An investigation into the potential of crude and partially separated material of selected non-crop plant species as control agents of root-knot nematodes (*Meloidogyne incognita*) in tomato

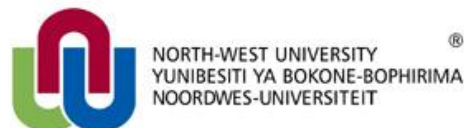
Mbokota Candy Khosa

Thesis submitted for the degree Doctor of Philosophy in Environmental Sciences at the Potchefstroom Campus of the North-West University South Africa

March 2013

Promoter: Prof A.H. Mc Donald

Co-promoter: Dr M.S. Daneel



PREFACE

I humbly express my gratitude to the Almighty Lord, besides whom none is worthy for being worshipped, who bestowed me the health, courage to compute and execute this manuscript. He blessed, guided, strengthened and helped me to endeavour my work.

I, therefore, fortified myself to join a doctorate course under the guidance of Prof. Alex Mc Donald, Department of Plant Protection, Unit of Environmental Sciences and Management (North-West University, Potchefstroom Campus, North West Province, South Africa). Expressing my sincere and deep sense of gratitude for his personal affection, unified approach, towering personality, constant embodiment and generosity bestowed on me without this, work would had been an uphill task for me. It is beyond my means and capacity to put in words my sincere gratitude to my co-advisor Dr. Mieke S. Daneel, Agricultural Research Council-Institute of Tropical and Subtropical Crops (ARC-ITSC), Mpumalanga Province, South Africa. Sincere gratitude and special thanks goes to Dr. Thomas A. Coudron, Research Chemist, Lead Scientist and Adjunct Associate Professor and Dr. Holly J. Popham, Adjunct Assistant Professor Division of Plant Sciences (Biological Control of Insects Research Laboratory, United State Department of Agriculture, Missouri State, USA); Dr. Mitchum Melissa Goellner, Assistant Professor and Mrs. Christy Copeland, Coordinator of International Agricultural Programs (University of Missouri, Missouri State, USA), Prof. Rodrigo Rodríguez-Kábana, Distinguished Professor of Nematology (Auburn University, Alabama State, USA) whose invaluable suggestions incredibly supported me throughout the perusal of this programme.

I express a deep sense of gratitude to members of my advisory committee Mr. Frikkie Calitz, Unit Manager Biometry Division., Mrs. Cynthia B. Nghwane, Junior Biometrician and Mr. Eric T. Mathebula, Junior Biometrician (Biometry Division, Agricultural Research Council-Head Office, Pretoria, Gauteng Province, South Africa) for giving me constructive opinions, which have gone in the completion of my task.

Words of thanks go to all those who provided laboratory assistance, especially Dr. Vinesh Maharaj, Chief Scientist, Council of Scientific and Industrial Research (CSIR) Biosciences' bio-

prospecting research group and Dr. Malefa Tselanyane, Research Scientist CSIR, Pretoria, South Africa; Prof. P.W. Mashela, Senior Researcher, Department of Plant Production, Soil Science and Agricultural Engineering, University of Limpopo; Mr. Mafemani W. Mabunda, Extension Officer (Department of Agriculture, Limpopo Province, South Africa); Mr. Charles S. Arries, Nematology Technician (ARC-ITSC, Mpumalanga Province, South Africa); Dr. Sonia Steenkamp, Senior Researcher, Agricultural Research Council-Grain Crop Institute (ARC-GCI), North West Province, South Africa, for their help and availability during my course of research. Special thanks to Agricultural Research Council, National Research Foundation, Agri-SETA and the Norman E. Borlaug Scholarship/Foundation for funding.

It gives me fathomless pleasure to place on record my heartfelt gratitude to my brothers Magigwana I. Khosa, Njhan'wa J. Khosa, Tinjombo M. Khosa and Nhlalala M. A. Khosa; my daughters Mmatseketla M.N. Khosa., Mkateko C. Khosa and Makungu K. Khosa; my friends Mr. Thulani Nkuna, Mr. Kholekile V. Tshaka, Mr. Nkosinathi Mnyaka, Mr. Phukuile J. Masudubele, Mr. Nhlamulo J. Maluleke, Mr. Dikiza D. Makaula, Mr. Safika. Makaula, Mr. Loyiso Ncoko and also to Ms. Andiswa C. Tshele for their encouragement and moral support, which enabled me to complete the work successfully.

I would not forget to express my thanks towards my uncles, Dr. Arnold K., Judge Winston M., Mr. Thembi and Khensani D. Msimeki; my cousins Mr. Themba Mashengete, Mr. Tinyiko G. Msimeki, Dr. Aubrey M. K. Msimeki, Dr. Khawulani Msimeki and Hanyani Msimeki; my friends and all my family members whose affection, sacrifice and blessings have always been the vital source of my inspiration.

Parents are the most precious and valuable gift of God. I feel blessed and lucky in the world to have the most lovable parents. I think words are insufficient to express my deep sense of feeling towards my mother Ms. Tintswalo C. Msimeki, Mr. Colbert M. Khosa and my late wife Mashobo W. Khosa for their concern, patience, and dedication during the course of my study.

ABSTRACT

Plant-parasitic nematodes (PPN) are a serious problem in vegetable production and can cause severe damage to several crops. In rural, low-input farming nematode damage is much higher and yields can be completely destroyed. Some Commercial nematicides have been withdrawn from the market due to health and environment concerns. These need to be replaced by alternative nematode control strategies of which soil amendments is one alternative. Nine non-crop plant species used in various forms in traditional healing, viz. *Cassia abbreviata*, *Cissus cactiformis*, *Euphorbia ingens*, *Ipomoea kituiensis, Maerua angolensis, Senna petersiana*, *Synadenium cupulare*, *Tabernaemontana elegans* and *Urginea sanguinea* were screened under glasshouse conditions for their effect on the plant-parasitic nematode (PPN) (*Meloidogyne incognita*) on tomato. Subsequent assessments in microplots and in the field supported the glasshouse results in terms of suppression of root-knot nematode numbers with crudely milled soil amendments of *C. cactiformis, M. angolensis* and *T. elegans.* Tomato growth responses in these trials showed a tendency of phytotoxic effects after treatment of soil with crude leaf meal of *E. ingens* and *S. cupulare.* In the microplot study, the overall soil-amendment treatment effect was greater than that of three soil types on the performance of the tomato, although soil type might have had an effect on nematode suppression. Due to lack of correspondence between tomato leaf nutrient contents and the nutrient contents of the soil amendments it is suggested that these non-crop materials had negligible soil fertilization effects.

In vitro bioassay studies confirmed that extracts of varying polarity of both plant products *M. angolensis* and *T. elegans* might be toxic to J2 stages of the root-knot nematode *M. incognita*. All extracts tested of *M. angolensis* caused immobility of J2, whereas only three extracts of *T. elegans* affected mobility of J2 adversely. Duration to 50 % effect, as well as extract concentration to cause immobility of the J2 varied but where movement ceased the J2 did not recover for up to 98 hours.

This study has demonstrated the potential of locally available botanical materials for use as amendments in plant-parasitic nematode management and tomato growth and productivity improvement. This would particularly be true for small-scale application in subsistence

agriculture. It is believed that these amendments could be used as control measures in integrated nematode control strategies. Their potential use could be adopted by small-scale farming communities, domestic gardeners and commercial farmers in the Mpumalanga, Limpopo and Kwazulu/Natal Provinces of South Africa where the relevant materials are available in useful quantities. Over-exploitation of natural resources should be avoided at all cost, however.

Key words: Amendment, botanical, extracts, *Meloidogyne incognita*, nutrients, soil, tomato

TABLE OF CONTENTS

CHAPTER 1. INTRODUCTION **1**

1.1. Small-scale-farming communities in South Africa 1

1.2. Tomato 2

1.3. Root-knot nematodes 3

1.4. Root-knot nematode management strategies 4

1.4.1. Fallowing 4

1.4.2. Soil solarization and heating 5

1.4.3. Plant- parasitic nematode control by synthetic agent 5

1.4.4. Bio-fumigation 6

1.4.5. Organic amendments 7

1.5. Non-crop plant species with herbal or medicinal properties 10

1.5.1. *Cassia abbreviata* Oliv. (Sjambok pod) 10

1.5.2. *Cissus cactiformis* Gilg. (Cucumber cactus) 11

1.5.3. *Euphorbia ingens* E.Mey. (L.C. Wheeler,'naboom' or candelabra tree) 12

1.5.4. *Ipomoea kituiensis* Vatke (Morning glory) 13

1.5.5. *Maerua angolensis* DC. (Bead-bean) 14

1.5.6. *Senna petersiana* (Bolle) Lock. (Wild Senna) 15

1.5.7. *Synadenium cupulare* (Boiss.) Wheeler ex A.C. White, R.A. Dyer and B. Sloane (Bead-man's tree) 16

1.5.8. *Tabernaemontana elegans* Stapf. (Toad tree) 16

1.5.9. *Urginea sanguinea* Shinz. ('Slangkop') 17

1.6. Aims of this study 18

CHAPTER 2. THE EFFECT OF MILLED MATERIAL OF SELECTED NON-CROP PLANT SPECIES ON THE GROWTH OF TOMATO AND ON POPULATION DENSITIES OF *MELOIDOGYNE INCOGNITA* IN THE GLASSHOUSE **20**

2.1. Introduction 20

2.2. Material and Methods 20

2.2.1. Collection and preparation of different plant leaf meals as soil amendments 21
2.2.2. Acquirement, multiplication, extraction and inoculation of root-knot nematodes 22
2.2.3. Treatments, trial layouts and glasshouse conditions 22
2.2.4. Growth of tomato and nematode assessments 23
2.2.5. Data analysis 23
2.3. Results 24
2.3.1. Tomato plant development 24
2.3.2. Nematode population development 27
2.4. Discussion 29

CHAPTER 3. EVALUATION OF SELECTED CRUDE PLANT MEALS AND DIFFERENT SOIL TYPES ON *MELOIDOGYNE INCOGNITA* AND LEAF-TISSUE NUTRIENT ELEMENTS IN TOMATO UNDER MICROPLOT CONDITIONS 31

3.1. Introduction 31
3.2. Material and Methods 32
3.2.1. Collection and preparation of different plant-leaf meals as soil amendments 32
3.2.2. Acquirement, multiplication, extraction and inoculation of root-knot nematodes 32
3.2.3. Treatments, trial layout and microplot conditions 32
3.2.4. Growth of tomato and nematode assessments 33
3.2.5. Nutrient-element analyses from tomato leaf tissue 33
3.2.6. Data analysis 34
3.3. Results 34
3.4. Discussion 46

CHAPTER 4. A FIELD ASSESSMENT OF THE EFFECT OF CRUDE PLANT-LEAF MEALS ON ROOT-KNOT NEMATODES, SELECTED LEAF TISSUE NUTRIENT ELEMENTS AND TOMATO CROP PERFORMANCE 48

4.1. Introduction 48
4.2. Material and Methods 49
4.2.1. Collection and preparation of different plant-leaf meals as soil amendments 49
4.2.2. Acquirement, multiplication, extraction and inoculation of root-knot nematodes 49
4.2.3. Treatments, trial layout and field conditions 49
4.2.4. Growth of tomato and nematode assessments 49
4.2.5. Nutrient-element analyses from tomato leaf tissue 50
4.2.6. Nutrient element analyses from crude leaf-meals used in this study 50
4.2.7. Data analysis 50
4.3. Results 50
4.4. Discussions 56

CHAPTER 5. *IN VITRO* EFFECT OF LEAF-MEAL EXTRACTS OF *MAERUA ANGOLENSIS* AND *TABERNAEMONTANA ELEGANS* ON THE MOTILITY OF SECOND-STAGE-JUVENILE ROOT-KNOT NEMATODES 59

5.1. Introduction 59
5.2. Material and Methods 60
5.2.1. Collection, preparation of different plant-leaf meals and extraction technique of root-knot nematodes 60
5.2.2. Extracts prepared from *M. angolensis* and *T. elegans* leaf meal 60
5.2.3. Preparation of stock solutions 61
5.2.4. Root-knot nematode J2 motility-inhibition assays 61
5.2.5. Reversible nature of J2 motility inhibition 62
5.3. Data analysis 63
5.4. Results 65
5.4.1. Root-knot nematode J2 motility-inhibition assays 65
5.4.1.1. *Maerua angolensis* 65
5.4.1.2. *Tabernaemontana elegans* 69

5.4.2. LC_{50} of plant-leaf extracts 73

5.4.2.1. *Maerua angolensis* 73

5.4.2.2. *Tabernaemontana elegans* 76

5.4.3. Reversible nature of J2 motility inhibition 79

5.4.3.1. *Maerua angolensis* 79

5.4.3.2. *Tabernaemontana elegans* 81

5.5. Discussion 83

CHAPTER 6. SUMMATIVE CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH ON THE BOTANICAL SOIL AMENDMENTS TESTED DURING THIS STUDY 85

BIBLIOGRAPHY 89

LIST OF FIGURES

Figure 1.1. *Cassia abbreviata* (Sjambok pod) 11

Figure 1.2. *Cissus cactiformis* (Cucumber cactus) 12

Figure 1.3. *Euphorbia ingens* ('naboom' or candelabra tree) 13

Figure 1.4. *Ipomoea kituiensis* (Morning glory) 14

Figure 1.5. *Maerua angolensis* (Bead-bean) 15

Figure 1.6. *Senna petersiana* (Wild Senna) 15

Figure 1.7. *Synadenium cupulare* (Bead-man's tree) 16

Figure 1.8. *Tabernaemontana elegans* (Toad tree) 17

Figure 1.9. *Urginea sanguinea* (Slangkop) 18

Figure 5.1. Diagram to illustrate five different extractions of crude leaf meals of *M. angolensis* and *T. elegans* to test *in-vitro* for their effects on the motility of *M. incognita* second-stage juveniles 64

Figure 5.2 a-j. Motility inhibition of *Meloidogyne incognita* J2 by means of five extracts with 10 concentrations of crude *M. angolensis* leaf meal under *in- vitro* conditions. Treatments A = Freeze-dried crude extract, B = Methanol/dichloromethane (1:1) extract, C = Evaporated hexane extract, D = Evaporated dichloromethane extract, E = Freeze-dried water extract, K1 = Pure suspended solution of pluronic gel and deionised water, K2 = 10 % methanol, K3 = Pure salicylic acid 66- 68

Figure 5.3 a-j. Motility inhibition of *Meloidogyne incognita* J2 by means of five extracts with 10 concentrations of crude *T. elegans* leaf meal under *in vitro* conditions. Treatments A = Freeze-dried crude extract, B = Methanol/dichloromethane (1:1) extract, C = Evaporated hexane extract, D = Evaporated dichloromethane extract, E = Freeze-dried water extracts, K1 = Pure suspension solution of pluronic gel and deionised water, K2 = 10% methanol, K3 = Pure salicylic acid 70- 72

Figure 5.4 a-e. LC_{50} graphs of five *Maerua angolensis* leave extracts on *M. incognita* J2 motility inhibition at three different times of exposure *in-vitro*. 74- 75

Figure 5.5 a-e. LC_{50} graphs of five *Tabernaemontana elegans* leave extracts on *M. incognita* J2 motility inhibition at three different times of exposure *in-vitro* 77- 78

Figure 5.6 a-c. Reversible nature of J2 motility inhibition of plant extracts in *Maerua angolensis* under 8-10 mg ml^{-1} bio-assay conditions. Treatments A = Freeze-dried crude extract, B = Methanol/dichloromethane (1:1) extract, C = Evaporated hexane extract, D = Evaporated dichloromethane extract, E = Freeze-dried water extracts, K1 = Pure suspension solution of pluronic gel and deionised water, K2 = 10 % methanol, K3 = Pure salicylic acid 80

Figure 5.7 a-c. Reversible nature of J2 motility inhibition of plant extracts in *Tabernaemontana elegans* under 8-10 mg ml^{-1} bio-assay conditions. Treatments A = freeze-dried aqueous, B = dried methanol/ dichloromethane (1:1), C = Dried hexane D = dried dichloromethane, E = freeze-dried partitioned extracts, K1 = pure mixture of pluronic gel and deionised water, K2 = 10 % methanol, K3 = pure salicylic acid 82

LIST OF TABLES

Table 2.1. Treatment means, least significant differences (LSD), P probabilities and F ratio of stem height, shoot and root mass on tomato during 2006 and 2007 glasshouse conditions 24

Table 2.2. Treatment means, least significant differences (LSD), P probabilities and F ratio of stem height, shoot and root mass of tomato during 2008 and 2009 under glasshouse conditions 25

Table 2.3. Treatment means, least significant differences (LSD), P probabilities and F ratio of stem height, shoot and root mass on tomato during 2011 under glasshouse conditions 26

Table 2.4. Treatment means, least significant differences (LSD), P probabilities and F ratio of root-knot-nematode egg and second-stage juvenile (J2) numbers on tomato during 2006, 2007, 2008 and 2009 under glasshouse conditions 27

Table 2.5. Treatment means, least significant differences (LSD), P probabilities and F ratio of root-knot nematode egg and second-stage juvenile (J2) numbers on tomato at three treatment application rates during 2011 under glasshouse conditions 28

Table 3.1. Treatment means, least significant differences (LSD), P probabilities and F ratio of stem height, shoot mass, fruit number, fruit mass, root mass and root-knot nematode on tomato under microplot conditions 36

Table 3.2. Treatment means, least significant differences (LSD), P probabilities and F ratio of selected leaf tissue nutrient elements on tomato leaves under microplot conditions in the first trial of this study 38

Table 3.3. Main effect of soil-type and treatments, least significant differences (LSD), P probabilities and F ratio of stem height, shoot mass, root mass, fruit number, fruit mass and root knot nematode numbers on tomato 40

Table 3.4. Main effect of soil-type and treatments, least significant differences (LSD), P probabilities and F ratio of selected leaf-tissue nutrient elements on tomato under microplot conditions 42

Table 3.5. Significant soil type x treatment interaction means, least significant differences (LSD), P probabilities and F ratio of shoot mass, root mass, root knot nematode numbers and nutrient elements extracted from leaf tissue on tomato under microplot conditions. 45

Table 4.1. Treatment means, least significant differences (LSD), P probabilities and F ratio of stem height, shoot mass, root mass, fruit number, fruit mass and log_{10}-transformed root-knot nematode egg and J2 numbers on tomato in a field trial at Agricultural Research Council-Institute of Tropical and Sub-tropical Crops during 2008 52

Table 4.2. Treatment means, least significant differences (LSD), P probabilities and F ratio of leaf-tissue nutrient elements on tomato under field conditions in the first trial of this study 54

Table 4.3. Leaf tissue nutrient element analysis of the crudely milled organic amendments used in the field trials 55

INTRODUCTION

1.1. Small-scale-farming communities in South Africa

South Africa is a fair-sized country at the southern end of the continent, with climatic conditions ranging from subtropical, desert to Mediterranean conditions (Labadarios *et al*., 2012). It has ca. 51 million inhabitants of which 48 % live in rural areas (Labadarios *et al*., 2012). A large portion (35 %) of this rural population lives below the poverty line (Labadarios *et al*., 2012). The majority of these communities depend on vegetables and other food produced in household gardens or communal gardens (Fourie and Schoeman, 1999; Van der Berg, 2006; Aliber, 2009; Coyne *et al.*, 2009; Fourie *et al.*, 2012; Ntidi *et al.*, 2012). Available land is often limited and, therefore, frequently re-used, which aggravates soil pest and disease problems, as well as degradation of the agricultural soil (Van der Berg, 2006; Aliber, 2009). All these factors have a direct and negative effect on food security and reinforce the need for sustainable farming (Aliber, 2009; Ntidi *et al.*, 2012). In agricultural production more than 10 % of crops are lost due to pest and diseases (Kleynhans *et al*., 1996; Sikora *et al*., 2005a; Ntidi *et al.*, 2012). However, in rural areas these percentages are much higher and pests or diseases can cause total destruction of a crop (Kleynhans *et al*., 1996; Sikora *et al*., 2005a). Additionally, land can be rendered unsuitable for vegetable production, especially when pests such as plant-parasitic nematodes are present in high numbers in the soil (Coyne *et al.*, 2009; Ntidi *et al.*, 2012).

Crop failure as a result of nematode infestation is frequently reported from resource-poor areas and is a major constraint in household food security in South Africa (Fourie and Schoeman, 1999; Mtshali *et al.*, 2002; Fourie *et al*., 2012; Ntidi *et al.*, 2012). Many cases are reported where vegetable production had to be terminated despite acceptable production and cultivation practices by these producers (Mtshali *et al.*, 2002; Ntidi *et al.*, 2012). Communities and households depend on these crops for food and dietary supplementation and the impact on the people is, therefore, real and comprehensive (Fourie and Schoeman, 1999; Mtshali *et al.*, 2002; Coyne *et al*., 2009; Ntidi *et al.*, 2012). Most crops grown by rural and peri-urban communities are highly susceptible to plant-parasitic nematodes (PPN) and maintain exceptionally high nematode populations (Fourie and Schoeman, 1999; Mtshali *et al.*, 2002; Fourie *et al.*, 2012; Ntidi *et al.*, 2012). The problem is further aggravated by the

lack of knowledge about nematodes and other pests by farmers and extension officers (Chitwood, 2002; Sikora *et al*., 2005a; McSorley, 2011; Ntidi *et al.*, 2012). Effective nematode management and pest control is impossible without raising awareness by the smallholder farmers and increasing the knowledge of extension officers (Fourie and Schoeman, 1999; Mtshali *et al.*, 2002; Fourie *et al.*, 2012; Ntidi *et al.*, 2012).

The use of pesticides is effective but unsuitable in small-scale farming as these chemicals are expensive, highly toxic to animals and humans and could pose a serious threat to the environment (Chitwood, 2002; Sikora *et al*., 2005a; McSorley, 2011). In addition, many of these commercial chemicals will not be available within the near future (McSorley, 2011). To alleviate the nematode-pest problem and secure food production successfully, alternative low-input, cost-effective and environmentally-friendly nematode management strategies need to be developed urgently to provide the disadvantaged rural people with technology to regain and maintain acceptable levels of food production (Stirling 1991; Chitwood, 2002; Sikora *et al*., 2005a; McSorley, 2011).

1.2. Tomato

Tomato (*Solanum lycopersicon* L.) is one of the most common vegetables grown by small-scale farmers in most rural and peri-urban areas in South Africa (Fourie and Schoeman, 1999; Mtshali *et al.*, 2002). Tomatoes are used fresh in salads, cooked as a vegetable and can also be dried (Anon., 2003). Tomatoes are rich in vitamins A and C and the crop is gaining importance because its fruit contains lycopene, a food component known to reduce the incidence of prostate cancer, as well as heart and age-related diseases (Anon., 2003). Some of the major varieties grown in South Africa by small-scale and commercial farmers are FA 1410, Floradade, Heinz, Moneymaker, Primepak, Rhapsody, Rodade, Roma and Star 9030, (Fourie *et al*., 2012). Tomato is fairly adaptable but grows best under warm conditions with optimum average temperatures of 15 °C to 25 °C. High humidity and extreme temperatures reduce fruit set and yields (Waiganjo *et al.,* 2006). Very low temperatures delay colour formation and ripening, while temperatures above 30 °C inhibit fruit set, lycopene development and flavour (Anon., 2003; Waiganjo *et al.,* 2006). Tomato thrives best in low-medium rainfall, with supplementary irrigation during the off-season (Waiganjo *et al.*, 2006). Wet conditions increase incidence of disease and affect fruit ripening (Anon.,

2003; Waiganjo *et al.,* 2006). Tomato grows well in a wide range of soil types but prefer those that are high in organic matter, well-drained and have a pH (H_2O) range of 5 - 7.5 (Anon., 2003; Waiganjo *et al.*, 2006).

The major tomato production constraints include diseases (bacterial wilt, blossom end-rot, early and late blight, leaf curl, tomato spotted wilt virus, leaf spot and powdery mildew), insect and other arthropod pests (spider mites, thrips, white flies and African bollworm), nematodes and poor crop management practices, especially lack of crop rotation and or in-effective crop rotation (Sikora and Fernandez, 2005a; Bridge and Starr, 2007; Ntidi *et al.*, 2012). Tomato grown informally in rural and peri-urban South Africa is irrigated during all growing seasons by means of flood or hand-held irrigation systems (Fourie and Schoeman, 1999; Mtshali *et al.*, 2002; Fourie *et al.*, 2012). Fertilizer sources used by small- scale farmers generally include kraal manure, chicken litter, organic waste and compost (Masarirambi *et al.*, 2012).

1.3. Root-knot nematodes

Tomato hosts a wide variety of PPN, but predominantly the root-knot nematode (RKN) or *Meloidogyne* species (Mtshali *et al.*, 2002; Sikora and Fernandez, 2005; Bridge and Starr, 2007; Ntidi *et al.*, 2012). Severe infestation of host crops by these pests usually causes significant yield loss and may result in total crop failure (Mtshali *et al.*, 2002; Ntidi *et al.*, 2012). *Meloidogyne incognita* (Kofoid and White) Chitwood is the predominant root-knot nematode species that parasitize tomato worldwide, ranking second to *M. javanica* (Treub) Chitwood on this crop in tropical and subtropical regions (Nono-Womdim *et al*., 2002). Both species attack tomato crops wherever they are grown and cause major yield reductions when proper nematode management strategies are not applied (Sikora and Fernandez, 2005; Bridge and Starr, 2007; McSorley, 2011). Estimated yield losses of between 20 % and 40 % (Bridge and Starr, 2007) and in excess of 50 % (Nono-Womdim *et al*., 2002) have been reported in tomato because of infestation by *Meloidogyne* species. A nematode survey in rural and peri-urban home, community and school gardens, as well as small fields in South Africa showed that RKN are the predominant biotic constraint in vegetable production, including tomato, where 48 of 51 sites sampled in rural and peri-urban areas in South Africa showed the presence of RKN (Mtshali *et al*., 2002).

Many producers purchase commercial seed for planting at some stage, although they regularly use second- and even third-generation seed in areas of the home or communal gardens dedicated to tomato production, where nematodes thrive (Fourie *et al.,* 2012). Most of the commercial varieties used by these producers are susceptible to RKN (Fourie *et al.,* 2012). Seedlings are often infected before they are even planted in the field. However, depending on biotic, abiotic or management factors the impact of root-knot nematode infection on tomato globally is highly variable (Nono-Womdim *et al*., 2002; Bridge and Starr, 2007). Should constraints such as PPN be managed efficiently and consistently, though, tomato production might be increased, ensuring greater food supply in these affected areas and communities. Other nematode-susceptible crops produced by them might also benefit and this will increase daily minimum nutrient intake for the poor and might provide for additional income through retail or even wholesale marketing.

1.4. Root-knot nematode management strategies

Resource-poor rural and peri-urban tomato growers do not have the means to purchase expensive nematicides or fertilizers. This makes the need to find alternative, affordable but effective methods to control nematodes in these crops critical. These communities often are constrained in what are transportable and by what distance, which further highlights the problem of finding suitable and accessible nematode control measures. In addition to this there has recently been substantial pressure on the crop production sector to use methods of pest control that do not pollute or degrade the environment (Duncan, 1991; Stirling, 1991; Akhtar and Malik, 2000; Chitwood, 2002; McSorley, 2011). A number of strategic reviews have been published that concentrate on nematode management in vegetable production (Johnson and Fassuliotis, 1984; Noling and Becker, 1994; Johnson, 1998; Sikora, 2002; Sikora *et al*., 2005a; McSorley, 2011) and could be referred to for additional information.

1.4.1. Fallowing

Bare fallowing is an effective means of managing RKN, especially when it can be applied during hot, dry periods between crops when alternative weed hosts are seldom a problem (Johnson and Fassuliotis, 1984; Sikora *et. al*., 2005b). In such areas where the climate is

characterized by prolonged and severely hot, dry seasons, fallowing during the dry season, tillage to dry out the soil, followed by growing non-host crops during the wet season will result in a significant reduction in *Meloidogyne* populations (Sikora *et. al*., 2005b). Bare fallowing needs to be economical and acceptable to the grower; therefore, it is most effective when other control techniques are used simultaneously (Kinloch and Dunavin, 1993). Soil-water conservation should not be a critical factor when considering applying fallowing. Another problem with this approach is the wide host ranges of *M. incognita* and *M. javanica* (Coyne *et al.,* 2009), which makes options for unsuitable host crops to these RKN species very limited.

1.4.2. Soil solarization and heating

The lethal temperature for plant-parasitic nematodes is considered to be around 45 °C (McSorley and McGovern, 2000; Wang *et al*., 2006). Increasing soil temperatures either by dry or by steam heating has been used for many years in protected cultivation to manage RKN (McSorley and McGovern, 2000; Wang *et al*., 2006). The high cost of heating soil has limited its use almost to the intensive cropping level only (McSorley and McGovern, 2000; Wang *et al*., 2006). Soil solarization with plastic mulches, which leads to the development of lethal temperature levels in the soil, is used for the control of RKN and soil-borne diseases (Katan, 1981; McSorley and McGovern, 2000; Wang *et al*., 2006). Solarization applied during summer before the next tomato crop in plastic greenhouses led to a 99 % reduction in *M. javanica* and *M. incognita* densities when compared with the controls (Eddaoudi and Ammati, 1995; Reddy *et al*., 2001). Soil solarization combined with application of the chemicals dazomet or calcium cyanamide also gave good control of RKN and increased tomato yield (Fiume and Parasi, 1995). Solarization for two to four weeks, combined with application of the commercial pesticides cadusafos or fenamiphos, was further considered a sustainable alternative for methyl bromide fumigation in greenhouse tomato (Ioannou *et al*., 2002). The scale of implementation again is limited by the accessibility, cost and simplicity of application.

1.4.3. Plant- parasitic nematode control by synthetic agents

Nematicides (fumigants and non-fumigants) have been used extensively since the late 1900s (Ferraz and Brown, 2002) as the major nematode control strategy for high-value or bulk

crops such as flowers, vegetables (Sikora and Fernandez, 2005), tobacco (Johnson *et al*., 2005) legumes (Sikora *et. al*., 2005b) or cereals (Mc Donald and Nicol, 2005). However, these chemicals are costly and pose environmental hazards such as contamination of underground water (Zureen and Khan, 1984; Alam and Jairaijpuri, 1990). Toxicity to beneficial fauna and flora in the soil, the development of nematicide resistance in parasitic nematodes and environmental degradation often result from their continuous or injudicious use (Akthar, 1991). Since the number of available nematicides is progressively declining because of the increase in production costs as well as impact on the environmental and non-target organisms (Ferraz and Brown, 2002), environmentally-friendly and effective nematode control methods are becoming increasingly important and popular, in particular in subsistence farming systems (Bridge, 1996). Nematicides will, however, consistently play a major role in the reduction of nematode populations in a variety of crops, as well as for use in regulatory and quarantine procedures (Johnson, 1985; Sikora and Fernandez, 2005).

1.4.4. Bio-fumigation

This term normally refers to the suppression of soil-borne pests and pathogens through the release of biocidal compounds, principally isothiocyanates in the soil (Kirkegaard *et al*., 1998). Soils amended with fresh or dried cruciferous residues at 38 °C day and 27 °C night temperatures reduced *M. incognita* galling by 95 to 100 % after seven days of incubation, with a simultaneous reduction in *Sclerotium rolfsii* Saccardo and *Pythium ultimum* Pringsheim under controlled-environment tests (Stapleton *et al*., 1998). However, many cruciferous plants are good hosts to *Meloidogyne* species. The term bio-fumigation is used more freely whenever volatile substances are produced through microbial degradation of organic amendments that result in significant toxic activity towards a soil-borne pest or disease (Bello, 1998). Control due to any form of bio-fumigation is probably the result of multifaceted mechanisms, including: (i) non-host or trap cropping depending on the host status of the plant used; (ii) lethal temperature due to solarization; (iii) nematicidal action of toxic by-products produced during the degradation of organic matter and (iv) stimulation of antagonists in the soil after bio-fumigation (Sikora and Fernandez, 2005).

