MECHANISM OF RESISTANCE TO *MELOIDOGYNE INCOGNITA* AND *MELOIDOGYNE JAVANICA* IN *CUCUMIS AFRICANUS* AND *CUCUMIS MYRIOCARPUS* SEEDLINGS

by

MUKONDELELI NDIVHUWO RAMATSITSI

MINI-DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

MASTER OF SCIENCE

in

AGRICULTURE (HORTICULTURE), DEPARTMENT OF PLANT PRODUCTION

in the

FACULTY OF SCIENCE AND AGRICULTURE

SCHOOL OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES

at the

UNIVERSITY OF LIMPOPO

SUPERVISOR : DR. K.M. POFU (ARC)

CO-SUPERVISOR : PROF. P.W. MASHELA (UL)

2017

TABLE OF CONTENTS

DECLARATION

I declare that this mini-dissertation hereby submitted to the University of Limpopo for the degree Master of Science in Agriculture (Horticulture) has not previously been submitted by me or anybody for a degree at this or any other University, that this is my work in design and in execution and that all materials contained herein had been acknowledged.

Ramatsitsi M.N. (Miss) Date

DEDICATION

To my loving mother and sisters

ACKNOWLEDGEMENTS

The past two years of my Master's study have been a period of intense learning and fulfilment, not only in the scientific arena, but also on a personal level. Writing this mini-dissertation has had a big impact on me. I owe my gratitude to all those who have made this mini-dissertation possible, the graduate experience that I acquired has been one that I will cherish forever. As such, I would like to reflect on the people who have supported and helped me throughout this period. I would like to express my heartfelt appreciation to my supervisor, Doctor K.M. Pofu, for granting me the opportunity to study my Master degree under her supervision. I feel I have been remarkably privileged to have a supervisor who gave me the freedom to explore on my own and at the same time the guidance to recover when my direction wavered. My deepest gratitude goes to my co-supervisor, Professor P.W. Mashela, for his continuous support in my research, for his patience, motivation, and immense knowledge in plant protection. Professor Mashela taught me how to question my thoughts and express abstract ideas. His patience and support helped me to overcome many predicament situations and finish this mini-dissertation in record time. I am also thankful to the supervisory team for all the editorial suggestions and recommendations. I could not have imagined having the best advisors and mentors for my Master degree. I am grateful to them for holding me to a high research standard, therefore, teaching me how to conceptualise ideas and then subject them to empirical methodologies. The office-door to Doctor Z.P. Dube, the research assistant at the Green Bio-Technologies Research Centre of Excellence (GBTRCE), was always open whenever I ran into a trouble spot or had a question about my research or writing. Doctor Dube had always been there to listen and give advice. I am deeply grateful to him for the long discussions that helped me sort out the

technical details of my work. I would like to extend my appreciation to the GBTRCE team for assisting me in the experiments I carried out. Most importantly, none of this would have been possible without the love and patience of my family, who have been a constant source of energy and strength, all these years. I will forever be grateful to the Agricultural Research Council (ARC) and the National Research Foundation of South Africa for their generous scholarship grants in my Master degree and the ARC-Universities Collaboration Centre for Smallholder Farmers for providing research funds. I will forever remain grateful to God the Almighty for taking me through this stage in life. This is definitely a dream come true.

LIST OF TABLES

LIST OF LEGENDS

LIST OF APPENDICES

ABSTRACT

Root-knot (*Meloidogyne* species) nematodes are economically destructive pathogens of over 3000 species, whereas others have resistance to *Meloidogyne* species. Wild watermelon (*Cucumis africanus*) and wild cucumber (*Cucumis myriocarpus*) are highly resistant to *Meloidogyne* species, particularly *M. incognita* and *M. javanica.* The two *Cucumis* species are used in inter-generic grafting with watermelon (*Citrullus lanatus*) as nematode resistant rootstocks. Also, the two *Cucumis* species are used in traditional medicine and in plant-parasitic nematode management as phytonematicides. The form of nematode resistance, which is essential in plant breeding, is not documented for the two *Cucumis* species. The objective of this study was to determine the form of nematode resistance in the two *Cucumis* species to *M. incognita* and *M. javanica* under greenhouse conditions. Four parallel experiments were each conducted under greenhouse conditions. Uniform six-week old *Cucumis* seedlings were transplanted into 250 ml polystyrene cups filled with 200 ml growing medium of steam-pasteurised fine sand. A week after transplanting, *Cucumis* seedlings were each infested by dispensing approximately 100 *M. incognita* second-stage juveniles (J2) or *M. javanica* J2 using a 20 ml plastic syringe by placing into 5-cm-deep furrow around the seedling stem and covered with growing medium. Treatments (periodic harvest intervals) were arranged in a randomised complete block design, replicated five times. Five seedlings from each experiment were harvested every second day, for 30 days, with stained roots being assessed for necrotic spot (suberised cells) number, giant cell number, proliferation of rootlet interference number and root gall number. Periodic harvest intervals were highly significant ($P \leq 0.01$) on necrotic spot number, proliferation of rootlet interference number and root gall number in *C. africanus-M. incognita* relations, but

were not significant for giant cell number. Treatments contributed 59, 64 and 50% in total treatment variation (TTV) of necrotic spot number, proliferation of rootlet interference number and root gall number, respectively. Harvest period had highly significant effects on necrotic spot number, giant cell number, proliferation of rootlet interference number and root gall number in *C. africanus-M. javanica* relations. Treatments contributed 55, 71, 63 and 59% in TTV of necrotic spot number, giant cell number, proliferation of rootlet interference number and root gall number, respectively. Periodic harvest intervals were significant ($P \leq 0.05$) on giant cell number and highly significant on root gall number in *C. myriocarpus-M. incognita* relations. However, there were no significant treatment differences on necrotic spot number and proliferation of rootlet interference number. Treatments contributed 57 and 57% in TTV of root gall number and giant cell number, respectively. Harvest period had highly significant effects on giant cell number, proliferation of rootlet interference number and root gall number, but were not significant on necrotic spot number in *C. myriocarpus-M. javanica* relations. Treatments accounted for 67, 49 and 53% in TTV of giant cell number, proliferation of rootlet interference number and root gall number, respectively. In conclusion, the mechanism of resistance to *M. incognita* and *M. javanica* in both *C. africanus* and *C. myriocarpus* was post-infectional nematode resistance, which has attributes for introgression into commercial nematode-susceptible *Cucumis* cultivars.

CHAPTER 1
RESEARCH PROBLEM

1.1 Background

Global withdrawal of the highly effective synthetic chemical fumigant nematicides, which had been relied upon for over a century in the management of plant-parasitic nematodes, has had severe economic consequences and created a serious void in most crop production systems (Caboni *et al*., 2015; Mashela, 2002, 2007; Mashela *et al*., 2008). Parasitism by root-knot (*Meloidogyne* species) nematodes is considered one of the main factors responsible for reduced productivity in various cultigens (Mashela *et al*., 2015). Infection by *Meloidogyne* species induces the formation of root galls, causing stunted growth, decreased water uptake, imbalances of essential nutrient elements, low evapotranspiration and increased root exudation of amino acids, which invariably reduces soil pH (Anwar and Javid, 2010; Curtis, 2008; Mashela, 2002; Saikia *et al*., 2013; Ullah *et al*., 2011).

Botlokwa in Limpopo Province, South Africa, is the centre of bio-diversity for wild watermelon (*Cucumis africanus*) and wild cucumber (*Cucumis myriocarpus*) (Kristkova *et al*., 2003). The two *Cucumis* species were highly resistant to all *Meloidogyne* species in South Africa (Mofokeng, 2005; Pofu, 2012; Pofu and Mashela, 2011; Pofu *et al*., 2009, 2010, 2011, 2012), namely, *M. incognita* races 2 and 4 and *M. javanica* (Kleynhans *et al*., 1996; Onkendi *et al*., 2014). Among the available alternative techniques to methyl bromide, plant resistance is the most investigated technique in plant-parasitic nematology (Sikora and Fernandez, 2005; Thurau *et al*., 2010). Host-plant resistance techniques are the most eco-friendly, cost effective and integratable nematode intervention strategies available for reducing

crop losses due to infection by plant-parasitic nematodes (Roberts, 1992; Silva *et al*., 2013; Sone, 2010; Starr *et al*., 2002; Zhang *et al*., 2016).

Nematode resistant plants are more effective than other alternatives because they do not permit nematode development or its reproduction (Silva *et al*., 2013). Interaction between the host plant and nematodes, especially those belonging to the genus *Meloidogyne*, is highly specialised (Rocha *et al*., 2015) and suited for nematode resistance (Williamson and Hussey, 1996; Mashela *et al*., 2016). A number of nematode-plant interactions are necessary for the success of nematode infection, which include stimulation for second stage juveniles (J2) hatching, attraction, penetration of suitable tissues, migration to feeding site, induction of feeding site and modification of cells (Davis and Mitchum, 2005; Mashela *et al*., 2016).

1.1.1 Description of the research problem

Plants that are resistant to root-knot nematodes may exhibit pre- or post-infectional nematode resistance. The use of genetic materials from one plant to another is referred to as introgression and may become important in climate-smart agriculture for the introduction of pathogen resistance in resilient cultivars (Haussmann *et al*., 2004). Only post-infectional nematode resistance can be introgressed (Kaplan and Davis, 1987; Thurau *et al*., 2010), dictating the need to establish the mechanism of nematode resistance in any nematode resistant plant species in order for it to serve as a candidate of introgression. On the basis of disproportionate numbers of J2 in the soil and in the roots, Pofu (2012) suggested that *C. africanus* and *C. myriocarpus* had post- and pre-infectional nematode resistance, respectively. However, the

proposed mechanisms of nematode resistance in the two *Cucumis* species had not yet been empirically verified.

1.1.2 Impact of the research problem

Prior to the cut-off date of the use of methyl bromide, the estimated yield losses due to nematodes were at US$125 billion (Chitwood, 2003). Three years (Abad *et al.,* 2008) and eight years (Elling, 2013) after the withdrawal the yield losses were estimated at US$157 and US$173 billion, respectively, which were relative yield losses of 25 and 37%, respectively.

1.1.3 Possible causes of the research problem

In most cases, cultigens do not have the genotypes that have resistance to *Meloidogyne* species as observed in four commercial genera of *Cucumis*, *Citrullus*, *Cucurbita* and *Lagenaria* within the Cucurbitaceae family and potato (*Solanum tuberosum*) cultigens (Davis, 2005; Marais *et al*., 2015; Thies and Levi, 2003, 2006, 2007). The development of genotypes with nematode resistance in certain cultigens without nematode resistance had produced seedless fruits, which could not be propagated using conventional sexual propagation methods (Thies and Levi, 2007). Pathogenic variability of root-knot nematodes with multiple biological races (Sasser, 1979; Taylor and Sasser, 1978) also contributed to the observed challenges in the use of nematode resistance (Castagnone-Sereno, 2002; Faghihi *et al*., 1995). Plant breeding in certain cultivars with resistance to plant-parasitic nematodes promoted the resurgence of virulent new nematode races (Thurau *et al*., 2010). In addition to scarcity of high-resistant genotypes, certain abiotic factors like high temperature (Dropkin, 1969a) and salinity (Mashela and Nthangeni, 2002) were shown to

contribute in breaking the nematode resistance. Also, honeydew-producing insects broke nematode resistance in *C. africanus* (Pofu *et al*., 2011).

1.1.4 Possible solutions to the research problem

The study would result in a better understanding of nematode resistance mechanism in *C. africanus* and *C. myriocarpus*. Understanding the mechanism of nematode resistance in the two *Cucumis* species would enhance the uses of these plant species in introgression since only post-infectional nematode resistance can be introgressed.

1.2 Problem statement

In all commercial *Cucumis* species, nematode resistant genotypes are not known (Montalvo and Esnard, 1994; Thies and Levi, 2003, 2006, 2007). Lack of information on the mode of nematode resistance in *C. africanus* and *C. myriocarpus* to *Meloidogyne* species reduces the potential uses of these plant species in plant breeding against nematodes. Generally, only post-infectional mechanism of nematode resistance can be used in plant breeding (Kaplan and Davis, 1987; Thurau *et al*., 2010).

1.3 Rationale of the study

The study would provide empirically-based information on mechanisms of root-knot nematode resistance in *C. africanus* and *C. myriocarpus*. Generally, should post-infectional nematode resistance be available in any of the two *Cucumis* species, the information would be relayed to plant breeders for use as source of introgression in

various commercial *Cucumis, Citrullus*, *Cucurbita* and *Lagenaria* species where nematode resistant genotypes do not exist.

1.4 Purpose of the study

1.4.1 Aim

The aim of the study was to generate empirically-based information on mechanisms of nematode resistance in wild *Cucumis* species to *Meloidogyne* species.

1.4.2 Objective

To determine whether the mechanism of nematode resistance to *M. incognita* and *M. javanica* in the two *Cucumis* species were post-infectional.

1.4.3 Hypothesis

The mechanism of nematode resistance to *M. incognita* and *M. javanica* in *C. africanus* and *C. myriocarpus* were post-infectional.

1.5 Reliability, validity and objectivity

The reliability of data was based on statistical analysis of data at the probability level of 5%. Validity was achieved through harvesting the experiments overtime, while objectivity was achieved by ensuring that the findings were discussed on the basis of empirical evidence, in order to eliminate all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

Bias was minimised by ensuring that the experimental error in each experiment was reduced through replications, and by assigning treatments randomly within the selected research designs (Leedy and Ormrod, 2005).

1.7 Ethical considerations

Commercial uses of the acquired protocols in *Cucumis* seedlings were in accordance to the legal rights entered between the researcher and the University of Limpopo, which was in line with the research policies and the appropriate legislative framework in South Africa. The University policies, appropriate legal framework and ethical considerations as outlined here, were endured beyond the completion of this study.

1.8 Structure of mini-dissertation

The title page would be followed by the description and detailed outlining of the research problem (Chapter 1) and literature review of the work done and not yet done on the research problem (Chapter 2). The subsequent chapter (Chapter 3) would address the objective. In the final chapter (Chapter 4), empirically-derived findings were summarised and integrated to provide the significance of the findings and recommendations with respect to future research, culminating in a conclusion which tied up the entire study together. The Harvard style of citation and referencing in alphabetical order, as approved by the Senate of the University of Limpopo, was adopted.

CHAPTER 2
LITERATURE REVIEW

Based on nematode-chemical interaction, mechanisms in nematode resistance had been reduced to two, namely, pre-infectional and post-infectional nematode resistance. The nematode bodies are covered with sensory organs, which are used to detect chemicals in small quantities, therefore influencing the direction to which nematode should move. Chemo-attractants and chemo-repellents attract and repel nematodes, respectively (Wuyts *et al*., 2006; Zhao *et al*., 2000).

2.1 Pre-infectional nematode resistance

Pre-infectional nematode resistance is the form of nematode resistance that occurs prior to nematodes coming into contact with the root systems (Ferraz and Brown, 2002). The pre-infectional nematode resistance prevents penetration of second-stage juveniles (J2), and includes pre-existing morphological factors or the production of root exudates that either attract or repel J2 (Huang, 1985). A number of experiments on pre-infectional mechanism demonstrated that root exudates played an important role in both attracting and repelling nematodes (Huang, 1985; Kaplan and Keen, 1980; Veech, 1981). Root exudates such as dhurrin, sorgoleone, glucosinolate, monocrotaline and alpha-terthienyl were released in certain plants and were shown to have nematicidal effects on root-knot (*Meloidogyne* species) nematodes (Chitwood, 2002; Czarnota *et al*., 2003; Gommers and Bakker, 1988; Ntalli and Caboni, 2012).

