RESPONSES OF ROOT-KNOT NEMATODE DENSITIES TO AQUEOUS EXTRACTS OF CHILLI AND TAMBOTI

by

LUFUNO PATRICK THOVHAKALE

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Supervisor: Professor C. van der Westhuizen, Ph.D.

Co-supervisor: Professor P.W. Mashela, Ph.D.

BLOEMFONTEIN
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Declaration of independent work

I, LUFUNO PATRICK THOVHAKALE, hereby declare that this dissertation, submitted for the degree MAGISTER TECHNOLOGIAE: AGRICULTURE, is my own independent work that has not been submitted before to any institution, by me or any other person, as part of any qualification.

L Thovhakale 17-01-2006

Signature Date

Foreword

This research is an effort towards finding alternatives to methyl bromide with regard to plant protection against root nematodes. In addition, it serves as a base for further research in the control of nematodes using plant extracts. The dissertation is presented in the form of five chapters. Chapter 1 is the general introduction that reflects the crop losses caused by plant parasitic nematodes and discusses the phasing out and replacement of chlorofluorocarbons due to the continuous and hazardous damage to the ozone layer, with life-threatening consequences on earth. Chapter 2 is the literature review, which focuses on crude plant extracts, the use of plant extracts for crop protection, the release of potent chemicals by plants, the effects of plant extracts on pests, plant extract residues in crops and the environment and the potential use of plant extracts. Chapter 3 comprises materials and methods. Chapter 4 contains the results obtained for all the variables measured in the study as well as a discussion thereof, and Chapter 5 provides an overall assessment of the results obtained in this study, with recommendations for future research.

Care was taken to avoid repetition through the use of secondary data from previous research. However, repetition was unavoidable in Chapter 3 (Materials and Methods), since the experiments were conducted under two different conditions (greenhouse and micro-plot).

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- The Central University of Technology, for supporting me and enabling me to perform my work properly.

The Lord promises in the Bible that He will bless us and make us prosperous. I thank and praise Him for giving me strength, perseverance and guidance throughout this study.

Lufuno Thovhakale

Dedication

I would like to dedicate this study to my father, Takalani, mother, Mulatedzi, wife, Mbengeni and son, Hulisani Lufuno. You are the driving force behind my success. Also a posthumous dedication to my late grandfathers, Jim and Tshińanga, and my grandmother, Luvhengo. To my special grannies, Muofhe, Tshinakaho and the late Mutshinya - I am here because of your efforts.

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ABBREVIATIONS

ANOVA	: Analysis of Variance
C:N	: Carbon to Nitrogen
cm	: centimetre
CFCs	: Chlorofluorocarbons
CUT	: Central University of Technology
ºC	: Degrees Celsius
EC	: Electrical Conductivity
g	: gram
GLT	: Ground Leaching Technology
J2s	: Second-stage juveniles
kg/ha	: kilogram per hectare
kg/l	: kilogram per litre
km	: kilometre
l	: litre
LSD	: Least Significant Difference
μm	: micrometre
ml	: millilitre
mm	: millimetre
NaOCl	: Sodium hypochlorite
NRF	: National Research Foundation
ODP	: Ozone Depletion Potential

P_i	: Initial population density
P_f	: Final population density
%	: Percentage
pH	: Log $_2$ of reciprocal concentration of Hydrogen ions
t/ha	: ton per hectare
SAS	: Statistical Analysis System
TTV	: Total Treatment Variation
UNIN	: University of the North
USA	: United States of America
v/v	: volume per volume
Zn	: Zinc

Chapter 1

GENERAL INTRODUCTION

Globally, more than 40 % of crop losses can be attributed to pest damage (Pedigo, 1996). Yield losses due to plant-parasitic nematodes range from 8–20 % in various crops (Table 1.1). However, in developing countries the loss may be as high as 25 to 50 %. The losses due to the root-knot nematode (*Meloidogyne species)* are heavy, and amount to millions of dollars worldwide. In practice, this implies that many people who go hungry, especially in developing countries, would have been fed if this plant pest had been controlled (Lucas, Campbell and Lucas, 1985).

Table 1.1: Annual crop losses caused by plant-parasitic nematodes (Ferraz and Brown, 2002).

	Crops								
	Banana	Citrus	Coffee	Maize	Peanut	Potato	Sugar-cane	Tea	Cotton
Yield losses (%)	20	14	15	10	12	12	15	8	11
Losses in million US$	180	4 000	2 500	6 800	1 100	5 800	16 500	510	4 200

Root-knot nematodes have a widespread distribution, which increases the cost of crop protection. Root-knot nematodes are a more serious and complex problem in most developing countries than in developed countries. In developing countries, small-scale farmers produce 70 % of the food crops. These small-scale farmers are poorly resourced and use low-cost production practices, characterised by indigenous knowledge with little or no advanced technology. With limited land available, moving from one piece of land to the

next is no longer practical (Lucas et al., 1985). In view of these circumstances, infestation of farms by root-knot nematodes brings production to a standstill.

Methyl bromide, which was the only fumigant at the farmers' disposal, is being suspended because of its high toxicity and harmful environmental impact (and possible effect on the ozone layer). The phasing out of this fumigant means that farmers are now left with fewer and more expensive options for the control of soil-borne pests, including root-knot nematodes. Root-knot nematodes infect more than 3 000 plants species, all of which are main crops. The *Meloidogyne* species are the most important economic crop pest.

Indirect losses associated with root-knot nematodes are caused by (a) secondary attacks by other pathogens, (b) the increased cost of weed control and reduced crop competition for weeds, (c) loss of usefulness of cultivars resistant to other pests and diseases, but susceptible to root-knot nematodes, (d) inefficient utilisation of fertilizers and water, (e) the cost of chemical control and (f) the loss of use of fields due to the necessity of crop rotation. The increase in production costs adversely affects crop yield, quality and profit.

In a recent scientific assessment, a connection was found between global ozone depletion and the emission of chlorofluorocarbons (CFCs) into the atmosphere. As a result, developed countries signed the Montreal Protocol containing interim reductions, and the final date for the phasing out of CFCs was set for 1 January 2005 (Wilcut and Thomas, 2002). If the developed countries accelerate the CFCs schedule, the developing countries will have to follow suit in order to retain their share in the global market. The phasing out of the CFCs has stimulated a considerable amount of nematode control research at international and national level. The research undertaken includes evaluations of organic amendments, soil solarization, biorational nematode suppressant compounds, cultural practices and the development of resistant crop cultivars (Noling and Gilreath, 2000). It is important that intensive research is conducted to ensure that effective, affordable, user- and environmentally friendly alternatives are available and ready for use by small-scale crop farmers before the final phasing out of CFCs in developing countries in 2015. Scientists still consider methyl bromide a significant ozone-depleting substance. It has an ozone

depletion potential (ODP) of 0.4 (Wilcut and Thomas, 2002). Continuous addition of methyl bromide to the atmosphere by humans may upset the delicate balance that allows life to exist on this planet. Depletion of the ozone layer will result in an increased level of radiation reaching the earth's surface, with a potential impact on human health, the environment and agricultural activities. There is a valid reason for the initiation of a phasing-out programme, since about 50 to 90% of the methyl bromide injected into the soil will eventually enter the atmosphere (Wilcut and Thomas, 2002).

The objective of this study is to analyse the responses of root-knot nematode densities to aqueous extracts of Tamboti and Chilli, under both greenhouse and micro-plot conditions.

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Chapter 2

LITERATURE REVIEW

2.1 Introduction

Literature on the suppression of plant-parasitic nematodes by organic amendments is replete with promising but inconsistent results (McSorley and Gallaher, 1995a, b). The efficacy of organic amendments against nematodes depends on the amount applied, the C: N ratio and the decomposition rate. Large quantities of organic amendments are needed and the efficacy will vary, depending on soil microbiology at various locations. The economics of implementation are a concern with regard to factors such as availability and transportation. Most organic amendments are phytotoxic during the first 2-4 weeks after application, which delays planting after application (McSorley and Gallaher, 1995a).

The Department of Plant Production at the University of the North, Limpopo Province, is evaluating various toxic plant organs used in the suppression of plant-parasitic nematodes. Mashela and Mphosi (2001) developed a plant extract technology using toxic plant organs, which attained nematode suppression at 0.71 t/ha. Conventional organic amendments with confirmed nematicidal effects are applied at much higher rates of 10 to 500 t/ha in order to achieve nematode suppression (McSorley and Gallaher, 1995a; Stirling, 1991). Mashela and Mphosi's (2001) promising result at a low application rate indicates that plant extracts could represent a breakthrough with regard to nematode management since one of the major constraints in this regard is high application rates, which translate into availability and transport problems.

Organic amendments have a short-lived residual effect against nematodes, and are preferable to synthetic nematicides (Rechcigl and Rechcigl, 2000). In order to counteract the instability of organic amendments, certain plant extracts are combined with antioxidants to improve their persistence. For long-term storage, they are kept in the dark

at 25°C (Rechcigl and Rechcigl, 2000). Nevertheless, plant extracts annually constitute a very small portion of the overall volume of protectants used globally. However, they remain crucial in pest management for four reasons: (a) they provide effective control over pests that have developed resistance to other synthetic pesticides, (b) they pose relatively low risks to non-target organisms as they are short-lived in the soil and crops, (c) they occur naturally and (d) their preparation requires only simple technology (Rechcigl and Rechcigl, 2000).

The literature review in this study will be limited to the specific use of plant extracts, the processes involved in the release of potent chemicals, the effect of plant extracts and their potential use for nematode control.

2.2 Crude extracts

Crude extracts are crude preparations of plant organs ground to produce a dust or powder. These are used at full strength or diluted in a carrier such as clay, talc or diatomaceous earth (Rechcigl and Rechcigl, 2000). Such preparations include dust from the pyrethrum daisy flower (*Chrysanthemum cinerariefolium* and *C. coccincum*), cube roots of rotenone, sabadilla seeds, ryania stems or the leaves and bark of the neem (*Azadirachta indica*) plant (Rechcigl and Rechcigl, 2000). Only slightly more sophisticated are water extracts or organic solvent extracts of the insecticidal components of plants. Crude plant extracts have been used traditionally for several centuries, and were known in developing countries around the world before being introduced in developed countries. Plant extracts with a long history of traditional use include neem in India, rotenone in East Asia and South America, pyrethrum in Iran and sabadilla in Central and South America (Radwanski, 1977a, b, c).

