



**Screening of the indigenous African vegetable,
Corchorus olitorius, against root-knot
nematodes**

DH Mcunu



orcid.org/0000-0002-2624-0673

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Supervisor: Prof. Sydney Mavengahama

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Student number : 36074578

Table of Contents

DECLARATION.....	v
DEDICATION	vi
ACKNOWLEDGEMENTS	vii
ABSTRACT	viii
LIST OF TABLES	ix
LIST OF APPENDICES.....	x
CHAPTER 1	1
1.1. Background	1
1.1.1 Description of the research problem	1
1.1.2 Impact of the research problem	2
1.1.3 Proposed solution to the research problem	4
1.1.4 General focus on the study	4
1.3 Rationale of the study.....	4
1.2 Problem Statement.....	5
1.4 Purpose of the study	6
1.4.1. Aim	6
1.4.2 Objectives	6
1.4.3 Hypotheses	6
1.5 Reliability, viability and objectivity.....	7
1.6 Bias	7

1.7 Scientific significance of the study	7
1.8 Structure of full-dissertation.....	7
1.9 References.....	8
CHAPTER 2	13
LITERATURE REVIEW	13
2.1 Food security and nutritional constraints in Sub-Saharan Africa.....	13
2.2. Indigenous leafy vegetables	17
2.2.1. <i>Corchorus</i> species	17
2.3 Utilisation of indigenous leafy vegetables	19
2.4 Production constraints of indigenous leafy vegetables	20
2.5 Pests and diseases affecting indigenous leafy vegetable production	23
2.6 Root knot nematodes.....	25
2.7 Impact of root-knot nematodes on traditional leafy vegetable production	25
2.8 Management of root-knot nematodes	27
2.8.1 Chemical control	28
2.8.2 Biological control	29
2.8.3 Cultural control	30
2.9 Role of phytonematicides and soil amendments in plant defence mechanism	33
2.10 References.....	34
CHAPTER 3	58
HOST-STATUS AND HOST SENSITIVITY OF <i>CORCHORUS OLITORIUS</i> TO <i>MELOIDOGYNE INCOGNITA</i>	58

3.1 Introduction	58
3.2 General materials and methods	59
3.2.1 Study site	59
3.2.2 Experimental design and cultural practices	59
3.2.3 Nematode inoculum preparation	59
3.2.4 Procedure	60
3.2.5 Data collection	60
3.2.5 Data analysis	61
3.3 Results	61
3.4 Discussion	65
3.5 Conclusion	71
3.6 References	71
CHAPTER 4	81
PHYTONEMATICIDAL EFFECTS OF <i>MAERUA ANGOLENSIS</i> LEAF MEAL EXTRACTS ON POPULATION DENSITIES OF <i>MELOIDOGYNE INCOGNITA</i> AND YIELD OF <i>CORCHORUS OLITORIUS</i>	81
4.1 Introduction	81
4.2 Materials and methods	82
4.2.1 Study site	82
4.2.2 Experimental design and cultural practices	82
4.2.3 Medicinal plant material collection and preparation	82
4.2.4 Procedure	83
4.2.5 Data collection	83

4.3 Results	83
4.4 Discussion	86
4.5 Conclusion	87
4.6 References.....	87
CHAPTER 5	93
SUMMARY, SIGNIFICANCE OF FINDINGS, RECOMMENDATION AND CONCLUSIONS	93
5.1 Summary	93
5.2 Significance of findings	93
5.3 Recommendation.....	93
5.4 Conclusions.....	94
5.4 References.....	95

DECLARATION

I declare that the full dissertation entitled **Screening of the indigenous African vegetable, *Corchorus olitorius*, against root-knot nematodes** hereby submitted to the North-West University, for the degree Master of Science (Crop Science) has not been submitted previously by me for a degree at this or any other University; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

Mcunu DH

2022/06/01

Date

DEDICATION

To my loving mother, Maria Mchunu, my nephew, Brooklyn Mchunu and late sister,
Thokozile Mchunu.

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ABSTRACT

Corchorus olitorius (Jute mallow) belongs to the genus of about 40-100 species of flowering plants in the family *Malvaceae*. Jute mallow is an important food for many families. It is famous for its sturdy natural fibre in the Middle East, Africa and Asia. The leaves are a rich source of iron, protein, calcium, thiamin, riboflavin, niacin, folate and dietary fibre. The yield and productivity of *C. olitorius* are plagued by poor cropping systems, poor soil health and pests or disease incidence. Information on the impact of root-knot nematodes (*Meloidogyne incognita*) on Jute mallow is scant. Hence, the objectives of the study were to: (i) test the host-status and host-sensitivity of *Corchorus olitorius* accessions to *Meloidogyne incognita* and (ii) determine the potential nematicidal effects of *Maerua angolensis* on population densities of *M. incognita* and yield of *C. olitorius* accessions. The two experiments were conducted under greenhouse conditions, in a randomized complete block design (RCBD), with five replications with two *C. olitorius* vegetable accessions, Manguzi 2 and Manguzi 3. First objective treatments were exposed to eight *M. incognita* eggs and J2 levels, namely, 0, 25, 50, 100, 200, 400, 800, 1600 and 3200 and for the second objective the treatments consisted of three levels of *M. angolensis* leaf meal powders, 5, 10 and 15 g plus a standard synthetic, commercial nematicide, Ne macur 10GR at a rate of 5 g per plant, as well as an untreated control. The results for objective 1 showed that both cv. *Corchorus olitorius* accessions had reproductive factor (RF) greater than 1, while nematode levels did not affect plant variables, except the dry root mass of Manguzi 2. For objective 2, *Maerua angolensis* decreased the total nematode population found in roots and soil for both cv. *Corchorus olitorius* accessions. In conclusion, both accessions of *C. olitorius* were tolerant to *M. incognita* because plant variables were not damaged by the infestation and *M. angolensis* managed to reduce the nematode population densities on both cv.. *C. olitorius* accessions, however, the plant extracts had no significant effect on the plant growth variables.

Keywords: *Corchorus Olitorius*, *Maerua angolensis*, *Meloidogyne incognita*, African vegetables, Nematodes

LIST OF TABLES

Table 3.1 Response of two accessions of <i>Corchorus olitorius</i> (Manguzi 2 and Manguzi 3) to inoculum levels of <i>Meloidogyne incognita</i>	63
Table 3.2 Plant growth responses of two cultivars of <i>Corchorus olitorius</i> (Manguzi 2 and Manguzi 3) to initial inoculum levels of <i>Meloidogyne incognita</i>	63
Table 4. 1 Response of two accessions of <i>Corchorus olitorius</i> (Manguzi 2 and Manguzi 3) towards application rates of <i>Maerua angolensis</i> and inoculum levels of <i>Meloidogyne incognita</i>	84
Table 4. 2 Response of <i>Maerua angolensis</i> on growth variables of two accessions of <i>Corchorus olitorius</i> (Manguzi 2 and Manguzi 3)	85

LIST OF APPENDICES

Appendix A.1 Shapiro-Wilk normality test for measured variables on <i>Corchorus olitorius</i> cv. Manguzi 2 exposed to <i>Meloidogyne incognita</i>	96
Appendix A.2 Analysis of variance (ANOVA) for nematode juveniles in roots of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Meloidogyne incognita</i>	96
Appendix A.3 Analysis of variance for nematode juveniles in the soil of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Meloidogyne incognita</i>	96
Appendix A.4 Analysis of variance (ANOVA) for total nematode population of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Meloidogyne incognita</i>	97
Appendix A.5 Analysis of variance (ANOVA) for <i>Meloidogyne incognita</i> reproductive factor on <i>Corchorus olitorius</i> cv. “Manguzi 2” accession	97
Appendix A.6 Analysis of variance (ANOVA) for plant height of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Meloidogyne incognita</i>	97
Appendix A.7 Analysis of variance (ANOVA) for fresh shoot mass of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Meloidogyne incognita</i>	97
Appendix A.8 Analysis of variance (ANOVA) for fresh root mass of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Meloidogyne incognita</i>	98
Appendix A.9 Analysis of variance (ANOVA) for stem diameter of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Meloidogyne incognita</i>	98
Appendix A.10 Analysis of variance (ANOVA) for dry shoot mass of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Meloidogyne incognita</i>	98
Appendix A.11 Analysis of variance (ANOVA) for dry root mass of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Meloidogyne incognita</i>	98
Appendix A.12 Analysis of variance (ANOVA) for chlorophyll content of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Meloidogyne incognita</i>	99
Appendix A.13 Shapiro wilk normality test for measured variables on <i>Corchorus olitorius</i> cv.. Manguzi 3 exposed to <i>Meloidogyne incognita</i>	99

Appendix A.14 Analysis of variance (ANOVA) for nematode juveniles in roots of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Meloidogyne incognita</i>	99
Appendix A.15 Analysis of variance (ANOVA) for nematode juveniles in soil of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Meloidogyne incognita</i>	100
Appendix A.16 Analysis of variance (ANOVA) for total nematode population of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Meloidogyne incognita</i>	100
Appendix A.17 Analysis of variance (ANOVA) for reproductive factor of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Meloidogyne incognita</i>	100
Appendix A.18 Analysis of variance (ANOVA) for plant height of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Meloidogyne incognita</i>	100
Appendix A.19 Analysis of variance (ANOVA) for fresh shoot mass of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Meloidogyne incognita</i>	101
Appendix A.20 Analysis of variance (ANOVA) for fresh root mass of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Meloidogyne incognita</i>	101
Appendix A.21 Analysis of variance (ANOVA) for stem diameter of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Meloidogyne incognita</i>	101
Appendix A.22 Analysis of variance (ANOVA) for dry shoot mass of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Meloidogyne incognita</i>	101
Appendix A.23 Analysis of variance (ANOVA) for dry root mass of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Meloidogyne incognita</i>	102
Appendix A.24 Analysis of variance for chlorophyll content of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Meloidogyne incognita</i>	102
Appendix B.1 Shapiro wilk normality test for measured variables on <i>Corchorus olitorius</i> cv.. Manguzi 2 exposed to <i>Maerua angolensis</i>	102
Appendix B.2 Analysis of variance (ANOVA) for juveniles in roots of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Maerua angolensis</i>	103
Appendix B.3 Analysis of variance (ANOVA) for juveniles in soil of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Maerua angolensis</i>	103

Appendix B.4 Analysis of variance (ANOVA) for TOTAL of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Meloidogyne incognita</i>	103
Appendix B.5 Analysis of variance (ANOVA) for reproductive factor of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Maerua angolensis</i>	103
Appendix B.6 Analysis of variance (ANOVA) for plant height of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Maerua angolensis</i>	104
Appendix B.7 Analysis of variance (ANOVA) for fresh shoot mass of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Maerua angolensis</i>	104
Appendix B.8 Analysis of variance (ANOVA) for fresh root mass of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Maerua angolensis</i>	104
Appendix B.9 Analysis of variance (ANOVA) for number of leaves of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Meloidogyne incognita</i>	104
Appendix B.10 Analysis of variance (ANOVA) for dry shoot mass of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Maerua angolensis</i>	105
Appendix B.11 Analysis of variance (ANOVA) for dry root mass of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Maerua angolensis</i>	105
Appendix B.12 Analysis of variance (ANOVA) for chlorophyll content of <i>Corchorus olitorius</i> cv. “Manguzi 2” exposed to <i>Meloidogyne incognita</i>	105
Appendix B.13 Shapiro wilk normality test for measured variables on <i>Corchorus olitorius</i> cv.. Manguzi 3 exposed to <i>Maerua angolensis</i>	105
Appendix B.14 Analysis of variance (ANOVA) for juveniles in roots of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Maerua angolensis</i>	106
Appendix B.15 Analysis of variance (ANOVA) for juveniles in soil of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Maerua angolensis</i>	106
Appendix B.16 Analysis of variance (ANOVA) for total nematode population of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Maerua angolensis</i>	106
Appendix B.17 Analysis of variance (ANOVA) for reproductive factor of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Maerua angolensis</i>	107

Appendix B.18 Analysis of variance (ANOVA) for plant height of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Maerua angolensis</i>	107
Appendix B.19 Analysis of variance (ANOVA) for fresh shoot mass of <i>Corchorus olitorius</i> cv.. “Manguzi 3” exposed to <i>Maerua angolensis</i>	107
Appendix B.20 Analysis of variance (ANOVA) for fresh root mass of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Maerua angolensis</i>	107
Appendix B.21 Analysis of variance (ANOVA) for number of leaves of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Maerua angolensis</i>	108
Appendix B.22 Analysis of variance (ANOVA) for dry shoot mass of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Maerua angolensis</i>	108
Appendix B.23 Analysis of variance (ANOVA) for dry root mass of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Maerua angolensis</i>	108
Appendix B.24 Analysis of variance (ANOVA) for chlorophyll content of <i>Corchorus olitorius</i> cv. “Manguzi 3” exposed to <i>Maerua angolensis</i>	108

CHAPTER 1

RESEARCH PROBLEM

1.1. Background

1.1.1 Description of the research problem

Indigenous African leafy vegetables (ALVs) or traditional leafy vegetables are important sources of food for most rural communities of Southern Africa (Muhanji *et al.*, 2011). These leafy vegetables are considered to be of good nutritional quality, with higher protein, vitamins, iron and other nutrients than most exotic crops in developing countries, contributing to about 54 per cent of the daily nutritional needs (Schonfeldt *et al.*, 2011). With Africa faced with challenges of malnutrition and general food insecurity, the potential of these vegetables can never be overemphasized (Afari-Sefa *et al.*, 2011).

Leafy indigenous vegetables are also tolerant abiotic and biotic factors such as drought and pests respectively (Cernansky, 2015). The major limiting factors in the production of ALVs is lack of breeding effort, ineffective or nonexistent seed supply systems, inadequate information on nutritive and medicinal values, production guidelines, marketing channels, regulatory and policy framework that have all contributed to the widespread occurrence of malnutrition on the continent (Abukutsa-Onyango, 2010; Afari-Sefa *et al.*, 2011).

The available research on the cultivation practices of these crops focuses on production systems, including optimum plant and harvest times, spacing, propagation and harvest methodology (ARC, 2014). Despite their noted good nutritional value, indigenous vegetables have not been widely domesticated and are not cultivated on a wide scale, especially in South Africa.

1.1.2 Impact of the research problem

Corchorus species production and productivity are hampered by number of abiotic and biotic stresses (Chakraborty *et al.*, 2013). Among them pests (i.e. yellow mite, *Polyphagotarsonemus latus* (Banks), semilooper, bihar hairy caterpillar, grey weevil, yellow mite, stem weevil and root-knot nematodes) causes devastating yield losses (Rahmana *et al.*, 2012; Rahman & Khan, 2011). During the winter, when temperature are low and days are short, vegetative growth is restricted (DAFF, 2012; Hadid *et al.*, 1994), limiting supply of fresh jews mallow. It also performs poorly in drought and low rainfall, cannot tolerate waterlogged conditions and grows poorly in shade (DAFF, 2012).

Major limiting factors in indigenous African vegetables such as lack of breeding effort, ineffective or nonexistent seed supply systems, inadequate information on nutritive and medicinal values, production guidelines, marketing channels, regulatory and policy framework that have all contributed to the widespread occurrence of malnutrition (Abukutsa-Onyango, 2010; Afari-Sefa *et al.*, 2011). Furthermore, ALVs are faced with several diseases that have been identified so far resulting in significant food waste and economic losses. Previously ALVs have been described as resistant to pests (Achigan-Dako, *et al* 2014), but recently, there has been an increase in literature on incidences of pests, like nematodes affecting their growth and reducing yields (Atungwu *et al.*, 2013; Nchore *et al.*, 2013; Kimaru *et al.*, 2014; Othim *et al.*, 2018; Ntidi *et al.*, 2019).

The impact of pests, such as nematodes as constraints in the production of traditional African leafy vegetables cannot be ignored. Yield losses due to root-knot nematode on commercial crops amount to at least 90% of all damage caused by pests (Ateeq-ur-Rehman, 2009). In commercialized crops, yield losses due to nematode infection were reported to be as much as 50%, while in some cases there is complete crop failure (Manju & Sankari, 2015). In South Africa, estimated overall crop losses due to nematodes stand at 14% (Mashela *et al.*, 2016). Nematodes do not only affect yield quantitatively but also have the potential to affect nutrient uptake by plants (Mashela *et al.*, 2016). Currently, there is limited information on the diversity of nematodes in traditional African leafy

vegetables of South Africa. The potential impact of the economically important nematodes of cultivated vegetables on yield quality and quantity of traditional African leafy vegetables is also scant.

