EFFECT OF CRUDE EXTRACTS OF *TULBAGHIA VIOLACEA* (WILD GARLIC) ON GROWTH OF TOMATO AND SUPPRESSION OF *MELOIDOGYNE* SPECIES

BY

MOLEBATSI MLUNGISI FLORAH MALUNGANE

MINI-DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE

REQUIREMENTS FOR THE DEGREE OF **MASTER OF SCIENCE**

IN

AGRICULTURE (PLANT PRODUCTION)

IN THE

FACULTY OF SCIENCE AND AGRICULTURE (SCHOOL OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES)

AT THE

UNIVERSITY OF LIMPOPO

SUPERVISOR: DOCTOR B. NZANZA

CO-SUPERVISOR: PROFESSOR P.W. MASHELA

2014

TABLE OF CONTENTS

DECLARATION

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

Malungane, MMF (Miss) **Date**

DEDICATION

To my beloved daughter Lethabo and nephew Kabelo Malungane

ACKNOWLEDGEMENTS

I would like to thank God for being with me through everything and for His protection all these years for His strength, comfort and wisdom. I am very grateful for the support, encouragement, guidance and supervision accorded to me by my supervisory team, Dr. B. Nzanza and Prof. P.W. Mashela, especially their guidance, patience and critical evaluation of this work.

I also want to thank Bertie van Zyl (PTY) LTD (ZZ2) for the appointment and experience in research, allowing me to further my studies and for registering this work as one of their research projects. Special thanks to Mr Solly Maluleke, Mr Richard Maake, Miss Esther Seunane, Mr Thapelo Malapane, Mr Pontsho Tseke and Mr Godfrey Rakgatji, for their technical assistance. Special thanks to Dr. Osvaldo Pelinganga for his guidance and technical assistance.

Special appreciation is also extended to my parents for their sponsorship and the financial support that I received during the entire duration of my study, along with their understanding and patience during the period of my absence.

LIST OF TABLES

LIST OF FIGURES

APPENDICES

ABSTRACT

The management of root-knot nematodes (*Meloidogyne* spp.) has become a challenging task in tomato (*Solanum lycorpesicum*) production, due to the withdrawal of effective chemical nematicides. Currently, crude extracts of different plant species are being researched as alternative to chemical nematicides, with promising results. The objective of this study was to determine the effect of crude extracts of wild garlic (*Tulbaghia violacea*) on the growth of tomato under greenhouse conditions, and the suppression of *M. incognita* race 2 population densities. Treatments consisted of four levels of crude extracts *viz.* 0, 2, 4 and 8 g per pot, were arranged in a randomised complete block design with 10 replicates. Seedlings were inoculated with 1000 juveniles of *M. incognita* race 2 at transplanting and treated with crude extracts two days later. At 56 days, the crude extract of *T. violacea* increased plant height, stem diameter, number of cluster, flowers, fruits and leaves by 43-73%, 108-200%, 57-81%, 55-110%, 170-223% and 51-66%, respectively. It also increased the root mass and shoot mass by 95% and 96%, respectively. Crude extracts of *T. violacea* did not have any effect on soil pH and electrical conductivity (EC). Crude extracts of *T. violacea* consistently reduced population densities of *M. incognita* race 2 by 50, 64 and 73% in roots at 2, 4 and 8 g crude extracts, respectively and by 21, 30 and 58% in soil at similar levels, respectively. In conclusion, crude extracts of *T. violacea* have the potential to improve growth of tomato plants and suppress population densities of *M. incognita* race 2 and could be used as botanical nematicide in tomato production.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Introduction

Plant-parasitic nematodes are among the most difficult soil-borne pests to manage. In addition, withdrawing fumigant nematicides such as methyl bromide from agrochemical markets following the adoption of the Montreal protocol (1997) increased the incidence of crop failures in most active agricultural soils (Pelinganga *et al.,* 2011). Plant-parasitic nematodes are particularly damaging in vegetable production in tropical and subtropical regions (Sikora and Fernandez, 2005) and cause complete crop failure in heavily infested fields (Kaskavalci, 2007). Economic damage in tomato (*Solanum lycorpersicum*) can occur with root-knot nematode densities of 0.1 ï 1.0 nematodes per cm^3 soil at planting (Sikora and Fernandez, 2005).

The genus *Meloidogyne* is among the main soil-pathogens of tomato with estimated yield losses ranging from 28% to 68% (Adesiyan *et al.,* 1990) but can cause suppression in yield of tomato as high as 85% (Sasser, 1979). Chitwood (2003) reported that global crop losses per annum due to plant-parasitic nematodes prior to the 2005 cut-off withdrawal date of methyl bromide had been estimated at US$ 125 billion. Currently, the estimated annual crop losses due to nematode damage in South Africa stand at 14% (Swart, 2010). Four major species of *Meloidogyne* include

M. incognita, *M. javanica*, *M. arenaria* and *M. hapla* (Esfahani, 2009), with known races readily attacking tomato crops in outdoor as well as in indoor cultivations.

Management of plant-parasitic nematodes is a difficult task and has mainly depended on chemical nematicides for decades, with remarkable reduction of nematode population densities (Akhtar and Malik, 2000). However, the detrimental environmental effects of synthetic nematicides redirected focus to the use of alternatives that improve soil health, resulting in the introduction of the Ground Leaching Technology (GLT) system (Mashela, 2002). The technology comprises the use of crude extracts from selected plant organs in suppression of plant-parasitic nematodes. The uniqueness of the GLT system is that it uses much smaller quantities (0.2ï 0.7 t/ha) of ground materials when compared with 10 ï 250 t/ha of conventional organic amendments (Mashela, 2002). Other alternative strategies include the application of soil organic amendments of crop residues and animal manures, heat treatment, soil solarisation and crop rotation with nematode-resistant plants (Oka *et al.,* 2007).

Effective phyto-pesticides have been developed from garlic extracts (Singh and Singh, 2008). The biocidal properties of garlic and other *Allium* spp., such as leek and onions are attributed to sulfur volatiles produced during degradation of *Allium* tissues (Auger *et al*., 2004). The most active compounds isolated from *Allium* spp. included methypropyl trisulfide, dipropyl disulphide, diallyl disulphide, dipropyl Ti, methyl propenyl Ti and dipropyl thiosulfonate (Tada *et al.,* 1988). Bio-nematicidal

activity of *Allium* spp. had been reported against the root-knot nematodes (Auger and Thibout, 2004). Singh and Singh (2008) found that 200 and 100 ppm concentrations of allicin as bare root dips for 30 minutes reduced *M. incognita* on tomato seedlings by 83% and 87%, respectively.

Although the biocidal properties of *Allium* spp. are well-documented in literature, information regarding the potential use of wild garlic (*Tulbaghia violacea*) for nematode control is currently lacking. Therefore, the development of crude extracts of *T. violacea* using the GLT system could be beneficial since the application of ground materials fruits of *Cucumis* spp. have been tested with great success.

1.2 Problem statement

The withdrawal of fumigant nematicides due to environmental hazards and human health, has led to the build-up of initial nematode population (Pi) in many agricultural active soils with limited management options. Currently, the focus of nematode management is based on developing alternatives that are environment-friendly, without detrimental residues on produce. Certain locally available plants such as *T. violacea* could potentially serve an important role in expanding the existing plant materials for use in nematode management. However, their efficacy on growth of plants and nematode suppression is not documented.

1.3 Motivation

Development of crude extracts of *T. violacea* for the GLT system would be beneficial to both commercial and small-scale farmers in tomato production. The product is ecologically friendly and not toxic to non-target organisms including human beings.

1.4 Aim

The aim of the study was to asses if crude extracts of *T. violacea* could be used as alternative to synthetic nematicides in the management of nematode population densities of plant-parasitic nematodes in order to supplement the locally available materials for the GLT system.

1.5 Objective

To determine the effect of crude extracts of *T. violacea* on growth of tomato and suppression of *M. incognita* race 2 population densities under greenhouse conditions.

1.6 Structure of mini-dissertation

The mini-dissertation was designed using the Senate-approved technical format of the University of Limpopo. Findings were summarised in the abstract, followed by detailed background to the research problem (Chapter 1), which was in turn followed by a review of relevant literature on the research problem (Chapter 2). Empirical study comprised that for achieving Objective 1 (Chapter 3). Finally, findings were

summarised, with related recommendations being provided regarding the use of crude extracts of *T. violacea* as a bio-nematicide (Chapter 4).

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Root-knot nematodes are difficult pests to manage and of great concern to both small-holder and commercial farmers involved in crop production. Following the withdrawal of synthetic fumigant nematicides from agrochemical markets, limited options are available for nematode management (Mashela *et al.,* 2011). However, plant extracts offer an alternative and economically feasible option. This review focuses on what has already been written on the research problem including the findings and contradictions, existing gaps on the research problem and explanation on how the existing gaps will be addressed.

