LIPPIA JAVANICA, MELOIDOGYNE INCOGNITA AND *BACILLUS* INTERACTIONS ON TOMATO PRODUCTIVITY AND SELECTED SOIL PROPERTIES

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DECLARATION

I hereby declare that the information recorded herein was originated and compiled by myself and that it was never presented by anybody else in this University or any other University.

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ABSTRACT

Greenhouse, micro plot and field experiments were conducted at the Horticultural Research Unit of the University of the North in fall, spring and summer 2000, 2001 and 2002, respectively, (i) to evaluate effects of *Lippia javanica* leaves on densities of *Meloidogyne incognita*, tomato plant growth, soil pH and electrical conductivity (EC) and, (ii) to establish the role of *Bacillus* species on the efficacy of *L. javanica* leaves on suppression of densities of *M. incognita*, tomato plant growth, soil pH and EC in field micro plots. The greenhouse, field and micro plot experiments comprised four, two and eight treatments, respectively. The treatments were arranged in a completely randomized block design, with 10 replicates each. Second-stage nematode inoculation comprised ± 4 600-5 100, ± 6 758-7 069, and ±7 053- 8 354 during 2000, 2001 and 2002, respectively, with ground *Lippia* applied at 0.71 mt/ha. Compared with nematode alone, *L. javanica* leaves reduced nematode densities in roots by 75-80%, 62-65% and 76-89% during 2000, 2001 and 2002, respectively. The organic amendment increased fresh fruit weight, dry shoot weight, stem diameter and soil EC, but reduced soil pH. *Bacillus* species had no effect on the efficacy of *L. javanica*. This observation indicated that leaching with irrigation water was responsible for the efficacy of *L. javanica* on nematode suppression. The results showed that ground *L. javanica* leaves have the potential of serving as an organic nematicide and fertilizer in tomato production, particularly, in semi-arid smallholder farming communities of Limpopo Province, which have generally alkaline soils.

CHAPTER 1

INTRODUCTION

Globally, halogenated fumigant nematicides such as EDB (ethyl dibromide), DBCP (dibromochloropropane), MB (methyl bromide), D-D (dichloropropane-dichloropropene), DDT (dichloro-diphenyl-trichloroethane) and related compounds were previously viewed as being indispensable in the management of plant-parasitic nematodes. The widespread use, persistence and broad spectrum of these materials in the first 20 years after their introduction caused various ecological problems. The main problems were the development of resistance to pests, elimination of natural enemies, persistent residues throughout different components of the food chain, their accumulation in the living systems and their ozone-breaking nature (Brent, 1987). These resulted in the phasing out of halogenated pesticides (Martindale, 1993).

A change in perception of priorities in pest control occurred from the early 1980. The concept of nematode pest management based on the adoption of organic amendments with nematicidal characteristics arose (Grossman and Liebman, 1995). Research nematologists had, with few exceptions, exclusively focused on conventional organic amendments with C:N ratios lower than 20:1 as alternatives to halogenated fumigant nematicides (Mankau and Minteer, 1962; Mankau,1968; Muller and Gooch, 1982). However, the major drawbacks in the use of conventional organic amendments as nematicides is the large quantities (about 10-500 mt/ha) that are required for these materials to be effective, their lowering of soil pH, negative (waiting) period after their application for decomposition to occur and their inherent inconsistent suppression of nematode densities (Rodriguez-Kabana and Pope, 1981).

Currently, at the University of the North, Discipline of Plant Production, a new organic amendment technology is being developed and evaluated (Mashela, 2002; Mashela and Mphosi, 2001; Mashela and Nthangeni, 2002). The technology referred to as ground-leaching technology (GLT) involves the use of traditional medicines in suppression of plant-parasitic nematodes. The technology is called ground-leaching technology because it is believed that water is required to leach out chemical compounds with nematicidal characteristics. The GLT involves spreading 5-g (about 0.71 mt/ha) ground organic amendment per 15-cm radius in a shallow hole around the base of the stem at transplanting and covering the materials with soil, followed by irrigation. The 5-g quantity translates to 20 kg for 4 000-tomato plants/ha (Mashela, 2002; Mashela and Nthangeni, 2002). The technology mitigates the conventional organic amendment drawbacks in that it eliminates the use of excessively large quantities, thus, reducing transport costs. Wild cucumber (*Cucumis myriocarpus*) and castor bean (*Ricinus communis*) ground fruits suppressed nematode densities at levels comparable to those of most synthetic nematicides (Mashela, 2002; Mashela and Mpati, 2001; Mashela and Mposi, 2001; Mashela and Nthangeni, 2002). Fever tea (*Lippia javanica*), another widely used "muti" is traditionally used as a fly and mosquito repellent (Hutchings, 1996). However, its effect on nematode suppression has not been tested.

The objective of this study is to evaluate the effects of ground *L. javanica* leaves on population densities of the root-knot nematode (*Meloidogyne incognita*), tomato plant growth, soil pH and electrical conductivity (EC) under greenhouse and field conditions. Also, the role of *Bacillus* species on the efficacy of ground *L. javanica* leaves was evaluated to establish if the efficacy of the GLT was strictly dependent on irrigation water.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Pest control, as practiced today in most developing countries, relies mainly on the use of imported synthetic chemical pesticides, which often lead to contamination of terrestrial and aquatic environments, damage to beneficial fauna and flora, accidental poisoning of humans and livestock and the twin problems of pest resurgence and resistance (Grossman, 1988; Gunther, 1982). A 1989 World Health Organization report estimated 1.2 million pesticide poisoning cases each year, and around 25 000 deaths directly associated with these poisonings. Clearly, there is a need for pest control agents that are pest specific, non-toxic to humans and beneficial organisms, biodegradable, less prone to pest resistance and resurgence, and relatively less expensive than synthetic chemicals (Quarles and Grossman, 1995).

Since the 1995 Methyl Bromide Technical Options Committee Assessment report, a great deal of research had been undertaken, developed and is continuing worldwide to replace halogenated fumigant nematicides for soil treatments. In the past, these materials were considered irreplaceable for soil treatments, but now these materials are considered fully replaceable as shown by certain alternatives that are being implemented (Weaver and King, 1996). Major achievements have been realized in the development of alternatives for the management of plant-parasitic nematodes.

The current void caused by non-availability of selective fumigant nematicides for nematode pest control could be significantly filled in part by the use of conventional organic amendments with volatile oil properties and narrower C: N ratios. Research efforts are being invested, developed and transferred globally with the aim of replacing halogenated fumigant nematicides without loss of economic crop yields and quality (Bello, 1998; Gamliel and Stepleton, 1993; Hoitink, 1988).

The mechanisms of release of nematicidal compounds into the environment include (i) microbial degradation, (ii) biofumigation, (iii) root exudation, and (iv) leaching. Various materials, which have been used to suppress plant-parasitic nematodes using the above mechanisms of chemical release, are being reviewed.

2.2 Microbial decomposition

The soil comprises diverse populations of microorganisms in the form of bacteria, actinomycetes and fungi. These microorganisms are capable of breaking down organic materials in soils. Breakdown of organic matter in the soil releases substantial amounts of water-soluble carbon compounds, which may affect the biological and chemical processes in the soil. These water-soluble carbon compounds may enhance or suppress plant growth, depending on the identities of the target plants, soil and the climatic conditions, and other related factors (Erick and Trusty, 1997).

In decomposition, the ideal C:N ratio for bacteria is 5:1, for actinomycetes is 6:1 and that for fungi is 10:1 (Jordan, Kremer, Bergfield, Kim and Cacnio, 1995). Inoculating organic matter with degradative microorganisms may enhance the efficacy of organic amendments. Microbial population growth generally increases immediately following the addition of organic matter, and subsequently, as part of the community succession, there is an increase in populations of nematode-trapping fungi (Hoitink, 1988).

Stirling (1991) proposed systems in which amendments could be inoculated with specific microorganisms as they were applied to the soil. The general hypotheses regarding the beneficial effects of organic amendments center around the stimulation of the saprotrophic growth phase of nematophagus fungi, and stimulation of other general microorganisms, which may be detrimental to nematodes. A good rise in enzymatic levels also occurs following soil amendment and the enzymes may attack the structural proteins in nematode cuticle or eggshell.

Organic amendments such as compost and kraal manure can control plant-parasitic nematodes in a wide range of crops (Akhtar and Mashkoor, 1993). Compost has substituted the use of MB in a number of commercial nurseries in California. Compost from crop residues is used by the Colombian flower industry to amend soil and suppress problems caused by soil-borne plant-parasitic pests and pathogens (Renkow, Safely and Chafin, 1994; Rodriguez-Kabana, 1986). At the same time, these crop residues provide plant nutrient elements and reduce production costs. Compost residues could be used for the control of root-knot nematodes in tomato and pepper (Bello, Lopez, Sanz, Escuer and Herrero, 1998; Marull, Pinochet and Rodriguez-Kabana, 1997).