2.1.1 Sorghum

Sorghum-sudangrass (*Sorghum bicolor* × *Sorghum bicolor var.* '*Sudanense*') is a highly productive grass grown for its biomass (Clark, 2007; Slanev and Enchev, 2014). Sorghum-sudangrass is popular for its nematode-suppressive attributes and had been frequently used as a cover crop and rotational crop (McSorley and Gallaher, 1991; McSorley *et al*., 1994). This hybrid produces allelochemicals referred to as dhurrin ($C_{14}H_{17}NO_7$), which has the ability to suppress nematodes (Chitwood, 2002; Wang *et al*., 2002). Suppressive activities of the sorghum-sudangrass are primarily due to the production of natural nematicidal compounds (Clark, 2007), its poor host-status, general stimulation of microbial antagonists and the release of toxic products during decomposition (Magdoff and Van Es, 2009). *Sorghum-sudanense* cv. 'SX-17' did not support reproduction of *M. incognita* races 1 and 3, *M. arenaria* race 1 or *M. javanica* (McSorley and Gallaher, 1991; McSorley *et al*., 1994).

2.1.2 Sweet stem sorghum

Sweet stem sorghum (*Sorghum bicolor*) is indigenous to Africa (Bryan, 1990; Saballos, 2008) and has the potential of serving as an ethanol-producing alternative crop (Mashela and Pofu, 2016). Failure of J2 to penetrate roots in *S. bicolor* cv. 'Ndendane-X1' led to the conclusion that the cultivar had pre-infectional mechanism of nematode resistance to both *M. incognita* race 2 and *M. javanica* (Mashela and Pofu, 2016). *Sorghum bicolor* produces chemical compound, sorgoleone ($C_{22}H_{29}O_4$), which has nematicidal properties that result in inhibition of nematode mobility (Czarnota *et al*., 2003; Dayan *et al*., 2010).

2.1.3 Asparagus

Asparagus (*Asparagus officinalis*) also possesses pre-infectional nematode resistance (Gommers, 1981). *Asparagus officinalis* contains a nematode toxic compound, asparagusic acid ($C_4H_6O_2S_2$) isolated from the roots (Chitwood, 2002; Dudash and Barker, 1992; Esmenjaud *et al*., 1990). *Trichodorus christiei* nematode populations were observed to decline rapidly around roots of *A*. *officinalis* (Esmenjaud *et al*., 1990). Lack of root galls on *A. officinalis* grown in *M. incognita*- and *M. hapla*-infested soil led to the conclusion that the plants were resistant to the two *Meloidogyne* species (Castillo *et al.*, 1977).

2.1.4 Cabbage

Out of 30 cabbage (*Brassica oleracea*) cultivars tested for nematode resistant, seven white head cabbage cultivars were reported to be highly resistant to *Heterodera cruciferae* (Aydinli and Mennan, 2012). Rotating with *B. oleracea* supported growth of the nematophagous fungus (*Pochonia chlamydosporia*) in their rhizospheres but limited reproduction of root-knot nematodes (Puertas and Hidalgo-Diaz, 2007). Cruciferous plants from Brassicaceae family contain glucosinolate ($C_{17}H_{32}O_{11}NS_3$) chemical compounds which release nematode toxic products such as thiocyanate (SCN-) and isothiocyanate (C_4H_5NS) when decomposing (Brown *et al*., 1991; Matthiesen and Kirkegaard, 2006; Ntalli and Caboni, 2012) which are toxic to nematodes (Larkin, 2013; Petersen *et al*., 2001). The resistance of Brassicaceae plants to root-knot nematodes had also been reported in cultivars of cauliflower (*B. oleracea*) which were found to be resistant to *M. incognita* races 1, 2, 3 and 4 and *M. javanica* (Clark, 2007; Khan and Khan, 1991).

2.1.5 Velvet bean

The genus *Mucuna* which contains two velvet bean types, namely, *Mucuna pruriens* and *M. deeringiana*, is an African legume that had been used in the southern United States as a forage and cover crop (Weaver *et al*., 1993). The value of velvet bean as a crop for managing *Meloidogyne* species had been recognised since the late 1980s (Vincente and Acosta, 1987). Velvet bean contains in its roots a nematode toxic chemical compound L-3,4-dihydroxyphenylalanine ($C_9H_{11}NO_4$) (Tomita-Yokotani *et al*., 2004). Root exudates from *M. pruriens* and *M. deeringiana* suppressed *Meloidogyne* species (Vincente and Acosta, 1987). The rhizosphere bacteria in the two *Mucuna* species were also markedly different from those in soybean and other legume crops (Kloepper *et al*., 1991). *Mucuna* species are non-host to *M*. *arenaria*, *M*. *incognita*, *M*. *javanica* and *Heterodera glycines* (McSorley, 2011; Rodriguez-Kabana *et al*., 1992a). The plants had been effective as rotation crops for management of *M*. *arenaria* in groundnuts (*Arachis hypogaea*), increasing yield by 47% when compared with groundnuts monoculture (Rodriguez-Kabana *et al*., 1992b).

2.1.6 Sunn hemp

Sunn hemp (*Crotalaria juncea*) released chemicals into the rhizosphere that prevented infection by *M*. *incognita* J2 prior to penetration (McSorley and Gallaher, 1991; Roberts, 1992). *Crotalaria juncea*, a tropical legume, had been used as a cover crop to suppress *M*. *incognita* nematodes and produced the allelochemical called monocrotaline ($C_{16}H_{23}NO_6$) (Chitwood, 2002; Wang *et al*., 2002). The monocrotaline chemical compounds had been shown to be nematostatic, but were capable of paralysing certain plant-parasitic nematodes (Wang *et al*., 2002).

2.1.7 Alfalfa

Medicago sativa cv. ‘Moapa 69’ inhibited penetration of *M. incognita* (Postnikova *et al*., 2015). Earlier, Potenza *et al*. (1996, 2001) observed that J2 of *M. incognita* were clumped onto root tips of cv. ‘Moapa 69’. Griffin and Waite (1971) noted that certain varieties of alfalfa (*Medicago sativa*) released exudates that were repellent to the tulip-root nematode (*Ditelynchus dipsaci*). *Medicago* species release large quantities of medicarpin ($C_{16}H_{14}O_4$), a phytoalexin known to be toxic to plant-parasitic nematodes (Baldridge *et al*., 1998).

2.1.8 Marigold

Marigolds (*Tagetes erecta*) have impressive abilities to suppress plant-parasitic nematodes and had been used in nematode management for many years (Hooks *et al*., 2010). *Tagetes erecta* exudes chemical compounds with nematicidal properties into the rhizosphere, preventing J2 to penetrate roots (Huang, 1985). The main active ingredient had been identified as alpha-terthienyl, one of the most toxic occurring naturally chemical compounds (Gommers and Bakker, 1988). This chemical compound contains nematicidal, insecticidal, antiviral and cytotoxic properties (Marles *et al*., 1992). Alpha-terthienyl inhibited J2 hatching of *M. javanica* (Dhangar *et al.*, 1996; Walia and Gupta, 1997) and *M. incognita* (Siddiqui and Alam, 1988). Second-stage juveniles of *Meloidogyne* species failed to develop fully in roots of *T. erecta* (Ploeg, 2000; Ploeg and Maris, 1999). El Allagui (2007) reported on 67 and 71% mortality of *Meloidogyne* species when inoculated of *T. patula*. Marahatta *et al*. (2012) demonstrated that *T. erecta* can, in their vegetative stage, suppress *Meloidogyne* species when planted immediately after a nematode susceptible host. The effects of *T. erecta* on *Pratylenchus penetrans* population in soil could last for

two years when it is included in crop rotation (Reynolds *et al*., 2000). Marigolds can be used against nematodes as a cover crop in rotation, as an intercrop, or as a crop residue amendment (Adekunle, 2011; Hooks *et al*., 2010; Wang *et al*., 2007; Xie *et al*., 2016).

2.1.9 Garlic

Fadzirayi *et al*. (2010) reported on the indirect effects of garlic (*Allium sativum*) on nematode populations, the reason being that it interrupts nematode's mobility, food absorption and subsequent reproduction. *Meloidogyne incognita* and reniform (*Rotylenchulus* reniformis) J2 were significantly reduced in garlic monoculture or when garlic was intercropped with cowpea (*Vigna unguiculata*) or tomato (*Solanum lycopersicum*) (Ameen, 1996). Nigh (1985) reported that garlic possesses biochemical substances that had allelopathic and nematicidal properties. The main active ingredient in garlic is allicin ($C_6H_{10}OS_2$) (McRae, 2005).

2.1.10 Tomato

Tomato cv. 'Nemared' was reported to be resistant to *M. incognita* and *P. penetrans* (Hung and Rohde, 1973). The J2 of *M. incognita* and *P. penetrans* could not penetrate roots of the cultivar (Ohri and Pannu, 2010). Failure of J2 to penetrate roots was due to a chemical compound, chlorogenic acid ($C_{16}H_{18}O_9$), which is a phenolic chemical compound (Malik *et al*., 1989).

2.1.11 Bean

Generally, different bean (*Phaseolus* species) cultivars responded differently when inoculated with different races of *M. incognita* (Ferreira *et al*., 2010; Pedrosa *et al*., 2000). Nematode races are morphological identical within the same species, but can be separated using differential hosts and/or molecular approaches (Mashela *et al.,* 2015). Dry bean (*P. vulgaris*) cultivars 'Apore' and 'Talisma' were highly resistant to *M*. *javanica*, whereas snap bean cultivars 'Macarrao atibaia' and 'Macarrao preferido' were moderately resistant to *M. javanica* (Ferreira *et al*., 2010). Common bean cv. 'Polder' was shown to be resistant to *M. chitwoodi* and *M. fallax* (Wesemael and Moens, 2012). Resistant lima bean (*P. lunatus*) inoculated with *P. penetrans* produced the phytoalexin, coumestrol ($C_{15}H_8O_5$) (Veech, 1982).

2.1.12 Turkey berry

Turkey berry (*Solanum torvum*), a wild relative of eggplant (*S. melongena*), was shown to be resistant to *Meloidogyne* species (Yamaguchi *et al*., 2010). Worldwide, *S. torvum* is being used as nematode-resistant rootstock for eggplants because of its vigour and resistance and/or tolerance to other soil-borne diseases such as bacterial and fungal wilts (Gousset *et al*., 2005). Kusirisin *et al*. (2009) identified phenol, flavonoid and tannin chemical compound in various parts of *S. torvum.* Dhivya *et al.* (2016) also reported on resistant of *S. torvum* to *M. incognita*.

2.1.13 Strawberry

Strawberry (*Fragaria ananassa*), cultivars ‘Allstar’, ‘Camarosa’, ‘Chandler’, ‘Dimante’ and ‘Firecracker’ were shown to be highly resistant to *M. hapla* (Pinkerton and Finn, 2005). Curi *et al.* (2016) also reported that *F. ananassa* cultivars ‘Oso grande’ and ‘Albion’ had nematode resistance to *M. hapla*. Rosaceae species release chemical compounds known as cyanogenic glycosides ($C_2N_2C_{47}H_{74}O_{15}$) which break down into hydrogen cynide and aglycones (Oslo, 2010).

2.1.14 Rapeseed

Rapeseed (*Brassica napus*) contains sulphur-containing chemicals (glucosinolates) ($C_{17}H_{32}O_{11}NS_3$) (Brown *et al*., 1991; Matthiesen and Kirkegaard, 2006; Ntalli and Caboni, 2012) which interfere with nematodes reproductive cycles (Brown and Morra, 1997). Rapeseed cultivars 'Dwarf Essex’, ‘Elena’, ‘Indore’, ‘Jupiter’, ‘Cascade’, ‘Bridger’, and ‘Humus’ were shown to suppress root-knot nematodes (Bernard and Montgomery-Dee, 1993).

2.1.15 Black-eyed Susan

Most plant species in the Asteraceae family have polyacetylene-imitative chemical compounds, which include the thiophenes and thiarubrines that have nematicidal properties (Freeman *et al*., 1993; Gomez-Barrios *et al*., 1992; Lu *et al*., 1993; Sanchez de Viala *et al*., 1998). Black-eyed Susan (*Rudbeckia hirta*), one of such species, contains in its roots the thiarubrines (thiarubrine A and thiarubrine C) (Freeman *et al*., 1993), chemical compounds which were toxic to plant-parasitic nematodes (Gommers and Voor in't Holt, 1976). Sanchez de Viala *et al.* (1998) observed that thiarubrine C was toxic to *M. incognita* and *P. penetrans*, killing 90%

M. incognita and 50% *P. penetrans* juveniles. Suppression of *P. penetrans* by *R*. *hirta* and *R*. *serotina* was also observed (McKeown and Potter, 1994; McKeown *et al*. 1994.

2.1.16 Rhodes grass

Rhodes grass (*Chloris gayana*) cv. 'Katambora' had been used to control root-knot nematodes in tobacco rotations for many years to date (York, 1990). Rhodes grass is used in rotation programmes where it improved soil structure, fertility and reduced population densities of *Meloidogyne* species (Cook *et al*., 2005). Rhodes grass root exudates inhibited hatching and nematode mobility of *Rotylenchulus reniformis* (Caswell *et al*., 1991).

2.2 Post-infectional resistance

In post-infectional nematode resistance J2 are allowed to penetrate the root systems (Kaplan and Davis, 1987), with passive chemicals previously called elicitors (Kuc, 1995), activated to form the phytoalexins (Veech, 1981), which have nematicidal properties (Chitwood, 2003; Kaplan and Keen, 1980). The phytoalexins are produced after infection, often affecting the nematode metabolic activities and causing death of the nematode (Huang, 1985). Some of the phytoalexins induce hypersensitivity, where cells around the nematode wither (Harborne, 1999), thereby preventing feeding, development of J2 and reproduction (Anwar and McKenry, 2000; Harborne, 1999). Nematode resistance under this category could fail under high soil temperature (Dropkin, 1969a), high salinity (Mashela and Nthangeni, 2002) and under attack from honeydew-producing insects like the greenhouse whiteflies (Pofu *et al*., 2011).

2.2.1 Cucumber

Fig-leafed gourd (*Cucumis ficifolia*) and African horned cucumber (*C. metuliferus*) were shown to have post-infectional resistance to *M. incognita acrita* (Fassuliotis, 1967). Although *M. incognita acrita* J2 penetrated roots of *C. ficifolia* and *C. metuliferus* as in the susceptible *C. melo*, few developed to the adult female stage, suggesting the existence of post-infectional resistance (Fassuliotis, 1967). In *C. ficifolia* and *C. metuliferus* resistance, responses were associated with hindrance of juvenile development beyond the second-stage juvenile, delayed development of juveniles to adults and increased stimulation toward maleness (Fassuliotis, 1967). Moon *et al*. (2010) observed undeveloped root galls in six *C. annuum* cultivars inoculated with *M. incognita.* Walters *et al*. (1990, 1993) also observed small, poorly formed giant cells in resistant *Cucumis* exposed to *M. hapla*.

2.2.2 Cotton

Veech and McClure (1977) and Veech (1977) observed that the expression of incompatibility in cotton (*Gossypium hirsutum*) to *M. incognita* was post-infectional resistance, which was stimulated by increasing concentration of methoxy-substituted terpenoid aldehydes. The terpenoid aldehydes accumulated at the nematode infection site (Veech, 1979).