2.2 Work done on the problem statement

Work done on the research problem included (i) alternative use to nematicides, (ii) crude extracts as bio-nematicides, (iii) bioactivity of *Tulbaghia violacea* and (iv) ground leaching technology.

2.2.1 Alternative use of nematicides

Withdrawal of highly effective synthetic fumigants used in the management of plant-parasitic nematode populations had economic consequences in many crop production systems (Mashela *et al.,* 2008). Recently, alternative management tactics have been investigated and developed (Pakeerathan *et al.,* 2009). Several researchers have suggested that naturally occurring bio-chemical and plant

allelochemicals, incorporated in an integrated pest management (IPM) system, could achieve effective reduction in target phytopathogens, while minimising environmental risks (Ferguson and Alford, 1985; Hedin, 1991). Certain plant organs contain biologically active compounds, some of which have antimicrobial and nematicidal properties (Mitscheret *et al.,* 1987). Extracts of certain plants with anthelminthic and antimicrobial properties had been proven effective in reducing soil population of plant-parasitic nematodes (Ferris and Zheng, 1999). Plants antagonistic to nematodes are those considered to produce anthelminitic compounds with different modes of action (Pandey *et al.,* 2003). One of the prominent advantages of using bio-pesticides is their environment friendliness, a criterion that demerits pesticides (Ballesteros *et al.,* 1992).

Much work had been done to develop non-chemical and environmental friendly nematode management practices such as the use of botanical and organic amendments (Bello, 1998; Mashela *et al.,* 2011). Application of plant extracts for nematode management in agricultural crops is gradually gaining ground. Costa *et al*. (2003) reported that *Artemisia vulgaris* rhizome extracts inhibited egg hatch, caused second stage juvenile mortality and reduced root gall of root-knot nematode (*Meloidogyne megadora)* on *Phaseolus vulgaris*. *Chrysanthemum coronarium* was used as an organic amendments and green manure reduced nematode infection on tomato roots and improved plant-top fresh weight both under greenhouse and micro plot conditions (Bar-Eyal *et al.,* 2006).

2.2.2 Crude extract as bio-nematicides

Initial screening of plants for possible antimicrobial activities usually begin with crude aqueous or alcohol extractions, followed by various organic fractionation methods (Yazdani *et al.,* 2011). The review by Yazdani *et al.* (2011) on plant secondary metabolites against plant pathogenic fungi showed that most investigations were performed by crude extracts instead of specific fractions. The toxicity of root extracts of different plants against nematodes had been reported by many researchers (Adegbite and Adesiyan, 2005). Crude extracts of *Cucumis myriocarpus* fruits suppressed the plant-parasitic nematodes by 90% and 80% in greenhouse and field trials, respectively (Mofokeng *et al.,* 2004; Mashela *et al.,* 2011). It also increased soil electric conductivity by 79% but had no significant effect on soil pH. Hassan *et al.* (2001) tested powder and extract of ginger against root-knot nematodes and observed a better growth of plant with lower root galling index on brinjal. Adegbite and Adesiyan (2005) found that root extracts of *Chromolaena odorata* and *Azadirachta indica* exhibited 100% inhibition of egg hatch and larval mortality of root-knot nematodes.

2.2.3 Bioactivity of *Tulbaghia violacea*

Certain plant organs contain concentrated toxic chemical compounds that are lethal to animals in small quantities (Van Wyk *et al.,* 1997). In ground form, these plant organs are referred to as ómutisô they are widely used as traditional medicines for human beings and livestock in South Africa (Van Wyk *et al.,* 1997). *Tulbaghia violacea* originated in KwaZulu Natal, Free state and Eastern Cape Provinces (Van

Wyk *et al.*, 1997). It is a bulbous plant, which grows to a height of 0.5 m (Harris, 2004). Leaves are narrow, hairless and strap-shaped, and grow to 30 cm long and 1.5 cm wide, arising from several white bases which are dark green, leathery in texture and smell strongly of garlic (Davison, 2002). It has been used by South African communities as herb for many years (Harris, 2004) and is one of the most important medicinal plants that are used by traditional health practitioners (Van Wyk *et al.,* 1997). *Tulbaghia violacea* plant extracts serve as natural sources for several active compounds (Borris, 1996). It has medicinal properties because of their antifungal, antibacterial and antiviral activities (Nteso and Pretorius, 2006). Auger *et al.* (2004) reported that crops belonging to Alliaceae family have insecticidal, fungicidal, acaricidal, nematicidal and bactericidal properties.

Aqueous and ethanolic extracts of *T. violacea* tubers have previously been shown to have anthelmintic activity (McGraw *et al.,* 2000). Natural compounds accumulated in plant extracts serve as growth regulators or have ecological role such as protection against fungal, viral and bacterial diseases (Pretorious *et al.,* 2003). One of organo-sulphur compounds that accumulate in *T. violacea* plant extracts has been identified as allicin (Baustista *et al.,* 2005). Its active ingredients are the breakdown of allicin, including diallytrisulphide and ajoene, which have greater antifungal effect than allicin (Corzo-Martinez *et al.,* 2007). Allicin is generated by a chemical reaction catalysed by the vascular enzyme, alliinase (Baustista *et al.,* 2005). It is active against microbial infections caused by fungus, virus and bacteria both in human and

plant pathogens. It restrains the growth of microbial organisms through its chemical reaction with the thiol groups of various enzymes (Baustista *et al.,* 2005).

2.2.4 Ground leaching technology

The ground leaching technology (GLT) involves the application of ground materials from selected plant organs in small quantities (Mashela, 2002; Mashela *et al.,* 2011). In GLT systems, *C. myriocarpus* fruit were used as an alternative organic amendment to control intial nematode population (Pi) of *M. incognita* on tomato under greenhouse conditions (Mashela, 2002). In GLT systems, active ingredients are leached-out of crude extracts through irrigation water (Mashela, 2002). Generally, the efficacy of GLT system in nematode suppression is independent of microbial activities (Mashela and Nthangeni, 2002a). The small quantities precluded high transport costs to haul the materials to the preparation site and then to the field (Mashela *et al.,* 2011). Also, when used at transplanting, the waiting period for microbial decomposition was not necessary and the material hardly reduced soil pH (Mashela, 2002).

2.3 Work not yet done on the problem statement

The use of *T. violacea* as a crude extract on suppression of *Meloidogyne* species in tomato production constitutes part of work not yet done with respect to management of plant-parasitic nematodes. The phytotoxicity of *T. violacea* (crude extracts) as post emergent nematicides and the standard rate of application for plant-parasitic nematodes suppression are still not well-documented. Similarly, the mode of action

of *T. violacea* and the part of the plants responsible for reducing *M. incognita* race 2 initial populations are not well documented.

2.4 Addressing the identified gaps

In nematode management, it is important to keep the initial population (Pi) level low at planting, if economic crop yields are to be produced. Indigenous plants such as *C. africanus* and *C. myriocarpus* have been widely used for nematode suppression under the GLT system with great success. Therefore, there is a need to broaden the use of GLT system with other indigenous plants such as *T. violacea*. In literature review of this study, two areas had been identified as still having gaps with respect to the use of crude extracts in management of plant-parasitic nematodes, *viz*. standard rate of application of crude extracts for stimulation of growth and inhibition for nematode population densities. The closing of the identified gaps enhance the use of *T. violacea* as crude extracts for both large and small scale farmers because the product is readily available, easy to use and cheap.

CHAPTER 3
RESPONSES OF TOMATO GROWTH AND NEMATODE NUMBERS TO CRUDE EXTRACTS OF *TULBAGHIA VIOLACEA*

3.1 Introduction

Tomato (*Solanum lycorpersicum*) is the second most important vegetable crop grown in South Africa (Anon, 2011) and is susceptible to various fungal, bacterial, viral and nematode pathogens (Lin *et al.,* 2009). Root-knot nematodes (*Meloidogyne*) are among the main pest of tomato plants all over the world (Jacquet *et al.,* 2005). They have a wide host range and are considered to be the greatest threat in plant production. A short life cycle of six to eight weeks enables them to survive well in the presence of a suitable host (Pakeerathan *et al.,* 2009). In susceptible plants, nematode populations build up to a maximum usually as a crop reach maturity and in some cases the plants die before reaching maturity (Singh and Khurma, 2007). Infested plants show symptoms of stunting, chlorosis, wilting under sufficient soil moisture, and increased susceptibility of plants to other diseases (Taylor and Sasser, 1978) with aberrant development of root system characterized by formation of galls and limited fruit production, ultimately causing reduced yield ranging from 28% to 68% (Williamson and Hussy, 1996).

The management of plant parasitic nematodes was mainly depended on chemical nematicides for decades with remarkable reduction of nematode population densities (Akhtar and Malik, 2000). Although soil nematicides are effective, they are currently being phased-out due to the environmental hazards and human health (Wachira *et al.,* 2009). Developing alternative strategies for management of root-

knot nematodes have been emphasized to researchers, farmers and scientists that do not pollute the environment (Mashela *et al.,* 2008). Botanical pesticides have found favour as alternative to pesticides in recent years (Taye *et al.,* 2012). Some of these botanicals are already being used commercially in insect pest management (Agnihotri *et al.,* 1999).