A considerable amount of research had been undertaken to validate the use of kraal manure and compost materials against plant-parasitic nematodes. What is prominent in these research reports is that the two materials can suppress nematodes to the extent that may be comparable to those of most synthetic nematicides (Mashela, 2002; Mashela and Nthangeni, 2002; Quarles and Grossman, 1995). However, their use is localized and limited by the availability of raw materials and transport costs. Also, these materials must be held to appropriate quality control standards to maintain aerobic conditions to prevent them from fermentation. Therefore, producing a sour substance containing organic acids that can be phytotoxic to plants (Hoitink, 1988; Quarles and Grossman, 1995).

In the process of composting, microorganisms break down organic matter and produce carbon dioxide, water, heat and humus, the relatively stable organic end-product (Renkow *et al.*, 1994). Different communities of microorganisms predominate during the various composting phases. Members of the genus *Bacillus* dominate the microbial populations during this phase. The heat they produce causes the compost temperature to rapidly rise and produce ozone, which suppress nematode populations. In Spain, the application of organic matter in vineyards is normally made by farmers on alternating sides of the row each year so that the quantity of manure or compost required for nematode suppression is reduced by 50% (Bello, 1998).

The use of kraal manure as conventional organic amendment has led to a significant reduction in the number of nematicide applications during the lifespan of solanaceous crops (Dube, 2001). In Morocco, cattle manure at 60 mt/ha reduced the incidence of soil-borne plant-parasitic pests and pathogens in tomato production (Besri, 1997).

Successful organic amendment generally requires large quantities (10-500 mt/ha) of materials to be added to the soil to serve as organic nematicides and as a result transport costs are high. Consequently, their use is localized, limited by the availability of raw materials and transport costs. These limitations can be overcome by using local by-products of agriculture and agro-industry (Akhtar and Mashkoor, 1993).

D'Addabbo (1995) reviewed the literature on organic amendments from 1982-1994. He found a total of 221 papers demonstrating continuing interest in this approach to nematode control. Most of this work is still concentrated in developing countries. Oil cakes were the most frequently used efficacious organic amendments, but also, green manures and agro-industrial wastes were well studied. Most attention had been focused on determining the underlying mechanisms of the nematicidal action of these materials.

Soil amendments such as chitin suppressed *Rhizoctonia solani* and plant-parasitic nematodes (Rodriguez-Kabana, 1986). Chitin materials in the soil are known to increase beneficial soil populations of bacteria and actinomycetes. Tilling in chitinous materials such as crushed shells of crustaceans significantly reduces nematode species. Chitin is effective because several species of fungi, which "feed" on chitin, also attack chitin-containing nematode eggs and nematodes. Increasing the amount of chitin in the soil also increases the population of nematophagus fungi, which will move on to nematodes when the crushed shell is gone (Akhtar, and Mashkoor, 1993). The addition of chitin to soil is followed by a relatively long-term (4-10 weeks) rise in chitinase activity in the soil (Rodriguez-Kabana, Boube and Young, 1989).

Chitin is the principal structural component of nematode eggshells, and the increase in chitinase activity may be accompanied by decreased survival of nematode eggs. However, the decomposition of chitin also releases ammonia, which may contribute to its beneficial effects. Addition of chitin in soils resulted into higher changes in the micro flora, which affected nematode activities. The population of nematode trapping fungi increased, resulting into suppression of plant-parasitic nematodes (Rodriguez-Kabana, 1986). Speigel, Chet, Cohn, Galper and Sharon (1988) postulated that the beneficial effects of chitin amendments resulted from the action of specialized microorganisms. At present, limitation of the implementation of amendments for nematode control is that they must be applied at10 mt/ha to be effective. The use of local resources for such amendments will keep transport costs minimal. One product, the chitin-based Clandosant is being commercially marketed (Renkow *et al.*, 1994).

Breakdown of organic matter releases compounds into the soil that may or may not be toxic to plant-parasitic nematodes. The most widely studied of the released compounds is ammonia. Because nitrogen is a constituent of nearly all soil amendments, ammonia is produced during decomposition (Huebner, Rodriguez-Kabana and Patterson, 1983). A careful balance must be maintained in the C:N ratio, together with sufficient concentrations of ammonia to provide optimal effects without phytotoxicity (Stirling, 1991). Chemical compounds may have stimulation of nematophagus or antagonistic organisms. Also, the addition of organic amendments may provide a substrate, which may stimulate nematophagus-fungal spore germination (Sitaramaiah and Singh, 1974). Mechanisms through which organic materials suppress nematode numbers are as complex as the compounds that are being released.

2.3 Biofumigation

Biofumigation is the amendment of soil with organic matter, which releases a gas such as hydrogen sulfide that controls or eliminates soil-borne plant-parasitic pests such as nematodes. Biofumigation may be combined with plastic or other soil covers to trap the heat from solar radiation, to raise the soil temperature and to retain gases generated during the process (Gamliel and Stapleton, 1993). A good example of biofumigation is the incorporation of residues of some *Brassicae* and various *Compositae* into the soil (Bello, 1998; Bello, Lopez, Sanz, Escuer and Herrero, 1998). These materials give off volatile chemicals such as methyl isothiocyanate and phenethyl isothiocyanate, which have herbicidal, fungicidal, insecticidal and nematicidal properties (Gamliel and Stapleton, 1993).

Additions of organic matter in the soil often stimulate activities of microorganisms that are antagonistic to plant pathogens (Ingham, 1990). Gamliel and Stapleton (1993) suggested the incorporation of organic amendments as a nonchemical approach to improving the efficacy and predictability of plant pathogen control through soil biofumigation. In crop rotations, crops that follow *brassicas* benefit from the "mustard effect". The mustard effect may be due to the release of volatile, fungicidal and nematicidal chemical compounds from the decomposing residues of *brassicas*. Allelochemicals produced by *brassicas*, and later released from the plant upon decomposition, include glucosinolates and isothiocyanates (Walker, 1997).

Sulfur, a natural component of isothiocyanate, is also released during decomposition. Glucosinolates occur in many agronomically important crops, but particularly in the mustard family (Walker, 1997). Although more than 100 different glucosinolates had been identified. Their exact role and the possible participation of other compounds with them are not yet understood. Toxicity is not attributed to intact glucosinolates, but to products released by enzymatic degradation and biofumigation. These breakdown products are similar to the synthetic chemical fumigant VAPAM. They also include the compounds that are responsible for the pungent flavors and odors of cruciferous plants (Gamliel and Stapleton, 1993).

Research had been undertaken to improve biofumigation techniques and to develop a greater understanding of action of various by-products from organic amendments (Angus, Gardner, Kirkegaard and Desmarchelier, 1994). Research efforts had shown biofumigation technique to be effective for the control of certain soil-borne plant-parasitic pests in the broader range of crops. However, the potential disadvantages of biofumigation are not clearly understood, but they could include the release of phytotoxic compounds, lack of available organic amendments and negative period waiting for the amendment to decompose (Mathiessen and Kirkegaard, 1993).

Preliminary results utilizing *brassica* biofumigation for tobacco seedbeds in Australia had shown responses similar to those of methyl bromide (Angus *et al.*, 1994; Kirkegaard, Gardner, Angus and Desmarchelier, 1993; Mathiessen and Kirkegaard, 1993). The possibilities for developing biofumigation techniques are as diverse as the types of available by-products for the preparation of amendments. The nature of active gases clearly depends on the type of organic matter added to soil.

Biofumigation considerably shortens the time necessary to accomplish acceptable pest control and had been used successfully in the production of bananas, tomatoes, grapes, melons, peppers and other vegetables (Bello, 1998; Sanz, Escuer and Lopez, 1998). Biofumigation results in the stimulation of soil microbial activity with increased population of microbivorous and predatory nematodes.

Volatiles released from *brassica* species, seed meal extract of *B. juncea* and pelletized formulations of *B. juncea* were inhibitory *in vitro* to the growth of nematodes. Nematodes were reduced by amendment of the mentioned materials, but only completely with seed meal. Volatiles were inhibitorier from *B. juncea* and *B. napus* and, within all *brassica* species, leaf and root tissues were inhibitorier than stems (Harding and Wicks, 2000). Following the trial on nematode suppression using *brassica* species, isothiocyanate compounds were reported to be highly biocidal to a diverse range of plant-parasitic pests and pathogens (Bello, 1998). Kirkegaard *et al.* (1993) reported that the ploughing back of crops such as *B. napus* or *B. juncea* at stage of maturity provided a principal source of glucosinolates biofumigation in the soil.

