# Developing Phytonematicides Using Indigenous *Cucumis africanus* and *Cucumis myriocarpus* Fruits for Tomato Production Systems

By

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DECLARATION

I declare that the thesis hereby submitted to the University of Limpopo, for the degree of Doctor of Philosophy 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, and related materials contained herein had been duly acknowledged.

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DEDICATION

É com imensa satisfação que dedico este trabalho cientifico a minha amada esposa,

Maria Pelinganga e as minhas adoraveis filhas Graciete e Ester Pelinganga.

Obrigado!

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ABSTRACT

Global withdrawal of synthetic fumigant and non-fumigant nematicides due to their eco-unfriendly impacts and high toxicity to non-target organisms, respectively, increased the research and development of alternatives for managing population densities of plant-parasitic nematodes, particularly the root-knot (*Meloidogyne* species) nematodes. Although *Meloidogyne* species had been managed using genotypes that are resistant to plant-parasitic nematodes in various crops, various challenges negate the available or introgressed nematode resistance. In tomato (*Solanum lycopersicum*) production, nematode races and instability of nematode resistant genotypes under certain conditions necessitated the continued research and development of alternatives since most of the existing commercial tomato cultivars are highly susceptible to various biological races of *Meloidogyne* species. The aim of the study was to research and develop appropriate dosages of two phyto- nematicides which could be applied through drip irrigation system in open field tomato production systems, while the specific objectives were to: (1) determine whether a computer-based model could provide non-phytotoxic concentrations to tomato plants using fresh fruits of wild watermelon (*Cucumis africanus)* and wild cucumber (*C. myriocarpus*) under greenhouse conditions, (2) determine whether computer-based concentrations from the two plant species when using dried fruits would be less phytotoxic and more suppressive to nematodes, (3) investigate application time intervals for the two products, (4) determine responses of plant growth in tomato and nematode suppression in respect to the derived dosages, and and (5) validate dosages of fermented crude extracts from the two plant species with respect to plant growth of tomato and suppression of nematode numbers.

Greenhouse, microplot and field studies were set to test the hypotheses intended to achieve the stated objectives, with reliability of measured variables being ensured by using statistical levels of significance ($P \leq 0.05$) and coefficients of determination ($R^2$), while validity was ensured by conducting experiments at the same location over two seasons and/or by setting up factorial treatments. Firstly, fermented plant extracts of fresh fruits from *C. africanus* and *C. myriocarpus* consistently reduced population densities of *Meloidogyne* species by 80-92% and 50-90%, respectively. Tomato plants were highly sensitive to the two products as shown by the total degree of sensitivities ($\sum k$) and biological index of 0 and 3, respectively. Also, the mean concentration stimulation range (MCSR) of 11% and 7% concentrations, respectively, attested to this phytotoxicity. Secondly, fermented crude extracts of dried fruits from *C. africanus* and *C. myriocarpus* also reduced population densities of *Meloidogyne* species by 78-97% and 87-97%, respectively. Tomato plants were highly tolerant to the two products in dried form as shown by the total degree of sensitivities ($\sum k$) and biological index of 4 and 3, respectively. The MCSR values for *C. africanus* and *C. myriocarpus* dried fruits on tomato were 2.64% and 2.99%, respectively, which for the purpose of this study were individually adjusted to 3%, which translated to 36 L undiluted material/ha of 4 000 tomato plants. In subsequent studies, 3% concentration was used as the standard, along with double strength concentration, namely, 6% concentration. Thirdly, the MCSR values derived in Objective 4, namely 3% and 6% concentration for both *Cucumis* species using the CARD model were used in the optimisation of application time interval using the innovative concept of weeks (0, 1, 2, 3 and 4) in a 30-day month period. Application time interval for 3% and 6% concentrations of *C. africanus* fruits was

optimised at 2.40 and 2.61 weeks in a 30-day month period, respectively, which translated to 18 days [(2.4 weeks/4 weeks) × 30 days] and 20 days [(2.6 weeks/4 weeks) × 30 days], respectively. In contrast, for both concentrations from fermented crude extracts of *C. myriocarpus* fruits, application time interval was optimised at 16 days for 2.2 and 2.1 weeks, respectively. During optimisation of application frequencies, fermented crude extracts from *C. africanus* and *C. myriocarpus* reduced final population densities of *M. incognita* race 2 by 70-97% and 76-96%, respectively. Fourthly, optimum application intervals (time), allowed computation of dosage, which is a product of concentration and application frequency (dosage = concentration × application frequency). Fifthly, validation of the dosages under open field conditions suggested that 6% × 16-day dosage under crude extracts from *C. myriocarpus* fruit significantly ($P \leq 0.05$) improved growth of tomato plants when compared with those of either 0% (untreated control) or 3% at 16 days. In contrast, dosages of *C. africanus* fruit at two application frequency had no effect on growth of tomato plants – suggesting that either of the dosages was suitable for use in tomato production since both reduced nematode numbers. During validation, the materials reduced nematode numbers by margins similar to those observed previously under other environments. In conclusion, crude extracts of the two *Cucumis* species have stimulatory concentrations which have potential similar reductive effects on population densities of *Meloidogyne* species and could serve as botanical nematicides. However, since plant responses to the two products differed in terms of their respective dosages and active ingredients, it implied that for further improvement of the two, the overriding focus should be on their interaction with the protected plants and nematode numbers. Ideally, future research

should include environmental impact studies, especially on the influence of the products fruit quality of tomato, earthworms, fish and bees.

