CUCURBITACIN CHEMICAL RESIDUES, NON-PHYTOTOXIC CONCENTRATION AND ESSENTIAL MINERAL ELEMENTS OF NEMARIOC-AL AND NEMAFRIC-BL PHYTONEMATICIDES ON GROWTH OF TOMATO PLANT

BY

HAPPY BANGO

MINI-DISSERTATION SUBMITTED IN PARTIAL FULLFILMENT OF THE

REQUIREMENTS FOR THHE DEGREE OF

MASTER OF SCIENCE

IN

HORTICULTURE

IN THE

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

AT THE

UNIVERSITY OF LIMPOPO

SUPERVISOR : DR K.G. SHADUNG

CO-SUPERVISOR : PROFESSOR P.W. MASHELA

2019

TABLE OF CONTENTS

DECLARATION

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

Candidate: Happy Bango	Signature	Date
Supervisor: Dr K.G. Shadung	Signature	Date
Co-Supervisor: Professor P.W. Mashela	Signature	Date

DEDICATION

To my late aunt, Miss Maite Maria Bango

ACKNOWLEDGEMENTS

All thanks to Jehovah, the Almighty God, the Alpha and Omega, the Father of my Lord Christ Jesus, Father of all the nations, for provision of life, good health, celestial protection, knowledge, understanding and wisdom during conducting of experiments for this mini-dissertation. Without Him, what has been achieved would not have been possible. Heavenly Father, You deserve all the praises. You are the creator of heaven and earth, and for that, I will honour You forever. Amen. To my supervisory team, Dr K.G. Shadung and Professor P.W. Mashela, I feel like words are not enough to explain how grateful I am. First and foremost, I thank you for the encouragement, unlimited support and training in various areas of scientific writing, more especially the 'KUW' philosophy that had worked for me. I am magnanimously honoured to have worked under the Green Biotechnologies Research Centre of Excellence (GBRCE). Special gratitude to Mrs Z. Ngubane from the Limpopo Agro-food Technology Station (LATS) for providing the much-needed technical and analytical expertise in Microwave digester (Titan MPS) and ICPE-9000 (Shimadzu) operations during analysis. I am much better because of your help. I would like to thank the GBRCE for my consumables in this work and the department of Plant Production, Soil Science and Agricultural Engineering for sponsoring my conference trips during the MSc programme to Faculty Research Day, Bolivia Lodge in 2017 and Fusion Boutique Hotel in 2018 where I presented parts of my MSc results and to the African Combined Congress, Cape Town, Western Cape Province, in 2018, where I presented an oral paper and a poster. My love goes to my family and friends, especially to my parents (Lydia Setagane and David Bango), my younger sister and brother (Nthabiseng and Duncan Bango), my cousin (Ronel Pilusa), my uncle (David Malungane),

and my best friend (Nikra Mokhomole) for their encouragement and solid support throughout the study. I give thanks to the GBRCE team, Mr P.E. Tseke, Mr M.K. Ralefatana, Mr L.T. Letswalo, Mr E.M. Letswalo, Ms M.A. Mawasha, Ms S.R. Mawasha, Ms S.M. Seabela and all the postgraduate students who assisted me throughout the course of this research. I am greatly humbled by your infinite humanity and in return, I thank you infinitely, and above all, infinitely once more!

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Worldwide, tomato (*Solanum lycopersicum* L.) is one of the most important crops grown for nutritional value and health benefits, and are highly susceptible to root-knot (*Meloidogyne* species) nematodes. Following the withdrawal of synthetic chemical nematicides, Nemarioc-AL and Nemafric-BL phytonematicides have been researched and developed as alternatives to synthetic chemical nematicides. However, Nemarioc-AL and Nemafric-BL phytonematicides contains allelochemicals namely, cucurbitacin A ($C_{32}H_{46}O_9$) and cucurbitacin B ($C_{32}H_{46}O_8$) as their active ingredients. Therefore, the objective of this study was to determine whether increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides would result in cucurbitacin residues in tomato plant, to generate mean concentration stimulation point (MCSP) values, overall sensitivity ($\sum k$) and selected foliar mineral elements of tomato plant. Two parallel trials of Nemarioc-AL and Nemafric-BL phytonematicides were conducted under field conditions, with each validated the next season. Each trial had seven treatments, namely, 0, 2, 4, 8, 16, 32 and 64% of Nemarioc-AL or Nemafric-BL phytonematicide concentrations, arranged in a randomised complete block design (RCBD), with five replications. In each trial, the seasonal interaction on variables was not significant and therefore data were pooled across the two seasons (n = 70). In both phytonematicides, the cucurbitacin residues were not detected in soil and tomato fruit. Plant variables and selected foliar nutrient elements were subjected to the Curve-fitting Allelochemical Response Data (CARD) model to generate biological indices which allowed for the calculation of MCSP of phytonematicides on tomato and their $\sum k$ values of tomato to Nemarioc-AL and Nemafric-BL phytonematicides. In Nemarioc-AL phytonematicide experiment, MCSP for tomato

plant variables was at 1.13%, with the ∑k of 60 units, while the MCSP for selected tomato nutrient elements in leaf tissues was at 2.49%, with the ∑k of 21 units. Plant height, chlorophyll content, stem diameter, number of fruit, dry fruit mass, dry shoot mass and dry root mass each with increasing concentration of Nemarioc-AL phytonematicide exhibited positive quadratic relations with a model explained by 95, 82, 96, 89, 83, 83 and 92%, respectively. Similarly, K, Na and Zn each with increasing Nemarioc-AL phytonematicide concentration exhibited positive quadratic relations with a model explaining a strong relationship by 91, 96 and 89%. In Nemafric-BL phytonematicide experiment, MSCP for tomato plant variables was at 1.75%, with the ∑k of 45 units, whereas MCSP for selected tomato nutrient elements in leaf tissues was at 3.72% with the ∑k of 33 units. Plant height, chlorophyll content, stem diameter, number of fruit, dry fruit mass, dry shoot mass and dry root mass and increasing Nemafric-BL phytonematicide concentration exhibited positive quadratic relations with the model explaining a strong relationship by 92, 83, 97, 96, 87, 94 and 96%. Likewise, Na and Zn each with increasing Nemafric-BL phytonematicide concentration exhibited positive quadratic relations with a model explaining their relationship by 93 and 83%, respectively. In contrast, K with increasing Nemafric-BL phytonematicide concentration exhibited negative quadratic relations with a model explaining the relationship by 96%. In conclusion, tomato plant variables and selected foliar nutrient elements over increasing concentration of phytonematicides exhibited DDG patterns, characterised by three phases, namely, stimulation, neutral and inhibition. The developed non-phytotoxic concentration would be suitable for successful tomato production under field conditions.

CHAPTER 1
RESEARCH PROBLEM

1.1 Background

1.1.1 Description of the research problem

Worldwide, tomato (*Solanum lycopersicum* L.) plants had been regarded as one of the most important vegetables cultivated for nutritional and health benefits. However, tomato plants had been shown to be highly susceptible to plant-parasitic nematodes, although there were those that contain *Mi* gene which were said to be resistant mainly wild tomato species (Abad *et al*., 2003). Root-knot (*Meloidogyne* spp.) nematodes had been one of the major pathogens in tomato production and limit the production of fruit (Sikora and Fernandez, 2005). Estimates of nematode damage to specific crops ranged from 3.3% to 20.6%, with a global mean damage of 12.3% (Chitwood, 2003). Developing countries reported greater yield loss percentages than developed countries, probably due to disparity in resources (Chitwood, 2003). Following the withdrawal of synthetic chemical nematicides from agrochemical markets, alternatives had to be researched and developed (Mashela *et al.*, 2011; Mashela *et al.*, 2015).

In South Africa, two cucurbitacin-containing phytonematicides, namely, Nemarioc-AL and Nemafric-BL phytonematicides have been researched and developed to rectify the incidence of synthetic chemical nematicides (Mashela *et al*., 2017a). The two phytonematicides successfully suppressed the population of plant-parasitic nematodes on both roots and soil (Pelinganga, 2013; Pelinganga *et al.*, 2012). Mahmood *et al*. (1979) also argued that phytotoxicity had been one of the major limiting factors in the successful adoption of phytonematicides in management of nematode population densities.

1.1.2 Impact of the research problem

Large parts of South Africa had been reported to be infested with plant-parasitic nematodes (De Waele and Jordaan, 1988; Fourie *et al*., 2001; Hugo and Malan, 2010). The damage caused by plant-parasitic nematodes on roots could reduce the ability of plants to absorb the available soil moisture and nutrients, which could result in lack of vigour and yield loss (Anwar and McKenry, 2010; Mashela *et al.*, 2015; Trudgill, 1992). Nemarioc-AL and Nemafric-BL phytonematicides had been effective in controlling plant-parasitic nematode population densities under several conditions (Mashela *et al*., 2017a). According to Liu *et al.* (2003), allelochemicals contained in most phytonematicides are invariably phytotoxic, and could lead to yield loss.

1.1.3 Possible causes of the research problem

Nemarioc-AL phytonematicide contains the active ingredient cucurbitacin A which decomposes to cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) and Nemafric-BL phytonematicide contains cucurbitacin B ($C_{32}H_{48}O_8$) (Chen *et al*., 2005; Jeffrey, 1978). However, the two phytonematicides regardless of their capability to manage root-knot (*Meloidogyne* spp.) nematodes, if applied inappropriately could reduce growth of different plant species by approximately 50% to complete crop failure (Mashela *et al*., 2015). Phytotoxicity remains a predicament in adoption of phytonematicides, and it was observed on different crops at high concentration (Mafeo and Mashela, 2009).

1.1.4 Proposed solutions

Using the Curve-Fitting Allelochemical Response Data (CARD) computer-based model (Liu *et al*., 2003), two of the seven biological indices, namely, threshold stimulation (D_m) and saturation point (R_h) would be adapted in developing the mean concentration stimulation point (MCSP) values. The MCSP [= D_m + (R_h/2)] had since been used widely in botinemagation technology to manage the population of *Meloidogyne* species (Mashela *et al*., 2015; Pelinganga and Mashela, 2012; Pelinganga, 2013). The MCSP had been described as the concentration of a phytonematicide that was not phytotoxic, while stimulating plant growth and suppressing nematode population densities (Mashela *et al*., 2017a; Pelinganga, 2013).

1.1.5 General focus of the study

The study focused on assessing cucurbitacin residues, non-phytotoxic concentration and selected foliar mineral elements of tomato plant to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides under field.

1.2 Problem statement

Locally developed Nemarioc-AL and Nemafric-BL phytonematicides have been successfully evaluated on nematode suppression, phytotoxicity on crops and on residues of tomato fruits using 3%. However, there is dearth information on chemical residues of Nemarioc-AL and Nemafric-BL phytonematicides under high increasing concentration in soil and plant produce. Also, the behaviour of essential mineral of tomato leaf tissues to increasing concentration of the two phytonematicide had not been documented. It was

therefore crucial for the researcher to assess residues of the two phytonematicides under increasing concentration in soil and tomato fruit.

1.3 Rationale of the study

Worldwide, tomato is one of the most important crops grown for their nutritional value and health benefits and are highly susceptible to *Meloidogyne* species (Pelinganga, 2013). Following the withdrawal of synthetic chemical nematicides, Nemarioc-AL and Nemafric-BL phytonematicides had been developed as alternatives to synthetic nematicides (Mashela *et al*., 2017a). However, the two phytonematicides contain allelochemicals as their active ingredients, which were shown to be highly phytotoxic to various crops protected against nematodes. Therefore, assessing cucurbitacin residues, tomato plant growth and foliar nutrient elements accumulation under increasing concentration would provide information required about the efficacy of the two phytonematicides under field conditions.

1.4 Purpose of the study

1.4.1 Aim

To determine potential cucurbitacin residues, non-phytotoxic concentration and foliar nutrient elements to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides on tomato plant growth under field conditions.

1.4.2 Objective

To determine whether increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides would result in cucurbitacin residues in tomato plant, to generate mean concentration stimulation point (MCSP) values, overall sensitivity (∑k) and selected foliar mineral elements of tomato plant.

1.4.3 Hypothesis

Increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides would result in cucurbitacin residues in tomato plant, generate MCSP values, ∑k and selected foliar mineral elements of tomato plant.

1.5 Reliability, validity and objectivity

The reliability of data was achieved through statistical analysis of data at the probability level of 5%, validity was achieved by repeating the experiments in time, and while objectivity was achieved by ensuring that the findings were discussed based on empirical evidence, as to eliminate all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

Bias was reduced through minimising the experimental error by increasing the number of replications on the experiments conducted. The treatments were also randomised within the selected experimental design (Leedy and Ormrod, 2005).

1.7 Scientific significance of the study

The study was intended to assess cucurbitacin residues, growth and selected nutrient elements on leaf tissues of tomato when exposed to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides under field conditions. Eventually the information from the CARD computer-based model would be useful concerning the concentration that would not be phytotoxic to tomato plant growth and accumulation of nutrient elements on leaf tissues, while assessing also their level of sensitivity to the two products. The MCSP values would suppress nematode number while stimulating plant growth and maintaining nutrient element accumulation. Synthetic nematicides were expensive and detrimental, therefore, the use of botanicals in tomato production to control nematodes will be affordable and friendly to the environment.

