INTERACTIVE EFFECTS OF NEMARIOC-AL AND NEMAFRIC-BL PHYTONEMATICIDES ON GROWTH AND FOLIAR NUTRIENT ELEMENTS OF TOMATO CULTIVAR 'HTX 14' PLANTS

BY

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DECLARATION

I declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Agriculture (Horticulture) has not been submitted previously by me or anybody for a degree at this or any other University. Also, this is my work in design and in execution, and related materials contained herein had been duly acknowledged.

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DEDICATION

To my beloved son, my parents and my siblings

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I wholeheartedly thank my God, the creator of Heavens and earth, God the Father, God the Son and God the Holy Spirit, for giving me wisdom and strength to complete this programme. If it was not for the Lord, Our God, I would not have made it this far. I would also like to thank my parents, Mr M.Z. and Mrs M.D. Maake, my sisters, Linah, Martha and Mokgadi, and also my brothers, William, Wilson and Frans. I would also like to acknowledge Tebatso and Kgaogelo. I thank all of you for your support, for being there for me and also for taking care of my son, while I was forever at the University of Limpopo, busy with my studies! To my son, Tumisho, I know I was not always there for you, but I was doing all this for you so that you could have a brighter future and also for you to have academic footsteps that you could emulate. I love you all, Bakone…! I would sincerely like to express my heartfelt gratitude to my supervisory team, Dr K.G. Shadung and Professor P.W. Mashela, for their unwavering support, encouragement and training in various concepts and for inspiring in me the passion for hard work. I now have more knowledge on scientific writing and all thanks go to you, my supervisors. I would like to direct my warm appreciation to the National Research Foundation (NRF) of South Africa, the Land Bank Chair of Agriculture and the Agricultural Research Council-Universities Collaboration Centre for kindly funding various aspects of my Master of Science in Agriculture. I am greatly honoured to have worked under the Green Biotechnologies Research Centre of Excellence (GBRCE) at University of Limpopo, where I was introduced to the amazing world of scientific research and writing. Special thanks to Dr Z. Dube and my fellow post-graduate students for their inputs in my research project. I am grateful for the assistance I received from the service workers,

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LIST OF ABBREVIATIONS

ANOVA – Analysis of variance

Ca - Calcium

CARD- Curve-fitting Allelochemical Response Data

DDG- Density-dependent growth

K - Potassium

MCSP - Mean Concentration Stimulation Point

Mg - Magnesium

Mn - Manganese

MSS – Mean Sum of Squares

Na - Sodium

P - Phosphorus

S - Sulphur

TTV – Total Treatment Variation

Zn - Zinc

ABSTRACT

The production of tomato (*Solanum lycopersicum* L.) plants had been crucial in various parts of the world since tomato fruit contribute widely to human health. However, most tomato cultivars had been shown to be highly susceptible to plant-parasitic nematodes, especially the root-knot (*Meloidogyne* species) nematodes. Two cucurbitacin-containing phytonematicides, namely, Nemarioc-AL and Nemafric-BL phytonematicides, manufactured from fruits of *Cucumis* species, are being researched and developed in South Africa as an alternative for management of *Meloidogyne* species. Most trials on tomato plants and cucurbitacin-containing phytonematicides had been under greenhouse conditions, with limited information on their interactive effects under microplot and field conditions. The objectives of this study were: (1) to determine the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides on growth and accumulation of nutrient elements in leaf tissues of tomato plants under microplot conditions and (2) to investigate the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides on growth and accumulation of nutrient elements in leaf tissues of tomato plants under field conditions. In the microplot study, uniform four-week-old tomato cv. 'HTX 14' seedlings were transplanted in 4 L plastic bags containing loam soil and Hygromix-T at the 3:1 ratio (v/v). Plastic bags were inserted into holes at 0.50 m inter-row spacing and 0.60 m intra-row spacing. The 2 x 2 factorial trial, with the first and second factors being Nemarioc-AL and Nemafric-BL phytonematicides, respectively, each at two levels. The four treatments, namely, AL_0BL_0, AL_0AL_1, BL_0BL_1 and AL_1BL_1, were arranged in a randomised complete block design. Treatments were

applied seven days after transplanting and repeated weekly until harvest. Under field conditions, uniform four-week-old tomato cv. 'HTX 14' seedlings were transplanted into the field at 0.50 m inter-row spacing and 0.60 m intra-row spacing. Treatments, experimental designs and application interval were as those under microplot conditions. At 60 days after the treatments, seedlings AL × BL interaction was not significant on all plant variables in Experiment 1 under microplot conditions, whereas in Experiment 2 the interaction was highly significant ($P \leq 0.01$) on dry shoot mass, contributing 72% in total treatment variation (TTV) of the variable. Relative to untreated control, the two-way matrix showed that the interaction reduced dry shoot mass by 8%. Nemarioc-AL phytonematicide had a significant ($P \leq 0.05$) effect on stem diameter in Experiment 1 under field conditions, whereas Nemafric-BL phytonematicide had significant effects on plant height in Experiment 2, contributing 39 and 56% in TTV of the respective variables. Relative to untreated control, Nemarioc-AL phytonematicide increased stem diameter by 4%, whereas Nemafric-BL phytonematicide increased plant height by 2%. The interaction was also significant ($P \leq 0.05$) on Na and S and highly significant ($P \leq 0.01$) on Zn, contributing 76, 26 and 6%, respectively, in TTV of the respective variables in Experiment 1 under field conditions. Using a two-way matrix, the interaction increased Na and S by 12 and 41%, respectively, but reduced Zn by 52%. In Experiment 2, the interaction was highly significant ($P \leq 0.01$) on P alone, contributing 16% in TTV of the variable, with the interaction reducing P by 76%. Nemarioc-AL phytonematicide had significant effects ($P \leq 0.05$) on Ca and highly significant effects ($P \leq 0.01$) on S, contributing 31 and 58% in TTV of the respective variables in Experiment 1. Relative to untreated control, Nemarioc-AL phytonematicide increased P by 39%. In

Experiment 2, Nemarioc-AL phytonematicide had significant effects on Ca and highly significant effects ($P \leq 0.01$) on S, contributing 66 and 49% in TTV of the respective variables. Relative to untreated control, Nemarioc-AL phytonematicide reduced Ca by 19% and S by 36%, respectively. Nemafric-BL phytonematicide had a significant effect ($P \leq 0.05$) on P, contributing 33% in TTV of the variable in Experiment 1. Relative to untreated control, Nemafric-BL phytonematicide increased P by 41%. In Experiment 2, Nemafric-BL phytonematicide had significant effects ($P \leq 0.05$) on S, contributing 40% in TTV of the variable. Relative to untreated control, Nemafric-BL phytonematicide reduced S by 33%. At 74 days after initiating the treatments under field conditions, the interaction of Nemarioc-AL and Nemafric-BL phytonematicides were not significant for plant height, stem diameter, fresh fruit and dry shoot mass in both experiments. Nemarioc-AL phytonematicide was also not significant in all plant variables in both experiments. Effects of Nemafric-BL phytonematicide were highly significant on dry shoot mass in Experiment 1 and stem diameter in Experiment 2, contributing 60 and 67% in TTV of the respective variables. Relative to untreated control, Nemafric-BL phytonematicide reduced dry shoot mass by 28% and increased stem diameter by 11% in Experiment 1 and Experiment 2, respectively. The AL × BL interaction had significant effects ($P \leq 0.05$) on P, contributing 57% in TTV of the variable in Experiment 1. Relative to untreated control, the interaction increased P by 12%. In Experiment 2, the interaction had significant effects ($P \leq 0.05$) on K, Mg, S and Mn, contributing 78, 65, 74 and 68% in TTV of the respective variables. Using a two-way matrix, relative to untreated control, the interaction increased K by 8%, but reduced Mg, Mn and S by 14, 82 and 1%, respectively. Nemarioc-AL phytonematicide was not significant in both the

experiments, whereas Nemafric-BL phytonematicide had significant effects on Mg in Experiment 1, contributing 68% in TTV of the variable. Relative to untreated control, Nemafric-BL phytonematicide increased Mg by 15%. In conclusion, the interaction of Nemarioc-AL and Nemafric-BL phytonematicides were not compatible with each other as they had undesirable effects on growth of tomato plants and accumulation of most essential nutrient elements in leaf tissues of this plant.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

Production of tomato (*Solanum lycopersicum* L.) is important in agriculture since tomato contributes to human health globally and yield of tomato might be drastically reduced by plant-parasitic nematodes (Mashela *et al.,* 2015). *Meloidogyne* species are one of the most damaging pests to both quality and quantity in tomato production causing stunted growth, reduced water uptake, nutrient elements imbalance and low evapotranspiration resulting in constraints on global food security (Mashela *et al.,* 2015). Negative impacts of synthetic pesticides on environment, human health, emergence of resistant pests and consumer's concern over pesticide residues in foods have called for new approaches to manage pests in crop husbandry (Isman and Seffrin, 2014). The global withdrawal of environmental-unfriendly synthetic nematicides from agro-chemical markets resulted in the emergence of various alternatives for managing plant-parasitic nematodes (Chedekal, 2013). Nemafric-BL and Nemarioc-AL phytonematicides, which are cucurbitacin-containing phytonematicides had been researched and developed as alternatives to methyl bromide to manage nematode population densities on tomato plants in Limpopo Province, South Africa (Mashela *et al.,* 2015).

1.1.1 Description of research problem

Agricultural inputs are potentially significant contributors to ozone-depletion, formation of greenhouse gases and global warming (Pollan, 2006). Suspension of halogenated fumigant nematicides due to their environment-unfriendliness, particularly breakdown of

ozone layer and high levels of toxicity, increased research and development of organic amendments for suppression of plant-parasitic nematodes (Bello, 1998). Nemarioc-AL and Nemafric-BL phytonematicides are being developed to manage plant-parasitic nematodes in crops with the hope that they would not be harmful to the environment and consumers (Mashela *et al*., 2015). Nemafric-BL phytonematicide has cucurbitacin B ($C_{32}H_{48}O_8$) while Nemarioc-AL phytonematicide contains two active ingredients which are cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) that accounts 80% global use of botanical pesticides. In purified form, most active ingredients of phytonematicides are not effective on nematode suppression while they are phytotoxic to crops (Wuyts *et al*., 2006).

1.1.2 Impact of research problem

Increased withdrawal of synthetic chemical nematicides from the agrochemical markets aggravated effects of plant-parasitic nematodes in crop husbandry (Mashela *et al*., 2016b). Globally, three years prior to the withdrawal of methyl-bromide in 2005, estimated yield losses due to nematode damage were at US$126 billion (Chitwood, 2003). Three and eight years after the withdrawal, yield losses were estimated at US$157 billion and US$173 billion, respectively. Relative to three years prior to the withdrawal, yield losses three and eight years after the withdrawal, therefore had increased by 25 and 37%, respectively (Mashela *et al*., 2016b). Consequently, alternative management strategies had to be researched and developed.

Two cucurbitacin-containing phytonematicides, namely, Nemarioc-AL and Nemafric-BL phytonematicides, had been researched and developed in South Africa for management of root-knot (*Meloidogyne* species) nematodes and the citrus nematode (*Tylenchulus semipenetrans*) (Mashela *et al*., 2015). Most phytonematicides lose their nematode suppression capabilities and are accompanied by high phytotoxicity levels on crops being protected against nematodes (Mashela *et al*., 2015; Okwute, 2012). Nemarioc-AL and Nemafric-BL phytonematicides were shown to be highly phytotoxic to tomato seedlings at above 10% concentration when applied as post-planting treatments (Pelinganga and Mashela, 2012; Pelinganga *et al*., 2012). Similarly, Mafeo and Mashela (2010) demonstrated that Nemarioc-AG phytonematicide was highly phytotoxic to seedlings of both dicotyledonous and monocotyledonous seedlings, with emergence prevented from as high as 60% to complete failure. In crop production, estimations of yield losses due to phytotoxicity induced by phytonematicides were from 24 to 50% (Mashela *et al*., 2015) and thus products with phytotoxicities cannot be registered for use in agriculture.

1.1.3 Possible causes of the research problem

The synthetic nematicides that were used in managing plant-parasitic nematodes had environmental problems in many crop production systems, which led to their withdrawal from the agrochemical markets (Mashela *et al.,* 2008). Currently, a lot of research is focused on environmental-friendly management strategies such as the use of botanical pesticides, phytonematicides and certain level of resistance to nematodes (Mashela *et al*., 2011). Most phytonematicides lose their nematode suppression capabilities and are

accompanied by high phytotoxicity levels on crops being protected against nematodes (Okwute, 2012). Nemarioc-AL and Nemafric-BL phytonematicides were highly phytotoxic to tomato seedlings when applied at 10% concentrations after transplanting (Pelinganga and Mashela, 2012). Phytotoxicity in phytonematicides limits the widespread use of these products in research and development of alternative products for managing population densities of nematodes (Mashela *et al.,* 2015).

1.1.4 Possible solutions of research problem

Nemarioc-AL and Nemafric-BL phytonematicides consistently suppressed population densities of *Meloidogyne* species under diverse environments in tomato production. Pelinganga *et al.* (2012) suggested that Nemarioc-AL and Nemafric-BL phytonematicides could be used as an alternative to methyl bromide. Fermented crude extracts of *C. africanus* and *C. myriocarpus* fruits were tested separately and reduced nematode population densities by 89 and 69%, respectively (Pelinganga *et al*., 2011). At low dilutions both materials had fertiliser effect on tomato plants, while at high dilutions each was phytotoxic. Results of the study (Pelinganga *et al*., 2011) demonstrated that the two materials could serve as potent bio-nematicides at low concentrations. In tomato plants, Mean Concentration Stimulation Point (MCSP) for Nemarioc-AL and Nemafric-BL phytonematicides were at 2.99 and 2.64%, respectively (Pelinganga *et al*., 2012) derived using Curve-fitting Allelochemical Response Data (CARD) model (Liu *et al*., 2003). In both Nemafric-BL and Nemarioc-AL phytonematicides, MCSP values were viewed as being equivalent to 3% for both products (Pelinganga *et al*., 2013a).

After establishing the stimulatory concentrations, Pelinganga and Mashela (2012) devised the concept of a “30-day week-month” to determine the application intervals of Nemarioc-AL and Nemafric-BL phytonematicides. The optimum application interval of Nemarioc-AL phytonematicide (3%) was at 16 days, whereas that of Nemafric-BL phytonematicide (3%) was at 18 days, with the average for both products being 17 days. At this interval, the products would be able to disrupt the life cycle of *Meloidogyne* species in tomato production, without reducing growth of tomato plants (Pelinganga *et al*., 2013b).

1.1.5 General focus of the study

The study focused on the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides on growth of tomato plants and accumulation of nutrient elements in leaf tissues.

