POTENTIAL CUCURBITACIN CHEMICAL RESIDUES AND NON-PHYTOTOXIC CONCENTRATION OF TWO PHYTONEMATICIDE FORMULATIONS IN NIGHTSHADE

AGREEMENT LEAGO MALEBE

MINI-DISSERTATION SUBMITTED FOR THE DEGREE MASTER OF PLANT PROTECTION, DEPARTMENT OF PLANT PRODUCTION, SOIL SCIENCE AND AGRICULTURAL ENGINEERING, SCHOOL OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES, FACULTY OF SCIENCE AND AGRICULTURE, UNIVERSITY OF LIMPOPO, SOUTH AFRICA

SUPERVISOR : DR K.G. SHADUNG

CO-SUPERVISOR : PROFESSOR P.W. MASHELA

2019

TABLE OF CONTENTS

DECLARATION

I, Agreement Leago Malebe, declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Agriculture in Plant Protection 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.

Candidate: Agreement Leago Malebe	Signature	Date
Supervisor: Dr K.G. Shadung	Signature	Date
Co-Supervisor: Prof. P.W. Mashela	Signature	Date

DEDICATION

To my mother, sister and brother.

ACKNOWLEDGEMENTS

I wholeheartedly give thanks to my God, Jehovah-Jireh-my provider, who provided me with ‘KUW’ (Knowledge, Understanding and wisdom), positive attitude and strength throughout the execution of this master’s programme. Where would I be? What would I be, if it had not been for the Lord who was on my side? Well, I believe I would have not made it this far. I would like to express my sincere and heartiest gratitude to my supervisory team, Dr K.G. Shadung and Professor P.W. Mashela, for their unshakeable support, patience and training in various concepts of scientific research and most importantly, for instilling in me the attitude of working hard. If it was not for you my supervisors, I would not be knowledgeable in terms of scientific writing, may the good Lord increase your “KUW” in the research world so that you will continue training other students after me and finding solutions for Africa. I would also like to thank my lovely mother, my pillar of strength, Mavis Mahlake, my younger sister, Amazement Malebe and my younger brother, Itumeleng Malebe, for their endless support and prayers.

I extend my special gratitude to the University of Limpopo, National Research Foundation (NRF) of South Africa and the Land Bank Chair of Agriculture for funding various aspects of my studies. I consider myself to be blessed and honoured to have worked under the Green Biotechnologies Research Centre of Excellence (GBRCE) at University of Limpopo, where I was introduced to the real meaning of the words “hard work” and the fascinating world of scientific research and writing. Special thanks to Dr Z.P. Dube, my friends and my fellow post-graduate students for their inputs towards the success of my research project. I will forever be grateful for the help I received from the service workers, Ms S.M. Seabela, Ms S.R. Mawasha, Ms M.A. Mawasha,

Mr M.K. Ralefatana, Mr L.T. Letsoalo and Mr E.M. Letsoalo, at the GBRCE, I can't start to imagine how challenging field work would have been for me, had you not assisted, I Thank You!

I would like to express my heartfelt gratitude to the University of Limpopo and the Land Bank Chair of Agriculture−University of Limpopo for sponsoring my conference trips during the Master's programme to (1) Bolivia Lodge, Polokwane, where I presented one abstract at the Faculty Research Day in 2017, (2) Ratanga Junction, Cape Town, where I presented two abstracts at the African Combined Congress in 2018 and (3) Fusion Boutique, Polokwane, where I presented one abstract at the Faculty Research Day in 2018.

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The successful cultivation of nightshade (*Solanum retroflexum*) as a leafy vegetable with the nutritional potential of contributing to food security in marginalised communities of Limpopo Province could be limited by high population densities of root-knot (*Meloidogyne* species) nematodes. However, the use of Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides in suppressing nematodes and not being phytotoxic requires the empirically-developed non-phytotoxic concentration, technically referred to as Mean Concentration Stimulation Point (MCSP). The MCSP, developed using the Curve-fitting Allelochemical Response Data (CARD) computer-based model, is crop-specific, hence it should be developed for every crop. The objective of this study was to investigate the influence of Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides on growth of nightshade, accumulation of essential nutrient elements and cucurbitacin residues in nightshade leaves. Microplots were established by inserting 20-cm-diameter plastic pots into 10-cm-deep holes at 0.6 m intra-row and 0.6 m inter-row spacing. Each pot was filled with 10 000 cm^3 steam-pasteurised river sand and Hygromix at 3:1. After establishment, Nemarioc-AL and Nemafric-BL phytonematicides were applied at 7-day interval, whereas, Nemarioc-AG and Nemafric-BG phytonematicides were only applied at planting. Two separate experiments for Nemarioc-AL and Nemafric-BL phytonematicides were conducted in summer (November-January) 2017/2018 under microplot conditions with each comprising treatments namely; 0, 2, 4, 8, 16, 32 and 64%, similarly, two separate experiments for the following phytonematicides, Nemarioc-AG and Nemafric-BG comprised treatments namely; 0, 2, 4, 6, 8, 10 and 12 g arranged in a randomised complete block design (RCBD), with 12 replications. The nutrient elements in leaf tissues of nightshade were analysed using the inductively coupled plasma optical emission spectrometry (ICPE-9000) while, cucurbitacin A and B were

each quantified using the isocratic elution Shimadzu HPLC Prominence with Shimadzu CTO-20A diode array detector. Plant growth and nutrient elements variables were subjected to the CARD computer-based model to generate biological indices to generate the curves, quadratic equations and the related biological indices (D_m, R_h, k) (Liu *et al*., 2003). The MCSP values were calculated using the biological indices of plant or nutrient element variables which, along with increasing concentration of Nemarioc-AL, Nemafric-BL, Nemarioc-AG and Nemafric-BG phytonematicides, exhibited positive quadratic relations, with $R^2 \geq 25$. Using cucurbitacin A and B standards, residues of Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides, were not detected in nightshade leaves, respectively. Dry root mass and dry shoot mass of nightshade over increasing concentration of Nemarioc-AL phytonematicide each exhibited a quadratic relationship, with the models explained by 93 and 61%, respectively. Dry root mass, dry shoot mass, plant height, chlorophyll content and stem diameter against increasing concentration of Nemafric-BL phytonematicide each exhibited positive quadratic relationships with the models explained by 95, 72, 65, 78 and 62%, respectively. Plant height, stem diameter and dry root mass against increasing concentration of Nemarioc-AG phytonematicide each exhibited positive quadratic relationships with their models explained by 93, 88 and 91%, respectively. Dry shoot mass and stem diameter against increasing concentration of Nemafric-BG phytonematicide each exhibited positive quadratic relationships with their models explained by 94 and 84%, respectively. Na, Fe and K over increasing concentration of Nemarioc-AL phytonematicide each exhibited positive quadratic relationships with their associations explained by 96, 91 and 95%, respectively. Zn over increasing concentration of Nemafric-BL phytonematicide exhibited positive quadratic relationship with the model explained by 98%. Fe over increasing concentration of Nemarioc-AG phytonematicide exhibited positive quadratic

relationship with the association explained by 91%. Fe, Na, K and Zn over increasing concentration of Nemafric-BG phytonematicide each exhibited positive quadratic relationships with their associations explained by 81, 90, 80 and 89%, respectively, whereas, on the contrary, Zn over increasing concentration of Nemarioc-AG phytonematicide exhibited negative quadratic relationship with the association explained by 96%. Significant ($P \leq 0.05$) plant variables were subjected to CARD, to generate biological indices which were used to compute the MCSP using the relation: $MCSP = D_m + R_h/2$ and the overall sensitivity value ($\sum k$). In Nemarioc-AL phytonematicide trial, MCSP = 3.02% and $\sum k = 1$ for plant variables, whereas, MCSP and $\sum k$ for nutrient elements were 12.09% and 1, respectively. In Nemafric-BL phytonematicide trial, MCSP = 3.08% and $\sum k = 0$ for plant variables, while MCSP = 2484.14% and $\sum k = 0$ for nutrient elements. In Nemarioc-AG phytonematicide trial, MCSP = 3.47 g and $\sum k = 0$ for plant variables, whereas, for nutrient elements MCSP = 8.49 g and $\sum k = 1$. In Nemafric-BG phytonematicide trial, MCSP = 4.70 g and $\sum k = 0$ for plant variables, whereas, MCSP =723.75 g and $\sum k = 1$ for nutrient elements. In conclusion, the application of Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides had the ability to stimulate the growth of nightshade and enhance the accumulation of the selected nutrient elements without leaving cucurbitacin chemical residues in leaf tissues of nightshade.

CHAPTER 1
RESEARCH PROBLEM

1.1 Background

1.1.1 Description of research problem

In commercial agricultural production system, more than 10% of crop yields can be lost because of diseases and pests, with plant parasitic nematodes included (Kleynhans *et al*., 1996; Sikora *et al*., 2005). Nightshade is indigenous to South Africa, drought tolerant and among the top indigenous leafy vegetables in Vhembe District, Limpopo Province, rich in vitamin A, E, folic acid, iron, beta-carotene, ascorbic acid, calcium, iron, proteins and fibre (Van Averbeke and Juma, 2006). This leafy vegetable could contribute to food security and job creation as explained in the Presidential Outcomes reserved for the agricultural sector (RSA, 2012). Nightshade is highly susceptible to *Meloidogyne* species, unfortunately, the withdrawal of synthetic nematicides from agro-chemical markets resulted in limited strategies available to manage nematodes (Mashela *et al.,* 2015). Following the withdrawal of synthetic nematicides, Nemarioc-AL, Nemarioc-AG, Nemafric-BL and Nemafric-BG phytonematicides were researched and developed at the Green Biotechnologies Research Centre of Excellence to serve as substitutes of synthetic nematicides in the context of climate-smart agriculture (Mashela *et al.,* 2015). However, according to Mashela *et al*. (2015), the success of these phytonematicides is dependent on the allelochemicals as active ingredients which are naturally phytotoxic to plants from different plant species during interference (Okwute, 2012). As a result, phytonematicides chance of being recognised as potential alternatives to synthetic nematicides becomes limited because of exhibiting high phytotoxicity levels on crops being protected against nematodes and leaving trace of residues on fruits and

vegetables, which eventually enter the food chain and are consumed by humans (Donkor *et al*., 2016; Okwute, 2012).

1.1.2 Impact of research problem

Phytotoxicity is among the important factors delaying the adoption of the four phytonematicides developed as alternatives for management of nematode population densities (Mashela *et al*., 2015). Phytotoxicity limits the use of phytonematicides because high yield losses may be unintentionally induced. Setia *et al*. (2007) reported that in some cases phytonematicides have killed plants being protected from nematodes, which is uneconomic in terms of profit making for commercial producers. Furthermore, it was noted that phytotoxicity is usually not suspected as the cause of poor crop production because phytotoxicity induced imbalances often look like signs attributed to deficiencies and toxicities of nutrient elements in many crops. Pelinganga (2013) reported that Nemarioc-AL and Nemafric-BL phytonematicides exhibited high phytotoxicity to tomato seedlings at above 10% when applied as post-planting treatments. Similarly, Mafeo and Mashela (2010) observed that Nemarioc-AG phytonematicide showed phytotoxic effects to dicotyledonous and monocotyledonous seedlings, resulting in inhibition of emergence as high as 60% to complete failure. In crop production, estimations of yield losses caused by phytotoxicity of phytonematicides were from 24 to 50% (Mashela *et al*., 2015). Chemical residue traces in edible parts of crops is another factor delaying the adoption of the four phytonematicides. Globally, there are increasing concerns in the public health concerning consumption of chemical residues in agricultural produce (Berrada *et al*., 2010). Chemical residues in food are an important food safety issue both in terms of consumer concern and food trade (O'Keeffe and Farrell, 2000). The trace amounts of

pesticide residues affect human health ranging from short-term (e.g. headaches and nausea) to long-term (e.g. cancer, reproductive harm and endocrine disruption) (Berrada *et al*., 2010). Hence, it is necessary that the non-phytotoxic concentration for every crop be empirically-established and chemical residue levels within edible parts of crops be determined since phytonematicides with phytotoxicity tendencies and high residue levels cannot be registered.

1.1.3 Possible causes of the research problem

Phytotoxicity is one of the limiting factors in the successful adoption of the four phytonematicides for management of nematode population densities. This is attributed to the allelochemicals contained within the phytonematicides, of which the phytonematicides' successfulness is dependent upon. The possible causes of chemical residue traces could be because of sole reliance on synthetic pesticides during production which leads to problems of pesticide residues, environmental and human contamination (Monfankye, 2014). Inappropriate application of pesticides affects the whole ecosystem by leaving residues in food chain and polluting the soil, air and surface water (Abhilash and Singh, 2009). On the other hand, increased use of chlorinated non-degradable pesticides results in accumulation of residues in various living systems for prolonged periods of their span and are responsible for a variety of toxic symptoms (Abhilash and Singh, 2009). Furthermore, many pesticides can persist for long periods in an environment such as organochlorine insecticides which were still noticeable in surface waters 20 years after their use had been banned and once a persistent pesticide has invaded the food chain, it can undergo ‘‘biomagnification’’ i.e., accumulation in the body tissues of organisms, where it may reach concentrations higher than in the surrounding environment (Arias-estévez *et al*., 2008).