1.8 Structure of mini-dissertation

The research problem of the study was introduced in Chapter 1, the work done, and the work not done on the problem statement being reviewed in Chapter 2. The research related to the objective was addressed in Chapter 3. The summary of findings, significance, recommendations with respect to future research and with the conclusions that were intended to provide a take home message regarding the current study are in Chapter 4. The citation and references followed the Harvard style of author-alphabet as approved by the Senate of the University of Limpopo.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Internationally, synthetic chemical nematicides had been withdrawn from the agrochemical markets due to their toxicity nature to the environment, non-target organisms and chemical residues in produce (Aktar *et al*., 2009; Chitwood, 2003). Following the withdrawal of synthetic chemical nematicides, nematode damage escalated to as high as 37% (Mashela *et al*., 2017a), necessitating the development of the alternative nematode management options. Most research workers revisited the concept of nematode resistance in crops (Fuller *et al.*, 2008; Williamson and Kumar, 2006). However, due to challenges of nematode races (Pofu *et al.*, 2012; Robertson and Diez-Rojo, 2008; Winter *et al.*, 2006), high temperatures (Karuri *et al.*, 2017), increasing salinity (Zaki *et al.*, 2012) and sucking insects (Wondafrash *et al.*, 2013), which break nematode resistance in various crops, other management options had to be developed. Among the options were phytonematicides, which were introduced as a mitigation strategy to drawbacks of conventional organic amendments in suppression of nematodes in context of climate-smart agriculture (Mashela, 2002). Nemarioc-AL and Nemafric-BL phytonematicides developed from dried fruits of wild cucumber (*Cucumis myriocarpus* Naudin) and wild watermelon (*Cucumis africanus* L.F.), respectively, had been highly effective in suppressing root-knot (*Meloidogyne* species) nematodes on various crops (Mafeo and Mashela, 2009; Mashela *et al*., 2017a; Mathabatha *et al*., 2016; Pelinganga *et al*., 2012; Sithole, 2016). The products were, in other crops, shown to be phytotoxic,

which was resolved through the computer-based model (Mashela *et al.*, 2017a), namely, the Curve-Fitting Allelochemical Response Data (CARD) model (Liu *et al*., 2003).

In successful production systems involving any innovative inputs, nutrient elements, whether macro- or micro-nutrient elements are required and could play a vital role in the successful use of the products. Generally, any detrimental effect related to the products' stimulating or inhibiting physiological activities related to nutrient elements, could be viewed unfavourably by the registration authorities of such inputs in agriculture (Mashela *et al.*, 2017a). Information on responses of plants to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides under field conditions is limited.

2.2 Work done on the problem statement

2.2.1 Chemical residues in produce from phytonematicides

The presence of chemical residues in food and feed had been a major problem during the pesticide era (Keikotlhaile and Spanoghe, 2011). The use of botanicals has been a breakthrough in management of pests since synthetic pesticides had been withdrawn from agrochemical markets. Two cucurbitacin-containing phytonematicides namely, Nemarioc-AL and Nemafric-BL phytonematicides had been tested for residues in tomato fruit (Dube, 2016; Shadung *et al*., 2017). Nemarioc-AL and Nemafric-BL phytonematicides were applied at a concentration of 3%, according to their respective application intervals, withholding a period of 15 days no cucurbitacin residues in tomato fruit were traced (Shadung *et al*., 2017). Similary, when neem products were used on strawberry (*Fragaria ananassa*), azadirachtin chemical residues in berries were not

detected at 7 days after application (Caboni *et al*., 2006). In contrast, Caboni *et al.* (2002), reported that in 1 to 7-day withholding period, azadirachtin ($C_{35}H_{44}O_{16}$) residues were traced in olive (*Olea europaea*) but the residues declined rapidly from 0.35 ppm in Day-1 to less than 0.02 ppm in Day-7 after application. Simeone *et al.* (2009) postulated that although azadirachtin was one of the chemicals used in the trials, it was not detected on the olives treated, even when sampling was made within 24 hours after application, possibly because of the inadequate sensitivity of the method and the rapid decay of this plant extracted chemical. However, rotenone among azadirachtin and pyrethrins was found to be a very stable compound on olives, its decay was quite rapid in the early days after its application (Simeone *et al.*, 2009). Botanicals have recently attracted many scientists, moreover with chemical properties they carry.

2.2.2 Efficacy of phytonematicides in liquid formulation

The use of fermented plant extracts with nematicidal properties had been a success in managing plant-parasitic nematodes as alternatives to synthetic chemical nematicides. Olabiyi and Ayeni (2016) assessed neem (*Azadirachta indica*) and Asian spider flower (*Cleome viscosa*) in liquid formulations as bio-nematicides, where results suggested that the two plant extracts could reduce nematode population densities within the nematode genera *Meloidogyne, Trichodorus, Paratrichodorus, Helicotylenchus and Xiphinema*. Also, the results indicated that *A. indica* and *C. viscosa* liquid formulations significantly improved growth and yield of okra planted on nematode-infested soil when compared to untreated nematode-infested control (Olabiyi and Ayeni, 2016).

Roots of marigold (*Calendula officinalis*) and basil (*Ocimum basilicum*) were collected to develop aqueous root extracts to control population of *Meloidogyne* species (Olabiyi, 2008). Overall, the aqueous plant root extracts reduced nematode population densities in soil and root galls when compared with those on untreated control. Additionally, the treatments also increased plant height, leaf size and fruit yield (Olabiyi, 2008). Increasing concentration (2 to 10 ppm) of leaf extracts of *Ageratum conyzoide* reduced population of *M. incognita* on mung bean (*Vigna mungo)* crop, with resultant improved plant growth (Pavaraj *et al.,* 2010). Similarly, at 20, 40, 60, 80 and 100% neem leaf liquid formulation on nematode population densities in cowpea (*Vigna unguiculata* L. Walp) were better when compared to similar concentration of synthetic chemical nematicide carbofuran (Nwankwo *et al*., 2016), with plant height, pod number, leaf number and pod mass versus increasing concentration of neem exhibiting significant relations.

In liquid formulation, Nemarioc-AL and Nemafric-BL phytonematicides had been suppressing nematode population densities of *Meloidogyne* species (Chokoe, 2017; Mathabatha *et al*., 2016; Pelinganga *et al*., 2012; Sithole, 2016) and the citrus nematode (*Tylenchulus semipenetrans*) (Maile, 2013; Mashela *et al.*, 2017b) under various conditions. Nemarioc-AL phytonematicide reduced population densities of *M. incognita* race 2 in roots and soil under field conditions by 79-85% and 79-85%, respectively (Pelinganga, 2013), under microplot conditions by 61% and 52%, respectively (Pelinganga, 2013) and under greenhouse conditions by 97-99% and 47-90%, respectively (Pelinganga *et al*., 2012; Pelinganga, 2013). Nemafric-BL phytonematicide was also applied under the three respective conditions, where *M. incognita* race 2 was

reduced under field conditions in roots and soil by 79-85% and 79-85%, respectively, under microplot conditions by 72% and 77%, respectively and under greenhouse conditions by 85-97% and 45-96%, respectively (Pelinganga *et al*., 2012; Pelinganga, 2013).

Chokoe (2017) observed that Nemarioc-AL phytonematicide reduced the second-stage juveniles (J2's) on roots and soil under microplot conditions by 65-100% and 52-100%, respectively, whereas Nemafric-BL phytonematicide reduced J2 and eggs on roots by 87-100% and 64-100%, respectively. Consubstantial results were reported when fermented lantana (*Lantana camara* L.) was used as a phytonematicide, with nematode numbers on roots and soil under microplot conditions being reduced by 53-85% and 47-73%, respectively, and under greenhouse conditions by 42-71% and 47-73%, respectively (Malatji, 2017). According to Taye *et al*. (2013), inhibitory effects of botanical extract on *M. incognita* J2 were dependent on concentration, as previously observed in other entity-allelopathic relations (Liu *et al*., 2003; Mashela *et al*., 2017a).

2.2.3 Phytotoxicity in phytonematicides

Plants produce a wide range of chemical compounds, which are responsible for various physiological functions, some of which had not been identified (Mashela *et al.*, 2017a). According to Macheix *et al.* (1990), allelochemicals could be highly compartmentalised in plant organs, with most being in roots, barks and fruits, whereas at most leaf tissues serve as temporary storage areas (Weston, 1996). In *C. myriocarpus*, for instance, which is used for the manufacturing of Nemarioc-AL phytonematicide, the active ingredient

cucurbitacin A is stored in roots and fruit (Jeffrey, 1978), where in *C. africanus*, used for Nemafric-BL phytonematicide, the active ingredient cucurbitacin B is stored in all plant organs (Jeffrey, 1978).

Phytotoxicity is one of the major constraints in the development of the phytonematicides, and it had been reported as a major setback in the potential registration of the locally developed phytonematicides (Mafeo and Mashela, 2009; Mashela *et al.*, 2015; Pelinganga, 2013). Susan *et al*. (2008) assessed phytotoxicity of clove oil derived from the clove plant (*Syzygium aromaticum*) at 0.1, 0.2 and 0.3% concentration, where at 0.3% clove oil was the most phytotoxic, with increased mortalities in comparison to plants on untreated control in all crops except for pepper (*Capsicum annuum*) (Susan *et al*., 2008). Similarly, Ghasemi *et al.* (2012) reported that aqueous extracts (leaf, stem and roots) of milkweed (*Calotropis procera*) application at maximum concentration (40%) reduced all the variables. Therefore, it was shown that aqueous extracts of *C. procera* were allelopathic to growth of weed and yield of wheat (*Triticum aestivum*) (Ghasemi *et al*., 2012).

Similarly, when African geranium (*Pelargonium sidiodes* DC.) was exposed to Nemarioc-AL and Nemafric-BL phytonematicides, the products phytotoxic effects at high concentration on the test crop (Sithole, 2016). At low concentration root latches from golden crown beard (*Verbesina encelloides* Cav.) had stimulation effects on growth of various plant species consistently (Inderjit *et al.*, 1999). Similarly, Mashela (2002)

observed that Nemarioc-BG phytonematicide stimulated growth of tomato plants which gave the impression that the phytonematicide has a 'fertilizer effect' at low concentration.

Yu and Matsui (1997) evaluated the role of cucumber-exuded allelochemicals on the uptake of several nutrients (N, P, S, K, Ca, Mg) by intact seedlings of cucumber, while cinnamic acid inhibited the uptake of nearly all nutrients. Similar observations were reported when Kobza and Einhellig (1987) treated sorghum seedlings with ferulic acid (0.25 and 0.5 mM) to evaluate its effects on tissue concentration of nutrients (K, Mg, P, Zn, Fe, Ca). Roots and shoots of treated plants had lower concentration of K, Mg, Fe, and P than the control. Consequently, water extracts of weeds-little seed canary grass (*Phalaris minor* Retz*.),* water pepper (*Polygonum hydropiper*) and lamb's quarters (*Chenopodium album*) reduced uptake of P and Zn in wheat roots and shoots with little seed canary grass having the greatest effect (Chakraverty *et al*., 2005).

2.2.4 Managing phytotoxicity

Curve-fitting Allelochemical Response Data (CARD) computer-based model: Liu *et al*. (2003) developed CARD model which involves the use of data collected to assess stimulation-inhibition the response of organisms to increasing concentration of allelochemicals. According to Mashela *et al.* (2017a), the CARD model quantifies three phases, namely, stimulated, neutral and inhibited, which had been described as the nematicidal, neutral and herbicidal zones (Mashela *et al*., 2017a). Generally, growth of organisms could be stimulated and inhibited by phytonematicides at stimulating (D_m-R_h) and inhibiting (D_0-D_{100}) concentration, respectively (Mashela *et al.*, 2015). Similarly, when

plant organs were subjected to phytonematicide, the three phases were observed in several crops. However, the three phases on plants tested were dependent on the concentration applied.

Mean concentration stimulation point (MCSP): The MCSP had been referred to as the concentration of a phytonematicide which could stimulate plant growth, while suppressing nematode population densities (Pelinganga *et al*., 2012). However, the CARD model characterises seven biological indices and the latter included (1) threshold stimulation (D_m), (2) saturation point (R_h), (3) 0% inhibition (D_0), (4) 50% inhibition (D_{50}), (5) 100% inhibition (D_{100}), (6) degree of sensitivity in stimulation (k) and (7) coefficient of determination (R^2) (Liu *et al*., 2003). Pelinganga (2013) developed MCSP values of 3% for Nemarioc-AL and Nemafric-BL phytonematicide under greenhouse and use it as a constant concentration for tomato cv. ′Floradade′ under field conditions.

Overall sensitivity index (∑k): The MCSP values should be interpreted with the overall k-values of the phytonematicide on the crop (Liu *et al.*, 2003; Mashela *et al*., 2015). The relationships generated through the dosage model could depend mainly on the k-values, with k being the number of *In*(D + 1) transformations that indicated the degree of sensitivity of the variables to the allelochemicals. The closer the (∑k) of the plant is to zero, the higher the sensitivity of the variable to the test allelochemical, while the opposite is true (Liu *et al*., 2003).

2.2.5 Responses of selected nutrient elements

A mineral element is considered essential to plant growth and development if the element is involved in plant metabolic functions and the plant cannot complete its life cycle without the element (Martens and Westerman, 1991). Normally the plant displays a visual symptom indicating a deficiency in a specific nutrient, which can be corrected by supplying the nutrient. However, the conditions which crops are being produced play a vital role in accumulation or absorption by the plants grown. There are factors which can contribute to low accumulation of nutrient elements by crops such as soil type, rainfall, temperature and other, more especially in an open field production.

Potassium (K) in plants is needed in copious quantities in the meristematic tissues, buds, leaves and root tips (Ashley *et al.*, 2006). Potassium helps to maintain an anion-cation balance in cells and is involved in protein synthesis, opening and closing of stomata, activation of enzymes and in the maintenance of the turgidity of cells (Shabala and Lew, 2002). According to Marschner (1995), K has high mobility in plants and they are richly contained in young leaves and fruits, since solutes of phloem can be translocated both upwards and downwards (Mengel and Kirkby, 2001). Potassium deficiency could slow down growth of crops as first sign and the plant stop growing completely, thus leading to stunted plant growth.