Some of the management strategies invented were the use of the ground leaching technology (GLT) system, which relies on irrigation water to leach potent bio-chemicals into the soil (Mashela and Nthangeni, 2002a). Screening trials were conducted on ground wild cucumber (*Cucumis myriocarpus*) fruit, castor bean (*Ricinus cummunis*) fruit and fever tea (*Lippia javanica*) leaves with consistent reduction in the densities of *Meloidogyne incognita* race 2 in root and soil samples (Mashela, 2002; Mashela and Nthangeni, 2002a). The advantages of using crude extracts are the additive or synergetic effect of the mixtures, the increase in the antimicrobial spectrum of the extract and the decreased risk for pathogen resistance to mixture (Yazdani *et al.,* 2011).

The phytotoxicity of plant residues which reduce growth of plants has been associated with the presence of various organic compounds including phenolic compounds which are widely produced in various plant species (Rice, 1984). These compounds are either leached from plant residues or are formed as by-products during residue decomposition in soil (Radziah *et al.,* 1997). The activities of various compounds in the soil are often attributed to the water-soluble fractions (Rice, 1984).

Growth is adversely affected as these water-soluble compounds are taken up by plant roots which inhibit nutrient uptake and affect various physiological processes (Whitehead *et al.,* 1983). Zasada *et al.* (2010) reported that garlic suppressed nematode numbers, while phytotoxicity on tomatoes was not observed. However, the aqueous extract and volatile compounds of the wild garlic bulbs were found to be strong inhibitors of seed germination and seedling growth compared to those in the leaves (Djurdjevic *et al.,* 2004).

Tulbaghia violacea, which is commonly known as wild garlic, wilde knoffel, isihaqa or itswele lomlambo, is indigenous in South Africa and is widely used as a herbal remedy for various ailments (Olorunnisola *et al.,* 2011). Aqueous and ethanolic extracts of *T. violacea* tubers have previously shown to have anthelmintic activity (McGraw *et al.,* 2000). Studies on the identification and use of local plant materials for the control of nematodes or integrated with other methods of control, are current areas of research in plant nematology (Agbenin *et al.,* 2005). There are less scientific studies on the use *T. violacea* for the suppression of root-knot nematodes. The objective of this study was to investigate the effect of crude extracts of *T. violacea* on the growth of tomato and suppression of *M. incognita* race 2 population densities under greenhouse conditions.

3.2 Materials and methods

3.2.1 Experimental site and growth conditions

The study was conducted under greenhouse conditions at the Horticultural Skills Centre, University of Limpopo (23°53′10″S, 29°44′15″E) in the summer season of 2012. Ambient day and night temperatures were averaged to 28°C and 21°C, respectively, with maximum temperatures controlled using thermostatically activated fans. Other greenhouse variables, such as relative humidity, photosynthetically active radiation and solar radiation, were not measured.

Tulbaghia violacea was collected at maturity from ZZ2 herb garden, Mooketsi (23°56′51″S, 30°15′83″E). All the plant parts, *viz*., roots, bulbs, leaves and flowers were washed and chopped into pieces and dried in a forced-air oven at 52°C for a maximum of five days to minimise the loss of volatile phytochemicals (Makkar, 1999). Dried materials were ground in Wiley mill through 1-mm-opening sieves. Prior to use, the ground material was stored at room temperature in sealed plastic bags. Nematode inoculums were prepared by extracting eggs and second-stage juveniles (J2S) of *M. incognita* race 2 from the roots of greenhouse grown nematode susceptible Kenaf (*Hibiscus cannabinus*) by the blending and maceration method for 30 seconds in 1% NaOCl (Hussey and Barker, 1973).

3.2.2 Experimental design and cultural practices

Forty 20-cm-diameter plastic pots, arranged at 0.3 m inter-row spacing and 0.25 m intra-row spacing, were each filled with 1 800 măsteam-pasteurised sand mixed with Hygromix (Hygrotech, Pretoria North, South Africa) at 3:1 (v/v). Six-week-old tomato seedlings cv. ôFloradadeôwere transplanted and inoculated with 1000 eggs and J2S of *M. incognita* race 2 into a 5-cm-deep hole around the base of each stem at two days after transplanting, while appropriate rates of crude extracts of *T. violacea* were placed in similar but separate holes. Four treatments, *viz*. 0, 2, 4 and 8 g crude extracts of *T. violacea* were arranged in a randomised complete block design with ten replications (Figure 3.1), as described for the GLT system (Figure 3.2). Three days after transplanting, each plant was fertilised with 20 g multi-feed applied for two weeks followed by 5 g of N-P-K 2:3:2 (22) to provide a total of 310 mg N, 210 mg P and 260 mg K per mă of water. Plants were irrigated with 500 mă tap water every other day. Plants were monitored for pest and disease incidence and when necessary, control measures were applied. Biomectin was applied at the rate of 0.6 ă ha $^{-1}$ for the control of leafminer, while benomyl was applied weekly at the rate of 0.8 ăha $^{-1}$ for the control of powdery mildew.

Figure 3.1: Greenhouse experiment using crude extracts of *T. violacea* on tomato plant.

Figure 3.2: Application of crude extracts of *T. violacea* using the GLT system.

3.2.3 Data collection

During harvest, 56 days after inoculation, plant height, stem diameter, number of leaves, number of flowers, number of fruits and number of clusters per plant were measured. Plant height was measured from the soil surface to the tip of the plant. Stems were cut off at the soil surface and the stem diameter was measured at 5 cm above the root area using a vernier caliper. Roots were removed from pots, immersed in water to remove soil particles. Fruit yield, fresh shoots and roots were weighed. Shoots were oven dried at 70ęC for 72 hours and weighed. Soil sample per pot was collected, shade dried and 15 g soil mixed with 75 mă distilled water and shaken for 1 hour at 175 cycles per minute (cpm). The solution was filtered using Whatman no. 42 into 100 măbeakers and electrical conductivity (EC) of the filtrates was measured with EC meter (model WTW CF 318) using Longenecker and Lyerlys (1964) method. Five grams soil sample was mixed with 25 măof distilled water plus 75 mă KCă for 50 minutes while being stirred and pH meter (model 420 A) was placed in a solution for 10 minutes prior to measuring the pH.

Roots were weighed to facilitate the calculation of nematode density/total roots/plant. Root galling was based on the scale of 0 to 5, in which 0 = no galls, 1 = 1 to 2 galls, 2 = 3 to 10 galls, 3 = 11 to 30 galls, 4 = 31 to 100 galls and 5 = >100 galls/root system (Taylor and Sasser, 1978). Nematodes were extracted from total root system/plant by maceration and blending for 30 seconds in 1% NaOCl (Hussey and Barker, 1973). The material was passed through nested 61- and 38 εm mesh sieves. The contents of the 38-εm mesh sieve were collected for further separation

of nematodes from debris using the sugar-floatation and centrifugation method (Jenkins, 1964). Eggs and juveniles from root and soil samples were each counted using a stereomicroscope and converted to total root system per plant and total soil per pot, respectively. Final nematode population density (Pf) allowed calculation of reproductive factor (RF = Pf/Pi) values, where Pi was initial nematode population density.

3.2.4 Data analysis

Data was subjected to analysis of variance (ANOVA) through SAS software (SAS Institute Inc, 2004). Sum of squares were partitioned, while treatment mean separation was achieved using Tukey test. Unless stated otherwise, treatments discussed were different at 5% level of probability. Significant variables were further assessed using relative impact, which was expressed as: Relative impact (RI) = $(\frac{extract}{control} - 1) \times 100$, where increased RI was depicted through positive (+) and decreased RI through negative (-) sign. Mean plant variables were subjected to Microsoft office excel (2007), generating the regression curve estimations using quadratic equation: $Y = b_2x^2 + b_1x + a$, where Y = plant variable value and x computed from $x = -b_1/2b_2$, where x = the optimum extract level, which is a concentration value where saturation sets in (Salisbury and Ross, 1992).

3.3 Results

3.3.1 Effect on plant growth

Crude extracts of *T. violacea* significantly affected the measured tomato growth variables (Table 3.1). Using the partitioning of the degrees of freedom and their associated sum of squares, levels of crude extracts of *T. violacea* explained 70, 87, 50, 55, 30 and 52% of the total treatment variation (TTV) on plant height, stem diameter, number of leaves, number of flowers, number of fruits and number of clusters of tomato, respectively. All levels of crude extracts of *T. violacea* stimulated growth of tomato plants by 43-73% on the plant height; 108-200% on the stem diameter; 51-66% on the number of leaves; 55-110% on the number of flowers; 170-223% on the number of fruits and 57-81% on the number of clusters (Table 3.2).

