DEGREE OF NEMATODE RESISTANCE IN ROSEMARY ON *MELOIDOGYNE* SPECIES AND RELATED MECHANISM OF RESISTANCE

by

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RECOMMENDATIONS AND CONCLUSIONS

DECLARATION

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

Surname, Initials (title)	Date

DEDICATION

To my grandmother (Ms Wehleminah Midally Gaila), mother (Mrs Dipuo Elizabeth Shalang) and lovely siblings (Oratile Shalang, Koketso Shalang and Boikhutso Shalang).

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ABSTRACT

Tropical root-knot nematodes, *Meloidogyne enterolobii*, *M. incognita* and *M. javanica*, are highly damaging and difficult pests to manage in agricultural crops. The use of resistant cultivars had been the most promising strategy, which is currently among the proposed alternative nematode management strategies when appropriate cultivars are available in the management of nematode population densities. However, the main challenge associated with the estimation of the degree of nematode resistance is the absence of consistent guidelines for testing resistance. In order to identify plants with a source of resistance to *Meloidogyne* species, usually specific protocols are employed. Current methods for assessing resistance, such as the reproductive factor (RF), egg-mass index (EI), gall index (GI) and index of reproduction (IR), have associated limitations. The concept of relative susceptibility (rs) is increasingly used as a standardised quantitative method for testing the degree of nematode resistance. Therefore, the objective of this study was to determine whether rs of rosemary to tomato for *M. enterolobii, M. incognita* and *M. javanica* would either be above one or affect relative accumulation of selected mineral nutrient elements. Rosemary cuttings and tomato seedlings were raised in polystyrene trays containing Hygromix-T and transplanted into 20-cm diameter plastic pots containing steam-pasteurised soil. At two weeks after transplanting, plants were inoculated with 250 eggs of each *Meloidogyne* species, arranged in a randomised complete block design, replicated 10 times. Data were collected at 56 days after inoculation using maceration and blending method. Relative susceptibility, expressed as the ratio of final egg population density of a nematode population on a test variety (unknown resistance) compared to the population density of a standard reference variety (susceptible) was used for assessing the relative susceptibility of rosemary to cv. tomato 'Floradade'. Generally, since root sizes of different plants differ, nematodes in test plants were first standardised using reproductive potential (RP = total Pf/ g fresh roots) and when $rs_a > 1$, the test plant (*i.e*., rosemary) was relatively susceptible, but when $rs_a < 1$, the test plant was relatively resistant. Inoculation with *M. enterolobii* on rosemary/tomato resulted in $rs_a = 0.16$, *M. incognita* $rs_a = 0.37$ and *M. javanica* $rs_a = 0.16$. The infection by *M. javanica* slightly affected the relative accumulation of Mg nutrient element in leaf tissues of rosemary. In conclusion, rs_a values on rosemary were less than unity, suggesting that rosemary was relatively resistant to the three test *Meloidogyne* species.

CHAPTER 1

RESEARCH PROBLEM

1.1 Background

1.1.1 Description of the research problem

Rosemary (*Rosmarinus officinalis* L.) is a medicinal plant native to the Mediterranean region and is cultivated in most parts of the world (de Macedo *et al*., 2020). It has been used for thousands of years for both culinary and medicinal purposes due to its aromatic properties and health benefits (Szumny *et al*., 2010). In the agricultural industry, rosemary is known to possess insect-repellent and antimicrobial properties beneficial in growing and storage of crops (Sasikumar, 2012). Additionally, oil extracts from rosemary have been found to be very effective in controlling some nematodes species (El-Nagdi and Youssef, 2021). Many other countries including South Africa conform to the demand of functional foods from plants through harvesting certain organs which exposes most plants to extinction. Successive harvesting of leaves for the essential oil used in various products, resulted in increased need to cultivate the plant under field conditions (Katar *et al*., 2019). The reduction of any introduced crop under field conditions could be challenged by root-knot (*Meloidogyne* species) nematodes, which are polyphagous and widely distributed in tropical and subtropical regions (Costa *et al*., 2019; Sasser *et al*., 1984). Since the degree of nematode resistance in rosemary to tropical *Meloidogyne* species that occur in South Africa is not known, it would be imperative to assess this parameter prior to introducing the crop in the region.

1.1.2 Impact of the research problem

Nematodes have gained prominence among phytosanitary issues, owing to the significant damage they cause in production and the lack of effective control strategies (Mafessoni *et al*., 2019). Ghaderi and Karssen (2020) reported that 105 *Meloidogyne* species had been discovered as of February 2020. Given the ongoing discovery of new species, the listed figure would likely rise yearly (Blok and Powers, 2009). In South Africa the most common thermophilic root-knot nematodes are *Meloidogyne enterolobii (*Yang and Eisenback) (Yang and Eisenback, 1983), *Meloidogyne incognita* (Kofoid and White) (Kofoid and White, 1919) and *Meloidogyne javanica* (Treub) (Treub, 1885). These species are the most dispersed species in the tropical and subtropical regions (Coyne *et al*., 2018; Perry *et al*., 2009), with *M. enterolobii* emerging as a major threat due to its aggressiveness, short life span and wide host range (Collett, 2020). Additionally, the genus can reproduce on tomato genotypes that carry Mi resistant genes (Castagnone-Sereno, 2012). Generally, *Meloidogyne* species are highly damaging and a difficult pest to manage (Collett, 2020; Mashela *et al*., 2015; Taylor and Sasser, 1978). Collett (2020) indicated that in 2020, worldwide *M. enterolobii* was already having over 200 plant hosts. Infection by *Meloidogyne* species in various crops induce five different symptoms, namely: knobbling which occurs when galls appear along the length of the roots, tippling occurs when galls appear at the tip of the roots, clumping occurs when root forms clumps, cracking occurs on sweet potatoes as surface cracks and root deformation occurs mainly on carrots as they become forked (Coyne *et al*., 2014). The multiple symptoms are associated with reduced quality and quantity of crop produce (Anwar and Javed, 2010; Curtis, 2008; Mashela, 2002). Furthermore, resultant wounds, lesions and giant cells adjacent to the vascular bundle reduce the ability of roots to absorb water and mineral nutrients, affecting the overall growth and productivity of the plant (Costa *et al*., 2019). Plant nematodes do not only cause direct crop yield losses, but also contribute to aggravating the disease complexes that involve other pathogens (Kumar *et al*., 2020). Yield loss of economic crops escalated to 37% after the suspension of methyl bromide from the agrochemical markets which was used as a major chemical for suppressing nematode populations (Chitwood, 2003; Mashela *et al*., 2016). The total crop loss (per annum) in South Africa as a result of plant parasitic nematode infection has been estimated to be over R1.9 billion for both horticultural and agricultural crops, while the yearly crop loss due to plant parasitic nematodes has been estimated to be around US$157 billion worldwide (Sikandar *et al*., 2020; Singh *et al*., 2015; Steyn *et al*., 2014). In South Africa, plant nematodes are projected to inflict 14% of the yearly global output loss in grain, vegetable and fruit crops (ARC, 2014). Generally, nematode yield losses in crops without nematode resistance can be from as high as 60% to complete crop failure (Yadav and Patil, 2021).

