RESPONSES OF TOMATO PLANT GROWTH AND ROOT-KNOT NEMATODES TO PHYTONEMATICIDES FROM FERMENTED FRESH FRUITS OF TWO INDIGENOUS *CUCUMIS* SPECIES

BY

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DECLARATION

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

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DEDICATION

To the Lord, God of all mankind, my family and my friends.

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I would like to thank my supervisory team, Professor P. W. Mashela and Professor N. M. Mokgalong, for their endless support in this project, particularly the training that I received on various aspects, specifically in the awesome skills of scientific writing and oral presentation of scientific work. Let me also thank my mentor, Dr O. M. Pelinganga – a former PhD student in Plant Protection Laboratory, for his guidance, dedication, motivation and assistance, all of which made my project a sounding success. To my parents and all close relatives and friends, who provided a shoulder to lean on when their support and encouragement were much needed – I will remember you and your support, forever. To K.D. Maile, you have been a source of inspiration in the VLIR Nematology Laboratory – to all of us. To all the people, who one way or the other, contributed to the successful completion of this work - physically and emotionally, I say, “Thank you – forever.” To my future wife, Miss Keletso Latakgomo, who stood by me through thick and thin, may the Almighty God bless you always! Peace and my love be unto your heart! Although thanks are not enough, but many thanks. I am grateful to the Land Bank Chair of Agriculture – University of Limpopo, Limpopo Agro-processing Technology Station and the Flemish Interuniversity Council of Belgium for funding sections of this project as part of the Indigenous Cucurbitaceae Technologies Research Niche. I would like to thank my heavenly Father for His power over all that I have achieved in this project. He granted me knowledge and wisdom, His Grace was sufficient and His Promises were true. I will forever be grateful for everything You have done, everything You are doing and everything You are going to do.

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ABSTRACT

Two phytonematicides were researched and developed from fermented crude extracts of wild watermelon (*Cucumis africanus*) and wild cucumber (*Cucumis myriocarpus*) fruits for use as alternatives to methyl bromide in managing root-knot (*Meloidogyne* species) nematodes in tomato (*Solanum lycopersicum*) production. Fruits of *C. africanus* contain cucurbitacin B ($C_{32}H_{48}O_8$), while those of *C. myriocarpus* contain cucurbitacin A, which comprises cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$). Phytonematicides from *C. africanus* and *C. myriocarpus* fruits are referred to as nemafric-B and nemarioc-A, respectively. The two phytonematicides, due to their origin from plant species with allelochemicals, have high potential of being phytotoxic to crops. The use of the Curve-fitting Allelochemical Response Dosage (CARD) computer-based model assisted in the establishment of concentrations which were stimulatory to growth of tomato (*Solanum lycopersicum*) plants, while exhibiting nematoxic properties to *Meloidogyne* species. The two phytonematicides were developed from crude extracts of fruits dried at 52˚C in air-forced ovens and ground in a Wiley mill through 1-mm-opening sieves. However, equipment for drying and grinding fruits would not be accessible to smallholder farmers who wished to prepare their own products on-farm. The objective of this study therefore, was to determine whether nemafric-BL and nemarioc-AL produced from fresh fruit of the two *Cucumis* species would be suitable for use (i.e. non-phytotoxic) in tomato production for managing population densities of *M. incognita* race 2. In order to distinguish the products of fresh (F) fruits from those of dried (D) fruits, they were code-named nemafric^{F}-BL or nemarioc^{F}-BL and nemafric^{D}-BL or nemarioc^{D}-AL, respectively, where G and L denoted granular and liquid formulations, respectively. Tomato cv. ‘Floradade’ seedlings were infested with 3 000 eggs and second-stage

juveniles of *M. incognita* race 2. An equivalent of 40 g and 80 g dried fruit mass of nemafric-B and nemarioc-A, namely, 284 g and 411 g fresh fruit mass for nemafric-B and nemarioc-A, respectively, were separately fermented using EMROSA effective micro-organisms mixed with 16 L chlorine-free tapwater in 20 L container for 14 days at ± 25˚C, allowing pH to gradually decline to ± 3.7. Separate experiments for each product run concurrently. Treatments, namely, 0, 2, 4, 8, 16, 32 and 64% concentrations, where for instance, 2% = 20 ml/1000 ml x 100, were arranged in a randomised complete block design, with 10 replications. Blocking in the greenhouse was done for wind direction which was regularly erected by fans for cooling down the greenhouse. At 56 days after weekly application of each treatment, flower number, fruit number, dry shoot mass, dry root mass, dry fruit mass, plant height, stem diameter and nematode numbers were each subjected to analysis of variance. Nematode data were, prior to analysis, transformed using $\log_{10}(x + 1)$, but untransformed data were reported. Using the sum of squares, nemafric-BL and nemarioc-AL treatments affected dry root mass, dry shoot mass, flowers number, fruit number, plant height and stem diameter. Nemafric-BL contributed 67%, 78%, 58%, 43%, 60% and 26%, while nemarioc-AL contributed 71%, 61%, 19%, 35%, 34% and 24% to total treatment variation of the six respective variables. Plant variables with significant ($P \leq 0.05$) treatment effects were further subjected to the CARD model to generate seven biological indices, with three distinct phases, namely, stimulation, neutral and inhibition phases. Using the quantified stimulation phase, the mean concentration stimulation range (MCSR) was computed for each variable using two biological indices, namely, threshold stimulation point (D_m) and saturation point (R_h). The CARD model explained 98%, 99%, 98% and 98% of the quadratic models of dry root mass, dry shoot mass, plant height and stem diameter,

respectively, against increasing concentrations of nemarioc-AL. Similarly, the CARD model explained 99%, 96%, 84% and 93% of total treatment variation in the respective plant variables. The integrated MCSR [MSCR = D_m + (R_h/2)] for nemafric-BL on tomato plants was 7%, while that for nemarioc-AL was 4%. In the CARD model, the overall sensitivities (∑k) of tomato plants exposed to nemafric-BL and nemarioc-AL were 3 units and 5 units, respectively. Tomato plants were therefore, less sensitive to nemarioc-AL since it had higher ∑k value than nemafric-BL. At 4% nemarioc-AL and at 7% nemafric-BL, the two phytonematicides were each highly suppressive to population densities of *M. incognita* race 2. In conclusion, on the basis of non-phytotoxicity of the computed MCSR values and their suppressive effects on population densities of *M. incognita* race 2, the smallholder farmers could produce nemafric-BL and nemarioc-AL phytonematicides on-farm. However, the production of the two products from fresh fruits would not be sustainable since fruits of the two *Cucumis* species are highly seasonal due to the high incidence of post-harvest decays.

PICTURES OF INDIGENOUS *CUCUMIS* SPECIES

Legend A: Plants and fruit of wild cucumber (*Cucumis africanus*).

Legend B: Plants and fruit of wild watermelon (*Cucumis myriocarpus*).

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

In South Africa, among other alternatives to methyl bromide, uses of fruits from indigenous *Cucumis* species were widely tested for managing population densities of *Meloidogyne* species under diverse environments with promising results (Mashela, 2002, 2007; Mafeo and Mashela, 2009a,b; Mashela and Nthangeni, 2002; Mashela *et al*., 2008; Pelinganga and Mashela, 2012; Pelinganga *et al*., 2012, 2013). The resultant research niche was code-named the Indigenous Cucurbitaceae Technologies (ICT), since the technologies focused on using plant organs from the Cucurbitaceae Family (Mashela *et al*., 2011). The ICT comprised five subniches, namely, (a) ground leaching technology (GLT), (b) intergeneric grafting technology, (c) agronomics technology, (d) chemical technology and (e) botinemagation technology (Mashela *et al*., 2011). The major feature of the ICT research niche is its focus on inclusivity in terms of multidisciplinary approach and accommodation of all farming systems – from subsistence to commercial farmers. In order to enhance the inclusivity of the farming systems, the application equipment for the products in all cases had been simple – manually placing materials in the planting hole or applying products through irrigation systems (Mashela, 2002; Pelinganaga *et al*., 2012a,b).

The two phytonematicides from wild watermelon (*Cucumis africanus* L. F.) and wild cucumber (*Cucumis myriocarpus* Naude.) fruits are code-named nemafric-B and nemarioc-A, respectively (Mashela *et al*., 2011). In granular formulation, the products

are nemafric-BG and nemarioc-AG, while in liquid formulation they are nemafric-BL and nemarioc-AL. Incidentally, when produced in liquid formulation from dried and fresh fruit of *C. africanus* the products are nemafric^{D}-BL and nemafric^{F}-BL, respectively, while those from *C. myriocarpus* fruits are nemarioc^{D}-AL and nemarioc^{F}-AL, respectively. The superscripts D and F denote dried and fresh forms, respectively, A and B being cucurbitacin A and cucurbitacin B, respectively, while G and L granular and liquid formulations, respectively. However, in this study, unless otherwise stated, nemafric-BL and nemarioc-AL would be used to connote the phytonematicides produced from fresh fruits of the two *Cucumis* species.

The major limiting factor in the use of nemafric-BL and nemarioc-AL had been the phytotoxicity of the products to the crops being protected against nematodes and the high cost of the equipment, particularly for fruits drying (Mashela *et al*., 2011). Using the Curve-fitting Allelochemical Response Dosage (CARD) computer-based model (Liu *et al*., 2003), Pelinganga *et al*. (2012a) adapted two of the seven biological indices to develop stimulatory concentrations to plant growth, with nematoxic properties. Stimulatory concentrations had been referred to as mean concentration stimulation range (MCSR), which had since been used widely in botinemagation technology (Pelinganga, 2013; Pelinganga and Mashela, 2012; Pelinganga *et al*., 2011; Pelinganga *et al*., 2012, 2013). In dried form, fresh fruits have to be chopped into pieces, dried in air-forced ovens at 52℃ for 72 h and then ground in a Wiley mill through 1-mm-pore-sieve.

1.2 Problem statement

The important tenets of the ICT include inclusivity in terms of use in various farming systems. However, the drying and grinding equipment may limit the use of the ICT for some of the smallholder farmers who might be interested in producing the products on-farm. Thus, the researcher intended evaluating options which would minimise reliance on specialised equipment in the ICT, in order to enhance production of the two phytonematicides on-farm.

1.3 Motivation

The use of fresh fruits from *Cucumis* species in the fermentation process for botinemagation may eliminate the need to use drying and grinding equipment, which may not be accessible to subsistent and smallholder farmers in marginal communities. Therefore, the researcher intended to assess whether in fresh form fruits for nemafric-B and nemarioc-A would be effective for use in tomato production for managing population densities of *M. incognita* race 2.

1.4 Aim

The aim of the study was to use the CARD computer-based model to develop the MCSR values from fermented fresh fruits of two *Cucumis* species for use in tomato production for managing population densities of nematodes.

1.5 Objectives

1. To determine whether nemafric-BL phytonematicide would stimulate growth of

tomato plants and suppress population densities of *M. incognita* race 2.

2. To investigate whether nemarioc-AL phytonematicide would stimulate growth of tomato plants and suppress population densities of *M. incognita* race 2.

1.6 Significance of the study

Protocols developed in this study would allow the production of nemafric-BL and nemarioc-AL on-farm using fermented fresh fruits from the two *Cucumis* species without drying and grinding the fruits. The on-farm production of the two phytonematicides would allow for the widespread adoption of the botinemagation technology. Also, results of this study would provide baseline information for comparisons of MCSR values and the overall sensitivities of fresh and dried fruits on equivalent basis.

1.7 Structure of the mini-dissertation

The mini-dissertation was designed using the Senate-approved format of the University of Limpopo. Findings were summarised in the abstract, followed by detailed background to the research problem (Chapter 1), which was in turn followed by a review of relevant literature on the research problem (Chapter 2). The empirical studies comprised those for achieving objective 1 and objective 2 (Chapter 3). Finally, findings were summarised, with related recommendations and conclusions being provided (Chapter 4).

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

International withdrawal of methyl bromide as a synthetic fumigant nematicide increased research and development efforts on alternatives for managing the root-knot (*Meloidogyne* species) nematodes in tomato (*Solanum lycopersicum*) production (Mashela *et al*., 2011). In Limpopo Province, South Africa, alternatives to methyl bromide in managing *Meloidogyne* species focused on the use of allelochemicals from crude extracts of fruits from selected indigenous plants, primarily the wild *Cucumis* species in the Indigenous Cucurbitaceae Technologies (ICT) research niche (Mashela *et al*., 2011).