1.4.5. Organic amendments

Organic amendments offer an alternative or supplementary control tactic to chemical or cultural control of nematode parasites on agricultural crops (Stirling, 1991; Akhtar and Mahmood, 1993). Considerable progress has been made in the utilization of organic materials as soil amendments for the control of plant-parasitic nematodes (Muller and Gooch, 1982; Rodríguez-Kábana, 1986; Trivedi and Barker, 1986; Stirling, 1991; Akhtar, 1993; Akhtar and Alam, 1993; Akhtar and Mahmood, 1993; Akhtar, 1997; Akhtar and Malik, 2000; Litterick *et al*., 2004; Oka, 2010). Nematoxic compounds have been isolated from a great number of plant species (Ferraz and de Freitas, 2004). Neem (*Azadirachta indica* A. Juss) has been widely studied for its nematoxic properties and has been used as plant extracts, oil cakes or whole plant materials in a large number of studies, particularly in India (Stirling, 1991; Akhtar and Malik, 2000; Ferraz and de Freitas, 2004; Oka, 2010). Studies with neem oil cake conducted between 1971 and 1981 gave positive results in terms of nematode control (Muller and Gooch, 1982). Neem extracts also enhanced the performance of other organic amendments when used in combination (Oka *et al*., 2007).

Amendments prepared from a number of other plants, including castor bean (*Ricinus communis* L.) and velvetbean (*Mucuna pruriens* (L.) DC.) may have some potential against PPN (Stirling, 1991; Ritzinger and McSorley, 1998; Zasada *et al*., 2006; Oka, 2010). Zasada *et al*. (2006) found that *M. incognita* eggs were less sensitive to crude aqueous extracts (1:15 dry mass per volume of water) of velvet bean than in J2 stage. They also found that for RKN management, extracts from the aboveground parts of the plants were much more toxic than those derived from the roots. Meyer *et al*. (2006) tested *Plantago lanceolata* L. and *P. rugelii* Decne extracts against *M. incognita* and they found that all were toxic to eggs and J2, with *P. lanceolata* shoot extract tending to have the highest level of activity against this nematode species. At lower concentrations, J2 were found to be more sensitive when exposed to the extracts than eggs, while at higher concentrations (75 % and 100 %) the extracts were equally toxic to both life stages. The suppression of PPN by marigold (*Tagetes* spp.) and *Crotalaria* species including sunn hemp (*Crotalaria juncea* L.) have also been much studied (Wang *et al*., 2001; 2002; Hooks *et al*., 2010). Tannins and phenolic compounds released from some plant residues are also toxic to RKN (Miami and Rodríguez-Kábana, 1982; Kokalis- Burelle *et al*., 1994). Several more botanically-derived organic amendments

to soil have been studied and still are being investigated (Stirling, 1991; Chitwood, 2002, McSorley, 2011).

Organic matter decomposes and when liberally watered releases many compounds such as phenols, polythienyls, glucosinolates, cyanogenic glycosides, alkaloids, terpenoids, steroids, triterpenoids, aldehydes and several gases, including ammonia (Chitwood, 2002). Kirkegaard *et al*. (1993) indicated that an advantage of using plant extracts for PPN suppression is that various compounds have synergistic effects with the same end-result. Plant extracts and phytochemicals in general have a potential for PPN control. Many of them are selective, possibly biodegradable, some are non-toxic to humans but they could be applied in a similar way than commercial nematicides (Zasada *et al*., 2006).

Plant residues and organic amendments, however, also may release nitrogen compounds, organic acids and other substances that may have direct adverse effects on PPN and or increase plant-growth potential (Stirling, 1991; Oka, 2010; Thoden *et al*., 2011). Ammonia (NH_3) is a common and much-studied by-product of the decomposition of organic materials (Rodríguez-Kábana, 1986; Rodríguez-Kábana *et al*., 1987). Concentrations of NH_3 released from compost in pot experiments were determined to be well above the lethal limit needed for *M. javanica* suppression (Oka and Yermiyahu, 2002). When examining a range of 15 different amendments, Miami and Rodríguez-Kábana (1982) found that galling by *M. arenaria* decreased as the N % in the amendments increased. Plant materials with C:N ratios in the range of 15 - 20 were considered most effective (Miami and Rodríguez-Kábana, 1982). Oil cakes tested had low C:N ratios (C:N = 7.0-7.1) and reduced RKN galling but were also phytotoxic (Miami and Rodríguez-Kábana, 1982). A sewage sludge (very low C:N = 5.8) applied to soil in pots decomposed quickly and released maximum levels of ammoniacal N within seven days after application (Castagnone-Sereno and Kermarrec, 1991). Efficacy against PPN increases as N % in amendments increases and as C:N ratio decreases (Rodríguez-Kábana *et al*., 1987). Nematicidal activity usually does not occur with amendments with a C:N ratio of more than 20, possibly due to slow decomposition and inadequate concentrations of released NH_3 and other toxins. Materials with low C:N (ca. < 10) can, however, cause phytotoxicity (Rodríguez-Kábana *et al*., 1987). Rodríguez-Kábana and co-workers pioneered work with mixes of different kinds of amendments to add

additional C sources and ameliorate the phytotoxic effects of rapid NH_3 release from materials with very low C:N ratios (Rodríguez-Kábana and King, 1980; Miami and Rodríguez-Kábana, 1982; Rodríguez-Kábana *et al*., 1987). Urea seems to be a more reliable source of NH_3 than various types of amendments. It was more consistent than several plant materials in reducing RKN numbers and was effective at lower rates (Chavarria-Carvajal and Rodríguez-Kábana, 1998). Urea and NH_3 were effective against PPN and RKN at rates as low as 300-400 mg kg^{-1} soil (0.03 - 0.04 %) (Rodríguez-Kábana and King, 1980; Rodríguez-Kábana *et al*., 1981; 1986; 1989). Research also showed that NH_3 is much more toxic to PPN and RKN than the ammonium ion, NH_4^+ (Oka and Yermiyahu, 2002) but NH_3 is ionized to NH_4^+ under acidic soil conditions (Rodríguez-Kábana *et al*., 1989; Oka *et al*., 2007). Increasing soil pH can shift the equilibrium in favour of NH_3 and thus improve nematicidal activity (Oka, 2010). This may explain the level of PPN suppression achieved with materials that greatly increased soil pH by Zasada *et al.* (2006).

Organic amendments also improve soil structure and water-holding capacity, reduce diseases and limit weed growth, which ultimately leads to stronger plants and improve their tolerance to nematode attack by PPN (Fortunum *et al.*, 1991; Stirling, 1991; Sikora, 1992; McSorley, 2011). Addition of organic matter to the soil produces an ecological succession of micro-organisms and successive phases of biochemical degradation. It also controls the orderly arrangement of natural enemies of PPN (Stirling, 1991; Yadav and Alam, 1993; Riegel *et al*., 1996; Akhtar and Malik, 2000; Chavarria-Carvajal *et al*., 2001; Oka, 2010).

Literature on the suppression of PPN by organic amendments presents both promising and inconsistent results (Mashela, 2002; Mashela *et al*., 2011; McSorley, 2011). Major limiting factors in the use of organic amendments of soils for suppression of PPN or crop growth enhancement include the large quantities (10-500 t ha^{-1}) that are sometimes required for these materials to be effective, long waiting periods for results to be evident, a reduction in soil pH (Stirling, 1991) and inconsistency in effectiveness mentioned above. Mashela and Mphosi (2002) developed an alternative organic amendment technology, using selective plant organs. *Meloidogyne incognita* suppression was consistently attained using small quantities of organic amendment. Since, soil amendment with wild cucumber (*Cucumis myriocarpus* E. Mey. Ex Naud), castor bean (*Ricinus communis*) fruit and fever tea (*Lippia*

javanica (Burm.f.) Spreng) leaves consistently suppressed *M. incognita* numbers and improved tomato yield (Mashela, 2002; Mashela and Mpati, 2002; Mashela and Mphosi, 2002; Mashela and Nthangeni, 2002; Mphosi *et al*., 2002; Ngobeni *et al*., 2002; Pofu *et al*., 2010; Mashela *et al*., 2011). Additional studies found that dried meal of such products seems to be more effective than fresh material (Rodríguez-Kábana *et al*., 1981; 1986; 1989). Dried products also have a much longer shelf life and can be kept for a long period (Rodríguez-Kábana *et al*., 1981; 1986; 1989). These authors' consistency in positive results, the small quantities they used and the fact that no waiting period is apparently required before crops could be utilized after application of such products all are factors that make investigation into similar botanical material appropriate.

1.5. Non-crop plant species with herbal or medicinal properties

Nine different plant species belonging to eight families were selected in this study for assessment of nematoxic and growth-promoting properties on tomato. The plant remedies are locally known as "muti" as they are regarded to have certain medicinal properties. Traditional healers in the areas concerned frequently use these mutis to treat human and domestic animals for various ailments. Living specimens of these plant species, as well as supplies of dried and finely ground material made from them are found in abundance in the rural communities of the lowveld in Mpumalanga, Limpopo and Kwa-ZuluNatal provinces of South Africa. Should these plant materials prove to be useful as bionematicides on tomato it would be a significant contribution towards sustainable crop production by ensuring better yields and increased income for large parts of the local rural, resource-poor communities. The selected plant species and their known properties are hence discussed.

1.5.1. *Cassia abbreviata* Oliv. (Sjambok pod)

This tree belongs to the family *Leguminosae* (IPNI, 2012) usually grow in open woodland or in wooded grassland and are also common on termitaria in the arid lowveld of South Africa (Venter and Venter, 1996). *Cassia abbreviata* is used in traditional medicine (Venter and Venter, 1996). Among the Shangana it is believed that when venison is cooked with the bark of *C. abbreviata* success in future hunting is ensured (Venter and Venter, 1996). The Zulus use the leaves and stalks against body vermin (Venter and Venter, 1996). The seed is purgative, powdered bark is used for treating abscesses and powdered root is taken for the

relief of backache. An infusion made from the bark and roots is used for the relief of abdominal pains, constipation and as a remedy for toothache. Relief from headache is obtained by inhaling the smoke of burnt branches (Venter and Venter, 1996).

Figure 1.1. *Cassia abbreviata* (Sjambok pod) (Photo by M.C. Khosa).

1.5.2. ***Cissus cactiformis* Gilg. (Cucumber cactus)**

This plant species belongs to the family *Vitaceae* (IPNI, 2012) and is indigenous to the tropical east of South Africa and Swaziland (Van Wyk *et al*., 1997; Wink and Van Wyk, 2008). The plant materials are used as a drench to treat horse sickness (Marloth, 1932). The Mapulana tribe in Mpmumalanga Province, South Africa uses juice from the bulbous root of *C. cactiformis* diluted in water as a gargle, an internal remedy and an application to glandular swellings or creeping sores (Marloth, 1932). Leafs are used to treat ulcerations and wounds. The roots serve as remedy for myalgia and the juice for earache. In central Africa a decoction is taken orally for blennorrhagia and to calm palpitations. The stem is used as a local antiphlogistic application for muscular pains and taken orally as an anthelmintic (Sim, 1907; Marloth, 1932). The plant is regarded as narcotic to fish (Sim, 1907). The roots and leaves of *C. cactiformis* are said to contain the tannin Procyanidin C1 (Steyn, 1949). Fresh leaves contain 7.19 % oxalate, calculated as oxalic acid on a dry mass basis (Burtt Davy, 1904; Marloth, 1932; Steyn, 1949; Wink and Van Wyk, 2008). The fruit is said to be irritant and is certainly highly astringent (Sim, 1907; Marloth, 1932). In spite of this, these fruits are eaten by children who seem to become habituated to the astringency. Although the ripe fruits of a number of *Cissus* species are edible, unripe fruit has been suspected of being poisonous (Steyn, 1949). Extracts of the plant species have a favourable

effect on gastrointestinal evacuation in humans and is recommended in cases of digestion problems, dyspepsia and gastritis (Burtt Davy, 1904; Marloth, 1932; Steyn, 1949).

This plant species was found to contain a steroid that can be separated into two fractions (Burtt Davy, 1904; Marloth, 1932; Steyn, 1949). A water-soluble glycoside has been obtained from it, which on oral administration had no toxic effect in mice, rats or guinea pigs at a dosage rate of 2 mg kg^{-1} body mass for 10 days. Upon intravenous administration, however, the animals showed convulsions and died within five minutes (Burtt Davy, 1904; Marloth, 1932). Extracts from the plant species containing resins and sterols acted on isolated intestines and the uteri of rabbits and albino rats in a manner comparable to that of acetylcholine (Marloth, 1932). The oral LD_{50} was 15.5 mg kg^{-1} in guinea pigs (Marloth, 1932). Toxicity of the leaves and immature fruits has been ascribed to the presence of acid-oxalate content (Burtt Davy, 1904; Marloth, 1932).

Figure 1.2. *Cissus cactiformis* (Cucumber cactus) (Photo by M.C. Khosa).

1.5.3. *Euphorbia ingens* E.Mey. (L.C. Wheeler, 'Naboom' or Candelabra tree)

The tree prefers warm, dry areas (Van Wyk *et al*., 1997; Wink and Van Wyk, 2008) and belongs to the family *Euphorbiaceae* (IPNI, 2012). It usually grows on rocky outcrops or in deep sand among lowveld vegetation (Van Wyk *et al*., 1997; Wink and Van Wyk, 2008). The species is distributed throughout the Gauteng, KwaZulu-Natal, Limpopo and North West provinces of South Africa and also throughout Mozambique, Swaziland and Zimbabwe,

further into tropical Africa (Van Wyk *et al*., 1997; Wink and Van Wyk, 2008). *Euphorbia* species contain irritant and toxic latex and several of them have been implicated in both human and livestock poisoning cases (Steyn, 1949; Vahrmeijer, 1981; Evans and Taylor, 1983; Van Wyk *et al*., 1997; Wink and Van Wyk, 2008). *Euphorbia ingens* is extremely toxic and its latex can cause severe injuries to the face, eyes, tongue and mouths of humans or animals that come into contact with it (Steyn, 1949; Van Wyk *et al*., 1997; Wink and Van Wyk, 2008). From a medical perspective the latex can be used as a purgative, for treatment of ulcers or as a cure for cancer (Van Wyk *et al*., 1997). Branches are cut and put in streams or pools to poison fish for easier catching in South Africa and Zimbabwe (Van Wyk *et al*., 1997). The principle irritant compounds are diterpenoids and various esters of ingenol (Evans and Taylor, 1983).

Figure 1.3. *Euphorbia ingens* ('naboom' or candelabra tree) (Photo by M.C. Khosa).

1.5.4. *Ipomoea kituiensis* Vatke (Morning glory)

This species belongs to the family *Convolvulaceae* (IPNI, 2012). The leaves and roots of *I. kituiensis* are used to make a decoction that is used as a lotion for eczema and abscesses. Boiled roots from these decoctions are applied as dressings, purgatives or emerics (Van Wyk *et al*., 1997). Cords or charms made of the runners of *I. kituiensis*as are believed to protect foetuses against abortion, to relieve uterine pain and to calm foetal movements. The cord is worn around the lower abdomen (Van Wyk *et al*., 1997). *Ipomoea kituiensis* is not particularly poisonous but their seeds contain toxic alkaloids and cases of fatal poisoning

have been recorded (Van Wyk *et al*., 1997). Several indole alkaloids had been isolated from *Ipomoea* species (Van Wyk *et al*., 1997).

Figure 1.4. *Ipomoea kituiensis* (Morning glory) (Photo by M.C. Khosa).

1.5.5. *Maerua angolensis* DC. (Bead-bean)

This plant is indigenous to the tropical east of South Africa and Swaziland (Van Wyk *et al*., 1997; Wink and Van Wyk, 2008) and belongs to the family *Capparaceae* (IPNI, 2012). It is used in traditional medicine to treat psychosis, ecthyma, epilepsy, diarrhoea, dysentery, jaundice, hepatitis, insomnia, dyspepsia, neurasthenia, liver diseases and is also used as a sedative (Adjanohoun *et. al*., 1989). In other cases it is useful for treating vomiting, skin rash, nasal infection, stomach ulcers, boils, pimples, miscarriages, bad spirits and also to prevent abortion (Adjanohoun *et al*., 1989; Chhabra *et al*., 1989). Nkunya (1985) has isolated several fatty acids and esters from the plant species and most of these compounds showed antifungal activity. Aqueous methanolic extracts of *M. angolensis* contain substances with anti-inflammatory properties (Adamu *et al*., 2007).

Figure 1.5. *Maerua angolensis* (Bead-bean) (Photo by M.C. Khosa).

1.5.6. *Senna petersiana* (Bolle) Lock. (Wild Senna)

This species belongs to the family *Leguminosae* (IPNI, 2012). Commercial laxative medicine is produced from roots, dried leaves and pods of senna, which originates from North Africa and the Middle East (Van Wyk and Wink, 2004). These trees grow in the northern and eastern parts of South Africa (Van Wyk *et al*., 1997). In the Venda region, a root decoction of *S. petersiana* is a traditional treatment of epilepsy (Arnold and Gulumian, 1984). Ethanolic extracts of the whole plant have anti-inflammatory, antipyretic, weak analgesic activity and inhibit prostaglandin release (Jain *et al*., 1997). *Senna petersiana* has antimicrobial, as well as some antiviral activity (Tsikalange *et al*., 2005) and was claimed to inhibit HIV enzymes (Tsikalange *et al*., 2008).

Figure 1.6. *Senna petersiana* (Wild Senna) (Photo by M.C. Khosa).

1.5.7. *Synadenium cupulare* (Boiss.) Wheeler ex A.C. White, R.A. Dyer and B. Sloane (Bead-man's tree)

This plant is indigenous to the tropical east of South Africa and Swaziland and belongs to the family *Euphorbiaceae* (IPNI, 2012). It is most frequently encountered along riverbanks, in coastal forests and savannah woodlands (Van Wyk *et al*., 1997; Wink and Van Wyk, 2008). The milky latex of *S. cupulare* is a notorius irritant. It may cause severe burning and itching of the skin, eyelids, nostrils and lips that often last for several hours. In more serious cases the latex may cause permanent blindness by completely destroying the eye (Verdcourt and Trump, 1969; Spoerke *et al*., 1985; Wink and Van Wyk, 2008). The principle skin irritant is 12-O-tigloyl-4-deoxyphorbol-13-isobutyrate (Kinghorn, 1980) and the plant has various uses in traditional medicine (Verdcourt and Trump, 1969; Wink and Van Wyk, 2008). Domestic animals, adult people and especially children may be at risk when *Synadenium* plants are cultivated in or near homes as a result of its skin irritation effect (Spoerke *et al*., 1985). *Synadenium cupulare* contains several tigliane-type diterpene esters of the 4-deoxyphorbol type (Kinghorn, 1980; Bagavathi *et al*., 1988).

Figure 1.7. *Synadenium cupulare* (Bead-man's tree) (Photo by M.C. Khosa).

1.5.8. *Tabernaemontana elegans* Stapf. (Toad tree)

The species belongs to the family *Apocynaceae* and is indigenous to tropical east Africa through to South Africa and Swaziland. In the latter two countries it is most commonly encountered along riverbanks, in coastal forests and savannah woodlands (Van Wyk *et al*., 1997; Wink and Van Wyk, 2008). The coagulated latex is rubber-like but of inferior quality

and is used as a styptic (Van Wyk *et al*., 1997). The seeds are baked, ground to a powder and mixed with tobacco for chewing or smoking (Van der Heijden *et al*., 1986). Root infusions are drunk as an aphrodisiac, as well as a remedy for lung ailments and stomach ache. In addition, a maceration of the roots is taken twice daily to treat tuberculosis (Van Wyk *et al*., 1997; Wink and Van Wyk, 2008). Some venereal diseases are treated with a potpourri of plant material including roots of *T. elegans*. The inner layer of the fruit wall (endocarp) is dried, pulverised and boiled in water, then filtered and taken orally to treat cancer (Van Wyk *et al*., 1997; Wink and Van Wyk, 2008). Apart from the yellow pulp being eaten on its own, the Zulu people add it to milk to speed up the curdling process (Van der Heijden *et al*., 1986; Van Wyk *et al*., 1997; Wink and Van Wyk, 2008). Some of the plant organs or extracts as administered by traditional healers in their preparation have, however, been recorded to be toxic (Van Wyk *et al*., 1997). The toxic compounds in *T. elegans* are terpenoid indole alkaloids (Van der Heijden *et al*., 1986; Van Wyk *et al*., 1997). Symptoms of toxic indole alkaloids from *T. elegans* include supraventricular tachycardia, cardiac fibrillation, hypotension, cerebral spasms, coma and cardiac and respiratory arrest (Wink and Van Wyk, 2008).

Figure 1.8. *Tabernaemontana elegans* (Toad tree) (Photo by M.C. Khosa).

1.5.9. *Urginea sanguinea* Shinz. (Slangkop)

This species, belonging to the family *Hyacinthaceae* (IPNI, 2012) is a very important traditional medicine in South Africa and is commonly used as expectorant, emetic, diuretic, heart tonic, to treat asthma and for wound healing (Van Wyk *et al*., 1997; IPNI, 2012).

Accidental deaths and stock losses have been caused when people used traditional medicine prepared from the bulb of *Urginea* species (McVann *et al*., 1992). Cardiac glycosides are responsible for both human and animal fatalities and are considered the toxic compound (McVann *et al.,* 1992). *Urginea sanguinea* bulb causes human and mammal gastrointestinal systems to malfunction, followed by nausea and vomiting. As a result, cardiac glycosides are sometimes overlooked in post mortems of persons that died from use of traditional medicine (McVann *et al*., 1992). In 41 fatal cases in South Africa over a one-year period, 44 % showed clear signs of cardiac glycosides during autopsy (McVann *et al*., 1992). Livestock poisoning is also well documented, especially for *U. sanguinea* (McVann *et al*., 1992).

Figure 1.9. *Urginea sanguinea* (Slangkop) (Photo by M.C. Khosa).

1.6. Aims of this study

More research is needed to explore the potential of botanically-derived materials as soil amendments as part of integrated nematode control strategies and to demonstrate possible application by rural, poor communities. The main objective of this study was to investigate potential nematoxic and growth-promoting activity on tomato of dried and crudely-milled leaves and bulbs of the selected non-crop plant species *C. abbreviata*, *C. cactiformis*, *E. ingens*, *I. kituiensis, M. angolensis, S. petersiana*, *S. cupulare*, *T. elegans* and *U. sanguinea*. These nine plant remedies were selected based on their common use in traditional healing in parts of South Africa and because they contain chemicals such as oxalic acid, terpenoids, alkaloids, fatty acids, diterpenes, esters, ingenol and diterpenoids that have been tested

previously, as indicated above. The general effects of these remedies on humans at prescribed dosage rates raised the suspicion that they might be toxic to small organisms such as PPN. After screening and further selection under greenhouse, microplot and field conditions, the extracts with significant nematoxic and/or growth-stimulating potential will be tested at a more advanced level of chemical separation to verify initial *in vivo* effects and explore further potential of the materials *in vitro* in bio-assays.

CHAPTER 2

THE EFFECT OF MILLED MATERIAL OF SELECTED NON-CROP PLANT SPECIES ON THE GROWTH OF TOMATO AND ON POPULATION DENSITIES OF *MELOIDOGYNE INCOGNITA* IN THE GLASSHOUSE

2.1. Introduction

Fresh or dried, crudely milled, ground or infused plant material such as oil cake of various oil- or protein-seed crops, coffee (*Coffea* spp. L.) husks, neem (*Azadirachta indica* A. Juss.), marigold (*Tagetes* spp. L.), castor bean (*Ricinus communis* L.) and wild cucumber, a.k.a. paddy melon (*Cucumis myriocarpus* Naudin), have been used to control root-knot nematodes (RKN) (Singh and Sitaramaiah, 1966, 1967; Sikora *et al*., 1973; Muller and Gooch, 1982; Stirling, 1991; Sikora, 1992; Mashela, 2002). Success in terms of nematode control by agents such as these may be due to (i) toxic compounds present in the material such as neem (Akhtar, 1998); (ii) non-toxic compounds such as residual sugar in bagasse (Sikora and Fernandez, 2005); (iii) toxic metabolites produced during microbial degradation after application to the soil (Sikora and Fernandez, 2005) and or (iv) enhancement of microbial nematode antagonists (Sikora and Fernandez, 2005).

The initial part of this study focused on crude powders of different organs of *Cassia abbreviata*, *Cissus cactiformis*, *Euphorbia ingens*, *Ipomoea kituiensis, Maerua angolensis, Senna petersiana*, *Synadenium cupulare*, *Tabernaemontana elegans* and *Urginea sanguinea*. The objective of this part of the study was to apply dried, crudely-milled leaf meals of selected wild-plant species as soil amendments in pots in glasshouses to evaluate and compare their effects on the growth of tomato and on the suppression of a population of the RKN species, *Meloidogyne incognita* race 2.

2.2. Material and methods

Glasshouse trials were conducted during 2006, 2007, 2008, 2009 and 2011, respectively, in a fully functional glasshouse at the Agricultural Research Council-Institute of Tropical and Subtropical Crops (ARC-ITSC) in Nelspruit, South Africa (approx. 25°27’06.18” S, 30°58’05.21” E).

2.2.1. Collection and preparation of different plant leaf meals for use as soil amendments

Leaf, bulb and stem parts of *C. abbreviata, C. cactiformis, E. ingens, I. kituiensis, M. angolensis, S. cupulare, S. petersiana, T. elegans* and *U. sanguinea* were collected from selected traditional healers from the Mopani and Vhembe districts in the Limpopo Province. The materials for this study were selected from those healers who could provide sufficient amounts of freshly collected, air-dried leaf tissue of the respective plant species. The healers all store and display these materials they have in stock in amply ventilated and well-kept stores of similar traditional design. All these enterprises are located within reasonable reach of fresh plant material and sufficient stock is collected for annual demand in the area that is serviced by a particular healer. *Cucumis myriocarpus* material used as a standard soil amendment (Mashela, 2002) in this study was obtained from the Nematology Laboratory of the University of Limpopo, Sovenga, South Africa.

After all selected plant species had been collected and had arrived at the nematology laboratory at ARC-ITSC in Nelspruit, the respective plant materials were chopped into pieces the next day and oven-dried for seven days at 52 °C prior to grinding in a Wiley mill through a 1-mm sieve (Makkar, 1999). The crudely-milled plant materials were stored in bulk in appropriately marked, air-tight glass containers in a laboratory at the ARC-ITSC as sources of the respective plant species for soil amendments while stocks lasted. The containers were kept away from direct sunlight and in an area where temperature fluctuations were minimal. When certain materials were depleted due to initial undersupply, fresh stock was collected from the same healer it was originally acquired from. In cases where certain materials were not available, they were replaced by other suitable plant species' materials from other healers for the duration of the study.

At the onset of each trial, each appropriate plant meal was spread thinly by hand in prepared and fixed aliquots around the base of tomato seedlings on the soil surface of every pot of each respective treatment. Each treatment was done separately on the same day that RKN inoculation was done, and hands, gloves and other appropriate utensils were thoroughly cleaned before commencing with the next treatment. After the application of

the respective plant materials, the different meals were lightly worked into the soil of each pot.

2.2.2. Acquirement, multiplication, extraction and inoculation of root-knot nematodes

RKN, confirmed as *M. incognita* race 2, acquired from the ARC-Grain Crops Institute, Potchefstroom, South Africa were multiplied over at least two months in a separate glasshouse on the susceptible tomato (var. Rodade). For the inoculation of each trial, sufficient numbers of nematode eggs and second-stage juveniles (J2) were extracted from these tomato roots by shaking in 3.5 % NaOCl and sieving (Hussey and Barker, 1973). Two-week-old tomato seedlings (var. Rodade) were transplanted into 4-l plastic pots filled with a 1:3 mixture of sterile, commercial sand and compost. After the transplanting of seedlings, each appropriate tomato plant was inoculated with ca. 3 000 nematode eggs and J2 suspended in tap water by injecting aliquots with a 1-ml plastic syringe in 10-mm-deep holes at the base of the seedlings. After nematode inoculation, the holes were filled with soil from the same pot and appropriate plant meal applied.

2.2.3. Treatments, trial layouts and glasshouse conditions

The soil used in all the glasshouse trials was the same and contained 84 % sand, 14 % silt, 2 % clay and had a pH(H_2O) 5.75. The trials conducted during 2006 and 2007 consisted of seven treatments, *viz*. 5 g dried, crude meal of each of *C. abbreviata, C. cactiformis, E. ingens, I. kituiensis, S. cupulare, S. petersiana* and *U. sanguinea* per pot and an untreated control. The trials were arranged in a randomized-complete block design (RCBD), with each treatment and control replicated eight times. The glasshouse trials conducted during 2008 and 2009 consisted of seven treatments each, *viz*. 5 g dried, coarse-meal material of each of *C. cactiformis, E. ingens, M. angolensis, S. cupulare*, *T. elegans,* fenamiphos at 5 g (Nemacur 15 G®) (South Africa, 2007) and untreated control. Each treatment was replicated six times.

An additional glasshouse trial was conducted during 2011. The treatment included the soil-amendment reference *C. myriocarpus* applied at a standard rate of 5 g dried, milled leaf meal per plant and a standard synthetic, commercial nematicide fenamiphos (Nemacur 15 GR®) at rate of 5 g per plant, as well as an untreated control. The trial has a RCBD, with three application rates (5, 10 and 15 g) of five treatments (milled plant-organ material of *C.*

cactiformis, E. ingens, M. angolensis, S. cupulare and *T. elegans*) and three controls (*Cucumis myriocarpus* and fenamiphos at rate of 5 g per pot and untreated pots). The 18 treatments were randomly assigned within each of the six block replicates.

Irrigation was applied in all trials by pouring ca. 300 ml tap water every second day into the tray of each pot from a watering-can. Plants in all trials were sprayed with mercaptothion (Malasol®/Malathion®) and tetradifon (Redspidercide®) alternatively every two weeks as preventative control of aphid and red spider mite, respectively.

2.2.4. Growth of tomato and nematode assessments

All the glasshouse trials were terminated 65 days after tomato transplanting, nematode inoculation and treatment application. Stem height and fresh shoot mass were recorded per plant. Stems were cut off at the soil surface and the arial plant growth was discarded. The roots were removed from the soil in each pot by carefully overturning the pots and shaking each root system free of adhering soil and also recorded. Each system was then separately immersed in a bucket of clean water to wash them free of remaining soil particles. Root samples were collected to test for nematode J2 and egg densities by cutting each root system into 1-cm pieces, mixing each plant's chopped roots separately and thoroughly and shaking a sub-sample of 50 g of each replicate for four minutes in 300 ml of a 3.5 % NaOCl solution (Hussey and Barker, 1973; Hussey and Boerma, 1981). The suspension was poured directly onto a set of nested sieves with apertures from top to bottom of 150, 63, 53, 38 and 25 µm, respectively. Nematode eggs and J2 contained on the 38- and 25-µm-aperture sieves were washed with a spout into a plastic bottle filled up to the 100-ml mark each, which were stored in a cold room at ca. 11 °C until the samples were counted under a compound microscope.

2.2.5. Data analysis

The data from each trial were subjected to appropriate analysis of variance (ANOVA) using SAS/STAT statistical software (SAS, 1999). The standardized residuals of each variable and transformed nematode data were tested for deviations from normality using Shapiro-Wilk's test. Fisher's protected t-LSD (least significant difference) was calculated at a 5 % level of

significance to compare means of significant effects (Snedecor and Cochran, 1980). Nematode numbers were $log_{10}(x+1)$ transformed before ANOVA was performed.

2.3. Results

2.3.1. Tomato plant development

With regard to stem height the *C. cactiformis, E. ingens* and *S. cupulare* treatments had significantly longer stems and were superior to those of the controls in 2006 and 2007, while *I. kituiensis, S. petersiana* and *U. sanguinea* showed possible phytotoxicity of the plants treated with these respective soil amendments in 2006 (Table 2.1). Shoot mass in the 2006 and 2007 glasshouse trials followed the same trend than stem height, in the sense that the *C. cactiformis, E. ingens* and *S. cupulare* treatments performed significantly better than the control and other treatments (Table 2.1).

Table 2.1. Treatment means, least significant differences (LSD), P probabilities and F ratios of stem height, shoot and root mass on tomato during 2006 and 2007 glasshouse conditions.