Plant extracts such as those from *Tagetes, Ruta, Cineraria or Pelargnium* were effective in killing plant-parasitic nematodes, but results refer mainly to *in vitro* or pot experiments (Brown and Morra, 1997). The combination of calcium cyanamide and shredded wheat straw applied with biofumigation resulted in more than 90% decrease in soil-borne plant-parasitic pests and pathogens in Greece (Bourbos, Skoudridakis, Darakis and Koulizakis, 1997).

Manageable quantities of organic amendments with volatile oil properties and narrower C:N ratios were required for the effective suppression of plant-parasitic nematodes (Bello, 1998; Gamliel and Stepleton, 1993; Hoitink, 1988). One of the promising compounds, furfuraldehyde, a by-product of sugar processing, which releases large quantities of volatile nematicidal chemical compounds, is effective in the management of plant-parasitic nematodes and some phytopathogenic fungi (Canullo, Rodriguez-Kabana and Walters, 1992; Rodriguez-Kabana, 1986; Rodriguez-Kabana and Kloepper, 1992; Steyn, Van Biljon and Du Toit, 2001). Addition of this volatile material in the soil increases populations of fungi and bacteria that are known to be antagonists of many plant pathogens (Rechcigl, 1995; Papavizas, 1971). Pressmud, a by-product from the sugar industry, may serve as an alternative to fumigant nematicides (Sundararaju, Selverajan and Santhiomoorthy, 2001). Benzaldehyde had also shown activities similar to that of furfuraldehyde (Soler-Serratosa *et al.*, 1996).

Under natural conditions, effectiveness of nematode suppression by biofumigant materials depended much upon the values of C:N ratios and the state of decomposition. For example, under anaerobic conditions of flooded rice fields in India, bacteria produced toxic hydrogen sulfide gas from the decomposition of organic matter, which eventually killed nematodes. Biofumigation is already in practice in some countries and is a promising method that needs further development for wider acceptance.

2.4 Root exudation

Root exudation happens in plants that are alive, and it involves the release of secondary metabolites (terpernoids, phenols, etc.) into the soil solution, which are capable of suppressing population densities of plant-parasitic nematodes, but, had no effect on the plant that is releasing them (Mcleod and Da Silva, 1994; Luna, 1993). Root exudates influence nematophagus fungal spore germination and the growth of certain nematode-trapping microorganisms in the soil. The effect is a selective support of the growth of certain microorganisms and inhibition of others (Mcleod and Da Silva, 1994).

Sometimes root exudates may stimulate plant resistance to certain plant-parasitic pests and pathogens, depending on the nature of the root exudates released (Walker, 1997). The quantity and quality of root exudates may change depending on light, temperature and moisture conditions. Plants readily utilize root exudates released by plants growing in non-sterile soil and only small quantities can be collected in the rhizosphere. The nature and chemical composition of root exudates are unknown because the metabolic products of microorganisms mask them.

Eisenburg (1987) pointed out that phytonematicides are released as root exudates by some marigolds and a few varieties of chrysanthemums. French marigold (*Tagetes patula*) was reported as the most effective type in lowering root-knot nematode populations (Hackney and Dickerson, 1975). Apparently, nematodes are attracted to marigold roots, but when a root was attacked, it released ozone, killing the nematodes. This occurs only when nematodes feed on living marigold roots, therefore, there is no residual benefit from tilling them in (Hackney and Dickerson, 1975).

Also, planting just a few marigolds will not be effective. To get the full benefit, a cover crop of marigolds, free of weeds, must be planted for a full season (Luna, 1993). Research is currently underway on the nematicidal properties of other *Tagetes* species (Siddiqui and Alam, 1987). The most effective cultivars are those that germinate quickly grow vigorously and have deep root penetration. The cultivar 'Single Gold' ('Nema-gone®') provided 99% nematode control in Dutch tests.

Wallace and Terry (1998) suggested that asparagus has the ability to release root exudates, which contain a nematicidal glycoside, which may protect plants from nematodes. Glucosinolates are hydrolyzed by myrosinase enzyme, which is present in *brassica* tissues to release a range of hydrolyzing products including oxazolidinethionates, nitrites, thiocyanates and various forms of volatile isothiocyanates (Brown and Morra, 1997). The hydrolyzed products, particularly isothiocyanates, are known to have a broad biocidal activity (Kirkegaard *et al.*, 1993; Walker, 1997). However, the potential disadvantage of using this technology is that a crop that is of no economic use has to be on the field for some time, suppressing nematode densities, before an economic crop is sown.

2.5 Leaching

A variety of substances may be leached from plants into the soil solution. In most cases, the observed leachates may include essential plant nutrient elements, plant amino acids, free sugars such as sucrose, pectic substances, alkaloids, tannins, terpernoids and phenolic substances (Grossman, 1988; Kocke, 1987). Leachates may thus serve as plant growth stimulants and suppress soil-borne plant-parasitic pests and pathogens, which is depended on the amount, concentration of nutrients and chemical compounds involved (Wallace and Terry, 1998).

Using the concept of "muti" (traditional medicines), Mashela and Mphosi (2001) developed an alternative and affordable new organic amendment technology, which is being referred to as ground-leaching technology (GLT). In this technology, effectiveness on nematode suppression was consistently attained using ground small quantities of wild cucumber (*Cucumis myriocarpus*) fruits. The GLT involves spreading 5-g ground organic amendment per 15-cm radius in a shallow hole around the base of the stem at transplanting and covering the materials with soil, followed by irrigation. The 5-g quantity translates to 20 kg for 4 000-tomato plants/ha (Mashela, 2002). The technology mitigates the conventional organic amendment drawbacks such as: (i) the use of excessively large quantities, thus, high transport costs, (ii) waiting period for thorough microbial decomposition to occur, (iii) reduction in soil pH and (iv) inconsistent results on nematode suppression (Mashela, 2002; Mashela and Mphosi, 2001; Mashela and Nthangeni, 2002).

Mashela and Mpati (2001) conducted a greenhouse experiment using the GLT to test the effects of castor bean (*Ricinus communis*) fruits on population densities of *M. incognita*, soil pH, soil EC and growth of tomato plants. Castor bean fruit reduced population densities of *M. incognita* by 73% and 75% in Experiment 1 and Experiment 2, respectively. The organic amendment increased dry shoot weight, plant height, stem diameter soil EC and fresh fruit yield on tomato plants, but had no effect on soil pH. Generally, mechanisms involved and the amount of organic amendment necessary for consistent suppression of nematode densities are unknown (McSorley and Gallaher, 1995). Ground-leaching technology regardless of the material used, increased soil EC (Mashela, 2002; Mashela and Mphosi, 2001; Mashela and Nthangeni, 2002).

In the two completed studies, *C. myriocarpus* and *R. communis* ground fruits consistently suppressed nematode densities at levels comparable to those of most synthetic nematicides (Mashela, 2002; Mashela and Nthangeni, 2002). The potential for using organic amendments for nematode suppression through the GLT appears high. In this study, fever tea (*Lippia javanica*) is being evaluated using the GLT, for its effects on nematode densities.

Lippia javanica is rich in volatile oils and numerous monoterpernoids, which include myrcene, caryophyllene, p-Cymene, linalool and ipsdienone (Neidlein and Staehle, 1974). Iridoid glycosides and highly toxic triterpernoids (icterogenins) had been detected in *L. javanica* and related family species (Vahmeijer, 1981; Watt and Breyer-Branderijk, 1962). *Lippia javanica* had been widely used in the past by traditional healers in a number of home-made remedies and medicines to treat coughs, colds, fever, bronchitis, chest ailments, influenza, measles, rashes, malaria, headaches and stomach problems. Plant parts used include: leaves, twigs and less often, roots (Van Wyk and Gericke, 2000).

Currently, *L. javanica* leaves are sold commercially as a herbal tea by various pharmacists and chemists. The commercial value of *L. javanica* oil as a fly and mosquito repellent had been investigated (Hutchings, 1996; Watt and Breyer-Branderijk, 1985). However, the role of *L. javanica* leaves on suppression of nematode densities had not been evaluated. Therefore, in this study, the GLT (Mashela and Mphosi, 2001) will be used to evaluate the effects of *L. javanica* leaves on population densities of *M. incognita,* growth of tomato and selected soil properties. Also, the role of effective microbes on the efficacy of *L. javanica* will be evaluated.

CHAPTER 3

LIPPIA JAVANICA ORGANIC AMENDMENT UNDER GREENHOUSE CONDITIONS

3.1 Introduction

The literature concerning suppression of plant-parasitic nematodes by organic amendments is full with both promising and inconsistent results (Mashela, 2002). The major inhibiting factor in the use of organic amendments in nematode suppression is the large quantities (10-500 mt/ha) that are required for these materials to be effective. Recently, Mashela and Mphosi (2001) developed an alternative organic amendment technology using traditional medicines, whereby *Meloidogyne incognita* suppression was consistently attained using small quantities of organic amendment. When applied at 0.71 mt/ha, ground wild cucumber (*Cucumis myriocarpus*) fruits suppressed root and soil densities of *M. incognita* by 90-98% in the greenhouse. Fruits of another traditional medicine, castor bean (*Ricinus communis*), also reduced densities of *M. incognita* in the greenhouse (Mashela and Nthangeni, 2002).