Crude extracts of *T. violacea* significantly affected the fresh root mass, shoot mass and dry shoot mass with no positive effect on fruit yield mass (Table 3.3). The levels of crude extracts of *T. violacea* explained 56, 59 and 27% of the TTV on root mass, fresh shoot and dry shoot mass (Table 3.3). The crude extracts of *T. violacea* increased the root mass, shoot mass by 95% and 96%, respectively.

The R^2 values for all the models ranged from 21 to 99% (Table 3.5). Plant variables tested had significant ($P < 0.05$) quadratic relationship when regressed against the four levels of crude extracts of *T. violacea*. The calculated optimum response dosage (CORD) values, derived from the quadratic relationships for plant height,

stem diameter, number of leaves, flowers, fruits, clusters, fresh shoot mass and ranged from 3.25, 3.52, 3.81, 3.52, 5.83, 3.81, 6.51, 4.03, while that of dry shoot mass was -3.89 (Table 3.5).

3.3.2 Effect on soil EC and pH

Treatment effects on soil EC and pH were not significant at the probability level of 5% (Table 3.6).

Table 3.1: Partitioning of sum of squares of crude extracts of *Tulbaghia violacea* on tomato growth variables.

Source of variation	DF	Plant height (cm)		Stem diameter (mm)		No. of leaves		No. of flowers		No. of fruits		No. of clusters	
		SS	%	SS	%	SS	%	SS	%	SS	%	SS	%
Block	9	3280.1	15	7.864	5	152.000	24	94.600	11	51.225	20	12.225	11
Treatment	3	14949.6	70*	13.001	87*	322.400	50*	466.100	55*	79.875	30*	56.075	52*
Error	27	3207.9	15	12.031	8	165.600	26	289.400	34	131.875	50	40.675	37
Total	39	21437.6	100	152.896	100	640.000	100	845.100	100	262.975	100	108.975	100

[ns] Indicates that the factor (s) were not significant and * indicates that the factors were significant at $P < 0.05$.

Table 3.2: Relative impact (%) of crude extracts of *Tulbaghia violacea* on tomato growth variables at 56 days after inoculation.

Extract level (g)	Plant height (cm)		Stem diameter (mm)		No. of leaves		No. of flowers		No. of fruits		No. of clusters	
	Variable	%	Variable	%	Variable	%	Variable	%	Variable	%	Variable	%
0	73.3c	-	2.24b	-	11.2b	-	8.5c	-	1.7b	-	3.8b	-
2	107.1b	46	6.29a	108	17a	51	13.2b	55	4.6a	170	6a	57
4	108.3b	47	6.26a	179	17.2a	53	15ab	76	4.3ab	152	6.4a	68
8	126.9a	73	6.73a	200	18.6a	66	17.9a	110	5.5a	223	6.9a	81

The means within each column followed by the same letter were not significantly different when means were separated by using the Tukey test range ($P < 0.05$).

Impact (%) = [(extract/control) ï 1) x 100].

Table 3.3: Partitioning of sum of squares of crude extracts of *Tulbaghia violacea* on tomato fresh and dry mass and its percentage impact.

Source of variation	DF	Fresh root mass (g)		Fresh shoot mass (g)		Dry shoot mass (g)	
		SS	%	SS	%	SS	%
Block	9	4240.8	23	5799.6	20	839.10	31
Treatment	3	10305.8	56*	17564.1	59*	716.42	27*
Error	27	3924.3	21	6349.8	21	1143.36	42
Total	39	18470.9	100	29713.5	100	2698.88	100

[ns] Indicates that the factor (s) were not significant and * indicates that the factor (s) were significant at $P < 0.05$.

Table 3.4: Relative impact (%) of crude extracts of *Tulbaghia violacea* on tomato fresh fruit-, root-, fresh shoot- and dry shoot mass at 56 days after inoculation.

Extract level (g)	Fresh root mass (g)		Fresh shoot mass (g)		Dry shoot mass (g)	
	Variable	%	Variable	%	Variable	%
0	59.28b	-	77.54c	-	21.84b	-
2	89.79a	51	111.44b	43	28.44ab	30
4	89.63a	51	111.40b	44	25.91ab	19
8	103.04a	74	136.48a	76	33.52a	53

The means within each column followed by the same letter were not significantly different when means were separated by using the Tukey test range ($P < 0.05$).

Impact (%) = [(extract/control) ï 1) × 100].

Table 3.5: Quadratic relationship, coefficient of determination and computed optimum response dosage for tomato variables against crude extracts of *Tulbaghia violacea* at 56 days after inoculation.

Plant variable	**Quadratic Relationship**	**R^2**	**CORD (x)**	**P <**
Plant height (cm)	$-0.8952x^2 + 5.8203x - 2.4558$	0.92	3.25	0.05
Stem diameter (mm)	$-0.8967x^2 + 5.8296x - 2.4693$	0.92	3.25	0.05
Number of leaves	$-1.1x^2 + 7.74x + 4.9$	0.92	3.52	0.05
Number of flowers	$-0.45x^2 + 5.25x + 3.9$	0.98	5.83	0.05
Number of fruits	$-0.425x^2 + 3.235x - 0.875$	0.86	3.81	0.05
Number of clusters	$-0.675x^2 + 4.245x + 0.225$	0.99	3.14	0.05
Fresh shoot mass (g)	$-2.205x^2 + 28.703x + 53.995$	0.90	6.51	0.05
Fresh root mass (g)	$-4.275x^2 + 34.487x + 31.28$	0.90	4.03	0.05
Dry shoot mass (g)	$0.254x^2 + 1.9798x + 20.573$	0.74	-3.89	0.05

Calculated optimum response dosage (x) = $-b_1/2b_2$.

Table 3.6: Partitioning of sum of squares of crude extracts of *Tulbaghia violacea* on soil pH and soil electrical conductivity (EC).

Source of variation	DF	EC (ms/dm)		pH	
		SS	%	SS	%
Block	9	0.02077	12	1.37355	23
Treatment	3	0.01383	8[ns]	0.69871	11 [ns]
Error	27	0.14058	80	4.02492	66
Total	39	0.17518	100	6.09718	100

[ns] Indicates that the factor (s) were not significant and * indicates that the factor (s) were significant at $P < 0.05$.

3.3.3 Effect on nematodes

The crude extracts of *T. violacea* significantly affected the final nematode (*M. incognita* race 2) population density (Pf) (Table 3.7). Using the partitioning of the degrees of freedom and their associated sum of squares, levels of crude extracts of *T. violacea* explained 65% of TTV of Pf for both root and soil. Crude extracts of *T. violacea* applied at the rates of 2, 4 and 8 per plot reduced the number of nematodes by 50, 64 and 73% in roots and by 21, 30 and 58 % in soil, respectively (Table 3.8).

Crude extracts of *T. violacea* had a significant effect on the number of root galls per root system (Table 3.7). The levels of crude extracts of *T. violacea* contributed 68% of the TTV of number of root galls of *M. incognita* race 2. In untreated plots,

root galls were well-developed whereas at 2ï 8 g crude extracts levels; they were poorly-developed (Table 3.8). Application of extracts of *T. violacea* at the rates of 2, 4 and 8 g reduced the number of root galls by 50, 53 and 59%, respectively.

Relative to the initial population (Pi), the Pf at all extracts levels was reduced (Table 3.9). The impact of crude extracts on Pf at all levels ranged from 42 to 69%, with the highest impact observed at the higher application level.

Table 3.7: Partitioning of sum of squares for final nematode (*M. incognita* race 2) population density (Pf) as affected by crude extracts of *Tulbaghia violacea*.

Source of variation	DF	Pf/total root		Pf/total soil		Total root galls	
		SS	%	SS	%	SS	%
Block	9	32299	15	1709.4	8	1.6	5
Treatment	3	144504	65**	13410.2	65**	23	68**
Error	27	44660	20	5636.8	27	9	27
Total	39	221463	100	20756.4	100	33.6	100

[ns] Indicates that the factor (s) were not significant and ** indicates that the factor (s) were significantly different at $P < 0.05$.

Table 3.8: Relative impact (%) effects of crude extracts of *Tulbaghia violacea* on final nematode (*M. incognita* race 2) population density.

Extracts levels (g)	Pf/total root		Pf/total soil		Total root galls	
	Nematode	%	Nematode	%	Gall	%
0	212.7a	-	87.9a	-	3.2a	-
2	106.2b	-50	68.8b	-21	1.6b	-50
4	76.1b	-64	61.3b	-30	1.5b	-53
8	57.3b	-73	36.8c	-58	1.3b	-59

The means within each column followed by the same letter were not significantly different when means were separated by using the Tukey test range ($P < 0.05$).

Impact (%) = [(extract/control) ï 1) × 100].

Table 3.9: Influence of crude extracts of *Tulbaghia violacea* on final nematode (*M. incognita* race 2) population density (Pf) and percentage impact.