# CHAPTER 1
# GENERAL INTRODUCTION

## 1.1 Introduction

Worldwide, the withdrawal of highly effective synthetic fumigant nematicides used in managing plant-parasitic nematode populations has had economic consequences in many crop production systems (Mashela, 2007, 2002; Mashela *et al*., 2008). Yield losses due to nematode infection in certain crops increased as much as 10-fold and in some cases complete crop failures were recorded (Ferraz and Brown, 2002; Fourie and Mc Donald, 2003). Global crop losses per annum caused by plant-parasitic nematodes have been estimated at 12% (Ferraz and Brown, 2002), whereas the South African estimate is 14% (Anon., 2011). Crop losses due to infection by nematodes have been estimated at US$125 billion (Chitwood, 2003).

The world leader in tomato (*Solanum lycopersicum* L.) production is China, followed by the USA, while South Africa is at number 40 position (Anon., 2012). Tomato plants are highly susceptible to infection by root-knot (*Meloidogyne* species) nematodes, with yield losses estimated at 24-38% in tropical and sub-tropical regions (Sasser, 1979a). Although nematode resistance in tomato genotypes is available (Gilbert and McGuire, 1956), the use of nematode resistance technology is limited by the existence of nematode races in *Meloidogyne* species (Riggs and Winstead, 1959; Sasser, 1979b, 1966). *Meloidogyne* races are morphologically similar and can be separated through differential host tests and molecular markers (Devran and Sogut, 2011; Hartman and Sasser, 1985). Four widely distributed *M. incognita* races 1, 2, 3 and 4 occur in tropical areas with sandy soils (Hartman and Sasser, 1985; Robertson *et al*., 2006).

*Meloidogyne incognita* races 5 and 6 had since been identified using molecular markers (Devran and Sogut, 2011; Robertson and Diez-Rojo, 2008). Also, it was previously recorded that certain nematode resistance in tomato was broken through exposure to soil temperature at 28$^{o}$C (Roberts and Thomason, 1989), which may be a common occurrence with the current high soil temperatures.

Use of organic amendments as alternative to methyl bromide (MB) received much attention after the adoption of the Montreal protocol (Bello, 1998). Unfortunately, large quantities (10-500 mt t/ha) of organic amendments were required to effectively suppress nematode numbers and therefore, the technology was uneconomic in terms of availability and transport of the materials (Mankau, 1968; Mankau and Minter, 1962; McSorley and Gallaher, 1995a; Muller and Gooch, 1982; Rodriguez-Kabana, 1986; Stirling, 1991). In addition to inconsistent results in nematode suppression, most organic amendments reduced soil pH (Mashela *et al*., 2010; Muller and Gooch, 1982; Stirling, 1991), resulting in unintended consequences of unavailability and/or supra-availability of certain elements in the soil (Bohn *et al*., 1985). The induced imbalances invariably manifested as deficiencies and/or toxicities of nutrient elements in crops.

The health benefits and the importance of tomato production in job creation and wealth generation are well-documented (Nzanza *et al*., 2013), particularly in South Africa where agriculture is one of the three important drivers of the economy (Mashela and Morudu, 2009). Attempts to mitigate drawbacks of conventional organic amendments in nematode suppression led to the widespread research and development of the ground

leaching technology (GLT) system in marginal communities of Limpopo Province, South Africa (Mashela, 2002; Mashela and Mphosi, 2002; Mashela and Nthangeni, 2002; Mashela *et al*., 2011). Unfermented small quantities (0.20 to 0.71 t/ha) of crude extracts of wild cucumber (*Cucumis myriocarpus* Naude.) fruit were successfully used to suppress *M. incognita* race 2 in tomato production, with evidence of stimulating plant growth (Mashela, 2002; Mashela and Mphosi, 2002; Mashela *et al*., 2011; Mphosi *et al*., 2004). In addition to consistent results, when using crude extracts of *C. myriocarpus* fruit, the technology did not reduce soil pH at the dosages used. However, the GLT system as currently used by small-scale farmers is labour-intensive and therefore, would not be cost-effective in large-scale commercial farming systems.