Fever tea (*Lippia javanica*), another traditional medicine in Limpopo Province is widespread. Traditionally, *L. javanica* is used as a fly and mosquito repellent (Hutchings, 1996; Neidlein and Staehle, 1974). However, its effect on nematode suppression had not been tested. The objective of this study is to use the technology developed by Mashela and Mphosi (2001) to evaluate the effects of ground *L. javanica* leaves on population densities of *M. incognita*, growth of tomato plants, soil pH and electrical conductivity in the greenhouse.

3.2 Materials and methods

A greenhouse experiment was established at the Horticultural Research Unit of the University of the North (23° 53 10 S, 29° 44 15 E) on 3 March 2000. The root-knot nematode isolate, confirmed as *M. incognita* race 1, was multiplied on tomato (*Lycopersicon esculentum* var. Floradade) plants in the greenhouse.

Nematode eggs when required were extracted from the tomato roots in 1% NaOCl (Hussey and Barker, 1973), and second-stage nematode juveniles (J2s) for inoculation were obtained by incubating eggs for five days using the modified Baermann tray method (Rodriguez-Kabana and Pope, 1981). *Lippia javanica* leaves for use as organic amendment were collected from the University of the North campus, shade-dried for 2 days and further dried for 72 hours at 52°C in air-forced ovens in order to minimize volatilization of nematicidal chemical compounds (Makkar, 1999). Dried leaves were ground in a Wiley mill through a 1-mm pore sieve. The dry matter contained 13.0-g Ca, 1.0-g S, 261-g Cl, 12-g Mg, 17.6-g K, 2.45-g P, 0.48-g Na, 13-mg Cu, 462-mg Fe, 99-mg Mn, 2.5-mg Mo, 41-mg Zn, 4.21-g C and 216-g N/Kg. The calculated C:N ratio of leaves was 8:1.

Forty tomato seedlings at 3 week-old were transplanted into 3 200-cm^3 plastic pots containing 2 800-cm^3 1:1 (v/v) steam-pasteurized sand:loam soil constituting a growing mixture of 36% sand, 50% clay, 14% silt, 1.3% organic C, soil EC 0.175 (dS/m) and pH (H_2O) 6.4 on 10 March 2000. Ambient greenhouse day/night temperatures averaged 27/18°C throughout the study.

A week after seedling establishment, half were inoculated with nematode juveniles using a 20-ml plastic syringe to place ± 4 600 J2s in four 3-cm deep holes around the stem of each seedling. On inoculation day, powdered *L. javanica* leaves were thinly spread on the surface of the potting mixture of half inoculated and uninoculated seedlings at 5-g per pot (0.71 mt/ha). The tomato seedlings were hand-irrigated with 1 λ municipal water soon after applying the treatments. Untreated control, nematode, *Lippia* and *Lippia* + nematode treatments were arranged on a greenhouse bench in a randomized complete block design, with 10 replicates each.

Two tensiometers were inserted to half the depth of randomly selected pots for scheduling irrigation and plants were hand-irrigated with 1 λ municipal water when readings averaged between 10 to 15 kPa/plant in order to return the average tension to 0 kPa/plant. Fertilization was once biweekly with a total of 2.5-mg 2:3:2 (22) + 0.5 % Zn and 1.0-mg Multifeed P (43) (Plaaskem: Houghton, RSA) per 1-cm^3 municipal water. Multifeed P (43) provided 0.9-mg Mg, 0.75-mg Fe, 0.075-mg Cu, 0.35-mg Zn, 1.0-mg B, 3.0-mg Mn and 0.07-mg Mo per 1-cm^3 municipal water. Dithane M-45, copper oxychlorite WP and Bravo EC were applied as recommended to control fungal diseases, whereas metasystox R was used for aphids and Comite EC for mites.

At harvest, 13 weeks after initiating the treatments, the tomato plants were removed from the soil and plant height, stem diameter, fruit fresh weight, root fresh weight and shoot dry weight were recorded. Root galls were rated according to Taylor and Sasser (1978) root-knot index, where 0 = 0, 1 =1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, and 5 = more than 100 galls/ root system. Infected roots were placed in jars for seven days to determine nematode densities (Rodriguez-Kabana and Pope, 1981).

Nematode eggs and juveniles were extracted in 1% NaOCl (Hussey and Barker, 1973) using the Baermann tray method, and incubated for five days to obtain juveniles (Rodriguez-Kabana and Pope, 1981). Sieves nested from 1 000, 150 to 45-µm-pore sieve were used for sieving debri and soil particles. Nematode eggs and juveniles were collected using a 25-µm-pore sieve. Nematodes were counted from a 10-mλ aliquot in a counting petri dish using a light microscope (Leica 2000 model) to determine nematode densities.

The growing mixture per pot was thoroughly mixed and 100-cm^3 representative soil sub-samples removed. The soil sub-samples were air-dried for the quantification of soil pH and soil EC. Soil pH was determined by suspending 5-g soil samples in 25-cm^3 de-ionized water in 100-mλ beakers, stirred for five seconds with a glass rod, incubated for 50 minutes, re-stirred and re-incubated for 10 minutes (Bohn, McNeal and O'Connor, 1985). The pH was quantified using a no. 420 A model pH meter. Soil EC was quantified by suspending 15-g soil samples in 75-cm^3 de-ionized water in a beaker (Bohn *et al.*, 1985). Samples were shaken for an hour on a mechanical shaker at 180-rpm cycles per minute. The samples were filtered through Whatman no. 42 into 100-cm^3 beakers and 3 drops of Na_2PO_3 added. The EC meter was calibrated with 0.01 M KCl and when the reading on the EC meter was 0.143 dS/m, soil EC was quantified using a WTW LF no. 318 model EC meter.

Data were subjected to statistical analysis using MSTAT-C software (Michigan State University, East Lansing, MI). Discrete nematode numbers were transformed by $\log_2 (x+1)$ prior to analysis using a two-sample t-test in order to homogenize the variances, although untransformed arithmetic means are reported. Plant and soil data were subjected to analysis of variance (ANOVA), and Fisher's least significant difference (LSD) among means was calculated when F-values were at $P \leq 0.05$ or lower levels of probability.

The sum of squares was partitioned to assess the percentage contribution of the sources of variation to the total treatment variation (TTV) observed. Unless otherwise stated, sources of variation discussed were significant at $P \leq 0.05$ or lower levels of probability.

The experiment was repeated on 28 August 2000. Each plant was inoculated with ±5 100 J2s. Ambient greenhouse day/night temperatures averaged 28/19^{0}C throughout the study. Treatments and other conditions were as described in Experiment 1.

3.3 Results

Relative to nematode treatment, *Lippia* + nematode reduced nematode juvenile densities in roots by 75% and 80% per gram fresh roots in Experiment 1 and Experiment 2, respectively (Table 3.1). Relative to nematode treatment, *Lippia* + nematode reduced gall rating by 40% and 60% per root system in Experiment 1 and Experiment 2, respectively.

Table 3.1: Effects of ground *Lippia javanica* leaves on juveniles of *Meloidogyne incognita* and gall formation on tomato roots in the greenhouse.

	Experiment 1		Experiment 2	
Treatment rating	Juveniles/g fresh roots	Gall rating	Juveniles/g fresh roots	Gall
Nematode	17	5	16	5
Lippia + nematode	4	3	3	2
Effect (%)	–75***	–40***	–80***	–60***

Effect (%) = (1– *Lippia* + nematode/ nematode) x 100
***Significant at $P \leq 0.01$.

Compared to untreated control, *Lippia* + nematode treatment increased tomato dry shoot weight by 42% and 36% in Experiment 1 and Experiment 2, respectively (Table 3.2). Relative to untreated control, *Lippia* treatment increased tomato dry shoot weight by 26% in Experiment 1. In Experiment 2, dry shoot weight under *Lippia* + nematode and *Lippia* treatments did not differ.

In Experiment 1, *Lippia* + nematode treatment increased stem diameter by 18% when compared to that of untreated control, whereas in Experiment 2, mean stem diameters were not affected by treatments (Table 3.2). Compared to untreated control, *Lippia* + nematode treatment increased fresh fruit weight by 83% and 102% in Experiment 1 and Experiment 2, respectively. Treatments had no effect on plant height in both experiments.