Extracts levels (g)	(Pi)	Pf/total root	Pf/total soil	Pf	Impact (%)
0	1000	212.7	87.9	300.6	-
2	1000	106.2	68.8	175	42**
4	1000	76.1	61.3	137.4	54**
8	1000	57.3	36.8	94.1	69**

Impact (%) = [1.0 ï (treatment/control) × 100], where ** implied that Pi and Pf were significantly different at 5% level of probability according to Tukey test range. Pf = Pf/total root + Pf/total soil.

3.4 Discussion

3.4.1 Effect on plant growth

The increased plant growth under crude extracts of *T. violacea* was different to that in plants infected with *Meloidogyne* species (Siddiqui and Alam, 1987). Generally plants heavily infected with *Meloidogyne* species exhibit stunted shoot growth and increased root growth. The latter is due to root galls (Dropkin, 1980; Siddiqui and Alam, 1987). The increased root growth in tomato plants under crude extracts of *T. violacea* when compared to those under untreated controls could not be ascribed to root galls since the used product reduced populations of *Meloidogyne* species, and therefore the incidence of root galls. Also, general plant growth for plants

treated with crude extracts could not have been due to increased uptake and transportation of water and nutrients as described for other systems elsewhere (Agbenin *et al.,* 2005). Generally, in GLT systems, used quantities are too small and have negligible effect on the accumulation of nutrient elements in leaves of plants (Mashela, 2002; Mashela *et al.,* 2011).

Generally, crude plant extracts have allelochemicals as active ingredients (Rice, 1984; Inderjit and Malik, 2002). Plants and microorganisms respond to increasing concentrations of allelochemicals in accordance to the density-dependent growth (DDG) patterns (Liu *et al.,* 2003). The DDG patterns have three responses, (i) the stimulation growth phase (ii) levelling-off (neutral) phase and (iii) inhibition growth phase (Salisbury and Ross, 1992; Liu *et al.,* 2003) with the relations being quantified through quadratic relationships (Salisbury and Ross, 1992; Liu *et al.,* 2003; Mamphiswana *et al.,* 2010). The increased plant growth observed in this study suggested that the concentrations of crude extract levels of *T. violacea* were still in the stimulation range for growth of tomato plants. Similar responses were observed when tomato plants were exposed to fermented crude extracts of fruits from wild *Cucumis* species (Pelinganga, 2013). This is in agreement with Agbenin *et al.* (2005) who did not find any phytotoxicity on tomato plants when plants were exposed to 20% concentration of garlic extracts. However, in another study conducted by Sukul *et al.* (1974), 50% concentration of garlic extracts negatively affected the growth of tomato. Obviously, the effect of crude extracts of *T. violacea*

on plant growth, in accordance with the DDG principles, is dependent on the concentration.

The CORD values derived from the quadratic relationships for plant variables were positive, while one variable (dry shoot mass) was negative. According to Pelinganga *et al.* (2011) a positive linear relationship between variables and increasing concentrations of a particular phytonematicide, suggests that the concentrations used were within the stimulation range and a negative linear relationship suggests that concentrations of allelochemicals were already in inhibition range. Using the curve-fitting allelochemical response dosage (CARD) model, the variables and the concentrations of the extracts are characterised by quadratic relationships (Salisbury and Ross, 1992). This shows the increase dry shoot mass was not dependent on the crude extract levels but the relationship was constant. The r^2 in plant variables suggested strong density-dependent relationships between growth of tomato plants and increased concentration of crude extracts of *T. violacea*.

The reduced stem diameter in tomato plants was not unique to the plants which were infected by *Meloidogyne* species, but not treated with crude extracts of garlic. Generally, nematode infection (Eisenback *et al.,* 1991; Mashela, 2002; Mashela and Nthangeni, 2002a), root rot due to *Phytophthora cinnamomi* (Podger, 1972), drought stress (Mafeo, 2005), salinity stress (Mashela and Nthangeni, 2002b) and

root pruning (Mashela and Nthangeni, 2002b), each reduced stem diameter. The reduction in stem diameter had been associated with the physical reduction of the route for osmotic sugars which are being channelled towards the roots for the re-establishment of the normal root/shoot ratios when roots are reduced due to various stresses (Mashela and Nthangeni, 2002b). In nematode infected roots, more sucrose is channelled towards roots, resulting in the reduced (constricted) stem diameter as seen in this study. Obviously, the removal of the stresses results in normal stem diameter (Gommers *et al.,* 1982).

3.4.2 Effect on soil EC and pH

Generally, crude extracts which reduce soil pH are not suitable since this effect results in the disturbance of alkaline-loving nutrient elements (McLean and Lawrence, 2000). The reduction of soil pH by crude extracts of fever tea (*Lippia javanica*) resulted in this material being discontinued as a phytonematicide (Mashela *et al.,* 2010). In contrast, like crude extracts of wild garlic, those from fruits of wild *Cucumis* species had no effect on soil pH (Mashela, 2002; Mashela *et al.,* 2011). However, unlike wild garlic, *Cucumis* species increased soil EC (Mashela, 2002; Mashela *et al.,* 2011). The mechanism involved in the latter is still not understood.

3.4.3 Effect on nematodes

Crude extracts of *T. violacea* reduced *M. incognita* race 2 population density at all treatment levels. The Pf for both root system and soil system decreased with an

increase in crude extracts levels. Many wild and cultivated medicinal plants have nematicidal properties against several plant-parasitic nematodes (Khan, 1990). For instance, Alashalaby and Noweer (2003) reported that aqueous neem (*Azadirachta indica*) extract significantly reduced the total number of root-knot nematode juveniles and inhibited egg hatch in peanut roots and soil. Adegbite and Adesiyan (2005) found that root extracts of *A. indica*, *Ricinus communis* and *Jatropha curcas* increased inhibition of egg hatch with the increase of concentration of the extract. In GLT systems, *Cucumis* fruit consistently reduced population densities of nematodes (Mashela, 2002; Mashela *et al.,* 2011).

The inhibitory effect of crude extracts of *T. violacea* on nematode population densities in the roots was high while in the soil was low, suggesting that the material had the ability to penetrate through the roots. The inhibitory effect might be due to the chemical properties present in the extract that possess nematicidal properties (Agbenin *et al.,* 2005). According to Adegbite and Adesiyan (2005), botanicals with nematicidal properties affect the embryonic development or kill the eggs.

Agbenin *et al.* (2005) reported that garlic extracts might have been lethal to the nematode juvenile. The reduction of root-knot nematodes could be attributed to poor root penetration and later retardation of activities such as feeding and reproduction (Bunt, 1975). Allicin inhibited the hatching of *M. incognita* at concentration as low as 5% and was toxic to juveniles at 25% (Gupta and Sharma,

1993). Marban-Mendoza *et al.* (1987) found that immersion of roots in allicin solutions as a prophylactic measure was beset with problems of phytotoxicity and lack of nematotoxicity but a five minute immersion in 2.5% allicin inhibited penetration on the roots by juveniles by 50% and was not phytotoxic. Most reports on the use of garlic and neem leaf extracts in nematode control used high application rates and concentrations (Sukul *et al.,* 1974; Agbenin *et al.,* 2005). Kali and Gupta (1980) reported that efficacy of plant extracts depends on the concentration and duration of exposure of the nematode to the extracts. Olorunnisola *et al.* (2012) found that oil extract of rhizome of *T. violacea* was cytotoxic and this toxicity was concentration dependant.

Galling and reproductive responses are more reliable indicators of host plant reaction than just root-knot galling index (Fassuliotis, 1985). In this study, crude extracts of *T. violacea* reduced number of galls on tomato plants with fewer galls being recorded on the treated plants than untreated plants. Agbenin *et al.* (2005) found that garlic bulb extracts gave a significant reduction in root-knot nematode galling index. In a similar study, Sukul *et al.* (1974) found that garlic extract was highly effective in reducing root-knot infection on tomato plants. This phenomenon might be due to the action of the extract releasing substances into the soil which inhibit the entry of root-knot nematodes into the roots of the plants (Gommers *et al.*, 1982) or toxic compounds released by wild garlic (Mian and Rodriguez-Kabana, 1982).

3.5 Conclusions

Crude extracts of *T. violacea* stimulated the growth of tomato plants. However, the material had no effect on soil pH and EC. The high root mass indicates that crude extracts of *T. violacea* stimulate root growth and vegetative growth of the plants. The growth of a plant is inversely proportional to the initial population density of *Meloidogyne* species. Crude extracts of *T. violacea* didn't show to have phytotoxicity effect. It has also shown the potential to reduce *M. incognita* race 2. It reduced *M. incognita* race 2 population densities with fewer root galls recorded on the treated plants.

