

**HOST STATUS AND HOST SENSITIVITY OF CANCER BUSH (*SUTHERLANDIA
FRUTESCENS*) TO *MELOIDOGYNE ENTEROLOBII***

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**A dissertation submitted in fulfilment of the requirements for the Master of Science Degree
in Agriculture**

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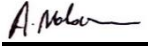
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**UNIVERSITY OF
MPUMALANGA**

DECLARATION

I, the undersigned, declare that this research project report titled '**Host Status and Host Sensitivity of Cancer bush (*Sutherlandia frutescens*) to *Meloidogyne enterolobii***' submitted to the University of Mpumalanga for the degree of Master of Science in Agriculture in the Faculty of Agriculture and Natural Sciences, School of Agricultural Sciences, and the work contained herein is my original work with exemption to the citations and that this work has not been submitted to any other University in partial or entirely for the award of any degree.

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DEDICATION

To my late mom, Mmamotsumi Violet Mabuela and grandfather, Jerry Sello Mabuela.

ACKNOWLEDGEMENT

I would like to express my heartfelt gratitude to the Almighty God for making this journey possible and for granting me the strength to achieve this significant milestone.

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LIST OF ABBREVIATIONS

AIDS –Acquired Immunodeficiency Syndrome

ANOVA– Analysis of variance

DF –Degree of Freedom

EPPO– European and Mediterranean Plant Protection Organization

GI –Gall Index

HIV– Human Immuno Virus

IPM –Integrated Pest Management

MS –Mean Squares

NPK –Nitrogen, Phosphorus, and Potassium

PF– Final Population

PPN– Plant Parasitic Nematodes

RCBD– Randomized Complete Block Design

RF– Reproductive Factor

RKN– Root Knot Nematodes

SS –Sum of Squares

SSA –Sub-Saharan African

TTV –Total Treatment Variable

USA –Unites States of America

USD –United States Dollar

ABSTRACT

Cancer bush (*Sutherlandia frutescens*) is an indigenous medicinal plant with significant bioactive chemicals that stimulate the human immune system. Its demand is rising in South Africa and globally due to its medicinal properties and the growing interest in plant-based remedies. However, pests such as nematodes are threatening its production. The pest challenges have been exacerbated by climate change, that has led to the emergence of new host-pest relationships. *Meloidogyne javanica* is the first root-knot nematode to be detected on *S. frutescens*, and there is still limited information on other potential nematode host of cancer bush with the potential to reproduce and affect its growth and yield. Therefore, the objectives of the study were to determine whether *Meloidogyne enterolobii* root-knot nematode will reproduce on *S. frutescens* roots and affect plant growth during summer and winter seasons. To achieve this objective, *S. frutescens* seedlings were subjected to 0, 25, 50, 125, 250, 625, 1250, and 3125 *M. enterolobii* eggs and second-stage juveniles (J2) under microplot and shade net conditions in two different seasons, 2023 (summer) and 2024 (winter). At 56 days post-inoculation, plant and nematode variables were measured, and the reproductive factor (RF) was calculated. The RF during summer and winter seasons under microplot experiments had similar trends, greater than 1 at lower nematode level, and decreased as it approached the equilibrium level with every increment of *M. enterolobii* inoculum level. While the RF under shade net was statistically the same for all nematode levels, the gall index (GI) was greater than 2, and the plant growth variables were not affected by the treatments. The nematode and plant variable responses indicated that *S. frutescens* was tolerant to *M. enterolobii*, irrespective of season and growing conditions. Microplot conditions was found to be a favorable environment for *M. enterolobii* infestation, and shade net has shown to be a less favorable environment for *M. enterolobii* in both seasons.

Keywords: Gall index, plant parasitic nematodes, root-knot nematodes, reproductive factor,

RESEARCH OUTPUT

1. **Mabuela, A., Masenya, T.A., Dube, Z.P., Maila, M.Y. and Zulu, N.F. 2024.** Host Response of Cancer Bush (*Sutherlandia frutescens*) to *Meloidogyne Enterolobii*. Oral Presentation, *One health, Bucharest, Romania 2024*.

CHAPTER ONE

GENERAL INTRODUCTION

1.1. Background

Cancer bush [*Sutherlandia frutescens* (L.R.) Br.] is an indigenous medical plant with a wide diversity, also found in places like Namibia, Botswana, and Zimbabwe (Masenya *et al.*, 2020). The leguminous plant contains many essential bioactive chemicals with clinically verified pharmacological activities, such as cancer inhibitors and pharmacological uses in stimulating immune system balance in Human Immune Virus (HIV) patients (Shaik *et al.*, 2011). Globally, large-scale medical cultivation is being adapted with current modern technologies and techniques to help meet the need for plant-based medicinal products and ingredients for food (Makgato *et al.*, 2020). South Africa's need for indigenous medicinal plants is projected to rise above 20 000 tonnes per year, and the international community's desire for alternative medical items rises as the population continues to grow (Noorhosseini *et al.*, 2017; Asong *et al.*, 2019; Nsibanyoni *et al.*, 2023).

Due to climate variability, new pests, such as nematodes with reduced lifecycles, have emerged, raising concerns for pest management strategies (Nkosi, 2019). Plant-parasitic nematodes (PPNs) are classified as agriculture's most damaging pests (Jones *et al.*, 2013, Nkosi, 2019), causing a drastic reduction in yield. *Meloidogyne enterolobii* has a 15-day life cycle (Collett, 2021), is becoming an increasingly major threat to most agricultural crops across the globe, with limited solutions for managing nematodes. Currently, despite the existence of Mi resistance genes, *M. enterolobii* is among the most destructive and dominant root-knot nematodes (RKN) species (Silva *et al.*, 2017). In South Africa, the nematode has been detected in guava (*Psidium guajava*) and potato (*Solanum tuberosum*), and it has proven to be destructive in both crops (De Waele and Elsen,

2007; Collett *et al.*, 2021). *Meloidogyne javanica* is the first RKN to be detected on *Sutherlandia frutescens*, and it is said to reproduce and affect the growth and yield of *S. frutescens* (Raselabe, 2017). There is a need for the evaluation of more PPNs, such as *M. enterolobii* in the production of cancer bush, to evaluate whether the RKN species will reproduce and affect the growth of the plant. This will allow for the use of the crop as an alternative strategy for the management of the selected RKN species in rotational systems or to decide what management strategies can be deployed to control the selected RKN species in the test crop (Talwana *et al.*, 2016).

The malpractices in the use of synthetic fumigants, which were formerly widely utilized to control *Meloidogyne* species, have led to their withdrawal from agricultural-chemical market due to their harmful effect on the environment (Mashela *et al.*, 2015; Mashela *et al.*, 2017; Nkosi, 2019). There has been extensive research and development of alternative methods to reduce RKN populations (Mashela *et al.*, 2011; Nkosi, 2019). In recent years, nematode-resistant cultivars have emerged as a viable alternative strategy and the most preferred method for managing RKN populations (Mashela *et al.*, 2011; Nkosi, 2019). The use of resistant varieties is the most reliable method for controlling RKN populations. To select cultivars with a degree of tolerance to the selected RKN species, tests for host status and host sensitivity to the species of nematodes are utilized (Pofu *et al.*, 2017). Several nematode levels, as well as the nematode's reproductive factor (RF) and the quantity of plant damage caused by the nematode, are assessed in nematode resistance trials (Pofu *et al.*, 2017; Nkosi, 2019). Tolerance, susceptibility, and resistance in each host are determined using the RF, which is calculated as a ratio of the final nematode population (PF) in relation to the initial nematode population (Pi) (Nkosi, 2019). The evaluation of whether plants are resistant to nematodes in agricultural research, development and breeding is necessary to determine which nematode species and/or races are present in each plant.

1.2. Problem statement

Meloidogyne enterolobii is a PPN that was recently reported in South African soils to be particularly aggressive in different agricultural systems (Collett *et al.*, 2021). Root-knot nematodes like *M. enterolobii* are particularly problematic in subtropical and tropical areas because of their capacity to bypass the defenses of a diversity of plant hosts like in banana and tomato (Silva *et al.*, 2017). *Meloidogyne enterolobii* is the pathogen that emerges in various parts of the world and causes a drastic reduction in yield linked with the development of a significant number of root galls on the host plant, causing 65% of the losses alone (Castagnone-Sereno, 2012). *Sutherlandia frutescens* is said to be a host to other *Meloidogyne* species that are thermophilic like *M. enterolobii*, such as *M. javanica* and *M. incognita* (Rashidifard *et al.*, 2019; Masenya, 2022). The elimination of hazardous nematicides that increased global warming, harmed human health, and non-target species prompted the development of viable alternatives to restore lost crop yields (Makhado, 2020). Mashela *et al.* (2016:2017) postulated that the available alternatives such as organic amendments are met with numerous challenges in terms of efficacy maintenance over time. Currently, there is no documentation on the prevalence of *M. enterolobii* in *S. frutescens* populations or its effects on the plant's development and growth. This lack of knowledge represents a significant gap in the understanding of the interactions between this plant and RKNs. To improve the production of *S. frutescens* for both agricultural and medicinal uses and to inform appropriate management practices, the evaluation of the host status and host sensitivity can help categorize the inclusion or exclusion of *S. frutescens* to rotational crop systems with an attempt to manage RKNs impact.

1.3. Significance of the study

Sutherlandia frutescens is presently in high demand due to its pharmacological value, which prompted the intensive destructive harvesting with limited conservation measures, which might lead to its extinction (Makgato *et al.*, 2020). The need to safeguard indigenous medicinal plants is critical to improving the economy of the country by meeting the increasing demand for medicinal products (Masenya, 2022). *Meloidogyne* species are the most difficult and laborious pests to control in crop production systems worldwide (Mashela *et al.*, 2017). Many species of thermophilic RKN (*Meloidogyne*) have been found in South African samples of *S. frutescens* (Raselabe, 2017). The control of nematode population densities would be necessary for sustained and increased production of *S. frutescens* to constantly supply medicinal products to meet the need of the increasing population (Masenya *et al.*, 2020). The desire to find less harmful and environmentally acceptable alternatives to commercial nematicides opened the door for substitutes including the use of nematode-resistant varieties (Khosa *et al.*, 2020). The goal to ensure sustained production to safeguard adequate availability of medicinal products and increase conservation measures of *S. frutescens* are linked to management of RKN species, which drastically affects worldwide cultivation of crops.

1.4. Purpose of the study

1.4.1. Aim

This research was conducted to investigate the host-status and host sensitivity of *S. frutescens* to *Meloidogyne enterolobii*.

1.4.2. Objectives

- i. To determine whether *M. enterolobii* will reproduce on *S. frutescens* and reduce plant growth under microplot and shade net conditions in summer.

- ii. To determine whether *M. enterolobii* will reproduce on *S. frutescens* and reduce plant growth under microplot and shade net conditions in winter.

1.4.3. Hypothesis

- i. *Meloidogyne enterolobii* will reproduce on *S. frutescens* and cause a reduction in the plant's growth under microplot and shade net conditions in summer.
- ii. *Meloidogyne enterolobii* will reproduce on *S. frutescens* and cause a reduction in the plant's growth under microplot and shade net conditions in winter

1.4.4. Research questions

- i. Will *M. enterolobii* reproduce on *S. frutescens* and cause a reduction in the plant's growth in summer?
- ii. Will *M. enterolobii* reproduce on *S. frutescens* and cause a reduction in the plant's growth winter?

1.5. Reliability, objectivity and validity

Statistical analysis offers several data reliability assessments (Berenson and Levine, 1996). Data reliability was established using statistical analysis with a 5% probability. Validity is the degree to which the instrument measures what is meant to be measured (Leedy and Ormrod, 1980). Objectivity was attained by ensuring that the findings were discussed using empirical evidence.

1.6. Bias

To reduce bias, the number of replications and randomization were increased to control the experimental error and increase precision and accuracy (Johnson, 2006)

1.7. Structure of the dissertation

First, the research problem is described in Chapter 1, and then, in Chapter 2, the literature review addressing the research problem is discussed. Achievement of objective 1 is discussed in Chapter 3. Objective 2 was discussed in chapter 4 then a summary in chapter 5. Following approval by the University of Mpumalanga senate, the Harvard referencing style was utilized for both in-text citations and reference lists.

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CHAPTER TWO

LITERATURE REVIEW

2.1. Introduction

Sutherlandia frutescens, commonly known as cancer bush, is a perennial legume indigenous to the southern regions of Africa (Raselabe, 2017). This plant holds significant therapeutic value due to its pharmacological and ethnomedicinal properties (Masenya, 2022). It is one of the most significant botanical specimens and member of the Fabaceae family, as stated by Zonyane *et al.* (2020). The utilization of this remedy is prevalent in the field of traditional medicine for the treatment of a diverse range of ailments, including but not limited to immunodeficiency virus infection/acquired immune deficiency syndrome, tuberculosis, cancer, diabetes, and asthma (Aboyade *et al.*, 2014; Gouws *et al.*, 2021). *Meloidogyne* species have been found in several crops and plants; however, the current body of scientific literature lacks substantial empirical evidence or published studies regarding the precise relationship between cancer bush and nematodes, including any potential benefits or detriments.