Table 3.2: Effects of ground *Lippia javanica* leaves and *Meloidogyne incognita* on tomato dry shoot weight, plant height, stem diameter and fresh fruit weight in the greenhouse.

Experiment	Treatment	Dry shoot weight (g)	Plant height (cm)	Stem diam.(mm)	Fresh fruit weight (g)
1	Untreated control	101.30 b	85.210	8.237 b	201.78 b
	Nematode	87.47 c	79.260	8.687 b	156.44 b
	Lippia	108.39 b	87.680	8.540	198.69 b
	Lippia + nematode	143.87 a	84.984	10.985 a	286.91 a
	$LSD_{0.05}$	18.80	ns	2.480	89.13
2	Untreated control	88.92 b	77.100	6.575	217.71 b
	Nematode	84.46 b	79.700	5.894	157.83 c
	Lippia	111.89 a	8.047	7.452	325.07 a
	Lippia + nematode	120.57 a	82.93	6.449	18.70 a
	$LSD_{0.05}$	38.36	ns	ns	69.42

Column means (n =10) followed by the same letter were not different ($P \leq 0.05$) according to the least significant difference test.

Relative to untreated control and nematode treatment, *Lippia* and *Lippia* + nematode treatments had lower soil pH values and higher soil EC (Table 3.3). Compared to untreated control, *Lippia* + nematode reduced soil pH by 6% in both experiments. Compared to untreated control, *Lippia* + nematode increased soil EC by 44% and 69% in Experiment 1 and Experiment 2, respectively (Table 3.3).

Table 3.3: Effects of ground *Lippia javanica* leaves and *Meloidogyne incognita* infecting tomato plants on soil pH and electrical conductivity in the greenhouse.

	Experiment 1		Experiment 2	
Treatment	pH (H_2O)	EC (dS/m)	pH (H_2O)	EC (dS/m)
Untreated control	6.19 a	0.189 b	6.30 a	0.156 b
Nematode	6.22 a	0.171 b	6.42 a	0.179 b
Lippia	5.81 b	0.250 a	5.99 b	0.239 a
Lippia + nematode	5.82 b	0.272 a	5.95 b	0.264 a
$LSD_{0.05}$	0.41	0.069	0.38	0.073

Column means (n=10) followed by the same letter were not different ($P \leq 0.05$) according to the least significant difference test.

3.4 Discussion

Reduced root-knot nematode juvenile densities under *Lippia* + nematode treatment suggested that the root-knot nematode juveniles were sensitive to the nematicidal compounds that were released from the leachates of *L. javanica* leaves. However, little is known about the mechanism through which powdered leaves of *L. javanica* reduce population densities of *M. incognita*. *Lippia javanica* contains highly toxic triterpernoids in the form of icterogenins, which are released as volatile oils (Watt and Breyer-Branderijk, 1985; Vahmeijer, 1981).

Organic amendments with volatile oil properties have the potential of suppressing population densities of nematodes (Gamliel and Stapleton, 1993; Papavizas, 1974; Rechcigl; 1985). Thus, it is probable that reduced densities of *M. incognita* under *Lippia* + nematode was due to the volatile triterpernoids that were released from the leachates of *L. javanica* leaves. In other studies on tomatoes, *C. myriocarpus* and *R. Communis* fruit meal reduced population densities of *M. incognita* to levels comparable to those of methyl bromide (Mashela, 2002; Mashela and Mpati, 2001; Mashela and Mphosi, 2001; Mashela and Nthangeni, 2002).

The major inhibiting factor on the use of conventional organic amendments on nematode suppression is the large quantities that are required for these materials to be effective. Small quantities of *L. javanica* leaves (0.71 mt/ha) when compared to conventional organic amendment rates (10-500 mt/ha) had consistent effects on nematode suppression. Possibly, grinding increased the contact surface area of *L. javanica* leaves, whereby irrigation water leached out potential nematicidal compounds, which suppressed densities of *M. incognita.*

Because dried *L. javanica* leaves were used in this study, it may be deduced that water-soluble chemical compounds that were nematicidal to the nematode population densities were leached out through irrigation water into the rhizosphere. Leaching from plant organs is among the four mechanisms through which nematicidal chemical compounds are released into the rhizosphere (Inderjit and Keating, 1999). Other mechanisms through which phytochemicals are released into soil solution include microbial decomposition, biofumigation and root exudation.

In most conventional organic amendment studies, improved plant growths were explained in terms of microbial activities, release of nutrient elements to plants and suppression of plant-parasitic nematodes (Muller and Gooch, 1982; Stirling, 1991). However, the relative increase in tomato plant growth and fruit yield under *Lippia* + nematode cannot be explained in a conventional way. In this and related GLT studies, plants under nematode-organic amendment treatments consistently had improved growth (Mashela, Mashela and Mpati, 2001; Mashela and Mphosi, 2001; 2002; Mashela and Nthangeni, 2002). Reduced densities of *Pratylenchus projectus*, *Trichodorus christiei* and *Meloidogyne* species stimulated plant growth in various crops (Coursen and Jenkins, 1958; Wallace, 1973). In this study, the observation on tomato plant growth and fruit yield agree with those of Seinhorst (1965), who postulated that invasion of plant roots by plant-parasitic nematodes below the threshold damage level stimulates plant growth, leading to increased plant growth and fruit yield, whereas invasion by nematode numbers above the threshold reduced crop growth and fruit yield.

The amendment of soil with ground *L. javanica* leaves increased tomato stem diameter, dry shoot weight and fresh fruit weight, suggesting that this organic amendment contains plant nutrient elements that were beneficial to the tomato plants. Also, this organic amendment was not phytotoxic to the tomato plants at the applied rates. In other studies with tomatoes, ground castor bean and wild cucumber fruits increased tomato dry shoot weight, plant height, stem diameter and fresh fruit weight in tomato production (Mashela, Mashela and Mpati, 2001; Mashela and Mphosi, 2001; 2002; Mashela and Nthangeni, 2002).

The major drawback in the use of organic amendments in nematode suppression is their potential of reducing the soil pH, and therefore, upsetting essential plant nutrient elements in the soil leading to phytotoxicity (Mashela, 2001; Stirling, 1991). The reduction in soil pH under *Lippia* and *Lippia* + nematode treatments suggested that ground *L. javanica* leaves released chemical compounds of acidic nature. Also, the reduction in soil pH under *Lippia* + nematode and *Lippia* treatments showed that this organic amendment may serve as an organic fertilizer in semi-arid smallholder farming communities of Limpopo Province which generally have alkaline soils.

The data on soil EC showed that ground *L. javanica* leaves had positive effects, particularly on nutrient elements, which directly increased soil EC, which is directly promotional to osmotic potentials. Therefore, increased soil EC under *Lippia* + nematode treatment suggested that this organic amendment has chemical compounds or elements which are electrically charged.

In conclusion, the major effects emerging from the two experiments are that ground *L. javanica* leaves reduced population densities of *M. incognita* and soil. Also, the amendment of soil with ground *L. javanica* leaves increased tomato dry shoot weight, fresh fruit weight, stem diameter and soil EC. Thus, it may be deduced that ground *L. javanica* leaves have the potential of serving as an organic nematicide in managing population densities of *M. incognita* to levels below the economic damage-threshold in tomato production, particularly in poor-resource semi-arid smallholder farming communities of Limpopo Province.

CHAPTER 4

LIPPIA JAVANICA ORGANIC AMENDMENT UNDER FIELD CONDITIONS

4.1 Introduction

Greenhouse evaluations of ground *Lippia javanica* leaves using the "ground-leaching technology" demonstrated that these materials reduced densities of the root-knot nematode (*Meloidogyne incognita*) and gall rating (chapter 3). The organic amendment increased tomato dry shoot weight, stem diameter, fresh fruit weight and soil EC. However, the organic amendment reduced soil pH. The effects of this organic amendment had not been evaluated under field conditions. The objective of this study is to evaluate the effects of ground *L. javanica* leaves on nematode densities, productivity of tomato and selected properties of soil under field conditions.

4.2 Materials and methods

A field experiment was established on tomato (*Lycopersicon esculentum* L. var. Floradade) on different micro-plot sites at the Horticultural Skills Centre, University of the North. Holes were dug at 0.5-m x 0.5-m to a 50-cm depth and filled with dugout soil. The root-knot nematode, confirmed as *M. incognita* race 1 (Taylor and Sasser, 1978), was cultured on tomato plants in the greenhouse. Nematode eggs when required were extracted from heavily infected tomato roots in 1% NaOCl (Hussey and Barker, 1973) and J2s for inoculation were obtained by incubating eggs for five days using the modified Baermann tray method (Rodriquez-Kabana and Pope, 1981). Organic amendment materials of *L. javanica* leaves were prepared as described previously (chapter 3).