2.2.3 Coffee

Silva *et al*. (2010) observed an increase in the activities of peroxidases, polyphenoloxidases and phenylalanine-ammonia-lyases, along with a higher concentration of lignin and phenolic compounds in roots of resistant *Coffea canephora* infected by *M. exigua*. *Coffea canephora* cv. '*Apoata*', resistant to *M.*

exigua exhibited hypersensitive reaction, which inhibited formation of feeding site, aggravated J2 emigration from roots or inhibited nematode development and reproduction as a tactic of defence responses, which were all constitutive and induced after nematode penetration (Silva *et al*., 2013). Resistance to *M. exigua* was conferred at least by one dominant gene known as *Mex-1* (Noir *et al*., 2003). Advances in molecular approaches, as shown later in the current review, had since provided much information on genera associated with nematode post-infectional resistance (Mashela *et al*., 2016).

2.2.4 Soybean

Pedrosa *et al*. (1996) indicated that resistance to *M. arenaria* was expressed in soybean (*Glycine max*) as small, poorly formed giant cells, with reduced cell number and cell size of cells surrounding selected feeding cell. Herman *et al*. (1991) and Pedrosa *et al*. (1996) observed that root-knot nematode development was delayed in resistant *G. max* genotypes, resulting in fewer J2 advancing to subsequent stages of *Meloidogyne* species, when compared to those in susceptible genotypes. *Glycine max* infected by *Meloidogyne* species produced glyceollin ($C_20H_{18}O_5$), a nematode-toxic chemical compound (Veech, 1982).

2.2.5 Citrus rootstocks

Citrus rootstocks such as *Poncirus trifoliata* allowed nematode juveniles of the citrus nematode (*Tylenchulus semipenetrans*) to penetrate the root system, but prevented damage by having cells around the nematode undergoing hypersensitivity. Kaplan (1981) reported that the hybrid rootstock *Swingle citrumelo* (*Citrus paradisi* x

Poncirus trifoliata), when inoculated with *T. semipenetrans*, responded to infection by hypersensitive reaction, which occurred at least 14 days after inoculation.

2.2.6 Grape

Grape (*Vitis vinifera*) cv. 'RS-9' and cv. 'Teleki 5C' expressed biochemical defence mechanisms by developing root tip necrosis in response to invading J2. Roots of cv. 'RS-9' and cv. 'Teleki 5C' expressed resistance to *Meloidogyne* arenaria by reducing the number of J2 entering roots, with small giant cells being formed without any galls (Anwar and McKenry, 2000). Ferris *et al*. (1982) also observed reduced penetration of *M. arenaria* J2 into roots of cv. 'RS-9'. *Meloidogyne arenaria* J2 had delayed development to adult females, limited numbers of J2 developed to the adult female stage, with fewer eggs being produced per gram of root in cv. 'Teleki 5C', all suggesting the existence of post-infectional nematode resistant mechanism. Grape roots of cv. '10-17A' and cv. '6-19B' responded to *M. arenaria* infection by undergoing a hypersensitive reaction that resulted in prevention of *M. arenaria* J2 development to adult females (Anwar and McKenry, 2002).

2.2.7 Alyce clover

Alyce clover (*Alysicarpus ovalifolium*) is a tropical forage legume, with resistant attributes to *Meloidogyne* species (Powers *et al*., 1992). *Alysicarpus ovalifolium*, hybrids FL-1 and FL-3, had post-infectional resistance to *M. arenaria* (Powers *et al*., 1992). *Meloidogyne arenaria* J2 were able to penetrate roots of both FL-1 and FL-3 hybrids, but failed to develop to subsequent J3, J4 and adult female stages (Powers *et al*., 1992). The two resistant hybrids had attributes of hypersensitive responses with delayed development of *M. arenaria* (Powers *et al*., 1992), which were attributed

to activities of phenylalanine ammonia-lyase and phytoalexins (Huang, 1985). Responses observed in *A. ovalifolium* hybrids FL-1 and FL-3 suggested the existence of post-infectional nematode resistance against *M. arenaria* (Powers *et al*., 1992).

2.2.8 Carrot

Carrot (*Daucus carota*) cv. 'Brasilia' was shown to be resistant to *M. javanica* (Huang, 1986; Huang *et al.,* 1986). Generally, when *M. chitwoodi* J2 penetrated roots of carrot cv. 'Parmex' and cv. 'Berlanda', fewer egg masses were observed on each cultivar (Sone, 2010), with high male to female ratio and numerous rootlets. Wesemael and Moens (2008) also reported egg masses of less than 20% in cv. 'Parmex' and cv. 'Berlanda' infected with *M. chitwoodi*.

2.2.9 Chilli pepper

Different chilli pepper (*Capsicum annuum*) cultivars responded to nematode infection differently, for example, cv. 'Chilseongcho' was highly susceptible to *M. incognita* whereas cv. 'CM334' was highly resistant to *M. incognita* (Moon *et al*., 2010). *Meloidogyne incognita* penetrated roots of both susceptible and resistant cultivars, although more J2 penetrated cv. 'Chilseongcho' than cv. 'CM334'. Moon *et al*. (2010) could not observe giant cells on resistant cv. 'CM334', but there were extensive necrotic spots around the feeding cells; suggesting that cv. 'CM334' had post-infectional nematode resistance to *M. incognita*.

2.2.10 Tobacco

Ng'ambi *et al*. (1999) identified nematode resistance to *M. incognita* races 1 and 3 in tobacco (*Nicotiana tabacum*) cv. 'Speight G 28'. Nematode resistance in tobacco cultivars was shown to be conferred by the resistant gene Rk (Yi *et al.,* 1998). Tobacco cultivars with *Rk* gene responded to nematode infection through hypersensitive responses with fewer or no galls at all (Lucas, 1975).

2.2.11 Cowpea

According to Osei *et al*. (2010), leguminous plants contain numerous chemicals, some of which were nematostatic or influence nematode behaviour. The absence of galls on the roots of cowpea (*Vigna unguiculata*) varieties led to the conclusion that *V*. *unguiculata* varieties had the ability to inhibit the formation of feeding sites that are required to support the reproduction of females after penetration (Williamson and Kumar, 2006). *Vigna unguiculata* cv. 'Mississippi Silver' had been reported to be resistant to *M. arenaria* and to *M. incognita* (Hadisoeganda and Sasser, 1982). The gene responsible for resistance to *M. incognita* in *V. unguiculata* appeared to confer resistance to other *Meloidogyne* species (Fery, 1980).

2.2.12 Tomato

Resistant tomato cultivars 'Small Fry', 'Jetsetter' and 'Celebrity' were reported to be resistant to *M. arenaria*, *M. incognita* and *M. javanica* (Kwara *et al*., 2014). Roots of the three resistant cultivars had no observable galls which led to poor reproduction of the *Meloidogyne* species (Kwara *et al*., 2014). The *Mi* gene was identified in tomato resistant cultivars and conferred resistance against root-knot nematodes (Ho *et al*., 1992; Mehlenbacher, 1995; Milligan *et al*., 1998). Nematode resistance associated

with *Mi* gene is characterised by hypersensitive reaction at the site of infection resulting in failure of successful establishment of feeding site (Hwang *et al*., 2000). The *Mi* gene had been introgressed from *S. peruvianum* to commercial tomato cultivars and conferred resistance against *M. arenaria*, *M. incognita* and *M. javanica* (Martinez de Ilarduya *et al*., 2001; Nombela *et al*., 2003).

2.3 Molecular approaches to nematode resistance

In the above review, it was evident that within the same plant species mechanism of nematode resistance could either be pre- or post-infectional, and possibly both. Most of the molecular approaches focus on post-infectional nematode resistance (Mashela *et al*., 2016). In the ensuing review, three mechanisms involved in nematode resistance at a molecular level were briefly reviewed.

Anti-gene products strategy: Plant-parasitic nematodes secrete chemical compound called gene products through the sub-ventral and dorsal gland cells during migration and sedentary phases, respectively (Gheysen and Fenoll, 2002; Tripathi *et al*., 2015). The secretion of gene products is important, especially during the formation of nematode feeding sites, which allow for nematode development to subsequent stages (Curtis, 2008; Siddique *et al*., 2014). During migratory phases, roots are wounded upon which, chemical compounds referred to as defence plant genes, comprising peroxidase, chitinase, lipoxygenase, extension and proteinase inhibitors are activated (Gheysen and Fenoll, 2002; Hewezi and Baum, 2015). The anti-gene products strategy in nematode resistant plants, ranged from those during both migratory and sedentary phases, in respect to those that silence the expression of the gene products (Mashela *et al*., 2015).

Anti-plant gene strategy: In anti-plant gene strategy, the host plant genes that respond to nematode feeding and secretions to allow for successful partnerships between gene products and gene plants are silenced (Mashela *et al*., 2016). Thus, the phytotoxic chemical compounds that destroy the feeding structures, syncytium and giant cells, are upregulated (Mashela *et al*., 2016). Also, the plant releases certain plant genes in order to protect the nematode and such chemicals could be suppressed, thereby leaving the bodies of nematodes exposed (Hewezi and Baum, 2015). Failure to develop and maintain the feeding structures arrest the nematode development. The anti-plant gene strategy in certain transgenic plants had been successfully used (Mashela *et al*., 2016).

RNA-interference strategy: The RNA interference (RNAi) disrupts the nematode gene products through host-induced gene silencing approach (Hewezi and Baum, 2015; Williamson and Hussey, 1996). The RNAi genes had shown precise selectivity for the target organisms with slight side effects (McDowell and Woffenden, 2003). Cathepsin L-like cysteine proteinases, produced by R genes in nematode resistant transgenic plants, were shown to be an attractive group of candidate genes for RNAi-induced downregulation due to their high level of specificity to the target nematode gene products (McDowell and Woffenden, 2003), thereby resulting in silencing of host-induced gene products. Host-produced RNAi of *Mi-cpl-1* gene confers resistance to *M. incognita* by inducing negative effects on nematode infection, development and the subsequent reproduction (McDowell and Woffenden, 2003).

2.4 Nematode resistance in Cucurbitaceae family

Different studies (Fassuliotis, 1970; Francine *et al*., 2013; Mofokeng, 2005; Pofu *et al*., 2010) demonstrated that nematode resistance exists in some species of the Cucurbitaceae family, although there were many other species that had no known nematode resistance at all (Davis, 2005; Fassuliotis, 1967). A good example of the former and the latter are wild *Cucumis* species and commercial *Citrullus* species, respectively. From the reviewed literature, it was evident that chemical and structural responses occur in nematode resistant plants. The most common structural responses that could be used to study mechanisms of nematode resistance were (1) necrotic spots, (2) failure of development of giant cells, (3) proliferation of rootlets and (4) undeveloped small root galls.

The mechanisms involved in nematode resistance in *C. africanus* and *C. myriocarpus* to *M. incognita* races 2 and 4 and *M. javanica* had not been explored. Information on such mechanisms would be useful in development of nematode resistant hybrids in the Cucurbitaceae family. Any plant-induced mechanism that reduces reproductive potential of adult females in roots is termed resistance mechanism (Trudgill, 1985, 1992). Both pre-infectional and post-infectional concepts had been used as indicators of how the plants affected nematode behaviour in resistant crops. Introgression of genes from *C. africanus* and *C. myriocarpus* into species from the Cucurbitaceae family would require identification of resistance genes in the two *Cucumis* species and further introgression studies.

CHAPTER 3
NEMATODE RESISTANCE MECHANISMS IN *CUCUMIS* SPECIES

3.1 Introduction

Mechanisms of nematode resistance in *Cucumis africanus* and *C. myriocarpus* against root-knot (*Meloidogyne* species) nematodes were, without empirical tests, suggested as pre- and post-infectional, respectively (Pofu, 2012). The suggestion was based on the absence of hypersensitive reactions on roots and aggregated relative penetration indices, where the latter were all greater than one for both nematode tests species except for *M. javanica* in *C. africanus* (Pofu, 2012). Information on mechanism of nematode resistance in the two *Cucumis* species would improve their utility in plant breeding programmes (Pofu, 2012). The objective of this study was to determine whether *C. africanus* and *C. myriocarpus* seedlings would have post-infectional resistance to *M. incognita* and *M. javanica*.

3.2 Materials and methods

3.2.1 Location of the study

Experiments were conducted under greenhouse conditions at the Green Technologies Research Centre, University of Limpopo, South Africa (23°53′10″S, 29°44′15″E). The *C. africanus*-*M. incognita* and *C. africanus*-*M. javanica* during autumn (January-March) and *C. myriocarpus*-*M. incognita* and *C. myriocarpus*-*M. javanica* during spring (August-October) in 2016. Ambient day/night temperatures during each season averaged 28/21°C, with maximum temperatures controlled using thermostatically-activated fans.

3.2.2 Procedures

Cucumis africanus and *C. myriocarpus* seeds were each sown in seedling trays filled with pasteurised (300 ºC for one hour) fine sand and raised for six weeks. Uniform seedlings of each *Cucumis* species were transplanted into 250 ml polystyrene cups, filled with 200 ml pasteurised fine sand. Cups were placed on 0.4 m-high-greenhouse benches at 10-cm inter- and 10-cm intra-row spacing. Isolates of *M. incognita* and *M. javanica* each were raised on nematode-susceptible tomato cv. 'Floradade' seedlings and roots collected for egg masses when needed. Uniform egg masses were hand-picked using a tooth pick and put in tapwater for over 72 hours to allow for the development of all eggs to J2 (Powers *et al*., 1992). A day after transplanting, *Cucumis* seedlings were each inoculated by dispensing approximately 100 *M. incognita* J2 or *M. javanica* J2 using a 20 ml plastic syringe by placing into 5-cm-deep furrow around the seedling stem and covered with growing medium. Seedlings were irrigated with 30 ml tapwater every other day.

3.2.3 Treatment and experimental design

In each experiment the treatments, comprising 15 harvesting times, were laid out in randomised complete block design, with five replications (Legend 3.1). Harvesting was done every other day, for a period of 30 days.

Legend 3.1 *Cucumis africanus* seedlings inoculated with *Meloidogyne javanica*.

3.2.4 Data collection

At each harvest, seedling shoots were separated from roots. Roots were rinsed in tapwater to remove soil particles, with excess water removed using pieces of paper towel and stained (Byrd *et al.*, 1983). Briefly, total roots/seedling were soaked in 1.5% NaOCl solution for four minutes to remove any associated microbe rinsed in tapwater, followed by a 15 minutes immersion in tapwater to remove excess NaOCl. Root samples were each stained by covering with 30 ml tapwater mixed with 1 ml acid fuchsin and boiled for 30 seconds (Legend 3.2). The solution was cooled to room temperature and roots distained by putting in acidified glycerine with a few drops of 5 N HCl, which were heated to boiling, followed by cooling to room temperature. Root samples were each placed in a petri dish (Legend 3.3) and closed with the top lid for assessment under the stereomicroscope at 45 × magnification for necrotic spots, rootlet emergence, giant cells and root gall number.

Legend 3.2 Root sample of *Cucumis africanus* infected with *Meloidogyne javanica* stained with acid fuchsin.

Legend 3.3 Distained root sample of *Cucumis africanus* infected with *Meloidogyne javanica* at 18 days after inoculation.

3.2.5 Data analysis

Prior to analysis of variance (ANOVA), all data were transformed through $\log_{10}(x + 1)$ to normalise the variances (Gomez and Gomez, 1984). Data were subjected to ANOVA through the 2008 SAS software. The mean sum of squares was partitioned to provide the contribution of sources of variation in the total treatment variation (TTV) of variables (Gomez and Gomez, 1984). Treatment means were separated using Waller-Duncan Multiple Range test at 5% level of probability. Unless stated otherwise, all treatment effects were discussed at 5% level of probability.