International withdrawal of synthetic chemical nematicides due to high chemical residues has resulted in the need for research and development of better alternatives. During the past decade, there has been an increase in the development of botanicals as phytonematicides (botanical nematicides, bionematicides, biopesticides) (Chitwood, 2002; Ntalli & Caboni, 2012; Okwute, 2012; Caboni & Ntalli, 2014; Mashela *et al.*, 2017). As a result, a number of promising bionematicides have been identified (Manju & Sankari Meena 2015). In South Africa, traditional medicines known as "muti" use a variety of natural products derived from trees (*M. angolensis* DC. (bead-been tree) and *Tabernaemontana elegans* Stapf (toad tree) and other species (Khosa *et al.*, 2021a). Through application of crudely milled powders of stem, leaves and plant bulbs from plant species as soil amendments, root knot nematode suppression and improved plant growth were observed in tomato plant roots (Khosa *et al.*, 2021a).

However, there are still a lot of problems that need to be resolved before these bionematicides can be used effectively. One of the concerns is the argument that fermented crude extracted bionematicides is more effective than the processed active components from botanicals (Javed *et al.*, 2008; Okwute 2012; Chaudhary *et al.*, 2013). For instance, nematodes' motility and viability were unaffected by azadirachtin (a purified ingredient from neem seed) containing Azaformulation which had lesser impact on second-stage juveniles (J2) hatch than crude neem cake and leaf extracts (Javed *et al.*, 2008). The inactivity of some of the purified substances resulted in potential phytonematicides failing to move beyond in vitro bioassays, limiting progress in the development of phytonematicides.

1.1.3 Proposed solution to the research problem

International withdrawal of synthetic chemical nematicides due to high chemical residues in produce and food chains (Larsson *et al.*, 2017), a reduction of beneficial species (Sun *et al.*, 2012), contamination of ground and surface water (Kafilzadeh, 2015), increased development of resistance in primary and secondary pests (Ejaz *et al.*, 2017), depletion of the ozone layer and various health risks to workers, has resulted in the need for research and development of better alternatives. The use of plant extracts has been viewed in many reports as a viable alternative because they are environment-friendly and locally available making them a cheap alternative strategy for the poor rural farming communities.

1.1.4 General focus on the study

This study is primarily focused on determining the host status and sensitivity of *Corchorus olitorius* towards root-knot nematodes since there is little information regarding the effect of nematodes on *Corchorus olitorius*. The study also focuses on establishing *Maerua angolensis* medicinal plant leaf extracts in the management of nematodes under greenhouse conditions.

1.3 Rationale of the study

ALVs have largely been unrecognized and unacknowledged by agricultural policymakers and nutritionists (Mavengahama *et al.*, 2013). In Southern Africa, these vegetables have received recognition as opportunities to ensure food security and alleviate poverty, while increasing income and improving human health (ARC, 2014; Ajekiigbe *et al.*, 2018). Recent reports have identified pests and diseases, including nematodes as potential sources of production constraints (Atungwu *et al.*, 2013; Gogo *et al.*, 2018; Othim *et al.*, 2018; Rai *et al.*, 2018; Ntidi *et al.*, 2019). Kimaru *et al.*, (2018) observed root knot infestation on ALVs caused galls, deformed roots, yellowing and wilting of plants leading to low biomass yield. Presence of nematodes was responsible for stunted growth and wilting of ALVs plants. Furthermore, galls on roots interfere with nutrient and water

absorption resulting in leaves discoloring (Atkins *et al.*, 2004). However, knowledge on the effects of root knot nematodes on growth of indigenous vegetables is still scarce. (Kimaru *et al.*, 2018). For any nematode management strategy to be developed on traditional African vegetables, proper identification of their impact needs to be determined.

With most highly effective synthetic nematicides been withdrawn from the agrochemical market, environment-friendly, cheap and easily accessible control tactics need to be introduced in the management of nematodes (Dube, 2016), to increase production of traditional vegetables. The researchers intend on determining the impact of important nematodes on the growth and yield of *C. olerius*, an important traditional leafy vegetable and also test the potential of environment-friendly and locally available medicinal plant extracts in the management of these nematodes.

1.2 Problem Statement

Sub Saharan African countries are continuously faced with three interrelated challenges: poverty, food and nutritional insecurity of essential micronutrients and vitamins (WHO, 2019). According to WHO, (2019), about 37% of children under 5 years of age in South Africa are anaemic due to poverty, and poor dietary/micro-nutrient deficiencies. The challenge of poverty and malnutrition has led to the National Development Plan Commission identifying poverty and micronutrient deficiency as national challenges (NDP, 2011). South Africa is facing high climate variability, water scarcity and increased degradation of lands, resulting in agriculture failing to meet the food demand of the country (Msangi, 2014; Ashraf *et al.*, 2018). The World Health Organisation (WHO, 2019) global call emphasizes that for any country to ensure effective and sustainable food production, research must be focused on local foods and feeding practices, which involves bringing under cultivation all underutilised indigenous crops (WHO, 2019). Traditional African vegetables have been identified as potential future crops because of their high nutritional value, good adaptation to marginal degraded lands, their ability to reach maturity within a short period, which render them very useful in supporting nutritional and poverty alleviation programs (Adjatin *et al.*, 2012; Achigan-Dako *et al.*,

2014). Most research on traditional vegetables has focused on the development of appropriate cultivation practices, improved planting and harvesting methods, determination of optimal water and fertiliser requirements and marketing (ARC, 2014; Ngenoh *et al.*, 2019). Recently, the effect of pests and diseases have gained importance with some reports showing the potential reduction of crop yields due to nematodes infestation (Kimaru *et al.*, 2014; Ntidi *et al.*, 2019). Understanding the susceptibility of AVLS to pests such as nematodes will contribute appropriate knowledge in the development of sustainable and environmentally friendly management strategies.

1.4 Purpose of the study

1.4.1. Aim

The aim of the study was to establish the host status and host sensitivity of *Corchorus olitorius* accessions to *Meloidogyne incognita* and the potential of *Maerua angolensis* medicinal plant leaf meal extracts in the management of nematodes.

1.4.2 Objectives

The objectives of this proposed study were:

- i. To test the host status and host sensitivity of *C. olitorius* accessions to *M. incognita*.
- ii. To determine the potential nematicidal effects of *M. angolensis* on population densities of *M. incognita* and yield of *C. olitorius* accessions.

1.4.3 Hypotheses

- i. *M. incognita* causes damage and reduces the growth and yield of *C. olitorius*.
- ii. *M. angolensis* extracts will decrease the population densities of *M. incognita* and increase the growth and yield of *C. olitorius*.

1.5 Reliability, viability and objectivity

Data reliability was based on a statistical analysis of data at the probability level of 5%. Validity was achieved by involving experts, to obtain the required nematode inoculum, nematode eggs and infective second-stage juveniles (J2) extracted from the tomato roots using a previously described method (Hussey & Barker, 1973). Objectivity was achieved by ensuring that the results were discussed on the basis of empirical evidence, thereby, eliminating all attributes of subjectivity (Leedy & Ormrod, 2005).

1.6 Bias

Bias was minimized by ensuring that experimental error in each experiment was contained through sufficient replications (Leedy & Ormrod, 2005).

1.7 Scientific significance of the study

The findings of the study expand knowledge of host status and sensitivity of the *C. olitorius* and the impact of economically important nematodes on the growth and yields of traditional leafy vegetables. Furthermore, this will help develop an environment-friendly nematode management program that can be adopted by rural communities in the potential cultivation of traditional African vegetables. The results generated will be written up as scientific publications and published in SAPSE accredited journals. The information will also be made available to rural communities through on-farm experiments and field days with rural farmers and the Indigenous Plant Use Forum.

1.8 Structure of full-dissertation

Chapter 1 focuses on the background and description of the research problem, followed by Chapter 2 which addresses work done on the research problem and literature review. Chapters 3 and 4 focus on addressing Objectives 1 and 2, respectively. In the final Chapter (Chapter 5), findings in all Chapters were summarized and integrated to provide the significance of the findings and recommendations concerning future research,

followed by conclusions. The citation in text and listing of references adopted the Harvard style as approved by the Faculty of Natural and Agricultural Sciences higher degree office of the North-West University.

1.9 References

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CHAPTER 2

LITERATURE REVIEW

2.1 Food security and nutritional constraints in Sub-Saharan Africa

Sub-Saharan Africa (SSA) faces challenges such as food insecurity, rapid population growth, persistent economic inequality, climate change threats, droughts, youth unemployment, undernourishment, domestic terrorism, drug trafficking, maritime piracy, protracted political crises and low-intensity short-lived wars, and conflict-induced famine (Dodo, 2020). Consequently, these challenges stand in the way of Africa's pursuit to achieve food security and eradicate hunger (Dodo, 2020). Food security is defined as a state where all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for active and healthy life (FAO, 2006).

Sub-Saharan Africa countries are continuously faced with three interrelated challenges: poverty, food and nutritional insecurity of essential micronutrients and vitamins (WHO, 2019). Over 900 million people in developing countries do not have access to enough food and one-third of population suffers from malnutrition, including children under the age of 5 (*Maseko et al.*, 2018). Which contributes to slow growth, abnormal physical development, poor cognitive capabilities and infectious diseases (WHO, 2019).. However, the pursuit to achieve food security requires a continuous supply of quality foods, sufficient incomes or other resources to access food, as well as proper processing and storage of food, which is challenging to obtain due to the rapid population growth. It is said that food security requires individuals to have sound knowledge about nutrition and child care and have access to adequate health and sanitation services (FAO, 2008), which is still to date a challenge in Sub-Saharan Africa countries.

According to WHO, (2019), about 37% of children under 5 years of age in South Africa are anaemic due to poverty, and poor dietary/micro-nutrient deficiencies. The challenge of poverty and malnutrition has led to the National Development Plan Commission

identifying poverty and micronutrient deficiency as national challenges (NDP, 2011). Food demand has continuously outpaced the supply with agricultural demand to provide food and resources for a projected world population of over 9 billion with the majority in developing countries by 2050 being a mammoth task (UN, 2019).

The African population has the highest malnutrition prevalence in the world with almost 33 per cent of malnourished people, translating to about 200 million people (Boussard *et al.*, 2006). Malnutrition is considered the greatest risk factor inflicting a rise in disease burden (GBD Risk Factors Collaborators, 2017), with noticeable direct implications on child growth failure, neonatal disorders, immune function, cognitive function, diabetes, heart disease and cancer (Micha *et al.*, 2015; Akombi *et al.*, 2017; De Pee *et al.*, 2017; Pisa *et al.*, 2017; James, 2018; Prentice, 2018; Fraval, *et al.*, 2019).

Food insecurity is a multidimensional problem that is linked to healthcare, conflicts, policies, politics, leadership, strategic vision, trade and economic interests, agricultural production, food system, global food industry trade politics, and the environment (mother nature) (FAO, 2019; Mahamat, 2020). It is generally acknowledged that the problem is particularly acute in rural areas (Boussard *et al.*, 2006). Often, in many SSA countries, foods are available and plentiful but not accessible to everyone. Almost one in four people in SSA were estimated to be undernourished in 2017, representing about one-third of the 821 million people suffering from chronic hunger globally (FAO *et al.*, 2018).

Climate change has been a major challenge in food security. It impacts the temperature, precipitation, and droughts in a given community adversely affecting the agricultural production of that community (Dodo, 2020). Changes in rainfall, soil quality, weather patterns, and precipitations in many regions of Africa have become the drivers of production constraints faced by farmers in all regions of the continent (Dodo, 2020). Negative effects of climate change like floods and droughts destroy the agricultural production and inputs of the surrounding communities.

Farmers cannot bring their staple crops to the markets because of the lack of security. The challenges and conflicts mentioned above disrupt markets and affect development policies that are put in place to assist the neediest of the population, therefore, enhancing the state of food insecurity (Dodo, 2020). The majority of poor households commonly in rural areas, generate income by selling goods produced at home and selling labour. Failure for income to meet basic needs contributes to food insecurity (Boussard *et al.*, 2006).

The consequences of conflicts are linked to a decline in food production, also food prices rising, and therefore poor families and their children can no longer have access to healthy and balanced dietary foods (Dodo, 2020). Food insecurity has been an ongoing challenge in SSA countries due to the never-ending political instability and crises, persistent corruption, poor leadership and misdirected economic policies and neglect towards the farmers, including a lack of financial and economic investment in the agricultural sector (Dodo, 2020). There are many powerful reasons why food security and agriculture have been neglected which include; food insecurity is not perceived as a priority problem, agricultural projects are overlooked and considered difficult to implement and ministries of finance are less inclined to fund agriculture for future development (Boussard *et al.*, 2006).

2.2. Climate change on growth of indigenous leafy vegetables

Climate change can result in extreme weather events that can have an impact in agricultural production (Adamo *et al.*, 2012). Sub Saharan Africa (SSA) has been identified as one of the regions that are most vulnerable to impacts of climate change (Bryan *et al.*, 2013; IPCC, 2014). It is seen as one of the major factors limiting Africa's efforts to achieve food security (IPPC, 2014; Phirri *et al.*, 2016). Climatic conditions such as temperature, humidity, light, wind flow and precipitation (water) are the main factors that control and influence the plant production, development of plant diseases and pest infestation (rate of multiplication and inoculum disperse) on plants (Islam *et al.*, 2009; Ahanger *et al.*, 2013). It is said that SSA will experience increase in temperatures, change

in rainfall,, pests, weeds, and disease epidemics (FAO 2015;Connoly-Boutin &Smit, 2015). *Corchorus olitorius* species are threatened by climate change/global warming (Mollah *et al.*,2021). Global warming is one of the threatening problems due to emissions of greenhouse gases, that leads to the amplified sea level, monsoon shifting, melted glaciers and natural hazards, i.e. floods, cyclones and droughts (Islam *et al.*, 2009). Climate variability is likely to cause significant fluctuations in jute production and is likely to affect long term yields. *C.olitorius* species are tolerant to drought but prone to waterlogged conditions (Prodhan *et al.*, 2001;Mollah *et al.*, 2021). It's production is estimated lower due excessive rainfall (Mollah *et al.*,2021). Jute shows premature flowering in low temperatures resulting in low quality (Islam *et al.*,2009).The seed yielding is severely affected by natural disasters such as floods, water, heavy rainfall and pest infestation. Drought and floods cause crop destruction and reduce seed viability(Mollah *et al.*,2021). The uneven rainfall distribution causing drought is likely to inhibit nutrient acquisition by roots, limiting production (Geethalakshmi *et al.*,2009).

Unfortunately, increase in *C.olitorius* production has been linked with decline in fertiliser nutrient use efficiency, especially Nitrogen (N). The low nutrient usage results from inappropriate fertilizer application and reduced available atmosphere moisture and time of fertilizer application due to climatic factors.(Singh *et al.*, 2019) .Climate change has been reported to exacerbate pests and disease infestations resulting in yield losses of over fifty percent of major crops. According to Thornton *et al.*, (2014), changes in climate have substantial impact on the prevalence and distribution of pests, weeds and crop diseases Increased temperatures influence the growth and development of most plant pathogens. Pathogens that depend on temperature for development can be active due to high temperature,they can develop and start spreading on crops if uncontrolled (Etterson & Shaw, 2001; Ahanger *et al.*, 2013). Research shows that the impact of climate change will likely intensify in future due to increase in temperature, therefore impacting agricultural production, causing further crop decline. The consequences will further increase poverty and vulnerability of people who depend agriculture for livelihoods (IPCC, 2014; Kabubo-Mariara & Kabara, 2015; Adhikari *et al.*, 2015).

