RESISTANCE STATUS OF MINT TO *MELOIDOGYNE ENTEROLOBII* AND UTILIZATION OF MYCORRHIZAL FUNGI AS A CONTROL STRATEGY

TSHIRELETSO LESIBE NCHABELENG

202050351

orcid.org/ 0009-0004-3679-1681

A research dissertation submitted in fulfillment of the requirements for the Master of Science Degree in Agriculture

Supervisor : Dr T.A Masenya

Co-supervisor : Dr ZP. Dube

School of Agricultural Sciences

Faculty of Agricultural and Natural Sciences

2026

UNIVERSITY OF MPUMALANGA

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DECLARATION

I, hereby declare that the dissertation titled 'Resistance status of Mint to *Meloidogyne enterolobii* and utilization of Mycorrhiza fungi as a control strategy' submitted to the University of Mpumalanga for the Master of Science degree in Agriculture and that the content provided herein is my work with exception to the citations and that this work was not handed over or submitted for any degree or to other institution.

Name: Tshireletso Nchabeleng	Signature: TL Nchabeleng	Date:
Supervisor: Dr T.A. Masenya	Signature:	Date: 20/04/2026
Co-supervisor: Dr Z.P. Dube	Signature:	Date:

DEDICATION

To my late sister, Khutsiso Nchabeleng and supportive family.

ACKNOWLEDGEMENTS

Firstly, the supervisor, Dr. T.A. Masenya, for his incredible guidance, support and encouragement throughout my experimental and thesis report writing journey. Your expertise and insights have helped shape my work, and I am deeply thankful for the time and effort you have put into my academic progress. I appreciate my co-supervisor, Dr. Z.P. Dube for the crucial guidance, support, as well as providing fruitful feedback. It has been a privilege to learn under their guidance.

Secondly, my mother, Madigwe Nchabeleng, for her unflinching love and support which have served as the cornerstone for my life. Her faith and sacrifices in me has motivated me to pursue my goals with determination and persistence. I thank her for always being my best cheerleader. I also thank my loving and supportive family. Thirdly, Amogelang Mabuela for support and guidance. Your support helped me navigate the difficulties of my study. Also, Tshepiso Khoza for helping me with setting up my experiments. I humbled by his effort in dedicating time to assisting me.

Fourtly, The Dr EJ Mabuza/Thebe Foundation scholarship. This scholarship not only reduces the financial burden of my education, but it also served as a fantastic source of encouragement for me to pursue my academic and career goals. Last but not least, our Creator God for making this journey possible. Your love and direction enlightened my way, gave me courage in times of adversity and joy in times of rejoicing.

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LIST OF ABBREVIATIONS

ANOVA− Analysis of variance

AMF - Arbuscular Mycorrhizal Fungi

CC - Chlorophyll Content

DF −Degree of Freedom

GI −Gall Index

g - gram

IPM −Integrated Pest Management

MS −Mean Squares

MAPs -Medicinal and aromatic plants

NPK −Nitrogen, Phosphorus, and Potassium

NB - Number of Branches

PF− Final Population

PPN− Plant Parasitic Nematodes

PH - Plant Height

P - Probability

RCBD− Randomized Complete Block Design

RF− Reproductive Factor

RKN− Root Knot Nematodes

SS −Sum of Squares

STD - Stem Diameter

TTV −Total Treatment Variable

AMF - Vesicular Arbuscular Mycorrhiza

ABSTRACT

Mint (*Mentha spicata*) is an important aromatic and medicinal crop used raw or processed into oil for a variety of consumption purposes. Medicinal plants play a critical role in providing medications for humans, particularly in poor countries, where communities depend on such medicinal plants for traditional medicinal use. However, with global climate change, root-knot nematodes are often considered to be among the most devastating pests in agriculture, resulting in a major decline in productivity of such medicinal plants which are found to be susceptible to root knot nematodes. The objectives of the study were to determine host status and host sensitivity of *Mentha spicata* to *Meloidogyne enterolobii* and the evaluation of whether Arbuscular Mycorrhizal Fungi (AMF) will reduce *M. enterolobii* in the event the test plant is susceptible. A micro plot and shade net experiment was conducted in 2025. To assess the host status and sensitivity, *M. spicata* seedlings were inoculated with the following treatment levels; 0, 25, 50, 125, 250, 625, 1250 & 3125 eggs and J2s of *M. enterolobii,* with the treatments laid in a Randomized Complete Block Design (RCBD). At 56 days after initiating treatment, plant and nematode variables were measured. The nematode reproductive factors (Rf) were greater than one in all treatment levels under micro plot setting, while under the shade net it was above one at the lower treatment levels and below unity as the treatment levels increased. Results showed that plant parasitic nematodes negatively reduced number of branches, plant height, and chlorophyll content with relative impacts (RI) ranging from 13 to 42%, 12 to 69% and 30 to 93% in microplot setting. While for a shade net setting only plant height was reduced with a RI of 8 to 41%. The test plant was susceptible to root knot nematodes; therefore no assessment of resistant was necessary, AMF was utilized as potential bio-control strategy. Each mint seedling was inoculated with 3000 eggs+ J2s of *M. enterolobii*. At 56 days after nematodes inoculation, AMF treatment levels of 0, 10, 20, 30, 40g were initiated arranged in RCBD. The fungus employed was RRHIZ-UP, and the isolates identified in the product were *Funneliformis coronatum*, *Funneliformis mosseae*, *Claroideoglomus etunicatum*, and *Rhizophagus* irregularis species. All the Arbuscular Mycorrhizal Fungi (AMF) treatment levels reduced nematodes infestation. The results of the study indicated that AMF improved plant parameters such as plant height, stem diameter, chlorophyll content, and fresh shoot weight under micro plot settings with relative impact ranging from 22 to 79%, 19 to 88%, 83 to 219% and 25 to 69%. While under shade net settings stem diameter was improved by 12 to 43%. On nematode variables, AMF had significant effect, reducing plant parasitic nematodes with relative impact of 13 to 30% on root galls, 17 to 31% in J2 in roots, 20 to 31% for eggs in roots, 25 to 35% for J2 in soil, 31 to 46% for eggs in soil and 18 to 27% for the final population (Pf).While for shade net settings nematodes were reduced by 16 to 33% on root galls, 12 to 28% in J2 in roots, 12 to 25% for eggs in roots, 6 to 38% for J2 in soil, and 9 to 24% for the final population (Pf). In conclusion, mint was a host to *M. enterolobii* and AMF controlled nematode population densities. Arbuscular Mycorrhizal Fungi could be used as potential bio-control strategy to manage root knot nematodes infestation in plants.

Keywords: *Mentha spicata*, Arbuscular Mycorrhiza Fungi. Gall index, plant parasitic nematodes, root-knot nematodes, reproductive factor.

CHAPTER 1

1. INTRODUCTION

1.1 Background of the study

Mint (*Mentha spicata*) is a perennial herb that is known for its aroma and numerous health benefits. The pharmacological and medicinal properties of *M. spicata* have gained the crop attention, gaining increased use in informal and formal markets access. The essential oils and extracts demonstrated remarkable antimicrobial, antioxidant, anticancer, anti-inflammatory and hepatoprotective properties (Mahendran *et al*., 2021). This herb is said to have originated in the Mediterranean, specifically in the areas surrounding Greece and Italy but is cultivated in many regions with temperate climates, including South Africa (Mahendran *et al*., 2021; Best, 2022). Mint herbs are known for their strong, refreshing scent and flavor, which comes from the presence of menthol and other essential oils in their leaves. These oils have a variety of uses, including culinary, medicinal, and cosmetics. *Mentha* floral parts have traditionally been used to treat tuberculosis, sinusitis, and foodborne diseases due to their antimicrobial properties (Arshad *et al*., 2023).

Medicinal plants are a vital source of life-saving medications for humans, particularly in developing countries, where communities depend on such medicinal plants for traditional medicinal use. According to the World Health Organization, over 80% of the world's population in developing countries relies mainly on herbal medicine for basic health care (Mahendran *et al*., 2021). Mint production is on the rise as the result of the increasing population demand, projected at an average of 28000 tonnes per annum (Gupta *et al.,* 2023).

The overall market for mint oil along with related items is projected to grow by 3-5% per year. Globally, as the population grows, the demand for the use of chemically produced medications and antimicrobials as well as the renewed interest in the utilization of plant-based medication will certainly increase (Gupta *et al.,* 2023). The rise in urbanization and shifting consumer preferences have increased the demand for medicinal and aromatic herbs such as mint in the agricultural industry. Mint's versatility and numerous health benefits, make it a valuable plant-based product in the markets (Tafrihi *et al*., 2021).

However, with the global climate change that resulted in evolving, destructive infestation of new species of plant-parasitic nematodes, that have shorter life cycles, limiting production of such value medicinal plants globally (Mabuela *et al*., 2025). Plant-parasitic nematodes are often considered to be among the most devastating pests in agriculture, resulting in a major decline in productivity (Coyne *et al*., 2018). *Meloidogyne enterolobii* is becoming an increasingly serious threat to most crops and predominating nematode species, with less nematode mitigation strategies (Coyne *et al*., 2018). Mint herbs are reported to be susceptible to several root-knot nematode species, including *Meloidogyne hapla*, *M. javanica* and *M. incognita,* they reproduce on the plant inhibiting its growth and productivity (Caboni *et al*., 2015; Pandey & Patra, 2001). Plant parasitic nematodes like *M. enterolobii* are yet to evaluated in mint herbs to determine whether or not the root-knot nematode species can reproduce and affect crop growth.

Synthetic fumigants were extensively used previously to control nematodes in agricultural contexts. As a result of negative effects to the environment, most of them are restricted or prohibited in many agricultural markets of many countries. This has resulted in a call for the development of new ways to successfully manage nematode infestations in crops (Laquale *et al*., 2015; Oka, 2020). One of the strategies used to control nematodes in the absence of synthetic fumigants is the use of resistant crop types, which can provide an effective method of nematode control (Mukhtar *et al.*, 2014). The resistance of nematodes is evaluated by exposing cultivars to tests for host-status and host-sensitivity. A range of nematode levels is utilized in nematode resistance studies, comprising measures on nematode reproductive factor (Rf) and crop injury induced by infections of nematodes (Pofu *et al*., 2017). Reproduction factor is a method utilized in nematode-resistant experiments that derive from the percentage of the last nematode population size to the first nematode population size to count nematode into a particular host (Seinhorst, 1967). The estimation of host sensitivity and status of *M. spicata* will help in its cultivation in soil infested by *M. enterolobii.*

1.2 Statement of the problem

Meloidogyne enterolobii is a pathogenic nematode with a wide host range able to cause severe reduction in yield on infected crops. This nematode has been reported to be a worldwide problem for many beneficial and economically valuable crops (Collett *et al*., 2023). As aggressive as *M. enterolobii* is, managing these nematodes is challenging because of their broad variety of hosts, ability to develop resistance to chemical nematicides, and short lifespan of a 15 day-life cycle (Collett *et al*., 2023). *Mentha spicata* has been found to host other *Meloidogyne* species which are thermophilic, as *M. enterolobii* like *M. hapla*, *M. javanica* and *M. incognita* as mentioned above (Caboni *et al*., 2015). In order to exploit nematode resistance in root-knot nematode management, the crop must be exposed to existing *Meloidogyne* species and their biological races (Nkosi, 2019; Pofu *et al*., 2012). The study looks to evaluate if *M. enterolobii* is an invasive plant parasitic nematode like the other thermophilic species and in cases where necessary develop management strategies. After exposing the selected plant to host status and host sensitivity test against *M. enterolobii*, if the plant is found to be susceptible and there is a decline in plant yields, then management strategy such as using bio-control method will be implemented. Alternative bio-control strategies include the use of Arbuscular Mycorrhizal Fungi (AMF), which are a kind of symbiotic fungi that develop relationships with many plants and can improve plant growth and stress tolerance (Dar and Reshi, 2017). The withdrawal of environmentally harmful nematicides has driven the need for the establishment of suitable alternatives to protect the crop. Currently, there are no reports of the mint's host status and susceptibility to *M. enterolobii*.

