428571	10.86428571	4.34	0.0453
Error	32	80.10714286	2.50334821		
Corrected Total	34	97.88571429			

Appendix H.13 Analysis of variance for *Meloidogyne enterolobii* and *Meloidogyne javanica* reproductive factor on potato ‘Hertha’ treated with nematophagous fungi with compost and without compost at 110 days after inoculation (n = 35) under field conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	0.04054431	0.04054431		
Treatment	6	37.25864195	37.25864195	5.32	0.0278
Error	24	224.3101984	7.0096937		
Corrected Total	34	261.6093847			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	0.00891571	0.00891571		
Treatment	6	92.34064286	92.34064286	5.34	0.0274
Error	24	553.1063386	17.2845731		
Corrected Total	34	645.4558971			
<i>Meloidogyne javanica</i> + compost					
Rep	4	0.00040320	0.00040320		
Treatment	6	31.63565180	31.63565180	5.47	0.0358
Error	24	185.0843579	5.7838862		
Corrected Total	34	216.7204129			
<i>Meloidogyne javanica</i> – compost					
Rep	4	25.98359863	25.98359863		
Treatment	6	22.95054183	22.95054183	0.86	0.0313
Error	24	856.3563242	26.7611351		
Corrected Total	34	905.2904647			

Appendix H.14 Analysis of variance for potato ‘Hertha’ plant height under *Meloidogyne enterolobii* and *Meloidogyne javanica* treated with nematophagous fungi with and without compost at 110 days after inoculation (n = 35) under field conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	9.5238914	9.5238914		
Treatment	6	153.1762400	153.1762400	11.52	0.0019
Error	24	425.4168286	13.2942759		
Corrected Total	34	588.1169600			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	0.5142857	0.5142857		
Treatment	6	130.5645714	130.5645714	12.14	0.0015
Error	24	344.1565714	10.7548929		
Corrected Total	34	475.2354286			
<i>Meloidogyne javanica</i> + compost					
Rep	4	1850.400143	1850.400143		
Treatment	6	3211.216071	3211.216071	3.61	0.0563
Error	24	28429.40779	888.41899		
Corrected Total	34	33491.02400			
<i>Meloidogyne javanica</i> – compost					
Rep	4	1850.400143	1850.400143		
Treatment	6	3211.216071	3211.216071	3.61	0.0563
Error	24	28429.40779	888.41899		
Corrected Total	34	33491.02400			

Appendix H.15 Analysis of variance for potato ‘Hertha’ stem diameter under *Meloidogyne enterolobii* and *Meloidogyne javanica* treated with nematophagous fungi with and without compost at 110 days after inoculation (n = 35) under field conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	0.48057143	0.48057143		
Treatment	6	2.63314286	2.63314286	3.67	0.0544
Error	24	22.95771429	0.71742857		
Corrected Total	34	26.07142857			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	0.30228571	0.30228571		
Treatment	6	1.28257143	1.28257143	1.20	0.0218
Error	24	34.25057143	1.07033036		
Corrected Total	34	35.83542857			
<i>Meloidogyne javanica</i> + compost					
Rep	4	0.00000000	0.00000000		
Treatment	6	6.00714286	6.00714286	18.52	0.0001
Error	24	10.37857143	0.32433036		
Corrected Total	34	16.38571429			
<i>Meloidogyne javanica</i> – compost					
Rep	4	0.00000000	0.00000000		
Treatment	6	6.00714286	6.00714286	18.52	0.0001
Error	24	10.37857143	0.32433036		
Corrected Total	34	16.38571429			

Appendix H.16 Analysis of variance for potato ‘Hertha’ chlorophyll content under *Meloidogyne enterolobii* and *Meloidogyne javanica* treated with nematophagous fungi with compost and without compost at 110 days after inoculation (n = 35) under field conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	62.776630	62.776630		
Treatment	6	9414.748035	9414.748035	17.64	0.0002
Error	24	17078.32572	533.69768		
Corrected Total	34	26555.85039			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	22.743000	22.743000		
Treatment	6	4688.743143	4688.743143	15.48	0.0004
Error	24	9690.78529	302.83704		
Corrected Total	34	14402.27143			
<i>Meloidogyne javanica</i> + compost					
Rep	4	0.000000	0.000000		
Treatment	6	8729.501786	8729.501786	85.36	<.0001
Error	24	3272.48393	102.26512		
Corrected Total	34	12001.98571			
<i>Meloidogyne javanica</i> – compost					
Rep	4	0.000000	0.000000		
Treatment	6	8729.501786	8729.501786	85.36	<.0001
Error	24	3272.48393	102.26512		
Corrected Total	34	12001.98571			