The discovery and increased development of synthetic pesticides in the 1940's and 1950's led to the abandonment of plant extracts in commercial agriculture in the developing world (Rechcigl and Rechcigl, 2000). Newer, synthetic pesticides were less expensive, more

effective and longer-lasting, and received state support through the promotion of their use among crop farmers. In the 1980's and 1990's interest in the use of organic amendments increased, with certain markets selling organically produced food. This interest was driven primarily by concerns about the longer-lasting residues of synthetic pesticides in crops (Rechcigl and Rechcigl, 2000). Plant extracts are still expensive to use, due to limited technological knowledge of the extraction of these materials (Rechcigl and Rechcigl, 2000). More research on extraction techniques that will produce large quantities of these materials will also increase their affordability.

2.3 The use of plant extracts

Plant extracts are reported to suppress a wide range of crop pests. Although the focus of this review is nematode suppression, a brief overview is given of the suppression of diseases and insect pests using plant extracts.

2.3.1 Suppression of diseases

Plant extracts are toxic and effective against certain bacteria and fungal pathogens. Onion (*Allium cepa*) repels many potential pathogenic fungi due to the protocatechuic acid (aldehyde) contained in its roots and shoots (Kaufman, 1989). Garlic (*Allium sativa*) leaves and bulbs contain high levels of *Alliin* (dialkyl sulphide), which has a bactericidal action when it enzymatically breaks down into *Allicin* (Kaufman, 1989). Apart from its insecticidal properties, the neem derivative *Azadirachtin* exhibits antiviral and antifungal properties (Mordue and Blackwell, 1993).

2.3.2 Suppression of insect pests

Chilli fruit powder is considered useful for home garden and commercial producers who want to avoid synthetic pesticides (Briggs, Zhender and Witt, 1996). A leaf dip bioassay with permethrin and thiodicard indicated that extracts of *Oleoresis* did not synergise these insecticides for control of *Spodoptera exigua* (Reed and Ramaswamy, 1995). Important factors to be considered in the control of soil pests are life cycle and habitats (Hassal,

1980). Many species seasonally migrate to different soil depths. Effectiveness is likely to be achieved by treatment during the stage of shallow occupation in the soil. Cutworm larvae avoided droplets containing permethrin extracts in a laboratory test (Reed and Ramaswamy, 1995). Crude pyrethrum powder was introduced to Europe around 1800, and it was in use to control insect pests worldwide by around 1850 (Casida, 1973; Casida and Quistad, 1995; Matsumura, 1985). Pyrethrum is a powder produced by grinding the dried flowers of the pyrethrum daisy. Pyrethroids are synthetic insecticides with core chemical structures that resemble natural pyrethrins (Rechcigl and Rechcigl, 2000). Although plant-derived pyrethrins are very toxic and effective against insects, they are not very toxic to mammals via dermal or oral routes. However, they are very toxic by inhalation, since this provides a more direct route to the blood (Rechcigl and Rechcigl, 2000). Due to their rapid breakdown rate, extracts of pyrethrins are ineffective in most outdoor applications, particularly where some degree of residual activity is needed to protect crops (Rechcigl and Rechcigl, 2000).

Neem is the primary source of *Azadirachtin*, a broad-spectrum plant extract pesticide that is pungent and prophylactin-based and controls nematodes and other soil-borne insect pests (Rechcigl and Rechcigl, 2000). The value of neem as a pesticide and deterrent has been known in India for centuries. It is one of several liminoids extracted from seeds. This is a steroid-like tetranortriterpernoid, which is basically a water-soluble terpernoid (Schmutterer, 1990). Neem extracts proved to be a growth regulator, interfering in the larval and pupal stages of many insects and thus effectively blocking the metamorphosis process (Rechcigl and Rechcigl, 2000). *Eucalyptus* and rhubarb leaf extracts are used to protect seedlings from cutworm (Kaufman, 1989; Munday, 1988). Pyrethrins suppress insect activities through interference with nerve transmission, by slowing down or preventing the shutting of sodium channels in the nerve axons (Bloomquist, 1996).

Extraction in methanol is a common method of obtaining *Azadirachtin* from seeds. Neem is reported to provide nitrogen in slow-release form; it also acts as an insect repellent, affects insect feeding, acts as an oviposition deterrent, reduces growth and development and interferes with reproduction in insect pests (Schmutterer, 1990; Yespen, 1984). It is

effective against the soft-bodied, immature stages of plant insect pests, including nematodes and cutworms (Rechcigl and Rechcigl, 2000). The products of neem are marketed for use in the USA as sprays, dust and systemic soil applications (Rechcigl and Rechcigl, 2000).

2.3.3 Suppression of nematodes

The application of the foliage, fruit and seed meal of castor bean meal (*Ricinus communis*) consistently reduced nematode population densities (Mashela and Mpati, 2001; Mashela and Nthangeni, 2002; Ritzinger and McSorley, 1998). Castor bean leaf extracts suppressed nematode population densities on tomato at a rate of 4-6 g per pot (Ritzinger and McSorley, 1998). When P_i was kept constant, each unit increase in *R. communis* fruit meal reduced final nematode population densities by 2.95 units (Mashela and Nthangeni, 2002).

Wild cucumber (*Cucumis myriocarpus)* fruit meal and fever tea (*Lippia javanica*) leaf meal consistently suppressed nematode numbers (Mashela and Mphosi, 2001; Ngobeni, 2003). Mashela and Nthangeni (2002) suggested that nematode numbers were suppressed by *Ricin*, a highly toxic compound from *Ricinus* fruit meal. In *C. myriocarpus*, nematodes are suppressed by large quantities of highly toxic *Cucumin* (C_{27} H_{40} O_9) *and Leptodermin* (C_{27} H_{38} O_8), collectively referred to as *Cucurbitacins.* They are water-soluble, have strong cutaneous adsorptive properties and are among the bitterest known substances (Mashela, 2002; Van Wyk, Van Oudtshoorn and Gericke, 1997). *In L. javanica,* nematodes are suppressed by oil that contains toxic active ingredients, including myrcene, caryophyllene, linalool, *p*-cymene and ipsdienone (Mashela and Ngobeni, 2001; Van Wyk and Gericke, 2000). These are active repellents. In another study, *L. javanica x Cucumis* and *Ricinus* acted accumulatively in the suppression of root-knot nematodes on tomato plants, suggesting that each active ingredient acted independently (Mabitsela, Mashela and Mphosi, 2003). Olive pomace extract resulted in low nematode population numbers in the greenhouse and micro-plot experiments, which also confirmed the finding on nematicidal properties (Rodriguez-Kabana, Estaún, Pinochet and Marfa, 1995).

2.4 The release of potent chemicals from plants

Potent chemicals from plants are by-products of plant metabolism. The materials may either be stored in or released by plant organs. Generally, most are released after the plant organ has withered in order to prevent self-poisoning. Four mechanisms of release, especially those that affect nematode suppression, are reviewed.

2.4.1 Volatilisation

Volatilisation is the process whereby organic extracts and amendments are applied and incorporated into the soil and then broken down by microbial decomposition, thereby releasing volatile gases that are lethal to plant-pathogenic microbes (Bello, 1998). Volatile gases like ammonia, acetaldehyde, ethanol, ethane, acetone and methanol are released as a result of microbial decomposition from plant extracts (Bennet and Wallsgrove, 1994). The volatile gases quickly kill certain pathogens and soil insect stages before they are released from the soil into the atmosphere. *Eucalyptus* species yield a volatile oil that is toxic to insect pests (Munday, 1988). The toxicity of garlic leaves to insect pests can be ascribed to the presence of a highly volatile substance (*Alliin*), which is a dialkyl sulphide compound (Kaufman, 1989). The volatile gases released in the soil increase the population of certain fungi and bacteria that are antagonistic to many soil-borne plant pathogens (Khan, 1990; Rechcigl, 1995; Walia and Gupta, 1995).

The breakdown of *Brassica's* plant materials releases glucosinolates and isothiocyanates (Walker, 1997). The toxicity arises not from the glucosinolates and isothiocyanates per se, but from the volatile gases that are released as a result of the decomposition of these compounds. The use of *Brassica* plant extracts in the suppression of nematodes through volatile gases in tobacco seedbeds had similar effects to those obtained with methyl bromide (Walker, 1997). Ploughing back *B. napus* or *B. juncea* upon maturity provided a principal source of glucosinolates biofumigation in the soil (Mathiessen and Kirkegeraad, 1993). In comparison to untreated soil, *Brassica* green manure reduced the nematode population of *Tylenchulus semipenetrans* by 76 % (Walker and Morey, 1999). Large quantities of volatile nematicidal chemical compounds from decomposing organic

amendments were effective in the management of plant-parasitic nematodes and some phytopathogenic fungi (Janzen and McGinn, 1991). The decomposition of yard waste compost released biocidal chemical compounds that reduced a large number of parasitic nematodes (McSorley and Gallaher, 1995a). Furfuraldehyde, a by-product of sugar processing, appears to have nematicidal properties. Furfuraldehyde was purported to have reduced the nematode population in highly-infested soil (Steyn, Van Biljon and Du Toit, 2001).

2.4.2 Microbial decomposition

Generally, the release of active ingredients from plant extracts is dependent upon microbial activities in certain plants (McSorley and Gallaher, 1995a, b; Stirling, 1991). The pasteurisation of growth media renders these plant extracts ineffective with regard to nematode suppression. Pasteurisation inevitably kills the soil actinomycetes, bacteria and fungi responsible for decomposition. Effective microbes may play a vital role during decomposition and the release of nematicidal compounds from plant extracts and organic amendments (Stirling, 1991). During decomposition, the plant extract and organic amendment tissues release simple organic acids such as acetic, propionic and butyric acids (Muller and Gooch, 1982). These acids remain effective against root-knot nematodes for several weeks, but are non-toxic to the free-living nematodes. Sudan grass and sorghum contain a chemical, *dhurrin,* which degrades into hydrogen cyanide, a powerful nematicide (Widmer and Abawi, 2000). Several oils immobilise root-knot nematode juveniles and reduce the hatching of eggs. Most of these plants are also aromatic and culinary herbs, which contain the nematicidal compounds carvanol and thymol. Caraway, fennel, apple-mint, spearmint, Syrian oregano and oregano contain essential oils with high levels of nematicidal activities (Yespen, 1984). Decomposing plant extracts and organic amendments are a food source for most microbes (fungi, bacteria and actinomycetes), and some of them are natural enemies of root-knot nematodes and soil pests (Meyer, 1997). Walker and Morey (1999) concluded that the decomposition processes of plant extracts and organic amendments appear to reduce the damage caused by root parasites.