2.3. Indigenous leafy vegetables

African or indigenous leafy vegetables are described as vegetables that are native or have been around long enough to have evolved and adapted to a production site that is in Sub-Saharan Africa (SSA) (Jan van Rensburg & Venter, 2007). SSA is a natural habitat for more than 45 000 species of indigenous leafy vegetables, of which about 1 000 can be eaten as green leafy vegetables (Wehmeyer & Rose, 1983; Muhanji *et al.*, 2011). African indigenous leafy vegetables are often referred to as morogo (Sesotho) or imfino (Isizulu or Isixhosa) (Jan van Rensburg & Venter, 2007). They are commonly grown in rural areas in home gardens or used as intercrops with staple foods (Abukutsa-Onyango, 2014). Production of these vegetables goes back to 12 000 years ago when Khoisan people lived in Africa and collected these vegetables from the wild (Fox & Norwood, 1982; Jan van Rensburg *et al.*, 2007). Furthermore, the introduction of these vegetables in South Africa was due to the Bantu-speaking people 2000 years ago who also relied on the collection of these vegetables from the wild for consumption (Burdy, 1988). They are seasonal vegetables, plant parts used include leafy parts, young succulent stems, flowers and young fruits, with leafy parts being most considered plant parts (Jan van Rensburg *et al.*, 2007). The leafy vegetable species commonly consumed in Africa include; *Amaranthus species* (cheke, thepe, tepe); *Vigna unguiculata* L. Walp., (Cowpea, msoni, monawa), *Cleome gynandra* L., (cleome, lerotho, spider flower), *Solanum species* (African vegetable nightshades), *Brassica carinata* (African kale), *Cucurbits species* (tinwembe, mophotse, intanga) and *Corchorus olitorius* (Jews mallow, thelele, ligushe) (Van den Heever & Coertze 1996,1996b; Chweya & Mnzava 1997; Schippers 2000; Van Wyk & Gericke 2000; Van Zijl *et al.*, 2002 Mutune *et al.*, 2014).

2.3.1. *Corchorus* species

Corchorus species are from the family *Tiliaceae*, the species include *Corchorus asplenifolius*, *C. trilocularis* and *C. olitorius* (Jan van Rensburg *et al.*, 2003). They are found in North and South America and Australia, but mainly in Asia and different parts of Africa. Commonly collected from the wild, with a potential of being cultivated (DAFF, 2012). *Corchorus spp* grow in a hot, humid environment with a lot of rainfall (600–2

000mm). They favour temperatures above 15 °C, while temperatures below 15 °C are damaging to the crop. *Corchorus spp* thrive in a variety of soils but grows best in rich, well-drained loam soils. The most popular amongst these species is *C.olitorius* also known as guxe, ligusha wild jute, delele or thelele. This leafy vegetable is an annual and herbaceous plant, found across all the tropics and subtropic regions. It has long beed and consumed as a vital vegetable and used for its medicinal benefits in arid parts of the Middle East, found in the eastern and northern regions of South Africa, with major production in SA found in KwaZulu-Natal, Limpopo, Eastern Cape and Mpumalanga provinces (Van Wyk & Gericke 2000; Schippers *et al.*, 2002a; DAFF, 2012; Kitis &Kaya, 2018). In South Africa, it grows well in summer, warm, humid conditions with high temperatures (30 °C/day and 25 °C/night), including high rainfall (600 to 2000 mm), with plants flowering from August to October and seeds ripening in October (DAFF, 2012). It prefers various types of soil with pH ranging from 4, 5 to 8,2, preferably sandy loam and clay soils that are moist and rich in organic matter (Facciola, 1990; DAFF, 2012). It is cultivated by seed in the backyard in most rural communities and consumed as a vegetable served as a traditional dish (sauce or soup delicacies) paired with starchy foods to enhance swallowing (Velempini *et al.*, 2013; Larnyo and Atitsogbui, 2020).

According to Holm *et al.* (1997), it is also considered a weed in 50 countries. It was discovered as a weed in summer crops like sesame, citrus and olive groves in the southern provinces such as Antalya, Adana and Hatay of the Mediterranean Region of Turkey (Incekara, 1979; Uremis, 2005; Kitis, 2009; Cagirgan *et al.*, 2014). The leafy vegetable grows erect, with alternate leaves, strong branches, hermaphrodite flowers, yellow petals and cylindrical fruit with a capsule with a short beak (DAFF, 2012; Kitis & Kaya, 2018). Seeds are dark grey or black, small (2-3mm), angular, irregularly, with a hard seed coat which causes physical dormancy and delays the germination of the seeds (Tareq *et al.*, 2015), affecting field performance. Furthermore, several advantages and potential uses of these African leafy vegetables, including delayed seed germination, necessitate the need for production in many African communities (Kitis &Kaya, 2018; Schipper, 2000).

2.4 Utilisation of indigenous leafy vegetables

Indigenous leafy vegetables are an important part of farming and consumption systems throughout SSA (Jan van Rensburg *et al.*, 2004). Most of the traditional vegetables are grown by low-income smallholder farmers. They provide another opportunity to diversify farming systems that play a crucial role in household food security and poverty alleviation while also raising income and improving human health and nutrition (Mavengahama *et al.*, 2013; Jan van Rensburg *et al.*, 2004). They once played important customary, traditional and food cultural roles in the lives of many rural people of Africa (Opabode *et al.*, 2017). According to studies, leafy vegetables help alleviate hunger during natural disasters such as droughts, which are two concerns that SSA countries face regularly (Jan van Rensburg, *et al.*, 2004).

Indigenous leafy vegetables are now being recognized for their importance in nutritional provision (Adebooye & Opabode, 2004; Abukutsa-Onyango, 2007; Jizan *et al.*, 2017), food supply (Balderman *et al.*, 2016; Hughes & Ebert, 2017), ethnobotanical properties (nutritional, medicinal, antioxidants and phenolic attributes) (Mateetee *et al.*, 2019; Mokganya & Tshisikhwa, 2019) and plant breeding properties (Cernasky, 2015; Kiebre *et al.*, 2015). Many of the indigenous vegetables contain more nutritional (vitamins and minerals) and medicinal attributes than exotic vegetables (Abukutsa-Onyango, 2007). The leaves of these vegetables are a rich source of vitamins (A and C), minerals (calcium, phosphorus and iron), proteins and carbohydrates, more than some exotic vegetables like tomato and cabbage (Manoko & Van der Weerden, 2004; Misihairabgwi *et al.*, 2019). The medicinal value of these leafy vegetables has several benefits. It is believed that regular consumption of the leaves by pregnant women will ease childbirth, by reducing the length of labour, and aid recovery (Van Zijl *et al.*, 2002). According to Schipper (2000; 2002a), the spider plant aids postpartum recovery, during breastfeeding; and stomach ailments (Schipper 2000, Schippers *et al.*, 2002a). They also contain phytochemicals (anticarcinogenic, antioxidant, antifungal, antibacterial and antiviral properties) which promote health by reducing the risk of chronic diseases like cancer (Abukutsa-Onyango, 2007). These vegetables are also well adapted to harsh environments, including biotic

and abiotic stresses that exotic vegetables cannot grow in (Abukutsa-Onyango, 2007). They require few inputs, are cheap and affordable, have a short maturity period, produce a high yield per unit area (Jan van Rensburg *et al.*, 2004; Abukutsa-Onyango, 2004), and can be used in intercropping systems with staple crops (Jan van Rensburg *et al.*, 2004). Production of leafy vegetables in rural areas provides employment and serves as a source of household income (Schipper, 2000). To prepare relish, the leaves are usually cleaned and boiled in water with or without salt, with a bit of bicarbonate of soda added, or cooked and blended with onions, tomatoes, minced peanuts, or peanut butter and served with a stiff porridge (Van Zijl *et al.*, 2002). Unfortunately, the introduction of exotic vegetables, colonization and globalization-driven trade policies has led to traditional indigenous vegetables being almost entirely neglected and considered ‘poor people’ plants (Muhanji *et al.*, 2011; Honfoga *et al.*, 2018).

2.5 Production constraints of indigenous leafy vegetables

Indigenous vegetables continue to face challenges due to a lack of information or research on their production and wide gaps between producers (farmers) with financial aid, policymakers and researchers (Onyango, 2021; Towns & Shackleton, 2018; Padulosi *et al.*, 2013; Afari-Sefa *et al.*, 2012). These vegetables lack community support, research institutions and incubation centres that are essential to determine their economic and nutritional value (Onyango, 2021; Padulosi *et al.*, 2013). The lack of policy interventions, cause the neglect of these vegetables, creating a further disconnect between producer and the banks, making it harder for farmers to gain credit from these financial institutions (Onyango, 2021; Towns & Shackleton, 2018). The lack of investments makes it extremely hard for farmers to continue or sustain production, and the lack of funds makes it hard to train people on the application and use of pesticides on these vegetables (Onyango, 2021).

The absence of cultivation guidelines, limited awareness of their value, potential and a lack of agronomic research also hamper the cultivation of indigenous vegetables in various production aspects (Mavengahama *et al.*, 2013). The Agricultural Research

Council Vegetable Industrial and Medicinal Plants (ARC-VIMP) and various provincial departments of agriculture have attempted to address most of these production constraints in South Africa, although other African countries' research continues to focus on mainly marketing the vegetables, which contributes to everlasting production challenges (ARC, 2014; Mathews, 2014; Ngenoh *et al.*, 2019). Studies also show that there is still a slow introduction of biotechnology focusing on the production of leafy vegetables, hindering the development of new cultivars of these vegetables. Opabode and Adebooye (2005) reported biological constraints hindering germplasm conservation efforts, including morphotypes for *Chocorus* spp and *Celosia argentea*, with only a few species (*C. olitorius*, *Solanecio biafrae* and *Telfairia occidentalis*) said to be receiving research attention. However, molecular biology has not been used to characterize their germplasm (Opsbode & Adebooye, 2005). Also, extension officers advising smallholder farmers often have limited knowledge of nematode pests. Despite the significance of these vegetables, more than 80% of the farmers have not attended training or workshops on vegetable cultivation (Schippers, 2000; Onyango, 2021).

According to Onyango (2021), about 90% of traditional African vegetables are still planted using farmer-saved seeds from the field, which adds to factors affecting the seed viability and poor crop quality. Farmers collect seeds from season to season. Most of the seeds are poor in quality and purity, which creates dormancy issues (Abukutsa-Onyango, 2007). Seed dormancy and improper seed processing in leafy vegetable species contribute to low seed germination, exacerbating the need for seed treatments (Debieu *et al.*, 2013; Mavengahama *et al.*, 2013; ARC, 2014). So far, physical and physiological dormancy are the main dormancy cases reported in leafy vegetable species (Baskin *et al.*, 2003). Similarly, Jute mallow seeds are known to have a high degree of dormancy due to impermeable seed coat, loss of viability and poor germination of fresh and old seeds. According to Schippers (2002), jute mallow seed viability slows down considerably when the temperature drops below 15 °C or when the plants are subjected to a prolonged period of water deficit. However, it can be broken through seed treatments, most considerably the hot water treatment (Schippers, 2000; Schippers *et al.*, 2002). According to Mwai and Schippers, (2004) in order to ensure uniform seed germination of leafy vegetables, it is

recommended that seeds are treated before sowing, to break seed dormancy. When seed germination of *Solanum scabrum* appeared to be problematic due to improper seed extraction, seed treatments were considered to improve vigour (Mwai & Schippers, 2004).

The lack of awareness of the benefits of leafy vegetables hinders opportunities for improved production. Neglect and stigmatization are also common factors hindering production. Often these vegetables are associated with low-income groups, regarded as old-fashioned or considered poor man's food. Another challenge is the short-shelf life which makes it undesirable to consumers, because of its high perishable nature reducing the quality of produce (Abukutsa-Onyango, 2007). The other greatest challenge is poor marketing channels. The monetary values of leafy vegetables in South Africa are not adequately established (Shackleton *et al.*, 1999; Shackleton *et al.*, 2001; Twine *et al.*, 2001; Magasela *et al.*, 2001). The lack of information and knowledge of yield also causes low distribution and production of leafy vegetables. Farmers are unable to track or monitor production, resulting in yields as low as 1-3 tons per hectare (Abukutsa-Onyango, 2007). The poor marketing channels further result in low consumption and utilization by consumers, due to less comprehensive data found on vegetable consumption (Shackleton *et al.*, 2009). As it stands most of the traditional vegetables are grown mostly in rural areas by farmers (Jan van Rensburg *et al.*, 2004). Furthermore, most rural farmers face financial issues due to rapidly increasing input/production costs and low sales costs, contributing to low income produced in households (Jan van Rensburg *et al.*, 2004). It is generally believed that the introduction of exotic vegetable varieties in Africa contributed to the decline in the production and consumption of African indigenous vegetables (Smith & Eyzaguirre, 2007) in the African countries. According to Sithanantham *et al.* (2005), there have been yield losses observed due to insect pests found on *Cleome gynandra*, *Amaranthus lividus*, *C. olitorius* and *Crotalaria brevidens*. The damage caused by insect pests on indigenous leafy vegetables carries disease-causing organisms to other plants thereby reducing crop yield (Shackleton *et al.*, 2009).

2.6 Pests and diseases affecting indigenous leafy vegetable production

Pests and disease outbreaks have been identified as some of the limiting factors in crop production, especially under current global change conditions (Thomson *et al.*, 2013; Macfadyen *et al.*, 2016). With increased production, pest and disease importance also increases affecting the optimum productivity of indigenous vegetables (Gogo *et al.*, 2018; Othim *et al.*, 2018; Rai *et al.*, 2018; Ntidi *et al.*, 2019).. Research conducted on indigenous leafy vegetables, about ten insect species were found to affect leafy vegetables; these vegetables included African nightshade (*Solanum villosum*) and *Cleome gynandra*, grown in Keiyo District, Kenya (Kipgosgei, 2004). The pests observed included cutworms (*Agrotis spp.*), black aphids (*Aphis fabae*), cotton aphids (*Aphis gossypii*), leaf miner (*Lyriomyza spp.*), red spider mite (*Tetranychus spp.*), nematodes (*Meloidogyne spp.*), flea beetles (*Chrysomelidae spp.*), *Epilachna* beetles (ladybird), diamondback moth (*Plutella xylostella*) and short-horned grasshoppers (Shackleton *et al.*, 2009). Several diseases have also been observed to affect *Corchorus olitorius*, such as seedling blight, stem rot, leaf blight, root rot (*Macrophomina phaseolina*), anthracnose and leaf mosaic (Roy *et al.*, 2008). According to Das *et al.* (2008), *M. phaseolina* is the causal agent of stem and root rot and it is considered to be one of the most devastating diseases affecting species of jute *C. olitorius* and *C. capsularis* (Ashraf & Javaid, 2007), as it can affect any stage the plant's growth.

Abiotic and biotic factors are the main factors affecting pest and disease abundance and distribution (Phophi *et al.*, 2020). These factors include climate change (temperature, humidity, rainfall, winds), season/time of the year, production practices, host status, sensitivity and availability, symbiosis or mutualism by ants, population density and competition (Phophi *et al.*, 2020). Climate change is considered a critical factor in predicting the potential distribution of pests/species (Dang *et al.*, 2021). According to Bale *et al.* (2008), climate change is most likely to alter insect pests and the relationship between the host and insect can be affected (Bale *et al.*, 2002), which can contribute to the difficulty to predict the effect of pest management, resulting in low crop yields. Examples of insect pests that are influenced by climate i.e. high temperatures include,

aphids, whitefly and stem borers (Sharma, 2014), diamondback moth (*Plutella xylostella*), and African bollworm (*Helicoverpa armigera*) (most common pest affecting agricultural production). These insects can overwinter due to reduced temperatures in the winter season (Reddy *et al.*, 2015) and, stink bugs (*Nezara viridula*: heteroptra: pentatomidae) (Musolin *et al.*, 2009) showed a high survival rate in winter season due to increased warmer conditions. High nematode prevalence is also associated with increased temperatures. High temperatures also affect plant phenology, therefore enhancing plant growth rates and yields, providing a greater food source for plant pathogenic nematodes (Colagiero *et al.*, 2011). High rainfall and moisture are also associated with induced changes in the selection of plant varieties which is likely to increase the incidents of nematodes, by switching species composition or increasing natural antagonists, or/also reducing available predators (i.e. *Pasteuria* spp.) (Colagiero *et al.*, 2011).