2.2 Work done in the research problem

The *Cucumis* studies have since resulted in the establishment of the ICT Research Niche, comprising five subniches, which are reviewed in detail.

2.2.1 Ground leaching technology

In the ground leaching technology (GLT) systems, mature fruit from *C. africanus* or *C. myriocarpus* are cut into pieces, dried at 52°C in air-forced ovens (Makkar, 1999) for 72 h and then ground in a Wiley mill to pass through a 1-mm-pore sieve (Mashela, 2002). The materials are applied at transplanting without being allowed to undergo any microbial degradation (Mashela, 2002). Crude extracts are generally spread in small quantities (2-5 g/plant) in a shallow hole around the base of the stem of the transplant,

which translates to 20-71 kg ground material/ha for 4 000 tomato plants (Mashela, 2002). The small quantities used in the GLT systems preclude high transport costs to haul the materials to the fields when compared to conventional organic amendments (Mashela *et al*., 2011). Also, when applied at transplanting, the waiting period for microbial decomposition was not an issue and the materials from the two plant species did not reduce soil pH (Mashela *et al*., 2011). High transport costs, availability of materials, waiting period for microbial degradation and reduction of soil pH – are all often cited as drawbacks of conventional organic amendments (Mashela and Nthangeni, 2002).

The GLT system consistently improved crop yield and reduced nematode numbers up to 56 days after application (Mashela *et al*., 2011). The materials which had been successfully used at transplanting without having any phytotoxicity include *C. myriocarpus* fruit, castor bean (*Ricinus communis* L.) and fever tea (*Lippia javanica* Burm. F.) leaves (Mashela, 2002; Mashela and Nthangeni, 2002; Mashela *et al*., 2010). Ground crude extracts from *C. myriocarpus* fruit have been promising to the extent that attempts to develop a phytonematicide are at an advanced stage (Mashela *et al*., 2011). Mashela *et al*. (2008) demonstrated that products from *C. myriocarpus* fruits were comparable to aldicarb ($C_7H_{14}N_2O_2S$) and fenamiphos ($C_{13}H_{22}NO_3PS$) in suppression of plant-parasitic nematodes and tomato productivity. Crude extracts of *C. myriocarpus* fruit suppressed plant-parasitic nematodes in greenhouses and microplot trials by over 90% (Mashela, 2002; Mofokeng *et al*., 2004; Shakwane *et al*., 2004), while in field trials over 80% suppression was observed (Mashela *et al*., 2011).

2.2.2 Intergeneric grafting technology

Host-status and host-sensitivity of *C. africanus* and *C. myriocarpus* to *Meloidogyne* species were investigated in greenhouse, microplot and field trials (Pofu *et al.,* 2009; 2010a,b). Both *C. africanus* and *C. myriocarpus* seedlings were highly resistant to *M. incognita* races 2 and 4 and *M. javanica* (Pofu *et al*., 2012). The two nematode species and their two races are dominant in South Africa (Kleynhans *et al*., 1996). Intergeneric grafting technology has had incompatibility challenges due to different stem diameter sizes of watermelon (*Citrullus lanatus* Thunb.) cultivars and *Cucumis* seedling rootstocks (Pofu *et al*., 2011a). Grafts of watermelon with relatively thick stem diameters and *Cucumis* seedling rootstocks with relatively thin stem diameters had survival rates of 36% (Pofu and Mashela, 2011). Through research and development, procedures were developed to optimise the stem diameters of *Citrullus* and *Cucumis*, resulting in 100% survival of the grafts (Pofu and Mashela, 2011). In a subsequent greenhouse study (Pofu *et al*., 2011b), all intergeneric grafted seedlings survived and *Cucumis* seedling rootstocks retained their capabilities to reduce population densities of *M. incognita* race 2. Under field conditions the procedure was also successful, with grafts flowering earlier and producing higher fruit yield than these of intact plants (Pofu *et al*., 2011a).

2.2.3 Agronomics technology

The agronomics technology involves all aspects of the agronomy of the two *Cucumis* species. Originally, fruits used in GLT systems were collected from the wild, since plants were difficult to propagate due to auto-allelopathy. Mafeo (2005) developed sexual propagation protocols and determined fertilisation requirements of *C. myriocarpus*. The

protocols included the leaching of allelochemicals in running tapwater to improve germination (Mafeo and Mashela, 2009b). Nkgapele *et al*. (2011a,b) also investigated irrigation and fertilisation requirements of *C. africanus* and *C. myriocarpus* in pot trials, while Mafeo (2005) tested these requirements for *C. myriocarpus* under field conditions. In both trials, results suggested that moderate irrigation and fertilisation were required for achieving optimum fruit yield. Attempts are being made to use *in vitro* propagation in order to eliminate auto-allelopathic effects and the resultant poor emergence and therefore, non-uniformity in plant population stands (Maila *et al.*, 2013).

2.2.4 Chemical technology

Aspects of the chemistry of the two *Cucumis*, which include the chemistry of the active ingredients, toxicities and residues of the products are investigated under this technology. The molecular structures of active ingredients in the two *Cucumis* species had been established in the late 1930s. Allelochemicals from *C. africanus* fruit are collectively called cucurbitacin B ($C_{32}H_{48}O_8$), while those from *C. myriocarpus* fruit are cucurbitacin A, which comprises cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Jeffrey, 1978; Rimington, 1938). Cucurbitacin A and cucurbitacin B are soluble and insoluble in water, respectively (Chen *et al*., 2005). Under the auspices of the Land Bank Chair of Agriculture – University of Limpopo, not much has been done in chemical technology except for LC_{50} for *Meloidogyne* species and the citrus nematode (*Tylenchulus semipenetrans* Cobb), which was 7 µg/ml water for each nematode species (Muedi *et al*., 2005).

2.2.5 Botinemagation technology

Botinemagation is the application of botanicals in the management of population densities of nematodes through irrigation water (Mashela *et al*., 2011). Incidentally, active ingredients from fruits of *Cucumis* species are extracted through a fermentation process which involves the use of pieces of dried fruits and EMROSA effective micro-organisms (EM) over a period of 14 days (Pelinganga and Mashela, 2012). Fermented crude extracts of 500 g fresh fruits from *Cucumis* species per 16 L of water were tested and reduced *M. incognita* race 2 population levels in roots and soil by 89% (range 80 – 100%) and 69% (range 52 – 79%) (Pelinganga *et al*., 2012). At low concentrations, both products had stimulatory effects on tomato plant growth, while at high concentrations they inhibited plant growth. Pelinganga and Mashela (2012) developed the mean concentration stimulation range (MCSR) using a series of dilutions from 40 g and 80 g dried fruits of *C. africanus* and *C. myriocarpus,* respectively. From both *Cucumis* species, the MCSR was approximately 3%, while nematode numbers were suppressed from 78-97% from *C. africanus* fruit and from 87-97% in *C. myriocarpus* fruit.

2.3 Role of effective micro-organisms

Components of EMROSA EM and their respective roles had already been reviewed in detail elsewhere (Ncube, 2008). The current review intended to simply provide highlights of Ncube's (2008) review.

Yeast

The role of this bacterium in decomposition is changing the pH of organic matter to acidic medium. Yeast can only break organic matter during respiration up to the end of

glycolysis, where pyruvic acid is produced as an end - product. Most fungal pathogens cannot grow under acid conditions, whereas this is the best medium under which various bacteria multiply and operate. Also, yeast releases antimicrobial chemicals, which help to sterilize the fermented material.

Lactic acid bacteria

Lactic acid bacteria are responsible for breaking down cellulose and lignin, which are the toughest materials in organic matter. The end-products in cellulose and lignin breakdown are lactic acids, which further reduce the pH of fermented materials.

Photosynthetic bacteria

Bacterial decomposition releases toxic gases, which include SO_2, which may be lethal and also result into H_2SO_4, which is a very strong acid, resulting in corrosion of containers in which fermentation is carried out. The photosynthetic bacteria break down H_2SO_4 where the H^+ from H_2SO_4 is used during the light phase of photosynthesis instead of H^+ from water. Also, during photosynthesis, much heat is released, which makes the fermented material to be quite warm. The end-products in photosynthetic bacteria include S, which is required as an essential nutrient element by plants.

Actinomycete bacteria

The actinomycete bacteria release chitinase, which breaks down the chitin in the exoskeleton of insects, insect eggs, nematodes and nematode eggs. Because the cell wall of fungi is also made of chitin (Campbell, 1990), actinomycetes also break down all fungal pathogens during fermentation

The other active ingredients in EMROSA EM (Yeast and lactic acid bacteria) all release acids which reduce the pH of the fermented product (Campbell, 1990). Generally, fungi can only grow under alkalinic conditions. Thus, this low pH prevents the growth of fungal pathogens. The above illustrated how crude extracts of *Cucumis* fruits are sterilized through using EMROSA EM.

2.4 Mean concentration stimulation range

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

The MCSR, which is half the sum of two biological indices, namely D_m and the adjusted saturation point (aR_h) (Pelinganga and Mashela, 2012; Pelinganga *et al*., 2013).

According to the adjusted aR_h, $aR_h = D_m + R_h$, with MCSR = $(D_m + aR_h)/2 = (D_m) + (D_m + R_h)/2 = (2D_m + R_h)/2 = D_m + R_h/2$. The practical importance of MCSR is that it is the concentration of the phytonematicide which stimulates plant growth, while at the same time suppressing population densities of nematodes (Pelinganga and Mashela, 2012; Pelinganga *et al*., 2012, 2013).

2.5 Work not yet done on the research problem

In botinemagation, use of fermented fresh fruits of the two *Cucumis* species was done on equivalent mass basis to those of dried fruits with results suggesting that in fresh form nemafric-BL and nemarioc-AL were highly phytotoxic to tomato plants than their counterparts in dried form nemafric-BL and nemarioc-AL (Pelinganga and Mashela, 2012; Pelinganga *et al*., 2013). However, since the inputs were quantitatively not equivalent, effects were therefore, not comparable. Use of fermented fresh fruits of *C. africanus* and *C. myriocarpus* on equivalent mass bases with dried fruits on phytotoxicity to tomato plants and suppression of *Meloidogyne* species constitute part of the work not yet done in botinemagation with respect to nemafric-BL and nemarioc-AL.

CHAPTER 3
RESPONSES OF TOMATO GROWTH AND NEMATODE NUMBERS TO TWO PHYTONEMATICIDES

3.1 Introduction

The major limiting factor in research and development of nemafric-B and nemarioc-A, like most other phytonematicides, had been the high phytotoxicity of the products (Mashela *et al*., 2011). Generally, conventional methods for assessing phytotoxicities in plants are cumbersome (Inderjit and Malik, 2002), with inconsistent bioactivity results (Mashela *et al*., 2011). Generally, responses of plant growth to increasing concentrations of allelochemicals are characterised by density-dependent growth patterns (Liu *et al*., 2003), which have three growth phases: stimulation, neutral and inhibition phases (Liu *et al*., 2003; Salisbury and Ross, 1992). Liu *et al*. (2003) developed the Curve-fitting Allelochemical Response Dosage (CARD) model, which quantifies the three growth phases using seven biological indices, providing information on where each phase starts and ends.

Mafeo *et al*. (2011) used the CARD model to establish the concentration stimulation range of nemarioc-A on various crops, which originates at the beginning of the stimulation phase and ends at the beginning of the neutral phase. Half the concentration stimulation range, referred to as the mean concentration stimulation range (MCSR), is the concentration where the product should stimulate growth of the protected plant, while suppressing plant-parasitic nematodes (Mafeo *et al*., 2011). Pelinganga and Mashela (2012) studied biological indices of nemafric$^{\mathbf{D}}$-BL on tomato production and then used two indices to develop the mean concentration stimulatory range (MCSR).

The MCSR is the concentration which should stimulate growth of tomato plants, while suppressing population densities of nematodes. However, biological indices and MCSR of nemafric-BL and nemarioc-AL produced from fresh fruits are not documented. The objective of the study was two-fold: (1) To determine whether nemafric-BL produced from fresh fruit would stimulate growth of tomato plants and suppress population densities of *M. incognita* race 2, (2) To investigate whether nemarioc-AL produced from fresh fruit would stimulate growth of tomato plants and suppress population densities of *M. incognita* race 2.