1.3 Rationale

Medicinal and aromatic plants (MAPs) such as *Mentha spicata* are in high demand due to a broad variety of reliable and affordable, therapeutic and effective treatments (Nurhussen and Philipos, 2014). The protection of indigenous medicinal plants is vital to the country's economic development to meet the growing demand for medical products (Masenya, 2022). General aspects of sustainability and unsustainable medicinal plant harvesting, as well as lack of knowledge in cultivation to increase medicinal plant populations for the medicinal plant trade market, mandate increasing of production of such valuable medicinal plant throughout the country. *Meloidogyne* species are considered the most challenging pests to manage in crop production systems globally (Mashela *et al*., 2017). Many species of thermophilic nematodes were discovered in South Africa on *M. spicata* samples. Controlling nematode population densities is required for continued and enhanced production of mint, towards the continuous delivery of medical and aromatic goods to the growing population (Masenya *et al*., 2020). The need to develop less destructive and environmentally acceptable alternatives to commercial nematicides opened the door to alternative solutions, such as the utilizing of nematode-resistant cultivars and biological control methods.

1.4 Purpose of the study

1.4.1 Aim

This study was carried out to determine *Mentha spicata* host status and host sensitivity to *Meloidogyne enterolobii* and the evaluation of whether Arbuscular Mycorrhizal Fungi will reduce *M. enterolobii* in the event the test plant is susceptible.

1.4.2 Objectives

i. To determine whether *Meloidogyne enterolobii* will reproduce on *Mentha spicata* and result in reduction of the plant's growth.

ii. To establish the mechanism of resistance in *Mentha spicata* to *M. enterolobii.*

iii. To investigate whether Arbuscular Mycorrhiza Fungi will reduce *M. enterolobii* in the event the test plant is susceptible.

1.4.3 Hypothesis

i. *Meloidogyne enterolobii* will reproduce on *Mentha spicata* and result in a reduction in the plant's growth.

ii. The mechanism of resistance in *Mentha spicata* to *M. enterolobii* will be established.

iii. Arbuscular Mycorrhiza Fungi will reduce *M. enterolobii* in the in the event the test plant is susceptible.

1.4.4 Research questions

i. Will *M. enterolobii* reproduce on *Mentha spicata* and results in a reduction in the plant's growth?

ii. Will the mechanism of resistance in *Mentha spicata* to *M. enterolobii* be established?

iii. Arbuscular Mycorrhiza Fungi will reduce *M. enterolobii* in the event *M*. species is susceptible?

1.5 Reliability, objectivity and validity

The reliability of the data was verified using statistical analysis at a 5% significance level. The validity of data was confirmed by replication of the experimental treatments. The acquisition of objectivity was reached through a thorough examination of empirical evidence, which eliminated any elements of subjectivity from the analysis.

1.6 Bias

To minimize bias, each experiment was carried out with adequate replications and randomization to control for potential errors according to Duflo *et al*. (2007).

1.7 Scientific contribution

The knowledge of *Mentha spicata*'s nematode resistance to *Meloidogyne enterolobii* would aid decision-making regarding the inclusion of *M. spicata* as an alternative crop for potential utilization as a combined nematode control strategy in South African farming systems. The obtained information can be useful in cultivating *Mentha spicata* plants and producing enough medicinal products and food for people in relation to the increasing population. The root knot nematodes can cause more damage and yields decline on important plants, effective management strategies such as using biological strategies like AMF will be adopted to reduce the damage and ensure sustainable production.

1.8 Structure of the dissertation

Chapter 1 contains a detailed background, statement of the problem, rationale, aim and objectives, while Chapter 2 provides a full review of the available literature on the research topic. Chapters 3 and 4 focus on achieving objectives 1 and 3, respectively. Chapter 5 consolidates and combines the findings from all previous chapters, providing a complete summary of their significance and recommendations for further research. The presentation of the conclusions follows this. The University of Mpumalanga Senate has approved the use of the Harvard referencing style for both in-text citations and reference lists.

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CHAPTER 2

2. LITERATURE REVIEW

2.1 Introduction

Mint (*Mentha spp*.) is an aromatic herb from the Lamiaceae family and is widely considered as the most valuable cash crop (Pandey *et al*., 2019). The herb is widely utilized in culinary, medicine and beverage goods due to its flavor, aroma, and medical benefits (Scherer *et al*., 2013). Mints are among the most important medicinal and aromatic crops for farmers, not only because of their pharmaceutical value, but also because of their numerous applications (Salehi *et al*., 2018). Herbal medicines such as mint contain a variety of compounds, some of which are highly complex (Patel & Sharma, 2019). The widespread use of medicinal plants and plant-derived remedies in traditional cultures around the world has encouraged the inclusion of phytochemicals into modern health-care products (Ghosh *et al*., 2024).

Medicinal plant constituents extracted from roots and above-ground crop sections contain both primary and, plant secondary metabolites, which also known as phytochemicals (Nazem *et al*., 2019). Flavonoids and phenolic acids are the most abundant and well researched phytochemicals produced by plants. They offer a wide range of industrial applications in cosmetics, medicines, and nutrition (Park *et al*., 2021). Their natural characteristics make them a desirable alternative for integrative health practices (Hedayati *et al*., 2024).

The cultivation of medicinal and aromatic plants (MAPs) has risen dramatically in tropical and subtropical countries around the world in order to meet the demands of the pharmaceutical, perfumery, cosmetic, and food industries (Pandey, 2017). Yet the main source of raw materials is particularly susceptible to numerous infections caused by a variety of pests and pathogens such as nematodes, which are responsible for significant losses in crop productivity or yield as well as the loss of medicinal and aromatic plants (MAP)' major genetic resource (Abtahi & Bakooie, 2017; Avato *et al*., 2017). Studies have shown that infestations can lead to yield losses ranging from 30% to over 80% in susceptible crops like guava, tomatoes, soybeans etc. (Khan *et al*., 2022). The marketable yield loss on all these crops estimated at R64.44 billion annually (Rutter *et al*., 2025). As a result, infections caused by such pests and pathogens are a significant challenge to the successful production of important medicinal and aromatic plants.

Mint cultivation is affected by root knot nematodes, especially *Meloidogyne* species just like any other plants and which can dramatically influence plant health and productivity (Santos *et al*., 2019). An effective root knot nematode strategy is based on understanding the relationship between the test nematode and the plant. The host status and sensitivity of medicinal plant like mint to *Meloidogyne enterolobii is* unknown*. Meloidogyne enterolobii* is one of the most dangerous harmful nematode species because of its wide host range, pathogenicity, and ability to establish and reproduce on multiple plants containing resistance genes (Castagnone-Sereno, 2012). Root knot nematodes such as *M. enterolobii* attack plant roots and cause galls or root knots (Costa *et al*., 2025). These galls affect normal root activity, reducing water and nutrient intake. Damage to the root system can cause nutrient deficits in the plant because the roots are unable to absorb important minerals and nutrients adequately (Ye *et al*., 2021). Even when soil moisture is adequate, a damaged root system might impair the plant's ability to absorb water, causing drought stress symptoms (Silva *et al*., 2019). The impacts of this nematode can lead to chlorosis, underdevelopment, and decreased yields on host plants (Sikandar *et al.*, 2023).

The impact of root knot nematodes seen on susceptible host plants has resulted in extensive crop loses and threaten food security. For sustainable food production sustainable agriculture practices are needed to develop or come up with biological strategies that can be utilized to reduce the impact

(Timper, 2014). The beneficial microorganisms such as AMF are used to manage the nematodes population (Dar & Reshi, 2017). Arbuscular Mycorrhizal Fungi are root symbionts that absorb nutrients from plants and supply important minerals such as nitrogen, phosphorus, and others to the host plant (Abbasi *et al.*, 2015). The influence of AMF in alleviating nematode stress incidences is yet to be documented on mint.

2.2 *Meloidogyne enterolobii* influence on plants

The genus *Meloidogyne* comprises more than 98 species globally with *Meloidogyne enterolobii* being one of the most well-known (Makhado, 2020). Using genetic and differential host plant techniques, *M. enterolobii* has been identified as one of the biological races among these *Meloidogyne spp*. (Makhado, 2020). *Meloidogyne enterolobii* has been seen to infect a wide range of plant species like medicinal, legumes and solanaceous crops (Salazar-Mesta *et al*., 2023). The nematode's capacity to thrive in a variety of environmental conditions has enabled its spread across geographical regions, making it a global agricultural problem (Tapia-Vázquez *et al.,* 2022; Dutta & Phani, 2023). Root knot nematodes infection is a complex process that begins with the nematode's entry into the root system. *Meloidogyne enterolobii* enters the root and stimulates the development of specialized feeding structures known as giant cells, which are crucial for its survival and replication (Siddique & Jones, 2021). These giant cells are created by changing host plant cells, causing major changes in plant metabolism and physiology. The nematode secretes a number of effector proteins that influence host plant defenses, allowing it to establish and reproduce (Przybylska & Obrępalska-Stęplowska, 2020).

Plants respond to RKN infection in a variety of ways, including activation of defense mechanisms (Molinari & Leonetti, 2019). The responses can be classified as physical barriers, such as the formation of root galls, and biochemical defenses, such as the generation of reactive oxygen species and pathogen-related proteins (Lone *et al*., 2024). However, *M. enterolobii* has developed tactics to reduce these defenses, allowing it to overcome plant immune responses (Feng *et al*., 2023). Some plant species have resistance to root knot nematodes which are frequently connected with specific resistance genes (Carrillo-Fasio *et al*., 2020). The study by Verssiani *et al*. (2023), discovered that several soybean cultivars have a high level of resistance to *M. enterolobii*, which is related to the presence of resistant genes that cause localized cell death and limit nematode development. Similarly, found root knot disease in menthol mint and bergamot mint in India and the two major nematode species, *M. incognita* and *M. javanica*, were discovered to be associated with menthol mint. But the information on the influence of the *M. enterollobii* to mint remains unknown.

2.3 Host range and pathogenicity

The relationship of plants with various root knot nematodes is determined by assessing their host status with the various test nematodes, this determines their host range. The concepts of host-status and host-sensitivity, which were first used by Seinhorst (1967) to describe nematode-plant interaction, have since been applied in plant-parasitic nematology. The reproductive factor (RF = Pf/Pi), which Seinhorst defined as the host-status, was calculated by dividing the initial nematode population density (Pi) by the percentage of the final nematode population density (Pf). Nematodes face intense competition for resources when Pf equals Pi because the RF is less than unity, which approaches an equilibrium (E) point (Makhado. 2020; Seinhorst, 1967). In addition, it was explained that if all the RF values are less than one, it means the nematode could not reproduce on that hostplant, and vice versa, for values bigger than one. The term host-sensitivity describes a plant's reaction to a nematode infestation and is influenced by a number of factors including the kind of plant, age, biotic and abiotic circumstances, nematode species and inoculum level.