Treatment	Stem height (cm)		Shoot mass (g)		Root mass (g)	
	2006	2007	2006	2007	2006	2007
Control	29.0 c[1]	24.5 c	3.4 d	4.1 c	2.2 a	1.8 d
C. abbreviata	25.8 cd	23.9 c	3.7 d	3.7 c	2.8 a	3.0 b
C. cactiformis	45.6 a	44.9 a	9.2 a	8.6 a	2.9 a	2.8 bc
E. ingens	44.8 a	41.1 b	7.2 b	7.7 a	2.0 a	1.8 d
I. kituiensis	24.1 d	25.5 c	3.3 d	3.7 c	3.2 a	4.6 a
S. cupulare	40.4 b	44.1 ab	5.3 c	6.4 b	2.4 a	2.1 cd
S. petersiana	24.5 d	25.5 c	4.0 d	3.8 c	2.6 a	1.8 d
U. sanguinea	23.9 d	26.8 c	4.0 d	3.9 c	2.5 a	2.5 bcd
$LSD_{p=0.05}$	3.3870	3.1450	0.9470	1.0230	n.s[2]	0.8410
P	<0.0010	<0.0010	<0.0010	<0.0010	0.2790	<0.0010
F	65.4000	73.5300	40.1000	31.8100	1.2800	10.6100

[1]Means within the same column followed by the same letter or letters do not differ significantly at a 5 % level of significance. [2]n.s· = non-significant.

No crude soil amendment treatment differed significantly from one another with regard to root mass (g) or from the control during 2006 (Table 2.1). However, in the 2007 glasshouse trial, the *C. abbreviate*, *C. cactiformis* and *I. kituiensis* treatments significantly improved tomato root mass compared to the control (Table 2.1).

With regard to stem height all treatments were superior to the control in 2008, while in the 2009 trial all treatments except *M. angolensis* had significantly longer stems than the control (Table 2.2). *C. cactiformis* and *T. elegans* performed similar to the standard nematicide treatment, fenamiphos in terms of the stem height of tomato in 2008, while the latter two plant amendments and *E. ingens* showed stem heights similar to fenamiphos in 2009. Similar trends for stem height were observed for shoot and root mass (Table 2.2), particularly *C. cactiformis* and *T. elegans* which consistently yielded better than the control for both parameter in both trials.

Table 2.2. Treatment means, least significant differences (LSD), P probabilities and F ratio of stem height, shoot and root mass on tomato during 2008 and 2009 glasshouse conditions.

Treatment	Stem height (cm)		Shoot mass (g)		Root mass (g)	
	2008	2009	2008	2009	2008	2009
Control	27.3 c[1]	20.5 c	7.9 c	6.0 c	21.6 c	14.6 b
C. cactiformis	39.9 a	28.2 ab	9.9 ab	10.0 a	30.2 a	20.6 a
E. ingens	31.6 b	28.4 ab	8.6 bc	8.1 abc	23.3 bc	19.0 a
M. angolensis	33.2 b	21.3 c	8.7 bc	7.1 bc	25.3 b	14.9 b
S. cupulare	31.1 b	27.9 b	7.6 c	9.2 ab	22.1 bc	20.2 a
T. elegans	40.0a	27.1 b	10.0 a	9.8 a	30.8 a	19.5 a
Fenamiphos	38.6 a	31.7 a	10.0 a	10.4 a	30.7 a	22.3 a
$LSD_{p=0.05}$	3.7400	3.7100	1.5760	2.5700	3.4400	3.4000
P	<0.0010	<0.0010	<0.0010	0.0080	<0.0010	<0.0010
F	14.5200	9.7400	12.0500	6.0200	4.7200	3.4100

[1]Means within the same column followed by the same letter or letters do not differ significantly at a 5 % level of significance.

In 2008 and 2009, *C. cactiformis*, and *T. elegans* did not differ significantly from the nematicide treatment in terms of shoot and root mass (Table 2.2). In 2008 *C. cactiformis* and *T. elegans* and in 2009 all the crude soil-amendment treatments except *M. angolensis* had a root-mass improvement above the control, similar to that of the standard nematicide. In 2009 all plant amendments as well as the nematicide treatment were associated with significantly higher root masses of tomato plants compared to the untreated control. In the 2011 trial all the treatments performed better than the controls in terms of stem height (Table 2.3) and no distinct application rate responses were apparent.

Table 2.3. Treatment means, least significant differences (LSD), P probabilities and F ratio of stem height, shoot and root mass on tomato during 2011 under glasshouse conditions.

Treatment	Treatment application rate (g)	Stem height (cm)	Shoot mass (g)	Root mass (g)
Control	0	65.1 i[1]	61.5 g	11.2 g
C. cactiformis	5	94.4 a	83.7 b	14.1 f
	10	80.5 gh	73.7 def	18.8 bc
	15	92.4 abcd	78.2 bcde	22.0 a
E. ingens	5	87.8 abcdef	80.5 bcd	15.2 f
	10	91.3 abcde	72.0 ef	14.5 f
	15	87.1 cdefg	72.5 ef	16.0 def
M. angolensis	5	88.6 abcdef	81.5 bcd	17.6 cde
	10	87.5 bcdef	83.2 bc	15.3 f
	15	85.0 efgh	91.9 a	18.2 bcd
S. cupulare	5	87.6 abcdef	70.2 f	19.2 bc
	10	80.3 h	72.3 ef	17.9 bcde
	15	85.4 efgh	75.5 cdef	17.9 bcde
T. elegans	5	92.9 abc	79.2 bcde	15.1 f
	10	85.9 defgh	75.3 cdef	15.9 ef
	15	88.0 abcdef	79.7 bcde	18.6 bc
C. myriocarpus	5	83.1 fgh	68.1 fg	20.0 ab
Fenamiphos	5	94.1 ab	72.3 ef	15.9 ef
$LSD_{p=0.05}$	-	6.8100	7.9400	2.2500
P	-	<0.0010	<0.0010	<0.0010
F	-	7.7200	5.9300	9.9700

[1]Means within the same column followed by the same letter or letters do not differ significantly at a 5 % level of significance.

All the treatments performed better than the controls in terms of stem height. At lower dosages *C. cactiformis, M. angolensis, S.cupulare* and *T. elegans* performed better as compared to higher dosages. *C. cactiformis, M. angolensis* and *T. elegans* showed differences between application rates. Similar to its root mass, fenamiphos performed poorly relative to some other treatments in terms of shoot mass. This is contrary to *M. angolensis* where all three rates in this treatment were relatively superior (Table 2.3). No distinct application responses were apparent in terms of the shoot mass of the different application rates of the respective soil amendment treatments. All the treatments performed better than the controls in terms of root mass. *Cissus cactiformis, M. angolensis* and *T. elegans* showed differences between application rates. At some or all rates all the

crude soil-amendment treatments, except *E. ingens* had higher root masses than the standard nematicide, fenamiphos. *C. cactiformis* at a rate of 15 g per plant performed similar to the standard soil amendment *C. myriocarpus*, which was far superior to all other treatments and rates in terms of root mass in this trial (Table 3.2). There was no signifant interaction amongst the treatment effect.

2.3.2. Nematode population development

In the 2006 and 2007 glasshouse trials (Table 2.4) all the crude plant-meal soil amendments significantly reduced the numbers of *M. incognita* eggs and J2 50 g roots relative to the control. The soil amendments significantly suppressed nematode numbers below that of the control. In the 2008 and 2009 glasshouse trials (Table 2.4) all the soil–amendment treatments significantly reduced the numbers of *M. incognita* eggs and J2 relative to the control. The nematoxic activity of all soil amendments was significant and compared favourably to the standard nematicide, fenamiphos.

Table 2.4. Treatment means, least significant differences (LSD), P probabilities and F ratio of root-knot-nematode egg and second-stage juvenile (J2) numbers on tomato during 2006, 2007, 2008 and 2009 under glasshouse conditions.

	Eggs and J2 numbers/ 50 g roots			
Treatment	**2006**	**2007**	**2008**	**2009**
Control	4.1 (12 225) a[1]	4.10[2] (13 238) a	3.7 (5 388)a	3.7 (5 188) a
C. abbreviata	3.6 (3 925) b	3.3 (2 050) d	-	-
C. cactiformis	3.4 (2 500) cd	3.2 (1 700) d	2.8 (662) c	3.0 (1 000) b
E. ingens	3.4 (2 500) cd	3.5 (3 562) b	2.9 (862) bc	2.9 (788) cd
I. kituiensis	3.4 (2 575) cd	3.5 (3 000) bc	-	-
M. angolensis	-	-	2.8 (650) c	2.8 (688) cd
S. cupulare	3.3 (2 125) d	3.4 (2 538) cd	2.8 (762) bc	2.7 (562) d
S. petersiana	3.5 (3 125) bc	3.3 (2 088) d	-	-
T. elegans	-	-	2.8 (712) c	2.8 (650) bcd
U. sanguinea	3.4 (2 775) cd	3.5 (3 600) b	-	-
Fenamiphos	-	-	2.3 (1 050) b	2.9 (875) bc
$LSD_{p=0.05}$	0.1950	0.1645	0.1757	0.1738
P	<0.0010	<0.0010	<0.0010	<0.0010
F	12.9700	24.2300	26.4100	31.7300

[1]Means within the same column followed by the same letter or letters do not differ significantly at a 5 % level of significance.

[2]Log_{10} [(eggs+J2)+1] (untransformed means in parentheses)

In the 2011 glasshouse trial, all treatments at all rates except *M. angolensis* at 10 g significantly reduced the numbers of *M. incognita* eggs and J2 compared to the control (Table 2.5). *Maerua angolensis* at application rates of 5 g and 10 g on average had significantly more eggs and J2 than *C. cactiformis* at 15 g, *E. ingens* at all three rates, *S. cupulare* at 15 g and *T. elegans* at 15 g in this trial and did not differ significantly from the control treatment. None of the crude plant amendment treatments and rates differed significantly from the crude amendment control *C. myriocarpus*, except for *M. angolensis* at 5 and 10 g. However, all the plant amendment treatments and rates resulted in significantly higher eggs and J2 numbers/ 50 g tomato roots compared to the fenamiphos treatment.

Table 2.5. Treatment means, least significant differences (LSD), P probabilities and F ratio of root-knot nematode egg and second-stage juvenile (J2) numbers on tomato during 2011 under glasshouse conditions

Treatment	**Application rate (g)**	**Eggs and J2 numbers/ 50 g roots**
Control	0	4.42[2] (30 063) a[1]
C. cactiformis	5	3.20 (1 688) bcd
	10	3.20 (1 950) bcd
	15	2.82 (2 975) cd
E. ingens	5	3.10 (1 838) cd
	10	3.04 (1 350) cd
	15	2.63 (1 113) d
M. angolensis	5	3.77 (6 688) b
	10	3.79 (6 625) ab
	15	3.37 (3 763) bc
S. cupulare	5	3.25 (2 588) bcd
	10	3.20 (1 838) bcd
	15	3.01 (1 300) cd
T. elegans	5	3.44 (3 588) bc
	10	3.32 (2 275) bc
	15	3.09 (1 375) cd
C. myriocarpus	5	2.86 (2 550) cd
Fenamiphos	5	1.17 (138) e
$LSD_{p=0.05}$	-	0.6426
P	-	<0.0010
F	-	7.7900

[1]Means within the same column followed by the same letter or letters do not differ significantly at a 5 % level of significance.

[2]Log_{10}[(eggs+J2)+1] (untransformed means in parentheses).

2.4. Discussion

Nine crude plant-meal soil amendments were tested in at least two trials each during this part of the study. When a crude-plant soil amendment is to be selected for further testing, it is important that it does not inhibit crop growth and therefore yield. Even when such amendments reduce nematode numbers, a producer still needs a yield increase. Four of the materials initially tested (2006 and 2007) had no enhancement effect on plant growth and were therefore, not tested further in the following trials. Instead, two different plant species were included in these trials.

The most pertinent observation with regard to tomato stem-height reaction to crude plant-meal soil-amendment treatments in this part of the study is the lack of any correspondence between the latter parameter and root mass reaction to treatments. This is despite the fact that tomato stem height was generally substantially improved by the amendments, except by the amendment control, *C. myriocarpus*. Inconsistencies in plant stem height reaction to different application rates of the amendments highlight the crudeness of these materials and their need for refinement to improve sustainability in their performance. Although all the soil amendments tested at all rates, except *C. myriocarpus* at the standard 5-g rate improved tomato shoot mass together with the standard nematicide treatment, this growth parameter turned out to be the most inconsistent of the three crop reactions measured.

Tomato root mass reaction to all the crude plant amendments was either stimulatory or neutral but never suppressive in any of the five glasshouse trials. Some treatments enhanced tomato growth more than others did, especially in terms of root and shoot mass. Previous studies have shown that organic amendments have differences in efficacy of nematode control (Akhtar and Malik, 2000; Chitwood, 2002). The effects on root development and growth of substances of these amendments different from those discussed in Chapter 1 are not excluded, based on significant and sometimes repeated enhanced-growth reaction reflected in these results. Again, the crudeness of the materials showed some levels of inconsistency in tomato root growth reaction. A tendency, however, could be observed when all three plant growth parameters are considered together, in that some treatments resulted in somewhat lanky plants, whereas the correspondent root and shoot masses were on the low side. Where all three parameters were on the high side for

treatments such as *M. angolensis* (15-g application rate, 2011 trial) strong, healthy plants could be imagined. This also demonstrates the value of the use of various parameters in these kinds of investigative studies.

Crude plant-meal amendments of all plant species used in all five glasshouse trials showed potential as nematicides for *Meloidogyne incognita* on tomato because from these results of the respective glasshouse trials it is evident the treatments tested can reduce nematode populations significantly. Mashela (2002) showed that ground fruit of wild cucumber suppressed the numbers of RKN under glasshouse conditions. This material increased the productivity of tomato and improved soil electrical conductivity without affecting soil pH. These and our results are also confirmed by similar results with botanical soil amendments (Akhtar, 2000; Akhtar and Malik, 2000; Sikora and Fernandez, 2005).

Nematicidal action of all crude plant amendments in relation to the untreated control was consistent in all five trials. These glasshouse trials on the effect on tomato plant development, growth and the nematicidal potential of the selected crude-plant soil amendments have provided sufficient evidence of their suitability for further testing under less-controlled conditions. Although only *I. kituinsis, S. petersiana* and *U. sanguinea* materials showed signs of possible phytotoxicity or allelopathy (Kadioglu *et al*., 2005), other unknown, detrimental effects on treated crops, humans or animals could have been masked by the controlled nature of this study. It is, therefore, essential to proceed to follow-up experimentation with these materials under microplot and field conditions.

CHAPTER 3

EVALUATION OF SELECTED CRUDE PLANT MEALS AND DIFFERENT SOIL TYPES ON *MELOIDOGYNE INCOGNITA* AND LEAF-TISSUE NUTRIENT ELEMENTS IN TOMATO UNDER MICROPLOT CONDITIONS

3.1. Introduction

Microplots provide a basis for more realistic ambient conditions for plants to grow and develop than the highly controlled environments in which plants are grown in glasshouses (Mc Donald, 1998). Therefore, while it might still be possible to control certain variables in a specific microplot study, it could be expected that under these semi-controlled conditions crop reaction to specific treatments could be closer to most common conditions occurring in subsistence or small-scale crop production (Johnson, *et al*., 1981; Caswell, *et al*., 1985). Bhattacharya and Goswani (1988) claimed that organic plant extracts contain N, P and K and their use along with synthetic nematicides might be an economical and effective way of nematode population management, with an additional benefit of plant nutrition. In conventional organic amendments improved crop productivity has been ascribed to reduced PPN numbers, as well as essential nutrient elements that were released (Stirling, 1991).

Crude leaf meals of five plant species that were screened as soil amendments in five glasshouse trials (Chapter 2) showed potential as nematicides for *Meloidogyne incognita* on tomato. The amendments selected for this study reduced PPN populations significantly and consistently in the abovementioned glasshouse study. The reference soil-amendment product, wild cucumber used in the abovementioned glasshouse study was also tested in microplots by Mashela (2002). The objective of this study, therefore, was to assess the efficacy of dried leaf-meal soil amendments of *Cissus cactiformis*, *Euphorbia ingens*, *Maerua angolensis*, *Synadenium cupulare* and *Tabernaemontana elegans* on *M. incognita* race 2, leaf-tissue nutrient element contents of tomato and the growth and productivity of tomato under microplot conditions. The extent to which three different soil types might affect variables measured in this study, or their interaction was also investigated.

3.2. Material and Methods

Microplot trials were conducted simultaneously during 2008 in three soil types (sand, clay and loam) under microplot conditions at the ARC-ITSC in the Mpumalanga Province, South Africa (approx. 25°27’32.31” S; 30°58’17.77” E).

3.2.1. Collection and preparation of plant-leaf material as soil amendments

The procedures and material used in this part of the study were the same than those described in Chapter 2. The non-crop plant species used for preparing dried, milled leaf material for soil amendments were *C. cactiformis*, *E. ingens*, *M. angolensis*, *S. cupulare* and *T. elegans*. Crudely milled *C. myriocarpus* material was obtained from the Nematology Laboratory of the University of Limpopo, Sovenga, as described in Chapter 2.

3.2.2. Acquirement, multiplication, extraction and inoculation of root-knot nematodes

The procedures and material used in this part of the study were the same than those described in Chapter 2.

3.2.3. Treatments, trial layout and microplot conditions

The microplots in this study consisted of 0.35-m-long, 75-cm-diameter PVC pipes buried vertically in rows, with 0.30 m space between plots and 1.8 m between rows. The aboveground end of each plot protruded 5 cm above the surrounding soil level to prevent contamination in case of excess rain. The soil used to fill the plots in the one trial was a steam-pasteurised, loamy soil (80 % sand, 11 % silt, 9 % clay) with a pH(H_2O) 6.15. The microplot complex was fitted with a drip-irrigation system, with a dripper placed in the middle of each plot. Four two-week-old tomato seedlings (var. Rodade) were transplanted into the microplots at the onset of each trial, as described in Chapter 2. The plants were spaced 15 cm from each other, 30 cm from the centre of each plot. Plants in all trials were sprayed with mercaptothion (Malasol®/Malathion®) and tetradifon (Redspidercide®) alternatively every two weeks as preventative control of aphid and red spider mite, respectively.

The first trial was arranged in a randomized-complete block design (RCBD). The treatment combinations were randomly assigned to plots in each of the six block replicates. The

treatments included the soil-amendment reference *C. myriocarpus* applied at a standard rate of 5 g dried, milled leaf meal per plant and a standard synthetic, commercial nematicide fenamiphos (Nemacur 15 GR®) at rate of 5 g per plant, as well as an untreated control. Three application rates (5, 10 and 15 g) of five soil-amendment treatments (*C. cactiformis, E. ingens, M. angolensis, S. cupulare* and *T. elegans*) were used. Measurements and assessments on the four tomato plants per plot were pooled in each replicate to reduce variation further.

In the second microplot trial the same 75-cm microplots as described above were filled with three different soil types. The sandy soil contained 88 % sand, 3 % silt, 9 % clay and its pH (H_2O) 8.58; the clay soil contained 40 % sand, 13 % silt and 47 % clay with a pH (H_2O) 7.15 and the sandy loam soil contained 80 % sand, 11 % silt, 9 % clay and pH (H_2O) 6.00. This microplot trial consisted of seven treatments, *viz*. 5 g dried, crude-powder leaf material of each of *C. cactiformis, E. ingens, M. angolensis S. cupulare* and *T. elegans,* as well as 5 g fenamiphos per plot and an untreated control each. This trial was arranged in a RCBD, with each treatment and control replicated six times.

3.2.4. Growth of tomato and nematode assessments

Both trials were terminated 120 days after tomato transplanting, at peak fruit maturity. The procedures and materials used in this part of the study were similar to those described in Chapter 2. The mean fruit number, fruit mass, shoot mass, root mass, number of eggs and J2/ 50 g root were calculated from the respective measurements of four plants per pot. Stem height was recorded from the taller of two plants per pot.

3.2.5. Nutrient-element analyses from tomato leaf tissue

The nutrient-element analyses were done in the ARC-ITSC Soil Laboratory. To determine B, Ca, Cu, Fe, K, Mg, Mn, P and Zn content, tomato leaf samples were oven-dried at 60 ˚C for 48 h and then passed through a Wiley mill with a 1-mm-apperture sieve. A 0.500-g tomato leaf sample from each microplot (replicate) was weighed, put in calibrated test tubes and digested for six hours at 180 ˚C using a 6-ml solution of 55 % nitric acid and 70 % perchloric acid in a v:v mixture of 2:1. The samples were cooled overnight and each was filled to the 25-ml mark with de-ionized water. Calcium, Cu, Fe, K, Mg, Mn, and Zn were determined

using a Varian 250 Plus atomic absorption spectrophotometer. Boron and P were determined using a colorimetric AAIII Auto Analyzer (Williams, 1984). For N analysis leaf samples were oven-dried at 60 ˚C for 48 hours passed through a Wiley mill with a 1-mm-apperture sieve. A 0.250-g tomato leaf sample from each microplot (replicate) was weighed, put in calibrated test tubes and digested for six h at 300 ˚C using a 5-ml, 4:1 solution of 98 % sulphuric acid and 30 % hydrogen peroxide. Samples were cooled overnight and each was filled to the 100-ml mark with water. Nitrogen was determined using a colorimetric AAIII Auto Analyzer (Williams, 1984).

3.2.6. Data analysis

The procedures used in this part of the study were the same as those described in Chapter 2. The data from the first and second trial were subjected to ANOVA using SAS/STAT statistical software (SAS, 1999). The standardized residuals of each variable and transformed data were tested for deviations from normality using Shapiro-Wilk's test. Fisher's protected t-LSD (least significant difference) was calculated at a 5 % level of significance to compare means of significant effects (Snedecor and Cochran, 1980). Nematode numbers were $\log_{10}(x+1)$ transformed before ANOVA was performed.

3.3. Results

Euphorbia ingens and *S. cupulare* soil amendments, both at the smallest and medium rates had significantly shorter stems than the untreated control and standard *C. myriocarpus* soil amendment (Table 3.1). No significant response in application rate in terms of longer stems of any of the crude plant-leaf meals was observed, however. There was not much correspondence in fruit mass and shoot mass response of the tomato plants to the respective treatments, except for the highest *C. cactiformis* rate that resulted in a sign of reduction in shoot mass compared to the untreated control and *C. myriocarpus* as well as *E. ingens* (5 g), *M. angolensis* (10 and 15 g), *S. cupulare* (15 g) and *T. elegans* (15 g). Only *C. cactiformis* at its highest rate had significantly less shoot mass than the untreated control (Table 3.1). Shoot mass response to different application rates was either insignificant or inconsistent (*cf*. *E. ingens*). *C. myriocarpus*-treated tomato in this trial did not differ from the untreated control but outperformed some of the other treatments in terms of shoot mass, including the standard nematicide, fenamiphos. Tomato shoot mass response to most

treatments did not correspond with shoot height response of the same treatment or application rate. No significant differences were observed between the untreated control and the treatments in terms of root mass (Table 3.1). Relatively, the higher dosages of *C. cactiformis, E. ingens* and *S. cupulare*, as well as the standard soil amendment *C. myriocarpus* had superior root mass but fenamiphos-treated root masses were the lowest. Generally the higher application rates (15 g) of the plant-meal soil amendments tested in this study had higher root masses than the 5-g application rates but increases did not correspond with application rates.

The treatments in this trial under microplot conditions had no effect on numbers of tomato fruits (Table 3.1). Variation in fruit mass was high and neither treatment (including the standard nematicide) nor different application rates differed significantly from the untreated control in this regard. *Cissus cactiformis*, particularly at its highest rate and fenamiphos had the lowest fruit mass of all treatments and the untreated control. Some rates of the *E. ingens*, *M. angolensis, S. cupulare* and *T. elegans* treatments had higher fruit mass and compared well to the soil-amendment control, *C. myriocarpus*.

$Log_{10}(x+1)$ numbers of nematode eggs and J2 (Table 3.1) showed that only the *C. cactiformis* and *T. elegans* treatments at all application rates reduced the *M*. *inocgnita* population in this trial significantly below those of the untreated control. The higher rates of *M. angolensis* also differed significantly from the control. These abovementioned plant-soil-amendment treatments also compared significantly with the standard synthetic nematicide, fenamiphos in terms of nematode number reduction, with the exception of one rate of *T. elegans* (Table 3.1). All the plant materials tested in this study compared favourably with the standard plant-meal soil amendment *C. myriocarpus*, which also reduced nematode numbers significantly below those in the untreated control. The highest rate of crudely milled *S. cupulare* as well as the 5 and 10 g application rates of *E. ingens* leaf material, however, had significantly higher numbers than the standard amendment.

Table 3.1. Treatment means, least significant differences (LSD), P probabilities and F ratio of stem height, shoot mass, fruit number, fruit mass, root mass and root-knot nematode on tomato under microplot conditions.

Treatment	Application rate (g)	Stem height (cm)	Shoot mass (g)	Fruit number	Fruit mass (g)	Root mass (g)	Eggs and J2 numbers/ 50 g roots
Control	0	49.0 ab[1]	425.4 abcd	46.7 a	2254 abcde	48.4 abcde	5.8[3] (608 667) a
C. cactiformis	5	41.6 bcdef	242.8 de	31.3 a	1676 de	41.5 de	2.9 (30 310) ef
	10	45.2 abcdef	374.8 abcde	38.3 a	1933 cde	53.3 abcde	2.2 (14 133) f
	15	46.8 abcde	214.9 e	42.0 a	1322 e	65.9 ab	4.0 (35 610) bcde
E. ingens	5	37.7 f	561.3 a	35.7 a	2569 abcde	53.4 abcde	5.2 (204 868) abc
	10	40.3 def	353.7 bcde	46.8 a	3441 a	68.5 a	5.1 (211 783) abc
	15	39.7 ef	561.3 a	44.3 a	3171 abc	67.1 ab	5.0 (176 200) abcd
M. angolensis	5	50.4 a	305.3 cde	36.5 a	1536 e	46.2 bcde	4.2 (23 033) abcde
	10	49.0 ab	444.6 abc	42.7 a	2027 bcde	59.7 abcd	3.5 (14 650) def
	15	48.4 abc	493.8 abc	53.2 a	2850 abcd	56.0 abcde	3.1 (37 135) f
S. cupulare	5	37.7 f	347.0 bcde	40.8 a	2127 bcde	43.7 cde	4.1 (161 133) bcde
	10	41.2 cdef	336.5 bcde	36.0 a	2313 abcde	60.7 abcd	5.0 (105 526) abcd
	15	41.8 bcdef	450.5 abc	50.0 a	3483 a	65.9 ab	5.3 (296 517) ab
T. elegans	5	42.7 bcdef	336.9 bcde	48.3 a	2578 abcde	52.1 abcde	3.3 (14 428) ef
	10	46.5 abcde	380.7 abcde	40.5 a	2582 abcde	58.8 abcd	4.0 (34 617) bcde
	15	47.5 abcd	532.0 ab	59.5 a	3257 ab	56.6 abcde	3.1 (6 767) ef
C. myriocarpus	5	52.3 a	572.9 a	56.0 a	3076 abc	64.7 abc	3.7 (23 100) def
Fenamiphos	5	44.9 abcdef	239.9 de	34.6 a	1347 e	36.7 e	2.2 (2 209) f
$LSD_{p=0.05}$	-	7.5390	199.5000	n.s[2]	1263.5000	21.0400	1.6160
P	-	0.0020	0.0080	0.2510	0.0040	0.0440	<0.0010
F	-	2.7300	2.2700	1.2500	2.4400	1.8000	5.0300

[1]Means within the same column followed by the same letter or letters do not differ significantly at a 5 % level of significance.

[2]n.s. = not significant.

[3]Log_{10}[(eggs+J2)+1] (untransformed means in parentheses).

With regard to macro-elements the 5 g and 10 g rates of *E. ingens*, the middle-rate application rate of *S. cupulare* had significantly lower N, P and K levels than the untreated control (Table 3.2). Phosphorus and K levels of *C. myriocarpus*-treated tomato were also significantly lower than the control. Calcium levels in the different treatments, although significantly different, did not show any noticeable tendencies. Iron and B were the only among the micro-elements that showed tendencies among the treatments (Table 3.2). *Euphorbia ingens* 5 g and 10 g rate treated tomato had significantly higher Fe levels than the untreated control plants, as had the highest rate of *C. myriocarpus*. All treatments had significantly higher B levels except for fernamiphos and the control, with one application rate of *M. angolensis* (5 g) two rates of *T. elegans* (10 and 15 g) topping the table. As in the other corresponding trials no tendencies in dosage response were noticeable in any of the plant-meal soil amendment treatments.

Table 3.2. Treatment means, least significant differences (LSD), P probabilities and F ratio of selected leaf tissue nutrient elements on tomato leaves under microplot conditions in the first trial of this study.

Treatment	**Application rate (g)**	**B mg/kg**	**Ca mg/kg**	**Cu mg/kg**	**Fe mg/kg**	**K %**	**Mg mg/kg**	**Mn mg/kg**	**N %**	**P %**	**Zn mg/kg**
Control	0	28.3 e[1]	5.6 a	56.2 a	1 001 cd	0.6 bc	0.8 a	1 647 fghi	2.6 ab	0.8 ab	38.3 abcd
C. cactiformis	5	31.9 cde	5.4 ab	51.3 a	1 080 c	0.7 b	0.8 a	1 820 cdefg	2.6 abc	0.8 ab	36.8 abcd
	10	34.1 bcde	4.5 abcdef	51.1 a	903 cd	0.7 b	0.7 a	1 394 hi	2.1 def	0.7 abc	33.8 cd
	15	29.0 e	4.2 cdef	64.7 a	1 808 ab	0.3 def	0.7 a	2 124 abcd	2.0 ef	0.5 cd	41.5 abc
E. ingens	5	34.7 bcde	4.4 bcdef	61.7 a	1 587 b	0.5 bcdef	0.8 a	1 931 bcdef	2.0 ef	0.5 d	33.2 cd
	10	28.6 e	4.1 cdef	57.8 a	1 661 ab	0.4 cdef	0.8 a	2 046 abcde	1.8 f	0.5 d	41.8 abc
	15	43.7 a	5.1 abcd	65.2 a	1 074 c	0.8 b	0.7 a	1 808 defg	2.5 abcd	0.8 ab	36.0 bcd
M. angolensis	5	41.5 ab	5.4 ab	46.3 a	981 cd	0.7 bc	0.9 a	1 645 fghi	2.6 abc	0.9 ab	40.0 abc
	10	29.4 de	5.2 abc	48.70 a	785 d	0.8 b	0.8 a	1 371 i	2.7 ab	0.8 ab	29.8 d
	15	30.2 de	5.0 abcde	50.8 a	930 cd	0.7 b	0.8 a	1 537 ghi	2.5 abc	0.8 ab	31.2 d
S. cupulare	5	35.3 bcde	5.6 a	53.8 a	1 010 cd	0.6 bcde	0.9 a	1 740 efgh	2.7 a	0.9 a	33.5 cd
	10	35.7 abcde	3.5 f	61.8 a	1 772 ab	0.3 def	0.7 a	2 309 a	2.0 ef	0.4 d	37.1 abcd
	15	31.5 cde	5.1 abcd	62.0 a	994 cd	0.8 b	0.8 a	1 652 fghi	2.6 abc	0.8 ab	41.8 abc
T. elegans	5	30.9 cde	4.6 abcdef	49.2 a	1 027 cd	1.1 a	0.7 a	1 433 hi	2.2 cdef	0.6 bcd	44.8 a
	10	38.7 abc	4.8 abcde	53.0 a	1 128 c	0.6 bcd	0.8 a	1 856 bcdefg	2.3 bcde	0.8 ab	40.0 abc
	15	37.7 abcd	4.0 def	66.8 a	1 857 a	0.6 f	0.8 a	2 183 abc	2.1 def	0.5 d	43.8 ab
C. myriocarpus	-	31.1 cde	4.0 ef	66.0 a	1 764 ab	0.3 ef	0.8 a	2 195 ab	2.0 ef	0.4 d	36.3 abcd
Fenamiphos	-	19.4 f	4.4 bcdef	57.8 a	970 cd	0.7 bc	0.8 a	1 801 defg	2.5 abcd	0.9 a	35.7 bcd
$LSD_{p=0.05}$	-	8.3900	1.1000	n.s[2]	263.9000	0.2700	n.s	364.0000	0.4400	0.2400	8.6700
P	-	<0.0010	0.0030	0.0700	<0.0010	<0.0010	0.5130	<0.0010	<0.0010	<0.0010	0.0250
F	-	3.5200	2.4900	1.6500	16.0300	4.4800	0.9600	4.8300	3.6400	4.5000	56.9300

[1]Means within the same column followed by the same letter or letters do not differ significantly at a 5 % level of significance.

