Influence of *Maerua angolensis* and *Tabernaemontana elegans* extracts application time on tomato growth and nematode management

Nkosingiphile Fortunate Zulu

201995204

orcid.org/ 0000-0002-1523-549X

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Supervisor: Dr. Z.P. Dube (UMP)

Co- supervisor: Dr. N. Sithole (ARC- VOPI)

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DEDICATION

To my mother, Lindiwe Masina, my grandfather Mr. T.K. Masina and grandmother Mrs. A.B.S. Masina. My siblings Lethukuthula Masondo, Kagiso Segami, Sgsana and Sphelele Zulu, and my two nieces Simthandile and Langelihle Yende and to the whole Masina family.

DECLARATION

I, Nkosingiphile Fortunate Zulu, hereby declare that the dissertation titled " **Influence of *Maerua angolensis* and *Tabernaemontana elegans* extracts application time on tomato growth and nematode management**" submitted for the Master of Science in Agriculture at the University of Mpumalanga has not been submitted for consideration toward any other degree or examination at any other university. All the sources utilized in this document have been appropriately identified and cited.

Student: Ms N.F Zulu (201995204)

Signature  Date: April 2026

Supervisor: Dr. Z.P Dube

Signature  Date: April 2026

Co-supervisor: Dr. N. Sithole

Signature Date: April 2026

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LIST OF ABBREVIATIONS

ARC- TSC - Agricultural Research Council - Tropical and Subtropical Crops

DDT- Dichloro- diphenyl- trichloroethane

MITC- Methyl isothiocyanate

EPNs- Entomopathogenic nematodes

PPNs- Plant parasitic nematodes

J2- Second- stage juveniles

IJ- Infective juveniles

RKN- Root-knot nematodes

RCBD- Randomised complete block design

FAO- Food and agriculture organisation

TB- Tuberculosis

CARD- Curve-fitting allelochemical response dosage

DDG- Density- dependent growth

M. angolensis- Maerua angolensis

T. elegans- Tabernaemontana elegans

M. javanica- Meloidogyne javanica

M. enterolobii- Meloidogyne enterolobii

M. incognita- Meloidogyne incognita

RESEARCH OUTPUT

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N. Zulu, Z. Dube, N. Sithole, N. Mnyambo, A. Mabuela. Evaluating the impact of *Maerua angolensis* extract application timing on the suppression of mixed nematode populations in field-grown tomato crops. One Health International Student Conference. 2024, 4-6 December, Bucharest, Romania.

N.F. Zulu, Z.P. Dube, N.M. Mnyambo and A. Mabuela. Effect of application timing of *Maerua angolensis* and *Tabernaemontana elegans* plant extracts on *Meloidogyne javanica* and *Meloidogyne enterolobii* population densities and tomato plant growth under greenhouse condition. 25th Symposium of the Nematological Society of Southern Africa. 2025, 28- 02 September, Malelane, Pestana Kruger Lodge, Mpumalanga.

Nkosingiphile F. Zulu, Zakheleni P. Dube, Nicholus M. Mnyambo, Amogelang Mabuela. Effects of *Maerua angolensis* and *Tabernaemontana elegans* Application Time on the Management of Rook-knot Nematodes. Accepted for publication in *Acta Agriculturae Scandinavica Section B- Soil and Plant Science.*

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ABSTRACT

Tomato (*Solanum lycopersicon* L.) is amongst the most common vegetable crops grown in South Africa and an essential part of a healthy diet. Unfortunately, its production is negatively impacted by various pests, including the root-knot nematodes, *Meloidogyne javanica* and *Meloidogyne enterolobii*, which cause high yield losses. The use of synthetic chemical nematicides has been very successful as a control method. Unfortunately, there are many challenges associated with it, including the negative impact on the environment, people and animals, hence most have been removed from the agrochemical markets. An alternative method of plant extracts has recently received a lot of research and development, however, the method comes with inconsistent results from several factors, among which is time of application, hence the current study seeks to determine the effect of time of application of the two medicinal plant extracts, *M. angolensis* and *T. elegans* on nematode population densities and tomato plant growth under greenhouse and field conditions. Two experiments were established to achieve the objective, a greenhouse experiment of 2 x 2 x 3 + 2 factorial arrangement with the first factor being two plant extracts (*M. angolensis* and *T. elegans*) applied at a recommended rate of 5g per plant, and the second factor consisted of two nematodes (*M. javanica* and *M. enterolobii*) at 5000 eggs and J2 per plant. The third factor was made up of three application and inoculation times (plant extract applied before nematode inoculation; plant extract applied simultaneously with nematode inoculation and plant extract applied after nematode inoculation) plus positive experiment control (Crop Guard, a.i. furfural, aldehyde 900g/L) and negative control (plants only inoculated with nematodes) with 5 replications. While a field experiment consisted of five treatments namely, the application interval of plant extracts and positive control (Crop Guard) weekly, every second, third and fourth week. Field treatments were laid out in a randomized complete block design (RCBD) with 15 replications. Greenhouse experiment was done over two seasons: summer and autumn, 2023 and 2024, respectively, and

field experiment was done once in 2024. At 56 days after the last treatment application, nematode and plant growth variables were measured from both experiments. Under greenhouse conditions, all second order interactions of season, nematode and time of application had no significant ($P > 0.05$) effects on all measured variables, except on juveniles in soil and juveniles in tomato root. While the first-order interactions of season and plant extracts, season and time of application were only significant for eggs in tomato root, juveniles in tomato root, total number of nematodes in root and final nematode populations. Generally, all three plant extract applications had a significant effect on the two nematodes, more nematodes were observed in summer than in winter across all treatments, also more nematodes were in tomato roots than in soil. Across all other factors, *M. angolensis* reduced nematode variables more than *T. elegans*. Applying plant extracts after inoculation yielded the lowest nematode numbers, followed by simultaneous application, with plant extract applied before nematode inoculation having the highest numbers. Across all measured variables, positive control consistently achieved lower nematode numbers than negative control. Under field conditions, nematode suppression was highest when plant extracts were applied weekly, as evidenced by a significantly decreased numbers of eggs in tomato root, juveniles in soil, total nematode in tomato root and final nematode population relative to the negative control. In conclusion, both plant extracts can be used in the suppression of nematodes even though best results can be obtained from *M. angolesis* applied weekly after nematode infestations.

Key words: *Meloidogyne incognita*, *Meloidogyne enterolobii*, *Maerua angolensis*, *Tabernaemontana elegans*, Furfural (Aldehyde), Phytonematicides.

CHAPTER 1

GENERAL INTRODUCTION

1.1. Background of study

Tomato (*Solanum lycopersicon* L.) is amongst the most common vegetable crops grown in South Africa and an essential part of a healthy diet (Bihon, Ognakossan, Tignegre, Hanson, Ndiaye and Srinivasan, 2022). It is globally consumed as food and a cash crop (Gatahi, 2020). Unfortunately, its production is negatively impacted by many pests, including the root-knot nematodes (RKNs); *Meloidogyne javanica* and *Meloidogyne enterolobii*, which infest the root of the crop, causing damage resulting in huge yield losses (Khosa, Dube, De Waele and Daneel, 2020a). Furthermore, Pakeerathan, Mikunthan and Tharshani (2009) reported that root-knot nematodes are the most severe of pathogens in many economic vegetables, causing yield losses ranging from 26 to 68 % on tomato plants. Globally, Fourie, Mc Donald, Mothata, Ntidi, De Waele (2012) reported that *Meloidogyne* spp. infection in tomato has been linked to estimated yield losses of more than 50% and between 20 -40%. In terms of monetary value, Forghani and Hajihassani (2020) estimated that plant parasitic nematode genera for many crops worldwide can cause an annual yield loss of approximately US$100 billion globally.

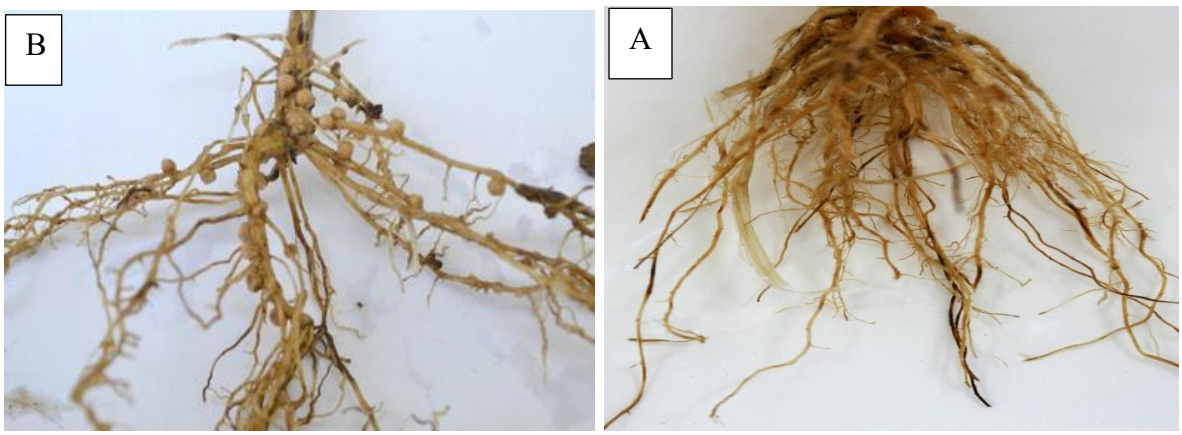

Figure 1.1: Tomato root, healthy (A) and nematode galled (B) (soilquality.org.au, 2020)

Recently, the use of chemicals has been the dominant method for managing plant-parasitic nematodes (Dutta, Khan and Phani, 2019; Khan, Asif, Khan, Tariq, Ansari, Shari and Siddiquli, 2019; Seong, Shin, Kim and Cho, 2021). However, some of these chemical nematicides have been taken off the agrochemical market due to their negative impact which include high risk to the applicators which (human beings), causing harm to animals and non-targeted soil organisms, environmental toxicity, as well as their high cost (Mashela, De Waele, Dube, Khosa, Pofu, Tefu, Daneel and Fourie, 2017; Hajihassani, Davis and Timper, 2019). Research is shifting away from synthetic nematicides and toward more environmentally-friendly, cost-effective alternatives, easily accessible, human and animal-friendly alternatives (Desaeger, Wram and Zasada, 2020; Khosa *et al*., 2020a). Several methods have received much attention, including the use of plant extracts. According to Khosa *et al*. (2020a), plant extracts have traditionally been used as natural alternatives to synthetic chemicals for nematode control. These extracts contain bioactive chemicals with nematicidal characteristics, allowing for a reduction in crop damage caused by nematodes (Abd-Elgawad, 2021). Reports have been made on the effectiveness of plant extracts, such as *Maerua angolensis* and *Tabernaemontana elegans*, in managing root-knot nematodes (Khosa *et al*., 2020a). The efficiency of these plant extracts against nematodes, however, has been inconsistent. These inconsistencies have been associated with different factors such as plant extract origin, stability, seasonal changes, method of extraction, application rate and application time (El Boukhari, Barakate, Bouhia and Lyamlouli, 2020).

1.2. Problem statement

Tomato is amongst the most widely produced crops in South Africa, with high demands and commercial significances (Kepenekci, Hazir, Oksal, and Lewis, 2018). However, it is largely infected by pests and diseases, including *Meloidoygne* spp., which causes infections

and reduces yield. Understanding agricultural management cultures like the use of plant extract will improve the manner in which they can be used and can suppr

ess the damage caused by these organisms, specifically nematodes. The root-knot nematodes *M. javanica* and *M. enterolobii* are sedentary endoparasites and are among the most damaging pests to agricultural crops. They attack a diversity of crops, particularly vegetables, and they significantly reduce yields in terms of both quality and quantity, primarily in tropical and subtropical agriculture (Fourie, McDonald, Steenkamp and De Waele, 2017). These plant-parasitic nematodes (PPNs) are a significant issue in the production of vegetables and can severely harm a number of crops; their global economic impact is estimated to be around R1500 billion (Hassan, Pham, Shi and Zheng, 2013). Due to health and environmental concerns, several nematicides sold commercially have been taken off from the market. New methods of nematode control are required as an alternative nematode control strategy, of which plant extracts are one alternative strategy, hence this research looks at the most appropriate time of application of plant extracts in managing , *Meloidogyne* nematode spp.

1.3. Rationale

Plant extracts are readily available, inexpensive, and environmentally friendly nematode control options for farmers (Mashela *et al*., 2017). However, variations in efficacy have threatened the adoption of this nematode management strategy. Many factors, such as application time, seasonal changes, plant extract origin, plant extract stability, and preparation methods can contribute to the inconsistencies of the effectiveness against nematodes (Naserinasab, Sahebani and Etebarian, 2011). This study will identify the challenge of the application time of these plant extracts as a potential source of inconsistency. In contrast to synthetic compounds, where the time of application is clearly known and mostly determined by the chemical mechanism of action, crude plant extracts have diverse modes of action; hence

the need for accurate determination of application time is always desirable (Abdel-Rahman, Clark and Saleh, 2008). Therefore, the current research seeks to determine and validate the time of application for *M. angolensis, T. elegans and* mixed nematode populations in the management of nematodes.

1.4. Purpose of the study

1.4.1. Overall aim

Determining the time of application of *M. angolensis* and *T. elegans* in the management of *M. javanica* and *M. enterolobii* on tomato plants.

1.4.2. Research objectives

i. To assess whether the time of application of *M. angolensis* and *T. elegans* will reduce *M. javanica* and *M. enterolobii* population densities and improve tomato plant growth under greenhouse conditions.

ii. To test whether the time of application of *M. angolensis* will reduce mixed nematode population densities and improve tomato plant growth under field conditions.

1.4.3. Research questions

i. Will the time of application of *M. angolensis* and *T. elegans* reduce *M. javanica* and *M. enterolobii* population densities and improve tomato plant growth?

ii. Will the time of application of *M. angolensis* reduce mixed nematode populations densities and improve tomato plant growth under field conditions?

1.4.4. Research hypothesis

i. Time of application of *M. angolensis* and *T. elegans* will reduce *M. javanica* and *M. enterolobii* population densities and improve tomato plant growth.

ii. Time of application of *M. angolensis* will reduce mixed nematode populations and improve tomato plant growth under field conditions.

1.5. Reliability, validity and objectivity

Reliability can be defined as the degree to which a measurement tool produces a certain result, even in the absence of change in the object being measured. In other words, the instruments ought to produce accurate measurements (Kimberlin and Winterstein, 2008). Statistical analysis does provide a wide range of data reliability tests (Berenson and Levine, Szabat and Krehbiel, 1996). Hence statistical analysis was thus employed to evaluate the dependability of the data at a 5% probability level. The validity was achieved by repeating the experiment; the Agricultural Research Council - Tropical and Subtropical Crops (ARC-TSC) were used for plant extract extraction while plant-parasitic nematode extraction from the soil and tomato root was done at the University of Mpumalanga laboratory. Objectivity was attained by eliminating any subjectivity from the process and ensuring that the conclusion was debated considering empirical data (Leedy and Ormrod, 2005).

1.6. Bias

Leedy and Ormrod (2005) describe state that any influence, circumstance, or rather combination of circumstances which, alone or in concert, skew the data gathered or conclusion reached is generally referred to as bias. In this present study, bias was minimized by guaranteeing that the number of replications and randomisations was increased to lower the experimental error in each experiment.

1.7. Structure of the dissertation

This dissertation is made up of 5 chapters, with first chapter (Chapter 1) briefly outlining the research problem, stating the work that was already done on the problem and work not yet

done. The chapter culminates into the purpose of this study and its relevance under the heading's rationale and justification. Chapter 2 entails the theory and literature of the work that has been done so far in addressing the research problem, identification of the research gap, and work which have not been done. The next two chapters, Chapter 3 & 4, address the research objectives 1 – 2, respectively. In the finale chapter, Chapter 5, summarises the research findings from all research chapters and makes recommendations for future work. Harvard style was used in the referencing both in text and in the list of references, as authorized by the University of Mpumalanga Senate. Every chapter has its own list of references and functions as a stand-alone piece.