1.1.4 Possible solutions of research problem

Generally, many allelochemicals affect bio-systems through density-dependent growth (DDG) patterns. Liu *et al*. (2003) developed the Curve-fitting Allellochemical Response Data (CARD) computer-based model, which helps with the development of non-phytotoxic concentrations of phytonematicides (Mashela *et al*., 2015). The CARD model has three phases, namely, stimulation, neutral and inhibition phases (Liu *et al*., 2003). Biological indices for stimulation phase (D_m and R_h) had been used for generating Mean Concentration Stimulation Point (MCSP) of phytonematicides for various commercial crops (Mashela *et al*., 2015). The MCSP was observed to be useful in generation of non-phytotoxic concentrations for the environmental-friendly phytonematicides because they will reduce nematodes population densities without being phytotoxic to protected plants.

1.1.5 General focus of the study

The study was focused on the potential improvement of nightshade plant growth, possible accumulation of cucurbitacin chemical residues and essential nutrient elements in leaf tissues of nightshade plants when treated with two phytonematicide formulations.

1.2 Problem statement

Most synthetic chemical pesticides were withdrawn from agrochemical markets because of their environment-unfriendliness and chemical residues in produce. This resulted in the research and development of Nemarioc-AL, Nemafric-BL, Nemarioc-AG and Nemafric-BG phytonematicides of which their success is dependent upon allelochemicals as active ingredients which are naturally phytotoxic to plants from

different plant species (Mashela *et al*., 2015). However, Nemarioc-AL and Nemafric-BL phytonematicides have been reported to be highly effective on suppression of nematode population densities at 3% without leaving any residues in the crop, but the claims have only been tested in tomato fruits and not in other crops. The researcher intends to empirically evaluate whether the use of Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides will have an influence on growth of nightshade, accumulation of essential nutrient elements and cucurbitacin residues in nightshade leaves post application.

1.3 Rationale of the study

The successful cultivation of nightshade as a leafy vegetable with the nutritional potential of contributing to food security in marginalised communities of Limpopo Province could be limited by high population densities of root-knot (*Meloidogyne* species) nematodes. However, the use of Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides in suppressing nematodes and not being phytotoxic requires the empirically-developed non-phytotoxic concentration, technically known as MCSP. Additionally, the effect on human health, including deaths, associated with synthetic pesticide poisoning has been documented (Monfankye, 2014). It is the expectation that the reduction in the use of synthetic chemicals on crops will persistently reduce the negative health and environmental impacts, especially in less developed countries where pesticide pollution is on the increase (Wilson and Tisdell, 2001). Phytonematicides represent a significant part of climate-smart agriculture because they are eco-friendly (Mashela *et al*., 2015). Therefore, it is of great importance to develop non-phytotoxic concentrations of Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides that will improve the growth and nutrition status of crops while

suppressing nematode population densities without leaving traces of cucurbitacin residues in the edible parts of crops leading to reduction of health hazards and environmental problems that result from the use of synthetic pesticides.

1.4 Purpose of the study

1.4.1 Aim

Assessment of potential improvement of nightshade plant growth, accumulation of essential nutrient elements and cucurbitacin residues in nightshade leaves post-treatment with phytonematicides at two formulations.

1.4.2 Objective

To investigate the influence of Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides on growth of nightshade, accumulation of essential nutrient elements and cucurbitacin residues in nightshade leaves.

1.4.3 Hypothesis

Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides will influence growth of nightshade, accumulation of essential nutrient elements and cucurbitacin residues in nightshade leaves.

1.5 Reliability, validity and objectivity

The reliability of data was achieved by using the suitable statistical probability level of ($P \leq 0.05$). Validity was ensured through repeating the experiments in time. Objectivity was attained by discussing the findings of the study based on empirical evidence as shown by statistical analyses, with findings compared to observations made in other

studies, to eliminate all forms of subjectivity (Leedy and Ormrod, 2005; Little and Hills, 1981).

1.6 Bias

In this study, bias was reduced by ensuring that the experimental error in each experiment was minimised through replications and randomisation of treatments.

1.7 Scientific significance of the study

Findings of this study will provide empirical information on whether increasing concentrations of Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides would result in improved growth of nightshade plant, accumulation of essential nutrient elements and cucurbitacin residues in nightshade leaves.

1.8 Structure of the mini-dissertation

Following the description and detailed outline of the research problem (Chapter 1), the work done and not yet done on the research problem were reviewed (Chapter 2), then the subsequent chapter (Chapter 3) addressed the single objective of this report. In the final chapter (Chapter 4), 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 worldwide phasing-out of synthetic nematicides from agrochemical markets due to health hazards and human health (Wachira *et al*., 2009), left an observable void in the management strategies of nematodes which resulted in the intensification of research and development of alternative nematicides (Mafeo and Mashela, 2009a, b; Mashela, 2007; Mashela *et al*., 2008). Numerous works is being done to produce environment-friendly nematicides by using botanicals (Mashela, 2002; Mashela and Mphosi, 2002; Sukul *et al*., 2001). Mashela and Mphosi (2002) opted to use crude extracts of wild cucumber (*Cucumis myriocarpus* Naud.) to suppress nematode populations in pot trials, which resulted in 90% suppression of nematodes. However, the successful use of botanicals with nematicidal characteristics for nematode management are restricted by their own degree of phytotoxicity to the protected crops and traces of residues in edible parts of the test crops. This is attributed to active ingredients known as allelochemicals which are contained within botanicals (Mashela *et al*., 2015). This review focuses on trials that have already been done on the research problem along with the findings and contradictions, existing gaps on the research problem and explanation on possible ways to address the existing gaps.

2.2 Work done on the problem statement

2.2.1 Chemical residues in produce from phytonematicides

The presence of pesticide residues in foods has always been a concern, particularly in fruits and vegetables consumed fresh (Nakano *et al*., 2016). Exposure to pesticide residues through the diet is assumed to be up to five times the magnitude of exposure through other routes such as air and drinking water (Donkor *et al*., 2016). Nakano *et*

al. (2016) reported that during analysis of 24 pesticides in 460 samples of Italian Tarocco oranges from 2003 to 2007, the presence of chlorpyrifos-ethyl (23%), chlorpyrifos-methyl (8%), dicofol (0.7%), fenitrothion (0.9%) and malathion (0.4%) among other pesticides were discovered. Similarly, Caboni *et al*. (2002) reported that in olives (*Olea europaea* L.) treated with azadirachtin ($C_{35}H_{44}O_{16}$), residues were detected but the residues declined rapidly from 0.35 ppm in day 1 to less than 0.02 ppm in day 7 after application. In agreement, Simeone *et al*. (2009) reported that pyrethrins were detected in olives but in amounts lower than the maximum residue limit (MRL), whereas, rotenone residues in olives exceeded the maximum residue limit. However, in contrast, when treating strawberry (*Fragaria ananassa* L.) with neem products, azadirachtin chemical residues in berries were not detected at 7 days after application (Caboni *et al*., 2006). Similarly, Shadung (2016) and Dube (2016) reported that in tomato fruit samples treated with Nemarioc-AL phytonematicide, cucurbitacin A residues were not detected in tomato fruit samples and likewise, in tomato plants treated with Nemafric-BL phytonematicide, cucurbitacin B residues were not detected in tomato fruit samples.

2.2.2 Efficacy of phytonematicides on plant growth

The worldwide expulsion of halogenated fumigant nematicide due to their harmful impact, intensified the assessment of botanicals with the potential to suppress the populations of plant parasitic nematodes (Mashela *et al*., 2008). The Land Bank Chair of Agriculture−University of Limpopo introduced, researched and developed the Indigenous Cucurbitaceae Technologies (ICT) for management of plant parasitic nematodes. Moreover, within the ICT, a system called ground leaching technology (GLT) was introduced as one of the post-planting alternative measures in the

management of root-knot nematodes, whereby, crude extracts are applied on a hole of 2 cm depth around the base of the plant (Maile, 2013; Mashela and Nthangeni, 2002). However, Mashela *et al*. (2011) reported that the release and leaching of the active ingredients within the crude extracts to the rhizosphere is dependent on rain or irrigation water. Mashela *et al*. (2011) further highlighted that the success of the GLT system is not determined by biodegradation but is rather dependent on the solubility of the crude extracts in water. The use of the GLT system was successful when crude extracts of *C*. *myriocarpus* were tested on tomato (*Lycopersicon esculentum* L.)*,* castor bean (*Ricinus communis* L.) and fever tea (*Lippia javanica* F. Burm.) (Mashela, 2002; Mashela and Nthangeni, 2002; Mashela *et al.,* 2007; Mashela *et al.*, 2010). Mashela and Nthangeni (2002) referred to stimulation of tomato plant growth attributed to application of crude extracts of *C. myriocarpus* fruits in the GLT system as fertiliser effect. At low concentration crude extracts of neem (*Azadirachta indica*) leaf stimulated growth of maize (*Zea mays* L.) and tomato seedlings, whereas, at high concentration inhibition was observed (Egunjobi and Afolamin, 1976; Rossner and Zebitz, 1986). Malungane (2014) reported that crude extracts of wild garlic (*Tubaghia violacea*) promoted plant growth of tomato. Khosa (2013) also observed growth stimulating effects on tomato plants when treated with cucumber cactus (*Cissus cactiformis* Gilg.), bead-bean (*Maerua angolensis* DC.) and toad tree (*Tabernaemontana elegans* Stapf.). Similarly, when using fermented crude extract of *C. myriocarpus* (Nemarioc-AL phytonematicide) fruits on tested plants through the technology or process called ‘botinemagation’, plant growth was enhanced. Botinemagation refers to the use of phytonematicides through irrigation systems (Mashela *et al*., 2011). The success of botinemagation technology was observed in previously conducted studies whereby Nemarioc-AL phytonematicide stimulated plant growth in green beans (*Phaseolus*

vulgaris L.) (Chokoe, 2017), tomato (Mashela, 2002), tomato (Pelinganga, 2013), potato (*Solanum tuberosum* L.) (Seshweni, 2017), African geranium (*Pelargonium sidoides* DC.) (Sithole *et al*., 2016). Similarly, when fermented crude extract of wild watermelon (*Cucumis africanus* LF.) known as, Nemafric-BL phytonematicide were applied, plant growth stimulation was observed on beetroot (Mashitoa, 2017) and African geranium (Sithole, 2017). Tseke (2013) also observed that when Nemafric-BL phytonematicide was applied on tomato plants, stimulatory effects on all plant variables occurred, whereas, Shadung (2016) reported that Nemarioc-AL and Nemafric-BL phytonematicides had no significant effects on all the measured tomato plant variables, except for chlorophyll content.

2.2.3 Phytotoxicity

The degree of phytotoxicity within phytonematicides is the most significant constraint in the development of any plant-based nematicide (Mashela *et al*., 2015). The challenge of phytotoxicity from plants with nematicidal properties is because of the allelochemicals they possess as active ingredients which are naturally phytotoxic to other plant species during interference interactions (Okwute, 2012). Allelochemicals are known to have the ability to pose different negative effects on plants such as suppression of seedling growth (Bhatt and Todoria, 1990) and germination inhibition (Mafeo *et al*., 2011a, b). Many allelochemicals affect biological systems through density-dependent growth (DDG) patterns (Liu *et al*., 2003), which comprise three phases, stimulation, neutral and inhibition phase (Salisbury and Ross, 1992) depending on the level of concentration and the degree of sensitivity of the test plant organs (Liu *et al*., 2003; Mashela *et al*., 2015). Crude extracts of garlic bulb at 50% concentration were able to manage nematode densities, but were phytotoxic to tomato

seedlings (Sukul *et al*., 1974). Similarly, Nemarioc-AG phytonematicide was reported to have exhibited high phytotoxicity to eight monocotyledonous and ten dicotyledonous crops when applied as drenches at planting with many crops failing to emerge (Mafeo and Mashela, 2010; Mafeo and Mashela, 2009b). *In vitro*, germination trial indicated that at 5 g crude extracts of *C. myriocarpus* fruits strongly inhibited the process of germination in tomato, watermelon (*Citrullus lanatus* Thunb.) and butternut squash (*Cucurbita moschata*) (Mafeo and Mashela, 2009a), including maize (*Zea mays* L.), finger millet (*Eleusine coracana* L.), sorghum (*Sorghum bicolor* L.) and onion (*Allium cepa* L.) (Mafeo and Mashela, 2009b). Pelinganga and Mashela (2012) reported that Nemarioc-AL and Nemafric-BL phytonematicides exhibited phytotoxicity to tomato seedlings when applied at high concentration post transplanting. Moreover, inhibition of growth was observed on tomato plants treated with naboom (*Euphorbia ingens* E. Mey.) and bead-man's tree (*Synadenim cupulare* (Boiss) L.C.) under both microplot and glasshouse trials (Khosa, 2013).

Managing phytotoxicity

Curve-Fitting Allelochemical Response Model: To manage the phytotoxicity challenge posed by phytonematicides, Liu *et al*. (2003) developed the CARD computer-based model. The CARD model is used to quantify three phases (i.e. stimulation, neutral and inhibition) and three zones (i.e. nematicidal, neutral and herbicidal). Mashela (2014) conceptualised the three phases based on plant growth responses to increasing concentration of phytonematicides using mean values of biological indices obtained from the card model. Mashela *et al*. (2015) showed that plant responses can either be stimulated, neutral or inhibited, with the degree of the response relying upon the concentration of the phytonematicides. At stimulation phase

the phytonematicides stimulate the growth of plants while at neutral phase the growth of treated and untreated plants cannot be statistically differentiated leading to the conclusion that the phytonematicides have no effects, but with continuous application of increasing concentration of phytonematicides, the phytonematicides eventually responds by inhibiting plant growth.