Nematode hostility differs significantly among species, some are more hostile than others (Seinhorst, 1967). The host range of *M. enterolobii* is varied among various crop species, with some showing mechanism of resistances and other tolerances (Collett *et al.*, 2021). The highly polyphagous pathogen *M. enterolobii* is capable of dispersing a wide range of crop species, including weeds, ornamentals, and other plants that may be found growing in temperate, tropical, or subtropical climates worldwide (Collet, 2020). These pathogenic nematodes penetrate plant roots and cause the production of large cells and root knots or galls (Selvam *et al*., 2025). This process is facilitated by nematode effectors that control plant cell growth. The galls affect the plant's vascular system, reducing water and nutrient absorption. The enormous cells operate as nutrient sinks, drawing nutrients away from the plant and toward the nematode (Favery *et al*., 2020). These impacts cause stunted growth, lower yields, and greater susceptibility to other diseases. A few crops such as groundnuts and maize are either regarded as non-hosts or do not exhibit any damage from nematodes (Ralmi *et al.*, 2016). Study conducted in Brazil and South Africa revealed that adult *M. enterolobii* females were present in peanut roots, but there were no egg clusters. This proved that although *M. enterolobii* infected the crop, it was unable to grow within its roots (Collet, 2020).

2.4 *Meloidogyne enterolobii* mechanisms to break host plant resistance

Meloidogyne enterolobii is well-known for its ability to overcome host plant resistance, which presents problems for sustainable agriculture (Masenya *et al*., 2023). The plant parasitic nematodes use a variety of tactics to overcome host plant resistance (Bernard *et al*., 2017). In several studies conducted, root knot nematodes showed a notable ability to change their behavior in response to resistant host plants. According to study Escobar & Fenoll (2015), the nematode has the capacity to change its penetration behavior, allowing it to more efficiently infiltrate resistant cultivars. For instance, it may boost its mobility or alter its host finding techniques in order to identify and enter resistant roots. Root knot nematodes and host plants have a complex molecular relationship, which various among crop species (Costa *et al*., 2021). The nematode secretes a number of effector proteins that alter host plant defenses. These effectors can inhibit the plants' immune response, allowing nematodes to establish and reproduce (Sato *et al*., 2019). For example, the production of proteins that resemble plant hormones can disrupt normal plant signaling pathways, increasing vulnerability.

Root knot nematodes can cause changes in the host plant's physiological responses, such as root structure and defense-related gene expression (Gomes *et al*., 2014). This nematode has been proven to manipulate the host's biological processes in order to generate large cells that serve as feeding sites (Rutter *et al*., 2022). The creation of these cells is frequently related with the suppression of local defense mechanisms, which allows the nematode to thrive (Molinari & Leonetti, 2019). *Meloidogyne enterolobii* populations' genetic diversity can also help them overcome resistance. Variability in the nematode's genetic makeup may result in virulence variances, allowing some populations to effectively overcome the resistance mechanisms of specific host plants (Barbary *et al.*, 2015). According to Onkendi *et al.* (2014), resistance typically develops during or in a later adult stage.

This genetic flexibility is an important feature in the nematode's adaptability and persistence in agricultural systems. Environmental factors such as soil type, moisture, and temperature can all influence the effectiveness of host plant resistance and the nematode's ability to overcome it (Reddy, 2013; Sorribas *et al*., 2020). Certain environmental pressures may reduce plant defenses, rendering them more vulnerable to invasion by *M. enterolobii* (Culbreath, 2022). The food security of the continent is at risk because resistance-breaking *Meloidogyne* species have made many of the pest management methods now in place ineffective (Philbrick *et al*., 2020).

2.5 Nematodes amendment strategies

Effective management of root-knot nematodes is crucial for minimizing the loss of crop yields. (Khan *et al*., 2021). Plant parasitic nematodes (PPNs) are major threats to food security and plant health (Kantor *et al*., 2024). Plant parasitic nematodes (PPNs) causes harm to crop growth, symptoms are mainly non-specific and are usually misdiagnosed as abiotic stress, hence PPNs infection goes untreated (Pandey, 2017). This can result in excessive population densities that are difficult to lower to an acceptable, non-damaging threshold once established in the field. Usually when nematodes are identified as the cause of damage and they are beyond the threshold resulting in economic losses, several strategies are deployed.

2.5.1 Chemical strategies

Chemical control with synthetic nematicides is the most effective strategy for controlling PPNs. Chemical nematicides are ideally applied before planting as a liquid or gas that rapidly volatilizes into a gas with upward movement (Dittmar *et al*., 2021). Nematicides are often general biocides with ability to kill a range of soilborne microorganisms and plants, and as a result, it needs to be utilized prior to planting, with enough time for the product to off-gas before the main crop is planted (Rutter *et al*., 2025). In Florida and North Carolina, it has been discovered that spraying, especially with 1,3-dichloropropene, effectively controls *M. enterolobii* symptoms in such as sweet potatoes (Grabau *et al*., 2024). The non-fumigant nematicides such as oxamyl and fluopyram were utilized. These nematicides can contaminate soil and water, harming beneficial organisms and disrupting ecosystems (Tiwari, 2024). Oxamyl’s high toxicity can affect aquatic life, while fluopyram’s persistence may impact soil health and biodiversity (Muñoz-Bautista *et al*., 2025).

Exposure to these chemicals poses health risks to farmworkers, including neurotoxic effects and potential endocrine disruption (Macieira *et al*., 2023). Their use can also contribute to nematode resistance, reducing long-term efficacy and necessitating integrated pest management approaches (Tiwari, 2024). However, due to their broad spectrum of activity, environmental toxicity, and significant legislative pressure to restrict them, they are gradually being phased out, necessitating the development of alternatives (Abd-Elgawad, 2025). Also, as the chemical levels decrease, the nematodes develop resistance and become more aggressive (Seid *et al*., 2015).

2.5.2 Physical and cultural pest management strategies

Physical and cultural pest management strategies, such as crop rotation have been shown to be successful against nematodes (Swaruparani & Shanmugam, 2024). Cultural methods are non-chemical management tactics, such as crop rotation with non-host crops or resistant cultivars, that are cost-effective for nematode control (Philbrick *et al*., 2020). Rotation with non-host crops reduces *M. enterolobii* populations by preventing reproduction and population growth in the absence of a suitable host (Dareus *et al*., 2021). Crop rotation with non-hosts species for at least one year can assist to minimize nematode numbers (Mukhtar *et al*., 2014). Tomato for example, on the other hand should be rotated with non-hosts species for at least three years. Unfortunately, crop rotation has limitations due to *M. enterolobii*'s wide host range and shorter life cycles (Wong *et al*., 2024). Peanut, corn, and wheat have proved to be poor hosts for this nematode species and can be used as rotation crops (Jordan *et al*., 2017). Despite this, biological management of nematodes utilizing plant-beneficial bacteria and fungi is gaining popularity due to their bio-efficiency against nematodes and deficit in negative environmental impact (Sivasabari *et al*., 2023).

2.5.3 Use of resistant varieties

The use of resistance cultivars as nematode management techniques is thought to be the most affordable and ecologically favorable (Sivasubramaniam *et al*., 2020). Cultivars resistant to root knot nematodes have been available for many years in a variety of crops and have assisted in the management of several of the most common root knot nematodes (RKN) spp. including *M. incognita*, *M. javanica*, and *M. arenaria* (Saucet *et al*., 2017; Subedi *et al*., 2020). Unfortunately, *M. enterolobii* can infect and harm several of the most commonly used RKN resistance genes. These include those found in cotton, soybeans (Rmi1), cowpeas (Rk), potatoes (Mh), sweet potatoes, peppers (N, Me1, Me3, and Tabasco), tomatoes (Mi1.2), and cowpeas (Rutter *et al*.,2025).

Many crops that have traditionally depended on resistant types to help manage these nematodes are seriously threatened by *M. enterolobii's* capacity to overcome RKN resistance (Yadav *et al*., 2025). In response, researchers are working to find the causes of *M. enterolobii* resistance in a number of vulnerable host crops. Breeders are currently working on a strategy to develop new crop varieties resistant to *M. enterolobii* by incorporating these resistance resources (Diniz *et al*., 2016). The frequency of genetic resistance to *M. enterolobii* varies greatly throughout host crops, similar to resistance to other RKN species (Shi *et al*., 2025).

For instance, despite the susceptibility of common commercial sweet potato cultivars in the US to *M*. *enterolobii*, their Department of Agriculture's germplasm collection contains numerous resistant lines. Sweet potato has over 35 accessions with strong resistance to *M. enterolobii*, making it a desirable crop for resistance breeding (Rutter *et al*.,2025). The achievement of food security is not linked to resistances of one crop but diversity of crops to meet the society`s needs. Resistance breeding in many solanaceous and cucurbit crops is less promising due to minimal or no resistance in the basic gene pool (Swarnapriya, 2021; Dias & Ortiz Rios, 2014). However, studies have shown some progress finding *M. enterolobii* resistance in wild cousins of pepper, tomato, and watermelon. Breeding for *M. enterolobii* resistance across plant species can be time-consuming and costly, but it can improve the prospects for many crops.

2.5.4 Use of biological control agents

In the aspect of biological control of plant parasitic nematodes, the focus is mainly on finding the organisms that can be applied whether to the seeds, growing mediums or furrow that will minimize or suppress nematode to optimum threshold level (Timper, 2014). The use of bio-pesticides is one of the vital tool in the integrated nematodes management including utilizing microorganisms and their metabolites (Migunova & Sasanelli, 2021). Certain group of plants such as tagetes and Brassicaceae are known to produce metabolites which suppress nematode populations (Sikder & Vestergård, 2020). The use of plants high in nematode inhibitory compounds into cropping systems as a health strategy has received a lot of research attention and is also used in practice (Lopes *et al*., 2019).

The Allium species, such as onion, and garlic also shows their potential as natural nematicides due to the presence of sulfur amino-acid precursors (Sikder & Vestergård, 2020). When these compounds are broken down by the enzyme allinase, they produce dimethyl disulfide (DMDS), a volatile organic compound with significant biological activity (Jivishov, 2016; Lancaster & Boland, 2020). The study by Sharma *et al*. (2023) indicated that DMDS exhibits nematicidal properties, effectively killed J2 juveniles of the root-knot nematode, *M. incognita*. Additionally, DMDS has been shown to reduce the formation of egg masses and galling on tomato roots, which are common symptoms of nematode infestation. This suggests that DMDS could be a viable option for managing nematode populations in agricultural fields (Yan *et al*., 2019). With all being said, using purified nematode-inhibitory plant-derived compounds may be an effective nematode management strategy.

2.5.4.1 Bacteria as biological control agents

Nematodes in soil are prone to bacterial and fungal diseases (Sayre & Starr, 2019). This opens up an opportunity of utilizing soil bacteria to control plant-parasitic nematodes (Nyaku *et al*., 2017). Nematophagous bacteria, which are effective natural enemies of nematodes, are widely distributed and have diverse host ranges (Ahmad *et al*., 2021). These bacteria have been found in soil, plant tissues, and the cysts and eggs of nematodes. The bacteria suppress nematodes by producing antibiotics, toxins, and enzymes, while also competing for nutrients and space through parasitism (Antil *et al*., 2023). This interaction enhances systemic resistance in plants, promotes growth by aiding rhizosphere colonization, and increases microbial antagonism (Topalović *et al*., 2020).

The bacteria such as plant growth-Promoting rhizobacteria in the rhizosphere create bioactive compounds that help plants grow and protect them from pathogens (Hassan *et al*., 2019). There is most information on utilizing rhizobacteria to reduce *Meloidogyne spp*. on crops such as tomatoes, with *Bacillus* and *Pseudomonas* being the most widely investigated bacterial genera (Zhou *et al*., 2016). The study by Rani *et al*. (2022) under field conditions, treated bottle gourd seeds with *Bacillus amyloliquefaciens*, *Bacillus megaterium*, *Pseudomonas fluorescens* and *Pseudomonas putida*. The bacteria significantly reduced the root gall index and the number of nematode juveniles in the soil and roots compared to untreated controls. This indicates their potential for controlling root knot nematodes.