Atungwu *et al.* (2013), reported the population density of *Helicotylenchus*, *Tylenchus*, *Pratylenchus*, *Rotylenchus* and *Meloidogyne* nematodes as potential factors in the yield reduction of indigenous vegetables. *Meloidogyne* species are by far the most important pests in reducing the yields of indigenous African vegetables (Nchore *et al.*, 2013; Kimaru *et al.*, 2014), due to their widespread and destructive nature (Fontem & Schippers, 2004; Nchore *et al.*, 2012a). The production problem is also aggravated by the lack of knowledge on the identification and management of pests and diseases among smallholding farmers, including plant-parasitic nematodes (PPN). Most farmers commonly confuse the damage caused by PPN with symptoms of nutrient deficiency, drought or other abiotic harmful factors resulting in the wrong management strategies being employed (Chitwood, 2002; Sikora *et al.*, 2005; McSorley 2011; Ntidi *et al.* 2012). Othim *et al.* (2018) reported that the only currently available management strategy to farmers is insecticides that are expensive and may pose health and environmental concerns.

2.7 Root knot nematodes

Root-knot nematodes (RKNs) belong to the order *Tylenchida*, the family of *Heteroderidae* and the genus of *Meloidogyne* (Decraemer & Hunt, 2013; Eisenback, 2020; Wendimu *et al.*, 2021). *Heterodera marioni* was the binomial name for the RKN until 1949, because of its morphological differences from cyst nematodes described by Chitwood, the genus name was changed to *Meloidogyne* (Moens *et al.*, 2009). There are about ninety species of the genus *Meloidogyne spp.* that have been reported worldwide (Hunt *et al.*, 2005), with 22 of these species reported in Africa (Onkedi *et al.*, 2014). Root-knot nematodes are round-plant parasitic worms, that are distinguished as common and emerging species, the four most common *Meloidogyne* species include; *M. incognita*, *M. arenaria*, *M. hapla*, *M. javanica* (Ziljstra *et al.*, 2000), and five emerging *M. chitwoodi*, *M. fallax*, *M. enterolobii*, *M. minor* and *M. paranaensis* and several other species that are economically important (Onkedi *et al.*, 2014). *Meloidogyne incognita* is the most economically important plant- parasitic nematode species on horticultural and field crops in tropical, subtropical and warmer regions of the world (Eisenback, 2020; Wendimu 2021). RKNs are polyphagous and obligate parasites that attack the roots of a wide host range of plants (Vieira & Gleason, 2014; Wendimu, 2021). They are known for having four races, which are namely: races 1, 2, 3, and 4. Pofu *et al.* (2011) postulated that *M. incognita* races 2 and 4 are the major races in SA. Distribution is found in many Asian, African, European, Oceania, and American countries (Eisenback, 2020). Usually, these nematodes can complete their generation within three to four weeks under suitable environmental conditions (Jaraba-Navas *et al.*, 2007). Root-knot nematodes can survive in a variety of conditions, an optimum temperature range of 15–30°C is required for the hatching of *M. incognita* egg and soils that are moist, low in clay content, or sandy will support the survival of nematodes, as it moves and aerates easily into roots of plants (Monfort, 2005; Jaraba-Navas *et al.*, 2007).

2.8 Impact of root-knot nematodes on traditional leafy vegetable production

Meloidogyne is the most widely spread PPNs genus worldwide and is liable for annual estimated yield and quality crop losses of approximately US \$ 100- 157 billion (Onkendi

et al., 2014; Coyne *et al.*, 2018). Root-knot nematodes rank as one of the most economically important pests because of their wide host range (Jones *et al.*, 2013; Dutta *et al.*, 2015; Sikora *et al.*, 2018), complex relationship with the host plants (Rocha *et al.*, 2015; Cabrera *et al.*, 2016; Hewezi & Baum, 2017), level of damage caused by infection (Bernard *et al.*, 2017) and high yield losses (Elling, 2013; Seid *et al.*, 2015). Damage caused by nematodes has been recorded with about a 40% reduction in the quantity and quality of harvested vegetables (Anwar & Mckenry, 2010). These nematodes are predominantly damaging on vegetable crops in tropical and subtropical countries (Sikora & Fernandez, 2005). Nematodes, including other biotic factors (diseases, insect pests) can cause up to 80% losses in vegetable yield (Cetintas & Yarba, 2010). Yield losses due to RKN amount to at least 90% of all damage caused to plants by RKN (Ateeq-ur-Rehman, 2009). In 1989, SA alone experienced about 17% annual loss accounting for \$7 million due to RKN (Keetch, 1989). Severe infection of good host crops by root-knot nematodes usually results in significant yield and/or quality loss and total crop failure (Mtshali *et al.*, 2002; Tefu *et al.*, 2005). Although globally plant-parasitic nematodes have been described as important pests of vegetable and horticultural crops (Johnson *et al.*, 1996; Palomares-Rius *et al.*, 2007), the gross impact of RKN in Africa is underestimated due to a lack of comprehensive assessment, information and resources. This means the overall annual loss in regions of Africa could be higher than stated (Onkedi *et al.*, 2014). However, they are the most difficult, widespread and destructive pathogens to the African vegetables (Fontem & Schippers, 2004; Nchore *et al.*, 2012).

The kind and nature of PPN interactions are primarily determined by the composition of PPN communities, the presence of a plant host, and the physiochemical factors of environments (Brinkman *et al.*, 2005). Plant parasitic nematodes life cycle is influenced by a variety of factors, namely climate, soil type and texture, and the host plant it infects; as a result, PPN populations are influenced by environmental diversity and variations in their food sources, both of which are influenced by agricultural practices. Vegetable crops are predominantly susceptible to RKN, feeding causes damage which can result in the quantity and quality reduced of harvested vegetables in nursery and field establishments (Schippers, 2000). These damages cause characteristics of swelling and disruption of

vascular tissues of the plant (Daramola *et al.*, 2015). There are known indirect and direct damages caused by these species. The common symptoms are categorized as above and below ground, and they include; reduced plant vigour, root lesions, rotting and disruption of vascular system, slow or stunted growth, yellowing of leaves, plant-wilting and restricted root development of seedlings (Onkedi *et al.*, 2014). Infected plants show swelling or galls on roots, due to nematode expansion of root cells and root's vascular tissue being damaged, which interfere with the normal movement of nutrients and water within the plant, resulting in patchy or poor/stunted crop development, reduced or delayed or total crop failure. *Meloidogyne* spp. have been reported to attack a variety of vegetables; sweet potato, tomato, cabbage, potato, pineapple, cassava, maize, tobacco, cowpea, okra, African spinach, (Onkedi *et al.*, 2014), *Amaranthus* (Castillo *et al.*, 2008; Ntidi *et al.*, 2013), black nightshades, *cucurbitacins* (Dube *et al.*, 2019), *Cleome gynandra* (Ntidi *et al.*, 2013) and several other vegetables (Castillo *et al.*, 2008). The presence of RKN continues to pose a huge challenge for most farmers who have little to no knowledge of the *Meloidogyne* spp. and their impact on agricultural production (Onkedi *et al.*, 2014). Unfortunately, these species will continue to cause crop yield losses due to the ongoing worldwide withdrawal of synthetic nematicides, increased cost of production in the field due to increased fertilizer application and reduced quality of harvest (Onkedi *et al.*, 2014). Effective, sustainable and eco-friendly management strategies of RKN have become imperative (Pimentel, 2014; Zhong *et al.*, 2015; Terra *et al.*, 2018), with the implementation of sustainable nematode management practices.

2.9 Management of root-knot nematodes

When considering the best management of RKN it is best to know the crop's history and correct pest diagnosis to select the best management control (Moens *et al.*, 2009). There needs to be a thorough assessment of genetic diversity, population structure and parasitism mechanism and factors that contribute to an outbreak of *Meloidogyne* spp. (Onkedi *et al.*, 2014). The goal of managing root-knot nematode has to be to keep the population levels below the economic threshold for effective management (Moens *et al.*, 2009). The principles of managing plant pathogens include; exclusion, eradication,

avoidance, protection, resistance and therapy (Moens *et al.*, 2009). Common management generally focused on is synthetically driven nematicides, which are now being internationally withdrawn due to the high toxicity they have on the environment and humans (Onkedi *et al.*, 2014). The international withdrawal has opened a gateway for integrated management. Currently, the most common nematode control strategies focus on an integrated pest management (IPM) plan. Integrated pest management requires three areas of competence that normally include prevention, observation and intervention (Heerden, 2000). Prevention is a secondary strategy, observation attempting to determine when and how something happens, and intervention focusing on bringing the impacts of economically damaging pest populations under control (Dobson *et al.*, 2002). strategies Integrated pest management to control nematodes include chemical, biological (including entomopathogens) and cultural.

2.9.1 Chemical control

Chemical control refers to the use of pesticides that are derived from plant/botanical or synthetic products (Okolle *et al.*, 2014). These pesticides include all inorganic groups of substances destined to destroy or control crop pests, disease vectors and species that are undesirable to plants and animals or negatively affect the entire value chain of agricultural products (Okolle *et al.*, 2014). These chemicals destroy the RKN by interfering with its reproductive potential. Nematicides have always been the main and most effective management of *Meloidogyne* (Onkendi *et al.*, 2014), however, these nematicides could be harmful to man, animals or the environment if not properly used or disposed of. The residues of some pesticides or phytosanitary products are likely to accumulate in organisms after repeated applications, resulting in diseases and subsequently death (Okolle *et al.*, 2014).

A wide variety of synthetically-derived nematicides are available as fumigants and non-fumigants in granule and/or liquid formulations (Moens *et al.*, 2009; Karssen *et al.*, 2013). Many plant products used include; neem (*Azadirachta indica*) and its relative Persian lilac (*Melia azedarach*), and wild sunflower (*Tithonia diversifolia*) leaves (Sithanantham *et al.*,

2005), which are known to have pesticide properties. Nematicides containing active ingredients of methyl bromide, Aldi-carb (Temik) and other harmful compounds have been banned in various parts of the world (Onkendi *et al.*, 2014). However, it is said that these products will be phased out by farmers to use softer products. Soft products will include seed coat products such as Avicta® (a.i. abamectin) used on Maize in SA (Syngeta, 2012), Velum® Prime (a.i. fluopyram) used on tobacco in Zimbabwe (Bayer, 2015) and Nimitz™ (a.i. fluensulfone) on peppers in SA (ADAMA USA, 2015). When considering the application of chemical treatments, good agricultural practices issued by the competent authority need to be followed. To help protect humans, animal health and the environment from hazards arising from accumulation of pesticide residues (Okolle *et al.*, 2014). Although nematicides are most effective, consistent use can lead to resistance (Onkedi *et al.*, 2014) and control also can be reduced, when nematodes are already in the root tissues and symptoms are visible (Sirias, 2011).

2.9.2 Biological control

A biological control refers to the management of plant diseases and pests through the use of living organisms or antagonists such as predators and parasites of organisms that damage or kill their hosts (Agenbag, 2016). Biocontrol is considered to be less detrimental to the environment than pesticides. It offers a good alternative to chemical control and provides efficient control with no or little hazard to the soil environment (Noling & Becker, 1994). A variety of microorganisms and natural enemies antagonistic to soil nematodes exist in the soil, including bacteria, fungi, predatory nematodes and mites (Park *et al.*, 2014). *Meloidogyne* spp. can be controlled biologically by using living organisms/microorganisms. There are several microorganisms used to control *Meloidogyne* spp, such as *Bacillus firmus*, *Pasteuria penetrans*, *Pasteuria hartismeri*, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Trichoderma* spp. (Bishop *et al.*, 2007; Admasu-Hailu & Admasu-Hailu, 2019). These microorganisms kill nematodes by damaging nematode cuticles or feeding on female eggs (Bishop *et al.*, 2007; Kariuki & Dickson, 2007).

According to Oka *et al.* (2000), the application of antagonistic soil microbes is effective in the control of nematodes. Khosa *et al.* (2020b), reported the suppression of *M. incognita* eggs and second juvenile population densities in tomato roots due to beneficial soil microorganisms released from soil amended with crude milled powders of *Cassia abbreviata*, *Maerua angolensis*, *Cucumis myriocarpus* and *Tabernaemontana elegans* (Dube *et al.*, 2019) and *Pseudomonas aeruginosa* (Putten *et al.*, 2006). Soil amendments of these plant species did not only suppress nematodes in tomato plants but also increased the growth and yield of the plant. The suppressive effect of these plant species when applied as soil amendments was consistent and comparable to commercial nematicide, Fenamiphos (Khosa *et al.*, 2021). The key to soil amendments is for applications to be at a high rate to increase the microorganisms released into the soil (Putten *et al.*, 2006; Onkedi *et al.*, 2014). Furthermore, biopesticides are not only cheap, they provide essential nutrients to antagonist bacteria and are also efficient (Onkedi *et al.*, 2014).

2.9.3 Cultural control

Cultural control is one of the oldest pest control methods and is based on changing how the crop is grown or altering its habitat to reduce the likelihood of damage from pests and diseases. It includes, crop rotation, use of companion crops (considered for smallholder farmers who do not have resources to manage insect pests that are damaging their main crops) (Calumpang & Navasero, 2013) or cover crops (involves transplanting a susceptible plant, exposure to nematode), improving soil health, discarding the source or plant material infected, not growing in areas with history or likeness of nematodes, without first treating the soil (Noling, 1999), cleaning machinery before use is recommended, and using resistant and tolerant varieties (Brown *et al.*, 2006; Moens *et al.*, 2009).

Crop rotation is one of the oldest environmental methods available. To limit the build-up of RKN densities, crop rotation concentrates on using host/susceptible with non-host/resistant to allow the host crop to develop effectively and provide satisfactory/expected yields (Reddy, 2017). Previous research observed rotating garlic with okra, tomato and cucumber (*Cucumis sativus* L.) reduced RKN population densities

(Dhillon *et al.*, 2019), as well as tomato and sesame rotation (*Sesamum indicum* L.) was found to considerably reduce nematode populations by up to 75% (Hallmann & Meressa, 2018). In South Africa the use of crop rotation can be considered an alternative, however, it is unsuccessful in some areas due to the wide host range (Onkendi *et al.*, 2014; Fourie *et al.*, 2015; Agenbag, 2016). The benefits do not only focus on nematodes but are also linked with weed management, improved soil nutrients/health, increasing natural enemies, and managing other known pests. Furthermore, the main purpose of growing companion crops in cultural control is to protect main crops from insect pests that will cause damage and therefore result in high yield loss (Calumpang & Navasero, 2013). These crops can be grown as either trap crops or intercrops and referred to as the push-pull strategy, i.e. *Desmodium spp* crop can repel insect pests of maize at the same time it can be used to improve soil fertility through biological nitrogen fixation and organic matter improvement (Calumpang & Navasero, 2013). Other cultural methods (Moens *et al.*, 2009; Karssen *et al.*, 2013), include soil solarization (use of solar energy as a source of heat to kill nematodes in the upper soil layers, although it is commonly used in areas with consistent solar power); ploughing (during dry seasons to expose eggs and J2 moults to desiccation); leaving land fallow and flooding (to 10cm depth for several months), soil tillage (ploughing, with intervals, recommended during the dry season to expose and kill eggs through heat) and flooding (reduces nematode population densities due to a lack of oxygen) (Moens *et al.*, 2009; Karssen *et al.*, 2013; Hallmann & Meressa, 2018), list of species as quarantine organisms and addition of soil organic amendments (reduces nematode- pest population densities as a result of various mechanisms reported (Akhtar & Malik, 2000; Litterick *et al.*, 2004; Moens *et al.*, 2009; Oka, 2010).

2.9.3.2 Resistant varieties used in nematode management

Host plant resistance can be either genetically or systemically acquired (Moen *et al.*, 2009). Genetically host plant resistance are plants that can resist the development and reproduction of nematodes, whereas systemic acquired resistance (SAR) are plants that develop resistance post-root-knot nematode exposure, preventing nematode attack (Moens *et al.*, 2009). The resistant host can be alternated or rotated in production fields

crops with susceptible hosts to reduce the build-up of nematode population and improve crop production (Talavera *et al.*, 2009; Hallmann & Meressa, 2018), and reduce the practice of monoculture by most rural South African farmers. In this manner, the decline in crop yield and quality is less and *Meloidogyne* species development and reproduction are reduced or inhibited in the presence of both these types of host resistance (Moen *et al.*, 2009; Karseen *et al.*, 2013). Furthermore, resistance can be through single (monogenic) or several species (polygenic) of genetic host plant might, monogenic (e.g. coffee cultivars found with resistance to only *M. exigua*) or polygenic (e.g. tomato cultivars with the *Mi* gene that exhibits resistance to *M. arenaria*, *M. incognita* and *M. javanica*) (Karseen *et al.*, 2013).