Forty uniform three-week old Floradade seedlings were transplanted on sandy loam soil with soil electrical conductivity 0.186-dS/m and pH (H_2O) 6.72 on 03 January 2002. All seedlings were inoculated with *M. incognita* using a 20-mP plastic syringe to place ±6 758 J2s on 10 January 2002. The nematode juveniles were placed adjacent to the stem in four 3-cm deep holes on cardinal quadrants of each seedling. On inoculation day, ground *L. javanica* leaves were applied as described previously (chapter 3). The two treatments, unamended control and *Lippia* amended were arranged in a completely randomized block design, with 10 replicates each.

The tomato plants were hand-irrigated with 4 P municipal water of EC 0.026 dS/m when tensiometer readings averaged between 20 to 25 kPa/plant in order to return the average tension to 0 kPa/plant. Fertilization, disease and pest control were done as described previously (chapter 3). Weeds were manually controlled.

At harvest, 13 weeks after initiating the treatments, nematode and plant data were collected as described previously (chapter 3). Soil samples for soil pH and EC determination were collected from each plant using a 2.5-cm diam. auger. Each soil sample consisted of 10 soil cores collected at 20-cm depths. The cores were thoroughly mixed and 100-cm^3 soil samples removed. Soil pH and EC were quantified in the Soil Science laboratory as described previously (chapter 3).

Discrete nematode numbers were transformed by $\log_2 (x + 1)$ prior to analysis in order to homogenize the variances, although untransformed arithmetic means are reported. All data were subjected to two-sample t-test using MSTAT-C software and means were calculated when F-values were at $P \leq 0.01$ or lower levels of probability.

The experiment was repeated on 5 March 2002. Each plant was inoculated with ±7 069 J2s on 12 March 2002. Treatments and other conditions were as described in Experiment 1. The experiment was terminated on 5 June 2002.

4.3 Results

Relative to unamended control; *Lippia* reduced nematode juvenile numbers in roots by 89% and 76% in Experiment 1 and Experiment 2, respectively (Table 4.1). Also, the amendment of soil with ground *L. javanica* leaves reduced gall rating by 80% and 60% in Experiment 1 and Experiment 2, respectively.

Table 4.1: Effects of ground *Lippia javanica* leaves on juveniles of *Meloidogyne incognita* and gall formation on tomato roots in the field.

	Experiment 1		Experiment 2	
Treatment	Juveniles/g fresh roots	Gall rating	Juveniles/g fresh roots	Gall rating
Unamended control	44	5	53	5
Lippia	5	1	13	2
Effect (%)	–89***	–80***	–76***	–60***

Effect (%) = (1– *Lippia*/ unamended control) x 100
***Significant at $P \leq 0.01$.

In Experiment 1, relative to unamended control, *Lippia* increased tomato dry shoot weight, stem diameter, plant height and fresh fruit weight by 58%, 23%, 22% and 60%, respectively (Table 4.2). In Experiment 2, *Lippia* increased tomato dry shoot weight, stem diameter, plant height and fresh fruit weight by 62%, 23%, 27% and 41%, respectively.

Table 4.2: Effects of ground *Lippia javanica* leaves on tomato dry shoot weight, plant height, stem diameter and fresh fruit weight on plots infested with *Meloidogyne incognita* in the field.

Experiment	Treatment	Dry shoot weight (g)	Plant height (cm)	Stem diam. (mm)	Fresh fruit weight (g)
1	Unamended control	94	57	10	202
	Lippia	221	73	13	500
	Effect (%)	58***	22***	23***	60***
2	Unamended control	82	54	10	286
	Lippia	216	74	13	484
	Effect (%)	62***	27***	23***	41***

_Effect (%) = (1– *Lippia*/ unamended control) x 100
***Significant at P≤ 0.01.

Relative to unamended control, *Lippia* reduced soil pH by 6% in both experiments (Table, 4.3). Relative to unamended control, *Lippia* increased soil EC by 44% and 69% in Experiment 1 and Experiment 2, respectively (Table 4.3).

Table 4.3: Effects of ground *Lippia javanica* leaves on soil pH and electrical conductivity on plots infested with *Meloidogyne incognita* in the field.

	Experiment 1		Experiment 2	
Treatment	pH (H_2O)	EC (dS/ m)	pH (H_2O)	EC (dS/m)
Unamended control	6.83	0.127	6.64	0.143
Lippia	5.34	0.269	5.52	0.277
Effect (%)	−6***	44***	−6***	69 ***

Effect (%) = (1− *Lippia*/ unamended control) x 100
***Significant at P≤ 0.01.

4.4 Discussion

The reduction of *M. incognita* population densities and gall rating in soils amended with ground *L. javanica* leaves confirmed greenhouse observations (chapter 3). Icterogenous triterpene, a highly toxic compound in *L. javanica* leaves (Watt and Breyer-Branderijk, 1985; Vahmeijer, 1981) had been identified and tested on juvenile nematode densities under greenhouse conditions with promising suppressive results (chapter 3). In this study, the observation on nematode suppression agree with those of Rich, Rahi and Opperman (1989), Mashela (2002), Mashela and Mpati (2001) and Mashela and Nthangeni (2002), whereby the GLT had been tested on juvenile densities of nematodes with promising suppressive results. Also, the observation supported the ground-leaching hypothesis, which postulates that nematicidal compounds in GLT systems are released through irrigation water (Mashela, 2002; Mashela and Mphosi, 2001; Mashela and Nthangeni, 2002).

Under conventional organic amendment systems, large quantities (10-500 mt/ha) are required for suppression of nematodes, but the results are inconsistent. However, under GLT systems, grinding increases the contact surface area of organic amendment with irrigation water, so that much lower quantities (0.20-0.71 mt/ha) are required for consistent suppression of nematodes (Mashela, 2002; Mashela and Mphosi, 2001; Mashela and Nthangeni, 2002).

In this study, the increase in tomato plant growth and fruit yield in soils amended with *Lippia* agreed with the observations in chapter 3 and related GLT studies. Compared with controls, plants under nematode-organic amendment treatments consistently had improved growth and fruit yield (Mashela, 2002; Mashela and Mpati, 2001; Mashela and Mphosi, 2001; Mashela and Nthangeni, 2002). This abnormality, established by Seinhorst (1965) is consistent in the GLT studies. In all studies it could not be explained by nutrient accumulation because the effects of *Lippia* + nematodes are higher than those of *Lippia* alone on various variables.

Most organic nematicidal compounds in plants originate as secondary metabolites, which occur as organic acids, with the potential status of reducing the soil pH (Stirling, 1991). Relative to unamended control as in chapter 3, *Lippia* reduced soil pH under micro-plot conditions. Therefore, the reduction in soil pH suggested that ground *L. javanica* leaves released chemical compounds of acidic nature. Also, the reduction in soil pH in soils amended with ground *L. javanica* leaves showed that this organic amendment may serve as an organic fertilizer in semi-arid smallholder farming communities of Limpopo Province, which have generally alkaline soils.

Increased soil EC in this and related GLT studies supported the view that organic amendments released nutrient elements into the soil solution (Mashela, 2002; Mashela and Mposi, 2001; Mashela and Nthangeni, 2002; Stirling, 1991). However, due to small quantities used, in GLT studies, treatment effects on nutrient elements in plant tissues did not differ.

The results of this study agree with those of chapter 3 and related GLT studies in terms of nematode suppression, increase in tomato shoot dry weight, stem diameter, fruit fresh weight and soil EC (Mashela, 2002; Mashela and Mphosi, 2001; Mashela and Nthangeni, 2002). Thus, the ground-leaching hypothesis also holds under field conditions.

CHAPTER 5

LIPPIA JAVANICA WITH AND WITHOUT *BACILLUS* SPECIES UNDER FIELD CONDITIONS

5.1 Introduction

The soil comprises diverse population of microorganisms in the form of bacteria, actinomycetes and fungi that play a vital role during the decomposition of organic amendments. Breakdown of organic materials releases large quantities of compounds into the soil that may be toxic to plant-parasitic nematodes. Under natural systems, effectiveness of nematode suppression by organic amendments depend much on the values of C:N ratio and state of decomposition (Rodriguez-Kabana and Pope, 1989; Rodriguez-Kabana and Walters 1992). Organic amendments with C:N ratios lower than 20:1 have higher degradation rates and generally, higher nematicidal chemical characteristics (Stirling, 1991). In decomposition, the ideal C:N ratio for bacteria is 5:1, for actinomycetes is 6:1, whereas that of fungi is 10:1 (Jordan, Kremer, Bergfield, Kim and Cacnio, 1995).

Organic amendments are reported to enhance the availability of beneficial microorganisms, predacious nematodes, nematode-trapping fungi and availability of plant nutrient elements (Speigel, Chet, Cohn, Galper and Sharon, 1988). McSorley and Gallaher (1995) noted that further research on the quantity and quality of organic amendments was needed to stimulate their widespread use in suppression of population densities of plant-parasitic nematodes.