1.2 Problem statement

Pelinganga *et al.* (2012) suggested that Nemarioc-AL and Nemafric-BL phytonematicides could be used as an alternative to methyl bromide since it was observed that under different environments the two products suppressed population densities of *M*. species. At low dilutions both materials had fertiliser effect on tomato plants, while at high dilutions each was phytotoxic, therefore the results of the study (Pelinganga *et al*., 2011) demonstrated that the two materials could serve as potent bio-nematicides at low concentrations. In efficacy trials of Nemarioc-AL and Nemafric-BL phytonematicides the following was ensured: (a) phytotoxicity in crops is avoided, (b)

the product suppresses nematodes consistently and (c) product does not leave the chemical residues in edible parts of the crops. However, the efficacy of Nemarioc-AL and Nemafric-BL phytonematicides on plant growth and accumulation of nutrient elements in leaf tissues of tomato plants was not documented when the two products were applied together. Therefore, the current study intended to investigate the interactive effect of the two phytonematicides on the growth and nutrient uptake of tomato plants under microplot and field conditions.

1.3 Rationale

Following the withdrawal of synthetic nematicides, Nemarioc-AL and Nemafric-BL phytonematicides are being researched and developed, to serve as substitutes for methyl bromide, which was a common synthetic fumigant nematicide that was used in various crop farming systems (Mashela *et al*., 2015). Most of the available environment−friendly phytonematicides are still at the research and developmental stages (Mashela *et al*., 2011), with phytotoxicities limiting the successful registration of most tested phytonematicides (Mashela *et al*., 2015). The use of products locally derived materials is important, since this would improve their accessibility to resource-poor farmers in South Africa. The interactive studies of the two phytonematicides would provide some information whether these locally-produced products could be used alone or combined in production of tomato. Therefore, the current study intends to investigate the interactive effects of the two products on growth of tomato plants and accumulation of nutrient elements under microplot and field conditions.

1.4 Purpose of the study

1.4.1 Aim

Establishment of interactive abilities of Nemarioc-AL and Nemafric-BL phytonematicides on growth and accumulation of nutrient elements of tomato cv. 'HTX 14'.

1.4.2 Objectives

1. To determine the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides on growth and accumulation of nutrient elements in leaf tissues of tomato plants under microplot conditions.
2. To investigate the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides on growth and accumulation of nutrient elements in leaf tissues of tomato plants under field conditions.

1.5 Reliability, validity and objectivity

Reliability was ensured by using appropriate statistical levels of significance ($P \leq 0.05$). A factorial set of treatments would be another way of increasing the range of validity. Validity was ensured by conducting the experiment at the same location during one season and by setting up factorial treatments, whereas the objectivity was attained by ensuring that the findings were discussed on the basis of empirical evidence, as shown in the statistical analyses, in order to eliminate all forms of subjectivity. Objectivity was achieved by discussing the findings on the basis of empirical evidence as shown by statistical analyses, with findings compared and contrasted with findings in other studies (Little and Hills, 1981).

1.6 Bias

Bias was described as any influence, conditions or set of conditions that singly or altogether distort the data (Leedy and Ormrod, 2005). In this study, bias was minimised by ensuring that the experimental error in each experiment was reduced through increased replications and randomisation.

1.7 Scientific significance of the study

Findings of this study would indicate whether Nemarioc-AL and Nemafric-BL phytonematicides would be suitable for growth and accumulation of nutrient elements in leaf tissues of tomato plants when applied separately and combined. Also, the findings will provide empirical information on the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides in tomato plants.

1.8 Structure of mini-dissertation

Following the description and detailed outlining of the research problem (Chapter 1), the work done and not yet done on the research problem was reviewed (Chapter 2). Then, each of the two subsequent chapters (Chapter 3, 4) addressed each of the two objectives, sequentially. In the final chapter (Chapter 5), findings in all chapters were summarised and integrated to provide the significance of the findings and recommendations with respect to future research, culminating in a conclusion which tied the entire study together. Literature citation and referencing followed the Harvard style using author-alphabets as prescribed by the relevant University of Limpopo Senate-approved policy framework.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

The global withdrawal of environment-unfriendly synthetic fumigant nematicides from the agrochemical markets resulted in the emergence of a wide range of alternatives for managing plant-parasitic nematodes (Chedekal, 2013). Nemarioc-AL and Nemafric-BL phytonematicides, which contain cucurbitacin A ($C_{32}H_{46}O_9$) and cucurbitacin B ($C_{32}H_{48}O_8$) active ingredients, respectively, are being researched and developed to manage nematode population densities (Mashela *et al*., 2015). Phytonematicides, due to their allelochemical active ingredients, could be highly phytotoxic to the protected crops and had been viewed in certain quarters as having 'inconsistent results' in nematode suppression (Mashela *et al*., 2015). The 'inconsistent results' had been shown to be consistent with the three phases of density-dependent growth (DDG) patterns, which occur with specific concentration ranges of allelochemical-containing phytonematicides.

2.2 Work done on problem statement

Global withdrawal of highly effective synthetic nematicides from the agrochemical markets used in management of plant-parasitic nematode populations has had economic consequences in many crop production systems (Mashela, 2007; Mashela *et al*., 2008). The withdrawal resulted in the emergence of numerous alternatives for management of plant-parasitic nematodes (Chedekal, 2013). Studies had shown that synthetic fumigant chemicals were potentially the highest contributors to ozone-depletion and the subsequent contributor to global warming (Pollan, 2006).

Consequently, there had been a surge in research on the use plant extracts and plant natural products for pest management (Castillo-Sánchez *et al*., 2010). An increasing number of studies had been assessing the efficacy of phytochemicals in an effort to address some of the challenges imposed by global warming (Joseph and Sujatha, 2012).

2.2.1 Cucurbitacin-containing phytonematicides

Nemarioc-AL and Nemafric-BL phytonematicides are being researched and developed as alternatives to synthetic fumigant nematicides for management of nematodes. Fruits collected from wild cucumber (*Cucumis myriocarpus* Naude.) and wild watermelon (*Cucumis africanus* L.f.) are being used to produce Nemarioc-AL and Nemafric-BL phytonematicides, respectively, through fermentation technology (Pelinganga, 2013). The two phytonematicides consistently suppressed nematode population densities, including the notorious root-knot (*Meloidogyne* species) nematodes (Pelinganga and Mashela, 2012; Pelinganga *et al*., 2012). The two *Cucumis* species, which are indigenous to Botlokwa in Limpopo Province (Kristkova *et al*., 2003), South Africa, contain allelochemicals in fruits which have been used as phytonematicides (Mashela *et al*., 2015). Nemarioc-AL and Nemafric-BL phytonematicides contain active ingredients cucurbitacin A and B, respectively. Due to their allelochemical nature, cucurbitacins could be highly phytotoxic to the protected crops with inconsistent results in crop production systems (Mashela *et al*., 2015). Shadung *et al.* (2016) observed that the quality of phytonematicides was dependent upon the concentration of active ingredient, which is directly associated with their performance. Furthermore, the storage period

suggested that cucurbitacin B in Nemafric-BL phytonematicide increased during the first three months of storage and decreased in the fifth month (Shadung *et al.*, 2016).

2.2.2 Cucurbitacins in *Cucumis* species

Fruits of *C. myriocarpus* and *C. africanus* are internationally used in medicinal systems, nutraceutical, pharmaceutical, cosmeceutical and pesticidal industries (Lee *et al*., 2010; Mashela *et al*., 2011; Van Wyk and Wink, 2012). The two *Cucumis* species have achieved international distribution since cucurbitacin A and B have the economic potential for use in industries that included the ten medicinal systems (Van Wyk and Wink, 2012). Cucurbitacins are known to have cancerous activities at low concentrations (Lee *et al*., 2010) due to their ability to stimulate cell division (Chen *et al*., 2005), whereas at high concentrations these chemical compounds are highly cytotoxic to healthy cells (Lee *et al*., 2010).

Cucumis myriocarpus and *C. africanus* fruits contain cucurbitacin A and B, respectively, as potent active ingredients (Chen *et al*., 2005; Jeffrey, 1978). Cucurbitacin A ($C_{32}H_{46}O_9$), which is a partial polar molecule, is slightly soluble in water (Jeffrey, 1978) and oxidises readily to cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) chemical compounds and is compartmentalised mainly in roots and fruit. In contrast, cucurbitacin B ($C_{32}H_{46}O_8$) is non-polar and therefore insoluble and stable chemical compound that occurs in all plant organs (Jeffrey, 1978). Because only cucurbitacin A is soluble in water due to its partial polarity (Chen *et al*., 2005), it had been uncertain whether crude extracts of *C. africanus* fruit could also serve as fermented crude extracts in

suppression of nematodes since cucurbitacin B, without any polarity, was insoluble in water (Mashela *et al*., 2015).

2.2.3 Management of nematodes using phytonematicides

Mashela and Mphosi (2002) used crude extracts of *C. myriocarpus* fruit to suppress population levels of *Meloidogyne* species and the citrus nematode (*Tylenchulus semipenetrans* Cobb) in pot trials, with results showing at least 90% suppression of the nematodes. Crude extracts of *C. myriocarpus* fruit suppressed the plant-parasitic nematodes in greenhouse and microplot trials by over 90% (Mashela, 2002; Mofokeng *et al*., 2004), with field trials being over 80% (Mashela, 2007). Fermented crude extracts of *C. africanus* and *C. myriocarpus* fruits were tested separately and reduced nematode population densities by 89% (range 80 to 100%) and 69% (range 52-79%), respectively, with the reproductive factor values being below one (Pelinganga *et al*., 2011). At low dilutions both crude extracts had fertiliser effect on tomato plants, while at high dilutions each was phytotoxic. Results of the study (Pelinganga *et al*., 2011) demonstrated that the two crude extracts could serve as effective bio-nematicides at low dilutions in botinemagation, which is the application of botanicals for suppression of nematode through irrigation water.

Nemarioc-AL phytonematicide reduced population density of *M. incognita* race 2 in roots and soil under greenhouse conditions by 97-99% and 47-90%, respectively, under microplot conditions by 61 and 52%, respectively, and under field conditions by 79-85% and 79-85%, respectively (Pelinganga, 2013; Pelinganga *et al*., 2012). Malungane

(2014) also observed that crude extracts of wild garlic (*Tulbaghia violacea)* stimulated growth of tomato plants and reduced *M. incognita* race 2 population densities. In granular (G) or liquid (L) formulation, the two phytonematicides consistently suppressed nematode numbers to as high as from 80 to 100% (Mafeo, 2012; Pelinganga, 2013), with recent results suggesting that reduction of nematode population could go as high as 100% (Seshweni, 2017; Sithole, 2016).

2.2.4 Phytotoxicity of phytonematicides

Phytotoxicity in phytonematicides limits the widespread use of these products in research and development of alternative products for managing population densities of nematodes (Mashela *et al.,* 2015). Due to their active ingredients which are allelochemicals, phytonematicides could be highly phytotoxic to the protected crops and had been viewed in certain cases as having 'inconsistent results' in crop production systems (Mashela *et al*., 2015). According to Pelinganga and Mashela (2012), both Nemafric-BL and Nemarioc-AL phytonematicides were highly phytotoxic to tomato seedlings when applied at 10% concentrations after transplanting. The major challenge in using fermented crude extracts would be phytotoxicity of the materials to protected crops as previously shown that potent chemicals in *Cucumis* species were highly phytotoxic to most commercial crops (Mafeo, 2012). Nemarioc-AG phytonematicide was highly phytotoxic to eight monocotyledonous and ten dicotyledonous crops when applied as drenches at planting with most crops failing to emerge (Mafeo and Mashela, 2010; Mafeo and Mashela, 2009b). *In vitro*, seed germination assays suggested that at 5 g crude extracts of *C. myriocarpus* fruit were highly phytotoxic to tomato, watermelon

and butternut squash (Mafeo and Mashela, 2009a), along with maize (*Zea mays* L.), finger millet (*Eleusine coracana* L.), sorghum (*Sorghum bicolor* L.) and onion (*Allium cepa* L.) (Mafeo and Mashela, 2009b). Similarly, Nemarioc-AL and Nemafric-BL phytonematicides were both highly phytotoxic to tomato seedling when applied at transplanting in high concentrations (Pelinganga and Mashela, 2012; Pelinganga *et al*., 2012).

2.2.5 Managing phytotoxicity

Mean Concentration Stimulation Point (MCSP): Non-phytotoxic concentrations of Nemarioc-AL and Nemafric-BL phytonematicides were researched and developed using density-dependent growth (DDG) patterns, which have three distinct growth responses: stimulation, saturation and inhibition phases (Mashela *et al*., 2015). At saturation, the plant no longer responds to phytonematicides, but with continuous application the phytonematicide becomes inhibitory to plant growth (Pelinganga, 2013). Mashela *et al*. (2015) introduced the concept of the dosage model in the management of phytotoxicity and consistent suppression of nematode numbers. In the model, Mean Concentration Stimulation Point (MCSP) was the concentration of a phytonematicide which would stimulate plant growth, while suppressing nematode numbers (Mashela *et al*., 2015). In tomato plants, MCSP for Nemarioc-AL and Nemafric-BL phytonematicides were at 2.99 and 2.64%, respectively (Pelinganga *et al*., 2012) and were empirically-derived using the Curve-fitting Allelochemical Response Data (CARD) model (Liu *et al*., 2003). In both Nemarioc-AL and Nemafric-BL phytonematicides, MCSP values were viewed as being equivalent to 3% for both products (Pelinganga *et al*., 2013a). At the MCSP values,

Nemarioc-AL and Nemafric-BL phytonematicides would not induce phytotoxicity to tomato plants, but would be consistent in suppression of nematode numbers (Mashela *et al*., 2015). In *Pelargonium sidoides,* MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides were 6.18 and 2.87%, respectively (Sithole *et al*., 2016), whereas for *Citrus volkameriana* were 8.6 and 6.3%, respectively (Mathabatha *et al*., 2016). Lebea (2017) observed that MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides on squash (*Cucurbita* pepo) were 11.85 and 2.83%, respectively, under microplot and greenhouse conditions, respectively.

Application interval: After empirically-deriving the MCSP, the result is then used to derive the application interval (T_a), where the concept of day-week-month in relation to the nematode life cycle was introduced (Mashela *et al*., 2015). Pelinganga and Mashela (2012), after establishing the stimulatory concentrations, devised the concept of a “30-day week-month” to determine the application intervals 16 and 17 days for *C. myriocarpus* and *C. africanus* fruits, respectively. At this interval, the material would be able to disrupt the life cycle of *M. incognita* race 2 in tomato production, without reducing growth of tomato plants (Pelinganga *et al*., 2013b).

Application frequency: Once the application interval was derived, the application frequency (T_f), which is the proportion of the crop cycle to the application interval [T_f = crop cycle (days)\ application interval (days)], was computed (Mashela *et al*., 2015). Non-phytotoxicity at the MCSP values depended on the number of times the product was applied per growing season, which was referred to as the application frequency

(Pelinganga *et al*., 2012). The application frequency is the unit-less factor which is empirically derived.