According to Welch (2002), all crops respond positively to application of zinc (Zn) and its deficiency could be found in every part of the world. Trace elements such as Zn are inherited normally from the soil which are formed from the rocks through geochemical

and pedochemical weathering processes (Hafeez *et al*., 2013). High pH, organic matter, clay and phosphate can fix Zn in the soil and risen the reduction level of available Zn. Generally, the deficiency of Zn is expected in calcareous soils, sandy soils, peat soils and soils with high phosphorus (Alloway, 2008). Zinc deficiency in soil may cause plants to die, while those which recover will have a delayed maturity and yield reduction. However, the lower the amounts of Zn in the soils, the lower the absorption rate and synthesis in plant organs. By contrast, high Zn concentration in plants can cause phytotoxicity, thus resulting in reduced yields.

Sodium (Na) can be regarded to as an essential element for crops although is required in smaller quantities in crops (Maathuis, 2009), it has been observed many times that during deficiency of potassium many plants respond positively to Na (Subbarao *et al*., 2003). However, Na in some plants can be used as a substitute for potassium and helps in opening and closing of stomata, which regulate internal water balance. Sodium has high level of mobility as potassium in crops. Amtmann and Sanders (1999) postulated that Na^+ and K^+ are chemically and structurally very similar, in hydrated form. According to (Maathuis, 2013) sodium compete with potassium, calcium, magnesium and ammonium for uptake by the plant. Low levels of sodium can be beneficial in many conditions, whereas moderate and prominent levels of salt are said to be harmful to most of plants (Flowers and Colmer, 2008).

Recently, few studies have been conducted to evaluate the response of mineral nutrient when exposed to naturally developed bio-pesticides/bio-nematicides as one of the control

to targeted pests. Mashela and Pofu (2017) assessed the influence of cucurbitacin containing phytonematicides, namely, Nemarioc-AL and Nemafric-BL phytonematicides on plant growth variables, nematode suppression and nutrient elements (Ca, K, Na, Fe, Zn) in leaf tissues of green bean (*Phaseolus vulgaris*) at 56 days after initiating the treatments. However, Ca, K, Na and Fe each with increasing concentration of Nemafric-BL exhibited positive quadratic relationship. In contrast, Na and Zn each with increasing concentration of Nemarioc-AL phytonematicide exhibited negative quadratic relationship, whereas K and Fe, each with increasing concentration of Nemarioc-AL phytonematicide displayed positive quadratic relationship. Rathore *et al.* (2009) evaluated the effect of seaweed (*Kappaphycus alvarezii* Doty) extracts on the growth, yield and uptake of nutrient uptake of soybean (*Glycine max* L.), whereby increasing concentration of *K. alvarezii* improved uptake of nutrients (N, P, K and S) on soybean crop under rain-fed conditions.

2.3 Work not yet done on the problem statement

Assessment of cucurbitacin residues, tomato plant growth and behaviour of selected nutrient elements in tomato leaf tissues when exposed to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides under field conditions remains undocumented. Therefore, assessment of growth and nutrient elements of tomato will fulfil the gap observed, as outlined in the problem statement.

2.4 Addressing the identified gaps

Further studies should be conducted to assess growth and behaviour of nutrient elements in tomato fruit treated with increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides. Furthermore, it is necessary to evaluate the potential cucurbitacin residues of the two phytonematicides on different commercial crops under wide range of conditions.

CHAPTER 3
INFLUENCE OF PHYTONEMATICIDES ON CUCURBITACIN RESIDUES, GROWTH AND SELECTED NUTRIENT ELEMENTS IN LEAF TISSUES OF TOMATO CULTIVAR 'FLORADADE'

3.1 Introduction

Worldwide, the protection of crops against plant-parasitic nematodes had previously relied much on synthetic fumigant chemical nematicides (Aktar *et al*., 2009; Mashela, 2007; Mashela *et al*., 2008). Due to environment-unfriendliness of these products, most were withdrawn from the agrochemical markets, with methyl bromide eventually withdrawn internationally in 2005 (Mashela *et al*., 2017a). The focus had since been on alternative nematode management strategies, such as the use of phytonematicides, with much caution being on ensuring that such alternatives did not repeat the challenges of synthetic chemical pesticides.

Nemarioc-AL and Nemafric-BL phytonematicides, had been researched and developed for various crops (Mashela *et al*., 2017a), with limited information on cucurbitacin residues in soil and edible tomato organs. However, their adoption had setbacks which had been phytotoxicity and inconsistent results in terms of nematode suppression (Mashela *et al.*, 2015). To ameliorate the incident of phytotoxicity in phytonematicide, Mashela *et al.* (2017a) used the Curve-Fitting Allelochemical Response Data (CARD) model by Liu *et al*. (2003) to determine the non-phytotoxic concentration. Therefore, the objective of this study was to determine whether increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides would result in cucurbitacin residues in tomato plant, to generate mean concentration stimulation point (MCSP) values, overall sensitivity (∑k) and selected foliar mineral elements of tomato plant.

3.2 Materials and methods

3.2.1 Description of the study site

Two parallel experiments of Nemarioc-AL and Nemafric-BL phytonematicides were conducted at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, Limpopo Province, South Africa (23°53'10"S, 29°44'15"E) in autumn (March-May) and repeated in spring (August-October) in 2017. The location has summer (November-January) rainfall with mean annual rainfall of less than 500 mm, whereas maximum/minimum temperatures average 38 /5°C. The soil at the site is predominantly Hutton sandy loam, containing 65% sand, 5% silt, 30% clay, 1.6% organic carbon, with electrical conductivity (EC) 0.148 $dS.m^{-1}$ and pH (H_2O) 6.5.

3.2.2 Treatments and research design

Each trial had seven treatments namely, 0, 2, 4, 8, 16, 32 and 64% phytonematicide, arranged in a randomised complete block design (RCBD), with five replications.

3.2.3 Procedures

Cultural practices: Tomato cv. 'Floradade' seedlings were hardened-off for a week through intermittent withholding of irrigation water outside the greenhouse. Uniform four-week-old tomato seedlings were transplanted in the field with 0.6 m × 0.6 m inter- and intra-row spacing (Figure 3.1). Three days after transplanting, each plant was fertilised with 5 g NPK 2:3:2 (26) + 0.5% Zn + 5% S and 5% Ca and 1 g 2:1:2 (43) Multifeed (Nulandies, Johannesburg) which provided a total of 0.175 mg N, 0.16 mg K and 0.16 mg P, 0.45 mg, 0.378 mg Fe, 0.0375 mg Cu, 0.175 mg Zn, 0.5 mg B, 1.5 mg Mn and 0.035

mg Mo per ml water (Mashela, 2002), without Ca. The two phytonematicides were prepared as described previously (Mashela *et al*., 2017a). Treatments were initiated seven days after transplanting. Plants were irrigated every other day for 2 h using drip irrigation system which is equivalent to 2 L with a spacing of 0.6 m in between. Treatments were applied once weekly as a supplement to irrigation with 300 ml container per plant, whereas untreated control plants were each irrigated with chlorine-free tapwater with 300 ml cup. Pests were scouted and monitored daily, whereas disease was managed using Adama, Dithane M-45 and Copper-oxide as per label instructions.

Phytonematicides preparation: Approximately 80 and 40 g ground material of *Cucumis myriocarpus* and *Cucumis africanus*, respectively, was fermented in 20 L-hermetically sealed plastic containers with 16 L chlorine-free tapwater. Allowance for released CO_2 to escape from the 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 litre bottle half-filled with tapwater. Approximately 300 ml molasses, 100 g brown sugar and 300 ml ZZ2 effective microorganisms (EM) was added into the container (Pelinganga *et al*., 2012). After a 14-day incubation period, when pH was at least ±3.7 (Kyan *et al*., 1999), phytonematicides were applied once a week as substitute to irrigation. Therefore, the product from *C. myriocarpus* manufactured Nemarioc-AL phytonematicide, whereas *C. africanus* formulated Nemafric-BL phytonematicide. Treatments namely, 0, 2, 4, 8, 16, 32 and 64% phytonematicide were calculated as follows, for example in a 10 L bucket, 2% of phytonematicide is referred to as 200 mL of Nemarioc-AL or Nemafric-BL phytonematicide mixed with 9800 mL of water which makes it 10 L of solution.

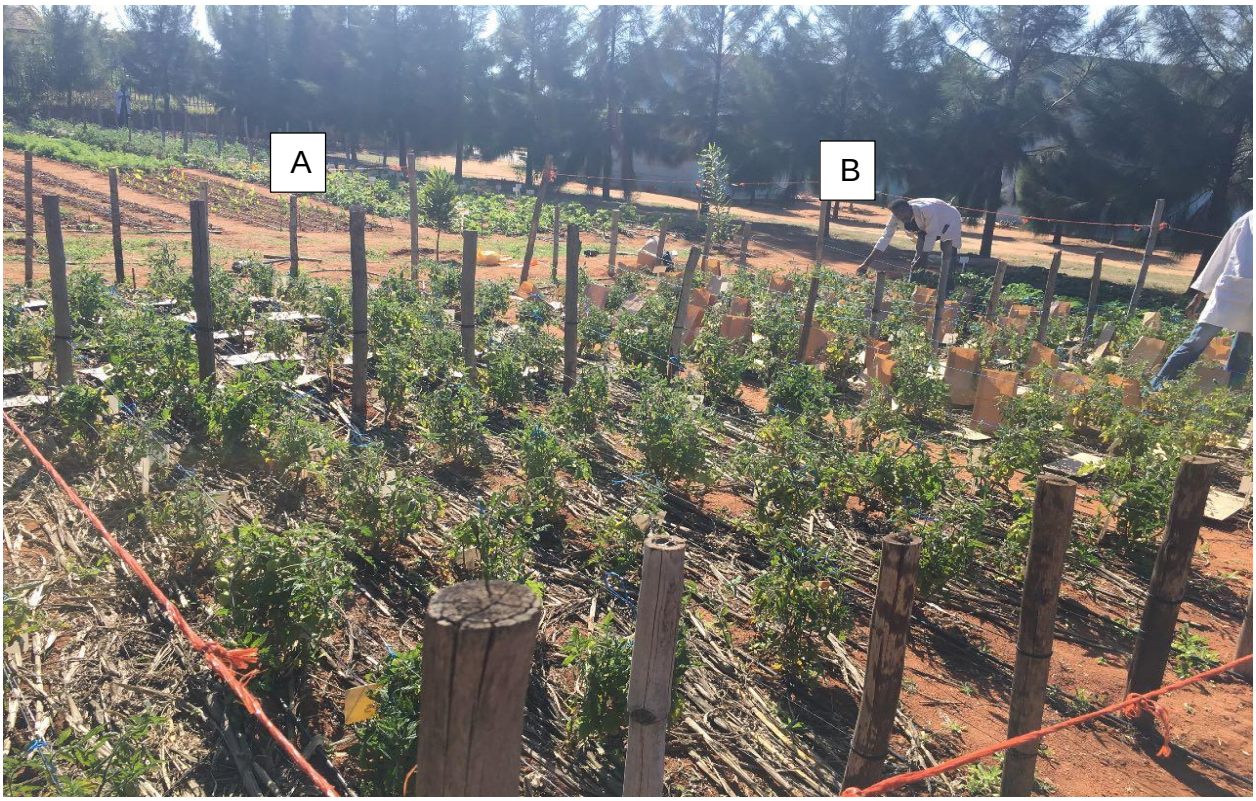

Figure 3.1 Layout of (A) Nemarioc-AL and (B) Nemafric-BL phytonematicide experiments at 64 days after initiation of treatments.

3.2.4 Data collection

Extraction and quantification of cucurbitacin residues: Fruit of tomato were cut into small pieces, oven-dried at 52°C for 72 h and ground in a Wiley mill. Approximately 250 mg soil sample was collected/planting station at harvest and thereafter at three months from soil depth of 0-25 cm and 25-50 cm for the determination of cucurbitacin residues. The soil samples were dried at 40°C for 24 h. A representative subsample 4 g of dried crude extracts of each organ and 10.0 g dried soil were mixed in 25:25 ml methanol and dichloromethane and allowed to run in water bath at 40°C at 45 rpm for 4 h (Rotary Evaporator). After extraction, subsamples were concentrated by reducing the volume

from 100 to 30 mL under reduced pressure on a rotary evaporator and then 1 ml aliquot of each treatment was centrifuged at 4500 rpm for 10 min before filtering through 0.22 µm-pore filter (Miller, Sigma, South Africa). Cucurbitacin A and B were each tested with the isocratic elution Shimadzu HPLC Prominence (PerkinElmer, Kyoto, Japan) with Shimadzu CTO-20A diode array detector. A wide pore reverse phase C18 (25 cm × 4.0 mm, 5 µm) discovery (Sigma-Aldrich, Milan, Italy) with 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 and the wavelengths monitored at 230 nm for 43 min was performed to quantify the cucurbitacins. Pure (≈ 98%) cucurbitacin A and B standards (Wuhan ChemFaces Biochemical Co. Ltd., Wuhan, China) were each dissolved in methanol and then diluted at 0.02, 0.04, 0.06, 0.08 and 1.0 $\mu g.ml^{-1}$ to compare the retention times and the peak areas of standards and samples, as described (Shadung, 2016).

Plant variables: At 64 days after the treatments, plant height was measured from the surface to the tip of the flag leaf and chlorophyll content measured with chlorophyll meter (Konica, Minolta Spad-502, Osaka, Japan). Stems were severed at the soil surface and stem diameter was measured at 5 cm above the severed end using Vernier calliper. Tomato fruit were harvested once during termination, and were counted per plant and weighed for fresh fruit mass and later dried at 52°C for 72 h and weighed for dry fruit mass. Shoots were dried in air forced oven at 70°C for 72 h for dry shoot mass. Root systems were removed from the field, immersed in water to remove soil particles, blotted dry and weighed, and dried in an air-forced oven at 70°C for 72 h for dry root mass.

Essential mineral nutrients: A microwave digester (PerkinElmer, Titan MPS) was used for preparation and approximately 0.5 g ground leaf materials of tomato plant 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. Tomato leaf samples were analysed for K, Na and Zn using inductively coupled plasma emission spectrometry (Shimadzu, ICPE 9000, Johannesburg, South Africa).