Meloidogyne species are regarded as the most significant plant-parasitic nematodes (PPNs) in the world and contribute to 5% of global crop losses (Walia and Bajaj, 2017; Khan *et al.*, 2022). *Meloidogyne enterolobii* is among the top 4 destructive species within the *Meloidogyne* genera. Its wide range of hosts makes it important to manage. In recent years, there has been significant global interest in *M. enterolobii*, a species of pathogenic root-knot nematode, which has garnered widespread attention and has been extensively documented (Collet *et al.*, 2021). This nematode has been reported worldwide and, in South Africa, has been first discovered in Mbombela, infesting a guava (*Psidium guajava* L.) tree (Willer 1997; Rashidifard, 2019). It has been reported to be a host of many crops, and only a few crops, such as garlic (*Allium sativum*), cabbage

(*Brassica oleracea*) and peanuts (*Arachis hypogaea*), have been reported as poor or non-hosts. *Meloidogyne enterolobii*, commonly known as the guava root-knot nematode, is a notorious and economically significant plant-parasitic nematode (Rashidifard, 2019).

2.2. History and importance of cancer bush (*Sutherlandia frutescens*)

Cancer bush is a medicinal plant native to Southern Africa, and it is classified as a legume crop. Its numerous traditional medicinal uses make it one of the most popular plants for treating illness in the Western Cape of South Africa (Zonyane *et al.*, 2020; Buthelezi *et al.*, 2022). The Khoi San and the Nama people are believed to have been the first to make use of cancer bush for medicinal purposes. Hartnett *et al.* (2005); Van Wyk and Albrecht (2008), and Raselabe (2017) reports how indigenous groups have traditionally used *S. frutescens* to treat a variety of medical issues, including cancer, HIV/AIDS, diabetes, stress, anxiety, inflammation, pain, and wounds. Based on its historical use, additional study of *S. frutescens*'s benefits may shed light on the ways it aids with these health problems and the need to preserve it.

The delicate little shrub, *S. frutescens* can reach a height of half a meter to one meter, and its blooming stems can be either flat or upright (Van Wyk *et al.*, 2008; Sishuba, 2022). The approximately 4-10 mm long leaves of this plant are pinnate. This medicinal plant has flowers with orange-red petals (Figure 2.1(a)), which grow every year from September to January (spring to summer) in the Southern Hemisphere, are 35 mm long (Sishuba, 2022). According to both Albrecht *et al.* (2012) and Sishuba (2022), the plant is known for its bitter flavor. *Sutherlandia frutescens* occurs naturally throughout the dry parts of Southern Africa, up the west coast as far north as Namibia and into Botswana, in the Western Cape region, and in the Western Karoo as far as the Eastern Cape Province as shown in Figure 2.1.(b) (South African National Biodiversity Institute, 2018; Korth, 2021; Sishuba, 2021). The medicinal plant has wide distribution found in the

Mpumalanga, Limpopo, KwaZulu-Natal provinces, Lesotho and Botswana, and it shows remarkable variation within its distribution. Among the most diverse plant communities on earth, Sutherlandia can be found in the Fynbos Biome. The fruit bearing Sutherlandia is a member of the Fabales order and the Magnoliopsida class (Aboyade *et al.*, 2014; Masenya 2021).

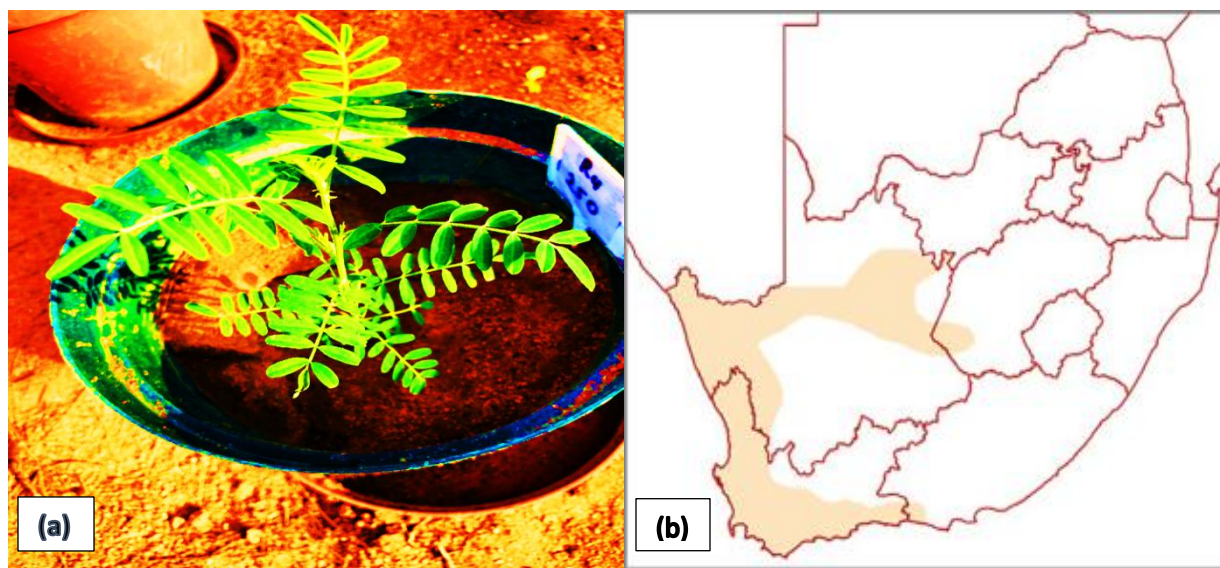


Figure 2.1. (a) A matured plant of *Sutherlandia frutescens* (b) The geographical distribution of *Sutherlandia frutescens* in South Africa indicated by the shaded region (SANBI.org).

Due to the overexploitation of medicinal natural populations and the loss of their habitats, the South African government has suggested that conservation efforts be taken for a number of medicinal plants, including the cancer bush, which is one of the many species that are threatened with extinction. (Raimondo *et al.*, 2009; SANBI, 2010-2012; Mkhwanazi *et.al.*, 2024). Research by Raghu *et al.* (2018) shows that just 10% of medicinal herbs are grown under cultivation, suggesting that the majority of these plants are sourced from the wild. In addition to providing a potential alternative to an unlimited supply, cultivating these species, particularly the ones that have been listed as endangered, may also help ensure their availability for generations to come

(Xego *et al.*, 2016). However, there are challenges to cultivation such as lack of knowledge on the agronomic practices, continued overharvesting, and among a multitude of others, and only a few studies have investigated that, opening a knowledge gap that is a limiting factor to successful commercialization (Nwafor, 2020).

2.3. The impact of plant parasitic nematodes

Mandal *et al.* (2021) assert that plant parasitic nematodes (PPNs) are small, worm-like organisms distinguished by their translucent organisms, bilateral symmetry, and pseudocoelomate morphology. These organisms are multicellular in nature and can be found in various settings, either as autonomous entities or as parasites. The organisms exhibit a diverse range of activities, including predatory tendencies, inhabiting both aquatic and terrestrial habitats, and displaying antipathogenic properties (Mukherjee, 2011). According to Mandal *et al.* (2021), parasites can be categorized into many groups according to their behavior, such as ectoparasites, endoparasites, semi-endoparasites, or stationary parasites. These nematodes provide significant challenges to important crops worldwide, affecting vegetables, fruits, and grain crops simultaneously. According to Sato *et al.* (2019), plant pathogens have the ability to infect a wide variety of economically significant crop families, including the *Solanaceae* (tomato), *Fabaceae* (soybean), *Malvaceae* (cotton), *Amaranthaceae* (sugar beet), and *Poaceae* (grasses). The root-knot and cyst nematodes are economically significant pests that can cause major harm to various crops. *Meloidogyne* species are the most common agricultural pests that reduce crop yields and quality and make hosts more vulnerable to environmental stresses.

Nevertheless, the destruction caused by nematodes is frequently not readily apparent, as it might be concealed by several other factors such as nutrient deficiency that hinder plant development (Vieira and Gleason, 2019).

Raselabe (2017) highlighted plant parasitic nematodes as one of the challenges that prevent successful development, mass cultivation and adoption of medicinal plants, especially where the observations on the roots revealed the presence of rotting roots like damage caused by root-knot nematodes. Raselabe (2017) also reported that *S. frutescens* were infested by nematode communities found in the soil, such as *Meloidogyne javanica*, with major above-ground symptoms of nematode damage such as stunted growth, wilting and chlorosis (yellowing), and the below-ground symptoms were root rotting, necrosis, and small black lesions on the medicinal plant. There aren't enough reports on the association of *Meloidogyne species* with *S. frutescens* and their damage has not been extensively reported in South Africa.

Agricultural output losses amounting to billions of rands per year are blamed on plant parasitic nematodes, even though these organisms are tiny (usually about 1 mm in length). A study conducted by Kumar *et al.* (2020) shows that overall, plant-parasitic nematodes caused 21.3% crop losses amounting to Rs. 102,039.79 million (1.58 billion USD) annually; the losses in 19 horticultural crops were assessed at Rs. 50,224.98 million, while for 11 field crops it was estimated at Rs. 51,814.81 million. The *Meloidogyne graminicola*, commonly known as the rice root-knot nematode, resulted in economical yield losses of Rs. 23,272.32 million in rice (*Oryza sativa*). Citrus (Rs. 9828.22 million), banana (*Musa acuminata*) (Rs. 9710.46 million) among fruit crops; and tomato (*Solanum lycopersicum*) (Rs. 6035.2 million), brinjal (*Solanum melongena L.*) (Rs. 3499.12 million) and okra (*Abelmoschus esculents L.*) (2480.86 million) among the vegetable crops suffered comparatively more losses in India.

2.3.1. Mode of infestation by root-knot nematodes in plants

The life cycle of a root-knot nematode (RKN; *Meloidogyne* spp.) can't be completed without infecting a host plant (Singh and Phulera, 2015). A brief overview of the RKN life cycle is shown

in Figure (2.2). The process starts with the female depositing her eggs in preexisting soil or plant tissues. Juveniles infected with the virus penetrate the roots of sensitive plants near the tips of their roots after hatching from these eggs (Singh and Phulera, 2015). In order to start and establish their permanent feeding sites, the second-stage juveniles (J2) RKNs move intercellularly towards the vascular bundles. These nematode feeding grounds consist of numerous multinucleate large cells that, in retrospect, stand out as "knots" or "galls" on the roots. Next, the mother bugs release her eggs, which hatch into fresh, contagious young (Singh and Phulera, 2015). The root-knot nematode begins a complex interaction connection with the host cell when it infects plant roots.

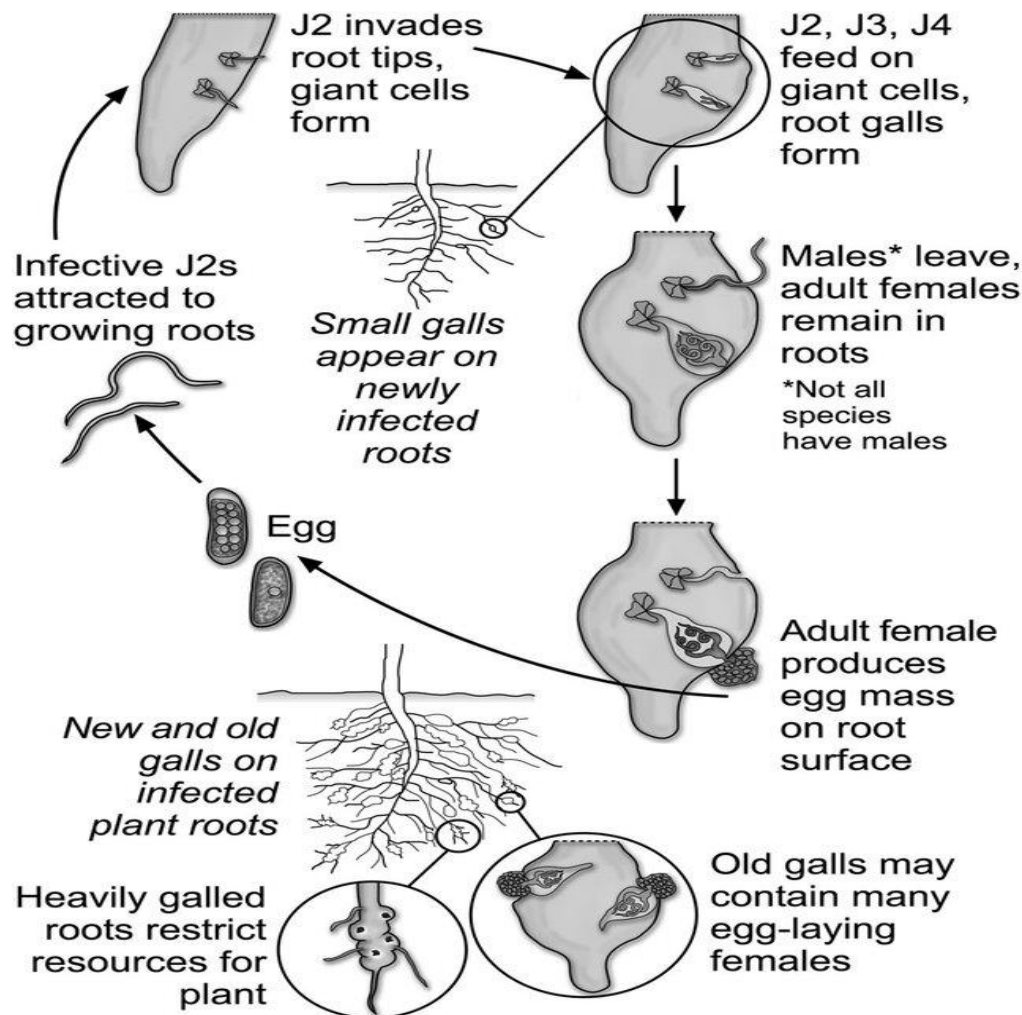


Figure 2.2 Life cycle of a root-knot nematode (Hunt, 2018).