Dosage model review: The curve-fitting allelochemical response data (CARD) computer-based model was developed to quantify density-dependent growth patterns in biological systems (Liu *et al*., 2003). In the CARD models, density dependent growth patterns are characterised by seven biological indices, namely: (1) threshold stimulation (D_m) - the dosage at which the allelochemical starts to have a measurable stimulating effect on plant growth, (2) saturation point (R_h) - the dosage at which growth remains constant before decreasing, (3) 0% inhibition (D_0) - the end-point dosage of R_h where the allelochemical has a zero effect on growth reduction, (4) 50% inhibition (D_{50}) - the dosage where the allelochemical inhibits growth by 50%, (5) 100% inhibition (D_{100}) - the dosage where the allelochemical inhibits growth by 100%, (6) k - the number of In (D + 1) transformations that serve as a biological indicator of the degree of sensitivity with relation to stimulation or inhibition to allelochemicals and (7) R^2 - the coefficient of determination (Liu *et al*., 2003; Pelinganga *et al*., 2012).

The concept of the dosage model in phytonematicides in managing phytotoxicity and consistent suppression of nematode numbers was introduced by Mashela *et al*. (2015). Dosage model = MCSP (%) × T_f (application frequency) (Mashela *et al*., 2015). Dosage was then defined as the amount of the total active ingredient that would have been put into a given soil by the end of the crop cycle (Mashela *et al*., 2015). At the dosage of 2 g/plant, crude extracts of ground *C. myriocarpus* fruit suppressed *M. incognita* race 2,

when applied as a pre-plant bio-nematicide the material had either 50 or 100% inhibition of growth in chive (*Allium schoenoprasum* L.), leek (*Allium porrum* L.) and onion (*Allium cepa* L.) during the 18-day testing period (Mafeo *et al*., 2011). Shadung (2016) observed that at 3% concentration of Nemarioc-AL and Nemafric-BL phytonematicides applied separately at 17 days, there was no significant effects on number of fruits, plant height, stem diameter and dry shoot mass under field conditions.

2.3 Work not done on phytonematicides

Nemarioc-AL and Nemafric-BL phytonematicides have been developed for management of plant-parasitic nematodes in crop production. Phytotoxicity, efficacy and consistency trials for Nemarioc-AL and Nemafric-BL phytonematicides had been completed under different conditions (Mashela *et al*., 2011), with limited information on growth and accumulation of nutrient elements on tomato plants when the two phytonematicides were combined. This current study focused on identifying the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides on growth and accumulation of nutrient elements in tomato plants when phytonematicides were applied separately and combined under microplot and field conditions.

2.4 Addressing the identified gaps

In order to address the identified gaps, this study focused on reviewing the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides on growth and accumulation of nutrient elements in plants. Although interactive studies using the two

phytonematicides were still limited under microplot and field conditions, most of the work had been focusing on the efficacy of the two products when used alone.

2.5 Summary of identified gaps

The interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides under microplot and field conditions were identified as the existing gaps. Findings in the study would provide information on whether the two products should be used separately or in combination.

CHAPTER 3
INTERACTIVE EFFECTS OF CUCURBITACIN-CONTAINING PHYTONEMATICIDES ON GROWTH AND FOLIAR NUTRIENT ELEMENTS OF TOMATO CULTIVAR 'HTX 14' UNDER MICROPLOT CONDITIONS

3.1 Introduction

Two cucurbitacin-containing phytonematicides, namely, Nemarioc-AL and Nemafric-BL phytonematicides developed from fruits of *Cucumis* species, are being researched and developed in South Africa as an alternative for management of root-knot (*Meloidogyne* species) nematodes and the citrus nematode (*Tylenchulus semipenetrans* Cobb) (Mashela *et al*., 2015). The two products reduced nematode population densities by 89% and 69%, respectively (Pelinganga *et al*., 2011). Recent results suggested that in certain crops such as *Solanum tuberosum* and *Pelargonium sidoides*, nematode population densities could be reduced by as high as 100% (Seshweni, 2017; Sithole, 2016).

In both Nemarioc-AL and Nemafric-BL phytonematicides, the mean concentration stimulation point (MCSP) for tomato (*Solanum lycopersicum* L.) plants was at approximately 3%, with the application interval (T_a) optimised at approximately 17 days (Pelinganga *et al*., 2013a). Most trials on tomato plants and cucurbitacin-containing phytonematicides had been separately conducted under greenhouse conditions, with limited information on their interactive effects under microplot conditions. The objective of this study was to determine the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides on growth of tomato plants and accumulation of nutrient elements in leaf tissues under microplot conditions.

3.2 Materials and methods

3.2.1 Description of the study design

The study was conducted on an open field system at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, South Africa (23º53''10'S, 29º44''15'E). The location has summer (October-December) rainfall with mean annual rainfall of less than 500 mm, with hot and dry summers having maximum temperatures ranging from 28°C to 38°C. Two experiments were conducted in April to June 2016 and repeated in September to November 2016 under microplot conditions. Holes were dug and 4 L plastic bags containing steam-pasteurised loam soil and Hygromix-T at the ratio 3:1 (v/v) respectively, inserted into holes (Legend 3.1).

Legend 3.1 Tomato plants cv. 'HTX 14' planted under microplot conditions.

3.2.2 Treatments and research design

The 2 x 2 factorial trial, with first and second factors being Nemarioc-AL (AL) and Nemafric-BL (BL) phytonematicides each at two levels and four treatments namely; AL_0BL_0, AL_1BL_0, AL_0BL_1 and AL_1BL_1, were arranged in a randomised complete block design, with 15 replications.

3.2.3 Procedures

Nemarioc-AL and Nemafric-BL phytonematicides were prepared using the locally-developed method (Mashela *et al*., 2015). Briefly, for Nemarioc-AL and Nemafric-BL phytonematicides the method comprised filling a 20 L container with 16 L chlorine-free tapwater, with 40 g and 80 g dried and ground fruit from wild cucumber (*Cucumis myriocarpus* Naude.) and wild watermelon (*Cucumis africanus* L.f*.),* respectively, with 300 ml effective microorganisms (EM), 300 ml molasses each added into the container. After adding the listed ingredients, the container was shaken to mix the materials. The container had an outlet dangling into a bottle half-filled with water in order to provide for the escape route of gasses generated during the fermentation process. The airtight system was placed at room temperature for 14 days to allow for the fermentation-induced pH to drop to approximately 3.7 units (Kyan *et al*., 1999).

In both experiments, uniform four-week-old tomato seedlings cv. 'HTX 14' seedlings were transplanted in 4 L plastic bags containing loam soil and Hygromix-T at the ratio 3:1 (v/v), respectively. Tomato seedlings were fertilised three days after transplanting with 5 g 2:3:2 (26) NPK + 0.5% Zn + 5% S + 5% Ca which provided 74.3 g N, 111.4 g P,

74.3 g K, 5 g Zn and 50 g Ca per seedling. Irrigation was achieved through a drip irrigation system, at 2 h with 1 h in the morning and the other in the afternoon every other day. Weekly sprays for disease management comprised alternating Mycoguard®, Bravo®, Funginex® and Dithane M45®, whereas insect pests were scouted and monitored on a daily basis.

3.2.4 Data collection

Plant variables: At 60 days after initiating the treatments, fruit were collected and weighed to obtain fresh mass, plant height was measured from the soil surface to the tip of the flag leaf and stem diameter was measured at the soil surface using a digital vernier caliper. Shoots were oven dried at 70 °C for 72 h and weighed. Fresh fruits were oven dried at 52 °C. Matured dried leaves and tomato fruits were finely ground through a Wiley™ mill to pass through a 1 mm opening sieve.

Essential nutrient analysis: Approximately 0.10 g dried materials were digested in 40 ml 5% nitric acid (HNO_3), followed by placing the container on a vortex to allow for complete wetting of the mixture. The materials were magnetically stirred, thereafter incubated in a 95 °C water-bath for 60 minutes, allowed to cool down at room temperature, filtered, decanted into 50 ml tubes which were covered with a foil and then Calcium (Ca), Magnesium (Mg), Phosphorus (P), Potassium (K), Manganese (Mn), Sodium (Na), Sulphur (S) and Zinc (Zn) in leaf tissues were analysed at Limpopo Agro-Food Technology Station (LATS) using the Inductively Coupled Plasma Optical Emission Spectrometry (ICPE-9000).

3.2.5 Data analysis

Data were subjected to analysis of variance (ANOVA) procedure using SAS software (SAS Institute Inc., 2008). When the treatments were significant at the probability level of 5%, the degrees of freedom and their associated mean sum of squares were partitioned to determine the percentage contribution of sources of variation to the total treatment variation (TTV) among the treatment means. Mean separation was achieved using Fisher's Least Significant Difference test when treatment means were significant at the probability level of 5%. Unless otherwise stated, treatment means were discussed at 5% level of probability.

3.3 Results

3.3.1 Plant variables

Interaction of Nemarioc-AL and Nemafric-BL phytonematicides was not significant on all plant variables in Experiment 1, whereas in Experiment 2 the interaction was highly significant on dry shoot mass, contributing 72% in TTV of the variable (Table 3.1). Relative to untreated control, the two-way matrix showed that the AL × BL interaction and Nemafric-BL phytonematicide alone reduced dry shoot mass by 8 and 10%, respectively, whereas Nemarioc-AL phytonematicide increased dry shoot mass by 4% (Table 3.4).

Nemarioc-AL phytonematicide had a significant effect on stem diameter in Experiment 1 and Nemafric-BL phytonematicide had significant effect on plant height in Experiment 2, contributing 59 and 56% in TTV of the variable, respectively (Table 3.1). Relative to

untreated control, Nemarioc-AL phytonematicide increased stem diameter by 4% (Table 3.2), whereas Nemafric-BL phytonematicide increased plant height by 2% (Table 3.3).

Table 3.1 Responses of plant height, stem diameter, fresh fruit mass and dry shoot mass of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions (n = 60).

		Plant height		Stem diameter		Fresh fruit mass		Dry shoot mass	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
				Experiment 1					
Replication	14	133.768	48	3.1231	14	42904.2	30	229.120	25
AL	1	1.441	1^{ns}	13.7282	59**	6861.7	5^{ns}	30.831	3^{ns}
BL	1	38.240	14^{ns}	3.0375	13^{ns}	8171.3	6^{ns}	377.545	41^{ns}
AL×BL	1	0.840	1^{ns}	0.2201	1^{ns}	5155.0	4^{ns}	4.444	1^{ns}
Error	42	104.352	36	3.0118	13	78435.0	55	281.018	30
Total	59	278.641	100	23.1207	100	141527.2	100	922.958	100
				Experiment 2					
Replication	14	87.953	8	4.93517	33	119258	29	150.55	10
AL	1	285.144	26^{ns}	5.76600	39^{ns}	3680	1^{ns}	62.59	4^{ns}
BL	1	624.683	56**	0.15000	1^{ns}	215593	54^{ns}	60.32	4^{ns}
AL×BL	1	1.873	1^{ns}	0.29400	2^{ns}	439	1^{ns}	1061.26	72^{***}
Error	42	107.253	9	3.75679	25	59916	15	145.26	10
Total	59	1106.906	100	14.90196	100	395574	100	1479.98	100

^{ns}Not significant at P ≤ 0.05, **Significant at P ≤ 0.05, ***Highly significant at P ≤ 0.01.

TTV = Total treatment variation.

MSS = Mean Sum of Squares.

Table 3.2 Effect of Nemarioc-AL phytonematicide on stem diameter of tomato plants under microplot conditions in Experiment 1.

Nemarioc-AL	Stem diameter	Relative impact (%)
AL_0	14.347^{b}	-
AL_1	14.967^{a}	4

Relative impact (%) = [(treatment/control) − 1] × 100.

Table 3.3 Effect of Nemafric-BL phytonematicide on plant height of tomato plants under microplot conditions in Experiment 2.

Nemafric-BL	Plant height	Relative impact (%)
BL_0	9.681^{b}	-
BL_1	67.670^{a}	2

Relative impact (%) = [(treatment/control) − 1] × 100.

Table 3.4 Two-way matrix for dry shoot mass as affected by first order interaction of Nemarioc-AL and Nemafric-BL phytonematicides under microplot conditions in Experiment 2.

	Nemafric-BL			
Nemarioc-AL	BL_0	Relative impact (%)	BL_1	Relative impact (%)
AL_0	46.269^{ab}	-	41.797^{b}	−10
AL_1	48.247^{a}	4	42.686^{b}	−8

Relative impact (%) = [(treatment/control) − 1] x 100.

3.3.2 Essential nutrient elements

The AL × BL interaction was significant on Na and S in leaf tissues of tomato plants, but was highly significant on Zn, contributing 76, 26 and 6% in TTV of the respective variables in Experiment 1 (Table 3.5). Using a two-way matrix, Nemarioc-AL and Nemafric-BL phytonematicides reduced Na in leaf tissues by 30 and 39%, respectively, whereas the interaction increased Na by 12% (Table 3.9). Also, in the two-way matrix Nemarioc-AL and Nemafric-BL phytonematicides separately increased S in leaf tissues by 11 and 19%, respectively, whereas the AL × BL interaction increased S by 41% (Table 3.8). Also, Nemarioc-AL and Nemafric-BL phytonematicides each reduced Zn by 9 and 72%, respectively, whereas the interaction reduced the variable by 52% (Table 3.10). In Experiment 1, Nemarioc-AL phytonematicide alone had significant effects on P and highly significant on S in leaf tissues, contributing 31 and 58% in TTV of P and S, respectively (Table 3.5). Relative to untreated control, Nemarioc-AL phytonematicide increased P by 39% (Table 3.6). Nemafric-BL phytonematicide had significant effects on P and Zn, contributing 33 and 91% in TTV of the respective variables in Experiment 1 (Table 3.5). Relative to untreated control, Nemafric-BL phytonematicide increased P by 41% (Table 3.7).

In Experiment 2, the interaction was highly significant on P, contributing 16% in TTV of the variable (Table 3.11). Using a two-way matrix, Nemarioc-AL and Nemafric-BL phytonematicides each reduced P by 57 and 69% with the interaction reducing P in leaf tissues of tomato plants by 76% (Table 3.14).

In Experiment 2, Nemarioc-AL phytonematicide had significant effects on Ca and highly significant on P and S in leaf tissues of tomato plants, contributing 66, 27 and 49% in TTV of the respective variables (Table 3.11). Relative to untreated control, Nemarioc-AL phytonematicide reduced Ca by 19% (Table 3.12) and S by 36% (Table 3.13). Similarly, Nemafric-BL phytonematicide had significant effects on P and S, contributing 53 and 40% in TTV of the variable (Table 3.11). Relative to untreated control, Nemafric-BL phytonematicide reduced S by 33% in leaf tissues of tomato plants (Table 3.15).

Table 3.5 Responses of mean sum of squares of essential nutrient elements Calcium (Ca), Magnesium (Mg), Phosphorus (P), Potassium (K), Sulphur (S), Sodium (Na), Manganese (Mn) and Zinc (Zn) to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined on tomato plants under microplot conditions in Experiment 1 (n = 60).