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CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Nematode pests have a negative impact on tomato production, especially in tropical and subtropical regions (Matlala, 2020). In the last two decades, plant-produced phytonematicides have gained recognition as desirable components of nematode management techniques (Khosa, Dube, Tselanyane, Senabe, Fouche, De Waele and Daneel, 2021). Nematicide applications are typically effective in lowering the population densities of plant-parasitic nematodes (PPNs) beneath damage threshold level in the short term. However, their negative environmental effects and their high costs have made them beyond the reach of resource-limited small-scale and rural tomato growers (Khosa, Dube, De Waele and Daneel, 2020). The use of *Maerua angolensis* and *Tabernaemontana elegans* plant extracts, traditional medicinal plants used to treat a variety of domestic animals and human ailments, has shown potential in managing *Meloidogyne* spp. on tomatoes grown in glasshouses, microplot, and in field trials (Khosa, Dube, Tselanyane, Fouche, De Waele and Daneel, 2019).

A study conducted by Khosa *et al.* (2021) reported that the two plant extracts, *M. angolensis* and *T. elegans*, inhibited *Meloidogyne incognita* race 2 second-stage juvenile (J2) hatching and caused high mortality of J2 under *in vitro* conditions. Additionally, Erdoğuş (2022) using *Thymbra spicata* L. (Lamiales: Lamiaceae) (Mediterranean thyme), *Plantago lanceolata* L. (Lamiales: Plantaginaceae) (ribwort plantain) and *Rosmarinus officinalis* L. (Lamiales: Lamiaceae) (Rosemary) plant extracts against root-knot nematodes observed the similar. The use of plant extracts for biotechnical management of root-knot nematodes is gaining significant attention due to their potential as a sustainable and eco- friendly alternative to chemical nematicides. Therefore, it is feasible that these plant extracts be used in root-knot nematode

management programs; nevertheless, further investigation is necessary to confirm their efficacy in practical applications. In this present study, we will determine the time of application of *M. angolensis* and *T. elegans* in managing *Meloidogyne* spp.

2.2. Nutritional importance of tomato

Tomato contributes significantly to the global diet, accounting for lycopene in the American diet and are a significant element of the Mediterranean diet (Perveen, Suleria, Anjum, Butt, Pasha, and Ahmad, 2015). The beneficial effect of tomato on health includes providing vitamins, minerals, and antioxidants (Frusciante, Carli, Ercolano, Pernice, Di Matteo, Fogliano and Pellegrini, 2007). Antioxidant metabolites encompass vitamins, carotenoids, phenolic compounds, and phenolic acid, which confer health benefits to the human body. The cumulative antioxidant activity of tomato is usually categorized into two, which are hydrophilic and lipophilic (Raiola, Rigano, Calafiore, Frusciante and Barone, 2014). The primary contribution comes from soluble phenolic chemicals and vitamin C, it significantly influences overall antioxidant activity (83%), with the latter derived from carotenoids, vitamin E, and lipophilic phenols (17%) (Raiola *et al.,* 2014).

2.3. Production status of vegetables in South Africa

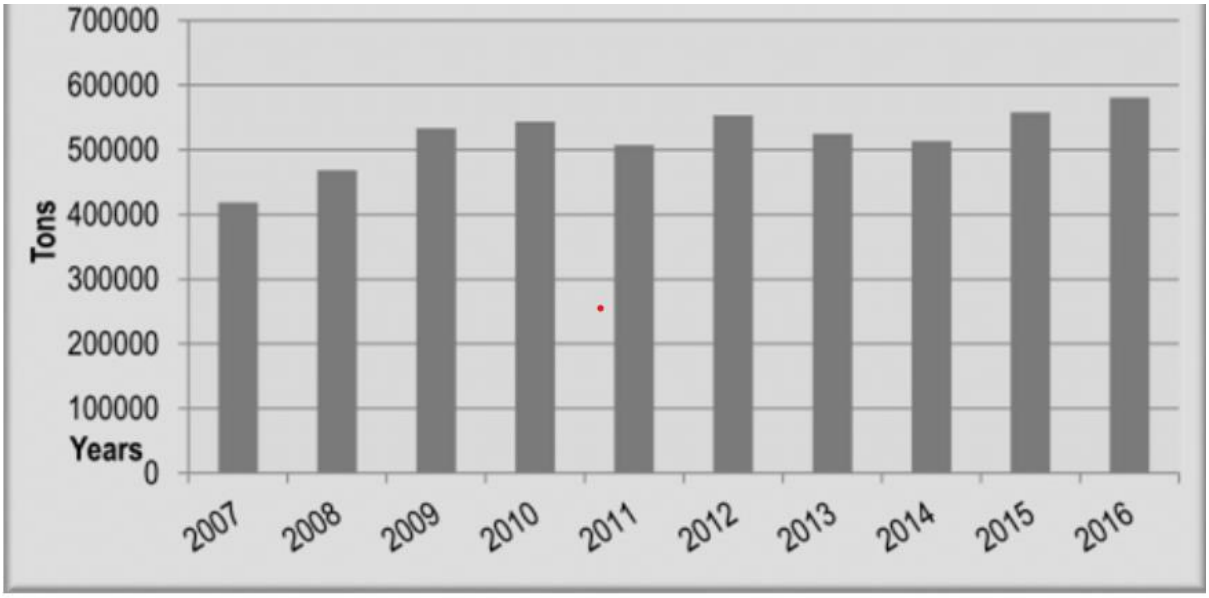
Vegetables are a collection of crops grown all over the world containing a vital element of the human diet (Ebert, 2020). They have been identified to improve food security, meet local consumer demand and satisfy export market standards (Xaba and Masuku, 2013).

South Africa is considered one amongst others that leads in term of food security due to its developed agricultural sector, robust trade capacity and stable food production (Global Food Security Index (GFSI), 2022). The nation produced over 13.1 million tons of maize in the 2023-2024 season, of which nearly 700,000 tons have already been exported for the current growing season.

Lately, it has been shown that about 45.6% of South African people have adequate nutrition; meanwhile, 28.3% are experiencing hunger, and 26% are at risk of facing food insecurity, which means they go hungry (Tibesigwa and Visser, 2016).

The primary production of vegetables in 2021 was 2.7 million tons. South Africa`s primary vegetable grew at an average yearly rate of 1.73% between 1972 and 2021, from 1.23 million tons to 2.7 million tons. It is estimated that by 2025, the world population will reach approximately 10 billion, so the goal is to increase global food production by at least 70 - 100% to meet this demand (Ghos, 2014). According to Walsh and Van Rooyen (2015), 43% of vegetables are cultivated in rural areas compared to 37% in urban areas, with carrots (*Daucus carita*), leafy vegetables and beetroot (*Beta vulgaris*) being mostly grown by rural people. It is predicted that in the next 10 years, if subsistence agriculture does not improve their level of food production output or proficiency in obtaining greater yields per land area in African land, 60% of people will be undernourished (Giller, 2020). Vegetables are a good source of nutrition that offers minerals, vitamins, protein, and fiber that are crucial for a healthy diet. They are utilized in different ways which include being cooked, boiled, or eaten raw or fried (Slavin and Lloyd, 2012).

In January 2021, there was an increase of 56% in the South African tomato export. Over the same time frame, exports to Botswana, Namibia and Eswatini had increased by 105%, 70% and 39%, respectively, while that of Zimbabwe increased by 199% (Rambau, Moatshe and Admin, 2024).



Total production of tomatoes in South Africa (DAFF, 2017)

Figure 2.1: Graph showing the total production of tomatoes in South Africa (DAFF, 2017).

2.4. Agricultural production constraints

Food production is strained by the increase in the world population, which causes competition for resources, land degradation and water scarcity. These may lead to food shortages, increased food costs, and unequal access to nutritious food (Dinar, Tieu and Huynh, 2019). According to Khosa, *et al.* (2017), there are approximately 56 million people living in South Africa, with 48% of them dwelling in the country`s rural areas. About 35% of this rural population is experiencing hunger and lives below the poverty line (Labadarios, Mchiza, Steyn, Gericke, Maunder, Davis and Packer, 2011). Majority of these communities are dependent on leguminous crops, cereals and vegetables mostly grown in private or communal gardens for their food (Ntidi, Fourie, Mc Donald, De Waele and Mienie, 2012). Diseases, pests and plant parasitic nematodes cause huge damage where crop yields can drop by more than 10% in commercial agricultural production. However, these percentages are substantially greater in rural regions, and in some cases, plant diseases and pests can eradicate crops (Sikora and Fernandez, 2005).

De Waele, Dube, Khosa, Pofu, Tefu, Daneel, and Fourie (2017) highlighted that, among all nematodes, RKN (*Meloidogyne* spp.) are the major constraint to food production. Thus far, only 101 species in this genus that have been described globally and according to reports, the four primary species of root-knot nematodes, *M. javanica, M. incognita*, *Meloidogyne arenaria* and *Meloidogyne hapla,* have been observed causing extensive economic crop damage, which hinders agricultural production (Gowda, Rai and Singh, 2017). Among all the species, *M. incognita* is recognized as the one that damages crops the most globally, causing an estimated $100 billion in losses every year (Hassan *et al.,* 2013). Significantly, the yield losses that are a result of this polyphagous pest have been reported to be an average of 10% in vegetable crop losses and are about 30% on vulnerable crops like tomato, eggplant (*Solanum melongena*), and melons (*Cucumis melo*) (Gowda, Rai and Singh, 2017).

The agricultural sector is naturally climate-sensitive and among the most vulnerable sectors to the risk and consequences of climate change (Maponya and Mpandeli, 2012). Scientist's predictions are that by 2050, the already risen by 0.9°C average temperature is anticipated to increase by 1.5°C or even more due to anthropogenic activities (Arora, 2019). According to the yearly report on weather, climate and catastrophe insight natural disasters alone resulted in economic losses of USD 225 billion worldwide in 2018, and the losses that are of natural calamities have exceeded USD 200 billion annually since the year of 2016. According to the data obtained from the Food and Agriculture Organization (FAO) which was released in the year 2016, should the present state of climate change persist, crop losses could reach previously unheard-of levels in the very near future, significantly lowering agricultural products. This will lead to difficulty in keeping up with the escalating food security demands of the expanding population.

2.5. Impact of plant parasitic nematodes on agricultural crops

Plant-parasitic nematodes are known as soil- dwelling creatures which devour plant tissues for their survival (Garcia, Buisson and Folcher, 2022). Few of these nematodes cause visual damage to the crops, which lowers production resulting in financial losses as the affected product becomes unsuitable for sale (Tileubayeza, Avdeenko, Avdeenko, Stroiteleva and Kondrashev, 2021). Most of these pests are expensive cost in agricultural crop production and about 4100 species of PPNs have been identified. Collectively, it is estimated that nematodes cause yield losses ranging between $80 -$118 billion dollars per year (Bernard, Egnin and Bonsi, 2017).

The most economically aggressive ones attack the plant root of major cash crops under production, preventing the absorption of nutrient uptake and water, and this results in lower crops' agronomic performance and overall quality (Bernard, Egnin and Bonsi, 2017). Yield losses related to nematodes differed in the tropical and sub-tropical climates with estimates of 14.6% and 8.8% in developing and developed countries, respectively, with an average of 12.3% (Gowda, Rai and Singh, 2017).

2.6. Factors influencing survival of *Meloidogyne* spp.

Plant-parasitic nematodes (*Meloidogyne* spp.) can thrive under unfavorable circumstances, such as during the dry and cold seasons between host harvests, and they are persistent. *Meloidogyne* spp. are soil dwelling aquatic animals that are dependent on external sources of heat and water for their development. They are influenced by soil edaphic and climate factors which includes temperature, humidity and soil moisture (Nisa, Tantray, Kouser, Allie, Wani, Alamri, Alyemeni, Wijaya and Shah, 2021). Temperature hinders the survival and activity of *Meloidogyne* spp. which the activities include hatching, reproduction, movement and

development (Teshita, Khan, Ullah, Iqbal and Ahmad, 2024). At a lower and higher temperature ranging between 5 to 15 °C and 30 to 40 °C, PPNs become inactive, and so they prefer an optimum range of 15 to 30 °C. Soil moisture is one of the significant components that is crucial to nematodes for their mobility and survival. Entomopathogenic nematodes (EPNs) need adequate soil moisture to move and survive (Nouh, 2022). However, excessive amounts of moisture content can result in deprive oxygen and restrict or limit their mobility. Once water is lost substrate, EPNs survival becomes poor (Teshita *et al.,* 2024). Nematodes are protected to varied degrees of dehydration in the soil environment (Grant and Villani, 2003). The recommended moisture content for J2 to grow and develop to the next stage is said to be between 10 and 30% (Wendimu, 2021)

2.7. Genus *Meloidogyne*

The members of the genus *Meloidogyne* are the most well-known harmful nematodes globally (Mantelin, Bellafiore and Kyndt, 2017). Furthermore, other species of this genus, such as *M. incognita* and *M. javanica* have huge range of host, and they produce asexually without undergoing meiosis (Kaloshian and Teixeira, 2019). The existence of the large diversity within these polyphagous species is determined by their ability to adapt to and feed on a variety of plant species from diverse taxa (Kaloshian and Teixeira, 2019).

Minelli (2002) stated that there are 3 primary sections to the taxonomy history of the genus *Meloidogyne*: A time when observations of a link between nematodes and gall root were made; thereafter, a protracted and somewhat perplexing period during which root nematodes were placed in the same genus as cyst nematodes; and lastly, the rival era, during which time root nematodes were assigned to a different genus. Though they make up a small portion of all plant parasitic nematodes, the genus *Meloidogyne* contains the most reported and harmful species of sedentary endoparasites. Pambuka (2014) mentioned that Barkeley (1855) discovered galls on

the root of cucumber plants in a glasshouse, which led him to be the first person to identify the presence of root-knot nematodes.

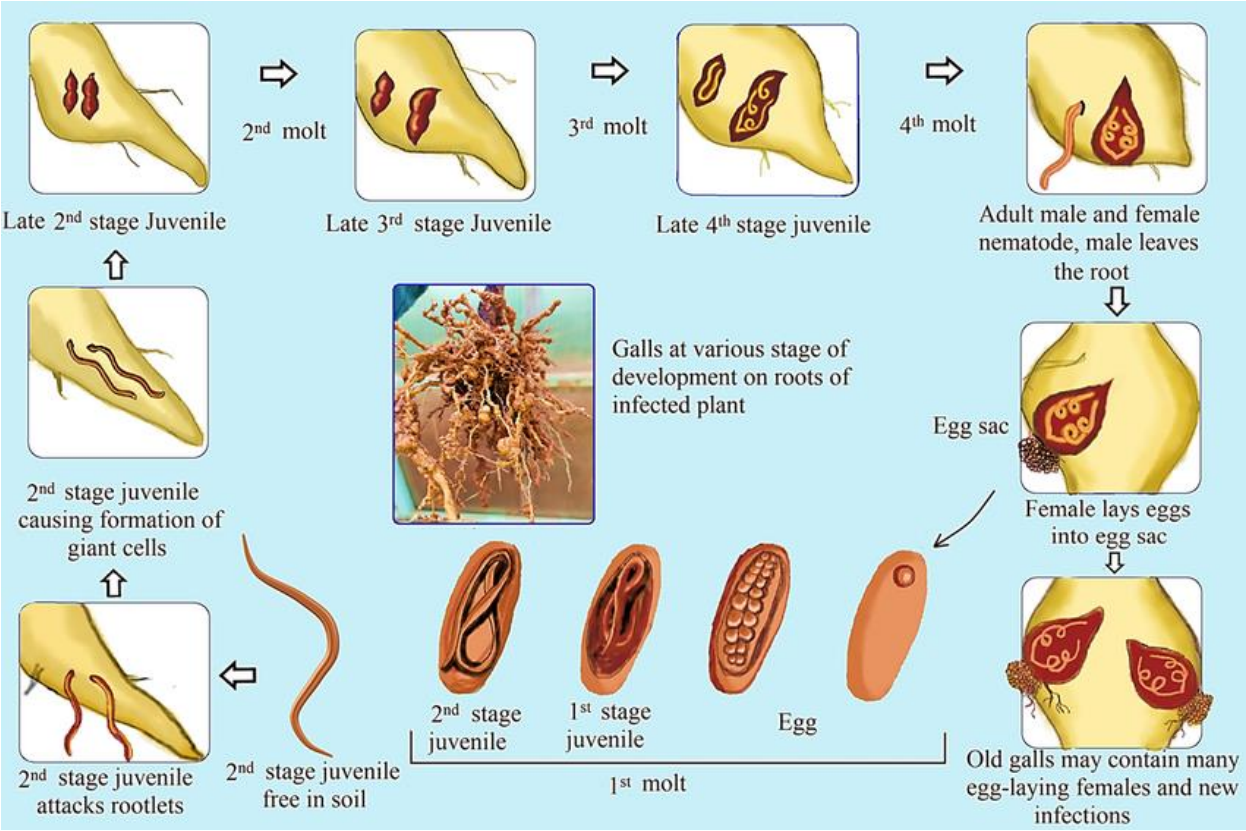


Figure 2.2: developmental stages of a root-knot nematode. (Pradhan, 2023).