Nematodes are special obligate biotrophs that feed on plant cells by penetrating them with their extendible stylets (Vieira and Gleason, 2019). Another common purpose for stylets is the secretion of chemicals into host cells or tissues; these molecules are called effectors. Effectors, which are secreted by nematodes, assist parasitism by targeting critical host molecular components and processes (Vieira and Gleason, 2019).

2.3.2. The widespread of *Meloidogyne enterolobii*

A thermophilic root-knot nematode species known as *Meloidogyne enterolobii* (Xu *et al.*, 2004; Karssen *et al.*, 2012) is a danger to the horticulture and agriculture sectors worldwide (EPPO, 2014), particularly in Africa (Coyne *et al.*, 2018) and Sub-Saharan Africa (SSA, 2018). A root infection of the pacara earpod tree [*Enterolobium contortisiliquum* (Vell.) Morong] was described in 1983 with the name *Meloidogyne enterolobii* from Hainan Island, China (Collett, 2020). The initial records of *M. enterolobii* in sub-Saharan Africa were from Côte d'Ivoire and Togo in 1987 (Fargette, 1987), followed by South Africa (Willers, 1997), and Senegal (Duponnois *et al.*, 1997; Gueye *et al.*, 1997) in 1997. In Mpumalanga province of South Africa, *M. enterolobii* was first discovered in 1997 and its distribution spread to the year 2019 in Gauteng, Limpopo, Northwest, and Northern Cape provinces. The presence and rising identification of *M. enterolobii* in several Sub-Saharan African nations indicate the threat it poses to agricultural productivity and, by extension, food security in this developing region.

Meloidogyne enterolobii is capable of feeding on a variety of plant types infecting ornamental plants, agricultural crops and weed species, across different regions worldwide. Collett (2020) reported that the presence of *M. enterolobii* in plants poses a challenge for farmers in Sub-Saharan Africa where it was found in the roots of several crops. The spread of *M. enterolobii* is expected to continue across parts of Africa, including Eastern, Western, Central and Southern regions based

on observations, by several researchers (Pagan *et al.*, 2015; Janssen *et al.*, 2016; Coyne *et al.*, 2018; Pretorius, 2018; Visagie *et al.*, 2018; dos Santos *et al.*, 2019; Rashidifard *et al.*, 2019a; Rashidifard *et al.*, 2019c; Collet, 2020).

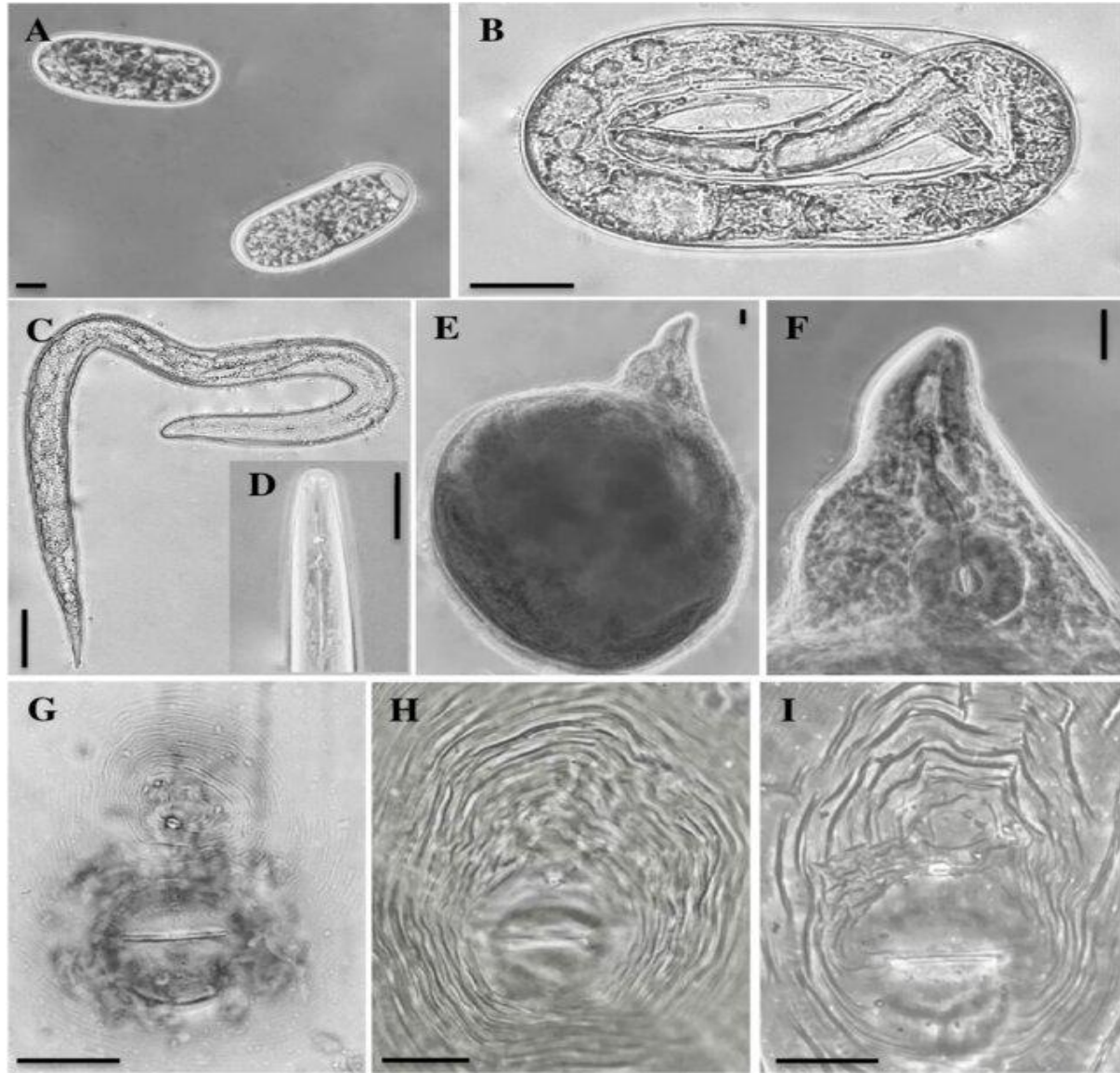


Figure 2. 3. Morphology of *Meloidogyne enterolobii*. A. Eggs. B. Second-stage juvenile (J2) inside the egg. C. Complete body of second-stage juvenile (J2). D. Anterior region in lateral view. E. Complete body of female. F. Pharyngeal region of mature female in lateral view. G-I. Representative Perineal Patterns. Scale bars = 20 μ m. (Retes-Manjarrez, 2024)

Like other root-knot nematode species, galls formed on roots or other below-ground sections of infected host plants are used to identify *M. enterolobii* infection. The root-knot nematode J2 and its females feed in the large cells, which reduces the amount of water and nutrients absorbed and transferred to the above-ground part of plant (Khan *et al.*, 2023). Like symptoms induced by other root-knot nematode species, chlorosis, stunting, and wilting are often evident symptoms of plants infected with *M. enterolobii* (Moens *et al.*, 2009). A microplot research conducted in Florida, USA, found that tomato ('Florida 47') fruit output was 65% lower in infected crops than in the non-inoculated control plants (Cetintas *et al.*, 2007). In addition, the nematode has infected seedlings purchased from nurseries, leading to mortality rate of up to 80% of the plants (da Silva and Santos, 2017). Crops such as parsley (*Petroselinum sativum* L.) which rely on the above-ground parts for production can suffer significant losses when exposed to high densities of *M. enterolobii* for extended periods of time (up to 57% yield loss compared to 39% loss when exposed to lower densities and shorter periods) (Sangronis *et al.*, 2014).

2.4. Root-knot nematodes on medicinal plants

Nematode infections, particularly those of the genus *Meloidogyne* (root-knot-nematodes), have the potential to adversely affect the qualitative and quantitative aspects of pharmacological and productive capabilities of medicinal plants. Several research have been conducted to assess the reproductive capabilities of *Meloidogyne* spp. in medicinal plants (Costa *et al.*, 2019). These investigations enable the identification and utilization of resistant species as sources of resistance genes and their incorporation into crop rotation systems to reduce nematode populations in afflicted regions.

Ansari *et al.* (2019) observed in their assessment, the existence of *M. javanica* in several medicinal plant rhizospheres in the Boyer-Ahmad area of Iran. In a controlled greenhouse setting, the ten (10) medicinal plants were tested on their ability to resist the RKN, *M. javanica*. The tested plants included alkakengy (*Physalis alkekengi* L.), chamomile (*Matricaria chamomilla* L.), English plantain (*Plantago lanceolata* L.), fennel (*Foeniculum vulgare* Mill.), garden anchusa (*Anchusa italica* Retz.), horehound (*Marrubium vulgare* L.), lovage (*Levisticum officinale* L.), sorrel (*Rumex acetosella* L.), thistle (*Echinops adenocaulos* Boiss.), and woundwort (*Stachys pilifera* Benth.). Canto-Saenz (1983) determined that seven species garden anchusa (*Anchusa azurea*), fennel (*Foeniculum vulgare*), horehound (*Marrubium vulgare* L.), alkakengi (*Physalis alkekengi*), english plantain (*Musa paradisiaca*), woundwort (*Stachys sylvatica*), and sorrel (*Rumex acetosa*) were classified as susceptible hosts if their gall index (GI) is above two (2) and their reproduction factor (RF) is also above one (1). On the other hand, thistle (*Cirsium vulgare*), lovage (*Levisticum officinale*), and chamomile (*Matricaria chamomilla*) are hyper-susceptible if their GI is greater than 2 and their RF is less than or equal to 1.

Some medicinal plants have been used as plant extract to control root-knot nematodes (RKNs) as so of these plant species produce volatile organic compounds (VOCs) that are toxic to PPNs. In a study by de Freitas Silva *et al.* (2020) the activities of VOCs emitted from medicinal plant leaves against the RKN, *M. incognita* was explored in vitro. The results revealed that thirteen (13) different medicinal plant leaves contained VOCs that inhibited the growth of *M. incognita* in its second stage of development. Tomato plants treated with these VOCs had less RKN galls and eggs, symbolizing its effectiveness. Isopulegol, dodecane, and α -ionone from the volatilomes of *Dysphania ambrosioides* and *Cymbopogon nardus* did not harm root-knot nematode, but

citronellal and ascaridole from these two species were poisonous (Silva *et al.*, 2020). There is potential for these chemicals to be used in commercial goods and as biofumigant plants.

Moreira *et al.* (2017) evaluated the susceptibility of thirty (30) species, comprising twenty (20) ornamental and ten (10) medicinal plants to *M. incognita*. The assessment of the plants and their response to the nematode was conducted by the quantification of galls and eggs, egg mass index, RF and its reduction. In relation to medicinal plants, it was observed that the species *M. villosa*, *L. alba*, *C. citratus*, *C. winterianus*, and *P. boldus* did not exhibit galls inside their root systems (Moreira *et al.*, 2017). Conversely, the other species had a moderate susceptibility, with a limited number of galls and/or the presence of females isolated at their roots.

Understanding the origins of resistance in medicinal plants is a crucial strategy for effectively managing nematode infestations in cultivated regions. Within cultivation systems, these species have the potential to be employed in consortium or rotational arrangements with other farmed species, with the objective of diminishing the concentration of inoculum in the field and thus mitigating agricultural losses (Costa *et al.*, 2019). Furthermore, understanding these potential sources of resistance can serve as a financial support for breeding initiatives aimed at selecting materials that exhibit nematode resistance in the *Meloidogyne* genus, both in the short and long term (Costa *et al.*, 2019). The existing body of literature suggests that the resistance observed in medicinal plants can be attributed to various substances that are derived from the secondary metabolism of these species. These substances include phenolic compounds, steroids, triterpenes, anthraquinones, flavonoid glycosides, saponin glycosides, condensed tannins, hydrolysable tannins, and sugars (Costa *et al.*, 2019). By identifying the specific chemicals implicated in resistance to root-knot nematodes, it becomes feasible to isolate these compounds for further utilization in the development of solutions that possess the ability to effectively manage this

disease. However, not all medicinal plants possess the ability to manage the root-knot nematodes, hence the need to determine host response of the test plant in the current study.