3.2 Materials and methods

3.2.1 Location of study and preparation

Nemafric-BL and nemarioc-AL experiments were conducted simultaneously at the greenhouse of the Plant Protection Skills Centre, University of Limpopo, South Africa (23°53′10″S, 29°44′15″E) in spring (August-October) 2012. Ambient day/night temperatures averaged 28/21^{o}C, with maximum temperatures controlled using thermostatically-activated fans. Fruits for nemafric-BL and nemarioc-AL phytonematicides were harvested from field-grown plants, washed and cut into pieces (Mafeo and Mashela, 2009a). An equivalent of 40 g dried fruit mass of nemafric-B, namely, 284 g fresh fruit mass was fermented using effective micro-organisms (EM) in 16 L chlorine-free tapwater in 20 L container for 14 days at room temperature (Kyan *et al*., 1999). Also, an equivalent of 80 g dried fruit for nemarioc-A, namely, 411 g fresh fruit was fermented using EMROSA (EM). The EMROSA EM culture comprised South African strains of yeast, lactic acid bacteria, photosynthetic bacteria, actinomycete bacteria and minor strains of fungi (Higa and Parr, 1994). 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 1-L bottle half-filled with chlorine-free tapwater.

When required, nematode inocula were prepared by extracting eggs and second-stage juveniles (J2s) of *M. incognita* race 2 from roots of greenhouse-grown nematode-susceptible kenaf (*Hibiscus cannabinus* L.) in 1% NaOCl solution (Hussey and Barker, 1973). Twenty-cm-diameter plastic pots, at 0.3 m inter-row spacing and 0.25 m intra-row spacing, were each filled with 2 700 ml steam-pasteurised river sand and Hygromix (Hygrotech, Pretoria North, South Africa) at 3:1 (v/v). Uniform four-week-old tomato cv. 'Floradade' seedlings were transplanted and each inoculated with 3 000 eggs and J2s of *M. incognita* race 2.

3.2.2 Experimental design and cultural practices

Seven treatments, namely, 0, 2, 4, 8, 16, 32 and 64% concentrations of nemafric-BL or nemarioc-AL were arranged in a randomised complete block design, with 10 replicates. The concentrations were applied weekly at 250 ml/pot until harvest, while the untreated control received 250 ml tapwater. Three days after transplanting, each plant was fertilised with 3 g 2:3:2 (22) to provide 186 N, 126 K and 156 P, with 2 g 2:1:2 (43) - providing 0.35 N, 0.32 K and 0.32 P, 0.9 Mg, 0.75 Fe, 0.075 Cu, 0.35 Zn, 1.0 B, 3.0 Mn and 0.07 Mo mg/ml water. Four sets of Hadeco Moisture Meter (Hadeco, New Delhi, India) were inserted to 10-cm depths in randomly selected pots to monitor soil moisture tension. Plants were irrigated to full capacity using 250 ml chlorine-free tapwater as

soon as 50% moisture meter readings were below 2 units. Scouting for the greenhouse whitefly (*Trialeurodes vaporariorum* West) was done weekly with plants sprayed with 1.33 ml Lebaycid (a.i. fenthion 50%) water when population densities increased above 10 whiteflies per five randomly selected plants.

3.2.3 Data collection

Flowers were counted weekly with pedicels tagged to avoid recounting. At 56 days after initiating the treatments, fruit of all sizes were collected with plant height measured from the soil surface to the tip of the flag leaf and recorded. Stems were severed at the soil surface and the stem diameter measured at 5 cm above the severed end using a digital vernier caliper. Shoots were dried in air forced ovens at 70^{o}C for 72 h for dry shoot mass. Root systems were removed from pots, immersed in water to remove soil particles, blotted dry and weighed to facilitate the calculation of nematode density/total roots/plant. Nematodes (eggs + juveniles) were extracted from total root system/plant by maceration and blending for 30 s in 1% NaOCl solution (Hussey and Barker, 1973). The material was passed through nested 75- and 25-µm-opening sieves. Contents of the 25-µm-opening-sieve were collected for separation of nematodes from debris using the sugar-flotation and centrifugation method (Jenkins, 1964). Soil in each pot was mixed and a 250 ml soil sample collected for nematode extraction using the sugar centrifugation and flotation method (Jenkins, 1964). Eggs and juveniles from root and juveniles from soil were each counted using a stereomicroscope and converted to total root system per plant and total soil per pot, respectively. Root and soil nematodes from samples were converted to final nematode population densities (Pf) in root, soil and then combining the two to generate final nematode population densities (Pf).

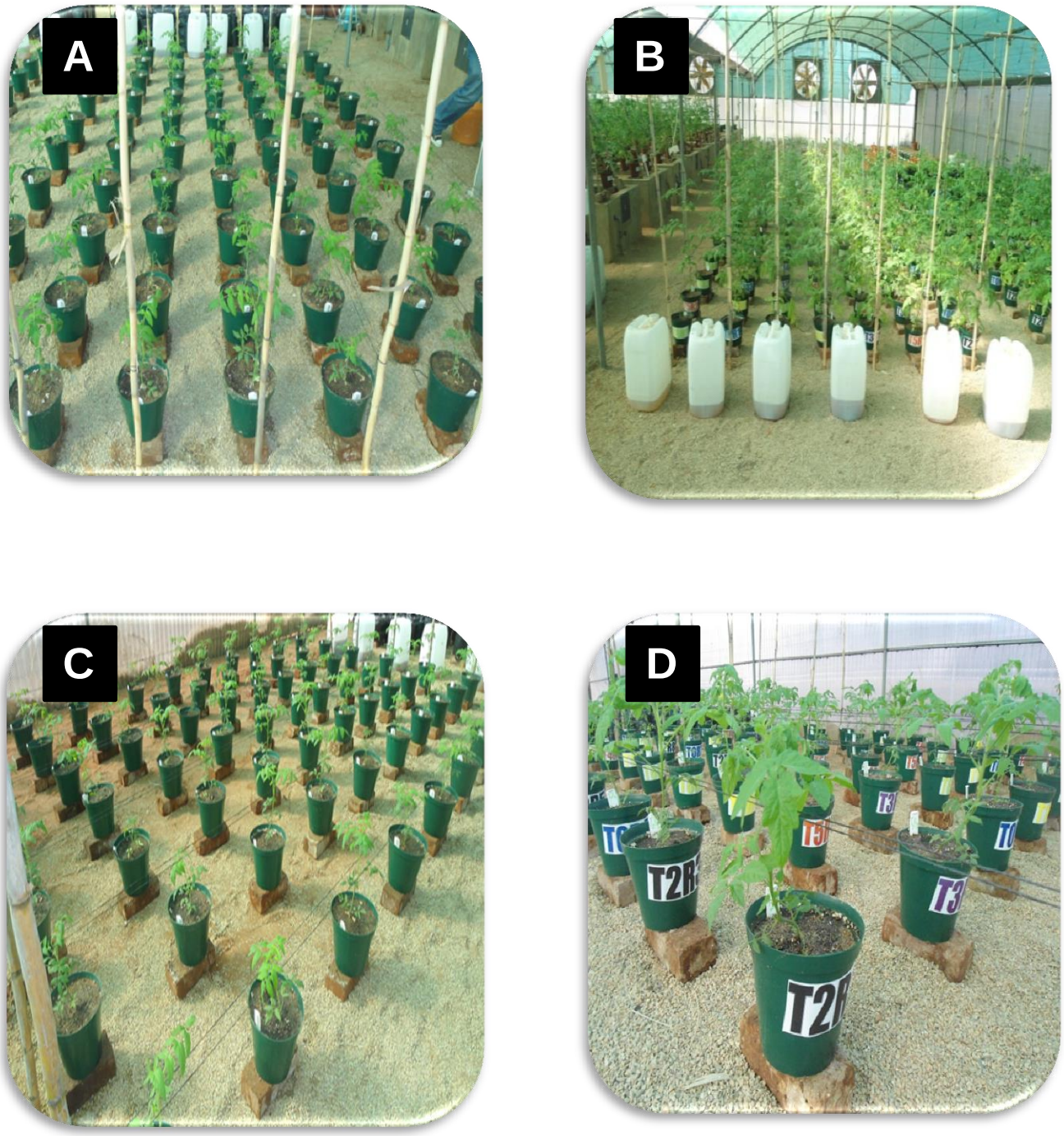

Figure 3.1.1 Greenhouse experiments using concentrations from fresh fruits of (**A** and **B)** nemafric-BL and (**C** and **D**) nemarioc-AL phytonematicides, respectively.

3.2.4 Data analysis

Data were subjected to analysis of variance (ANOVA) through the 2008 SAS software (SAS Institute, 2008). Flower and nematode numbers were each transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984), but untransformed means were reported. The sum of squares was partitioned to determine the contribution of sources of variation to the total treatment variation (TTV) in plant and nematode variables (Gomez and Gomez, 1984). Treatment mean separation was achieved using Waller-Duncan multiple range test at the probability level of 5%. Significant ($P \leq 0.05$) plant variables were further subjected to the CARD model (Mafeo *et al*., 2011; Pelinganga *et al*., 2013) to generated biological indices and then allow for the calculation of the MCSR for nemafric-BL or nemarioc-AL. Unless otherwise stated, only treatment effects which were significant at 5% level of probability were discussed.

3.3 Results

3.3.1 Responses to nemafric-BL phytonematicide

Nemafric-BL treatments had effects on dry root mass, dry root mass, plant height, number of flowers, number of fruit, plant height and stem diameter, contributing 67%, 78%, 58%, 43%, 60% and 26% to the total treatment variation, respectively (Table 3.1.1). Significantly different treatment means of dry root mass, dry shoot mass, plant height and stem diameter (Table 3.1.2), were subjected to the CARD model since they represented actual plant growth (Pelinganga, 2013). In contrast, number of flowers and fruits were not included in the CARD model since they did not constitute actual plant growth data (Pelinganga *et al*., 2012).

The CARD model, being a reiterative curve fitting tool, produced ideal density-dependent growth curves for dry root mass (Figure 3.1.2), dry shoot mass (Figure 3.1.3), plant height (Figure 3.1.4) and stem diameter (Figure 3.1.5), along with their biological indices. The CARD model explained 98%, 99%, 98% and 98% of quadratic curves of in dry root mass, dry shoot mass, plant height and stem diameter, respectively, against increasing concentrations of nemafric-BL (Table 3.1.3). The two biological indices, namely, the threshold stimulation point (D_m) and the saturation point (R_h) for each plant variable, provided concentration stimulation ranges of nemafric-BL on tomato plants, which allowed the computation of the adjusted R_h and thereafter, the integrated MCSR (Mafeo, 2012), which was approximately 7% (Table 3.1.4). The overall sensitivity ranking of tomato plants exposed to nemafric-BL was equivalent to 3 units. The R^2 values for all variables in the CARD model ranged from 97 to 99% (Table 3.1.5).

Treatment effects were highly significant ($P \leq 0.01$) for nematode in root, soil and total (root + soil), contributing 67%, 80% and 80%, respectively, to the total treatment variation (Table 3.1.6). Relative to untreated control, increasing concentrations of nemafric-BL reduced nematodes in root, soil and Pf by 87-97%, 49-96% and 70-97%, respectively (Table 3.1.7).

Table 3.1.1 Partitioning sum of squares for dry root mass, dry shoot mass, number of flowers, number of fruits, plant height and stem diameter of tomato cv. 'Floradade' under increasing concentrations of nemafric-BL at 56 days after initiation of treatments (n = 70).

		Dry root mass		Dry shoot mass		Number of flower		Number of fruit		Plant height		Stem diameter	
Source	DF	SS	%	SS	%	SS	%	SS	%	SS	%	SS	%
Replication	9	5.75	3^{ns}	24.40	1^{ns}	138.13	6^{ns}	22.357	17^{ns}	2949.80	10^{ns}	3.46	10^{ns}
Treatment	6	125.45	67^{**}	1354.50	78^{**}	1277.77	58^{**}	46.286	35^{**}	17279.86	60^{**}	8.89	26^{**}
Error	54	55.97	30	357.33	21	781.37	36	63.143	48	8548.90	30	22.00	64
Total	69	187.18	100	1736.23	100	2197.27	100	131.786	100	28777.80	100	34.34	100

^{ns} = Means that the factor was not significant at $P \leq 0.05$; while ** means that the factor was significant at $P \leq 0.01$.