[2]n.s. = not significant.

Tomato grown in sand, clay or loam in this trial showed no significant differences between soil types with regard to stem height, number of fruits per plant and fruit mass (Table 3.3). However for shoot mass, root mass and nematode numbers (log_{10}-transformed) significant differences were observed between the different soil types.

The treatment effects were more distinct with regard to the plant variables measured than soil type effects (Table 3.3) as significant differences were observed for all the variables measured. For most variables the fenamiphos and *M. angolensis* treatments were significantly different than for the other treatments except for eggs and J3 numbers. The values were lower than the untreated control probably showing some phytotoxicity. *T. elegans* of the plant-meal soil amendments had the lowest nematodes at tomato harvesting.

Table 3.3. Main effect of soil-type and treatments, least significant differences (LSD), P probabilities and F ratio of stem height, shoot mass, root mass, fruit number, fruit mass and root knot nematode numbers on tomato.

	Stem height (cm)	Shoot mass (g)	Root mass (g)	Fruit number	Fruit mass (g)	Eggs and J2 numbers/ 50 g roots
Soil type						
Sand	43.9	211.3 b[1]	32.0 a	45.3	1 857	2.7 (8 123) a[2]
Clay	42.9	271.8 a	26.2 b	45.9	1 764	2.7 (8 835) a
Loam	44.4	317.5 a	30.4 ab	48.7	1 765	1.8 (1 776) b
$LSD_{p=0.05}$	3..11	48.809	4.2577	8.8998	337.1900	0.7107
P	0.6289	0.0002	0.0236	0.7245	0.7942	0.0149
F	0.4700	9.8000	3.9500	0.3200	0.2300	4.4600
Treatment						
Control	49.5 a	279.43 b	35.1 a	50.0 a	1 944 a	2.1 (4 850) b
C. cactiformis	46.8 a	364.9 a	34.5 a	48.3 a	2 143 a	3.4 (8 742) a
E. ingens	47.3 a	300.4 ab	30.9 ab	50.4 a	1 981 a	3.1 (18 613) ab
M. angolensis	36.7 c	175.4 c	24.8 bc	30.6 b	1 110 b	2.6 (5 112) ab
S. cupulare	50.2 a	281.1 b	31.5 a	58.3 a	2 451 a	2.9 (3 306) ab
T. elegans	41.5 b	293.1 ab	31.0 ab	59.1 a	1 966 a	0.6 (293) c
Fenamiphos	35.2 c	184.5 c	22.0 c	30.4 b	1 019 b	2.2 (4 019) b
$LSD_{p=0.05}$	4.7745	74.9180	6.5352	13.7020	520.0500	1.0909
P	<0.0001	<0.0001	0.0013	<0.0001	<0.0001	<0.0001
F	13.6500	6.6800	4.1200	6.4400	9.1400	0.1909

[1]Means within the same column followed by the same letter or letters do not differ significantly at the 5 % level of significance.

[2]Log_{10}[(eggs+J2)+1] (untransformed means in parentheses).

The main effect of soil type at harvesting time was significant for all the elements extracted from tomato leaf tissue (Table 3.4). Tomato grown in the sandy and clay soils had similar levels of most nutrient elements in their leaves, except for B and Zn where loam and clay soils seemed to react similary and Ca and Fe, which were different for each soil type. The tomato in the loamy soil had either the highest or lowest level of elements in their leaves (Table 3.4), with the exception of B in this trial. The crude leaf-meal treatment of *E. ingens* was associated with either a stimulatory or suppressive effect relative to the untreated control on nutrient-element levels in the leaves of tomato in this trial, except for B and Ca, regardless of soil type (Table 3.4) *E. ingens* was the only milled plant-leaf soil amendment that had significantly greater N levels in the tomato leaves than the untreated control. Some treatments and the standard fenamiphos may have had effects on some elements but no other clear patterns could be distinguished.

Table 3.4. Main effect of soil-type and treatments, least significant differences (LSD), P probabilities and F ratio of selected leaf-tissue nutrient elements on tomato under microplot conditions.

	B (mg/kg)	Ca (%)	Cu (mg/kg)	Fe (mg/kg)	K (%)	Mg (%)	Mn (mg/kg)	N (%)	P (%)	Zn (mg/kg)
Soil type										
Sand	23.7 b[1]	3.0 c	38.8 b	659 c	1.1 a	0. 7 a	1 017 b	3.0 a	0.5 b	28.3 b
Clay	30.6 a	3.8 b	36.9 b	762 b	1.2 a	0.7 a	1 045 b	3.0 a	0.5 b	31.8 ab
Loam	31.3 a	4.3 a	47.2 a	994 a	0.7 b	0.6 b	1 402 a	2.7 b	0.6 a	32.9 a
$LSD_{p=0.05}$	2.3131	0.2929	3.8064	82.8750	0.1666	0.0352	84.5900	0.1295	0.0560	3.5734
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	<0.0001	<0.0001	<0.0001	0.0291
F	26.6600	30.0200	15.3300	32.2500	19.4900	8.5700	48.0600	14.6900	13.7000	3.70
Treatment										
Control	25.6 bc	3.7 abc	42.2 ab	834 ab	0.8 b	0.7 ab	1 223 a	2.8 c	0.5 bc	26.8 b
C. cactiformis	27.7 b	3.8 ab	44.4 a	745 bc	1.2 a	0.7 a	1 211 a	3.0 abc	0.6 abc	27.6 b
E. ingens	28.9 b	3.5 bc	35.2 c	689 c	1.2 a	0.6 b	1 003 b	3.1 a	0.6 a	36.1 a
M. angolensis	38.3 a	4.0 a	42.8 a	831 ab	0.9 ab	0.7 a	1 211 a	2.9 bc	0.5 bc	30.6 a
S. cupulare	26.2 bc	3.3 c	36.8 bc	817 ab	1.2 a	0.6 b	1 030 b	2.9 bc	0.6 ab	36,2 a
T. elegans	28.0 b	3.7 abc	44.0 a	897 a	1.0 ab	0.7 a	1 236 a	2.9 bc	0.5 bc	27.9 b
Fenamiphos	22.9 c	3.4 bc	39.6 abc	740 bc	0.9 b	0.7 a	1 078 b	3.0 ab	0.5 ab	29.7 b
$LSD_{p=0.05}$	3.5312	0.4471	5.8107	126.5200	0.2544	0.05138	129.1300	0.1977	0.0855	5.4551
P	<0.0001	0.0342	0.0019	0.0200	0.0447	0.0046	<0.0001	0.0055	0.0207	0.0060
F	15.5800	2.4100	3.8900	2.6800	2.2700	3.4300	6.4800	3.3400	2.6700	3.2900

[1]Means within the same column an effect followed by the same letter or letters do not differ significantly at a 5 % level of significance

The only tomato growth variable that showed significant interaction between soil type and the treatments applied in this trial was shoot and root mass, respectively (Table 3.5). Stem height of the tomato plants grown in the untreated controls in the different soil types did not differ significantly. Stem height of tomato in the *E. ingens* and *S. cupulare* treatments was smaller than those in the untreated control in all three soil types. However, stem height was significantly greater in sand and clay than in loam in tomato treated with crudely milled leaves of *T. elegans*. Tomato plants treated with fenamiphos and grown in loamy soil had a greater stem height than their counterparts grown in sand but this standard treatment did not differ from the untreated control in this respect.

Boron, Ca, Cu, Fe, K, Mg, Mn, N, P and Zn were the only nutrient elements extracted from tomato leaf tissue in this trial that showed the possibility of interaction between soil type and the different treatments applied (Table 3.5). Boron levels in tomato leaves did not differ between the untreated controls in the three respective soil types. This element was, however, present at lower levels in tomato grown in sand and treated with *E. ingens* and *M. angolensis* than their counterparts in clay or loamy soil. Boron levels were higher in *M. angolensis*-treated tomato than in the untreated control in all three soil types. *T. elegans*-treated plants in sand had lower B levels than those in clay or loam. Plants treated with the standard nematicide fenamiphos in sand had significantly lower levels of B in their leaves than those treated with this agent in clay or loam but fenamiphos plants did not differ from the control in either sand, clay or loam in this respect.

Calcium levels in tomato leaves grown in untreated loamy sand in this trial were generally greater for all treatments in loam than sand or clay (Table 3.5). In sandy and clay soils none of the treatments differed significantly from the control but in loamy soil tomato treated with *S. cupulare* crude leaf meal had lower levels of Ca than those in the untreated microplots. Fenamiphos-treated tomato in sand had lower Ca levels than those of the same treatment in clay and loamy soils but the fenamiphos treatments in none of the soil types tested differed from their respective untreated controls.

Tomato plants in the untreated controls in the three soil types in this trial had similar levels of Mg in their leaves at harvesting of the tomato (Table 3.5). Magnesium levels were smaller

in tomato treated with crude *E. ingens* or *S. cupulare* meal than the control plants in sandy soil, while *M. angolensis* treatment had significantly greater levels than the untreated tomato. In clay and loam soil the differences in Mg levels in tomato did not differ significantly between the treatments. Fenamiphos-treated plants had significantly greater levels of Mg in sand than in clay soil, but not in loamy soil.

Manganese levels in tomato leaves in untreated loamy soil were greater than in sand or clay (Table 3.5). In clay there were no differences between the respective treatments and the control in Mn levels but in sandy soils *E. ingens* and *S. cupulare* meals were associated with smaller levels of Mn in tomato leaves than untreated plants. Manganese levels, on the contrary, were greater in than latter two treatments than the control in loamy soil. *Cissus cactiformus*-treated tomato had lower Mn levels than the control in loamy soil. Tomato grown in loamy soil and having been treated with fenamiphos had greater Mn levels in their leaves than those of their counterparts in sand or clay.

Table 3.5. Significant soil type x treatment means, least significant differences (LSD), P probabilities and F ratio of shoot mass, root mass, root knot nematode numbers and nutrient elements extracted from leaf tissue on tomato under microplot conditions.

Soil type	Treatment	Shoot mass (g)	Root mass (g)	Eggs and J2 numbers/ 50 g roots	B (mg/kg)	Ca (%)	Cu (mg/kg)	Fe (mg/kg)	K (%)	Mg (%)	Mn (mg/kg)	N (%)	P (%)	Zn (mg/kg)
Sand	Control	209.9 def[1]	40.1 a	1.8 (4 483) cdefgh	22.8 fghi	3.1 efgh	42.2 bcde	717.5 cdefg	1.1 abcdef	0.7 bcdefg	1 147.5 def	2.8 bcdef	0.4 fg	26.0 cde
	C. cactiformis	383.7 ab	39.0 ab	4.0 (13 900) ab	22.4 hi	3.1 efgh	47.0 abc	637.7 efg	1.4 ab	0.7 bcdefg	1 142.8 def	3.0 abcd	0.6 bcdef	27.0 cde
	E. ingens	209.2 def	32.3 abcde	4.1 (21 400) a	23.6 fgh	2.4 h	28.5 h	522.8 g	1.3 abcd	0.6 hi	761.0 i	3.2 a	0.6 abcd	39.4 ab
	M. angolensis	113.9 f	19.7 f	3.1 (5 640) abcde	33.5 bc	3.6 cdef	41.7 bcde	696.8 cdefg	1.2 abcde	0.8 a	1 056.8 efgh	3.1 abc	0.5 cdef	26.0 cde
	S. cupulare	226.6 cdef	35.7 abc	3.0 (4 117) abcdef	22.5 hi	2.7 gh	30.7 fgh	703.0 cdefg	1.1 abcdef	0.6 hi	925.0 ghi	3.1 ab	0.5 cdef	30.3 bcde
	T. elegans	178.7 ef	33.8 abc	0.0 (0) h	22.8 ghi	3.3 defg	37.0 cdefgh	626.3 fg	1.1 abcdef	0.8 ab	986.7 fgh	3.0 abcd	0.5 cdef	23.8 e
	Fenamiphos	140.7 f	21.7 def	3.2 (9 117)abcde	17.6 i	2.9 fgh	40.3 bcdef	643.8 defg	0.9 defgh	0.8 abc	995.5 fgh	3.1 abc	0.5 cdef	27.2 cde
Clay	Control	304.4 bcde	25.1 cdef	3.7 (8 467) abc	27.9 cdefgh	3.5 cdefg	39.0 bcdefg	757.7 cdef	0.9 defg	0.7 bcdefg	1 050.3 efgh	3.0 abcd	0.5 cdef	25.0 de
	C. cactiformis	292.9 bcde	30.1 abcdef	2.8 (4 675) abcdef	32.4 bc	4.1 bc	41.0 bcde	815.2 cdef	1.2 abcdef	0.7 abcd	1 233.2 cde	3.0 abcd	0.5 cdef	27.3 cde
	E. ingens	303.7 bcde	27.4 bcdef	3.5 (34 560) abcd	30.9 bcd	3.8 bcde	30.2 hg	700.7 cdefg	1.5 a	0.7 efghi	909.3 hi	3.2 a	0.5 cdef	35.5 abc
	M. angolensis	206.5 def	27.8 bcdef	3.2 (8 383) abcde	39.7 a	4.1 bc	39.0 bcdefg	684.0 cdefg	1.2 abcde	0.7 abcde	1 019.7 fgh	3.0 abcd	0.5 cdef	31.7 abcde
	S. cupulare	305.5 bcde	28.4 abcdef	3.5 (4 533) abcd	32.7 bc	4.0 bcd	35.0 efgh	779.7 cdef	1.5 a	0.7 bcdef	1 003.0 fgh	3.1 abc	0.5 cdef	40.2 a
	T. elegans	314.6 bcd	24.0 cdef	1.6 (750) defgh	26.4 defgh	3.5 cdefg	40.7 bcdef	864.7 cd	1.3 abcd	0.7 cdefgh	1 139.2 def	2.8 bcdef	0.3 g	30.2 bcde
	Fenamiphos	218.1 cdef	21.0 ef	1.0 (500) fgh	24.4 efgh	3.2 defg	36.0 defgh	758.8 cdef	1.0 cdefg	0.6 fghi	993.0 fgh	3.0 abcd	0. 5 cdef	31.5 abcde
Loam	Control	347.9 abc	35.1 abc	1.4 (3 120) efgh	28.5 cdefg	4.4 ab	48.3 ab	1169.0 a	0.5 h	0.6 fghi	1 573.7 ab	2.8 cdef	0.5 cdef	29.8 bcde
	C. cactiformis	452.3 a	29.9 abcdef	3.1 (1 400) abcde	29.6 cde	5.2 a	47.0 abc	855.5 cde	0.9 defgh	0.7 efghi	1 345.5 cd	2.8 cdef	0.8 a	30.0 bcde
	E. ingens	373.6 ab	32.6 abcde	1.8 (3 000) cdefgh	30.3 cd	3.9 bcde	43.5 bcde	771.8 cdef	0.8 efgh	0.6 ghi	1 240.7 cde	3.0 abcd	0.7 abc	34.0 abcd
	M. angolensis	195.6 def	26.2 cdef	1.7 (1 400) cdefgh	41.8 a	4.3 bc	47.7 ab	1113.2 ab	0.6 gh	0.6 efghi	1 558.7 ab	2.7 ef	0.5 cdef	34.2 abcd
	S. cupulare	311.5 bcde	30.6 abcdef	2.1 (1 267) bcdefg	23.7 efgh	3.6 cdef	41.8 bcde	906.7 bc	0.8 fgh	0.6 i	1 125.3 efg	2.6 f	0.6 bcde	30.0 bcde
	T. elegans	413.4 ab	33.1 abcd	0.6 (280) gh	36.2 ab	4.6 ab	55.0 a	1202.5 a	0.8 fgh	0.7 efghi	1 623.8 a	2.7 def	0.7 abc	30.0 bcde
	Fenamiphos	199.7 def	24.2 cdef	2.5 (1 650) abcdefg	28.7 cdef	4.5 ab	45.8 bcd	891.0 bc	0.7 gh	0.7 cdefgh	1 389.0 bc	2.8 cdef	0.7 abc	30.8 abcde
	$LSD_{p=0.05}$	133.9100	11.681	16 270	5.8908	0.8001	10.076	225.99	0.401	0.0887	213.01	0.3282	0.1457	9.7204
	P	0.0002	0.0236	0.0149	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	<0.0001	<0.0001	<0.0001	0.0291
	F	9.8000	3.9500	4.4600	26.6600	30.0200	15.3300	32.2500	19.4900	8.5700	48.0600	14.6900	13.7000	3.7000

[1]Means within the same column followed by the same letter or letters do not differ significantly at a 5 % level of significance.

3.4. Discussion

Tomato growth response in microplots to the treatments applied in this study showed a tendency of possible phytotoxic effects by crude leaf-meal applications of *E. ingens* and *S. cupulare* in both trials. The crudeness of the plant meals applied as soil amendments could be a reason why tomato growth and yield response was insignificant in this study, as had been the case in the glasshouse study (Chapter 2). In this study where no known critical stress was applied on the tomato plants for the duration of the trials it was not surprising that overall treatment effect was greater than that of the respective soil types on the performance of the plants. The latter effects seemed to have been more indirect on tomato growth by affecting the amendments differently due to differences in inherent characteristics. These would include texture, water potential, adsorption capacity and colloidal particles present in a particular soil type (Bidwell, 1974). Miami and Rodríguez-Kábana (1982) demonstrated that improved growth responses could be due to the absorption of carbon compounds that leached from plant residues into soil-water solutions. Improved growth response of pineapple to fenamiphos application was partly due to increased accumulation of indole-acetic acid in the plant tissue, which stimulates growth regardless of nematode infection (Milne *et al.*, 1977).

Suppression of RKN numbers associated with soil amendment of crudely milled leaf meals of *C. cactiformes, M. angolensis and T. elegans* in this study supports the previous glasshouse results. However, the poorer performance of the leaf meals in this regard, particularly in the second trial could be ascribed to the overall effect that soil type could have had. In addition to the effect that differences in inherent soil characteristics mentioned above might have had on the soils x treatments interactions, root-knot nematodes might also have been affected by soil type (Greco and Di Vito, 2009). Previous studies have also shown that organic amendments differ in their in efficacy of RKN control (Akhtar and Malik, 2000; Chitwood, 2002).

Variable but significant effects of plant-leaf-meal soil amendments on the macro- and micro-nutrient element levels extracted from tomato leaves at harvesting in this study support similar studies mostly on some of the macro-elements (Rodríguez-Kábana *et al*., 1981, 1982; Rodríguez-Kábana, 1986; Bhattacharya and Goswami, 1988; Stirling, 1991). The

different pH levels of the soils tested in this study could have had a substantial effect on the nutrient levels and their uptake by the tomato plants (Bidwell, 1974), as well as on the breakdown and uptake of nutrients and active substances contained in the different crude plant-leaf meals applied in this study. This is supported by the significant interaction between soil type and treatment with regard to some of the nutrient element levels in tomato leaves in this study. This is a situation that should be continuously kept in mind when further developing or testing these or similar material is done. Some elements are needed in greater quantities by tomato plants than others, while others might be toxic to plants when present in soil at too high levels. Similarly the availability for uptake of some elements by plants could be influenced by soil properties, as well as by the presence or absence of certain other elements in the soil (Bidwell, 1974).

Apart from the effects that different soils could have had on the desirability and efficacy of plant leaf-meal soil amendments this study has also provided motivation for the elimination of some of the materials for consideration as nematicides, even in their crude form. Negative or doubtful effects of *E. ingens and S. cupulare* cast scepticism on their possible large-scale use as soil amendments in crop production. However, confirmation of the usefulness of *C. myriocarpus*, as well as the positive results on *M. angolensis and T. elegans* of the previous glasshouse studies (Chapter 2), provide motivation for further testing of these materials under field conditions.

CHAPTER 4

A FIELD ASSESSMENT OF THE EFFECT OF CRUDE PLANT-LEAF MEALS ON ROOT-KNOT NEMATODES, SELECTED LEAF TISSUE NUTRIENT ELEMENTS AND TOMATO CROP PERFORMANCE

4.1. Introduction

Crude plant-leaf meal of five non-crop plant species as soil amendments evaluated under glasshouse (Chapter 2) and microplot (Chapter 3) conditions showed potential as nematicides for *Meloidogyne incognita* race 2 on tomato. There is a continuous interest in the development of nematoxic compounds from botanical products (Rodríguez-Kábana *et al*., 1989; Chitwood, 2002; Crow, 2005; Zasada *et al*., 2010; Thoden *et al*., 2011) such as these evaluated in this study. Nematode–toxic or antagonistic plants are considered those that produce substances that inhibit the growth and development of or are lethal to plant-PPN in plant tissue as well as in soil and they reduce nematode population densities (Sano, 2005). Mashela (2002) showed that ground fruit of wild cucumber (*Cucumis myriocarpus*) suppressed RKN numbers under glasshouse and microplot conditions (Mashela and Nthangeni, 2002; Mashela and Mpati, 2002; Mashela and Mphosi, 2002). This material also increased the productivity of tomato and improved soil electrical conductivity without affecting soil pH (Mashela, 2002). Similar results with botanical soil amendments were obtained by Akhtar (2000), Akhtar and Malik (2000) and Sikora and Fernandez (2005).

Information about the performance of botanical soil amendments as nematode control agents under field conditions is hard to obtain (McSorley, 2011), particularly on local non-crop materials such as those evaluated thus far in this study. The objective of this particular study, therefore, was to evaluate the efficacy of dried-meal soil amendments of *Cissus cactiformis*, *Euphorbia ingens, Maerua angolensis, Synadenium cupulare* and *Tabernaemontana elegans* on RKN (*M. incognita* race 2), selected tomato leaf-tissue nutrient elements and productivity of the crop under field conditions.

4.2. Material and Methods

A field trial was conducted during 2008 at the ARC-ITSC in Mpumalanga Province, South Africa (approx. 25°27'32.31" S; 30°58'17.77" E).

4.2.1. Collection and preparation of different plant-leaf meals as soil amendments

The procedures and material used in this part of the study were the same than those described in Chapters 2 and 3, respectively.

4.2.2. Acquirement, multiplication, extraction and inoculation of root-knot nematodes

The procedures and material used in this part of the study were the same than those described in Chapter 2 and Chapter 3, respectively.

4.2.3. Treatments, trial layout and field conditions

Two-week-old tomato seedlings (var. Rodade) were transplanted in ridges 1.8 m apart. Rows were 50 m long, with 30 cm between each plant within a row. Before seedbed preparation the area was fumigated with Tellone II at a dosage g 5 ml/ 1 m furrow to render the field nematode-free. The soil in this trial was a sandy loam (80 % sand, 11 % silt, 9 % clay with a pH (H_20) 6.15. The procedures and material used for tomato transplanting, irrigation and pest control were the same than those described in Chapter 3. The trial was arranged in a randomized-complete block design (RCBD). The treatments were randomly assigned to plots consisting of six rows of tomato plants in each of the six block replicates. The treatments included the soil-amendment reference *C. myriocarpus*, a standard synthetic, commercial nematicide fenamiphos and an untreated control. The main factors were three application rates (5, 10 or 15 g per plant) of five soil-amendment treatments as described in Chapters 2 and 3. Measurements and assessments on the four tomato plants per plot were pooled in each replicate to further reduce variation. Crudely milled *C. myriocarpus* fruits were btained from the Nematology Laboratory of the University of Limpopo, Sovenga as described in Chapters 2 and 3.

4.2.4. Growth of tomato and nematode assessments

The procedures and material used in this part of the study were the same than those described in Chapter 3.

4.2.5. Nutrient-element analyses from tomato leaf tissue

The procedures and material used in this part of the study were the same than those described in Chapter 3.

4.2.6. Nutrient element analyses from crude leaf-meals used in this study

Nutrient-element analyses were done in the ARC-ITSC Soil Laboratory to determine their relative contents in the crude leaf meals used in this study. Sub-samples of 5 g each were taken from the stock materials that were kept in the laboratory as described in Chapter 2. The analysis procedures for the different nutrient elements in the leaf-meal material were the same than the respective procedure described in Chapter 3. The extraction procedure for S was the same than for B, Ca, Cu, Fe, K, Mg, Mn, P and Zn. Quantification of S was done similar to B and P, using a colorimetric AAIII Auto Analyzer (Chapter 3). The N:K and C:N ratios were also determined for each of the dry leaf meals in this analysis.

4.2.7. Data analysis

The procedures used in this part of the study were the same as those described for the appropriate glasshouse or microplot trials in Chapter 2 and 3, respectively.

4.3. Results

Tomato stem height in soil amended with milled *M. angolensis* (10 g) and *T. elegans* leaves (5 g and 10 g) was significantly greater than untreated tomato stem height (Table 4.1). *Euphorbia ingens* and *S. cupulare* all rates treated tomato tended to have shorter stems, although not significantly compared to those of the untreated control. Differences in shoot mass of tomato in this field trial were highly variable, with a range of 602 g between the heaviest and lightest root systems. Differences between treatments and the untreated control did not show any clear trend. *E. ingens* and *S. cupulare* treatments generally tended to have the weakest root systems in this trial, as well as the lowest number of fruits (Table 4.1). The *M. angolensis* and *T. elegans* treatments gave the largest number of fruits, except at the highest application rate of *M. angolensis*. Fruit mass was highly variable in this trial, especially within some treatments, e.g. *S. cupulare*. Contrary to the corresponding glasshouse and microplot trials (Chapters 2 and 3) tomato root mass did not differ

significantly between any of the treatments or the untreated control in this field trial. However, an overall trend in growth reaction to treatments in this trial was that *C. cactiformes*, *M. angolensis* and *T. elegans* seemed stimulatory to tomato growth, while *E. ingens* and *S. cupulare* seemed growth inhibitory in most cases (Table 4.1). No clear application rate responses were visible in any of the soil-amendment treatments applied in this trial.

Nematode egg and J2 numbers were highest in the untreated-control tomato in this trial (Table 4.1). The numbers increased to almost half a million per 50 g roots at harvesting (120 days) after inoculation with ca. 3 000 eggs and J2 per plant at transplanting. *M. angolensis* (all three dosages), *E. ingens* (15 g) and *T. elegans* (all three dosages) crude leaf-meal treatments gave significant control, even under these conditions of high population pressure by the RKN. These leaf-meal soil amendments compared well with the standard synthetic nematicide treatment, fenamiphos (Table 4.1). The other soil-amendment treatments gave variable results in this regard. The *E. ingens* and *S. cupulare* treatments had high nematode numbers in the tomato roots at harvesting of the trial, except at their highest respective application rates. However, nematode population reaction to the plant-leaf-meal soil amendments did not correspond with application rate of these materials.

Table 4.1. Treatment means, least significant differences (LSD), P probabilities and F ratio of stem height, shoot mass, root mass, fruit number, fruit mass, root-knot nematode egg and J2 numbers on tomato in a field trial at ARC-ITSC during 2008.

Treatment	Application rate (g)	Stem height (cm)	Shoot mass (g)	Fruit number	Fruit mass (g)	Root mass (g)	Eggs and J2 numbers/ 50 g roots
Control	0	40.0 bcde[1]	427.0 cde	21.8 de	1 637 cde	34.9 a	5.6[3] (474 433) a
C. cactiformis	5	41.8 abcd	637.1 abc	23.5 cde	1 628 de	32.9 a	4.7 (79 083) bcdef
	10	47.3 ab	608.0 abc	34.1 ab	2 332 abc	45.2 a	4.7 (77 950) bcde
	15	47.7 ab	545.6 abcd	27.7 abcde	2 458 ab	45.7 a	5.0 (142 900) abcd
E. ingens	5	33.0 efg	218.0efg	9.0 g	499 f	32.8 a	5.3 (220 067) abc
	10	27.5 g	141.0 g	11.7 fg	390 f	34.7 a	5.1 (202 083) abcd
	15	38.8 def	438.6 cde	20.6 ef	1 531 e	54.4 a	3.9 (73 717) f
M. angolensis	5	47.3 abc	743.3 a	31.5 abc	2 147 abcde	33.7 a	4.5 (71500) cdef
	10	48.3 a	626.8 abc	31.7 abc	2 298 abcd	32.6 a	4.2 (46 500) ef
	15	45.5 abcd	454.1 bcd	29.6 abcde	1 880 bcde	30.3 a	4.5 (52 450) cdef
S. cupulare	5	39.2 cdef	377.0 def	24.6 bcde	2 015 abcde	38.3 a	4.9 (122 167) abcde
	10	31.5 fg	185.1 fg	9.7 g	250 f	26.7 a	5.4 (251 517) ab
	15	41.5 abcd	534.6 abcd	21.6 de	1 756 cde	31.5 a	4.7 (84 750) bcdef
T. elegans	5	48.8 a	625.9 abc	29.8 abcd	2 091 abcde	33.4 a	4.6 (62 267) bcdef
	10	48.5 a	674.8 ab	28.0 abcde	1 961 abcde	35.0 a	4.4 (31 017) def
	15	46.8 abcd	485.1 bcd	33.1 ab	2 618 a	29.6 a	4.8 (76 667) bcde
C. myriocarpus	5	43.8 abcd	370.8 defg	27.8 abcde	1 847 bcde	30.5 a	4.8 (97 683) abcde
Fenamiphos	5	42.2 abcd	421.3 cde	24.9 bcde	1 827 bcde	28.1 a	4.5 (67 683) def
$LSD_{p=0.05}$	-	8.1630	229.9000	9.0310	695.9000	n.s[2]	0.7967
P	-	<0.0010	<0.0010	<0.0010	<0.0010	0.2710	0.0120
F		4.7400	4.3900	5.7700	7.7300	1.2100	2.1500

[1]Means within the same column followed by the same letter or letters do not differ significantly at a 5 % level of significance.

[2]n.s. = not significant.

[3]Log_{10}[(eggs+J2)+1] (untransformed means in parentheses)

Only the tomato-leaf elements that showed significant differences between treatments applied in this study are shown in Table 4.2. All nutrient-element contents in the tomato leaves, though significantly different, were highly variable and showed no clear response to leaf-meal application rate. Boron was present at significantly higher levels than those in untreated control plants in tomato where the soil was treated with *M. angolensis* (10 g) and *T. elegans* (10 g) (Table 4.2) but not from *C. myriocarpus*-treated plants had the highest B levels in their leaves in this field trial. *Euphorbia ingens* (5 g) and *S. cupulare* (10 g) had significantly lower Ca levels than the untreated control in tomato plants, while other dosage rates for those amendments did not differ from the untreated tomato. Copper levels were higher at some or all rates of the plant-leaf meals, along with fenamiphos-treated tomato than those of untreated control. Iron levels were higher only at some rates of *C. cactiformes*, *E. ingens* and *S. cupulare*. Potassium, N and P levels in the tomato leaves in this trial showed very few differences between treatments (Table 4.2) and Zn levels were higher in tomato plants where the soil was treated with different rates of *E. ingens* (15 g)*, M angolensis* (all three dosage rates)*, S. cupulare* (10 g) and *T. elegans* (all three dosage rates).

Table 4.2. Treatment means, least significant differences (LSD), P probabilities and F ratio of leaf-tissue nutrient elements on tomato under field conditions in the first trial of this study.