2.5. The interaction of legumes and nematodes

The *Fabaceae*, often called *Leguminosae*, inhabits more than 20,000 species and is the third biggest family of the flowering plants. Through their symbiotic relationship with microbes like rhizobia, legumes increase soil fertility by fixing atmospheric nitrogen (N). Rhizobia and legumes work together to fix molecular nitrogen in root nodules, which helps the legumes meet their N needs (Concha and Doerner, 2020). In response to microbial signals, legumes develop two distinct forms of root organs, namely nodules and root galls. Within the domain, these interactions manifest simultaneously and frequently engage in mutual influence. The results of these interactions show variability and are contingent on inherent fluctuations in rhizobia and nematode populations within the soil, and abiotic factors (Costa *et al.*, 2021). Rhizobia are symbiotic organisms that provide fixed N to their hosts, whereas parasitic RKN induces the formation of galls as feeding structures, which deplete plant resources without making any significant contributions to the plant. However, there are some parallels between the two relationships (Costa *et al.*, 2021). These commonalities include the signaling activity in the rhizosphere, the suppression of host defensive responses, the activation of host cell division and differentiation, the flow of nutrients, and the modification of root architecture. Rhizobia induce alterations in defense mechanisms and developmental processes via the activation of Nod factor signaling, accompanied by the involvement of effector proteins and exopolysaccharides (Costa *et al.*, 2021). Type III secretion system (T3SS) is a technique that involves the injection of a substantial quantity of protein effectors into plant cells, which directly inhibit immunological signals and influence developmental pathways. Khan *et al* (2017) studied the potential effect of rhizobial inoculation on RKNs in chickpea, mung bean and pigeon pea were

studied under field conditions. The three legumes had their root systems infested with oval galls caused by the nematode (1500 juveniles/kg soil), which stunted their development and reduced their production. Without rhizobium treatment, plants had more galling, egg mass production, and nematode soil population. The test plant in the current study is a legume, so the ability to nodulate may reduce nematode population density. Nodulation, bacteroid population, leghemoglobin concentrations of nodules, and NPK absorption by the plants were all negatively impacted by the nematode infection. This shows the negative impact the nematodes have on legumes to perform their duty (Khan *et al.*, 2017). The results of Khan *et al.* (2017) are in line with a study conducted by Wangmare *et al.* (2022) where they investigated RKN, *M. incognita*, on mung bean (*Vigna radiata*) cultivation. The experiment was conducted in a controlled environment to examine how varying inoculum loads of the RKN, *M. incognita*, affected the growth, nodulation, nematode development, and nutritional status of mung beans. As the inoculum level increased, the results showed that the plant growth metrics, including shoot length, fresh and dried shoot weight, and overall growth rate, decreased. The inverse pattern, however, held true for both wet and dry root weights. Inoculum levels ranging from 100 to 6000 J2/pot were likewise associated with a decline in leaf chlorophyll content (Wangmare *et al.* 2022). The plant's nutrient content, including N, P, K, Ca, and Mg, was shown to be much lower in the shoots as inoculum levels grew, but higher in the roots. The maximum inoculum level, which was 6,000 J2/pot, had an 80% impact on nodulation. Additionally, when the nematode inoculum level increased, leghemoglobin, bacteroid concentration, and nitrogenase activity all decreased (Wangmare *et al.* 2022). Produce quality was negatively impacted because the RKN, *M. incognita*, disrupted the symbiotic N fixation process between the mung bean host and rhizobium.

2.6. Host-status and host-sensitivity studies of nematodes

According to Seinhorst (1967), there are two main ideas in plant-parasitic nematology that assist to understand nematode interactions namely, host status and host sensitivity. The ability of a nematode to reproduce on a specific hostplant is measured by the reproductive factor (RF) ($RF = PF/Pi$) where PF is the final population density and Pi is initial population density, which provides insight into the host-status (Windham and Williams, 1988; Makhado, 2020). 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. There was high intra-specific competition for resources beyond the equilibrium (E) point, according to Seinhorst (1967), and RF would always be less than one when the final nematode population density (PF) was equal to the initial nematode population density (Pi). Seinhorst (1965) used host status and plant response to nematode infection to describe host sensitivity. Host-sensitivity can be influenced by numerous factors, including the type of nematode, the amount of inoculum, the type of plant, the age of the plant, and both biotic and abiotic variables (Seinhorst, 1965). In his study, it was found that host plants could be classified as susceptible hosts when yields are reduced; tolerant hosts when yields are not affected, and resistant hosts where there was no infection (Nkosi, 2019).

Different plants respond to nematode infestations differently depending on the species and cultivar. The damage levels are also influenced by temperature, crop rotations, nematode type, soil properties, and soil moisture content (Pulavarty *et al.*, 2021). Numerous plant species are susceptible to PPN infection. The host ranges of nematode species vary greatly, and each nematode species has distinct preferences when it comes to which plant species and variations are suitable as hosts. Some RKNs are restricted to one or a small number of hosts like *Meloidogyne graminicola*, but many of the most well-studied RKNs that is, those species that are widely spread

and seriously harm the world economy have very wide host ranges like *M. enterolobii* (Sasser, 1977; Trudgill and Blok, 2001; Abad and Williamson, 2010).

2.7. Nematode management strategies

The removal of synthetic nematicides from the agrochemical markets, due to their negative impact on the environment, has led to an increase in the utilization of alternative products such as biocontrol agents and phytonematicides for the purpose of managing nematode population densities (Mashela *et al.*, 2017; Masenya *et al.*, 2021). The utilization of integrated pest management (IPM) strategies effectiveness in mitigating RKNs is location and resource specific, meaning not all farmers may have the resources needed to comply. Alternative strategies in management of RKN encompass four key components: biological, cultural, chemical, and genetic host plant resistance approaches.

2.7.1. Biological control of nematodes

The utilization of biological methods has demonstrated efficacy in the management of PPNs, and there is a growing interest in research aimed at identifying potential biocontrol agents. Promising results were observed in the management of *M. enterolobii* through the utilization of the fungi *Trichoderma harzianum* (Jindapunnapat *et al.*, 2013) which effectively controlled several other soil-borne pathogens and was shown to induce disease resistance and stimulate adventitious root growth in plants and *Athrobotrys oligospora* (Gueye *et al.*, 1997). In a study conducted by Ferriera *et al.* (2011), the researchers investigated the indirect impacts of toxic metabolites that are released by the symbiotic bacterium *Photorhabdus luminescens*, which is associated with *Heterorhabditis baujardi* (Phan *et al.*, 2003). Specifically, the study examined the effects of these metabolites on the hatching and motility of J2, a developmental stage of the nematode. J2 hatching was delayed, probably because of the eggs permeability to noxious metabolites released by *Photorhabdus*

luminescens, which was the bacterial symbiont of *H. baujardi*. Nevertheless, previous research conducted by Trudgill *et al.* (2000) and Carneiro *et al.* (2004) demonstrated that the utilization of *Pasteuria penetrans* and rhizobacteria, as means of controlling *M. enterolobii*, was less effective.

According to Collett (2020) the effectiveness of naturally derived nematicides, which are not chemically synthesized, such as those obtained from secondary metabolites of biological control agents (e.g., bacteria, fungi, etc.) or various components of animals and plants (e.g., leaves, roots, stems, fruits) containing aldehydes, essential oils, glucosinolate derivatives, and other compounds, has been demonstrated in combating *M. enterolobii*. Two examples of plant extracts with potential effects on nematode populations are the aqueous and ethanolic extracts of *Calotropis procera*, which resulted in a 6.4% hatching rate of *M. enterolobii* J2 after 72 hours (Vegas *et al.*, 2010), and *Chenopodium ambrosioides* (L.), which caused mortality rates ranging from 51.25% to 100% of J2 within 24 to 48 hours of exposure (Quevedo *et al.*, 2010; Friere and Santos, 2018).

2.7.2. Cultural control of nematodes

Cultural practices depend on the host status of crop genotypes and non-essential plants, such as weeds, to facilitate their incorporation into crop rotation strategies (Collett, 2020). An equally significant aspect pertains to the identification and detection of the *Meloidogyne* species within a particular agricultural area, ensuring precision in the process (Collett, 2020). The cultivation of immune, non-hosts, or resistant crops in crop rotation cycles, such as oat (*Avena sativa*), sorghum (*Sorghum bicolor*), or wheat (*Triticum aestivum*), has been found to be an effective strategy for managing *M. enterolobii* (de Brida *et al.*, 2018). These practices are part of IPM strategies, which sometimes are met with resources challenges. The exact stipulation of this is access to varieties by all farmers.

2.7.3. Chemical control of nematodes

Chemical control is a widely employed management strategy owing to its prompt response and efficacy in achieving desired outcomes. A limited number of chemically derived nematicides with classical properties have been assessed for their impact on population densities of *Meloidogyne* species. Specifically, the nematicides containing 1,3 dichloropropene, commonly referred to as 1,3-D, were examined in a study conducted by (Coyne *et al.*, 2009) which proved effectiveness against *M. enterolobii*.

Chemical nematicides have long been the major strategy for controlling nematode pests in South Africa since 1982 (Jones, 2017), many products, including organophosphates, fumigants, furfural and carbamates, have been studied and approved for commercial usage. However, growing consumer and governmental concerns about environmental and health dangers have resulted in tighter limits. The discontinuation of aldicarb and the planned phase-out of methyl bromide reflect this trend. Furthermore, nematicide resistance is a rising issue. In response, the chemical industry has created safer, lower-dose alternatives with less environmental effect (Jones, 2017). Meanwhile, biological control methods, notably bacterial and fungal-based therapies, are gaining ground, but with limited use in South Africa.

2.7.4. Genetic host plant resistance to nematodes

Plant genetic host resistance can manifest as the nematode pests' inability to locate a feeding site within the internal structure of the host plant, thereby preventing their reproductive and developmental processes (Mbatyoti, 2018). According to Venter (2013), genetic host resistance is a highly recommended economic strategy for mitigating yield losses on indigenous seed cultivars due to its environmentally friendly nature and convenient implementation.

To effectively identify resistance within a cultivar, it is necessary to obtain and evaluate the following factors: i) The reproductive characteristics of the nematode, ii) The progression of nematode life-stages and iii) The process of J2 penetration (Collett, 2020). While certain genotypes may demonstrate resistance responses to *M. enterolobii*, contrasting responses may occur for other species of *Meloidogyne*. Examples of potential responses that could be considered include the application of genotypes resistant to *M. enterolobii*, as well as the utilization of oat variety 'IPR-126' (Machado *et al.* (2015); Riede *et al.* (2015), and de Brida *et al.* (2018)). A study conducted by Freitas *et al.* (2013) reported that species such as *Psidium cattleianum* (yellow guava), *Psidium friedrichsthalianum* (Costa Rican guava), *Acca sellowiana* (feijoa) and *Psidium rufum* (purple guava) were resistant to *M. enterolobii*. Although the species showed late resistance with giant cell deterioration and nematodes showed arrested development. Costa *et al.* (2020) conducted a study evaluating seven macadamia walnut varieties for resistance to *M. enterolobii* and *M. javanica*, it was found that all the varieties were resistant to the nematode species.

Despite the well-documented impact of *M. enterolobii* on various crops, limited research exists on its interaction with *S. frutescens*, particularly under different environmental conditions. The influence of cultivation systems, such as microplots and shade nets, on nematode infestation levels remains unclear, leaving a gap in understanding optimal management strategies. Additionally, the economic implications of nematode infestation on medicinal plants like *S. frutescens* are not well explored, highlighting the need for further investigation into its resistance mechanisms and potential control measures.

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CHAPTER THREE

HOST STATUS OF CANCER BUSH (*SUTHERLANDIA FRUTESCENS*) TO

MELOIDOGYNE ENTEROLOBII DURING SUMMER

3.1. Introduction

Root-knot (*Meloidogyne* species) nematodes (RKNs) are among the most economically significant pests because of their intricate relationship with host plants, wide host range, and the level of damage ensured by the infection (Azlay *et al.*, 2023). The above makes RKNs to be a costly burden on crop production and represent a constraint on global food security (Bernard *et al.*, 2017). The most effective way of managing nematodes is both environmentally and economically friendly by using cultivars that are resistant (Akinsanya *et al.*, 2020). To select cultivars with a degree of tolerance to the selected RKNs species, tests for host status and host sensitivity to the species of nematodes are utilized (Pofu *et al.*, 2017). The yield losses associated with PPNs in *S. frutescens* in South Africa are undocumented and the relationship between the two is very important to improve the production for the commercialization of the crop.

The utilization of the crop has expanded beyond the local market but has entered the international market as well (Raselabe, 2017). The heightened need to increase production could be limited by the RKN species. A study conducted by Raselabe (2017) recorded that the *Meloidogyne spp.* also infests the *S. frutescens* in South Africa. There is scanty to no information on the resistant genotypes available in South Africa; hence the objective was to determine whether *M. enterolobii* will reproduce on *S. frutescens* and reduce plant growth under microplot and shade nets during summer.

3.2. Materials and methods

3.2.1. Description of the study area

Two separate experiments were carried out at the University of Mpumalanga's (25.4371° S, 30.9818° E) Mbombela campus farm under microplot-field and shade-net conditions from November 2023 to January 2024 with temperature ranging between 26°C and 33°C, with an average of 162 mm precipitation per annum.

3.2.2. Preparation and collection of plant material and inoculum

Cancer bush seeds were purchased from the Mountain Herb Estate Nursery in Gauteng province, South Africa (25°43'27.6"S 27°57'54.8"E). For both experiments a total of 112, 20-cm-diameter plastic pots were filled with 27000 ml steam pasteurized (300 °C for one hour) sand and loam soil at a 3:1 (v/v) ratio, and then placed under a microplot-field (Figure 3.1.a) and shade-net (Figure 3.1.b) conditions using a randomized complete block design (RCBD). Under the microplot conditions, blocking was necessary against the shade, and under shade nets, blocking was against the uneven coverage of the nets on the side of the nets where wind was causing variation in plant development. Pots were spaced at 0.5 m x 0.5 m inter-row and intra-row spacing in both planting systems, whereas under microplot conditions, pots were inserted at 30 cm depth.

Scarification of the seeds was done in hot water (80°C) over-night to enhance germination before planting, 5 seeds were directly planted per pot and 2 weeks after germination, cancer bush seedlings were then thinned to one seedling per pot. Seedling were hardened for seven days through intermittent withdrawal of water before transplanting, seven days after transplanting, eight treatments of 0, 25, 50, 125, 250, 625, 1250, and 3125 *M. enterolobii* eggs and second-stage juveniles (J2) were applied to respective seedlings, with each replicated seven times. Roots of

nematode-susceptible kenaf (*Hibiscus cannabinus* L.) grown in a greenhouse were harvested for J2 and eggs of *M. enterolobii* in preparation for the inoculum. The inoculum was applied on the cardinal points of the plant with 3-cm-deep holes around the test plant. Plants were irrigated with 250 ml of tap water when soil moisture level was below 60%. Daily inspections for pests and diseases were conducted the whole duration of the study.