		Ca		Mg		P		K	
Source	DF	MSS	%	MSS	%	MSS	%	MSS	%
Rep	14	1199.9	14	2490.7	41	10.815	5	95.156	15
AL	1	34.20	0^{ns}	994.71	16^{ns}	67.700	31**	85.443	13^{ns}
BL	1	85.68	1^{ns}	1505.0	25^{ns}	71.317	33**	36.504	6^{ns}
AL×BL	1	5744.8	68^{ns}	30.39	1^{ns}	50.942	24^{ns}	272.21	43^{ns}
Error	42	1430.8	17	1071.3	17	14.624	7	144.49	23
Total	59	8495.4	100	6092.3	100	215.39	100	633.80	100
		S		Na		Mn		Zn	
Rep	14	123.2	4	2.235	7	37.055	27	0.029	1
AL	1	1890.6	58***	1.609	5^{ns}	42.392	31^{ns}	0.032	1^{ns}
BL	1	275.2	8^{ns}	0.018	1^{ns}	27.382	20^{ns}	2.821	91***
AL×BL	1	838.5	26**	23.019	76**	1.070	1^{ns}	0.195	6**
Error	42	129.0	4	140.18	11	27.766	21	0.038	1
Total	59	3256.5	100	167.06	100	135.67	100	3.115	100

^{ns}Not significant at P ≤ 0.05, **Significant at P ≤ 0.05, ***Highly significant at P ≤ 0.01.

TTV (%) = Total treatment variation.

MSS = Mean Sum of Squares.

Table 3.6 Response of phosphorus (P) to Nemarioc-AL phytonematicide applied on tomato plants under microplot conditions in Experiment 1.

Nemarioc-AL	Mean	Relative impact (%)
AL_0	5.4045^{b}	-
AL_1	7.5290^{a}	39

Relative impact (%) = [(treatment/control) − 1] × 100.

Table 3.7 Response of phosphorus (P) to Nemafric-BL phytonematicide applied on tomato plants under microplot conditions in Experiment 1.

Nemafric-BL	Mean	Relative impact (%)
BL_0	7.5290^{a}	-
BL_1	5.4045^{b}	41

Relative impact (%) = [(treatment/control) − 1] × 100.

Table 3.8 Two-way matrix for sulphur (S) as affected by first order interaction of Nemarioc-AL and Nemafric-BL phytonematicides under microplot conditions in Experiment 1.

	Nemafric-BL			
Nemarioc-AL	BL_0	Relative impact (%)	BL_1	Relative impact (%)
AL_0	16.797^{c}	-	19.990^{b}	19
AL_1	35.500^{a}	11	23.740^{ab}	41

Relative impact (%) = [(treatment/control) – 1] x 100.

Table 3.9 Two-way matrix for sodium (Na) as affected by first order interaction of Nemarioc-AL and Nemafric-BL phytonematicides under microplot conditions in Experiment 1.

Nemarioc-AL	Nemafric-BL			
	BL_0	Relative impact (%)	BL_1	Relative impact (%)
AL_0	3.0820[a]	-	1.8777[b]	−39
AL_1	2.1707[ab]	−30	3.4440[a]	12

Relative impact (%) = [(treatment/control) – 1] x 100.

Table 3.10 Two-way matrix for zinc (Zn) as affected by first order interaction of Nemarioc-AL and Nemafric-BL phytonematicides under microplot conditions in Experiment 1.

Nemarioc-AL	Nemafric-BL			
	BL_0	Relative impact (%)	BL_1	Relative impact (%)
AL_0	0.7522[ab]	-	0.2046[c]	−72
AL_1	7.5290[a]	−9	0.3647[b]	−52

Relative impact (%) = [(treatment/control) – 1] x 100.

Table 3.11 Responses of mean sum of squares of essential nutrient elements Calcium (Ca), Magnesium (Mg), Phosphorus (P), Potassium (K), Sulphur (S), Sodium (Na), Manganese (Mn) and Zinc (Zn) to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined on tomato plants under microplot conditions in Experiment 2 (n = 60).

		Ca		Mg		P		K	
Source	DF	MSS	%	MSS	%	MSS	%	MSS	%
Rep	14	1884.9	10	16.265	26	29.895	2	634.2	24
AL	1	12255.1	66**	9.777	15^{ns}	424.2	27***	1403.6	54^{ns}
BL	1	1707.7	9^{ns}	3.730	6^{ns}	831.2	53***	152.9	6^{ns}
AL×BL	1	137.7	1^{ns}	12.078	19^{ns}	254.9	16***	2.4	0^{ns}
Error	42	2545.6	14	21.821	34	28.738	2	412.4	16
Total	59	18531	100	63.671	100	1568.9	100	2605.4	100
		S		Na		Mn		Zn	
Rep	14	112.36	4	0.437	13	0.057	19	0.112	16
AL	1	1560.42	49***	1.637	51^{ns}	0.033	11^{ns}	0.022	3^{ns}
BL	1	1264.26	40**	0.664	21^{ns}	0.041	14^{ns}	0.081	12^{ns}
AL×BL	1	47.59	1^{ns}	0.073	2^{ns}	0.072	25^{ns}	0.299	43^{ns}
Error	42	194.13	6	0.422	13	0.091	31	0.181	26
Total	59	3178.76	100	3.232	100	0.294	100	0.696	100

^{ns}Not significant at P ≤ 0.05, **Significant at P ≤ 0.05, ***Highly significant at P ≤ 0.01.

TTV (%) = Total treatment variation.

MSS = Mean Sum of Squares.

Table 3.12 Response of calcium (Ca) to Nemarioc-AL phytonematicide applied on tomato plants under microplot conditions in Experiment 2.

Nemarioc-AL	Mean	Relative impact (%)
AL_0	150.44[a]	-
AL_1	121.868[b]	−19

Relative impact (%) = [(treatment/control) – 1] x 100.

Table 3.13 Response of sulphur (S) to Nemarioc-AL phytonematicide applied on tomato plants under microplot conditions in Experiment 2.

Nemarioc-AL	Mean	Relative impact (%)
AL_0	29.419	-
AL_1	18.973	−36

Relative impact (%) = [(treatment/control) – 1] x 100.

Table 3.14 Two-way matrix for phosphorus (P) as affected by first order interaction of Nemarioc-AL and Nemafric-BL phytonematicides under microplot conditions in Experiment 2.

	Nemafric-BL			
Nemarioc-AL	BL_0	Relative impact (%)	BL_1	Relative impact (%)
AL_0	16.683[a]	-	5.117[b]	−69
AL_1	7.243[ab]	−57	3.921[c]	−76

Relative impact (%) = [(treatment/control) – 1] x 100.

Table 3.15 Response of sulphur (S) to Nemafric-BL phytonematicide applied on tomato plants under microplot conditions in Experiment 2.

Nemafric-BL	Mean	Relative impact (%)
BL_0	28.897[a]	-
BL_1	19.495[b]	−33

Relative impact (%) = [(treatment/control) – 1] x 100.

3.4 Discussions

3.4.1 Plant variables

In most plant variables, the AL × BL interactions on tomato plant growth and accumulation of nutrient elements in leaf tissues of tomato plants were not significant. Apparently the concentrations of the two phytonematicides were within the saturation phase, which is characterised by treatment effects that are not significant in context of density-dependent growth (DDG) patterns (Dube, 2016). Generally, when the interactions were significant in context of DDG patterns, the concentration could either be within the stimulation range or the inhibition phases (Mashela *et al*., 2015).

Most phytonematicides have the ability to stimulate and inhibit plant growth under the auspices of DDG growth patterns as shown with Nemarioc-AL and Nemafric-BL phytonematicides (Mashela *et al*., 2015). The DDG patterns, with three phases, namely, stimulation, neutral and inhibition phases, are phytonematicide- and plant-specific (Mashela *et al*., 2016a). The interaction of phytonematicides showed that by the time of harvest they were actually operating at inhibition phase which is shown by reduction in

dry shoot mass. The fact that other variables were not affected by the phytonematicides in the current study, suggested that the organs at harvest time were still on the saturation phase (Mashela *et al*., 2015), which is a common phenomenon in phytonematicides (Dube and Mashela, 2016). Since the interaction reduced dry shoot mass, should the trial have continued longer, the phytonematicides could have inhibited tomato plant growth.

In the current study, the interaction reduced dry shoot mass of tomato plants, which contradicted other observation where the second order interaction of Nemarioc-AL phytonematicide and crude extracts of fever tea (*Lippia javanica* L.) and castor fruit (*Ricinus communis* L.) which increased dry shoot mass, along with fruit yield, stem diameter and plant height under microplot conditions (Mashela *et al*., 2007).

Nemarioc-AL and Nemafric-BL phytonematicides increased stem diameter and plant height in tomato plants, respectively, which agreed with other observations where combination of Nemarioc-AL phytonematicide, crude extracts of fever tea and castor fruit increased stem diameter and plant height (Mashela *et al*., 2007). Alone, Nemarioc-AL and Nemafric-BL phytonematicides affected some plant variables and that agreed with observations by Sithole *et al*. (2016), where Nemarioc-AL and Nemafric-BL phytonematicides affected growth of *P. sidoides.* Observations from other studies showed that the Nemarioc-AL phytonematicide stimulated plant growth in tomato (Mashela, 2002), tomato (Pelinganga, 2013), potato (Seshweni, 2017), green beans (Chokoe, 2017) and *P. sidoides* (Sithole *at al*., 2016). Nemafric-BL phytonematicide on

the other hand stimulated plant growth in beetroot (Mashitoa, 2017) and *P. sidoides* (Sithole, 2017).

Malungane (2014) demonstrated that in ground leaching technology (GLT) crude extracts of wild garlic (*Tulbaghia violacea* L.) had significant effects on plant height, stem diameter, fresh root mass, fresh shoot mass, dry shoot mass, number of leaves, number of fruits, number of flowers and number of clusters in tomato plant variables. Generally, the product stimulated plant height, stem diameter, fresh root mass, fresh shoot mass, dry shoot mass, number of leaves, number of fruits, number of flowers and number of clusters in tomato plants (Malungane, 2014). Mashela *et al*. (2010) observed that in GLT *L. javanica* leaf tissues tomato plant growth was significantly increased under microplot conditions. Sithole (2016) found that Nemarioc-AL phytonematicide had significant effects on plant height and dry root mass on *P. sidoides,* whereas Nemarioc-AL phytonematicide had effects on gall rating and chlorophyll content of potato (Seshweni, 2017). Nemarioc-AL phytonematicide in the current study did not have any significant effect on plant variables except for stem diameter, which agreed with observations whereby Nemarioc-AL phytonematicide was not significant on all variables of *Cleome gynandra* (Rabothata, 2017). Significant effects of Nemarioc-AL phytonematicide on stem diameter in the current study contradicted those of Shadung (2016) where the product did not have any significant effects on stem diameter. Mashela and Nthangeni (2002) explained that the stimulation of tomato plant growth due to the application of crude extracts of C. *myriocarpus* fruit species in the GLT

system was referred to as a fertiliser effect. Consequently, Nemarioc-AL phytonematicide could be applied alone on the tomato cultivar used in the current study.

The increase in plant height by Nemafric-BL phytonematicide agreed with the findings of Malungane (2014) where crude extracts of *T. violacea* increased plant height of tomato. Nemafric-BL phytonematicide in the current study had positive effects on growth of tomato plants which contradicted observations where the phytonematicide was shown to be phytotoxic to tomato plants (Pelinganga and Mashela, 2012). Under greenhouse conditions, Tseke (2013) observed that Nemafric-BL phytonematicide when applied alone on tomato plants could have positive effects on all plant variables, whereas Shadung (2016) found that Nemafric-BL phytonematicide had significant effect on chlorophyll content whereby the phytonematicide increased chlorophyll content in tomato plants. In the current study, phytotoxicity effects of Nemafric-BL phytonematicide to tomato plants was not observed and could therefore be applied on tomato plants as shown previously (Mashela *et al*., 2015).

3.4.2 Essential nutrient elements

Interaction of Nemarioc-AL and Nemafric-BL phytonematicides was significant and reduced Na, Zn, P and increased S, which agreed with recent observations where the cucurbitacin-containing phytonematicides were shown to improve the accumulation of nutrient elements in leaf tissues of green beans (*Phaseolus vulgaris* L.) (Mashela and Pofu, 2017). Findings agreed with those of Pelinganga (2013), where the interaction of Nemafric-BL phytonematicide and application frequency were significant on foliar Ca,

Mg, Mn, Na, P, S and Zn. In the current study, the interactions did not have any effects on nutrient element such as K which is required for activating starch synthase that hydrolyses sucrose into glucose and fructose (Mashela and Nthangeni, 2002). Mashela and Nthangeni (2002) explained that, as more sucrose reaches the roots, K^+ from leaves is further excreted into the root apoplast, where it leaches into the soil, then follow the route of minimal resistance which then result in both leaf and root K^+ deficits. In green beans study Mashela and Pofu (2017) observed that increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides exhibited positive quadratic relations whereby accumulation of nutrient elements in leaf tissues of green beans was stimulated. The stimulation range for accumulation of the selected nutrient elements in leaf tissues of green beans was within approximately 3% concentration of each phytonematicide (Mashela and Pofu, 2017), which were empirically-established concentration for nematode management in tomato (Mashela *et al*., 2016).

Generally, various organs in tomato plants have different sensitivities to crude extracts of *C. africanus* and *C. myriocarpus* (Mafeo, 2012), with findings in this study suggesting that nutrient elements in leaf tissues could also be responding differently to various interactions. Rabothata (2017) found that the interaction of VAM and Nemafric-BL phytonematicide increased Zn, which contradicted with findings of the current study, where the interaction reduced Zn. Rabothata (2017) also observed that the interaction of VAM and Nemarioc-AL phytonematicide did not have significant effects on foliar nutrient elements. *Trichoderma harzianum* × VAM interaction had significant effects on

leaf tissues of Mn and Zn in tomato plants (Nzanza *et al*., 2011). The observations suggested that the different products affected pants differently.

The interaction of Nemarioc-AL and Nemafric-BL phytonematicides reduced Na which is an undesirable effect on growth of tomato plants since Na is required for improvement of fruit quality in tomato plants (Salisbury and Ross, 1992). Contradictory, increasing concentrations of Nemafric-BL phytonematicide increased the accumulation of Na in leaf tissues of green beans (Mashela and Pofu, 2017). The findings in the current study can be desirable in C3 plants where this element does not serve any essential nutrient element role except for participating as an osmotically active ion (Salisbury and Ross, 2005). Phosphorous is an important element in the establishment of crops and it encourages the formation, development and establishment of roots, particularly the secondary roots (Brady and Weil, 2000). Deficiency of P leads to stunted growth, with older foliage having purple venation, especially on the ventral sides (Brady and Weil, 2000). In the current study the interaction of the two phytonematicides reduced P in leaf tissues of tomato plants. Increase in S in the current study was important since S is required for synthesis of S-containing amino acids, such as cystine, cystein and methionine which are essential components of protein. Sulphur deficiency might lead to increased susceptibility to pathogens (Dubuis *et al.,* 2005). Interaction of Nemarioc-AL and Nemafric-BL phytonematicides reduced Zn which is a component of protein in plants and its deficiency might result in reduction of elongation of internodes and therefore, the formation of smaller leaves with brown necrotic spot on leaves with slight chlorosis and downward curling of the petioles (Sainju *et al.,* 2003). In other

observation, increasing concentrations of Nemarioc-AL phytonematicide increased accumulation of Zn in leaf tissues of green beans (Mashela and Pofu, 2017).