2.8. Mode of *Meloidogyne* spp. reproduction

Triantaphyllou (1987) described the mode of reproduction for *Meloidogyne* in a range of publication (Van der Beek, 1997). Typically, the life stages of a PPN include an egg, four juvenile stages, and adult (male and female). Eggs are either deposited in egg-masses that are covered in a protective gelatinous matrix (*Meloidogyne* spp.) or individually (*Pratylenchus* spp)for example) (Ntidi, 2016). Just like other species of PPNs, the species of the *Meloidogyne* are comprises of male and female individual hence are said to be bisexual. The sex character of these individuals is easily recognised (Onkendi, Kariuki, Marais and Moleleki, 2014). *Meloidogyne* reproduce either sexually through amphimixis or asexually through apomixis

(Figure 2.3). Different forms of sexual reproduction known as apomixis occur when there is no fusing of the gametes (Calderon- Urrea, Vanholme, Vangestel, Kane, Bahaji, Pha, Garcia, Snider and Gheysen, 2016).

Parthenogenetic species of *Meloidogyne* can be categorised as facultative or obligatory parthenogenones based on their manner of reproduction. An individual can brand parthenogenones *Meloidogyne* as either mitotic or meiotic somatic based on cytological data (Van der Beek, Los and Pijnacker, 1998; Philbrick, Adhikari, Louws, and Gorny, 2022).

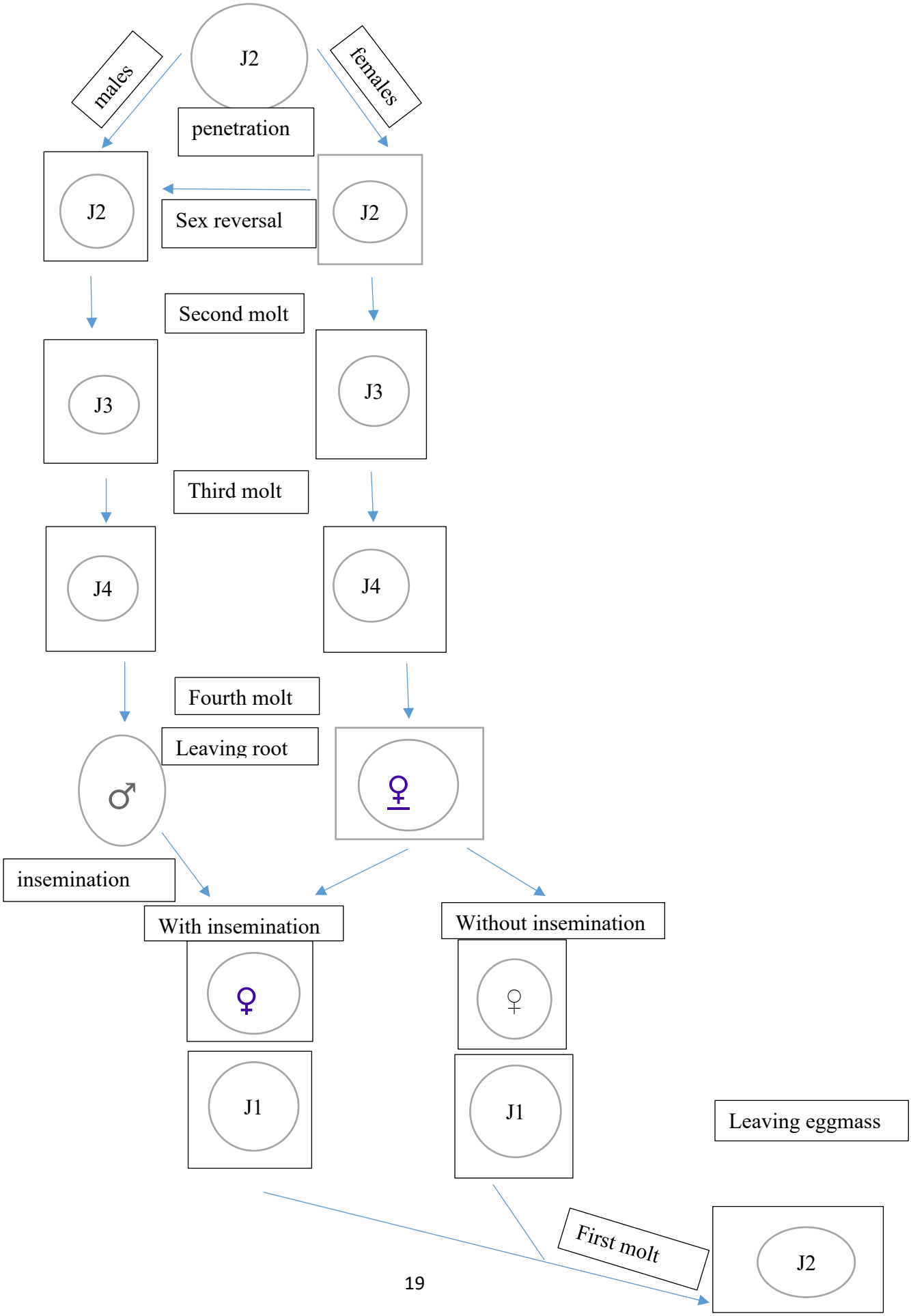

J2
males
females
penetration
J2
Sex reversal
J2
Second molt
J3
J3
Third molt
J4
J4
Fourth molt
Leaving root
♂
♀
insemination
With insemination
Without insemination
♀
♀
J1
J1
Leaving eggmass
First molt
J2

Figure 2.3: Life cycle of root-knot (*Meloidogyne* species) nematode. Legend: circle = nematode stage; double and triple circle = nematode stage within cuticula of previous stage; J1 to J4= successive juvenile stage, solid lined box= root; dotted lined box= egg mass (Walia, 2023).

2.9. Management strategies of root-knot nematodes

2.9.1. Biological control of nematodes

Natural enemies are one of the effective and easy-to-apply nematode management tactics (Akhtar, 2008). The creation of suppressive soil involves the use of specific microbial antagonists, which, when at high levels, provide instant nematode control (Collange, Navarrete, Peyre, Mateille and Tchamitchian, 2011). Long-term effects result from antagonizers can colonize the soil and continue to be active. These antagonizers include fungi and bacteria or parasitic nematodes that rely on compounds that are being released by other organisms, such as nematocidal plants or fungi (Collange *et al.,* 2011). For example, hyperparasites are a biological control agent that has been investigated by several researchers for plant-parasitic nematodes; they are rhizobacteria plant growth promoters, which grow on, attack, and disintegrate the cell wall or cell membrane of the targeted organisms through enzyme action (Anandakumar and Rajadurai, 2023).

Pseudomonas aeruginosa is one kind of bacteria that has been found to affect tomato development and decrease galling (Shankar, Pavaraj, Umamaheswari, Prabhu, Baskaran, 2011). By enhancing root zone micro-ecology, where nematicidal-activity *Pseudomonas beteli* bacteria grew in the root-zone soil, *Streptomyces* species have been identified as promising agents for enhancing tomato defenses against RKN infection, root levels of growth promoting bacteria, *Enterobacter asburiae (*growth-promoting bacteria) rose while root levels of *Pseudomonas brassicacearum (*plant-pathogenic bacteria *)* disappeared (Ma, Li, Lai, Guo, and Xue, 2017). Beneficial effects of endo-root-derived bacteria in nematode control have been

demonstrated in single-isolate with the African marigold species *Tagetes erecta* and *Tagetes patula* (Makhubu, Khosa and McGaw, 2021). Also, the main group of fungi that has been studied and is used as a biological control agent against nematodes as resistance inducers is the genus *Tricoderma mycorrhizal*. These groups of fungi can directly lessen the impact caused by plant parasitic nematodes by either parasitism, antibiosis and paralysis (Poveda, Abril-Urias and Escobar, 2020). However, to isolate them, one must assess or determine whether the soil`s suppressive effect is biological in nature. Then, the biological contaminants are separated from the soil and identified. Lastly, screening is done to determine whether they have the ability to control nematodes (Bent, Loffredo, McKenry, Becker and Borneman, 2008).

2.9.2. Nematodes used as biological control of pests

Most nematodes, such as the EPNs, have successful biocontrol activities against various economically important insect pests and hence are used as biological control of those pests (Belien, 2018). The EPNs, which belong to the family *Heterorhabditidae* and *Steinernematidae* inhabit soil and parasitise insects, including important crop pests (Patidar, Sumita, Kumawat, Pathak, Singh, Shakywar and Yadav, 2017). Nevertheless, they have no harmful impact to farm animals or either to human, and they are, therefore, environmentally safe biocontrol agents (Sobhy, Abdel-Bary, Harras, Faragalla and Husseinen, 2020).

Just like *Rhadbitida* orders, the EPNs, family Heterorhabditidae and Steinernematidae exhibit infective juvenile (IJ) phases. These IJs have about 0- 250 cells within the region of their intestine and are specialised for long-term survival (Türköz And Kaşkavalci, 2016). The EPNs from Heterorhabditidae and Steinernematidae families are obligatory parasites of numerous diseases and pathogens. Their intestine is a host to symbiotic bacteria (*Photorhabdus* sp. and

Xenorhabdus sp.) which can kill pests (Jaffue, Krishnamani, Machado, Campos-Herrera and Turlings, 2022).

Third-stage IJs that do not feed, enter the host hemocoel by means of natural openings like mouth, anus and stigma. In certain species, such as *Heterorhadbitis* spp., they also enter through the cuticle (Bula, Etana, Abdisa and Getu, 2023). Once inside, they release their symbiotic bacteria host`s haemolymph and these bacteria multiply and create poisons and other metabolites to weaken the host`s defenses (Türköz and Kaşkavalci, 2016). The host typically dies 48 hours after the nematode invades (Gang and Hallem, 2017)

It has previously been noted and documented that EPNs have antagonistic effect on PPNs. Entomopathogenic nematodes management against various nematode spp. including *Criconemoides* spp., *Rotylenchulus reniformis*, *Globodera rostochiensis*, *Belonolaimus longicaudatus*, and *Meloidogyne* spp has been demonstrated in both greenhouse and field conditions (El Aimani, Houari, Laasli, Mentag, Iraqi, Diria, Khayi, Lahlali, Dababat and Mokrini, 2022). These nematodes have the potential to successfully manage PPNs by lowering nematode populations and it is achieved through targeting the soil dwelling stages of PPNs which then disrupt their life cycle and prevent further damage to crops. This results in higher crop yield, health plant and less need for chemical nematicide (Sushma, Shanthi and Anita, 2024).

2.9.3. Synthetic chemical control of nematodes

The synthetic chemicals that have toxicity within them should be incorporated into the soil at least a month prior to seedlings. These chemicals are methyl isothiocyanate (MITC) precursors. The nematicidal fumigants are extremely volatile; during their application to the soil, they evaporate and fill the voids in the soil as gases (Bui and Deseager, 2021). It is required to close the surface of the soil with plastic film to contain the gaseous molecules intact as they diffuse into the atmosphere in significant quantities. This will increase the treatment's effectiveness

and lessen its negative effects on the environment (Sasanelli, Konrat, Migunova, Toderas, Lurcu-Straistaru, Rusu, Bivol, Andoni and Veronico, 2021). These substances include dazomet, metam-potassium, and metam-sodium. Additionally, non-fumigant chemicals with extremely low phytotoxicity can be used during the crop cycle, during sowing or transplanting. These formulations fall within the classes of phosphorganic compounds, avermectins, carbamates, and benzamides, each of which has a unique mode of action for killing nematodes (Sasanelli *et al.,* 2021).

2.9.4. Limitations to using synthetic chemical nematicides

Nematicides with high toxicity are no longer suitable for modern agriculture, due to the increase in demand of food safety and environmental protection (Heiken, 2017). For example, two synthetic substances that have already been burned from the market are methyl bromide and dibromochloropropane. Another highly toxic substance is fosthiazate. While the volatility of the avermectins resulted in inadequate field control (Heiken, 2017; Abd-Elgawad, 2016; Khan, Sayed, Shaukat and Handoo, 2008). The regular, long-term use of synthetic nematicides has induced the resistance of the nematodes, making their control even more difficult. Furthermore, a report by Chen, Li and Song (2020) has shown that the use of organophosphate and carbamate chemical will likely be restricted due to their negative impact on the environment, which can result in fewer nematicides being available for use.

Nematicides that have high toxicity are no good for the modern agriculture, this is because of the higher requirements for food safety and environmental protection. For example, 2 substances that have been burned from the market already are methyl bromide and dibromochloropropane. Also, another highly toxic substance is fosthiazate. The volatility of the avermectins resulted in inadequate field control (Heiken, 2017; Abd-Elgawad, 2016; Khan, Sayed, Shaukat and Handoo, 2008). Their regular, long-term use boosted the resistance of the

nematodes, making nematode control even more difficult. The use of organophosphate and carbamate chemical substances will be restricted because of the negative impact that they have on the environment; this will result in a lower number of nematicides available for use (Chen, Li and Song, 2020).

According to reports, synthetic dichloro- trichloroethane (DDT) is hazardous as it contaminates groundwater and the environment, where it builds up (by biomagnification) into trophic levels and stays inside the bodies of the species, building up poison (Pandya, 2018). “In addition, the synthetic chemicals that are responsible for controlling plant-parasitic nematodes are of high costs and therefore subsistence farmers in Africa do not afford them”, said Ibraham, Elderiny, Ansari, Rizvi, Sumbul and Mahmood (2020).

2.10. Plant extracts in nematodes management

There is a myriad of compounds within the plant extracts which indicate the suppressive properties of nematodes. For example, compounds produced from plants extracts, such as allicin from garlic and azadirachtin from neem, have demonstrated nematicidal effects (Salim, Salman, Majeed and Hussein, 2016). A study by Bernard, Egnin, and Bonsi (2017) found that *M. incognita, Helicotylenchus multicinctus* and *Hoplolaimus* were supressed by the nematicidal activities of ethanolic extracts of *Azadirachta indica* (neem), *Withania somnifera* (ashwagandha), *Tagetes erecta* (marigold) and *Eucalyptus citrodora* (eucalyptus) which showed to work the same as chemical nematicidal control. According to Khosa, Dube, Tselanyane, Fouche, De Waele and Daneel (2020b), milled leave, stem or bulb extracts had high efficacy in reducing nematode population of *M. angolensis*, as a potential tactic of controlling and managing the root-knot disease. *Maerua angolensis* and *T. elegans* (phytonematicide) stand out as they show nematicidal properties when they were used to suppress *M. incognita* (Khosa *et al*., 2021). These plants repel, deterrent and or poison the pests

when they come into contact with them since they produce phytochemicals such as alkaloids, steroids, phenols and other compounds which have nematicidal activity (Sithole, Kulkarni, Finnie and Van Staden, 2021).

According to various research by Khosa *et al.,* (2020a) and Khalil (2014), concentrated plant extracts from the leaves, root or stem of the plant have been discovered to contain chemicals that are active bioagents are often ground into powder. Furthermore, Khosa *et al.* (2020a) demonstrated that plant extracts can suppress plant parasitic nematodes by nematicidal action. A 5g crude powder was found to reduce eggs developing into J2 nematodes significantly.

According to reports by Khosa *et al*. (2020b), plants like *M. angolensis* and *T. elegans* found throughout the Limpopo province (SA) that are used by indigenous healers for their medicinal properties suppress nematode infestation in plants, especially in tomato.

2.10.1. *Maerua angolensis* (Bead-bean) in PPNs management e

Maerua angolensis (Figure 2.4) belongs to the Capparaceae family, originates from the East coast of South Africa and Swaziland (Benneh, Biney, Mante, Tandoh, Adongo and Woode, 2017). *Maerua angolensis* is used as a treatment for many diseases, such as ecthyma, epilepsy, diarrhoea and insomnia (Khosa *et al.,* 2020a). Moreover, the plant can be used in treating skin rashes, nose infections and vomiting, also in preventing miscarriage (Benneh *et al.,* 2017).