Treatment	**Application rate (g)**	**B mg/kg**	**Ca mg/kg**	**Cu mg/kg**	**Fe mg/kg**	**K %**	**Mg (%)**	**Mn (mg/kg)**	**N %**	**P %**	**Zn mg/kg**
Control	0	29.7 defg[1]	6.5 a	31.5 e	780 c	1.1 a	0.9 a[2]	1652 a	2.2 b	0.5 cde	31.7 f
C. cactiformis	5	32.4 cdefg	6.1 a	40.2 cd	916 bc	1.1 a	0.8 a	1855 a	2.1 b	0.5 def	32.8 f
	10	35.7 bcdef	6.3 a	38.2 cde	899 bc	1.3 a	0.8 a	1676 a	2.2 b	0.5 def	35.5 def
	15	34.9 bcdef	6.1 ab	41.5 cd	1978 b	1.3 a	0.8 a	1976 a	2.1 b	0.4 f	37.2 def
E. ingens	5	24.6 fg	4.8 c	53.7 a	1510 a	0.5 b	0.8 a	1691 a	2.2 b	0.4 ef	38.8 cdef
	10	24.5 fg	5.1 bc	52.7 a	1574 a	0.5 b	1.0 a	1636 a	2.2 b	0.5 bcde	40.3 bcdef
	15	39.5 bcd	6.4 a	38.2 cde	814 bc	1.4 a	0.8 a	1706 a	2.2 b	0.6 abc	48.0 abc
M. angolensis	5	38.2 bcde	6.7 a	36.5 cde	917 bc	1.3 a	1.0 a	1778 a	2.2 b	0.6 abc	43.8 abcde
	10	46.1 ab	6.2 a	40.0 cd	1006 bc	1.3 a	0.9 a	1879 a	2.0 b	0.6 a	48.5 abc
	15	36.5 bcdef	6.4 a	36.2 cde	954 bc	1.4 a	0.9 a	1792 a	2.3 b	0.6 ab	45.0 abcd
S. cupulare	5	35.8 bcdef	6.4 a	42.5 bc	997 bc	1.2 a	0.9 a	1798 a	2.2 b	0.5 abcde	34.7 ef
	10	22.9 g	4.4 c	49.2 ab	1517 a	0.6 b	0.9 a	1548 a	2.7 a	0.4 ef	52.8 a
	15	31.4 defg	6.4 a	40.8 cd	1015 bc	1.3 a	0.8 a	1868 a	2.1 b	0.6 abcd	31.3 f
T. elegans	5	35.7 bcdef	6.6 a	36.2 cde	921 bc	1.3 a	0.9 a	1815 a	2.1 b	0.5 bcde	49.3 ab
	10	44.3 abc	6.6 a	39.7 cd	922 bc	1.4 a	0.9 a	1801 a	2.2 b	0.6 abcd	44.2 abcde
	15	34.2 bcdefg	6.9 a	38.2 cde	1022 bc	1.3 a	0.9 a	1969 a	2.2 b	0.6 abcd	49.7 ab
C. myriocarpus	5	51.5 a	6.2 a	34.3 de	824 bc	1.4 a	0.8 a	1531 a	2.1 b	0.5 bcde	40.7 bcdef
Fenamiphos	5	26.9 efg	6.4 a	39.3 cd	906 bc	1.1 a	0.8 a	1916 a	2.2 b	0.5 bcde	33.3 f
$LSD_{p=0.05}$	-	12.0300	0.9200	7.2600	271.1000	0.1100	n.s	n.s	0.2390	0.1040	9.8800
P	-	<0.0010	<0.0010	<0.0010	<0.0010	<0.0010	0.2520	0.3070	0.0040	<0.0010	<0.0010
F		3.1900	4.3300	5.2300	6.3700	7.4000	1.2400	1.1700	2.4300	2.9600	3.9100

[1]Means within the same column followed by the same letter or letters do not differ significantly at a 5 % level of significance

[2]n.s. = not significant.

Agreement between nutrient-element levels in tomato leaves in this (Table 4.2) and the microplot study (Table3.2) was not strong. While B, Fe, N and Zn levels were similar in the respective corresponding treatments the rest were either higher or lower in the field than in the microplot study. Potassium levels, for example, in the field were twice or more times higher in the field untreated control, *C. myriocarpus* and fenamiphos treatments than in their corresponding treatments in the microplots. Sometimes some of the element levels in the tomato leaves were similar in certain corresponding treatments, while they differed greatly between others in the respective microplot (Table 3.2) and field (Table 4.2) studies.

Table 4.3. Leaf tissue nutrient element analysis of the crudely milled organic amendments used in the field trials

	Soil amendments				
Nutrient elements	***C. cactiformis***	***E. ingens***	***M. angolensis***	***S. cupulare***	***T. elegans***
B mg/kg	40.60	25.80	101.10	34.40	45.70
Ca %	3.48	1.35	1.94	1.37	1.16
Cl %	0.46	0.32	0.68	0.40	0.87
Cu mg/kg	11.00	6.00	6.00	15.00	8.00
Fe mg/kg	1160.00	324.00	172.00	580.00	382.00
K %	2.20	1.42	1.73	4.42	1.64
Mg %	0.91	0.59	0.93	0.40	0.93
Mn mg/kg	241.00	54.00	45.00	96.00	233.00
N %	1.71	0.68	4.62	1.27	2.78
Na %	0.22	0.01	0.04	0.03	0.10
P %	0.23	0.09	0.14	0.21	0.20
S %	0.23	0.26	0.25	0.16	0.28
Zn mg/kg	29.00	65.00	13.00	69.00	23.00
N:K	0.79	0.48	2.67	0.29	1.70
C:N	22.00	63.00	9.:00	30.00	15.00

Contents of the different nutrient elements analyzed during this study differed considerably between the respective leaf meals (Table 4.3). *Cissus cactiformis* had the highest or second highest contents of most of the elements, *viz.* Ca, Cu, Fe, Mn and Na. Only Zn was relatively high in content in *E. ingens* leaf meal, while it's Na and P levels were very low, also relative to that of the other leaf meals. *Maerua angolensis* had by far the highest B and N contents but relatively poor Fe and Zn levels. Its N:K ratio was highest among the leaf meals and its C:N ratio the lowest. Copper, K and Zn contents were the highest in *S. cupulare*, while in *T. elegans* only Mn levels were higher than in the other leaf meals. The N:K ratio was highest

and the C:N ratio lowest to those of *M. angolensis*. *E. ingens* had by far the highest C:N ratio of the five plant-leaf meals in this study (Table 4.3).

The nutrient element contents in the tomato leaves in this trial (Table 4.2) and those of the leaf meals of the soil-amendment treatments in this trial are comparable because the quantity of material analysed as well as the procedures were the same. Elements that were present in similar contents in the tomato leaves (Table 4.2) and some leaf meals (Table 4.3) were B, Fe, K, and Zn. Calcium, Cu and P levels were considerably higher in tomato than in the other plant leaf meals. Nitrogen levels in tomato leaves were either higher or lower in the non-crop plants than in tomato. The nutrient levels in dried *C. myriocarpus* material were more comparable with those in tomato (Table 4.2) than with those in the non-crop plant leaves (Table 4.3). There was no obvious agreement between higher or lower nutrient-element levels in the tomato leaves in this study and levels of the corresponding elements in the respective non-crop-plant leaf materials tested in this study (Tables 4.2 and 4.3).

4.3. Discussion

Growth stimulating effects by *C. cactiformis*, *M. angolensis* and *T. elegans* on tomato and inhibition of growth by *E. ingens* and *S. cupulare* in this part of the study confirm the results observed in the glasshouse (Chapters 2) and microplot (Chapter 3) studies. Dried and crudely-milled wild cucumber fruit (Mashela, 2002) and castor bean (*Ricinus communis*) were also associated with tomato growth enhancement in similar studies (Mashela and Nthangeni, 2002). Closer correspondence between the various growth parameters of tomato in this study, as in the microplots (Chapter 3) demonstrates the value of testing plant growth reaction in conditions that are more similar to regular practices (McSorley, 2011) with specific crops. The postulation that the crudeness of the leaf-meal soil amendments masked the effect of increments in application rates was also supported by the lack of tomato growth reaction in the field, as it did in the respective glasshouse and microplot studies in Chapters 2 and 3.

The comparative superior efficacy of *M. angolensis* and *T. elegans* over the other plant soil amendments tested in this study in the suppression of egg and J2 numbers of *M. incognita*, as well as the former's similar performance to the standard nematicide (fenamiphos)

provide further evidence of the potential usefulness of these materials in PPN management. This would particularly be true for small-scale application in subsistence agriculture (Akhtar and Malik, 2000; Chitwood, 2002; Mashela, 2002; Mashela and Nthangeni, 2002; Zasada *et al*., 2006; Meyer *et al*., 2006; Coyne *et al*., 2009; McSorley, 2011).

The results in this study on nutrient-element contents of tomato leaves largely support those in the previous study that differences in tomato response to the respective crude plant-leave meals could exist. However, as indicated in Chapter 3 the variability in these nutrient element levels seems more likely to be the effect of soil physical or chemical factors than direct treatment effects (Akhtar and Malik, 2000; Oka and Yermiyahu, 2002). Once again the lack of treatment rate responses, in addition to the lack of agreement between element levels in the soil-amendment treatments and those in the corresponding tomato leaves indicate that these plant-leaf materials in their crude form might have other effects on the crop than primary amelioration of soil fertility (Bhattacharya and Goswami, 1988). Soil amendments of organic nature could have multiple effects to the soil environment and everything associated with it (McSorley, 2011). The latter author also highlighted the discrepancy between glasshouse and field results concerning soil organic amendments, which were the case between our microplot (Chapter 3) and field results too, especially concerning nutrient-element contents of tomato leaves. The lack of clear tendencies in levels of nutrient-element contents of the respective non-crop plant leaf meals used in this study, as well as the correspondent tomato leaf contents the latter was treated with, provide further support to the abovementioned conclusions that soil amendment with these materials had negligible soil fertilization effects.

The substantially higher N:K rates in dried and milled leaves of *M. angolensis* and *T. elegans* (Table 4.3) might have some significant value, however. According to Campbell (2000) the optimum N:K rate for greenhouse and trellis tomato in the southern United States of America is between 1.2 and 1.8. Concomitantly the C:N ratio (Table 4.3) of *T. elegans* leaf meal fit within the range of 15 to 20, which is considered the range where the most effective plant materials fall in terms of soil amendment for PPN control (McSorley, 2011). The C:N ration of *M. angolensis* is on the low side, which implies that it falls in the risk range of causing phytotoxicity (Rodríguez-Kábana *et al*., 1989). In our study, however, there was

little evidence of this. The latter authors stated that organic soil amendment material that have C:N ratios in excess of 20 usually lacks efficacy in nematode control, which at least in part could explain the performance in this regard of *E. ingens* and *S. cupulare* in this part of the study and the previous ones (Chapters 2 and 3). The C:N ratio of *C. myriocarpus*, which proved to control RKN effectively is 14:1 (Mashela, 2002).

Consistent nematode-suppressive effects by particularly *M. angolensis* and *T. elegans* in this part of the study and the preceding ones (Chapters 2 and 3), along with non-substantial evidence of plant-nutrient amelioration of soil (as discussed above) may contradict popular views that organic soil amendments enhance plant growth by improving soil conditions (Muller and Gooch, 1982; McSorley and Gallaher, 1995; Noling, 1999; Akhtar and Malik, 2000; Powers and McSorley, 2000; McSorley, 2011). Apart from effects such as improving plant tolerance (McSorley, 2011) or resistance (Stirling, 1991) to nematodes, at least some of the non-crop leaf meals tested in our study may contain substances that possess nematoxic properties (Stirling, 1991; Chitwood, 2002; Ntalli and Menkissoglu-Spiroudi, 2011). Different substances with possible direct effect on PPN have been isolated by other researchers from the non-crop plant species that we have used in this study.

It is still possible that with these non-crop materials applied as soil amendments more mechanisms may play a role concomitantly (Akhtar and Malik, 2000; McSorley, 2011), however, in reducing PPN numbers and enhancing tomato growth. Therefore it remains important to continue the study by trying to get closer to isolating possible active substances for nematode control. There may be more substances that are involved than those mentioned in the introduction to this thesis. *In vitro* studies on nematode suppressive effects of extracts of these materials might provide direction towards their possible modes of action and practicality of their use in crop production (McSorley, 2011). Therefore, *in vitro* trials were conducted and will be elaborated on in Chapter 5.

CHAPTER 5

IN VITRO EFFECT OF LEAF-MEAL EXTRACTS OF *MAERUA ANGOLENSIS* AND *TABERNAEMONTANA ELEGANS* ON THE MOTILITY OF SECOND-STAGE-JUVENILE ROOT-KNOT NEMATODES

5.1. Introduction

Primary or secondary metabolites derived from botanical sources have been and are still widely used in herbal medicines, poisons and pharmaceutical products or as additives to other manufactured products (Yoshikawa, 2002). Plant-derived metabolites have attracted the most attention of all prospective alternatives to pesticides (Chitwood, 2002), either as plant extracts, formulated phytochemicals or as organic amendments to soil (Akhtar and Malik, 2000). Several plant species have been reported to contain metabolites with nematoxic activity (Gonzalez and Estevez-Braun, 1997; Bar-Eyal *et al*., 2006; Kong *et al*., 2006; Batish *et al*., 2008; Shakil *et al.,* 2008) and there is continuous effort in discovering new plant sources with such characteristics (Rodríguez-Kábana *et al*., 1989; Chitwood, 2002; Crow, 2005; Zasada *et al*., 2010).

Nematoxic chemicals can attract, repel and/or inhibit movement or cause the death of nematodes (Wuyts *et al*., 2006). Nematode motility inhibition could allow a plant host to 'buy time' and escape early damage or perhaps provide time for the induction or expression of defence mechanisms such as induced or antibiosis resistance (Akhtar and Mahmood, 1994a; Bos and Parlevliet, 1995; Chitwood, 2002; Wuyts *et al*., 2006; McSorley, 2011). Crude leaf meals of *Maerua angolensis* and *Tabernaemontana elegans* consistently showed reduction in RKN numbers on tomato in glasshouse, micro-plot and field trials (Chapters 2, 3 and 4). These two materials can be purchased from traditional healers in the Limpopo Province for medicinal and related uses. Although leaf material of these two non-crop plant species are known to contain substances that might have nematoxic properties (Chapter 4) our results thus far also indicated some plant-growth enhancement in tomato after soil amendment with these materials.

In this part of the study, the efficacy of abovementioned crude plant-leaf meals on RKN was investigated *in vitro*. The objective was to assess whether partly separated extracts of the two plant species' leaf meals would cause *in vitro* nematode J2 motility inhibition over a limited period of exposure. This information would provide further insight into the usefulness of these materials in RKN control in small-scale cropping systems.

5.2. Material and methods

The *in vitro* trials were initiated in 2011 under laboratory conditions at the Biosciences Unit at the Council for Scientific and Industrial Research (CSIR) in Pretoria, South Africa (approx. 25°44'47.96" S, 28°16'47.01" E).

5.2.1. Collection of different plant organs and extraction technique of root-knot nematodes

The procedures and material used in this part of the study were similar to those in Chapters 2, 3 and 4.

5.2.2. Extracts prepared from *M. angolensis* and *T. elegans* leaf meals

Five stock extract preparations were made of each crude plant meal at the Biosciences Unit, CSIR, by means of the method described below and shown diagrammatically in section 5.1.

Five hundred gram of crude leaf meal of *M. angolensis* and *T. elegans*, respectively, were separately and independently from each other left to soak in 1 l de-ionized water for 24 hours at room temperature. These relatively large amounts of the ground materials were used in order to have a sufficient supply of sample material for analysis. The extraction process followed of the fractionated extracts of these two non-crop plant species are outlined in figure 5.1. The extracts were filtered and the filtrates were freeze-dried to remove the water from the material (Treatment A). Another 500 g ground material of both plant species was extracted by means of shaking in a methanol/dichloromethane (1:1 volume/volume) mixture for eight hours. The residue was discarded and the liquid from the extracted material evaporated at 60 °C to remove all the solvents. This extract was called Treatment B. Fifteen gram of the latter dry material was then taken and partitioned between one part 10 % deionised water to one part 90 % methanol and two parts 100 %

hexane. After 24 hours two layers were formed, namely the hexane layer on top, which was poured off and evaporated (Treatment C). The aqueous methanol layer at the bottom was evaporated and then further partitioned in a 1:1 dichloromethane and de-ionized water mixture. The water layer on top was poured off and the dichloromethane layer was evaporated to dryness (Treatment D). The water layer was freeze-dried and the remnant was Treatment E.

5.2.1. Preparation of stock solutions

Ten stock solutions were prepared for each of the 10 plant-extract treatments explained above. Using an analytical balance, aliquots from 10 mg to 100 mg in increments of 10 mg were measured from each extract and put separately into sterile 1.5-ml Eppendorf tubes. This was followed by adding a solution of pluronic gel and de-ionized water to fill each tube to the 1 ml mark, after which the tubes were tightly closed. The extract was brought into suspension in each tube by thoroughly shaking it for 45 minutes. These stock solutions were stored at -20 °C in a freezer so that the stability of the extracts would not be affected. A 10 % dilution of each extract was required from the stock solutions for the RKN bioassay tests. These were obtained by preparing a solution of pluronic gel and de-ionized water and pouring 900 µl of that in sterile 1.50-ml Eppendorf tubes. To make up 1 ml of each dilution, 100 µl of each stock solution was added to the above-mentioned Eppendorf tubes. Each was again thoroughly mixed and stored at 4 °C in a refrigerator. The dilutions were prepared for the repetitions of the experiments.

5.2.4. Root-knot nematode J2 motility-inhibition assays

The abovementioned dilutions of the extracts of *M. angolensis* and *T. elegans* were tested for nematicidal activity in terms of nematode J2 motility inhibition at 10 different concentrations (0.1-1.0 mg ml^{-1}) each in 96-well test plates. This was achieved in the following way: A suspension of 20 ml pure pluronic gel and de-ionized water was put into 50-ml Eppendorf tubes. This was followed by adding a 10-ml suspension of J2 of *M. incognita* race 2 in water containing approximately 35 000 J2. The suspension was thoroughly shaken by hand for 5 s. From this a 90-µl suspension containing 100 ± 20 J2 was transferred to the 96-well plate using a pipette. Ten microlitre extract of each concentration

was subsequently put in each well plate in a specific direction and order. Thus the final volume in each well was 100 µl.

Four independent trials for each plant extract concentrate were arranged in a randomized-complete block design (RCBD), with the eight treatments in each test replicated four times each. Every treatment was randomly assigned to four rows (replicate blocks). Each test included two negative controls, namely a diluted solution of pure pluronic gel and de-ionized water (Treatment K1), 10 µl of 10 % methanol (MeOH) added to diluted solution (Treatment K2) and a positive control of 10 µl of 1.2 mg ml^{-1} salicylic acid added to the 90 µl mixture of pluronic gel and de-ionized water (Treatment K3).

After having sealed each well plate tightly with plastic wrapping to avoid any cross-well contamination, each well plate was shaken for 10 min at 1 000 revolutions min^{-1} on an Orbit 1000 (Labnet) laboratory shaker. After J2 inoculation, on the same day, the total numbers of motile and immotile J2 in each well were counted under an inverted compound microscope at 32× magnification. After that, the plates were incubated at 22 °C in the dark and the number of immotile J2 per well was counted at 24, 48 and 72 hours after the addition of the different diluted extract concentrations.

5.2.5. Reversible nature of J2 motility inhibition

All the abovementioned extracts from crude meals of *M. angolensis* and *T. elegans* leaf material were tested to determine whether the J2 motility inhibition for *M. incognita* race 2 is reversible. Five concentrations of each extract, ranging from 0.6 to 1.0 mg ml^{-1} were applied in a 24-well plate. The same two negative controls and the positive control as described above under the J2 motility inhibition assay were used. Aliquots of 100 ± 20 J2 of *M. incognita* were suspended in 360 µl pluronic gel and inoculated in each well prior to the addition of the respective extract concentrations. Forty microlitre of each of the five concentrations of *M. angolensis* and *T. elegans* mentioned above were added to each well. The final volume in each well was 400 µl. These plates were incubated at 22 °C in the dark and the numbers of immotile J2 were counted after 24, 48 and 72 hours under an inverted microscope at 32× magnification as described above.

Four independent trials for each plant extract concentrate were arranged in a RCBD, with the eight treatments in each test replicated four times each. Every treatment was randomly assigned to four rows (replicate blocks). After 72 hours, the content of each well was diluted by 1 200 µl pluronic gel and de-ionized water solution into each well, the plates were incubated for an additional day. After after a total of 96 hours since the experiments commensed, the total numbers of motile and immotile J2 in each well were counted for verification of any reversible mechanisms.

5.3. Data analysis

The data from each trial were subjected to appropriate ANOVA using SAS/STAT statistical software (SAS, 1999). The standardized residuals of each variable were tested for deviations from normality using Shapiro-Wilk's test. Fisher's protected t-LSD (least significant difference) was calculated at a 5 % level of significance to compare means of significant effects (Snedecor and Cochran, 1980).

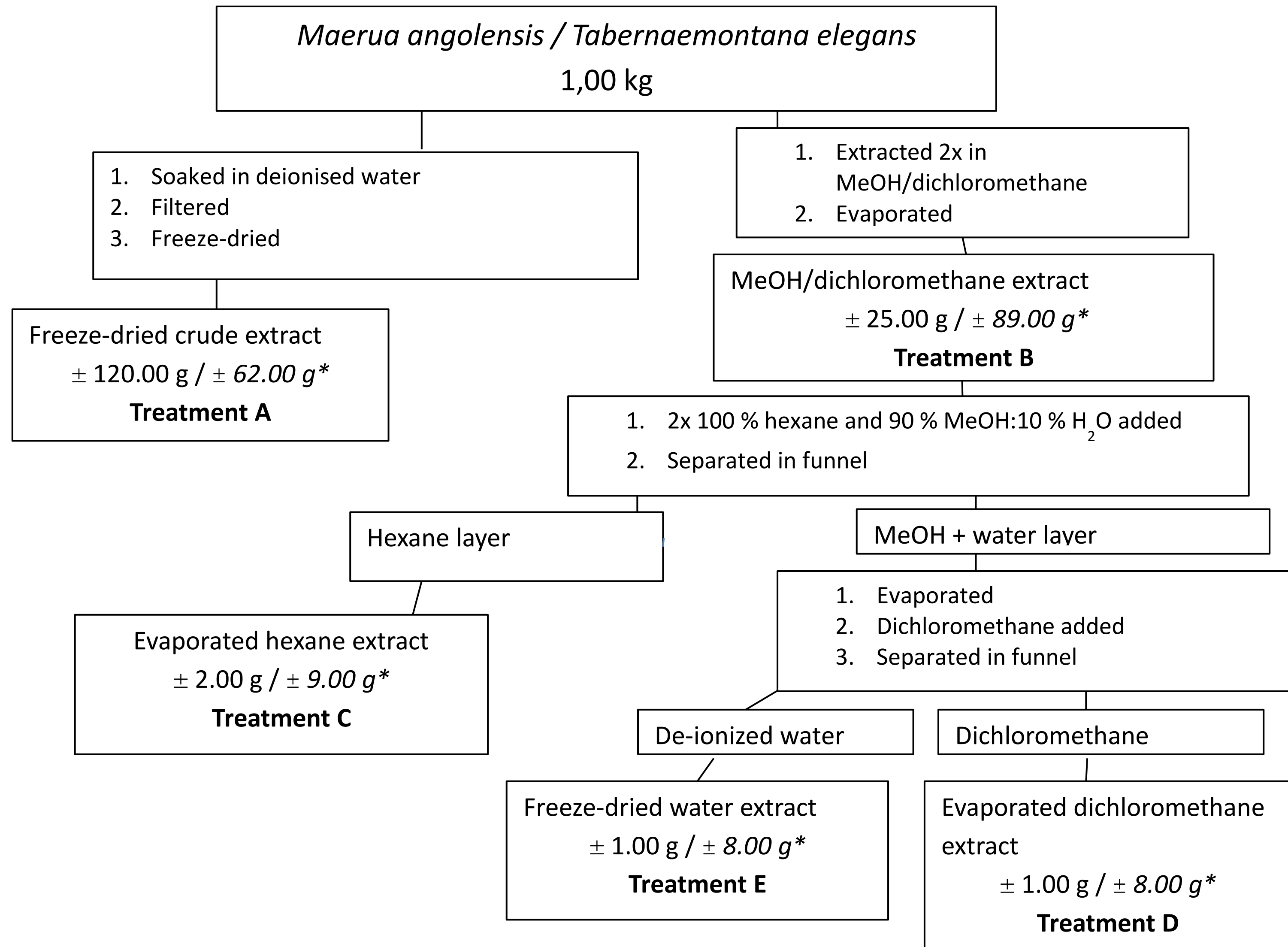


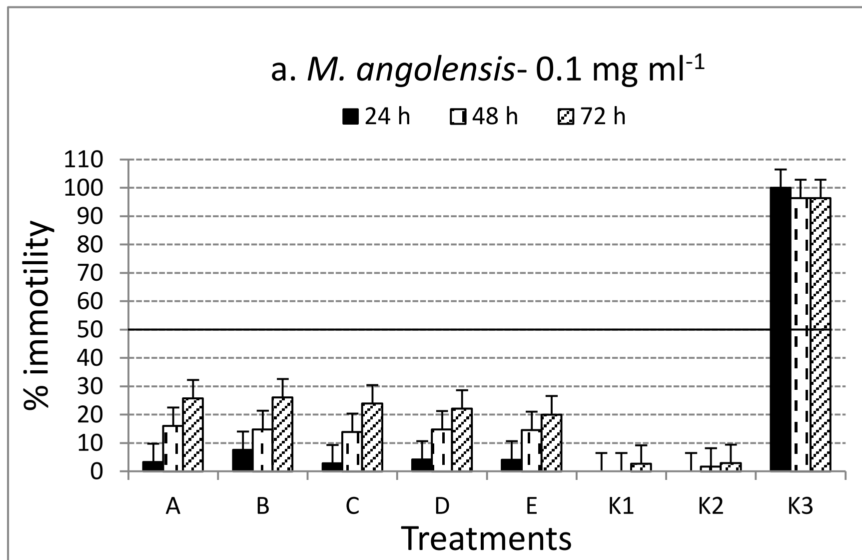
Figure 5.1. Diagram to illustrate five different extractions of crude leaf meals of *M. angolensis* and *T. elegans* to test *in vitro* for their effects on the motility of *M. incognita* second-stage juveniles. *Values in normal font are for *M. angolensis* while those in italics are for *T. elegans.*

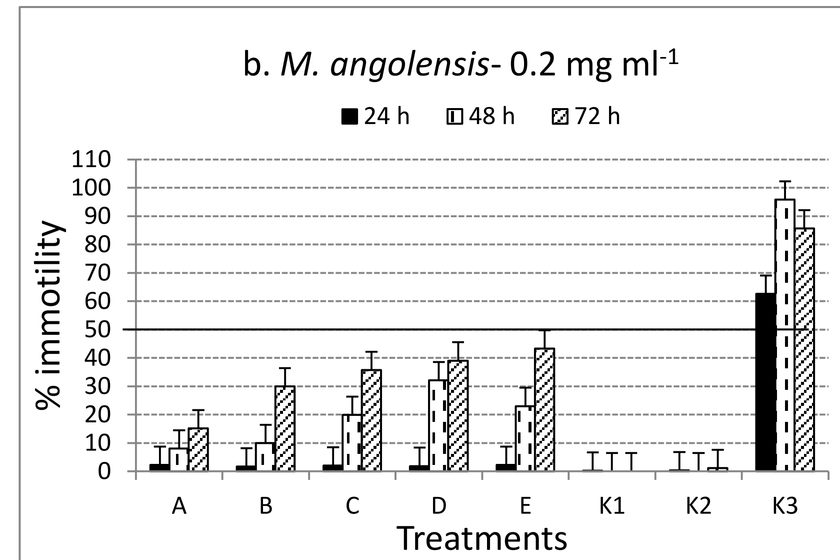
5.4. Results

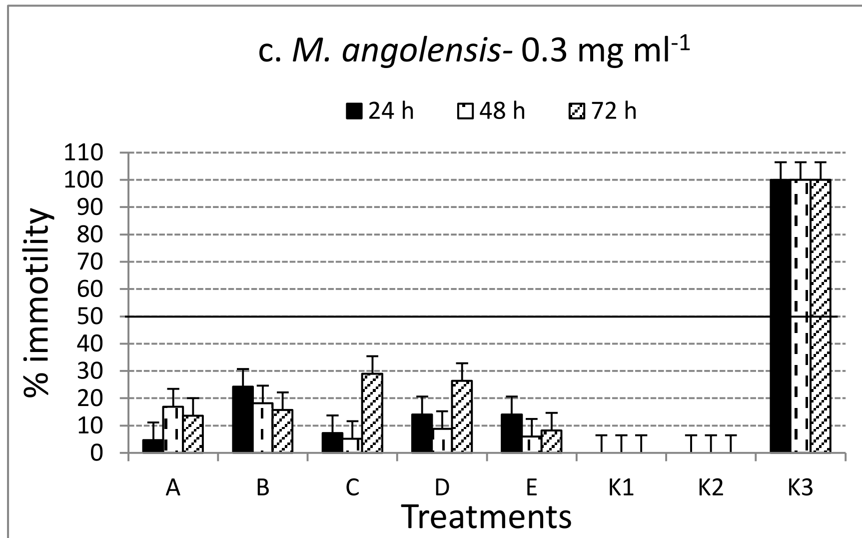
5.4.1. Root-knot nematode J2 motility-inhibition assays

5.4.1.1. *Maerua angolensis*

After 24 hours only pure salicylic acid (K3) showed a high percentage of J2 immotility (Figures 5.2 a-j). All concentrations of *M. angolensis* (A- E) showed a much slower reaction because, even at a rate of 1.0 mg ml^{-1}, 50 % immotility of J2 was not reached in 24 hours, except in the freeze-dried crude extract (Treatment A) (Figure 5.2 j). Forty-eight and 72 hours after treatment greater percentages of J2 immotility were observed, especially for the higher *M. angolensis* extract concentrations. From 0.8 mg ml^{-1} and higher all *M. angolensis* extract concentrations showed J2 immotility percentages in excess of 50 % after exposure times of 48 to 72 hours (Figures 5.2. h-j). It also seems that the J2 reactions at 48 and 72 hours were fairly similar in most of the extracts. At the 1.0 mg ml^{-1} rate (Figure 5.2 j) all five of the latter *M. angolensis* extracts seemed to be able to cause immotility of J2 nematodes at levels comparable to salicylic acid (K3), particularly after 48 and 72 hours exposure as well as for the 24 hours exposure for *M. angolensis* Treatment A.