More nematicidal and pesticidal activities take place in plant materials with a C:N ratio of less than 20:1 (McSorley and Gallaher, 1995a). Plant extracts with a high C:N ratio release nematicidal compounds too slowly to be effective, as a result of slow decomposition. The decomposition of olive pomace extract is accelerated by a low C:N ratio that enhances its nematicidal activities and reduces its phytotoxicity (Rodruguez-Kabana et al., 1995). Inadequate ecological knowledge has often led to failed attempts to use microbial agents against plant-parasitic nematodes.

Other factors that affect the decomposition rate of plant extracts are soil type, soil moisture and climate (Ritzinger and McSorley, 1998). Nematicidal compounds in most plant extracts exist in ineffective form. Generally, these plant extracts must decompose before they can release effective compounds. A high decomposition rate in plant extracts has been associated with increasing numbers of nematodes and soil-borne antagonistic organisms (Muller and Gooch, 1982; Stirling, 1991; Trivedi and Baker, 1986). Mid-season nematode population reduction suggested that some toxic substances may have been released during decomposition (Chindo and Khan, 1990).

The control achieved with microbial agents is often slow-acting, less robust and more species-specific, and therefore demands integration with other pest management strategies (Davies, De Leij and Kerry, 1991). The non-chemical pest management strategies may include rotation with non-host crops, the use of resistant cultivars and / or solarization.

2.4.3 Exudation

Exudation is the release of toxic substances into the soil solution by living plant organs that act against any form of attack by their enemies and suppress pest population densities, without any adverse effect on the plant that releases them (Kaufman, 1989; McLeod and Da Silva, 1994). Secondary metabolites such as flavanoids, terpernoids, alkaloids and sulphides are commonly released through root exudation. Some of these allelochemicals (exudates) are toxic and lethal to plant-parasitic nematodes.

Marigold (*Tagetes minuta)* exudes nematicidal chemicals. Marigold roots release ozone that attracts and kills nematodes when they feed on roots. Research indicated that the cultivar "Single Gold" provides 99% effective nematode control, and it is now being sold commercially. However, the most effective marigold species (*T. patula)* for lowering nematode populations is the French marigold (Hackney and Dickerson, 1975). Sellami and Cheifa (1997) reported that *T. erecta* reduced nematode infestation in soil and increased tomato yield when grown two and half months prior to tomato. Research is under way on the nematicidal properties of other *Tagetes* species.

Sun hemp (*Crotolaria zanziberica*) contains the alkaloid *Crotolisin*, which is thought to be a trap crop for nematodes and has been recommended in rotation programmes (Kaufman, 1989). The radish plant (*Raphanus sativus*) contains mustard oils called *Glucoraphenin,* a glucose that is believed to protect the plant against insect attack (Kaufman, 1989). *Glucoraphenin* gives radish the characteristic "hot" pungent taste. The onion plant repels many insect predators and potential fungi because of the presence of thiocyanic acid (HSCN), its salts and protocatechuic acid (aldehyde) (Kaufman, 1989). These compounds are important natural insecticides that play a vital role in the defence mechanism of plants against natural predators (Kaufman, 1989). Garlic is used as a companion plant with flowers and vegetables to deter insect pests, due to the presence of the highly pungent leaves. Asparagus (*Asparagus officinalis*) releases root exudates that contain a nematicidal glycoside, which defend the plant against several attacking nematodes (Wallace, 1973). However, soil conditions such as temperature and moisture affect the quality and quantity of exudates.

2.4.4 Leaching

Generally, water can leach chemicals out of withered plant organs (Schmutterer, 1990). Leachates dissolve in water, provided that an adequate quantity of water is present to dissolve and leach them.

Plant organs used at the University of the North for nematode suppression depend on leaching as a mechanism for releasing nematicidal chemicals. In ground-leaching technology, when P_i was kept constant, final numbers were reduced by 2.95 units for each unit increase in *R. communis* (Mashela and Nthangeni, 2002). In spring, with regard to nematodes alone, introducing a *C. myriocarpus* amendment reduced *M. incognita* densities in soil by 49 %, and in roots by 89 % (Mashela, 2002). The efficacy of extracts such as *R. communis* regarding the suppression of nematodes and insect pests is independent of microbial decomposition (Mashela and Nthangeni, 2002). The fruits and roots of *C. myriocarpus* contain large amounts of highly toxic *Cucumin* and leptodermin. The chemical compounds are cucurbitacins, which are water-soluble, have strong cutaneous adsorptive properties and are among the bitterest known substances (Van Wyk, Van Oudtshoorn and Gericke, 1997). Using ground leaching technology (GLT), nematodes were effectively and consistently suppressed by 5 g per plant (0.71t/ha) of *Cucumis* fruit meal (Mashela and Mphosi, 2001).

Bacillus species did not play a role in the efficacy of *C. myriocarpus* and *L. javanica* fruit and leaf meal with regard to the suppression of nematode population densities (Mafeo, Ngobeni and Mashela, 2003; Mofokeng, Ndove and Mashela, 2003; Mphosi, Mashela and Mabitsela, 2003). Neem powder is worked into the soil and irrigated for controlling nematodes and soil-borne insect pests, whereas an infusion is prepared from 2 kg/10 l of water and soaked for twelve hours for the control of aerial insect pests (Schmutterer, 1990). Grinding increases the contact surface area with the water, and a much lower quantity of less than 20 kg/ha will then be applied for the consistent suppression of nematodes and insect pests (Mashela and Nthangeni, 2002).

2.5 Effects of plant extracts

Plants extracts may have either a positive or negative impact on the environment. Some of the possible effects are reviewed briefly.

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Plants extracts may have either a positive or negative impact on the environment. Some of the possible effects are reviewed briefly.

2.5.1 Soil nutrient elements

At the University of the North (UNIN), all plant materials used increased soil electrical conductivity (EC). However, the increase did not result in an accumulation of nutrients in leaves. Nutrient elements are released from plant extracts as a result of decomposition (Brady and Weil, 1999; Muller and Gooch, 1982; Stirling, 1991). The increase in soil EC in various experiments at UNIN suggested that the compounds released from plant extracts were ionic, which does not necessarily mean that they are nutrient elements. Previous reports on decomposition studies demonstrated that various acids were released (Muller and Gooch, 1982). The acids are known for their ability to conduct electrons.

2.5.2 Soil pH

Most plants do well at a soil pH that is favourable for nematodes. Cyst nematode eggs do not hatch in acid soils of pH 4, or alkaline soils of pH 8 (Yespen, 1984). Extracts of *C. myriocarpus* and *R. communis* fruit meal had no effect on soil pH, whereas an extract of *L. javanica* consistently reduced soil pH (Mashela and Mphosi, 2001; Mashela and Nthangeni, 2002; Ngobeni 2003). Thus, in the case of *C. myriocarpus* and *R. communis*, it could not have been an indirect effect of pH that reduced nematodes.

2.5.3 Plant growth

Plant extracts that are efficient in the suppression of nematode and insect pest populations result in good plant growth, development and yield. Fruit meal of *C. myriocarpus, L. javanica* and *R. communis* consistently improved the growth and yield of tomato plants (Mashela and Mpati, 2001; Mashela and Mphosi, 2001; Mashela and Nthangeni, 2002). In Experiment 1 and Experiment 2, *R. communis* predicted the variability in the total treatment variation (TTV) of tomato plants' height, accounting for 65 % and 82 % respectively (Mashela and Nthangeni, 2002). In Experiment 2, *R. communis* accounted for 67 % of the TTV in the increase in fruit weight (Mashela and Nthangeni, 2002). With regard to nematodes only, *C. myriocarpus* increased fruit weight by 538 % and 226 % in the spring and autumn respectively (Mashela, 2002).

The best plant growth responses on tomato were recorded at 4-6 g/pot of *R. communis* fruit meal extract (Ritzinger and McSorley, 1998). With regard to nematodes alone, *C. myriocarpus* increased plant height by 34 % and 13 % respectively (Mashela, 2002). The highest values with regard to plant height, stem diameter, fruit weight and yield were recorded on peanut hay amended with okra (McSorley and Gallaher, 1998). Some plant extracts that release volatile nematicides and pesticides are also phytotoxic during the 2nd to 4th weeks, which delays plant growth and development. Therefore, additional time must be allowed before planting to give plant extracts enough time to decompose and release volatile gases that kill nematodes (Mathiessen and Kirkegaard, 1993).

Due to a lack of direct nutritional effect with regard to *C. myriocarpus*, the growth increase of nematode-infected plants in *C. myriocarpus*-amended soils was primarily attributed to the suppression of nematodes (Mashela, 2002). This applies to *R. communis* and *L. javanica,* applied in very small quantities.

2.5.4 Non-target organisms

Most of the synthetic pesticides that are still in use today have an extended effect on non-target organisms, thus breaking the natural food cycle. Therefore it is important to determine the plant extract toxicity to non-target organisms once efficacy on the target insect pests has been proved. Unlike synthetic pesticides and fumigants, which reduce the overall number of soil microbes, certain plant extracts increase the total number of soil fungi and bacteria 10, 000 times, but also decrease the number of pathogens (Rechcigl and Rechcigl, 2000). After treatment initiation, *C. myriocarpus* did not demonstrate phytotoxicity throughout the growing season (Mashela, 2002). If used repeatedly, plant extracts are toxic to pests and beneficial insects and can disrupt biotic control of insect pests by their natural enemies (Rechcigl and Rechcigl, 2000). However, their limited persistence in the environment helps to minimise their adverse effect. Some plant extracts - rotenone and nicotine in particular - are more toxic to humans than many common synthetic pesticides. Pyrethrin extracts are moderately to highly toxic to fish, but their

unstable nature in the environment reduces the potential hazard. Pyrethrin extracts are highly unstable in higher concentrations of 90% (Rechcigl and Rechcigl, 2000). Synthetic pyrethroids are more stable, and their toxicity to fish is therefore a much greater threat (Rechcigl and Rechcigl, 2000). While neem derivatives have been reported to provide broad-spectrum control of pest species, they appear to be less toxic to natural enemies of insect pests than to the pests themselves (Banken and Stark, 1997; Schmutterer, 1990). Neem extract is non-toxic to humans, other mammals and insects such as honeybees and spiders, as well as earthworms (Schmutterer, 1990).