## 1.2 Problem statement

Alternative technologies to methyl bromide technology in the management of plant-parasitic nematode are replete with challenges. The researcher intends using the perceived challenges in GLT system to develop phytonematicides using fermented crude extracts of fruits from indigenous *Cucumis* species for use in large-scale tomato production for managing *Meloidogyne* species in a cost-efficient manner.

## 1.3 Motivation of the study

The development of an efficient and effective bio-nematicide would ensure that the South African tomato industry remains competitive in job creation and wealth creation, with improved soil health, human health and environmental health.

## 1.4 Aim and objectives

The aim of the study was to research and develop two botanical nematicides which could be applied through drip irrigation system in tomato production, while the objectives were:

1. To investigate whether EM-fermented crude extracts from fresh fruits of wild watermelon (*Cucumis africanus)* and wild cucumber (*C. myriocarpus*) would suppress population densities of *M. incognita* race 2 and the non-phytotoxic concentration for these materials on tomato plants under greenhouse conditions.
2. To determine whether fermented crude extracts from dried fruits of *C. africanus* and *C. myriocarpus* would be suitable for use in tomato production for the management of *M. incognita race 2.*
3. To optimise application time intervals for fermented crude extracts from dried fruits of *C. africanus* and *C. myriocarpus* at 3% and 6% concentrations for use in tomato production and suppression of *M. incognita* race 2 under microplot conditions.
4 To compare the efficacy of dosages of individual products in suppression of population densities of *M*. *incognita,* growth of tomato plants and accumulation of nutrient elements in tomato leaves under field conditions.
5. To validate dosages of fermented crude extracts from *C. africanus* and *C. myriocarpus* fruits for growth of tomato plants and suppression of *M. incognita* numbers under field conditions.

## 1.5 Reliability, validity and objectivity

Reliability is described as the extent to which a measuring instrument yields consistent results when the variable being measured repeatedly had not changed (Leedy and Ormrod, 2005). Statistical analyses provide various reliability checks on the data (Berenson and Levine, 1996). In this study, reliability in various experiments was ensured by using appropriate levels of statistical significance for mean separation and when evaluating the variance explained by models as measured by coefficients of determination ($R^2$). Validity is described as an extent to which the instrument measures what was actually intended to be measured (Leedy and Ormrod, 2005). In empirical research, experiments are either replicated in time or space in order to increase the range of validity of conclusions drawn from it (Little and Hills, 1981). A factorial set of treatments would be another way of increasing the range of validity. Validity is ensured by conducting the experiment at the same location over two seasons, or during one season at different locations or by setting up factorial treatments (Little and Hills, 1981). Objectivity is described as striving, as far as possible or practicable, to reduce or eliminate biases, prejudices or subjective evaluations by relying on verifiable data (Leedy and Ormrod, 2005). Objectivity is achieved by discussing the findings on the basis of empirical evidence as shown by statistical analyses, with findings compared and contrasted with findings in other studies (Little and Hills, 1981).

## 1.6 Bias

Bias is described as any influence, conditions or set of conditions that singly or altogether distort the data (Leedy and Ormrod, 2005). In this study, bias was minimised

by ensuring that the experimental error in each experiment was reduced through increased replications and randomisation (Little and Hills, 1981).

## 1.7 Significance of the study

The study was intended to produce a bio-nematicide from *C. africanus* and *C. myriocarpus* fruits, whereby potent chemicals could be extracted using EM technology. Eventually, the material would be applied in large-scale commercial tomato production systems through drip irrigation system in order to reduce labour costs incurred in the previously described ground leaching technology.

## 1.8 Format of thesis

Following the description and detailed outlining of the research problem (Chapter 1), the work done and not done on the research problem was reviewed (Chapter 2). Then, each of the five objectives constituted a separate chapter (Chapters 3-7). In the final chapter (Chapter 8), findings in all chapters were summarised and integrated to provide the significance of the findings and recommendations with respect to future research, ending with a conclusions that were intended to provide a take home message regarding the entire study.

# CHAPTER 2
# LITERATURE REVIEW

## 2.1 Introduction

Tactics which had been crafted for the management of plant-parasitic nematodes, each has its own pros and cons. Although the environmental impact of other tactics could not match those of synthetic chemical nematicides, it could be due to limited empirically-based information on these tactics. Limitations in using nematode-resistance were introduced as the widespread existence of nematode races, particularly among the southern root-knot nematodes [(*Meloidogyne incognita* (Kofoid and White) Chitwood]. *Meloidogyne incognita* race 1 contributes 44% to the total *M. incognita* populations, while certain economically important crops such as cotton (*Gossypium hirsutum* L.), tobacco (*Nicotiana tabacum* L.), tomato (*Solanum lycopersicum* L.) and watermelon (*Citrullus lanatus* L.) have no resistant genotypes to this race (Hartman and Sasser, 1985). In contrast, *M. incognita* races 2, 3 and 4 contribute 13%, 4% and 2% to the total *M. incognita* populations, respectively (Sasser, 1979b). Inter- and intra-continental differences in *M. incognita* races have been reported, with *M. incognita* races 3 and 4 having the highest frequencies in Central and South America (Sasser, 1979b). In contrast, *M. incognita* race 1 is dominant in Europe (Robertson *et al*., 2006); while in the USA *M. incognita* races 1, 2 and 3 are the most frequent (Sasser, 1989). In Africa, *M. incognita* race 4 is widely distributed in West Africa (Olowe, 2010), while *M. incognita* races 2 and 4 are common in South Africa (Kleynhans *et al*., 1996).