Figure 3.1. (a) Cancer bush trial under microplot-field condition (b) and under shade net condition

3.2.3. Data collection

Plant Variables

The height of plants was measured using a measuring tape to the nearest millimeter, 56 days after plants were inoculated. The counting of branches was achieved manually, while the measurement of dry shoot mass was performed by subjecting the shoots cut at soil line to an oven set at a temperature of 52 °C for a duration of 72 hours (Makhado, 2020). Chlorophyll was measured using the chlorophyll meter MC-100 manufactured at Apogee, Utah. The stem diameter was measured at 5 cm above the cut end using a Vernier caliper manufactured at Tock Craft, Johannesburg. The root systems were removed from the pots, put under tap water to remove any residual soil, bloated dry using laboratory paper towel (Makhado, 2020), and then fresh root mass was measured. Root

galls were assessed using a 5-point scale, where 0 represented no galls, 1 represented 0.5 galls, 2 represented 3-10 galls, 4 represented 31-100 galls, and 5 represented more than 100 galls (Taylor and Sasser, 1978).

Extraction of nematodes from the roots

The maceration and blending method (Hussey and Barker, 1973) was employed to extract nematodes from root material. The infected roots were cut into small 1 cm pieces and placed for 90 seconds in a solution of 1% sodium hypochlorite (NaOCl). The mixture was then filtered through a series of sieves with pore diameters of 125 μ m, 75 μ m and 25 μ m using a pressured flow of tap water to collect mostly eggs. The remaining roots were then macerated in a blender, roots fragments were then washed in stacked sieves with tap water before the eggs and J2 are collected in the 25 μ m sieve. Nematodes obtained from the 25 μ m sieve were quantified utilizing a light microscope and subsequently analyzed for morphological identification.

Extraction of nematodes from the soil

Nematode extraction from soil samples was achieved using the modified sugar floatation and centrifugation method (Kleynhans, 1997). Briefly, a soil sample of 200g was placed in a bucket containing five litres of tap water and stirred to allow for suspension of nematodes. When the swirl had stopped, an aliquot was quickly passed through a 125 μ m, 75 μ m and 25 μ m nest of sieves. Nematodes on the bottom sieve were washed into 100 ml centrifuge tubes. A teaspoon of kaolin was then added to each tube and centrifuged at 2000 rpm for 5 min. The top liquid aliquot was discarded, the tubes were then filled with sugar solution of 624 g sugar/L and stirred to bring the solutes into suspension prior to centrifuging for 1 min at 2000 rpm to suspend nematodes in the sugar solution. The aliquot was then passed through a 25 μ m mesh sieves. The sugar was rinsed

off with tap water and nematodes collected in a 25 µm mesh sieves, were poured into a 100 ml container for counting under a stereomicroscope and examined for morphological identification.

Nematode variables

The second-stage juveniles (J2) in root, J2 in soil and eggs in root were counted using the compound microscope under x40 magnification. Final population (PF) was calculated as J2 in soil + eggs in roots+ J2 in roots. Reproductive factor was calculated by dividing the PF with initial population (Pi), (PF/Pi). To calculate the Total Treatment Variance (TTV), the total number of components, replication, treatment, and error, was first determined by summing the individual values. The percentage contribution of each component was then calculated by dividing each value by the total and multiplying by 100.

3.3. Data analysis

The plant growth and nematode variable data were subjected to analyses of variance (ANOVA), through Statistix10 software. Prior to analysis, data from all experiments were analyzed separately. The Shapiro-Wilk normality test was used to test for deviation from normality in each standardized residual variable (Gomez and Gomez, 1984). To minimize variation amongst variables the data was transformed using $\log_{10}(x+1)$ transformation. Fisher's Least Significant Difference Test ($P \leq 0.05$) was used to achieve the mean separation.

3.4. Results

3.4.1. Plant growth variables

In both the microplot (Appendix 3.9-3.14) and shade net (Appendix 3.21-3.26) experiments, treatments were not statistically significant ($P > 0.05$) for all plant growth variables measured.

3.4.2. Nematode variables

Under microplot conditions, all the nematode variables, were significant ($P \leq 0.05$) except J2 in soil (Table 3.1). The root galls, J2 in roots, eggs in roots, PF and RF had a total treatment variable (TTV) of 82%, 70%, 64%, 94%, and 91%, respectively (Table 3.1). Under shade net conditions, the root galls, J2 in soil, and RF, were not significant (Appendix 3.15-3.20), whereas the J2 in roots, eggs in roots, and PF were significant ($P < 0.05$) with TTVs of 71%, 78%, and 76%, respectively (Table 3.1). The root galls were above two (2) under microplot experiment with PF decreasing with an increment in nematode inoculum levels (table 3.2). The RF was greater than one (1) at nematode inoculum levels that are ≤ 50 , then decreased to less than 1 at inoculum levels ≥ 125 (Table 3.2). Under shade net experiment, the PF increased with the increase in nematode inoculum level, then decreased when reaching nematode inoculum level 625 (Table 3.2).

Table 3.1. Partitioning mean sum of squares of root galls, second-stage juveniles (J2) in soil, J2 in roots, eggs in roots, final population (PF) and reproductive factor (RF) of *Meloidogyne enterolobii* to Cancer bush under 2 different conditions.

Source	Root		J2 in		J2 in		Eggs in		PF		RF		
	Galls		soil		Roots		Roots						
	DF	MS	TTV	MS	TTV	MS	TTV	MS	TTV	MS	TTV	MS	TTV
			(%)		(%)		(%)		(%)		(%)		(%)
Microplot experiment													
Replication	6	0.03	9.46	0.98	17.25	1.32	17	1.62	21.41	0.25	2.97	0.14	4.71
Treatment	7	0.29	82.41**	3.54	62.42ns	5.42	69.75**	4.85	64.26**	7.92	94.45**	2.70	91.15**
Error	42	0.03	8.12	1.15	20.34	1.03	13.25	1.08	14.33	0.22	2.58	0.12	4.14
Total	55	0.35	100	5.68	100	7.78	100	7.55	100	8.38	100	2.97	100
Shade net experiment													
Replication	6	0.05	18.66	1.29	40.08	1.25	13.79	0.58	6.35	0.83	8.52	0.13	21.48
Treatment	7	0.16	60.16ns	1.42	44.37ns	6.40	70.76**	7.16	77.93**	7.38	75.83**	0.30	49.16 ^{ns}
Error	42	0.06	21.18	0.50	15.55	1.40	15.45	1.45	15.73	1.52	15.65	0.18	29.35
Total	55	0.27	100	3.21	100	9.05	100	9.19	100	9.73	100	0.62	100

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

Table 3. 2. Response of root galls, second-stage juveniles (J2) in soil, J2 in roots, eggs in root, final nematode population density (PF) and reproductive factor (RF) of Cancer bush to *Meloidogyne enterolobii* under 2 different conditions.

Treatment	Root Galls	J2 in Roots	Eggs in Roots	PF	RF	J2 in Roots	Eggs in Roots	PF
Microplot experiment					Shade net experiment			
25	0.61 ^a (3.29)	2.42 ^a (885.71)	2.38 ^{ab} (942.86)	3.11 ^{ab} (2114.40)	1.72 ^a (84.57)	0.90 ^{bcd} (57.14)	0.66 ^{cd} (57.14)	1.36 ^{bc} (157.14)
50	0.63 ^a (3.29)	2.47 ^a (1214.30)	2.15 ^{ab} (702)	3.12 ^{ab} (2273.40)	1.45 ^a (45.47)	0.71 ^{cd} (142.86)	0.62 ^{cd} (42.86)	1.11 ^{cd} (200)
125	0.56 ^{ab} (2.71)	2.42 ^a (900)	1.47 ^b (342.86)	2.79 ^b (1285.70)	0.82 ^b (10.29)	1.10 ^{bcd} (371.43)	0.80 ^{cd} (600)	1.81 ^{bc} (1028.60)
250	0.48 ^{ab} (2.29)	2.09 ^a (357.14)	2.03 ^{ab} (300)	2.71 ^b (814.29)	0.53 ^{bc} (3.26)	1.52 ^{bc} (500)	1.58 ^{bc} (471.43)	2.00 ^{bc} (971.43)
625	0.51 ^{ab} (2.57)	2.70 ^a (1200)	1.32 ^b (128.57)	2.86 ^{ab} (1471.40)	0.41 ^c (2.35)	1.54 ^{bc} (142.86)	1.14 ^{bcd} (300)	1.80 ^{bc} (442.86)
1250	0.43 ^b (2.14)	1.82 ^a (285.71)	1.71 ^{ab} (314.29)	2.69 ^b (714.29)	0.17 ^{cd} (0.57)	3.16 ^a (2442.90)	3.09 ^a (2200)	3.45 ^a (4657.10)
3125	0.58 ^{ab} (2.86)	2.60 ^a (1171.40)	2.69 ^a (1100)	3.32 ^a (2885.70)	0.25 ^{cd} (0.92)	2.10 ^{ab} (1471.40)	2.33 ^{ab} (1371.40)	2.64 ^{ab} (2914.30)
LSD _{0.05}	0.18	1.10	1.12	0.50	0.38	1.28	1.30	1.33

^yColumn means ± standard error followed by the same letter were not different (P ≤ 0. 05) according to Fisher's Least Significant Different test.

3.5. Discussion

The RF assesses a nematode's reproductive capacity in a host plant (Windham and Williams, 1988). The RF helps evaluate whether a plant is a host or non-host to a given nematode (Seinhorst, 1967). Reproduction factor values less than one show that the test nematode was unable to feed and reproduce on the test plants. In contrast, values larger than one indicate that the nematodes effectively established feeding sites and reproduced on the test plants (Windham and Williams, 1988; Makhado, 2020). In the present study, at the inoculation level ≤ 50 the RF was greater than 1 while at the inoculation level of ≥ 125 the RF was less than 1 under microplot conditions. Similar results were observed by El- Sherif *et al.* (2013) where RFs of *Meloidogyne incognita* decreased at higher inoculation levels. They further explained that the increased competition and stress on host plants, lead to the reduced nematode reproduction levels. In the current study it was observed that the RF under shade net conditions was not statistically significant. The non-significance of the RF can be attributed to various factors. A study by Khanal and Land (2023) indicates that increased soil temperatures reduce nematode reproduction, but the non-significance may arise from species specific responses and variability in experimental conditions requiring further investigation.

The population of the *M. enterolobii* eggs and J2 accelerated with the increase in nematode inoculum up to level 50, then rapidly decreased at nematode inoculum levels above 125. This indicates that the initial *M. enterolobii* were able to penetrate and reproduce in the roots of *S. frutescens*. *Meloidogyne enterolobii*'s ability to reproduce in the roots has been proven due to its ability to multiply in host plants having resistance against major tropical RKN (Koutsovoulos *et al.*, 2020; Sikandar *et al.*, 2023). This shows that the nematodes have reached the equilibrium point at inoculum 50, whereby the resources are no longer available to sustain the larger nematode numbers due to the increased population density that have resulted from damage that the

nematodes have caused to the plants. The increased rates of nematodes are usually described by the negative exponential models, indicating that as the population increases the growth rate decreases due to the limit of resources (Ferris, 1985). The decrease in final population when the levels of nematode inoculum increases were also observed by Kayani *et al.* (2018) where a rate of nematodes build up decreased with the increase in inoculum densities.

Root galls are abnormal growth on the plant caused by various pests, including nematodes which result in nutrients deficiencies. In the present study the root gall index (GI) is greater than 2 in all treatment levels under microplot conditions, which indicates that the plant has been damaged by the nematodes (Taylor and Sasser, 1978; Sasser *et al.*, 1984). The galls formation on the roots show susceptibility and host sensitivity to the nematodes (Favery *et al.*, 2020). A similar observation was observed by Park *et al.* (2007) where the medicinal plants were susceptible with the GI of 2.7-5.0. the ability of nematode to attack medicinal plants severely makes a threat to the highly susceptible plants. Using the Seinhorst model, the RF indicates that *M. enterolobii* was able to reproduce on cancer bush without causing a reduction in the plant's growth variables. Masenya *et al.* (2023) reports similar results on Kickapoo white tepary bean (*Phaseolus acutifolius* A. Gray) variety where *M. enterolobii* was able to infest the plant without causing a reduction in the plant growth variables.

3.6. Conclusion

The reproduction factor (RF) is used to measure the host status. As the inoculum levels increased the RF was below 1 but the measured plant variables relative to the control were not significantly different. The RF indicates that *M. enterolobii* was able to reproduce on cancer bush without causing a reduction in the plant's growth variables. The increase in the RF as the inoculum increase suggest that the plant could have reached the equilibrium where the root sources is not sufficient

to the nematode population density inoculated. The observation made in the study indicate that *S. frutescens* is tolerant to *M. enterolobii* and the status was maintained in both microplot and shade net conditions in summer.