2.5.4.2 Fungi as biological control agents

Currently, the use of a fungal bio-control agents (BCAs) is regarded as a fast-evolving natural phenomenon in the field of research, with implications on plant yield and food supply (Savita & Sharma, 2019). Because of their complicated mode of action, BCAs have no negative impact on the environment and do not build resistance in diverse types of pests and pathogens (Ayaz *et al*., 2023). They have been proven as an alternative to the undesired use of chemical pesticides. There are many natural enemies that attack nematodes and are capable of reducing their population to optimal level but the number of organisms that can be utilized for bio-control is limited (Moosavi & Zare, 2020).

The use of Arbuscular Mycorrhizal Fungi (AMF) is a potential management method that has received recent interest by farmers and researches. Arbuscular Mycorrhizal Fungi are symbiotic microbes that create mutualistic interactions with plant roots, supplying nutrients and increasing resistance to a variety of stressors, including nematode infection (Campos, 2020). Several researches have studied the potential of AMF as a bio-control agent for *Meloidogyne* species in medicinal and aromatic plants. The study by Pandey (2017) discovered that *Meloidogyne incognita* and *M. javanica* are globally important for the menthol mint damage, however *M. incognita* occurs more frequently than *M. javanica*. Pandey *et al*. (2019) found that *M. incognita*, a root-knot nematode, multiplies rapidly in the absence of AMF, resulting in decreased plant growth and yield. Also, root-knot nematode infection was significantly reduced when plants were inoculated with three AMF at the same time, as opposed to inoculating them separately. This investigation found that AMF may effectively limit nematode infection in menthol mint, in addition to boosting plant growth and biomass. Similarly, the study by Kedar & Pandey (2020) highlights the complex interactions between the root-knot nematode, *M. incognita*, and AMF. Their findings suggest that

M. incognita can thrive in the absence of AMF, leading to significant reductions in plant growth and yield.

2.6 Mechanisms of action of AM fungi in reducing nematodes densities.

Arbuscular Mycorrhizal Fungi constitute a group that forms advantageous symbiotic connections with the roots of numerous plants (Wani *et al*., 2017). They serve an important role in improving plant health, growth and one of its lesser-known advantages is their capacity to lower nematode populations in the soil (Gough *et al*., 2020). Several mechanisms have been proposed to explain how AMF can reduce nematode infestation in plants (Bagyaraj, 2018). Arbuscular Mycorrhizal Fungi-mediated bio-control involves directs effects on pathogens, such as competition for space and nutrients, or indirect effects through plant-mediated mechanisms (Kaur & Rani, 2022). Arbuscular Mycorrhizal Fungi function as an extension of the root system, establishing a complex network that enhances nutrients absorption, strengthens defenses, and increases overall plant resilience to environmental stresses (Nafady *et al*., 2022).

2.6.1 Competition for nutrients and space

One of the primary mechanisms is competition for resources (Abbasi *et al*., 2025). Microorganisms with similar physiological requirements may compete for nutrients, space, and infection sites in an ecological niche, particularly when resources like carbon are scarce (Vos *et al*., 2014). Arbuscular Mycorrhizal Fungi generate a network of hyphae that extends into the soil, increasing the available surface area for nutrient absorption (Khanday *et al*., 2016). This can outcompete nematodes for critical nutrients like nitrogen and phosphorus, lowering their population and limiting the damage they do to plant roots. Arbuscular Mycorrhizal Fungi and plant parasitic nematodes interactions may involve competition for space due to their same root location (Patel *et al*., 2022). Space constriction can negatively impact plant parasitic nematodes by causing mycorrhizal arbuscules to develop exclusively in the cortex, where migrating nematodes feed (Siddiqui & Aziz, 2024). AMF and sedentary endoparasite nematodes may compete for space if feeding cells reach the cortex (Schouteden *et al*., 2015).

2.6.2 Changed rhizosphere interactions

The effects of Arbuscular Mycorrhizal Fungi (AMF) on plants extend beyond mycorrhiza-induced resistance (Mitra *et al*., 2021). AMF symbiosis alters the composition and level of root exudation, affecting plant parasitic nematodes in the rhizosphere, including hatching, motility, chemotaxis, and host location (Schouteden *et al*., 2015). The AMF modify rhizosphere interactions by producing extracellular enzymes. These enzymes are released by fungi and serve an important function in breaking down organic matter in the soil, releasing nutrients that the plant absorbs (Bao *et al*., 2022). Arbuscular Mycorrhizal Fungi for instance, create enzymes such as phosphatases and proteases, which aid in the release of phosphorus and nitrogen from organic molecules in the soil, making these nutrients more available to plants (Wahab *et al*., 2023). Another major way by which AMF influence rhizosphere interactions is the alteration of root exudates (Shahzad *et al*., 2015). Root exudates are organic chemicals released by plant roots into the soil. They play a significant role in structuring the microbial population in the rhizosphere (Balasubramanian *et al*., 2021). Arbuscular Mycorrhizal Fungi may change the composition and quantity of root exudates, affecting the abundance and activity of other soil microbes (Hugoni *et al*., 2018). The AMF can induce the development of certain root exudates that attract helpful bacteria while suppressing the growth of harmful species, thereby improving plant health (Jain *et al*., 2020). Also, AM fungi can affect the rhizosphere's physical structure by forming hyphal networks (Sylvia, 2019). These networks extend from the fungal mycelium into the soil, generating a huge network of interwoven hyphae that can boost nutrient intake and plant water absorption (Wang *et al*., 2022). The hyphal networks also act as conduits for nutrient exchange between the plant and the fungus, allowing carbon compounds from the plant to be transferred to the fungi in return for nutrients such as phosphorus and nitrogen (Basiru *et al*., 2023). Variety of spores from the rhizosphere of various host species, mainly *Glomus*, *Gigospora*, *Acaulospora*, *Entrophospora* and *Sclerocystis*, are found in AMF listed In Table 2.1 below (Laka *et al*., 2024).

Table 2.1**:** The major category of Arbuscular Mycorrhizal Fungi isolates (Laka *et al*., 2024)

Genus	Example of species
Glomus	*G. fassiculatum, G. mosseae*
Gigaspora	*Gigaspora nigra*
Acaulospora	*A. scrobiculata*
Sclerocystis	*S. clavispora*
Endogone	*E. increseta*

2.6.3 Enhanced plant tolerance

Many studies have been conducted recently to examine the tactics by which Arbuscular Mycorrhizal Fungi (AMF) provide increased tolerance to their host plants (Branco *et al*., 2022). Arbuscular Mycorrhizal Fungi boost plant tolerance by enhancing nutrient uptake (Bahadur *et al*., 2019). These fungi have been proven to improve the uptake of critical nutrients like phosphorus, nitrogen, and micronutrients, which are frequently scarce in soil settings (Schouteden *et al*., 2015). Arbuscular Mycorrhizal fungi aid plant growth and development by enhancing nutrient uptake, even under stressful situations (Garg *et al*., 2017).

Apart from nutrient uptake, AMF improve plant-water connections (Bowles *et al*., 2018). According to several research studies, AMF can improve host plant water uptake efficiency, allowing them to resist drought stress more effectively (Hashem *et al*., 2018; Tang *et al*., 2022). This is achieved by a variety of factors, including increased root surface area, enhanced root hydraulic conductivity, and changes in plant hormone levels (Fernández-Lizarazo & Moreno-Fonseca, 2016). Arbuscular Mycorrhizal Fungi improve plant tolerance to biotic and abiotic stressors, including pathogens, heavy metal toxicity and salt (Diagne *et al*., 2020). This is assumed to be accomplished by a variety of direct and indirect methods, including the activation of plant defense responses, the generation of antifungal chemicals, and heavy metal detoxification (Dowarah *et al*., 2022).

2.6.4 Induced systemic resistance

Systemic resistance is the condition in which a plant's immune system is biased to respond more effectively to pathogen attacks (Chen *et al*., 2021). Important microorganisms such as AMF can contribute to increased resistance (Pieterse *et al*., 2014). The processes by which AMF cause systemic resistance in plants are not fully known, although several major pathways have been found. Arbuscular Mycorrhizal Fungi produce systemic resistance by activating plant defensive responses (Nair *et al*., 2015). These fungi colonizes plants and creates a variety of defense-related chemicals including phytoalexins, pathogenesis-related proteins, and reactive oxygen species (Cameron *et al*., 2013). These chemicals defend the plant from pathogens by reducing their growth and spread.

Arbuscular Mycorrhizal Fungi induced systemic resistance (ISR) also involves the modification of plant hormone signaling pathways (Bedini *et al*., 2018). Arbuscular Mycorrhizal Fungi colonization has been demonstrated to affect the levels of several plant hormones, especially salicylic acid, and ethylene, which are important regulators of plant defense responses (Kamle *et al*., 2020). By regulating these hormone levels, these fungi can improve the plant's ability to defend it against diseases (Dreischhoff *et al*., 2020). The fungus can increase systemic resistance by priming the plant's immune system to respond more quickly and robustly to pathogen attacks (Weng *et al*., 2022). This priming effect involves the activation of certain signaling pathways, allowing the plant to recognize and respond more effectively to pathogen invasion (Müller & Harrison, 2019). This increased preparedness can help the plant establish a faster and more efficient defense against pathogens, decreasing the severity of disease symptoms (Schouteden *et al*., 2015).

2.7 Challenges of bio-control strategies

Bio-control strategy offer a more ecological way for the management of nematodes, but they also face several challenges such as many barriers for the use of biological products, a lack of knowledge of agricultural biological products and lack of global availability (Stirling, 2014; Stenberg *et al*., 2021). As much as this bio-control strategy is eco-friendly and favorable over the chemical strategy, there are several challenges pertaining to this strategy. This include expensive production methods, narrow action spectra, susceptibility to environmental conditions, short shelf life, poor storage stability, legislation registry constraints, and general lack of knowledge are slowing down their adoption (Hamrouni *et al*., 2025).

Root-knot nematodes have a complex life cycle consisting of several stages namely eggs, juveniles, and adults and can reproduce quickly (Subedi *et al*., 2020). Most second stage juveniles are known as the most damaging stage but finding potential biological agents that have ability to reduce or prevent reproduction in all life stages will be very vital tool in achieving optimum nematode population level (Norton & Niblack, 2020). However, because of its complexity, bio-control agents have difficult efficiently targeting all life stages. Many BCAs, such as nematophagous fungus or bacteria, may be extremely specific to individual nematode species (Timper, 2014). This specialization may restrict their efficacy against a wide range of root-knot nematodes, because many agents can be available to control certain type of nematodes species and fail to control other species (Lawal *et al*., 2022).

The environmental conditions such as temperature, moisture, and soil type can all have an impact on bio-control agents' efficacy (Ferreira & Musumeci, 2021). These factors can have an effect on the agents' survival and activity, causing their performance to vary depending on the conditions (Bonaterra *et al*., 2022). Most microbes thrive in warm temperature, some prefer moist areas and certain type of soils; if such agents are found in cold areas, it might affect they potential in controlling nematodes or even die (Fătu *et al*., 2020).

Bio-control agents, such as beneficial microorganisms introduced to control pathogens in agricultural fields, can compete with native soil microbes (Bonaterra *et al*., 2022). This competition may limit the development and effectiveness of both introduced and native microbial populations (Lahlali *et al*., 2022). Because both bio-control agents and native soil bacteria need resources to thrive and reproduce. When native bacteria are added, they may compete with bio-control agents for critical nutrients (Mazzola & Freilich, 2017). This can results in a decline in the population and activity of bio-control organisms. The introduction of bio-control agents has the potential to change resource dynamics in the soil ecosystem (Knudsen & Dandurand, 2014). If introduced agents spread quickly, they may dominate limited resources, leaving little for native bacteria (Young *et al*., 2017). This can disturb established microbial communities and limit functional diversity, which is critical for soil health and resilience (Ambrosini *et al*., 2016).