3.2.5 Data analysis

Data were subjected to the CARD computer-based model. To avoid curve-fitting challenges with observations being crowded at lower concentration (Lui and An, 2005), equidistance's between observations were generated by transforming the geometric concentration using $\log_2$2-transformation (Causton, 1977). A $\log_2$2-transformation of the exponential series 2^0, 2^1, 2^2, 2^3, 2^4, 2^5 and 2^6% phytonematicide to result in 0, 1, 2, 3, 4, 5 and 6% phytonematicide, was used to generate biological indices (Tseke and Mashela, 2017). Plant variables and selected foliar nutrient elements were subjected to the CARD model to generate biological indices which allowed for the calculation of MCSP of phytonematicides on tomato and the related sensitivity values of tomato to the products (Pelinganga, 2013). MSCP and ∑k values were developed and reported with plant variables and foliar nutrient elements which exhibited positive quadratic relations, unless

stated otherwise. Therefore, plant variables and foliar nutrient elements exhibiting negative quadratic relations were subjected to the lines of best fit.

3.3 Results

3.3.1 Nemarioc-AL phytonematicide

Cucurbitacin residues: Using cucurbitacin A standard, residues of Nemarioc-AL phytonematicide were not detected in both soil and tomato fruit samples.

Plant growth variables: The seasonal interaction of variables was not significant and therefore data were pooled across the two seasons (n = 70). Plant height, chlorophyll content, stem diameter, number of fruit, dry fruit mass, dry shoot mass and dry root mass over increasing concentration of Nemarioc-AL phytonematicide exhibited positive quadratic relations (Figure 3.2 A, B). The models were explained by 95, 82, 96, 89, 83, 83 and 92% associations for plant height, chlorophyll content, stem diameter, number of fruit, dry fruit mass, dry shoot mass and dry root mass with increasing Nemarioc-AL phytonematicide concentration, respectively (Table 3.1). Two biological indices (D_m and R_h) were used in this study to obtain the MCSP value by using the formula $MCSP = D_m + (R_h/2)$, the MSCP in this study was established at 1.13% (Table 3.1). Plant variables had sensitivity (k) values of 0, 0, 0, 0, 20, 20 and 20 units, respectively, with the $\sum k$ of tomato cv. 'Floradade' being equivalent to 60 units (Table 3.1).

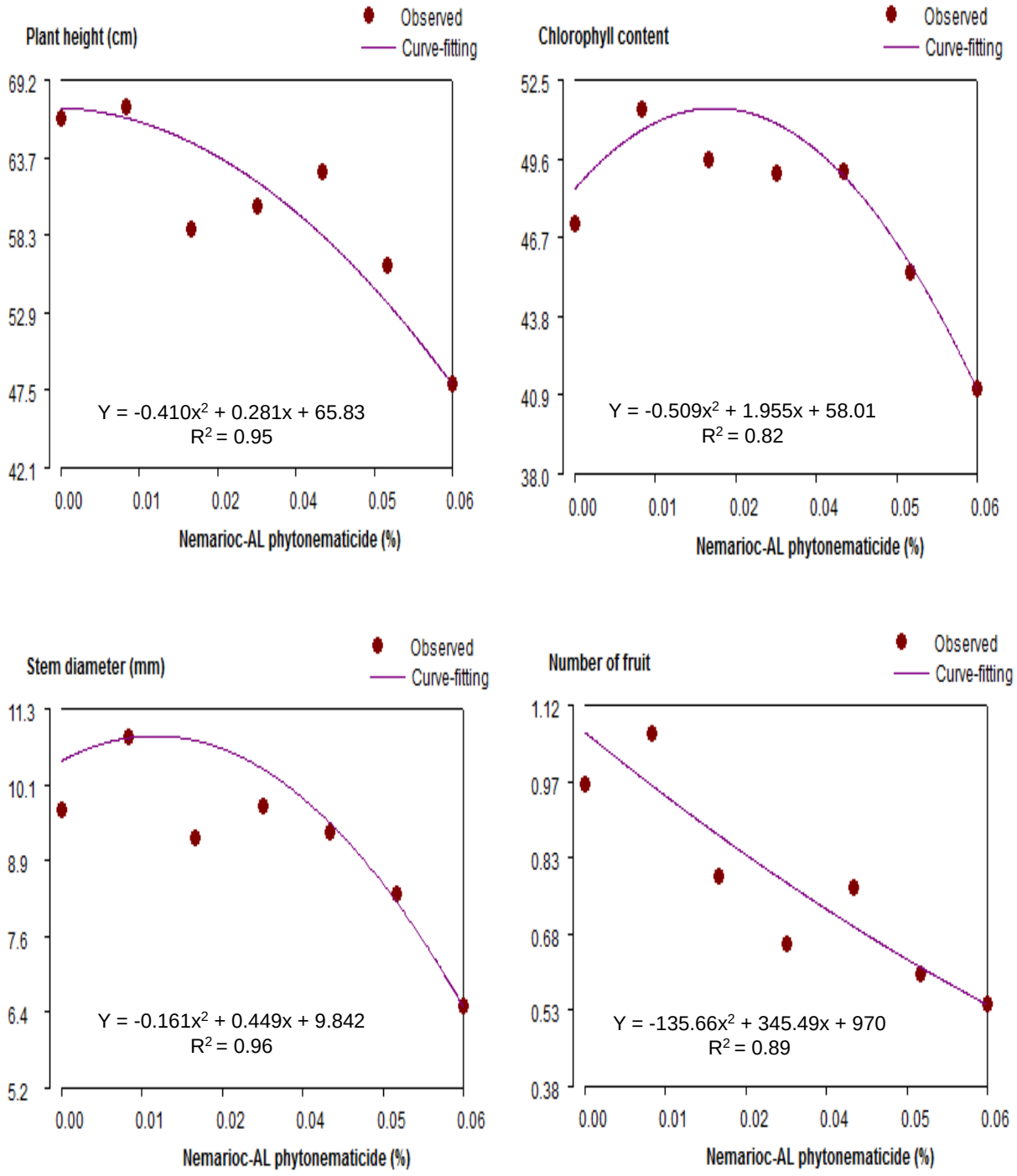

Figure 3.2 (A) Response of plant height, chlorophyll content, stem diameter and number of fruit of tomato cv. 'Floradade' to increasing concentration of Nemarioc-AL phytonematicide (n = 70).

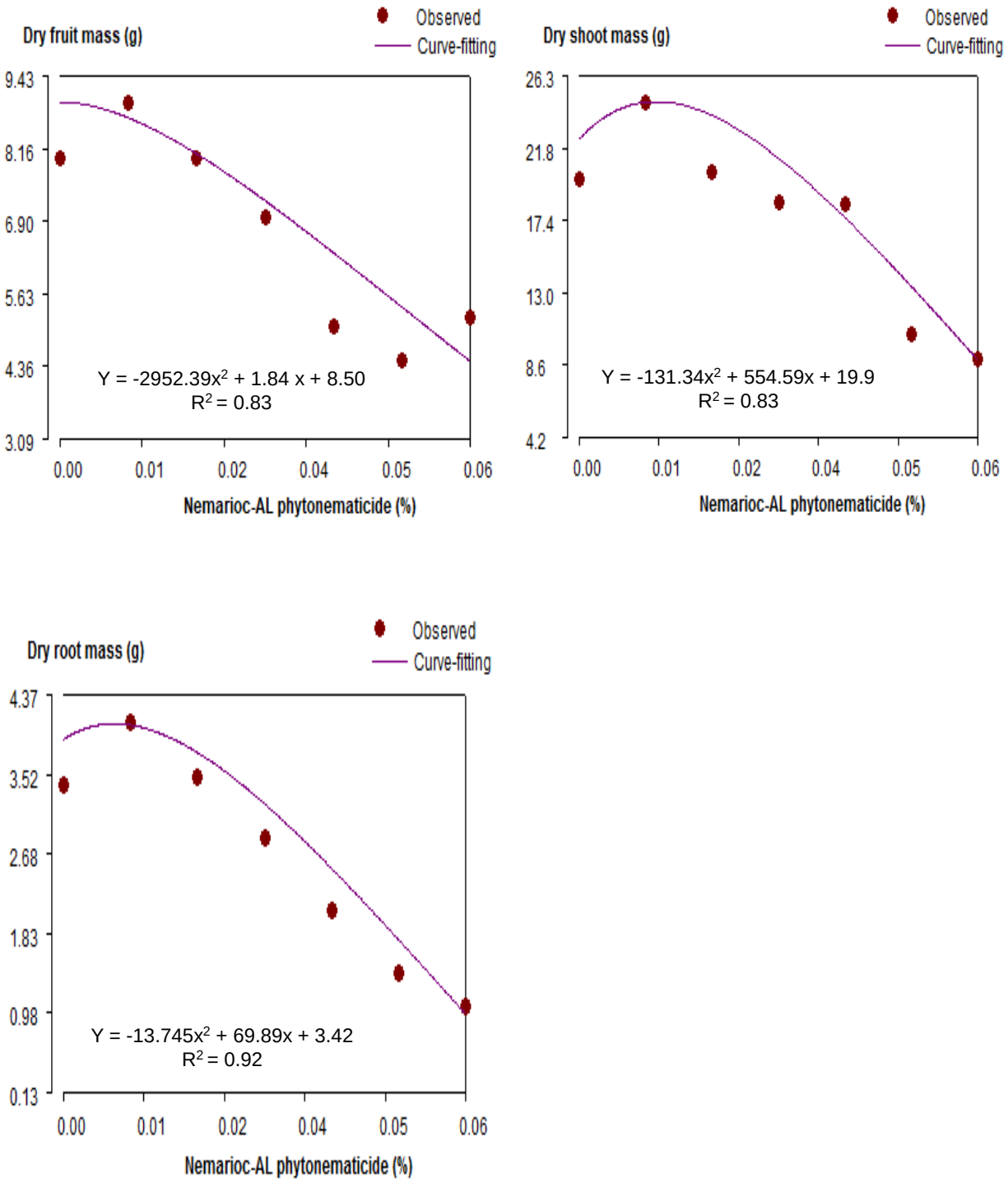

Figure 3.2 (B) Response of dry fruit mass, dry shoot mass and dry root mass of tomato cv. 'Floradade' to increasing concentration of Nemarioc-AL phytonematicide (n = 70).

Table 3.1 Biological indices for plant height (PHT), chlorophyll content (CHL), stem diameter (STD), number of fruit (NOF), dry fruit mass (DFM), dry shoot mass (DSM) and dry root mass (DRM) of tomato cv. 'Floradade' exposed to increasing concentration of Nemarioc-AL phytonematicide at 64 days after treatment initiation (n = 70).

Biological indices	PHT (cm)	CHL	STD (mm)	NOF	DFM (g)	DSM (g)	DRM (g)	Mean
Threshold stimulation (D_m)	0.26	0.21	0.41	0.20	0.01	0.13	0.08	0.19
Saturation point (R_h)	1.90	2.64	0.51	0.86	4.14	2.09	0.98	1.87
0% inhibition (D_0)	0	0.42	0.28	0	0.02	0.27	0.16	0.15
50% inhibition (D_{50})	0.87	0.87	0.71	0.65	0.06	0.55	0.47	0.53
100% inhibition (D_{100})	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.11
R^2	0.95	0.82	0.96	0.89	0.83	0.83	0.92	–
k – value	0	0	0	0	20	20	20	–

Overall sensitivity ($\sum k$) = 60.

MCSP = $D_m + (R_h/2)$ = 0.19 + (1.87/2) = 1.13%.

Selected nutrient elements in leaf tissues: Potassium, Na and Zn when exposed to increasing concentration of Nemarioc-AL phytonematicide exhibited positive quadratic relations (Figure 3.3), with the models were explained by 91, 96 and 89%, of the respective mineral nutrient elements (Table 3.2). Two biological indices (D_m and R_h) were used to obtain the MCSP value by using the formula MCSP = D_m + (R_h/2), the MSCP was equivalent to 2.49% (Table 3.2). Tomato foliar nutrient elements had k-values of 2, 19 and 0 units, respectively, with the ∑k of tomato foliar nutrient elements being equivalent to 21 units when exposed to increasing concentration of Nemarioc-AL phytonematicide (Table 3.2).