3.3 Results

3.3.1 *Cucumis africanus-Meloidogyne incognita* relations

Periodic harvest intervals were highly significant ($P \leq 0.01$) on necrotic spot number, proliferation of rootlet interference number and root gall number, contributing 59, 64 and 50% in TTV of the respective variables (Table 3.1). Harvest intervals had no effects on development of giant cell number. Starting from 2 to 18 days after inoculation, necrotic spot, rootlet interference and root gall numbers were not noticeable, but were noticeable for necrotic spot and root gall number from 20 to 28 days, whereas for rootlet interference from 22 to 28 days (Table 3.2).

3.3.2 *Cucumis africanus-Meloidogyne javanica* relations

Harvest period had highly significant effects on necrotic spot number, giant cell number, proliferation of rootlet interference number and root gall number, contributing 55, 71, 63 and 59% in TTV of the respective variables (Table 3.1). From 2 to 22 days, necrotic spot, giant cell number and root gall number were not noticeable, whereas rootlet interference was not noticeable from 2 to 14 days.

Necrotic spot, giant cell number and root gall number were noticeable from 24 to 30 days after inoculation, whereas rootlet interference was noticeable from 16 to 30 days (Table 3.2).

3.3.3 *Cucumis myriocarpus-Meloidogyne incognita* relations

Periodic harvest intervals were highly significant on root gall number and significant on giant cell number. Treatments contributed 57% in TTV of root gall number and 57% in TTV of giant cell number (Table 3.3). Harvest period had no effects on necrotic spot and rootlet interference (Table 3.3). Giant cell number was noticeable from 18 to 28 days, whereas root gall number was noticeable from 18 to 30 days (Table 3.4).

3.3.4 *Cucumis myriocarpus-Meloidogyne javanica* relations

Harvest period had highly significant effects on giant cell number, proliferation of rootlet interference number and root gall number, contributing 67, 49 and 53% in TTV of the respective variables (Table 3.3). There were no treatment effects on necrotic spot (Table 3.3). Giant cell number and root gall number were noticeable from 24 to 30 days, whereas rootlet interference was noticeable from 16 to 30 days (Table 3.4).

3.3. 5 Nematode juveniles in *Cucumis* species

In all experiments, nematode juveniles at various stages of development were not detected. Even under higher magnification (× 100), when using oil emersion, nematode juveniles in stained roots were undetectable.

Table 3.1 Total treatment variation (TTV) on necrotic spot, giant cell number, rootlet interference and root gall number in *Cucumis africanus* seedlings infected by *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions at 30 days after inoculation (n = 75).

		Necrotic spot		Giant cell number		Rootlet interference		Root gall number	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Meloidogyne incognita									
Rep	4	0.02927	21	0.05015	42	0.01023	11	0.02477	32
Treatment	14	0.08161	59***	0.04106	34ns	0.05811	64***	0.03799	50***
Error	56	0.02714	20	0.02963	24	0.02232	25	0.01355	18
Total	74	0.13802	100	0.12084	100	0.09066	100	0.07631	100
Meloidogyne javanica									
Rep	4	0.02154	28	0.02091	16	0.02704	21	0.02719	24
Treatment	14	0.04213	55***	0.09017	71***	0.08175	63***	0.06584	59***
Error	56	0.01294	17	0.01595	13	0.02028	16	0.01950	17
Total	74	0.07661	100	0.12703	100	0.12907	100	0.11253	100

nsNot significant at P ≤ 0.05, **Significant at P ≤ 0.05, ***Significant at P ≤ 0.01.

Table 3.2 Mean separation for necrotic spot, giant cell number, rootlet interference and root gall number in *Cucumis africanus* infected by *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions at 30 days after inoculation (n = 75).

Days	*Meloidogyne incognita*			*Meloidogyne javanica*			
	Necrotic spot[z]	Rootlet interference[z]	Root gall number[z]	Necrotic spot[z]	Giant cell number[z]	Rootlet interference[z]	Root gall number[z]
2	0.0000[c]	0.0000[c]	0.0000[c]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
4	0.0000[c]	0.0000[c]	0.0000[c]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
6	0.0000[c]	0.0000[c]	0.0000[c]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
8	0.0000[c]	0.0000[c]	0.0000[c]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
10	0.0000[c]	0.0000[c]	0.0000[c]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
12	0.0000[c]	0.0000[c]	0.0000[c]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
14	0.0000[c]	0.0000[c]	0.0000[c]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
16	0.0000[c]	0.0000[c]	0.0000[c]	0.0000[b]	0.0000[b]	0.0954[b]	0.0000[b]
18	0.0000[c]	0.0000[c]	0.0000[c]	0.0000[b]	0.1204[b]	0.0954[b]	0.1556[b]
20	0.0954[bc]	0.0000[c]	0.0954[bc]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
22	0.3908[a]	0.1806[abc]	0.1556[b]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
24	0.1398[bc]	0.0602[bc]	0.0602[bc]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
26	0.2760[ab]	0.3362[a]	0.3113[a]	0.0954[b]	0.0602[b]	0.0000[b]	0.0602[b]
28	0.2408[ab]	0.2408[ab]	0.0602[bc]	0.3496[a]	0.5169[a]	0.4919[a]	0.4292[a]
30	0.0000[c]	0.0000[c]	0.0000[c]	0.0602[b]	0.0954[b]	0.0954[b]	0.000[b]
P ≤	0.01	0.01	0.01	0.01	0.01	0.01	0.01

[z]Column means followed by the same letter were not different (P ≤ 0.005) according to Waller-Duncan Multiple Range test.

Table 3.3 Total treatment variation (TTV) on necrotic spot, giant cell number, rootlet interference and root gall number on *Cucumis myriocarpus* seedlings infected by *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions at 30 days after inoculation (n = 75).

		Necrotic spot		Giant cell number		Rootlet interference		Root gall number	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
				Meloidogyne incognita					
Rep	4	0.04740	32	0.00851	16	0.02043	20	0.01802	18
Treatment	14	0.06163	41^{ns}	0.03122	57**	0.05276	50^{ns}	0.05524	57***
Error	56	0.03991	27	0.01498	27	0.03151	30	0.02400	25
Total	74	0.14894	100	0.05471	100	0.10470	100	0.09726	100
				Meloidogyne javanica					
Rep	4	0.05804	53	0.03624	21	0.07155	34	0.04581	28
Treatment	14	0.03077	28^{ns}	0.11481	67***	0.10314	49***	0.08462	53***
Error	56	0.02066	19	0.02135	12	0.03669	17	0.03079	19
Total	74	0.10947	100	0.17240	100	0.21138	100	0.16122	100

^{ns}Not significant at P ≤ 0.05, **Significant at P ≤ 0.05, ***Significant at P ≤ 0.01

Table 3.4 Mean separation for giant cell number, rootlet interference and root gall number in *Cucumis myriocarpus* seedlings infected by *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions at 30 days after inoculation (n = 75).

	Meloidogyne incognita		*Meloidogyne javanica*		
Days	Giant cell number[z]	Root gall number[z]	Giant cell number[z]	Rootlet interference[z]	Root gall number[z]
2	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
4	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
6	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
8	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
10	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
12	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
14	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
16	0.0000[b]	0.0000[b]	0.0000[b]	0.0602[b]	0.0000[b]
18	0.2760[a]	0.3715[a]	0.0000[b]	0.0000[b]	0.0000[b]
20	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
22	0.0954[b]	0.0000[b]	0.0000[b]	0.0602[b]	0.0000[b]
24	0.0000[b]	0.0000[b]	0.0602[b]	0.0602[b]	0.0954[b]
26	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]	0.0000[b]
28	0.1398[ab]	0.1908[ab]	0.5823[a]	0.5405[a]	0.4937[a]
30	0.0000[b]	0.0954[b]	0.1398[b]	0.2083[b]	0.1398[b]
P ≤	0.05	0.01	0.01	0.01	0.01

[z]Column means followed by the same letter were not different (P ≤ 0.005) according to Waller-Duncan Multiple Range test.

.

3.4 Discussion

3.4.1 Necrotic spots

Harvest period had significant effects on necrotic spots for *C. africanus* relations with both *M. incognita* and *M. javanica.* Necrotic spots were observed at least 20 days after inoculation for *C. africanus-M. incognita* relations and 26 days after inoculation for *C. africanus-M. javanica* relations. According to Nicholson and Hammerschmidt (1992), the presence of necrotic spots may indicate the presence of phenols that could have played a role in plant defence. Marini *et al.* (2016) observed similar results for resistant oats (*Avena sativa*) when exposed to *M. incognita* at 15 days after inoculation. The necrosis of plant cells in contact with juveniles were clearly observed in roots of *A. sativa* at 15 to 18 days after inoculation, which probably resulted in arresting of J2 development as observed in acid fuchsin stained roots (Marini *et al.,* 2016).

Coffea canephora cv. '*Apoata*', resistant to *M. exigua* exhibited necrotic spots, which inhibited formation of feeding site (Silva *et al*., 2013). Kaplan (1981) observed necrotic spots at 14 days after inoculation when *Swingle citrumelo* hybrid was inoculated with *T. semipenetrans.* Moon *et al.* (2010) also observed necrotic spots in resistant *C. annuum* cultivars exposed to *M. incognita.* Two resistant *A. ovalifolium* hybrids, FL-1 and FL-3, showed attributes of hypersensitive responses to *M. arenaria* (Powers *et al*., 1992). In nematode resistant *C. metuliferus,* resistant to root-knot *M. incognita acrita* J2 was not associated with nematode resistance. No hypersensitive reaction was observed on roots of *C. metuliferus* after J2 penetration (Fassuliotis, 1970). However, Dropkin (1969b) proposed that the hypersensitive response was not entirely necessary for expression of plant resistance. Localised

necrosis resembled those described for other resistance genes (Dangl *et al.,* 1996; Hammond-Kosack and Jones, 1996).

From penetration sites through migration pathways to the feeding sites, roots are subjected to physical and chemical activities of the gene products from the hyperactive sub-ventral gland cells (Dutta *et al*., 2015; Wang *et al*., 1999). Some of the gene products secreted and verified from the sub-ventral gland cells of migratory phases of root-knot J2 included β-1,4 endoglucanase, pectate lyase (Duncan *et al.,* 1996) and polygalacturonase (Gheysen and Fenoll, 2002). The listed gene products are all responsible for the degradation of plant cell walls. Necrotic spots are known to be a common response to root-knot nematode infection in resistant crops (Kaplan, 1981; Lucas, 1975; Powers *et al*., 1992; Silva *et al*., 2013), resulting in cell death and prevention of nematode feeding site formation and nematode development (Morel and Dangl, 1997; Postnikova *et al*., 2015), leading to subsequent nematode death (Dropkin, 1969a; Paulson and Webster, 1972). Necrotic spots, in nematode-infected cells, are representatives of hyperactive responses in nematode resistant plants (Mashela *et al*., 2016).

3.4.2 Failure of giant cell development

Harvest period had significant effects on undeveloped giant cell number for *C. africanus-M. incognita* relations and *C. myriocarpus-M. incognita*. At 18 days after inoculation, undeveloped giant cells were observed in *C. africanus-M. javanica* relations and *C. myriocarpus-M. incognita*. However, for *C. myriocarpus-M. javanica* relations, giant cells were not observed until 24 days after inoculation. The observation could be due to delayed response, which had been widely reported in

nematode resistant trials using molecular approaches (Escobar and Fenoll, 2015; Gheysen and Fenoll, 2002; McIntyre, 1980). The giant cells occurred as deeper stained spots with multiple nuclei that failed to develop beyond the zygote-like size.

At 18 days after inoculation, Marini *et al.* (2016) also observed that *M. incognita* gradually initiated undeveloped giant cells in resistant roots of *A. sativa* cv. 'IPR Afrodite'. Observation of the undeveloped giant cells also agreed with observations in resistant *G*. *max* cultivars exposed to *M. arenaria* (Pedrosa *et al.,* 1996), in resistant chili pepper *C. annuum* cultivars '02G132' and '03G53' (Moon *et al.,* 2010) and in resistant *G*. *hirsutum* cultivars (Carneiro *et al.,* 2005). Pedrosa *et al*. (1996) indicated that resistance to *M. arenaria* was expressed in *G. max* as small, poorly formed giant cells. Walters *et al*. (1990, 1993) also observed small, poorly formed giant cells in resistant *Cucumis* exposed to *M. hapla*. In all the cited examples, the cultivars had post-infectional nematode resistance.

In nematode-susceptible plant species, giant cells are formed as multinucleate structures formed when the feeding cell and those around it responds to nematode infection by undergoing repeated mitosis without cytokinesis (Huang *et al.,* 2003; Van der Eycken *et al.,* 1996). The successful establishment of feeding cells is essential for nematode development. *Meloidogyne* species evolved strategies that enable them to induce feeding cell formation on thousands of plant species by manipulating important factors of plant cell development (Caillaud *et al.,* 2005). The secretion of gene products is important in the formation of nematode feeding site and nematode development to subsequent reproductive stages (Curtis, 2008; Siddique *et al.,* 2014). The giant cell serves as a source of nutrients for the developing nematode

(Bartlem *et al*., 2013). The post-penetration compatibility in susceptible crops is usually associated with optimal development of giant cells that form a large multinucleate structure which, however, fail to develop in nematode resistant crops (Orion *et al.,* 1980).

Koltai and Bird (2000) proposed that expression of the two plant genes, *Phantastica* and *Knotted1*, in giant cells could relate to the alteration in cytokinin and auxin levels, in agreement with the observed production of biologically active cytokinins by the dorsal gland cells in *Meloidogyne* species (Bird and Loveys, 1980) and the proposed function of cytokinins as a primary inductive signal for the formation of giant cells (Bird, 2004). However, in the highly resistant chili pepper cv. 'CM334', no giant cells were observed (Moon *et al.,* 2010), which supported observations for *C. africanus-M. incognita* relations in the current study. *Cucumis africanus* contains cucurbitacin B, which is distributed throughout all organs (Chen *et al*., 2005) and had different chemical properties to those of cucurbitacin A that occurs in roots and fruits of *C. myriocarpus* (Chen *et al*., 2005).

3.4.3 Proliferation of rootlet interference

The formation of excessive rootlets in plants infected by *Meloidogyne* species is common in carrots (*Daucus carota*), with the phenomenon referred to as rootlet interference (Sone, 2010). Sone (2010) observed numerous rootlets on *D. carota* when exposed to *M. chitwoodi*. Rootlet interference was observed in *C. africanus-M. incognita* relations from 22 days after inoculation, *C. africanus-M. javanica* relations from 16 days after inoculation and *C. myriocarpus-M. javanica* at 24 days. The observations, supported those in nematode-resistant *G. max* that was exposed to *M.*

javanica (Doyle and Lambert, 2003) and on nematode-resistant white clover (*Trifolium repens*) that was exposed to *M. trifoliophila* (Mecer *et al*., 2004).

During the sedentary phases, for plant-nematode interactions to be compatible, most of the gene products from the dorsal gland cells of nematodes mimic plant genes by producing plant growth regulators, especially the cytokinins and the auxins (Mashela *et al*., 2016). For example, the cytokinins (Lohar *et al.,* 2004; Siddique *et al.,* 2014) and auxins (Domingo *et al.,* 1998; Duncan *et al.,* 1996; Huyangura *et al.,* 1999), produced by the dorsal gland cells in sedentary adult nematodes, are known to play a role in the initiation of lateral roots (Benkova and Bielach, 2010). Plant growth regulator manipulation is known to be an important process during the initiation and development of the feeding sites of sedentary plant-parasitic nematodes (Mashela *et al*., 2016). The auxin pathway is responsible for root initiation, development and lateral root formation (De Smet *et al*., 2010). In the current study, rootlets were observed originating adjacent to the undeveloped giant cells, which confirmed observations of improved lateral root initiation adjacent to root galls in other studies (Goverse *et al*., 2000; Karczmarek *et al*., 2004).