Table 3.1.2 Responses of dry root mass, dry shoot mass, number of flowers, number of fruits, plant height and stem diameter of tomato cv. 'Floradade' to increasing concentrations of nemafric-BL at 56 days after initiation of treatments (n = 70).

Treatment (%)	Dry root mass	Dry shoot mass	Number of flower	Number of fruit	Plant height	Stem diameter
0	4.56[a]	23.98[a]	13.6[a]	1.3[bc]	95.2[a]	5.15[a]
2	4.51[a]	23.16[a]	11.8[a]	3.0[a]	87.9[a]	5.12[a]
4	4.25[ab]	24.27[a]	15.9[a]	1.9[ab]	91.0[a]	5.12[a]
8	3.78[ab]	22.67[ab]	13.4[a]	2.4[a]	92.8[a]	5.13[a]
16	3.03[bc]	22.28[ab]	12.4[a]	1.9[ab]	102.7[a]	5.09[a]
32	2.11[c]	19.27[b]	10.9[a]	1.8[ab]	90.1[a]	5.09[a]
64	0.68[d]	10.81[c]	1.6[b]	0.2[c]	35.7[b]	4.10[b]

Column means with the same letter were not different according to Waller-Duncan multiple range test at 5% level of probability.

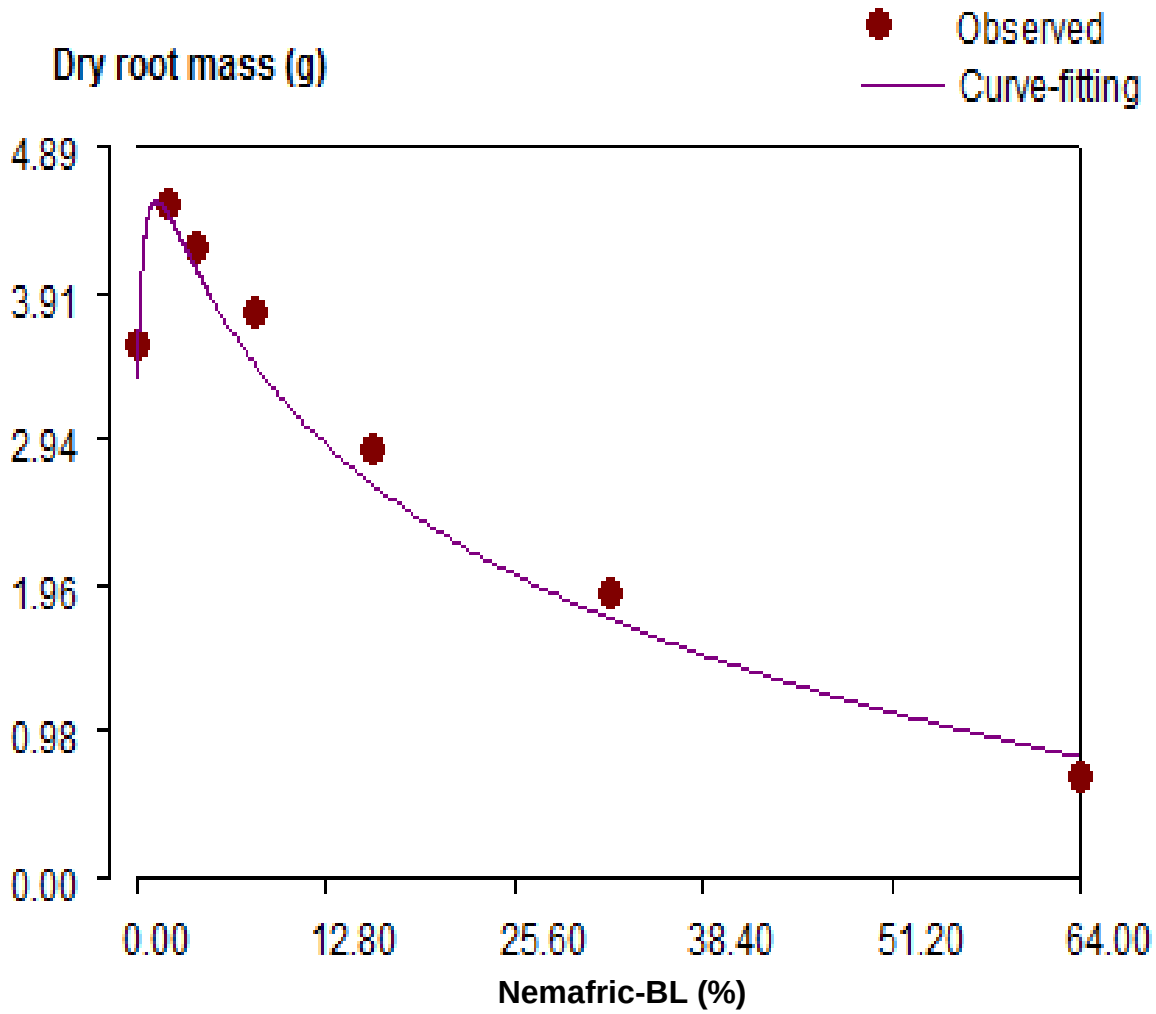

Figure 3.1.2 Response of dry root mass to increasing concentrations of nemafric-BL at 56 days after initiation of treatments (n = 70).

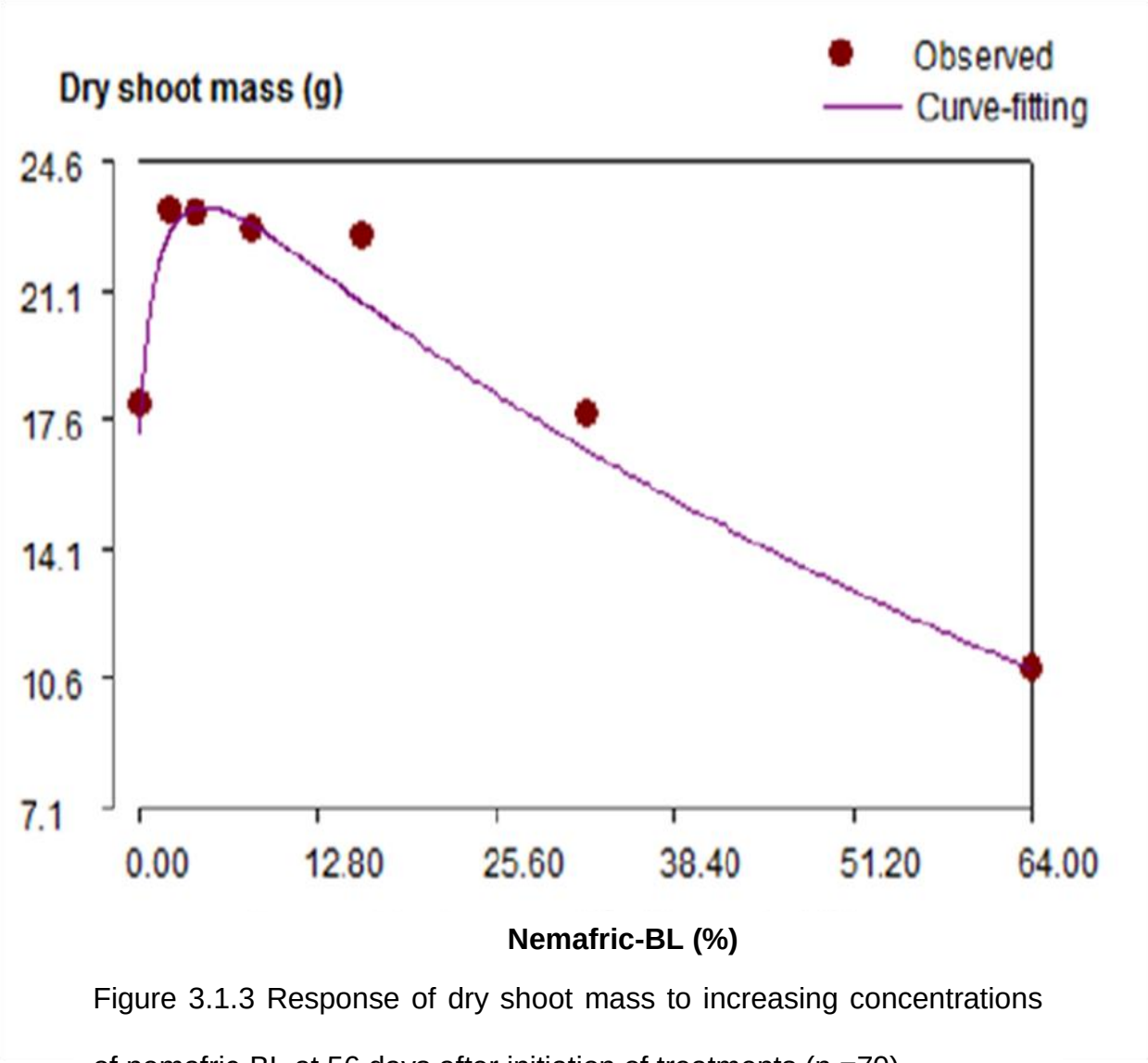

Figure 3.1.3 Response of dry shoot mass to increasing concentrations of nemafric-BL at 56 days after initiation of treatments (n =70).

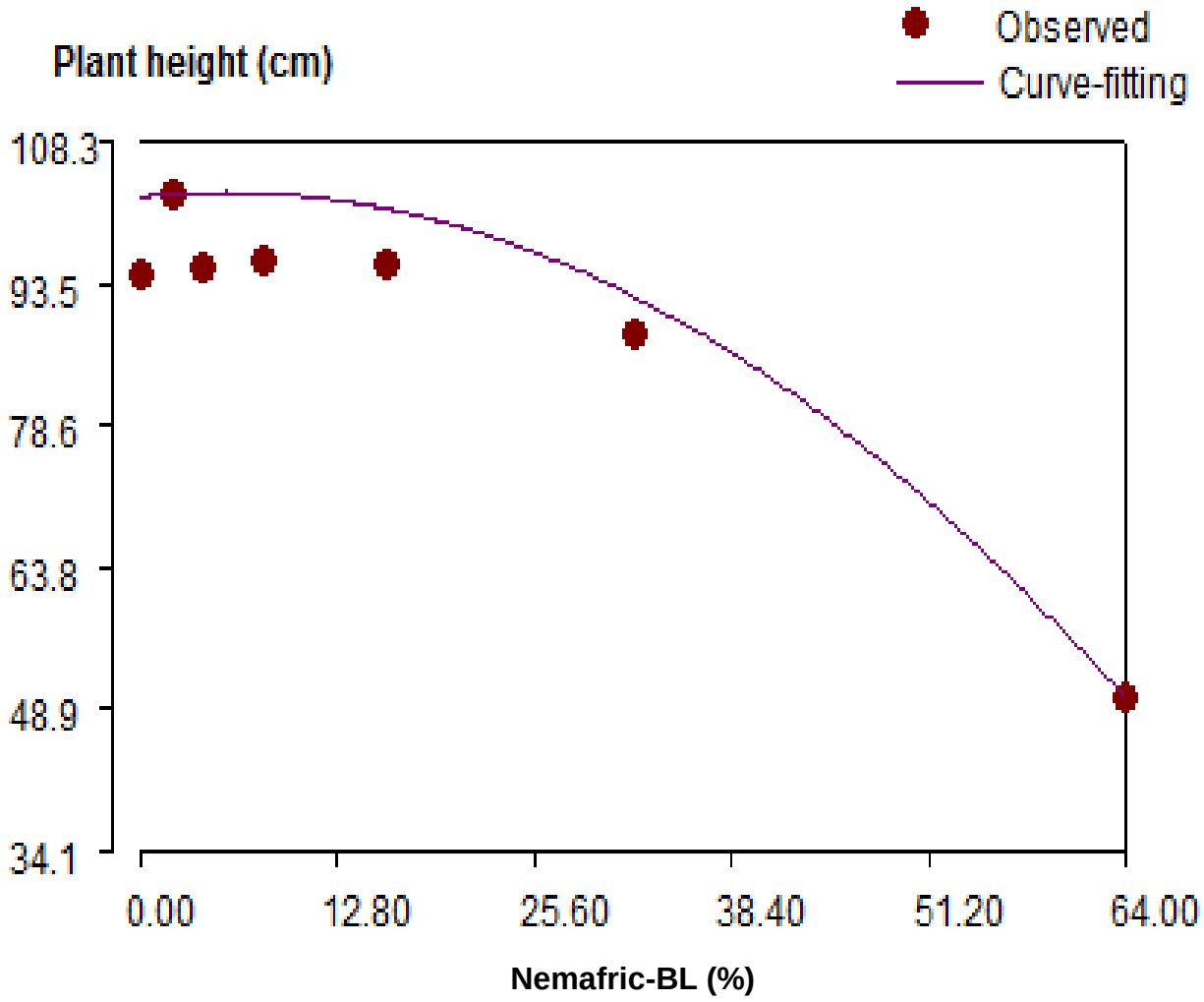

Figure 3.1.4 Response of dry plant height to increasing concentrations of nemafric-BL at 56 days after initiation of treatments (n =70).