The mechanism of action of these materials against plant-parasitic nematodes, although poorly understood, may include the release of nematoxic chemical compounds, stimulation of antagonistic microorganisms, predator and production of degradation enzymes and antibiotics. Direct effect is thought to operate in the case of nitrogen-rich amendments such as ammonia (Stirling, 1991; Rodriguez-Kabana, 1986). Processes such as microbial decomposition, biofumigation, root exudation and leaching are responsible for the release of nematicidal compounds from organic matter into soil solutions.

Mashela and Nthangeni (2002) demonstrated that *Bacillus* species referred to, as effective microbes (EM) were not essential in the release of nematicidal compounds by ground castor bean fruits. The two researchers proposed that water was responsible for leaching out nematicidal chemical compounds in what Mashela and Mphosi (2001) referred to as a ground-leaching technology (GLT). The objective of this study is to evaluate the role of *Bacillus* species on the efficacy of ground *Lippia javanica* leaves on suppression of densities of *Meloidogyne incognita*, tomato plant growth, soil pH and EC in field micro-plots.

5.2 Materials and methods

Tomato (*Lycopersicon esculentum* L. var. Floradade) seedlings were established in the Horticultural Research Unit micro plots of the University of the North. Organic amendment materials of *L. javanica* leaves were collected and prepared as described previously (chapter 3).

Sandy and loamy soils were steam-pasteurized at 300°C for 30 minutes, and 3 200-cm^3 plastic pots filled with 2 800-cm^3 Hutton soil. Holes were dug at 0.5-m x 0.5-m to a depth of 45-cm and pots set in and covered with dugout soil, ensuring that there is no re-contamination of the pasteurized growing media. The soil was irrigated to field capacity using municipal water. A day later, 80 uniform 3-week old tomato seedlings were transplanted.

The root-knot nematode, confirmed as *M. incognita* race 1 (Taylor and Sasser, 1978), was cultured on tomato (*Lycorpersicon esculentum* L. var. Floradade) plants in the greenhouse. Nematode eggs when required were extracted from heavily infected tomato roots in 1% NaOCl (Hussey and Barker, 1973) and J2s for inoculation were obtained by incubating eggs for five days using the modified Baermann tray method (Rodriquez-Kabana and Pope, 1981).

Eighty seedlings were inoculated with *M. incognita* using a 20-ml plastic syringe to place ±8 354 J2s on 4 March 2001. The nematode juveniles were placed adjacent to the stem in four 3-cm deep holes on cardinal quadrants of each seedling. On inoculation day, ground *L. javanica* leaves were spread on the surface of the potting mixture of half inoculated and uninoculated seedlings at 5-g per pot (0.71 mt/ha). *Bacillus* species commercially available as Biostart® (Microbial Solutions Ltd, Strubens Valley, RSA), with active ingredients *B. chitinosporus*, *B. laterosporus* and *B. litcheniformis* were used at the strength of 10^9 CFU/mλ. Concentrated Biostart® microbes were diluted 10 times using municipal water and applied into appropriate pots using a 20-mP plastic syringe.

A 2^3 factorial experiment, comprising initial population nematode density (P_i), *Lippia*, *Bacillus*, *Lippia* + P_i, *Lippia* + *Bacillus*, *Bacillus* + P_i, *Lippia* + *Bacillus* + P_i and untreated control treatments was established. The treatments were arranged in a completely randomized block design, with 10 replicates each. Irrigation scheduling, fertilization, disease, weed and pest control was as described previously (chapter 3). At harvest, 12 weeks after initiating the treatments, data collection, nematode extraction, nematode counting, soil pH and soil EC determinations were as described previously (chapter 3).

Discrete nematode numbers were transformed by $\log_2 (x + 1)$ before analysis in order to homogenize the variances. Data were subjected to factorial analysis of variance in order to evaluate first and second order interactions (Petersen, 1994). However, *Lippia* x *Bacillus* interactions were not significant ($P \geq 0.05$) for all variables measured. Thus, each variable was regressed on P_i, *Lippia*, *Bacillus*, soil EC and pH using stepwise regression model. The model segregated treatments that significantly ($P \leq 0.05$) contributed to the total treatment variation (TTV) in a particular variable (Petersen, 1994). Soil EC was regressed on P_i, *Lippia*, *Bacillus* and pH, whereas, soil pH was regressed on P_i, *Lippia*, *Bacillus* and EC. Unless otherwise stated, only significant ($P \leq 0.05$) predictive models are discussed.

The experiment was repeated on 25 August 2001 under similar conditions. Each plant was inoculated with ±7 053 J2s. The experiment was terminated on 10 December 2001.

5 3 Results

In Experiment 1, P_i and *Lippia* predicted the variability in P_f, accounting for 62% of the TTV in P_f (Table 5.1). The model showed that when *Lippia* was held constant, for each additional unit of P_i, P_f increased on average by 39.53 units. Alternatively, when P_i was held constant, for each unit increase in *Lippia*, P_f decreased on average by 24.68 units. In Experiment 2, the two treatments accounted for 65% of the TTV in P_f. When *Lippia* was held constant, for each unit increase in P_i, P_f increased on average by 68.53 units. Similarly, when P_i was held constant, for each additional unit of *Lippia*, P_f decreased on average by 95.00 units. In this and subsequent variables measured, the coefficients of segregated treatments were different ($P \leq 0.05$) from zero.

Table 5.1: Predictive models for *Meloidogyne incognita* final population density (P_f) based on its initial population density (P_i) and *Lippia javanica* leaves (n = 10).

Experiment	Variable	Coefficient	SE	t-value	P≤
1	Constant	12.34	3.55	3.47	0.01
	P_i	39.53	4.10	9.64	0.01
	Lippia	–24.68	4.10	–6.02	0.01
2	Constant	111.50	7.95	14.02	0.01
	P_i	68.53	5.85	6.78	0.01
	Lippia	–95.00	11.25	–8.45	0.01

Exp. 1: $P_f = 12.34 + 39.5\ P_i - 24.68$ *Lippia*, $R^2 = 0.62$
Exp. 2: $P_f = 111.5 + 68.53\ P_i - 95.00$ *Lippia*, $R^2 = 0.65$

In Experiment 1, P_i and *Lippia* predicted the variability in tomato dry shoot weight, accounting for 90% of the TTV in shoot weight (Table 5.2). The model showed that when *Lippia* was held constant, for each additional unit of P_i, shoot weight decreased on average by 62.89 units. Alternatively, when P_i was held constant, for each unit increase in *Lippia*, shoot weight increased on average by 159.79 units. In Experiment 2, the two treatments accounted for 87% of the TTV in shoot weight. When *Lippia* was held constant, for each unit increase in P_i, shoot weight decreased on average by 18.97 units. Similarly, when P_i was held constant, for each additional unit of *Lippia*, shoot weight increased on average by 244.25 units.

Table 5.2: Predictive models for tomato dry shoot weight based on *Meloidogyne incognita* initial population density (P_i) and *Lippia javanica* leaves (n = 10).

Experiment	Variable	Coefficient	SE	t-value	P≤
1	Constant	128.15	5.49	23.35	0.01
	P_i	−62.89	6.03	−4.63	0.01
	Lippia	159.79	6.34	25.21	0.01
2	Constant	73.44	6.55	11.21	0.01
	P_i	−18.97	6.34	−2.99	0.01
	Lippia	244.25	9.27	26.35	0.01

Exp. 1: Dry shoot weight = 128.15 −62.89 P_i +159.79 *Lippia*, $R^2 = 0.90$
Exp. 2: Dry shoot weight = 73.44 −18.97 P_i +244.25 *Lippia*, $R^2 = 0.87$

In Experiment 1, P_i and *Lippia* predicted the variability in tomato stem diameter, accounting for 20% of the TTV in stem diameter (Table 5.3). The model showed that when *Lippia* was held constant, for each additional unit of P_i, stem diameter decreased on average by 3.29 units. Alternatively, when P_i was held constant, for each unit increase in *Lippia*, stem diameter increased on average by 2.84 units. In Experiment 2, the two treatments accounted for 34% of the TTV in stem diameter. When *Lippia* was held constant, for each unit increase in P_i, stem diameter decreased on average by 2.45 units. Similarly, when P_i was held constant, for each additional unit of *Lippia*, stem diameter increased on average by 3.38 units.

Table 5.3: Predictive models for tomato stem diameter based on *Meloidogyne incognita* initial population density (P_i) and *Lippia javanica* leaves (n = 10).