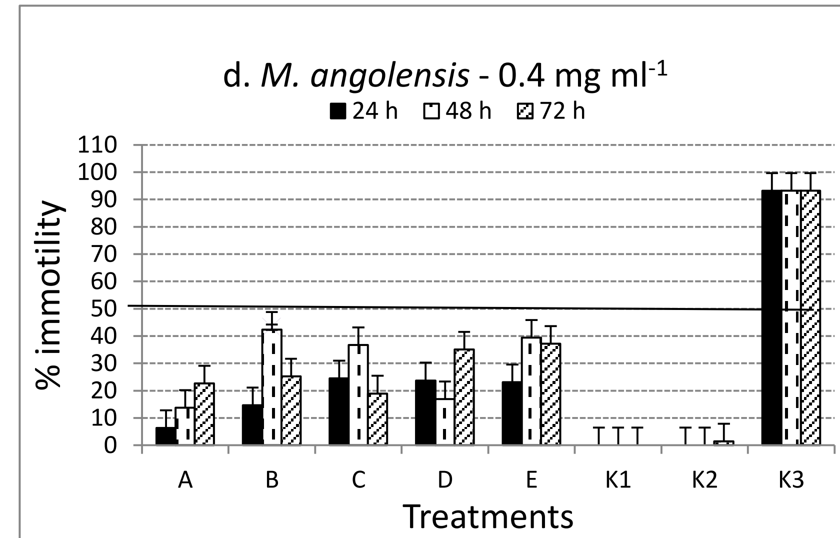
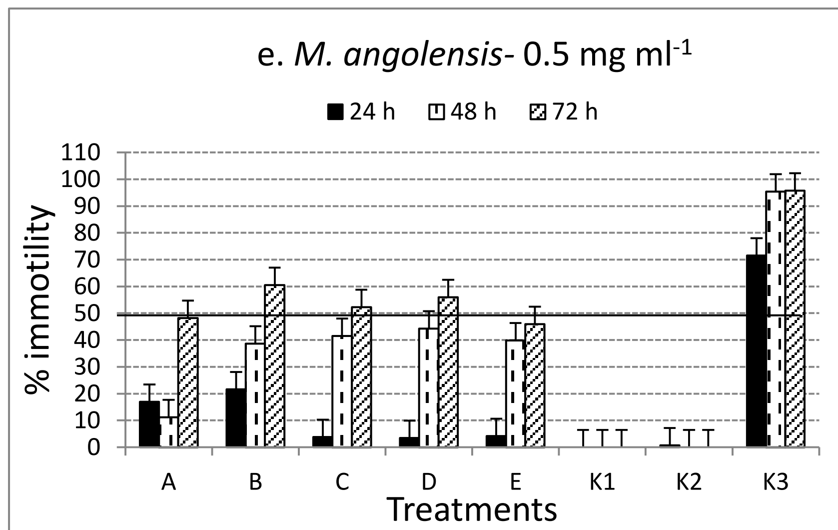
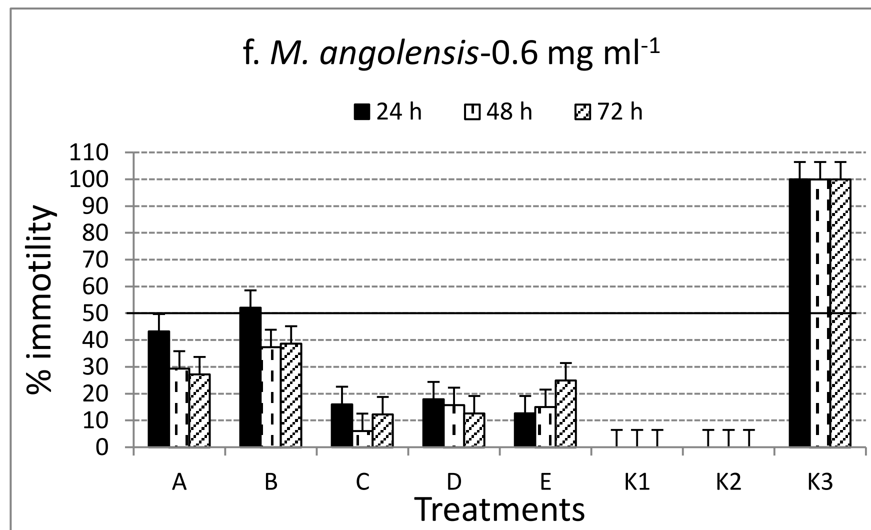
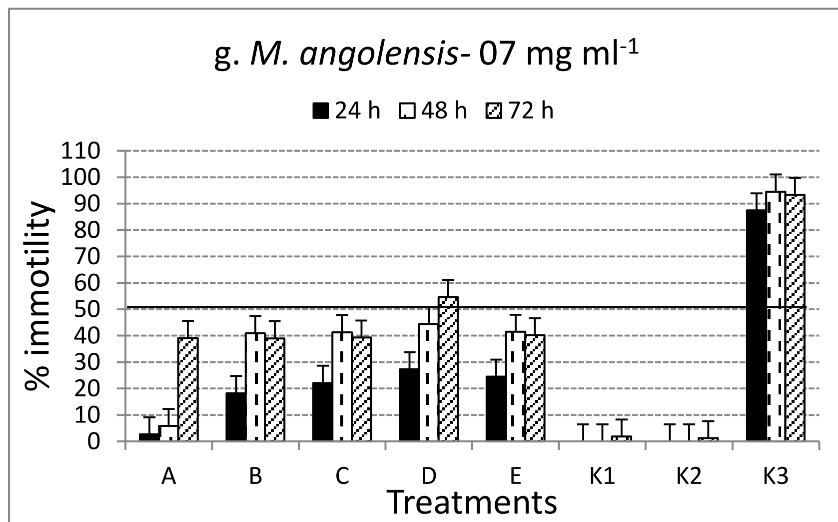


Figure 5.2 a-d. Motility inhibition of *Meloidogyne incognita* J2 by means of five extracts with 10 concentrations of crude *M. angolensis* leaf meal under *in vitro* conditions. Treatments A = Freeze-dried crude extract, B = Methanol/dichloromethane (1:1) extract, C = Evaporated hexane extract, D = Evaporated dichloromethane extract, E = Freeze-dried water extract, K1 = Pure suspended solution of pluronic gel and deionised water, K2 = 10 % methanol, K3 = Pure salicylic acid. Fisher's protected t-test's least significant difference (LSD) was 6.4643.







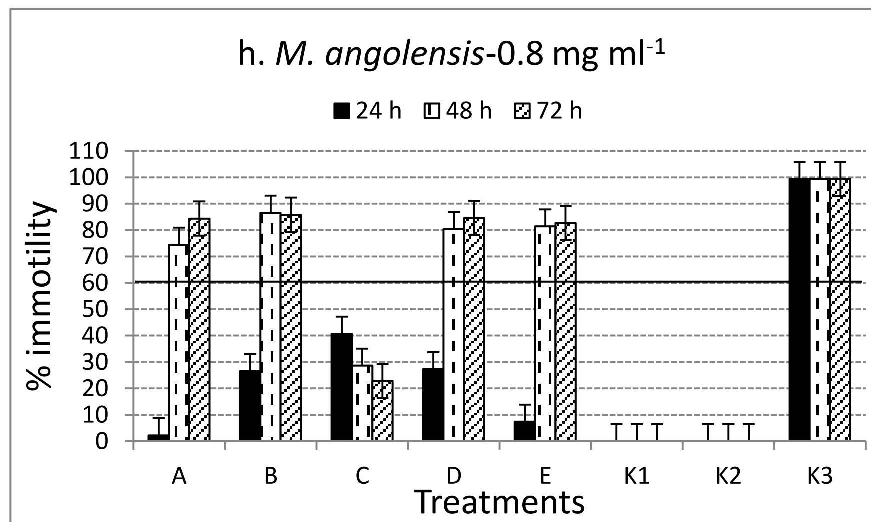
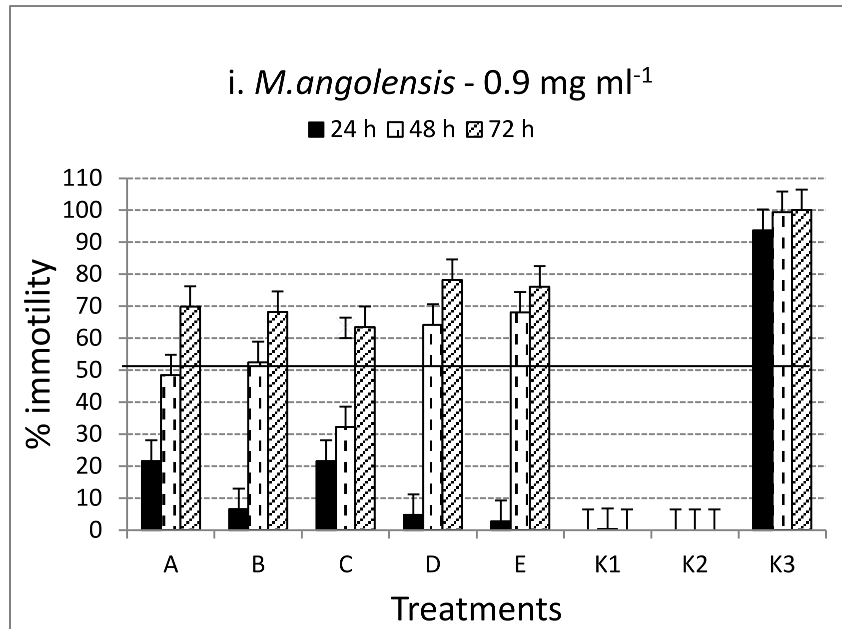


Figure 5.2 e-h. Motility inhibition of *Meloidogyne incognita* J2 by means of five extracts with 10 concentrations of crude *M. angolensis* leaf meal under *in vitro* conditions. Treatments A = Freeze-dried crude extract, B = Methanol/dichloromethane (1:1) extract, C = Evaporated hexane extract, D = Evaporated dichloromethane extract, E = Freeze-dried water extract, K1 = Pure suspended solution of pluronic gel and deionised water, K2 = 10 % methanol, K3 = Pure salicylic acid. Fisher's protected t-test's least significant difference (LSD) was 6.4643.



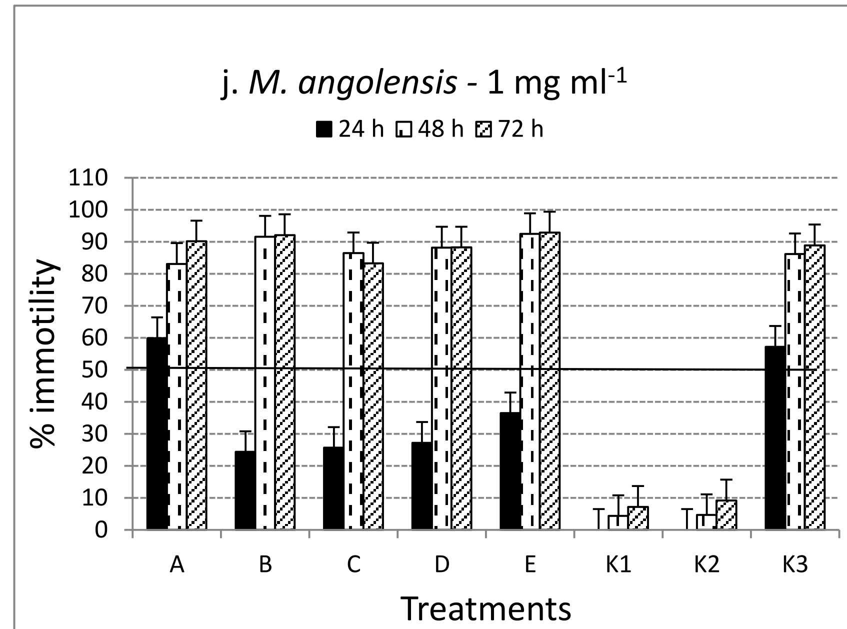
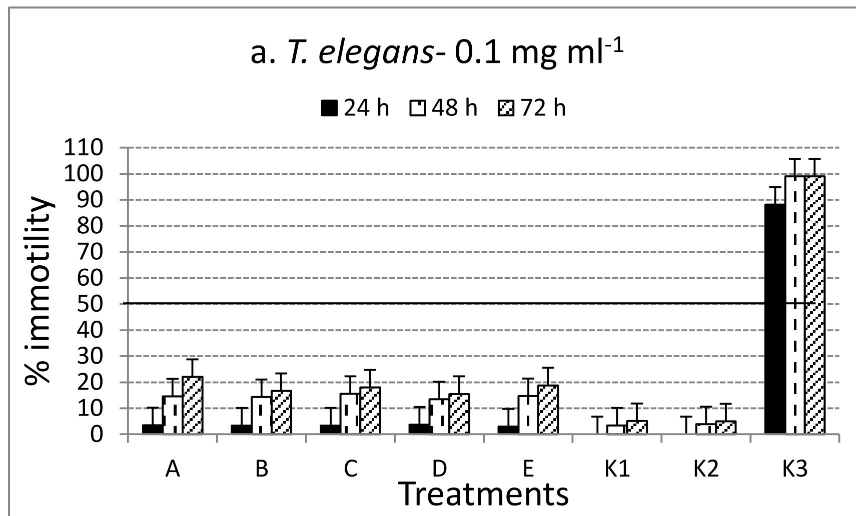
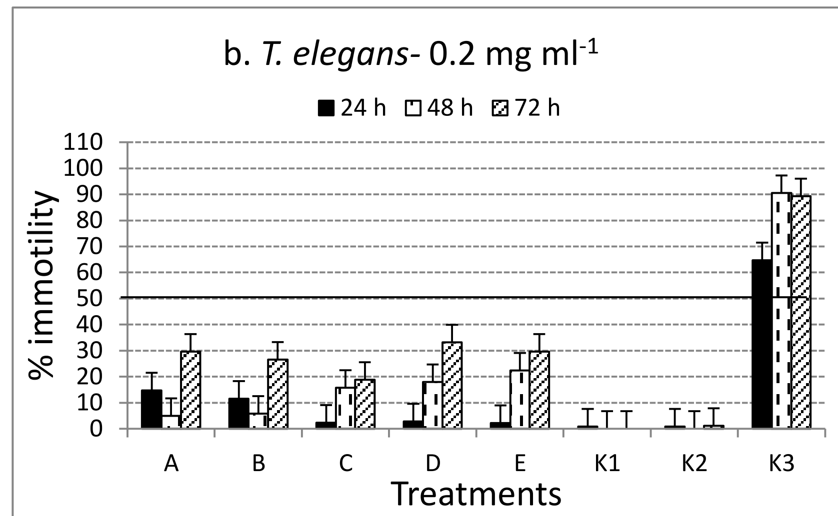


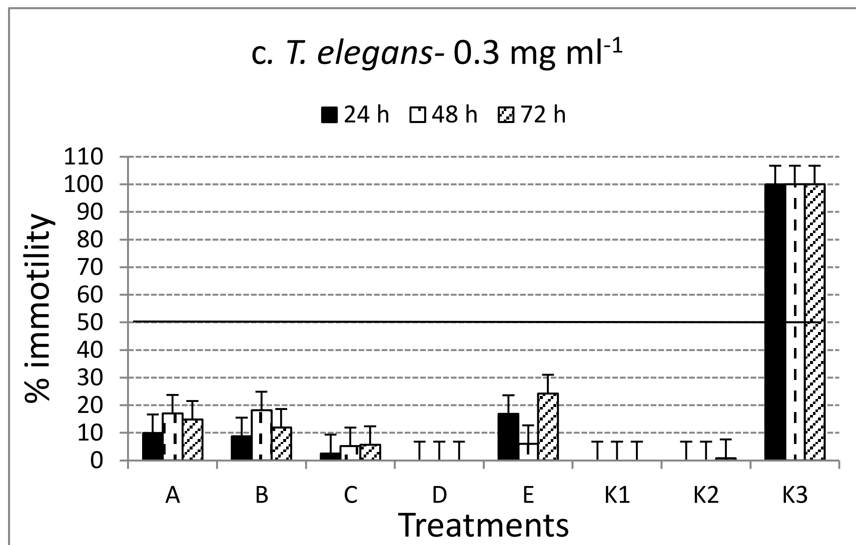
Figure 5.2 i-j. Motility inhibition of *Meloidogyne incognita* J2 by means of five extracts with 10 concentrations of crude *M. angolensis* leaf meal under *in vitro* conditions. Treatments A = Freeze-dried crude extract, B = Methanol/dichloromethane (1:1) extract, C = Evaporated hexane extract, D = Evaporated dichloromethane extract, E = Freeze-dried water extract, K1 = Pure suspended solution of pluronic gel and deionised water, K2 = 10 % methanol, K3 = Pure salicylic acid. Fisher's protected t-test's least significant difference (LSD) was 6.4643.

5.4.1.2. *Tabernaemontana elegans*

According to Figures 5.3 a-j salicylic acid (K3) again showed the largest percentage J2 immotility after 24, 48 and 72 hours up to the *T. elegans* dosage rates of 0.9 mg ml^{-1}. The respective extracts and rates of *T. elegans* did not seem to be more effective in causing immotility of J2 than those of *M angolensis* (Fig. 5.2). At concentrations of 0.1 – 0.6 mg ml^{-1} no *T. elegans* extracts at any concentration was able to cause 50 % J2 immotility, even after exposure periods of 72 hours. At rates of 0.7 mg ml^{-1} and higher, only freeze-dried water extracts (Treatment E) was able to induce more than 50 % immobility after 48 and 72 hours exposure. At rates of 0.8-1.0 mg ml^{-1} freeze-dried crude extract (Treatment A), methanol/dichloromethane (B) and freeze-dried water extracts (Treatment E) of *T. elegans* were able to induce J2 immobility of more than 50 % after 48 and 72 hours (Fig 5.3 h-j). This excluded the methanol/dichloromethane extract (Treatment B) at 0.9 mg ml^{-1} (Fig 5.3 i), which was still less than the required 50 % J2 motility. Only freeze-dried water (Treatment E) extracts of *T. elegans* at 1.0 mg ml^{-1} were sufficient to cause immotility greater than 50 % at 24 hours and longer exposure *in vitro* (Figure 5.3 j). Evaporated dichloromethane extract (Treatment D) was not tested at 0.8-1.0 mg ml^{-1} since the concentrate of the extract made the solution too dark and nothing could be seen in the solution.







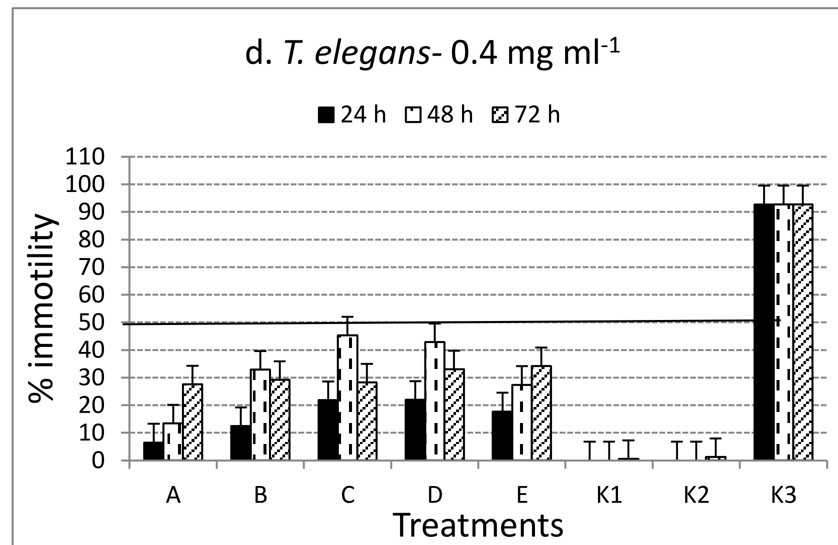
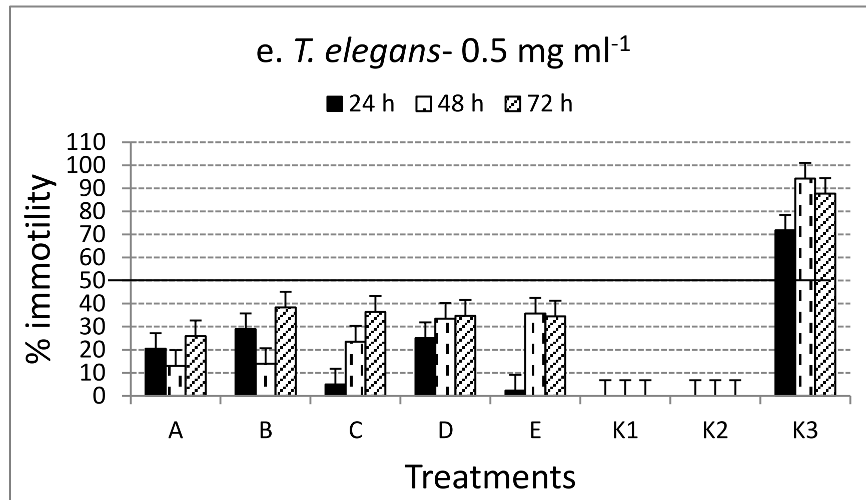
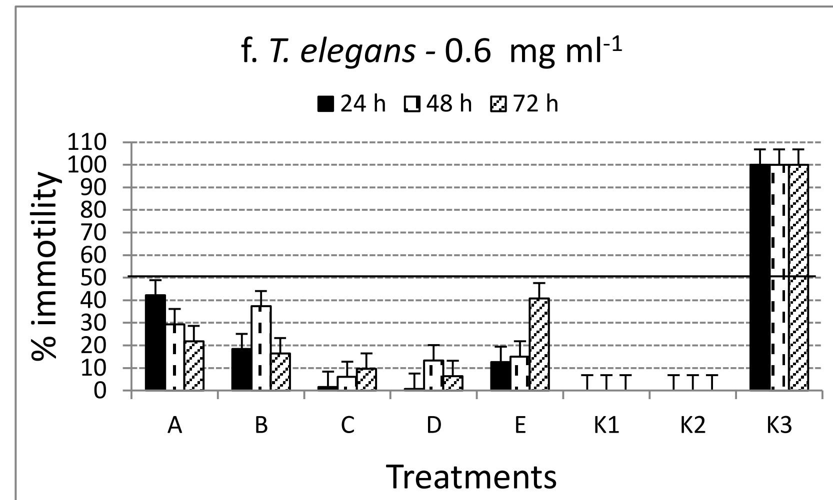
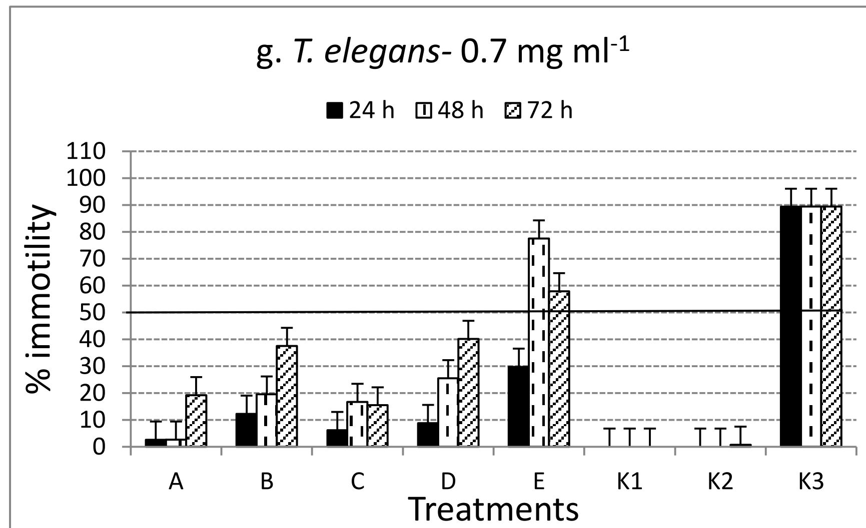


Figure 5.3 a-d. Motility inhibition of *Meloidogyne incognita* J2 by means of five extracts with 10 concentrations of crude *T. elegans* leaf meal under *in vitro* conditions. Treatments A = Freeze-dried crude extract, B = Methanol/dichloromethane (1:1) extract, C = Evaporated hexane extract, D = Evaporated dichloromethane extract, E = Freeze-dried water extracts, K1 = Pure suspension solution of pluronic gel and deionised water, K2 = 10% methanol, K3 = Pure salicylic acid. The Fisher's protected t-test's least significant difference (LSD) was 6.7590.







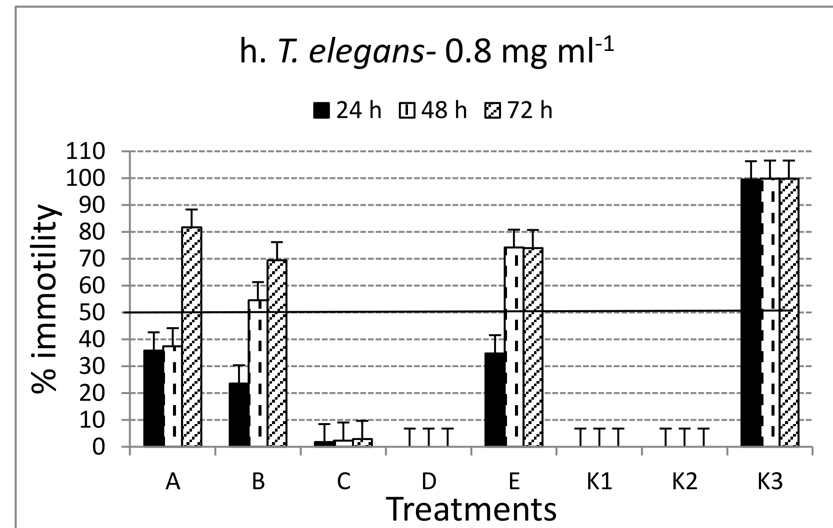
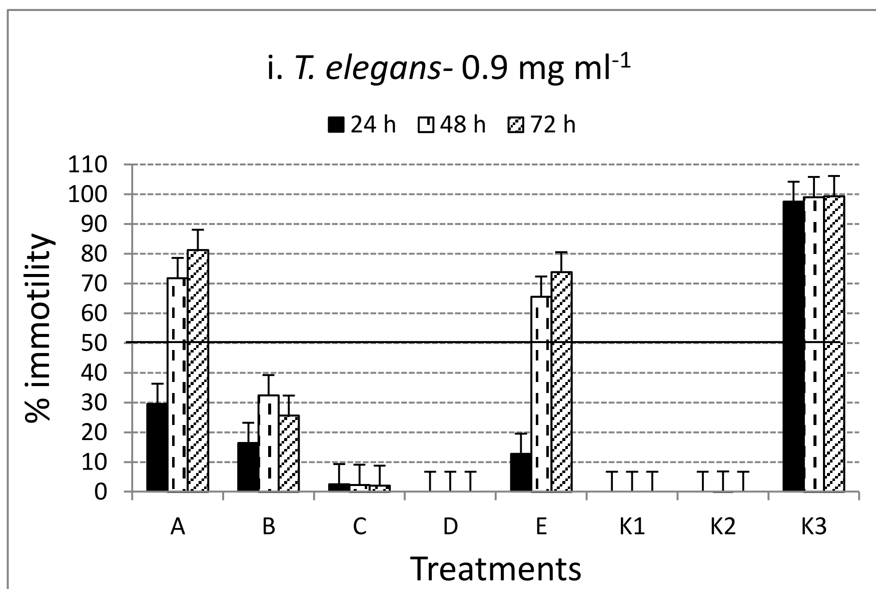


Figure 5.3 e-h. Motility inhibition of *Meloidogyne incognita* J2 by means of five extracts with 10 concentrations of crude *T. elegans* leaf meal under *in vitro* conditions. Treatments A = Freeze-dried crude extract, B = Methanol/dichloromethane (1:1) extract, C = Evaporated hexane extract, D = Evaporated dichloromethane extract, E = Freeze-dried water extracts, K1 = Pure suspension solution of pluronic gel and deionised water, K2 = 10% methanol, K3 = Pure salicylic acid. The Fisher's protected t-test's least significant difference (LSD) was 6.7590.



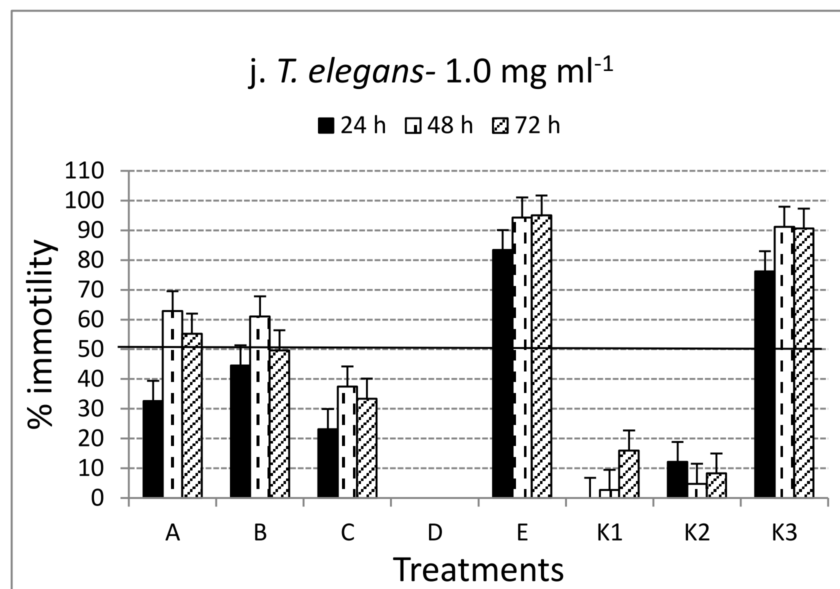
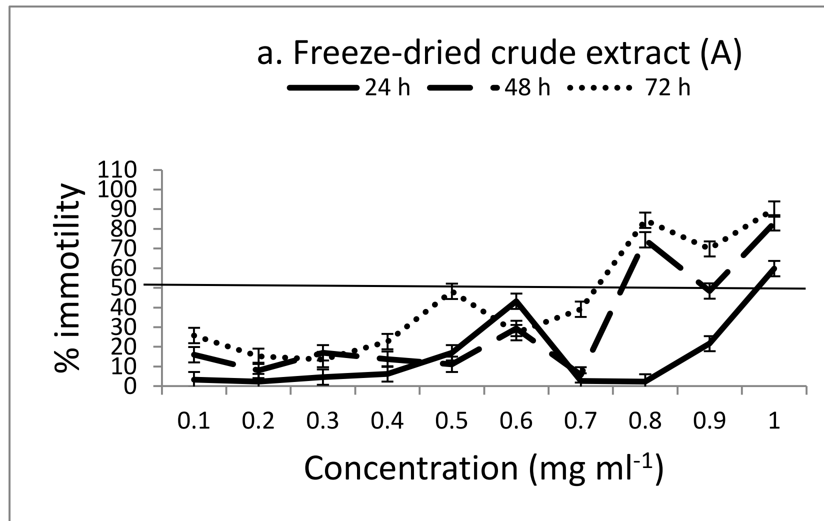


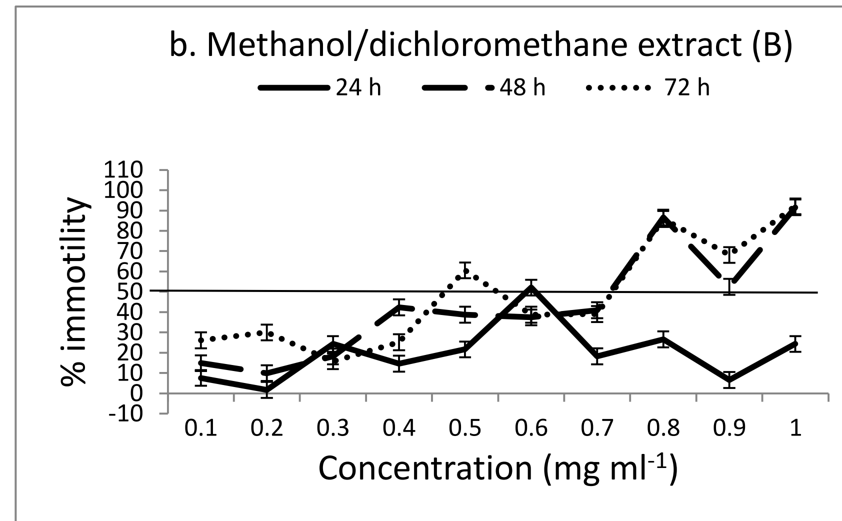
Figure 5.3 i-j. Motility inhibition of *Meloidogyne incognita* J2 by means of five extracts with 10 concentrations of crude *T. elegans* leaf meal under *in vitro* conditions. Treatments A = Freeze-dried crude extract, B = Methanol/dichloromethane (1:1) extract, C = Evaporated hexane extract, D = Evaporated dichloromethane extract, E = Freeze-dried water extracts, K1 = Pure suspension solution of pluronic gel and deionised water, K2 = 10% methanol, K3 = Pure salicylic acid. The Fisher's protected t-test's least significant difference (LSD) was 6.7590.

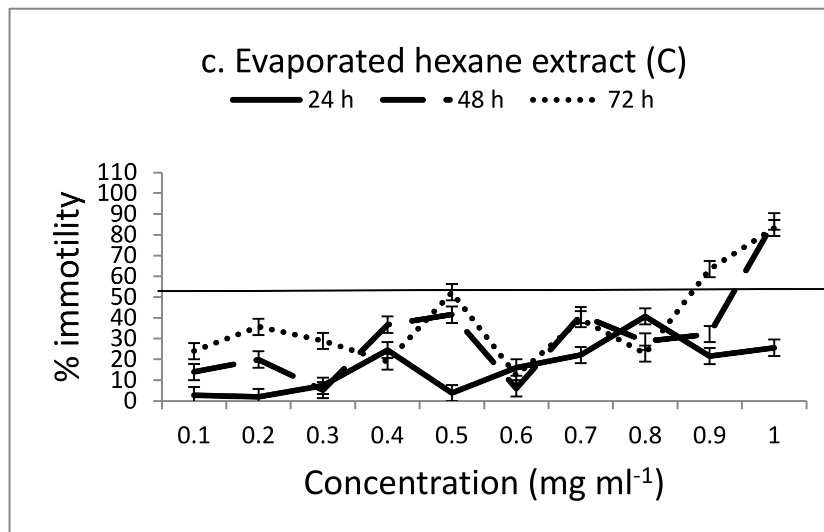
5.4.2. LC_{50} of plant-leaf extracts

5.4.2.1. *Maerua angolensis*

The LC_{50} results of *M. angolensis* (Fig. 5.4 a-e) largely correspond with the results on J2 motility inhibition in Figure 5.2 a-j. The LC_{50} application rate for freeze-dried crude (Treatment A) and methanol/dichloromethane (Treatment B) extracts of *M. angolensis* was in the region of 0.70-0.75 mg ml^{-1} after 48 and 72 hours exposure (Fig. 5.4 a). Treatment A is the only extract of *M. angolensis* that had an LC_{50} after 24 hours exposure, at a rate of almost 1.0 mg ml^{-1}. The LC_{50} for evaporated hexane (Treatment C) extract of *M. angolensis* was ± 0.85-0.95 mg ml^{-1} at 72 and 48 h exposure periods, respectively (Fig. 5.4 c). Those for evaporated dichloromethane (Treatment D) and freeze-dried water (Treatment E) extracts were in the region of 0.68-0.72 mg ml^{-1} after 72 and 48 hours exposure, respectively (Fig. 5.4 d and e). These results show that longer exposure to higher concentrations of all the *M. angolensis* extracts was more effective in the inhibition of *M. incognit*a J2 motility. A slight peak occurred at around 0.50 mg ml^{-1} for all *M. angolensis* extracts after 48 and 72 hours exposure.