1.1.3 Possible causes of the research problem

Managing population densities of *Meloidogyne* species is difficult due to the polyphagous nature of the genus, along with the existence of survival strategies in nematodes. As a form of survival strategy, *Meloidogyne* species produce gelatinous matrix (egg masses) covered in chitin found outside of galled roots and the first-stage juveniles (J1) in eggs enter the survival stage known as dauer stage, in which metabolic activities are discontinued when it is gradually subjected to unfavourable conditions like those of nematicide and/or botanicals. Chemicals are ineffective at killing nematodes while they are adapting and exiting the dauer phase is also sequential as a survival strategy (Mashela, 2007; Zhang *et al*., 2020). Over the years, farmers relied on a wide range of synthetic chemical nematicides, especially the fumigant nematicides, as one of the main primary tool for managing nematode population densities due to their ability to act faster (Ntalli and Caboni, 2012). Fumigant synthetic chemical nematicides, due to their high levels of phytotoxicity, were applied as pre-planting products, with a wide range of risks, especially those associated with the depletion of ozone layer, resulting in large quantities of ultraviolet rays reaching the earth (Strajnar and Sirca, 2011). Thus, fumigant chemical nematicides had, from 2005, internationally been withdrawn from the agrochemical markets. In contrast, non-fumigant nematicides such as organophosphates (*e.g.* phenamiphos), carbamates (*e.g*. aldicarb) and oxycarbamates (*e.g*. carbaryl) were hardly phytotoxic, but had own set of challenges (Rich *et al*., 2004). Primarily, the challenges arise from two different translocation movements: acropetal and basipetal. Acropetal translocation involves the movement of applied chemicals from roots to leaves or fruits, resulting in insect control and increased growth response, which occurred whether nematodes were present or absent. The products did not kill nematodes, but changed their behaviours, and were referred to as nematostatic products (Rich *et al*., 2004). On the other hand, basipetal translocation involves the movement of applied chemicals from leaves to roots, originally intended for insect control but has been found to control nematodes instead. In order to achieve effective control using non-fumigant nematicides, multiple applications are necessary. However, the non-fumigant nematicides have also been excluded from agrochemical markets due to high chemical residues in edible produce, which then poisoned man and his animals (Desaeger *et al*., 2017). Various nematode management strategies have since been tested, including the use of biological control agents (Migunova and

Sasanelli, 2021) and various cultural methods, which included crop rotations with plants that contain some degree of nematode resistance (Chiuta *et al*., 2021; Khan *et al*., 2021; Pofu *et al*., 2019). Despite the various methods employed, nematodes have not been effectively eradicated due to cryptobiosis. Cryptobiosis is a state of dormancy where the metabolic processes of an organism are paused, enabling it to survive in lethal conditions. There are six types of cryptobiosis, namely, anhydrobiosis, cryobiosis, osmobiosis, anoxybiosis, chemobiosis and thermobiosis (Clegg, 2001). Anhydrobiosis allows the organism to survive in a completely dry state while cryobiosis allows it to survive in a frozen state, osmobiosis enables it to survive in a high solute concentration state, anoxybiosis allows it to survive in a low or zero oxygen state, chemobiosis allows it to thrive in high levels of toxins or chemicals, and thermobiosis allows it to survive in extreme conditions such as drought and high temperatures. Additionally, the previously used methods in the management of nematodes, were often associated with high costs and labour requirements (Chitwood, 2003; Tapia-Vázquez *et al*., 2022). The use of resistant crops (Roberts, 1992) and phytonematicides (Mashela *et al*., 2017) to manage nematode populations is one of the strategies that had since been accepted with some attributes that would counter the challenges observed in conventional synthetic chemical nematicides.

1.1.4 Proposed solutions

Since nematicides have also not been effective in controlling *Meloidogyne* populations (Desaeger *et al*., 2017; Roze *et al*., 2008), particularly in terms of long-term nematode population reduction (Starr *et al*., 2002), management of nematodes has moved towards the use of resistance cultivars (Roberts, 1992). A resistant plant permits little or no nematode reproduction (Pofu *et al*., 2012), but damage may still occur if nematode densities at planting (Pi) exceed the damage tolerance limit. In crop rotation systems, it appears that most resistant crops fall short of reducing nematode population densities, particularly when the succeeding crops are vulnerable to nematode parasitism (McSorley, 2011; Pofu and Mashela, 2017). The main challenge associated with the estimation of the degree of nematode resistance is the absence of consistent guidelines for testing resistance posed a difficulty in the late 1980s, when resistance became a crucial factor for control purposes (Teklu, 2018). This was a significant goal of the international *Meloidogyne* consortium (1975-1984) supported by USAID, which aimed to establish a protocol for root-knot nematodes (Sasser *et al*., 1984). Despite various enhancements since then, a universally applicable method that can be replicated and implemented in various locations with consistent outcomes and no limitations is necessary for the desired outcomes (Teklu, 2018). The relative susceptibility estimator, which is based on population dynamic parameters, is a tool that can be utilised to measure the impact of rotation plans in managing nematodes. It has been effectively employed for potato (*Solanum tuberosum* L.) and potato cyst nematode (*Globodera pallida* Behrens.) (Stone, 1973) and resulted in a significant decrease in the use of agricultural chemicals and reduction of yield losses in The Netherlands (Teklu, 2018).

1.1.5 General focus of the study

The study focused on the use of rs in establishing the degree of nematode resistance in rosemary with respect to *M. incognita*, *M. javanica* and *M. enterolobii*.

1.2. Problem statement

Reliable and effective degree of nematode resistance procedures for identifying resistant plants are necessary for the identification of plants with a novel source of resistance against the three destructive root-knot *Meloidogyne* species (Norshie *et al*., 2011). In order to identify plants with a source of resistance to *Meloidogyne* species, usually specific protocols are employed. Current methods for assessing resistance, such as the reproduction factor, egg-mass index, gall index and index of reproduction have limitations (Teklu, 2018). Reproductive factor (RF), first proposed by Oostenbrink in 1966 does not designate a certain degree of resistance, such as resistant, tolerant or susceptible (Sasser *et al*., 1984). The egg-mass index (EI), which Taylor and Sasser explained (Taylor and Sasser, 1978) was found to be non-empirical in assessing degree of resistance given different number of eggs in different egg masses (Sasser *et al.,* 1984). Similar to the EI, the gall index (GI) was not logic, since it uses the same scale as the EI. Index of reproduction (IR) scale could not be accurately applied to other crops, additionally, Sasser *et al*. (1984) indicated that IR is merely a comparative and relative assessment of host effectiveness rather than an accurate indicator of host resistance. Seinhorst (1984) and Phillips and Trudgill (1984) proposed the concept of relative susceptibility (rs) as a result of the necessity of a standardised quantitative method based on actual nematode reproduction in the host and damage to the plant. The idea of rs was found to be necessary and used successfully in potato cyst nematode investigations as it has promoted an increased uniformity of host-resistance designations and thereby allowed a broader interpretation and comparison of results. Relative susceptibility is calculated by comparing the multiplication rate of reproduction (a) of the variety under consideration to the multiplication rate of a reference crop. The plant is considered resistant to the test nematode if rs values at all levels of inoculation are below one and the nematode did not cause damage to the test plant and if rs is greater than one and the plant suffered from nematode damage, the plant is susceptible (Seinhorst, 1967).