Maerua angolensis comprises of compounds that exhibit nematicidal action, and are alkaloids, flavonoids, tannins and saponins. These chemicals disrupt nematode membrane, hinder egg hatching, and affect movement and feeding. Flavonoids may potentially interfere with nematode signaling and reproduction (Khosa, Mashela and Dube, 2019). This combined biochemical effect results in enhanced plant health and reduced nematode infection (Khosa *et al.,* 2020). This actions applies to *Tabernaemontana elegans* as well.

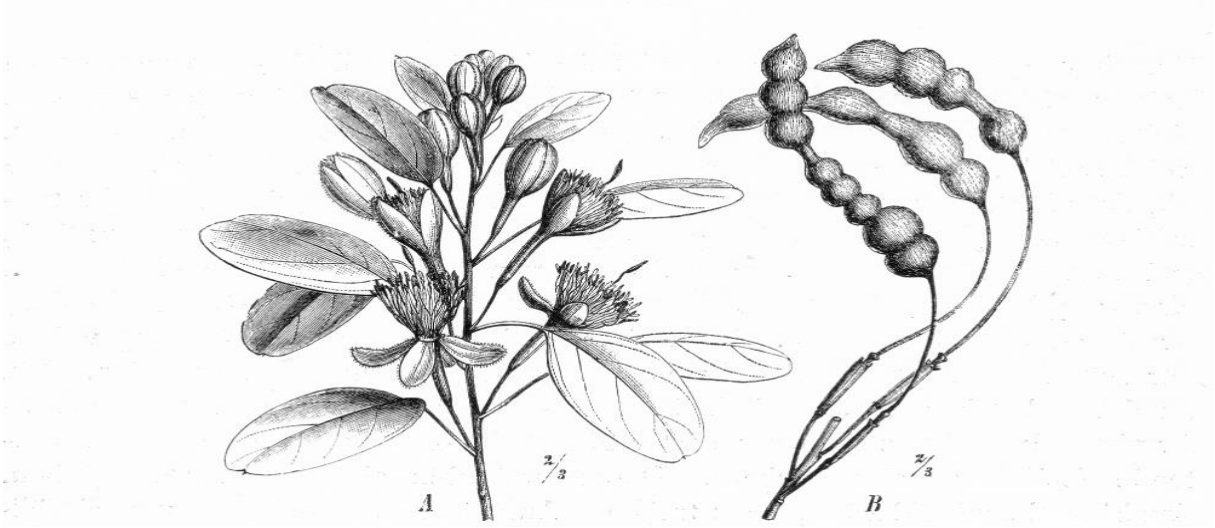


Figure 2.4: *Maerua angolensis* reproductive parts. A- Flowers, B- Seeds (Alamy Stock Photo,1887).

2.10.2. *Tabernaemontana elegans* (Toad tree) in PPNs management

Tabernaemontana elegans is native to Africa, specifically in South Africa and Swaziland. This plant belongs to the Apocynaceae family and is found along the riverbanks in savannah woodlands (Pallant, Cromarty and Steenkamp, 2012). This plant can also be smoked as in cigarettes, where the seeds are cooked, ground to a powder and mixed with tobacco (Mansoor, Borralho, Dewanjee, Mulhovo, Rodrigues and Ferreira, 2013). Lung cancer and stomach disorders can be treated with root infusions of this extract (Dahlin and Hallman, 2020). Again, the root can also be used in treating Tuberculosis (TB) (Mansoor *et al.,* 2013). In addition, a part of this material, "potpourri", which includes the root of the *T. elegans* is utilized to treat some venereal illnesses (Pallant *et al.,* 2012). The endocarp of this seed is dried, cooked and administered to treat cancer (Pallant *et al.,* 2012). Like *Maerua angolensis*, the aqueous methalic of this extract is composed of qualities that are anti-inflammatory (Khosa *et al.,* 2020a).

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CHAPTER 3

EFFECTS OF APPLICATION TIMING OF *MAERUA ANGOLENSIS* AND *TABERNAEMONTANA ELEGANS* ON *MELOIDOGYNE JAVANICA* AND *MELOIDOGYNE ENTEROLOBII* POPULATION DENSITIES AND TOMATO PLANT GROWTH UNDER GREENHOUSE CONDITIONS

3.1. Introduction

The nematicidal efficacy of *Maerua angolensis* and *Tabernaemontana elegans* extracts have been researched extensively with models developed to explain the relationship between extracts and nematodes (Khosa *et al.,* 2020a,b; Khosa *et al*., 2021; Khosa *et al.,* 2022). Khosa *et al.* (2020b) used the Curve-fitting Allelochemical Response Dosage (CARD) model to explain the inhibition rate of *M. angolensis* and *T. elegans* plant extracts on juvenile hatch and mortality in glasshouse. According to the CARD model, the effect of the two plant extracts on *M. incognita* J2 mortality and the J2 hatch were comparable to the commercial synthetic nematicide (Khosa *et al.,* 2020b). The model was able to establish the lowest nematode juvenile inhibition rates as well as the sensitivity of *Meloidogyne incognita* to *M. angolensis* and *T. elegans* plant extracts. The generated model explained 74%, 93% and 97% of the juvenile hatch inhibition DDG patterns due to *M. angolensis* exposure periods of 7, 14 and 21 days, respectively, and 87%, 98% and 99% for *T. elegans* at the same exposure (Khosa *et al.,* 2020b). The derived model also explained juvenile mortality due to *M. angolensis* at 92%, 85% and 96% of the effect at 24-, 48- and 72-hours exposure periods, respectively, and for *T. elegans*, they explained 89%, 94% and 70% at the same exposure. Khosa, *et al.* (2021) demonstrated that five-meal plant extracts, including those from *M. angolensis* and *T. elegans*, could reduce J2 hatch percentage by 9.3% - 18.2 % after 7 day exposure and 46.2%- 65.5% after 21 days of exposure. Also, the effect on the J2 mortality increased as exposure time increased for all extracts, from 13.1-19.8% after 24 h exposure to 38.1-49.7% after 72 h exposure (Khosa *et al.,*

2021). Under field and microplot conditions, *M. angolensis*, *T. elegans and C. cactiformis* in were able to manage *M. incognita* race 2 (Khosa *et al.,* 2022). The total number of eggs and J2 of *M. incognita* race 2 in the roots of infected plants was significantly supressed by 82–97% under microplot and 83–87% under field conditions (Khosa *et al.,* 2022).

This demonstrated the possible high potency of *T. elegans* and *M. elegans* in controlling nematodes.

There is little information on the effects of application timing on nematode management. . When it comes to pest control, time of application directly influences the pesticides' maximum effect on the targeted pests. While minimizing adverse impact on non-target organisms and environment (Parween and Jan 2019). Also, application of pesticides is impacted by environmental factors, thus the time of application is crucial to the effectiveness of these botanical extracts (Lengai, Muthomi and Mbega, 2020). Botelho, da Silva and Ávila (2018) assessed the effectiveness of two concentrations of the Chin-IA (I-A) isolate (ChinSNPV) in controlling *Chrysodeixis includens,* a soyabean looper. A 70% loss in the control of the pest was observed when the application was separated by four days (Melo and Swarowsky, 2022). Mnyambo, Rantho, Dube and Timana (2024), demonstrated that plant extracts timing is important in managing *M. incognita*. Applying *M. angolensis* and *T. elegans* plant extracts prior or post planting affected the nematode management differently, with prior application being comparable to a commercial synthetic nematicide, Nemacur (Mcunu, 2023)). Even though application of plant extract relative to crop planting is important, the relationship between plant extract application and nematode inoculum might bring a better picture of the way in which plant extracts control nematodes. Therefore, the objective of this study was to assess the time of application of *M. angolensis* and *T. elegans* in controlling nematodes population densities and improve tomato plant growth under greenhouse conditions.

3.2. Material and methods

3.2.1. Description of the study site

The current experiment was conducted under greenhouse conditions at the University of Mpumalanga farm (25.4365° S, 30.9818° E), Nelspruit, South Africa. Greenhouse temperatures were set at 25 ± 30 °C, with thermostatically controlled fans and wet wall at opposite ends. The experiment was conducted during the summer and autumn seasons, 2023 and 2024, respectively.

3.2.2. Plant extracts and inoculum preparation

Plant extract powders were prepared from *M. angolensis* and *T. elegans* leaves as previously described (Khosa *et al.,* 2020). *Meloidogyne javanica* and *M. enterolobii* inocula (J2 and eggs) were obtained from Agricultural Research Council- Tropical and Subtropical Crops (ARC-TSC), Nelspruit, S.A. The population of *M. javanica* and *M. enterolobii* were previously identified and verified using sequence-characterized amplified region-polymerase chain reaction (SCAR-PCR) (Fourie, McDonald and Loots, 2001). The book of Fourie *et al.,* (2017) explains that the population of the *M. javanica and M. enterolobii* was established using single egg mass principle from an infected tomato cultivars.

3.2.3. Treatment and experimental design

Thirty-centimetre diameter plastic pots were filled with a mixture of steam-pasteurized (250 °C for 4½ h) loamy and sandy soil at a 1:3 (v/v) ratio. The pots were then placed on greenhouse benches at a spacing of 0.3 m intra- and 0.8 minter-row spacing. Each pot received two tomato seedlings, cultivar ‘Star 9009’ (Ezigro company, Whiteriver). Two weeks after transplanting, the seedlings were thinned to one per pot before being separately inoculated with 5000 *M. javanica* and *M. enterolobii* eggs and second-stage juvenile (J2), using a 20 ml plastic syringe.

The treatments were arranged as 2 x 2 x 3 + 2 factorial experiment, laid out in a randomized complete block design (RCBD) with 5 replications (blocking was done to reduce experimental errors and any other variation in the environmental condition that might have affected the experimental outcome).

The first factor consisted of two plant extracts (*M. angolensis* and *T. elegans*) at 5g per plant, and the second factor was made up of two nematodes species (*M. javanica* and *M. enterolobii*) at 5000 eggs and J2 per plant. The third factor was made up of three application and inoculation times [(1. Plant extract applied before nematode inoculation; 2. Plant extract applied simultaneously with nematode inoculation; 3. Plant extract applied after nematode inoculation). Two controls were also added as treatments, positive control (Crop Guard [Furfural (Aldehyde)]) at a recommended rate of 5g per plant, and negative control (plants inoculated with nematode only)]. Plant extract application/ nematode inoculation treatments were separated by a week. Plants were monitored, and irrigation was done using 250ml of water when necessary to maintain 60% water capacity. Other than the nematodes, which we were working on , none of the pests were controlled as they were not the focus on the experiment.

Figure 3.1**:** Experimental trial under greenhouse conditions**.** Photo by Nkosingiphile Zulu, 2024.

3.3. Data collection

At 56 days after the last treatment application, the greenhouse experiment was terminated, nematode and plant growth variables were measured.

3.3.1. Nematode variables

Using the maceration and blending procedure, nematodes were extracted from the entire root system (Fourie, Mc Donald, Steenkamp and De Waele, 2017). Soil from each pot was thoroughly mixed, and a 250 mL sample taken for nematode extraction. Sugar floatation and centrifugation method was used to extract nematodes from the soil samples (Fourie *et al*., 2017). Ten 1 ml aliquot of each sample was counted under a stereomicroscope (Olympus Corporation Tokyo 163-0914, CX23RTFS2) for eggs and second-stage juveniles (J2) from the

tomato root and J2 from the soil, with averages calculated. The total number of eggs and J2 from the tomato root system was added to the total J2 from the soil to calculate the final nematode population density per pot.

3.3.2. Plant growth variables

The height of plants was measured from the soil surface to flag leaf tip before cutting the plant at soil line using a measuring tape. A digital vernier calliper (Model no.: 464-9952, RS PRO, South Africa) was used to measure the stem diameter 3 cm above cut end of the stem. The chlorophyll meter (Model no.: 900PDL, GA SPAD 502q, United State of America) was employed to ascertain the concentration of chlorophyl on the fully developed upper leaves. The plant shoots mass were by first drying the shoot in an oven at 70 °C for 72 hours and weighed them. After removing the root system from the soil, it was cleaned to eliminate any remaining dirt particles, blotted dry with a paper towel, and weighed.

3.4. Data analysis

The data on nematode and plant growth variables were subjected to an analysis of variance (ANOVA) using Statisticx 10 software. Normality distribution was ascertained using the Shapiro-Wilk normality test prior to ANOVA. $Log_{10}(x+1)$ was used to convert non-continuous data to homogenous variance (Gomez and Gomez, 1984). Mean separation was achieved using Fisher's least significant difference at 5 % probability level.

3.5. Results

Shapiro-Wilk normality tests showed that none of the nematode and plant growth variables were normally distributed ($P \leq 0.05$), except for stem diameter, chlorophyll content, plant height and fresh shoot mass (Appendix 3.1). As a results, the variables were therefore transformed accordingly.

3.5.1. Nematode variables

Second-order interaction between season, nematode species, and time of plant extract application were not statistically significant ($P > 0.05$) on all measured variables, except for J2 in soil and tomato root (Appendices 3.3 and 3.4). However, first-order interactions of season-plant extracts and season-time of application significantly influenced eggs, total nematodes in tomato root, and the final nematode populations (Appendices 3.2, 3.4, 3.5, and 3.6). When each factor was examined independently, season significantly impacted J2 in tomato roots, the total number of nematodes in tomato roots and the final nematode populations. Similarly, the time of plant extract application influenced J2 in tomato root and soil, total nematode numbers in tomato root, and the final nematode populations (Appendices 3.2-3.6).

In summer, the time of plant extract application demonstrated variable effects depending on the nematodes and season (Table 3.1). When extracts were applied before nematode inoculation, *M. javanica* populations in the soil were lower compared to *M. enterolobii*, the trend reversed when plant extracts were applied after nematode inoculation (Table 3.1). Furthermore, simultaneous application of plant extracts with nematode inoculation resulted in higher *M. javanica* J2 in tomato root compared to *M. enterolobii* (Table 3.1). For juvenile numbers in soil, there were differences observed between the positive control (Crop Guard) and the negative control (Table 3.1). The crop guard had lower number of J2 in soil than negative control while for J2 in tomato root, there were no differences observed (Table 3.1)

In autumn, the time of plant extract application; whether before, simultaneously, or after nematode inoculation did not significantly influence J2 in tomato root but plant extract applied after nematode inoculation significantly differed from the two plant extracts application in J2 in soil. (Table 3.1). Second stage juvenile numbers in soil did not differ between the two nematode species for plants treated with either positive or negative control. However, plants

treated with crop guard consistently exhibited lower J2 numbers in soil compared to those under the negative control treatment and no differences were observed in J2 in tomato root.

Table 3.1: Interactive effects of season, nematode species and time of application on nematode second-stage juveniles in soil and in tomato root.

Season	Nematode species	Time of application	Juveniles in soil	Juveniles in tomato root
Summer	*Meloidogyne javanica*	PF	2.538^{j} (440)	3.875^{a} (8470)
Summer	*Meloidogyne enterolobii*	PF	2.875^{bcdefg} (1010)	3.767^{ab} (6890)
Summer	*Meloidogyne javanica*	P+N	2.644^{hij} (520)	3.517^{defg} (5290)
Summer	*Meloidogyne enterolobii*	P+N	2.679^{ghij} (550)	3.754^{abc} (8040)
Summer	*Meloidogyne javanica*	PL	2.882^{bcdefg} (970)	3.532^{cdef} (4090)
Summer	*Meloidogyne enterolobii*	PL	2.641^{hij} (550)	3.611^{bcde} (4550)
Summer	No control (*M. javanica & M. enterolobii*)	-a	2.971^{abcde} (1470)	3.447^{efgh} (3210)
Summer	Crop guard (*M. javanica & M. enterolobii*)	+b	2.653^{hij} (500)	3.412^{efgh} (3150)
Autumn	*Meloidogyne javanica*	PF	3.001^{abcd} (1030)	3.336^{fghi} (2230)
Autumn	*Meloidogyne enterolobii*	PF	2.911^{abcdef} (830)	3.311^{fghi} (2150)
Autumn	*Meloidogyne javanica*	P+N	2.788^{defgh} (670)	3.252^{hij} (1940)
Autumn	*Meloidogyne enterolobii*	P+N	2.809^{cdefgh} (680)	3.300^{ghi} (2060)

Autumn	*Meloidogyne javanica*	PL	2.564^{ij} (380)	3.132^{ij} (1450)
Autumn	*Meloidogyne enterolobii*	PL	2.563^{ij} (380)	3.058^{j} (1190)
Autumn	No control (*M. javanica & M. enterolobii*)	-a	3.057^{ab} (1150)	3.325^{fghi} (2570)
Autumn	Crop guard (*M. javanica & M. enterolobii*)	+b	2.664^{ghij} (470)	3.296^{ghi} (2110)
P Value			0.0301*	0.0333*
$LSD_{0.05}$ Value			0.2201	0.2303
F Value			2.75	2.69

*Significant difference($P \leq 0.05$). PF- Plant extracts applied before nematode inoculation. P+N- Plant extracts and nematode inoculation applied simultaneously. PL- Plant extracts applied after nematode inoculation. +b- positive control (Crop Guard); -a- negative control (no control treatments). Numbers in brackets are untransformed means.