There are several studies available on resistance. Although, the use of resistance as an alternative depends on the target species. Resistance has been recorded in several vegetables, e.g., tomatoes and wild potatoes (*Solanum bulbocastanum*) due to the *Mi-1* gene. The gene *Rmc-1* on wild potatoes is said to confer resistance against *Meloidogyne* spp; *M. chitwoodi*, *M. fallax*, *M. hapla* (Gebhardt & Valkonen, 2001; Brown *et al.*, 2006). Norshie *et al.* (2011) recently showed that certain potato lines are capable of partially resisting *M. chitwoodi* during infection. Furthermore, Pofu *et al.* (2009) demonstrated that two wild *Cucumis* species were highly resistant to tropical *Meloidogyne* species in South Africa. Resistant cultivars should be recommended for use in crop rotations since they prevent nematode build-up for the successor susceptible crops (Pofu *et al.*, 2010a). Crop rotation has been successful with host and non-host over the years, Talavera *et al.* (2009), observed population reduction of the major *Meloidogyne* spp by 90%, when *Mi* resistant tomato 'Monika' were rotated with susceptible 'Durinta' over three years. Similarly, Dhillon *et al.* (2019), observed root-knot nematode reduction in vegetable crops when okra, tomato and cucumber (*Cucumis sativus* L.) were alternated with garlic (*Allium sativum* L.). Tomato rotation with sesame (*Sesamum indicum* L.) was also found to reduce nematode population by 75% (Hallmann & Meressa (2018), however, the success of alternating resistant varieties with non-hosts is not always successful due to the wide host range of nematodes.

2.10 Role of phytonematicides and soil amendments in plant defence mechanism

According to Hammerschidt (2005), the majority of the plants have been proven to produce a large range of secondary metabolites and some of them are accurate on high concentrations in bark, roots and needles. These metabolites are involved in plant response to numerous biotic stress factors, including fungal pathogens (Chonf *et al.*, 2009). Phenolic metabolites have been confirmed to be frequently linked to the defence mechanisms of plants against natural enemies, such as pathogenic fungi (Hammerschmidt, 2005). The presence of phytochemicals such as alkaloids, phenols, diterpenes, glucosinolates, fatty acids, isothiocyanates, tannins, steroids and thienyls has been associated with nematicidal activity (Sithole *et al.*, 2021). When organic matter decomposes, it releases phenols, polythienyls, glucosinolates, cyanogenic glycosides, alkaloids, terpenoids, steroids, triterpenoids, aldehydes, and several gases, including ammonia (Chitwood, 2002). In a comparable way to synthetic pesticides, these molecules act as repellents, deterrents, attractants, respiratory poisons and contact pesticides (Hikal *et al.*, 2017). According to Mashela *et al.* (2015), phytonematicides have four key mechanisms that affect and reduce the population of RKN: juvenile motility, chemotaxis, egg hatch inhibition, and juvenile death.

These phytonematicides are considered efficient and environmentally friendly. The use of soil amendments made from plants is practised by many farmers throughout the world (Ntalli *et al.*, 2020). Numerous authors have proved the efficacy of this approach in lowering nematode population densities (Oka, 2010; Renco, 2013; Daneel *et al.*, 2018; Dutta *et al.*, 2019). In SA, Mashela *et al.* (2010), had tomato sites treated with plant-based amendments. Furthermore, *M. incognita* densities were decreased over four seasons in two years when dried leaves of *Lippia javanica* L. were incorporated into the soil before planting. Similarly, Khosa *et al.* (2013;2020a) and Mashela *et al.* (2017) observed that plants such as *Maerua angolensis*, *Tabernaemontana elegans*, *Synadium cupulare*, *Cucumis myriocarpus*, and other medicinal plants have nematicidal compounds that suppress root-knot nematode populations, in particular, *M. incognita* race 2. A variety of extracts derived from various plant components, including *Myrtus communis* L.,

Capsicum frutescens L., *Hyoscyamus niger*, *Melia azedarach* L., *Xanthium strumarium* L., and *Achillea wilhelmsii* Koch have been reported to also reduce RKN population densities to varying degrees of effectiveness (Oka *et al.*, 2012; Kepenekci *et al.*, 2016). Organic soil amendments have shown to be effective in reducing a variety of soilborne diseases over the years. Furthermore, Raviv *et al.* (2005) observed that using orange peel-separated cow manure (OP-SCM) and wheat straw-separated cow manure (WS-SCM) in Israel suppressed *M. javanica* population densities in tomatoes. These amendments not only suppress the RKN population, but they also help increase soil structure and water-holding capacity, as well as minimize illnesses and limit weed growth, leading to stronger plants that are much more resistant to PPN nematode infestation (McSorley, 2011).

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CHAPTER 3

HOST-STATUS AND HOST SENSITIVITY OF *CORCHORUS OLITORIUS* TO *MELOIDOGYNE INCOGNITA INCOGNITA* RACE 2 UNDER MICROPLOT CONDITIONS

3.1 Introduction

Root-knot nematodes (*Meloidogyne* species) are considered to be the economically highest ranking pest with the potential to cause total crop failure (Jones et al., 2013; Dutta et al., 2015; Sikora et al., 2018). Yield losses due to RKN on vegetables have been recorded to be as high as 90% (Ateeq ur-Rehman, 2009), while some 40% reduction in the quality of harvested vegetables and 80% losses in vegetable production have been observed (Anwar & McKenry, 2010). Root-knot nematodes damage is associated with root vascular tissue lesions and disruption (Daramola et al., 2015), reduced plant vigour, restricted movement of nutrients and water within the plant resulting in yellowing of leaves, plant wilting and restricted root development of plants (Schippner et al., 2000).

Plant resistance is considered the method of choice for managing root-knot nematodes due to the negative impact associated with the use of chemical nematodes (Roberts, 2002). Root-knot nematode resistance has been observed in traditional leafy vegetables; however, the type of nematode resistance in these plants has not been recorded (Ramatsitsi & Dube, 2020). The commercially available tomato resistant cultivars carry the dominant Mi gene, which confers resistance to the three main species of nematodes, *M. arenaria*, *M. incognita* and *M. javanica* (Roberts, 2015). Previous studies also observed the resistance of the wild vegetable, *C. myriocarpus* to RKN (Pofu et al., 2010b, 2011, 2012; Onkendi et al., 2014).

Resistance was discovered in *L. peruvianum*, a wild relative of the cultivated tomato, and was transferred into the cultivated tomato, *L. esculentum*. Gene Mi-1 was transferred from *L. peruvianum* to *L. esculentum* conferring effective resistance towards *Meloidogyne* species (*incognita*, *chitwood*, *aneria*, *javanica*). (Sharma & Sharma, 2015). However,

there are several gaps in host plant resistance available. As a result, the goal of this study is to assess the host status and sensitivity of *Corchorus olitorius* to *Meloidogyne incognita* race 2.

3.2 General materials and methods

3.2.1 Study site

The study was conducted under microplot conditions (18-22°C and average 934mm rainfall per year) at the University of Mpumalanga, South Africa (25°26'11.4" S; 30°58'54.48" E). Trials were conducted for two seasons, Summer (November 2020-January 2021) and Spring (September 2021- November 2021), .

3.2.2 Experimental design and cultural practices

The two *Corchorus olitorius* vegetable accessions, Manguzi 2 and Manguzi 3, used in this study were obtained from ARC-VOPI. Each plant accession was exposed to eight *M. incognita* population levels of, 0, 25±20, 50±20, 100±20, 200±20, 400±20, 800±20, 1600±20 and 3200±20 eggs and J2, which were arranged in a randomised complete block design, with five replications. A susceptible tomato cv. 'Floradade' was included as an indicator plant of nematode inoculum viability in all replications. Plants were irrigated with 250 ml of water when required.

3.2.3 Nematode inoculum preparation

The populations of *M. incognita* were acquired from the Agricultural Research Council, Nelspruit, Mbombela South Africa. Before inoculation, a population of *M. incognita* was identified and confirmed by sequence-characterized amplified regions-polymerase chain reaction (SCAR-PCR) (Zijlstra *et al.*, 2000; Fourie *et al.*, 2001; Khosa *et al.*, 2021). The *M. incognita* were multiplied by introducing a sample of confirmed nematodes on greenhouse-grown nematode-susceptible tomato (*Solanum lycopersicum*) cv. 'Floradade' for two months. Nematode eggs and J2 used were extracted from roots of tomato plants by blending and maceration of plant roots in a 1% NaCl solution (Marais *et*

al., 2017). The aliquot was then sieved through several nested sieves with different apertures: 150, 63, and 25 μm , with inoculum collected from the smallest sieve.

3.2.4 Procedure

Soil (river sand) was collected from the University of Mpumalanga farm. The plastic plant pots were filled with a mixture of river sand (steam-pasteurized (300 °C for 1h) and Hygromix (Hygrotech, Pretoria North, South Africa) at a ratio of 2:1 (v/v) respectively. Artificial micro-plots were established by placing 30-cm-diameter plastic pots into 20-cm-deep holes at 1.0 m intra-row and inter-row spacing. Treated seeds were directly sown in approximately 2-cm deep holes into each of the 30 cm plastic plant pots. Five weeks after establishment, seedlings were each inoculated with a specific number of nematode eggs and J2 using a 20-ml plastic syringe by placing it into 2½-cm-deep holes on cardinal points of the stems. The plants were individually fertilized as recommended by the ARC, Traditional Leafy vegetables program (ARC, 2014).

3.2.5 Data collection

Plant variables: At 56 days after inoculation, the number of leaves (NOL) per plant was determined, plant height was measured from the crown to the terminal end of the flag leaf, stems were cut at the crown and stem diameters measured at 3 cm above the cut ends using a digital vernier calliper (DC – 515, Bangkok, Thailand). Chlorophyll content was measured on two top most mature leaves using the chlorophyll meter (Minolta spad – 502, Hangzhou, China). Fresh shoots were used as an indicator of fresh leaf yield before drying. Shoots were then oven-dried at 55 °C for 72 h and weighed again to obtain dry mass. Fresh root systems were removed from pots, immersed in water to remove soil particles, blotted dry and weighed to obtain root mass.

Nematode variables: Root systems were removed from pots, immersed in water to remove soil particles and blotted dry. Root galls were assessed using the North Carolina Differential Rating Scale, 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls and 5 = ≥ 101 galls per root system (Taylor and Sasser, 1978). Nematodes

were extracted from the whole root system per plant using the maceration and blending method (Hussey and Barker, 1973). Soil per pot was thoroughly mixed and a 250 ml soil sample was collected. From the sample, nematodes were extracted using the sugar-floatation and centrifugation method (Jenkins, 1964). Eggs and J2 from root and J2 from soil were counted from a 10 ml aliquot of each sample with the use of a stereomicroscope (Zeiss Stemi 508, Suzhou, China). Final nematode population (pf) density was determined by adding total eggs and J2 from the root system to the total J2 from the soil. Reproductive factor (Reproductive factor (rf) = final population (pf)/ initial population (pi)), which is the capability of the nematode to reproduce in each plant per unit gram of root was also counted (Sasser, 1984).

3.2.5 Data analysis

Data for plant and nematode variables were subjected to analysis of variance (ANOVA) using SAS (SAS Institute Inc., Cary, NC, USA). Before ANOVA, Shapiro-Wilk's normality test was conducted to test deviation from normality in each standardized residuals variable (Gomez & Gomez, 1983). Discrete data values were transformed through $[\log_{10}(x + 1)]$ to homogenize the variances (Gomez & Gomez, 1984), but untransformed means were reported. Mean separation was achieved through the Fisher's Least Significant Difference test at a 5% level of probability.

3.3 Results

Shapiro-Wilk normality test indicated that all nematode variables for both accessions of cv. *C. olitorius* were not normally distributed ($P \geq 0.05$) (Appendix A.1 & A.13), furthermore, most plant growth variables for both cv. *C. olitorius* accessions were normally distributed ($P \geq 0.05$), variables that were not normally included were plant height, fresh roots and fresh shoots for Manguzi 2 and dry root mass for Manguzi 3 (Appendix C.1& C.13), hence they were transformed accordingly.

In both accessions, very small, underdeveloped galls were observed on the roots, hence gall rating as an indicator of host status was not necessary. All nematode variables for both Manguzi 2 and Manguzi 3 were highly significant ($P < 0.01$) (Table 3.2), however, all inoculum levels applied on Manguzi 2 and Manguzi 3 cv. *C. olitorius* accessions were not significantly different ($P \geq 0.05$), except for the dry root mass of the Manguzi 2 which showed a significant difference ($P \leq 0.05$) between inoculum levels (Table 3.2).

The total nematode population increased with the increase in initial inoculum levels of *Meloidogyne incognita* eggs and J2, the case was the same for all significant nematode variables for both cv. *C. olitorius* accessions (Table 3.1). There was generally, an increase in final total nematodes in roots of both accessions with an increase in inoculum levels, up to an inoculum level of 800 *M. incognita* eggs and J2. An increase in inoculum of *M. incognita* from 800 to 1600 eggs and J2 resulted in a decrease in the final nematode population in roots (Table 3.1). However, there was a decrease in juvenile populations found in soil when compared to roots for both cv. *C. olitorius* accessions (Table 3.1).

Nematode inoculum of 800 *M. incognita* eggs and J2 had the highest number of final total nematode population densities, which were not significantly different from 400 and 1600 (Table 3.1). Manguzi 2 plants exposed to inoculum level of 25 *M. incognita* eggs and J2 had the least total final nematodes, which was not statistically different from those exposed to an inoculum of 50. Manguzi 3, with an inoculum level of 100 had the least total nematodes which were not different from those exposed to inocula levels 25 and 400 (Table 3.1).

The reproductive factor for both accessions decreased with an increase in *M. incognita* inoculum levels. The RF were highest at inoculum level of 400 *M. incognita* eggs and J2 for Manguzi 2 and inoculum level of 25 *M. incognita* for Manguzi 3, then started to decrease with an increase in inoculum level. In Manguzi 2 treatments, all RF were greater than one except when exposed to inoculum levels of 1600 *M. incognita* eggs and J2, while for Manguzi 3, all inoculum levels above 400 *M. incognita* eggs and J2 had RFs below one (Table 3.1), this serves as an indication for equilibrium position.

Table 3.1: Response of two accessions of *Corchorus olitorius* (Manguzi 2 and Manguzi 3) to inoculum levels of *Meloidogyne incognita*

Manguzi 2					Manguzi 3			
TRT	J2R	J2S	TT	RF	J2R	J2S	TT	RF
0	0.0 ^e	0.0 ^e	0.0 ^e	0.0 ^e	0.0 ^b	0.0 ^d	0.0 ^e	0.0 ^e
25	1.6 (39.2) ^a	0.8 (11.8) ^c	1.7 (51.0) ^d	0.5 (2.0) ^{ab}	1.7 (65.4)	1.9 (92.8) ^{bc}	2.2 (158.3) ^{bcd}	0.8 (6.3) ^a
50	1.8 (65.8) ^{cd}	1.1 (13.6) ^b	1.9 (79.4) ^{cd}	0.4 (1.6) ^{abc}	1.7 (84.0)	1.9 (88.8) ^{bc}	2.2 (172.8) ^{bcd}	0.6 (3.5) ^b
100	1.9 (89.2) ^c	1.2 (16.3) ^b	2.0 (105.5) ^c	0.3 (1.1) ^{cd}	1.7 (86.8)	1.8 (67.9) ^c	2.10 (156.3) ^d	0.4 (1.6) ^c
200	2.5 (222.5) ^a	1.8 (60.3) ^a	2.4 (282.8) ^a	0.4 (1.4) ^{bc}	2.0 (131.1)	2.2 (174.7) ^{ab}	2.4 (307.7) ^{abc}	0.4 (1.5) ^c
400	2.9 (898.3) ^a	1.4 (32.4) ^{ab}	3.0 (930.8) ^a	0.5 (2.3) ^a	1.7 (88.4)	1.8 (699.3) ^c	2.1 (157.7) ^{cd}	0.1 (0.4) ^{de}
800	3.1 (1476.7) ^a	1.7 (48.6) ^a	3.0 (1515.3) ^a	0.4 (1.9) ^{abc}	2.1 (282.8)	2.3 (222.4) ^a	2.6 (505.3) ^a	0.2 (0.6) ^d
1600	2.9 (790.0) ^a	1.5 (33.3) ^{ab}	2.9 (823.3) ^a	0.2 (0.5) ^d	1.1 (2466.6)	2.2 (228.9) ^{ab}	2.5 (475.2) ^{ab}	0.1 (0.4) ^{de}
F _{value}	55.14	7.64	52.39	8.14	1.01	2.95	2.76	22.24
LSD _{0.05}	0.23	0.35	0.23	0.018	0.59	0.31	0.23	0.10
P _{value}	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*

Column means with the same letters (a, b, c, d, e) do not differ significantly ($P \leq 0.05$) according to Fisher's protected least significant difference test. Values in brackets are untransformed means [$\log_{10}(x + 1)$]. RF = Pf/ Pi. * Highly significant ($P \leq 0.01$). TRT treatment; J2R juveniles in roots; J2S juveniles in soil; TT total; RF reproductive factor.