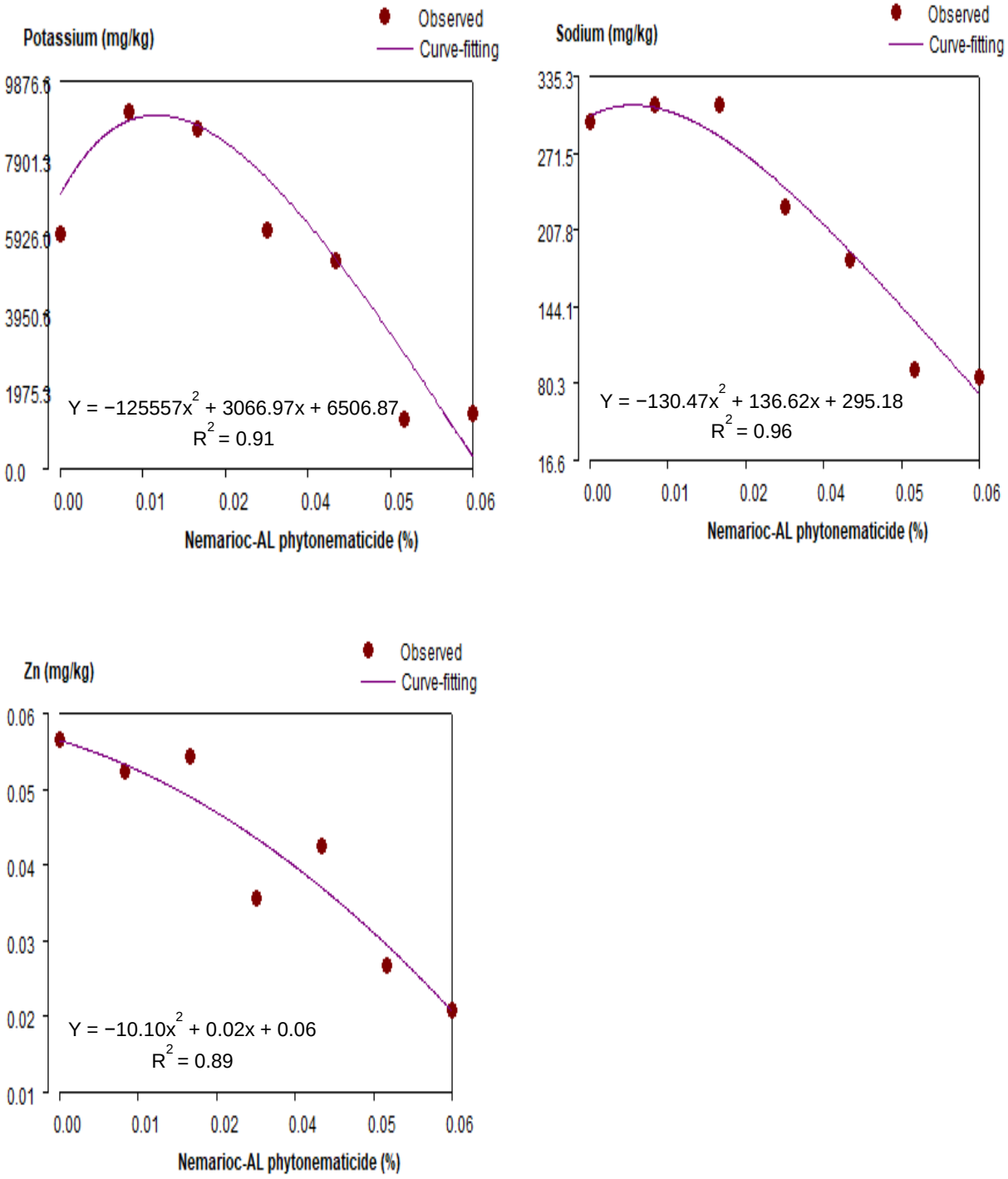

Figure 3.3 Response of K, Na and Zn leaf content of tomato cv. 'Floradade' to increasing concentration of Nemarioc-AL phytonematicide (n = 70).

Table 3.2 Biological indices for potassium (K), sodium (Na) and zinc (Zn) of tomato foliar nutrient elements exposed to increasing concentration of Nemarioc-AL phytonematicide at 64 days after treatment initiation (n = 70).

Biological indices	K (mg/kg)	Na (mg/kg)	Zn (mg/kg)	Mean
Threshold stimulation (D_m)	0.70	0.01	0.08	0.26
Saturation point (R_h)	3.74	9.60	0.05	4.46
0% inhibition (D_0)	2.83	0.01	0	0.95
50% inhibition (D_{50})	4.57	0.05	0.03	1.55
100% inhibition (D_{100})	6.80	0.10	0	2.30
R^2	0.91	0.96	0.89	–
k – value	2	19	0	–

Overall sensitivity ($\sum k$) = 21.

MCSP = $D_m + (R_h/2)$ = 0.26 + (4.46/2) = 2.49%.

3.3.2 Nemafric-BL phytonematicide

Cucurbitacin residues: Using cucurbitacin B standard, residues of Nemafric-BL phytonematicide were not detected in both soil and tomato fruit samples.

Plant growth variables: The seasonal interaction of variables was not significant and therefore data were pooled across the two seasons (n = 70). Plant height, chlorophyll content, stem diameter, number of fruit, dry fruit mass, dry shoot mass and dry root mass each with increasing Nemafric-BL phytonematicide concentration exhibited positive quadratic relations (Figure 3.4 A, B). The models were explained by 93, 83, 97, 96, 87, 94 and 96% associations for plant height, chlorophyll content, stem diameter, number of fruit, dry fruit mass, dry shoot mass and dry root mass with increasing Nemafricc-BL phytonematicide concentration, respectively (Table 3.3). Two biological indices namely, threshold stimulation and saturation point were used in the current study to develop the MCSP value by means of the formula MCSP = $D_m + (R_h/2)$, the MSCP in this study was established at 1.75%. Plant variables had k-values of 0, 0, 20, 5, 16, 4 and 0 units, respectively, whereas the $\sum k$ of 45 units (Table 3.3).

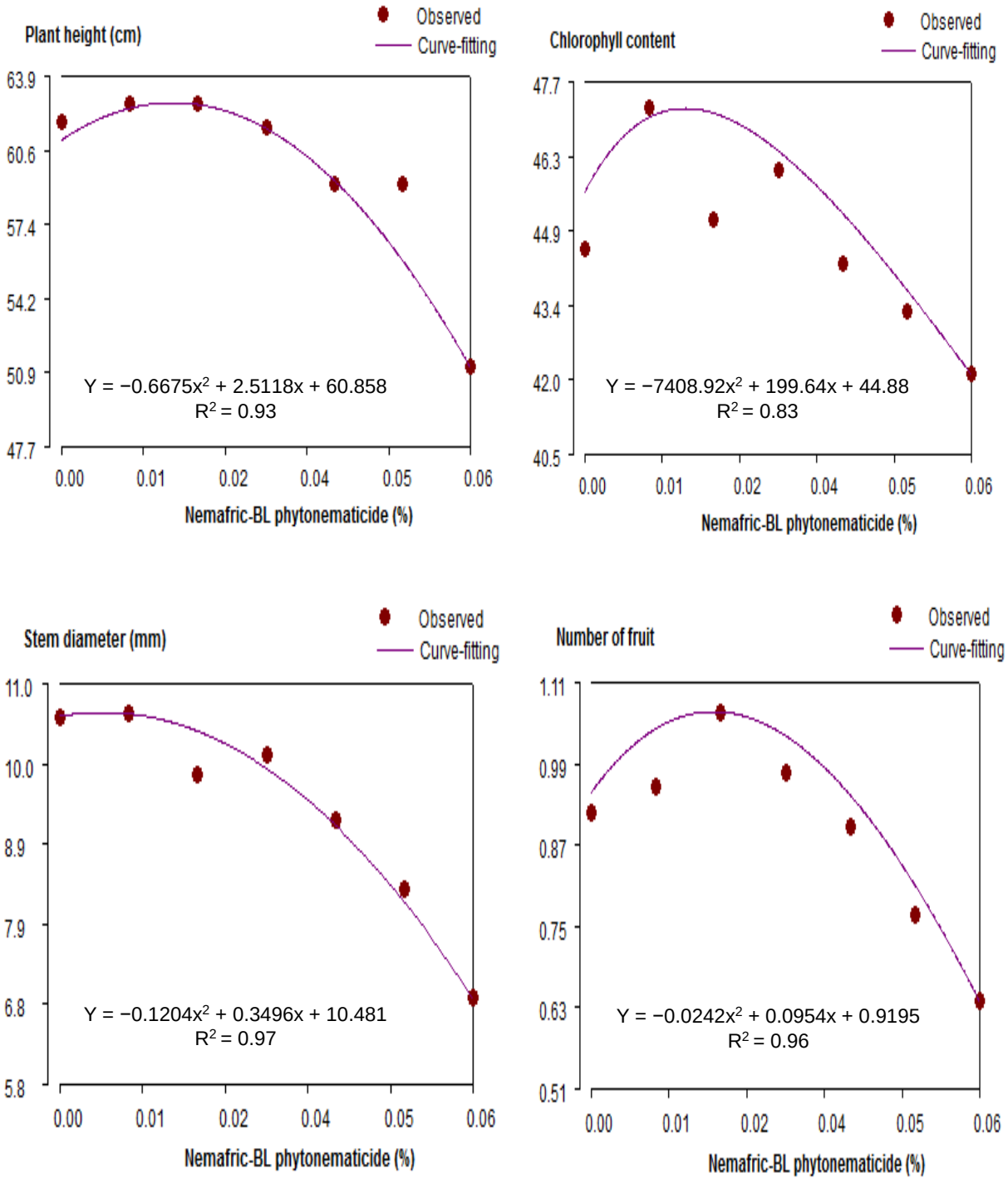

Figure 3.4 (A) Response of plant height, chlorophyll content, stem diameter and number of fruit of tomato cv. 'Floradade' to increasing concentration of Nemafric-BL phytonematicide (n = 70).

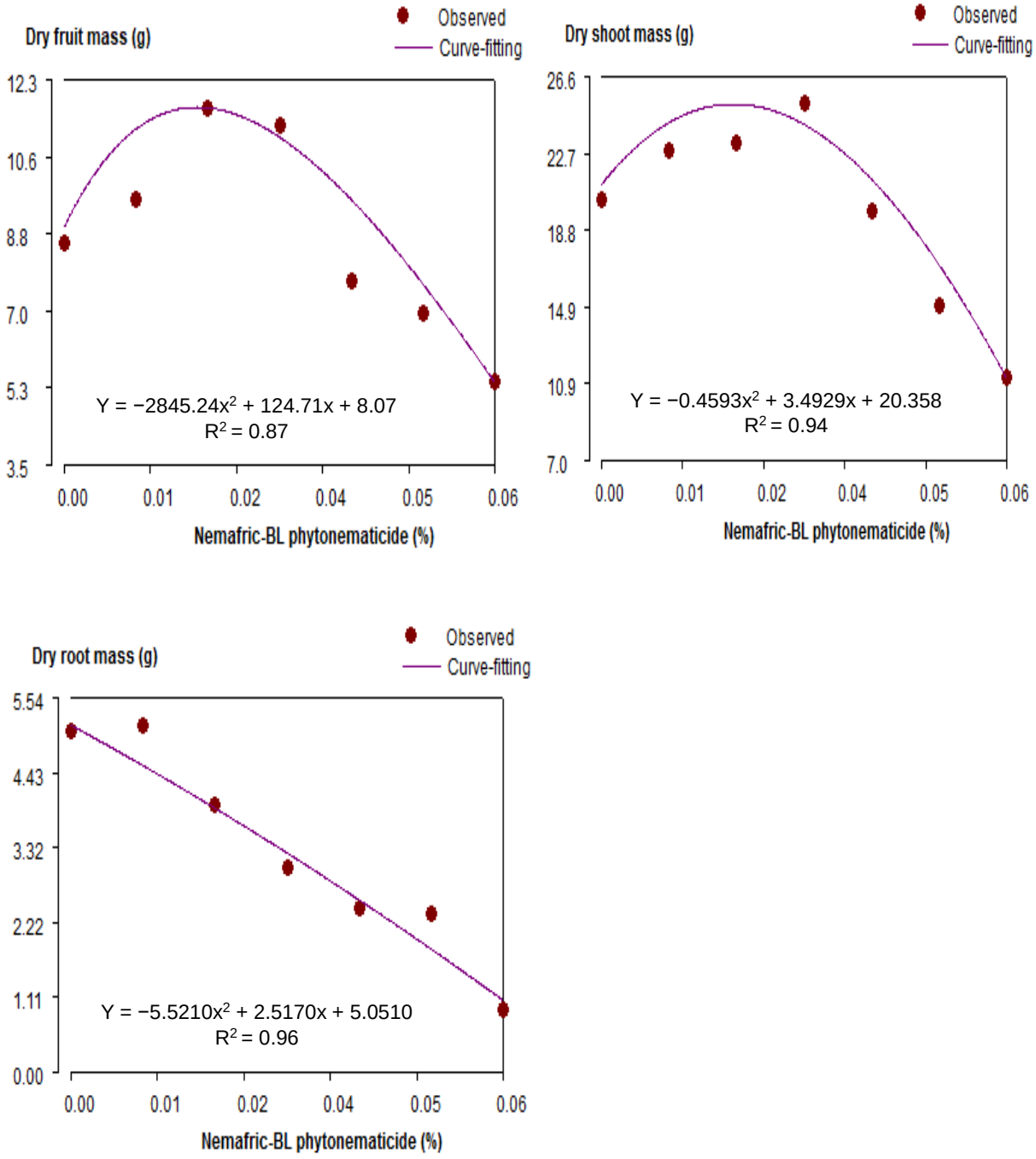

Figure 3.4 (B) Response of dry fruit mass, dry shoot mass and dry root mass of tomato cv. 'Floradade' to increasing concentration of Nemafric-BL phytonematicide (n = 70).

Table 3.3 Biological indices for plant height (PHT), chlorophyll content (CHL), stem diameter (STD), number of fruit (NOF), dry fruit mass (DFM), dry shoot mass (DSM) and dry root mass (DRM) of tomato cv. 'Floradade' exposed to increasing concentration of Nemafric-BL phytonematicide at 64 days after treatment initiation (n = 70).

Biological indices	PHT (cm)	CHL	STD (mm)	NOF	DFM (g)	DSM (g)	DRM (g)	Mean
Threshold stimulation (D_m)	0.16	0.12	0.06	0.22	0.28	0.20	0.18	0.17
Saturation point (R_h)	1.50	1.35	0.47	2.81	6.58	3.96	5.43	3.16
0% inhibition (D_0)	0.03	0.04	0.01	0.04	0.04	0.43	0	0.08
50% inhibition (D_{50})	0.92	0.23	0.72	0.70	0.06	0.06	0.40	0.40
100% inhibition (D_{100})	0.10	0.90	0.10	0.10	0.10	0.10	0.10	0.20
R^2	0.92	0.83	0.97	0.96	0.87	0.94	0.96	–
k – value	0	20	0	5	16	4	0	–

Overall sensitivity ($\sum k$) = 45.

MCSP = $D_m + (R_h/2)$ = 0.17 + (3.16/2) = 1.75%.

Selected nutrient elements in leaf tissues: Sodium and Zn when exposed to increasing concentration of Nemafric-BL phytonematicide exhibited positive quadratic relations, whereas K exhibited negative quadratic relations (Figure 3.5). The models were explained by a strong relationship of 93 and 83% of the respective mineral nutrient elements (Table 3.4). Two biological indices (D_m and R_h) were used to obtain the MCSP value by using the formula $MCSP = D_m + (R_h/2)$, the MSCP was equivalent to 3.72% (Table 3.4). Tomato foliar nutrient elements had k-values of 20 and 13 units, respectively, with the $\sum k$ of tomato foliar nutrient elements being equivalent to 33 units (Table 3.4).