3.7. References

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CHAPTER FOUR

HOST STATUS OF CANCER BUSH (*SUTHERLANDIA FRUTESCENS*) TO *MELOIDOGYNE ENTEROLOBII* DURING WINTER

4.1. Introduction

Seasons play a role in the ability of plant parasitic nematodes (PPNs) to infect the plant and reproduce. *Sutherlandia frutescens* have been reported to be tolerant to *Meloidogyne enterolobii* in Chapter 3. Since it is known as a perennial crop, it is important to know the ability of the nematodes to affect the crop in different seasons. Temperature has a critical role in root-knot nematode (RKN) infectivity, with different ideal circumstances for each species (Teklu *et al.*, 2018). Understanding the distribution and possible effects of these nematodes in the context of climate change requires an understanding of their variability in response to temperature and incubation time. Velleso *et al.* (2022) reported that lower (+8) and intermediate temperatures (25 – 30) decreased the reproduction and developmental cycles of *M. enterolobii*.

Researchers support larger initiatives to create adaptation and mitigation measures by comprehending how nematodes react to shifting environmental conditions and using this information into climate models. These tactics support global food security by enhancing ecosystem resilience and protecting agricultural productivity (Esterlin, 2024).

4.2. Materials and methods

4.2.1. Description of the study area

Two separate experiments were carried out at the University of Mpumalanga's (25.4371° S, 30.9818° E) Mbombela campus farm under microplot-field and shade-net conditions from May to

August 2024 with temperature ranging between 10°C and 27°C, with an average of 162 mm precipitation per annum.

4.2.2. Preparation of plant materials and inoculum

The plant materials and inoculum were prepared as explained in Chapter 3.

4.2.3. Data collection and analysis

Data was collected and analyzed following the same procedures as documented in Chapter 3 for both plant and nematodes variables, similarly with extractions.

4.3. Results

4.3.1. Plant growth variables

In both the microplot and shade net experiment, the plant growth variables, namely number of branches, chlorophyll, stem diameter, plant height, fresh shoot mass, and dry shoot mass, were not significant ($P>0.05$) to the treatment (Appendix 4.9 -4.15 and Appendix 4.21-4.26).

4.3.2. Nematode variables

Under microplot conditions, the nematode variables, namely root galls, second-stage juveniles (J2) in roots, eggs in roots, final population (PF), and reproductive factor (RF), were significant ($P\leq 0.05$) except J2 in soil (Appendix 4.3-4.8). The nematodes variables had a total treatment variable (TTV) of 81%, 86%, 98%, 99%, and 98%, respectively (Table 4.1). Under shade net conditions, three of the variables, namely root galls and J2 in roots were not significant (Table 4.2). J2 in soil, eggs in roots, PF and RF were significant (Appendix 4.15-4.20) with TTVs of 76%, 83%, 92% and 90% respectively (Table 4.1). Under microplot experiment, the root galls were less than 2 (Table 4.2). The PF increased with an increase in nematode inoculum levels (Table 4.2). The RF was greater than 1 in nematode inoculum levels that are ≤ 50 (Table 4.2), then decreased

to less than 1 at nematode inoculum levels ≥ 125 (Table 4.2). Under the shade net experiment, RF was greater than 1 only at nematode inoculum level 25 then decreased at nematode inoculum levels that are ≥ 50 . (Table 4.2).

4.3.3. Interactive effect of treatments, season and growing conditions on nematodes variables

Microplot conditions show to host *M. enterolobii* better than shade net conditions in both seasons (Table 4.3). The highest PF was observed under microplot at nematodes inoculum level of 3125 and the lowest number under shade net at 50 inoculum level (Table 4.3), in terms of RF, microplot conditions again showed a favorable outcome with the highest RF observed at nematode inoculum level of 25 and the lowest number recorded under shade net conditions at nematode inoculum of 3125 (Table 4.3). The highest number of PF was observed during summer under microplot conditions, followed by winter under microplot conditions (Table 4.4). The treatment, season and growing conditions had a significant impact on the RF (Table 4.5).

Table 4.1. Partitioning mean sum of squares of root galls, second-stage juveniles (J2) in soil, J2 in roots, eggs in roots, final population (PF) and reproductive factor (RF) of *Meloidogyne enterolobii* on Cancer bush under 2 different conditions.

Source		Root		J2 in		J2 in		Eggs in		PF		RF	
		Galls		soil		Roots		Roots					
	DF	MS	TTV	MS	TTV	MS	TTV	MS	TTV	MS	TTV	MS	TTV
			(%)		(%)		(%)		(%)		(%)		(%)
Microplot experiment													
Replication	6	0.02	10.94	0.75	13.38	0.64	10.61	0.04	0.69	0.02	0.21	0.01	0.54
Treatment	7	0.16	81.10**	4.13	74.07 ^{ns}	5.15	85.99**	5.56	98.19**	7.56	99.50**	1.99	98.85**
Error	42	0.02	7.96	0.70	12.55	0.20	3.40	0.06	1.12	0.02	0.29	0.01	0.61
Total	55	0.20	100	5.58	100	5.99	100	5.66	100	7.60	100	2.01	100
Shade net experiment													
Replication	6	0.00	0.00	0.68	11.57	0.80	17.44	0.45	8.44	0.28	4.45	0.05	5.35
Treatment	7	0.00	0.00	4.47	75.92**	2.87	62.80 ^{ns}	4.42	83.25**	5.88	92.43**	0.80	90.98**
Error	42	0.00	0.00	0.74	12.51	0.90	19.76	0.44	8.31	0.20	3.12	0.03	3.67
Total	55	0.00	0.00	5.88	100	4.57	100	5.31	100	6.36	100	0.88	100

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

Table 4.2. Response of root galls, *Meloidogyne enterolobii* second-stage juveniles (J2) in soil, J2 in roots, eggs in root, final nematode population density (PF) and reproductive factor (RF) on Cancer bush under 2 different conditions.

Treatment	Root Galls	J2 in Roots	Eggs in Roots	PF	RF	J2 in soil	Eggs in Roots	PF	RF
Microplot Experiment					Shade Net Experiment				
25	0.38 ^a (1.57)	2.45 ^{ab} (300)	2.35 ^b (257.14)	2.84 ^b (714.29)	1.46 ^a (28.57)	0.28 ^c (14.29)	1.85 ^a (142.86)	2.35 ^b (285.71)	1.01 ^a (11.43)
50	0.35 ^a (1.29)	2.47 ^{ab} (357.14)	2.48 ^{ab} (385.71)	2.95 ^{ab} (971.43)	1.28 ^b (19.43)	1.23 ^b (85.71)	0.86 ^b (42.86)	1.76 ^c (214.29)	0.60 ^b (4.29)
125	0.48 ^a (2.14)	2.15 ^{ab} (285.71)	2.36 ^b (271.43)	2.87 ^b (785.71)	0.84 ^c (6.29)	1.80 ^{ab} (114.29)	1.80 ^a (114.29)	2.50 ^{ab} (328.57)	0.55 ^b (2.63)
250	0.44 ^a (1.86)	2.42 ^{ab} (300)	2.46 ^{ab} (371.43)	2.88 ^b (814.29)	0.61 ^d (3.26)	1.47 ^{ab} (85.71)	2.11 ^a (142.86)	2.48 ^{ab} (314.29)	0.35 ^c (1.26)
625	0.42 ^a (1.71)	2.44 ^{ab} (300)	2.59 ^{ab} (414.29)	2.94 ^{ab} (885.71)	0.38 ^e (1.42)	1.85 ^{ab} (128.57)	2.09 ^a (128.57)	2.54 ^{ab} (357.14)	0.19 ^{cd} (0.57)
1250	0.44 ^a (1.86)	2.11 ^b (257.14)	2.65 ^a (457.14)	3.00 ^{ab} (1100)	0.26 ^c (0.90)	1.31 ^b (114.29)	2.29 ^a (214.29)	2.60 ^{ab} (442.86)	0.12 ^{de} (0.35)
3125	0.35 ^a (1.43)	2.61 ^a (428.57)	2.61 ^{ab} (457.14)	3.04 ^a (1142.90)	0.13 ^f (0.37)	2.38 ^a (257.14)	2.09 ^a (271.43)	2.84 ^a (714.29)	0.09 ^{de} (0.23)
LSD _{0.05}	0.14	0.47	0.27	0.16	0.12	0.93	0.72	0.48	0.19
Column means $\pm$ standard error followed by the same letter were not different ($P \leq 0.05$) according to Fisher's Least Significant Different test.									

Table 4.3: Interactive effect of the treatments and growing condition on *Meloidogyne enterolobii* final population (PF) and reproductive factor (RF)

Treatments	Growing Condition	PF	RF
25	Microplot	2.98 ^a	0.41 ^a
50	Microplot	3.03 ^a	0.36 ^a
125	Microplot	2.83 ^a	0.26 ^b
250	Microplot	2.79 ^a	0.19 ^{cd}
625	Microplot	2.90 ^a	0.14 ^{def}
1250	Microplot	2.85 ^a	0.08 ^{fgh}
3125	Microplot	3.18 ^a	0.07 ^{gh}
25	Shade net	1.85 ^{cd}	0.24 ^{bc}
50	Shade net	1.43 ^d	0.16 ^{de}
125	Shade net	2.16 ^c	0.17 ^{de}
250	Shade net	2.24 ^{bc}	0.13 ^{def}
625	Shade net	2.17 ^c	0.07 ^{gh}
1250	Shade net	3.03 ^a	0.12 ^{efg}
3125	Shade net	2.74 ^{ab}	0.06 ^{hi}
Column means $\pm$ standard error followed by the same letter were not different ($P \leq 0.05$) according to Fisher's Least Significant Different test			

Table 4.4: Interactive effect of season and growing condition on *Meloidogyne enterolobii* final population (PF)

Season	Growing Condition	PF
Summer	Microplot	2.57 ^a
Summer	Shade net	1.77 ^c
Winter	Microplot	2.57 ^a
Winter	Shade net	2.14 ^b
Column means $\pm$ 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 Interactive effect of treatments, season and growing conditions on *Meloidogyne enterolobii* reproductive factor (RF)

Treatments	Season	Growing Condition	RF
25	Summer	Microplot	0.43 ^a
50	Summer	Microplot	0.38 ^{ab}
125	Summer	Microplot	0.25 ^{cde}
250	Summer	Microplot	0.18 ^{efgh}
625	Summer	Microplot	0.14 ^{fghij}
1250	Summer	Microplot	0.07 ^{klm}
3125	Summer	Microplot	0.09 ^{ijkl}
25	Summer	Shade net	0.17 ^{efghi}
50	Summer	Shade net	0.12 ^{ghijk}
125	Summer	Shade net	0.14 ^{fghij}
250	Summer	Shade net	0.14 ^{fghij}
625	Summer	Shade net	0.07 ^{klm}
1250	Summer	Shade net	0.19 ^{defg}
3125	Summer	Shade net	0.07 ^{klm}
25	Winter	Microplot	0.39 ^a
50	Winter	Microplot	0.36 ^{ab}
125	Winter	Microplot	0.27 ^{cd}
250	Winter	Microplot	0.21 ^{def}
625	Winter	Microplot	0.14 ^{fghij}
1250	Winter	Microplot	0.10 ^{hijkl}
3125	Winter	Microplot	0.05 ^{klm}
25	Winter	Shade net	0.30 ^{bc}
50	Winter	Shade net	0.19 ^{defg}
125	Winter	Shade net	0.19 ^{defg}
250	Winter	Shade net	0.13 ^{fghijk}
625	Winter	Shade net	0.08 ^{klm}
1250	Winter	Shade net	0.05 ^{klm}
3125	Winter	Shade net	0.04 ^{lm}

Column means $\pm$ 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

Seinhorst (1965) explains nematode resistance in terms of two fundamental concepts which are host status and host sensitivity. The host-status and host-sensitivity ideas are the starting point for the identification of plant nematode resistance status (Seinhorst, 1967). According to Ngobeni *et al.* (2012), the reproductive factor measures the nematode's capacity for reproduction on a particular host, and it is used to characterize host status. In the present study, at nematodes inoculation level of ≤ 50 the RF was greater than 1 while at the inoculation level of ≥ 125 the RF was less than 1 under microplot conditions (Table 4.2). Under shade net conditions, the RF was greater than 1 at inoculation level of ≤ 25 and less than one at inoculation level of ≥ 50 (Table 4.2). This is an indication that the nematode population level has reached the equilibrium point. Similar results were observed by Timana (2023), whereby the RF rate was the highest at lower level of inoculum and then decreased with an increase in inoculum at higher levels when he was testing the host response of cassava cv. “Mbonisweni” to *Meloidogyne incognita*. The expected trend in susceptible plants is that the final nematodes population (PF) density increases as the initial population (Pi) increases (Gine *et al.*, 2016; Pofu *et al.*, 2020; Timana, 2023). It is believed that the factors such as competition for infection sites in roots and food scarcity causes a decline in PF and stabilizes around the equilibrium density at which the plant can supply enough food to maintain the population density at planting (Gine *et al.*, 2016; Timana, 2023).