Mean Concentration Stimulation Point (MCSP): Mashela *et al*. (2015) adapted the CARD model to develop the concept of MCSP. Two biological indices, the threshold stimulation (D_m) and the saturation point (R_h) were used in the development of MCSP which is considered as the concentration that will not exhibit phytotoxicity on protected plants while consistently suppressing nematode population densities (Mashela, 2014). Using the relation $MCSP = D_m + (R_h/2)$, MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides on tomato were obtained at 2.63 and 2.89%, respectively, (Pelinganga and Mashela, 2012; Pelinganga *et al*., 2013). When the MCSP concept is properly used, it is likely that the concentration of phytonematicides will consistently suppress nematode population densities without imposing phytotoxicity to protected plants. However, MCSP values are plant-specific. For African ginger, MCSP values when treated with Nemarioc-AL and Nemafric-BL phytonematicides were 6.2 and 2.9%, respectively (Sithole *et al*., 2016). Similarly, MCSP for *Citrus volkameriana* seedling rootstocks were 8.6 and 6.3%, respectively (Mathabatha *et al*., 2016). Lebea (2017) also observed that MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides on squash (*Cucurbita pepo*) were 11.9 and 2.8% under microplot and greenhouse conditions, respectively. Pelinganga (2013) indicated that although these MCSP values were high when compared to those obtained for tomato, they were still able to stimulate growth. In these three studies, the lowest MCSP values were

reported to have consistently suppressed nematode population densities and were therefore adopted instead of the highest empirically derived MCSP values.

Application interval: Maile *et al*. (2013) indicated that the response of plants to phytonematicides, in addition to being concentration specific, was also application time interval specific. After empirically-deriving MCSP values, the values are then used to derive the application interval (T_a), where the concept of day-week-month relating to the nematode life cycle was introduced (Mashela *et al*., 2015). Experiment findings demonstrated that Nemarioc-AL and Nemafric-BL phytonematicides should be applied at 17 and 19-day intervals, respectively on tomato plants infected with root-knot nematodes.

Application frequency: Application frequency is an empirically derived unit-less factor. Mashela *et al*. (2015) highlighted that after deriving the application interval, the application frequency (T_f) defined as the proportion of the crop cycle to the application interval T_f = crop cycle (days)/application interval (days) was computed. In order for the derived MCSP values to be non-phytotoxic, they were applied per growing season, which was defined as the application frequency (Pelinganga *et al*., 2012).

Dosage model: Dosage (D) is the product of concentration and the application frequency (T_f) summarised as D (%) = C (%) × T_f. Mashela *et al*. (2015) defined dosage as the amount of the total active ingredient that would have been placed into a given soil by the end of the crop cycle. Shadung (2016) found that at 3% concentration of Nemarioc-AL and Nemafric-BL phytonematicides applied separately at 17 days, there were no significant effects on number of fruits, plant height, stem diameter and dry

shoot mass under field conditions. However, Mafeo *et al*. (2011b) observed that at the dosage of 2 g/plant, crude extracts of ground *C. myriocarpus* fruit suppressed *M. incognita* race 2 and when applied as a pre-plant bio-nematicide the material had either 50 or 100% inhibition effect on growth of chive (*Allium schoenoprasum* L.), leek (*Allium porrum* L.) and onion (*Allium cepa* L.) during the 18-day testing period.

2.2.4 Efficacy of phytonematicides on nutrient elements

In phytonematicides, the centre of interest had always been on the success in management of nematodes and determination of concentration that would not induce phytotoxicity in crops (Mashela *et al*., 2017), with less attention to phytonematicides' interaction with plants. However, Mashela *et al.* (2017) found that the accumulation of nutrient elements also follows the DDG pattern characterised by the three phases namely, stimulation and neutral and inhibition phases. According to Mashela and Pofu (2017) cucurbitacin-containing phytonematicides stimulated the accumulation of nutrient elements in the leaf tissues of green beans. Rabothata (2017) observed that the interaction of VAM and Nemafric-BL phytonematicide increased Zn, whereas, the interaction of VAM and Nemarioc-AL phytonematicide did not have significant effects on foliar nutrient elements. Shadung (2016) reported that Nemarioc-AL phytonematicide promoted the accumulation of most nutrient elements in leaf tissues of tomato plants but decreased Fe. Similarly, Pelinganga (2013) observed that crude extracts of *C. myriocarpus* and *C. africanus* fruits had a significant effect on some foliar nutrient elements of tomato. Khosa (2013) reported that crude extracts of *E*. *ingens* and *S. cupulare* significantly decreased N, P and K in leaf tissues of tomato, whereas, in contrast, an increase in Fe was observed in *E*. *ingens* treated tomato leaf tissues.

Nzanza *et al*. (2011) found that *Trichoderma harzianum* × VAM interaction had significant effects on foliar Mn and Zn of tomato plants

2.3 Work not yet done on the problem statement

The developed phytonematicides, Nemarioc-AL, Nemarioc-AG, Nemafric-BL and Nemafric-BG have been used for management of plant parasitic nematodes in crop production. Studies have proven their effectiveness in managing nematodes in production of a variety of crops but studies on their effect on growth of nightshade plants have not been documented. Similarly, the influence of these phytonematicides on accumulation of essential nutrient elements and cucurbitacin residues in leaves of nightshade plants are not documented.

2.4 Addressing the identified gaps

To address the identified gaps, the current study focused on reviewing the influence of Nemarioc-AL, Nemafric-BL, Nemarioc-AG and Nemafric-BG phytonematicides on growth of nightshade plants, potential accumulation of cucurbitacin residues and nutrient elements in nightshade leaves.

CHAPTER 3
CUCURBITACIN RESIDUES, PLANT GROWTH AND FOLIAR ESSENTIAL NUTRIENT ELEMENTS IN NIGHTSHADE PLANTS TREATED WITH TWO PHYTONEMATICIDE FORMULATIONS

3.1 Introduction

Internationally, phytonematicides are constantly gaining recognition in modern agriculture mainly because of their environment-friendliness (Mashela *et al*., 2015). Considering the harmful effects synthetic chemical pesticides posed to non-target organisms and trace of chemical residues in produce, many were withdrawn from agrochemical markets, thus increasing the need for development of environment-friendly phytonematicides (Krol *et al*., 2000; Maile, 2013; Mashela *et al*., 2015). As a result, Nemarioc-AL (L = Liquid formulation), Nemafric-BL, Nemarioc-AG and Nemafric-BG (G = Granular formulation) phytonematicides serving as alternatives to synthetic nematicides were developed and came with numerous successes, which includes nematode suppression and not leaving chemical residues in tomato (Mashela *et al.,* 2015*;* Shadung, 2016).

However, the most significant challenge that limit the commercialisation of Nemarioc-AL, Nemafric-BL, Nemarioc-AG and Nemafric-BG phytonematicides is that they exhibit phytotoxicity (Tseke *et al.,* 2013), which may affect farmers profit in case of complete inhibition of emergence of tested crops (Mafeo and Mashela, 2010). Additionally, the indiscriminate and sole use of synthetic chemicals has not only led to resistant strains but accumulation of toxic residues in fruits and vegetables, which eventually enters the food chain and are consumed by humans, thereby affecting human health (Berrada *et al*., 2010; Donkor *et al*., 2016). The cause of trace of residues in food was attributed by the slow degradation of synthetic pesticides (Kumar, 2012). However, the problem of

pesticide residues may be solved by using botanicals as alternative pesticides which are biodegradable due to their natural origin and are less harmful to the environment and human health (Dubey *et al*., 2008). However, the phytotoxicity challenge was solved through the development of the Curve-fitting Allelochemical Response Data (CARD) computer-based model (Liu *et al*., 2003). The outcomes of this study will reduce the existing gap of scant documented information on the influence of phytonematicides on nightshade plant growth, the required non-phytotoxic concentration of Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides for nightshade, the status of essential nutrient elements and potential cucurbitacin chemical residues in edible parts of nightshade after treatment with phytonematicides. Therefore, the objective of this study was to investigate the influence of Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides on growth of nightshade, accumulation of essential nutrient elements and cucurbitacin residues in nightshade leaves.

3.2 Materials and methods

3.2.1 Description of the study area

The experiments for Nemarioc-AL, Nemafric-BL, Nemarioc-AG and Nemafric-BG phytonematicides were conducted simultaneously at the Green Bio-technologies Research Centre of Excellence (GBRCE), University of Limpopo, South Africa (23°53'10"S, 29°44'15"E) under microplot conditions (Figure 3.1−3.4). The location has summer rainfall with mean annual rainfall of 500 mm and minimum/maximum temperature averages of 19/38°C. The site was characterised by Hutton soil (65% sand, 30% clay, 5% silt, 1.6% organic C, ECe 0.148 $dS.m^{-1}$ and pH (H_2O) 6.5).

Figure 3.1 Nightshade (*Solanum retroflexum*) plants under microplot conditions treated with Nemarioc-AL phytonematicide.

Figure 3.2 Nightshade (*Solanum retroflexum*) plants under microplot conditions treated with Nemafric-BL phytonematicide.

Figure 3.3 Nightshade (*Solanum retroflexum*) plants under microplot conditions treated with Nemarioc-AG phytonematicide.

Figure 3.4 Nightshade (*Solanum retroflexum*) plants under microplot conditions treated with Nemafric-BG phytonematicide.

3.2.2 Treatments and research design

Separate experiments for Nemarioc-AL and Nemafric-BL phytonematicides were conducted in summer (November-January) 2017/2018 under microplot conditions with each comprising treatments namely: 0, 2, 4, 8, 16, 32 and 64%. Similarly, separate experiments for each of the following phytonematicides, Nemarioc-AG and Nemafric-BG comprised treatments namely: 0, 2, 4, 6, 8, 10 and 12 g, arranged in a randomised complete block design (RCBD), with 12 replications.

3.2.3 Procedures

Liquid formulations preparation: Matured fruits of *C. myriocarpus* and *C. africanus* were collected locally, washed in tap water, cut into small pieces and dried in air-forced oven at 52°C for 72 h (Mashela *et al*., 2017). The materials were ground in a Wiley mill through a 1-mm-mesh sieve and then finely powdered using A43 Monlinex coffee grinder. Ground material were stored at room temperature in hermitically sealed plastic bags for future use. Approximately, 80 and 40 g ground material of *C. myriocarpus and C. africanus*, respectively, were fermented in separate 20 L-hermetically sealed plastic containers with 16 L chlorine-free tap water. Approximately, 300 ml molasses, 100 g brown sugar and 300 ml effective microorganisms (EM) were added into each container (Mashela *et al*., 2017; Pelinganga *et al*., 2012). Allowance for released CO_2 to escape from each container was provided through an airtight 5 mm diameter tube with one end glued to a hole on the lid of the 20 L container, while the outlet end dangled into a 2-litre bottle half-filled with tap water. After a 14-day incubation period, when pH was at least ±3.7 (Mashela *et al*., 2017), the phytonematicides were applied once a week as substitute to irrigation.

Granular formulation preparation: In the ground leaching technology (GLT) systems, mature fruits of *C. myriocarpus* or *C. africanus* were cut into pieces, dried at 52°C (Makkar, 1999) for 72 h and ground as described previously (Mashela, 2002). The powder was packaged in brown bags and weighed according to treatments prior to application. The materials were applied seven days after transplanting nightshade seedlings without first undergoing any microbial degradation (Mashela, 2002).

Cultural practices: Microplots were established by inserting 20-cm-diameter plastic pots into 10-cm-deep holes at 0.6 m intra-row and 0.6 m inter-row spacing. Each pot was filled with 10 000 cm^3 steam-pasteurised river sand and Hygromix at 3:1. The seeds of nightshade were raised under greenhouse conditions in seedling trays for four weeks until they were ready to be transplanted. Uniform four-week-old seedlings of nightshade were hardened for seven days before transplanting. The seedlings were fertilised three days after transplanting with 2.5 g of NPK 2:3:2 (22) per plant to provide 155 mg N, 105 mg P and 130 mg K per ml water. Multifeed (Nulandies, Johannesburg) of approximately 5 g was applied three days after transplanting which provided N, P, K, Mg, Fe, Cu, Zn, B, Mn and Mo per ml water (Mashela, 2002). All the liquid phytonematicides were applied on the plants seven days after transplanting. Thereafter, Nemarioc-AL and Nemafric-BL phytonematicides were applied at 7 days interval, whereas, Nemarioc-AG and Nemafric-BG phytonematicides were applied only once, at transplanting. Irrigation was done every other day with 500 ml tap water.

3.2.4 Data collection

Extraction and quantification of cucurbitacin chemical residues: A representative subsample 4 g of dried crude extracts of nightshade leaves were extracted with 100 ml

methanol and dichloromethane [1:1 (v/v)] solution on a rotary evaporator set at 60 rpm at 40°C for 4 h. After extraction, sub-samples were concentrated by reducing the volume to 30 ml under reduced pressure on a rotary evaporator and then 1 ml aliquots centrifuged at 4500 rpm for 10 minutes before filtering through 0.22 µm pore filter (Miller, Sigma). Concentration of cucurbitacin were quantified using the isocratic elution Shimadzu HPLC Prominence while for detection, Shimadzu CTO-20A diode array detector was used. Quantification was performed in a wide pore reverse phase C18 (25 cm × 4.0 mm, 5 µm) discovery (Sigma-Aldrich) using 2:3 (v/v) methanol and deionised water as a mobile phase at a flow rate of 1.0 ml/min in an oven at 35°C, with wavelengths monitored at 230 nm for 43 minutes.