Development of nematode-resistant cultivars is nematode-specific and oftentimes, it is also race-specific. Inter-continental differences limit the use of *M. incognita*-resistant cultivars bred in developed countries in developing countries. In many countries, certain crops are poorly supported through research, resulting in a situation where farmers are persuaded to use nematode-resistant exotic cultivars without testing them against the existing local biological races. The objective of this study was to review literature in tactics that could ameliorate the existence of nematode races in the management of *M. incognita* race 2 in tomato production.

### 2.1.1 Search for alternatives in nematode management

Poisonous plants in South Africa had been classified as being non-poisonous, poisonous, very poisonous, deadly, causing skin allergies and poisonous to animals, with each class constituting 4%, 23%, 18%, 7%, 4% and 44%, respectively (Van Wyk *et al*., 2002). These poisonous plants overlap those that had been classified as medicinal plants of South Africa (Van Wyk *et al*., 1997). For instance, while wild cucumber (*Cucumis myriocarpus* Naude.) and wild watermelon (*Cucumis africanus* L.F.) are classified as poisonous plants (Van Wyk *et al*., 2002), the two are widely used in traditional medicines (Mphahlele *et al*., 2012). The two *Cucumis* species, along with *Aster bakeranus* (Burtt Dave ex C.A.Sm.), *Cotyledon orbiculata* (L.), *Embelia ruminate* (E.Mey. ex A.D.C), *Punica granatum* (L.), *Rumex lanceolatus* (L.) *and Sansevieria hyacinthoides* (L.) had been used to control intestinal roundworms in humans *(Ascaris lumbricoides*), dogs (*Toxocara canis, Toxascaris leonine*), chickens (*Ascaridia galli*) and other domesticated animals (Watt and Breyer-Brandwijk, 1962). All the listed intestinal

worms are zoo-parasitic nematodes (Mashela, 2007). Motivated by the use of these materials in zoo-parasitic nematodes, Mashela and Mphosi (2002) opted to use crude extracts of *C. myriocarpus* fruit to suppress population levels of *Meloidogyne* species and the citrus nematode (*Tylenchulus semipenetrans* Cobb 1913) in pot trials, with results suggesting at least 90% suppression of the nematodes (Mashela, 2007; Mashela, 2002; Mashela and Mphosi, 2002). During that time, the Land Bank Chair of Agriculture – University of Limpopo was developing strategies to ameliorate the drawbacks of conventional organic amendments in managing plant-parasitic nematodes as alternatives to nematode resistance.

### 2.1.2 Cucurbitaceae family

The family Cucurbitaceae comprises 115 genera (Pitrat *et al*., 1999) and have been widely used in South Africa for centuries as food and traditional medicine (Pitrat *et al.*, 1999; Rimington, 1938). *Cucumis africanus* and *C. myriocarpus* are actually indigenous to South Africa (Kristkova *et al*., 2003). Crude extracts of *C. myriocarpus* fruit and roots, along with the whole plant of *C. africanus,* contain pharmacological properties used in the treatment of liver damage, weakening of the immune system, lumps, jaundice, acute and chronic viral hepatitis, hepatocirrhosis, persistent dyspepsia, epilepsy due to wind-phlegm, cancer, gonorrhea, boils and infections by intestinal roundworms (Mphahlele *et al*., 2012). In South Africa, much more work in management of plant-parasitic nematodes in the Cucurbitaceae family was done using *C. africanus* and *C. myriocarpus*, which resulted in the development of a research niche called Indigenous Cucurbitaceae Technologies (ICT).

## 2.2 Indigenous Cucurbitaceae Technologies

The Indigenous Cucurbitaceae Technology (ICT) Research Niche was initiated, researched and developed by the Land Bank Chair of Agriculture – University of Limpopo under five themes: (1) ground leaching technology, (2) inter-generic grafting technology, (3) chemical technology, (4) agronomics technology and (5) botinemagation technology.