3.4.4 Small undeveloped root galls

Periodic harvest intervals had significant effects on undeveloped small root galls towards the last day within the 30-day cycle in all *Cucumis* and *Meloidogyne* relations. Cervantes-Flores (2000) also found significant effects on root galling of resistant sweet potatoes cultivars ‘Excel’ and ‘Hernandez’ that were exposed to *M. incognita* and *M. javanica.* Out of 39 cultivars of *C. annuum* screened for nematode resistance, six were resistant to *M. incognita*, with few undeveloped root galls (Moon

et al., 2010). Similar small galls were observed on resistant sugar beet (*Beta vulgaris*) exposed to *M. incognita*, while majority of J3 and J4 were observed at 16 days after inoculation (Yu, 1995).

Fassuliotis (1967) reported on hindrance of juvenile development beyond the second-stage juvenile, delayed development of juveniles to adults when *C. ficifolia* and *C. metuliferus* were exposed to *M. incognita acrita.* Herman *et al*. (1991) and Pedrosa *et al*. (1996) observed fewer J2 advancing to subsequent stages of *Meloidogyne* species. Ferris *et al*. (1982) also observed limited numbers of *M. arenaria* J2 developing to the adult female stage when inoculated in *V. vinifera* cv. 'RS-9'. There were no root galls observed on roots of *V. unguiculata* varieties when inoculated with *Meloidogyne* species (Williamson and Kumar, 2006). Generally, in nematode-susceptible plant species, when root-knot J2 develop through J3, J4 and adult female stages, the adjacent root cells bulge to form a root gall (Mashela *et al*., 2016). Small and undeveloped root galls had been reported in various trials of *C. africanus* and *C. myriocarpus* (Mabuka, 2015; Pofu, 2012; Pofu and Mashela, 2011).

3.4.5 Nematode juveniles in *Cucumis* roots

In a host-parasitic interaction study, tomato host reactions to *Meloidogyne* species parasitism was initiated during the first 12 hours after infection (Williamson *et al.*, 1994). However, in the two *Cucumis* species against the *Meloidogyne* species in the current study, there was no evidence of rapid host reactions. The result of these interactions is generally influenced by responses of plants to plant genes and gene products (Gheysen and Fenoll, 2002; Siddique *et al.,* 2014).

Findings by Fassuliotis and Dukes (1972) explained and supported the findings in the current study wherein there were no detectable nematode juveniles in roots at 30 days after inoculation even though they were observed earlier after inoculation. At 30 days after inoculation, Marini *et al.* (2016) also found a decrease in nematode numbers inside the roots of a resistant *A. sativa* cv. 'IPR Afrodite' that was exposed to *M. incognita.* At the onset of feeding, the nematode becomes sedentary, going through three moults before becoming a mature adult female, with males migrating out of the plant without playing any role in reproduction (Caillaud *et al.,* 2005). Due to conversion of juveniles to male when feeding site is not established, it was possible that the converted males migrated to the soil. In another trial Pofu and Mashela (2011) observed that in *C. myriocarpus-M. incognita* inter-relation, more male juveniles were in the soil than inside the roots.

3.5 Conclusion

Responses in roots of the two *Cucumis* species to infection by the two *Meloidogyne* species were more or less similar as depicted by (1) necrotic spots, (2) failure of giant cell development, (3) proliferation of rootlet interference and (4) proliferation of small undeveloped root gall. All these responses suggested that post-infectional nematode resistance was in place in the two wild indigenous *Cucumis* species. The observations agree with the previous observations that cucurbitacins, which are active ingredients of *Cucumis* species, are also localised in roots. Due to their large sizes, it could be argued that cucurbitacin A and B in *C. myriocarpus* and *C. africanus*, respectively, are too large to be exuded through the membranes into the rhizosphere and thereby conferring pre-infectional nematode resistance.

CHAPTER 4
SUMMARY, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

4.1 Summary

The study focused on two major objectives, namely, to determine whether the mechanism of resistance in (1) *C. africanus* seedlings to *M. incognita* and *M. javanica* and (2) *C. myriocarpus* seedlings to *M. incognita* and *M. javanica* was pre- or post-infectional. Results from this study confirmed that *C. africanus* and *C. myriocarpus* were resistant to *Meloidogyne* species (Mofokeng, 2005; Pofu, 2012). The four responses that were observed, namely, (1) necrotic spots, (2) failure of giant cell development, (3) proliferation of rootlet interference and (4) proliferation of small undeveloped root gall, all provided evidence of interactions of active chemicals produced by nematode and plant cells. These agreed with the recent literature review on chemical interactions in nematode-resistant transgenic plants (Mashela *et al*., 2016). Results of this study suggested that the mechanism of resistance to *Meloidogyne* species in *C. africanus* and *C. myriocarpus* was post-infectional, which could be important in plant breeding programmes.

4.2 Significance of findings

The identified nematode resistance in *C. africanus* and *C. myriocarpus* to *Meloidogyne* species was post-infectional. The form of resistance could be introgressed into economically important cultivars that do not have resistance to *Meloidogyne* species (Mashela *et al*., 2016). The significance of findings in the current study was that most commercially available *Cucumis* species and the two *Cucumis* species confer a unique opportunity to South African plant breeders.

4.3 Recommendations

Nematode resistance in plants is conferred chemical compounds referred to as plant genes (Mashela *et al*., 2016). Since post-infectional nematode resistance had been identified in *C. africanus* and *C. myriocarpus,* it would be necessary to investigate the related plant genes in the two *Cucumis* species. Additionally, attempts should be made to introgress the plant genes into nematode-susceptible hosts in commercial *Cucumis* species along within *Citrullus lanatus* cultivars (Thurau *et al*., 2010).

4.4 Conclusions

Cucumis africanus and *C. myriocarpus,* with biodiversity centres in Botlokwa, Limpopo Province, South African, have fruits that contain cucurbitacins, which are used in various industries, including traditional medicines and pest management as alternative products. The identified post-infectional nematode resistance to *Meloidogyne* species would most probably further promote the use of the two *Cucumis* species in plant breeding, thereby expanding the uses and economic importance of the two *Cucumis* species.

REFERENCES

ABAD, P., GOUZY, J., AURY, J.M., CASTAGNONE-SERENO, P., DANCHIN, E.G., DELEURY, E., PERFUS-BARBEOCH, L., ANTHOUARD, V., ARTIQUENAVE, F., BLOK, V.C., *et al.,* 2008. Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita. Nature Biotechnology* 26:909-915.

ADEKUNLE, O.K. 2011. Amendment of soil with African marigold and sunn hemp for management of *Meloidogyne incognita* in selected legumes. *Crop Protection* 30:1392-395.

AMEEN, H.H. 1996. Influence of garlic (*Allium sativum*) on populations of *Rotylenchulus reniformis* and *Meloidogyne incognita* infecting cowpea and tomato. *Journal of Agricultural Research* 23:77-85.

ANWAR, S.A. and M.V. McKENRY. 2000. Penetration, development and reproduction of *Meloidogyne arenaria* on two new resistant *Vitis* species. *Nematropica* 30:9-17.

ANWAR, S.A. and M.V. McKENRY. 2002. Penetration and development of *Meloidogyne arenaria* on two new grape rootstocks. *Journal of Nematology* 34:143-145.

ANWAR, S.A. and N. JAVID. 2010. *Meloidogyne incognita* infecting Dhalia. *Pakistan Journal of Zoology* 42:348-350.

AYDINLI, G. and S. MENNAN. 2012. Screening resistance level of Brassicaceae plants to cabbage cyst nematode, *Heterodera cruciferae* Franklin, 1945 (*Tylenchida*: Heteroderidae). *Turkish Journal of Entomology* 36:3-10.

BALDRIDGE, G.D., O'NEILL, N.R. and D.A. SAMAC. 1998. Alfalfa (*Medicago sativa*) resistance to the root-lesion nematode, *Pratylenchus penetrans*: Defense response gene mRNA and isoflavonoid phytoalexin levels in roots. *Plant Molecular and Biology* 38:999-1010.

BARTLEM, D.G., JONES, M.G.K. and U.Z. HAMMERS. 2013. Vascularisation and nutrient delivery at root-knot nematode feeding sites in host roots. *Journal of Experimental Botany* 65:1789-1798.

BENKOVA, E. and A. BIELACH. 2010. Lateral root organogenesis: From cell to organ. *Current Opinion Plant Biology* 13:677-683.

BERNARD, E.C. and M.E. MONTGOMERY-DEE. 1993. Reproduction of plant-parasitic nematodes on winter rapeseed (*Brassica napus* cv. 'Oleifera'). *Journal of Nematology* 25:863-868.

BIRD, A.F. and B.R. LOVEYS. 1980. The involvement of cytokinins in a host parasite relationship between the tomato (*Lycopersicon esculentum*) and a nematode, *Meloidogyne javanica*. *Parasitology* 80:497-505.

BIRD, D. 2004. High society (of nematologists). *Genome Biology* 5:353.

BROWN, P.D. and M.J. MORRA. 1997. Control of soil-borne plant pests using glucosinolate-containing plants. In: Sparks, D.L. (ed.). Advances in agronomy. Academic Press: San Diego, California.

BROWN, P.D., MORRA, M.J., MCCAFFREY, J.P., AULD, D.L. and L. WILLIAMS. 1991. Allelochemicals produced during glucosinolate degradation in soil. *Journal of Chemical Ecology* 17:2021-2034.

BRYAN, W.L. 1990. Solid-state fermentation of sugars in sweet stem sorghum. *Enzyme Microbiology and Technology* 12:27-29.

BYRD, D.W., KIRKPATRICK, T. and K.R. BARKER. 1983. An improved technique for clearing and staining plant tissue for detection of nematodes. *Journal of Nematology* 15:142-143.

CABONI, P., AISSANI, N., DEMURTAS, M., NTALLI, N. and V. ONNIS. 2015. Nematicidal activity of acetophenones and chalcones against *Meloidogyne incognita* and structure-activity considerations. *Pest Management Science* 72:125-130.

CAILLAUD, M.C., DUBREUIL, G., QUENTIN, M., PERFUS-BARBEOCH, L., LECOMTE, P., DE ALMEIDA ENGLER, J., ABAD, P., ROSSO, M.N. and B. FAVERY. 2005. Root-knot nematodes manipulate plant cell functions during a compatible interaction. *Journal of Plant Physiology* 165:104-113.

CARNEIRO, R.M.D.G., DAS NEVE, D., FACAO, R., PAES, N.S., CIA, E. and M.F. GOSSE. 2005. Resistance of cotton genotypes to *Meloidogyne incognita* race 3: Reproduction and histopathology. *Brazilian Nematology* 299:1-10.

CASTAGNONE-SERENO, P. 2002. Genetic variability of nematodes: A threat to the durability of plant resistance genes? *Euphytica* 124:193-199.

CASTILLO, M.B., ALEJAR, M.S. and J.A. LITSINGER. 1977. Nematodes in cropping patterns. Composition and populations of plant-parasitic nematodes in selected cropping patterns in Batangas. *Philippine Agriculture* 60:285-292.

CASWELL, E.P., TANG, C.S., DE FRANK, J. and W.J. APT. 1991. The influence of root exudates of *Chloris gayana* and *Tagetes patula* on *Rotylenchulus reniformis. Review of Nematology* 14:581-587.

CERVANTES-FLORES, J.C. 2000. Root-knot nematode resistance in sweet potato and development of sweet potato differential host genotypes for *Meloidogyne*

species. Master dissertation submitted to North Carolina State University, North Carolina, United States of America.

CHEN, J.C., CHIU, M.H., NIE, R.L., CORDELL, G.A. and S.X. QIU. 2005. Cucurbitacins and cucurbutane glycosides: Structures and biological activities. *Journal of Natural Products Report* 22:386-399.

CHITWOOD, D.J. 2002. Phytochemical based strategies for nematode control. *Annual Review of Phytopathology* 40:221-249.

CHITWOOD, D.J. 2003. Research on plant-parasitic nematode biology conducted by the United States Department of agricultural research services. *Pest Management Science* 59:748-753.

CLARK, A. 2007. Managing cover crops profitably. National SARE outreach handbook series book 9. National Agriculture Laboratory: Beltsville, Maryland.

COOK, B.G., PENGELLY, B.C., BROWN, S.D., EAGLES, D.A. and J. HANSON. 2005. Tropical Forages: An interactive selection tool. CD-ROM publisher. Brisbane, Australia.

CURI, P.N., PECHE, P.M., PIO, R., CAPRONI, C.M. and M. SOUZA de OLIVEIRA. 2016. Relationship between production, nematodes and "redness" in strawberries. *Crop Protection* 46:1309-1325.

CURTIS, R.H.C. 2008. Plant nematode interactions: Environmental signals detected by the nematode’s chemosensory organs control changes in the surface cuticle and behaviour. *Parasite* 15:310-316.

CZARNOTA, M.A., RIMANDO, A.M. and L.A. WESTON. 2003. Evaluation of root exudates of seven sorghum accessions. *Journal of Chemical Ecology* 29: 2073-2083.

DANGL, J.L., DIETRICH, R.A. and M.H. RICHBERG. 1996. Death don't have no mercy: Cell death programs in plant-microbe interactions. *Plant Cell* 8:1793-1807.

DAVIS, E.L. and M.G. MITCHUM. 2005. Nematodes: Sophisticated parasites of legumes. *Plant Physiology* 137:1182-1188.

DAVIS, R.F. 2005. Effect of the southern root-knot nematode on watermelon yield. In: Kelly, W.T. and D.R. Langston (eds.). Georgia vegetable research extension report 2004. University of Georgia: Tifton, Georgia.

DAYAN, F.E., RIMANDO, A.M., PAN, Z., BAERSON, S.R., GIMSING, A.L. and S.O. DUKE. 2010. Sorgoleone. *Phytochemistry* 71:1032-1039.

DE SMET, I., LAU, S., VOSS, U., VANNESTE, S., BENJAMINS, R., RADEMACHER, E.H., SCHLERETH, A., DE RYBEL, B., VASSILEVA, V., GRUNEWALD, W., *et al.*, 2010. Bimodular auxin response controls organogenesis in *Arabidopsis. Proceedings of the National Academy of Sciences* 107:2705-2710.

DHANGAR, D.S., GUPTA, D.C. and R.C. JAIN. 1996. Studies on aqueous extract of different parts of *Tagetes erecta* on hatching, larval mortality and larval penetration of *Meloidogyne javanica*. *Indian Journal of Nematology* 26:1.

DHIVYA, R., SADASAKTHI, A. and M. SIVAKUMAR. 2016. Reaction of wild Solanum rootstocks and tomato scions against root-knot nematode (*Meloidogyne incognita*). *Advanced Research Journal of Crop Improvement* 7:10-13.

DOMINGO, C., ROBERTS, K., STACEY, N.J., CONNERTON, I., RUIZ-TERAN, F. and M.C. McCANN. 1998. A pectase lyase from *Zinnia elegans* is auxin inducible. *Plant Journal* 13:17-28.

DOYLE, E.A. and K.N. LAMBERT. 2003. *Meloidogyne javanica* chorismate mutase 1 alters plant cell development. *Molecular Plant Microbe Interaction* 16: 123-131.