Plant variables: After eight weeks of transplanting, heights of nightshade plants were measured from the soil surface to the tip of the flag leaf. Stem was cut off at the soil surface and its diameter measured at 5 cm using a digital Vernier calliper. Chlorophyll content was measured using chlorophyll meter. Shoots and roots were oven dried at 52°C for 72 h and weighed.

Essential nutrient elements: Mature leaves were ground using a Wiley mill to pass through a 2 mm sieve for analysis of essential nutrients. A microwave digester (PerkinElmer, Titan MPS) was used for preparation and approximately 0.4 g of ground leaf tissues of nightshade were digested in 75 ml vessel with 5.0 ml of nitric acid (70%) and 3.0 ml of peroxide (30%). The mixture was vortexed for 2 min and samples allowed to cool for at least 10 min prior to closing the vessels, which were then inserted into the microwave digester to run for 46 min under temperature ranging up to 260°C. Thereafter, the vessels were allowed to cool at room temperature for 20 min. Samples

from the vessels were decanted into 50 ml tubes and stored in the cold room to avoid evaporation of samples prior analytical work. Nightshade samples were then analysed for K, Na, Fe and Zn using the inductively coupled plasma optical emission spectrometry (ICPE-9000).

3.2.5 Data analysis

Plant growth and nutrient elements variables were subjected to the CARD computer-based model to generate biological indices for the development of curves, quadratic equations and the related biological indices (D_m, R_h, k) (Liu *et al*., 2003). Before running the means in the CARD model, the concentration with the geometric series 0, 2, 4, 8, 16, 32 and 64% of the phytonematicides were transformed using $\log_2 2^x$ to generate 0, 1, 2, 3, 4, 5 and 6%. This process was done to avoid curve-fitting challenges such as observations being overcrowded at lower concentration and this allowed equidistances between observations. The MCSP values were calculated using the biological indices of plant or nutrient element variables that, along with increasing concentration of Nemarioc-AL, Nemafric-BL, Nemarioc-AG and Nemafric-BG phytonematicides, exhibited positive quadratic relations, with $R^2 \geq 25$.

3.3 Results

The seasonal effects for the plant variables measured were not significant and hence, the data were pooled for Nemarioc-AL (n = 84), Nemafric-BL (n = 84), Nemarioc-AG (n = 84) and Nemafric-BG (n = 84) phytonematicides trials.

3.3.1 Cucurbitacin chemical residues

Using cucurbitacin A and B standards, residues of Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides, were not detected in nightshade leaves, respectively.

3.3.2 Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides on plant variables

Mean Concentration Stimulation Point: Dry root and dry shoot mass of nightshade over increasing concentration of Nemarioc-AL phytonematicide exhibited quadratic relationships, with the associations explained by 93 and 61%, respectively (Figure 3.5). Similarly, dry shoot mass, chlorophyll content, dry root mass, stem diameter and plant height over increasing concentration of Nemafric-BL phytonematicide exhibited quadratic relationships, with the models explained by 72, 78, 95, 62 and 65%, respectively (Figure 3.6). In granular trials, plant height and stem diameter over increasing concentration of Nemarioc-AG phytonematicide each exhibited positive quadratic relationships, with the associations explained by 93 and 88%, respectively (Figure 3.7). Similarly, dry shoot mass, dry root mass and stem diameter over increasing concentration of Nemafric-BG phytonematicide exhibited positive quadratic relationships, with the models explained by 94, 91 and 84%, respectively (Figure 3.8). Using MCSP = $D_m + (R_h/2)$ relation, the MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides were found to be 3.02 and 3.08%, respectively (Table 3.1). The

MCSP values for Nemarioc-AG and Nemafric-BG phytonematicides were found to be 3.47 and 4.70 g, respectively (Table 3.2).

Sensitivity: Dry root and dry shoot mass had sensitivity values of k = 0 and 1, respectively, with the ∑k of nightshade being 1, when treated with Nemarioc-AL phytonematicide (Table 3.1). In contrast, when nightshade was treated with Nemafric-BL phytonematicide, all plant variables had sensitivity values of k = 0, with ∑k of nightshade being 0 (Table 3.1). Plant height and stem diameter had sensitivity values of k = 1 and 0, respectively, with the ∑k of nightshade being 1, when treated with Nemarioc-AG phytonematicide (Table 3.2). In contrast, when nightshade was treated with Nemafric-BG phytonematicide, dry shoot mass, dry root mass and stem diameter had sensitivity values of k = 0, with ∑k of nightshade being 0 (Table 3.2).

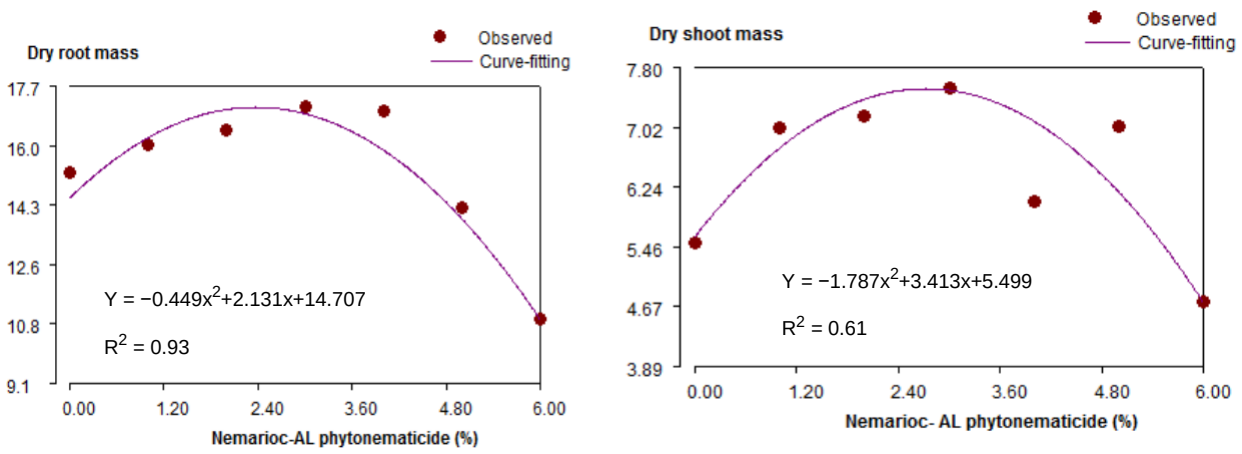

Figure 3.5 Responses of dry root mass and dry shoot mass of nightshade to increasing concentration of Nemarioc-AL phytonematicide.

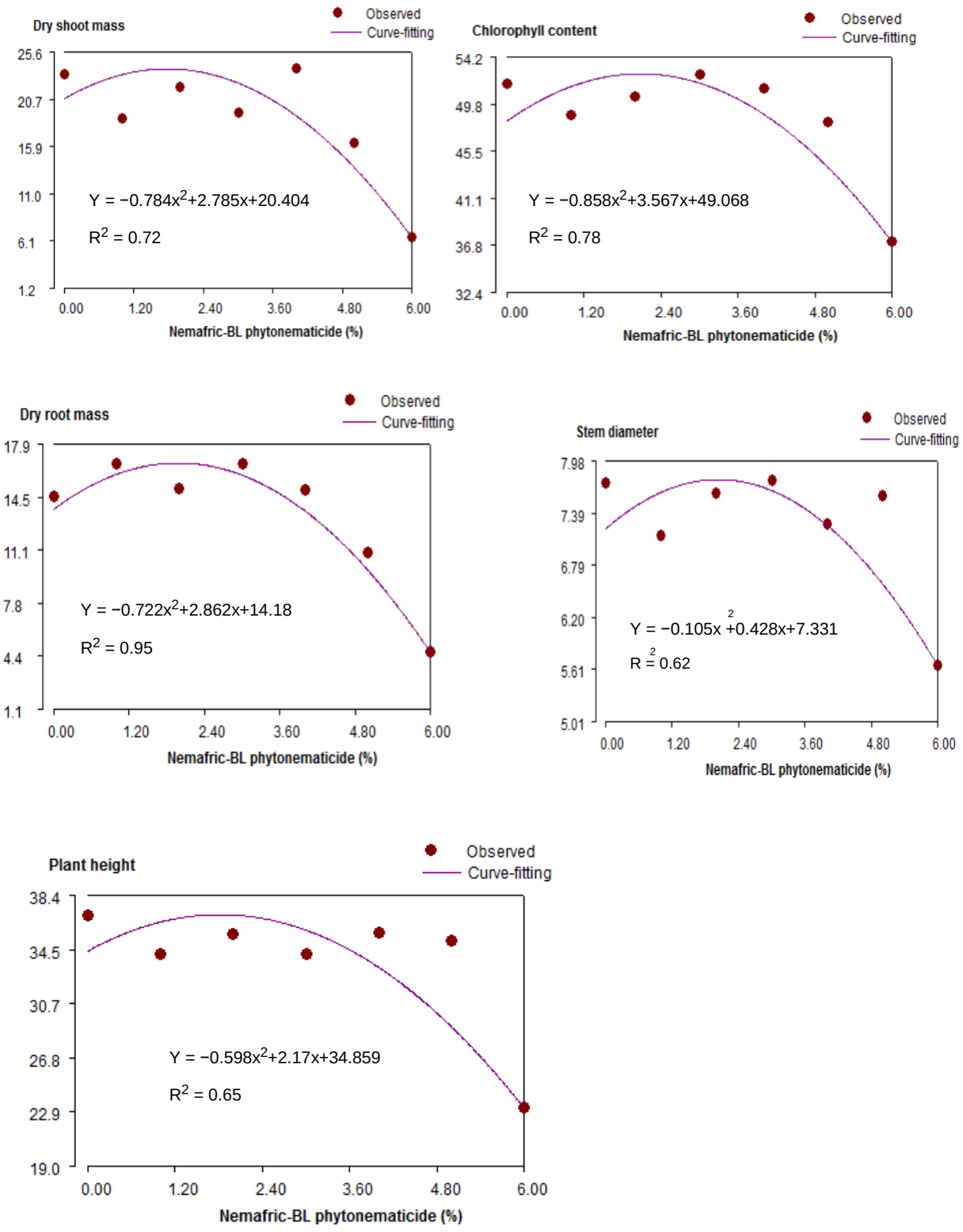

Figure 3.6 Responses of dry root mass, dry shoot mass, chlorophyll content, plant height and stem diameter of nightshade to increasing concentration of Nemafric-BL phytonematicide.

Table 3.1 Biological indices for dry root mass (DRM), dry shoot mass (DSM), plant height (PHT), chlorophyll content (CC) and stem diameter (STD) of nightshade to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides.

	Nemarioc-AL			Nemafric-BL					
Biological index	DRM	DSM	Mean	DRM	DSM	PHT	CC	STD	Mean
Threshold stimulation (D_m)	2.371	1.599	1.985	1.981	1.776	1.816	2.078	2.037	1.938
Saturation point (R_h)	2.526	1.63	2.078	2.835	2.472	1.971	3.706	0.436	2.284
0% inhibition (D_0)	4.742	5.7531	5.248	3.962	3.551	3.632	4.156	4.075	4.844
50% inhibition (D_{50})	7.061	11.433	9.247	5.688	5.795	7.514	7.814	8.287	8.775
100% inhibition (D_{100})	8.6	18.1	13.35	6.8	7.2	9.7	9.9	10.6	8.84
R^2	0.932	0.614		0.947	0.72	0.651	0.779	0.623	
k-value	0	1		0	0	0	0	0	
Overall sensitivity	$\sum k = 1$			$\sum k = 0$					
Nemarioc-AL MCSP = $D_m+(R_h/2)$ = 1.985 + (2.078/2) = 3.02%									
Nemafric-BL MCSP = $D_m+(R_h/2)$ = 1.968 + (2.855/2) = 3.08%									

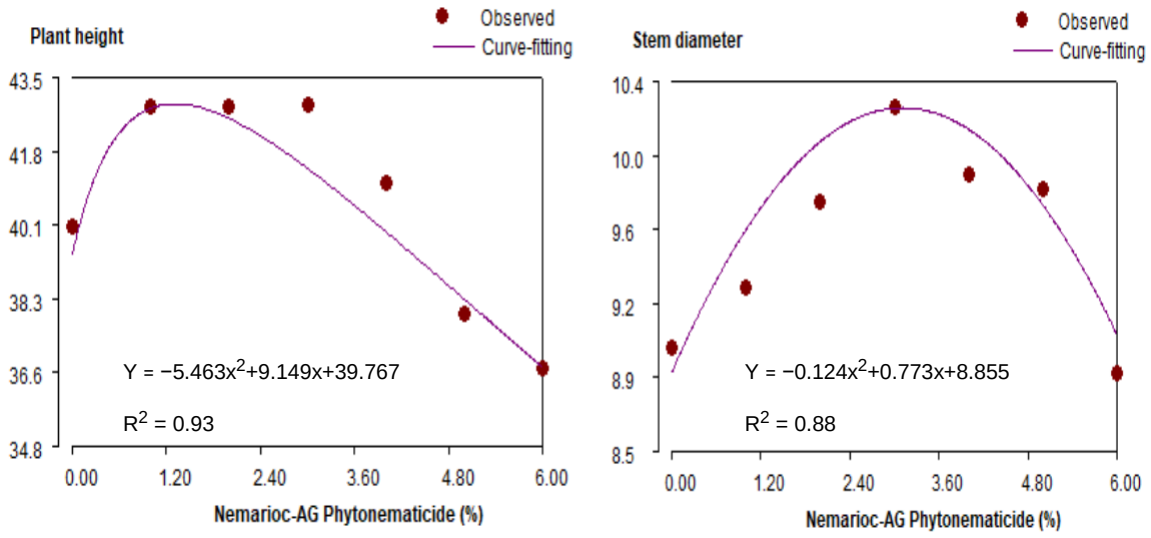

Figure 3.7 Responses of plant height and stem diameter of nightshade to increasing concentration of Nemarioc-AG phytonematicide.