Nemarioc-AL phytonematicide increased P, reduced S and Ca and this disagreed with observation in *C. gynandra,* where Nemarioc-AL phytonematicide did not have significant effects on nutrient elements (Rabothata, 2017). Shadung (2016) observed that Nemarioc-AL phytonematicide increased most nutrient elements in leaf tissues, but reduced Fe in tomato plants. Crude extracts from fruit of *C. myriocarpus* fruit species had no significant effect on nutrient elements in leaf tissues of tomato plants, which was then ascribed to the small quantities used in ground leaching technology (GLT) (Mashela, 2002). In another study (Mashela and Pofu, 2017), increasing concentrations of Nemarioc-AL phytonematicide stimulated the accumulation of K and Fe in leaf tissues of green beans.

Increase in P is essential and Nemarioc-AL phytonematicide had, therefore, positive effects on tomato plants. Concentration of phytochemicals such as ascorbic acid, flavonoids and lycopene in tomato fruits may also increase due to increasing P content in leaf tissues (Dorais *et al*., 2008). Generally, an increase in Ca in the soil increases Ca content in tomato fruit, thereby improving fruit firmness and shelf-life. Also, Ca ameliorates the physiological disorder referred to as blossom-end rot in tomato fruit (Taylor and Locascio, 2004). In the current study, Nemarioc-AL phytonematicide reduced Ca, which could be explained as the phytonematicide concentration operating at the inhibition phase. Mashela and Pofu (2017) suggested that the accumulation of

essential nutrient elements over increasing concentration of phytonematicides ascribed to the DDG patterns within the context of stimulation, neutral and inhibition phases.

As observed under Nemarioc-AL phytonematicide, Nemafric-BL phytonematicide increased P, which also agreed with observations in Shadung's study (2016), where Nemafric-BL phytonematicide increased Mg, Na, Ca and P, but reduced Fe in leaf tissues of tomato plants. Mashela and Pofu (2017) observed that Ca, K, Na and Fe in leaf tissues of green beans exhibited positive quadratic relations against increasing concentrations of Nemafric-BL phytonematicide. In the current study, Nemafric-BL phytonematicide was at the saturation phase for P and at the inhibition phase for S, which were shown by increase in P and decrease in S. However, contradictory responses of Ca, K, Na and Fe in leaf tissues of green beans against increasing concentrations of Nemafric-BL phytonematicide started from stimulation through the neutral to the inhibition phases (Mashela and Pofu, 2017). These observations contradicted those in the current study, where Nemafric-BL phytonematicide did not have any effect on Ca, K and Na in leaf tissues of tomato plants. Apparently, for certain nutrient elements, the responses could be plant-specific.

Increase in P is important in tomato production since it helps to initiate root growth of tomato and therefore helps in early establishment of the plant immediately after transplanting or seeding (Sainju *et al*., 2003). In contrast, P deficiency could result in short, underdeveloped root systems. Phosphorus is believed to increase the number of blossoms during early growth and early fruit set, thus, increasing tomato fruit yield

(Sainju *et al*., 2003). Pelinganga (2013) noted that crude extracts of *C. africanus* fruit had no effect on certain nutrient elements in leaf tissues of tomato, except that the concentration played a role in accumulation certain nutrient elements, particularly Ca, Mg, Mn, Zn, Na and S. Also, Pelinganga (2013) observed that *C. africanus* extracts, relative to the untreated control, increased foliar K, whereas in the current study Nemafric-BL phytonematicide did not have an effect on K.

3.5 Conclusion

Phytonematicides are being researched and developed for management of nematodes in tomato production. In the current study, Nemarioc-AL and Nemafric-BL phytonematicides were observed to promote tomato plant growth when applied separately. The interaction also had negative effects on some nutrient elements in leaf tissues and this could affect plant growth and yield. Nemarioc-AL and Nemafric-BL phytonematicides each promoted foliar nutrient elements in tomato plants. The interaction of Nemarioc-AL and Nemafric-BL phytonematicides in the current study was phytotoxic to tomato plants. Therefore, it was not advisable to combine Nemarioc-AL and Nemafric-BL phytonematicides in tomato husbandry.

CHAPTER 4

INTERACTIVE EFFECTS OF CUCURBITACIN-CONTAINING PHYTONEMATICIDES ON GROWTH AND FOLIAR NUTRIENT ELEMENTS OF TOMATO CULTIVAR 'HTX 14' UNDER FIELD CONDITIONS

4.1 Introduction

Most trials on tomato (*Solanum lycopersicum* L.) using Nemarioc-AL and Nemafric-BL phytonematicides had been conducted under greenhouse and microplot conditions (Chapter 3; Mashela *et al*., 2015), with limited information on their interactive effects under field conditions. Generally, under greenhouse conditions temperatures and relative humidity are high and more or less constant. However, root systems are confined to limited growing spaces, with root bounds limiting plant growth. Consequently, in some cases, greenhouse findings cannot be extrapolated to field conditions, *vice versa*. In contrast, although under microplot conditions the growth spaces could be limiting with aseptic growth medium, the day/night temperatures could be highly variable, thereby, affecting the efficacy of soil-drenched agricultural products such as phytonematicides.

Under field conditions, in addition to variable temperature conditions, the soil could also be heterogeneous in terms of soil particles and the existing edaphic microorganisms. Also, under field conditions, phytonematicides have unrestricted movements into the soil through percolation, which might be down-and side-ward, depending on soil type, slope, intensity of rainfall, irrigation intensity and/or cultural practices. In contrast, in plastic containers used under greenhouse and microplot conditions, the plastic walls might restrict the movements of phytonematicides, and consequently, increasing the

concentrations in the rhizosphere (Dube, 2016). The objective of this study was to determine the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides on growth of tomato plants and accumulation of nutrient elements in leaf tissues under field conditions.

4.2 Materials and methods

4.2.1 Description of the study design

The study was conducted on an open field system at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, South Africa (23^{o}53"10'S, 29^{o}44"15'E). The location has summer (October-December) rainfall with mean annual rainfall of less than 500 mm, with hot and dry summers having maximum temperatures ranging from 28 to 38°C. The site contained Hutton soil (65% sand, 30% clay, 5% silt) with 1.6% organic C, electrical conductivity (EC) 0.148 dS/m and pH(H_2O) of 6.5. Two experiments were conducted during late summer (January-March) 2016 and repeated during winter to spring (July-September) 2016 under field conditions (Legend 4.1).

Legend 4.1 Tomato plants cv. 'HTX 14' planted under field conditions.

4.2.2 Treatments and research design

The 2 x 2 factorial trial, with first and second factors being Nemarioc-AL and Nemafric-BL phytonematicides each at two levels were arranged in a randomised complete block design, with 18 replications.

4.2.3 Procedures

Nemarioc-AL and Nemafric-BL phytonematicides were prepared using the locally-developed method as explained previously (Chapter 3). Uniform four-week-old tomato cv. 'HTX 14' seedlings were transplanted directly into the field at 0.50 m inter-row spacing and 0.60 m intra-row spacing. Fertilisation, irrigation and disease management were as explained previously (Chapter 3).

4.2.4 Data collection

At 74 days after initiating the treatments, plant variables were collected as explained previously (Chapter 3), except that it was not feasible to sample total roots. Therefore, the variable was sampled for assessment of root galls.

4.2.5 Data analysis

Data were subjected to analysis of variance (ANOVA) procedure using SAS software (SAS Institute Inc, 2008). Unless otherwise stated, treatment effects were significant at probability level of 5%.

4.3 Results

The seasonal interactions were significant and therefore, data for the two seasons were not pooled.

4.3.1 Plant variables

The AL × BL interactions were not significant for plant height, stem diameter, fresh fruit and dry shoot mass in Experiment 1 and Experiment 2. Effects of Nemarioc-AL phytonematicide were also not significant for all plant variables in both experiments. However, effects of Nemafric-BL phytonematicide were highly significant ($P \leq 0.01$) on dry shoot mass in Experiment 1 and on stem diameter in Experiment 2, contributing 60 and 67% in total treatment variation (TTV) of the respective variables (Table 4.1). Relative to untreated control, Nemafric-BL phytonematicide reduced dry shoot mass by

28% (Table 4.2) and increased stem diameter by 11% in Experiment 1 and Experiment 2, respectively (Table 4.3).

Table 4.1 Responses of plant height, stem diameter, fresh fruit mass and dry shoot mass of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions (n = 72).

Source	DF	Plant height		Stem diameter		Fresh fruit mass		Dry shoot mass	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Experiment 1									
Replication	17	150.272	34	1.66965	20	74231	14	481.71	6
AL	1	124.294	28^{ns}	1.90125	23^{ns}	75784	14^{ns}	718.91	10^{ns}
BL	1	6.009	1^{ns}	0.82347	10^{ns}	88656	17^{ns}	4506.98	60***
AL×BL	1	14.045	3^{ns}	0.07347	1^{ns}	192603	37^{ns}	1191.12	16^{ns}
Error	51	152.722	34	3.74695	46	93227	18	618.88	8
Total	71	447.342	100	8.21479	100	524501	100	7517.6	100
Experiment 2									
Replication	17	265.056	37	6.5074	21	1478.0	20	1305.15	32
AL	1	75.031	11^{ns}	0.0868	1^{ns}	1506.0	20^{ns}	919.20	22^{ns}
BL	1	26.767	4^{ns}	20.5868	67**	1792.0	24^{ns}	356.09	9^{ns}
AL×BL	1	210.467	29^{ns}	0.2813	1^{ns}	1216.0	16^{ns}	1010.70	24^{ns}
Error	51	136.788	19	3.1928	10	1545.0	20	534.86	13
Total	71	714.109	100	30.6551	100	7537.0	100	4126	100

^{ns}Not significant at P ≥ 0.05, **Significant at P ≤ 0.05, ***Highly significant at P ≤ 0.01.

TTV = Total treatment variation.

MSS = Mean Sum of Squares.

Table 4.2 Effect of Nemafric-BL phytonematicide on dry shoot mass of tomato plants under field conditions in Experiment 1.

Nemafric-BL	Dry shoot mass	Relative impact (%)
BL_0	56.067[a]	-
BL_1	40.244[b]	−28

Relative Impact (%) = [(treatment/control) − 1] × 100.

Table 4.3 Effect of Nemafric-BL phytonematicide on stem diameter of tomato plant under field conditions in Experiment 2.

Nemafric-BL	Stem diameter	Relative impact (%)
BL_0	9.681[b]	-
BL_1	10.750[a]	11

Relative Impact (%) = [(treatment/control) − 1] × 100.

4.3.2 Essential nutrient elements

The interaction of Nemarioc-AL and Nemafric-BL phytonematicides was not significant on most nutrient elements except for P which was significant contributing 57% in TTV of the variable in Experiment 1 (Table 4.4). Using a two-way matrix the interaction increased P by 12%, whereas Nemarioc-AL and Nemafric-BL phytonematicides each reduced P by 19 and 8%, respectively (Table 4.6). Nemafric-BL phytonematicide was significant on Mg, contributing 68% in TTV of the variable (Table 4.4). Nemafric-BL phytonematicide increased the accumulation of Mg in leaf tissues of tomato by 15% (Table 4.5).

In Experiment 2, the interaction was significant Mg, K, S and Mn, contributing 65, 78, 74 and 68% in TTV of the respective variables (Table 4.7). Using a two-way matrix the interaction reduced Mg by 14%, whereas Nemarioc-AL and Nemafric-BL phytonematicides alone reduced Mg by 31% (Table 4.8). Using a two-way matrix the interaction increased K by 8%, whereas Nemarioc-AL and Nemafric-BL phytonematicides alone reduced K by 21 and 22%, respectively (Table 4.9). Using a two-way matrix the interaction reduced S by 1%, whereas Nemarioc-AL and Nemafric-BL phytonematicides alone increased S by 26 and 28%, respectively (Table 4.10). Also, using a two-way matrix, the interaction reduced Mn by 82%, whereas Nemarioc-AL and Nemafric-BL phytonematicides alone increased Mn by 64 and 79%, respectively (Table 4.11). Nemarioc-AL phytonematicide as the main factor was not significant in all nutrient elements in leaf tissues for both the experiments.

Table 4.4 Responses of mean sum of squares of Calcium (Ca), Magnesium (Mg), Phosphorus (P), Potassium (K), Sulphur (S), Sodium (Na), Manganese (Mn) and Zinc (Zn) to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined on tomato plants under field conditions in Experiment 1 (n = 72).

		Ca		Mg		P		K	
Source	DF	MSS	%	MSS	%	MSS	%	MSS	%
Rep	17	616.899	43	12.462	15	64.675	13	121.641	16
AL	1	68.445	5^{ns}	1.2641	1^{ns}	0.470	0^{ns}	86.023	12^{ns}
BL	1	19.220	1^{ns}	56.499	68**	97.730	20^{ns}	229.337	32^{ns}
AL×BL	1	9.827	1^{ns}	0.9203	1^{ns}	283.680	57**	161.700	23^{ns}
Error	51	726.732	50	12.224	15	49.316	10	117.883	17
Total	71	1441.12	100	83.368	100	495.871	100	716.584	100
		S		Na		Mn		Zn	
Source	DF	MSS	%	MSS	%	MSS	%	MSS	%
Rep	17	98.074	11	1.16056	44	1.76607	15	3.03750	19
AL	1	187.686	22^{ns}	0.02323	1^{ns}	1.04425	9^{ns}	2.92296	19^{ns}
BL	1	197.352	23^{ns}	0.29713	11^{ns}	3.94459	32^{ns}	4.40105	28^{ns}
AL×BL	1	295.557	34^{ns}	0.04915	2^{ns}	4.08456	34^{ns}	2.62243	17^{ns}
Error	51	92.922	10	1.09378	42	1.27749	10	2.68895	17
Total	71	871.591	100	2.62385	100	12.11696	100	15.67289	100

^{ns}Not significant at P ≤ 0.05, **Significant at P ≤ 0.05, ***Highly significant at P ≤ 0.01.

TTV (%) = Total treatment variation.

MSS = Mean Sum of Squares.

Table 4.5 Response of magnesium (Mg) to Nemafric-BL phytonematicide applied on tomato plants under field conditions in Experiment 1.

Nemafric-BL	Mg	Relative impact (%)
BL_0	13.767[a]	-
BL_1	11.995[b]	15

Relative Impact (%) = [(treatment/control) − 1] × 100.

Table 4.6 Two-way matrix for phosphorus (P) as affected by first order interaction of Nemarioc-AL and Nemafric-BL phytonematicides under field conditions in Experiment 1.