Appendix H.17 Analysis of variance for potato ‘Hertha’ fresh weight under *Meloidogyne enterolobii* and *Meloidogyne javanica* treated with nematophagous fungi with compost and without compost at 110 days after inoculation (n = 35) under field conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	5.9451429	5.9451429		
Treatment	6	402.5625714	402.5625714	15.46	0.0004
Error	24	833.155143	26.036098		
Corrected Total	34	1241.662857			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	1.0321429	1.0321429		
Treatment	6	183.0857857	183.0857857	10.36	0.0030
Error	24	565.7889286	17.6809040		
Corrected Total	34	749.9068571			
<i>Meloidogyne javanica</i> + compost					
Rep	4	0.00000000	0.00000000		
Treatment	6	0.00144643	0.00144643	0.99	0.0140
Error	24	808.7059821	25.2720619		
Corrected Total	34	808.7074286			
<i>Meloidogyne javanica</i> – compost					
Rep	4	0.00000000	0.00000000		
Treatment	6	0.00144643	0.00144643	1.99	0.0440
Error	24	808.7059821	25.2720619		
Corrected Total	34	808.7074286			

Appendix H.18 Analysis of variance for potato ‘Hertha’ dry weight under *Meloidogyne enterolobii* and *Meloidogyne javanica* treated with nematophagous fungi with compost and without compost at 110 days after inoculation (n = 35) under field conditions.

Source	Degrees of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	0.00104143	0.00104143		
Treatment	6	7.99694000	7.99694000	12.25	0.0014
Error	24	20.89753286	0.65304790		
Corrected Total	34	28.89551429			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	0.14904143	0.14904143		
Treatment	6	8.45257143	8.45257143	15.16	0.0005
Error	24	17.83905571	0.55747049		
Corrected Total	34	26.44066857			
<i>Meloidogyne javanica</i> + compost					
Rep	4	0.132124968	0.132124968		
Treatment	6	2.66064286	2.66064286	7.34	0.0107
Error	24	11.59535714	0.36235491		
Corrected Total	34	14.25600000			
<i>Meloidogyne javanica</i> – compost					
Rep	4	0.23765265	0.23765265		
Treatment	6	2.66064286	2.66064286	7.34	0.0107
Error	24	11.59535714	0.36235491		
Corrected Total	34	14.25600000			

Appendix H.19 Analysis of variance for potato ‘Hertha’ tuber number under *Meloidogyne enterolobii* and *Meloidogyne javanica* treated with nematophagous fungi with compost and without compost at 110 days after inoculation (n = 35) under field conditions.

Source	Degree of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	6.91428571	6.91428571		
Treatment	6	1.40000000	1.40000000	1.16	0.2897
Error	24	38.65714286	1.20803571		
Corrected Total	34	46.97142857			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	0.35714286	0.35714286		
Treatment	6	1.02857143	1.02857143	1.17	0.2882
Error	24	28.21428571	0.88169643		
Corrected Total	34	29.60000000			
<i>Meloidogyne javanica</i> + compost					
Rep	4	1.659146515	1.659146515		
Treatment	6	16.32168754	16.32168754	10.45	0.0013
Error	24	37.60000000	1.321364161		
Corrected Total	34	54.32163411			
<i>Meloidogyne javanica</i> – compost					
Rep	4	1.72857143	1.72857143		
Treatment	6	16.45714286	16.45714286	12.39	0.0013
Error	24	42.50000000	1.32812500		
Corrected Total	34	60.68571429			

Appendix H.20 Analysis of variance for potato ‘Hertha’ tuber weight under *Meloidogyne enterolobii* and *Meloidogyne javanica* treated with nematophagous fungi with compost and without compost at 110 days after inoculation (n = 35) under field conditions.

Source	Degree of freedom	Sum of Squares	Mean Square	F	P
<i>Meloidogyne enterolobii</i> + compost					
Rep	4	3.18862286	3.18862286		
Treatment	6	96.82817786	96.82817786	4.45	0.0428
Error	24	696.1278736	21.7539960		
Corrected Total	34	796.1446743			
<i>Meloidogyne enterolobii</i> – compost					
Rep	4	0.08228571	0.08228571		
Treatment	6	77.09380071	77.09380071	3.12	0.0370
Error	24	791.1309136	24.7228410		
Corrected Total	34	868.3070000			
<i>Meloidogyne javanica</i> + compost					
Rep	4	0.0000000	0.0000000		
Treatment	6	207.6446429	207.6446429	0.20	0.6597
Error	24	33629.12507	1050.91016		
Corrected Total	34	33836.76971			
<i>Meloidogyne javanica</i> – compost					
Rep	4	1165.90089	1165.90089		
Treatment	6	40267.11045	40267.11045	9.08	0.0050
Error	24	141981.9116	4436.9347		
Corrected Total	34	183414.9229			