### 2.2.1 Ground leaching technology

The ground leaching technology (GLT) was initially developed for use in ameliorating the drawbacks of using conventional organic amendments in managing plant-parasitic nematodes, that included the following: (1) inconsistent results in nematode suppression, (2) large quantities (10-500 mt/ha) required to effect nematode suppression, (3) unavailability of organic materials in sufficient quantities, (4) when high quantities were available far from the site of use, this translated to high transport costs, (5) waiting period to enhance microbial decomposition and, therefore, to avoid negative period, and (6) reduction of soil pH, which invariably increased the (un)availability of certain nutrient elements from the soil (Mankau, 1968; Mankau and Minteer, 1962; McSorley and Gallaher, 1995a, b; Muller and Gooch, 1982; Rodriguez-Kabana, 1986; Stirling, 1991). Consequently, originally the GLT system was developed and researched for use in post-emergent applications, but later on the system was adapted for pre-emergent applications.

In post-emergent application systems, mature fruits of *C. africanus* and *C. myriocarpus* were cut into pieces, dried at 52°C (Makkar, 1999) in air-forced ovens for 72 h, ground in a Wiley mill and passed through a 1-mm-pore sieve (Mashela, 2002). The material was then applied at transplanting without first undergoing any microbial degradation activity (Mashela, 2002; Mashela and Mphosi, 2002). Crude extracts were spread in a shallow hole around the base of the stem of the transplant at 2-5 g/plant and then covered with soil. The amount translates to 20-71 kg ground material/ha (0.20-0.71 mt/ha) for 4 000 tomato plants/ha, which is much less when compared with quantities (10-500 mt/ha) required in conventional organic amendment systems (Stirling, 1991). Incidentally, the small quantities precluded high transport costs to haul the materials to the fields. Also, when used at transplanting, the waiting period for microbial decomposition was not necessary and the material did not reduce soil pH (Mashela and Nthangeni, 2002). Most importantly, suppression of nematode numbers was consistently achieved, regardless of the environment where the study was conducted (Mashela *et al*., 2011).

Crude extracts of *C. myriocarpus* fruit suppressed plant-parasitic nematodes in greenhouse trials by over 90% (Mashela, 2002), in microplot trials by over 90% (Mofokeng 2005; Shakwane *et al*., 2005) and in field trials by over 80% (Mashela, 2007). Additionally, the crude extracts increased soil EC from 95 to 160%, but had no effect on soil pH. Also, materials improved fruit yield and growth of tomato and citrus in all studies (Mashela, 2007; 2002; Mashela *et al*., 2008; Mphosi, 2004). Efficacy of crude extracts from *C. myriocarpus* fruit, when compared with that of aldicarb (2 – methyl – 2

– (methylthio) propionaldehyde O – (methylcarbamoyl) oxyme) and fenamiphos (Ethyl 3 – methyl – 4 – (methylthio) phenyl (1 – methyllethyl) phosphoramidate (56)) suggested that the three materials did not have significant differences in their nematode suppression and fertiliser effect on growth of tomato plants (Mashela *et al*., 2008).

In GLT system, microbial decomposition had negligible role in the efficacy of crude extracts from *C. myriocarpus* fruit, as shown by lack of interaction between this material and *Bacillus* species (Mphosi *et al*., 2004). Shakwane *et al*. (2005) also demonstrated that the material promoted nodulation of *Bradyrhizobium japonicum* in cowpeas (*Vigna unguiculata*). Also, independence of GLT from microbial activity was demonstrated through elimination of *Bacillus* species in predictive models when using crude extracts of castor bean (*Ricinus communis* L.) fruit (Mashela and Nthangeni, 2002; Mofokeng 2005) and fever tea (*Lippia javanica* L.) leaves. The concept, ground leaching technology, emanated from the fact that the plant organ is ground (present tense: grind), with potent chemicals being leached out of plant residues through irrigation or rain water.

Crop yield losses are generally proportional to initial population densities (Pi) of nematodes (Seinhorst, 1967). Ideally, the use of a material in GLT should be as a pre-emergent bio-nematicide in order to keep Pi at the lowest level possible. *In vitro*, seed germination assays suggested that at 5 g crude extracts of *C. myriocarpus* fruit were not suitable for use in tomato, watermelon and butternut squash (Mafeo and Mashela, 2009a), along with maize, finger millet, sorghum and onion (Mafeo and Mashela,

2009b). In greenhouse trials, the material completely inhibited seedling emergence of all dicotyledonous crops tested (Mafeo and Mashela, 2010).