CHAPTER 4

SUMMARY, RECOMMENDATION AND CONCLUSION

4.1 Introduction

Tulbaghia violacea has been used for centuries as traditional medicinal plants, by South African traditional healers for the treatment of flu, fever, cold, tuberculosis, cancer of the oesophagus and asthma (Davison, 2002). There are few studies that have been conducted on the bioactivity of *T. violacea* crop. Demand for the use of local plant materials for the control of nematodes or integrated with other methods, are current areas of research in plant nematology. In Limpopo Province, South Africa, alternatives to methyl bromide in managing *Meloidogyne* species focused on the uses of allelochemicals from crude extracts of selected plants using the ground leaching technology (GLT) system (Mashela *et al.,* 2011). A greenhouse experiment was conducted to investigate the potential effect of crude extracts of *T. violacea* on the growth and development of tomato plants, and the suppression of *Meloidogyne incognita* race 2 population densities.

4.2 Summary

In this study, crude extracts of *T. violacea* significantly reduced *Meloidogyne incognita* race 2 population densities. It reduced the number of nematodes by 50ï 73% in roots and by 21ï 58% in soil, respectively. The final nematode population density (Pf) was reduced at all extract levels by 42ï 69%. The higher crude extracts concentration, the higher nematode reduction occurred. Crude extracts of *T.*

violacea significantly stimulated the growth of tested plant at different treatment levels. It stimulated the growth of tomato plants by 43ï 73% on the plant height, 108ï 200% on the stem diameter, 51ï 66% on the number of leaves, 55ï 110% on the number of flowers, 170ï 223% on the number of fruits and 57ï 81% on the number of clusters. Furthermore, it has both stimulatory and inhibitory activities; and is a concentration-dependant phenomenon. However, crude extracts of *T. violacea* did not have any effect on the soil pH and EC.

4.3 Recommendations

Recently, crude extracts from botanicals have been widely used in plant protection for sustainable agriculture. The use of crude extracts of *T. violacea* is highly recommended to both small-holder and a commercial farmer due to its affordability and it is also easy to prepare. Future studies on *T. violacea* can be investigated on other nematode species and economic crops. It can also be tested as fermented crude extracts. Furthermore, crude extracts of *T. violacea* has the potential to reduce nematode population densities. The use of *T. violacea* will be beneficial to these farmers due to its ability to suppress nematode population and at the same time improve the soil health status which will subsequently result in improved crop yield.

4.4 Conclusions

Sufficient data is provided in this study that crude extracts of *T. violacea* have the ability to reduce nematode population density. The use of crude extracts of *T. violacea* as a bio-nematicide is recommended as treatment on tomato. Further work should be done on this material since its mode of action is not documented and has stimulatory effect which can be ultimately be allowed to be registered as a bio-nematicide treatment for tomato production in South Africa.

REFERENCES

ADEGBITE, A.A. and S.O. ADESIYAN. 2005. Root extracts of plants to control root-knot nematode (*Meloidogyne incognita*) on edible soybean. *World Journal of Agricultural Science* 1: 18 ï 21.

ADESIYAN, S.O., CAVENESS, F.E., ADENYI, M.O. and B. FAWOLE. 1990. Nematode Pests of Tropical Crops. Heinemann Educational Books. Nigeria Limited.

AGBENIN, N.O., EMECHEBE, A.M., MARLEY, P.S. and A.D. AKPA. 2005. Evaluation of nematicidal action of some botanicals on *Meloidogyne incognita* in vivo and in vitro. *Journal of Agriculture and Rural Development in the Tropics and Subtropics*106: 29 ï 39.

AGNIHOTRI, N.P., WALIA, S. and V.T. GAJBHYIE. 1999. Green Pesticides, Protection and Safety Evaluation. Indian Agricultural Research Institute, New Delhi.

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

ALASHALABY, E.M. and E.M.A. NOWEER. 2003. Effect of the five plant extracts on the reproduction of root-knot nematode (*Meloidogyne incognita*) infested peanut under field condition. *Journal of Agricultural Science* 28: 7447 ï 7454.

ANON. 2011. A Profile of the South African Tomato Market Value Chain. Department of Agriculture, Forestry and Fisheries.Republic of South Africa.

AUGER, J. and E. THIBOUT. 2004. Sulfur Compounds Derived from *Allium* and Crucifers and their Potential Applications in Crop Protection. In: Biopesticides of plant origins. *Les derniers ouvrages de tec and doc* (edition), France.

AUGER, J., ARNAULT, I., DIWO-ALLAIN, S., RAVIER, M., MOLIA, F. and M. PETTITI. 2004. Insecticidal and fungicidal potential of *Allium* substances as biofumigants. *Agroindustria* 3: 367 ï 370.

BALLESTEROS, E., MARTIN, D. and M.J. URIZ. 1992. Biological activity of extracts from some Mediterranean macrophytes. *Botanical Marina* 35: 481 ï 485.

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

BAUSTISTA, D.M., MOVHED, P., HINMAN, A., AXELSSON, H.E., STERNER, O., HOGESTATT, E.D., JULLIUS, D., JORDT, S.E. and P.M. ZYGMUNT. 2005. Pungent products from garlic activate the sensory ion channel TRPA 1. *Clinical and Experimental Pharmacy* 102: 12248 ï 12252.

BELLO, A. 1998. Biofumigation and Integrated Crop Management. In: Bello, A, Gonzalez, JA, Arias, M, and R, Rodriguez-Kabana (eds.), Alternatives to

Methyl Bromide for the Southern European Countries. DG XI, EU, CSIC, Madrid, Spain.

BORRIS, R.P. 1996. Natural products research: perspectives from a major pharmaceutical company. *Journal of Ethnopharmacology* 51: 29 ï 38.

BUNT, J.A. 1975. Effect and mode of action of some systemic nematicides. Mecedlando Hogesch. *Wageningen* 75: 1 ï 128.

CHITWOOD, D.J. 2003. Research on plant-parasitic nematode biology conducted by the United States Department of Agricultural Research Services. *Pest Management Science* 59: 748 ï 753.

CORZO-MARTINEZ, M., CORZO, N. and M. VILLAMIEL. 2007. Biological properties of onions and garlic. *Trends in Food Science and Technology* 18: 609 ï 625.

COSTA, S.D., SANTOS, M.S.N.D. and M.F. RYAN. 2003. Effect of *Artemisia vulgaris* rhizome extracts on hatching, mortality and plant infectivity of *Meloidogyne megadora*. *Journal of Nematology* 35: 437 ï 442.

DAVISON, C. 2002. A biochemical study of the antidiabetic and anticoagulant effects of *Tulbaghia violacea*. PhD Thesis submitted to Nelson Mandela Metropolitan University, Port Elizabeth.

DJURDJEVIC, L., DINIC, A., PAVLOV, P., MITROVIC, M., KARADZIC, B. and V. TESEVIC. 2004. Allelopathic potential of *Allium ursinum*. *Biochemical Systematics and Ecology* 32: 533 ï 544.

DROPKIN, V.H. 1980. Introduction to Plant Nematology. Wiley, New York.

EISENBACK, J.D. CARMEL-GOREN, L., HAREVEN, D. and H.H. TRIANTAPHYLLOU. 1991. Root-knot Nematodes: *Meloidogyne* spp. and Races. *In*: Nickle, W.R. and M. Dekker (eds.), *Manual of Agricultural Nematology*.Inc, New York.

ESFAHANI, M.N. 2009. Distribution and identification of root-knot nematode species in tomato fields. *Myco-phytopathological Society of Pakistan* 7: 45 ï 49.

FASSULIOTIS, G. 1985. The Role of Nematologist in the Development of Resistant Cultivars. In: Sasser, JN, and CC, Carter (eds.), An advanced Treatis on *Meloidogyne*: Vol. 11, Methodology. North Carolina State University Department, Department of Plant Pathology and the United State Agency for International Development, Raleigh.

FERGUSON, M.P. and H.G. ALFORD. 1985. Microbial/Bio-rational Pesticides Registration. Special Publication No. 3318. University of California, Berkeley.

FERRIS, H. and L. ZHENG. 1999. Plant sources of Chinese herbal remedies: Effects of *Pratylenchus vulnus* and *Meloidogyne javanica*. *Journal of Nematology* 31: 241 ï 263.

GOMMERS, F.L., BARKER, J. and H. NYMBRG. 1982. Dithiophenes as single oxygen sensitizers. *Phytochemistry and Photobiology* 35: 615 ï 619.

GUPTA, R. and N.K. SHARMA. 1993. A study of the nematicidal activity of allicin - an active principle in garlic (*Allium sativum*) against root-knot nematodes (Kofoid and White, 1919) Chitwood, 1949. *International Journal of Pest Management* 39: 390 ï 392.

HARRIS, S. 2004. *Tulbaghia violacea*. Free State National Botanical Garden. South Africa.

HASSAN, S.M.E., RAHMAN, M., AMIN, M.R., MAJUMDAR, U.K. and M.U. AHMAD. 2001. Effect of neem (*Azadirachta indica*) on the root-knot (*Meloidogyne javanica*) of sweet gourd. *Pakistan Journal of Biological Sciences* 11: 1853 ï 1854.