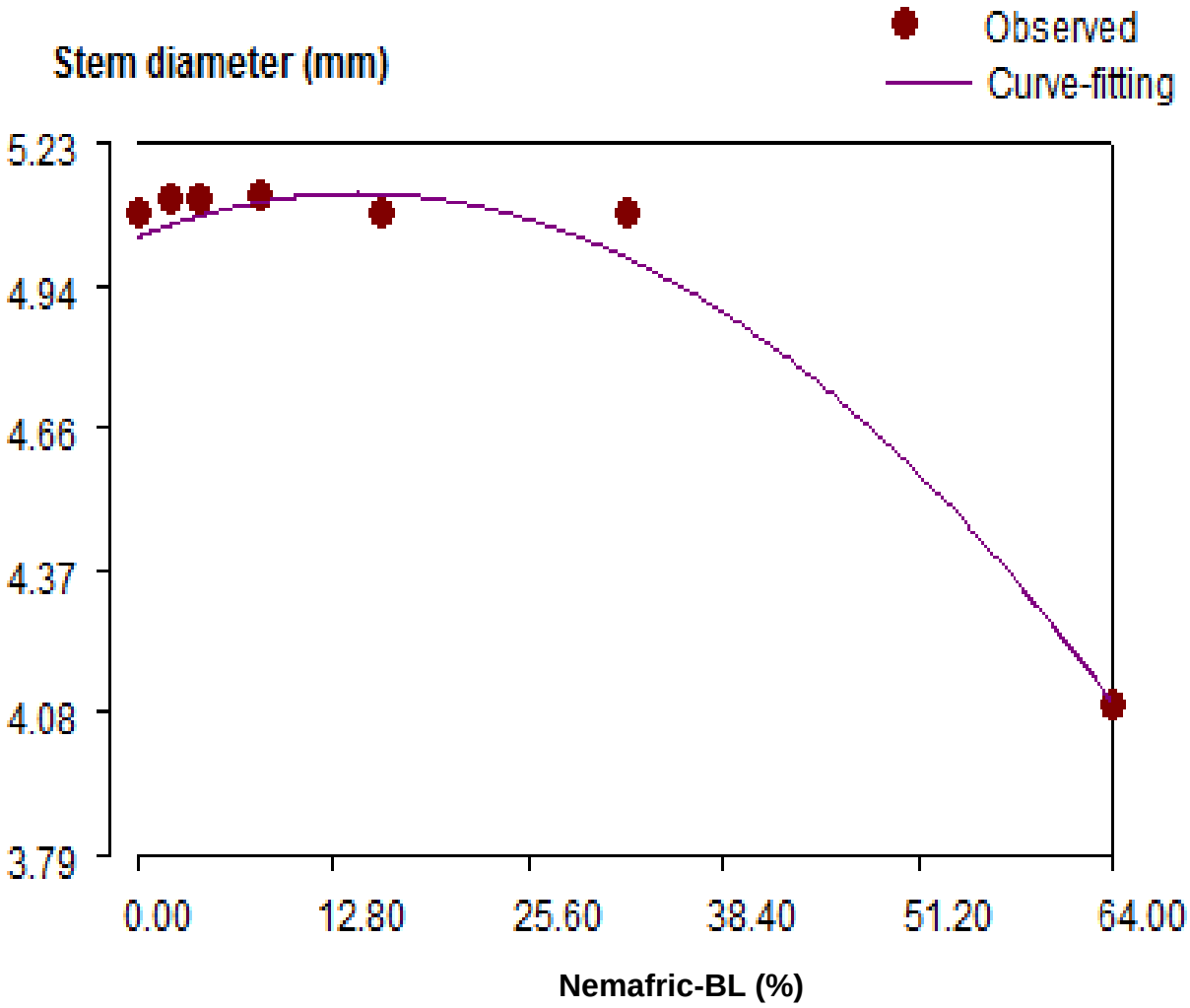

Figure 3.1.5 Response of stem diameter to increasing concentrations of nemafric-BL at 56 days after initiation of treatments (n =70).

Table 3.1.3 Biological indices for dry shoot, dry root mass, plant height and stem diameter of tomato cv. 'Floradate' exposed to increasing concentrations of nemafric-BL at 56 days after initiation of treatments (n = 70).

Biological indices	Dry root mass	Dry shoot mass	Plant height	Stem diameter
Threshold stimulation (D_m)	1.245	4.572	5.443	14.434
Saturation point (R_h)	1.244	6.154	0.409	0.089
0% inhibition (D_0)	8.696	30.045	10.889	28.868
50% inhibition (D_{50})	33.530	80.752	64.997	92.676
100% inhibition (D_{100})	116.500	164.000	89.500	128.398
R^2	0.99	0.98	0.98	0.98
Sensitivity (k)	2	1	0	0
Total sensitivity ($\sum k$) = 3				

Table 3.1.4 Mean concentration stimulation range of dry shoot mass (DSM), dry root mass (DRM), plant height (PHT) and stem diameter (SDR) of tomato exposed to increasing concentrations of nemafric-BL at 56 days after initiation of treatments (n = 70).

Biological indices	Dry root mass	Dry shoot mass	Plant height	Stem diameter	Mean
Threshold stimulation (D_m)	1.245	4.572	5.443	14.434	6.42
Adjusted saturation point $(R_h)^y$	2.49	10.73	5.85	14.52	8.40
Mean concentration stimulation range (MCSR)					7.41

Adjusted $R_h = D_m + R_h$, while MCSR = (D_m + adjusted R_h)/2

Table 3.1.5 Quadratic relationship, coefficient of determination and computed optimum response dosage (CORD) for variables of tomato from Curve-fitting Allelochemical Response Dosage to increasing concentrations of nemafric-BL at 56 days after initiation of treatments (n = 70).

Plant variables	Quadratic relationship	R^2	CORD (x)	P ≤
Dry shoot mass	$-2.083x^2 + 7.165x + 17.786$	0.98	1.720	0.05
Dry root mass	$-3.542x^2 + 4.199x + 3.517$	0.99	0.593	0.05
Flower number	$-0.005x^2 + 0.154x + 12.286$	0.97	15.400	0.05
Fruit number	$-0.526x^2 + 0.783x + 0.286$	0.97	0.744	0.05
Plant height	$-0.014x^2 + 0.150x + 96.982$	0.98	5.357	0.05
Stem diameter	$-0.001x^2 + 0.012x + 5.075$	0.98	6.000	0.05

CORD (x) = $-b_1/2b_2$

Table 3.1.6 Partitioning sum of squares for final *Meloidogyne incognita* population density in root and soil of tomato cv. 'Floradade' under increasing concentrations of nemafric-BL at 56 days after initiation of treatments (n = 70).

Source of	Juveniles & eggs in root		Juveniles in soil		Total juveniles & eggs	
Variation	SS	%	SS	%	SS	%
Replication	4.56	7^{ns}	1.32	2^{ns}	2.22	3^{ns}
Treatment	42.92	67^{***}	58.07	80^{***}	64.50	80^{***}
Error	16.63	26	13.26	18	13.95	17
Total	64.10	100	72.64	100	80.67	100

^{ns} = significant at P ≤ 0.10; while *** was highly significant at P ≤ 0.01.

Table 3.1.7 Influence of increasing concentrations of nemafric-BL on nematode juveniles *Meloidogyne incognita* in roots, soil and total population density (Pf) (n = 70).

Concentration (%)	Juveniles & eggs in root	(%)[z]	Juveniles in soil	(%)[z]	Pf	(%)[z]
0	3540 (3.11[a])	-	3366 (3.52[a])	-	6905 (3.78[a])	-
2	340 (2.44[ab])	-90	1728 (3.23[ab])	-49	2068 (3.30[ab])	-70
4	471 (2.60[ab])	-87	1350 (3.12[ab])	-60	1821 (3.25[ab])	-74
8	303 (2.35[b])	-91	954 (2.94[ab])	-72	1257 (3.07[b])	-82
16	337 (2.46[ab])	-90	1044 (3.00[ab])	-69	1381 (3.12[ab])	-80
32	557 (2.58[ab])	-84	774 (2.83[b])	-77	1331 (3.08[ab])	-81
64	94 (0.45[c])	-97	144 (0.57[c])	-96	238 (0.60[c])	-97

[z]Relative impact (%) =[(Treatment/Control) – 1] x 100

3.3.2 Responses to nemarioc-AL phytonematicide

Nemarioc-AL treatments had effects on dry root mass, dry root mass, plant height, number of flowers, number of fruit, plant height and stem diameter, contributing 71%, 61%, 19%, 35%, 34% and 24.% to the total treatment variation, respectively (Table 3.2.1). Different treatment means of dry root mass, dry shoot mass, plant height and stem diameter (Table 3.2.2), were subjected to the CARD model since they represented data that depicted actual plant growth number of flowers and fruit data were viewed as described previously (Section 3.3.1) (Pelinganga, 2013). The CARD model produced density-dependent growth curves for dry root mass (Figure 3.2.1), dry shoot mass (Figure 3.2.2), plant height (Figure 3.2.3) and stem diameter (Figure 3.2.4), along with their respective biological indices (Table 3.2.4). The CARD model explained 98%, 96%, 84% and 93% of the total treatment variation in dry root mass, dry shoot mass, plant height and stem diameter, respectively (Table 3.2.3). The threshold stimulation (D_m) and the saturation point (R_h) for each plant variable provided the concentration stimulation range of nemarioc-AL on tomato plants, which allowed the calculation of the adjusted R_h and thereafter, the integrated (MCSR) (Table 3.2.4) as described previously (Section 3.3.1). The MCSR for nemarioc-AL was 4% (Table 3.2.4). The overall sensitivity ranking (Σk) of tomato exposed to nemarioc-AL was equivalent to 5 units, while R^2 values for all variables in the CARD model ranged from 82 to 98% (Table 3.2.5).

Treatment effects were highly significant for nematodes in root, soil and final nematode population density (Pf), contributing 68%, 87% and 88%, respectively, to the total treatment variation (Table 3.2.6). Relative to untreated control, increasing

concentrations of nemarioc-AL reduced nematodes in roots, soil and final nematode population density (Pf) by 46-92%, 74-96% and 74-96%, respectively (Table 3.2.7).

Table 3.2.1 Partitioning sum of squares for dry root mass, dry shoot mass, number of flowers, number of fruits, plant height and stem diameter of tomato cv. 'Floradade' under increasing concentrations of nemarioc-AL at 56 days initiation of treatments (n = 70).

Source of variance		Dry root mass		Dry shoot mass		Number of flower		Number of fruit		Plant height		Stem diameter	
	DF	SS	%	SS	%	SS	%	SS	%	SS	%	SS	%
Replication	9	5.41	4^{ns}	50.48	5^{ns}	64.29	5^{ns}	16.63	15^{ns}	1430.90	13^{ns}	0.02	4.4^{ns}
Treatment	6	97.07	71^{**}	673.33	61^{**}	265.60	19^{**}	38.77	35^{**}	3855.50	34^{**}	0.12	24.4^{**}
Error	54	33.76	25	371.94	34	1034.11	76	56.37	50	6055.10	53	0.36	71
Total	69	136.24	100	1095.74	100	1364.00	100	111.77	100	11341.50	100	0.51	100

^{ns} = Means that the factor was not significant at $P \leq 0.05$; while ** means that the factor was significant at $P \leq 0.01$.

Table 3.2.2 Responses of dry root mass, dry shoot mass, number of flowers, number of fruits, plant height and stem diameter of tomato cv. 'Floradade' to increasing concentrations of nemarioc-AL at 56 days after initiation of treatments (n = 70).