1.3. Rationale of the study

The use of resistant varieties is by far the most promising method for managing plant parasitic nematodes (Khanzada *et al*., 2012). Among the plant-parasitic nematode genera, *Meloidogyne* is the most widely distributed nematode genus in the world (Karssen *et al.*, 2013). The information concerning the host status of many crops, currently regarded as hosts of *Meloidogyne* species is uncertain. Thorough research on the host status using protocols such as relative susceptibility might reveal that many crops, now designated as moderate or good hosts, are in fact bad hosts (Teklu, 2018). Apart from ranking varieties into different resistance classes, rs also permits the use of partially resistant varieties in rotation trials (Mwaura *et al*., 2015). Relative susceptibility is becoming increasingly used to measure the level of resistance since it offers a bridge between screening of multiple hosts and the degree of nematode resistance in a single crop but using one nematode level, where both host-status and host-sensitivity are determined. The South African Department of Science and Innovation has introduced a bio-economy strategy in 2013, which includes promoting the cultivation of commercially important indigenous medicinal plants as an adaptation strategy to protect the environment (Mofokeng *et al*., 2020). Rosemary amongst several other medicinal plants is identified as a possible crop that can be used in nematode population management strategies.

1.4 Purpose of the study

1.4.1 Aim

Assessment of the degree of resistance in rosemary to *Meloidogyne* species using relative susceptibility.

1.4.2. Objective

To determine whether relative susceptibility of rosemary to tomato for *M. enterolobii, M. incognita* and *M. javanica* would be above one and affect the relative accumulation of selected mineral nutrient elements.

1.4.3. Null hypothesis

Relative susceptibility of rosemary to tomato for *M. enterolobii, M. incognita* and *M. javanica* would neither be above one nor affect the relative accumulation of selected mineral nutrient elements.

1.5 Reliability, validity and objectivity

The degree to which a measuring instrument produces consistent results when the variable being measured is repeated without change is referred to as reliability (Leedy and Ormrod, 2005). The reliability of various experiments was ensured. The extent to which an instrument measures what was intended to be measured is referred to as validity (Leedy and Ormrod, 2005). Validity of the experiment was ensured by conducting the study at the same location over time (Little and Hills, 1981). Objectivity is defined as striving to eliminate biases, prejudices, or subjective evaluations as much as possible by relying on verifiable data (Leedy and Ormrod, 2005). Objectivity was achieved by discussing the findings and comparing them to the findings of other studies (Little and Hills, 1981).

1.6 Bias

Bias is defined as any influence, condition, or set of conditions that distorts data either singularly or collectively (Leedy and Ormrod, 2005). Bias was reduced in this study by ensuring that the experimental error in each experiment was reduced through increased replications and treatment randomisation (Little and Hills, 1981).

1.7 Scientific significance of the study

The study would provide useful information on the relative susceptibility of rosemary versus tomato to three *Meloidogyne* species. Large scale farming of this crop would not only meet the demands of big industries like the culinary and pharmaceutical, but it would also help in revenue generation, job and wealth creation.

1.8 Structure of mini-dissertation

Following the detailed outline of the research problem (Chapter 1), work done on the research problem was reviewed and work not done was clearly outlined in Chapter 2. Then, Chapters 3 addressed the single research objective. The findings from chapter 3 were summarised and integrated in the final chapter to give the relevance of the findings, as well as recommendations for future research and conclusions that connected the entire study together. This mini-dissertation followed the Harvard style using author-alphabets as approved by University of Limpopo Senate. Following a detailed appraisal of the research problem, the work done and work not done on the research problem would be thoroughly reviewed.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Meloidogyne genus is polyphagous, infecting a wide variety of agricultural crops as well as non-host crops (e.g. natural veld and weeds), causing catastrophic losses (Coyne *et al*., 2018; Onkendi *et al*., 2014; Varandas *et al*., 2020). In warm regions of South Africa, the most common root-knot nematode species identified as constraints to agricultural production generally are *M. incognita* and *M. javanica* (Kleynhans *et al*., 1996). Furthermore, the recent discovery of *M. enterolobii* in production areas in the Highveld of Mpumalanga and Limpopo Provinces, qualifies it as a potential threat species to be added to the list of common root-knot nematode species that may damage staple crops (Collett, 2020; Rashidifard *et al*., 2019). Karajeh (2008) reported that about 5% of the world's crop production is destroyed annually by *Meloidogyne* species depending on the level of infestation and the species planted (Costa *et al*., 2019). When crops are sown in locations with significant nematode populations, total crop failure is common (Grabau and Noling, 2019). Damage caused by nematodes to plants is directly proportional to their population densities in soil and their reproduction potentials on the plant (Barker and Olthof, 1976). The impact of nematodes in the plant is mostly through the management strategies used for their control (Costa *et al*., 2019) and majority of strategies used were associated with groundwater contamination, toxicity to living organisms and residues in food products which led to severe restrictions on chemical control (Moreira *et al*., 2017). Therefore, host plant resistance has been identified as the most affordable, safe and efficient method of nematode control (Bird and Kaloshian, 2003). The population and the reproduction of nematodes in the soil are inhibited using resistant plants secreting harmful substances during their secondary metabolism (Costa *et al*., 2019).

2.2 Work done on the research problem

2.2.1 Descriptors of nematode resistance

Seinhorst (1965) proposed a framework for characterising the relationships between nematodes and plants in order to identify susceptible, tolerant and resistant hosts. Host status is typically assessed in plant nematodes by measuring either the reproductive potential (RP = Pf/g fresh roots) of several cultivars inoculated at a single level or multiplication rate (MR = Pf/Pi), where Pf represents the final nematode population density and Pi represents the initial nematode population density (Pofu *et al*., 2010; Seinhorst, 1965). The determination of the degree of nematode resistance and interpretation of MR values requires an understanding of the equilibrium (E) point for nematode reproduction. Seinhorst (1967) noted that at the E point, where the final population is equal to the initial population (Pf = Pi), MR values may be either above or below unity. However, beyond the E point, MR values are always below one due to competition for infection sites and food resources. In contrast, if the plant is susceptible, MR values are consistently greater than one at the E point, as the nematode population is at its lowest competition level (Pofu, 2012). In general, susceptible hosts (MR > 1) are plants that allow the formation of giant cells by *Meloidogyne* species, thereby providing nourishment for nematode growth, development, and reproduction (Hussey and Grundler, 1998). Tolerant hosts (MR > 1) are plants that permit the penetration of J2 and subsequent development into adults, as well as feeding and reproduction, without suffering from nematode-induced damage

(Seinhorst, 1967; Trudgill, 1992). In contrast, resistant hosts (MR < 1) either prevent J2 from penetrating their roots with pre-infectional nematode resistance or, in plants with post-infectional nematode resistance, hinder the movement or feeding of J2, resulting in their failure to reach maturity (Pofu and Mashela, 2011; Seinhorst, 1967; Taylor and Sasser, 1978).

The host response of *Capsicum frutescens* (L) (García *et al*., 2016) cv. 'Capistrano' to *M. incognita* race 2 revealed MR less than one without nematode infection effecting plant development, the cultivar was considered resistant to *M. incognita* race 2 (Mashela and Pofu, 2012). Hussain *et al*. (2014) evaluated twelve okra cultivars for susceptibility and resistance to *M. incognita*. Maximum galls (> 100) were recorded on the roots of cv. 'Sharmeeli,' suggesting a high degree of susceptibility. Anmol and Okra Sindha cultivars were discovered to be susceptible (71-100 galls). The cultivars 'Sabz Pari', 'Super Star', 'PMS-55' and 'PMS Beauty' appeared to be moderately susceptible (31-70 galls). The cultivars 'Sanam', 'Dikshah', 'Arka Anamika', 'Ikra-1' and 'Ikra-2' (11-30 galls) were rated as moderately resistant because they showed less nematode damage than susceptible, moderately and highly susceptible cultivars. Moreover, Moreira *et al*. (2017) tested the vulnerability of 30 species, 20 ornamental and ten medicinal to *M. incognita*. Five species of medicinal plants had no galls in their root systems, whereas the remaining species, including rosemary, were somewhat vulnerable, with few galls and/or females isolated with their roots. Nkosi (2019) on the degree of nematode resistance in sweet potato cv. 'Mafutha' to *M. javanica*, *M. incognita* race 2 and *M. incognita* race 4 under greenhouse conditions exposed the vulnerability of sweet potato and revealed that nematode infection hampered the growth of sweet potato cv. 'Mafutha,'. Plants of the Y50 accession were found to be resistant to *M. enterolobii*, indicating variability of reaction of plants to the nematode within the *Psidium* genus (De Oliveira *et al*., 2019).