There were no clear trends on the effects of initial nematode inoculum on the dry root mass of Manguzi 2, inoculum level of 50 *M. incognita* had the highest dry root mass when compared to the control, 100 and 200 inoculum levels but was not different to all others (Table 3.2).

Table 3.2: Plant growth responses of two cultivars of *Corchorus olitorius* (Manguzi 2 and Manguzi 3) to initial inoculum levels of *Meloidogyne incognita*

Manguzi 2								Manguzi 3						
TRT	PH (mm)	FSM (g)	FRM (g)	SD (cm)	DSM (g)	DRM (g)	CC	PH (mm)	FSM (g)	FRM (g)	SD (cm)	DSM (g)	DRM (g)	CC
0	2.0 (111.3)	1.8 (74.2) ^{ab}	1.8 (62.6) ^{ab}	0.8	15.3 ^{ab}	10.7 ^{bc}	43.5	51.0	22.7 ^a	22.7 (22.3) ^a	0.4 ^a	6.5 ^a	0.7 (4.3)	16.3 ^{ab}
25	2.0 (110.7)	1.8 (54.8) ^{ab}	1.8 (64.6) ^{ab}	0.8	14.1 ^{ab}	11.3 ^{abc}	41.3	50.0	16.2 ^b	16.2 (20.2) ^b	0.4 ^{ab}	4.6 ^b	0.7 (3.9)	14.03 ^b
50	2.1 (112.1)	1.9 (62.8) ^{ab}	1.9 (79.6) ^{ab}	0.8	14.7 ^{ab}	12.7 ^a	41.6	50.3	16.4 ^b	16.4 (19.3) ^b	0.3 ^b	4.1 ^b	0.7 (3.8)	17.4 ^a
100	2.1 (120.9)	1.8 (57.2) ^b	1.8 (58.8) ^b	0.8	15.6 ^{ab}	10.2 ^c	41.7	54.9	20.1 ^{ab}	20.1 (120.8) ^{ab}	0.4 ^{ab}	5.8 ^{ab}	0.7 (4.0)	14.8 ^{ab}
200	2.1 (124.3)	1.8 (55.3) ^b	1.7 (56.9) ^b	0.8	15.7 ^a	10.3 ^c	41.9	52.7	20.1 ^{ab}	18.3 (20.4) ^{ab}	0.4 ^a	4.4 ^{ab}	0.7 (3.9)	17.6 ^a
400	2.0 (101.4)	1.8 (58.4) ^{ab}	1.8 (67.9) ^{ab}	0.8	13.6 ^b	11.9 ^{abc}	40.6	49.0	18.3 ^{ab}	19.7 (23.6) ^{ab}	0.4 ^{ab}	5.4 ^{ab}	0.8 (5.2)	17.8 ^a
800	2.0 (108.8)	1.8 (63.3) ^{ab}	1.81 (63.5) ^{ab}	0.8	14.9 ^{ab}	11.8 ^{abc}	39.3	50.3	19.7 ^{ab}	19.7 (21.1) ^{ab}	0.4 ^{ab}	5.1 ^{ab}	0.7 (4.0)	17.7 ^a
1600	2.0 (109.1)	2.0 (65.0) ^a	2.0 (188.9) ^a	0.8	15.2 ^{ab}	12.2 ^{ab}	41.5	53.9	19.7 ^{ab}	17.4 (20.5) ^{ab}	0.4 ^{ab}	4.8 ^{ab}	0.7 (3.9)	15.5 ^{ab}
F-value	0.72	3.20	1.09	0.89	1.07	2.40	0.58	0.95	0.28	0.35	1.20	1.83	0.62	1.57
LSD _{0.05}	0.08	0.10	0.21	0.08	2.00	1.65	4.45	6.15	6.21	6.48	0.07	1.67	0.13	3.37

P-value	0.65 ^{ns}	0.15 ^{ns}	0.39 ^{ns}	0.52 ^{ns}	0.40 ^{ns}	0.04 ^{**}	0.77 ^{ns}	0.48 ^{ns}	0.60 ^{ns}	0.92 ^{ns}	0.33 ^{ns}	0.11 ^{ns}	0.74 ^{ns}	0.18 ^{ns}
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Column means with the same letters (a, b) do not differ significantly ($P \leq 0.05$) according to Fisher's protected least significant difference test. Values in brackets are untransformed means $[\log_{10}(x + 1)]$. ^{ns} Not significant ($P > 0.05$). ^{**} Significant ($P \leq 0.05$). RF = P_f/P_i. TRT Treatment; PH plant height; FRM Fresh root mass; FSM fresh shoot mass, CC chlorophyll content; DRM dry root mass; DSM dry shoot mass Values in brackets are untransformed means. Rf= P_f/P_i

3.4 Discussion

The resistance or susceptibility of a plant to nematodes is usually defined using two indicators, host status and host sensitivity (Anwar & McKenry, 2010; Pofu *et al.*, 2010). The host status of a plant to nematodes is a measure of the nematodes' ability to reproduce in/on a plant measured using nematode variables while host sensitivity quantifies the plant's response to nematode infestation measured using the plant variables (Steyn *et al.*, 2013). In the current study, all nematode variables were significant for both cv. *C.olitorius* vegetable accessions.

According to Noling (1999), who reported that for RKN to feed on roots, young larval stages need to invade root tissues to establish permanent feeding sites within roots (which cause the presence of galls), and then mature into adult females to produce eggs. There is not much research done on the presence of galls on *C. olitorius*. Small to no root galls were observed in this study. This agrees with Fourie *et al.* (2015) who reported that the appearance of small and underdeveloped root galls in the current study does not imply that nematode reproduction and juvenile hatch did not occur. Orion *et al.* (1998), state that in nematode-resistant plants, root galls might be initiated but fail to fully develop (Orion *et al.*, 1980). Ndivhuwo *et al.* (2020) reported that the failure of giant cells to develop can contribute to the inhibition of nematode growth and reproduction after penetration, therefore restricting the reproductive factor of the nematodes. Mukhtar and Hussain, (2019), also revealed that on resistant cultivars, nematode juveniles cannot induce the development of giant cells to successfully parasitize the root cells.

Furthermore, the lack of parasitism of nematodes to plants leads to death or exit out of roots, therefore reducing the nematode's ability to develop into a mature adult, inhibiting reproduction. To further support this, Williamson and Kumar (2006), reported fewer galls on cowpeas varieties which may indicate that the ability of RKN to form feeding sites on roots was inhibited therefore reducing the reproduction of females after penetration.

In the current study, total final nematode densities increased with the increase in inocula. An increase in nematode population, triggers the photosynthetic products to move to the root forming galls, supporting nematode development and reproduction (Maleita *et al.*, 2012). According to Pofu *et al.*, (2020), the ability of *M. incognita* to reproduce in the roots of plants increasing the final nematode population in roots or soil is evident in susceptible and tolerant plants. Atungwu *et al.* (2013), also reported an increase in J2 of *M. incognita* in the sweet potato root system with an increase in initial inocula levels. This increase was explained as the ability of nematodes to break the plant's resistance mechanism and establish a feeding structure. Mukhtar and Kayani (2019), also observed that the initial inoculum population density of *M. javanica* affects the rate of nematode multiplication, higher reproduction rates were observed at lower inocula levels. Mashela *et al.* (2017) also observed in their study that the availability of a higher number of nematodes in roots of *Cucumis* species serves as a confirmation that nematodes were able to locate and penetrates roots. It is suggested that the short life cycle facilitates the RKN to prosper effectively when a suitable host is present causing the population to increase (Mukhtar & Kayani, 2020).

In the current study, the reproductive factor was above one for Manguzi 2 and Manguzi 3. The reproductive factor has previously been used to indicate the host status of the plant to nematodes (Anwar & McKenry, 2010). The reproductive factor of the nematode, quantifies the nematode establishment and reproduction through a ratio of initial versus final nematode populations on the plant, making this a perfect indicator under a closed environment where soil nematodes can be manipulated (Hadisoeganda & Sasser, 1982; Kayani & Mukhtar, 2018). When the reproductive factor on a particular host is less than one, it indicates that the nematode could not reproduce on that host hence that plant is not a host (Kayani & Mukhtar, 2018). If the reproductive factor is greater than one, the nematode has multiplied successfully on the plant (Pofu *et al.*, 2010a; Steyn *et al.*, 2013). According to Zhang and Schmitt (1994), host susceptibility is categorized using the following scale; as good [susceptible] when $Pf/Pi > 5.0$, fair [moderately resistant/tolerant] if $5.0 \geq Pf/Pi > 1$, poor if $1 > Pf/Pi > 0$, and non-host when $Pf/Pi = 0$. According to the results of the current study, nematodes failed to induce giant cell development but

managed to increase in number. These results then create uncharted territories for both viewpoints, hence for a proper conclusion on the host-status of *Corchorus olitorius*, reproductive factor had to be considered alone. Osunlola and Fawole (2015) demonstrated the importance of reproductive potential when screening a large number of sweet potato cultivars against *Meloidogyne* species, this is supported by Mukhtar and Kayani (2019) who demonstrated a direct relationship between the increased reproductive potential and the host status.

The greater than one reproductive factor at lower and higher nematode inocula levels provides a clear indication that the two cv. *C. olitorius* accessions were moderate-host/tolerant of the nematode. However, this observation is against Bendezy and Star (2003) along with Mukhtar and Kayani (2019), who critique that many plant cultivars are not hosting at higher inocula levels. It is common for susceptible plants to increase in nematode population with the increase in inocula, however, it is not always the case (Gina *et al.*, 2016; Pofu *et al.*, 2020). The high rate of multiplication of nematodes in susceptible hosts with low levels of initial inoculum is due to favourable factors like plenty of food, increased competition and the ability of hosts to support these populations, however as populations continue to increase, factors such as limited food resource and high competition of nematodes for infection sites in roots also increase (Seinhorst, 1967), causing a final population to decline while initial inoculum continues to increase, this is referred to as a plant's nematode equilibrium position (Seinhorst, 1967). The plant can no longer sustain the increase in population, which contributes to the decline in final nematode populations after a peak increase. In the current study, the equilibrium position for Manguzi 2 is at 1600 nematodes while for Manguzi 3 it is at 400 nematodes.

Host sensitivity explains whether the plant is damaged by the nematode infestation or not (Seinhorst, 1967). When the plant is damaged by the nematode infestation, that plant is referred to as sensitive while the one not damaged is said to be non-sensitive/resistant (Seinhorst, 1967). Durrant and Dong (2004), proved that gall number and degree of galling may be used to reflect the ability of a plant to overcome an RKN attack. The presence of giant cells serves as a source of nutrients for developing nematodes (Bartlem

et al., 2013) and are mostly formed in nematode-susceptible plant species (Huang *et al.*, 2003). Furthermore, the development of nematodes from J2 to an adult female causes the adjacent root cells to bulge (Mashela *et al.*, 2017), reducing root functionality. Reduced functionality of roots confers water stress and nutrient deficiency in plants, therefore leading to poor plant growth such as reduced plant height, number of leaves and reduced yield (Zwart *et al.*, 2019). In the current study, the lack of reduction response of all growth plant variables for Manguzi 3 and dry root mass increase for Manguzi 2, is an indication that the two *C. olitorius* accessions are tolerant to *M. incognita*. The susceptibility of vegetable crops to *Meloidogyne* has previously been reported worldwide (Sikora & Fernandez, 2005; Noling, 2012). However, only a few studies similar to the present are available. Daramola *et al.* (2015) and Gruben and Denton (2004) reported *C. olitorius* to be very susceptible to root-knot nematodes (*Meloidogyne* spp.), due to the galling of its roots and the presence of various developmental stages of nematodes found feeding on roots. In the present study, the dry root mass of *C. olitorius* cv. Manguzi 2 was higher than the control, which opposes the findings of Nacar and Ozarslandan, (2021), who observed a general reduction of plant parameters when compared to control. The increase in dry root mass is explained in previous okra varieties, as inhibition of nutrients through absorption, disrupts the distribution of photosynthesis products, therefore conferring build-up of products in the root contributing to an increase in root length and root weight (Nacar & Ozarslandan, 2021). Kankam *et al.* (2021), support the current study, as they observed some cowpea varieties producing a high number of pods with low gall index, conferring slight resistance of varieties. Similarly, Charenghani and Hamzehzarghani, (2012), observed that the increase in root weight is conferred by the formation of root galls. However, the present findings are inconsistent with some other studies, According to studies (Noling, 2012; Kayani *et al* 2018), plant growth parameters are directly linked to infestations in roots. *Meloidogyne incognita* as a root infecting nematode, it is expected that the roots are the most sensitive indicator of relationship status between nematodes and the plant (Mukhtar *et al*, 2017) as observed in the current study. The presence of nematodes is supposed to reduce the plant's development and yield (Noling, 2012; Nacar & Ozarslandan, 2021). A similar conclusion was made by Bhau

et al. (2016) and Adegbite (2017) working on some cassava cultivars in the presence of the same nematode. Kayani *et al.* (2018) supported the narrative of the relationship between plant growth variables and the level of nematode resistance on observations made on cucumbers. In sensitive plants, an increase in nematode populations is always associated with decreases in plant growth and productivity (Karseen & Moens, 2006; Vovlas *et al.*, 2008; Azam *et al.*, 2011). Pofu (2010a), using the same model, reported nematode resistance of two indigenous vegetables, *C. africanus* and *C. myriocarpus*, to *M. incognita* since RF values were less than unity and the two vegetables did not suffer any yield loss in response to nematode infection. The variations in reproductive rates observed between Manguzi 2 and 3 might partially be the result of genetic factors between the two accessions as reported previously for other indigenous vegetable accessions (Griffin, 1982; Jacquet *et al.*, 2005; Castagnone-serena, 2006).

Both host status and host sensitivity are used in determining whether the plant is susceptible, tolerant, or resistant to the presence of nematode. Susceptible plants will allow nematode reproduction and suffer growth and or yield loss due to them (Mukhtar *et al.*, 2013). Susceptible plants lack defence mechanisms against parasitism, hence the nematode can replicate on host plant species (Mukhtar *et al.*, 2013). According to the present study, *C. olitorius* cv. (Manguzi 2 and Manguzi 3) are tolerant, considering the reproductive factor and plants' response to nematode population. Tolerant host plants prevent nematode attack on the plant, but can allow nematode reproduction without causing growth and yield losses (Trudgill, 1991; Jones *et al.*, 2013). Which explains the increase in total final nematode population, small underdeveloped galls and $5 \geq \text{pf}/\text{pi} > 1$ with an increase in inoculum levels in the study. Resistant plants are those plants that are neither hosts nor sensitive to nematode attack (Ralmi *et al.*, 2016). Sasser *et al.* (1984) included the galling index together with the RF to describe the plant status, when the gall index is less than 2 and the reproductive factor is less than 1, the plant is said to be resistant. A plant is regarded as tolerant when the gall index is less than 2 and the reproductive factor greater than 1. In the current study, both accessions of *Corchorus olitorius* were hosts of *M. incognita* but were not damaged by the infestation, an indication that they are both tolerant to the nematode. Tolerant plants allow nematode production

causing minimum root damage, which is different when compared to susceptible plants. Dordas (2008) and Santana-Gomes *et al.* (2013) emphasised the importance of studying plant-pathogen nutrient interactions, as it is complex and important for the plant's defence mechanism. There has been no study reported on the nematode effect on concentrations of macro and micronutrients on the vegetables in focus. According to research, nematode infestation activates the plant defence mechanism increasing the energy demand, enhancing demand reduces the available nutrients in plant and soil, as it is used for protein synthesis, photosynthetic electron transfer, oxidative stress response, cell wall formation and hormonal structure (Dordas, 2008) to defend plants against nematodes, therefore decreasing essential micro and macro nutrients needed to support the plant's development, resulting in restricted growth. Similar studies have been conducted for tomatoes, soybeans, coffee and ridge gourd (Miamoto *et al.*, 2017).