Treatment (%)	Dry root mass	Dry shoot Mass	Number of flower	Number of fruit	Plant height	Stem diameter
0	3.89[a]	24.82[ab]	14.5[ab]	2.0[a]	96.5[ab]	5.17[a]
2	4.43[a]	25. 56[ab]	14.6[ab]	2.3[a]	94.9[ab]	5.21[a]
4	4.43[a]	24.80[ab]	14.9[ab]	2.0[a]	86.5[bc]	5.21[a]
8	3.84[ab]	26.06[a]	16.2[a]	2.8[a]	100.5[ab]	5.17[a]
16	2.77[bc]	22.46[bc]	15.0[ab]	2.6[a]	102.6[a]	5.16[a]
32	1.87[cd]	20.45[c]	13.1[ab]	1.5[ab]	90.8[abc]	5.10[b]
64	1.24[d]	16.83[d]	9.7[b]	0.4[b]	79.8[c]	5.10[b]

Column means with the same letter were not different according to Waller-Duncan multiple range test at 5% level of probability.

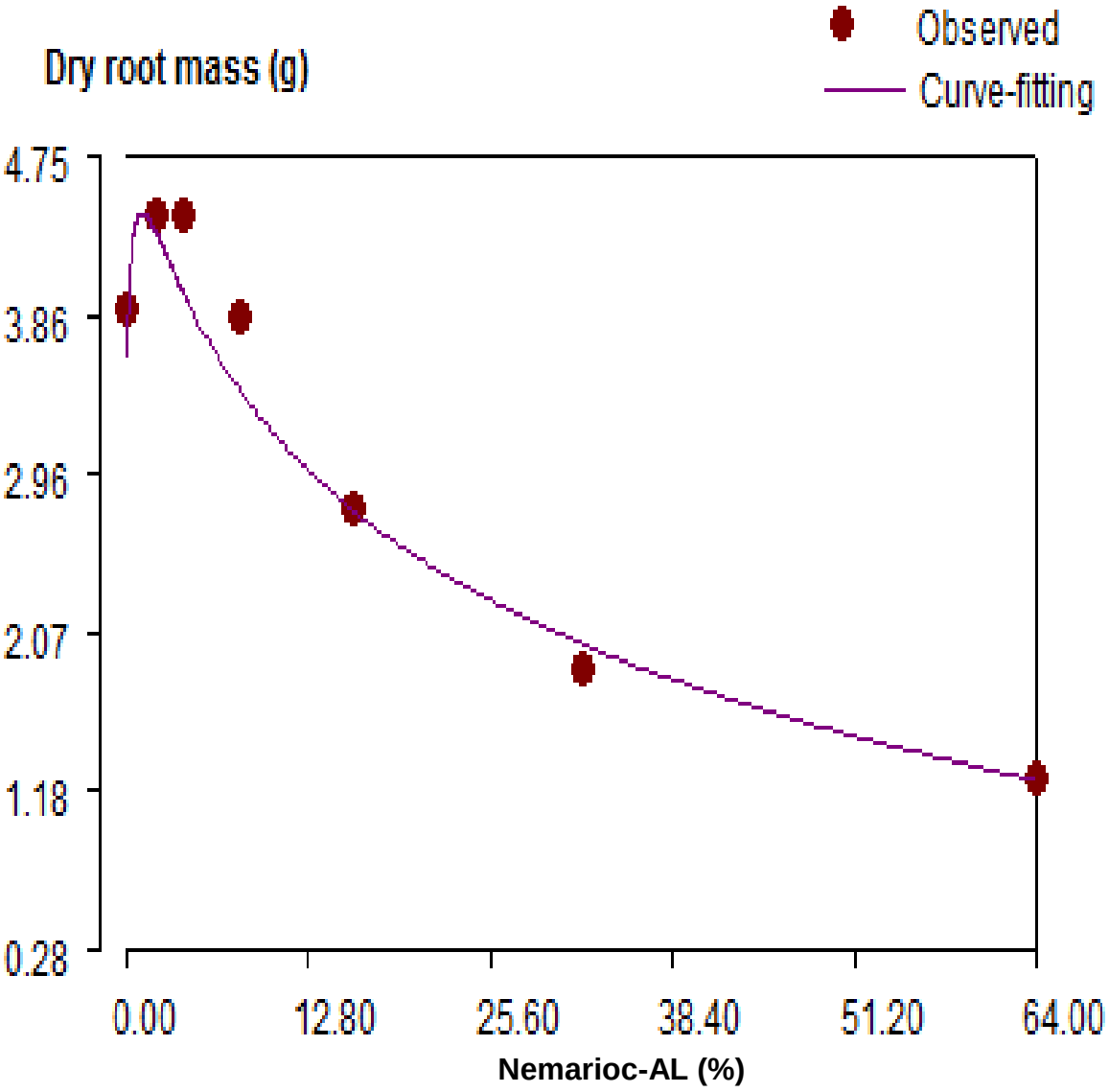

Figure 3.2.1 Response of dry root mass to increasing concentrations of nemarioc-AL at 56 days after initiation of treatments (n = 70).

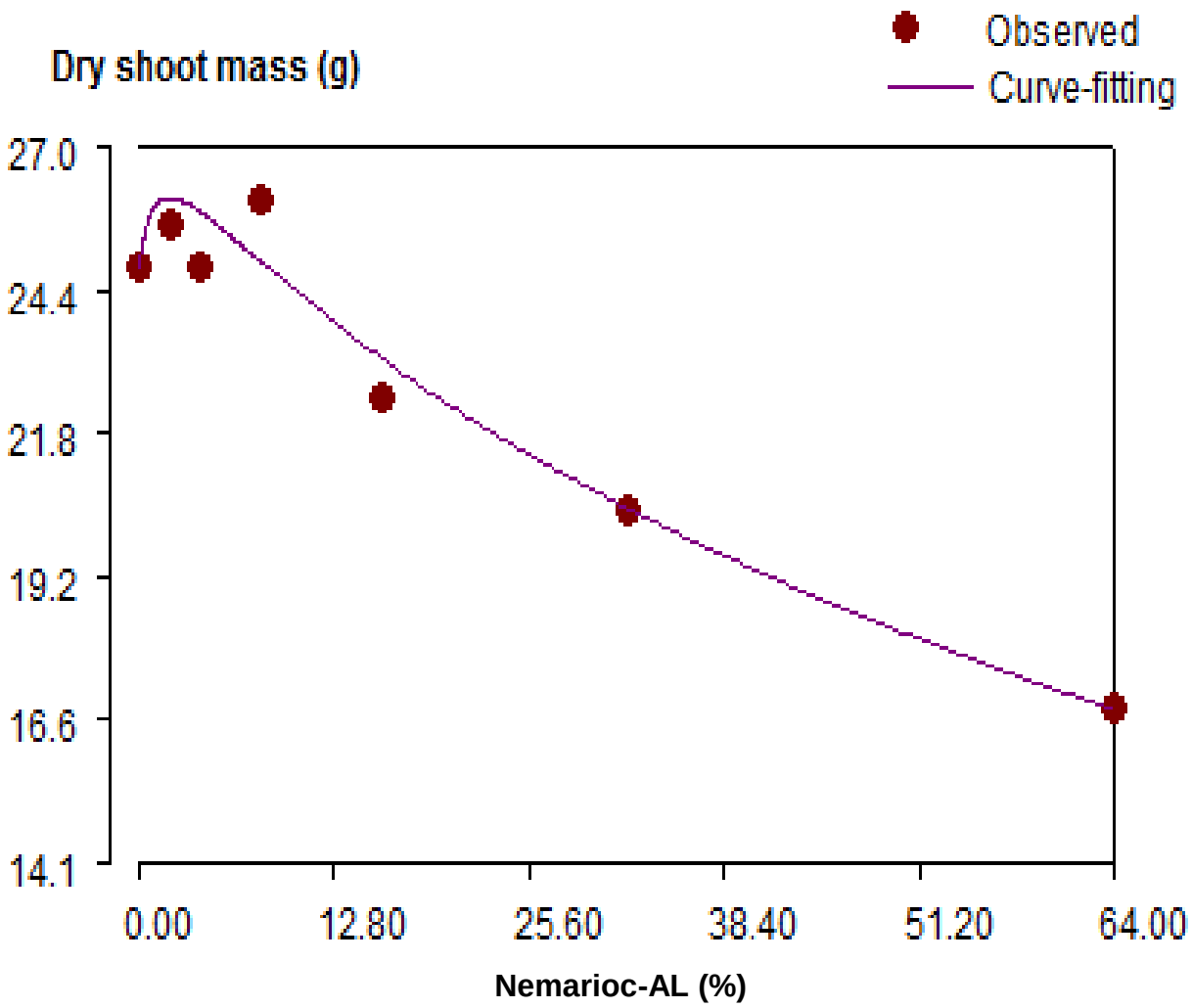

Figure 3.2.2 Response of dry shoot mass to increasing concentrations of nemarioc-AL at 56 days after initiation of treatments (n = 70).

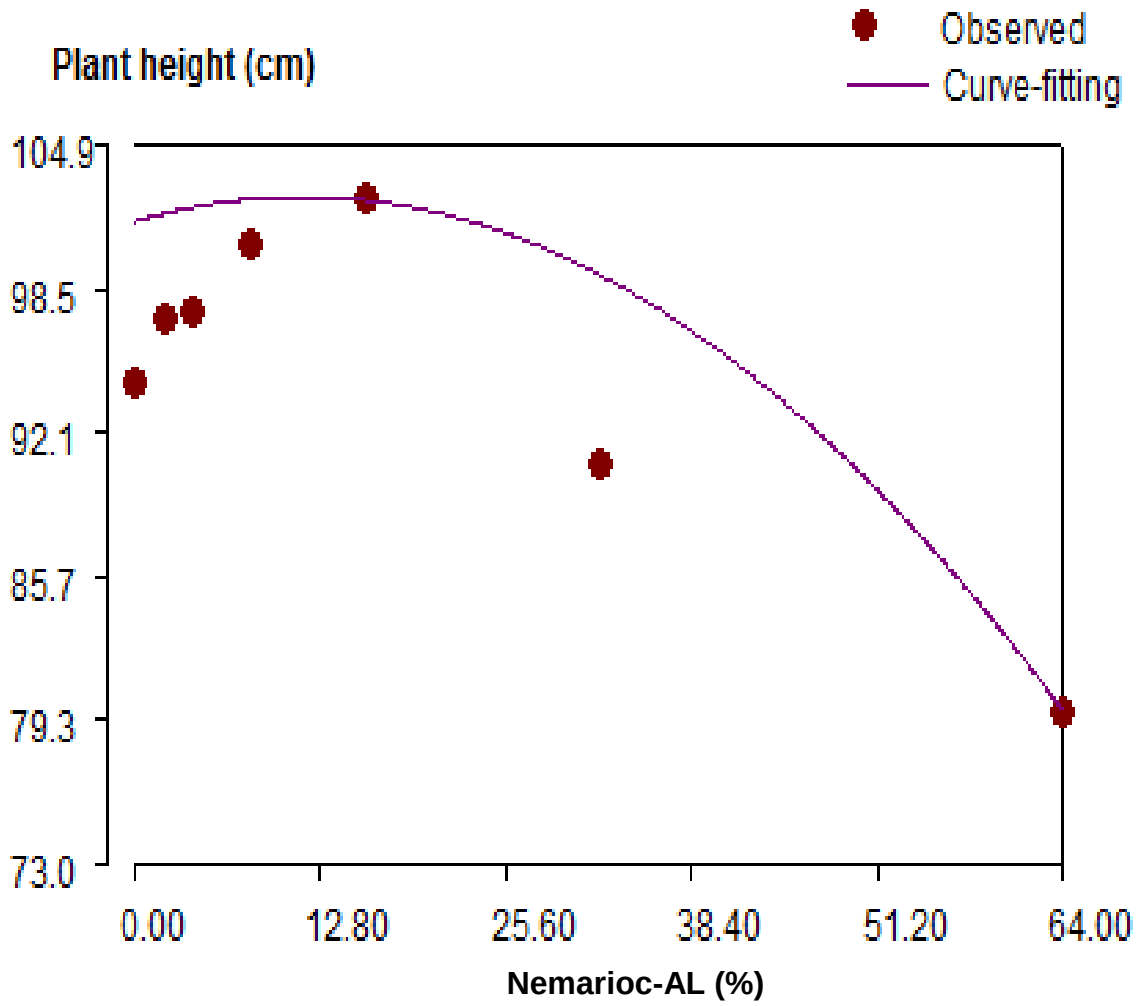

Figure 3.2.3 Response of dry plant height to increasing concentrations of nemarioc-AL at 56 days after initiation of treatments (n = 70).