Plant extracts such as *C. myriocarpus* had no effect on the non-target organism, *Rhizobium* species (Muedi and Mashela, 2003). Cowpea plants treated with *Rhizobium* + *Cucumis* exhibited higher dry shoot weight, higher root nodule numbers and nodule weight (Muedi and Mashela, 2003). However, since *R. communis* produces the highly toxic compound Ricin, which is widely used by terrorist groups, the research group at UNIN has shelved the use of this plant extract. On the other hand, *C. myriocarpus* - although highly toxic to mammals - is also used as a traditional medicine, which suggests that it might serve a role in plant protection.

2.6 Residues of plant extracts in crops and the environment

Apart from being non-toxic to non-target organisms, neem extracts are biodegradable within 25 days. This means that no toxic residues are left behind, which keeps the produce and the environment clean and safe. The limited persistence of plant extracts in the soil helps to minimise adverse environmental effects. The instability of pyrethrins is desirable, so that unwanted residues do not persist on treated crop produce (Rechcigl and Rechcigl, 2000). If a reliable breakthrough were made in the use of plant extracts, synthetic pesticides would be used only for pest outbreaks (Stonehouse, 1981).

2.7 Uses of Tamboti

Interesting and important information was gathered with regard to the uses of Tamboti. A number of researchers found the same information in different parts of Southern Africa. Based on carbon dating of the wood from the Zimbabwe ruins, it is estimated that this plant was already used more than 2000 years ago. Pieces of the wood are placed in clothing as insect repellents. In Namibia it is used as an arrow and fish poison, and in Zimbabwe as a fish poison. The latex is extremely irritating to the skin, and the sap may cause blindness if it gets into the eye. The plant contains cytotoxic phorbol esters. However, fresh latex is used for the treatment of toothache (Palgrave, 1983; Van Wyk and Gericke, 2000; Venter and Venter, 1996).

2.8 Uses of Chilli extracts

Chilli fruit powder is considered useful for home garden and commercial producers who want to avoid synthetic pesticides (Briggs, Zhender and Witt, 1996). It offers very effective protection from cutworms and larger insects. Chilli fruit powder is used as a counter-irritant to pests. African chilli is the best for crop protection, since it is very pungent. The pungence of chilli is derived from *Capsaicin*, C_{18} H_{27} NO_3 - a decylenic acid derivative of vanillylamine (Purseglove, 1979).

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Chapter 3

MATERIALS AND METHODS

3.1 Responses of root-knot nematode densities to aqueous extracts of Tamboti and Chilli in greenhouse experiments

In this chapter, the materials and methods used in the four experiments that were conducted, are discussed.

3.1.1 Introduction

The phasing out of many synthetic pesticides and fumigants, including methyl bromide, due to their long-term residual effect on the environment brings crop farmers to a dead end. Although methyl bromide is the most effective application against many plant insect pests, including nematodes, it is also the most expensive, and is not affordable for small-scale and emerging crop farmers. However, most of the known and unknown traditional pesticides and medicines are available in the bush and yards at no cost.

Few studies have been conducted to identify potential bio-fumigants and pesticides that are environmentally friendly. Alternative bio-fumigants and pesticides should be in place and developed before the last phasing-out date for methyl bromide. Studies had been conducted on the use of organic amendments to suppress nematode population densities. The efficacy of organic amendments depends on the quantity applied. Large quantities of organic amendments are required for the effective suppression of nematode densities (Rodriguez-Kabana, 1986). Recently, Mashela and Mphosi (2001) developed an alternative organic amendment technology using *mutis*, which consistently suppressed nematodes when applied in small quantities. Fruit meal of *C. myriocarpus* suppressed nematode population densities when applied at 0.71 t/ha (Mashela and Mphosi, 2001). *Lippia javanica* consistently reduced nematode population densities while increasing soil EC and reducing soil pH (Mashela and Ngobeni, 2001).

The objective of this study was to evaluate the responses of root-knot nematode population densities, tomato plant growth and soil EC and pH to Tamboti bark meal and Chilli fruit meal in pot experiments in the greenhouse.

3.1.2 Materials and methods

Greenhouse experiments were conducted at the Horticultural Skills Centre, University of the North, South Africa (23° 53'10"S, 29° 44'15"E), during winter and spring, 2003. Ripe fruits of Chilli (*Capsicum frutescens*), var. "Serrano", were harvested from the greenhouse during the spring and summer of 2002. Tamboti (*Spirostachys africana)* bark was collected from the bush, 30 km southeast of Tzaneen, Limpopo Province, during the autumn of 2003. Chilli fruits and Tamboti bark were cut into 10- to 15-mm pieces prior to drying in a forced-air oven (Scientific, Series 9000®) for 72 hours at 52°C, to minimise the loss of volatile phytochemicals (Mashela, 2002; Mashela and Nthangeni, 2002). Dried Chilli fruits and Tamboti bark were ground into powder using a Wiley mill, through 1 mm and 0.75 mm-diameter sieves (Mashela, 2002; Mashela and Mphosi, 2001).

Tomato (*Lycorpersicon esculentum* L., var. Floradade) seeds were sown in seedling trays using Hygromix® medium, and kept in the greenhouse. Fine sand was steam-pasteurised for 30 minutes at 300°C. Cement basins and spades were sterilised with a sodium hypochlorite (NaOCl) solution prior to thoroughly mixing 3 parts of sand with 1 part of Hygromix®, and filling up 30 cm-diameter plastic pots with the growth medium.

Three-week-old tomato seedlings were transplanted into 30 cm-diameter plastic pots on 4 April 2003. Each seedling was inoculated with 5 000 second-stage juveniles (J2) of *M. incognita* at a distance of 6 cm from the trunk. In other words, all plots (pots) were infested with nematodes, and nematodes were not part of the treatments. Four treatments, comprising unamended control, Tamboti-amended, Chilli-amended and Tamboti + Chilli-amended, were arranged in a randomised complete block design, with 10 replicates. Tamboti bark meal was applied at 14.8 g per plant (296 kg/ha), Chilli fruit meal at 13.6 g

per plant (272 kg/ha), and a mixture of Tamboti bark meal and Chilli fruit meal at 7.4 g per plant (148 kg/ha) and 6.8 g per plant (136 kg/ha) respectively.

Banded fertilizer 2:3:2 (22) 5% Zn was applied at 8.3 g per plant (166 kg/ha), 5 cm deep, at four-week intervals. Irrigation was applied at a rate of 0.5 l of tap water per plant every other day, in order to keep the media at field capacity and minimise leaching. Winter and spring ambient day / night temperatures averaged 26 / 17°C and 28 / 20°C, with maximum temperatures being controlled using thermostatically-activated fans. Weeds were physically uprooted. No pesticides and fungicides were applied to plants.

Stem diameters were recorded with a Digital Vernier Calibre® (mm) at the crown, and stem height was measured using a metal tape measure (cm), 60 and 108 days after initiating treatments. Flower numbers, including fruit numbers, were recorded 40 and 80 days after initiating treatments. Only open flowers were considered. Plant fruit weight (yield) was recorded with an Ohaus Standard Precision TS400® laboratory scale. Data was recorded 60 and 108 days after initiating treatments.

Upon harvesting, 108 days after initiating treatments, stems were cut from the base with pruning shears, placed in brown paper bags and weighed to determine fresh shoot weight. Shoots were air-dried at room temperature prior to being oven-dried at 60°C for 72 hours, and weighed for the dry shoot weight record. Soil around the root zone (20 to 30 cm deep) was loosened by shaking the pot. Root systems were carefully removed from the soil and immersed in water to remove soil particles. Roots were randomly sampled, placed in bottles filled with tap water and kept away from sunlight. Nematode eggs were extracted from the roots with 1% NaOCl (v/v) (Hussey and Barker, 1973), and incubated for five days in modified Baermann trays (Agrios, 1997; Rodriguez-Kabana and Pope, 1981; Van Gundy, 1986). Nematodes were separated from soil and debris, which was followed by rinsing through nested sieves (145 μm, 45 μm and 25 μm). Second-stage juveniles (J2s) were counted with a compound microscope. Root-sample weights were recorded per plant. Soil was removed from the pot and thoroughly mixed with a garden fork, prior to sampling for EC and pH analyses. Each sample consisted of 10 soil cores, collected at 20 cm depth

using a 2.5 cm-diameter auger. Soil samples were air-dried prior to determining EC and pH. Soil clods were broken up using a porcelain mortar and pestle. Stones and unwanted materials were separated from the soil by means of a 2 mm-diameter sieve. For soil EC, each sample consisted of 10 g of soil. Distilled water (50 mm) was added to the soil in 100 ml plastic bottles. An electric shaker was used to thoroughly mix the soil-water solution at 225 revolutions per minute, for an hour. The solution was left for 15 minutes to allow the suspension to settle. From each sample, 25 ml of water was drawn off into 100 ml plastic cups using a 50 ml glass pipette. A drop of analytical reagent, Sodium Hexametaphosphate $(NaPO_3)_6$, was added at 0.1 % (v/v), and stirred to mix it thoroughly into the solution. Soil electrical conductivity was determined using a Crison Basic 30 ® EC meter, calibrated by buffer solution 1314 μS/cm at 25°C (Brady and Weil, 1999; Singer and Munns, 1999).