Season influences on effects of plant extracts were also observed (Table 3.2). In both seasons, applying plant extracts after nematode inoculation resulted in significantly lower total nematode numbers in tomato root and final nematode populations, compared to applying the extracts before and simultaneously with nematode inoculation (Table 3.2).

All three plants extract application (before, simultaneously, and after nematode inoculation) showed significant differences for J2 in soil (Table 3.2). Applying plant extracts after inoculation yielded the lowest nematode numbers, followed by simultaneous application, with plant extract applied before nematode inoculation having the highest numbers. Across all measured variables, positive control consistently achieved lower nematode numbers than negative control.

Table 3.2**:** Interactive effects of season and time of application on nematodes population density

Season	Time of application	Eggs in tomato root	Total number of nematodes in tomato root	Final nematode populations
Summer	PF	3.485[a] (3565)	4.011[a] (11245)	4.039[a] (11970)
Summer	P+N	3.552[a] (5940)	3.992[ab] (12605)	4.019[ab] (13140)
Summer	PL	3.430[ab] (3350)	3.832[cd] (7670)	3.881[cd] (8430)
Summer	-a	3.505[a] (4520)	3.897[ab] (9120)	3.975[abc] 10775)
Summer	+b	3.494[a] (3860)	3.876[c] (8675)	3.919[bcd] (9295)
Autumn	PF	3.307[bc] (2080)	3.623[e] (4220)	3.710[e] (5150)
Autumn	P+N	3.211[cd] (1800)	3.577[e] (3800)	3.649[e] (4475)
Autumn	PL	3.082d (1295)	3.412[f] (2615)	3.472[f] (2995)
Autumn	-a	3.472[ab] (3025)	3.758[d] (5780)	3.836[d] (6895)
Autumn	+b	3.163[cd] (1545)	3.548[f] (3630)	3.609[e] (4155)
P Value		0.0338*	0.0022**	0.0039**
$LSD_{0.05}$		0.1668	0.1110	0.1019
F Value		2.68	4.37	4.03

*Significant difference (P≤0.05). **Highly significant difference (P≤0.01). PF- Plant extracts applied before nematode inoculation. P+N- Plant extracts and nematode inoculation applied simultaneously. PL- Plant extracts after applied after nematode inoculation. +b- positive control (Crop Guard). -a- negative control(no control treatments). Numbers in brackets are untransformed means.

In summer, plants treated with *M. angolensis* had a lower number of eggs found in tomato root (3396) and final nematode population (9286), when compared to those treated with *T. elegans* (5098 and 12158, respectively) (Table 3.3). In autumn, the effect of *M. angolensis* and *T.*

elegans were the same on both eggs found in tomato root and the final nematode populations (Table 3.3).

Table 3.3: Interactive effects of season and plant extracts on the number of eggs in tomato root and final nematode populations.

Season	Plant extract	Eggs in tomato root	Final nematode populations
Summer	*Tabernaemontana elegans*	3.562[a] (5098)	4.021[a] (12158)
Summer	*Marua angolensis*	3.424[b] (3396)	3.912[b] (9286)
Autumn	*Tabernaemontana elegans*	3.265[c] (1898)	3.657[c] (4766)
Autumn	*Maerua angolensis*	3.229[c] (2000)	3.653[c] (4702)
P Value		0.0221*	0.0239*
$LSD_{0.05}$ Value		0.1055	0.0644
F Value		5.34	5.20

*Significant difference($P \leq 0.05$). The numbers in brackets are untransformed.

3.5.2. Plant variables

Second-order interactions between season, nematode species, and time of plant extract application were not significant ($P > 0.05$) on plant variables, except for number of leaves, fresh shoot mass and root mass (Appendices 3.11, 3.13 and 3.15). Among first-order interactions, season and nematodes significantly impacted the number of fruits. Additionally, season and time of application had significant effects on variables such as the number of fruits, stem diameter, chlorophyll content, plant height, and dry shoot mass (Appendices 3.8, 3.9, 3.10, 3.112 and 3.14).

When factors were analysed individually, season significantly influenced flower and gall rating, time of application significantly influenced flower and nematodes significantly influenced stem diameter (Appendices 3.7, 3.9 and 3.16).

Plant extracts applied before, simultaneously with and after nematode inoculation had no differing effect on the number of leaves across both nematodes (Table 3.4).

Both the negative and positive control treatments did not vary in the number of leaves between nematodes The population of *Meloidogyne javanica* and *M. enterolobii* on No control were significantly the same, same applies to the treatment of Crop guard in terms of the nematode spp.

Table 3.4: Interaction of nematodes, plant extracts and application time on number of leaves.

Nematodes	Plant extracts	Time of application	Number of leaves
Meloidogyne javanica	*Maerua angolensis*	PF	0.313^{abcd} (10.7)
Meloidogyne javanica	*Tabernaemontana elegans*	PF	0.3111^{bcd} (10.3)
Meloidogyne enterolobii	*Tabernaemontana elegans*	PF	0.308^{bcd} (10.3)
Meloidogyne enterolobii	*Maerua angolensis*	PF	0.306^{bcd} (9.8)
Meloidogyne javanica	*Maerua angolensis*	P+N	0.328^{a} (12.9)
Meloidogyne javanica	*Tabernaemontana elegans*	P+N	0.317^{abcd} (11.1)
Meloidogyne enterolobii	*Tabernaemontana elegans*	P+N	0.314^{abcd} (10.7)
Meloidogyne enterolobii	*Maerua angolensis*	P+N	0.314^{abcd} (10.7)
Meloidogyne enterolobii	*Maerua angolensis*	PL	0.322^{ab} (11.7)
Meloidogyne javanica	*Tabernaemontana elegans*	PL	0.319^{abc} (11.6)
Meloidogyne javanica	*Maerua angolensis*	PL	0.316^{abcd} (10.9)
Meloidogyne enterolobii	*Tabernaemontana elegans*	PL	0.303^{cde} (9.7)
Meloidogyne javanica	No control	-a	0.320^{ab} (11.8)
Meloidogyne enterolobii	No control	-a	0.316^{abcd} (11.4)
Meloidogyne javanica	Crop guard	+b	0.287^{ef} (8.6)
Meloidogyne enterolobii	Crop guard	+b	0.280^{f} (8.6)
P Value			0.0040**
$LSD_{0.05}$			0.0171
F Value			4.01

**Highly significant difference. PF- Plant extracts applied before nematode inoculation. P+N- Plant extracts and nematode inoculation were applied simultaneously. PL- Plant extracts after applied after nematode inoculation -a: Negative control (no treatment). +b: Positive control (Crop Guard). Values in brackets are untransformed.

Within each season, the time of plant extract application before nematode inoculation did not significantly affect fresh shoot mass for either nematode (Table 3.5). However, when comparing seasons, differences emerged: plant extracts applied in summer increased fresh shoot mass, while those applied in autumn resulted in a reduction (Table 3.5). Similarly, plant extracts applied simultaneously with nematode inoculation showed no differences within each season, but across seasons, summer applications increased fresh shoot mass, whereas autumn applications reduced it (Table 3.5).

For plant extracts applied after nematode inoculation, no differences were observed within each season (Table 3.5). However, comparing the two seasons, plants treated with extracts in summer exhibited higher fresh shoot mass than those treated in autumn (Table 3.5). For the negative control, which is no control, differences in fresh shoot mass were observed between the two seasons. There was higher number of fresh shoot mass in summer than in autumn. A similar trend was observed with crop guard, mirroring the effects seen in negative control (Table 3.5).

Table 3.5: Interactive effects of season, plant extracts and time of application on fresh shoot mass.

Season	Plant extracts	Time of application	Fresh shoot mass
Summer	*Maerua angolensis*	PF	0.435^{a} (52.700)
Summer	*Tabernaemontana elegans*	PF	0.432^{a} (51.286)
Autumn	*Tabernaemontana elegans*	PF	0.386^{bcd} (26.858)
Autumn	*Maerua angolensis*	PF	0.382^{bcd} (25.994)
Summer	*Tabernaemontana elegans*	P+N	0.431^{a} (49.640)

Summer	*Maerua angolensis*	P+N	0.429^{a} (48.231)
Autumn	*Tabernaemontana elegans*	P+N	0.383^{bcd} (25.994)
Autumn	*Maerua angolensis*	P+N	0.383^{bcd} (26.005)
Summer	*Maerua angolensis*	PL	0.441^{a} (57.007)
Summer	*Tabernaemontana elegans*	PL	0.437^{a} (54.281)
Autumn	*Maerua angolensis*	PL	0.375^{cd} (22.875)
Autumn	*Tabernaemontana elegans*	PL	0.371^{cd} (22.372)
Summer	No control	-a	0.446^{a} (62.767)
Autumn	No control	-a	0.268^{f} (7.149)
Summer	Crop guard	+b	0.397^{bc} (31.780)
Autumn	Crop guard	+b	0.239^{g} (5.290)
P Value			0.0199**
$LSD_{0.05}$ Value			0.0237
F Value			3.01

**Highly significant difference. PF- Plant extracts applied before nematode inoculation. P+N- Plant extracts and nematode inoculation are applied simultaneously. PL- Plant extracts after applied after nematode inoculation -a: Negative control (no treatment). +b: Positive control (Crop Guard). Values in brackets are untransformed.

The effect of time of application of plant extracts applied first before nematode inoculation in autumn was not different for both nematode species; the same applies in summer (Table 3.6). There were no differences observed in the effect of application of plant extracts applied simultaneously with nematodes inoculation particularly in autumn and in summer (Table 3.6). This trend is the same as in the plant extracts applied after nematode inoculation. However, for the three- time of application of plant extracts, when comparing the two seasons, plants treated with plant extracts in autumn had higher root mass than those in summer (Table 3.6). The effect of no control resulted in differences amongst the two seasons. In autumn, there was a higher number of root mass than in summer. The trend was the in plants treated with crop for both seasons.

Table 3.6: Interaction of seasons, nematodes and time of application on root mass.

Seasons	Nematodes	Time of application	Root mass
Autumn	*Meloidogyne enterolobii*	PF	0.412[a] (38.703)
Autumn	*Meloidogyne javanica*	PF	0.401[ab] (32.272)
Summer	*Meloidogyne enterolobii*	PF	0.323[ef] (13.249)
Summer	*Meloidogyne javanica*	PF	0.340[e] (16.247)
Autumn	*Meloidogyne enterolobii*	P+N	0.400[abc] (34.292)
Autumn	*Meloidogyne javanica*	P+N	0.382[bcd] (26.341)
Summer	*Meloidogyne javanica*	P+N	0.318[ef] (11.300)
Summer	*Meloidogyne enterolobii*	P+N	0.311[fg] (10.815)
Autumn	*Meloidogyne javanica*	PL	0.395[abc] (30.163)
Autumn	*Meloidogyne enterolobii*	PL	0.380[bcd] (26.624)
Summer	*Meloidogyne enterolobii*	PL	0.321[ef] (11.851)
Summer	*Meloidogyne javanica*	PL	0.295[g] (8.614)
Autumn	No control *(M. javanica)*	-a	0.396[abc] (30.497)
Summer	No control *(M. enterolobii)*	-a	0.224[i] (4.000)

Autumn	Crop guard (*M. javanica)*	+b	0.368^{d} (21.479)
Summer	Crop guard *(M. javanica)*	+b	0.257^{h} (5.622)
P Value			0.024*
$LSD_{0.05}$			0.023
F Value			2.89

**Significant difference. PF- Plant extracts applied before nematode inoculation. P+N- Plant extracts and nematode inoculation applied simultaneously. PL- Plant extracts after applied after nematode inoculation -a: Negative control (no treatment). +b: Positive control (Crop Guard). Values in brackets are untransformed.

Differences were observed between the two species of nematodes in summer, with *M. enterolobii* exposed plants having a lower number of fruits compared to those exposed to *M. javanica* (Table 3.7). No differences in the number of fruits were observed amongst the plant exposed to the two nematodes in autumn (Table 3.7).

Table 3.7: Interactive effects of season and nematodes on fruits.

Season	Nematodes	Number of fruits
Summer	*Meloidogyne enterolobii*	0.016^{a} (0.140)
Summer	*Meloidogyne javanica*	$2.29E\text{-}03^{b}$ (0.020)
Autumn	*Meloidogyne enterolobii*	0.000^{b} (0.000)
Autumn	*Meloidogyne javanica*	0.000^{b} (0.000)
P Value		0.020*
$LDS_{0.05}$		8,177E-03
F Value		5.49

* significant differences. Values in the brackets are untransformed.

In summer, the time of plant extract application whether applied after nematode inoculation, first before inoculation, or simultaneously with nematode inoculation showed no differences in their effects on the number of leaves, stem diameter, chlorophyll content, plant height, and dry shoot mass (Table 3.8). However, for the number of fruits, differences were noted: plants treated with extracts applied after nematode inoculation produced more fruits than those treated with plant extracts applied simultaneously with nematode inoculation (Table 3.8). For the negative and positive controls, no differences were observed in their effects on fruit number, chlorophyll content, or plant height. Differences, however, were noted in stem diameter, leaf count, and dry shoot mass, with plants treated with the positive control showing lower values than those treated with the negative control (Table 3.8).

In autumn, the positive and negative controls did not differ in their effects on the number of fruits, stem diameter, plant height, or dry shoot mass. However, differences were observed in chlorophyll content and the number of leaves, with plants treated with the positive control exhibiting lower values than those treated with the negative control (Table 3.8). For plant extracts, no differences were observed in their effects on fruits number, stem diameter, leaves, plant height, or dry shoot mass. The only observed difference was in chlorophyll content, where plants treated with extracts applied first before nematode inoculation had lower chlorophyll content than those treated with extracts applied after or simultaneously with nematode inoculation (Table 3.8).

Table 3.8**:** Interactive effects of season and time of application on plant variables.

Season	Time of application	Number of fruits	Stem diameter	Chlorophyll content	Number of leaves	Plant height	Dry shoot mass
Summer	PL	0.028^{a} (0.250)	0.259^{ab} (5.627)	0.373^{a} (22.625)	0.326^{ab} (12.250)	0.411^{c} (37.710)	0.308^{a} (9.875)

Summer	PF	0.011[b] (0.100)	0.259[ab] (5602)	0.373[a] (22.285)	0.317[b] (11.050)	0.412[c] (37.550)	0.304[a] (9.561)
Summer	P+N	5.71E-03[b] (0.050)	0.261[a] (5.716)	0.380[a] (25.040)	0.321[b] (11.700)	0.410[c] (36.820)	0.298[a] (8.897)
Summer	-a	0.000[b] (0.000)	0.260[a] (5.696)	0.375[a] (23.105)	0.335[a] (13.750)	0.414[c] (39.055)	0.308[a] (10.112)
Summer	+b	0.000[b] (0.000)	0.253[bc] (5.223)	0.365[a] (20.445)	0.321[b] (11.650)	0.406[c] (34.825)	0.247[b] (5.065)
Autumn	-a	0.000[b] (0.000)	0.234[d] (4.320)	0.254[c] (6.425)	0.296[d] (8.650)	0.440[b] (57.225)	0.220[c] (3.886)
Autumn	+b	0.000[b] (0.000)	0.239[d] (4.520)	0.188[d] (2.740)	0.267[e] (6.850)	0.445[b] (62.850)	0.215[c] (3.750)
Autumn	P+N	0.000[b] (0.000)	0.257[ab] (5.500)	0.315[b] (11.295)	0.314[bc] (10.850)	0.468[a] (87.325)	0.253[b] (5.299)
Autumn	PF	0.000[b] (0.00)	0.255[abc] (5.340)	0.264[c] (6.530)	0.303[cd] (9.350)	0.468[a] (87.475)	0.255[b] (5.387)
Autumn	PL	0.000[b] (0.000)	0.248[c] (4.960)	0.312[b] (11.305)	0.304[cd] (9.700)	0.463[a] (83.000)	0.244[b] (4.755)
P Value		0.013**	0.000**	0.000**	0.000**	0.000**	0.000**
$LDS_{0.05}$		0.012	7.641E-03	0.021	0.012	7.915E-03	0.014
F Value		3.28	5.50	20.19	9.87	11.30	8.66

**Highly significant difference. PF- Plant extracts applied before nematode inoculation. P+N- Plant extracts and nematode inoculation applied simultaneously. PL- Plant extracts applied after nematode inoculation -a: Negative control (no treatment). +b: Positive control Crop Guard). Values in brackets are untransformed.