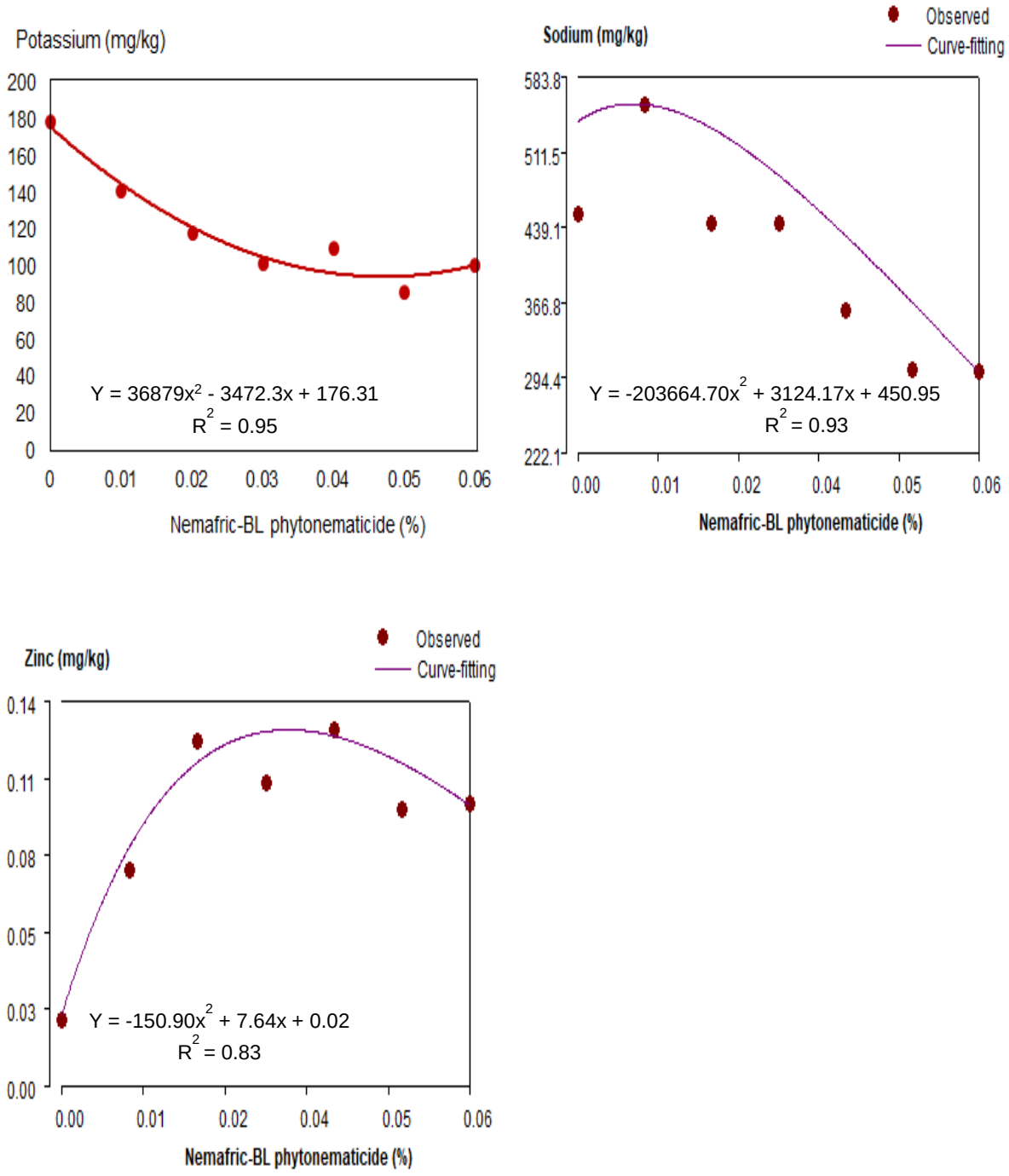

Figure 3.5 Response of K, Na and Zn leaf content of tomato cv. 'Floradade' to increasing concentration of Nemafric-BL phytonematicide (n = 70).

Table 3.4 Biological indices for sodium (Na) and zinc (Zn) of tomato foliar nutrients elements exposed to increasing concentration of Nemafric-BL phytonematicide at 64 days after treatment initiation (n = 70).

Biological indices	Na (mg/kg)	Zn (mg/kg)	Mean
Threshold stimulation (D_m)	0.01	0.04	0.03
Saturation point (R_h)	14.64	0.10	7.37
0% inhibition (D_0)	0.02	0.09	0.06
50% inhibition (D_{50})	0.07	0.10	0.09
100% inhibition (D_{100})	0.10	0.10	0.01
R^2	0.93	0.83	–
k – value	20	13	–

Overall sensitivity ($\sum k$) = 33.

MCSP = $D_m + (R_h/2)$ = 0.03 + (7.37/2) = 3.72%.

3.4 Discussion

3.4.1 Cucurbitacin residues

Nemarioc-AL and Nemafric-BL phytonematicides are contained in fruit of *C. myriocarpus* and *C. africanus*, with cucurbitacin A ($C_{32}H_{46}O_9$) and cucurbitacin B ($C_{32}H_{46}O_8$) as their active ingredients, respectively (Jeffrey, 1978). In the current study, cucurbitacin residues were not detected under increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides on soil and tomato fruit samples. Similar findings were observed when 3% of Nemarioc-AL and Nemafric-BL phytonematicides was used, where cucurbitacin A and cucurbitacin B residues in tomato fruit were not detected (Dube, 2016; Shadung *et al.*, 2017). The two allelochemicals contains large molecules which are non-polar, with cucurbitacin A being slightly soluble in water (Gry *et al*., 2006), whereas cucurbitacin B is insoluble in water (Jeffrey, 1978). According to Tykodi (1989), polar molecules interact freely with water, while non-polar molecules does not allow such movements. Campbell (1990) postulated that non-polar molecules, including glucose, glycine and stearic acid cannot be transported through bipolar membranes. Since phytonematicides are formulated from botanicals, it has been reported that chemicals formulated from botanicals or plant extracts has rapid degradation and lack of persistence than synthetic chemicals on the produce (Aktar *et al*., 2009). Similarly, Cavoski *et al*. (2008) observed that neem products were less persistent and even less toxic to natural enemies than synthetic pesticides, the same was observed by others (Naqvi *et al*., 2002).

According to Cavoski *et al*. (2008) soils are active filters where chemical compounds are degraded by physical, chemical and biological processes. Undetected cucurbitacin

residues in soil could be the environmental conditions, moreover the sunlight which encourages rapid degradation since the trials were conducted under field conditions. The withholding period of 30 days in the current study could have also contributed to the observed results. Similarly, rotenone was found to be a very stable compound on olives, its decay was rapid in the early days after its application, decreased when the concentration on the olives reached 0.4 - 0.5 mg kg^{-1} and continued slowly, without ever going below the maximum residue limit, not even 12 days after the treatment (Simeone *et al*., 2009). Since Nemarioc-AL and Nemafric-BL phytonematicides contains cucurbitacin as their active ingredient, lesser amounts of cucurbitacin in produce could be risky since they have a capability of stimulating cell division which can later result in cancer (Lee *et al*., 2010). Therefore, the use of Nemarioc-AL and Nemafric-BL phytonematicides, within the tested conditions, may be feasible with respect to the current needs of consumers and demands for environmental safety: given the rapid decay observed, these products can be applied also few days close to harvest, if necessary.

3.4.2 Influence of phytonematicides on tomato plant growth

Plant height, chlorophyll content, stem diameter, number of fruit, dry fruit mass, dry shoot mass and dry root mass with increasing Nemarioc-AL and Nemafric-BL phytonematicide concentration each exhibited positive quadratic relations, which is characterised by density-dependent growth (DDG) patterns. Increasing phytonematicides concentration and tomato plant variables incidentally suggested DDG patterns (Liu *et al*., 2003; Mashela *et al.*, 2015). However, the existence of the DDG patterns was observed by three distinct growth responses: stimulated, saturated (neutral) and inhibited growth on plant variables,

which confirms that biological entities respond to allelochemicals (Liu *et al*., 2003; Zasada and Ferris, 2003; Mashela *et al*., 2017a). Generally, the response of tomato plant variables to increasing concentration of phytonematicides supports the results of Liu and Lovett (1990), who postulated that stimulation occurs at lower concentration whereas inhibition appears at high concentration. Liu *et al*. (2003) postulated that allelochemicals invariably induce DDG patterns in living organisms.

In the current study the stimulation effects were observed at lower concentration of Nemarioc-AL and Nemafric-BL phytonematicides. Similarly, at low concentration, crude extracts of neem (*Azadirachta indica*) leaf were reported to stimulate growth of maize (Egunjobi and Afolami, 1976) and tomato (Rossner and Zebitz, 1986) plants. Furthermore, Taye *et al.* (2013) tested nine botanical plant extracts using two concentration (3 and 5%) where it was observed that neem seeds, neem leaves and pyrethrum flower extracts stimulated tomato growth at lower concentration (3%). Distinct results were obtained when Moremi *et al.* (2018) used fermented crude extracts of paintbrush flower (*Kleinia longiflora* DC.) under increasing concentration where it was observed that at lower concentration the product had phytotoxic effects, thus inhibiting growth of tomato plants under field conditions. At lower concentration it was observed that Nemarioc-AL and Nemafric-BL phytonematicides did not have toxic effects, instead they stimulated the division of healthy cells (Lee *et al.,* 2010) on tomato plant variables, thus leading to stimulated plant growth.

In contrast, at high concentration, Nemarioc-AL and Nemafric-BL phytonematicides were highly phytotoxic to tomato plant variables when applied specifically at 64%, thus inhibiting growth of tomato plant. Similarly, Kohli *et al*. (2001) observed that at 2% crude extracts of yellow nutsedge (*Cyperus esculentus* L.) had no effect on germination of lettuce, whereas at 5% the extracts inhibited germination. Also, similar observations were made, when African geranium (*Pelargonium sidiodes* DC.) (Sithole, 2016), tomato seedlings (Pelinganga, 2013; Tseke *et al*., 2013) and *Citrus volkameriana* seedling rootstocks (Mathabatha *et al*., 2016) were exposed at high concentration of Nemarioc-AL and Nemafric-BL phytonematicides. Similarly, when lettuce (*Lactuca sativa* L.) seeds were exposed to increasing concentration of banana (*Musa acuminata* L.) plant extracts, germination, and seedling growth was inhibited (Roy *et al*., 2006). In contrast, Olabiyi and Ayeni (2016) used increasing concentration (0, 3 and 5L) of *A. indica* and *Cleome viscosa* whereby the highest concentration (5L) improved the growth and yield of okra plant. However, at high concentration results of the current study revealed that allelochemicals were phytotoxic to tomato plant cells. Effects of increased allelochemicals might have led to death of tomato plant cells, which later caused malfunctioning of tomato plants thus affecting the overall performance of the tomato plants.

The observed positive quadratic models provided the phytonematicide concentration at which tomato plant growth would be stimulated rather than inhibited. Similarly, Mashela and Pofu (2017), observed positive quadratic models which provided optimum phytonematicide concentration at which the selected nutrient elements in leaf tissues of green bean would be at optimum. However, in the current study, two biological indices,

threshold stimulation (D_m) and saturation point (R_h) were used to obtain the mean concentration stimulation point (MCSP) value by using the formula MCSP = D_m + (R_h/2) (Pelinganga, 2013), the MSCP in this study was equal to 1.13 and 1.75% of Nemarioc-AL and Nemafric-BL phytonematicides, respectively. Using the Curve-Fitting Allelochemical Response Data (CARD) computer-based model, the collected data was used to assess the response of plant variables to increasing concentration of the two phytonematicides. Mashela *et al.* (2017a), stated that values between the actual D_m and R_h present a definition of concentration stimulation range (CSR), which is regarded as the concentration at which growth of plants is stimulated. Using MCSP, MSCP values for Nemarioc-AL and Nemafric-BL phytonematicides for tomato plants were generated at 1.13 and 1.75%, respectively. As for *C. volkameriana* seedling rootstocks, MCSP values were 8.60 and 6.30% for Nemarioc-AL and Nemafric-BL phytonematicides, respectively (Mathabatha *et al*., 2016). Similarly, Sithole (2016) calculated MCSP of 6.20 and 2.90% for Nemarioc-AL and Nemafric-BL phytonematicides on *P. sidiodes*, respectively. The calculated MCSP values on *C. volkameriana* seedling rootstocks and *P. sidiodes* were rather higher when compared to the values of the current study. According to Mashela *et al*. (2015; 2017a), the MCSP values should be interpreted by implementing the use of overall sensitivity (∑k) values of the phytonematicide on the crop. Mashela *et al.* (2017a) suggested that the ∑k was specific for many factors, which could include the phytonematicide concentration, application rate, plant species and growth phase and nematode life stage.

Plant height, chlorophyll content, stem diameter, number of fruit, dry fruit mass, dry shoot mass and dry root mass had sensitivity (k) values of 0, 0, 0, 0, 20, 20 and 20 units, of the respective plant variables when exposed to Nemarioc-AL phytonematicide, whereas similar plant variables had k-values 0, 20, 0, 5, 16, 4 and 0 units, respectively. According to Liu *et al*. (2003), the closer the k-value of the plant variable is to zero, the more sensitive the plant variable is to the phytonematicide, *vice versa*. However, the observed k-values of 0 units on plant height, chlorophyll content, stem diameter and number of fruit showed that tomato plant variables were highly sensitive to increasing concentration of Nemarioc-AL phytonematicide, whereas dry fruit mass, dry shoot mass and dry root mass were tolerant to the product. Similarly, the observed k-values of 0 units on Nemarioc-AL phytonematicide, were observed on plant height, stem diameter and dry root mass when exposed to increasing concentration of Nemafric-BL phytonematicide, whereas chlorophyll content, number of fruit and dry shoot mass were the least sensitive to the product. The extreme sensitivity observed when dry root mass was exposed to Nemafric-BL phytonematicide, it could be that the roots of tomato plant were directly exposed to the product.