Another factor that could have accounted for increased nematodes in roots is the age of plants. Age of plants has also been observed to affect the ability of the RKN to penetrate and reproduce in the plant, with young plants being more severely affected by nematodes than older plants (Kayani *et al.*, 2018). Younger plants are generally more sensitive than older plants because of undeveloped resistance tissues in them (Kayani *et al.*, 2018). In this study, inoculation was done 3 weeks after planting, as was done by Makumbi *et al.* (2000), but even though plants were young, they were not damaged by nematodes which is contrary to Kudza *et al.* (2000) findings. Several studies explain that the variations in a host can influence various stages in the life cycle of nematodes. The studies state that the nematode either fails to enter the resistant host or they are likely killed after penetration (Mukhtar *et al.*, 2013). According to the previous findings, only a few juveniles make their way into the roots of resistant cultivars and get to mature, which is revealed by the juveniles in roots and soil, and RF (Khan *et al.*, 2019). On the other hand, it is suggested that the production of maximum egg mass in highly susceptible cultivars explains the maximum number of juveniles that entered the roots and were successful in completing the life cycle in a host (Khan *et al.*, 2019). However, the minimum nematode density that can inflict a considerable reduction in the growth or yield of a plant depends on the kind of host, nematode, cultivar and environmental factors (Barker & Oithof, 1976).

3.5 Conclusion

The use of resistant varieties is the most economical and efficient method for the control of RKN. In the current study, reproductive factor was a good indicator, as it measured the establishment and reproduction in the *Corchorus olitorius* c.v. Therefore to conclude, both accessions of *C. olitorius* were hosts of *M. incognita*, however, plant variables were not damaged by the infestation, an indication that they are both cv. *C. olitorius* accessions are tolerant to the root-knot nematode in focus.

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CHAPTER 4

PHYTONEMATICIDAL EFFECTS OF *MAERUA ANGOLENSIS* LEAF MEAL EXTRACTS ON POPULATION DENSITIES OF *MELOIDOGYNE INCOGNITA* RACE 2 AND YIELD OF *CORCHORUS OLITORIUS* UNDER MICROPLOT CONDITIONS

4.1 Introduction

Corchorus olitorius has been reported to be tolerant to *Meloidogyne incognita* (Chapter 3). Severe infection of good host crops by RKN usually results in significant yield and/or quality loss and, in some cases even in total crop failure (Mtshali *et al.*, 2002; Tefu *et al.*, 2005). In a tolerant host, nematode management needs to be managed to prevent a continuous population build-up potentially affecting the next crop.

Synthetic nematicides application is currently the principal and most effective control method for nematodes, as it can reduce PPN population densities below damage threshold levels (Desaeger *et al.*, 2017). However currently, many nematicidal products have been withdrawn from the agriculture chemical market due to their high toxicity to human beings and the environment (Sousa *et al.*, 2015).

There is currently a huge demand for new alternatives that are safe and cost-effective in nematode management to replace synthetic nematicides (Subbotin & Chitambar, 2018; Gomes *et al.*, 2020). Alternative that require less input, easily accessible, pest-specific, not prone to resistance, beneficial to natural enemies, human and environmentally friendly (Subbotin & Chitambar, 2018; Gomes *et al.*, 2020). Medicinal plant compounds such as phenols, flavonoids, quinones, terpenes, tannins, alkaloids, lectins, polypeptides, saponins and sterols have been researched and found to have potential nematicidal effects (Halama & Van Haluwin, 2004). In smallholder, rural farming communities, methods that do not require a lot of processing will be ideal, such as phytonematicides, botanical nematicides and bionematicides (Ntalli & Caboni, 2012; Caboni *et al.*, 2013, Caboni & Ntalli, 2014). According to Akpheokhai *et al.* (2012), *Tagetes* species (Kalaiselvam & Devaraj, 2011), *Cucurbita maxima*, *Tithonia diversifolia*, *Azadirachta*

indica, *Zanthoxylum zanthoxyloides* and *Datura metel* and *Inula viscosa* (Ibrahim *et al.*, 2016) can be used as plant extracts to control root-knot nematodes. *M. angolensis* and *T. elegans* are plants indigenous to SA that have been used in the suppression of *M. incognita* populations in tomato plants (Khosa *et al.*, 2020b), but there has not been any empirical evidence of their ability to control nematodes on *C. olitorius* accessions (Khosa *et al.*, 2020b). Hence the objective of this study was to determine the potential nematicidal effects of *M. angolensis* leaf meal powder on population densities of *M. incognita* and the growth of *C. olitorius* accessions under microplot conditions.

4.2 Materials and methods

4.2.1 Study site

The study was conducted under microplot conditions at the University of Mpumalanga, (25°26'11.4"S; 30°58'54.48"E)Mbombela, SA. Trials were conducted for two seasons, Summer (November 2020- January 2021) and Spring (September 2021- November 2021).

4.2.2 Experimental design and cultural practices

The treatments in each of the experiments consisted of three levels of *M. angolensis* leaf meal powders, 5, 10 and 15 g plus a standard synthetic, commercial nematicide, Nemacur 10GR (a.i. fenamiphos) at a rate of 5 g per plant, as well as the untreated control (0 g), which is translated as 0kg/ha, 460kg/ha, 920kg/ha and 1380kg/ha. The experiments were arranged in a randomized complete block design, with five replications. Untreated *C. olitorius* plants were used as negative control while plants treated with Nemacur® were used as a positive control across all replications. In all replications, a nematode susceptible tomato cv. 'Floradade' was included as an indicator of nematode viability.

4.2.3 Medicinal plant material collection and preparation

Leaves of *M. angolensis* were collected from traditional healers in the Mopani and Vhembe districts, Limpopo Province, South Africa. The botanical origin of the plant parts (stems and leaves) were identified and confirmed by a botanist from the South African

National Biodiversity Institute (SANBI, Tshwane). The plant parts were chopped into 5-cm-long pieces and oven-dried for 4 days at 52 °C prior to grinding in a Wiley mill and passed through a 1-mm-aperture sieve (Makkar, 1999). Crude-milled plant material of 25 kg was stored in bulk in marked, air-tight glass containers at room temperature in the dark until required.

4.2.4 Procedure

The experimental procedures were as stated in Chapter 3 above except that crude-milled leaf meal powder of *M. angolensis* was evaluated for its effect on the growth of Manguzi 2 and 3 *C. olitorius* traditional leafy vegetable accessions and population densities of *M. incognita* race 2 under microplot conditions.

Inoculation

Four weeks after direct sowing, seedlings of *C. olitorius* were inoculated with 3 000 nematode eggs and J2 using a 20 ml plastic syringe introduced into 2½ cm deep holes on cardinal points of the stems.

4.2.5 Data collection

Data were collected and analyzed as previously described (Chapter 3).

4.3 Results

Shapiro-Wilk normality test indicates that all the nematode variables for both Manguzi 2 and Manguzi 3 were not normally distributed ($P \leq 0.05$) (Appendix B.1 and B.16). However, Manguzi 2 had only three plant variables that were normally distributed ($P \geq 0.05$), whereas plant height, fresh shoot mass, dry shoot mass and chlorophyll content, were not normally distributed ($P \leq 0.05$) (Appendix B.1), furthermore Manguzi 3 had only one plant variable (number of leaves), that was not normally distributed ($P \leq 0.05$) (Appendix B.12). Variables that were found not normal were transformed as previously stated. All nematode variables were highly significant ($P \leq 0.01$) (Appendices D.1- D.17), whereas, all plant variables were not significant ($P \geq 0.05$) for both cv.

C.olitorius accessions (Appendices D.1-D.17). In both accessions, very small, underdeveloped galls were observed on the roots. Increased plant extract application decreased the total nematode population found in both roots and soil for both cv. *C.olitorius* accessions (Table 4.1). There was an increased number of juveniles in soil than in roots for Manguzi 2, whereas increased juveniles in roots than in the soil for Manguzi 3 (Table 4.1).

According to the current study, Manguzi 2 control treatment had the highest total nematode population, when compared to the plant extracts. Plants of cv. *C. olitorius* applied with 5 g of plant extracts had the highest total nematode population for both accessions, however, Manguzi 2 total nematode population to 5 g was not significantly different from the other extracts and Nemacur (Table 4.1). Plants applied with 20 g of plant extracts had the lowest total nematode population for both cv. *C. olitorius*, which effects are not significantly different from 15 g plant extract and Nemacur for Manguzi 3 (Table 4.1).

The addition of plant extracts reduced nematodes in soil and roots with also a reduction in their ability to reproduce (Table 4.1). All plant extract treatments for Manguzi 2, including control had a reproductive factor (RF) below 1, with the control having the highest RF and Nemacur the least, which were not significantly different from other plant extract treatments, whereas, Manguzi 3, 5 g treatment level had the highest RF, with two of the plant extracts (5 g and 10 g), including control having a reproductive factor above one, while the rest of plant extracts that are not significantly different, had an RF below one (Table 4.1).

Table 4. 1: Response of two accessions of *Corchorus olitorius*(Manguzi 2 and Manguzi 3) towards application rates of *Maerua angolensis* and inoculum levels of *Meloidogyne incognita*

Manguzi 2					Manguzi 3			
TRT	J2R	J2S	TT	RF	J2R	J2S	TT	RF
0	3.6(4033.3) ^a	4.3(23467) ^a	4.4(26800) ^a	1.0(8.9) ^a	4.9(150295) ^b	3.7(58486) ^a	4.9(156143) ^b	1.4(50.51) ^b
5g	3.2(1555.6) ^c	3.8(5937) ^{bc}	3.9(7493) ^{bc}	0.6(2.5) ^{bc}	5.4(274743) ^a	4.0(10971) ^a	5.4(285714) ^a	2.0(95.24) ^a
10g	3.2(1929.2) ^c	4.0(10250) ^{bc}	4.0(12179) ^b	0.7(4.1) ^b	4.4(25371) ^c	3.8(7314) ^a	4.5(32686) ^c	1.1(10.90) ^b

15g	3.1(1361.1) ^c	3.7(5747) ^{bc}	3.8(7108) ^c	0.5(2.4) ^c	3.6(6743) ^d	2.8(1714) ^b	3.8(8457) ^d	0.5(2.82) ^c
20g	3.3(2165.7) ^{bc}	3.5(3926) ^c	3.7(6091) ^c	0.5(2.0) ^c	3.1(1478) ^e	2.0(503) ^b	3.3(1981) ^e	0.2(0.66) ^c
Nemacur	3.5(3733.3) ^{ab}	3.1(1700) ^d	3.7(5629) ^c	0.4(1.9) ^c	3.3(2643) ^{de}	2.7(617) ^b	3.5(3260) ^{de}	0.3(1.08) ^c
F-value	4.59	16.28	12.08	15.02	42.57	6.16	52.63	34.46
LSD _{0.05}	0.25	0.30	0.22	0.15	0.41	0.90	0.34	0.34
P-value	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*

Column means with the same letters (a, b, c, d, e) do not differ significantly ($P \leq 0.05$) according to Fisher's protected least significant difference test. Values in brackets are untransformed means $[\log_{10}(x + 1)]$. RF = Pf/ Pi. * Highly significant ($P \leq 0.01$). TRT treatment; J2R juveniles in roots; J2S juveniles in soil; TT total; RF reproductive factor.

The plant variables for both cv. *C. olitorius* accessions were all not significantly different from the other treatments, including the control. According to the results, the increase in plant extracts increased the fresh roots for *C. olitorius* cv. (Manguzi 2 and Manguzi 3) (Table 4.2).

Table 4. 2: Response of *Maerua angolensis* on growth variables of two accessions of *Corchorus olitorius* (Manguzi 2 and Manguzi 3)

TRT	Manguzi 2							Manguzi 3						
	PH (mm)	FSM (g)	FRM (g)	NOL	DSM (g)	DRM (g)	CC	PH (mm)	FSM (g)	FRM (g)	NOL	DSM (g)	DRM (g)	CC
0	2.3(190.0)	1.7(45.4)	44.3 ^a	97.6 ^{ab}	1.0(8.7) ^a	6.5 ^{ab}	1.8(67.3)	145.0	42.9	37.5 ^a	2.0(100.7)	7.5	4.6	69.0
5g	2.2(162.9)	1.7(50.7)	42.6 ^{ab}	116.7 ^a	1.0(8.2) ^{ab}	7.2 ^a	1.8(67.3)	157.9	36.4	39.9 ^a	2.0(93.4)	7.3	5.3	71.7
10g	2.2(180.7)	1.6(433.6)	33.1 ^{ab}	116.1 ^a	1.0(7.4) ^{ab}	5.1 ^{ab}	1.8(68.1)	147.1	32.7	34.4 ^a	1.8(80.7)	6.8	4.8	66.2
15g	2.2(167.9)	1.7(48.9)	43.4 ^a	118.4 ^a	1.0(8.7) ^a	6.7 ^{ab}	1.8(67.3)	135.0	33.4	34.1 ^a	1.8(95.0)	7.1	4.8	66.4
20g	2.3(191.4)	1.6(35.8)	29.3 ^b	78.4 ^b	1.0(8.0) ^b	4.7 ^b	1.8(66.0)	145.7	43.7	34.4 ^a	2.0(97.7)	8.1	5.2	64.9
Nemacur	2.2(179.3)	1.7(47.9)	39.1 ^{ab}	98.7 ^{ab}	0.9(5.8) ^{ab}	6.1 ^{ab}	1.8(68.9)	156.4	38.7	37.9 ^a	1.9(88.1)	6.7	5.6	64.4
F- value	0.77	1.03	1.6	1.7	1.20	1.6	0.10	0.53	0.36	0.19	0.54	0.48	0.15	0.98
LSD _{0.05}	0.08	0.16	13.9	34.8	2.42	2.28	0.08	33.36	22.39	16.04	0.26	3.74	2.25	8.04
P- value	0.58 ^{ns}	0.42 ^{ns}	0.18 ^{ns}	0.17 ^{ns}	0.33 ^{ns}	0.21 ^{ns}	0.99 ^{ns}	0.75 ^{ns}	0.87 ^{ns}	0.96 ^{ns}	0.74 ^{ns}	0.79 ^{ns}	0.98 ^{ns}	0.45 ^{ns}

4.4 Discussion

The application of *M. angolensis* plant extracts decreased *M. incognita* population densities for Manguzi 2, and the same was observed for Manguzi 3 except for the treatment level of 5 g. The reduction in nematode population densities with the application of plant extracts could be due to the toxic effects of chemicals contained in the leaf extracts of *M. angolensis*. Khosa *et al.* (2020a; 2020b) reported that *M. incognita* eggs and J2 were highly sensitive to *M. angolensis* under *in vitro* conditions. Literature is full of reports on the potential suppressive nature of plant extracts against root-knot nematodes (Hong *et al.*, 2010, 2011; Adomako & Kwosheh, 2013; Ullah *et al.*, 2014; Ntalli *et al.*, 2016; Oplos *et al.*, 2018; Tarraf *et al.*, 2019). Similar results were obtained by Husain *et al.* (1996) and Assif *et al.* (2013). However, more evidence indicates that the efficacy of plant extracts depends on the concentration and duration of exposure of nematodes to extract (Mshmoode *et al.*, 1979, Kali *et al.*, 1980, Jain *et al.*, 1993). The relationship between nematode management efficacy of plant extracts and concentration was observed in the current study on both Manguzi 2 and 3.