	Nemafric-BL			
Nemarioc-AL	BL_0	Relative impact (%)	BL_1	Relative impact (%)
AL_0	20.341[ab]	-	18.701[ab]	−8
AL_1	16.532[b]	−19	22.341[a]	12

Relative impact (%) = [(treatment/control) – 1] x 100.

Table 4.7 Responses of mean sum of squares of Calcium (Ca), Magnesium (Mg), Phosphorus (P), Potassium (K), Sulphur (S), Sodium (Na), Manganese (Mn) and Zinc (Zn) to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined on tomato plants under field conditions in Experiment 2 (n = 72).

		Ca		Mg		P		K	
Source	DF	MSS	%	MSS	%	MSS	%	MSS	%
Rep	17	361.296	33	19.720	12	159.170	27	424.20	10
AL	1	117.300	10^{ns}	9.396	6^{ns}	1.145	0^{ns}	90.17	2^{ns}
BL	1	42.473	4^{ns}	9.324	6^{ns}	253.275	43^{ns}	59.09	2^{ns}
AL×BL	1	279.661	26^{ns}	104.753	65**	9.074	2^{ns}	3225.70	78**
Error	51	292.545	27	18.443	11	164.480	28	320.95	8
Total	71	1093.275	100	161.636	100	587.144	100	4120.11	100
		S		Na		Mn		Zn	
Source	DF	MSS	%	MSS	%	MSS	%	MSS	%
Rep	17	52.537	13	8.6872	22	0.14442	8	20.3262	40
AL	1	2.083	1^{ns}	8.0936	20^{ns}	0.21683	12^{ns}	0.2393	1^{ns}
BL	1	2.383	1^{ns}	12.6169	31^{ns}	0.10644	6^{ns}	18.5054	37^{ns}
AL×BL	1	302.006	74**	3.4252	8^{ns}	1.19150	68***	1.0044	2^{ns}
Error	51	46.356	11	7.5365	19	0.10548	6	10.1074	20
Total	71	871.591	100	40.3594	100	1.76467	100	50.1827	100

^{ns}Not significant at P ≤ 0.05; **Significant at P ≤ 0.05, ***Highly significant at P ≤ 0.01.

TTV (%) = Total treatment variation.

MSS = Mean Sum of Squares.

Table 4.8 Two-way matrix for magnesium (Mg) as affected by first order interaction of Nemarioc-AL and Nemafric-BL phytonematicides under field conditions in Experiment 2.

	Nemafric-BL			
Nemarioc-AL	BL_0	Relative impact (%)	BL_1	Relative impact (%)
AL_0	10.008[a]	-	6.876[b]	−31
AL_1	6.873[b]	−31	8.566[ab]	−14

Relative impact (%) = [(treatment/control) – 1] x 100.

Table 4.9 Two-way matrix for potassium (K) as affected by first order interaction of Nemarioc-AL and Nemafric-BL phytonematicides under field conditions in Experiment 2.

	Nemafric-BL			
Nemarioc-AL	BL_0	Relative impact (%)	BL_1	Relative impact (%)
AL_0	53.356[ab]	-	41.781[b]	−22
AL_1	42.207[b]	−21	57.406[a]	8

Relative impact (%) = [(treatment/control) – 1] x 100.

Table 4.10 Two-way matrix for sulphur (S) as affected by first order interaction of Nemarioc-AL and Nemafric-BL phytonematicides under field conditions in Experiment 2.

Nemarioc-AL	Nemafric-BL			
	BL_0	Relative impact (%)	BL_1	Relative impact (%)
AL_0	14.792[b]	-	18.611[ab]	26
AL_1	18.956[a]	28	14.583[b]	−1

Relative impact (%) = [(treatment/control) – 1] x 100.

Table 4.11 Two-way matrix for manganese (Mn) as affected by first order interaction of Nemarioc-AL and Nemafric-BL phytonematicides under field conditions in Experiment 2.

Nemarioc-AL	Nemafric-BL			
	BL_0	Relative impact (%)	BL_1	Relative impact (%)
AL_0	0.2289	-	0.4093	79
AL_1	0.3764	64	0.0422	−82

Relative impact (%) = [(treatment/control) – 1] x 100.

4.4 Discussion

4.4.1 Plant variables

The interaction of Nemarioc-AL and Nemafric-BL phytonematicides were not significant for plant height, stem diameter, fresh fruit and dry shoot mass in both experiments, which did not confirm observations of significant interactions of the two products on

tomato under microplot on tomato (Chapter 3) and on potato (*Solanum tuberosum L.*) plants under field conditions (Seshweni, 2017). Apparently, AL × BL interactions are variable- and plant species-specific (Seshweni, 2017). The cited case contradicted the findings in the current study where AL × BL interaction was not significant on all plant variables.

Non-significant effects of the interaction in the current study contradicted observations in another study (Pelinganga, 2013) where the interaction of concentration and application interval of fermented crude extracts of *C. africanus* was significant on dry root mass under field conditions. Also, the effects of Nemarioc-AL phytonematicide were not significant on all tomato plant variables in both experiments, which contradicted observations under field conditions in other studies where the product had significant effects on dry shoot mass, dry root mass and plant height (Pelinganga, 2013). In another field study, Nemarioc-AL phytonematicide did not affect tomato plant variables, except for improving chlorophyll content (Shadung, 2016). However, it is common that the product could not have any effect on plant growth, since they serve as phytonematicides and at 3% the product had been shown it could hardly suppress plant growth (Mashela *et al*., 2015, 2016; Pelinganga, 2013).

Generally, when plant growth responses are under stimulation and/or inhibition phases, the analysis of variance (ANOVA) on affected variables was significant at the probability level of 5% (Mashela *et al*., 2015). Generally, when ANOVA is not significant on plant variables, it could also be explained as where the two phytonematicides have no effect

since the test organs were saturated by the active ingredients at the time of harvest (Dube, 2016; Pelinganga, 2013). In the current study, the results suggested that under field conditions the interaction of Nemarioc-AL and Nemafric-BL phytonematicides did not have effect on all plant variables, which was similar to Nemarioc-AL phytonematicide.

The increase in stem diameter by Nemafric-BL phytonematicide agreed with the findings in other studies (Mashela *et al*., 2010). Generally, infection by *Meloidogyne* species reduced stem diameter in plants (Mashela *et al*., 2011). Thus, improvement of stem diameter suggested that there were no effects from nematode infection, which were suppressed by the product. Generally, crude extracts of Nemafric-BL phytonematicide had been shown to be phytotoxic to various crops (Pelinganga and Mashela, 2012). In another field study (Pelinganga, 2013), Nemafric-BL phytonematicide had significant effects on plant height only, which was attributed to allelochemical properties of phytonematicides which were on a number of occasions shown to be highly phytotoxic when their uses were not based on empirical data (Mashela *et al.*, 2008; Pelinganga *et al.,* 2011). In the current study, the reduction in dry shoot mass by Nemafric-BL phytonematicide under field conditions suggested that this product had some phytotoxicity during the autumn trial, which was, however, not observed during the spring trial. This was the first report on the effects of Nemafric-BL phytonematicide on cv. 'HTX 14' under field conditions. The observation suggested that some inhibition attributed to phytonematicide application could occur in tomato plants during certain seasons. In other studies where the product improved or had no effect on

the variable, the tomato cv. 'Floradade' was used (Pelinganga, 2013; Shadung, 2016). Apparently, there could be cultivar differences in responses to the empirically-developed mean concentration stimulation point (MCSP) in Nemafric-BL phytonematicide, which had not been detected for Nemarioc-AL phytonematicide.

4.4.2 Essential nutrient elements

In the current study, Nemarioc-AL and Nemafric-BL phytonematicides when combined either increased or decreased the accumulation of certain nutrient elements in leaf tissues of tomato. The AL × BL interaction increased K, which plays a major role in cellular osmoregulation in plant cells (Mashela and Nthangeni, 2002). In another study (Mashela *et al*., 2016a) K was also reduced by nematode infections. Among the factors that strongly influence the quality of tomato, K plays an important role since it is involved in metabolic and transport processes, charge balance and generation of turgor pressure (Dorais *et al*., 2001).

The AL × BL interaction also increased P in leaf tissues, which is an essential nutrient that is in short supply in South Africa soils. According to Dorais *et al*. (2008), increasing P content in leaf tissues could also increase the concentration of chemical compounds such as ascorbic acid, flavonoids and lycopenes in tomato fruits. Pelinganga (2013) observed that concentration × time per 30-day month period interactions of fermented crude extracts of *C. africanus* dried fruit and application frequency was significant on foliar Ca, Mg, Mn, Na, P, S and Zn in leaf tissues of tomato plants except for K which was not significant under field conditions.

In the current study, the AL × BL interaction reduced Mn and Mg in leaf tissues of tomato plants. Manganese is an essential nutrient element and plays a role in the biosynthesis of chlorophyll, aromatic amino acids, secondary products like lignin and flavonoids (Lidon *et al*., 2004). In plants Mg is an essential nutrient element and plays an important role in structural construction of chlorophyll molecules and generally, deficiencies of Mg results in chlorophyll failing to carry out effective and efficient photosynthesis roles (Lidon *et al*., 2004).

In the current study the interaction increased foliar K, which is a non-structural element in plants (Salisbury and Ross, 1992), thereby contradicting other observations under field conditions where cv. 'Floradade' was used (Pelinganga, 2013). As indicated earlier in Nemarioc-AL phytonematicide, K plays an important role in osmoregulation in cells and it also plays a role in synthesis of starch synthase (Mashela and Nthangeni, 2002). A reduction in foliar S was observed in the current study and this could have an undesirable effect on tomato plants since lack of S could result in plants being highly susceptibility to diseases (Dubuis *et al.,* 2005). The increased disease susceptibility is caused by the specific effect of S deficiency on the accumulation of S-containing defense compounds such as phytoalexins, glucosinolates (GSL) and cysteine-rich antifungal polypeptides which can play important roles in disease resistance (Dubuis *et al.,* 2005).

Different responses in leaf nutrient elements in tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides were consistent with previous observations on tomato

plants under microplot conditions (Pelinganga, 2013) and in green beans under microplot and field conditions (Chokoe, 2017). Nemarioc-AL phytonematicide did not have significant effects on all the nutrient elements and the results confirmed observations by Rabothata (2017) on *Cleome gynandra* leaf tissues, where Nemarioc-AL phytonematicide had no significant effects on nutrient elements. The findings in the current study, however, contradicted those from Shadung (2016) study, where Nemafric-AL phytonematicide increased leaf Mg, Na, P and Ca under field conditions. These dynamics could be suggesting that the responses of essential nutrient elements in plants treated with phytonematicide are, in addition to other factors, also season-specific.

Among all the nutrient elements analysed, Nemafric-BL phytonematicide had significant effects on Mg only. Different results were obtained in another study (Shadung, 2016), where Nemafric-BL phytonematicide had significant effects on leaf Mg, Na, P and Ca in leaf tissues of tomato plants. Pelinganga (2013) observed that the concentration of Nemafric-BL phytonematicide increased foliar K. In other observations, Rabothata (2017) observed that Nemafric-BL phytonematicide did not have significant effects on accumulation of certain nutrient elements in leaf tissues of cleome which agreed with the findings of the current study, where Nemafric-BL phytonematicide did not have effects on P, K, Mn, Zn, Na, Ca and S in leaf tissues of tomato plants. In the current study, Nemafric-BL phytonematicide increased Mg, which confirmed previous findings (Shadung, 2016). Magnesium is the most important constituent of the chlorophyll molecule and an enzyme activator for a number of energy transfer reactions (Nzanza,

2006), hence the increase of foliar Mg in the current study was an important observation.

4.5 Conclusion

Nemarioc-AL and Nemafric-BL phytonematicides when combined did not have a significant effect on plant growth variables. In contrast, the two products when combined could either reduce or increase certain essential nutrient elements. Nemarioc-AL phytonematicide did not have significant effects on plant growth variables and essential nutrient elements, whereas Nemafric-BL phytonematicide resulted in reduction of dry shoot mass and increased foliar Mg. Findings in the current study suggested that it was not recommended to apply Nemarioc-AL and Nemafric-BL phytonematicides combined in the production of tomato cv. 'HTX 14'.

CHAPTER 5
SUMMARY, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary

Nemarioc-AL and Nemafric-BL phytonematicides, which are cucurbitacin-containing phytonematicides, are being researched and developed in South Africa for management of the notorious root-knot (*Meloidogyne* species) nematodes (Mashela *et al*., 2015). The study was carried out to determine the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides on growth and accumulation of nutrient elements in leaf tissues of tomato (*Solanum Lycopersicum* L.) cv. 'HTX 14' under microplot and field conditions.

Under microplot conditions, Nemarioc-AL and Nemafric-BL phytonematicides promoted tomato plant growth when applied separately, with stem diameter increased by Nemarioc-AL phytonematicide and plant height increased by Nemafric-BL phytonematicide. Nemarioc-AL and Nemafric-BL phytonematicides also promoted the accumulation of certain foliar nutrient elements in tomato plants. The interaction of Nemarioc-AL and Nemafric-BL phytonematicides in the current study was rather phytotoxic, which was shown by the combined effects on the reduction of dry shoot mass. The AL × BL interaction also had negative effects on accumulation of certain nutrient elements in leaf tissues of tomato plants.

Additionally, under field conditions, when Nemarioc-AL and Nemafric-BL phytonematicides were combined did not have any significant effects on plant growth variables. In contrast, the two products when combined reduced nutrient elements such

as Mg, S and Mn, which are essential in growth and development of tomato plants. Nemarioc-AL phytonematicide did not have any significant effect on plant growth variables and nutrient elements under field conditions; whereas Nemafric-BL phytonematicide reduced and increased dry shoot mass and foliar Mg, respectively, by 28 and 15%, respectively.

5.2 Significance of findings

The findings of the study indicated that Nemarioc-AL and Nemafric-BL phytonematicide when combined negatively or positively affected plant variables and nutrient elements than when applied separately. Evidence from the study suggested that each should be applied separately in tomato production. The two products have different active ingredients, namely, cucurbitacin A and B, respectively, which appeared to counter each other when applied together. The significance of the findings in the current study is that the two products, not at least on tomato cv. ‘HTX’, should be used separately in the management of population densities of *Meloidogyne* species.

5.3 Recommendations

This was the first report under microplot and field conditions where Nemarioc-AL and Nemafric-BL phytonematicides were used on tomato cultivar 'HTX 14'. The observations could suggest cultivar differences in response to application of the two phytonematicides. Further studies should be conducted to investigate the effects of Nemarioc-AL and Nemafric-BL phytonematicides on nematode management on other commonly used tomato cultivars in Limpopo Province.

5.4 Conclusions

The interaction of Nemarioc-AL and Nemafric-BL phytonematicides was not compatible and also showed undesirable effects in tomato plants, regardless of whether trials were conducted under aseptic or septic conditions. Also, for tested cv. 'HTX 14', Nemarioc-AL and Nemafric-BL phytonematicides should be applied separately for management of nematodes. The assessment of responses of various tomato cultivars to the two phytonematicides under field conditions with different climatic zones would enhance the understanding of how these products will behave when used for commercial production purposes.