In the present study there was a difference in the appearance of root galls in the experiments. The nematodes had a significant impact on root galls under microplot experiment while there were no root galls visible under shade net experiment. According to Eisenback and Triantaphyllou (2020) the absence of the root galls does not mean there is no nematodes present which is supported by a

study conducted by Ansari *et al.*, (2019) where 10 medicinal plants were tested as hosts and Chamomile (*Matricaria chamomilla* L.) was found to have least number of galls but was found to be hyper susceptible to *Meloidogyne javanica*. Multiple root activities, including water intake, are disrupted when galls develop (Engelbrecht *et al.*, 2021). According to Vilela *et al.*, (2023) they disorganize the vascular system, influencing the formation of cultures. The observation of root galls formation in the current study under microplot conditions might be due to the stress the plants undergo as the plants are exposed to the environment, making them susceptible to nematodes whereas under shade net conditions the environment is more balanced and offers less stress to the plants. Medicinal plants such as cancer bush contain secondary metabolites, such plants use the secondary metabolites such as phenolic compounds, steroids, triterpenes, anthraquinones, flavonoid glycosides, saponin glycosides, condensed tannins, hydrolysable tannins and sugars to kill or poison the threatening species (Van Wyk and Prinsloo, 2020). *Sutherlandia frutescens* under shade net conditions might have used the secondary metabolites as a resistance mechanism against the *M. enterolobii* hence the absence of the galls. However, the phenomenon needs to be investigated.

Root galling was also one of the symptoms observed by Raselabe (2017) when screening nematodes on the roots of *S. frutescens* on a field trial. The findings were similar to Ansari *et al.*, (2019) where the medicinal plants such as Sorrel (*Rumex acetosella* L.) and horehound (*Marrubium vulgare* L.) had significantly higher number of galls. Contradictory, this study had a root galling index (GI) that is less than 2 in all the nematodes inoculum level under microplot during winter. However, a study conducted by Esterlin (2024) showed that *M. enterolobii* can survive and remain infective at lower temperatures and its establishment in temperate regions is an eminent threat.

Host status and host sensitivity are shown by tolerance, sensitivity and resistance. *Sutherlandia frutescens* is a host to *M. enterolobii* as the nematodes were able to reproduce. The root system of *S. frutescens* are generally small and this was evident in all the experiments. The size had an impact of the decrease of the RF at higher inoculation levels. The feeding sites might have been too populated and not being able to provide food for the larger nematode populations hence the reduction in the final population (PF). Some medical plants are susceptible whereas some are resistant to RKNs, Mendonça (2016) investigated how seven different species of medicinal plants responded to *Meloidogyne paranaensis*, assessing the results using the RF and GI. *Melissa officinalis* (lemon balm), *Hypericum perforatum* (St. John's wort), and *Paffia glomerata* (Brazilian ginseng) were all highly susceptible to *M. paranaensis*. *Pogostemon cablin* (patchouli) was categorized as susceptible due to its intermediate response. *Cordia verbenacea*, also known as erva-baleeira in Brazil, was categorized as resistant, while *Artemisia annua* (sweet sage) and *Catharanthus roseus* (Madagascar periwinkle) were extremely resistant. *Catharanthus roseus* stood out due to its high gall index, which prevented the nematode from reproducing (Mendonça, 2016). Whereas the gall index in the present study was less than 2.

Sutherlandia frutescens was found to be tolerant of *M. enterolobii*. This reveals that *M. enterolobii* in both seasons (Summer: chapter 3 and winter: chapter 4) was able to reproduce in cancer bush but not beyond the threshold level as the plant growth was not affected.

The study also reveals that microplot conditions host *M. enterolobii* better than shade net conditions in both seasons (Table 4.3). Esterlin (2024) agrees with this phenomenon as *M. enterolobii* showed greater resilience to high temperatures over time, notably at 20°C and 25°C in his study.

The treatments and growing conditions significantly influence the final population and reproduction factor of *M. enterolobii*. The highest PF was observed under microplot at nematodes inoculum level of 50 and the lowest number under shade net at the same nematode inoculum level (Table 4.3), in terms of RF, microplot conditions again showed a favorable outcome with the highest RF observed at nematode inoculum level of 25 and the lowest number recorded under shade net conditions at nematode inoculum of 3125 (Table 4.3.). This result indicates that microplot condition supports a more conducive environment reproduction and growth of *M. enterolobii*, particularly at lower inoculum levels.

The results indicate that while shade net conditions, which probably have different environmental factors like light intensity, humidity, or temperature fluctuations, may not support nematode growth as effectively, microplots, which offer a more controlled environment, may offer better conditions for nematode reproduction. Understanding the dynamics of *M. enterolobii* in agricultural systems, especially regarding management options for nematode control, may be significantly impacted by this variation in nematode behaviour under various settings.

The season and growing condition played a significant role in influencing the final population (PF). As shown in Table 4.4, the highest number of PF was observed during summer under microplot condition, followed by winter under microplot condition. In contrast, the lowest PF was recorded in summer under shade net conditions. This suggests that microplot, particularly during summer season, provides more favorable conditions for population growth and reproduction of *M. enterolobii*.

With summer being especially favourable for *M. enterolobii* growth in microplot settings, the seasonal variation highlights the influence of environmental factors on nematode dynamics. The

conclusion can be reached that microplot circumstances are more beneficial for nematode population development than shade net settings, which further supports the notion that microplots provide a more stable and suitable environment for nematode reproduction.

As treatment levels increase, the RF decreases for both growing conditions and seasons (Table 4.5). In both seasons and growing conditions, lower nematode inoculum levels (25 and 50) generally show higher RF values than higher levels (625, 1250, and 3125). At the highest nematode inoculum levels, the RF value significantly decreases, indicating the equilibrium point. The results support chapter 3 and chapter 4 findings.

4.5. Conclusion

Sutherlandia frutescens is tolerant to *M. enterolobii* during winter as the gall index (GI) is less than 2 under microplot conditions and the reproductive factor (RF) is less than 1, particularly at high inoculum levels, while growth was not reduced relative to the control. *Sutherlandia frutescens* can be cultivated and produced on soils that are infested by *M. enterolobii* during winter.

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CHAPTER FIVE

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

5.1. Summary

This study evaluated the host status and host sensitivity of cancer bush (*Sutherlandia frutescens*) to *Meloidogyne enterolobii* across two different seasons, summer and winter. The research further examined how the interaction between the treatment, growing conditions and season influenced final population and reproductive factor of *M. enterolobii*. The findings revealed that microplot provided more conducive environment for nematode reproduction than shade net. In summer, the galling index was greater than 2, the reproductive factor was greater than 1, but the plant variables were not affected, which shows that the plant is tolerant. Conversely, in winter, the galling index was less than 2, however the reproductive factor was greater than 1, but with no effect on plant variables suggesting a tolerance response. The seasonal interactions show the advantage of growing *S. frutescens* under shade net conditions, as it proved less favourable conditions for *M. enterolobii* in both seasons.

5.2. Significance of the findings

The study demonstrated that *S. frutescens* is a tolerant host to *M. enterolobii* during summer and winter. The favourable shade net conditions may facilitate the release of secondary metabolites, which could help protect the plants from nematode attack. Furthermore, winter emerges as a favourable season to plant *S. frutescens* in nematodes infested areas, as nematode reproduction occurs at a reduced rate during this period compared to summer.

5.3. Future research

Sutherlandia frutescens was found as a host to *M. enterolobii*, indicating the need for the development and evaluation of effective management strategies to control *M. enterolobii* infestations. Under shade net conditions during winter, the plants did not develop the root galls, suggesting a potential seasonal interaction that influences the plant's resistance to nematode attack. Further investigation is required to better understand how *S. frutescens* interacts with *M. enterolobii* in different season, particularly regarding the plant's mechanism in fighting nematode infestations.

5.4. Conclusion

Sutherlandia frutescens is tolerant to *Meloidogyne enterolobii* in both warmer and cooler temperatures. However, microplot conditions have a more conducive environment for *M. enterolobii* compared to shade net. This knowledge can inform the optimization of cultivation practices and the implementation of targeted control measures to mitigate the impact of *M. enterolobii* infestation. Furthermore, the economic impact of nematode infestation on this medicinal plant underscores the importance of appropriate management methods to ensure its commercial viability and potential contribution to the local economy.

APPENDICES

Appendix 3.1: Shapiro-Wilk normality test for variables measured on *Sutherlandia frutescens* exposed to *Meloidogyne enterolobii* under microplot conditions during summer

Variable	N	W	P
Root galls	56	0.8464	0.0000
J2 in soil	56	0.5547	0.0000
J2 in roots	56	0.7092	0.0000
Eggs in roots	56	0.5633	0.0000
Final population	56	0.7064	0.0000
Reproductive factor	56	0.4408	0.0000
Chlorophyll	56	0.8762	0.0000
Plant height	56	0.9874	0.8227
Number of branches	56	0.7720	0.0000
Stem diameter	56	0.9216	0.0014
Fresh shoot mass	56	0.9059	0.0004
Dry shoot mass	56	0.9213	0.0013

Appendix 3.2: Shapiro-Wilk normality test for variables measured on *Sutherlandia frutescens* exposed to *Meloidogyne enterolobii* under shade net conditions during summer

Variable	N	W	P
Root galls	56	0.7157	0.0000
J2 in soil	56	0.5074	0.0000
J2 in roots	56	0.5360	0.0000
Eggs in roots	56	0.4791	0.0000
Final population	56	0.5172	0.0000
Reproductive factor	56	0.4672	0.0000
Chlorophyll	56	0.1439	0.0000
Plant height	56	0.9712	0.1999
Number of branches	56	0.6127	0.0000

Stem diameter	56	0.3209	0.0000
Fresh shoot mass	56	0.8585	0.0000
Dry shoot mass	56	0.8858	0.0001

Appendix 3.3: Analysis of variance for root galls on cancer bush under microplot condition in summer

Source	DF	SS	MS	F	P
Replication	6	0.19897	0.03316		
Treatment	7	2.02168	0.28881	10.14	0.0000
Error	42	1.19593	0.02847		
Total	55	3.41658			

Appendix 3.4: Analysis of variance for *Meloidogyne enterolobii* J2 in soil under microplot condition in summer

Source	DF	SS	MS	F	P
Replication	6	5.8753	0.97921		
Treatment	7	24.8038	3.54340	3.07	0.0106
Error	42	48.4896	1.15451		
Total	55	79.1687			

Appendix 3.5: Analysis of variance for *Meloidogyne enterolobii* J2 in roots of cancer bush under microplot condition in summer

Source	DF	SS	MS	F	P
Replication	6	7.9291	1.32151		
Treatment	7	37.9615	5.42307	5.26	0.0002
Error	42	43.2915	1.03075		
Total	55	89.1820			

Appendix 3.6: Analysis of variance for *Meloidogyne enterolobii* eggs in roots of cancer bush under microplot condition in summer

Source	DF	SS	MS	F	P
Replication	6	9.6941	1.61569		
Treatment	7	33.9408	4.84868	4.49	0.0008
Error	42	45.3996	1.08094		
Total	55	89.0345			

Appendix 3.7: Analysis of variance for *Meloidogyne enterolobii* final population on cancer bush under microplot condition in summer

Source	DF	SS	MS	F	P
Replication	6	1.4944	0.24906		
Treatment	7	55.4216	7.91737	36.57	0.0000
Error	42	9.0934	0.21651		
Total	55	66.0094			

Appendix 3.8: Analysis of variance for *Meloidogyne enterolobii* reproduction factor on cancer bush under microplot condition in summer

Source	DF	SS	MS	F	P
Replication	6	0.8376	0.13961		
Treatment	7	18.9267	2.70381	22.00	0.0000
Error	42	5.1612	0.12289		
Total	55	24.9255			

Appendix 3.9: Analysis of variance for chlorophyll of cancer bush under microplot condition in summer

Source	DF	SS	MS	F	P
Replication	6	3.5036	0.58393		
Treatment	7	2.2741	0.32487	1.19	0.3280

Error	42	11.4421	0.27243
Total	55	17.2198	

Appendix 3.10: Analysis of variance for plant height of cancer bush under microplot condition in summer

Source	DF	SS	MS	F	P
Replication	6	39637	6606.11		
Treatment	7	25068	3581.13	0.92	0.5022
Error	42	163819	3900.46		
Total	55	228524			

Appendix 3.11: Analysis of variance for number of branches of cancer bush under microplot condition in summer

Source	DF	SS	MS	F	P
Replication	6	1.8571	0.30952		
Treatment	7	6.6964	0.95663	1.17	0.3420
Error	42	34.4286	0.81973		
Total	55	42.9821			

Appendix 3.12: Analysis of variance for stem diameter of cancer bush under microplot condition in summer

Source	DF	SS	MS	F	P
Replication	6	0.00866	1.443E-03		
Treatment	7	0.01853	2.647E-03	0.40	0.8942
Error	42	0.27491	6.545E-03		
Total	55	0.30210			

Appendix 3.13: Analysis of variance for fresh shoot mass of cancer bush under microplot condition in summer

Source	DF	SS	MS	F	P
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Replication	6	0.50587	0.08431		
Treatment	7	0.14336	0.02048	0.33	0.9366
Error	42	2.61833	0.06234		
Total	55	3.26756			

Appendix 3.14: Analysis of variance for dry shoot mass of cancer bush under microplot condition in summer

Source	DF	SS	MS	F	P
Replication	6	0.32812	0.05469		
Treatment	7	0.20765	0.02966	0.80	0.5921
Error	42	1.55805	0.03710		
Total	55	2.09382			

Appendix 3.15: Analysis of variance for root galls in cancer bush under shade net condition in summer

Source	DF	SS	MS	F	P
Replication	6	0.30335	0.05056		
Treatment	7	1.14092	0.16299	2.84	0.0162
Error	42	2.41040	0.05739		
Total	55	3.85468			

Appendix 3.16: Analysis of variance for *Meloidogyne enterolobii* J2 in soil under shade net condition in summer

Source	DF	SS	MS	F	P
Replication	6	7.7199	1.28664		
Treatment	7	9.9690	1.42414	2.85	0.0158
Error	42	20.9631	0.49912		
Total	55	38.6520			