HEDIN, P.A. 1991. Use of Natural Products in Pest Control. Developing Research Trends. *In*: Hedin, P.A. (ed.), Naturally Occuring Pest Bioregulators. ASC Symposium Series, NO. 449. American Chemical Society, Washington, D. C.

HUSSEY, R.S. and K.R. BARKER. 1973. A comparison of methods of collecting inoculants *Meloidogyne* species including a new technique. *Plant Disease Reporter* 57: 1025 ï 1028.

INDERJIT, K. and A.U. MALIK. 2002. Chemical Ecology of Plants: Allelopathy in Aquatic and Terrestrial Ecosystems. Springer-Verlag, Heidelburg, Berlin.

JACQUET, M., BONGIOVANNI, M., MARTINEZ, M., VERSCHAVE, P., WAJNBERG, E. and P. CATAGNONE-SERENO. 2005. Variation in

resistance to the root-knot nematode *Meloidogyne incognita* in tomato genotypes bearing the Mi gene. *Plant Pathology* 54: 93 ï 99.

JENKINS, W.R. 1964. A rapid centrifugal-floatation technique for separating nematodes from soil. *Plant Disease Reporter* 48: 692 ï 695.

KALI, R. and D.C. GUPTA. 1980. A note on the efficiency of fresh neem leaf extract in the control of *Meloidogyne javanica* infecting chickpea. *Indian Journal of Nematology* 10: 96 ï 98.

KASKAVALCI, G. 2007. Effect of soil solarisation and organic amendment treatments for controlling *Meloidogyne incognita* in tomato cultivars in Western Anatolia. *Turkey Agricultural Journal* 31: 159 ï 169.

KHAN, A.F. 1990. Nematicidal potential of some naturally growing plants against *Pratylenchus* zeae. *Reveue Nematology* 13: 463 ï 465.

LIN, C.Y., ZHENG, C.R. and Z.H. CHENG. 2009. Inhibitory effects of freshly crushed garlic (*Allium sativum*) extract on seed-borne pathogens of cucumber (*Cucumis sativus*) and allelopathy functions. *Journal of Natural Science* 37: 140 ï 150.

LIU, D.L., AN, M., JOHNSON, I.R. and J.V. LOVETT. 2003. Mathematical modelling of allelopathy. III. A model for curve-fitting allelochemical dose responses. *Nonlinearity in Biology Toxicology Medicinal* 1: 37 ï 50.

LONGENECKER, D.E. and P.J. LYERLY. 1964. Some Relations Among Irrigation Water Quality, Soil Characteristics and Management Practices in the Trans-Pecos Area. Texas Agricultural Experiment Station Report MP-373.

MAFEO, T.P. 2005. Propagation, fertilisation and irrigation of *Cucumis myriocarpus*. *MSc Dissertation*, University of Limpopo, Sovenga, South Africa.

MAKKAR, H.P.S. 1999. Quantification of Tannins in Tree Foliage. IAEA Working Document, IAEA. Vienna, Austria.

MAMPHISWANA, N.D., MASHELA, P.W. and L.K. MDEE. 2010. Distribution of total phenolics and antioxidant activity in fruit, leaf, stem and root of *Monsonia burkeana*. *African Journal of Agricultural Research* 5: 2570 ï 2575.

MARBAN-MENDOZA, N., JEYAPRAKASH, A., JANSSON, H.B., DAMAN, R.A. and B.M. ZUCKERMAN. 1987. Control of root-knot nematode on tomato by lectins. *Journal of Nematology* 19: 331 ï 335.

MASHELA, P.W. 2002. Ground wild cucumber fruits suppress numbers of *Meloidogyne incognita* on tomato in microplots. *Nematropica* 32: 13 ï 19.

MASHELA, P.W. and M.E. NTHANGENI. 2002a. 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 ï 402.

MASHELA, P.W. and M.E. NTHANGENI. 2002b. Mechanism through which *Tylenchulus semipenetrans* imbalances osmoticum ions in Citrus. *Journal of Nematology* 32: 4 ï 19.

MASHELA, P.W., SHIMELIS, H.A. and F.N. MUDAU. 2008. Comparison of the efficacy of ground wild cucumber fruits, aldicarb and fenamiphos on suppression of the root-knot nematode on tomato. *Journal of Phytopathology* 156: 264 ï 267.

MASHELA, P.W., SHIMELIS, H.A., DE WAELE, D., MOKGALONG, M.N., MUDAU, N. and L.G. NGOBENI. 2010. Fever tea (*Lippia javanica*) as a root-knot nematode suppressant in tomato production. *African Plant Protection* 16: 1 ï 6.

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

McGRAW, L.J., JAGER, A.K. and J. VAN STADEN. 2000. Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. *Journal of Ethnopharmacol*ogy 72: 247 ï 263.

McLEAN, K.S. and G.W. LAWRENCE. 2000. A survey of plant-parasitic nematodes associated with cotton. *Journal of Nematology* 35: 508 ï 512.

MIAN, I.H. and R. RODRIGUEZ-KABANA. 1982. Survey of the nematicidal properties of some organic materials available in Alabama as amendments to soil for control of *Meloidogyne arenaria*. *Nematropica* 12: 235 ï 246.

MITSCHERET, L.A., DRAKE, S., GOLLOAPUDI, S.R. and S.K. OKWUTE. 1987. A modern look at folkloric use of anti-ineffective agents. *Journal of Natural Products* 50: 1025 ï 1040.

MOFOKENG, M.A., TSILO, T.J., NDOVE, T. and P.W. MASHELA. 2004. Role of Bacillus species in *Ricinus communis* suppression of *Tylenchulus semipenetrans*. *African Plant Protection* 10: 130.

NTESO, L. and J.C. PRETORIUS. 2006. *Tulbaghia violacea* I: *In* vitro antimicrobial properties towards plant pathogens. *Australian Journal of Agricultural Research* 57: 511 ï 516.

OKA, Y., SHAPIRA, N. and P. FINE. 2007. Control of root-knot nematodes in organic farming systems by organic amendments and soil solarization. *Crop Protection* 26: 1556 ï 1565.

OLORUNNISOLA, O.A., BRADLEY, G. and A.J. AFOLAYAN. 2012. Chemical composition, antioxidant activity and toxicity evaluation of essential oil of *Tulbaghia violacea*. *Journal of Medicinal Plants Research* 6: 2340 ï 2347.

OLORUNNISOLA, O.A., BRADLEY, G. and A.J. AFOLAYAN. 2011. Antioxidant properties and cytotoxicity evaluation of methanolic extract of dried and

fresh rhizomes of *Tulbaghia violacea*. *African Journal of Pharmacy and Pharmacology* 5: 2490 ï 2497.

PAKEERATHAN, K., MIKUNTHAN, G. and N. THARSANI. 2009. Eco-friendly management of root-knot nematode *Meloidogyne incognita* (Kofid and White) chitwood using different green leaf manures on tomato under field conditions. *American-Eurasian Journal of Agricultural and Environment Science* 6: 494 ï 497.

PANDEY, R., SIKORA, R.A., KALRA, A., SINGH, H.B. and S. PANDEY. 2003. Plants and their products act as major inhibitory agents. In: Trivedi, P.C. (ed.), Nematode Management in Plants. Scientific Publishers: New Delhi.

PELINGANGA, O., NZANZA, B., MAMPHISWANA, N. and P.W. MASHELA. 2011. Influence of fermented fruit extracts of *Cucumis africanus* and *Cucumis myriocarpus* on nematode numbers and tomato productivity. *Symposium of Nematological Scociety of South Africa* 20: 71.

PELINGANGA, O. 2013. Developing bio-nematicides using indigenous *Cucumis africanus* and *Cucumis myriocarpus* fruits for tomato production systems. PhD Thesis, University of Limpopo, Sovenga, South Africa.

PODGER, F.D. 1972. *Phytophthora cinnamomi*, a cause of lethal disease in indigenous plant communities in Western Australia. *Phytopathology* 62: 972 ï 981.

PRETORIUS, J.C., MAGAMA, S. and P.C. ZIETMAN. 2003. Growth inhibition of plant pathogenic bacteria and fungi by extracts from selected South African plant species. *South African Journal of Botany* 69: 186 ï 192.

RADZIAH, O., AZIZAH, H. and A.R. ZAHARAH. 1997. The phytotoxicity effects of palm oil dry soils on plant growth. *Pertanika Journal of Tropical Agricultural Science* 20: 91 ï 99.

RICE, E.L. 1984. Allelopathy. Academic Press. Orlando, FL, USA.

SALISBURY, F. B. and C.W. ROSS. 1992. Plant Physiology. Wadsworth, Belmont California.

SAS. 2004. SAS Institute, Inc., Cary, NC, U.S.A.