Cucumin, from crude extracts of *C. myriocarpus* fruit, was shown to also suppress the division of cancer cells in animals (Van Wyk *et al*., 1997). However, the suppression occurred at dosages which were toxic to healthy cells, whereas at reduced dosages, the material stimulated division of cancer cells. Quadratic relationships between cell divisions and dosages ascribed to cucumin characterised responses of biological systems to extrinsic factors (Salisbury and Ross, 1992). Using the observation of stimulation effect on cells, dosages were reduced *in vitro* from 0 to 2.25 g/plant, with germination of tomato, watermelon and butternut squash having quadratic relationships with the increasing concentrations of the material (Mafeo and Mashela, 2009a). In the trials, dosages of crude extracts of *C. myriocarpus* fruit explained 91, 97 and 91% of the total treatment variation in inhibition of seed germination in tomato, watermelon and butternut squash, respectively. Results suggested that crude extracts of *C. myriocarpus* fruit had allelopathic effect on seed germination of test plants and therefore, the material was not suitable for use as a pre-emergent phytonematice. Various studies were initiated using the Curve-Fitting Allelochemical Response Dosage (CARD) computer-based model (Liu *et al*., 2003) to determine dosages where crude extracts of *C. myriocarpus* fruit would stimulate and/or inhibit germination of various crops in order to establish the pre-emergent quantities (Mafeo and Mashela, 2009a; Mafeo and Mashela, 2010; Mafeo *et al*., 2010; Mafeo *et al*., 2011a,b,c). The detailed review of the model is deferred to section 2.4 of this chapter.

2.2.1.1 Potent chemicals in *Cucumis* species

Potent chemicals in *Cucumis* species have been isolated and identified as cucurbitacins (Rimington, 1938). Plants in the Cucurbitaceae family contain a total of 12 cucurbitacins, with cucurbitacin A in *C. myriocarpus* fruit and roots being the only water-soluble one (Chen *et al*., 2005). These chemical compounds are oxygenated tetracyclic triterpenes with glycosides, which originate from the mevalonic pathway (Inderjit and Malik, 2002). Generally, these compounds are used in plant defence against pest invasion (Inderjit and Malik, 2002; Inderjit *et al*., 1995; Mashela, 2002). Cucurbitacin A comprises two potent chemicals, *viz.* cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Jeffrey, 1978; Rimington, 1938), which have bigger but simpler molecular structures when compared with aldicarb ($C_7H_{14}N_2O_2S$) and fenamiphos ($C_{13}H_{22}NO_3PS$) synthetic nematicides. In contrast, *C. africanus* contains cucurbitacin B ($C_{32}H_{48}O_8$), which is insoluble in water (Chen *et al*., 2005).

2.2.1.2 Evidence of nematicidal properties in *Cucumis* species

Application of crude extracts in granular form or as aqueous solutions, reduced population density of *M. incognita* race 2 and the citrus nematode by 80-94% (Muedi *et al*., 2005). Similar effects in GLT systems were observed when using crude extracts of castor bean (*Ricinus communis* L.) fruit (Mashela and Nthangeni, 2002; Mashela *et al*., 2007) and fever tea (*Lippia javanica* L.) leaves (Mashela *et al*., 2010, 2008, 2007).

*In vitro* bioactivity effects of crude extracts of *C. myriocarpus* fruit on *M. incognita* race 2 and *T*. *semipenetrans* had nematode mortalities of 87-95% and 83-96%, respectively,

while minimum inhibition concentration of crude extracts for each nematode species was 7 µg/ml distilled water (Muedi *et al*., 2005). However, it should be indicated at this point that not all plant materials are suitable for use in GLT system. For instance, crude extracts of oleander (*Nerium indicum* Mill.) leaves, chilli pepper (*Capsicum annuum* L.) fruit and tamboti (*Spirostachys Africana* Sond.) bark did not suppress nematode numbers, while those of fever tea suppressed nematode numbers, but also reduced soil pH (Mashela *et al*., 2008).

Apparently, there is a link between the suitability of a plant material in GLT and its being suitable for use in aqueous form. Crude extracts of fruits from the two *Cucumis* species, *L. javanica* leaves and *R. communis* fruit were, as detailed in the review, highly successful in GLT systems. Crude extracts of *C. myriocarpus* fruit and *R. communis* fruit had been successful when used in aqueous form as phytonematicides in tomato and carrot (*Daucus carota* L.) production, respectively (Mafeo and Mashela, 2009b; Mashela and Nthangeni, 2002; Mashela *et al*., 2011).

The stimulation of tomato plant growth due to the application of crude extracts from fruit of *C. myriocarpus* fruit species in the ground leaching technology (GLT) system was referred to as a fertiliser effect (Mashela and Nthangeni, 2002). However, due to the small quantity used in GLT, the treatment had no significant effect on nutrient elements in leaf tissues (Mashela, 2002). Others also observed that in animal cells, the potent chemical cucumin had stimulatory and inhibitory effects on cell growth at low and high dosages, respectively (Lee *et al*., 2010). However, the material could not be used in

curing of cancer since at low dosages it stimulated division of non-cancerous cells, while at high dosages it was cytotoxic (Chen *et al*., 2005). Generally, the successful use of a botanical with nematicidal properties in nematode management is limited by the degree of its phytotoxicity to the protected crops (Agbenin *et al*., 2005).