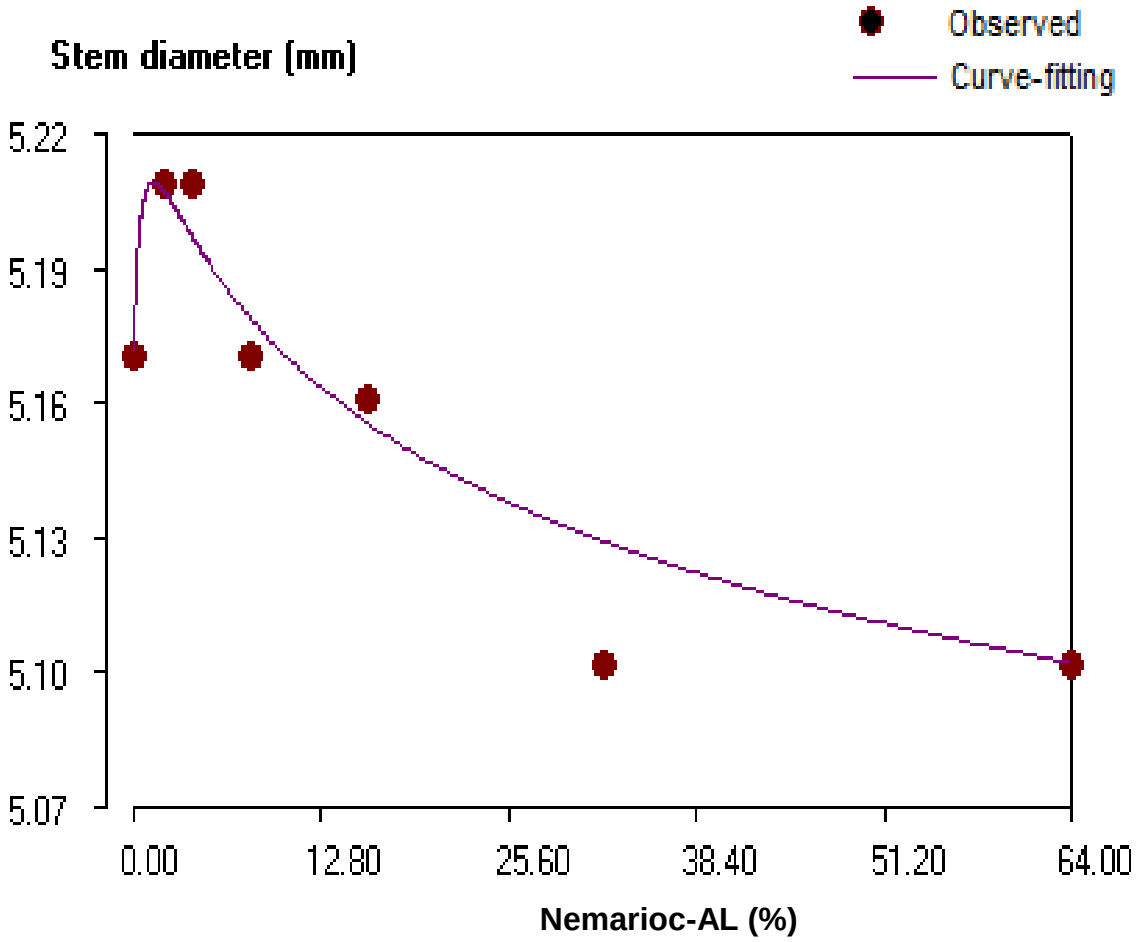

Figure 3.2.4 Response of stem diameter to increasing concentrations of nemarioc -AL at 56 days after initiation of treatments (n = 70).

Table 3.2.3 Biological indices for dry shoot, dry root mass, plant height and stem diameter of tomato cv. 'Floradate' exposed to increasing concentrations of nemarioc-AL at 56 days after initiation of treatments (n = 70).

Biological indices	Dry root mass	Dry shoot mass	Plant height	Stem diameter
Threshold stimulation (D_m)	1.077	2.035	11.613	1.315
Saturation point (R_h)	0.906	1.174	0.939	0.044
0% inhibition (D_0)	6.359	8.214	23.225	9.839
50% inhibition (D_{50})	34.504	129.884	95.956	n/a
100% inhibition (D_{100})	161.9	552.5	n/a	n/a
R^2	0.98	0.96	0.84	0.93
Sensitivity (k)	2	1	0	2
Total sensitivity ($\sum k$) = 5				

Table 3.2.4 Mean concentration stimulation range of dry shoot mass (DSM), dry root mass (DRM), plant height (PHT) and stem diameter (SDR) of tomato cv, 'Floradade' exposed to increasing concentrations of nemarioc-AL at 56 days after initiation of treatments (n = 70).

Biological indices	Dry root mass	Dry shoot mass	Plant height	Stem diameter	Mean
Threshold stimulation (D_m)	1.077	2.035	11.613	1.315	4.01
Adjusted saturation point $(R_h)^y$	1.983	3.209	12.552	1.359	4.776
Mean concentration stimulation range (MCSR)					4.4

Adjusted $R_h = D_m + R_h$, while MCSR = $(D_m$ + adjusted $R_h)/2$

Table 3.2.5 Quadratic relationship, coefficient of determination and computed optimum response dosage (CORD) for variables of tomato from Curve-fitting Allelochemical Response Dosage to increasing concentrations of nemarioc-AL at 56 days after exposure (n = 70).

Plant variables	Quadratic relationship	R^2	CORD (x)	P ≤
Dry shoot mass	$-24.641x^2 + 2.115x + 0.953$	0.96	49.282	0.05
Dry root mass	$-3.863x^2 + 3.304x - 3.012$	0.98	0.428	0.05
Flower number	$-0.005x^2 + 0.154x + 12.286$	0.97	15.400	0.05
Fruit number	$-2.257x^2 + 0.009x + 0.001$	0.82	0.002	0.05
Plant height	$-97.187x^2 + 0.162 – 0.007$	0.96	8.334	0.05
Stem diameter	$-5.171x^2 + 0.144x – 0.118$	0.93	0.014	0.05

CORD (x) = $-b_1/2b_2$

Table 3.2.6 Partitioning sum of squares for final *Meloidogyne incognita* population density in root and soil of tomato cv. 'Floradade' under increasing concentrations of nemarioc-AL at 56 days after initiation of treatments (n = 70).

Source of variation	Juveniles & eggs in root		Juveniles in soil		Total juveniles & eggs	
	SS	%	SS	%	SS	%
Replication	0.5217	3.4^{ns}	0.2156	2^{ns}	0.1964	1.4^{ns}
Treatment	10.5301	68^{***}	12.6227	87^{***}	12.3428	88^{***}
Error	4.3546	28.3	1.5997	11	1.4313	10.2
Total	15.4065	100	14.4380	100	13.9704	100

^{ns} = significant at P ≤ 0.10; while *** was highly significant at P ≤ 0.0.

Table 3.2.7 Influence of increasing concentrations of nemarioc-AL on nematode juveniles *Meloidogyne incognita* in roots, soil and total population density (Pf) (n = 70).

Concentration (%)	Juveniles & eggs in root	(%)[z]	Juveniles in soil	(%)[z]	Pf	(%)[z]
0	163 (2.19[a])	-	9540 (3.97[a])	-	9703 (3.98[a])	-
2	88 (1.93[ab])	-46	2448 (3.37[b])	-74	2536 (3.39[b])	-74
4	79 (1.88[abc])	-52	1800 (3.25[bc])	-81	1879 (3.27[bc])	-81
8	74 (1.86[abc])	-55	1584 (3.18[bc])	-83	1659 (3.21[bc])	-83
16	63 (1.78[bc])	-61	1188 (3.04[c])	-88	1251 (3.07[c])	-87
32	36 (1.50[c])	-78	630 (2.74[d])	-93	666 (2.78[d])	-93
64	13 (0.89[d])	-92	396 (2.56[d])	-96	409 (2.57[d])	-96

[z]Relative impact (%) = [(Treatment/Control – 1)] x 100

3.4 Discussion

3.4.1 Density-dependent growth patterns

Nemafric-BL and nemarioc-AL each had the attributes of other allelochemicals in terms of inducing density-dependent growth patterns in tomato plants as concentrations increased. Although this feature characterises most biological responses when exposed to intrinsic and or extrinsic increasing concentrations of allelochemicals (Salisbury and Ross, 1992). In the early stages of the ground leaching technology (GLT) system, Mashela (2002) referred to the observed stimulated growth in tomato plants as a "fertiliser effect", which was, however, not supported by empirical evidence of accumulated essential nutrient elements. Later, it was confirmed that at small quantities of crude extracts from *Cucumis* species, the materials invariably stimulated growth of tomato plants (Mafeo, 2012; Pelinganga, 2013), which confirmed the existence of the stimulation phase in density-dependent growth patterns in response to low concentrations of allelochemicals (Lui *et al*., 2003).

Density-dependent growth patterns in allelochemicals from the two *Cucumis* species are not restricted to plant species only. Cucumin ($C_{27}H_{40}O_9$), which is one of the consitituents of cucurbitacin A was observed to reduce cancerous cells in human, although it was toxic at high concentrations (Lee *et al*., 2010). This was a clear evidence of the existence of density-dependent growth patterns in animal cells in response to cucurbitacin A. Currently, cucurbitacin B is also being widely investigated in medicine for use in suppression of cancer (Lee *et al*., 2010). Density-dependent growth patterns in cancer studies have also demonstrated that the effect of cucurbitacin A and B are at

cellular level, the information which had not been forth coming in plant and other studies (Lui *et al*., 2003; Mafeo, 2012; Pelinganga, 2013).

3.4.2 Overall sensitivity of tomato plants

Overall sensitivity (∑k) of tomato plants to nemafric^{F}-BL was 3 units, when compared with that of 5 units from the nemafric^{D}-BL product (Pelinganga and Mashela, 2012). Incidentally, a ∑k value of nemarioc^{F}-AL was 5 units, while that of nemarioc^{D}-AL was 3 units (Pelinganga *et al*., 2012). Conventionally, sensitivities of a plant to allelochemicals are inversely proportional to ∑k values (Liu *et al*., 2003). In other words, when ∑k values approach zero, the more sensitive is the plant to the allelochemical (Liu *et al*., 2003). In this study, tomato plants were more sensitivity to nemafric^{F}-BL than nemafric^{D}-BL, while plants were more sensitive to nemarioc^{D}-AL than nemarioc^{F}-AL. However, the ∑k values should not be viewed in isolation to the concentrations of the products.

In this study nemafric^{F}-BL with ∑k = 3 units had MCSR of 7% for tomato plants, while nemafric^{D}-BL at ∑k = 5 units had MCSR of 3% for the same plants (Pelinganga and Mashela, 2012). In contrast, nemarioc^{F}-AL with ∑k = 5 units for tomato, had MCSR of 4% while nemarioc^{D}-AL with ∑k = 3 units had MCSR of 3% (Pelingnga, 2013). Apparently, there was no consistent relationship between ∑k and MCSR as shown below:

Table 3.2.8 Relationship between sensitivity value (∑k) and mean concentration stimulation range (MCSR) on tomato plant growth.

Products	∑k value	MCSR	References
Nemafric^{F}-BL	3	7%	This study
Nemafric^{D}-BL	5	3%	(Pelinganga, 2013)
Nemarioc^{F}-AL	5	4%	This study
Nemarioc^{D}-AL	3	3%	(Pelingnga, 2013)

Ideally, when ∑k values are low, in order to minimise a, which is common when using allelochemicals, lower concentrations should be used, as was evident in nemarioc^{D}-AL.

Using dried fruits of *C. africanus* and *C. myriocarpus* in producing nemafric-BL and nemarioc-AL appeared to be ideal for use as phytonematicides for both products. However, since phytonematicides have multiple active ingredients (Wuyts *et al*., 2006), it is currently not clear which ones are affected by drying at 52^{o}C, where Makkar (1999) observed the optimum concentration of chemicals in dried materials. Importantly, the remaining active ingredients were still nonphytotoxic to tomato plants.