Alternatively, in the screening study to investigate the reaction of 11 medicinal and aromatic plant species to approximately 3 000 newly hatched J2 of *M. javanica* under greenhouse conditions, rosemary was classified as being susceptible with the highest values of nematode in eggs produced and final population among the test plants (El-Mesalamy *et al*., 2020). The host status of 17 medicinal plant species was assessed to the root-knot nematode (*M. incognita*) susceptibility. Most tested plant species were incompatible with nematode infection according to nematode reproduction and ranked them as follows, periwinkle (*Catharanthus roseus* L.) (Jacobs *et al*., 2004), geranium (*Pelargonium peltatum* L.) (Hutchinson, 1969), peppermint (*Mentha piperita* L.) (Tucker and Naczi, 2007) and sage (*Salvia officinalis* L.) (Webb *et al*., 1964) were immune (I), common mint (*Mentha spicata* L.) (Ali-Shtayeh *et al*., 2019), horsemint (*Monarda puctata* L.), marjoram (*Origanum majorana* L.) (Shirley, 2012), rosemary and goldenrod (*Giant goldenrod* Aiton.) (Weber and Jakobs, 2005) were highly resistant (HR), coriander (*Coriandrum sativum* L.) (Hedge and Lamond, 1972), dill (*Anethum graveolens* L.) (Mozaffarian, 2007) and thyme (*Thymus vulgaris* L.) (Stahl-Biskup and Venskutonis, 2004) were ranked as resistant (R), caraway (*Carum carvi* L.) (Ruszkowska, 1998), parsley (*Petroselinum crispum* Mill.) (Petropoulos *et al.*, 2004) and chamomile were moderately susceptible, sweet basil was susceptible and marigold (*Calendula arvensis* L.) (Heyn and Joel, 1983) was the highly susceptible host. Five plant species were classified as highly resistant (common mint, horsemint, marjoram, rosemary and goldenrod) (Al-Sayed *et al*., 2022).

2.2.2 Relative susceptibility

Phillips (1984) and Seinhorst (1984) proposed a mathematical model or equation to summarise and describe the relationship between plant yield of susceptible and non-host plants in the presence of nematode species with only one generation per season (monocyclic). The model identifies two crucial parameters: the maximum rate of reproduction (α) at very low initial population densities and a theoretical maximum density (M) of eggs that would have been produced per unit weight of soil at very high initial nematode densities if the plant size had not been reduced by the nematodes (Seinhorst, 1993). Initially, the model was developed for the potato cyst nematode (*Globodera pallida* Behrens.) (Stone, 1973) on partially resistant potato cultivars (*Solanum tuberosum* L.) (Phillips, 1984; Seinhorst and Oostrom, 1984). Relative susceptibility (rs) is calculated as the ratio of nematode populations on a tested cultivar and a susceptible reference cultivar (αresistant/αsusceptible) or the equivalent ratio of M on these cultivars (Mresistant/Msusceptible) (Phillips, 1984). These ratios are unaffected by external conditions that may influence both α and M and provide two measures of relative susceptibility or partial resistance (Been *et al*., 1995). Although rs_{α} and rs_{M} are usually identical, it is recommended that rs be determined through a simple pot test at a sufficiently low population density of eggs to obtain estimates of both α on the tested and susceptible controls, while still obtaining a high enough final population to obtain accurate Pf estimates (Norshie *et al*., 2011). Forrest and Holliday (1979) recommended that Pf/Pi be quoted in terms of eggs rather than cysts, as eggs are the survival stage in nematodes (Müller, 1953). Some nematode species, such as *Meloidogyne*, have multiple generations per season and can lay a high number of eggs, leading to a significant population increase within a year (Ehwaeti *et al*., 2000; Eurofins Agro, 2023). At low inoculation levels, for instance, 250 eggs, the maximum rate of reproduction (a) of both the susceptible crop and the reference crop is higher due to the lack of competition for resources, allowing for accurate estimations of nematode resistance. The known nematode-susceptible crop used is entirely susceptible and produces a 100% multiplication rate. For example, if a partially resistant variety has a 10% relative susceptibility, its multiplication rate will be ten times greater than that of a susceptible crop (Eurofins Agro, 2023).

Phillips *et al*. (1979) proposed that different multiplication rates of reproduction are the result of interactions between genotype and environment. This method has been mainly used in the Netherlands since 1999 and has also been introduced to the European Union (Norshie *et al*., 2011). Recently, Norshie *et al.* (2011) utilised the concept of rs to investigate the population dynamics of the polycyclic *Meloidogyne* species (*M. chitwoodi* Golden, O'Bannon.) (Santo and Finley, 1980), whereby the European resistance ranking system was utilised in a manner such that a given rs value was assigned. Plants that demonstrated high levels of resistance were assigned the top rank of 1, which corresponded to *a* rs of over 100%. Those with a rs value between 50.1-100% were assigned rank 2, while those with a rs value between 25.1-50% were assigned rank 3. Similarly, plants with a rs value between 15.1-25%, 10.1-15%, 5.1-10%, 3.1-5%, and 1.1-3% were assigned ranks 4, 5, 6, 7, and 8 respectively. Finally, plants that demonstrated a rs value less than 1% were assigned rank 9. Since the population dynamics of *Meloidogyne* species are distinct from that of *Globodera* species. The findings provided the first valuable insight into the population dynamic model for polycyclic nematodes, describing the relationship between initial nematode density (Pi) and the nematode densities at harvest (Pf) on susceptible and tested partially resistant cultivars. This allows for the same simple test as developed for the potato cyst nematode. Given the limited literature available on the relative susceptibility of rosemary (*Rosmarinus officinalis* L.) (de Macedo *et al*., 2020) to tomato (*Solanum lycopersicum* L.) (Melomey *et al*., 2019), the present review relied on studies that employed the concept of relative susceptibility.

Seinhorst *et al*. (1995) on the rs of eleven potato cultivars and breeders clones to *G. pallida* pathotype Pa 3, revealed that the rs_a and rs_M, of the tested cultivars and breeders clones ranged between 0.50 and 0.15, respectively. Norshie *et al*. (2011) tested the three new potato cultivars, designated 'AR 04-4107', 'AR 04-4096' and 'AR 04-4098' for resistance towards *M. chitwoodi* and revealed that cv. 'AR 04-4107', 'AR 04-4096' and 'AR 04-4098' had rs_a values of 1.7%, 0.8% and 2.8%, respectively. Partial resistance expressed as rs_M was 0.2%, 0.2% and 0.1%, respectively. It was concluded that 'AR 04-4107', 'AR 04-4096' and 'AR 04-4098' are strongly partially resistant to *M. chitwoodi*. The fodder radish (*Raphanus raphanistrum* L.) (Schroeder, 1989) cultivars 'Anaconda', 'Contra', 'Defender', 'Doublet' and 'Terranova', known to have some partial resistance, were compared to the standard cultivar, 'Radical', to estimate their relative susceptibility (rs) in which 'Radical' proved to be a bad host for *Meloidogyne* species with rs values of 0.17, 0.10, 0.42, 0.32 and 0.14%, respectively reducing high nematode population by more than 98% (Teklu *et al*., 2014).