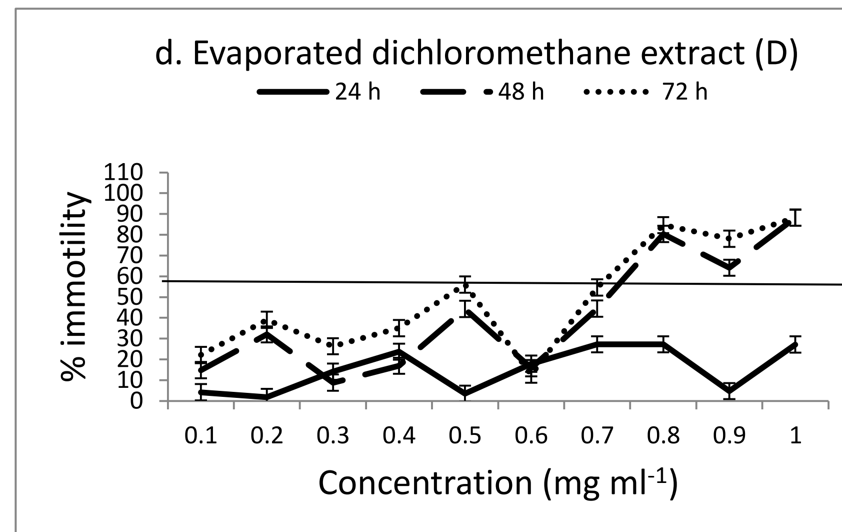


Figure 5.4 a-d. LC_{50} graphs of five *M. angolensis* leave extracts on *M. incognita* J2 motility inhibition at three different times of exposure *in vitro*. The Fisher's protected t-test's least significant difference (LSD) was 7.8080.

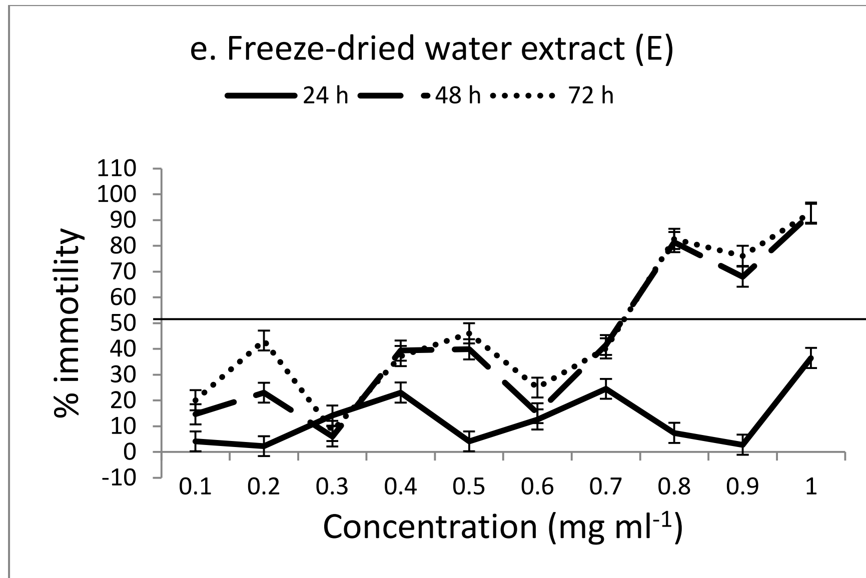
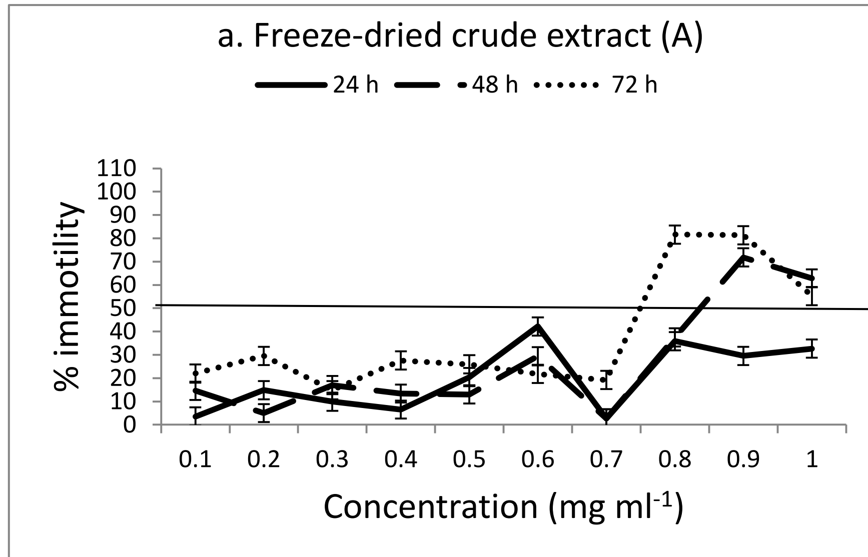
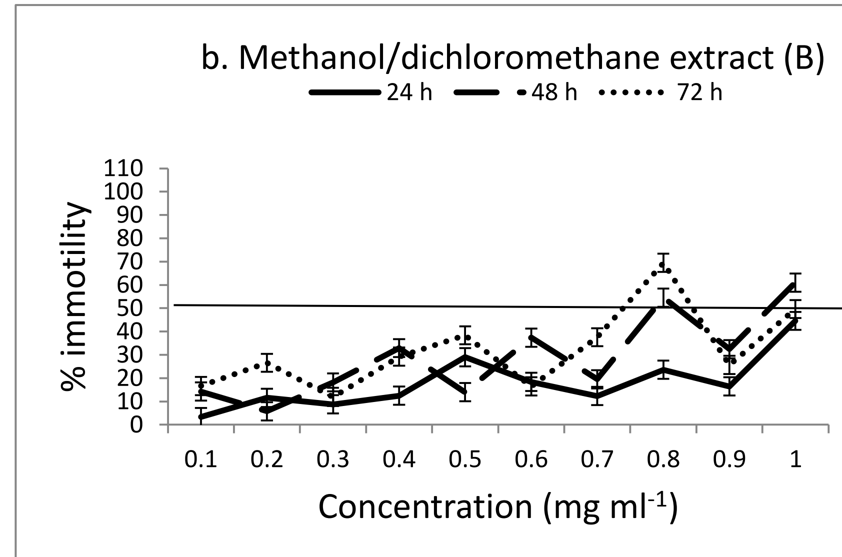


Figure 5.4 e. LC_{50} graphs of five *M. angolensis* leave extracts on *M. incognita* J2 motility inhibition at three different times of exposure *in vitro*. The Fisher's protected t-test's least significant difference (LSD) was 7.8080.

5.4.2.2. *Tabernaemontana elegans*

Only three of the extracts of *T. elegans* induced motility inhibition of nematode J2 above 50 % (Fig. 5.5 a-e). The freeze-dried crude extract (Treatment A) reached the LC_{50} at ± 0.76 mg ml^{-1} after 72 hours and at ± 0.85 mg ml^{-1} after 48 hours. Evaporated methanol/dichloromethane extract (Treatment B) reached LC_{50} at ± 0.73 mg ml^{-1} but dipped again under the set line at ± 0.85 mg ml^{-1} after 72 hours of exposure. After 48 hours exposure this extract only really passed the 50 % mark at close to 0.96 mg ml^{-1} (Fig. 5.5 b). Evaporated hexane (Treatment C) and evaporated dichloromethane (Treatment D) extracts of this crude plant leaf-meal did not inhibit motility of nematode J2 above 50 %, even at a rate of 1.0 mg ml^{-1} (Figure 5.5 c and d). This agrees with the results depicted in Figures 5.3 a-j. Freeze-dried water extract of *T. elegans* (Fig 5.5 e) reached the LC_{50} at ± 0.65 mg ml^{-1} at 48 and 72 hours and at ± 0.95 mg ml^{-1} at 24 hours after treatment.





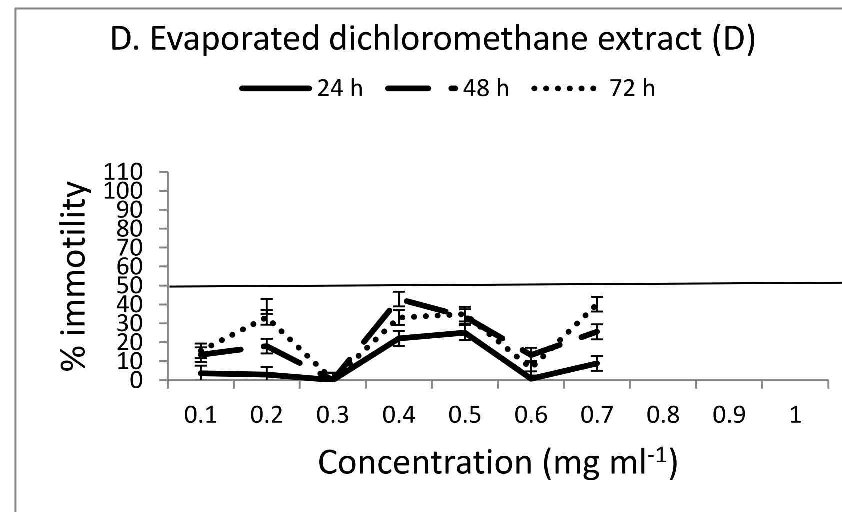


Figure 5.5 a-d. LC_{50} graphs of five *T. elegans* leave extracts on *M. incognita* J2 motility inhibition at three different times of exposure *in vitro*. The Fisher's protected t-test's least significant difference (LSD) was 7.8080

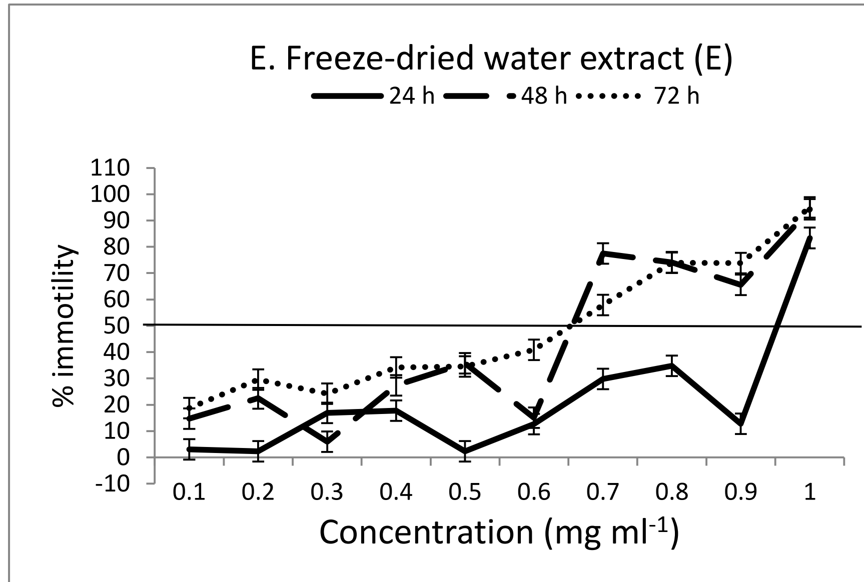
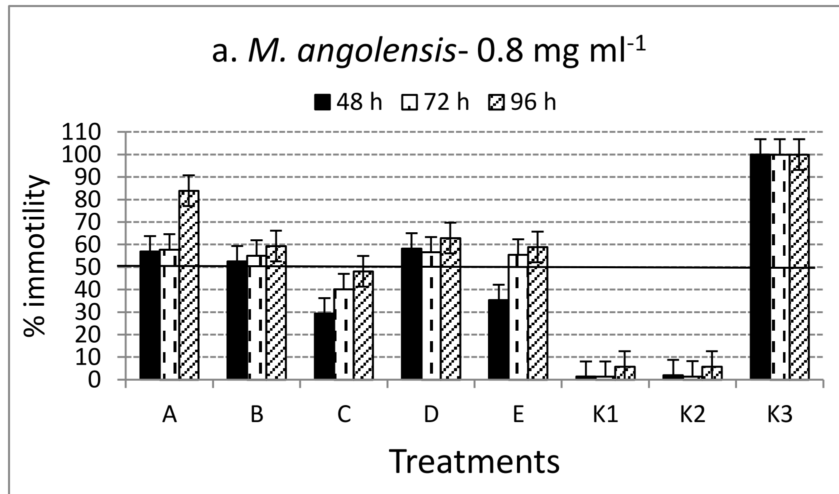


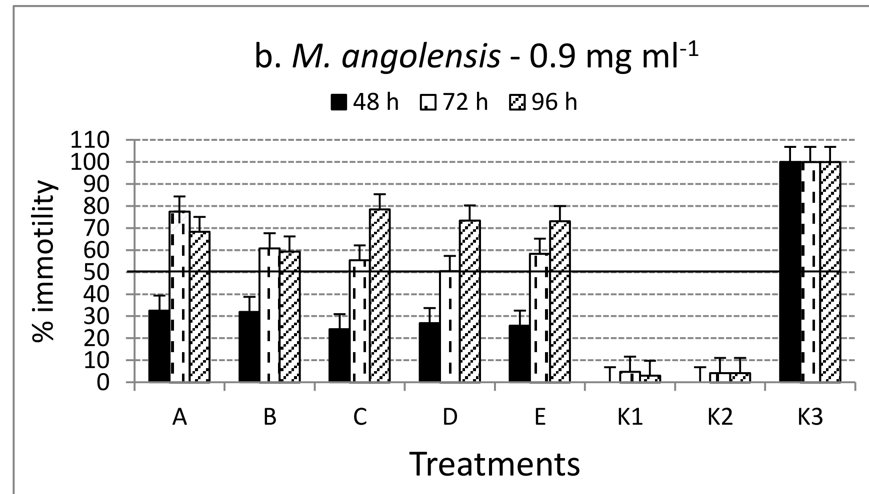
Figure 5.5. e. LC_{50} graphs of five *T. elegans* leave extracts on *M. incognita* J2 motility inhibition at three different times of exposure *in vitro*. The Fisher's protected t-test's least significant difference (LSD) was 7.8080.

5.4.3. Reversible nature of J2 motility inhibition

5.4.3.1. *Maerua angolensis*

According to Figures 5.6 a-c none of the *M. angolensis* extracts tested in this study showed reversibility in J2 motility inhibition at rates of 8 mg ml^{-1} and higher. *M. incognita* J2 immobility prevailed even when the solutions were diluted and rates of plant extracts were substantially reduced after 72 hours. The nematode-suppressive nature of these extracts seemed to be effective for a prolonged period as the immobility obtained by the concentrations of the extracts was persistent.





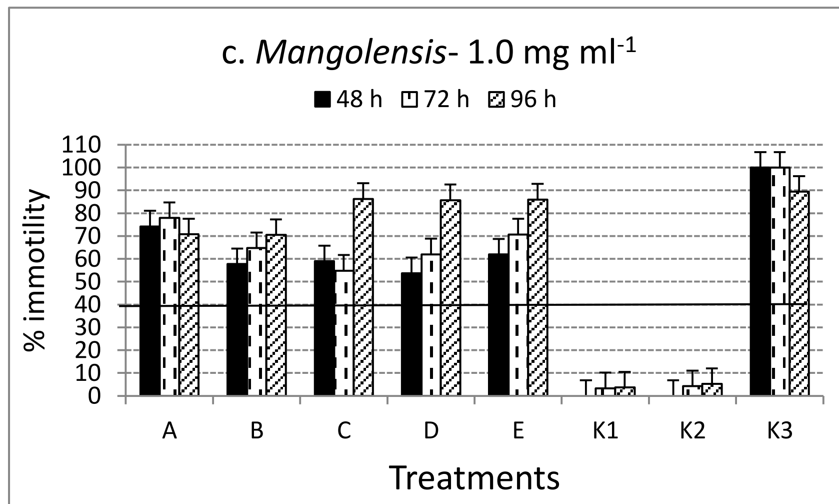
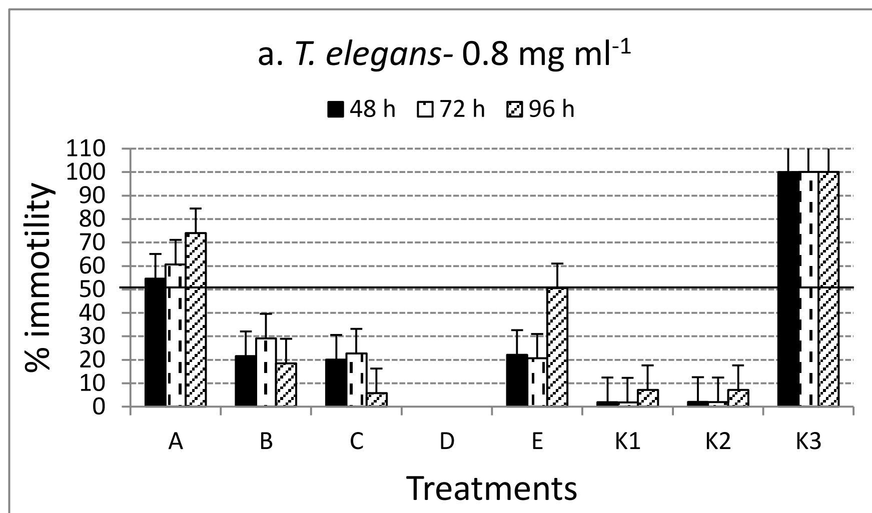
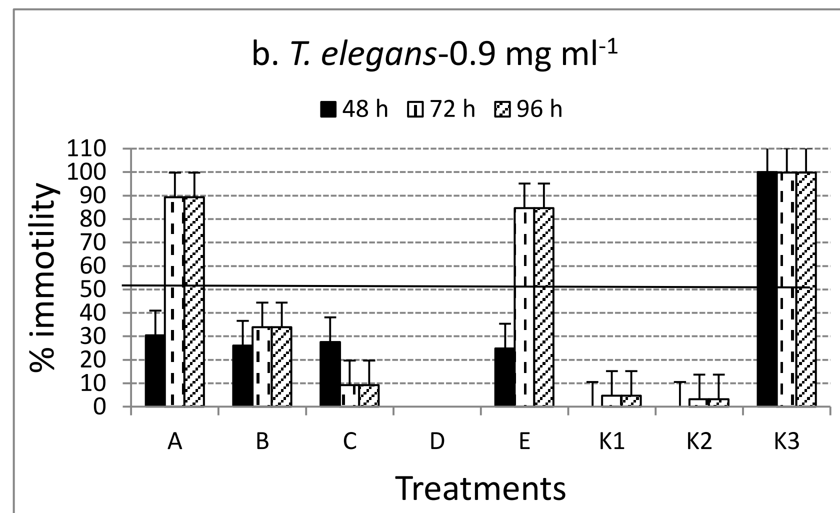


Figure 5.6 a-c. Reversible nature of J2 motility inhibition of plant extracts in *T. elegans* under 0.8-1.0 mg ml^{-1} bio-assay conditions. Treatments A = Freeze-dried crude extract, B = Methanol/ dichloromethane (1:1) extract, C = Evaporated hexane extract, D = Evaporated dichloromethane extract, E = Freeze-dried water extracts, K1 = Pure suspension solution of pluronic gel and de-ionized water, K2 = 10 % methanol, K3 = Pure salicylic acid. The Fisher's protected t-test's least significant difference (LSD) was 6.7590.

5.4.3.2. *Tabernaemontana elegans*

At 0.8, 0.9 and 1.0 mg ml^{-1} rates only *T. elegans* extracts A and E suppressed nematode J2 motility (Fig. 5.7 a-c). The motility in these treatments was not reversible after 96 h of exposure *in vitro*, even after dilution in water. Extracts B and C were only effective above 50 % at a rate of 1.0 mg ml^{-1} but nematode J2 motility seemed to have been reversible after 72 hours, even at lesser rates where they were not effective (Fig. 5.7 a-c). These assay results also agreed with those of *T. elegans* extracts in the previous tests (Figs. 5.3 and 5.5) in terms of efficacy in nematode J2 motility inhibition.





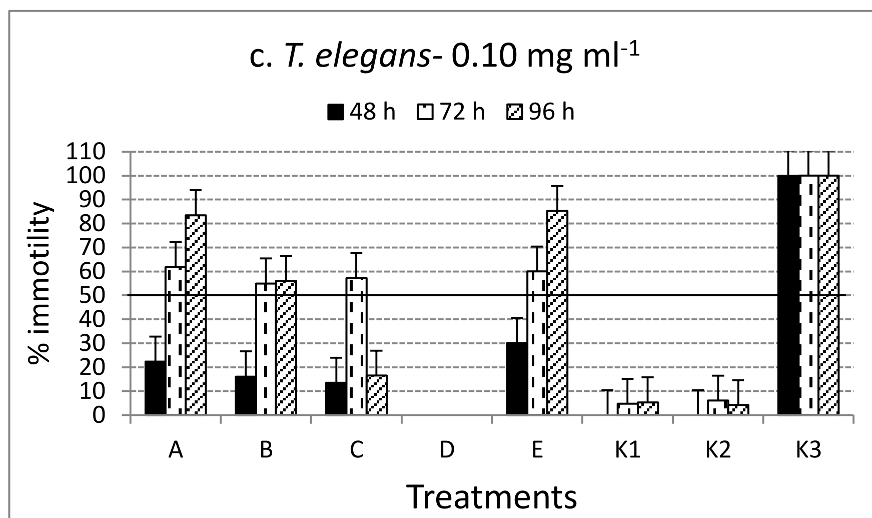


Figure 5.7 a-c. Reversible nature of J2 motility inhibition of plant extracts in *T. ele gans* under 0.8-1.0 mg ml^{-1} bio-assay conditions. Treatments A = Freeze-dried crude extract, B = Methanol/ dichloromethane (1:1) extract, C = Evaporated hexane extract, D = Evaporated dichloromethane extract, E = Freeze-dried water extracts, K1 = Pure suspension solution of pluronic gel and deionised water, K2 = 10 % methanol, K3 = Pure salicylic acid. The Fisher's protected t-test's least significant difference (LSD) was 10.5370.

5.5. Discussion

This part of the study confirmed that extracts of both plant products of *M. angolensis* and *T. elegans* might be toxic to J2 of the RKN *M. incognita* under *in vitro* laboratory conditions. This *in vitro* efficacy at least in part explains the performance of these and other crude leaf meals in controlling the nematode in the roots of tomato plants under glasshouse, microplot and field conditions (Chapters 2, 3 and 4). This part of the study also gave an indication of the concentration levels that should be aimed at when extracts from *M. angolensis* and *T. elegans* plants are prepared for further development as substances for the control of RKN in crop production. Comparative LC_{50} values in this part of the study also gave an indication of different modes of action that might be involved, both in terms of time and at least in level of *in vitro* nematode J2 inhibition. Since the same spectrum of polar to non-polar solvents were used for both plant extracts (Hansen, 2007) it can be safely stated that substances active as nematode toxicants in the two plant species tested differed in efficacy. Although one or two substances from both plant species showed promise after 24 hours, most activity was only visible after 48 hours and longer. Some concentrations seemed adequate in the region of 0.65 mg ml^{-1} but most became more stable in nematode motility inhibition at higher rates. The rates could be refined when specific compounds or molecules are isolated in future studies. The mild peak in all *M. angolensis* extracts at lower rates cannot at this stage be explained.

In a study by Wuyts *et al*. (2006) motility inhibition of *Radopholus similis* Cobb and *M. incognita* J2 occurred in 20 to 40 % of the nematodes and was reversible or only temporary for most substances tested. After 48 hours of treatment in their study *M. incognita* J2 were paralyzed but for all active substances except salicylic acid the effect was lost after 72 hours (Wuyts *et al*., 2006). Diluted in water after 72 hours of treatment, motility was completely restored in 71 % of the active substances (Wuyts *et al*., 2006). In this part of our study the difference in reversibility of effect of substances on nematode J2 motility between *M. angolensis* and *T. elegans* supports the notion that there is more than one active substance in each of the plant species that might be at least partly be involved in RKN population suppression on tomato. We suggest that

further studies into isolation of active substances should focus on those extracts that showed persistent immobilization up to 96 hours, even after dilution in water.

The specific modes of action of these nematoxic substances in extracts of these two crude leaf meals is still not known but this study has contributed substantially to the existing knowledge base by confirming that the leaf extracts might contain substances that have a significant effect on reduction of the nematode population and an increase in the growth and productivity of tomato. The effect of plant material in crude leaf-meal form may still be of multiple nature (Chapter 4) but we maintain that the potential of these plant materials as soil amendments at least at small scale could be worth further investigation and exploitation. Possible human, animal and environmental risks of their use in plant production should be determined, preferably in consultation with the traditional healers who use these materials for other purposes. A wide spectrum of new and exciting research opportunities has been revealed, however.

CHAPTER 6

SUMMATIVE CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH ON THE BOTANICAL SOIL AMENDMENTS TESTED DURING THIS STUDY

This study focused on the potential of crudely milled leaf material of selected non-crop plant species as control agents of root-knot nematodes (RKN) (*Meloidogyne incognita* race 2) applied as soil amendment to tomato. The nine selected plant species, viz. *Cassia abbreviata*, *Cissus cactiformis*, *Euphorbia ingens*, *Ipomoea kituiensis, Maerua angolensis, Senna petersiana, Synadenium cupulare*, *Tabernaemontana elegans* and *Urginea sanguinea* grow abundantly in the Mopani and Vhembe districts of the Limpopo Province. They also occur in the lowveld regions of the KwaZulu-Natal and Mpumalang provinces of South Africa. Parts of these plants are regularly collected by traditional healers for their specific potential to treat certain human and domestic animal diseases and disorders. The general effects of these plant parts on humans and livestock at controlled dosage rates, as well as some known substances contained by some of the plant species invoked a hypothesis that they might be toxic to small soil-dwelling organisms such as plant-parasitic nematodes (PPN).

Nematoxic action of almost all of the crude botanical soil amendments was consistent in all five glass house trials in contrast with several studies where results with other plant-derived soil amendments were inconsistent in this regard, according to recent literature. Some of the treatments enhanced tomato growth in the glasshouse more than others. Tomato stem height was generally substantially improved, while shoot mass seemed to be the most inconsistent growth parameter we have measured. The glasshouse study provided sufficient evidence of the suitability of our selected materials for further testing under less controlled conditions in microplots. The crude leaf meals of *C. abbreviata, I. kituinsis, S. petersiana* and *U. sanguinea* showed signs of possible phytotoxicity or allelopathy. Other unknown, detrimental effects on treated crops could have been masked by the controlled nature of this study. It was, therefore,

essential to proceed with follow-up experimentation with these materials under microplot and field conditions.

Suppression of RKN numbers associated with soil amendment of crudely milled plant leaves of *C. cactiformis, M. angolensis* and *T. elegans* in our microplot study supported the glasshouse results. The crudeness of the plant meals applied as soil amendments could be a reason why tomato growth and yield response was insignificant under microplot conditions. In the microplot study where no known critical stress was applied on the tomato plants for the duration of the trials it was not surprising that overall treatment effect was greater than that of the respective soil types on the performance of the plants, although soil type might have had an effect on nematode suppression by the experimental material.

The different pH levels of the soils tested in the microplot study could have had a substantial effect on the breakdown and uptake of nutrients and active substances contained in the different crude plant-leaf meals applied, as well as on the quantity of different nutrients taken up by the tomato plants. This suggestion is supported by the significant interaction in this study between soil type and treatment in terms of some of the nutrient element levels in tomato leaves. This is a situation that should be continuously kept in mind when further developing or testing these or similar material is done. Some elements are needed in greater quantities by growing plants than others, while others might be toxic to plants when present in the soil at too high concentrations. The presence or absence of some nutrient elements in the soil also affects the uptake of some essential elements by plants.

The field study also supported the results of the preceding glasshouse and microplot studies, particularly in terms of suppression of RKN populations in tomato by some of the botanical soil amendments tested. Suggestions that soil amendment with crude leaf meals of *E. ingens* and *S. cupulare* could be phytotoxic or allelopathic to tomato were reinforced. Contrary to this, soil amendment with *C. cactiformis*, *M. angolensis* and *T. elegans* persistently enhanced growth of the crop. However, because of the lack of correspondence between the leaf nutrient contents

of the tomato and the nutrient contents of the soil amendments we suggest that these botanical materials had negligible soil fertilization effects. The comparative superior efficacy of *M. angolensis* and *T. elegans* over the other plant soil amendments tested in the glasshouse, microplot and field studies in the suppression of egg and J2 numbers of *M. incognita*, as well as the former's similar performance to the standard nematicide fenamiphos, provided motivation for further testing of these materials under *in vitro* bioassay studies.

The *in vitro* bioassay study confirmed that extracts in solvents with different polarities of both *M. angolensis* and *T. elegans* might be toxic to J2 of the RKN *M. incognita*. This part of the study also gave an indication of the concentration levels that should be aimed at when extracts from *M. angolensis* and *T. elegans* plants are prepared for further development. Comparative LC_{50} values in the *in vitro* study also gave an indication that different modes of action might be involved, both in terms of time after exposure and in the level of *in vitro* nematode J2 inhibition. Although substances from both plant species showed some action after 24 hours, most motility inhibition occurred after 48 hours and longer. Some extracts seemed to become potent at concentrations in the region of 0.65 mg ml^{-1} but most were more effective at higher concentrations. Application rates could be refined as specific compounds or molecules are isolated in future studies. In the reversibility part of the study some extracts of both plant species showed persistent immobilization of nematode J2 for up to 96 hours, even after dilution in water. This supports the notion that there might be more than one active substance in each of the plant species that are at least partly involved in RKN population suppression on tomato. We suggest that further studies into the isolation of active substances should focus on those extracts in our study that showed persistent J2 immobilization.

The specific modes of action of the nematoxic substances in extracts of these two crude plant amendments are still not known but this study has contributed substantially to the knowledge base on botanical soil amendments by confirming that more plant materials might contain substances that have a significant effect on reduction of PPN populations and increased growth and productivity of tomato. We, therefore, suggest that the potential of these plant materials

as soil amendments are worth further investigation and exploitation. Possible human, animal and environmental risks of their use in plant production should be determined, preferably in consultation with the traditional healers that use these materials for other purposes. These materials could be used as soil amendments in integrated nematode control strategies and that their potential could be developed for small-scale farming communities, domestic gardeners and commercial farmers in the Mpumalanga, Limpopo and Kwazulu/Natal Provinces of South Africa. Over-exploitation of the resources should be kept in consideration from the start, however. It must also be noted that inconsistencies, especially in growth response of tomato to the amendments highlight the crudeness of these materials and their need for refinement to improve sustainability in their performance.

BIBLIOGRAPHY

ADAMU, A., ABDURAHMAN, E.M., IBRAHIM, H., ABUBAKAR, M.S., MAGAJI, M.G. AND YARO, A.H. 2007. Effect of aqueous methanolic stem bark extract of *Maerua angolensis* DC. on acute and sub-acute inflammations. *Nigerian Journal of Pharmaceutical Sciences*, 6 (2): 1-6.

ADJANOHOUN, E., ADJAKIDJE, V. AND AHYI, M.R.A. 1989. Contribution aux études ethnobotaniques et floristiques en République Populaire du Bénin. Paris: Agence de Coopération Culturelle et Technique. 895 pp.

AKHTAR, M. 1991. Studies on the management of plant parasitic nematodes with organic soil amendments. PhD Thesis. Aligarh Muslim University, Aligarh, India. 195 pp.