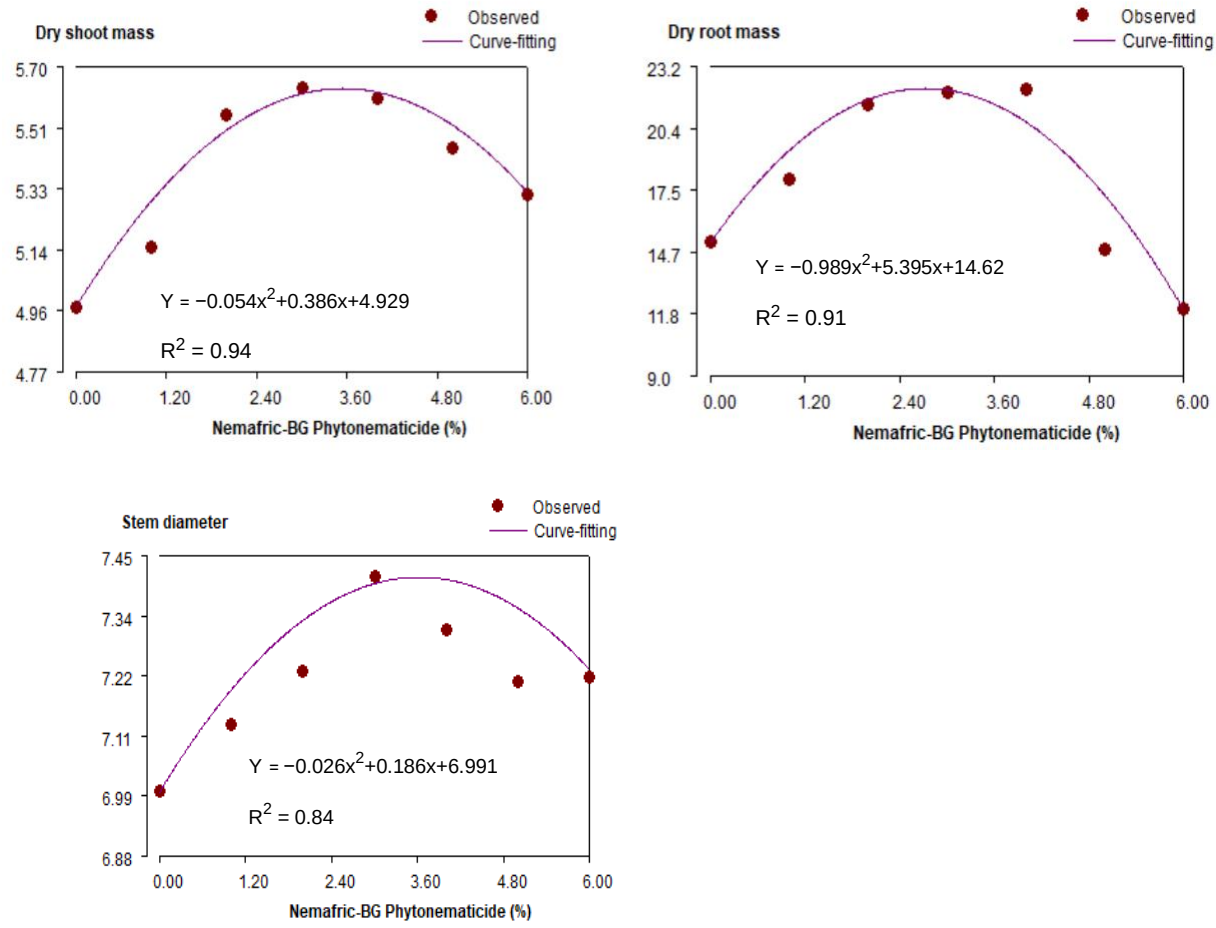

Figure 3.8 Responses of dry shoot mass, dry root mass and stem diameter of nightshade to increasing concentration of Nemafric-BG phytonematicide.

Table 3.2 Biological indices for dry root mass (DRM), dry shoot mass (DSM), plant height (PHT) and stem diameter (STD) of nightshade to increasing concentration of Nemarioc-AG and Nemafric-BG phytonematicides.

	Nemarioc-AG			Nemafric-BG			
Biological index	PHT	STD	Mean	DSM	DRM	STD	Mean
Threshold stimulation (D_m)	1.31	3.11	2.212	3.55	2.73	3.63	3.30
Saturation point (R_h)	3.83	1.20	2.52	0.69	7.36	0.34	2.80
0% inhibition (D_0)	4.34	6.23	5.28	7.10	5.46	7.25	6.60
50% inhibition (D_{50})	17.56	9.85	13.70	11.16	6.58	15.83	11.19
100% inhibition (D_{100})	38	12.10	25.05	13.70	7.40	20.50	13.87
R^2	0.93	0.88		0.94	0.91	0.84	
k-value	1	0		0	0	0	
Overall sensitivity	$\sum k = 1$			$\sum k = 0$			

Nemarioc-AG MCSP = $D_m+(R_h/2)$ = 2.212 + (2.517/2) = 3.47 g

Nemafric-BG MCSP = $D_m+(R_h/2)$ =3.301 + (2.795/2) = 4.70 g

3.3.3 Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides on nutrient elements Mean Concentration Stimulation Point: In liquid phytonematicides trials, Fe and Na over increasing concentration of Nemarioc-AL phytonematicide exhibited positive quadratic relationships, with the associations explained by 91 and 96%, respectively (Figure 3.9). Similarly, K and Zn over increasing concentration of Nemafric-BL phytonematicide exhibited positive quadratic relationships, with the models explained by 95 and 98%, respectively (Figure 3.10). In granular phytonematicides trials, Fe exhibited negative quadratic relationship, whereas, Zn exhibited a positive quadratic relationship over increasing concentration of Nemarioc-AG phytonematicide, with the models explained by 91 and 86%, respectively (Figure 3.11). Similarly, K, Na, Zn and Fe over increasing concentration of Nemafric-BG phytonematicide exhibited positive quadratic relationships, with the models explained by 80, 90, 89 and 81%, respectively (Figure 3.12). Using the relation of MCSP = D_m+ (R_h/2), the MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides were found to be 12.09 and 2484.14%, respectively (Table 3.3). Similarly, using the relation MCSP = D_m+ (R_h/2), the MCSP values for Nemarioc-AG and Nemafric-BG phytonematicides were 8.49 and 723.75 g, respectively (Table 3.4).

Sensitivity: Fe and Na had sensitivity values of k = 1 and 0, respectively, with the ∑k of nutrients in nightshade leaf tissues being 1, when treated with Nemarioc-AL phytonematicide (Table 3.3). In contrast, when nightshade was treated with Nemafric-BL phytonematicide, both K and Zn had sensitivity values of k = 0, with ∑k of nightshade leaf tissues being 0 (Table 3.3). Fe and Zn had sensitivity values of k = 1 and 0, respectively, with the ∑k of nutrients in nightshade leaf tissues being 1, when treated with Nemarioc-AG phytonematicide (Table 3.4). In contrast, when nightshade was treated with Nemafric-BG phytonematicide, Fe had sensitivity value of k = 1, whereas, Na, K and Zn had sensitivity values of k = 0, with ∑k of nightshade leaf tissues being 1 (Table 3.4).

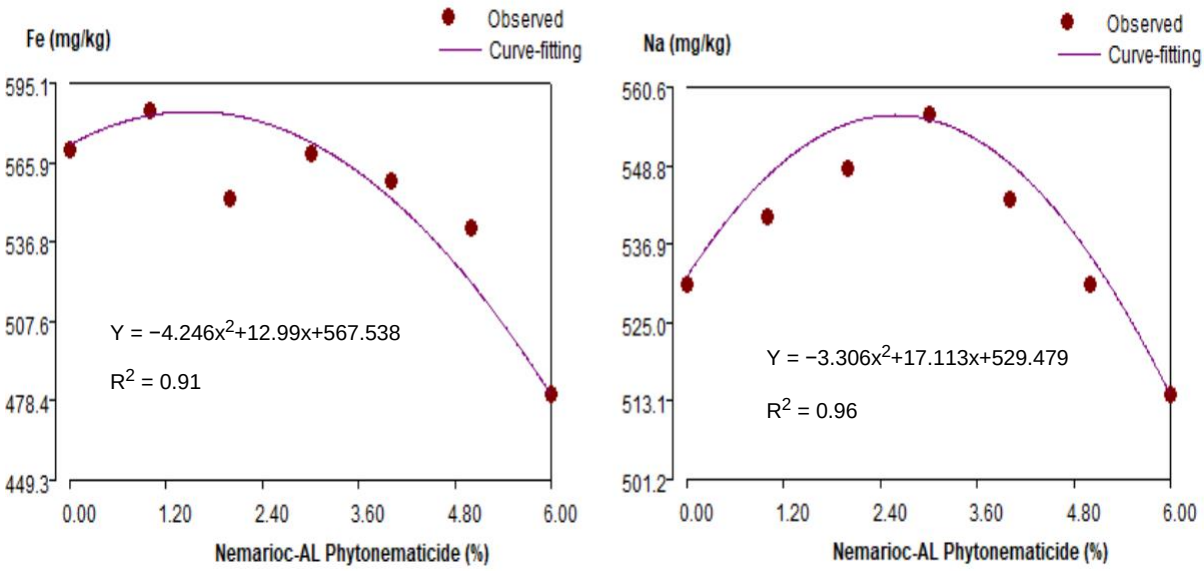

Figure 3.9 Responses of iron (Fe) and sodium (Na) in leaf tissues of nightshade to increasing concentration of Nemarioc-AL phytonematicide.

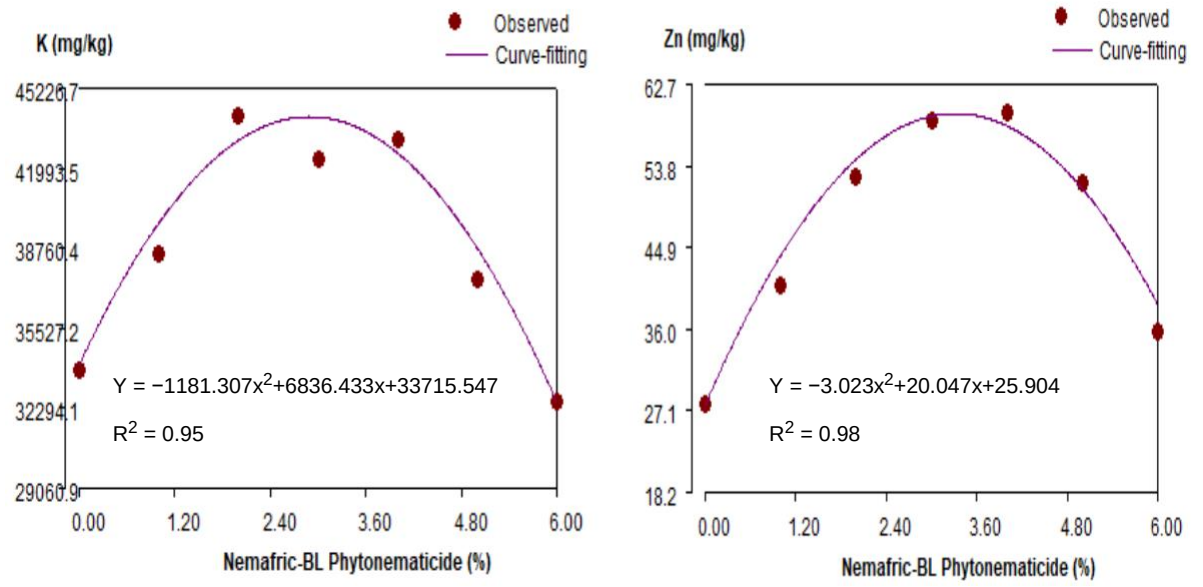

Figure 3.10 Responses of potassium (K) and zinc (Zn) in leaf tissues of nightshade to increasing concentration of Nemafric-BL phytonematicide.

Table 3.3 Biological indices for iron (Fe), sodium (Na), potassium (K) and zinc (Zn) in leaf tissues of nightshade treated with increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides.