There were no differences in effect amongst the application interval of three plant extracts for both nematodes while differences were observed within plants treated with negative and positive control (Crop Guard) for both nematodes (Table 3.9).

Table 3.9: Interaction of nematodes and time of application on fresh shoot mass.

Nematodes	Time of application	Fresh shoot mass
Meloidogyne enterolobii	PF	0.412^{a} (40.454)
Meloidogyne javanica	PF	0.405^{a} (37.965)
Meloidogyne javanica	P+N	0.408^{a} (37.972)
Meloidogyne enterolobii	P+N	0.405^{a} (36.722)
Meloidogyne enterolobii	PL	0.407^{a} (39.177)
Meloidogyne javanica	PL	0.404^{a} (39.090)
No control (*M. javanica*)	-a	0.372^{b} (35.879)
No control (*M. enterolobii)*	-a	0.350^{c} (34.853)
Crop guard (*M. javanica)*	+b	0.345^{c} (23.285)
Crop guard (*M. enterolobii)*	+b	0.322^{d} (17.026)
P Value		0,0234*
$LSD_{0.05}$		0,0167
F Value		2,91

*Significant difference. PF- Plant extracts applied before nematode inoculation. P+N- Plant extracts and nematode inoculation are applied simultaneously. PL- Plant extracts after applied after nematode inoculation -a: Negative control (no treatment). +b: Positive control (Crop Guard). Values in brackets are untransformed

Season affected the number of flower and gall rating differently. The number of flowers in summer were lower than those of in autumn while vice versa in effect of season was observed in gall rating (Table 3.10).

Table 3.10: Effects of season on the number of flower and gall rating.

Season	Number of flower	Gall rate
Summer	0.050^{b} (0.600)	0.100^{a} (5.950)
Autumn	0.086^{a} (1.060)	0.000^{b} (0.000)
P Value	0.0004**	0.0000**
$LDS_{0.05}$	0.0197	1.788E-03

F Value	13.19	12384.79

**Highly significant difference. Values in brackets are untransformed.

There were no differences in effect in the number of flowers amongst the application interval of three plant extracts. Also, for the two controls, no differences in the effect of the treatment were observed (Table 3.11).

Table 3.11: Effects of application time on number of flowers.

Time of application	Number of flowers
P+N	0.105^{a} (1.225)
PF	0.102^{a} (1.200)
PL	0.092^{a} (1.175)
+b	0.024^{b} (0.325)
-a	0.017^{b} (0.225)
P Value	0.000**
$LDS_{0.05}$	0.031
F Value	15.29

**Highly significant difference. PF- Plant extracts applied before nematode inoculation. P+N- Plant extracts and nematode inoculation are applied simultaneously. PL- Plant extracts after applied after nematode inoculation -a: Negative control (no treatment). +b: Positive control (crop guard). Values in brackets are untransformed

Differences were observed between *M javanica* and *M. enterolobii*. Stem diameter was recorded higher in plants infected with *M. javanica* rather than *M. enterolobii* (Table 3.12).

Table 3. 12: Effects of nematodes on stem diameter.

Nematodes	Stem diameter

Meloidogyne javanica	0.2549[a] (5.337)
Meloidogyne enterolobii	0.2512[b] (5.164)
P Value	0.0365*
$LDS_{0.05}$	3.417E-03
F Value	4.45

* Significant difference. Values in brackets are untransformed

3.6. Discussion

The experiments conducted was to ascertain the effect of the plant extracts in controlling root-knot nematode, *M. javanica* and *M. enterolobii* during the summer and autumn growing seasons. It is apparent from the current results that amending soil with plant extracts in summer increased juveniles in tomato root and the final nematode population density. *Tabernaemontana elegans* and *M. angolensis* plant extract levels were applied 3 times at different times: plant extract application first before nematode inoculation (PF); plant extracts application simultaneously with nematodes application (P+N); and plant extracts application after nematodes inoculation (PL); and the positive and the negative control.

The current findings demonstrated an interaction between season, nematodes and application time. According to these results, there is little to no impact on nematode, *M. javanica* and *M. enterolobii* caused by the application of plant extracts in the summer season. The tomato plants had a higher number of total nematodes after termination compared to the number of nematodes that were initially inoculated; this means that the application of plant extract could not control the nematodes. However, in details, comparing the application time, plant extracts applied at the same time with nematodes inoculation and plant extracts applied after nematode inoculation had a lower number of nematodes compared to plant extracts applied before nematodes inoculation. The numeral reasons contributing to the rise in nematode population during the summer season include warmer temperatures; summer`s high temperatures cause the soil

temperature to be high, which accelerate the metabolic process of nematodes fostering faster development, reproduction and population growth (Sagar, Banakar, Harshman and Khanal, 2025). In warmer condition, many nematodes, notably the root- knot nematode have a shorter life cycle, and this shorter life cycle enable them to reproduce more (leading to high population) quicky and inflict greater effects to crops (Khanal and Land, 2022).

However, according to the second results of this chapter (Chapter 3), our investigation has successfully revealed that in autumn, the interaction of nematode, season and time of application was effective because there was a huge reduction in the number of juveniles found in soil, tomato root, total number of nematodes in tomato root and the final nematode populations. In this trial, when the crop guard was applied, it was effective also but less compared to the application of plant extracts that were applied after nematodes inoculation. Therefore, in detail, when contrasting the time of applications, plant extracts applied after nematodes inoculation were more effective compared to the other application times, including the positive and negative control. Based on these results, a more effective nematode management technique may be achieved by applying *T. elegans* and *M. angolensis* as a protective treatment to plants after nematode inoculation. These results align with the report made by Khosa *et al.* (2021; 2020a; 2020b; 2013).

Khosa *et al.* (2020a; 2020b) demonstrated that *M. incognita* eggs and second- stage juveniles were highly sensitive to *M. angolensis* under *in vitro* and greenhouse conditions. Using a CARD Model, Khosa *et al.,* (2020b) showed that *M. angolensis* has a very high efficacy on hatching and mortality rates of *M. incognita.* These results contradict the report made by Timana, Mnyambo, Sebati, Kgotse, Ubisi, Mkhwanazi, Khosa, Mbatyoti, De Waele and Dube (2023) that higher nematode levels were observed in soil with plants exposed to plant extracts compared to the negative controls. They also align with the findings of Aydinli and Mennan

(2014), who reported that amending the soil with some plant extracts, namely *V. album*, suppressed the populations of *Meloidogyne arenaria* both in the soil and on the roots of tomatoes with a concomitant increase in the growth and yield of tomato. In a study conducted by Hussaini, Rao and Pandu (1996), it was observed that the leaf extracts of eleven different plant species induce a mortality rate of 90% among the juveniles and prevent egg hatching of *M. incognita, M. javanica and M. arenaria.* Our current findings on the autumn season are consistence with those of Hussaini, Rao and Pandu (1996) and Haroon, Hassan, Hamad and Rady (2018), who observed that several plant extracts exhibited notable nematicidal capabilities.

From the results of this chapter, taking into consideration phytochemical screening done by Dubale, Kebebe, Zeynudin, Abdissa and Suleman (2023), the plant extract *M. angolensis* outperformed *T. elegans*, and this observation reenforces the suggestion that the reason *M. angolensis* outperformed *T. elegans* is that the compound causing J2 in *T. elegans* could be present in lower concentration than in *M. angolensis* or maybe are totally different from the compound causing mortality in *M. angolensis.* These results agree with the results of Khosa, Dube, Tselanyane, Senabe, Fouche, De waele and Daneel (2021), who reported that the application of both *M. angolensis* and *T. elegans* was successful in reducing *M. incognita* J2 number and egg production in the greenhouse. However, after 7 days, when compared to *M. angolensis,* the extracts from *T. elegans* resulted in a slightly greater J2 hatching. Moreover, prior research has demonstrated that the effectiveness of organic amendments in controlling root-knot nematodes varies (Akhtar and Malik, 2000; Chitwood, 2002). According to Karuri (2022), it is very important to take into consideration the climatic condition and soil during the implementation of the management strategy for plant parasitic nematodes as these factors play a huge role in influencing the effectiveness of the control method. This is because some nematode species thrive well under different soil conditions, like sandy-loam soil, and others

thrive very well in climatic conditions such as temperature and moisture. The use of natural substances extracted from some plants is a recently rising approach in nematode control that is directed at reducing the population of plant parasitic nematodes in soil. These techniques do not cause any disruption to the natural biological equilibrium (fouriw *et al.,* 2018). Globally, the use of antagonistic plants or their by-products is a standard practice to minimize the risks associated with typical chemical nematicides (Ogumo, 2014).

Given the environmental issues brought forth by chemical control methods, it has become increasingly important in pest management to use botanical extracts derived from different portions plants in recent years (Mamun and Ahmed, 2011). In the current investigation, the different times of application of *M. angolensis* and *T. elegans* plant extracts on soil amendments before nematode inoculation, applied at the same time with nematode inoculation, and lastly applied after nematode inoculation had an impact on the plant growth densities. All these times of application significantly improved plant growth densities. The plant extracts, compared to the positive control on plant variables: number of leaves, fresh shoot mass, roots mass, chlorophyll content and dry shoot mass of tomato, had a greater effect than the those which received crop guard as treatment. This indicates that the plant extracts were acting as fertilizers to the plant. Seshweni (2016) demonstrated that because most phytonematicides perform a dual role in boosting plant growth and impending nematode development, plants treated with phytochemicals saw a considerable rise in fruit weight. These findings are consistent with the findings from previous research, indicating that most plant extracts have the ability to improve plant growth. Our summer results, using extracts made from *T. elegans* and *M. angolensis*, are consistent with published research by Khosa, Dube, Tselanyana, Fouche, De Waele and Daneel (2020) reported a higher shoot weight in plants treated with *M. angolensis* and *T. elegans* in the years 2008 and 2011. Hussain, Kasinadhuni and Arioli (2021) discovered that various plant growth variables were greatly boosted by the application of

seaweed extracts (SWE) to plants, and these parameters were the number of flower clusters and flowers, fruit number, dry weight of foliage and root length. The results of this chapter (Chapter 3) are in line with Mavuze, Birgen and Te (2021) who found that the use of plant extracts resulted in an increase in plant height, plant root length, shoot height, number of leaves, dry root and shoot weight in plants treated with extracts. The nematicide applied as positive control in this study could not improve plant growth, rather, all the parameters had lower growth compared to those of plant extracts. These results contradict the results reported by El-Deeb, El-Sappah and Arisha (2018) where they noticed an increase in the dry and fresh shoot weight of tomato plants that were treated with Nemacur®. However, in regard to which application time of plant extracts outperformed the others, the effectiveness of these extract differed with plant parameters.

3.7. Conclusion

The efficacy of *M. angolensis* and *T. elegans* extracts was further confirmed in the current study with further evidence of season and time of application being established. In terms of the time of plant extracts application, application of plant extracts after nematode infestation showed better nematode management effects when compared to application of plant extracts before and simultaneously with nematode infestation. This indicates that the extracts can be used as post infection treatment in tomato plants. In summer-grown crops, the effect of the application of plant extracts in reducing nematode population density was lower, however, plant growth was better. The trend were reversed for autumn-grown crops.

3.8. References

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CHAPTER 4

APPLICATION TIME OF *MAERUA ANGOLENSI*S ON MIXED NEMATODE POPULATIONS INFECTING TOMATO PLANTS UNDER FIELD CONDITIONS

4.1. Introduction

Application of plant extracts relative to nematode inoculation varied according to the season and nematodes (Chapter 3). When plant extracts were applied before nematode inoculation in summer, *M. javanica* populations in the soil were lower than *M. enterolobii*; however, this trend was reversed when plant extracts were applied simultaneously with nematode inoculation. Furthermore, simultaneous application of plant extracts with nematode inoculation resulted in higher *M. javanica* J2 in tomato root compared to *M. enterolobii.* In autumn, the time of plant extract application; whether before, simultaneously, or after nematode inoculation did not significantly influence J2 in tomato root for both *M. javanica* and *M. enterolobii* but plant extract applied after nematode inoculation significantly differed from the two plant extracts application time in J2 found in soil (Chapter 3). Applying plant extracts at the right time is essential for managing nematodes and this is due to the fact that it increases the efficacy of these plant materials combating the population density of nematodes and resolves the inconsistent results of plant extracts that have been reported in multiple articles (Mnyambo, Rantho, Dube and Timana, 2024).

Both single and mixed nematode population studies are crucial for a deeper understanding of nematological research and efficient agricultural control (Mohankumar, Balakrishnan and Samiyappan, 2014). Researchers can examine the distinct behaviour, life cycle, and targeted management methods of nematodes through field and greenhouse trials. Mixed nematode population experiments are essential to understanding complicated interspecies relationships and their cumulative effect on crops (Mondal, Purohit, Hazra, Das, Chakrabarti, Khan, Lopez-

Nicora, Chakraborti and Mukherjee, 2024). Considering these two kinds of trials, scientists and farmers can develop more practical nematode management strategies that considers the complexity of natural nematode ecosystem. Several studies screened the phytochemicals of *M. angolensis* mostly using a single species population (Mcunu, 2023). Hence this study intended on evaluating whether the time of application of *M. angolensis* will reduce mixed nematode population densities and improve tomato plant growth under field conditions.

4.2. Materials and methods

4.2.1. Description of the study conditions

The experimental study was conducted at the same location as identified in Chapter 3 except that instead of greenhouse condition with plants inoculated with a single nematode it was established under field conditions with a mixture of nematodes.

4.2.2. Plant extracts and inoculum preparation

The preparation of plant extract and inoculum were as previously described (Chapter 3).

4.2.3. Treatments and experimental design

The seedlings of the tomato, cultivar "Star 9009" were collected from the nearer company, Ezigro seedlings in White River, South Africa. The experiment was carried out on a university farm that was deep ploughed prior to planting. Six weeks old tomato seedling were transplanted into 15 rows of 3 meters long, 50 cm wide with 50 cm between rows and 5 plants per row. Each plant was inoculated with 5000 mixed nematode populations (*M. incognita, M. javanica* and *M. enterolobii*, 2:1:3 ratio). The soil type in the field is sandy loam. Treatments consisted of four plant extract application levels of, weekly, every second, every third and every fourth week, all at 5 g of *M. angolensis,* applied into the soil around rhizosphere of each plant a week after nematode inoculation. Plants treated with nematicide; crop guard [Furfural (Aldehyde)]

were regarded as positive control. The experiment was laid out in a randomised complete block design for a duration of 56 days. All treatments were replicated 15 times.

4.3. Data collection and analysis

Plant growth and nematode variables data were collected and analysed as previously described in Chapter 3.

4.4. Results

Shapiro-Wilk normality tests showed that none of the nematode and plant growth variables were normally distributed ($P \leq 0.05$), except for eggs in tomato root, juveniles in soil, total number of nematodes in the tomato root and final nematode populations and thus were transformed accordingly (Appendix 4.1).

4.4.1. Nematode variables

The time of application of *M. angolensis* plant extracts showed significant ($P<0.05$) effects on nematode populations, specifically regarding eggs in tomato root, juveniles in soil, the total number of nematodes in tomato root, and final nematode populations (Appendix 4.2–4.5).

The impact of time of plant extract application varied across different weeks, increasing with number of applications (Table 4.1). Relative to the commercial nematicide, treatment applications increased the numbers of eggs in tomato root (12-277%), juveniles in soil (26-751%), total nematodes in tomato root (5-174%), and final nematode populations (12-380%). Weekly application of plant extracts demonstrated the most effective nematode suppression, with lowest numbers of eggs in tomato root (433), juveniles in soil (607), total nematodes in tomato root (913), and final nematode populations (1520) (Table 4.1).