For soil pH, each sample consisted of 20 g of soil. Distilled water (50 mm) was added to the soil in a 100 ml plastic cup. A glass stirring rod was used to thoroughly mix the soil-water solution. The solution was left for 15 minutes to allow the suspension to settle. Soil pH was determined using an Orion 420A® pH meter, calibrated by buffer solutions at pH 4.01 and pH 7 (Brady and Weil, 1999; Singer and Munns, 1999). Statistical analyses were conducted using Statistical Analysis System (SAS) software (SAS Institute, Inc., Cary, NC, U.S.A.). Nematode data were transformed using Log_2 (Nematode + 1), prior to analysis to homogenise the variance. Data were subjected to analysis of variance (ANOVA), whereas mean separation was accomplished using the Least Significant Difference (LSD) test when the F-values were significant at $P \leq 0.05$.

The experiment was repeated on 2 August 2003 under similar conditions, except that all plots (pots) were inoculated with 4 300 nematode juveniles.

3.2 Responses of root-knot nematode densities to aqueous extracts of Tamboti and Chilli in micro-plot experiments

Materials and methods for the greenhouse and micro-plot experiments were basically the same, except for a few minor differences.

3.2.1 Introduction

The phasing out of methyl bromide has stimulated a considerable amount of nematode control research at international and national level. Studies conducted range from the use of cultural practices and resistant varieties to allelochemical plants, organic amendments and plant extracts. In most of these studies, very few results are inapplicable or inconsistent. When poultry manure was applied at 4 t/ha and 8 t/ha in the field, it reduced nematode population densities, improved tomato plant growth and yield as a result of increased plant nutrient availability, improved soil conditions and brought about a change in the biotic and abiotic environment of the plant, which ultimately altered the host-parasitic relationship (Chindo and Khan, 1990).

Recently, Mashela and Mphosi (2001) developed an alternative organic amendment technology using *mutis*, which consistently suppressed nematodes when applied in small quantities. Fruit meal of *C. myriocarpus* suppressed nematode population densities when applied at 0.71 t/ha (Mashela and Mphosi, 2001). Nematode population densities were consistently reduced *by L. javanica,* while soil EC was increased and soil pH was reduced (Mashela and Ngobeni, 2001).

The objective of this study was to evaluate the responses of root-knot nematode population densities, tomato plant growth and soil EC and soil pH to Tamboti bark meal and Chilli fruit meal in micro-plot experiments in the field.

3.2.2 Materials and methods

Micro-plot experiments were conducted at the Horticultural Skills Centre, University of the North, South Africa (23° 53'10"S, 29° 44'15"E) during winter and spring, 2003. Ripe fruits of Chilli (*Capsicum frutescens*), var. "Serrano", were harvested from the greenhouse during the spring and summer of 2002. Tamboti (*Spirostachys africana*) bark was collected from the bush, 30 km southeast of Tzaneen, Limpopo Province, during the autumn of 2003. Chilli fruits and Tamboti bark were cut into 10- to 15-mm pieces prior to drying in a forced-air oven (Scientific, Series 9000®) for 72 hours at 52°C, to minimise the

loss of volatile phytochemicals (Mashela, 2002; Mashela and Nthangeni, 2002). Dried Chilli fruits and Tamboti bark were separately ground into powder using a Wiley mill, through 1 mm and 0.75 mm-diameter sieves (Mashela, 2002; Mashela and Mphosi, 2001).

Three-week-old tomato seedlings were transplanted into holes 60 cm deep and 50 cm in diameter, with an intra-row spacing of 0.5 m and an inter-row spacing of 1 m. All plots were infested with 3 000 juveniles each, as described for the greenhouse experiments. Treatments, replications and the design of the experiment were as described for the greenhouse experiments. The experiment was initiated on 4 April 2003.

Fertiliser and irrigation were as described for the greenhouse experiments. Winter and spring ambient day / night temperatures averaged 26 / 17 and 28 / 20°C. Weeds were physically uprooted. No pesticides and fungicides were applied to plants.

Stem diameters were recorded with a Digital Vernier Calibre® (mm) at the crown, and stem height was measured using a metal tape measure (cm), 60 and 108 days after initiating treatments. Flower numbers, including fruit numbers, were recorded 40 and 80 days after initiating treatments. Only open flowers were considered. Plant fruit weight (yield) was recorded with an Ohaus Standard Precision TS400® laboratory scale. Data were recorded 60 and 108 days after treatments.

Upon harvesting, 108 days after initiating treatments, stems were cut from the base with pruning shears, placed in brown paper bags and weighed to determine fresh shoot weight. Shoots were air-dried at room temperature prior to oven-drying at 60°C for 72 hours, and weighed to determine dry shoot weight record. Soil around the root zone (20 to 30 cm deep) was loosened by shaking the pot. Root systems were carefully removed from the soil and immersed in water to remove soil particles. Roots were randomly sampled, placed in bottles filled with tap water and kept away from sunlight. Nematode eggs were extracted from the roots with 1 % NaOCl (v/v) (Hussey and Barker, 1973), and incubated for five days in modified Baermann trays (Agrios, 1997; Rodriguez-Kabana and Pope, 1981;

Van Gundy, 1986). Nematodes were separated from soil and debris, which was followed by rinsing through nested sieves (145 μm, 45 μm and 25 μm). Second-stage juveniles (J2s) were counted with a compound microscope. Root-sample weights were recorded per plant. Soil was removed from the pot and thoroughly mixed with a garden fork prior to sampling for EC and pH analyses. Each sample consisted of 10 soil cores, collected at a 20 cm depth using a 2.5 cm-diameter auger. Soil samples were air-dried prior to determining EC and pH. Soil clods were broken up using a porcelain mortar and pestle. Stones and unwanted materials were separated from the soil by a 2 mm-diameter sieve. For soil EC, each sample consisted of 10 g of soil. Distilled water (50 mm) was added to the soil in 100 ml plastic bottles. An electric shaker was used to thoroughly mix the soil-water solution at 225 revolutions per minute, for an hour. The solution was left for 15 minutes to allow the suspension to settle. From each sample, 25 ml of water was drawn off into 100 ml plastic cups using a 50 ml glass pipette. A drop of analytical reagent, Sodium Hexametaphosphate $(NaPO_3)_6$, was added at 0.1 % (v/v) and stirred to mix it thoroughly into the solution. Soil electrical conductivity was determined using a Crison Basic 30 ® EC meter, calibrated by buffer solution 1314 μS/cm at 25°C (Brady and Weil, 1999; Singer and Munns, 1999).

For soil pH, each sample consisted of 20 g of soil. Distilled water (50 mm) was added to the soil in 100 ml plastic cup. A glass stirring rod was used to thoroughly mix the soil-water solution. The solution was left for 15 minutes to allow the suspension to settle. Soil pH was determined using an Orion 420A® pH meter, calibrated by buffer solutions at pH 4.01 and pH 7 (Brady and Weil, 1999; Singer and Munns, 1999). Statistical analyses were conducted using Statistical Analysis System (SAS) software (SAS Institute, Inc., Cary, NC, U.S.A.). Nematode data were transformed using Log_2 (Nematode + 1), prior to analysis to homogenise the variance. Data were subjected to analysis of variance (ANOVA), whereas mean separation was accomplished using the Least Significant Difference (LSD) test when the F-values were significant at $P \leq 0.05$.

The experiment was repeated on 2 August 2003 under similar conditions, except that all plots were inoculated with 4 300 nematode juveniles.

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Chapter 4

RESULTS AND DISCUSSION

4.1 Results

In greenhouse experiment 1 and 2, the total treatment variation (TTV) on final nematode numbers was 27 % and 21 % respectively (Table 4.1). However, on the micro-plot, only 18 % TTV was observed in Experiment 2. For reasons that cannot be explained, the experimental errors in micro-plots were quite high, namely 63 % and 70 % respectively. However, the results give a clear indication of the efficacy of the materials tested on nematode numbers. In greenhouse experiment 1, the suppression of nematodes using Tamboti did not differ ($P \leq 0.05$) from the untreated controls (nematodes alone). However, compared to the untreated controls, Chilli and Tamboti + Chilli suppressed nematode numbers by 55 % and 70 % respectively. In greenhouse experiment 2, the effects of Tamboti and Chilli on nematode numbers did not differ ($P \leq 0.05$) from those of the untreated controls. However, compared to the untreated controls, Tamboti + Chilli reduced nematode numbers by 54 %.

Table 4.1: Percentage variations in nematode numbers due to three sources of variation.

Source	Greenhouse				Micro-plot			
	Exp. 1		Exp. 2		Exp. 1		Exp. 2	
	SS	%	SS	%	SS	%	SS	%
Rep.	5.420	18ns	6.206	31*	14.970	26ns	2.100	12ns
Treatment	8.055	27***	4.245	21**	6.124	11ns	1.422	18**
Error	16.811	55	9.332	48	36.853	63	4.543	70
Total	30.286	100	19.963	100	57.947	100	8.065	100

ns = not significant at $P \leq 0.10$; * = significant at $P \leq 0.10$; ** = significant at $P \leq 0.05$; *** = significant at $P \leq 0.01$.

In Experiment 1 of the micro-plot studies, the effects of Tamboti and Chilli on nematode numbers did not differ ($P \leq 0.05$) from those of the untreated control. However, compared

to the untreated control, Tamboti + Chilli reduced nematode numbers by 59 %. In micro-plot experiment 2, the effects of Chilli and Tamboti + Chilli on nematode numbers did not differ (P≤ 0.05) from those of the untreated control. However, compared to the untreated control, Tamboti reduced nematode numbers by 37 %.

Table 4.2: Effect of Tamboti and Chilli organic amendments on *Meloidogyne incognita* numbers, 108 days after treatment initiation.

Treatment	Greenhouse		Micro-plot	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Nematode	2a (1.50)	2a (1.63)	2a (1.52)	1a (1.38)
Nematode + Tamboti	1ab (0.99)	1a (1.34)	1ab (0.75)	1b (0.87)
Nematode + Chilli	0b (0.46)	1a (1.47)	1ab (0.94)	1ab (1.10)
Nematode + Tamboti + Chilli	1b (0.67)	1b (0.75)	1b (0.63)	1ab (1.01)
LSD_{50}	0.08	0.54	0.80	0.38

Column means followed by the same letters did not differ (P=0.05) according to the Least Significant Difference (LSD) test.