	Nemarioc-AL			Nemafric-BL		
Biological index	Fe	Na	Mean	K	Zn	Mean
Threshold stimulation (D_m)	0.825	2.588	1.7065	2.894	3.316	3.105
Saturation point (R_h)	19.39	22.146	20.768	9890.92	33.239	4962.076
0% inhibition (D_0)	0	5.176	2.588	5.787	6.632	6.2095
50% inhibition (D_{50})	0	11.904	5.952	7.652	7.225	7.4385
100% inhibition (D_{100})	0	15.5	7.75	9	7.7	
R^2	0.914	0.962		0.952	0.98	
k-value	1	0		0	0	
Overall sensitivity	∑k = 1			∑k = 0		
Nemarioc-AL MCSP = $D_m+(R_h/2)$ = 1.7065 + (20.7675/2) = 12.09%						
Nemafric-BL MSCP = $D_m+(R_h/2)$ =3.105 + (4962.076/2) = 2484.14%						

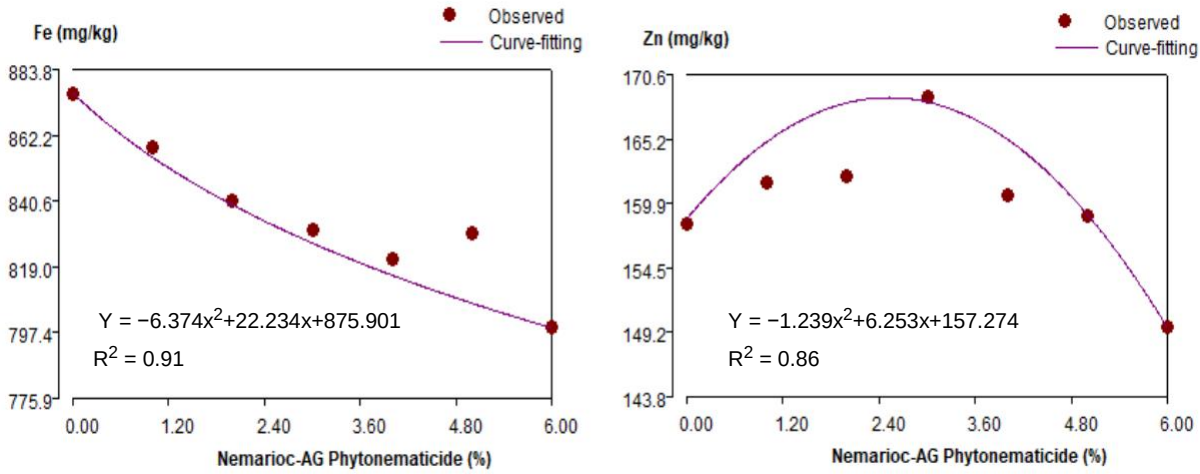

Figure 3.11 Responses of iron (Fe) and zinc (Zn) in leaf tissues of nightshade to increasing concentration of Nemarioc-AG phytonematicide.

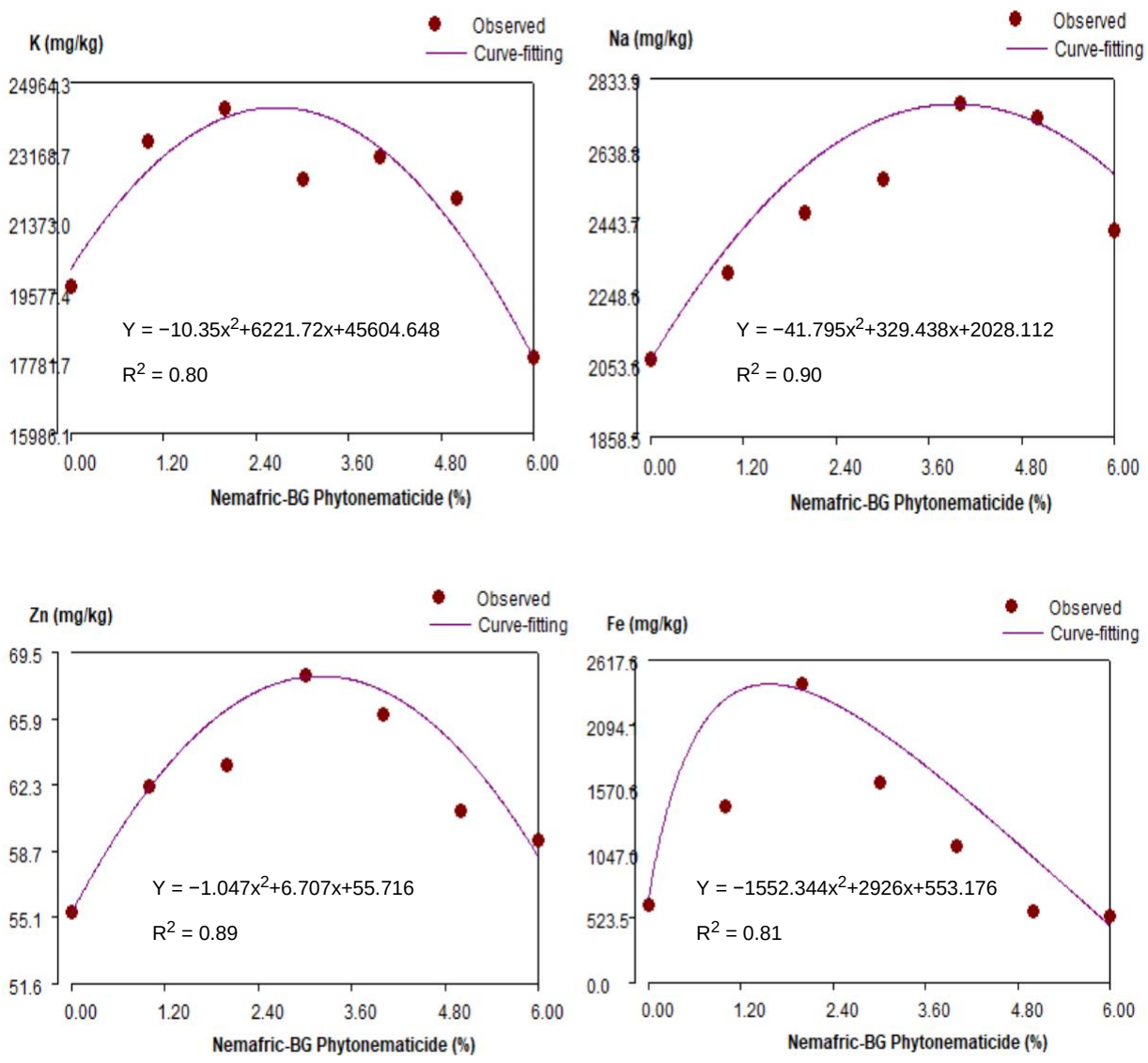

Figure 3.12 Responses of iron (Fe), sodium (Na), potassium (K) and zinc (Zn) in leaf tissues of nightshade to increasing concentration of Nemafric-BG phytonematicide.

Table 3.4 Biological indices for iron (Fe), sodium (Na), potassium (K) and zinc (Zn) in leaf tissues of nightshade treated with increasing concentration of Nemarioc-AG and Nemafric-BG phytonematicides.

	Nemarioc-AG			Nemafric-BG				
Biological index	Fe	Zn	Mean	Fe	K	Na	Zn	Mean
Threshold stimulation (D_m)	0.83	2.52	1.67	1.57	2.67	3.94	3.20	2.85
Saturation point (R_h)	19.39	7.89	13.64	1379.48	3727.85	649.17	10.74	1441.81
0% inhibition (D_0)	0	5.05	2.52	5.59	5.34	7.88	6.40	6.30
50% inhibition (D_{50})	0	10.88	5.44	6.21	7.82	10.25	9.27	8.39
100% inhibition (D_{100})	0	14.10	7.05	6.80	9.40	11.90	11.20	9.83
R^2	0.91	0.86		0.81	0.86	0.90	0.89	
k-value	1	0		1	0	0	0	
Overall sensitivity	$\sum k = 1$			$\sum k = 1$				
Nemarioc-AG MCSP = $D_m+(R_h/2)$ = 1.67 + (13.64/2) = 8.49 g								
Nemafric-BG MCSP = $D_m+(R_h/2)$ = 2.845 + (1441.809/2) = 723.75 g								

3.4 Discussion

3.4.1 Cucurbitacin chemical residues

In the current study, residues from the two phytonematicide formulations were not detected in all the leaf tissues of nightshade. Similar findings were observed by Dube (2016) and Shadung *et al*. (2017) where tomato plants were treated with Nemarioc-AL and Nemafric-BL phytonematicides and cucurbitacin chemical residues were not detected. The two phytonematicides contain cucurbitacin A and B as active ingredients. The two cucurbitacin molecules are non-polar, with cucurbitacin A being slightly polar and soluble in water (Gry *et al*., 2006), whereas, cucurbitacin B is insoluble (Jeffrey, 1978). Basically, non-polar molecules cannot be moved through the bipolar membranes of the symplastic pathway of the endodermis in roots into the vascular bundle (Campbell, 1990). However, Caboni *et al*. (2002) reported that in olives treated with azadirachtin, residues were detected but the residues declined rapidly from 0.35 ppm in day-1 to less than 0.02 ppm in day-7 after application. Similar findings were observed by Simeone *et al*. (2009) where pyrethrins were detected in olives but in amounts less than the Maximum Residue Limit (MRL), while rotenone residues in olives were found to have exceeded the acceptable limit. In contrast, azadirachtin ($C_{35}H_{44}O_{16}$) chemical residues were not detected at 7 days after application in strawberries (Caboni *et al*., 2006), olives (Simeone *et al*., 2009) and cabbage (Akbar *et al*., 2010) when treated with neem products. Although azadirachtin was one of the chemicals used in the experiments, in the olives experiment, it was not possible to detect it on the treated olives, even when sampling was performed within 24 hours after application, which could be because of the inadequate sensitivity of the method and the rapid decay of this chemical (Simeone *et al*., 2009). Furthermore, the actual concentrations of azadirachtin on the treated crops must have been below the

detection limit of the method (0.08 mg kg-1) and the MRL for this chemical is known to be 0.5 mg kg-1 (Commission Regulation (EC) No 149/2008). Hence, the amount of azadirachtin used with the described treatments applied to the above-mentioned crops did not result in residue levels of concern at any time after application. The azadirachtin and pyrethrins findings along with the undetected concentration of cucurbitacin A and B within nightshade edible parts, suggested that these active ingredients could be considered as being “safe” for the consumers, environment, operators, host-pest system and nightshade as the tested crop.

In context of density-dependent growth (DDG) patterns, the presence of minute amount of cucurbitacin residues in edible produce and products would be highly dangerous because they could stimulate cell division and that might result in cancer (Lee *et al*., 2010). On the other hand, at high concentration, where growth of cell would be inhibited, cucurbitacins could result in cytotoxicity (Lee *et al*., 2010). Therefore, the fact that cucurbitacin residues were not detected in this study, made a significant contribution to the nightshade industry in terms of commercialisation prospects of Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides.

3.4.2 Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides on plant variables

Mean Concentration Stimulation Point: Generally, observations on nightshade plant variables exposed to increasing concentration of the Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides confirmed the concept of density-dependent growth (DDG) patterns (Liu *et al*., 2003). Similarly, Lebea (2017) observed positive quadratic relationships when squash was exposed to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides under microplot and greenhouse conditions. In

contrast, Mathabatha *et al*. (2016) observed negative quadratic relations of certain variables of *Citrus volkameriana* seedling rootstocks when exposed to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides. Mafeo *et al*. (2011 a, b) also observed positive quadratic relations when maize, millet, sorghum and selected Alliaceae seedlings were exposed to Nemarioc-AG phytonematicide. Similar observations were made by Pelinganga (2013) when tomato was treated with Nemarioc-AG and Nemafric-BG phytonematicides. Mashela *et al*. (2015) provided a detailed explanation of potential outcomes when plants are exposed to increasing concentration of phytonematicides, which were concentration-dependent.

The DDG patterns are characterised by three phases known as stimulation, neutral and inhibition phases, with concentration found within the stimulation phase being approved as suitable for developing phytonematicides, whereas, the inhibition concentration was approved to be suitable for use in the development of herbicides. Therefore, in situations where nightshade plant variables and increasing concentration of the two phytonematicide formulations exhibited quadratic associations, the MCSP was computed using the CARD computer-based model. Furthermore, the plant variables of nightshade were only confirmed to be following the DDG patterns when the four scenarios explained by Mashela *et al*. (2015) were observed, of which their occurrence depends on the concentration range being used. Mashela *et al*. (2015) demonstrated that the relations could be (a) positive linear if stimulation concentration were involved, (b) neutral (ANOVA not significant at $P \leq 0.05$), (c) negative linear if inhibition concentration are involved and (d) positive or negative quadratic when the stimulation, neutral and inhibition concentration are involved.

The MCSP values for both Nemarioc-AL and Nemafric-BL phytonematicides in the current study were 3.02 and 3.08%, respectively, which were relatively closer to the MCSP values of 2.63 and 2.89% which were established for tomato when treated with Nemarioc-AL and Nemafric-BL phytonematicides, respectively, but they were all lower when compared with the MCSP values that were obtained when other plants were treated with Nemarioc-AL phytonematicide namely, 11.85% for squash under greenhouse conditions (Lebea, 2017), 9% for *C. volkameriana* under greenhouse conditions (Mathabatha *et al*., 2016) and 6.18% for African geranium plants under microplot conditions (Sithole, 2016).