Conversely, when the plant extracts were increased to every 2-, 3- or 4-weeks application intervals, the impact of the extracts in reducing nematode variables were lower, leading to increased nematode populations, with highest at 4 weeks interval. Interestingly, the positive control (Crop Guard) applied produced effects comparable to those of weekly intervals of plant extract application. Both treatments significantly reduced nematode populations (Table 4.1).

Table 4.1: Effects of *M. angolensis* application interval on nematode variables

Weeks	Eggs in tomato root	RI%	Juveniles in soil	RI%	Total number of nematodes in tomato root	RI%	Total nematode population	RI%
Weekly	2.58^{c}(433)	12	2.72^{d}(607)	26	2.93^{cd}(913)	5	3.15^{d}(1520)	13
Every second week	2.74^{b}(647)	67	3.01^{c}(1440)	200	3.05^{c}(1213)	40	3.39^{c}(2653)	665
Every third week	3.01^{a}(1160)	200	3.29^{b}(2287)	376	3.21^{b}(1827)	111	3.56^{b}(4113)	205
Every fourth week	3.14^{a}(1460)	277	3.51^{a}(4087)	751	3.35^{a}(2373)	174	3.76^{a}(6461)	380
Crop guard	2.57^{c}(387)	-	2.64^{d}(480)	-	2.91^{d}(867)	-	3.12^{d}(1347)	-
P Value	0.000**		0.000**		0.000**		0.000**	
LSD0.05 Value	6.88		7.31		5.55		4.43	
F Value	26.17		41.31		18.35		49.88	

**highly significant difference($P \leq 0.05$). From week 1- 4 (Application of *Maerua angolensis*). Week 5- Positive control (Crop Guard).
Relative impact (RI) % = [(treatment/control) – 1] x 100.

Optimum application interval for numbers of eggs in tomato root, juveniles in soil, total nematodes in tomato root, and final nematode populations were 5.9, -0.2, -1.6, and -0.2, respectively (Table 4.2). Average optimum application interval of 0.975 "weeks of 30-day-month" was observed, which is equivalent to 7 days (0.975/4 x 30) (Table 4.2).

Table 4.2: Quadratic relationship, coefficient of determination and computed optimum *M. angolensis* application interval for numbers of eggs in tomato root, juveniles in soil, total nematodes in tomato root, and final nematode populations.

Variable	Quadratic relation	R^2	x^z
numbers of eggs in tomato root	$y = 21.5x^2 - 251.9x + 134$	98	5.9
juveniles in soil	$y = 241.75x^2 + 80.05x + 492.25$	99	-0.2
total nematodes in tomato root	$y = 61.5x^2 + 191.9x + 640.5$	99	-1.6
final nematode populations	$y = 303.75x^2 + 109.55x + 1134.7$	100	-0.2
Mean			0.975

$^z x = -b_1/2b_2$, where x is optimum concentration (from the equation: $y = b_2x^2 + b_1x + c$).

4.4.2. Plant growth variables

All plant growth variables were not significantly different ($P \leq 0.05$) (Appendix 4.6- 4.13).

4.5. Discussion

The experimental results of this chapter (chapter 4) showed that repeated applications of *M. angolensis* plant extracts significantly reduced mixed nematode populations (*Meloidogyne javanica, Meloidogyne enterolobii and Meloidogyne incognita*) in the soil. All treatments led to a reduction in nematode variables, though the level of effectiveness varied. This highlights

the potential advantage of consistent, frequent application intervals in managing nematode populations. Weekly applications of plant extracts and the crop guard (positive control) yielded the most significant decreases in the final nematode population. In contrast, treatments applied at two, three, and four-week intervals were less effective. These findings align with earlier observations in Chapter 3, emphasizing the importance of time of application in maximizing nematicidal efficacy.

Similar studies have corroborated these results, Oluwatayo, Jidere and Nwankiti (2019) reported that weekly applications of *Moringa oleifera*, *Ricinus communis*, and *Jatropha curcas* extracts significantly impacted root-knot nematode populations. Likewise, Abo-Elyousr, Khan, Award and Abedel-Moneim (2010) found that plant extracts and bacterial suspensions applied two or three times at intervals of 15 to 25 days effectively controlled nematodes and enhanced yield. Agbenin, Emechebe, Marley and Akpa (2005) compared neem and garlic bulb extracts, observing that weekly applications of garlic bulb extract at a 20% concentration were particularly effective in reducing galling indices and reproductive factors such as egg masses and female nematode counts. The efficacy of these botanical extracts can be attributed to their biochemical composition. There are specific oxygenated compounds that are within the plant extracts enabling them to dissolve the cytoplasmic membranes of nematode cells (Humaira, Shad, Muhammad and Shau, 2020). Additionally, these compounds disrupt enzyme protein structures, interfering with nematode survival and reproduction (Njenge, 2019). In the case of this study, nematicidal action may have directly targeted the infective second-stage juveniles (J2) in the soil, reducing the number of J2 capable of penetrating plant roots. Beyond their practical application, this study validates the traditional knowledge of local farmers who have utilized botanical resources for pest management. By confirming the effectiveness of *M. angolensis* extracts, the findings provide scientific support for integrating these resources into sustainable agricultural practices. This finding underscores the importance of timing in

nematode management strategies and frequent applications appear to yield the best outcomes in reducing nematode density. The outcomes of this study showed that frequent application intervals play a crucial role in leveraging the nematicidal properties of *M. angolensis*. It also highlights the potential for integrating such plant extracts into sustainable nematode management programs, offering a natural alternative with comparable efficacy to chemical controls.

4.6. Conclusion

The study established an application interval of 7 days for effective suppression of mixed nematode populations of root-knot nematodes when 5 g *M. angolensis* is applied. This interval was comparable to the commercial nematicide, Crop Guard.

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CHAPTER 5

SUMMARY, SIGNIFICANCE OF FINDINGS, FUTURE RESEARCH, CONCLUSIONS AND RECOMMENDATION

5.1. Summary

Nematodes pose a significant challenge to sustainable agriculture, particularly in intensively cultivated areas due to their detrimental impact on crop yields (Phani, Khan and Dutta, 2021). The increasing concerns surrounding the environmental hazards of synthetic pesticides have driven researchers to explore botanical extracts as viable alternatives for pest management. Botanical materials, such as plant extracts, are advantageous due to their accessibility, cost-effectiveness, biodegradability, and environmentally friendly properties, making them an integral part of sustainable agricultural practices (Desaeger, Wram and Zasada, 2020; Khosa *et al*., 2020a; Godlewska, Ronga and Michalak, 2021). This study achieved two objectives, first objective was to assess whether the time of application of *M. angolensis* and *T. elegans* will reduce *M. javanica* and *M. enterolobii* population densities and improve tomato plant growth under greenhouse conditions. The second objective was to test whether the time of application of *M. angolensis* will reduce mixed nematode populations (*Meloidogyne* spp.) on crops under field conditions. The findings of the first experiment indicated the effectiveness of plant extracts depend on seasons. In summer, there were little or no effects of the plant extracts on nematode reduction whereas in autumn, there were higher number of nematodes reduced by the plant extracts. For both seasons, plant extracts applied after nematode inoculation showed to be the best application time, followed by plant extracts applied simultaneously with nematode inoculation.

The results of the second experiment indicated that weekly applications intervals of the extracts significantly reduced mixed nematode populations in the soil. Treatments applied weekly

demonstrated the greatest nematicidal effect, comparable to the performance of the commercial nematicide, crop guard. Conversely, applications at longer intervals, such as every two, three, or four weeks, were less effective. The findings align with previous studies and reinforce the importance of time of application in enhancing the effectiveness of botanical nematicides.

The nematicidal action of *M. angolensis* could be attributed to its biochemical properties, which include specific oxygenated compounds with lipophilic characteristics. These compounds disrupt the structural integrity of nematode cell membranes and interfere with enzyme functions, thereby reducing nematode survival and reproduction. This aligns with other studies that have highlighted the ability of plant extracts, such as those of Oluwatayo *et al*. (2019) where *Moringa oleifera*, *Ricinus communis*, and garlic bulbs suppressed nematode populations effectively. Moreover, the results validate traditional agricultural practices and local farmers' knowledge of using botanical resources for pest management. By scientifically confirming the efficacy of these extracts, the study supports the integration of such sustainable methods into modern agricultural systems.

5.2. Significance of findings

This research provides empirical evidence for the most suitable time to apply the *M. angolensis* and *T. elegans* plant extracts in the management of *Meloidogyne* species. It will potentially address the challenge of inconsistent results reported by different authors. However, more information is still required on such cases of inconsistencies. The information generated from this research is valuable to farmers producing tomato crops. The utilization of these extracts in tomato crops is important not only for the reduction of nematodes in root but also to improve the plant’s growth. More root and healthy root in tomato means more flowers will be formed thus more fruit yield obtained.

5.3. Future research

The current study demonstrated the potential of the two tested plant extracts in the management of two separate nematodes under greenhouse and a mixture of three nematodes under field conditions in Mpumalanga Province. The study did not establish the potential residuals of the two extracts in the tomato fruits and the impact of the concentration on beneficial soil dwelling organisms. The recommendation of the two plant extracts could benefit extensively from further test of the extracts in different crops under different soil and climatic conditions. The stability of the active ingredients of the two extracts could give potential users the potential shelf life of the two.

5.4. Conclusion

Controlling nematode population densities in tomato plants was successfully accomplished by using plant extracts of *M. angolensis* and *T. elegans*; however, it differed with seasons and time of application. The effect of administering plant extracts of *T. elegans* and *M. angolensis* at different times on nematode population densities varied. In the greenhouse, applying plant extracts after nematode infection decreased nematode population densities comparable to synthetic nematicide, Crop Guard. A weekly field application of 5 g of *M. angolensis* significantly reduced a mixed population of nematodes. This demonstrates that *T. elegans* and *M. angolensis* have the potential for use in nematode management.

5.5. Recommendation

The phytochemical included in *M. angolensis* and *T. elegans* extracts need to be identified further since identifying their nematicidal actions may be crucial for enhancing plant secondary metabolites or protecting crops as mentioned by Dubale, Kebebe, Zeynudin, Abdissa and Suleman (2023). Furthermore, as these extracts offer an affordable and widely available

alternative approach to managing wide species of nematodes, their use could assist especially communal farmers who cannot afford chemical synthetic nematicides.

5.6. References

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APPENDICES

Appendix 3.1: Shapiro- Wilk Normality test

Variable	N	W	P
Flower	200	0,7425	0,0000
Fruits	200	0,1937	0,0000
SD	200	0,9786	0,0038
Chlorophyll	200	0,9542	0,0000
Leaves	200	0,9745	0,0010
PH	200	0,8740	0,0000
FSM	200	0,9654	0,0001
DSM	200	0,9527	0,0000
RW	200	0,9245	0,0000
GR	200	0,7741	0,0000
ER	200	0,6307	0,0000
JR	200	0,7537	0,0000
JS	200	0,6857	0,0000
TNR	200	0,7925	0,0000
TNP	200	0,8056	0,0000

Appendix 3.2: Analysis of variance for eggs in tomato root

Source	DF	SS	MS	F	P
Rep	4	0,1945	0,04863		
Season	1	3,0307	3,03074	42,01	0,0000
Nema	1	0,0017	0,00170	0,02	0,8782
PE	1	0,1316	0,13163	1,82	0,1787
AT	4	1,1853	0,29632	4,11	0,0034
Season*Nema	1	0,0272	0,02719	0,38	0,5402
Season*PE	1	0,3810	0,38103	5,28	0,0229
Season*AT	4	0,7634	0,19086	2,65	0,0356
Nema*PE	1	0,0006	0,00063	0,01	0,9254
Nema*AT	4	0,1320	0,03300	0,46	0,7669
PE*AT	4	0,1553	0,03883	0,54	0,7078

Season*Nema*PE	1	0,0003	0,00034	0,00	0,9456
Season*Nema*AT	4	0,1705	0,04261	0,59	0,6699
Season*PE*AT	4	0,2912	0,07280	1,01	0,4046
Nema*PE*AT	4	0,3135	0,07838	1,09	0,3653
Season*Nema*PE*AT	4	0,1559	0,03898	0,54	0,7064
Error	156	11,2554	0,07215		
Total	199	18,1903			

Appendix 3.3: Analysis of variance for juveniles in tomato root

Source	DF	SS	MS	F	P
Rep	4	0,3379	0,08447		
Season	1	6,7113	6,71130	97,60	0,0000
Nema	1	0,0807	0,08072	1,17	0,2803
PE	1	0,1996	0,19960	2,90	0,0904
AT	4	1,1704	0,29260	4,26	0,0027
Season*Nema	1	0,0282	0,02816	0,41	0,5232
Season*PE	1	0,0044	0,00436	0,06	0,8016
Season*AT	4	0,6323	0,15808	2,30	0,0613
Nema*PE	1	0,0172	0,01724	0,25	0,6173
Nema*AT	4	0,5791	0,14477	2,11	0,0827
PE*AT	4	0,0912	0,02281	0,33	0,8564
Season*Nema*PE	1	0,0100	0,00995	0,14	0,7041
Season*Nema*AT	4	0,7306	0,18265	2,66	0,0350
Season*PE*AT	4	0,2087	0,05218	0,76	0,5536
Nema*PE*AT	4	0,1457	0,03643	0,53	0,7140
Season*Nema*PE*AT	4	0,1533	0,03833	0,56	0,6939
Error	156	10,7268	0,06876		
Total	199	21,8274			

Appendix 3.4: Analysis of variance for juveniles in soil

Source	**DF**	**SS**	**MS**	**F**
Rep	4	0,4752	0,11880	

Season	1	0,0718	0,07183	1,16	0,2828
Nema	1	0,0035	0,00354	0,06	0,8112
PE	1	0,0003	0,00031	0,01	0,9435
AT	4	3,6505	0,91263	14,76	0,0000
Season*Nema	1	0,0422	0,04217	0,68	0,4102
Season*PE	1	0,1622	0,16223	2,62	0,1073
Season*AT	4	1,1307	0,28268	4,57	0,0016
Nema*PE	1	0,0090	0,00897	0,15	0,7038
Nema*AT	4	0,3760	0,09400	1,52	0,1990
PE*AT	4	0,2081	0,05203	0,84	0,5009
Season*Nema*PE	1	0,0072	0,00717	0,12	0,7339
Season*Nema*AT	4	0,6834	0,17084	2,76	0,0296
Season*PE*AT	4	0,2710	0,06775	1,10	0,3608
Nema*PE*AT	4	0,5337	0,13343	2,16	0,0763
Season*Nema*PE*AT	4	0,2885	0,07213	1,17	0,3279
Error	156	9,6484	0,06185		
Total	199	17,5618			

Appendix 3.5: Analysis of variance for total nematodes in tomato root

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	4	0,2697	0,06743		
Season	1	5,7030	5,70300	178,70	0,0000
Nema	1	0,0057	0,00567	0,18	0,6740
PE	1	0,1997	0,19971	6,26	0,0134
AT	4	1,1815	0,29537	9,26	0,0000
Season*Nema	1	0,0033	0,00333	0,10	0,7470
Season*PE	1	0,1222	0,12220	3,83	0,0522
Season*AT	4	0,5518	0,13796	4,32	0,0024
Nema*PE	1	0,0003	0,00028	0,01	0,9251
Nema*AT	4	0,2921	0,07303	2,29	0,0623
PE*AT	4	0,1568	0,03919	1,23	0,3012
Season*Nema*PE	1	0,0129	0,01287	0,40	0,5263
Season*Nema*AT	4	0,2315	0,05788	1,81	0,1289

Season*PE*AT	4	0,1433	0,03582	1,12	0,3480
Nema*PE*AT	4	0,1151	0,02878	0,90	0,4645
Season*Nema*PE*AT	4	0,0762	0,01904	0,60	0,6656
Error	156	4,9786	0,03191		
Total	199	14,0437			