Bio-control agents often produce metabolites that hinder the growth of other bacteria (Köhl *et al*., 2019). While this is useful for managing pests and pathogens, it can also harm native soil bacteria (El-Saadony *et al*., 2022). The suppression of natural microbial populations can reduce their beneficial activities, such as organic matter decomposition and nutrient mineralization (Bhattacharyya *et al*., 2022). Many soil microorganisms, including mycorrhizal fungus and nitrogen-fixing bacteria, create symbiotic interactions with plants (Dellagi *et al*., 2020). The introduction of bio-control agents may disturb these connections by outcompeting beneficial organisms or modifying the soil environment in ways that are detrimental to these symbionts (Liu-Xu *et al*., 2024). This can have a negative influence on plant health and growth, limiting the efficacy of bio-control agents (Singh *et al*., 2017).

Bio-control techniques often take longer to produce results than chemical pesticides (Stirling, 2018). They often require time to establish themselves in the surroundings. This can entail reproduction and population increase before they can successfully control pest populations (Abd-Elgawad, 2020). The dynamics of ecosystems are complicated, and the introduction of bio-control agents can result in a variety of interactions with other species, including non-targets (Szewc *et al*., 2021). These interactions can impact the effectiveness and speed of insect control (Abd-Elgawad, 2016). Also, many bio-control agents have longer life cycles than chemical pesticides that are effective almost immediately after application (Norton & Niblack, 2020). This means that biological agents may take longer to reach a sufficient number to have an influence on pest populations (Van Zyl & Malan, 2014). Farmers that require immediate management of nematode populations may find this slow action disadvantageous.

Regulatory barriers, such as the requirement for extensive testing and approval processes, might hinder the development and commercialization of bio-control agents (Barratt *et al*., 2021).

Different countries have different regulatory systems for the use of bio-control agents (Lahlali *et al*., 2022). These restrictions are often intended to protect human health, non-target organisms, and the environment (QU *et al*., 2022).The regulatory framework for bio-control agents in South Africa is governed by Act 36 of 1974 (the Plant Protection Act) and related legislation administered by the Department of Agriculture, Land Reform and Rural Development (DALRRD) (Moela, 2024 ; Moeletsi *et al*., 2023). The Act requires that bio-control agents undergo rigorous efficacy, safety and environmental impact assessments before registration and commercial use, ensuring protection of human health, non-target organisms and the environment Van (Rooyen , 2021) . A bio-control agent is often subjected to intensive testing to determine its efficacy, safety, and potential environmental impact before being certified for use (Köhl *et al*., 2019). Laboratory studies, greenhouse trials and field trials are all examples of time and resource-intensive activities. This may delay down the availability of effective products in the market (Lacey *et al*., 2015).

Most farmers are still not familiar with bio-control techniques or unsure of their effectiveness when compared to standard chemical pesticides (Mkenda *et al*., 2020). Educating and training farmers on bio-control strategies can assist in closing the knowledge gap (Kinuthia, 2019). Workshops, field demonstrations, and extension services can all help to highlight biological control's benefits and practical applications (Barratt *et al*., 2018). The expense of developing and implementing biological control measures might be costly, especially for smallholder farmers (Grzywacz *et al*., 2014). Developing biological control measures sometimes necessitates substantial research and development effort (Bottrell & Schoenly, 2018). This comprises the expenses involved with laboratory investigations, field trials, and regulatory approvals (Stevenson *et al*., 2017). Also, some farmers may not have access to innovative biological control technology. This may limit their ability to fulfil effective pest management measures, which could otherwise increase yields and reduce losses (Soul-kifouly *et al*., 2021).

2.8 Identified gaps in the study area

There are more research studies done and excessive information on the impacts of popular *Meloidogyne* s*pp*. such as *Meloidogyne incognita* and *Meloidogyne javanica* on mint cultivars. There is little to no information on the impact of this test nematode on underutilized medicinal and aromatic herbs such as mint. There is no research study that directly addresses the effect of *M. enterolobii* on spearmint. There has been no specific research on resistance status of mint to *Meloidogyne enterolobii* yet. There is a lack of studies specifically addressing Arbuscular Mycorrhizal Fungi and *Meloidogyne* species interactions on mint. The utilization of AMF as a sustainable and effective nematode control strategy in agriculture is available with limited information on its use as a control strategy on root-knot nematodes on crops such as mint.

2.9 References

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CHAPTER 3

RESISTANCE STATUS OF MINT (*MENTHA SPICATA*) TO *MELOIDOGYNE ENTEROLOBII*

3.1. Introduction

Ensuring food security and feeding a growing global population will be a major challenge in coming decades because of aggressive root knot nematodes (Fróna *et al*., 2019). Root-knot nematodes, *Meloidogyne spp*. are economically important plant pathogens that are widely distributed (Eisenback & Triantaphyllou, 2020). They are obligate parasites that infect thousands of different plant species. *Meloidogyne spp*. are pests in major food crops (Azlay *et al*., 2023) with potential to lower yield quantity and quality (Reddy, 2021). Root-knot nematodes pose a substantial danger to global agricultural productivity (Khan *et al*., 2023).

The utilization of resistant cultivars is one of the most crucial environmentally and economically friendly methods to manage or control nematodes (Sivasubramaniam *et al*., 2020). In order to select plants with a degree of resistance to the specified root knot nematodes species, host status and susceptibility to the nematode species are tested (Rusinque *et al*., 2023). Currently there is no information about the yield losses associated with *Meloidogyne enterolobii* in *Mentha spicata* and understanding the interaction among the two is critical for improving production and commercializing the crop.

There is a need to sustainably increase agricultural production in response to increased resource demand in poor areas around the world, such as India and Africa (Khan *et al*., 2023). This could be difficult since the RKN species could limit the increase in production. Hence, the objective of the study is to determine whether *M. enterolobii* will reproduce in *Mentha spicata* and cause reduction of the plant growth.

3.2. Materials and methods

3.2.1. Study area

Two studies were simultaneously conducted at the University of Mpumalanga farm, Mbombela campus (25˚ 26' 11'' S 30˚ 58' 54'' E) under shade and micro plot conditions in 2025 (validated in space) with temperatures varying between 26°C and 33°C and an average annual precipitation of 667 mm (Masereka *et al*., 2018). While the daytime temperatures under the shade net ranged between 20°C and 29°C, nocturnal temperatures varied between 14°C and 18°C depending on the weather.

3.2.2 Cultural practices and experimental design

The two trials were conducted from July to September 2025 in twenty centimeters diameter plastic pots, filled with approximately 2700ml of steam-pasteurized (300°C for 1 hour) sand and loamy soil (3:1 v/v ratio) growing medium. Pots were spaced 15 cm inter-row and 15 cm intra-row under micro plot conditions (Figure 3.1). *Mentha spicata* seeds were planted in 200 cell seedling trays. Two weeks after the emergence, seedlings were transplanted into the pots. Seven days after transplanting, seedlings were separately inoculated with nematode treatments of 0, 25, 50, 125, 250, 625, 1250, and 3125 *Meloidogyne enterolobii* eggs and second-stage juveniles (J2s) per pot and replicated eight times. The pots were arranged in a randomized completely block design (RCBD).

The *M. enterolobii* inoculum was extracted from kenaf (*Hibiscus cannabinus*), a nematode-susceptible greenhouse-grown plant (Pofu *et al*., 2012). Nematode inoculums were injected in 5-centimeter-deep pit around the plant stem diameter. The pots were monitored for pests every other day for the duration of the research trial, when required, recommended pest management was implemented. Plants were irrigated every other day, and manual weeding was done.

Figure 3.1. Host status and AMF- nematodes on mint experiments under a micro plot (a), preparation of pots for two trials (b), host status and AMF- nematodes on mint experiments under shade net (c).

3.2.3 Data collection

Data were recorded 56 days after treatment initiation. Plant height was measured from the base of the stem up to the topmost part of the flag leaf. Shoots were separated from roots, after which the total quantity of branches was counted. The stem diameter was measured five centimetres above the cut end, utilizing a digital Vernier caliper before shoots were oven-dried at fifty-two degrees celsius for seventy-two hours to obtain the dry matter (Pofu *et al*., 2016). Root systems were taken out of pots, shaken in tap water to remove soil debris, then blotted dry with laboratory paper towel to eliminate any remaining moisture, and weighed. Root gall scores were taken following the North Carolina Differential Rating scale (Masenya *et al*., 2023), with 0 indicating no gall, 1 = 1−2 galls, 2 = 3−10 galls, 3 = 11−30 galls, 4 = 31−100 galls and 5 ≥ 100 galls. The roots were then weighed with fresh root mass recorded (Nkosi, 2019).

The maceration and blending process was used to extract nematodes from the complete root system of each plant in a one per cent sodium hypochlorite solution (Pofu *et al*., 2016). The materials were passed through a nested set of sieves of 75 µm, then after passed through 25 µm, with the contents of the 25 µm sieve taken for further nematode extraction utilizing the sugar floatation method (Pofu *et al*., 2016) and then after centrifuged to separate trash from nematodes. Each pot's soil was mixed, and a 250 ml soil subsample were obtained for extracting J2 utilizing centrifugation method (Makhado, 2020). Eggs plus J2 from roots and 250 mL soil subsamples was placed into one hundred millilitre bottles, topped with distilled water to the specified level and stored within a cool chamber at five degrees Celsius (°C) until further processing. Counting of nematodes were done from a five millilitres aliquot at sixty times magnifications using a stereomicroscope from Zeiss Stemi 2000, Model number: Z4SV240Vac (Leca Microsystems) (Nkosi, 2019). Final nematode population densities (Pf) were computed by adding number of eggs and J2 from roots to J2 from soil.

3.2.4 Data analysis

To verify the normality of data distribution, data were subjected to the Shapiro-Wilk test (Shapiro and Wilk, 1965; Ghasemi and Zahediasl, 2012). Nematode variables data was homogenized using a log10(x + 1) transformation prior to ANOVA (Gomez and Gomez, 1984). Data were analyzed using one-way ANOVA, utilizing Statistix 10.0 software. The degrees of freedom and their mean sum of squares (MSS) were separated to estimate the contribution of sources of variation to total treatment variation (TTV) for the variables. The Fisher's least significant difference (LSD) was employed to distinguish between means that were significant at a probability level of 5%. Unless stated otherwise, only variables that were significant at the five percent probability range were presented.

3.3 Results

3.3.1 Plant variables

The treatment under micro plot condition had significant effects on the number of branches, plant heights and chlorophyll content, contributing 57%, 93% and 83% of total treatment variation (TTV), respectively, while under the shade net condition, treatments had significant effect on plant height contributing 80% TTV of respective variable (Table 3.1). Relative to control, number of branches, plant heights and chlorophyll content were significantly reduced by 13-42, 12-69 and 30-93 %, respectively under micro plot conditions and plant height was reduced by 8-41 % under shade conditions (Table 3.2). Under both conditions all *M. enterolobii* inoculum levels significantly reduced plant variables relative to the control (Table 3.2).

Table 3.1: Source of variation for number of branches (NB), plant heights (PH) and chlorophyll content (CC) under two different conditions

		Micro plot Experiment						Shade net Experiment	
		NB		PH		CC		PH	
Source	DF	MS	TTV(%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Replications	7	9.34	23.01	14.35	5.33	77.71	15.40	16.84	17.02
Nematodes	7	23.12	56.98**	249.40	92.64***	418.10	82.87***	78.94	79.81***
Error	49	8.12	20.01	5.47	2.03	8.732	1.73	3.13	3.17
Total	63	40.58	100	269.22	100	504.55	100	98.90	100

** Significant $P \leq 0.05$, *** Highly significant at $P \leq 0.01$. TTV (%) = Total Treatment Variation.

Table 3.2 Relative impact (RI) of the treatments on number of branches (NB), plant heights (PH) and chlorophyll content (CC) under 2 different conditions.