In the granular phytonematicide trials, the MCSP values for both Nemarioc-AG and Nemafric-BG phytonematicides in the current study were 3.47 and 4.70%, respectively, which were higher than MCSP values that were discovered by Mafeo *et al*. (2011a) for maize (1.13%), millet (0.86%) and sorghum (1.12%) when treated with Nemarioc-AG phytonematicide. The differences observed between the current findings and observations from previous studies confirmed the theory that MCSP is crop-specific (Mashela *et al*., 2015), with the recent findings highlighting that the environmental conditions under which plants are being tested could also have contributed to differences observed in the already developed MCSP values. This was explained by Shadung (2016), that the movements of phytonematicides in pot trials are restricted, unlike under field conditions where the movements of phytonematicides in the soil are unconstrained, it can be sidestream or downstream, which can be determined by factors such as irrigation intensity, soil type and slope. Furthermore, the differences could also have been observed because nightshade and the crops in comparison are not physiologically the same, in a sense that nightshade is a leafy

vegetable and the other crops are fruity vegetables (i.e. their edible parts are fruits). This was confirmed by Sharifi and Zebarth (2006) who reported that there are significant differences in pesticides and fertilizer uptake between a variety of plant species and cultivars too, but it can also be influenced by environmental conditions (Wheeler *et al*., 1998).

Sensitivity: In liquid phytonematicide trials, the overall sensitivities of nightshade to Nemarioc-AL and Nemafric-BL phytonematicides were low, $\sum k = 1$ and $\sum k = 0$, respectively, both suggesting that nightshade would be highly sensitive to both products when used for nightshade production. Similar observations were made on tomato (Pelinganga, 2013) and African geranium (Sithole, 2016) where the overall sensitivities for both crops were found to be $\sum k = 3$ when treated with Nemarioc-AL phytonematicide. Similarly, in granular phytonematicide trials, lower overall sensitivities were observed when nightshade was treated with Nemarioc-AG and Nemafric-BG phytonematicides with values of $\sum k = 1$ and 0, respectively. The current findings agree with those found by Pelinganga (2013) where tomato plant organs sensitivities to Nemarioc-AG and Nemafric-BG phytonematicides ranged from 0-1. On the contrary, Mafeo *et al*. (2011a, b) reported that 18 seedlings of different crops displayed different overall sensitivity values to Nemarioc-AG phytonematicide and the values were high as compared to the sensitivity values observed for nightshade. The differences in sensitivity values among the studies confirms the hypothesis of Mashela *et al*. (2011), that the nearer the value of 'k' is to zero, the greater the sensitivity of the crop to the phytonematicide and the findings are further supported by Liu *et al.* (2003) who explained that as the overall sensitivity becomes less, the sensitivity of the crop to the phytonematicide becomes greater and vice versa. Additionally, these

differences agree with the report by Rice (1984) who discovered that the extent to which plants are sensitive to allelochemicals was plant specific, with seedlings stage demonstrated to be highly tolerant than at other stages in the life of a given plant species (Mafeo *et al*., 2011a, b). Finally, the differences observed among the studies highlights that k values are affected by various factors, which may include, fermented versus unfermented phytonematicides, age of the test plant and/or organ of the test plant (Pelinganga, 2013).

3.4.3 Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides on nutrient elements

Mean Concentration Stimulation Point: In the liquid phytonematicide trials, the selected nutrient elements against the increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides exhibited quadratic relations. Based on the evidence found in the current study, responses of Fe and Na in leaf tissues of nightshade against increasing concentration of Nemarioc-AL phytonematicide along with those of K and Zn against increasing concentration of Nemafric-BL phytonematicide followed the DDG pattern. These results agree with those observed by Mashela and Pofu (2017) who observed positive quadratic relations for Fe in leaf tissues of green beans against increasing concentration of Nemarioc-AL phytonematicide, whereas, in contrast, Na and Zn exhibited negative quadratic relations. Similarly, with the current findings, Mashela and Pofu (2017) also reported positive quadratic relations for K in leaf tissues of green beans over increasing concentration of Nemafric-BL phytonematicide.

In granular phytonematicide trials, Fe exhibited negative quadratic relationship, whereas, Zn exhibited a positive quadratic relationship over increasing concentration of Nemarioc-AG phytonematicide. Similarly, Fe, Na, K and Zn, over increasing

concentration of Nemafric-BG phytonematicide exhibited positive quadratic relations. In liquid phytonematicide trials, using the relation MCSP = D_m + (R_h/2), the MCSP value for Nemarioc-AL phytonematicide was found to be 12.09%, while MCSP value for Nemafric-BL phytonematicide was 2484.14%. Using the relation MCSP = D_m + (R_h/2), the MCSP value for Nemarioc-AG phytonematicide was found to be 8.49 g, while, MCSP value for Nemafric-BG phytonematicide was 723.75 g.

Sensitivity: In liquid phytonematicide trials, Fe displayed sensitivity value of k = 1, whereas, Na had sensitivity value of k = 0, with the ∑k of nutrients in nightshade leaf tissues being 1, when treated with Nemarioc-AL phytonematicide, whereas, when nightshade was treated with Nemafric-BL phytonematicide, both K and Zn had sensitivity values of k = 0, with ∑k of nightshade leaf tissues being 0. In granular phytonematicide trials, Fe had sensitivity value of k = 1, whereas, Zn had sensitivity value of k = 0, with the ∑k of nutrients in nightshade leaf tissues being 1, when treated with Nemarioc-AG phytonematicide. In contrast, when nightshade was treated with Nemafric-BG phytonematicide was observed to have sensitivity value of k = 1, whereas, Na, K and Zn had sensitivity values of k = 0, with ∑k of nightshade leaf tissues being 1.

The effect of phytonematicides on accumulation of nutrients in the leaf tissues of tested plants, nightshade included, forms an important portion in the success of using phytonematicides from the two-plant species, *C*. *myriocarpus and C*. *africanus*, regardless of the formulation. However, in phytonematicides context, the focal point had always been success of phytonematicides on management of nematodes and development of concentration that would not induce phytotoxicity on tested crops

(MCSP), with less attention on how the nutrients within the tested crops would respond to the products. Hence, there is no documented information in all phytonematicide-plant relation studies as reviewed recently on granular phytonematicides (Mashela *et al*., 2016). In the recently reviewed document Mashela and Pofu (2017) highlighted that more studies are needed because the stimulated accumulation of the nutrient elements could be very helpful in the interpretation of the previously observed “fertiliser effect” of phytonematicides (Mashela, 2002). Moreover, the observed positive quadratic models could also be useful in providing optimum phytonematicide concentration at which the selected nutrient elements would be at optimum amount. Apparently, this is the first report where the influence of Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides on accumulation of nutrient elements within leaf tissues of leafy vegetables was empirically researched and documented.

3.5 Conclusion

At 3.02% for Nemarioc-AL, 3.08% for Nemafric-BL, 3.47 g for Nemarioc-AG and 4.70 g for Nemafric-BG phytonematicides, enhanced the growth of nightshade, essential nutrient elements status in the leaves of nightshade plants with no detection of cucurbitacin residue traces in the leaves. Therefore, the current findings together with consistent efficacy reports on nematode suppression and non-phytotoxicity effects, intensifies the potential commercialisation of the two phytonematicide formulations for use in nightshade crop protection against nematodes.

CHAPTER 4
SUMMARY OF FINDINGS, SIGNIFICANCE, RECOMMENDATIONS AND CONCLUSIONS

4.1 Summary of findings

The study focused on assessing the influence of four phytonematicides in different formulations on growth of nightshade plant, accumulation of essential nutrient elements and cucurbitacin residues in nightshade leaf tissues. This study was carried out because botanicals were discovered to have great potential to replace 'the' harmful synthetic nematicides. Nemarioc-AL and Nemafric-BL phytonematicides were produced using fermented crude extracts of fruits from wild cucumber (*Cucumis myriocarpus* Naude.) and wild watermelon (*Cucumis africanus* LF.), respectively, while, Nemarioc-AG and Nemafric-BG phytonematicides were manufactured using only the ground dried material of the respective fruits. Chemical residues of cucurbitacin A and B were tested in nightshade leaves where plant growth and essential nutrient elements status were improved using Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides, without traceable residues of the two cucurbitacins. In Nemarioc-AL phytonematicide trial, dry root mass and dry shoot mass were observed to have exhibited positive quadratic relationships with the models explained by high coefficients of determination values, 93 and 61%, respectively. Similar trend was observed under Nemafric-BL phytonematicide for dry root mass, dry shoot mass, plant height, chlorophyll content and stem diameter with the associations explained by 95, 72, 65, 78 and 62%, respectively (Chapter 3). These high coefficients of determination values suggested the existence of strong allelopathic interactions. In both trials, it was shown by the figures found in Chapter 3 that nightshade growth was stimulated at lower concentrations of these materials. Nightshade leafy vegetable was

found to be highly sensitive to Nemarioc-AL phytonematicide as shown by ∑k = 0 and similarly, nightshade leafy vegetable was found to be highly sensitive to Nemafric-BL phytonematicide as reflected by ∑k = 0. The generated MCSP for Nemarioc-AL and Nemafric-BL phytonematicide were 3.02 and 3.08%, respectively.

In Nemarioc-AL phytonematicide trial, Na and Fe over increasing concentration of Nemarioc-AL phytonematicide each exhibited positive quadratic relationships with the associations explained by 96 and 91%, respectively. Similarly, K and Zn over increasing concentration of Nemafric-BL phytonematicide exhibited positive quadratic relationships with the model explained by 95 and 98%, respectively. Nightshade leafy vegetable nutrient elements were found to be highly sensitive to Nemarioc-AL phytonematicide with the ∑k = 1 and similarly, nightshade nutrient elements were reported to be sensitive to Nemafric-BL phytonematicide as shown by ∑k = 0. The MCSP derived for Nemarioc-AL and Nemafric-BL phytonematicide were 12.09 and 2484.14%, respectively, for nutrient elements stimulation.

In Nemarioc-AG phytonematicide trial, plant height and stem diameter exhibited positive quadratic relationships with the models explained by high coefficients of determination values, 93 and 88%, respectively. Similar trend was observed under Nemafric-BG phytonematicide for dry root mass, dry shoot mass and stem diameter with the relationships explained by 91, 94 and 84%, respectively (Chapter 3). The CARD model figures in Chapter 3 show that nightshade growth was stimulated at lower concentrations of these two products. Nightshade leafy vegetable was observed to be highly sensitive to Nemarioc-AG phytonematicide as indicated by ∑k = 0 and similarly, nightshade leafy vegetable was found to be highly sensitive to Nemafric-BG

phytonematicide as shown by ∑k = 0. The generated MCSP for Nemarioc-AG phytonematicide was 3.47 g, while, the MCSP for Nemafric-BG phytonematicide was found to be 4.70 g (Chapter 3).

In Nemarioc-AG phytonematicide trial, Fe over increasing concentration of Nemarioc-AG phytonematicide exhibited positive quadratic relationships with the model explained by 91%, whereas, on the contrary, Zn over increasing concentration of Nemarioc-AG phytonematicide exhibited negative quadratic relationship with the association explained by 96%. In Nemafric-BG phytonematicide trial, Fe, Na, K and Zn over increasing concentration exhibited positive quadratic relationships with the associations explained by 81, 90, 80 and 89%, respectively. Nightshade leafy vegetable nutrient elements were found to be highly sensitive to Nemarioc-AG phytonematicide as shown by ∑k = 1, similarly, nightshade leafy vegetable nutrient elements were observed to be sensitive to Nemafric-BG phytonematicide as shown by the ∑k = 1. The MCSP derived for Nemarioc-AG phytonematicide was 8.49 g while the computed MCSP for Nemafric-BG phytonematicide was obtained at 723.75 g for nutrient elements stimulation.

4.2 Significance

Plant-based products were discovered to be the most environment-friendly nematicides to use for management of nematodes. Unfortunately, chemical residues and phytotoxicity have been observed to be the most significant barrier to the success of the phytonematicides as substitutes to synthetic nematicides in the management of nematode population densities. However, in the current study, the trace of residues challenge was solved using DDG patterns which helped with the determination of non-

phytotoxic concentration for Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides using the Curve-fitting Allelochemical Response Dosage (CARD) computer-based model. The CARD model among other biological indices, it provided the two biological indices (D_m and R_h) to calculate the Mean Concentration Stimulation Point (MCSP) for nightshade leafy vegetable (*Solanum retroflexum*) when exposed to the four products in the management of nematodes, while the K-values provided were used to determine the ∑k of nightshade leafy vegetable towards the four materials. The non-phytotoxic concentration which did not leave traces of cucurbitacin residues in nightshade leaves for Nemarioc-AL, Nemafric-BL, Nemarioc-AG and Nemafric-BG phytonematicides were 3.02%, 3.08%, 3.47 g and 4.70 g, respectively, with the ∑k of the respective phytonematicides being 1, 0, 0 and 0.

4.3 Recommendations

Nemarioc-AL, Nemafric-BL, Nemarioc-AG and Nemafric-BG phytonematicides could be applied at 3.02%, 3.08%, 3.47 g and 4.70 g, respectively, under microplot conditions since they were able to enhance nightshade plant growth, the status of essential nutrient elements in the leaves of nightshade without leaving any traces of cucurbitacin residues in the leaf tissues. The computed MCSP values of those products could be used to establish the application interval and thereafter, the dosage model for the four phytonematicides on nightshade leafy vegetable. Additionally, the current study was the first one conducted to investigate the response of nightshade plant to phytonematicides under micro-plot conditions. The findings of the current study could have been as observed due to nightshade responding to application of the four phytonematicides in different formulations whose efficacy might have been influenced by environmental conditions. Therefore, after deriving the dosage model, it

would be imperative to assess the environmental effect of the four products in terms of persistence of their active ingredients in the soil. Finally, it would be necessary to establish further studies to help in assessing whether the four phytonematicides potential to influence the growth of nightshade, accumulation of essential nutrient elements and cucurbitacins within nightshade leaf tissues will differ under different growing conditions such as field and greenhouse conditions which were not covered in the current study.