Teklu *et al*. (2016) tested the population dynamics of *M. chitwoodi* on eight potato cultivars compared to the susceptible cv. Desiree in four glasshouse experiments and revealed that seven cultivars had average rs_a and rs_M smaller than 0.29%. Relative susceptibility and tolerance of thirteen Egyptian wheat cultivars to the cereal cyst nematode (*Heterodera avenae* Woll.) (Nicol and Rivoal, 2008) were tested under greenhouse conditions, revealed that cv. 'Misr-1', 'Misr-2', 'Misr-3' and 'Shandaweel' were highly susceptible to *H. avenae* with rs more than 92% (Korayem and Mohamed, 2019).

The relative susceptibility to root-knot nematode *M. incognita* indicated that two fodder beet cultivars 'Beta Rozsa' and 'Jamon' were the best with high yield and resistant reaction, in contrast cv. 'Starmon' had highly susceptible reaction, whereas cultivars 'Jary', 'Mnro' and 'Vorosch' had moderately resistant reactions (El-Nagdi *et al*., 2021). The host responses of six commercial watermelon (*Citrullus lanatus* Thunb.) cultivars assessed under glasshouse conditions to single-species populations of *Meloidogyne incognita, M. javanica* and *M. enterolobii* based on the number of egg masses, Pf, RF and rs revealed that all watermelon cultivars were susceptible to the predominant single-species populations of *Meloidogyne* species (Bello *et al*., 2021).

2.2.3 Accumulation of mineral nutrient elements as affected by *Meloidogyne* species

According to Chávez-Servín *et al*. (2017), the availability of nutrients in plants can be impacted by various factors, including the cultivar and environmental conditions such as soil type, temperature, rainfall, solar radiation, pests and diseases. The presence of nematode infection can cause significant alterations in the accumulation of nutrient elements in the leaf tissues of different crops (Mashela 1992; Melakeberhan *et al*. 1987; Santana-Gomes *et al*., 2013). Such changes in nutrient accumulation might have implications on the quality of produce. Root-knot nematodes can penetrate the plant, feed on its tissues and extract nutrients, leading to mechanical damage and physiological changes. Nutrients can influence disease severity, affect the environment in terms of attracting or deterring pathogens and induce resistance or tolerance in the host plant, as noted by Santana-Gomes *et al*. (2013) and Agrios (2005).

In the study by Carneiro *et al*. (2002), it was observed that infection of the two soybean (*Glycine max*) cultivars ‘Ocepar' and 'BR 16' by *M. incognita* and *M. javanica* interferes with Ca, N and P uptake and translocation. The concentrations of Ca, Fe, K, Mg and Zn on hybrid Sorghum-Sudan (*Sorghum drummondii* L.) grass resistant to *M. javanica*, *M. incognita* race 2 and *M. incognita* race 4 was affected by nematode infection (Selapa, 2021). Mahapatra and Nayak (2020), revealed that, of the seventeen bitter gourd plants infected with *M. incognita*, the shoot K content of both susceptible and resistant plants increased whereas shoot and root concentration of Zn decreased by 19.00-21.76% and 9.53-23.93%, Fe by 3.11-4.1% and 14.35- 33.85%, respectively. *Phaseolus vulgaris* plants infected with *Meloidogyne incognita* under controlled conditions revealed the concentration of K increased, whereas Ca and Fe decreased, with duration of infection in all treatments. Zinc decreased in the highest nematode treatments (Melakeberhan *et al*., 1987).

2.3 Work not done on problem statement

The degree of nematode resistance in relation to relative susceptibility of *M. incognita* and *M. javanica* on rosemary and the effect of nematodes on the nutrient element concentration have not been investigated and there is a need to study the development of nematodes in the roots of rosemary. Screening studies for *M.*

enterolobii host suitability have not yet been conducted in rosemary at all. Also, a recent study by Al-Sayed *et al*. (2022) suggested a need for further research on the resistance of rosemary against root knot nematodes.

2.4 Addressing the identified gaps

Relative susceptibility of rosemary to tomato under the infection of *Meloidogyne* species have not been investigated as well as the effect of *Meloidogyne* species on the nutrient concentration of Ca, Fe, K, Mg and Zn. Experiments on the relative susceptibility of rosemary to tomato infected with *M. enterolobii*, *M. incognita* and *M. javanica* under greenhouse conditions is sufficient to fill all the identified gaps. After the provided review of literature, Chapter 3 addresses the relative susceptibility of rosemary to the three thermophilic *Meloidogyne* species.

CHAPTER 3

RELATIVE SUSCEPTIBILITY OF ROSEMARY TO THE THREE THERMOPHILLIC *MELOIDOGYNE* SPECIES

3.1 Introduction

The only way to compare multiplication rates of a specific nematode on different plants is to grow them all at the same low initial egg density of the nematode in a properly randomised experiment (Seinhorst, 1984). When resistant cultivars are grown fewer females will mature than on susceptible cultivars, also the number of eggs produced may be smaller (Been *et al*., 1995). Therefore, nematodes multiply less strongly on these cultivars than on susceptible ones and sustain a smaller maximum population density. The degree of resistance is determined based on the relative susceptibility (rs_a) according to the criteria established by Seinhorst (1984) and Phillips (1984) calculated as the percentage of eggs per plant on rosemary compared to that on the susceptible tomato. To assess host-status, degree of nematode resistance descriptors is utilised to indicate relative susceptibility on a specific host. Therefore, the objective of this study was to determine whether relative susceptibility of rosemary to tomato for *M. enterolobii, M. incognita* and *M. javanica* would either be above one or affect relative accumulation of selected mineral nutrient elements. The null hypothesis stated that rs of rosemary to tomato for *M. enterolobii, M. incognita* and *M. javanica* would neither be above one nor affect relative accumulation of selected mineral nutrient elements.

3.2 Materials and methods

3.2.1 Description of the study site

The study was conducted at the Green Biotechnologies Research Centre of Excellence (GBRCE) at the University of Limpopo, Limpopo Province, South Africa (23˚53'10"S, 29˚44'15"E). The greenhouse was 20 m × 100 m in area, with a green net covering the ceiling to allow 65% photosynthetically active radiation to flow through. Relative humidity was maintained at 60-70% using the wet wall situated on the southern side wall and temperature kept at 21-28 ˚C Day and night using thermostatically activated fans situated on the northern side wall. The trial was conducted during from January-March 2022.

3.2.2 Treatments and research design

Treatment 250 eggs of *M. enterolobii*, *M. javanica* and *M. incognita* were arranged in a randomised complete block design (RCBD) to reduce variation, with 10 replications for *M. javanica* and 12 replications for *M. enterolobii* and *M. incognita*. The highly nematode susceptible tomato (*Solanum lycopersicum* L.) (Melomey *et al*., 2019) cv. 'Floradade' and rosemary (*Rosmarinus officinalis* L.) (de Macedo *et al*., 2020) with unknown resistance were planted in each replication.