DROPKIN, V.H. 1969a. Cellular responses of plants to nematode infections. *Annual Review of Phytopathology* 7:101-122.

DROPKIN, V.N. 1969b. The necrotic reaction of tomatoes and other hosts resistant to *Meloidogyne*: Reversal by temperature. *Phytopathology* 59:1632-1637.

DUDASH, P.J. and K.R. BARKER. 1992. Host suitability and response of asparagus cultivars to *Meloidogyne* species and races. *Journal of Nematology* 24: 109-116.

DUNCAN, L.H., ROBERTSON, W.M., KUSEL, R.J. and M.S. PHILLIPS. 1996. A putative nematode auxin binding protein from the potato cyst nematode *Globodera pallida*. *Nematropica* 26:259.

DUTTA, T.K., BANAKAR, P. and U. RAO. 2015. The status of RNAi-based transgenic research in plant nematology. *Frontiers in Microbiology* 5:1-7.

EL ALLAGUI, N., TAHROUCH, S., BOURIJATE, M. and A. HATIMI. 2007. Action of plant extracts on root-knot nematodes (*Meloidogyne* species) mortality 503. *Acta Botanica Gallica* 154:503-509.

ELLING, A.A. 2013. Major emerging problems with minor *Meloidogyne* species. *Phytopathology* 103:1092-1102.

ESCOBAR, C. and C. FENOLL. 2015. Plant nematode interactions: A view on compatible interrelationships. In advances in botanical research. Elsevier, New York.

ESMENJAUD, D., VOISIN, R., MINOT, J.C. and J.G. NOURRISSEAU. 1990. Multiplication of several *Meloidogyne* species on cultivated asparagus. *Nematologica* 36:304-312.

FADZIRAYI, C.T., MASAMHA, B. and I. MUKUTIRWA. 2010. Efficacy of Garlic (*Allium sativum*) in controlling nematode parasites in sheep. *International Journal of Research* 8:3.

FAGHIHI, J., VIERLING, R.A., HALBRENDT, J.M., FERRIS, V.R. and J.M. FERRIS. 1995. Resistance genes in a 'Wasiams 82' x 'Hartwig' soybean cross to an inbred line of *Heterodera glycine. Journal of Phytopathology* 61:395-399.

FASSULIOTIS, G. 1967. Species of *Cucumis* resistant to the root-knot nematode, *Meloidogyne incognita acrita*. *Plant Disease Reporter* 51:720-723.

FASSULIOTIS, G. 1970. Resistance of *Cucumis* species to the root-knot nematode, *Meloidogyne incognita acrita*. *Journal of Nematology* 2:174-177.

FASSULIOTIS, G. and P.D. DUKES. 1972. Disease reactions of *Solanum melongena* to root-knot nematode, *Meloidogyne incognita*. *Plant Disease Reporter* 57:606-608.

FERRAZ, L.C.C.B. and D.J.F. BROWN. 2002. An introduction to nematodes: Plant nematology. Pensoft: Sofia, Bulgaria.

FERREIRA, S., GOMES, L.A.A., MALUF, W.R., CAMPOS, V.P., CARVALHO, J.L.S.F. and D.C. SANTOS. 2010. Resistance of dry bean and snap bean cultivars to root-knot nematodes. *Horticultural Science* 45:320-322.

FERRIS, H., SCHNEIDER, S.M. and M.C. STUTH. 1982. Probability of penetration and infection by root-knot nematode, *Meloidogyne arenaria*, in grape cultivars. *Journal of Nematology* 33:31-35.

FERY, R.L. 1980. Genetics of *Vigna*. *Horticulture Review* 2:311-394.

FRANCINE de, S.G., FRANCO, A.J., ITO, L.A., HAMILTON DE, O.C., GAION, L.A. and L.T. BRAZ. 2013. Rootstocks resistant to *Meloidogyne incognita* and compatibility of grafting in net melon. *Journal of Nematology* 60:432-436.

FREEMAN, F., AREGULLIN, M. and E. RODRIGUEZ. 1993. Naturally occurring 1,2 dithiins. *Review of Heteroatom Chemistry* 9:1-19.

GHEYSEN, G. and C. FENOLL. 2002. Gene expression in nematode feeding sites. *Annual Review of Phytopathology* 40:191-219.

GOMEZ, K.A. and A.A. GOMEZ. 1984. Statistical procedures for agricultural research. Wiley: New York.

GOMEZ-BARRIOS, M.L., PARODI, F.J., VARGAS, D., QUIJANO, L., HJORTSO, M.A., FLORES, H.E. and N.H. FISCHER. 1992. Studies on the biosynthesis of thiarubrine A in hairy root cultures of *Ambrosia artemisiifolia* using 13C labelled acetates. *Phytochemistry* 31:2703-2707.

GOMMERS, F.J. 1981. Biochemical interactions between nematodes and plants and the irrelevance to control: A review. *Helminthological Abstracts* 50:9-24.

GOMMERS, F.J. and D.J.M. VOOR IN'T HOLT. 1976. Chemotaxonomy of Compositae related to their host suitability for *Pratylenchus penetrans*. *Netherlands Journal of Plant Pathology* 82:1-8.

GOMMERS, F.J. and J. BAKKER. 1988. Physiological diseases induced by plant responses or products. In: Poinar, G.O. and H.B. Jansson (eds.). Diseases of nematodes. Chemical Rubber Company Press: Boca Raton, Florida.

GOUSSET, C., COLLONNIER, C., MULYA, K., MARISKA, I., ROTINO, G.L., BESSE, P., SERVAES, A. and D. SIHACHAKR. 2005. *Solanum torvum*, as a useful source of resistance against bacterial and fungal diseases for

improvement of eggplant (*Solanum melongena*). *Plant Science* 168: 319-327.

GOVERSE, A., OVERMARS, H., ENGELBERTINK, J., SCHOTS, A., BAKKER, J. and J. HELDER. 2000. Both induction and morphogenesis of cyst nematode feeding cells are mediated by auxin. *Molecular Plant Microbe Interaction* 13:1121-1129.

GRIFFIN, G.D. and W.W. WAITE. 1971. Attraction of *Ditelynchus dipsaci* and *Meloidogyne hapla* by resistant and susceptible alfalfa seedlings. *Journal of Nematology* 3:215.

HADISOEGANDA, W.W. and J.N. SASSER. 1982. Resistance of tomato, bean, southern pea and garden pea cultivars to root-knot nematodes based on host suitability. *Plant Disease* 66:145-150.

HAMMOND-KOSACK, K.E. and J.D.G. JONES. 1996. Resistance gene-dependent plant defense responses. *The Plant Cell* 8:1773-1791.

HARBORNE, J.B. 1999. The comparative biochemistry of phytoalexin induction in plants. *Biochemistry System Ecology* 27:335-367.

HAUSSMANN, B.I.G., PARZIES, H.K., PRESTERL, T., SUSIC, Z. and T. MIEDANER. 2004. Plant genetic resources in crop improvement. *Plant Genetic Resources* 2:3-21.

HERMAN, M., HUSSEY, R.R. and H.R. BOERMA. 1991. Penetration and development of *Meloidogyne incognita* on roots of resistant soybean genotypes. *Journal of Nematology* 23:155-161.

HEWEZI, T. and T.J. BAUM. 2015. Gene silencing in nematode feeding sits. In: Escobar, C. and C. Fenoll (eds.). Advances of botanical research, plant

nematode interactions: A view on compatible interrelationships. Elsevier: New York.

HO, J.Y., WEIDE, R., MA, H.M., VANWORDRAGEN, M.F., LAMBERT, K.N., KOORNEEF, M., ZABEL, P. and V.M. WILLIAMSON. 1992. The root-knot nematode resistance gene (*Mi*) in tomato: construction of a molecular linkage map and identification of dominant cDNA markers in resistant genotypes. *The Plant Journal* 2:971-982.

HOOKS, C.R.R., WANG, K.H., PLOEG, A. and R. McSORLEY. 2010. Using marigold (*Tagetes* species) as a cover crop to protect crops from plant parasitic nematodes. *Applied Soil Ecology* 46:307-320.

HUANG, C.S. 1985. Formation, anatomy and physiology of giant cells induced by root-knot nematodes. In: Sasser, J.N. and C.C. Carter (eds.). Advanced treatise of *Meloidogyne* species. University Graphics: Raleigh, United States of America.

HUANG, G., GAO, B., MAIER, T., ALLEN, R., DAVIS, E.L. and T.J. BAUM. 2003. A profile of putative parasitism genes expressed in the oesophageal gland cells of the root-knot nematode *Meloidogyne incognita. Molecular Plant Microbe Interaction* 16:376-381.

HUANG, S.P. 1986. Penetration, development, reproduction and sex ratio of *Meloidogyne javanica* in three carrot cultivars. *Journal of Nematology* 18:408-412.

HUANG, S.P., DELLAVECCHIA, P.T. and P.E. FERREIRA. 1986. Varietal response and estimates of heritability of resistance to *Meloidogyne javanica* in carrots. *Journal of Nematology* 18:496-501.

HUNG, C.L. and R.A. RHODE. 1973. Phenol accumulation related to resistance in tomato to infection by root-knot and lesion nematodes. *Journal of Nematology* 5:253-258.

HUYANGURA, P., MATHESIUS, U., JONES, M.G.K. and B.G. ROLFE. 1999. Auxins induction is a trigger for root gall formation caused by root-knot nematodes in white clover and is associated with the activation of the flavonoid pathway. *Australian Journal of Physiology* 26:221-231.

HWANG, C.F., BHAKTA, A.V., TRUESDELL, G.M., PUDLO, W.M. and V.M. WILLIAMSON. 2000. Evidence for a role of the N terminus and leucine-rich repeat region of the *Mi* gene product in regulation of localised cell death. *Plant Cell* 12:1319-1329.

KAPLAN, D.T. 1981. Characterisation of citrus rootstock responses to *Tylenchulus semipenetrans*. *Journal of Nematology* 13:492-498.

KAPLAN, D.T. and E.L. DAVIS. 1987. Mechanisms of plant incompatibility with nematodes. In: Veech, J.A. and D.W. Dickson (eds.). Vistas on nematology. Society of Nematologists: Hyattsville, Maryland.

KAPLAN, D.T. and N.T. KEEN. 1980. Mechanisms conferring plant incompatibility to nematodes. *Journal of Nematology* 3:123-134.

KARCZMAREK, A., OVERMARS, H., HELDER, J. and A. GOVERSE. 2004. Feeding cell development by cyst and root-knot nematodes involves a similar early, local and transient activation of a specific auxin-inducible promoter element. *Molecular Plant Pathology* 5:343-346.

KHAN, A.A. and M.W. KHAN. 1991. Reaction of cauliflower cultivars to *Meloidogyne javanica* and races of *Meloidogyne incognita*. *Nematropica* 21:161-166.

KLEYNHANS, K.P.N., VAN DER BERG, E., SWART, A., MARAIS, M. and N.H. BUCKLEY. 1996. Plant nematodes in South Africa. Handbook number 8. Plant Protection Research Institute: Pretoria.

KLOEPPER, J.W., RODRIGUEZ-KABANA, R., McLNROY, J.A. and D.J. COLLINS. 1991. Analysis of populations and physiological characterisation of microorganisms in rhizospheres of plants with antagonistic properties to phytopathogenic nematodes. *Plant and Soil* 136:95-102.

KOLTAI, H. and D.M.C.K. BIRD. 2000. Recovery and sequence validation of the histological signal following in-situ RT-PCR localisation of plant gene transcription. *Plant Molecular and Biology Reporter* 20:391-397.

KRISTKOVA, E., LEBEDA, A., VINTER, V. and O. BLAHOUSEK. 2003. Genetic resources of genus *Cucumis* and their morphological description. *Journal of Horticultural Science* 30:14-42.

KUC, J. 1995. Phytoalexins, stress metabolism and disease resistance. *Annual Review of Phytopathology* 33:275-297.

KUSIRISIN, W., JAIKANG, C., CHAIYASUT, C. and P. NARONGCHAI. 2009. Effect of polyphenolic compounds from *Solanum torvum* on plasma lipid peroxidation, superoxide anion and cytochrome P450 2E1 in human liver microsomes. *Medicinal Chemistry* 5:583-588.

KWARA, B.K., KWOSEH, C.K. and J.L. STARR. 2014. Effectiveness of root-knot nematode (*Meloidogyne* species) resistant tomato (*Solanum lycopersicum*) and pepper (*Capsicum* species) cultivars in Ghana. *Nematropica* 44:130-136.

LARKIN, R.P. 2013. Green manures and plant disease management. *CAB Review* 8:1-10.

LEEDY, P.D. and J.E. ORMROD. 2005. Practical research: Planning and design. Pearson Education: New Jersey.

LOHAR, D.P., SCHAFF, J.E., LASKEY, J.G., KIEBER, J.J., BILYEU, K.D. and D.M. BIRD. 2004. Cytokinins play opposite roles in lateral root formation and nematode and rhizobial symbioses. *Plant Journal* 38:203-214.

LU, T., PARODI, F.J., VARGAS, D., QUIJANO, L., MERTOOETOMO, E.R., HJORTSO, M.A. and N.H. FISCHER. 1993. Sesquiterpenes and thiarubrines from *Ambrosia trifida* and its transformed roots. *Phytochemistry* 33:113-116.

LUCAS, G.B. 1975. Diseases of tobacco. Fuquay-Varina: North Carolina.

MABUKA, K.L. 2015. Integrated management strategies for *Meloidogyne* species in *Solanum lycopersicum* production systems. Master dissertation submitted to University of Limpopo, Sovenga, South Africa.

MAGDOFF, F. and H. VAN ES. 2009. Building soils for better crops: Sustainable soil management. Sustainable Agriculture Publications: Waldorf, Maryland.

MALIK, M.S., PAL, V., SANGWAN, N.K., DHINDSA, K.S., VERMA, K.K. and D.S. BHATTI. 1989. Nematicidal efficacy of substituted phenols, phenoxyacetic acid esters and hydrazides: A structure-activity relationship study. *Nematology* 35:366-370.

MARAHATTA, S.P., WANG, K.H., SIPES, B.S. and C.R.R. HOOKS. 2012. Effects of *Tagetes patula* on active and inactive stages of root-knot nematodes. *Journal of Nematology* 44:26-30.

MARAIS, M., SWART, A., KNOETZE, R. and K. POFU. 2015. Plant nematodes in South Africa. 13: A checklist of nematodes associated with potatoes. Technical News 34-43.

MARINI, P.M., GARBUGLIO, D.D., DORIGO, O.F. and A.C.Z. MACHADO. 2016. Histological characterisation of resistance to *Meloidogyne incognita* in *Avena sativa*. *Tropical Plant Pathology* 41:203.

MARLES, R.J., HUDSON, J.B., GRAHAM, E.A., BREAU, C.S., MORAND, P., COMPADRE, R.L., COMPADRE, C.M., TOWERS, G.H.N. and J.T. ARNASON. 1992. Structure-activity studies of photoactivated antiviral and cytotoxic thiophenes. *Phytochemistry and Phytobiology* 56:479-487.

MARTINEZ de ILARDUYA, O., MOORE, A.E. and I. KALOSHIAN. 2001. The tomato Rme1 locus is required for *Mi*-1-mediated resistance to root-knot nematodes and the potato aphid. *Plant Journal* 27:417-425.

MASHELA, P.W. 2002. Ground wild cucumber fruits suppress numbers of *Meloidogyne incognita* on tomato in microplots. *Nematropica* 32:13-19.