REFERENCES

BELLO, A. 1998. Biofumigation and Integrated Crop Management. *In*: Bello, A., González, J.A., Arias, M. and R. Rodriguez-Kabana (eds.). Alternatives to Methyl Bromide for the Southern European Countries. International Workshop, Arona: Tenerife, Spain.

BRADY, N.C. and R.R., WEIL 2000. Elements of the Nature and Properties of Soils. Prentice Hall: Upper Saddle River, NJ, USA.

CASTILLO-SÁNCHEZ, L.E., JIMÉNEZ-OSORNIO, J.J. and M.A. DELGADO-HERRERA. 2010. Secondary metabolites of the *Annonaceae*, *Solanaceae* and *Meliaceae* families used as biological control of insects. *Tropical and Subtropical Agroecosystems* 12:445–462.

CHEDEKAL, A.N. 2013. Effect of four leaf extracts on egg hatching and juvenile mortality of root knot nematode *Meloidogyne incognita. International Journal of Advanced Life Science* 6:68–74.

CHEN, J.C., CHIU, M.H., NIE, R.L., CORDELL, G.A. and S.X. QIU. 2005. Cucurbutacins and cucurbitane glycosides: Structures and biological activities. *Nature Product Reports* 22:386–399.

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

CHOKOE, M.F. 2017. Non-phytotoxic concentration of Nemarioc-AL and Nemafric-BL phytonematicides on green bean cultivar ˈtahoeˈ Msc. dissertation. University of Limpopo. Sovenga, South Africa.

DORAIS, M., PAPADOPOULOS, A.P. and A. GOSSELIN. 2001. Greenhouse tomato fruit quality. *Horticultural Review* 26:239–350.

DORAIS, M., EHRET, D.L. and A.P. PAPADOPOULOS. 2008. Tomato (*Solanum lycopersicum*) health components, from the seed to the consumer. *Phytochemistry Reviews* 7:231–250.

DUBE, Z.P. 2016. Density-dependent growth patterns of nematode egg hatch exposed to active ingredients of Nemarioc-AL and Nemafric-BL phytonematicides. PhD thesis submitted to the University of Limpopo. Sovenga, South Africa.

DUBE, Z.P. and P.W. MASHELA. 2016. Nemafric-BL phytonematicide induces egg hatch inhibition in *Meloidogyne incognita. Acta Agriculturae Scandinavica Section B − Soil and Plant Science* 66:384–386.

DUBUIS, P.H., MARAZZI, C., STADLER, E. and F. MANCH. 2005. Sulphur deficiency causes a reduction in antimicrobial potential and leads to diseases susceptibility of oil seed. *Journal of Phytopathology* 153(1):27–36.

ISMAN, M.B. and R. SEFFRIN. 2014. Natural insecticides from the *Annonaceae*: A unique example for developing biopesticides. *Advances in Plant Biopesticides* 401:21–33.

JEFFREY, C. 1978. Cucurbitaceae. *In*: Heywood, V.H. (ed.). Flowering Plants of the World. Oxford University Press: Oxford, UK.

JOSEPH, B. and S. SUJATHA. 2012. Insight of botanical based biopesticides against economically important pest. *International Journal of Pharmacy and Life Sciences* 3(11):2138–2148.

KRISTKOVA, E., LEBEDA, A., VINTER, V. and O. BLAHOUSEK. 2003. Genetic resources of genus *Cucumis* and their morphological description. *Horticultural Science* 30:14–42.

KYAN, T., SHINTANI, M., KANDA, S., SAKURAI, M., OHASHI, H., FUJISAWA, A. and S. PONGDIT. 1999. Kyusei Nature Farming and the Technology of Effective Microorganisms. Asia Pacific Natural Agriculture Network: Bangkok, Thailand. 44.

LEBEA, M.P. 2017. Mean Concentration Stimulation Point of Nemarioc-AL and Nemafric-BL phytonematicides on *Cucurbita pepo* cultivar 'Caserta'. Msc. dissertation. University of Limpopo. Sovenga, South Africa.

LEE, D.H., LWANSKI, G.B. and N.H. THOENNISSEN. 2010. Cucurbitacin: Ancient compound shedding new light on cancer treatment. *Scientific World Journal* 10:413–418.

LEEDY, P.D. and J.E. ORMROD. 2005. Practical Research: Planning and Design. Merrill Prentice Hall: Upper Saddle River, NJ, USA.

LIDON, F.C., BARREIRO, M.G. and J.C. RAMALHO. 2004. Manganese accumulation in rice: Implications for photosynthetic functioning. *Journal of Plant Physiology* 161:1235–1244.

LITTLE, T.M. and F.J. HILLS. 1981. Statistical Methods in Agricultural Research. University of California: Davis, California.

LIU, D.L., JOHNSON, I.R. and J.V. LOVETT. 2003. Mathematical modeling of allelopathy. III. A model for Curve-fitting Allelochemical Dose responses. *Non-Linearity in Biology, Toxicology and Medicine* 1:37–50.

MAFEO, T.P. 2012. Responses of economically important crops to crude extracts of *Cucumis* fruit when used as pre-emergent bio-nematicide. PhD Thesis submitted to the University of Limpopo. Sovenga, South Africa.

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

MAFEO, T.P. and P.W. MASHELA. 2009b. Responses of monocotyledonous crops to crude extracts of *Cucumis myriocarpus* fruit as a pre-emergent bionematicide. *African Crop Science Conference Proceedings* 9:631–634.

MAFEO, T.P. and P.W. MASHELA. 2010. Allelopathic inhibition of seedling emergence in dicotyledonous crops by *Cucumis* bio-nematicide*. African Journal of Biotechnology* 9:8349−8354.

MAFEO, T.P., MASHELA, P.W. and M.S. MPHOSI. 2011. Sensitivity of selected *Allliaceae* seedlings to crude extracts of *Cucumis myriocarpus* fruits. *African Journal of Agriculture Research* 6(1):158–164.

MALUNGANE, M.M.F. 2014. Effect of crude extracts of *Tulbaghia violacea* (wild garlic) on growth of tomato and suppression of *Meloidogyne* species. Msc. dissertation. University of Limpopo. Sovenga, South Africa.

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

MASHELA, P.W. 2007. Undefeatable Enemies: Answering Questions with Questions. University of Limpopo Press: Sovenga, South Africa.

MASHELA, P.W. and M.E. NTHANGENI. 2002. Response of plant hosts and microflora to the citrus nematode-osmolyte allocation in response to *Tylenchulus semipenetrans* infection, stem girdling, and root pruning in citrus. *Journal of Nematology 34*(3):273.

MASHELA, P.W. and M.S. MPHOSI. 2002. Wild cucumber fruit residues reduce population densities of *Meloidogyne incognita* on tomato plants. *African Plant Protection* 8:84.

MASHELA, P.W. and K.M. POFU. 2017. Influence of cucurbitacin-containing phytonematicides on selected nutrient elements in leaf tissues of green bean under greenhouse conditions. *Acta Agriculturae Scandinavica, Section B-Soil and Plant Science* doi.org/10.1080/09064710.2017.1343379.

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

MASHELA, P.W., DUBE, Z.P. and K.M. POFU. 2015. Managing the Phytotoxicity and Inconsistent Nematode Suppression in Soil Amended with Phytonematicides. *In*: Meghvansi, M.K. and A. Varma (eds.). Organic Amendments and Soil Suppressiveness in Plant Disease Management. Springer International Publishing: Heideberg, Switzerland.

MASHELA, P.W., MPHOSI, M.S., SHIMELIS, H. and M.N. MOKGALONG. 2007. Interactions of *Cucumis myriocarpus*, *Lippia javanica* and *Ricinus communis* organic amendments on suppression of *Meloidogyne incognita*. *Journal of Phytopathology* 55:690–693.

MASHELA, P.W., POFU, K.M., ARAYA, H.T. and Z.P. DUBE. 2016a. Response of mineral malnutrition elements in African ginger pseudo-stems to nematode infection. *Acta Agriculturae Scandinavica, Section B-Soil and Plant Science* 66:387–390.

MASHELA, P.W., NDHLALA, A.R., POFU, K.M. and Z.P. DUBE. 2016b. Transgenesis and Secondary Metabolism. *In*: Sumita, J. (eds.). Phytochemicals of Nematode Resistant Transgenic Plants. Springer Publishers: Verlag, Germany.

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

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

MASHITOA, M.F. 2017. Development of non-phytotoxic concentration of Nemarioc-AL and Nemafric-BL phytonematicides on *Beta vulgaris* cultivar 'detroit dark red'. Msc. dissertation. University of Limpopo. Sovenga, South Africa.

MATHABATHA, R.V., MASHELA, P.W. and N.M. MOKGALONG. 2016. Sensitivity of Nemarioc-AL and Nemafric-BL phytonematicides to *Citrus volkameriana* seedling rootstock. *Transylvanian Review* 24:969–972.

MOFOKENG, M.A., MASHELA, P.W. and N.M. MOKGALONG. 2004. Host-status of wild cucumber to the root-knot nematode. *Proceedings of the Nematological Society of Southern Africa Symposium* 17:17.

NZANZA, B. 2006. Yield and quality of tomato as influenced by differential Ca, Mg and K nutrition. Msc. Dissertation. University of Pretoria. South Africa.

NZANZA, B., MARAIS, D. and P. SOUNDY. 2011. Tomato (*Solanum lycopersicum* L.) seedling growth and development as influenced by *Trichoderma harzianum* and arbuscular mycorrhizal fungi. *African Journal of Microbiology Research* 5:425–431.

OKWUTE, S.K. 2012. Plants as Potential Sources of Pesticidal Agents: A Review. *In*: Soundararajan, R.P. (ed.). Pesticides: Advances in Chemical and Botanical Pesticides in Technology. Intech: Rijeka.

PELINGANGA, O.M. 2013. Developing phytonematicides using indigenous *Cucumis africanus* and *Cucumis myriocarpus* fruits for tomato production systems. PhD Thesis submitted to the University of Limpopo. Sovenga, South Africa.

PELINGANGA, O.M and P.W. MASHELA. 2012. Mean dosage stimulation range of allelochemicals from crude extracts of *Cucumis africanus* fruit for improving growth of tomato plant and suppressing *Meloidogyne incognita* numbers. *Journal of Agricultural Sciences* 4(12):8–11.

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

PELINGANGA, O.M., MASHELA, P.W., NZANZA, B. and M.S. MPHOSI. 2012. Baseline information on using fermented crude extracts from *Cucumis africanus* fruit for suppression of *Meloidogyne incognita* and improving growth of tomato plant. *African Journal of Biotechnology* 11:11407–11413.

PELINGANGA, O.M., MASHELA, P.W., MPHOSI, M.S. and B. NZANZA. 2013a. Optimising application frequency of diluted (3%) fermented *Cucumis africanus* fruit in tomato production and nematode management. *Acta Agriculturae Scandinavica, Section B – Soil and Plant Science* 63: 278–282.

PELINGANGA, O.M., MASHELA, P.W., MPHOSI, M.S., MAFEO, T.P. and Z.P. DUBE. 2013b. Using density-dependent growth patterns of tomato plants to establish application intervals for 3% Nemarioc-A phytonematicide. *African Crop Science Conference Proceedings* 11:343–347.

POLLAN, M. 2006. The Omnivore's Dilemma: A Natural History of Four Meals. Penguin Press: New York.

RABOTHATA, M.R. 2017. Interaction of vesicular arbuscular mycorrhiza, nematode and phytonematicides on growth and nutritional content of *Cleome gynandra.* Msc. dissertation. University of Limpopo. Sovenga, South Africa.

SAINJU, U.M., DRIES, R. and B. SINGH. 2003. Mineral nutrition of tomato. *Journal of Food, Agriculture and Environment* 1:176–183.

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

SALISBURY, F.B. and C.W. ROSS. 2005. Plant Physiology. Wiley: New York.

SAS Institute Inc. 2008. SAS/STAT® 9.2 User's Guide. SAS Institute Inc: Cary, North Carolina.

SESHWENI, M.D. 2017. Integrated system for the management of population densities of *Meloidogyne javanica* in potato production. Msc. dissertation. University of Limpopo. Sovenga, South Africa.

SHADUNG, K.G. 2016. Quality protocols for Nemarioc-AL and Nemafric-BL phytonematicides and potential chemical residues in tomato fruits. PhD thesis submitted to the University of Limpopo. Sovenga, South Africa.

SHADUNG, K.G., MASHELA P.W., and M.S. MPHOSI. 2016. Responses of cucurbitacin B concentration in Nemafric-BL phytonematicide to increasing storage period. *Journal of Stored Products and Post-harvest Research* 7(3):32–36.

SITHOLE, N.T. 2016. Mean concentration stimulation point and overall sensitivity of Nemarioc-AL and Nemafric-BL phytonematicides on *Pelargonium sidoides*: An indigenous future cultigen. Msc. dissertation. University of Limpopo. Sovenga, South Africa.

SITHOLE, N.T., POFU, K.M., MASHELA, P.W., DUBE, Z.P. and H. ARAYA. 2016. Overall sensitivity of *Pelargoniun sidoides* and root-knot nematodes to Nemarioc-AL phytonematicide. *Transylvanian Review* 24:2996–3001.

TAYLOR, M.D. and S.J. LOCASCIO. 2004. Blossom-end rot: A calcium deficiency. *Journal of Plant Nutrition*. 27:123–139.

TSEKE, P.E. 2013. Responses of tomato plant growth and root-knot nematodes to phytonematicides from fermented fresh fruits of two indigenous *Cucumis* species. Msc. dissertation. University of Limpopo. Sovenga, South Africa.

Van WYK, B.E. and M. WINK. 2012. Medicinal Plants of the World: An Illustrated Scientific Guide to Important Medicinal Plants and Their Uses. Briza Publications: Pretoria, South Africa.

WUYTS, N., SWENNEN, R. and D. DE WAELE. 2006. Effects of plant phenilpropanoid pathway products and selected terpenoids and alkaloids on the behavior of the plant-parasitic nematodes *Radopholus similis, Pratylenchus penetrans* and *Meloidogyne incognita*. *Nematology* 1:89–101.