	Micro plot condition						Shade net condition	
Nematodes	NB	RI (%)	Plant height (PH)	RI (%)	Chlorophyll content	RI (%)	Plant height	RI (%)
0	13.25^{a}	0	24.25^{a}	0	22.96^{a}	0	23.11^{a}	0
25	11.50^{ab}	-13	21.31^{b}	-12	16.06^{b}	-30	21.29^{b}	-8
50	9.50^{bc}	-28	17.63^{c}	-27	11.33^{c}	-51	20.50^{b}	-11
125	10.25^{bc}	-23	15.68^{c}	-35	8.26^{d}	-64	19.84^{b}	-14
250	9.88^{bc}	-25	16.28^{c}	-33	6.85^{de}	-70	17.05^{c}	-26
625	9.13^{bc}	-31	11.82^{d}	-51	4.66^{ef}	-80	16.20^{c}	-30
1250	8.88^{bc}	-33	10.63^{d}	-56	2.88^{fg}	-87	17.00^{c}	-26
3125	7.75^{c}	-42	7.40^{e}	-69	1.59^{g}	-93	13.54^{d}	-41

Relative impact (RI %) = [(treatment/control) – 1] × 100. Column means ± standard error followed by the same letter were not different ($P \leq 0.05$) according to Fisher's Least Significant Different test.

3.3.2 Nematodes variables

In both micro plot and shade net conditions, all the nematode variables were highly significant ($P \leq 0.01$), under the micro plot, root galls, J2s in roots, eggs in roots, J2s in soils, eggs in soil, Pf and Rf contributed 99.18%, 99.85%, 99.68%, 99.41%, 95.91%, 99.94% and 99.64% of the TTV, respectively, while the root galls, J2s in roots, eggs in roots, J2s in soils, eggs in soil, Pf, Rf contributed 90.27%, 99.25%, 99.39%, 96.32%, 95.59%, 99.76%, 99.18% of TTV under the shade net conditions, respectively (Table 3.3).

Table 3.3**:** Sources of variation of root galls, second-stage juveniles (J2) in roots & soil, eggs in roots & soil, final population (Pf) and reproductive factor (Rf) of *Meloidogyne enterolobii* on mint under two different conditions.

Source	DF	Root galls		J2 in roots		Eggs in roots		J2 in soil		Eggs in soil		Pf		Rf	
		MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
							Micro plot condition								
Replications	7	0.00211	0.49	0.0126	0.10	0.0210	0.18	0.03254	0.32	0.20921	2.42	0.0049	0.03	0.00483	0.21
Nematodes	7	0.42739	99.18***	12.8301	99.85***	11.6757	99.68***	9.96894	99.41***	8.27623	95.91***	15.3207	99.94***	2.27135	99.64***
Error	49	0.00144	0.33	0.0065	0.05	0.0160	0.14	0.02713	0.27	0.14399	1.67	0.0037	0.03	0.00334	0.15
Total	63	0.43094	100	12.8492	100	11.7127	100	10.0286	100	8.62943	100	15.3293	100	2.279	100
							Shade net condition								
Replications	7	0.00908	3.38	0.05662	0.61	0.03192	0.38	0.15695	2.07	0.16073	2.13	0.0220	0.19	0.01455	0.64
Nematodes	7	0.24250	90.27**	9.17794	99.25***	8.34740	99.39***	7.31096	96.32***	7.21056	95.59***	11.8346	99.76***	2.25145	99.18***
Error	49	0.01707	6.35	0.01244	0.14	0.01934	0.23	0.12198	1.61	0.17213	2.28	0.0059	0.05	0.004	0.18
Total	63	0.26865	100	9.247	100	8.39866	100	7.58989	100	7.54342	100	11.8625	100	2.2701	100

Significant P ≤ 0.05, * Highly significant at P ≤ 0.01. TTV (%) = Total Treatment Variation

Both the root galls and Pf values under micro plot condition increased with increasing nematode inoculum levels (Table 3.4). The Rf values were greater than one in all treatment levels which means that the values were above unity (Table 3.4-3.5). The root galls and Pf values under the shade net had slight increase with increment in the inoculum levels (Table 3.5).

Table 3.4: Impact of the initial nematode level (Pi) on the root galls (RG), second stage juveniles (J2) in roots & soils, eggs in the roots & soils, final nematode population (Pf), as well as the reproductive factor (Rf) of *Meloidogyne enterolobii* on mint under micro plot conditions.

Treatment (Pi)	Root galls	J2 in roots	Eggs in roots	J2 in soil	Eggs in soil	Pf	Rf
25	0.51^{d}	2.74^{ef}	2.62^{e}	2.41^{de}	2.24^{b}	3.16^{f}	1.77^{a}
	(2.25)	(562.50)	(425)	(262.50)	(187.50)	(1437.50)	(57.50)
50	0.59^{c}	2.94^{e}	2.65^{e}	2.46^{de}	2.50^{b}	3.31^{ef}	1.62^{b}
	(2.88)	(875)	(500)	(337.50)	(350)	(2062.50)	(41.25)
125	0.61^{bc}	3.03^{e}	2.73^{de}	2.64^{de}	2.07^{b}	3.37^{e}	1.29^{c}
	(3.13)	(1125)	(550)	(462.50)	(287.50)	(2425)	(19.40)
250	0.64^{b}	3.30^{d}	3.03^{d}	2.79^{cd}	2.59^{b}	3.63^{d}	1.25^{cd}
	(3.38)	(2037.5)	(1125)	(650)	(462.50)	(4275)	(17.10)
625	0.67^{a}	3.60^{c}	3.45^{c}	3.01^{bc}	2.63^{b}	3.93^{c}	1.17^{d}
	(3.75)	(4075)	(2975)	(1087.50)	(487.50)	(8625)	(13.82)
1250	0.69^{a}	3.73^{b}	3.58^{b}	3.14^{b}	2.80^{b}	4.07^{b}	1.01^{e}
	(3.88)	(545487.5)	(3950)	(1600)	(650)	(11688)	(9.35)
3125	0.70^{a}	4.10^{a}	3.92^{a}	3.80^{a}	3.49^{a}	4.49^{a}	1.03^{e}
	(4.00)	(12550)	(8337.5)	(6425)	(3837.50)	(31150)	(9.76)
$LSD_{0.05}$	0.35	672.39	622.61	544.90	781.30	872.76	4.01

[y]Column means ± standard error followed by the same letter were not different ($P \leq 0.05$) according to Fisher's Least Significant Different test.

Table 3.5**:** Impact of the initial nematode level (Pi) on the root galls (RG), second stage juveniles (J2) in roots & soils, eggs in the roots & soils, final nematode population (Pf), as well as the reproductive factor (Rf) of *Meloidogyne enterolobii* on mint under shade net condition.

Treatment (Pi)	Root galls	J2 in roots	Eggs in roots	J2 in soil	Eggs in soil	Pf	Rf
25	0.34^{e} (1.38)	2.48^{ef} (312.50)	2.46^{c} (325)	2.21^{d} (187.50)	1.73^{c} (162.50)	2.99^{e} (987.50)	1.60^{a} (39.50)
50	0.40^{de} (1.63)	2.60^{de} (412.50)	2.49^{c} (312.50)	2.20^{d} (300)	2.35^{c} (262.50)	3.11^{de} (1287.50)	1.42^{b} (25.75)
125	0.46^{cde} (1.88)	2.74^{de} (575)	2.63^{c} (462.50)	2.64^{c} (462.50)	2.43^{c} (300)	3.25^{d} (1800)	1.18^{c} (14.40)
250	0.51^{bcd} (2.25)	2.87^{cd} (775)	2.90^{b} (812.50)	2.67^{bc} (500)	2.65^{b} (462)	3.40^{c} (2550)	1.04^{d} (10.20)
625	0.52^{bc} (2.38)	2.98^{c} (1012.50)	2.94^{b} (900)	2.76^{bc} (612.50)	2.63^{b} (462.50)	3.46^{c} (2987.50)	0.75^{e} (4.78)
1250	0.59^{ab} (2.88)	3.17^{b} (1525)	2.95^{b} (987.50)	2.77^{b} (625)	2.80^{a} (650)	3.56^{b} (3787.50)	0.60^{ef} (3.03)
3125	0.63^{a} (3.25)	3.45^{a} (2962.50)	3.20^{a} (1650)	2.95^{a} (900)	2.86^{a} (737.50)	3.78^{a} (6250)	0.47^{ef} (2)
$LSD_{0.05}$	0.69	391.68	254.25	152.43	150.47	668.55	3.14

[y]Column means ± standard error followed by the same letter were not different ($P \leq 0.05$) according to Fisher's Least Significant Different test.

3.4 Discussion

The degree of nematode resistance in a particular plant exposed to different levels of inoculation is often determined by host status and sensitivity in plant nematodes (Pofu *et al*., 2017). Seinhorst (1967) defined host status as the nematode's capacity to reproduce within a plant, and sensitivity as the host's response to nematodes. The Rf helps determine whether a plant is a host or non-host to a given nematode. Three concepts; susceptible, tolerant, and resistant plants of the plant-parasitic nematode interaction are then defined using the two phenomena; a reproduction factor (Rf) number less than one (Rf<1) indicates a non-host, which means the test nematode was not able to feed and reproduce on the plant being tested, while a value larger than one indicates a host, meaning the nematode successfully created feeding sites and reproduced (Masenya *et al*., 2023). In the current study, the Rf values under micro plot condition were above one in all inoculum levels. This indicated that the nematodes were able to penetrate and reproduce on the test plant in both trials (Table 3.4-3.5). The Rf values being above unity indicated that the test plant is host. While under shade net condition, the Rf values were greater than one at treatment inoculum levels that are ≤ 250 and then declined to below one at inoculum levels ≥ 625. Mabuela *et al*. (2025) found similar results where Rf values of *Meloidogyne enterolobii* showed decline with higher inoculum levels. Study by Oliveira *et al*. (2019) also had the same results where Rf values decreased with increment in inoculation levels. Therefore, it was further concluded that high inoculation levels hinder nematode reproduction by limiting feeding sites for J2 establishment and female development. The number of root knot nematode eggs and J2 increased with nematode inoculum up to level 250 before significantly decreasing above level 625. This demonstrates that the nematodes have reached the point at inoculum 250, whereby the resources are no longer sufficient to sustain the bigger nematode populations due to the increasing population density produced. Taking note that the plant can have Rf value above 1 but be tolerant to the nematodes and with no reduction in yield as compared to controls (Adamu *et al.,*2024). In this study, the yield components of the crop were significantly reduced by inoculated treatments.

Root galls are abnormal, knot-like growths or swellings on plant roots that are typically brought on by a wide range of pests, including root-knot nematodes (Das *et al*., 2024). Normally, the presence of root galls on the tested plants indicates that the penetrating second stage juveniles (J2) had created a feeding site, suggesting that the plant enabled the nematode to reproduce. In the current study, the root gall index (GI) is above 2 in all treatment levels under microplot conditions, indicating that the plant has sustained harm from the *M. enterolobii* (Zhang *et al.*, 2006; Mabuela *et al*., 2025). The establishment of galls on the roots indicates susceptibility (Olmo *et al*., 2020). Abdel-rahman (2020) made a comparable observation, noting that the medicinal herbs exhibited susceptibility with a gall index above 2(2.7-5.0) and nematode's potential to severely destroy medicinal and aromatic plants poses a threat to the most vulnerable plants. The finding showed that micro plot settings provide more favorable environment *for M. enterolobii* than shade net. According to the Seinhorst model, the Rf under micro plot conditions demonstrates that *M. enterolobii* successfully reproduced on mint, caused decline in the plants growth parameters. Carrillo-Fasio *et al*. (2020) reported similar findings for the pepper (*Capsicum annuum*) wherein *M. enterolobii* infested the crop and causing the decline in plant variables.