3.2.3 Procedures

Established rosemary cuttings from ARC-Vegetable, Industrial and Medicinal Plants (ARC-VIMP) were set in 20-cm diameter plastic pots filled with 2 700 ml loam soil, comprising of 47% sand, 38% clay and 15% silt from GBRCE plots. The soil was steam-pasteurised at 300°C for an hour and after night cooling, mixed with Hygromix-T (Hygrotech, Pretoria West, South Africa) at 3:1 (v/v) ratio. Pots were placed on greenhouse benches at 0.25 m × 0.30 m spacing. Eggs of *M. enterolobii*, *M. incognita* and *M. javanica* were extracted from the roots of a highly susceptible tomato cv. 'Floradade' using maceration and blending method in 1% NaOCl solution for 30 s (Marais *et al*., 2017). Since relative susceptibilities determined at large initial densities are unreliable (Seinhorst *et al*., 1995), each plant was therefore inoculated 5 days after transplanting by dispensing approximately 250 eggs of *M. enterolobii*, *M. incognita* and *M. javanica* using a 20-ml-plastic syringe into 5-cm deep holes around the cardinal points of plant stems. After inoculation, the growing media was used to cover the holes. Irrigation was done every other day, initially using 250 ml tapwater and later using 300 ml tapwater, always ensuring that there is no leaching out of nematodes.

Multifeed application was interchanged with fertilisation every 10 days. Approximately 5 g Multifeed (Multisol' N) was applied to provide 1.21 Mg, 0.43 K, 0.47 N, 0.43 P, 1 Fe, 4.02 Mg per ml, 0.47 Zn, 0.10 Cu, 1.34 B and 0.09 Mo mg per ml. The cuttings were also fertilised with 2.5 g N: P: K 2:3:2 (22) to provide a total of 155 mg N, 105 mg P and 130 mg K. Pest management comprised of regular monitoring, major pest and diseases observed included leaf miner (*Tuta absoluta* Meyrick.) (Rwomushana *et al*., 2019) and powdery mildew (*Leveillula taurica* Lév.) (Jin *et al*., 2021) only on tomato plants. Heavily infested tomato leaves were removed to reduce the spread of pest and diseases. Leaf minor was controlled using Complete 350 (40 ml/10 L water) every 7 days, Complete 350 (40 ml/10 L water) every 23 days and Copper flow fungicide Ideone (50 ml/10 L water) was used for powdery mildew every 25 days.

Figure 3.1 Established rosemary cuttings inoculated with *Meloidogyne enterolobii*.

Figure 3.2 Establishment of rosemary cuttings inoculated with *Meloidogyne incognita*.

Figure 3.3 Establishment of rosemary cuttings inoculated with *Meloidogyne javanica.*

3.2.4 Data collection

Data were collected at 56 days after inoculation after the nematode had completed three life cycles (Mashela *et al*., 2015), nematode and plant variables for each trial were recorded.

Plant variables: Variables measured included plant height using a measuring tape; chlorophyll content using chlorophyll meter (Konica Minolta, Beijing, China); stem diameter using a Digital Vernier Caliper ® (mm); fresh root mass; dry root mass; fresh shoots mass and dry shoot mass using a weighing balance. Plant shoots were dried at 60-70ºC for 72 hours in an oven.

Nematode variables: Roots were removed from the pots and rinsed with water to remove soil particles and paper towel was used to absorb excess moisture associated with the root surface. North Carolina differential scale index was used to count number of galls per root system where 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls and 5 ≥ 100 galls per root system (Taylor and Sasser, 1978). Nematodes were extracted using maceration and blending method in 1% NaOCl solution (Hussey and Barker, 1973). Each root was chopped into smaller pieces of around 0.5 cm and then 100 ml of water containing 1% NaOCl was added to the jar, followed by 60 s of shaking. The shaken material was then blended for 60 s in a 1.75 L Russel Hobbs household blender. Top-down nested 150 µm, 75 µm and 25 µm mesh sieves were used to filter the combined material. The contents of the 25 µm mesh sieve were placed into a 50 ml centrifuging tube. Inside the centrifuging tubes containing nematodes, two and a half teaspoons of Kaolin were mixed with tap water and centrifuged for 4 minutes at 1800 rpm (Jenkins, 1964). The nematodes were isolated from the aliquot debris using the sugar floatation and centrifugation procedure (Marais *et al*., 2017). After preparing a sugar stock solution (624 g sugar/L tapwater), 45 ml of the stock solution was added to the centrifuge tubes and swirled once before centrifuging for 3 minutes at 1800 rpm. The aliquot was then decanted onto a 25-m opening sieve with sugar washed off the nematodes under running tapwater, eggs and J2 were collected into a 100-ml plastic bottle for storage, nematodes were counted out of 5 ml aliquot using stereomicroscope.

Nutrient element variables: Rosemary leaves were ground using a blender, about 0.4 g ground healthy matured leaves of rosemary were digested in 75 ml vessel with 5 ml of 70% nitric acid (HNO_3) and 3 ml of 30% hydrogen peroxide (H_2O_2) using microwave digester (PerkinElmer, Titan MPS). The vessels were then inserted into the microwave digester to whirl for 46 minutes under temperature ranging up to 190°C. Subsequently the vessels were placed in the laminar flow hood and allowed to cool down for 20 minutes. Samples from the vessels were transferred into 50 ml centrifuging tubes and stored in the refrigerator before analysis of Ca, Fe, Mg, N and Zn. Calcium, Fe, K, Mg and Zn elements were analysed from leaf samples using the Inductively Coupled Plasma Optical Emission Spectrometry (Shimandzu, ICPE-9000).

3.2.5 Data analysis

Relative susceptibility (rs_a) measured as ratios of egg densities obtained on the tested plant and on a fully susceptible plant, both at the identical initial egg densities was used for assessing the degree of nematode resistance (Seinhorst, 1984). Average for the total eggs and each nutrient element in rosemary to tomato were used to calculate the related relative susceptibilities of rosemary in relation to a susceptible tomato.

3.3 Results

The relative susceptibility values of rosemary to tomato produced 0.21, 0.37 and 0.18 for rs_a *M. enterolobii*, *M. incognita* and *M. javanica,* respectively. The reproductive potentials of tomato and rosemary inoculated with *M. enterolobii* are 5.68 and 27.24, respectively while inoculation with *M. incognita* produced 1.52 and 3.14 RP for tomato and rosemary, similarly, inoculation with *M. javanica* produced 4.66 and 25.70 RP for tomato and rosemary, respectively (Table 3.1).

The relative nutrient concentration of rosemary to tomato after 56 days inoculated with *Meloidogyne* species shows that nematode infection influences nutrient concentration (Table 3.2). Inoculation with *M. enterolobii* produced 0.77, 0.71, 1.02, 0.81 and 0.39 rs$_{\alpha}$ for calcium (Ca), iron (Fe), magnesium (Mg), potassium (K) and zinc (Zn). In contrast, inoculation with *M. incognita* produced 0.55, 1.02, 0.69, 0.74 and 0.88 rs$_{\alpha}$ for Ca, Fe, Mg, K and Zn while *M. javanica* produced 0.79, 0.98, 1.30, 0.71 and 0.91 rs$_{\alpha}$ for Ca, Fe, Mg, K and Zn, respectively.

Table 3.1 Reproductive potential [eggs in gram (g) roots] of rosemary and tomato plants inoculated separately with *Meloidogyne enterolobii, M. incognita* and *M. javanica* and the related relative susceptibility (rs$_{\alpha}$) values at 56 days after inoculation with 250 eggs.