In GLT system, the material is applied for 56 days, thereafter, there is no guarantee that the material would still be effective in suppressing nematodes, since results beyond this period were inconsistent (Mashela, 2007). When harvested at 150 days after initiating treatment with unfermented fruits from *Cucumis* species in GLT system, *T. semipenetrans* numbers were significantly higher in *Cucumis*-treated soil than in untreated controls (Maile, 2013). This observation suggested that reapplication after 56 days is necessary, which adds to operational costs.

### 2.2.2 Inter-generic grafting technology

The family Cucurbitaceae contains four genera of economic importance in agriculture, namely, *Citrullus*, *Cucumis*, *Cucurbita* and *Lagenaria* (Pitrat *et al.*, 1999). South Africa is considered as the centre of diversity for wild *Cucumis* species (Kristkova *et al*., 2003), mainly *C. africanus* and *C. myriocarpus.* 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.,* 2010a,b; 2009). Both *C. africanus* and *C. myriocarpus* were shown to be highly resistant to *M. incognita* races 2 and 4 and *M. javanica* (Pofu *et al*., 2012a), which are dominant in South Africa (Kleynhans *et al*., 1996).

Mechanisms of resistance to *Meloidogyne* species in the two *Cucumis* species were established in the greenhouse trials (Pofu and Mashela, 2011). Generally, resistance in plant-parasitic nematodes had been broadly classified as pre-infectional and post-infectional resistance (Kaplan and Keen, 1980). Establishment of resistance type in plant nematology is essential since germplasm can be introgressed into breeding lines with post-infectional resistance only (Thurau *et al*., 2010). Resistance forms in *C. africanus* and *C. myriocarpus* were pre-infection and post-infection, respectively (Pofu *et al*., 2010a). Consequently, resistant germplasm in *C. myriocarpus* could be useful for introgression in highly nematode-susceptible genera such as *Citrullus* species. In another study, most second-stage juveniles of *M. javanica* on *C. africanus* were converted into males (Pofu and Mashela, 2011). Apparently, this was due to their failure to establish feeding sites in the test plant species since further development of juveniles from second to adult stage is dependent on the availability of food (Ferraz and Brown, 2002). The observation regarding conversion of juveniles to males confirmed results of Fassuliotis (1970) in other plant species with resistance to *Meloidogyne* species. The biological importance of conversion of juveniles into males is that the latter do not feed and are also not required for reproduction (Ferraz and Brown, 2002).

Inter-generic grafting technology has had incompatibility challenges due to different stem diameter sizes of scions and rootstocks in various crops (Tiederman, 1989). Grafts of watermelon with relatively thick stem diameters and *C. africanus* and *C. myriocarpus* 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 the two genera, resulting into 100% survival of the grafts (Pofu and Mashela, 2011). In a subsequent greenhouse study (Pofu, 2012), all inter-generic grafted seedlings also survived and *C. africanus* and *C. myriocarpus* seedling rootstocks retained their capabilities to reduce population densities of *M. incognita*. Under field conditions the procedure was also successful, with grafts flowering earlier and producing higher fruit yield than those of intact plants (Pofu *et al*., 2012b).

### 2.2.3 Agronomics technologies

Development of any technology to solve an identified problem, invariably results in a set of different unique limitations, which are briefly reviewed for the GLT system. In the current review, it has been clear that the GLT system had ameliorated the drawbacks of using conventional organic amendments in managing plant-parasitic nematodes with regard to inconsistent results in nematode suppression, large quantities, the corresponding high transport costs and elimination of a negative period. However, unavailability of organic materials in sufficient quantities, inconsistent results in nematode suppression and reduced soil pH limited the overall adoption of the technology.

Originally, fruits used in GLT system were collected from the wild, with initial attempts to propagate the two *Cucumis* species not being successful due to auto-allelopathy in seeds of these plant species (Mafeo, 2006). Mafeo (2006) developed propagation protocols for *C. myriocarpus*, where seeds were leached in running tapwater for 8 h or exposed to 45$^{o}$C for 45 minutes prior to planting. However, germination of *C.*

*myriocarpus* seeds remained erratic after the treatment, while those of *C. africanus* resulted in 100% germination (Pofu, 2012). Maila *et al*. (2012) developed *in vitro* mass propagation protocols of the *Cucumis* species, which have completely ameliorated this limitation. Also, since the materials are used in dried form, the GLT materials may have a longer shelf-life when compared to conventional organic amendments. Also, Nkgapele *et al*. (2011a,b) investigated irrigation and fertilisation requirements of *C. africanus* and *C. myriocarpus* in pot trials, with results suggesting that moderate irrigation and fertilisation were required for achieving optimum yields.