3.5 Conclusion

The Rf values under both trial conditions were greater than one, indicating that the mint is a host of *M. enterolobii*. *Meloidogyne enterolobii* reduced measured plant variables relative to the control. The host status and sensitivity indicators indicate that mint (*M. spicata*) is susceptible to *M. enterolobii.* Research is required to develop management strategies for the control of *M. enterolobii* in mint production.

3.6 References

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CHAPTER 4

UTILIZATION OF MYCORRHIZA FUNGI AS A CONTROL STRATEGY FOR *MELOIDOGYNE ENTEROLOBII* ON MINT (*MENTHA SPICATA)*

4.1. Introduction

Diverse physical, chemical and agronomic strategies have been recommended to manage root-knot nematodes (Krif *et al*., 2020). Chemical nematicides are the most effective at inhibiting nematode infestations, however the majority of the effective chemicals are banned due to their negative effects on human health and the environment (Oka, 2020). Cultural strategies such as crop rotations, fallow, and cover crops can help minimize disease incidence, however these methods are difficult to practice under particular settings due to limited land area and intensive farming systems (Sikora & Roberts, 2018). Biological management methods are advantageous due to their minimal environmental impact, allowing for continuing land use for economic gain (Mendoza-de Gives, 2022). Both Arbuscular Mycorrhizal Fungi (AMF) and root knot nematodes are soil dwelling organisms (Alvarado-Herrejón *et al*., 2019). Because of the positive impacts of AMF on host plants, AMF are thought to have the ability to act as biological fertilizers and bio-control agents in a variety of biotic and abiotic conditions (Liu *et al*., 2012; Vallejos-Torres *et al*., 2021). In most cases, inoculation with Arbuscular Mycorrhizal Fungi can enhance the tolerance of host plants to nematodes (Schouteden *et al*., 2015). Arbuscular Mycorrhizal Fungi was reported to reduce root-knot nematode levels by 33% on average (Zhang *et al*., 2009; Ahmad *et al*., 2021). However, there have been no studies on the link between AMF and root knot nematodes of mint (*Mentha spicata*). The purpose of this study was to investigate the interactions between AMF and *Meloidogyne enterolobii* in mint as this test plant was found susceptible.

4.2 Materials and methods

4.2.1 Study area

The experiments were carried out as previously outlined (Chapter 3) from July to September 2025 under micro plot and shade net settings.

4.2.2 Experimental design and cultural practices

Mint was found to be susceptible to *M. enterolobii*, Arbuscular Mycorrhiza Fungi (AMF) treatments was applied as a control strategy. Treatments ranging from 0, 10, 20, 30 and 40 g AMF were structured in RCBD, with eight replicates. The fungus employed was RRHIZ-UP (Meson Manufacturing, Randburg, South Africa, https;//www.metson.co.za), and the isolates identified in the product were *Funneliformis coronatum*, *Funneliformis mosseae*, *Claroideoglomus etunicatum*, and *Rhizophagus irregularis* species. Every seedling was inoculated with 3000 *M. enterolobii* eggs + J2 utilizing a twenty-milliliter syringe and inserted in 3-centimeter-deep pits surrounding the center of the stem. Throughout the study, crops were watered with 250 ml of tap water on an alternate day, with watering substituted treatments once a week. Scouting for pests was done every other day.

4.2.3 Data collection and analysis

Data were collected and analyzed in accordance with the methods outlined in Chapter 3 for both plant and nematode variables, as well as for sample extractions.

4.3 Results

4.3.1 Plant variables

The AMF treatment applied under micro plot experiment had a significant effect on the plant height, stem diameter, chlorophyll content, and fresh shoot weight with a total treatment variation (TTV) of 83.04%, 86.52%, 66.49, and 66.07%, respectively. Under shade net, the treatment had a highly significant effect only on the number of branches contributing 81.55% of TTV of the respective variables (Table 4.1).

Table 4.1: Partitioning mean sum of squares of plant height (PH), stem diameter (STD), Chlorophyll content (CC) and fresh shoot weight (FSW) of *Meloidogyne enterolobii* and AMF on mint under two different settings.

		Micro plot								Shade net	
		PH		STD		CC		FSW		STD	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Reps	4	24.94	14.32	6.84	11.34	37.04	23.39	15.19	10.75	2.18	12.89
AMF	4	144.62	83.04***	52.17	86.52***	105.32	66.49***	93.35	66.07**	13.79	81.55***
Error	31	4.59	2.64	1.29	2.14	16.03	10.12	32.75	23.18	0.94	5.56
Total	39	174.15	100	60.30	100	158.39	100	141.29	100	16.91	100

**Significant $P \leq 0.05$, ^{ns} Not significant, ***Highly significant $P \leq 0.01$

Under micro plot study, in comparison to the control, the experiments showed that plant height, stem diameter, chlorophyll content and fresh shoots weight increased from 22% to 79%, 19% to 88%, 118% to 167% and 69% of the respective variables. Also shade net experiment showed that stem diameter in all treatment levels out competed the controls (Table 4.2).

Table 4.2: Relative impact of the treatments on plant height (PH), stem diameter (STD), chlorophyll content (CC) and fresh shoot weight (FSW) on mint under two different settings.

					Micro plot				Shade net	
AMF (g)	PH	RI (%)	STD	RI (%)	CC	RI (%)	FSW	RI (%)	STD	RI (%)
0	13.80^{e}	0	6.86^{d}	0	4.41^{c}	0	13.86^{b}	0	7.42^{c}	0
10	16.79^{d}	22	8.13^{c}	19	9.60^{b}	118	17.33^{b}	25	7.43^{c}	0
20	19.22^{c}	39	9.36^{b}	36	8.05^{bc}	83	18.75^{ab}	35	8.32^{bc}	12
30	22.29^{b}	62	12.18^{a}	78	14.07^{a}	219	19.63^{ab}	42	9.19^{b}	24
40	24.70^{a}	79	12.92^{a}	88	11.78^{ab}	167	23.36a	69	10.58^{a}	43

Relative impact (RI %) = [(treatment/control) – 1] × 100. ^{y}Column means ± standard error followed by the same letter were not different ($P \leq 0.05$) according to Fisher's Least Significant Different test.

4.3.2 Nematodes variables

Under the micro plot experiment where *Meloidogyne enterolobii* and Arbuscular Mycorrhizal Fungi (AMF) interaction was evaluated, all the nematode variables were significant ($P \leq 0.05$). The root gall, J2 in roots, eggs in roots, J2 in soils, eggs in soil and Pf had total treatment variation (TTV) of 93.85%, 94.97%, 94.24%, 92.79%, 88.70% and 95.90%, respectively (Table 4.3). Also under the shade net experiment, all the nematode variables were significant ($P \leq 0.05$) except for eggs in soils. The root galls, J2s in roots, eggs in roots, J2s in soils, Pf, and Rf contributed 81.94%,71.16%, 66.01%,72.76%,45.14% and 61.54% of TTV (Table 4.3). Relative to controls, AMF treatment under micro plot setting showed notable impact on all nematodes variables with relative impact varying from 9 to 37% of respective variables (Table 4.4). While under shade net, the treatment had significant effects on all variables except eggs in soil with relative impact ranging from 6% to 38% of respective variables (Table 4.5).

Table 4.3: Sources of variation of root galls, second-stage juveniles (J2) in soil, J2 in roots, eggs in roots, final population (PF), as well as reproductive factor (RF) of *Meloidogyne enterolobii* on mint under two different conditions.

		Roots galls		J2s in roots		Eggs in roots		J2s in soil		Eggs in soil		Pf	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Micro plot condition													
Replication	4	0.00234	3.81	0.09979	4.25	0.09139	4.46	0.09940	5.16	0.20078	5.64	0.07857	3.81
AMF	4	0.05757	93.85***	2.22829	94.97***	1.93238	94.24***	1.78692	92.79***	3.16042	88.70**	1.97644	95.90***
Error	31	0.00143	2.33	0.01835	0.78	0.02666	1.30	0.03936	2.04	0.20165	5.66	0.00593	0.29
Total	39	0.06134	100	2.34643	100	2.05043	100	1.92568	100	3.56285	100	2.06094	100
Shade net condition													
Replication	4	0.00684	8.06	0.22099	14.74	0.10856	11.30	0.23718	12.84	0.29396	25.50	0.23211	17.78
AMF	4	0.06953	81.94***	1.06684	71.16***	0.63401	66.01**	1.34342	72.76***	0.52041	45.14ns	0.80356	61.54**
Error	31	0.00848	10.0	0.21134	14.10	0.21786	22.68	0.26589	14.40	0.33848	29.36	0.27003	20.68
Total	39	0.08485	100	1.49917	100	0.96043	100	1.84649	100	1.15285	100	1.3057	100

Significant P ≤ 0.05,* Highly significant P ≤ 0.05 , ns Not significant P ≥0.05

The roots galls and Pf values under both environmental conditions showed decline with increase in AMF concentration. Under the micro plot setting, the Rf value was one on the plants that were inoculated with 3000 eggs+J2s of *M. enterolobii* and 0g of AMF or control. The Rf decreased with increasing AMF concentration (Table 4.4). Rf values were below one in all treatment levels under shade net experiment (Table 4.5).

Table 4.4. The effect of Vesicular Arbuscular Mycorrhiza (AMF) on root galls, second-stage juveniles (J2s) in roots & soil, eggs in root & soil and final nematode population density (Pf) of mint under micro plot conditions.

AMF	Root Galls	RI (%)	J2 in Roots	RI (%)	Eggs in Roots	RI (%)	J2 in soil	RI (%)	Eggs in soil	RI (%)	Pf	RI (%)
0	0.69[a]	0	4.08[a]	0	3.89[a]	0	3.79[a]	0	3.66[a]	0	4.50[a]	0
10	0.63[b]	-9	3.37[b]	-17	3.11[b]	-20	2.86[b]	-25	2.53[b]	-31	3.69[b]	-18
20	0.60[b]	-13	2.99[c]	-27	2.94[bc]	-24	2.77[b]	-27	1.99[b]	-46	3.44[c]	-24
30	0.51[c]	-26	2.80[c]	-31	2.68[c]	-31	2.74[b]	-28	2.36[b]	-36	3.31[c]	-26
40	0.48[c]	-30	2.82[c]	-31	2.69[c]	-31	2.59[b]	-32	2.32[b]	-37	3.27[c]	-27

Relative impact (RI %) = [(treatment/control) – 1] × 100. [y]Column means ± standard error followed by the same letter were not different ($P \leq 0.05$) according to Fisher's Least Significant Different test.

Table 4.5: The effect of AMF on root galls, J2s in soil & roots, eggs in root and final nematode population (Pf) density of mint under shade net conditions.

AMF	Root Galls	RI (%)	J2 in Roots	RI (%)	Eggs in Roots	RI (%)	J2 in soil	RI (%)	Pf	RI (%)
0	0.64[a]	0	3.42[a]	0	3.11[a]	0	2.77[a]	0	3.70[a]	0
10	0.54[b]	-16	3.01[b]	-12	2.74[b]	-12	2.55[b]	-8	3.38[b]	-9
20	0.52[b]	-19	2.84[c]	-17	2.62[bc]	-16	2.60[b]	-6	3.23[c]	-13
30	0.45[c]	-30	2.64[c]	-23	2.63[bc]	-15	2.56[b]	-8	3.19[c]	-14
40	0.43[c]	-33	2.45[c]	-28	2.33[c]	-25	1.71[c]	-38	2.82[c]	-24

Relative impact (RI %) = [(treatment/control) – 1] × 100. [y]Column means ± standard error followed by the same letter were not different ($P \leq 0.05$) according to Fisher's Least Significant Different test.