Experiment	Variable	Coefficient	SE	t-value	P≤
1	Constant	14.03	0.45	31.05	0.05
	P_i	−3.29	0.43	−2.73	0.01
	Lippia	2.84	0.64	4.44	0.05
2	Constant	9.58	0.38	25.37	0.05
	P_i	−2.45	0.47	−3.49	0.01
	Lippia	3.38	0.54	6.32	0.05

Exp. 1: Stem diameter = 14.03 −3.29 P_i +2.84 *Lippia*, $R^2 = 0.20$

Exp. 2: Stem diameter = 9.58 −2.45 P_i +3.38 *Lippia*, $R^2 = 0.34$

In Experiment 1, P_i and *Lippia* predicted the variability in tomato fresh fruit weight, accounting for 66% of the TTV in fruit fresh weight (Table 5.4). The model showed that when *Lippia* was held constant, for each additional unit of P_i, fresh fruit weight decreased on average by 48.93 units. Alternatively, when P_i was held constant, for each unit increase in *Lippia*, fresh fruit weight increased on average by 240.82 units. In Experiment 2, the two treatments accounted for 83% of the TTV in fresh fruit weight. When *Lippia* was held constant, for each unit increase in P_i, fresh fruit weight decreased on average by 18.73 units. Similarly, when P_i was held constant, for each additional unit of *Lippia*, fresh fruit weight increased on average by 436.81 units. Treatments had no effect on tomato plant height and soil pH on both experiments.

Table 5.4: Predictive models for tomato fresh fruit weight based on *Meloidogyne incognita* initial population density (P_i) and *Lippia javanica* leaves (n = 10).

Experiment	Variable	Coefficient	SE	t-value	P≤
1	Constant	193.40	13.95	13.87	0.01
	P_i	−48.93	3.36	−9.74	0.01
	Lippia	240.82	19.72	12.21	0.01
2	Constant	179.87	19.88	9.05	0.01
	P_i	−18.73	16.21	−6.05	0.01
	Lippia	436.81	22.95	19.03	0.01

Exp. 1: Fresh fruit weight = 193.40 −48.93 P_i +240.82 *Lippia*, R^2 = 0. 66
Exp. 2: Fresh fruit weight = 179.87 −18.73 P_i +436.81 *Lippia*, R^2 = 0.83

In Experiment 1, P_i and *Lippia* predicted the variability in soil EC, accounting for 84% of the TTV in soil EC (Table 5.5). The model showed that when *Lippia* was held constant, for each additional unit of P_i, soil EC decreased on average by 0.27 units. Alternatively, when P_i was held constant, for each unit increase in *Lippia*, soil EC increased on average by 0.53 units. In Experiment 2, the two treatments accounted for 92% of the TTV in soil EC. When *Lippia* was held constant, for each unit increase in P_i, soil EC decreased on average by 0.16 units. Similarly, when P_i was held constant, for each additional unit of *Lippia*, soil EC increased on average by 0.36 units.

Table 5.5: Predictive models for soil EC based on *Meloidogyne incognita* initial population density (P_i) and *Lippia javanica* leaves (n = 10).

Experiment	Variable	Coefficient	SE	t-value	P≤
1	Constant	0.14	0.04	32.17	0.01
	P_i	−0.27	0.08	−8.75	0.01
	Lippia	0.53	0.06	20.45	0.01
2	Constant	0.19	0.34	43.02	0.01
	P_i	−0.16	0.29	−8.65	0.01
	Lippia	0.36	0.96	79.03	0.01

Exp. 1: Soil EC = 0.14 −0.27 P_i +0.13 *Lippia*, R^2 = 0. 84

Exp. 2: Soil EC = 0.19 −0.16 P_i +0.36 *Lippia*, R^2 = 0. 92

5.4 Discussion

Reduced *M. incognita* P_f by *L. javanica* organic amendment in GLT systems confirmed previous observations (Chapter 3 and 4; Mashela, 2002; Mashela and Mpati, 2001; Mashela and Mphosi, 2001; Mashela and Nthangeni, 2002). Generally, reduced P_f due to conventional organic amendments is explained in terms of either through the release of chemicals during microbial decomposition or increased nemaphagous-microbial activities (Muller and Gooch 1982; Stirling, 1991). Under GLT systems, efficacy of organic amendments on suppression of nematode numbers relied on irrigation water for releasing nematicidal chemical compounds (Mashela, 2002; Mashela and Mphosi, 2001; Mashela and Nthangeni, 2002). Grinding increased the contact surface area of L. *javanica* organic amendment with water, so that much smaller quantities (0.71 mt/ha) are required for consistent suppression of nematodes when compared with large quantities (10-500 mt/ha) used under conventional broadcasting methods with inconsistent results (Mashela, 2002; Mashela and Nthangeni, 2002; McSorley and Gallaher, 1995; Stirling, 1991).

In this study, the increase in soil EC in soils amended with *Lippia* confirmed previous observations (Chapter 3 and 4). Soil under nematode-organic amendment had increased soil EC (Chapter 3 and 4; Mashela, 2002; Mashela and Mphosi, 2001; Mashela and Nthangeni, 2002). However, because the dynamics involved in the increase in soil EC are not clear, the release of nematicidal substances are both electrically charged and not essential for plant growth. Like *C. myriocarpus* and *R. communis* fruit meal (Mashela and Mphosi, 2001; Mashela and Mpati, 2001; Mashela and Nthangeni, 2002), *L. javanica* leaves had no effect on soil pH, suggesting that this organic amendment has the potential for use in GLT systems.

The results of this study showed that commercial *Bacillus* species, referred to as effective microbes (EM), had no role on the efficacy of *L. javanica* leaves on suppression of *M. incognita* population densities nor any variable measured. Also, the results supported the hypothesis that the efficacy of ground organic amendments in GLT systems relied on irrigation water for releasing nematicidal chemical compounds (Mashela and Mphosi, 2001). In other study, Mashela and Nthangeni (2002), demonstrated that *Bacillus* species had no role in the efficacy of *R. communis*. The observations that Bacillus species had no role in the efficacy of organic amendments in the GLT systems are important for two reasons. First, this demonstrates that negative period is not important in the use of GLT systems. Second, it confirms the ground-leaching hypothesis in the GLT systems, which proposes that irrigation water serves as an agent for leaching out potential nematicidal compounds.

CHAPTER 6

SUMMARY AND CONCLUSION

Medicinal properties of *Lippia javanica* were reported in various literatures (Van Wyk and Gericke, 2000; Neidlein and Staehle, 1974). Extracts of *L. javanica* are being used as a fly and mosquito repellent (Hutchings, 1996; Watt and Breyer-Branderijk, 1985). However, the effects of *L. javanica* in Plant Protection are not documented.

The use of plant products in Plant Protection had been limited to the control of nematodes in a technology referred to as ground-leaching technology (GLT). This technology was developed within the Discipline of Plant Production at the University of the North (Mashela and Mphosi, 2001). Advantages of the GLT include: the use of small quantities for consistent suppression of nematodes, lack of negative (waiting) period and phytotoxicity, and increase in crop productivity (Mashela and Nthangeni, 2002).

Lippia javanica, at 0.71 mt/ha, consistently suppressed population densities of *Meloidogyne incognita* under greenhouse, micro plots and field conditions. The reduction effect under these conditions ranged from 62% to 89%. This range is comparable to that of highly toxic synthetic nematicides. Although the mechanism of nematode suppression is not yet understood, it is well documented that the toxic compound in *L. javanica* is the icterogenous triterpene (Hutchings, 1996; Watt and Breyer-Branderijk, 1985).

In all studies that were conducted, *L. javanica* bionematicide improved tomato plant growth and fresh fruit yield. Fresh fruit yield increase ranged from 41% to 102%, whereas dry shoot weight increases ranged from 36% to 90%. Also, in all studies, amendment of soil with *L. javanica* increased soil EC from 44% to 92%. The major disadvantage of *L. javanica* was its negative effect on soil pH. In all studies that were conducted, except one, *L. javanica* reduced soil pH by 6%. This study demonstrated for the first time that *L. javanica* leaves have nematicidal properties.

Generally, the efficacy of organic amendments on nematode suppression depends on the values of C:N ratios and the state of decomposition (Stirling, 1991). Using *Bacillus* species as decomposers, the study demonstrated that the efficacy of *L. javanica* on nematode suppression was not dependent on microbial decomposition. Thus, the observation demonstrated that *L. javanica* organic amendment can be applied at any time as opposed to pre-planting.

The reduction of soil pH suggested that *L. javanica* would not be suitable for use under high rainfall areas where the soil pH is generally low. However, in semi-arid areas of Limpopo Province, the use of *L. javanica* may assist in lowering soil pH.

Further studies are necessary to explain the mechanisms involved in suppression of population densities of *M. incognita* by *L. javanica.* Also, its residual impact in soil and plant organs should be evaluated to establish its effects on the environment and consumers.

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