Appendix 3.6: Analysis of variance for final nematode populations

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	4	0,2276	0,05691		
Season	1	4,8459	4,84587	180,71	0,0000
Nema	1	0,0019	0,00192	0,07	0,7892
PE	1	0,1618	0,16184	6,04	0,0151
AT	4	1,3511	0,33777	12,60	0,0000
Season*Nema	1	0,0057	0,00574	0,21	0,6443
Season*PE	1	0,1385	0,13846	5,16	0,0244
Season*AT	4	0,4285	0,10713	4,00	0,0041
Nema*PE	1	0,0004	0,00044	0,02	0,8981
Nema*AT	4	0,2429	0,06072	2,26	0,0647
PE*AT	4	0,1384	0,03460	1,29	0,2761
Season*Nema*PE	1	0,0115	0,01146	0,43	0,5142
Season*Nema*AT	4	0,2023	0,05057	1,89	0,1156
Season*PE*AT	4	0,1152	0,02881	1,07	0,3712
Nema*PE*AT	4	0,1441	0,03604	1,34	0,2561
Season*Nema*PE*AT	4	0,0742	0,01856	0,69	0,5986
Error	156	4,1833	0,02682		
Total	199	12,2734			

Appendix 3.7: Analysis of variance number of flower

Source	**DF**	**SS**	**MS**	**F**
Rep	4	0,5425	0,13562	
Season	1	0,5267	0,52670	12,17
Nema	1	0,0075	0,00746	0,17
PE	1	0,0024	0,00236	0,05

AT	4	2,3760	0,59400	13,72
Season*Nema	1	0,0009	0,00091	0,02
Season*PE	1	0,0082	0,00817	0,19
Season*AT	4	0,3767	0,09418	2,18
Nema*PE	1	0,0007	0,00068	0,02
Nema*AT	4	0,1198	0,02995	0,69
PE*AT	4	0,1076	0,02690	0,62
Season*Nema*PE	1	0,0030	0,00303	0,07
Season*Nema*AT	4	0,2561	0,06401	1,48
Season*PE*AT	4	0,0162	0,00405	0,09
Nema*PE*AT	4	0,0324	0,00811	0,19
Season*Nema*PE*AT	4	0,0328	0,00820	0,19
Error	156	6,7521	0,04328	
Total	199	11,1615		

Appendix 3.8: Analysis of variance for number of fruits

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	4	7,250E-03	1,812E-03		
Season	1	0,02900	0,02900	9,75	0,0021
Nema	1	0,01631	0,01631	5,48	0,0205
PE	1	0,00000	0,00000	0,00	1,0000
AT	4	0,03897	9,742E-03	3,28	0,0131
Season*Nema	1	0,01631	0,01631	5,48	0,0205
Season*PE	1	0,00000	0,00000	0,00	1,0000
Season*AT	4	0,03897	9,742E-03	3,28	0,0131
Nema*PE	1	1,812E-03	1,812E-03	0,61	0,4362
Nema*AT	4	0,01541	3,851E-03	1,29	0,2744
PE*AT	4	0,01359	3,398E-03	1,14	0,3386
Season*Nema*PE	1	1,812E-03	1,812E-03	0,61	0,4362
Season*Nema*AT	4	0,01541	3,851E-03	1,29	0,2744
Season*PE*AT	4	0,01359	3,398E-03	1,14	0,3386
Nema*PE*AT	4	0,01178	2,945E-03	0,99	0,4147
Season*Nema*PE*AT	4	0,01178	2,945E-03	0,99	0,4147

Error	156	0,46397	2,974E-03		
Total	199	0,69595			

Appendix 3.9: Analysis of variance for stem diameter

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	4	0,03022	0,00756		
Season	1	0,11542	0,11542	47,59	0,0000
Nema	1	0,01034	0,01034	4,26	0,0406
PE	1	0,00021	0,00021	0,09	0,7670
AT	4	0,08857	0,02214	9,13	0,0000
Season*Nema	1	0,00458	0,00458	1,89	0,1716
Season*PE	1	0,00008	0,00008	0,03	0,8542
Season*AT	4	0,05249	0,01312	5,41	0,0004
Nema*PE	1	0,00094	0,00094	0,39	0,5338
Nema*AT	4	0,01849	0,00462	1,91	0,1122
PE*AT	4	0,00535	0,00134	0,55	0,6985
Season*Nema*PE	1	0,00039	0,00039	0,16	0,6878
Season*Nema*AT	4	0,01464	0,00366	1,51	0,2023
Season*PE*AT	4	0,01435	0,00359	1,48	0,2113
Nema*PE*AT	4	0,00549	0,00137	0,57	0,6881
Season*Nema*PE*AT	4	0,01374	0,00243		
Error	156	0,37839			
Total	199	0,75369			

Appendix 3.10: Analysis of variance for chlorophyll content

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	4	0,15868	0,03967		
Season	1	12,3756	12,3756	602,13	0,0000
Nema	1	3,286E-05	3,286E-05	0,00	0,9682
PE	1	2,630E-03	2,630E-03	0,13	0,7210
AT	4	2,26421	0,56605	27,54	0,0000
Season*Nema	1	4,921E-04	4,921E-04	0,02	0,8772

Season*PE	1	1,567E-03	1,567E-03	0,08	0,7828
Season*AT	4	1,46593	0,36648	17,83	0,0000
Nema*PE	1	1,253E-03	1,253E-03	0,06	0,8053
Nema*AT	4	0,08267	0,02067	1,01	0,4065
PE*AT	4	0,06011	0,01503	0,73	0,5720
Season*Nema*PE	1	0,02207	0,02207	1,07	0,3017
Season*Nema*AT	4	0,14572	0,03643	1,77	0,1371
Season*PE*AT	4	0,04377	0,01094	0,53	0,7121
Nema*PE*AT	4	0,04352	0,01088	0,53	0,7143
Season*Nema*PE*AT	4	0,01388	3,471E-03	0,17	0,9540
Error	156	3,20630	0,02055		
Total	199	19,8885			

Appendix 3.11: Analysis of variance for number of leaves

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	4	0,08801	0,02200		
Season	1	0,75650	0,75650	110,67	0,0000
Nema	1	0,05802	0,05802	8,49	0,0041
PE	1	0,01597	0,01597	2,34	0,1284
AT	4	0,26872	0,06718	9,83	0,0000
Season*Nema	1	0,00032	0,00032	0,05	0,8292
Season*PE	1	0,00190	0,00190	0,28	0,5984
Season*AT	4	0,28806	0,07201	10,54	0,0000
Nema*PE	1	0,00723	0,00723	1,06	0,3053
Nema*AT	4	0,00486	0,00121	0,18	0,9497
PE*AT	4	0,00624	0,00156	0,23	0,9222
Season*Nema*PE	1	0,01646	0,01646	2,41	0,1227
Season*Nema*AT	4	0,00637	0,00159	0,23	0,9196
Season*PE*AT	4	0,05049	0,01262	1,85	0,1226
Nema*PE*AT	4	0,11216	0,02804	4,10	0,0034
Season*Nema*PE*AT	4	0,06110	0,01527	2,23	0,0677
Error	156	1,06635	0,00684		

Total	199	2,80876			

Appendix 3.12: Analysis of variance for plant height

Source	DF	SS	MS	F	P
Rep	4	0,03680	0,00920		
Season	1	4,21173	4,21173	652,50	0,0000
Nema	1	0,01030	0,01030	1,60	0,2084
PE	1	0,00048	0,00048	0,07	0,7864
AT	4	0,32908	0,08227	12,75	0,0000
Season*Nema	1	0,02020	0,02020	3,13	0,0788
Season*PE	1	0,00025	0,00025	0,04	0,8452
Season*AT	4	0,31098	0,07775	12,04	0,0000
Nema*PE	1	0,00358	0,00358	0,55	0,4574
Nema*AT	4	0,01563	0,00391	0,61	0,6593
PE*AT	4	0,03117	0,00779	1,21	0,3100
Season*Nema*PE	1	0,01889	0,01889	2,93	0,0891
Season*Nema*AT	4	0,00944	0,00236	0,37	0,8328
Season*PE*AT	4	0,00267	0,00067	0,10	0,9811
Nema*PE*AT	4	0,00733	0,00183	0,28	0,8881
Season*Nema*PE*AT	4	0,00926	0,00231	0,36	0,8378
Error	156	1,00694	0,00645		
Total	199	6,02472			

Appendix 3.13: Analysis of variance for fresh shoot mass

Source	DF	SS	MS	F	P
Rep	4	0,0432	0,0108		
Season	1	12,3245	12,3245	795,27	0,0000
Nema	1	0,0569	0,0569	3,67	0,0573
PE	1	0,0051	0,0051	0,33	0,5656
AT	4	4,6028	1,1507	74,25	0,0000
Season*Nema	1	0,0031	0,0031	0,20	0,6564
Season*PE	1	0,0205	0,0205	1,32	0,2517
Season*AT	4	2,8118	0,7030	45,36	0,0000

Nema*PE	1	0,0090	0,0090	0,58	0,4469
Nema*AT	4	0,1821	0,0455	2,94	0,0224
PE*AT	4	0,1185	0,0296	1,91	0,1113
Season*Nema*PE	1	0,0031	0,0031	0,20	0,6541
Season*Nema*AT	4	0,1313	0,0328	2,12	0,0811
Season*PE*AT	4	0,1600	0,0400	2,58	0,0395
Nema*PE*AT	4	0,0269	0,0067	0,43	0,7834
Season*Nema*PE*AT	4	0,0871	0,0218	1,41	0,2348
Error	156	2,4176	0,0155		
Total	199	23,0035			

Appendix 3.14: Analysis of variance for dry shoot mass

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	4	0,34483	0,08621		
Season	1	2,77444	2,77444	343,95	0,0000
Nema	1	0,00761	0,00761	0,94	0,3328
PE	1	0,00228	0,00228	0,28	0,5959
AT	4	1,08774	0,27193	33,71	0,0000
Season*Nema	1	0,00002	0,00002	0,00	0,9596
Season*PE	1	0,00009	0,00009	0,01	0,9174
Season*AT	4	0,34351	0,08588	10,65	0,0000
Nema*PE	1	0,00011	0,00011	0,01	0,9066
Nema*AT	4	0,03545	0,00886	1,10	0,3593
PE*AT	4	0,01569	0,00392	0,49	0,7458
Season*Nema*PE	1	0,00174	0,00174	0,22	0,6426
Season*Nema*AT	4	0,00796	0,00199	0,25	0,9114
Season*PE*AT	4	0,01088	0,00272	0,34	0,8526
Nema*PE*AT	4	0,02817	0,00704	0,87	0,4816
Season*Nema*PE*AT	4	0,01616	0,00404	0,50	0,7352
Error	156	1,25835	0,00807		
Total	199	5,93502			

Appendix 3.15: Analysis of variance for root mass

Source	DF	SS	MS	F	P
Rep	4	0,2197	0,0549		
Season	1	13,6604	13,6604	809,82	0,0000
Nema	1	0,0108	0,0108	0,64	0,4241
PE	1	0,0193	0,0193	1,14	0,2863
AT	4	2,2121	0,5530	32,78	0,0000
Season*Nema	1	0,0256	0,0256	1,52	0,2196
Season*PE	1	0,0003	0,0003	0,02	0,8883
Season*AT	4	1,5303	0,3826	22,68	0,0000
Nema*PE	1	0,0057	0,0057	0,34	0,5614
Nema*AT	4	0,0102	0,0026	0,15	0,9622
PE*AT	4	0,0814	0,0203	1,21	0,3106
Season*Nema*PE	1	0,0004	0,0004	0,02	0,8757
Season*Nema*AT	4	0,2070	0,0517	3,07	0,0182
Season*PE*AT	4	0,0494	0,0124	0,73	0,5713
Nema*PE*AT	4	0,0414	0,0104	0,61	0,6534
Season*Nema*PE*AT	4	0,1473	0,0368	2,18	0,0733
Error	156	2,6315	0,0169		
Total	199	20,8529			

Appendix 3.16: Analysis of variance for gall rating

Source	DF	SS	MS	F	P
Rep	4	0,2064	0,0516		
Season	1	34,3676	34,3676	6084,64	0,0000
Nema	1	0,0064	0,0064	1,14	0,2877
PE	1	0,0006	0,0006	0,11	0,7385
AT	4	0,0098	0,0024	0,43	0,7846
Season*Nema	1	0,0064	0,0064	1,14	0,2877
Season*PE	1	0,0006	0,0006	0,11	0,7385
Season*AT	4	0,0098	0,0024	0,43	0,7846
Nema*PE	1	0,0002	0,0002	0,04	0,8399
Nema*AT	4	0,0027	0,0007	0,12	0,9754

PE*AT	4	0,0111	0,0028	0,49	0,7411
Season*Nema*PE	1	0,0002	0,0002	0,04	0,8399
Season*Nema*AT	4	0,0027	0,0007	0,12	0,9754
Season*PE*AT	4	0,0111	0,0028	0,49	0,7411
Nema*PE*AT	4	0,0268	0,0067	1,18	0,3198
Season*Nema*PE*AT	4	0,0268	0,0067	1,18	0,3198
Error	156	0,8811	0,0056		
Total	199	35,5704			

Appendix 4.1: Shapiro- Wilk Normality test

Variable	**N**	**W**	**P**
Leaves	75	0,9680	0,0544
Fruits	75	0,9083	0,0000
Stem diameter	75	0,9728	0,1063
Chlorophyll	75	0,9493	0,0046
Plant height	75	0,9542	0,0085
Dry shoot mass	75	0,9420	0,0019
Fresh shoot mass	75	0,9613	0,0218
Root weight	75	0,9573	0,0128
Eggs in root	75	0,8706	0,0000
Juveniles in root	75	0,8561	0,0000
Juveniles in soi	75	0,7172	0,0000
Total number of nematodes in root	75	0,9147	0,0001
Total number of nematodes in pot	75	0,7931	0,0000

Appendix 4.2: Analysis of variance for eggs in tomato root

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	14	1,12943	0,08067		
Weeks	4	3,90608	0,97652	26,17	0,0000
Error	56	2,08975	0,03732		
Total	74	7,12526			

Appendix 4.3: Analysis of variance for juveniles in soil

Source	DF	SS	MS	F	P
Rep	14	3,6122	0,25801		
Weeks	4	8,1346	2,03365	41,31	0,0000
Error	56	2,7570	0,04923		
Total	74	14,5038			

Appendix 4.4: Analysis of variance for total number of nematodes in tomato root

Source	DF	SS	MS	F	P
Rep	14	0,89364	0,06383		
Weeks	4	2,15535	0,53884	18,35	0,0000
Error	56	1,64435	0,02936		
Total	74	4,69335			

Appendix 4.5: Analysis of variance for final nematode populations

Source	DF	SS	MS	F	P
Rep	14	1,40032	0,10002		
Weeks	4	4,50238	1,12560	49,88	0,0000
Error	56	1,26365	0,02257		
Total	74	7,16634			

Appendix 4.6: Analysis of variance for number of leaves

Source	DF	SS	MS	F	P
Rep	14	0,79987	0,05713		
APPT	3	0,04502	0,01501	1,12	0,3529
Error	42	0,56413	0,01343		
Total	59	1,40903			

Appendix 4.7: Analysis of variance for number of flowers

Source	DF	SS	MS	F	P
Rep	14	0,44353	0,03168		
APPT	3	0,01581	0,00527	0,20	0,8964
Error	42	1,11180	0,02647		

Total	59	1,57114			

Appendix 4.8: Analysis of variance for number of stem diameter

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	14	172,955	12,3539		
APPT	3	10,339	3,4464	0,84	0,4777
Error	42	171,576	4,0851		
Total	59	354,870			

Appendix 4.9: Analysis of variance for chlorophyll

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	14	1,22403	0,08743		
APPT	3	0,13714	0,04571	1,28	0,2922
Error	42	1,49506	0,03560		
Total	59	2,85622			

Appendix 4.10: Analysis of variance for plant height

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	14	0,04043	2,888E-03		
APPT	3	0,02895	9,649E-03	2,40	0,0811
Error	42	0,16873	4,017E-03		
Total	59	0,23811			

Appendix 4.11: Analysis of variance for dry shoot mass

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	14	0,39618	0,02830		
APPT	3	0,06517	0,02172	0,76	0,5215
Error	42	1,19666	0,02849		
Total	59	1,65800			

Appendix 4.12: Analysis of variance for fresh shoot mass

Source	DF	SS	MS	F	P
Rep	14	23321	1665,81		
APPT	3	9536	3178,65	1,50	0,2284
Error	42	88994	2118,91		
Total	59	121851			

Appendix 4.13: Analysis of variance for root mass

Source	DF	SS	MS	F	P
Rep	14	0,38862	0,02776		
APPT	3	0,12661	0,04220	2,40	0,0816
Error	42	0,73956	0,01761		
Total	59	1,25480			