4.4. Discussion

Root-knot nematodes can significantly reduce plant development and crop yield in a variety of crops (Singh *et al*., 2019). Despite the fact that the mint cultivar employed in the current trials was susceptible to *Meloidogyne enterolobii* infestation, plants treated just with nematodes showed substantially less development. Reduced plant development caused by nematode infestation is a typical observation. In the current experiments, mint seedlings were inoculated with 3000 eggs + J2s of *M. enterolobii* for 5 weeks, and the nematodes were expected to cause damage to plants with prolonged treatment as evidenced by the high gall numbers on the roots of *M. enterolobii* inoculated control plants. Mycorrhizal Fungi as natural root symbionts facilitate the uptake of important inorganic nutrients for host plants resulting in increased chlorophyll content and enhanced growth in mycorrhizal plants. In both infected and non-AMF inoculated plants, chlorophyll content and growth were dramatically reduced. AMF significantly increased plant height, stem diameter, chlorophyll content, and fresh shoot of mint plants and the impact became prevalent as treatment levels increased, indicating that AMF has the potential to impose better tolerance to root-knot nematode infection on mint.

In the analysis of different nematode variable responses of mint plants growing in the presence of *Meloidogyne enterolobii* to AMF inoculation, the variables decreased compared to plants infected with nematodes only. Similarly, the study by Bernardo *et al*. (2021), showed that there was a reduction in the nematodes variables in pepper plant growing in the presence of *N. aberrans* to AMF. The presence of root galls on examined plants shows that penetrating second stage juveniles (J2) developed a feeding site, implying that the plant allowed the nematode to reproduce (Jones & Goto, 2011; Vilela *et al*., 2019). In this study, the root galls on the roots suggest susceptibility.

Nematodes - Mycorrhizal plants had considerably fewer root galls compared to controls. Detrey *et al*. (2022) reported that tomato plants exhibited a reduced

Number of galls when inoculated with Arbuscular Mycorrhizal Fungi (AMF). Similarly, a study by Schouteden *et al*. (2015) reported enhanced resistance to root knot nematodes *M. javanica* and *M. incognita.* Also, in contrast to controls J2s in roots and soil, eggs in roots and soil and final population (Pf) were less. The significantly reduced root galling and nematode proliferation show that AMF had a suppressive impact on *M. enterolobii* in *Mentha spicata*. Our findings support the involvement of processes in the AMF-nematode interaction. Keshari *et al*. (2024) highlighted the potential processes by which AMF may affect nematodes, including changes in root exudates, decreased penetration by *Meloidogyne spp.* on AMF, and competition for photosynthesis and space.

4.5. Conclusion

The *M. enterolobii* poses negative impact on various crops, they limit plant growth and reduces yields. The application of AMF has showed the ability to mitigate the adverse effects of nematode infection on plant growth. Mycorrhizal fungus significantly reduced root-knot nematode infestation, improving mint (*Mentha spicata*) herb growth. Our findings support that Arbuscular Mycorrhizal Fungi as potential bio-control have ability to control *Meloidogyne enterolobii* in mint.

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CHAPTER 5

SUMMARY, SIGNIFICANCE OF FINDINGS, FUTURE RESEARCH, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

This study evaluated resistance status of mint (*Mentha spicata*) to *Meloidogyne enterolobii* in two different environmental settings (micro plot and shade net). The objectives of this study were to determine host status and host sensitivity of *Mentha spicata* to *Meloidogyne enterolobii* and the evaluation of whether Arbuscular Mycorrhizal Fungi (AMF) will reduce *M. enterolobii* in the event that test plant is susceptible .The results indicated that micro plot offered a more favorable environment for nematode proliferation compared to shade net setting. The reproductive factor (Rf) under micro plot setting was above unity in all treatment levels and plant variables namely, number of branches, plant height and chlorophyll content were negatively affected which indicated that test plant is susceptible. The Rf values under shade net conditions were above unity in the lower treatment levels and declined to below one with increasing treatment levels which can be caused by the nematodes population whereby they reach a point where they compete for space and feeding site which led to decline reproductive factor values. The only plant variable affected was plant height. In micro plot experiment, the galling index was greater than 2 in all treatment levels indicating that *M. enterolobii* was able to penetrate the roots of plant, find a feeding site and reproduced. The research project further investigated the interaction between AMF and root knot nematodes in mint crops. In this study, the Rf was above unity under micro plot condition only on non-Mycorrhizal controls. In all AMF-treated experiments, the Rf values were below unity and the values declined with increasing AMF treatment levels, indicating that AMF has the potential to impose better tolerance to root-knot nematode infection on mint.

5.2. Significance of the findings

The mint species susceptibility to plant-parasitic nematodes, *Meloidogyne enterolobii* has not been well studied. In the current study*, Mentha spicata* was found to be susceptible to *M. enterolobii*. Therefore, AMF was used as a bio-control strategy to manage nematodes population. The AMF treatment application had a significant impact on plant variables such as plant height, stem diameter, fresh shoot weight and chlorophyll content. This indicates that Arbuscular Mycorrhizal Fungi can be used as bio-control strategy to manage root knot nematodes infestation in crops. Root knot nematodes cause substantial yield losses and increase production costs. Therefore utilizing AMF as a bio-control agent, farmers can reduce nematode damage, enhance crop growth and improve mint quality leading to higher yields and economic returns. The strategy also supports sustainable agriculture by providing an environmentally friendly alternative to chemical nematicides, which can reduce input costs and minimize ecological impact. Adopting AMF treatments can therefore improve profitability for mint producers and contribute to sustainable pest management practices in the broader agricultural industry.

5.3. Future research

In this study, mint was found to be a host to *M. enterolobii* under different environmental settings. Mycorrhiza Fungi (AMF) was used as a mitigation strategy to reduce nematodes infection. AMF showed potential as bio-control strategy to manage nematode populations to optimum level. The findings of this study pave the way for further investigation into the mechanisms by which Mycorrhizal Fungi interact with nematodes and mint crops. Understanding these interactions at the molecular level could help to design suitable management strategies and identify certain Mycorrhizal strains that are most effective against root-knot nematodes.

5.4. Conclusion and recommendations

Mentha spicata is susceptible to *Meloidogyne enterolobii* in both environments. *M. enterolobii*, on the other hand, thrives better under micro plot conditions rather than under shade nets. The study's findings on mint nematode susceptibility and the utility of Mycorrhizal Fungi as a management technique are important for developing sustainable agriculture, improving crop resistance, and increasing farmer economic viability. Integrating these insights into practical applications can result in better agricultural practices that benefit both producers and consumers while promoting environmental sustainability. Also the farmers should adopt shade net conditions for mint cultivation because *M. enterolobii* thrives better in micro plot settings this may enhance the production.

APPENDICES

Appendix 3.1: Analysis of variance for number of branches of mint under shade net condition

Source	DF	SS	MS	F	P
Replication	7	59.250	8.4643		
AMF	7	98.750	14.1071	1.07	0.3985
Error	49	98.750	13.2194		
Total	63	805.750			

Appendix 3.2: Analysis of variance for stem diameter of mint under micro plot condition

Source	DF	SS	MS	F	P
Replication	7	4.7504	0.70532		
AMF	7	8.4006	0.90051	1.60	0.3120
Error	49	29.1143	0.71320		
Total	63	36.5207			

Appendix 3.3: Analysis of variance for J2 in roots of mint under micro plot condition

Source	DF	SS	MS	F	P
Replication	7	0.0880	0.0126		
Nematodes	7	89.8109	12.8301	1981.85	0.0000
Error	49	0.3172	0.0065		
Total	63	90.2161			

Appendix 3.4: Analysis of variance for eggs in roots of mint under micro plot condition

Source	DF	SS	MS	F	P
Replication	7	0.1472	0.0210		
Nematodes	7	81.7301	11.6757	730.11	0.0000
Error	49	0.7836	0.0160		
Total	63	82.6609			

Appendix 3.5: Analysis of variance for J2s in soil of mint under micro plot condition

Source	DF	SS	MS	F	P
Replication	7	0.2278	0.03254		
Nematodes	7	69.7826	9.96894	367.43	0.0003
Error	49	1.3294	0.02713		
Total	63	71.3399			

Appendix 3.6: Analysis of variance for eggs in soil of mint under micro plot condition

Source	DF	SS	MS	F	P
Replication	7	1.4645	0.20921		
Nematodes	7	57.9336	8.27623	57.48	0.0000
Error	49	7.0555	0.14399		
Total	63	66.4536			

Appendix 3.7: Analysis of variance pf of *Meloidogyne enterelobii* under micro plot condition

Source	DF	SS	MS	F	P
Replication	7	0.035	0.0049		
Nematodes	7	107.245	15.3207	4148.57	0.0000
Error	49	0.181	0.0037		
Total	63	107.461			

Appendix 3.8: Analysis of variance for reproductive factor of *Meloidogyne enterelobii* under micro plot condition

Source	DF	SS	MS	F	P
Replication	7	0.0338	0.00483		
Nematodes	7	15.8995	2.27135	679.75	0.0000
Error	49	0.1637	0.00334		
Total	63	16.0970			

Appendix 3.9: Analysis of variance for root galls of *Meloidogyne enterelobii* under micro plot condition

Source	DF	SS	MS	F	P
Replication	7	0.01477	0.00211		
Nematodes	7	2.99176	0.42739	297.35	0.0000
Error	49	0.07043	0.00144		
Total	63	3.07696			

Appendix 4.1: Analysis of variance for stem diameter of mint shade net condition

Source	DF	SS	MS	F	P
Replication	4	8.7321	2.1830		
AMF	4	55.1648	13.7912	14.60	0.0000
Error	31	29.2800	0.9445		
Total	39	93.1769			

Appendix 4.2: Analysis of variance for number of branches of mint under micro plot condition

Source	DF	SS	MS	F	P
Replication	4	33.600	8.40000		
AMF	4	37.055	9.26364	1.74	0.1670
Error	31	165.320	5.33292		
Total	39	235.975			

Appendix 4.3: Analysis of variance for dry shoot weight of mint under micro plot condition

Source	DF	SS	MS	F	P
Replication	4	43.702	10.9254		
AMF	4	78.790	19.6976	1.18	0.3368
Error	31	515.317	16.6231		
Total	39	637.809			

Appendix 4.4: Analysis of variance for root mass of mint under micro plot condition

Source	DF	SS	MS	F	P
Replication	4	6.270	1.56744		
AMF	4	5.891	1.47274	0.41	0.7972
Error	31	110.273	3.55719		
Total	39	122.433			

Appendix 4.5: Analysis of variance for fresh shoot weight of mint under micro plot condition

Source	DF	SS	MS	F	P
Replication	4	60.75	15.1877		
AMF	4	373.40	93.3496	2.85	0.0403
Error	31	1015.22	32.7490		
Total	39	1449.37			

Appendix 4.6: Analysis of variance for plant height of mint under micro plot condition

Source	DF	SS	MS	F	P
Replication	4	99.753	24.938		
AMF	4	578.486	144.621	31.52	0.0000
Error	31	142.237	4.588		
Total	39	820.476			

Appendix 4.7: Analysis of variance for stem diameter of mint under micro plot condition

Source	DF	SS	MS	F	P
Replication	4	27.372	6.8430		
AMF	4	208.682	52.1706	40.40	0.0000
Error	31	40.034	1.2914		
Total	39	276.088			

Appendix 4.8: Analysis of variance for chlorophyll content of mint under micro plot condition

Source	DF	SS	MS	F	P
Replication	4	148.16	37.040		
AMF	4	421.27	105.317	6.57	0.0006
Error	31	496.85	16.028		
Total	39	1066.28			