Plant height, chlorophyll content, stem diameter, number of fruit, dry fruit mass, dry shoot mass and dry root mass had $\sum k$ of 60 units when exposed to increasing concentration of Nemarioc-AL phytonematicide, whereas for Nemafric-BL phytonematicide similar plant variables had $\sum k$ of 45 units. The $\sum k$ of 60 and 45 units were reported in the current study for Nemarioc-AL and Nemafric-BL phytonematicides, respectively. Tomato plants had $\sum k$ of 60 and 45 units, thus indicating that tomato plants were highly tolerant to Nemarioc-AL

and Nemafric-BL phytonematicide, respectively. For *C. volkameriana* seedling rootstocks had ∑k of 2 units when exposed to increasing concentration of Nemarioc-AL phytonematicide, whereas increasing concentration of Nemafric-BL phytonematicide had ∑k of 4 (Mathabatha *et al*., 2016). Sithole (2016) observed ∑k of 3 on wild geranium (*P. sidiodes*) exposed to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides. However, the observed ∑k values in the current study denoted that plant variables were highly tolerant to the two products when compared to the one of *C. volkameriana* seedling rootstocks and *P. sidiodes* studies which were highly sensitive to the two products.

3.4.3 Influence of phytonematicides on selected nutrient elements in tomato leaf tissues

Potassium, Na and Zn when exposed to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides exhibited positive quadratic relations, except for K when exposed to increasing concentration of Nemafric-BL phytonematicide. Potassium, Na and Zn with increasing Nemarioc-AL phytonematicide concentration each exhibited positive quadratic relations. Similarly, Na and Zn with increasing concentration of Nemafric-BL phytonematicide concentration each exhibited positive quadratic relations. In contrast, K with increasing phytonematicide concentration exhibited negative quadratic relations. All the selected tomato leaf mineral nutrients had a strong relationship as indicated by the models.

In the present study, the behaviour of nutrient elements in tomato leaf tissues exhibited DDG patterns when exposed to increasing concentration of Nemarioc-AL and Nemafric-

BL phytonematicides, which contains secondary metabolites as their active ingredients. According to Jabran *et al*. (2013), secondary metabolites or allelochemicals, upon release into the rhizosphere may influence nutrient movement, availability and uptake by plants. Yu and Matsui (1997) postulated that secondary metabolites may restrict or improve the nutrients mobility to plants as observed recently in the current study. Nemarioc-AL phytonematicide cucurbitacin A comprises two potent chemicals, *viz*. cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) Nemafric-BL phytonematicide contain cucurbitacin B ($C_{32}H_{48}O_8$) as their secondary metabolites. Jabran *et al.* (2013) stated that secondary metabolites decrease uptake of mineral elements by altering the functions of plasma membrane in plant, thus depolarizing the electrochemical potential gradient across the membranes and this could be since Nemarioc-AL and Nemafric-BL phytonematicides at high concentration reduced uptake of nutrient elements in tomato leaf tissues.

In the current study, lower concentration of Nemarioc-AL and Nemafric-BL phytonematicides had stimulation effects on selected tomato foliar nutrient elements, except for K and Zn which responded differently when exposed to increasing concentration of Nemafric-BL phytonematicide. Shadung (2016) also reported that 3% of Nemarioc-AL phytonematicide stimulated Mg by 28%, Na by 38%, P by 27% and Ca by 25%. In contrast, high concentration of Nemarioc-AL and Nemafric-BL phytonematicides effects were phytotoxic at high concentration specifically at 64%. Distinct results were obtained when increasing concentration (0, 2.5, 5, 7.5, 10, 12.5, 15%) of sea weed (*K. alvarezii*) extracts was used, whereby it increased all nutrient elements tested on soybean

(*Glycine max* L.) with the highest treatment being significantly better (Rathore *et al*., 2009).

The reduction of K, Na and Zn uptake observed in the current study when tomato was exposed to high levels of Nemarioc-AL phytonematicide could lead to minimal uptake of other essential nutrient elements, thus leading to poor tomato quality and reduced shelf life. In the present study Nemarioc-AL and Nemafric-BL phytonematicides at lower concentration showed physiological synergism on tomato leaf nutrient elements, whereas at higher concentration displayed physiological antagonism, which revealed that the increase in concentration Nemarioc-AL phytonematicide concentration reduced Na uptake on tomato leaf tissues.

The MSCP for tomato foliar nutrient elements in this study was at 2.49 and 3.72% of Nemarioc-AL and Nemafric-BL phytonematicides, respectively. Potassium values were not adopted in developing MCSP of Nemafric-BL phytonematicide since it exhibited a negative quadratic relation. The reported concentration will not be phytotoxic to the selected tomato leaf nutrient elements, but at the same time will be expected to improve and maintain accumulation of tomato leaf mineral nutrients. Phytonematicides do not serve as fertilisers, although they have stimulation capabilities on plant growth, therefore the observed concentration of Nemafric-BL phytonematicide can be reduced to the range of 3%, as to avoid phytotoxicity. Potassium, Na and Zn had k-values of 2, 19 and 0, respectively, when exposed to increasing concentration of Nemarioc-AL phytonematicide. According to Marschner (1995), K is a nutrient element with high

mobility which helps it respond faster to any changes in the plant, therefore the observed sensitivity of K to Nemarioc-AL phytonematicide it's because of its poor structure in tomato crop. Zinc was highly sensitive to Nemarioc-AL phytonematicide, because it was obtained in lower amounts in the soil, Alloway (2008) postulated that the lower the amounts of Zn in the soils, the lower the absorption rate and synthesis in plant organs. Sodium was the least sensitive to the product. Sodium and Zn had k-values of 20 and 13, respectively, when exposed to increasing concentration of Nemafric-BL phytonematicide. The three mineral nutrient elements on tomato leaves when exposed to Nemafric-BL phytonematicides were least sensitive as compared to Nemarioc-AL phytonematicide. However, selected nutrient elements had ∑k of 21 and 33 units to Nemarioc-AL and Nemafric-BL phytonematicides, respectively, suggested that the selected nutrient elements were tolerant to the two products.

3.5 Conclusion

Cucurbitacin A and B chemical residues were not detected in soil and tomato fruit samples under increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides. Tomato plant growth and accumulation of selected essential nutrient element in tomato leaf tissues and increasing phytonematicide concentration exhibited density-dependant growth patterns with high tolerance to the two phytonematicides. However, 1.13 and 1.75% dilutions of Nemarioc-AL and Nemafric-BL phytonematicides, respectively, would be recommended to alleviate the incident of phytotoxicity to tomato plants. Nemarioc-AL phytonematicide in this study, was rather lower, therefore it can be applied at a range of 3% dilution, to effectively suppress nematode numbers without inducing phytotoxicity.

CHAPTER 4
SUMMARY OF FINDINGS, SIGNIFICANCE, RECOMMENDATIONS AND CONCLUSIONS

4.1 Summary of findings

Cucurbitacin A and cucurbitacin B chemical residues were not detected in soil and tomato fruit samples under increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides. Although the study was conducted under uncontrolled conditions, the two phytonematicides were developed from plant extracts which are normally less persistent and degrades quickly. Also, degradation from microbial organisms, sunlight and time taken to prepare and analyse the samples might have contributed to the observed results in the current study regarding cucurbitacin residues. The phytotoxic response of test crops when exposed to high concentration of Nemarioc-AL and Nemafric-BL phytonematicides, had been limiting their adoption as alternatives to synthetic chemical nematicides. However, mean concertation stimulation point (MCSP) derived from the Curve-fitting Allelochemical Response Data (CARD) computer-based model has been regarded as the non-phytotoxic concentration. This study was conducted to determine whether increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides would result in cucurbitacin residues in tomato plant, to generate mean concentration stimulation point (MCSP) values, overall sensitivity ($\sum k$) and selected foliar mineral elements of tomato plant. Under field conditions cucurbitacin residues of Nemarioc-AL and Nemafric-BL phytonematicides were not detected, however they affected plant height, chlorophyll content, stem diameter, number of fruit, dry fruit mass, dry shoot mass and dry root mass. Nemarioc-AL and Nemafric-BL phytonematicides had stimulation effects on tomato plant variables at lower concentration. Contrarily, reduction

of the affected tomato plant variables was observed as the concentration were increasing, which served as an evident that at high concentration, allelochemicals contained in phytonematicides are phytotoxic. Similar behaviour was observed on selected tomato foliar nutrient elements when exposed to increasing concentration of the two products, whereby at lower concentration, the accumulation of nutrient elements was improved. In contrast, at higher concentration there was inhibition or rather low accumulation of nutrients elements on tomato leaves. Although, Zn and K responded differently when exposed to Nemafric-BL phytonematicide concentration, Zn increased with increasing Nemafric-BL phytonematicide concentration, whereas K reduced with increasing Nemafric-BL phytonematicide concentration. Using the CARD model the MCSP values were at 1.13 and 1.75% of Nemarioc-AL and Nemafric-BL phytonematicides on tomato plant variables, respectively. On selected tomato foliar nutrient elements, the MCSP values were at 2.49 and 3.72% of Nemarioc-AL and Nemafric-BL phytonematicides, respectively. The sensitivity (k) values in this study were used to determine the ∑k of tomato to Nemarioc-AL and Nemafric-BL phytonematicides. The ∑k of tomato plant variables to Nemarioc-AL and Nemafric-BL phytonematicides was 60 and 45 units, respectively, while selected nutrient elements to Nemarioc-AL and Nemafric-BL phytonematicide was of 21 and 33 units, respectively. Tomato plant variables were rather highly tolerant to Nemarioc-AL phytonematicide when compared to Nemafric-BL phytonematicide, whereas with selected tomato foliar nutrient elements it was vice versa.

4.2 Significance

Nemarioc-AL and Nemafric-BL phytonematicides left no traces of cucurbitacin residues in soil and tomato fruit samples, the results indicated that the two phytonematicides can be adopted for control of nematodes under field conditions without leaving residues in soil and produce. The response of tomato indicated that high phytonematicide concentration inhibit growth, while lower concentration had stimulatory effects on tomato growth and essential nutrient elements on leaf tissues. Nemarioc-AL and Nemafric-BL phytonematicides exhibited density-dependent growth patterns which comprised with three phases, stimulation, neutral and inhibition. However, the CARD computer-based model was used to calculate the MCSP and to determine the overall sensitivity. Nemarioc-AL phytonematicide had an MCSP of 1.13 and 2.49% dilutions, on tomato growth and selected nutrient elements in tomato leaf tissues, respectively whereas Nemafric-BL phytonematicide had an MCSP value of 1.75 and 3.72% dilutions, on tomato growth and selected nutrient elements on leaf tissues, respectively. Nemarioc-AL phytonematicide had ∑k of 60 and ∑k of 21 units on tomato growth and selected nutrient elements on leaf tissues, whereas Nemafric-BL phytonematicide had ∑k of 45 and ∑k of 33 units on tomato growth and selected nutrient elements on leaf tissues, respectively. Tomato portrayed high tolerance to increasing phytonematicide concentration under field conditions.

4.3 Recommendations

Further studies are encouraged to investigate cucurbitacin residues of Nemarioc-AL and Nemafric-BL phytonematicides under varying growing conditions, also it will be advisable to analyse the samples immediately after harvesting or sampling to avoid degradation

over time. It may however, be feasible with respect to the current needs of consumers and demands for environmental safety: given the rapid decay observed, these products can be applied also close to harvest, if necessary. The derived MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides should be adopted in managing plant-parasitic nematodes while stimulating growth of tomato plants under field conditions. Through the developed MCSP it would be imperative to determine the application interval and dosage model as described elsewhere (Mashela *et al*., 2015), as to improve accumulation of nutrient elements in tomato leaf tissues since they play a vital role in crop's life cycle.

4.4 Conclusions

Recently, in this study it was observed that cucurbitacin residues of Nemarioc-AL and Nemafric-BL phytonematicides were not detected under increasing concentration on all the tested samples. Nemarioc-AL and Nemafric-BL phytonematicides at high concentration were highly phytotoxic to tomato growth and to the selected nutrient elements. Since phytonematicides contains allelochemicals as their active ingredients, they should be applied at lower concentration as shown by the developed MCSP values to avoid allelopathy. The inhibition of selected nutrient elements experienced in this study when exposed to high concentration of Nemarioc-AL and Nemafric-BL phytonematicides, should be avoided by using the appropriate developed phytonematicide concentration.

REFERENCES

Abad, P., Favery, B., Rosso, M. and P. Castagnone-Sereno. 2003. Root-knot nematode parasitism and host response: Molecular basis of a sophisticated interaction. *Molecular Plant Pathology* 4:217-224.

Aktar, M.W., Sengupta, D. and A. Chowdhury. 2009. Impact of pesticides use in agriculture: Their benefits and hazards. *Interdisciplinary Toxicology* 2:1-2.

Alloway, B.J. 2008. Micronutrients and Crop Production: An Introduction. In: Alloway, B.J. (ed.). Micronutrient Deficiencies in Global Crop Production. Springer: Dordrecht.

Amtmann, A. and D. Sanders. 1999. Mechanisms of Na^+ uptake by plant cells. *Advances in Botanical Research* 29:75-112.

Anwar, S.A. and M.V. McKenry. 2010. Incidence and population density of plant-parasitic nematodes infecting vegetable crops and associated yield losses in Punjab, Pakistan. *Pakistan Journal of Zoology* 44:327-333.

Ashley, M.K., Grant, M. and A. Grabov. 2006. Plant responses to potassium deficiencies: A role for potassium transport proteins. *Journal of Experimental Botany* 57:425-436.