M. enterolobii				
	RT(g)	**Egg**		
		PF	RP	rs$_{\alpha}$
Rosemary	1.93	1420	5.68	**0.21**
Tomato	54.36	6810	27.24	-
M. incognita				
Rosemary	1.99	288	1.52	**0.37**
Tomato	51.63	785	3.14	-
M. javanica				
Rosemary	1.07	1166	4.66	**0.18**
Tomato	36.76	6424	25.70	-

Relative susceptibility (rs$_{\alpha}$) = Reproductive potential on rosemary/reproductive potential on tomato, where rs$_{\alpha}$ < 1 depicts that rosemary was resistant.

Table 3.2 Nutrient elements in the dry shoot (DS) leaf tissues of rosemary and tomato inoculated with *Meloidogyne enterolobii, M. incognita* and *M. javanica* and related relative susceptibility (rs_a) at 56 days after inoculation.

	DS(g)	**Ca**		**Fe**		**K**		**Mg**		**Zn**	
		Average	rs_a	Average	rs_a	Average	rs_a	Average	rs_a	Average	rs_a
						M. enterolobii					
Rosemary	0.25	5237.29	**0.77**	186.12	**0.71**	1.28	**0.84**	2615.42	**1.02**	76.42	**0.39**
Tomato	34.63	6771.88	-	263.33	-	1.53	-	2563.54	-	194.27	-
						M. incognita					
Rosemary	0.33	4034.81	**0.55**	268.53	**1.02**	1.10	**0.74**	1902.81	**0.69**	168.93	**0.88**
Tomato	34.08	7326.04	-	263.02	-	1.50	-	2762.5	-	191.88	-
						M. javanica					
Rosemary	0.27	5811.25	**0.79**	239.5	**0.98**	1.12	**0.71**	3188.75	**1.30**	175	**0.91**
Tomato	25.92	7273.15	-	245.63	-	1.58	-	2457.5	-	193	-

Relative susceptibility (rs_a) = Reproductive potential on rosemary/reproductive potential on tomato, where $rs_a < 1$ depicts that rosemary was resistant.

Figure 3.4 Comparison of galls on rosemary (A) and tomato (B) roots inoculated with 250 eggs of *Meloidogyne enterolobii*.

Figure 3.5 Comparison of galls on rosemary roots inoculated with 250 eggs of *Meloidogyne javanica* (A) and the non-inoculated healthy (B) roots.

3.4 Discussion

3.4.1 Relative nematode susceptibility of rosemary to tomato

The concept of Seinhorst (1965) can be used to describe the nematode and plant results for all three test nematodes on rosemary. Plants are termed resistant if the nematodes numbers observed on the test plants are lower than those observed on susceptible plants. The only way to compare multiplication rates (rs_a) of a specific nematode on different cultivars is to grow them all at the same initial egg density of the nematode in a properly randomised experiment (Seinhorst, 1984). The host status of rosemary against the three *Meloidogyne* species has been the subject of a small number of investigations. The discussion was therefore based on the host status of other crops with the three *Meloidogyne* species, the focus of this discussion is based more on the relative susceptibility of the eggs since the use of eggs in pot experiments involving partially resistant cultivars is likely to reduce the multiplication rates of the nematodes there by decreasing the risk of host plant being damaged by the resulting nematodes. The biggest factor affecting the multiplication rates on partially resistant hosts is the gradation of female numbers that will develop (Phillips and Trudgill, 1984).

The fact that the rs_a values were less than unity at all levels of inoculation, with the test nematodes not affecting growth of rosemary, rosemary was then confirmed as being resistant to all test thermophilic *Meloidogyne* species. The relative susceptibility values less than one, indicated that the nematodes were unable to reproduce on the test plant. The known susceptible tomato cv. 'Floradade' exhibited a high mean nematode number indicating that the nematodes were viable, fed and reproduced on the provided host. Similar observations were seen in the study by Kokalis-Burelle and

Rosskopf (2013) in which nematode reproduction was higher on tomato and lowest on Celosia cultivar ‘Bombay’, which is believed to have been influenced by the very low root weights and high level of root disease for that Celosia cultivar. The reduced proportion of nematodes in rosemary roots contradicts the belief that nematodes detect and penetrate the roots of both nematode susceptible and nematode-resistant plants equally (Pofu *et al*., 2010).

This study revealed RP above unity for all the three *Meloidogyne* species evaluated. The reproductive potentials of *M. enterolobii* was significantly higher than for *M. incognita* and *M. javanica*, this finding contradicts with the observations of Agenbag (2016), in which single-species population of *M. javanica*, had a much higher reproduction potential (RP = 203) than the populations of *M. enterolobii*, with reproductive potentials ranging between 8 and 14, contrary to its reputation as an "aggressive" root-knot nematode species. A particular interesting result from this current study is the fact that the rs_a of eggs under the inoculation of *M. incognita* was relatively higher than the other two species indicating some sort of aggressiveness by this nematode towards rosemary. On the studies by Fourie *et al*. (2012); Agenbag (2016); Rashidifard *et al*. (2019) using the three *Meloidogyne* species, *M. javanica* populations were reported as being the most aggressive on tomato in greenhouse and microplot studies. This disagrees with the results of the current study in which *M. incognita* seems to be the most aggressive amongst the three species with the highest rs_a.

Another interesting outcome of the study relates to the egg production per root system by *Meloidogyne* species. Rosemary galls were sufficiently smaller in size and very few in number as compared to those on tomato (figure 3.5). The findings of this study support those of Hussain *et al*. (2014), on the discovery that the cultivar 'Sharmeeli' was highly susceptible to *M. incognita*, with maximum galls and egg masses observed on the roots, indicating that many juveniles penetrated the roots and successfully completed their life cycles, as in the highly susceptible tomato of the current study. On the other hand, rosemary only allowed a limited number of nematode juveniles to penetrate the roots, leading to maturity, as evidenced by the small number of galls and egg masses on their roots. Inoculation with *M. enterolobii* produced many eggs for both rosemary and tomato, even though maximum egg production was observed, the rs_a of eggs is lower than that on *M. incognita*. This phenomenon further supports that egg production counts for *Meloidogyne* species is not necessarily the most accurate indication of their reproductive potentials, as has been previously demonstrated by several authors (Ntidi *et al*., 2016; Steyn *et al*., 2014).

The total nematode number observed under the infection of *M. enterolobii* is relatively higher in both rosemary and the susceptible tomato. Even though it was previously mentioned that *M. incognita* seem to be more aggressive due to the overall highest rs_a values compared to rs_a of *M. incognita* and *M. javanica*, this demonstrates an interesting observation which may provide valuable insight into future studies regarding the aggressiveness of this specie as to how is *M. enterolobii* having highest egg production with a lower rs_a compared to *M. incognita* within the root system of rosemary.

According to the European resistance ranking system, rosemary is ranked at 3 for *M. enterolobii* and *M. javanica* whereas for *M. incognita* it is ranked at 4. Therefore, in terms of the host response suitability, rosemary is a non-host to the three *Meloidogyne* species. Low relative susceptibility values were also observed in watermelon cultivars to *Meloidogyne* species grown by Nigerian producers and it was recommended that the cultivars with low rs_{α} values should preferentially be cultivated by producers in order to discourage build-up of particularly *M. incognita* and *M. javanica* (Bello *et al.*, 2021). It is foreseen that growing such cultivars could discourage population density build-up of the prevalent *Meloidogyne* species in fields where these species occur.