In greenhouse experiment 1, compared to the untreated control, Chilli and Tamboti + Chilli reduced nematode numbers by 69 % and 55 % (P≤ 0.01) respectively. In greenhouse experiment 2, compared to the untreated control, Tamboti + Chilli reduced nematode numbers by 54 % (P≤ 0.05). In micro-plot experiment 1, compared to the untreated control, Tamboti + Chilli reduced nematode numbers by 59 % (P≥ 0.10), and in Experiment 2, Tamboti reduced nematode numbers by 37 % (P≤ 0.05). In greenhouse experiment 1 and 2 and micro-plot experiment 1, compared to the untreated control, Tamboti did not reduce nematode numbers (Table 4.2).

In both the greenhouse and micro-plot studies, first fruit weight showed an unchanging pattern in response to all the treatments (Table 4.3). In greenhouse experiment 1 and micro-plot experiment 1 and 2, treatments showed an unchanging pattern on second fresh fruit weight. In greenhouse experiment 2, second fruit weight showed a changing pattern

in response to treatments (P<0.01) (Table 4.4). Compared to the untreated control, Tamboti and Chilli increased second fruit weight by 107 % and 159 % respectively.

Table 4.3: Effect of Tamboti and Chilli organic amendments on fresh fruit weight (g), 60 days after treatment initiation.

Treatment	Greenhouse		Micro-plot	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Nematode	102.85a	206.29a	92.26a	152.88a
Nematode + Tamboti	99.58a	255.24a	117.01a	123.35a
Nematode + Chilli	70.55a	271.54a	56.02a	135.61a
Nematode + Tamboti + Chilli	77.44a	344.51a	140.79a	210.09a
LSD_{50}	98.52	154.86	117.72	108.12

Column means followed by the same letters did not differ (P=0.05) according to the Least Significant Difference (LSD) test.

Table 4.4: Effect of Tamboti and Chilli organic amendments on fresh fruit weight (g), 108 days after treatment initiation.

Treatment	Greenhouse		Micro-plot	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Nematode	5.48a	25.99c	6.10a	47.75a
Nematode + Tamboti	2.81a	53.85ab	4.91a	38.73a
Nematode + Chilli	2.52a	67.18a	3.69a	58.83a
Nematode + Tamboti + Chilli	1.61a	35.03bc	4.54a	52.13a
LSD_{50}	3.97	24.74	6.43	27.52

Column means followed by the same letters did not differ (P=0.05) according to the Least Significant Difference (LSD) test.

Table 4.5: Effect of Tamboti and Chilli organic amendments on stem diameter (mm), 60 days after treatment initiation.

Treatment	Greenhouse		Micro-plot	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Nematode	8.94a	10.71a	8.48b	10.69a
Nematode + Tamboti	8.12a	11.44a	9.37ab	10.76a
Nematode + Chilli	8.54a	11.14a	8.16b	10.88a
Nematode + Tamboti + Chilli	7.19a	10.80a	10.78a	10.70a
LSD_{50}	2.03	1.59	2.10	1.33

Column means followed by the same letters did not differ (P=0.05) according to the Least Significant Difference (LSD) test.

Table 4.6: Effect of Tamboti and Chilli organic amendments on stem diameter (mm), 108 days after treatment initiation.

Treatment	Greenhouse		Micro-plot	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Nematode	11.83ab	13.44a	11.88ab	12.77a
Nematode + Tamboti	11.34ab	12.86a	13.05ab	13.05a
Nematode + Chilli	12.53a	12.32a	11.09b	12.30a
Nematode + Tamboti + Chilli	9.58b	12.95a	14.02a	12.97a
LSD_{50}	2.27	1.36	2.19	1.46

Column means followed by the same letters did not differ (P=0.05) according to the Least Significant Difference (LSD) test.

In micro-plot experiment 1, compared to the untreated controls, Tamboti + Chilli increased first stem diameter by 27 % (Table 4.5). In greenhouse experiment 1 and 2, as well as micro-plot experiment 2, first stem diameter showed an unchanging pattern, and in greenhouse experiment 1 and 2, as well as micro-plot experiment 1 and 2, second stem diameter also showed an unchanging pattern, suggesting that there was no treatment effect (Table 4.6). In greenhouse experiment 1 and 2, as well as micro-plot experiment 2, first

stem height demonstrated an unchanging pattern (Table 4.7). In micro-plot experiment 1, compared to the untreated control, Tamboti + Chilli increased first stem height by 28 % (Table 4.7). In both the greenhouse and micro-plot studies, second stem height showed an unchanging pattern (Table 4.8).

Table 4.7: Effect of Tamboti and Chilli organic amendments on stem height (cm), 60 days after treatment initiation.

Treatment	Greenhouse		Micro-plot	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Nematode	47.51a	85.07a	41.69b	87.85a
Nematode + Tamboti	44.81a	80.13a	48.89ab	84.72a
Nematode + Chilli	45.65a	85.08a	41.39b	92.17a
Nematode + Tamboti + Chilli	37.42a	89.32a	53.49a	53.49a
LSD_{50}	10.66	11.52	9.91	11.17

Column means followed by the same letters did not differ (P=0.05) according to the Least Significant Difference (LSD) test.

Table 4.8: Effect of Tamboti and Chilli organic amendments on stem height (cm), 108 days after treatment initiation.

Treatment	Greenhouse		Micro-plot	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Nematode	63.00a	104.07a	61.62a	112.50a
Nematode + Tamboti	61.36a	104.76a	69.07a	105.22a
Nematode + Chilli	64.20a	104.06a	58.52a	112.23a
Nematode + Tamboti + Chilli	54.66a	104.03a	68.95a	114.20a
LSD_{50}	13.62	7.21	11.39	13.09

Column means followed by the same letters did not differ (P=0.05) according to the Least Significant Difference (LSD) test.

In greenhouse experiment 1 and 2 and micro-plot experiment 2, first flower numbers were not affected by treatments. In micro-plot experiment 1, compared to the untreated control, Tamboti + Chilli increased first flower numbers by 94 % (Table 4.9). The treatment effect

on second flower numbers was not statistically significant ($P \leq 0.05$) in greenhouse experiment 1 and micro-plot experiment 1 and 2. In greenhouse experiment 2, compared to the untreated control, Tamboti reduced second flower numbers by 27 % (Table 4.10).

Table 4.9: Effect of Tamboti and Chilli organic amendments on flower numbers, 40 days after treatment initiation.

Treatment	Greenhouse		Micro-plot	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Nematode	6.08a	15.70a	4.00b	15.70a
Nematode + Tamboti	5.46a	13.30a	6.03ab	13.30a
Nematode + Chilli	4.85a	15.90a	5.23ab	15.90a
Nematode + Tamboti + Chilli	4.46a	15.00a	7.77a	15.00a
LSD_{50}	3.46	4.20	2.97	4.20

Column means followed by the same letters did not differ (P=0.05) according to the Least Significant Difference (LSD) test.

Table 4.10: Effect of Tamboti and Chilli organic amendments on flower numbers, 80 days after treatment initiation.

Treatment	Greenhouse		Micro-plot	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Nematode	16.36ab	22.20a	10.15a	24.50a
Nematode + Tamboti	16.85ab	16.00b	15.77a	21.50a
Nematode + Chilli	18.15a	21.30a	11.69a	22.90a
Nematode + Tamboti + Chilli	9.15b	17.60ab	15.46a	26.00a
LSD_{50}	8.84	5.28	6.08	8.29

Column means followed by the same letters did not differ (P=0.05) according to the Least Significant Difference (LSD) test.

Table 4.11: Effect of Tamboti and Chilli organic amendments on fruit numbers, 60 days after treatment initiation.

Treatment	Greenhouse		Micro-plot	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Nematode	8.15a	12.50a	5.62a	18.50a
Nematode + Tamboti	7.62a	12.90a	8.62a	12.10b
Nematode + Chilli	6.00a	11.60a	6.00a	18.50a
Nematode + Tamboti + Chilli	5.31a	14.00a	9.00a	17.90a
LSD_{50}	4.52	5.34	4.03	4.63

Column means followed by the same letters did not differ (P=0.05) according to the Least Significant Difference (LSD) test.

In greenhouse experiment 1 and 2, as well as micro-plot experiment 1, fruit numbers were not affected by treatments. In micro-plot experiment 2, the effects of Tamboti on fruit numbers differed ($P<0.05$) from those for the untreated control. Compared to the untreated control, Tamboti reduced fruit numbers by 35 % (Table 4.11). In greenhouse experiment 1 and 2, as well as micro-plot experiment 2, dry shoot weight showed an unchanging pattern in response to treatments. However, in micro-plot experiment 1, compared to the untreated control, Tamboti increased dry shoot weight by 60 %. The effect of Chilli and Tamboti + Chilli on dry shoot weight did not differ ($P\leq 0.05$) from those of the untreated control (Table 4.12). In greenhouse and micro-plot studies, soil pH showed an unchanging pattern in response to treatments (Table 4.13). Soil electrical conductivity showed both changing and unchanging patterns. In greenhouse experiment 2, compared to the untreated controls, Tamboti + Chilli increased soil EC by 32 %. The effects of Tamboti and Chilli on soil EC did not differ ($P\leq 0.05$) from those of the untreated control (Table 4.14).

Table 4.12: Effect of Tamboti and Chilli organic amendments on dry shoot weight (g), 108 days after treatment initiation.

Treatment	Greenhouse		Micro-plot	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Nematode	82.01a	109.61a	67.90b	113.55a
Nematode + Tamboti	64.16a	104.26a	108.77a	103.04a
Nematode + Chilli	87.29a	101.11a	69.05b	118.60a
Nematode + Tamboti + Chilli	59.48a	98.98a	96.55ab	110.28a
LSD_{50}	29.90	15.91	35.45	16.92

Column means followed by the same letters did not differ (P=0.05) according to the Least Significant Difference (LSD) test.

Table 4.13: Effect of Tamboti and Chilli organic amendments on soil pH (water), 108 days after treatment initiation.

Treatment	Greenhouse		Micro-plot	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Nematode	5.83ab	5.61a	5.55a	5.67a
Nematode + Tamboti	6.07a	5.66a	5.71a	5.67a
Nematode + Chilli	5.96a	5.70a	5.18a	5.69a
Nematode + Tamboti + Chilli	4.97b	5.61a	5.62a	5.67a
LSD_{50}	0.89	0.12	0.64	0.11

Column means followed by the same letters did not differ (P=0.05) according to the Least Significant Difference (LSD) test.