Appendix 3.17: Analysis of variance for *Meloidogyne enterolobii* J2 in roots of cancer bush under shade net condition in summer

Source	DF	SS	MS	F	P
Replication	6	7.487	1.24785		
Treatment	7	44.826	6.40369	4.58	0.0007
Error	42	58.710	1.39785		
Total	55	111.022			

Appendix 3.18: Analysis of variance for *Meloidogyne enterolobii* eggs in roots of cancer bush under shade net condition in summer

Source	DF	SS	MS	F	P
Replication	6	3.500	0.58327		
Treatment	7	50.146	7.16372	4.96	0.0004
Error	42	60.714	1.44558		
Total	55	114.360			

Appendix 3.19: Analysis of variance for *Meloidogyne enterolobii* final population in cancer bush under shade net condition in summer

Source	DF	SS	MS	F	P
Replication	6	4.976	0.82934		
Treatment	7	51.665	7.38071	4.85	0.0005
Error	42	63.957	1.52278		
Total	55	120.598			

Appendix 3.20: Analysis of variance for *Meloidogyne enterolobii* reproduction factor in cancer bush under shade net condition in summer

Source	DF	SS	MS	F	P
Replication	6	0.7997	0.13328		

Treatment	7	2.1348	0.30498	1.67	0.1416
Error	42	7.6479	0.18209		
Total	55	10.5825			

Appendix 3.21: Analysis of variance for chlorophyll in cancer bush under shade net condition in summer

Source	DF	SS	MS	F	P
Replication	6	1121.1	186.845		
Treatment	7	1420.7	202.952	1.03	0.4221
Error	42	8241.4	196.225		
Total	55	10783.2			

Appendix 3.22: Analysis of variance for plant height of cancer bush under shade net condition in summer

Source	DF	SS	MS	F	P
Replication	6	209848	34974.7		
Treatment	7	68571	9795.9	1.36	0.2487
Error	42	303216	7219.4		
Total	55	581636			

Appendix 3.23: Analysis of variance for plant number of branches of cancer bush under shade net condition in summer

Source	DF	SS	MS	F	P
Replication	6	1.7500	0.29167		
Treatment	7	4.2143	0.60204	1.23	0.3076
Error	42	20.5357	0.48895		
Total	55	26.5000			

Appendix 3.24: Analysis of variance for plant stem diameter of cancer bush under shade net condition in summer

Source	DF	SS	MS	F	P
Replication	6	1.52467	0.25411		
Treatment	7	0.68702	0.09815	0.53	0.8059
Error	42	7.76013	0.18476		
Total	55	9.97182			

Appendix 3.25: Analysis of variance for fresh shoot mass of cancer bush under shade net condition in summer

Source	DF	SS	MS	F	P
Replication	6	0.43272	0.07212		
Treatment	7	0.32966	0.04709	1.16	0.3451
Error	42	1.70297	0.04055		
Total	55	2.46534			

Appendix 3.26: Analysis of variance for dry shoot mass of cancer bush under shade net condition in summer

Source	DF	SS	MS	F	P
Replication	6	0.28132	0.04689		
Treatment	7	0.22829	0.03261	1.38	0.2381
Error	42	0.99096	0.02359		
Total	55	1.50057			

Appendix 4.1: Shapiro-Wilk normality test for variables measured on Cancer bush exposed to *Meloidogyne enterolobii* under microplot conditions during winter

Variable	N	W	P
Root galls	56	0.8774	0.0000
J2 in Soil	56	0.6547	0.0000
J2 in Roots	56	0.9417	0.0092
Eggs in Roots	56	0.9522	0.0267
Final population	56	0.9314	0.0034

Reproductive factor	56	0.7134	0.0000
Chlorophyll	56	0.5695	0.0000
Plant height	56	0.9606	0.0647
Number of branches	56	0.2803	0.0000
Stem diameter	56	0.9213	0.0013
Fresh shoot mass	56	0.8724	0.0000
Dry shoot mass	56	0.9132	0.0007

Appendix 4.2: Shapiro-Wilk normality test for variables measured on Cancer bush exposed to *Meloidogyne enterolobii* under shade net conditions during winter

Variable	N	W	P
Root galls	56	M	M
J2 in soil	56	0.8335	0.0000
J2 in roots	56	0.8405	0.0000
Eggs in roots	56	0.8317	0.0000
Final population	56	0.9307	0.0032
Reproductive Factor	56	0.5293	0.0000
Chlorophyll	56	0.4652	0.0000
Plant height	56	0.9695	0.1661
Number of branches	56	0.2910	0.0000
Stem diameter	56	0.9838	0.6511
Fresh shoot mass	56	0.9617	0.0725
Dry shoot mass	56	0.9249	0.0019

Appendix 4.3: Analysis of variance for root galls on cancer bush under microplot condition in winter

Source	DF	SS	MS	F	P
Replication	6	4.3571	0.72619		
Treatment	7	21.1250	3.01786	6.18	0.0001

Error	42	20.5000	0.48810		
Total	55	45.9821			

Appendix 4.4: Analysis of variance for *Meloidogyne enterolobii* J2 in soil under microplot conditions in winter

Source	DF	SS	MS	F	P
Replication	6	196786	32797.6		
Treatment	7	596429	85204.1	1.48	0.2025
Error	42	2426071	57763.6		
Total	55	3219286			

Appendix 4.5: Analysis of variance for *Meloidogyne enterolobii* J2 in roots of cancer bush under microplot conditions in winter

Source	DF	SS	MS	F	P
Replication	6	226786	37798		
Treatment	7	757143	108163	6.05	0.0001
Error	42	750357	17866		
Total	55	1734286			

Appendix 4.6: Analysis of variance for *Meloidogyne enterolobii* eggs in roots of cancer bush under microplot conditions in winter

Source	DF	SS	MS	F	P
Replication	6	141071	23512		
Treatment	7	1132679	161811	4.54	0.0008
Error	42	1496071	35621		
Total	55	2769821			

Appendix 4.7: Analysis of variance for *Meloidogyne enterolobii* final population in cancer bush under microplot conditions in winter

Source	DF	SS	MS	F	P
Replication	6	478571	79762		
Treatment	7	6244107	892015	8.28	0.0000
Error	42	4527143	107789		
Total	55	1.125E+07			

Appendix 4.8: Analysis of variance for *Meloidogyne enterolobii* reproductive factor in cancer bush under microplot conditions in winter

Source	DF	SS	MS	F	P
Replication	6	68.88	11.480		
Treatment	7	5556.18	793.741	59.42	0.0000
Error	42	561.06	13.358		
Total	55	6186.12			

Appendix 4.9: Analysis of variance for chlorophyll in cancer bush under microplot conditions in winter

Source	DF	SS	MS	F	P
Replication	6	35.612	5.93536		
Treatment	7	20.876	2.98222	1.42	0.2225
Error	42	88.131	2.09835		
Total	55	144.618			

Appendix 4.10: Analysis of variance for plant height of cancer bush under microplot conditions in winter

Source	DF	SS	MS	F	P
Replication	6	1347.9	224.655		

Treatment	7	6691.8	955.977	1.86	0.1017
Error	42	21637.8	515.185		
Total	55	29677.6			

Appendix 4.11: Analysis of variance for number of branches of cancer bush under microplot conditions in winter

Source	DF	SS	MS	F	P
Replication	6	1.10714	0.18452		
Treatment	7	1.35714	0.19388	1.18	0.3340
Error	42	6.89286	0.16412		
Total	55	9.35714			

Appendix 4.12: Analysis of variance for stem diameter of cancer bush under microplot conditions in winter

Source	DF	SS	MS	F	P
Replication	6	16.0402	2.67336		
Treatment	7	1.5492	0.22132	0.57	0.7791
Error	42	16.4220	0.39100		
Total	55	34.0114			

Appendix 4.13: Analysis of variance for fresh shoot mass of cancer bush under microplot conditions in winter

Source	DF	SS	MS	F	P
Replication	6	0.22118	0.03686		
Treatment	7	0.37480	0.05354	2.77	0.0186
Error	42	0.81322	0.01936		
Total	55	1.40920			

Appendix 4.14: Analysis of variance for dry shoot mass of cancer bush under microplot conditions in winter

Source	DF	SS	MS	F	P
Replication	6	0.01234	2.057E-03		
Treatment	7	0.00976	1.395E-03	1.08	0.3961
Error	42	0.05449	1.297E-03		
Total	55	0.07659			

Appendix 4.15: Analysis of variance for root galls in cancer bush under shade net conditions in winter

Source	DF	SS	MS	F	P
Replication	6	0.00000	0.00000		
Treatment	7	0.00000	0.00000	M	M
Error	42	0.00000	0.00000		
Total	55	0.00000			

Appendix 4.16: Analysis of variance for *Meloidogyne enterolobii* J2 in soil under shade net conditions in winter

Source	DF	SS	MS	F	P
Replication	6	20000	3333.3		
Treatment	7	305714	43673.5	7.21	0.0000
Error	42	254286	6054.4		
Total	55	580000			

Appendix 4.17: Analysis of variance for *Meloidogyne enterolobii* J2 in roots of cancer bush under shade net conditions in winter

Source	DF	SS	MS	F	P
Replication	6	32500	5416.7		

Treatment	7	131429	18775.5	2.21	0.0521
Error	42	356071	8477.9		
Total	55	520000			

Appendix 4.18: Analysis of variance for *Meloidogyne enterolobii* eggs in roots of cancer bush under shade net conditions in winter

Source	DF	SS	MS	F	P
Replication	6	84643	14107.1		
Treatment	7	365000	52142.9	5.58	0.0001
Error	42	392500	9345.2		
Total	55	842143			

Appendix 4.19: Analysis of variance for *Meloidogyne enterolobii* final population in cancer bush under shade net conditions in winter

Source	DF	SS	MS	F	P
Replication	6	167143	27857		
Treatment	7	1999286	285612	13.10	0.0000
Error	42	915714	21803		
Total	55	3082143			

Appendix 4.20: Analysis of variance for *Meloidogyne enterolobii* reproduction factor in cancer bush under shade net conditions in winter

Source	DF	SS	MS	F	P
Replication	6	86.31	14.385		
Treatment	7	728.92	104.131	8.16	0.0000
Error	42	535.71	12.755		
Total	55	1350.94			

Appendix 4.21: Analysis of variance for chlorophyll in cancer bush under shade net conditions in winter

Source	DF	SS	MS	F	P
Replication	6	3828.8	638.137		
Treatment	7	858.6	122.662	0.61	0.7422
Error	42	8408.7	200.207		
Total	55	13096.2			

Appendix 4.22: Analysis of variance for plant height of cancer bush under shade net conditions in winter

Source	DF	SS	MS	F	P
Replication	6	46840	7806.7		
Treatment	7	80101	11443.1	1.48	0.1990
Error	42	323705	7707.3		
Total	55	450647			

Appendix 4.23: Analysis of variance for number of branches of cancer bush under shade net conditions in winter

Source	DF	SS	MS	F	P
Replication	6	1.1786	0.19643		
Treatment	7	3.2679	0.46684	1.30	0.2751
Error	42	15.1071	0.35969		
Total	55	19.5536			

Appendix 4.24: Analysis of variance for stem diameter of cancer bush under shade net conditions in winter

Source	DF	SS	MS	F	P
Replication	6	4.5321	0.75536		

Treatment	7	4.3027	0.61467	1.74	0.1257
Error	42	14.8336	0.35318		
Total	55	23.6684			

Appendix 4.25: Analysis of variance for fresh shoot mass of cancer bush under shade net conditions in winter

Source	DF	SS	MS	F	P
Replication	6	3.6634	0.61056		
Treatment	7	5.8379	0.83398	1.53	0.1823
Error	42	22.8291	0.54355		
Total	55	32.3304			

Appendix 4.26: Analysis of variance for dry shoot mass of cancer bush under shade net conditions in winter

Source	DF	SS	MS	F	P
Replication	6	0.24105	0.04017		
Treatment	7	0.21118	0.03017	1.21	0.3197
Error	42	1.04895	0.02498		
Total	55	1.50118			

Appendix 4.27: Interactive effect of treatment, season and growing season on final population (PF)

Source	DF	SS	MS	F	P
Replication	6	0.769	0.1281		
Treatment	7	175.824	25.1177	52.03	0.0000
Season	1	1.773	1.7726	3.67	0.0569
Growing condition	1	21.353	21.3530	44.23	0.0000
Treatment*Season	7	3.035	0.4336	0.90	0.5092

Treatment*Growing condition	7	16.004	2.2863	4.74	0.0001
Season*Growing condition	1	1.939	1.9394	4.02	0.0465
Treatment*Season*Growing condition	7	6.303	0.9004	1.87	0.0775
Error	186	89.796	0.4828		
Total	223	316.796			

Appendix 4.28: Interactive effect of treatment, season and growing season on reproductive factor (RF)

Source	DF	SS	MS	F	P
Replication	6	0.00948	0.00158		
Treatment	7	2.24605	0.32086	51.36	0.0000
Season	1	0.00046	0.00046	0.07	0.7870
Growing condition	1	0.29815	0.29815	47.72	0.0000
Treatment*Season	7	0.05354	0.00765	1.22	0.2914
Treatment*Growing condition	7	0.35498	0.05071	8.12	0.0000
Season*Growing	1	0.00166	0.00166	0.27	0.6073
Treatment*Season*Growing condition	7	0.11601	0.01657	2.65	0.0123
Error	186	1.16203	0.00625		
Total	223	4.24235			