3.4.2 Relative nutrient element accumulation of rosemary to tomato

Normal plant growth requires a sufficient supply, appropriate absorption and a balanced distribution of nutrient components inside a plant. The nutritional balance and host physiology change when nematodes infect plants. In most nematode resistance studies, nutrient elements are hardly assessed, which could explain the paucity of such data (Makhado, 2020). According to reports (Melakeberhan and Webster, 1993), the effect of nematodes on the uptake of nutrient components and distribution within the plant depends on the nematode species, host type and stage of infection (Oteifa, 1952), and whether the data were expressed as concentration or content (Price and Sanderson, 1984). For all three test nematodes on rosemary, nematode and plant results could be explained using the host-status and host-sensitivity concepts (Seinhorst, 1965), whereby $rs_{\alpha} < 1$ means that nematode infection did not have any effect on the concentration of nutrient elements within the plant, the opposite is true when $rs_a > 1$ meaning that nematode infection influenced nutrient element concentration.

The total shoot mass of rosemary under the infection of *M. incognita* and *M. javanica* had not been affected by nematode infection, this disagrees with the results of Moosavi (2015) and Wesemael *et al*. (2014) in which *M. javanica* reduced the total shoot mass on pepper plants. Infection by *M. enterolobii* resulted in a decrease in total shoot mass. According to Abad *et al*., 2009, auxin-like substances produced by nematode-infected plants keeps plant yields unaltered or even slightly boost them when nematode population density is significantly lower (Greco and Di Vito, 2009), this totally disagrees with the findings of the current study under the infection of *M. enterolobii*.

Cetintas *et al*. (2007) demonstrated that tomato plants infected with *M. enterolobii* and *M. incognita* populations from the United States (Florida) were significantly shorter than those infected with *M. arenaria*, *M. floridensis*, or *M. javanica* based on plant growth parameters. This disagrees with the findings of the current study in which the growth parameters in terms of dry shoot mass of *M. javanica* is lower on the susceptible tomato than on tomatoes inoculated with *M. enterolobii* and *M. javanica*, respectively. Nematode infection leads to drastic changes in accumulation of nutrient elements in leaf tissues of various crops (Santana-Gomes *et al*., 2013), which could affect the quality of the produce. Also, nematode species express different behaviours in resistant crops (Brida *et al*., 2017; Weston *et al*., 2013).

The availability of nutrients in the leaf tissues is typically unaffected when the root system is unharmed by the nematodes (Ames, 1997; Kaplan *et al*., 2008). The results of the current study indicated that inoculation with *M. enterolobii and M. incognita* did not have any negative effect on the nutrient concentration of rosemary in terms of the rs_{a} protocols, whereas *M. javanica* slightly affected the concentration of Mg within the leaves of rosemary. According to the findings of this study, Ca and K concentration was not affected by any of the *Meloidogyne* species. This disagrees with the belief that K is replaced by Ca under the infection of *M. incognita* which alters the balance of these elements (Melakeberhan *et al*., 1985). The high K concentration in the root system, thickens the epidermal cell wall, boosts the structural rigidity of tissues and plays a crucial part in many metabolic reactions in plants (Huber and Arny, 1985; Perrenoud, 1990), these might have been the main reason for low nematode numbers within the root system of rosemary.

Most nutrient elements are readily mobilised from organ to organ, depending on the prevailing conditions that affect the physiology of the plants (Santana-Gomes *et al*., 2013; Taiz and Zeiger, 2010). Inoculation with *M. javanica* slightly affected the rs_{a} nutrient element concentration of Mg in rosemary relative to tomato. This study demonstrated that the presence of *Meloidogyne* species in the soil is more likely to slightly have an impact on either Mg concentration within the plant. Magnesium and Fe are both intimately associated with the chlorosis of plants, the former is a constituent of chlorophyll, and the latter is probably involved at some step in the biosynthesis of chlorophyll (Agarwala and Mehrotra, 1984).

Even though *Meloidogyne* species did not have any significant effect on the leaf Zn nutrient concentrations, inoculation with *M. enterolobii* resulted in a very low rs_a observed for all the three species. In several studies, lower concentrations of Zn were observed on okra infected with *M. incognita* (Sharma *et al*., 2018) as compared to healthy plants. Farahat *et al*. (2013) also observed lower concentrations of Zn in nematode infected with *M. incognita* of several other plants including cowpea as compared to healthy plants. Infection by *M. enterolobii* is known to influence the level of micro- and macronutrients within the plant by decrease the foliar levels of either nitrogen, phosphorus or potassium (Gomes *et al*., 2008), this contradicts with the results of the current study in which *M. enterolobii* did not negatively affect the nutrient concentration within the plants. Plants deficient in Zn have shorter internodes resulting in rosette of leaves (Peck and McDonald, 2010). Additionally, Zn deficiency results in low levels of superoxide dismutase, which inhibits metabolic processes and leads to membrane integrity loss (Barker and Pilbeam, 2007). In contrast, the accumulation of Zn in plant tissues improves the amino acids in the root exudates, which might weaken the attraction of nematodes towards roots (Cakmak and Marschner, 1988; Santana-Gomes *et al*., 2013). Thus, fertilisation with zinc-oxide to provide Zn would be essential in areas where rosemary plants are infested by *M. enterolobii.*

3.5 Conclusion

The study shows that rosemary screened for host response to the predominant *Meloidogyne* species identified was resistant with all the rs*a* value being less than one. As a result, it is appropriate for use in crop rotations designed to manage the population densities of the three thermophilic *Meloidogyne* species. Nutrient concentration of rosemary is mostly not affected by nematode infection except for Mg. It is therefore recommended to supplement Mg levels in soils with infested particularly with *M. javanica*. However, more research is needed to ascertain the type of nematode resistance present in rosemary and whether it might be employed in breeding.

CHAPTER 4

SUMMARY, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

4.1 Summary

The study focused on establishing the degree of nematode resistance of rosemary using the concept of relative susceptibility which is a proportion of reproductive potential of plant with unknown nematode resistance to that with known degree of nematode resistance. In the current study tomato cv. 'Floradade' was used as a reference plant for *Meloidogyne enterolobii, M. incognita* and *M. javanica.* Results demonstrated that relative susceptibility of the test crops to the test *Meloidogyne* species was below unity, thereby suggesting that relative to the test tomato cv. 'Floradade', rosemary was resistant to the test *Meloidogyne* species. The study also demonstrated that Mg concentration in leaf tissues of rosemary relative to tomato leaf tissues in plants infected by *M. javanica* was less than unity, whereas other test *Meloidogyne* species had no effects on the variable and other nutrient elements.

4.2 Significance of findings

Since the relative susceptibility was less than one, this implied that in fields where *M. enterolobii, M. incognita* or *M. javanica* were identified as dominant, rosemary would perform better than tomato cv. 'Floradade'. The study suggested that rosemary could be produced successfully in regions with the three test thermophillic *Meloidogyne* species.

4.3 Recommendations

The relative susceptibility protocols do not provide information on the mechanisms of nematode resistance. Thus, it would be prudent to establish the mechanism of nematode resistance in rosemary against the three test *Meloidogyne* species. Also, it would be interesting to further investigate as to whether the concentration of Mg as affected by *Meloidogyne* species has any significant effects on the quality or quantity of rosemary oil.

4.4 Conclusions

Rosemary, relative to tomato cv. 'Floradade', could successfully be produced in regions with *M. enterolobii*, *M. incognita* and *M. javanica,* which are thermophillic *Meloidogyne* species. Additionally, relative to tomato cv. 'Floradade' rosemary is appropriate for use in crop rotation systems designed to control population densities of the three test *Meloidogyne* species.

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