3.4.3 Suppression of *Meloidogyne* species

Regardless of the form, nemafric-B and nemarioc-A reduced population densities of *M. incognita* race 2.various mechanisms have been described as being responsible for the observed suppression. Under chemotaxis, the effect of a phytonematicide can either be repellent or attractive to the nematode (Hewlett *et al*., 1997). Generally, mobility

inhibition allows the host plants to induce and express more powerful defense mechanisms, while egress inhibition and increased mortality have the potential ability of reducing overall nematode population densities in roots and soil (Agbenin *et al*., 2005; Wuyts *et al*., 2006). Second-stage juveniles get into contact with phytonematicides in soil solutions soon after egress since they have to migrate into the soil for re-infection of new roots (Wyss *et al*., 1992), where chemotaxis, mobility inhibition and mortality occur (Wuyts *et al*., 2006). Certain potent chemicals enter egg masses, where they interfere with development of stylets in J1s and therefore, inhibit egg hatch since stylets are required for this process to succeed (Hirschmann, 1985; Parmar, 1987). Actinomycete bacteria in the EM culture release chitinases, which hydrolysis chitin in exoskeleton of insects, insect eggs, nematode bodies and nematode eggs (Higa and Parr, 1994). However, the influence of EM in the reduction of plant-parasitic nematodes is not supported by empirical evidence.

The multi-site active ingredients in botanicals have also been identified in neem (*Azadirachta indica A. Juss*.) and wild garlic (*Tulbaghia violacea L.*) when used as insecticides (Nzanza and Mashela, 2012). For instance, azadirachtin from neem and allicin from wild garlic have multi-site active ingredients. Azadirachtin (i) acts as a strong antifeedant and repellent, (ii) delays and prevents moulting, (iii) reduces insect growth and development, (iv) interferes with oviposition and (v) can induce high mortalities in more than 200 insect species (Coudriet *et al*., 1985; Liu and Stansly, 1995; Kumar and Poehling, 2006; Kumar *et al*., 2005; Mitchell *et al*., 2004; Prabhaker *et al*., 1989). Similarly, wild garlic bulbs possess chemical compounds such as sacrid volatilic oil and

sulpho-oxides derived from allicin, which are responsible for antifeedant, repellent and toxicant properties against pests (Dhanalakshmi, 2006; Vijayalakshmi *et al*., 1996). Apparently, multiple-site active ingredients in phytopesticides are more advantageous than single active ingredients, which characterised the suspended synthetic nematicides.

In comparing results of the two products from dried (nemafric^{D}-BL; nemarioc^{D}-AL) and fresh (nemafric^{F}-BL; nemarioc^{F}-AL) forms, one is faced with the challenge of selecting the suitable product. First, drying *C. africanus* fruit at 52^{o}C for 72 h prior to fermentation appears to reduce phytotoxicity of the products to tomato, although the mechanism involved is not yet clear. Secondly, since fresh fruits of *C. africanus* and *C. myriocarpus* are highly susceptible to post-harvest decays (Mphahlele *et al*., 2012), in fresh form the raw inputs for nemafric-B and nemarioc-A would not be available during certain seasons, particularly in areas where tomatoes are produced all-year-round. Thirdly, since fruits of both *Cucumis* species can be produced in large quantities during their appropriate seasons (Mafeo, 2005), nemafric$^{\mathbf{D}}$-B and nemarioc$^{\mathbf{D}}$-A are ideal candidates for commercialisation, since raw inputs would not limit the production of the products. Fourthly, since cucurbitacin B is equally distributed in all organs of *C. africanus* plants (Jeffrey, 1978; Rimington, 1938), the whole plant could be dried and used as raw material in fermentation, thus, eliminating challenges faced in the disposal of by-products.

3.5 Conclusions

Produced from fresh fruits nemafric-BL and nemarioc-AL have the potential of serving as phytonematicides in tomato for on-farm production purposes. However, the two products would, as commercial products, inevitably face unavailability challenges of raw materials during off-seasons, since fruits are highly perishable (Mphahlele *et al*., 2012). Consequently, in this study, nemafric-B and nemarioc-A produced from dried fruits are being recommended as having potential attributes for serving as commercial phytonematicides from crude extracts of *C. africanus* and *C. myriocarpus* fruits.

CHAPTER 4
SUMMARY, RECOMMENDATIONS AND CONCLUSIONS

4.1 Summary

Two separate trials were conducted to investigate biological indices and mean concentration stimulation range (MCSR) of nemafric-BL and nemarioc-AL in tomato production and management of root-knot nematodes. Fruit of *C. africanus* and *C. myriocarpus* were widely used in management of the root-knot (*Meloidogyne incognita*) in Limpopo Province, South Africa. Generally, MCSR values from fermented nemafric-BL and nemarioc-AL fruits retained their capability to suppress numbers of *M. incognita* race 2. However, for technical reasons, nemafric-BL and nemarioc-AL are not recommended for commercialization purposes.

4.2 Recommendations

Generally, in fresh form, fruits of the two *Cucumis* species have high incidence of post-harvest fruit decay (Mphahlele *et al*., 2012). Thus, the materials may not be available during certain times of the year. In order to reduce costs, smallholder farmers could produce their own products on-farm. This is, however, not recommended since the quality of the products would not be guaranteed.

4.3 Conclusions

In conclusion, results of this study confirmed those of Pelinganga *et al*. (2012) in terms of k and MCSR values. Both fermented forms of nemafric^{F}-BL and nemafric^{D}-BL were more effective in suppressing population densities of *Meloidogyne* species. However, due to the higher phytotoxicity of nemafric^{F}-BL form (lower k value), nemafric^{D}-BL form is recommended for use in botinemagation. Both nemarioc^{D}-AL

and nemarioc-AL had good capabilities of suppressing numbers of *M. incognita* race 2 in tomato production. However, at their commended concentrations the materials should be used with caution in tomato production since ∑k is rather low.

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APPENDICES

Appendix 3.1.1 Analysis of variance for dry root mass of tomato cv. 'Floradade' under increasing concentrations of nemafric-BL.

Source of variance	DF	SS	MS	F	P ≤
Replication	9	5.750	0.6389	0.03	
Treatment	6	125.452	20.9087	20.17	0.01
Error	54	55.974	1.0366		
Total	69	187.176			

Appendix 3.1.2 Analysis of variance for dry shoot mass of tomato cv. 'Floradade' under increasing concentrations of nemafric-BL.

Source of variance	DF	SS	MS	F	P ≤
Replication	9	24.40	2.711	0.01	
Treatment	6	1354.50	225.751	34.12	0.01
Error	54	357.33	6.617		
Total	69	1736.23			

Appendix 3.1.3 Analysis of variance for number of flowers of tomato cv. 'Floradade' under increasing concentrations of nemafric-BL.

Source of variance	DF	SS	MS	F	P ≤
Replication	9	138.13	15.348	0.07	
Treatment	6	1277.77	212.962	14.72	0.01
Error	54	781.37	14.470		
Total	69	2197.27			

Appendix 3.1.4 Analysis of variance for number of fruits of tomato cv. 'Floradade' under increasing concentrations of nemafric-BL.

Source of variance	DF	SS	MS	F	P ≤
Replication	9	0.64098	0.07122	0.25	
Treatment	6	1.70610	0.28435	9.58	0.01
Error	54	1.60355	0.02970		
Total	69	3.95063			

Appendix 3.1.5 Analysis of variance for plant height of tomato cv. ‘Floradade’ under increasing concentrations of nemafric-BL.

Source of variance	DF	SS	MS	F	P
Replication	9	2949.8	327.76	0.11	
Treatment	6	17279.1	2879.86	18.19	0.01
Error	54	8548.9	158.31		
Total	69	28777.8			

Appendix 3.1.6 Analysis of variance for stem diameter of tomato cv. ‘Floradade’ under increasing concentrations of nemafric-BL.

Source of variance	DF	SS	MS	F	P ≤
Replication	9	3.4600	0.38444	0.26	
Treatment	6	8.8869	1.48114	3.64	0.01
Error	54	21.9960	0.40733		
Total	69	34.3429			

Appendix 3.1.7 Analysis of variance for *Meloidogyne incognita* population densities in roots of tomato cv. 'Floradade'.

Source of variance	DF	SS	MS	F	P ≤
Replication	9	4.5582	0.50647	0.07	
Treatment	6	42.9184	7.15307	23.23	0.01
Error	54	16.62554	0.30788		
Total	69	64.1020			

Appendix 3.1.8 Analysis of variance for *Meloidogyne incognita* population densities in soil of tomato cv. 'Floradade'.

Source of variance	DF	SS	MS	F	P ≤
Replication	9	1.3191	0.14656	0.02	
Treatment	6	58.0681	9.67802	39.42	0.01
Error	54	13.2560	0.24548		
Total	69	72.6432			

Appendix 3.1.9 Analysis of variance for *Meloidogyne incognita* population densities in total (root + soil) of tomato cv. 'Floradade'.

Source of variance	DF	SS	MS	F	P ≤
Replication	9	2.2233	0.2470	0.02	
Treatment	6	64.4961	10.7493	41.60	0.01
Error	54	13.9520	0.2584		
Total	69	80.6714			

Appendix 3.2.1 Analysis of variance for dry root mass of tomato cv. 'Floradade' under increasing concentrations of nemarioc-AL.

Source of variance	DF	SS	MS	F	P ≤
Replication	9	5.405	0.6005	0.04	
Treatment	6	97.074	16.1791	25.88	0.01
Error	54	33.757	0.6251		
Total	69	136.236			

Appendix 3.2.2 Analysis of variance for dry shoots mass of tomato cv. ‘Floradade’ under increasing concentrations of nemarioc-AL.

Source of variance	DF	SS	MS	F	P ≤
Replication	9	50.48	5.609	0.05	
Treatment	6	673.33	112.221	16.29	0.01
Error	54	371.94	6.888		
Total	69	1095.74			

Appendix 3.2.3 Analysis of variance for number of flowers of tomato cv. ‘Floradade’ under increasing concentrations of nemarioc-AL.

Source of variance	DF	SS	MS	F	P ≤
Replication	9	64.29	7.1429	0.16	
Treatment	6	265.60	44.2667	2.31	0.05
Error	54	1034.11	19.1503		
Total	69	1364.00			

Appendix 3.2.4 Analysis of variance for number of fruits of tomato cv. ‘Floradade’ under increasing concentrations of nemarioc-AL.

Source of variance	DF	SS	MS	F	P ≤
Replication	9	16.629	1.84762	0.29	
Treatment	6	38.771	6.46190	6.19	0.01
Error	54	56.371	1.04392		
Total	69	111.771			

Appendix 3.2.5 Analysis of variance for plant height of tomato cv. ‘Floradade’ under increasing concentrations of nemarioc-AL.

Source of variance	DF	SS	MS	F	P ≤
Replication	9	1430.9	158.990	0.25	
Treatment	6	3855.5	642.581	5.73	0.01
Error	54	6055.1	112.131		
Total	69	11341.5			

Appendix 3.2.6 Analysis of variance for stem diameter of tomato cv, ‘Floradade’ under increasing concentrations of nemarioc-AL.

Source of variance	DF	SS	MS	F	P ≤
Replication	9	0.02229	0.00248	0.12	
Treatment	6	0.12400	0.02067	3.09	0.05
Error	54	0.36171	0.00670		
Total	69	0.50800			

Appendix 3.2.7 Analysis of variance for *Meloidogyne incognita* population densities in roots of tomato ‘Floradade’.

Source of variance	DF	SS	MS	F	P ≤
Replication	9	0.5217	0.05797	0.03	
Treatment	6	10.5301	1.75502	21.76	0.01
Error	54	4.3546	0.08064		
Total	69	15.4065			

Appendix 3.2.8 Analysis of variance for *Meloidogyne incognita* population densities in soil of tomato ‘Floradade’.

Source of variance	DF	SS	MS	F	P ≤
Replication	9	0.2156	0.02396	0.01	
Treatment	6	12.6227	2.10378	71.02	0.01
Error	54	1.5997	0.02962		
Total	69	14.4380			

Appendix 3.2.9 Analysis of variance for *Meloidogyne incognita* population densities in roots of tomato ‘Floradade’.

Source of variance	DF	SS	MS	F	P ≤
Replication	9	0.1964	0.02182	0.01	
Treatment	6	12.3428	2.05713	77.61	0.01
Error	54	1.4313	0.02650		
Total	69	13.9704			