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

Caboni, P., Sarais, 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 Agriculture and Food Chemistry* 54:10026-10032.

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

Causton, D.R. 1977. A Biologist's Mathematics. Contemporary Biology. Edward Arnold: London.

Cavoski, I., Caboni, P., Sarais, G. and T. Miano. 2008. Degradation and persistence of rotenone in soils and influence of temperature variations. *Journal of Agricultural and Food Chemistry* 56:8066-8073.

Chakraverty, S., Sharma, K., Kumar, S., Sand, N.K. and P.B. Rao. 2005. Effect of three weed extracts on nutrient uptake in different varieties of wheat through radio-tracer technique and autoradiography. *Biological Journals and Abbreviations* 15:171-180.

Chen, J.C., Chiu, M.H., Nie, R.L., Cordell, G.A. and S.X. Qiu. 2005. The cucurbitacins and cucurbitane glycosides: Structures and biological activities. *Natural Products Report* 22:386-399.

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

Chokoe, F.M. 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.

De Waele, D. and E.M. Jordaan. 1988. Plant-parasitic nematodes of field crops in South Africa. *Maize Review Nematology* 11:62-74.

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

Egunjobi, O.A. and S.O. Afolami. 1976. Effects of neem (*Azadirrachta indica*) leaf extracts on population of *Pratylenchus brachyurus* and on the growth and yield of maize. *Nematologica* 22:125-132.

Flowers, T.J. and T.D. Colmer. 2008. Salinity tolerance in halophytes. *New Phytologist* 179:945-963.

Fourie, H., McDonald, A.H. and G.C. Loots. 2001. Plant-parasitic nematodes in field crops in South Africa. 6. Soybean. *Nematology* 3:447-454.

Fuller, V.L., Lilley, C.J. and P.E. Urwin. 2008. Nematode resistance. *New Phytologist* 180:27-44.

Ghasemi, S., Ghasemi, M., Moradi, N. and A.M. Shamili. 2012. Effect of *Calotropis procera* leaf extract on seed germination of some plants. *Journal of Ornamental and Horticultural Plants* 2:27-32.

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

Hafeez, B., Khanif, Y.M. and M. Saleem. 2013. Role of zinc in plant nutrition: A review. *American Journal of Experimental Agriculture* 3:374-391.

Hugo, H.J. and A.P. Malan. 2010. Occurrence and control of plant-parasitic nematodes in irrigation water. A Review. *South African Journal of Enology and Viticulture* 31:169-180.

Inderjit, K., Asakawa, C. and K.M.M. Dakshini. 1999. Allelopathic potential of *Verbesina encelloides* root leachate in soil. *Canadian Journal of Botany* 77:1419-1424.

Jabran, K., Farooq, M., Aziz, T. and K.H.M. Siddique. 2013. Allelopathy and Crop Nutrition. In: Cheema, Z.A., Faroog, M. and A. Wahid. (eds.). Allelopathy: Current Trends and Future Applications. Springer International Publishing: Heidelberg.

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

Karuri, H.W., Olago, D., Neilson, R., Enock, M. and J. Villinger. 2017. A survey of root-knot nematode and resistance to *Meloidogyne incognita* in sweet potato varieties from Kenyan fields. *Crop Protection* 92:114-121.

Keikotlhaile, B.M. and P. Spanoghe. 2011. Pesticide Residues in Fruits and Vegetables. In: Stoytcheva, M. (ed.). Pesticides-Formulations, Effects, Fate. Ghent University: Belgium.

Kobza, J. and F.A. Einhellig. 1987. The effects of ferulic acid on the mineral nutrition of grain sorghum. *Plant Soil* 98:99-109.

Kohli, R.K, Singh, H.P. and D.R. Batish. 2001. Allelopathy in agroecosystem. *Journal of Crop Production* 8:2-4.

Kyan, T., Shintani, M., Kanda, S., Sakurai, M., Ohashi, H., Fujisaswa, A. and S. Pongdit. 1999. Kyusei Future Farming and the Technology of Effective Microorganisms. Asia Pacific Natural Agriculture Network: Thailand.

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

Leedy, P.D. and J.E. Ormrod. 2005. Practical Research: Planning and Design. Pearson Education: New Jersey.

Liu, D.L. and J.V. Lovett. 1990. Allelopathy in Barley: Potential for Biological Suppression of Weeds. In: Bassett, C., Whitehouse, L.J. and J.A. Zabkiewicz. (eds.). Alternatives to the Chemical Control of Weeds. Ministry of Forestry, Forest Research Institute: Bulletin.

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

Lui, D.L. and M. An. 2005. Implementation of card: Curve-Fitting Allelochemical Response Data. *Non-linearity in Biology, Toxicology and Medicine* 3:235-244.

Maathuis, F.J.M. 2009. Physiological functions of mineral macronutrients. *Current Opinion in Plant Biology* 12:250-258.

Maathuis, F.J.M. 2013. Sodium in plants: Perception, signalling and regulation of sodium fluxes. *Journal of Experimental Botany* 65:849-858.

Macheix, J.J., Fleuriet, A. and J. Billot. 1990. Fruit Phenolics. Boca Raton, CRC Press: USA.

Mafeo, T.P. and P.W. Mashela. 2009. Responses of germination in tomato, watermelon and butternut squash to a *Cucumis* bio-nematicide. *Journal of Agricultural and Environmental Sciences* 6:215-219.

Mahmood, I., Masood, A., Saxena, S.K. and S.I. Hussain. 1979. Effect of some plant extracts on the mortality of *Meloidogyne incognita* and *Rotylenchulus reniformis*. *Acta Botanica Indica* 7:129-132.

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

Malatji, K.P. 2017. Development of mean concentration stimulation point for fermented *Lantana camara* phytonematicide on tomato production. MSc Mini-dissertation. University of Limpopo, Sovenga, South Africa.

Marschner, H. 1995. Mineral Nutrition in Plants. Academic Press. 2nd edition. San Diego, CA: USA.

Martens, D.C. and D.T. Westerman. 1991. Fertilizer Applications for Correcting Micronutrient Deficiencies. In: Mortvedt, J.J., Cox, F., Shuman, L.M. and R.M. Welch. (eds.). Micronutrients in Agriculture. Soil Science Society of America Book Series: Madison.

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

Mashela, P.W., De Waele, D., Dube, Z.P., Khosa, M.C., Pofu, K.M., Tefu, G., Daneel, M.S. and H. Fourie. 2017a. Alternative Nematode Management Strategies. In: Fourie, H., Spaul, 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 Publishing: Heidelberg.

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. Springer International Publishers: Heidelberg.

Mashela, P.W., Mphosi, M.S., Shimelis, H. and M.N. Mokgalong. 2007. Interactions of *Cucumis myriocarpus*, *Lippia javanica* and *Ricinus communis* organic amendments on suppression of *Meloidogyne incognita*. *Journal of Phytopathology* 55:690-693.

Mashela, P.W., Pofu, K.M. and J.T. Maduare. 2017b. Responses of the citrus nematode population densities post-application of Nemarioc-AL phytonematicide and aldicarb synthetic nematicide. *Research on Crops* 18:523-527.

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.

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* 7:969-972.

Mengel, K. and E.A. Kirkby. 2001. Principles of Plant Nutrition. Kluwer Academic Publishers: Dordrecht.

Moremi, M.G., Shadung, K.G. and P.W. Mashela. 2018. Response of tomato plant growth to dried crude extracts of *Kleinia longiflora*. African Combined Congress. Abstract No. 98.

Naqvi, S.S.M., Mumtaz, S. Shereen A. and M.A. Khan. 2002. Comparative performance of two methods for proline estimation. *Pakistan Journal of Botany* 34:355-358.

Nwankwo, E.N., Onuseleogu, D.C., Ogbonna, C.U. and A.O.E. Okorocha. 2016. Effect of neem leaf extracts (*Azadirachta indica*) and synthetic pesticide (Carbofuran) on the root-knot nematode (*Meloidogyne* spp) of cowpea (*Vigna unguiculata* L. Walp). *International Journal of Entomology Research* 1:1-6.

Olabiyi, T.I. 2008. Pathogenicity study and nematoxic properties of some plant extracts on the root-knot nematode pest of tomato, *Lycopersicon esculentum* (L.) Mill. *Plant Pathology Journal* 7:45-49.

Olabiyi, T.I. and B.P. Ayeni. 2016. Assessment of *Azadirachta indica* and *Cleome viscosa* liquid-formulations as bio-nematicides in the management of nematode pests of okra. *African Journal of Agricultural Research* 11:467-471.

Pavaraj, M., Karthikairaj, K. and M.K. Rajan. 2010. Effect of leaf extract of *Ageratum conyzoides* on the biochemical profile of blackgram *Vigna mungo* infected by root-knot nematode, *Meloidogyne incognita. Journal of Biopesticides* 3:313-316.

Pelinganga, O.M. 2013. Developing bio-nematicides 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 Science* 4:8-12.

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 plants. *African Journal of Biotechnology* 11:11407-11413.

Pelinganga, O.M., Nzanza, B., Mamphiswana, N. and P.W. Mashela. 2011. Influence of fermented fruit extracts of *Cucumis africanus* and *Cucumis myriocarpus* on nematode numbers and tomato productivity. *Symposium of Nematological Society of Southern Africa* 20:71.

Pofu, K.M., Mashela, P.W. and H. Shimelis. 2012. Inter-generic grafting in watermelon for managing *Meloidogyne* species: A review. *Scientific Research and Essays* 7:107-113.

Rathore, S.S., Chaudhary, D.R., Boricha, G.N., Ghosh, A., Bhatt, B.P., Zodape, S.T. and J.S. Patolia. 2009. Effect of seaweed extracts on the growth of yield and nutrient uptake of soybean (*Glycine max*) under rain-fed conditions. *South African Journal of Botany* 75:351-355.

Robertson, L. and M.A. Diez-Rojo. 2008. New host races of *Meloidogyne arenaria*, *Meloidogyne incognita* and *Meloidogyne javanica* from horticultural regions of Spain. *Plant Disease Journal* 93:180-184.

Rossner, J. and C.P.W. Zebitz. 1986. Effect of neem products on nematodes and growth of tomato (*Lycopersicon esculentum*) plants. Proceedings of the 3rd International Neem Conference: Nairobi, Kenya.

Roy, S., Asaduzzaman, M., Pramanik, M.H.R. and A.K.M.A. Prohan. 2006. Effects of banana plant extracts on germination and seedling growth of some vegetable crops. *Bangladesh Journal of Crop Science* 17:235-242.

Shabala, S.N. and R.R. Lew. 2002. Turgor regulation in osmotically stressed Arabidopsis epidermal root cells. Direct support for the role of inorganic ion uptake as revealed by concurrent flux and cell turgor measurements. *Plant Physiology* 129:290-299.

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 L.V. Mulaudzi. 2017. Study of chemical residues from Nemarioc-AL and Nemafric-BL phytonematicides in tomato fruit. *African Journal of Agricultural Research* 12:1164-1168.

Sikora, R.A. and E. Fernandez. 2005. Nematode Parasites of Vegetables. In: Luc, M., Sikora, R.A. and J. Bridge (eds.). Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CAB International: Embrapa.

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 of Nemarioc-AL and Nemafric-BL phytonematicides on *Pelargonium sidiodes*: An indigenous future cultigen. MSc Mini-dissertation. University of Limpopo, Sovenga, South Africa.

Subbarao, G.V., Ito, O., Berry, W.L. and R.M. Wheeler. 2003. Sodium: A functional plant nutrient. *Critical Reviews in Plant Sciences* 22:391-416.

Susan, L.F.M., Lakshman, D.K., Zasada, I.A., Vinyard, B.T. and D.J. Chitwood. 2008. Phytotoxicity of clove oil to vegetable crop needling and nematotoxicity to root-knot nematodes. *Horttechnology* 4:631-638.

Taye, W., Sakhuja, P.K. and T. Tefera. 2013. Root-knot nematode (*Meloidogyne incognita*) management using botanicals in tomato (*Lycopersicon esculentum*). *Academia Journal of Agricultural Research* 1:9-16.

Trudgill, P. 1992. Resistance and tolerance of plant-parasitic nematodes in plants. *Annual Review of Phytopathology* 29:167-192.

Tseke, P.E. and P.W. Mashela. 2017. Efficacy of Nemafric-BL phytonematicide from fresh *Cucumis africanus* on suppression of root-knot nematodes and growth of tomato plants. *Research on Crops* 18:289-293.

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.

Tykodi, R.J. 1989. Identifying polar and nonpolar molecules. *Journal of Chemical Education*. 66:1007.

Welch, R.M. 2002. The impact of mineral nutrients in food crops on global human health. *Plant and Soil* 247:83-90.

Weston, L.A. 1996. Utilization of allelopathy for weed management in agro-ecosystems. *Agronomy Journal* 88:860-866.

Williamson, V.M. and A. Kumar. 2006. Nematode resistance in plants: The battle underground. *Trends in Genetics* 7:396-403.

Winter, S.M.J., Rajcan, I. and B.J. Shelp. 2006. Soybean cyst nematode: Challenges and opportunities. *Canadian Journal of Plant Science* 86:25-32.

Wondafrash, M., Van Dam., N.M. and T.O.G. Tytgat. 2013. Plant systemic induced responses mediate interactions between root parasitic nematodes and aboveground herbivorous insects. *Font Plant Science* 4:87.

Yu, J.Q. and Y. Matsui. 1997. Effects of root exudates of cucumber (*Cucumis sativus*) and allelochemicals onion uptake by cucumber seedlings. *Journal of Chemical Ecology* 23:817-827.

Zaki, M.J., Khan, D. and M. Abid. 2012. Nematodes in the saline environment: A mini overview. *International Journal of Biology and Biotechnology* 9:99-113.

Zasada, I.A. and H. Ferris. 2003. Sensitivity of *Meloidogyne javanica* and *Tylenchulus semipenetrans* to isothiocyanates in laboratory assays. *Phytopathology* 93:747-750.