MASHELA, P.W. 2007. Undefeatable enemies: Answering questions with questions. Inaugural lecture. University of Limpopo Press: Sovenga, South Africa.

MASHELA, P.W. and K.M. POFU. 2016. Sweet stem sorghum (*Sorghum bicolor*) for ethanol production in areas with *Meloidogyne* species. *Transylvanian Review* 24:898-904.

MASHELA, P.W. and M.E. NTHANGENI. 2002. Efficacy of *Ricinus communis* fruit meal with and without *Bacillus* species on suppression of *Meloidogyne incognita* and growth of tomato. *Journal of Phytopathology* 150:399-402.

MASHELA, P.W., DUBE, Z.P. and K.M. POFU. 2015. Managing the phytotoxicity and inconsistent nematode suppression in soil amended with phytonematicides. In: Meghvansi, M.K. and A. Varma (eds.). Organic amendments and soil suppressiveness in plant disease management. Springer International Publishing: Heidelberg, Switzerland.

MASHELA, P.W., NDHLALA, A.R., POFU, K.M. and Z.P. DUBE. 2016. Transgenesis and secondary metabolism. In: Sumita, J (ed.). Phytochemicals of nematode resistant transgenic plants. Springer Publisher: Verlag, Germany.

MASHELA, P.W., SHIMELIS, H.A. and F.N. MUDAU. 2008. Comparison of the efficacy of ground wild cucumber fruits, aldicard and fenamiphos on suppression of the root-knot nematode in tomato. *Journal of Pathology* 156:264-267.

MATTHIESEN, J.N. and J.A. KIRKEGAARD. 2006. Biofumigation and biodegradation: Opportunity and challenge in soil-borne pest and disease management. *Critical Reviews in Plant Sciences* 25:235-265.

McDOWELL, J.M. and B.J. WOFFENDEN. 2003. Plant disease resistance genes: Recent insights and potential applications. *Trends Biotechnology* 27:178-183.

McINTYRE, J.L. 1980. Defenses triggered by previous invaders: Nematodes and insects. In: Horsfall, J.D. and E.B. Cowling (eds.). Plant disease. Academic Press: New York.

McKEOWN, A.W. and J.W. POTTER. 1994. Native wild grasses and flowers: New possibilities for nematode control. *Agrifood Research* 17:20-26.

McKEOWN, A.W., POTTER, J.W., GARTSHORE, M. and P. CARSON. 1994. A note on the occurrence of root-lesion nematodes under native sand-prairie plant species in the Regional Municipality of Haldimand-Norfolk, Ontario. *Phytoprotection* 75:139-142.

McRAE, M.P. 2005. A review of studies of garlic (*Allium sativum*) on serum lipids and blood pressure before and after 1994: Does the amount of allicin released from garlic powder tablets play a role. *Journal of Chiropractic Medicine* 4:82-90.

McSORLEY, R. 2011. Overview of organic amendments for management of plant parasitic nematodes, with case studies from Florida. *Journal of Nematology* 43:69-81.

McSORLEY, R. and R.N. GALLAHER. 1991. Nematode population changes and forage yields of six corn and sorghum cultivars. *Supplement to Journal of Nematology* 23:673-677.

McSORLEY, R., DICKSON, D.W. and J.A. DEBRITO. 1994. Host-status of selected tropical rotation crops to four populations of root-knot nematodes. *Nematropica* 24:45-53.

MECER, C.F., HUSSAIN, S.W. and K.K. MOORE. 2004. Resistance reactions to *Meloidogyne trifoliophila* in *Trifolium repens* and *Trifolium semipilosum. Journal of Nematology* 36:499-504.

MEHLENBACHER, S.A. 1995. Classical and molecular approaches to breeding fruit and nut crops for disease resistance. *Horticultural Science* 30:466-477.

MILLIGAN, S., BODEAU, J., YAGHOOBI, J., KALOSHIAN, I., ZABEL, P. and V.M. WILLIAMSON. 1998. The root-knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. *Plant Cell* 10:1307-1319.

MOFOKENG, M.A. 2005. Host-status of *Cucumis myriocarpus* to *Meloidogyne incognita.* Master Mini-dissertation submitted to University of Limpopo, Sovenga, South Africa.

MONTALVO, A.E. and J. ESNARD. 1994. Reaction of ten cultivars of watermelon (*Citrullus lanatus*) to a Puerto Rican population of *Meloidogyne incognita. Journal of Nematology* 26:640-643.

MOON, H.S., KHAN, Z., KIM, S.G., SON, S.H. and Y.H. KIM. 2010. Biological and structural mechanisms of disease development and resistance in chili pepper infected with the root-knot nematode. *Journal of Plant Pathology* 26:149-153.

MOREL, J.B. and J.L. DANGL. 1997. The hypersensitive response and the induction of cell death in plants. *Cell Death and Differentiation* 4:671-683.

NG'AMBI, T.B.S., RUFTY, R.C., BARKER, K.R. and T.A. MELTON. 1999. Identification of sources of resistance to four species of root-knot nematodes in tobacco. *Journal of Nematology* 31:272-282.

NICHOLSON, R.L. and R. HAMMERSCHMIDT. 1992. Phenolic compounds and their role in disease resistance. *Annual Review Phytopathol*ogy 30:369-380.

NIGH, E.L. 1985. Allelopathic activity of plants to nematode. *Journal of Nematology* 17:518.

NOIR, S., ANTHONY, F., BERTRAND, B., COMBES, M.C. and P. LASHERMES. 2003. Identification of a major gene (*Mex-1*) from *Coffea canephora* conferring resistance to *Meloidogyne exigua* in *Coffea arabica*. *Plant Pathology* 52:97-103.

NOMBELA, A.G., WILLIAMSON, V.M. and M. MUNIZ. 2003. The root-knot nematode resistance gene *Mi*-1.2 of tomato is responsible for resistance against the whitefly *Bemisia tabaci*. *Molecular Plant-Microbe Interactions* 16:645-649.

NTALLI, N.G. and P. CABONI. 2012. Botanical nematicides in the Mediterranean basin. *Phytochemistry Reviews* 11:351-359.

OHRI, P. and S.K. PANNU. 2010. Effect of phenolic compounds on nematodes: A review. *Journal of Applied and Natural Science* 2:344-350.

ONKENDI, E.M., KARIUKI, G.M., MARAIS, M. and L.N. MOLELEKI. 2014. The threat of root-knot nematodes (*Meloidogyne* species) in Africa: A review. *Plant Pathology* 68:727-737.

ORION, D., WERGIN, W.P. and B.Y. ENDO. 1980. Inhibition of syncytia formation and root-knot nematode development on cultures of excised tomato roots. *Journal of Nematology* 12:196-203.

OSEI, K., GOWEN, R.S., PEMBROKE, B., BRANDENBURG, R.L. and D.L. JORDAN. 2010. Potential of leguminous cover crops in management of a mixed population of root-knot nematodes (*Meloidogyne* species). *Journal of Nematology* 42:173-178.

OSLO, N.F. 2010. Bioactive compounds in plants-benefits and risks for man and animals. In: Bernhoft, A. (ed.). Proceedings from a symposium held at the Norwegian Academy of Science and Letters. National Veterinary Institute: Norway.

PAULSON, R.E. and J.M. WEBSTER. 1972. Ultrastructure of the hypersensitive reaction in roots of tomato (*Lycopersicon esculentum*) to infection by the root-knot nematode *Meloidogyne incognita. Journal of Physiology and Plant Pathology* 2:227-234.

PEDROSA, E.M., HUSSEY, R.S. and H.R. BOERMA. 1996. Cellular responses of resistant and susceptible soybean genotypes infected with *Meloidogyne arenaria* races 1 and 2. *Journal of Nematology* 28:225-232.

PEDROSA, E.M.R., MOURA, R.M. and E.G. SILVA. 2000. Responses from genotypes of *Phaseolus vulgaris* to *Meloidogyne* species and some mechanisms involved in the reaction. *Brazilian Phytopathology* 25:190-196.

PETERSEN, J., BELZ, R., WALDER, F. and K. HURLE. 2001. Weed suppression by release of isothiocyanates from Turniprape mulch. *Agronomy Journal* 93:37-43.

PINKERTON, J. and C.E. FINN. 2005. Responses of strawberry species and cultivars to root-lesion and northern root-knot nematodes. *Horticultural Science* 40:33-38.

PLOEG, A.T. 2000. Effects of amending soil with *Tagetes patula* cv. 'Single Gold' on *Meloidogyne incognita* infestation in potato. *Nematology* 2:489-493.

PLOEG, A.T. and P.C. MARRIS. 1999. Effect of temperature on suppression of *Meloidogyne incognita* by *Tagetes* cultivars. *Journal of Nematology* 31: 709-714.

POFU, K.M. 2012. Potential uses of indigenous *Cucumis africanus* and *Cucumis myriocarpus* as root-knot nematode-resistant rootstocks in watermelon (*Citrullus lanatus*) husbandry. PhD thesis submitted to University of Limpopo, Sovenga, South Africa.

POFU, K.M. and P.W. MASHELA. 2011. Using relative penetration and maleness indices in *Meloidogyne incognita* to establish resistance type in *Cucumis myriocarpus*. *African Journal of Biotechnology* 10:390-393.

POFU, K.M., MAFEO, T.P. and P.W. MASHELA. 2009. The responses of *Cucumis africanus* to *Meloidogyne incognita* race 2 infection. African *Journal of Crop Science* 9:659-661.

POFU, K.M., MASHELA, P.W. and H. SHIMELIS. 2012. Host-status and host-sensitivity of wild *Cucumis* species to *Meloidogyne incognita* race 4. *Acta Agriculturae* 62:329-334.

POFU, K.M., MASHELA, P.W. and M.N. MOKGALONG. 2010. Host-status and host-sensitivity of *Cucumis africanus* and *Cucumis myriocarpus* to *Meloidogyne incognita* race 2 under greenhouse conditions. *African Journal of Agricultural Research* 5:1504-1508.

POFU, K.M., MASHELA, P.W., MOKGALONG, M.N. and D. DE WAELE. 2011. Greenhouse whitefly infection on wild watermelon (*Cucumis africanus*) break resistance to the root-knot nematode. *African Crop Science Society* 10: 239-244.

POSTNIKOVA, O.A., HULT, M., SHAO, J., SKANTAR, A. and L.G. NEMCHINOV. 2015. Transcriptome analysis of resistant and susceptible alfalfa cultivars infected with root-knot nematode *Meloidogyne incognita*. PLOS ONE 10(3): e0123157. doi: 10.1371/journal.pone.0123157.

POTENZA, C.L., THOMAS, S.H. and C. SENGUPTA-GOPALAN. 2001. Genes induced during early response to *Meloidogyne incognita* in roots of resistant and susceptible alfalfa cultivars. *Plant Sciences* 161:289-299.

POTENZA, C.L., THOMAS, S.H., HIGGINS, E.A. and C. SENGUPTA-GOPALAN. 1996. Early root response to *Meloidogyne incognita* in resistant and susceptible alfalfa cultivars. *Journal of Nematology* 28:475-484.

POWERS, L.E., DUNN, R.A., McSORLEY, R., BALTENSPERGER, D.D. and D.S. WAFFORD. 1992. Effects of resistance in alyce clover (*Alysicarpus* species) on root-knot nematode (*Meloidogyne* species) populations. *Journal of Nematology* 26:30-39.

PUERTAS, A. and L. HIDALGO-DIAZ. 2007. Influence of the host plant and its interaction with *Meloidogyne incognita* on the effectiveness of *Pochonia*

chlamydosporia var. ‘Catenulata’. *Review of Vegetable Protection* 22:104-109.

REYNOLDS, L.B., POTTER, J.W. and B.R. BALL-COELHO. 2000. Crop rotation with *Tagetes* species is an alternative to chemical fumigation for control of root-lesion nematodes. *Agronomy Journal* 92:957-966.

ROBERTS, P.A. 1992. Current status of the availability, development and use of host plant resistance to root-knot nematodes. *Journal of Nematology* 24: 213-227.

ROCHA, R.O., MORAIS, J.K., OLIVEIRA, J.T., OLIVEIRA, H.D., SOUSA, D.O., SOUZA, C.E., MORENO, F.B., MONTEIRO-MOREIRA, A.C., de SOUZA JÚNIOR, J.D., de SÁ, M.F. and I.M. VASCONCELOS. 2015. Proteome of soybean seed exudates contains plant defense-related proteins active against the root-knot nematode *Meloidogyne incognita*. *Journal of Agricultural and Food Chemistry* 63:5335-5343.

RODRIGUEZ-KABANA, R., KLOEPPER, J.W., ROBERTSON, D.G. and L.W. WELLS. 1992a. Velvet bean for the management of root-knot and southern blight in peanut. *Nematropica* 22:75-79.

RODRIGUEZ-KABANA, R., PINOCHET, J., ROBERTSON, D.G. and L.W. WELLS. 1992b. Crop rotation studies with velvet bean (*Mucuna deeringiana*) for the management of *Meloidogyne* species. *Supplement to the Journal of Nematology* 24:662-668.

SABALLOS, A. 2008. Development and utilisation of sorghum as a bioenergy crop. Department of agronomy, Purdue University. West Lafayette: United States of America.

SAIKIA, S.K., TIWARI, S. and R. PANDEY. 2013. Rhizospheric biological weapons for growth enhancement and *Meloidogyne incognita* management in *Withania somnifera* cv. 'Poshita'. *Biological Control* 65:225-234.

SANCHEZ de VIALA, S., BRODIE, B.B., RODRIGUEZ, E. and D.M. GIBSON. 1998. The potential of thiarubrine C as a nematicidal agent against plant-parasitic nematodes. *Journal of Nematology* 30:192-200.

SAS INSTITUTE INC. 2008. SAS/STAT® 9.2 Qualification tools user's guide. Cary, North Carolina: SAS Institute Incorporation.

SASSER, J.N. 1979. Economic importance of *Meloidogyne* in tropical countries. In: Lamberti, F. and C.E. Taylor (eds.). Root-knot nematodes (*Meloidogyne* species) systematics, biology and control. Academic Press: London.

SIDDIQUE, S., RADAKOVIC, Z.S., DE LA TORRE, C.M., CHRONIS, D., NOVAK, O., RAMIREDDY, E., HOLBOIN, J., MATERA, C., HUTTEN, M., GUTBROD, P., *et al.,* 2014. Parasitic worms stimulate host NADPH oxidases to produce reactive oxygen species that limit plant cell death and promote infection. *Science Signal* 7:33.

SIDDIQUI, M.A. and M.M. ALAM. 1988. Toxicity of different plant parts of *Tagetes lucida* to plant-parasitic nematodes. *Indian Journal of Nematology* 18: 181-185.

SIKORA, R.A. and E. FERNANDEZ. 2005. Nematode parasites of vegetables. In: Luc, M., Sikora, R.A. and J. Bridge (eds.). Plant-parasitic nematodes in subtropical and tropical agriculture. Centre for Agriculture and Biosciences International: Wallingford.

SILVA, R.V., OLIVEIRA, R.D.L., FERREIRA, P.S., FERREIRA, A.O. and F.A. RODRIGUES. 2013. Defense responses to *Meloidogyne exigua* in resistant coffee cultivar and non-host plant. *Tropical Plant Pathology* 38:114-121.

SILVA, R.V., OLIVEIRA, R.D.L., NASCIMENTO, K.J.T. and F.A. RODRIGUES. 2010. Biochemical responses of coffee resistance against *Meloidogyne exigua* mediated by silicon. *Plant Pathology* 59:586-593.