APPENDICES

Appendix 3.1 Analysis of variance plant height of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	14	1872.75	133.768		
AL	1	1.44	1.441	0.01	0.9070
BL	1	38.24	38.240	0.37	0.5482
AL × BL	1	0.82	0.840	0.01	0.9289
Error	42	4382.78	104.352		
Total	59	6296.03			

Appendix 3.2 Analysis of variance stem diameter of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	14	43.7233	3.1231		
AL	1	13.7282	13.7282	4.56	0.0386
BL	1	3.03750	3.0375	1.01	0.3210
AL × BL	1	0.1667	0.0002	0.00	0.9941
Error	42	126.497	3.0118		
Total	59	186.986			

Appendix 3.3 Analysis of variance fresh fruit mass of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	14	600658	42094.2		
AL	1	6862	6861.7	0.09	0.7689
BL	1	8171	8171.3	0.10	0.7485
AL × BL	1	5156	5155.6	0.07	0.9941
Error	42	3294268	78435.0		
Total	59	3915115			

Appendix 3.4 Analysis of variance dry shoot mass of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	14	3207.7	229.120		
AL	1	30.8	30.831	0.11	0.7421
BL	1	377.5	377.454	1.34	0.2530
AL × BL	1	4.4	4.444	0.02	0.9005
Error	42	11802.8	281.018		
Total	59	15423.2			

Appendix 3.5 Analysis of variance plant height of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	14	1231.34	87.953		
AL	1	285.14	285.144	2.66	0.1105
BL	1	624.68	624.683	5.82	0.0202
AL × BL	1	1.87	1.873	0.02	0.8955
Error	42	4504.65	107.253		
Total	59	6647.69			

Appendix 3.6 Analysis of variance stem diameter of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	14	69.092	4.93517		
AL	1	5.766	5.76600	1.53	0.2223
BL	1	0.150	0.15000	0.04	0.8426
AL × BL	1	0.294	0.29400	0.08	0.7810
Error	42	157.785	3.75679		
Total	59	233.087	14.90196		

Appendix 3.7 Analysis of variance fresh fruit mass of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	14	1669606	119258		
AL	1	3680	3680	0.06	0.8055
BL	1	215593	215593	3.060	0.0647
AL × BL	1	439	439	0.01	0.9322
Error	42	2516471	59916		
Total	59	4405788			

Appendix 3.8 Analysis of variance dry shoot mass of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	14	2107.67	150.55		
AL	1	62.59	62.59	0.42	0.5151
BL	1	60.32	60.32	0.42	0.5228
AL × BL	1	1061.26	1061.26	7.31	0.0099
Error	42	6101.03	145.26		
Total	59	9392.86			

Appendix 3.9 Analysis of variance for calcium in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	14	16798.0	1199.86		
AL	1	34.2	34.20	0.02	0.8779
BL	1	85.7	85.68	0.06	0.8079
AL × BL	1	5744.8	5744.77	4.01	0.0516
Error	42	60094.9	1430.83		
Total	59	82757.6			

Appendix 3.10 Analysis of variance for potassium in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	14	1332.18	95.156		
AL	1	85.44	85.443	0.59	0.4462
BL	1	36.50	36.504	0.25	0.6178
AL × BL	1	272.21	272.214	1.88	0.1772
Error	42	6068.47	144.487		
Total	59	7794.81			

Appendix 3.11 Analysis of variance for magnesium in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	14	34869.6	2490.68		
AL	1	994.7	994.71	0.93	0.3408
BL	1	1505.0	1505.00	1.40	0.2426
AL × BL	1	30.4	30.39	0.03	0.8671
Error	42	44996.1	1071.34		
Total	59	82395			

Appendix 3.12 Analysis of variance manganese of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	14	518.77	37.0549		
AL	1	42.39	42.3915	1.53	0.2235
BL	1	27.38	27.3821	0.99	0.3264
AL × BL	1	1.07	1.0701	0.04	0.8453
Error	42	1166.18	27.7663		
Total	59	1755.80			

Appendix 3.13 Analysis of variance for sodium in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	14	31.289	2.2349		
AL	1	1.609	1.6085	0.48	0.4914
BL	1	0.018	0.0179	0.01	0.9420
AL × BL	1	23.019	23.0194	6.90	0.0120
Error	42	140.182	3.3377		
Total	59	196.117			

Appendix 3.14 Analysis of variance for phosphorus in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	14	151.406	10.8147		
AL	1	67.700	67.7004	4.63	0.0372
BL	1	71.317	71.3165	4.88	0.0327
AL × BL	1	50.942	50.9424	3.48	0.0690
Error	42	614.197	14.6237		
Total	59	955.562			

Appendix 3.15 Analysis of variance for zinc in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	14	0.40107	0.02865		
AL	1	0.03202	0.03202	0.83	0.3668s
BL	1	2.82100	2.82100	73.33	0.0000
AL × BL	1	0.19471	0.19471	5.06	0.0298
Error	42	1.61565	0.03847		
Total	59	5.06445			

Appendix 3.16 Analysis of variance for sulphur in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	14	1725.4	123.24		
AL	1	1890.6	1890.57	14.66	0.0004
BL	1	275.2	257.20	2.13	0.1516
AL × BL	1	838.5	838.51	6.50	0.0145
Error	42	5417.8	129.00		
Total	59	10147.4			

Appendix 3.17 Analysis of variance for calcium in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	14	26388	1884.9		
AL	1	12255	12255.1	4.810	0.0338
BL	1	1708	1707.7	0.67	0.4174
AL × BL	1	138	137.7	0.05	0.8172
Error	42	106915	2545.6		
Total	59	147403			

Appendix 3.18 Analysis of variance for potassium in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	14	8878.9	634.21		
AL	1	1403.6	1403.60	0.45	0.0721
BL	1	153.0	152.96	0.37	0.5458
AL × BL	1	2.4	2.40	0.01	0.9396
Error	42	17320.9	412.40		
Total	59	27758.8			

Appendix 3.19 Analysis of variance for magnesium in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	14	227.72	16.2654		
AL	1	9.78	9.7768	0.45	0.5069
BL	1	3.73	3.7300	0.17	0.6814
AL × BL	1	12.08	12.0781	0.55	0.4610
Error	42	916.49	21.8212		
Total	59	1169.79			

Appendix 3.20 Analysis of variance for manganese in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	14	0.79200	0.05657		
AL	1	0.03313	0.03313	0.36	0.5499
BL	1	0.04098	0.04098	0.45	0.5063
AL × BL	1	0.07197	0.07197	0.79	0.3794
Error	42	3.82999	0.09119		
Total	59	4.76807			

Appendix 3.21 Analysis of variance for sodium in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	14	6.1120	0.43657		
AL	1	1.6368	1.63680	3.88	0.0555
BL	1	0.6636	0.66360	1.57	0.2168
AL × BL	1	0.0728	0.07280	0.17	0.6800
Error	42	17.7236	0.42199		
Total	59	26.2088			

Appendix 3.22 Analysis of variance for phosphorus in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	14	418.52	29.895		
AL	1	424.22	424.217	14.76	0.0004
BL	1	931.20	831.197	28.92	0.0000
AL × BL	1	254.95	254.946	8.87	0.0048
Error	42	1207.00	28.738		
Total	59	3135.88			

Appendix 3.23 Analysis of variance for zinc in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	14	1.56868	0.11205		
AL	1	0.02200	0.02200	0.12	0.7288
BL	1	0.08148	0.08148	0.45	0.5054
AL × BL	1	0.29949	0.29949	1.66	0.2048
Error	42	7.58348	0.18056		
Total	59	9.55512			

Appendix 3.24 Analysis of variance for sulphur in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	14	1573.10	112.36		
AL	1	1560.42	1560.42	8.04	0.0017
BL	1	1264.26	1264.26	6.51	0.0146
AL × BL	1	47.59	47.59	0.25	0.6232
Error	42	7765.02	194.13		
Total	59	12210.37			

Appendix 4.1 Analysis of variance plant height of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	17	2554.6	150.272		
AL	1	124.3	124.294	0.81	0.3712
BL	1	6.0	6.009	0.04	0.8436
AL × BL	1	14.0	14.045	0.09	0.7629
Error	51	7788.8	152.722		
Total	71	10487.8			

Appendix 4.2 Analysis of variance stem diameter of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	17	28.384	1.66965		
AL	1	1.901	1.90125	0.51	0.4795
BL	1	0.823	0.82347	0.22	0.6412
AL × BL	1	0.073	0.07347	0.02	0.8892
Error	51	191.094	3.74695		
Total	71	222.277			

Appendix 4.3 Analysis of variance fresh fruit mass of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under microplot conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	17	1261919	74231		
AL	1	75784	75784	0.81	0.3715
BL	1	88656	88656	0.95	0.3341
AL × BL	1	192603	192603	2.07	0.1567
Error	51	4754597	93227		
Total	71	6373558			

Appendix 4.4 Analysis of variance dry shoot mass of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field in conditions Experiment 1.

Source	DF	SS	MS	F	P
Rep	17	8189.0	481.71		
AL	1	718.9	718.91	1.16	0.2862
BL	1	4707.0	4506.98	7.28	0.0094
AL × BL	1	1191.1	1191.12	1.92	0.1714
Error	51	31562.8	618.88		
Total	71	46168.9			

Appendix 4.5 Analysis of variance plant height of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	17	4506.0	265.056		
AL	1	75.0	75.031	0.55	0.4623
BL	1	26.8	26.767	0.20	0.6601
AL × BL	1	210.5	210.467	1.54	0.2205
Error	51	6976.2	136.788		
Total	71	11794.4			

Appendix 4.6 Analysis of variance stem diameter of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	17	110.626	6.5074		
AL	1	0.087	0.0868	0.03	0.8697
BL	1	20.587	20.5868	6.45	0.0142
AL × BL	1	0.281	0.2813	0.09	0.7678
Error	51	162.833	3.1928		
Total	71	294.413			

Appendix 4.7 Analysis of variance fresh fruit mass of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	17	2513.0	1478.0		
AL	1	15506.0	1506.0	0.97	0.3282
BL	1	1792.0	1792.0	1.16	0.2866
AL × BL	1	1216.0	1216.0	0.79	0.3793
Error	51	7881.0	1545.0		
Total	71	1085.0			

Appendix 4.8 Analysis of variance dry shoot mass of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	17	22187.5	1305.15		
AL	1	919.2	919.20	1.72	0.1957
BL	1	356.1	356.09	0.67	0.4183
AL × BL	1	1010.7	1010.70	1.89	0.1753
Error	51	27278.0	534.86		
Total	71	51751.5			

Appendix 4.9 Analysis of variance for calcium in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	17	10487.3	616.899		
AL	1	68.4	68.445	0.09	0.7602
BL	1	19.2	19.220	0.03	0.8715
AL × BL	1	9.8	9.827	0.01	0.9079
Error	51	37063.3	726.732		
Total	71	47.648.1			

Appendix 4.10 Analysis of variance for potassium in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	17	2067.89	121.641		
AL	1	86.02	86.023	0.73	0.3970
BL	1	229.34	229.337	1.95	0.1691
AL × BL	1	161.70	161.700	1.37	0.2470
Error	51	6012.02	117.883		
Total	71	8556.97			

Appendix 4.11 Analysis of variance for magnesium in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	17	211.846	12.4616		
AL	1	1.264	1.2641	0.10	0.7491
BL	1	56.489	56.4984	4.62	0.0363
AL × BL	1	0.920	0.9203	0.08	0.7849
Error	51	623.406	12.2237		
Total	71	893.936			

Appendix 4.12 Analysis of variance for manganese in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	17	30.023	1.76607		
AL	1	1.044	1.04425	0.82	0.3702
BL	1	3.945	3.94459	3.09	0.0849
AL × BL	1	4.085	4.08456	3.20	0.0797
Error	51	65.152	1.27749		
Total	71	104.248			

Appendix 4.13 Analysis of variance for sodium in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	17	19.7295	1.16056		
AL	1	0.0232	0.02323	0.02	0.8847
BL	1	0.2971	0.29713	0.27	0.6045
AL × BL	1	0.0491	0.04915	0.04	0.8330
Error	51	55.7828	1.09378		
Total	71	75.8818			

Appendix 4.14 Analysis of variance for zinc in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	17	51.637	3.03750		
AL	1	2.923	2.92296	1.09	0.3020
BL	1	4.401	4.40105	1.64	0.2066
AL × BL	1	2.622	2.62243	0.98	0.3280
Error	51	137.137	2.68895		
Total	71	198.721			

Appendix 4.15 Analysis of variance for phosphorus in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	17	1099.48	64.675		
AL	1	0.47	0.470	0.01	0.9226
BL	1	97.73	97.730	1.98	0.1653
AL × BL	1	283.68	283.680	5.75	0.0202
Error	51	2515.11	49.316		
Total	71	3996.47			

Appendix 4.16 Analysis of variance for sulphur in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 1.

Source	DF	SS	MS	F	P
Rep	17	1667.26	98.074		
AL	1	187.69	187.686	2.02	0.1613
BL	1	197.35	197.352	2.12	0.1512
AL × BL	1	295.56	295.557	3.18	0.0805
Error	51	4739.04	92.922		
Total	71	7086.90			

Appendix 4.17 Analysis of variance for calcium in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	17	6142.0	361.296		
AL	1	117.3	117.300	0.40	0.5294
BL	1	42.5	42.473	0.15	0.7048
AL × BL	1	279.7	279.661	0.96	0.3328
Error	51	14919.8	292.545		
Total	71	21501.3			

Appendix 4.18 Analysis of variance for potassium in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	17	7211.4	424.20		
AL	1	90.2	90.17	0.28	0.5984
BL	1	59.1	59.09	0.18	0.6697
AL × BL	1	3225.7	3225.70	10.05	0.0026
Error	51	16368.5	320.95		
Total	71	26954.8			

Appendix 4.19 Analysis of variance for magnesium in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	17	335.24	19.720		
AL	1	9.40	9.396	0.51	0.4786
BL	1	9.32	9.324	0.51	0.4803
AL × BL	1	104.75	104.753	5.68	0.0209
Error	51	940.60	18.443		
Total	71	1399.32			

Appendix 4.20 Analysis of variance for manganese in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	17	2.45513	0.14442		
AL	1	0.21683	0.21683	2.06	0.1577
BL	1	0.10644	0.10644	1.01	0.3199
AL × BL	1	1.19150	1.19150	11.30	0.0015
Error	51	5.37957	0.10548		
Total	71	9.34948			

Appendix 4.21 Analysis of variance for sodium in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	17	147.683	8.6872		
AL	1	8.094	8.0936	1.07	0.3049
BL	1	12.617	12.6169	1.67	0.2015
AL × BL	1	3.425	3.4252	0.45	0.5033
Error	51	384.359	7.5365		
Total	71	556.178			

Appendix 4.22 Analysis of variance for phosphorus in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	17	2705.9	159.170		
AL	1	1.1	1.145	0.01	0.9338
BL	1	253.3	253.275	1.54	0.2203
AL × BL	1	9.1	9.074	0.06	0.8152
Error	51	8388.5	164.480		
Total	71	11357.9			

Appendix 4.23 Analysis of variance for sulphur in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	17	893.13	52.537		
AL	1	0.08	0.083	0.00	0.9665
BL	1	1.38	1.383	0.03	0.8635
AL × BL	1	302.01	302.006	6.51	0.0137
Error	51	2364.14	46.356		
Total	71	3560.73			

Appendix 4.24 Analysis of variance for zinc in leaves of tomato plants to Nemarioc-AL and Nemafric-BL phytonematicides alone and combined under field conditions in Experiment 2.

Source	DF	SS	MS	F	P
Rep	17	345.546	20.3262		
AL	1	0.239	0.2393	0.02	0.8783
BL	1	18.505	18.5054	1.83	0.1822
AL × BL	1	1.004	1.0044	0.10	0.7539
Error	51	495.261	10.1074		
Total	71	860.555			