The lower rate of nematode multiplication in Nemacur® treated plants observed in this study could be due to the toxicity of the chemical on J2 (Mukhtar *et al.*, 2013). According to Khurma *et al.* (1997), the mechanism involved in the suppression of nematodes by plant extracts includes disruption of cell membrane permeability. Another mechanism could involve the exhaustion of oxygen in the soil by decomposing plant extracts starving migrating J2 of the vital gas (Khurma *et al.*, 1997). Khosa *et al.* (2020) reported *M. angolensis* suppressing the nematode population densities in tomato plants by over 80 % relative to untreated plants and this efficacy was comparable to that of Nemacur®. This is similar to what was observed in the current study, where high plant extract levels were

comparable to synthetic chemical nematicide. Compared to what was observed in Chapter 3, plant extracts reduced the reproductive ability of nematodes.

In the current study, the plant extracts had no effects on plant growth variables of the two *C. olitorius* accessions. This contradicts reports made by Khosa *et al.* (2020), where a higher shoot mass was observed when *M. angolensis* extracts were applied to tomato plants under field and greenhouse conditions. Claudius-Cole *et al.* (2010) found that plant extracts increased plant growth, vigour, and yield by decreasing nematode populations in cowpea. The current study contradicts the work by El-Deeb *et al.* (2018) who observed an increase in fresh and dry shoot mass of tomato plants treated with Nemacur®. Soybean plants treated with plant extracts produced higher grain mass, fresh root mass, and the number of root nodules (Akpheokhai *et al.*, 2012). The increase in plant growth and yield has been linked to increased nitrogen and organic matter content of the soil as is achieved when plants are mulched when plant extracts are added. An increase in plant vigour and an increase in yield were also observed in *tithonia*-mulched plantain plants (Coyne *et al.*, 2006).

4.5 Conclusion

Maerua angolensis managed to reduce the nematode population densities on both cv. *C. olitorius* accessions, however, the plant extracts had no significant effect on the plant growth variables. Further study is necessary to identify the phytochemicals that are causing the observed nematicidal effects of *M. angolensis*.

4.6 References

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CHAPTER 5

SUMMARY, SIGNIFICANCE OF FINDINGS, RECOMMENDATION AND CONCLUSIONS

5.1 Summary

This study assessed the host status and host sensitivity of two *C. olitorius* accessions, Manguzi 2 and Manguzi 3 to *M. incognita* and the nematicidal effects of *Maerua angolensis* leaf meal extracts on population densities of *M. incognita* and growth of *C. olitorius*. There was a clear pattern of how the final nematode population densities in roots and soil increased with the increase in initial inoculum levels of *M. incognita* eggs and J2. There were very small and underdeveloped galls found in the roots of the two accessions. The two accessions of *C. olitorius* had reproductive factors of greater than one at lower *M. incognita* inocula levels and lower than one reproductive factor at higher inocula, an indication of nematode reproduction in the roots of the two. The two accessions also were not sensitive to the *M. incognita* inocula levels, allowing a description of the two accessions in relation to nematode infestation as tolerant. The application of *Maerua angolensis* reduced nematode population and reproductive factors in both roots and soil, however, it did not affect the plant growth variables.

5.2 Significance of findings

The study provided the first report that the two *C. olitorius* accessions are tolerant to *M. incognita* and also that *M. angolensis* could potentially be used as plant extracts to reduce the nematode population on *C. olitorius*.

5.3 Recommendation

In the beginning, the study had more than six *Corchorus olitorius* accessions, a majority of these accessions failed to germinate and grow as anticipated. In almost all studies conducted on *Corchorus olitorius*, seed dormancy was observed (Heealy *et al.*, 2008; Maina *et al.*, 2011; Velempini *et al.*, 2013). Which is the reason mechanisms of breaking dormancy in these vegetables need to be investigated for gain to be obtained from the

crops' high nutritional and medicinal values. Chemical characterization of *M. angolensis* plant extracts needs to be conducted for clarification of the mechanism of nematode suppression by the plant. *Maerua angolensis* has a potential in the suppression of nematodes on *C. olitorius*.

5.4 Conclusions

Corchorus olitorius accessions, Manguzi 2 and Manguzi 3 are both tolerant to *M. incognita*. At the levels used in the current study, the fertilizer effects of plant extracts reported in many studies were not observed.

5.4 References

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List of Appendices

Appendix A.1 Shapiro-Wilk normality test for measured variables on *Corchorus olitorius* cv.. Manguzi 2 exposed to *Meloidogyne incognita*

Variables	N	W	P
Nematode juveniles in roots	42	0,5984	0,0000
Nematode juveniles in soil	42	0,9120	0,0034
Total nematode population	42	0,6095	0,0000
Reproductive factor	42	0,9010	0,0015
Plant height	48	0,8063	0,0000
Fresh shoot mass	48	0,8819	0,0002
Fresh root mass	48	0,2043	0,0000
Stem diameter	48	0,9812	0,6300
Dry shoot mass	48	0,9806	0,6060
Dry root mass	48	0,9696	0,2439
Chlorophyll content	48	0,9745	0,3751

Appendix A.2 Analysis of variance (ANOVA) for nematode juveniles in roots of *Corchorus olitorius* cv. "Manguzi 2" exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	0,0552		
Treatment	6	12,9560	55,14	0,0000
Error	30	1,1749		
Total	41	14,1861		

Appendix A.3 Analysis of variance for nematode juveniles in soil of *Corchorus olitorius* cv. "Manguzi 2" exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	1,33132		
Treatment	6	4,15176	7,64	0,0000
Error	30	2,71681		
Total	41	8,19989	41	

Appendix A.4 Analysis of variance (ANOVA) for total nematode population of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	0,0704		
Treatment	6	11,4630	52,39	0,0000
Error	30	1,0940		
Total	41	12,6274		

Appendix A.5 Analysis of variance (ANOVA) for *Meloidogyne incognita* reproductive factor on *Corchorus olitorius*, Manguzi 2 accession

Source	DF	SS	F	P
Replication	5	0,01250		
Treatment	6	0,33869	8,14	0,0000
Error	30	0,20797		
Total	41	0,55916		

Appendix A.6 Analysis of variance (ANOVA) for plant height of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	0,07191		
Treatment	7	0,02174	0,72	0,6537
Error	35	0,15045		
Total	47	0,24410		

Appendix A.7 Analysis of variance (ANOVA) for fresh shoot mass of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	0,04737		
Treatment	7	0,09211	1,65	0,1533
Error	35	0,27872		
Total	47	0,41820		

Appendix A.8 Analysis of variance (ANOVA) for fresh root mass of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	0,11562		
Treatment	7	0,23810	1,09	0,3915
Error	35	1,09365		
Total	47	1,44736		

Appendix A.9 Analysis of variance (ANOVA) for stem diameter of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	0,01825		
Treatment	7	0,02981	0,89	0,5244
Error	35	0,16742		
Total	47	0,21548		

Appendix A.10 Analysis of variance (ANOVA) for dry shoot mass of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	17,734		
Treatment	7	21,907	1,07	0,4024
Error	35	102,296		
Total	47	141,938		

Appendix A.11 Analysis of variance (ANOVA) for dry root mass of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	3,454		
Treatment	7	33,160	2,40	0,0406
Error	35	69,014		
Total	47	105,627		

Appendix A.12 Analysis of variance (ANOVA) for chlorophyll content of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	12,233		
Treatment	7	58,439	0.58	0.7672
Error	35	503,641		
Total	47	574,313		

Appendix A.13 Shapiro wilk normality test for measured variables on *Corchorus olitorius* cv.. Manguzi 3 exposed to *Meloidogyne incognita*

Variables	N	W	P
Nematode juveniles in roots	42	0,6004	0,0000
Nematode juveniles in soil	42	0,6835	0,0000
Total nematode population	42	0,6863	0,0000
Reproductive factor	42	0,7370	0,0000
Plant height	48	0,9783	0,5103
Fresh shoot mass	48	0,9875	0,8832
Fresh root mass	48	0,9779	0,4950
Stem diameter	48	0,9825	0,6863
Dry shoot mass	48	0,9557	0,0677
Dry root mass	48	0,9286	0,0060
Chlorophyll content	48	0,9691	0,2343

Appendix A.14 Analysis of variance (ANOVA) for nematode juveniles in roots of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	1,6810		
Treatment	6	1,5160	1,01	0,4364
Error	30	7,4921		
Total	41	10,6891		

Appendix A.15: Analysis of variance (ANOVA) for nematode juveniles in soil of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	0,14149		
Treatment	6	1,21183	2,95	0,0221
Error	30	2,05550		
Total	41	3,40882		

Appendix A.16: Analysis of variance (ANOVA) for total nematode population of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	0,46040		
Treatment	6	1,40055	2,76	0,0292
Error	30	2,53277		
Total	41	4,39373		

Appendix A.17 Analysis of variance (ANOVA) for reproductive factor of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	0,13104		
Treatment	6	1,93733	22,24	0,0000
Error	30	0,43558		
Total	41	2,50395		

Appendix A.18: Analysis of variance (ANOVA) for plant height of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	208,90		
Treatment	7	183,29	0,95	0,4804
Error	35	962,56		
Total	47	1354,74		

Appendix A.19: Analysis of variance (ANOVA) for fresh shoot mass of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	87,84		
Treatment	7	199,99	1,02	0,4356
Error	35	981,79		
Total	47	1269,62		

Appendix A.20: Analysis of variance (ANOVA) for fresh root mass of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	87,09		
Treatment	7	74,94	0,35	0,9246
Error	35	1070,89		
Total	47	1232,93		

Appendix A.21: Analysis of variance (ANOVA) for stem diameter of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	0,03346		
Treatment	7	0,03385	1,20	0,3297
Error	35	0,14129		
Total	47	0,20860		

Appendix A.22: Analysis of variance (ANOVA) for dry shoot mass of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	3,674		
Treatment	7	26,138	1,83	0,1122
Error	35	71,401		
Total	47	101,213		

Appendix A.23: Analysis of variance (ANOVA) for dry root mass of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	0,03996		
Treatment	7	0,05014	0,62	0,7393
Error	35	0,40735		
Total	47	0,49746		

Appendix A.24: Analysis of variance for chlorophyll content of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	5	9,974		
Treatment	7	90,800	1,57	0,1767
Error	35	289,043		
Total	47	389,817		

Appendix B.1: Shapiro wilk normality test for measured variables on *Corchorus olitorius* cv.. Manguzi 2 exposed to *Maerua angolensis*

Variables	N	W	P
Nematode juveniles in roots	42	0,8288	0,0000
Nematode juveniles in soil	42	0,7866	0,0000
Total nematode population	42	0,7785	0,0000
Reproductive factor	42	0,7785	0,0000
Plant height	42	0,8612	0,0001
Fresh shoot mass	42	0,9127	0,0035
Fresh root mass	42	0,9687	0,2984
Number of leaves	42	0,9771	0,5508
Dry shoot mass	42	0,8909	0,0008
Dry root mass	41	0,9582	0,1358
Chlorophyll content	42	0,8991	0,0014

Appendix B.2: Analysis of variance (ANOVA) for juveniles in roots of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	0,70629		
Treatment	5	1,20677	4,59	0,0032
Error	30	1,57788		
Total	41	3,49093		

Appendix B.3: Analysis of variance (ANOVA) for juveniles in soil of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	0,13195		
Treatment	5	6,18644	16,28	0,0000
Error	30	2,28025		
Total	41	8,59864		

Appendix B.4: Analysis of variance (ANOVA) for TOTAL of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	6	0,39203		
Treatment	5	2,40752	12,08	0,0000
Error	30	1,19594		
Total	41	3,99549		

Appendix B.5: Analysis of variance (ANOVA) for reproductive factor of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	0,17784		
Treatment	5	1,42435	15,02	0,0000
Error	30	0,56890		
Total	41	2,17109		

Appendix B.6: Analysis of variance (ANOVA) for plant height of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	0,30329		
Treatment	5	0,02137	0,77	0,5766
Error	30	0,16579		
Total	41	0,49044		

Appendix B.7: Analysis of variance (ANOVA) for fresh shoot mass of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	0,37086		
Treatment	5	0,11544	1,03	0,4190
Error	30	0,67369		
Total	41	1,15999		

Appendix B.8: Analysis of variance (ANOVA) for fresh root mass of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	61,23		
Treatment	5	1321,79	1,63	0,1833
Error	30	4875,93		
Total	41	6258,95		

Appendix B.9: Analysis of variance (ANOVA) for number of leaves of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	6	6739,0		
Treatment	5	8678,5	1,71	0,1636
Error	30	30515,9		
Total	41	45933,3		

Appendix B.10: Analysis of variance (ANOVA) for dry shoot mass of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	0,22457		
Treatment	5	0,08647	1,20	0,3323
Error	30	0,43182		
Total	41	0,74286		

Appendix B.11: Analysis of variance (ANOVA) for dry root mass of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	13,140		
Treatment	5	83,326	1,55	0,2053
Error	29	311,672		
Total	40			

Appendix B.12: Analysis of variance (ANOVA) for chlorophyll content of *Corchorus olitorius* cv. “Manguzi 2” exposed to *Meloidogyne incognita*

Source	DF	SS	F	P
Replication	6	0,01951		
Treatment	5	0,00228	0,10	0,9922
Error	30	0,14293		
Total	41	0,16473		

Appendix B.13: Shapiro wilk normality test for measured variables on *Corchorus olitorius* cv.. Manguzi 3 exposed to *Maerua angolensis*

Variables	N	W	P
Nematode juveniles in roots	42	0,6432	0,0000
Nematode juveniles in soil	42	0,8062	0,0000
Total nematode population	42	0,6568	0,0000
Reproductive factor	42	0,6526	0,0000
Plant height	42	0,9860	0,8797

Fresh shoot mass	42	0,9791	0,6258
Fresh root mass	42	0,9578	0,1229
Number of leaves	42	,9230	0,0076
Dry shoot mass	42	0,9453	0,0440
Dry root mass	42	0,9802	0,6687
Chlorophyll content	42	0,9552	0,0989

Appendix B.14: Analysis of variance (ANOVA) for juveniles in roots of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	0,4664		
Treatment	5	29,7382	42,57	0,0000
Error	30	4,1919		
Total	41	34,3965		

Appendix B.15: Analysis of variance (ANOVA) for juveniles in soil of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	2,4530		
Treatment	5	21,0826	6,16	0,0005
Error	30	20,5427		
Total	41	44,0783		

Appendix B.16: Analysis of variance (ANOVA) for total nematode population of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	0,2988		
Treatment	5	26,1852	52,63	0,0000
Error	30	2,9854		
Total	41	29,4695		

Appendix B.17: Analysis of variance (ANOVA) for reproductive factor of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	0,3203		
Treatment	5	16,5304	34,46	0,0000
Error	30	2,8781		
Total	41	19,7288		

Appendix B.18: Analysis of variance (ANOVA) for plant height of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	8282,1		
Treatment	5	2464,3	0,53	0,7533
Error	30	28010,7		
Total	41	38757,1		

Appendix B.19: Analysis of variance (ANOVA) for fresh shoot mass of *Corchorus olitorius* cv.. “Manguzi 3” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	1480,0		
Treatment	5	752,8	0,36	0,8731
Error	30	12621,6		
Total	41	14854,4		

Appendix B.20: Analysis of variance (ANOVA) for fresh root mass of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	2086,24		
Treatment	5	205,04	0,19	0,9641
Error	30	6477,82		
Total	41	8769,10		

Appendix B.21: Analysis of variance (ANOVA) for number of leaves of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	0,27012		
Treatment	5	0,15687	0,54	0,7436
Error	30	1,73956		
Total	41	2,16656		

Appendix B.22: Analysis of variance (ANOVA) for dry shoot mass of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	0,29490		
Treatment	5	0,14280	0,48	0,7868
Error	30	1,77735		
Total	41	2,21504		

Appendix B.23: Analysis of variance (ANOVA) for dry root mass of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	94,831		
Treatment	5	9,118	0,15	0,9772
Error	30	355,479		
Total	41	459,428		

Appendix B.24: Analysis of variance (ANOVA) for chlorophyll content of *Corchorus olitorius* cv. “Manguzi 3” exposed to *Maerua angolensis*

Source	DF	SS	F	P
Replication	6	311,86		
Treatment	5	266,18	0,98	0,4450
Error	30	1626,77		
Total	41	2204,81		