SLANEV, K. and S. ENCHEV. 2014. Influence of variety and density on crop productivity of sorghum x sudan grass hybrids in flowering stage. *Bulgarian Journal of Agricultural Science* 20:183-185.

SONE, N.N.A. 2010. Resistance of carrot cultivars to *Meloidogyne chitwoodi*. Nematology, Department of Biology. University of Ghent: Belgium.

STARR, J.L., COOK, R. and J. BRIDGE. 2002. Plant resistance to plant-parasitic nematodes. Biddles Guildford: United Kingdom.

TAYLOR, A.L. and J.N. SASSER. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). North Carolina State University Press: Raleigh, United States of America.

THIES, J.A. and A. LEVI. 2003. Resistance of watermelon germplasm to the peanut root-knot nematode. *Journal of Horticultural Science* 38:1417-1421.

THIES, J.A. and A. LEVI. 2006. Resistance of *Citrullus lanatus* var. 'Citroides' germplasm to root-knot nematodes. *Journal of Nematology* 38:258-303.

THIES, J.A. and A. LEVI. 2007. Characterisation of watermelon (*Citrullus lanatus* var. 'Citroides') germplasm for resistance to root-knot nematodes. *Journal of Horticultural Science* 42:1530-1533.

THURAU, T., YE, W. and D. CAI. 2010. Insect and nematode resistance. In: Widholm, J.M. and T. Nagata (eds.). Biotechnique in agriculture and forestry. Springer-Verlag: Heidelberg, Switzerland.

TOMITA-YOKOTANI, K., HASHIMOTO, H., FUJII, Y., NAKAMURA, T. and M. YAMASHITA. 2004. Distribution of L-DOPA in the root of velvet bean plant (*Mucuna pruriens*) and gravity. *Biological Science Space* 18:165-166.

TRIPATHI, L., BABIRYE, A., RODERICK, H., TRIPATHI, J.N., CHANGA, C., URWIN, P.E., TUSHEMEREIRWE, W.K., COYNE, D. and H.J. ARKINSON. 2015. Field resistance of transgenic plantain to nematode has potential for future African food security. *Science Republic* 5:1-10.

TRUDGILL, D.L. 1985. Concepts of resistance, tolerance and susceptibility in relation to cyst nematodes. In: Lamberti, F. and C.E. Taylor (eds.). Cyst nematodes. Plenum Press: New York.

TRUDGILL, D.L. 1992. Resistance to and tolerance of plant-parasitic nematodes in plants. *Annual Review of Phytopathology* 29:167-192.

ULLAH, Z., ANWAR, S.A., JAVED, N., KHAN, S.A. and M. SHAHID. 2011. Response of six eggplant cultivars to *Meloidogyne incognita. Pakistan Journal of Phytopathology* 23:152-155.

VAN DER EYCKEN, W., DE ALMEIDA ENGLER, J., INZE, D., VAN MONTAGU, M. and G. GHEYSEN. 1996. A molecular study of root-knot nematode-induced feeding sites. *Plant Journal* 9:45-54.

VEECH, J.A. 1977. The relationship between terpenoid aldehydes and resistance of cotton to *Meloidogyne incognita. Journal of Nematology* 9:287-288.

VEECH, J.A. 1978. An apparent relationship between methoxy-substituted terpenoid aldehydes and the resistance of cotton to *Meloidogyne incognita. Journal of Nematology* 9:222-229.

VEECH, J.A. 1979. Histochemical localisation and nematotoxicity of terpenoid aldehydes in cotton*. Journal of Nematology* 11:240-246.

VEECH, J.A. 1981. Plant resistance to nematodes. In: Zuckerman, B.M. and R.A. Rhode (eds.). Plant-parasitic nematodes. Academic Press: New York.

VEECH, J.A. 1982. Phytoalexins and their role in the resistance of plants to nematodes. *Journal of Nematology* 14:2-9.

VEECH, J.A. and M.A. McCLURE. 1977. Terpenoid aldehydes in cotton roots susceptible and resistant to the root-knot nematode, *Meloidogyne incognita*. *Journal of Nematology* 9:225-229.

VINCENTE, N.E. and N. ACOSTA. 1987. Effects *of Mucuna deeringiana* on *Meloidogyne incognita. Nematropica* 17:99-102.

WALIA, K.K. and D.C. GUPTA. 1997. Management of root-knot nematode *Meloidogyne javanica* on vegetable crops with *Tagetes* species. *Indian Journal of Nematology* 27:18-23.

WALTERS, S.A., WEHNER, T.C. and K.R. BARKER. 1990. Resistance of cucumber to the root-knot nematode, *Meloidogyne hapla. Cucurbit Genetics Cooperative Report* 13:10-11.

WALTERS, S.A., WEHNER, T.C. and K.R. BARKER. 1993. Root-knot nematode resistance in cucumber and horned cucumber. *Horticultural Science* 28:151-154.

WANG, K.H., SIPES, B.S. and D.P. SCHMITT. 2002. Suppression of *Rotylenchulus reniformis* by *Crotalaria juncea*, *Brassica napus*, and *Target erecta*. *Nematropica* 31:237-251.

WANG, Q., LI, Y., HANDOO, Z. and W. KLASSEN. 2007. Influence of cover crops on populations of soil nematodes. *Nematropica* 37:79-92.

WANG, X., MEYERS, D., YAN, Y., BAUM, T., SMANT, G., HUSSEY, R. and E. DAVIS. 1999. In planta localisation of a β-1,4-endoglucanase secreted by *Heterodera glycines*. *Molecular Plant Microbe Interaction* 12:64-67.

WEAVER, D.B., RODRIGUEZ-KABANA, R. and E.L. CARDEN. 1993. Velvet bean in rotation with soybean for management of *Heterodera glycines* and *Meloidogyne arenaria*. *Supplement to Journal of Nematology* 25:809-813.

WESEMAEL, W.M.L. and M. MOENS. 2008. Quality damage on carrots (*Daucus carota*) caused by the root-knot nematode *Meloidogyne chitwoodi*. *Nematology* 10:261-270.

WESEMAEL, W.M.L. and M. MOENS. 2012. Screening of common bean (*Phaseolus vulgaris*) for resistance against temperate root-knot nematodes (*Meloidogyne* species). *Pest Management Science* 68:702-708.

WILLIAMSON, V.M. and A. KUMAR. 2006. Nematode resistance in plants: The battle underground. *Trends in Genetics* 22:396-403.

WILLIAMSON, V.M. and R.S. HUSSEY. 1996. Nematode pathogenesis and resistance in plants. *Plant Cell* 8:1735-1745.

WILLIAMSON, V.M., LAMBERT, K.N. and I. KALOSHIAN. 1994. Molecular biology of nematode resistance in tomato. In: Lamberti, F., Giorgi, C.D. and D.M. Bird (eds.). Advances in molecular plant nematology. Plenum Press: New York.

WUYTS, N., SWENNEN, R. and D. DE WAELE. 2006. Effects of plant phenylpropanoid pathway products and selected terpenoids and alkaloids on the behaviour of the plant-parasitic nematodes *Radopholis similis*, *Pratylenchus penetrans* and *Meloidogyne incognita*. *Nematology* 8:89-101.

XIE, G., CUI, H., DONG, Y., WANG, X., LI, X., DENG, R., WANG, Y. and Y. XIE. 2016. Crop rotation and intercropping with marigold are effective for root-knot nematode (*Meloidogyne* species) control in angelica (*Angelica sinensis*) cultivation. *Journal of Plant Science* 97:1-6.

YAMAGUCHI, H., FUKUOKA, H., ARAO, T., OHYAMA, A., NUNOME, T., MIYATAKE, K. and S. NEGORO. 2010. Gene expression analysis in cadmium-stressed roots of a low cadmium-accumulating Solanaceous plant, *Solanum torvum*. *Journal of Experimental Botany* 61:423-437.

YI, Y.H., RUFTY, R.C. and E.A. WERNSMAN. 1998. Identification of RAPD markers linked to the wildfire resistance gene of tobacco using bulked segregant analysis. *Tobacco Science* 42:52-57.

YORK, P.A. 1990. Range of susceptibility within and between diploid and tetraploid strains of Rhodes grass (*Chloris gayana*) to *Meloidogyne javanica. Review of Nematology* 13:45-50.

YU, M.H. 1995. Identification of a *Beta maritima* source of resistance to root-knot nematode *Meloidogyne incognita* for sugar beet. *Journal of Crop Science* 35:5.

ZHAO, X., SCHMITT, M. and M. HAWES. 2000. Species-dependent effects of border cell and root tip exudates on nematode behaviour. *Nematology* 90:1239-1245.

ZHANG, H., LI, C., DAVIS, E.L., WANG, J., GRIFFIN, J.D., KOFSKY, J. and S. BAO-HUA. 2016. Genome-wide association study of resistance to soybean cyst nematode (*Heterodera glycines*) HG Type 2.5.7 in wild soybean (*Glycinesoja*). *Frontiers in Plant Science* 7:1214.

APPENDICES

Appendix 3.1 Analysis of variance for necrotic spot number of *Cucumis africanus* inoculated with *Meloidogyne incognita* under greenhouse conditions from 2 to 30 days after inoculation (n = 75).

Source	DF	SS	MS	F	P
Replication	4	0.11707	0.02927		
Treatment	14	1.14260	0.08161	3.01	0.0017
Error	56	1.51961	0.02714		
Total	74	2.77928			

Appendix 3.2 Analysis of variance for giant cell number of *Cucumis africanus* inoculated with *Meloidogyne incognita* under greenhouse conditions from 2 to 30 days after inoculation (n = 75).

Source	DF	SS	MS	F	P
Replication	4	0.20061	0.05015		
Treatment	14	0.57487	0.04106	1.39	0.1909
Error	56	1.65945	0.02963		
Total	74	2.43492			

Appendix 3.3 Analysis of variance for proliferation of rootlet interference of *Cucumis africanus* inoculated with *Meloidogyne incognita* under greenhouse conditions from 2 to 30 days after inoculation (n = 75).

Source	DF	SS	MS	F	P
Replication	4	0.04091	0.01023		
Treatment	14	0.81355	0.05811	2.60	0.0057
Error	56	1.24984	0.02232		
Total	74	2.10430			

Appendix 3.4 Analysis of variance for root gall number of *Cucumis africanus* inoculated with *Meloidogyne incognita* under greenhouse conditions from 2 to 30 days after inoculation (n = 75).

Source	DF	SS	MS	F	P
Replication	4	0.09909	0.02477		
Treatment	14	0.53192	0.03799	2.81	0.00331
Error	56	0.75852	0.01355		
Total	74	1.38954			

Appendix 3.5 Analysis of variance for necrotic spot number of *Cucumis africanus* inoculated with *Meloidogyne javanica* under greenhouse conditions from 2 to 30 days after inoculation (n = 75).

Source	DF	SS	MS	F	P
Replication	4	0.08616	0.02154		
Treatment	14	0.58979	0.04213	3.26	0.0008
Error	56	0.72451	0.01294		
Total	74	1.40045			

Appendix 3.6 Analysis of variance for giant cell number of *Cucumis africanus* inoculated with *Meloidogyne javanica* under greenhouse conditions from 2 to 30 days after inoculation (n = 75).

Source	DF	SS	MS	F	P
Replication	4	0.08364	0.02091		
Treatment	14	1.26233	0.09017	5.65	0.0000
Error	56	0.89337	0.01595		
Total	74	2.23934			

Appendix 3.7 Analysis of variance for proliferation of rootlet interference of *Cucumis africanus* inoculated with *Meloidogyne javanica* under greenhouse conditions from 2 to 30 days after inoculation (n = 75).

Source	DF	SS	MS	F	P
Replication	4	0.10816	0.02704		
Treatment	14	1.14447	0.08175	4.03	0.0001
Error	56	1.13586	0.02028		
Total	74	2.38848			

Appendix 3.8 Analysis of variance for root gall number of *Cucumis africanus* inoculated with *Meloidogyne javanica* under greenhouse conditions from 2 to 30 days after inoculation (n = 75).

Source	DF	SS	MS	F	P
Replication	4	0.10874	0.02719		
Treatment	14	0.92170	0.06584	3.38	0.0006
Error	56	1.09222	0.01950		
Total	74	2.12266			

Appendix 3.9 Analysis of variance for necrotic spot number of *Cucumis myriocarpus* inoculated with *Meloidogyne incognita* under greenhouse conditions from 2 to 30 days after inoculation (n = 75).

Source	DF	SS	MS	F	P
Replication	4	0.11707	0.02927		
Treatment	14	1.14260	0.08161	3.01	0.0017
Error	56	1.51961	0.02714		
Total	74	2.77928			

Appendix 3.10 Analysis of variance for giant cell number of *Cucumis myriocarpus* inoculated with *Meloidogyne incognita* under greenhouse conditions from 2 to 30 days after inoculation (n = 75).

Source	DF	SS	MS	F	P
Replication	4	0.20061	0.05015		
Treatment	14	0.57487	0.04106	1.39	0.1909
Error	56	1.65945	0.02963		
Total	74	2.43492			

Appendix 3.11 Analysis of variance for proliferation of rootlet interference of *Cucumis myriocarpus* inoculated with *Meloidogyne incognita* under greenhouse conditions from 2 to 30 days after inoculation (n = 75).

Source	DF	SS	MS	F	P
Replication	4	0.04091	0.01023		
Treatment	14	0.81355	0.05811	2.60	0.0057
Error	56	1.24984	0.02232		
Total	74	2.10430			

Appendix 3.12 Analysis of variance for root gall number of *Cucumis myriocarpus* inoculated with *Meloidogyne incognita* under greenhouse conditions from 2 to 30 days after inoculation (n = 75).

Source	DF	SS	MS	F	P
Replication	4	0.09909	0.02477		
Treatment	14	0.53192	0.03799	2.81	0.0031
Error	56	0.75952	0.01355		
Total	74	1.38954			

Appendix 3.13 Analysis of variance for necrotic spot number of *Cucumis myriocarpus* inoculated with *Meloidogyne javanica* under greenhouse conditions from 2 to 30 days after inoculation (n = 75).

Source	DF	SS	MS	F	P
Replication	4	0.23215	0.05804		
Treatment	14	0.43075	0.03077	1.49	0.1455
Error	56	1.15669	0.02066		
Total	74	1.81958			

Appendix 3.14 Analysis of variance for giant cell number of *Cucumis myriocarpus* inoculated with *Meloidogyne javanica* under greenhouse conditions from 2 to 30 days after inoculation (n = 75).

Source	DF	SS	MS	F	P
Replication	4	0.14494	0.03624		
Treatment	14	1.60741	0.11481	5.38	0.0000
Error	56	1.19543	0.02135		
Total	74	2.94778			

Appendix 3.15 Analysis of variance for proliferation of rootlet interference of *Cucumis myriocarpus* inoculated with *Meloidogyne javanica* under greenhouse conditions from 2 to 30 days after inoculation (n = 75).

Source	DF	SS	MS	F	P
Replication	4	0.28622	0.07155		
Treatment	14	1.44398	0.10314	2.81	0.0031
Error	56	2.05450	0.03669		
Total	74	3.78470			

Appendix 3.16 Analysis of variance for root gall number of *Cucumis myriocarpus* inoculated with *Meloidogyne javanica* under greenhouse conditions from 2 to 30 days after inoculation (n = 75).

Source	DF	SS	MS	F	P
Replication	4	0.18324	0.04581		
Treatment	14	1.18470	0.08462	2.75	0.0037
Error	56	1.72425	0.03079		
Total	74	3.09218			