4.4 Conclusions

The application of Nemarioc-AL, Nemafric-BL, Nemarioc-AG and Nemafric-BG phytonematicides had the ability to stimulate the growth of nightshade, enhance the accumulation of the selected nutrient elements in leaf tissues of nightshade and not leave cucurbitacin residues within the edible parts of nightshade at lower concentrations. However, at higher concentration of the materials, both growth and nutrient elements of nightshade were inhibited. Therefore, the use of Nemarioc-AL, Nemafric-BL, Nemarioc-AG and Nemafric-BG phytonematicides could be successful in managing nematode population densities in nightshade leafy vegetable production provided the products are used at the MCSP values of 3.02%, 3.08%, 3.47 g and 4.70 g, respectively. The mentioned MCSP values should be used on nightshade leafy vegetable mainly because at those concentration the four products would each be able to consistently suppress population densities of nematodes, without posing any phytotoxicity to the plants and not leave traces of cucurbitacins in nightshade leaves.

REFERENCES

Abhilash, P.C. and N. Singh. 2009. Pesticide use and application: An Indian scenario. *Journal of Hazardous Materials* 165:1-12.

Akbar, M.F., Haq, M.A., Parveen, F., Yasmin, N. and S.A. Sayeed. 2010. Determination of synthetic and bio-insecticides residues during aphid (*Myzus persicae* (Sulzer) control on cabbage crop through high performance liquid chromatography. *Pakistan Entomologist* 32:155-162.

Arias-estévez, M., López-Periago, E., Martínez-Carballo, E., Simal-Gándara, J., Mejuto, J.C. and L. García-río. 2008. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. *Agriculture, Ecosystems and Environment* 123:247-260.

Berrada, H., Fernández, M., Ruiz, M.J., Moltó, J.C., Mañes, J. and G. Font. 2010. Surveillance of pesticide residues in fruits from Valencia during twenty months (2004/05). *Food Control* 21:36-44.

Bhatt, B.P. and N.P. Todoria. 1990. Studies on the allelopathic effects of some agroforestry tree crops of Garhwal Himalaya. *Agroforestry System* 12:251-255.

Caboni, P., Cabras, M., Angioni, A., Russo, M. and P. Cabras. 2002. Persistence of azadiractin residues on olives after field treatment. *Journal of Agricultural and Food Chemistry* 50:3491-3494.

Caboni, P., Sarai, G., Angioni, A., Garcia, A.J., Lai, F., Dedola, F. and P. Cabras. 2006. Residues and persistence of neem formulations on strawberry after field treatment. *Journal of Agricultural and Food Chemistry* 54:10026-10032.

Campbell, N.A. 1990. Biology. Benjamin/Cummings Publisher: Redwood City, USA.

Chokoe, M.F. 2017. Non-phytotoxic concentration of Nemarioc-AL and Nemafric-BL

phytonematicides on green bean cultivar 'tahoe'. MSc. Mini-dissertation, University of Limpopo, Sovenga, South Africa.

Donkor, A., Osei-Fosu, P., Dubey, B., Kingsford-Adaboh, R., Ziwu, C. and I. Asante. 2016. Pesticide residues in fruits and vegetables in Ghana: A review. *Environmental Science and Pollution Research* 23:18966-18987.

Dube, Z.P. 2016. Nemarioc-AL and Nemafric-BL phytonematicides: Bioactivities in *Meloidogyne incognita*, tomato crop, soil type and organic matter. PhD Thesis, University of Limpopo, Sovenga, South Africa.

Dubey, N.K., Srivastava, B. and A. Kumar. 2008. Current status of plant products as botanical pesticides in storage pest management. *Journal of Biopesticides* 1:182-186.

Egunjobi, A. and O. Afolamis. 1976. Effects of neem (*Azadirachta indica*) leaf extracts on populations of *Pratylenchus bruchyurus* and on the growth and yield of maize. *Nematologica* 22:125-132.

Gry, J., Søborg, I. and H.C. Anderson. 2006. Cucurbitacins in Plant Food. Ekspressen Tryk and Kopicenter: Copenhagen, Denmark.

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

Khosa, M.C. 2013. An investigation into the potential of crude and partially separated material of selected non-crop plant species as control agents of root-knot nematodes (*Meloidogyne incognita*) in tomato. PhD Thesis, North-West University, Potchefstroom, South Africa.

Kleynhans, K.P.N., Van der Berg, E., Swart, A., Marais, M. and N.H. Buckley. 1996. Plant Nematodes in South Africa. ARC - Plant Protection Research Institute: Pretoria.

Krol, W.J., Arsenault, T.L., Pylypiw, H.M. and M.J.I. Mattina. 2000. Reduction of pesticide residues on produce by rinsing. *Journal of Agricultural Food Chemistry* 48:4666-4670.

Kumar, S. 2012. Biopesticides: A neem for food and environmental safety. *Journal of Biofertilisers and Biopesticides* 3:1-3.

Lebea, M.P. 2017. Mean concentration stimulation point of Nemarioc-AL and Nemafric-BL phytonematicides on *Cucurbita pepo* cultivar 'Caserta'. MSc. Mini-dissertation, University of Limpopo, Sovenga, South Africa.

Lee, D.H., Iwanski, G.B. and N.H. Thoennissen. 2010. Cucurbitacin: Ancient compound shedding new light on cancer treatment. *Journal of the Scientific World* 10:413-418.

Leedy, P.D. and J.E. Ormrod. 2005. Practical Research: Planning and Design. Merrill Prentice Hall: Upper Saddle River, New Jersey.

Little, T.M. and F.J. Hills. 1981. Statistical Methods in Agricultural Research. University of California: California.

Liu, D.L., Johnson, I.R. and J.V. Lovett. 2003. Mathematical modelling of allelopathy. A model for curve-fitting allelochemical dose responses. *Non-linearity in Biology, Toxicology and Medicine* 1:37-50.

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., Mphosi, M.S. and K.M. Pofu. 2011a. Modelling responses of maize, millet and sorghum seedlings to crude extracts of *Cucumis myriocarpus* fruit as pre-emergent bio-nematicide. *African Journal of Agricultural Research* 6:3678-3684.

Mafeo, T.P., Mashela, P.W. and M.S. Mphosi. 2011b. Sensitivity of selected Alliaceae seedlings to crude extracts of *Cucumis myriocarpus* fruits. *African Journal of Agricultural Research* 6:158-164.

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

Maile, K.D. 2013. Responses of *Tylenchulus semipenetrans* to crude extracts of indigenous *Cucumis* fruits with and without effective microorganisms in citrus production. MSc. Dissertation, University of Limpopo, Sovenga, South Africa.

Maile, K.D., Mashela, P.W. and P.E. Tseke. 2013. Responses of the citrus nematode to Nemarioc-AG phytonematicide with and without micro-organisms in citrus production. *African Crop Science Conference Proceedings* 11:333-337.

Makkar, H.P.S. 1999. Quantification of Tannins in Tree Foliage. FAO/IAEA Working Document, IAEA: Vienna.

Mashela, P.W. 2002. Ground wild cucumber fruits suppress numbers of *Meloidogyne incognita* on tomato in micro plots. *Nematropica* 32:13-19.

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

Mashela P.W. 2014. Soil allelochemical residue effects in a tomato cowpea rotation-nodulation and productivity of cowpea and nematode suppression. *Acta Agriculturae Scandinavica, Section B – Soil and Plant Science* 64:372-375.

Mashela, P.W., De Waele, D., Dube, Z.P., Khosa, M.C., Pofu, K.M., Tefu, G., Daneel, M.S. and H. Fourie. 2017. Alternative Nematode Management Strategies. In: Fourie, H., Spaull, V.W., Jones, R.K., Daneel, M.S. and D. De Waele (eds). Nematology in South Africa: A View from the 21st Century. Springer International Publishers: Heidelberg, Switzerland.

Mashela, P.W., De Waele, D. and K.M. Pofu. 2011. Use of indigenous *Cucumis* technologies as alternatives to synthetic nematicides in management of root-knot nematodes in low-input agricultural farming systems: A review. *Scientific Research and Essays* 33:6762-6768.

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. Vorma (eds.). Organic Amendments and Soil Suppressiveness in Plant Disease Management, Soil Biology 46. Springer International Publishers: Heidelberg, Switzerland.

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 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* 34:273.

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* 67:743-747.

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.

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.

Mashitoa, M.F. 2017. Development of non-phytotoxic concentration of Nemarioc-AL and Nemafric-BL phytonematicides on beetroot (*Beta vulgaris*) cultivar 'detroit dark red'. MSc. Mini-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 rootstocks. *Transylvanian Review* 24:969-972.

Monfankye, R. 2014. The Management of field pests on cowpea (*Vigna unguiculata* (L.) Walp) using botanicals [Tobacco (*Nicotiana tabacum*) leaves, neem (*Azadirachta indica*) leaves, ginger (*Zingiber officinale*) rhizomes and onion (*Allium cepa*) Bulbs]. PhD Thesis, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Nakano, V.E., KussumI, T.A., Lemes, V.R.R., Kimura, I.D.A., Rocha, S.B., Alaburda, J., Oliveira, M.C.C.D., Ribeiro, R.A., Faria, A.L.R. and K.C. Waldhelm. 2016.

Evaluation of pesticide residues in oranges from São Paulo, Brazil. *Food Science and Technology* 36:40-48.

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.

O'keeffe, M. and F. Farrell. 2000. The importance of chemical residues as a food safety issue. *Irish Journal of Agricultural and Food Research* 39:257-264.

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. InTechOpen: Rijeka.

Pelinganga, O.M. 2013. Developing phytonematicides using indigenous *Cucumis africanus* and *Cucumis myriocarpus* fruits for tomato production system. PhD Thesis, 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* 4:8-11.

Pelinganga, O.M., Mashela, P.W., Mphosi, M.S. and B. Nzanza. 2013. 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., 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.

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

Republic of South Africa (RSA). 2012. Executive summery-national development plan 2020. Our Future - make it work. South African government, Pretoria, South Africa.

Rice, E.L. 1984. Allelopathy. Academic Fresh: New York.

Rossner, J. and C.P.W. Zebits. 1986. Effects of Neem products on Nematodes on Tomato (*Lycorpersicon esculentum*) Plants. Proceedings of the 3rd International Neem Conference: Nairobi, Kenya.

Salisbury, F.B. and C.W. Ross. 1992. Plant Physiology. Wadsworth: Belmont, California.

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.

Setia, N., Batish, D.R., Singh, H.P. and R.K. Kohli. 2007. Phytotoxicity of volatile oil from *Eucalyptus citriodora* against some weedy species. *Journal of Environmental Biology* 1:63-66.

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

Shadung, K.G., Mashela, P.W., Mphosi, M.S. and V.L. Mulaudzi. 2017. Study of chemical residues from Nemarioc-AL and Nemafric-BL phytonematicides in tomato fruit. *African Journal of Agricultural Research* 12:1164-1168.

Sharifi, M. and B.J. Zebarth. 2006. Nitrate influx kinetic parameters of five potato cultivars during vegetative growth. *Plant and Soil* 288:91-99.

Simeone, V., Baser, N., Perrelli, D., Cesari, G., El Bilali, H. and P. Natale. 2009. Residues of rotenone, azadirachtin, pyrethrins and copper used to control *Bactrocera oleae* (Gmel.) in organic olives and oil. *Food Additives and Contaminants* 26:475-481.

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. Mini-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.

Sikora, R.A., Bridge, J. and J.L. Starr. 2005. Management Practices: An Overview of Integrated Nematode Management Technologies. In: Luc, M., Sikora, R.A. and J. Bridge (eds.). Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CAB International: Wallingford.

Sukul, N.C., Das, P.K. and G.C. DE. 1974. Nematicidal action of some edible crops. *Nematologica* 20:187-191.

Sukul, N.C., Sinhababu, S.P., Datta, S.C. and A. Sukul. 2001. Nematode effect of *Acacia auriculiformis* and *Artemesia nilagrica* against root-knot nematode. *Allelopathy Journal* 8:65-72.

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. Mini-dissertation, University of Limpopo, Sovenga, South Africa.

Tseke, P.E., Mashela, P.W. and N.M. Mokgalong. 2013. Responses of tomato plant growth and root-knot nematodes to Nemarioc-AL phytonematicide. *African Crop Science Conference Proceedings* 11:367-370.

Van Averbeke, W. and K.A. Juma. 2006. The cultivation of *Solanum retroflexum* Dun. in Vhembe, Limpopo Province, South Africa. Procurement international symposium on the nutritional value and water use of indigenous crops for improved livelihoods. 19-20 September, The Centre for Nutrition, University of Pretoria, Pretoria, South Africa.

Wachira, P.W., Kimenju, J.W., Okoth, S.A. and R.K. Mikey. 2009. Stimulation of nematode destroying fungi by organic amendments applied in management of plant parasitic nematode. *Asian Journal of Plant Science* 8:153-159.

Wheeler, E.F., Albright, L.D., Spanswick, R.M., Walker, L.P. and R.W. Langhans. 1998. Nitrate uptake kinetics in lettuce as influenced by light and nitrate nutrition. *Transactions of the American Society of Agricultural Engineering* 41:859-867.

Wilson, C. and C. Tisdell. 2001. Why farmers continue to use pesticides despite environmental, health and sustainability costs. *Ecological Economics* 39:449-462.