SASSER, J.N. 1979. Economic Importance of *Meloidogyne* in Tropical Countries. *In*: Lamberti, F. and C.E. Taylor (eds.), Root-knot Nematode (*Meloidogyne* spp.) Systematic, Biology and Control. Academic Press, London.

SIDDIQUI, M.A. and M.M. ALAM. 1987. Efficacy of seed dressing with extracts of neem and *Persia lilak* against *M. incognita* and *Rotylenchulus reniformis*. *Nematology Mediterrean* 15: 399 ï 403.

SIKORA, R.A. and E. FERNANDEZ. 2005. Nematode parasites of vegetables. *In*: Luc, M. Sikora, R.A. and J. Bridge (eds.), Plant-parasitic Nematodes in Subtropical and Tropical Agriculture. CABI Publishers, Cambridge.

SINGH, V.K. and D.K. SINGH. 2008. Pharmacological effects of garlic (*Allium sativum*). ARBS *Annual Review of Biochemical Sciences* 10: 6 ï 26.

SINGH, S.K. and R.K. KHURMA. 2007. Susceptibility o six tomato cultivars to root-knot nematode *Meloidogyne incognita*. *The South Pacific Journal of Natural Science* 13: 73 ï 77.

SUKUL, N.C., DAS, P.K. and G.C. DE. 1974. Nematicidal action of some edible crops. *Nematologica* 20: 187 ï 191.

SWART, A. 2010. Research on Nematodes. Agricultural Research Council, http://www.arc.agric.za/home.asp?pid=1798 (Accessed date 25/5/2012).

TADA, M., HIROE, Y., KIYOHARA, S. and S. SUZUKI. 1988. Nematicidal and antimicrobial constituents from *Allium grayi* Regel, *Allium fistulosum* and *var. caespitosum*. *Agricultural Biological Chemistry* 52: 2383 ï 2385.

TAYE, W., SAKHUJA, P.K. and T. TEFERA. 2012. Evaluation of plant extracts on infestation of root-knot nematode on tomato (*Lycopersicon esculentum*). *Journal of Agricultural Research and Development* 2: 086 ï 091.

TAYLOR, A.L. and J.N. SASSER. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyn*e species) Raleigh, North Carolina, North Carolina State University Press.

VAN WYK, B.E., VAN OUDTSHOORN, B. and N. GERICKE. 1997. Medicinal Plants of South Africa. Brizza Publications, Pretoria.

WACHIRA, P.W., KIMENJU, J.W., OKOTH, S.A. and R.K. MIKEY. 2009. Stimulation of nematode destroying fungi by organic amendments applied in

management of plant parasitic nematode. *Asian Journal of Plant Science* 8: 153 ï 159.

WHITEHEAD, D.C., DIBB, H. and R.D. HARTLEY. 1983. Bound phenolics compounds in water extracts of soils, plant root and litter. *Journal of Soil Biology and Biochemistry* 15: 133 ï 136.

WILLIAMSON, V.M. and R.S. HUSSY. 1996. Nematode pathogenesis and resistance in plants. *Plant Cell* 8: 1735 ï 1745.

YAZDANI, D., TAN Y.H., ZAINAL-ABIDIN, M.A. and B. JAGANATH. 2011. A review on bioactive compounds isolated from plants against plant pathogenic fungi. *Journal of Medical Plants Research* 5: 6584 ï 6589.

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

APPENDICES

Appendix 3.1 Analysis of variance (ANOVA) for four levels of crude extracts of *Tulbaghia violacea* on plant height of tomato plant.

Source of variance	DF	SS	MS	F	P
Block	9	3280.1	364.46		
Treatment	3	14949.6	4983.20	41.94	0.0000
Error	27	3207.9	118.81		
Total	39	21437.6			

Appendix 3.2 Analysis of variance (ANOVA) for four levels of crude extracts of *Tulbaghia violacea* on stem diameter of tomato plant.

Source of variance	DF	SS	MS	F	P
Block	9	7.864	0.8737		
Treatment	3	13.001	44.3338	99.49	0.0000
Error	27	12.031	0.4456		
Total	39	152.896			

Appendix 3.3 Analysis of variance (ANOVA) for four levels of crude extracts of *Tulbaghia violacea* on number of leaves of tomato plant.

Source	DF	SS	MS	F	P
Block	9	152.000	16.889		
Treatment	3	322.400	107.467	17.52	0.0000
Error	27	165.600	6.133		
Total	39	640.000			

Appendix 3.4 Analysis of variance (ANOVA) for four levels of crude extracts of *Tulbaghia violacea* on number of fruits of tomato plant.

Source	DF	SS	MS	F	P
Block	9	51.225	5.6917		
Treatment	3	79.875	26.6250	5.45	0.0046
Error	27	131.875	4.8843		
Total	39	262.975			

Appendix 3.5 Analysis of variance (ANOVA) for four levels of crude extracts of *Tulbaghia violacea* on number of flowers for tomato plant.

Source	DF	SS	MS	F	P
Block	9	94.600	10.511		
Treatment	3	466.100	155.367	14.75	0.0000
Error	27	284.400	10.533		
Total	39	845.100			

Appendix 3.6 Analysis of variance (ANOVA) for four levels of crude extracts of *Tulbaghia violacea* on number of cluster for tomato plant.

Source	DF	SS	MS	F	P
Block	9	12.225	1.3583		
Treatment	3	56.075	18.6917	12.41	0.0000
Error	27	40.675	1.5065		
Total	39	108.975			

Appendix 3.7 Analysis of variance (ANOVA) for four levels of crude extracts of *Tulbaghia violacea* on fresh shoot weight of tomato plant.

Source	DF	SS	MS	F	P
Block	9	5799.6	644.40		
Treatment	3	17564.1	5854.70	24.89	0.0000
Error	27	6349.8	235.18		
Total	39	29713.5			

Appendix 3.8 Analysis of variance (ANOVA) for four levels of crude extracts of *Tulbaghia violacea* on dry shoot weight of tomato plant.

Source	DF	SS	MS	F	P
Block	9	839.10	93.234		
Treatment	3	716.42	238.808	5.64	0.0039
Error	27	1143.36	42.347		
Total	39	2698.88			

Appendix 3.9 Analysis of variance (ANOVA) for four levels of crude extracts of *Tulbaghia violacea* on root weight of tomato plant.

Source	DF	SS	MS	F	P
Block	9	4240.8	471.20		
Treatment	3	10305.8	3435.28	23.64	0.0000
Error	27	3924.3	145.35		
Total	39	18470.9			

Appendix 3.10 Analysis of variance (ANOVA) for four levels of crude extracts of *Tulbaghia violacea* on fruit yield weight of tomato plant.

Source	DF	SS	MS	F	P
Block	9	826.45	91.8273		
Treatment	3	82.05	27.3489	0.35	0.7888
Error	27	2104.54	77.9458		
Total	39	3013.03			

Appendix 3.11 Analysis of variance (ANOVA) for four levels of crude extracts of *Tulbaghia violacea* on soil electrical conductivity (EC) of tomato plant.

Source	DF	SS	MS	F	P
Block	9	0.02077	0.00231		
Treatment	3	0.01383	0.00461	0.89	0.4612
Error	27	0.14058	0.00521		
Total	39	0.17518			

Appendix 3.12 Analysis of variance (ANOVA) for four levels of crude extracts of *Tulbaghia violacea* on soil pH of tomato plant.

Source	DF	SS	MS	F	P
Block	9	1.37355	0.15262		
Treatment	3	0.69871	0.23290	1.56	0.2214
Error	27	4.02492	0.14907		
Total	39	6.09718			

Appendix 3.13 Analysis of variance (ANOVA) for four levels of crude extracts of *Tulbaghia violacea* on number of root galls of tomato plant.

Source	DF	SS	MS	F	P
Block	9	1.6000	0.17778		
Treatment	3	23.0000	7.66667	23.00	0.0000
Error	27	9.0000	0.33333		
Total	39	33.6000			

Appendix 3.14 Analysis of variance (ANOVA) for four levels of crude extracts of *Tulbaghia violacea* on nematode numbers in root of tomato plant.

Source	DF	SS	MS	F	P
Block	9	32299	3588.7		
Treatment	3	144504	48168.0	29.12	0.0000
Error	27	44660	1654.1		
Total	39	221463			

Appendix 3.15 Analysis of variance (ANOVA) for four levels of crude extracts of *Tulbaghia violacea* on nematode numbers in soil of tomato plant.

Source	DF	SS	MS	F	P
Block	9	1709.4	189.93		
Treatment	3	13410.2	4470.07	21.41	0.0000
Error	27	5636.8	208.77		
Total	39	20756.4			

Appendix 3.16 Analysis of variance (ANOVA) for four levels of crude extracts of *Tulbaghia violacea* on total nematode numbers in root + soil of tomato plant.

Source	DF	SS	MS	F	P
Block	9	38002	4222.5		
Treatment	3	160583	53527.7	13.52	0.0000
Error	27	106915	3959.8		
Total	39	305500			