AKHTAR, M. 1993. Utilisation of plant-origin waste materials for the control of parasitic nematodes. *Bioresource Technology,* 46: 255-257.

AKHTAR, M. 1997. Current options in integrated management of plant parasitic nematodes. *International Pest Management Reviews,* 2: 187-197.

AKHTAR, M. 1998. Biological control of plant-parasitic nematodes by neem products in agricultural soil. *Applied Soil Ecology*, 7: 219-223.

AKHTAR, M. 2000. Nematicidal potential of the neem tree *Azadirachta indica* (A. Juss). *Integrated Pest Management Reviews*, 5: 57-66.

AKHTAR, M. AND ALAM, M.M. 1993. Control of plant-parasitic nematodes by ‘Nimin,’ a urea-coating agent and some plant oils. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, 100: 337-342.

AKHTAR, M. AND MAHMOOD, I. 1993. Utilization of fallen leaves as soil amendments for the control of plant-parasitic nematodes. *Pakistan Journal of Nematology*, 11: 131-38.

AKHTAR, M. AND MAHMOOD, I. 1994a. Control of root-knot nematode by bare-root dip in undecomposed and decomposed extracts of neem cake and leaf. *Nematologia Mediterranea,* 22: 55-57.

AKHTAR, M. AND MALIK, A. 2000. Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: A review. *Bioresource Technology*, 74: 35-47.

ALAM, M.M. AND JAIRAIJPURI, M.S. 1990. Natural enemies of nematodes. In: Jairajpuri, M.S., Alam, M.M. and Ahmad, I. *eds*. Nematode Bio-control (Aspects and Prospects). New Delhi, International Publishing House. 40 pp.

ALIBER, M. 2009. Exploring Statistics South Africa's national household surveys as sources of information about food security and subsistence agriculture. Unpublished Report, Centre for Poverty Employment and Growth: Human Sciences Research Council. Pretoria. 68 pp.

ANONYMOUS. 2003. Variations of anti-oxidants and their activity in tomato. Asian Vegetable Research and Development Corporation, Progress report. 115 pp.

ARNOLD, H.J. AND GULUMIAN, M. 1984. Pharmacopoeia of traditional medicine in Venda. *Journal of Ethnopharmacology*, 12: 35-74.

BAGAVATHI, R., SORG, B. AND HECKER, E. 1988. Tigliane-type diterpene esters from *Synadenium grantii*. *Planta Medica*, 54: 506-510.

BAR-EYAL, M., SHARON, E. AND SPIEGEL, Y. 2006. Nematicidal activity of *Chrysanthemum coronarium*. *European Journal of Plant Pathology*, 114: 427-433.

BATISH, D.R., SINGH, H.P., KOHLI, R.K. AND KAUR, S. 2008. Eucalyptus essential oil as a natural pesticide. *Forest Ecology and Management*, 256: 2166-2174.

BELLO, A. 1998. Biofumigation and integrated crop management. In: Bello, A., Gonzalez, J.A., Aries, M. and Rodríguez-Kábana, R. *eds*. Alternatives to methyl bromide for the Southern European Countries. International Workshop, Arona, Tenerife, Spain. 126 pp.

BHATTACHARYA, D. AND GOSWAMI, B.K. 1988. Effect of oil-cakes used alone and in combination with aldicarb on *Meloidogyne* infecting tomato. *Journal of Nematology,* 16: 139-141.

BIDWELL, R.G.S. 1974. Plant Physiology. New York: Macmillan. 641 pp.

BOS, L. AND PARLEVLIET, J.E. 1995. Concepts and terminology on plant/pest relationships: Towards consensus in plant pathology and crop protection. *Annual Review of Phytopathology*, 33: 69-102.

BRIDGE, J. 1996. Nematode management in sustainable and subsistence agriculture*. Annual Review of Phytopathology*, 34: 201-225.

BRIDGE, J. AND STARR, J.L. 2007. Plant nematodes of agricultural importance. A color handbook. Boston: Academic Press. 152 pp.

BURTT DAVY, J. 1904. Report of the botanist. Pretoria: Transvaal Department of Agriculture. 261 pp.

CAMPBELL, C.R. 2000. Reference sufficiency ranges for plant analysis in the southern region of the United States. *Southern Cooperative Series Bulletin* 394. 122 pp.

CASTAGNONE-SERENO, P. AND KERMARREC, A. 1991. Invasion of tomato roots and reproduction of *Meloidogyne incognita* as affected by raw sewage sludge. *Journal of Nematology,* 23: 724-728.

CASWELL, E.P., NELSEN, C.E. AND THOMASON, I.J. 1985. A microplot method for recovery of entire plant root systems and their associated endoparasitic and semi-endoparasitic nematodes. *Revue de Nématologie,* 8(1): 85-92.

CHAVARRIA-CARVAJAL, J.A. AND RODRÍGUEZ-KÁBANA, R. 1998. Changes in soil enzymatic activity and control of *Meloidogyne incognita* using four organic amendments. *Nematropica,* 28: 7-18.

CHAVARRIA-CARVAJAL, J.A., RODRÍGUEZ-KÁBANA, R., KLOEPPER, J.W. AND MORGAN-JONES, G. 2001. Changes in populations of microorganisms associated with organic amendments and benzaldehyde to control plant-parasitic nematodes. *Nematropica*, 31: 165-180.

CHHABRA, S.C., MAHUNNAH, R.L.A. AND MSHIU, E.N. 1989. Plants used in traditional medicine in Eastern Tanzania. 11. Angiosperms (Capparidaeae to Ebenaceae). *Journal of Ethnopharmacology*, 25: 339-359.

CHITWOOD, D.J. 2002. Phytochemical-based strategies for nematode control. *Annual Review of Phytopathology*, 40: 221-249.

COYNE, D., MOENS, M. AND FOURIE, H. 2009. Current and future management strategies in resource-poor farming. In: Perry, R.N., Moens, M., and Starr, J.L. *eds*. Root-knot Nematodes. Wallingford: CAB International. pp 444-475.

CROW, W.T. 2005. Alternatives to fenamiphos for management of plant-parasitic nematodes on bermudagrass. *Journal of Nematology*, 37: 477-482.

DUNCAN, L.W. 1991. Current options for nematode management. *Annual Review of Phytopathology*, 29: 467-490.

EDDAOUDI, M. AND AMMATI, M. 1995. Study on the effects of single and combined soil solarization and the use of resistant tomato varieties on the population of *Meloidogyne javanica* and production of tomato in South Morocco. *Afro-Asian Journal of Nematology*, 5: 28-33.

EVANS, F.J. AND TAYLOR, S.E. 1983. Pro-inflammatory, tumour-promoting and anti-tumour diterpenes of the plant families Euphorbiaceae and Thymelaeaceae. Fortschritte der Chemie Organischer Naturstoffe 44. 99 pp.

FERRAZ, L.C.C.B. AND BROWN, D.J.F. 2002. An introduction to nematodes: Plant Nematology. A student's textbook. Sofia: Pensoft. 220 pp.

FERRAZ, S. AND DE FREITAS, L.G. 2004. Use of antagonistic plants and natural products. In: Chen Z.X., Chen S.Y. and Dickson D.W. *eds.* Nematology Advances and Perspectives. Beijing: Tsinghua University Press. pp. 931-977.

FIUME, F. AND PARASI, B. 1995. La difesa del pomodora in coltura protetta contro nematode galligeni (*Meloidogyne* Goeldi) e *Pyrenochaeta lycopersici* (Schnaider et Gerlach) in associazione. *Informatore Fitopatologico*, 45: 44-50.

FOURIE, H. AND SCHOEMAN, M.J. 1999. Nematode management in home gardens and small-scale farming systems: a preliminary study. Proceedings of the 14th Symposium of Nematological Society of Southern Africa. *African Plant Protection*, 5: 1-20.

FOURIE, H., MC DONALD, A.H., MOTHATA, T.S., NTIDI, K.N. AND DE WAELE, D. 2012. Indications of variation in host suitability to root-knot nematode populations in commercial tomato varieties. *African Journal of Agricultural Research,* 7: 2344-2352.

FORTUNUM, B.A., KASPERBAUER, M.J., HUNT, P.G. AND BRIDGES, W.C. 1991. Biomass partitioning in tomato plants infected with *Meloidogyne incognita*. *Journal of Nematology*, 23: 291-297.

GONZALEZ, J.A. AND ESTEVEZ-BRAUN, A. 1997. Phytonematicidal activity of aromatic compounds related to shikimate pathway. *Pesticide Biochemistry and Physiology*, 58: 193-197.

GRECO, N. AND DI VITO, M. 2009. Population dynamics and damage levels. In: Perry. R.N., Moens M. and Starr J.L. *eds.* Root-knot Nematodes. Wallingford: CAB International. pp. 246-274.

HANSEN, C.M. 2007. Hansen solubility parameters: a user's handbook, 2nd Edition. Boca Raton: CRC Press. 197 pp.

HOOKS, C.R. R., WANG, K.H., PLOEG, A. AND MCSORLEY, R. 2010. Using marigold (*Tagetes* spp.) as a cover crop to protect crops from plant-parasitic nematodes. *Applied Soil Ecology*, 46: 307-320.

HUSSEY, P.S. AND BAKER, K.R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* species, including a new technique. *Plant Disease Reporter*, 57: 1025-1028.

HUSSEY, P.S. AND BOERMA, H.R. 1981. A greenhouse screening procedure for root-knot nematode resistance in soybeans. *Crop Science*, 21: 794-796.

IPNI. 2012. International Plant Name Index. http://www.ipni.org/. Accessed on 15 November 2012.

IOANNOU, N., IOANNOU, M. AND PAROUSI, G. 2002. Integrated management of soil-borne pathogens of green-house tomato in Cyprus. In: Voyiatzis, D. and Paroussis, E. *eds*. Proceedings of the 2nd Balkan Symposium of Vegetable and Potatoes. Thessaloniki, Greece, 2000. *Acta Horticulturae,* 579: 433-438.

JAIN, S.C., JAIN, R., SHARMA, R.A. AND CAPASSO, F. 1997. Pharmacological investigation of *Cassia italic*a. *Journal of Ethnopharmacology,* 58: 135-142.

JOHNSON, A.W. 1985. The role of nematicides in nematode management. In: Sasser, J.N. and Carter, C.C. *eds*. An Advanced Treatise on *Meloidogyne*. Volume 1. Biology and Control. Raleigh: North Carolina State Graphics. pp. 249-301.

JOHNSON, A.W. 1998. Vegetable crops. In: Baker, K. R., Pederson, G. A. and Windham, G. L. *eds.* Plant and Nematode Interactions. Madison: American Society of Agronomy. pp. 595-636.

JOHNSON, A.W. AND FASSULIOTIS, G. 1984. Nematode parasites of vegetable crops. In: Nickle, W. R. *ed.* Plant and Insect Nematodes. New York: Marcel Dekker Inc. pp. 323-372.

JOHNSON, J.T., RICH, J.R. AND BOATRIGHT, A.W. 1981. A technique for establishing microplots in the field. *Journal of Nematology,* 13(2): 233-235.

JOHNSON, C.S., WAY, J. AND BARKER, K.R. 2005. Nematodes parasites of tobacco. In: Luc, M., Sikora, R.A. and Bridge, J. *eds.* Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. Second Edition. Wallingford: CAB International. pp. 675-708.

KADIOGLU, I., YANAR, Y. AND ASAV, U. 2005. Allelopathic effects of weeds extracts against germination of some plants. *Journal of Environmental Biology*, 26: 169-173.

KATAN, J. 1981. Solar heating (solarization) of soil for control of soilborne pests. *Annual Review of Phytopathology,* 19: 211-236.

KINGHORN, A.D. 1980. Major skin-irritant principle from *Synadenium grantii*. *Journal of Pharmacology,* 69: 1446-1447.

KINLOCH, R.A. AND DUNAVIN, L.S. 1993. Summer cropping effects on the abundance of *Meloidogyne arenaria* race 2 and subsequent soybean yield. *Journal of Nematology*, 25: 806-809.

KIRKEGAARD, J.A. GARNDER, P.A., DESMARCHELIER, J.M. AND AGNUS. J.F. 1993. Biofumigation using *Brassica* species to control pests and diseases in horticulture and agriculture. In: Wratter, N., and Mailer, R. *eds*. Proceedings of the 9th Australian Assembly on Brassicas, Wagga Wagga 5-7 October. pp. 77-82.

KIRKEGAARD, J.A., SARWAR, M., MATTHIESSEN, J.N. THOMAS, G. AND MONTERIO, A.A. 1998. Assessing the biofumigation potential of crucifers. *Acta Horticulturae*, 459: 105-111.

KLEYNHANS, K.P.N., VAN DEN BERG, E., SWART, A., MARAIS, M. AND BUCKLEY, N.H. 1996. Plant nematodes in South Africa. Plant Protection Research Institute Handbook No 8. Pretoria: Agricultural Research Council. 165 pp.

KOKALIS-BURELLE, N., RODRÍGUEZ-KÁBANA, R., WEAVER, C.F. AND KING, P.S. 1994. Evaluation of powdered pine bark for control of *Meloidogyne arenaria* and *Heterodera glycines* on soybean. *Plant and Soil,* 162: 163-168.

KONG, J.O., LEE, S.M., MOON, Y.S., LEE, S.G. AND AHN, Y.J. 2006. Nematicidal activity of plant essential oils against *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae). *Journal of Asia-Pacific Entomology*, 9: 173-178.

LABADARIOS, D., MCHIZA, Z.J., STEYN, N.P., GERICKE, G., MAUNDER, E.M.W., DAVIDS, Y.D. AND PARKER, W. 2012. Food security in South Africa: a review of national surveys. *Bulletin of the World Health Organization,* 89(12): 853-928.

LITTERICK, A.M., HARRIER, L., WALLACE, P., WATSON, C.A. AND WOOD, M. 2004. The role of uncomposted materials, composts, manures, and compost extracts in reducing pest and disease incidence and severity in sustainable temperate agricultural and horticultural crop production – A review. *Critical Reviews in Plant Sciences,* 23: 453-479.

MAFEO, T.P. AND MASHELA, P.W. 2009. Responses of germination in tomato, watermelon and butternut squash to a C*ucumis* bio-nematicide. *American Eurasian Journal of Agricultural and Environmental Sciences,* 6: 215-219.

MAKKAR, H.P.S. 1999. Quantification of tannins in tree foliage: A laboratory manual for the FAO/IAEA Co-ordinated Research Project on Use of Nuclear and Related Techniques to develop simple tannin assay for predicting and improving the safety and efficiency of feeding ruminants on the Tanniniferous Tree foliage., Vienna: IAEA Working Document. 29 pp.

MARLOTH, R. 1932. The flora of South Africa. London: Wesley. 94 pp.

MASARIRAMBI, M.T., HLAWE, M.M., OSENI O.T. AND SIBIYA, T.E. 2012. Effects of kraal manure, chicken manure and inorganic fertilizers on growth and yield of lettuce (*Lactuca sativa* L. var Commander) in a semi-arid environment. *Asian Journal of Agricultural Sciences*, 4(1): 58-64.

MASHELA, P.W. 2002. Ground wild cucumber fruits suppress numbers of *Meloidogyne incognita* on tomato production. *Nematropica*, 22: 13-19.

MASHELA, P.W. AND MPATI, K.W. 2002. Effects of castor bean fruit residues on population densities of *Meloidogyne incognita*, soil properties and growth of tomato plants. *African Plant Protection*, 10: 125-142.

MASHELA, P.W. AND MPHOSI, M.S. 2002. Wild cucumber fruit residues reduce population densities of *Meloidogyne incognita* on tomato plants. *African Plant Protection*, 10: 125-142.

MASHELA, P.W. AND NTHANGENI, M.E. 2002. Efficacy of *Ricinus communis* fruit meal with and without *Bacillus* species on suppression of *Meloidogyne incognita* and growth of tomato. *Journal of Phytopathology,* 150: 399-402.

MASHELA, P.W., DE WAELE, D AND POFU, K.P. 2011. Use of indigenous *Cucumis* technologies as alternative to synthetic nematicides in management of root-knot nematodes in low-input agricultural farming systems: A review. *Scientific Research and Essays,* 6(33): 6762-6768.

MC DONALD, A.H. 1998. Management of *Ditylenchus africanus* (Nematoda: Tylenchidae) on groundnut (*Arachis hypogaea*). Ph.D Thesis, North-West University, Potchefstroom, South Africa.

MC DONALD, A.H. AND NICOL, J. 2005. Nematode parasites of cereals. In: Luc, M., Sikora, R.A. and Bridge, J. *eds.* Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. Second Edition. Wallingford: CAB International. 131-192.

MCSORLEY, R. 2011. Overview of organic amendments for management of plant-parasitic nematodes, with case studies from Florida. *Journal of Nematology,* 43: 69-81.

MCSORLEY, R., AND GALLAHER, R.N. 1995. Effect of yard waste compost on plant-parasitic nematode densities in vegetable crops. *Supplement to the Journal of Nematology*, 27: 545-549.

MCSORLEY, R. AND MCGOVERN, R.J. 2000. Effects of solarization and ammonium amendments on plant-parasitic nematodes. *Supplement to the Journal of Nematology,* 32: 537-541.

MCVANN, A., HAVLIK, I., JOUBERT, P.H. AND MONTEAGUDO, F.S.E. 1992. Cardiac glycoside poisoning involved in deaths from traditional medicine. *South African Medical Journal*, 81: 139-141.

MEYER, S.L.F., ZASADA, I.A., ROBERTS, D.P., VINYARD, B.T., LAKSHMAN, D.K., LEE, J.K, CHITWOOD, D.J. AND LYNN, K. 2006. *Plantago lanceolata* and *Plantago rugelii* extracts are toxic to *Meloidogyne incognita* but not to certain microbes. *Journal of Nematology*, 38: 333-338.

MIAMI, I.H. AND RODRÍGUEZ-KÁBANA, R., 1982. Organic amendments with high tannin and phenolic contents for control of *Meloidogyne arenaria* in infested soil. *Nematropica*, 12: 221-234.

MILNE, D.L., VAN LELYVELD, L.J. AND DE VILLIERS, E.A. 1977. The response of peroxidase and indoleacetic acid oxidase in pineapple roots to foliar application of phenamiphos. *Agrochemophysica,* 9: 65-70.

MPHOSI, M.J., MASHELA, P.W. AND SHAKWANE, M.K. 2002. Efficacy of *Cucumis myriocarpus* with and without *Bacillus* species on suppression of *Meloidogyne incognita. African Plant Protection*, 10: 125-142.

MTSHALI, M., FOURIE, H. AND Mc DONALD, A.H. 2002. Incidence of root-knot nematodes in resource-poor agriculture in South Africa. *African Plant Protection*, 10: 125-142.

MULLER, R. AND GOOCH, P.S. 1982. Organic amendments in nematode control: An examination of the literature. *Nematropica*, 12: 319-326.

NGOBENI, G.L., MAFEO, T.P. AND MASHELA, P.W. 2002. The effect of *Bacillus* species, *Lippia javanica* and *Meloidogyne incognita* on tomato growth and yield under field conditions. *African Plant Protection*, 10: 125-142.

NKUNYA, M.H.H. 1985. A search for potentially useful compounds from Tanzanian plants. Proceedings of the Science Symposium, University of Dar es Salaam Tanzanian Commission for Science and Technology. 75 pp.

NOLING, J.W. 1999. Plant resistance and soil amendments in Florida tomato and pepper. In: Obenauf, G.L. *ed*. Annual Research Conference on Methyl Bromide Alternatives and Emissions Reductions. Fresno: Methyl Bromide Alternatives Outreach. 35 pp.

NOLING, J.W. AND BECKER, J.O. 1994. The challenge of research and extension to define and implement alternatives to methyl bromide. *Journal of Nematology*, 26: 573-586.

NONO-WOMDIM, R., SWAI, I.S., MROSSO, L.K., CHADHA, M.L. AND OPENA, R.T. 2002. Identification of root-knot nematode species occurring on tomatoes in Tanzania and resistant lines for their control. *Plant Disease*, 86 (2): 127-130.

NTALLI, N.G. AND MENKISSOGLU-SPIROUDI, U. 2011. Pesticides of botanical origin: A promising tool in plant protection. In: Pesticides - Formulations, Effects Fate, M. Stoytcheva *eds.* InTech, 7: 307-532 Available from: http://www.intechopen.com/books/pesticides-formulations-effectsfate/ pesticides-of-botanical-origin-a-promising-tool-in-plant-protection

NTIDI, N.K., FOURIE, H., MC DONALD, A.H., DE WAELE, D. AND MIENIE, C.M.S. 2012. Plant-parasitic nematodes associated with weeds in developing agriculture in South Africa. *Nematology*, 14: 875-887.

OKA, Y. 2010. Mechanisms of nematode suppression by organic soil amendments- A review. *Applied Soil Ecology*, 44: 101-115.

OKA, Y. AND YERMIYAHU, U. 2002. Suppressive effects of composts against the root-knot nematode *Meloidogyne javanica* on tomato. *Nematology*, 4: 891-898.

OKA, Y., TKACHI, N., SHUKER, S. AND YERMIYAHU, U. 2007. Enhanced nematicidal activity of organic and inorganic ammonia-releasing amendments by *Azadirachta indica* extracts. *Journal of Nematology*, 39: 9-16.

POFU, K.M., MASHELA, P.W. AND MPHOSI, M.S. 2010. Responses of *Cucumis africanus* and *Cucumis myriocarpus* to *Meloidogyne incognita* race 2 under microplot conditions. *International Journal of Nematology,* 20: 113-118.

POWERS, L.E. AND MCSORLEY, R. 2000. Ecological principles of agriculture. Albany: Delmar Thomson Learning. 433 pp.

REDDY, G.S., RAO, V.K., SITARAMAIH, K. AND CHALAM, R.V. 2001. Soil solarization for control of the soil-borne pathogens complex due to *Meloidogyne incognita* and *Pythium aphanidermatum*. *Indian Journal of Nematology*, 31: 136-138.

RIEGEL, C., FERNANDEZ, F.A. AND NOE, J.P. 1996. *Meloidogyne incognita* infested soil amended with chicken litter. *Journal of Nematology,* 28: 369-378.

RITZINGER, C.H.S.P. AND MCSORLEY, R. 1998. Effect of castor and velvet bean organic amendments on *Meloidogyne arenaria* in greenhouse experiments. *Journal of Nematology*, 30: 624-631.

RODRÍGUEZ-KÁBANA, R. 1986. Organic and inorganic nitrogen amendments to soil as nematode suppressants. *Journal of Nematology*, 18: 129-135.

RODRÍGUEZ-KÁBANA, R. AND KING, P.S. 1980. Use of mixture of urea and blackstrap molasses for control of root-knot nematodes in soil. *Nematropica*, 10: 38-44.

RODRÍGUEZ-KÁBANA, R., KING, P.S. AND POPE, M.H. 1981. Combinations of anhydrous ammonia and ethylene dibromide for control of nematodes parasitic of soybeans. *Nematropica,* 11: 27-41.

RODRÍGUEZ-KÁBANA, R., SHELBY, R.A., KING, P.S. AND POPE, M.H., 1982. Combinations of anhydrous ammonia and 1,3-dichloropropene for control of root-knot nematodes in soybeans. *Nematropica,* 12: 61-69.

RODRÍGUEZ-KÁBANA, R., WEAVER, C.F., ROBERTSON, D.G. AND SNODDY, E.L. 1986. Population dynamics of *Meloidogyne arenaria* juveniles in a field with 'Florunner' peanut. *Nematropica,* 16: 185-196.

RODRÍGUEZ-KÁBANA, R., MORGAN-JONES, G. AND CHET, I. 1987. Biological control of nematodes: soil amendments and microbial antagonists. *Plant and Soil*, 100: 237-247.

RODRÍGUEZ-KÁBANA, R., BOUBE, D. AND YOUNG, R.W. 1989. Chitinous materials from blue crab for control of root-knot nematode. I. Effect of urea and enzymatic studies. *Nematropica,* 19: 53-74.

SANO, Z. 2005. Cultural control of the nematode damage. In: Cheema, Z.A., Farooq, M. and Wahid, A. *eds*. Allelopathy. Current Trends and Future Applications. New York, Springer. 113 pp.

SAS INSTITUTE, INC. 1999. SAS/STAT User's Guide, Version 9, 1st printing, Volume 2. Cary: SAS Institute Inc. 9 pp.

SHAKIL, N.A., PANKAJ KUMAR, J., PANDEY, R.K. AND SAXENA, D.B. 2008. Nematicidal prenylated flavanones from *Phyllantus niruri*. *Phytochemistry* 69: 759-764.

SIKORA, R.A. 1992. Management of the antagonistic potential in agricultural ecosystems for the biological control of plant parasitic nematodes. *Annual Review of Phytopathology*, 30: 245-270.

SIKORA, R.A. 2002. Strategies for biological system management of nematodes in horticulture crops: fumigate, confuse or ignore them. *Communications in Agricultural and Applied Biological Sciences*, 67: 5-18.

SIKORA, R.A. AND FERNANDEZ, E. 2005. Nematode parasites of vegetables. In: Luc, M., Sikora, R.A. and Bridge, J. *eds.* Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. Second Edition. Wallingford: CAB International. pp. 319-392.

SIKORA, R.A., SINGH, R.S. AND SITARAMAIAH, K. 1973. Control of root-knot nematodes through organic and inorganic soil amendments. Effects of rice husk and sugarcane bagasse. *Haryana Journal of Horticultural Sciences*, 2: 123-127.

SIKORA, R.A. BRIDGE, J. AND STARR, J.L. 2005a. Management practices: An overview of integrated nematode management technologies. In: Luc, M., Sikora, R.A. and Bridge, J. *eds.* Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. Second Edition. Wallingford: CAB International. pp. 793-826.

SIKORA, R.A., GRECO. N. AND SILVA J.F.V. 2005b. Nematode parasites of food legumes. In: Luc, M., Sikora, R.A. and Bridge, J. *eds.* Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. Second Edition. Wallingford: CAB International. pp. 259-318.

SIM, T.R. 1907. The forests and forest flora of the Cape of Good Hope. Aberdeen: Taylor and Henderson. 361 pp.

SINGH, R.S. AND SITARAMAIAH, K. 1966. Effect of decomposing green leaves, sawdust and urea on the incidence of root-knot to okra and tomato. *Indian Journal of Phytopathology* 20: 349-355.

SINGH, R.S. AND SITARAMAIAH, K. 1967. Influence of root-knot of okra and tomatoes in oil-cake amended soil. *Plant Disease Reporter* 50: 668-672.

SNEDECOR, G.W. AND COCHRAN, W.G. 1980. Statistical methods (Seventh Edition.). Ames: Iowa State University Press. 507 pp.

SOUTH AFRICA. 2007. A Guide for the Control of Plant Pests. 40th Edition. National Department of Agriculture, Directorate Food Safety and Quality Assurance, Sub-directorate Agricultural Production Inputs. Pretoria: Department of Agriculture. 275 pp.

SPOERKE, D.G., MONTANIO, C.D. AND RUMACK, B.H. 1985. Pediatric exposure to the houseplant *Synadenium grantii. Veterinary and Human Toxicology*, 28: 283-284.

STAPLETON, J., DUNCAN, R.A. AND JOHNSON, R. 1998. Soil disinfection with cruciferous amendments and sub-lethal heating: effects on *Meloidogyne incognita*, *Sclerotium rolfisii* and *Pythium ultimum*. Proceedings of the 7th International Congress of Plant Pathology, Edinburgh. *Journal of Plant Pathology*, 47: 737-742.

STEYN, D.G. 1949. Poisoning in man and animals with special reference to plant poisoning. Pretoria: J. L. van Schaik. 264 pp.

STIRLING, G.R. 1991. Biological Control of Plant Parasitic Nematodes. Wallingford: CAB International. 282 pp.

THODEN, T.C., KORTHALS, G.W. AND TERMORSHUIZEN, A.J. 2011. Organic amendments and their influences on plant-parasitic and free-living nematodes: a promising method for nematode management? *Nematology*, 13: 133-153.

TRIVEDI, P.C. AND BARKER, K.R. 1986. Management of nematodes by cultural practices. *Nematropica*, 16: 213-236.

TSHIKALANGE, T.E., VAN VUUREN, S.F. AND NAIDOO, D. 2005. Antimicrobial investigation of plants used traditionally in southern Africa to treat sexually transmitted infections. *Journal of Ethnopharmacology*, 96: 515-519.

TSHIKALANGE, T.E., MEYER, J.J.M., LALL, N., MUNOZ, E., SANCHO. R., VAN DE VENTER, M. AND OOSTHUIZEN, V. 2008. *In vitro* anti-HIV-1 properties of ethnobotanically selected South African plants used in the treatment of sexually transmitted diseases. *Journal of Ethnopharmacology*, 119: 478-481.

VAHRMEIJER, J.H. 1981. Poisonous plants of Southern Africa that Cause Stock Losses. Cape Town: Tafelberg Publishers. 168 pp.

VAN DER BERG, S., 2006. Public spending and the poor since the transition to democracy. In: Bhorat and Kanbur. *eds*. Poverty and Policy in Post-Apartheid South Africa. Pretoria: Human Sciences Research Council. pp. 201-231.

VAN DER HEIJDEN, R., BROUWER R.L., VERPOORTE R., WIJNSMA., VAN BEEK T.A., HARKES P.A.A. AND SVENDSEN A.B. 1986. Indole alkaloids from a callus culture of *Tabernaemontana elegans*. *Journal of Phytochemistry,* 25: 393-397.

VAN WYK, B.E., VAN OUDTSHOORN, B. AND GERICKE, N. 1997. Medical plants of South Africa. Pretoria: Briza. 273 pp.

VAN WYK, B-E. AND WINK, M. 2004. Medicinal Plants of the World. Pretoria: Briza. 480 pp.

VENTER, F. AND VENTER, J.A. 1996. Making the most of Indigenous Trees. Pretoria: Briza. 92 pp.

VERDCOURT, B. AND TRUMP, E.C. 1969. Common Poisonous Plants of East Africa. London: Collins. 254 pp.

WAIGANJO, M.M., WABULE, N.M., NYONGESA, D., KIBAKI, J.M., ONYANGO, I. WEPUKHULU, S.B. AND MUTHOKA, N.M. 2006. Tomato Production in Kirinyanga District, Kenya. A Baseline Survey Report. KARI/IPM-CRSP Collaborative Project. USAID. 43 pp.

WANG, K.H., SIPES, B.S. AND SCHMITT, D.P. 2002. *Crotalaria* as a cover crop for nematode management: a review. *Nematropica,* 32: 35-57.

WANG, K.H., MCSORLEY, R., AND KOKALIS-BURELLE, N. 2006. Effects of cover cropping, solarization, and soil fumigation on nematode communities. *Plant and Soil,* 286: 229-243.

WINK, M. AND VAN WYK, B.E. 2008. Mind-altering and poisonous plant of the world. Pretoria: Briza. 268 pp.

WUYTS, N., SWENNEN, R. AND DE WAELE, D. 2006. Effects of plant phenylpropanoid pathway products and selected terpenoids and alkaloids on the behaviour of the plant-parasitic nematodes *Radopholus similis*, *Pratylenchus penetrans* and *Meloidogyne incognita. Journal of Nematology,* 8: 89-101.

YADAV, A. AND ALAM, M.M. 1993. Control of plant parasitic nematodes with organic amendments. *Integrated Pest Management*. 149-164.

YOSHIKAWA, T. 2002. Introduction to a medicinal herb of Okinawa. Okinawa: Nirai-sha. 216 pp.

ZASADA, I.A., KLASSEN, W., MEYER, S.L.F., CODALLO, M. AND ABDUL-BAKI, A.A. 2006. Velvet bean (*Mucuna pruriens*) extracts: Impact on *Meloidogyne incognita* survival and on *Lycopersicon esculentum* and *Lactuca sativa* germination and growth. *Pest Management Science*, 62: 1122-1127.

ZASADA, I.A., HALBRENDT, J.M., KOKALIS-BURELLE, N., LAMONDIA, J., MCKENRY, M.V. AND NOLING, J.W. 2010. Managing nematodes without methyl bromide. *Annual Review of Phytopathology,* 48: 311-328.

ZUREEN, S. AND KHAN, M.I. 1984. Nematicidal activity of some plant lattices. *Pakistan Journal of Nematology*, 2: 69-77.