Table 4.14: Effect of Tamboti and Chilli organic amendments on soil electrical conductivity (EC) µS/cm, 108 days after treatment initiation.

Treatment	Greenhouse		Micro-plot	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Nematode	41.79a	88.14b	46.79a	88.47a
Nematode + Tamboti	51.32a	80.24b	40.89a	94.78a
Nematode + Chilli	42.62a	80.20b	47.35a	97.19a
Nematode + Tamboti + Chilli	40.31a	115.93a	47.32a	111.64a
LSD_{50}	14.81	24.36	1.14	38.82

Column means followed by the same letters did not differ (P=0.05) according to the Least Significant Difference (LSD) test.

4.2 Discussion

Nematode numbers were generally reduced by Tamboti and Chilli treatments. Mashela (2002) reported a reduction in nematode numbers in the soil and roots, attributed to *C. myriocarpus,* by 49 % and 83 % respectively. When the experiment was repeated in the autumn, nematode numbers were reduced in the soil and roots by 68 % and 73 % respectively. In another study, *L. javanica* accounted for a 62 % and 65 % reduction in nematode numbers in Experiment 1 and 2 respectively (Mafeo, Ngobeni and Mashela, 2003). In Experiment 2, leaf powder of *Inula viscosa* reduced nematode numbers by 93 % and 98 % when applied at 0.5 % and 1.0 % (v/v) of soil respectively (Oka, Ben-Daniel and Cohen, 2001). In Experiment 2, stem powder of *I. viscose* reduced nematode numbers by 46 % and 92 % when applied at 0.1 % and 0.2 % (v/v) of soil respectively (Oka, Ben-Daniel and Cohen, 2001). In Experiment 1 and 2, each unit increase in *R. communis* reduced nematode numbers by 2.95 and 4.35 units respectively (Mashela and Nthangeni, 2002). A study by Oka, Ben-Daniel and Cohen (2001) supported the GLT system with amendments ground to different particle sizes. In another experiment, *I. viscose* leaf powder with < 335 μm particles had the highest suppression effect on nematode numbers, i.e. 85 %. Particle sizes of 300 – 1000 μm and > 1000 μm reduced nematode numbers by

53 % and 49 % respectively (Oka, Ben-Daniel and Cohen, 2001). Grinding the amendment into powder supports the Ground Leaching Technology (GLT) system in that it provides a large surface area for the material to come into contact with water, which facilitates efficient leaching of the active ingredient. In Experiment 1 and 2, broccoli residues reduced nematode numbers by 24 % and 40 % respectively (Ploeg and Stapleton, 2001). The reduced nematode number obtained with conventional organic amendment technologies is explained in terms of increased concentrations of chemicals during microbial decomposition, increased nematophagous-microbial activities or the release of essential nutrients (Muller and Gooch, 1982; Stirling, 1991). The efficacy of Tamboti and Chilli with regard to the suppression of nematode numbers was independent of microbial activities (Mashela, 2002; Mashela and Nthangeni, 2002). Chilli fruit meal, which had the highest effect on suppressing nematode numbers, contains *Capsaicin*, a highly pungent and water-soluble compound that may have contributed to the suppression of nematode numbers.

Increased fruit weight in this and related GLT studies (Mashela and Mpati, 2001; Mashela and Mphosi, 2001; Mashela and Ngobeni, 2001; Mashela, 2002) supported the view that organic amendments release nematicides that suppress nematode numbers, thus providing a favourable rhizosphere for plant growth and development. Ground Leaching Technology eliminates the use of excessively large quantities of organic amendments, thus reducing transport costs as well as the waiting period for microbial decomposition and soil pH reduction (Mashela and Nthangeni, 2002). *Lippia javanica* accounted for a 66 % - 83 % increase in fresh fruit weight (Mafeo, Ngobeni and Mashela, 2003). In Experiment 1, *Ricinus* accounted for a 49 % increase in fresh fruit weight, while other factors remained constant (Mashela and Nthangeni, 2002). When P_i and EC were kept constant, fruit weight increased by 15.18 units for each unit increase in Ricinus. In Experiment 2, P_i and Ricinus accounted for 67 % of the TTV in fruit weight (Mashela and Nthangeni, 2002). Mashela (2002) reported that *C. myriocarpus* increased fruit weight by 538 % and 226 % in the spring and autumn respectively. Since the accumulation of nutrient elements in plant tissues was not related to any treatment, it is inferred that low nematode numbers stimulated increased fruit weight.

Nematode densities below and above the damage threshold levels stimulate and suppress plant growth respectively (Seinhorst, 1965; Wallace, 1973). However, in this study, stem diameter, plant height, flower numbers, fruit numbers and dry shoot weight were not affected by treatment in both the greenhouse and micro-plot experiments. In Experiment 1 and 2, *Ricinus* increased plant height, accounting for 65 % and 82 % of TTV respectively (Mashela and Nthangeni, 2002). For each unit increase in *Ricinus*, plant height increased by 8.88 units (Mashela and Nthangeni, 2002). Compared to the control, *C. myriocarpus* increased plant height by 13 - 34 % (Mashela, 2002).

In Experiment 1, shoot weight increased by 34.13 units for each unit increase in *Ricinus,* whereas *Ricinus* accounted for 89 % of the TTV in Experiment 2 (Mashela and Nthangeni, 2002). Compared to the control, *C. myriocarpus* increased shoot weight by 338 % and 135 % in the spring and autumn respectively (Mashela, 2002). In Experiment 1 and 2, *Lippia javanica* accounted for 87 % - 90 % (Mafeo, Ngobeni and Mashela, 2003). With the exception of fruit yield, the reduction in nematode numbers did not have a direct effect on other plant growth parameters. The limited changing patterns observed on flower and fruit numbers do not have a significant effect, and occurred in response to various treatments in each experiment. These patterns can therefore be regarded as inconsistent.

Like *C. myriocarpus* fruit meal (Mashela and Mphosi, 2001; Mashela, 2002) and *R. communis* fruit meal (Mashela and Nthangeni, 2002), Tamboti and Chilli had no effect on soil pH, suggesting that these organic amendments can potentially be used in GLT systems. Mashela (2002) reported that, compared to the untreated control, nematodes reduced soil pH by 3 % and 4 % in the spring and autumn respectively.

Generally, treatment had no effect on soil EC. The interaction of *Ricinus* x *Bacillus* also had no effect on soil EC (Mashela and Nthangeni, 2002). The combination of Tamboti + Chilli increased soil EC in greenhouse experiment 2. In other studies, organic amendments increased soil EC. *Cucumis myriocarpus* increased soil EC. Compared to the untreated control, *C. myriocarpus* increased soil EC by 68 % and 50 % in the spring and autumn

respectively (Mashela, 2002), whereas *C. myriocarpus* + *M. incognita* increased soil EC by 79 % and 65 % in the spring and autumn respectively (Mashela, 2002). Compared to the control, *L. javanica* accounted for an 84 % - 92 % increase in soil EC (Mafeo, Ngobeni and Mashela, 2003). The amendment quantity applied in the GLT system is too small to supply adequate nutrient ions to increase soil EC. However, in previous studies it was suggested that the compounds released through GLT give rise to both nematicidal activities and electrical ions (Stirling, 1981; Mashela, 2002; Mafeo, Ngobeni and Mafeo, 2003).

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Chapter 5

CONCLUSION AND RECOMMENDATIONS

Chilli fruit meal, which had the highest effect on suppressing nematode numbers, contains *Capsaicin*, a highly pungent and water-soluble compound that may have contributed to the suppression of nematode numbers. Tamboti fruit meal contains tetratriterpanoid, which is water-soluble and known to have nematicidal activities. The reason for an unchanging pattern in plant height, stem diameter and shoot weight could be that the suppression of nematode population densities was too slight to result in a significant effect on plant growth and development. A few plant parameters that responded to the treatment effect were regarded as inconsistent with regard to treatment and experiments. The fact that Tamboti had the lowest treatment effect regarding nematode suppression could be ascribed to relative microbial stimulation due to the high C:N ratio. Amendments with a C:N ratio of more than 20:1 have a nematicidal activity rate that is too slow to effectively suppress nematode numbers. Perhaps the toxins in Tamboti dissolve slowly in water, so that by the time they are released, nematodes have already infected the root system.

Therefore, grinding Tamboti bark and Chilli fruit into a powder increased the surface area that comes into contact with water, which made it possible to apply small quantities for the suppression of nematodes. The small quantities applied could not accumulate adequate nutrient elements to support plant growth and soil pH and increase soil EC. The reduction in nematode numbers is therefore solely attributed to the nematicidal compounds leached from the amendments.

The study revealed that Tamboti bark meal and Chilli fruit meal (a) suppress nematode numbers, (b) increase fruit yield and (c) do not affect soil pH and soil EC.

Therefore, based on the results as presented in Chapter 4, it is recommended that the following studies be conducted to accumulate additional knowledge and develop a better

understanding of the application of these two amendments for nematode suppression and plant response:

- The low efficacy of Tamboti and Chilli in the suppression of nematode numbers warrants a follow-up study to evaluate treatment with different application rates.
- Inconsistent treatment effects obtained with regard to nematode numbers and plant growth parameters in general also require further studies.
- The lack of direct effect on plant growth parameters by reduced nematode numbers should be investigated further.

On the basis of these promising results and provided that the studies recommended above and other research generate improved and definite results, the most important task in future would be to explore ways of making these amendments accessible to small-scale crop farmers, as well as applicable and effective.

RESPONSES OF ROOT-KNOT NEMATODE DENSITIES TO AQUEOUS EXTRACTS OF CHILLI AND TAMBOTI

by

LUFUNO PATRICK THOVHAKALE

Degree : **M. Tech.: Agriculture**

Department : **School for Environmental Development and Agriculture (Central University of Technology)**

Supervisor : **Prof C. van der Westhuizen**

Co-supervisor : **Prof P. W. Mashela**

Abstract

Root-knot nematodes (*Meloidogyne incognita*) infect major crops, reducing the overall crop production and the production area. Available nematicides are expensive for small-scale crop farmers, and not environmentally friendly. Tamboti (*Spirostachys africana*) bark meal and Chilli (*Capsicum frutescens*) var. Serrano fruit were evaluated with regard to the control of root-knot nematode population densities, plant parameters, soil electrical conductivity (EC) and soil pH, in tomato plants (*Lycorpersicon esculentum L.* var. Floradade). Greenhouse and micro-plot experiments were conducted in winter and spring 2003 at the Horticultural Skills Centre, University of the North, South Africa. Treatments comprised unamended control, Tamboti, Chilli and a mixture of Tamboti + Chilli amendments, and were arranged in a randomised complete block design with 10 replicates. Three-week-old Floradade tomato seedlings were transplanted, each inoculated with 5000 and 4300 second-stage nematode juveniles for Experiment 1 and Experiment 2, for greenhouse and micro-plot conditions respectively. On inoculation day, Tamboti bark meal was applied at 14.8 g / plant, Chilli fruit meal at 13.6 g / plant and a mixture of Tamboti and Chilli at 7.4 g / plant and 6.8 g / plant respectively. Upon harvesting, 108 days after treatment initiation, nematode eggs were extracted from the roots in sodium hypochlorite

(NaOCl) 1 % v/v, and incubated for five days in modified Baermann trays. Juveniles were separated from the soil and debris, followed by rinsing through nested sieves (145 μm, 45 μm and 25 μm), and counted using a compound microscope. Stem diameter, stem height, fruit weight, dry shoot weight, soil EC and soil pH were recorded using a Digital Vernier Calibre ®, metal tape measure, laboratory scale, EC and pH meter respectively. Flower and fruit numbers were counted and recorded. In greenhouse experiment 1, Chilli and Tamboti + Chilli reduced nematode numbers ($P \leq 0.05$) by 55 % and 70 % respectively. In greenhouse experiment 2, Tamboti + Chilli reduced nematode numbers by 54 %. In micro-plot experiment 1 and 2, Tamboti and Tamboti + Chilli reduced nematode numbers ($P \leq 0.05$) by 37 % and 54 % respectively. In greenhouse experiment 2, Tamboti increased second fruit weight by 107 % and Chilli by 159 %. In micro-plot experiment 1, Tamboti + Chilli increased first stem diameter by 32 %. Generally, results indicated that Tamboti bark meal and Chilli fruit reduced nematode numbers, did not have a significant effect on plant parameters and did not affect soil EC and pH. Thus, these organic materials would be suitable as a potential organic amendment to manage nematode numbers on tomato plants.

REAKSIE VAN WORTELKNOOPNEMATODE-GETALLE OP DIE TOEDIENING VAN WATERAGTIGE AFTREKSELS VAN BRANDRISSIES EN TAMBOTIE

deur

LUFUNO PATRICK THOVHAKALE

Degree	:	**M.Tech.: Landbou**
Department	:	**Skool vir Landbou en Omgewingswetenskappe (Sentrale Universiteit vir Tegnologie, Vrystaat)**
Supervisor	:	**Prof. C. van der Westhuizen**
Co-supervisor	:	**Prof. P.W. Mashela**

Uittreksel

Wortelknoopnematodes (*Meloidogyne incognita*) infekteer hoofgewasse deurdat dit algehele gewasproduksie inhibeer en die area van produksie beperk word. Beskikbare nematosides is nie omgewingsvriendelik nie en is ook baie duur, wat meebring dat kleinskaalse boere dit nie kan aankoop nie. Die gemaalde bas van Tambotie (*Spirostachys africana*) en die vrugte van Brandrissies (*Capsicum frutescens*, var. Serrano) is geëvalueer ten opsigte van die invloed daarvan op die voorkoms van wortelknoopnematodes, sekere plantparameters, elektriese geleidingsvermoë (EGV) en grond pH ten opsigte van tamatieplante (*Lycorpersicon esculentum L.* var. Floradade) en die grond waarop hulle verbou word. Kweekhuis- en mikro-proefperseeleksperimente is gedoen in die winter en lente van 2003 by die Hortologievaardigheidsentrum van die Universiteit van die Noorde in Suid-Afrika. Behandelings het bestaan uit 1) onbehandelde kontroles, 2) Tambotiebehandelings, 3) Brandrissiebehandelings en 4) 'n mengsel van Tambotie- en Brandrissiebyvoegings. Die behandeling het plaasgevind in 'n ewekansige volledige blokontwerp, waarvan 10 herhalings gerangskik is. Drie weke oue Floradade

tamatiesaailinge is oorgeplant, elkeen ingeënt met 5000 en 4300 tweedevlak (*stage*) jeugdige nematodes vir Eksperiment 1 en 2, vir die kweekhuis en mikro-proefperseel onderskeidelik. Tydens inokulasie is gemaalde Tambotiebas toegedien teen 14.8 g / plant, Brandrissievrugtemeel teen 13.6 g / plant, en die mengsel van Tambotie en Brandrissie teen 7.4 g / plant en 6.8 g / plant onderskeidelik. Gedurende die oes, 108 dae nadat behandeling begin is, is die eiers van nematodes uit die wortels geïdentifiseer/onttrek deur gebruik te maak van 'n hipochloriet (NaOCl) 1 % v/v oplossing, en vir vyf dae op gemodifiseerde Baermann-rakke geïnkubeer. Jongeling-nematodes is geskei van die grond en ander oorblyfsels en met behulp van gestapelde siwwe (145 μm, 45 μm en 25 μm) uitgespoel, waarna dit getel is deur middel van 'n saamgestelde mikroskoop. Stamdeursnee, stamhoogte, vrugmassa, droëlootgewig, grond-EGV en grond-pH is aangeteken deur gebruik te maak van 'n digitale Vernier® kalibreerder, 'n metaalmeetband en laboratoriumskaal, asook 'n EGV- en 'n pH-meter. Die aantal bloeisels en tamatievrugte is ook getel en aangeteken. In kweekhuiseksperiment 1 het die Brandrissiebehandeling en die Tambotie + Brandrissie mengsel nematodegetalle verminder ($P \leq 0.05$) met 55 % en 70 % onderskeidelik. In kweekhuiseksperiment 2 het die behandeling met Tambotie + Brandrissie mengsel nematodegetalle laat afneem met 54 %. In mikro-proefperseeleksperiment 1 en 2 het die Tambotiebehandeling en die behandeling met Tambotie + Brandrissie mengsel onderskeidelik nematodegetalle verminder ($P \leq 0.05$) met 37 % en 54 %. In kweekhuiseksperiment 2 het die Tambotiebehandeling die massa van die tweede tamatievrug laat toeneem met 107 %, terwyl die toediening van die Brandrissie-ekstrak die massa van die tweede tamatievrug met 159 % laat toeneem het. In mikro-proefperseeleksperiment 1 het die deursnee van die tamatiestam met 32 % toegeneem waar die Tambotie + Brandrissie mengsel toegedien is. Alhoewel gemaalde Tambotiebas en die Brandrissie vrugte-ekstrak nematodegetalle verminder het, het dit nie 'n betekenisvolle (negatiewe) invloed gehad op die elektriese geleidingsvermoë en pH van die grond in die direkte omgewing van die tamatieplante nie. In die algemeen dui die resultate dus daarop dat hierdie plante (Tambotie en Brandrissies) potensiële organiese materiaal is wat nematodegetalle by tamatieproduksie doeltreffend kan beheer sonder om die grondeienskappe negatief te beïnvloed.

Appendix

Conversion of amendments and fertilizer application rates

1. Amendments

Application per plant

Intra-row spacing	: 0.5 m
Inter-row spacing	: 1 m
Area occupied by a plant is	: 0.5 m x 1 m = 0.5 m ^{2}
A plant received	: 0.0148 kg of Tamboti
	: 0.0136 kg of Chilli
1 ha	: 10 000 m ^{2}
Number of plants in a ha	= Area (m ^{2}) x 1 plant ÷ area occupied by 1 plant (m ^{2})
	= 10 000 m ^{2} x 1 plant / 0.5 m ^{2}
	= 20 000 plants / ha
Tamboti applied per ha	= number of plants per ha x application rate (kg) ÷ 1 plant
	= 20 000 plants per ha x 0.0148 kg ÷ 1 plant
	= 296 kg / ha or 0.296 t / ha
Chilli applied per ha	= number of plants per ha x application rate (kg) ÷ 1 plant
	= 20 000 plants per ha x 0.0136 kg ÷ 1 plant
	= 272 kg / ha or 0.272 t / ha

In the above calculations, the Tamboti + Chilli mixture consisted of half of the original amount of each.

This brings Tamboti to 148 kg / ha or 0.148 t / ha, and Chilli to 136 kg / ha or 0.136 t / ha.

Application per area

Area of a circle : ½ $\prod r^2$

Radius : ½ of diameter (0.15 m) ²

$\prod$: 22 ÷ 7

The area of a pot = ½ x (22 ÷ 7) x (0.15 m) ²

= 0.5 x 3.14 x 0.0225 m ²

= 0.0353 m ²

The area of a ha : 10 000 m ²

Tamboti applied per ha = Area of a ha x application rate (kg) ÷ area of 1 plant

= 10 000 m ² x 0.0148 kg per plant ÷ 0.0353 per plant

= 4 193 kg / ha or 4.193 t / ha

Chilli applied per ha = Area of a ha x application rate (kg) ÷ area of 1 plant

= 10 000 m ² x 0.0136 kg per plant ÷ 0.0353 per plant

= 3 853 kg / ha or 3.853 t / ha

In the above calculations, the Tamboti + Chilli mixture consisted of half of the original amount of each.

This brings Tamboti to 2 096.5 kg / ha or ≈ 2.1 t / ha, and Chilli to 1 926.5 kg / ha or ≈ 2 t / ha.

2. Fertilizer applied

Application per plant

Application rate : 0.0083 kg (2:3:2) 5 % Zn / plant

Fertilizer applied per ha = number of plants per ha x application rate (kg) ÷ 1 plant

= 20 000 plants per ha x 0.0083 kg ÷ 1 plant

= 166 kg / ha or 0.166 t / ha

Application per area

Fertilizer applied per ha = Area of a ha x application rate (kg) ÷ area of 1 plant

= 10 000 m ² x 0.0083 kg per plant ÷ 0.0353 per plant

= 2 351 kg / ha or 2.351 t / ha