### 2.2.4 Chemical Technology

The use of botanicals in pest management has been increasing over the years not only for being environmental friendly but mainly due to the presence of potent chemicals which confer them resistance against pests and pathogens as those in synthetic pesticides. In *Cucumis* species the potent chemicals in the fruits have been extensively identified and isolated as cucurbitacins (Rimington, 1938). Cucurbitacins are used in plant protection against plant-parasitic nematodes, fungi and insects (Inderjit and Malik, 2002; Inderjit *et al*., 1995; Mashela, 2002). Cucurbitacin A, which is the only cucurbitacin soluble in water, is present in fruit and roots of *Cucumis myriocarpus* plant, whereas in *Cucumis africanus* cucurbitacin B is the potent chemical present in the whole parts of the plant (Chen *et al*., 2005). Cucurbitacin A comprises of two potent chemicals, namely, cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Jeffrey, 1978; Rimington, 1938) having similarities to the basic structure of aldicarb ($C_7H_{14}N_2O_2S$) and fenamiphos ($C_{13}H_{22}NO_3PS$). Cucumin has been extensively explored with a well

established acute dermal toxicities ($LD_{50}$) for rat and rabbit as $LD_{50}$ = 0.5 and 13 mg $kg^{-1}$ (Mashela, 2007).

### 2.2.5 Botinemagation

Botinemagation is defined as the use of phytonematicides through irrigation systems (Mashela *et al*., 2011). Crude extracts of *Cucumis myriocarpus* were successfully used in suppression of plant-parasitic nematodes when leached out through irrigation water in what has been termed as Ground Leaching Technology (GLT) system (Mashela, 2002). However, the GLT system was only suitable for small farming communities of Limpopo Province in South Africa. In order to accommodate large agricultural farming enterprises, the ground fruit material had to be improved and used through irrigation system as a way to enhance the existing GLT system, which is labour intensive. Effective microorganisms (EM) technology was brought in to extract the potent chemicals in *C. africanus* and *C. myriocarpus* crude extracts through fermentation process (Higa, 1999).

## 2.3 Other technologies used in managing plant-parasitic nematodes

Effective micro-organism (EM) technology is currently in use to mine out chemicals with the potential to suppress population nematode densities (Mashela *et al*., 2011). Components of EM which include effective micro-organisms (EM) technology originally developed in Japan for nature farming (Kyan *et al*., 1999), is currently available in the Republic of South Africa. Effective micro-organisms comprise a mixture of photosynthetic bacteria, lactic acid bacteria, yeast, actinomycetes, fermenting fungi and

other little known fungi (Higa, 1993), which were reviewed in detail elsewhere (Ncube, 2008). Degradation microbes used in EM technology are an approximation of degradation microbes in natural ecosystems, which implies that the EMROSA-EM had been specifically formulated for conditions in South Africa. Specific plant materials are fermented with the EM prior to application for reducing population densities of a particular pest (Kyan *et al*., 1999). In South Africa, although EM technology is purported to have achieved results of impressive proportions in various applications (Kyan *et al*., 1999), empirically-based trials to support these claims are scant. Fresh aboveground parts of lantana (*Lantana camara* L.) have been used in management of plant-parasitic nematodes in tomato production (Nzanza *et al*., 2013). The technology is not as labour-intensive as the GLT system since the materials are applied through irrigation systems.

## 2.4 Curve-fitting allelochemical response dosage

Botanicals used as pesticides originated as secondary metabolites (Inderjit *et al*., 1995), which are used in defence mechanisms by originator plants against invasion by various pests (Horsefall and Cowling, 1980). Incidentally, these secondary metabolites may inhibit growth and development of plant species different from the originator plant species, a phenomenon referred to as allelopathy (Inderjit, 2001), with the involved chemicals referred to as allelochemicals. When the originator plant is affected by its own allelochemicals, the phenomenon is referred to as auto-allelopathy. Both auto-allelopathy and allelopathy of *C. myriocarpus* had been reported (Mafeo, 2012). Generally, biological systems respond to extrinsic or intrinsic factors in accordance to density-dependent growth patterns, which are characterised by three growth responses,

namely, stimulation, saturation and inhibition phases (Salisbury and Ross, 1992). Conventional methods of determining density-dependent growth patterns are tedious and usually result in inconsistent results (Inderjit, 2001). The curve-fitting allelochemical response dosage (CARD) computer-based model was developed to quantify responses of biological entities toward increasing dosages of allelochemicals (Liu *et al*., 2003). In the CARD model, responses of biological entities (y-axis) and increasing dosages of a botanical nematicide (x-axis) are characterised by density-dependent growth patterns, which are quantified through six biological indices and quadratic curves (Liu *et al*., 2003).

In the CARD models, density-dependent growth patterns are characterised by seven biological indices, namely: (1) threshold stimulation ($D_m$) - the dosage at which the allelochemical begins to have a measurable stimulating effect on plant growth, (2) saturation point ($R_h$) - the dosage at which growth remains constant prior to decreasing, (3) 0% inhibition ($D_0$) - the end-point dosage of $R_h$ where the allelochemical has a zero effect on growth reduction, (4) 50% inhibition ($D_{50}$) - the dosage where the allelochemical inhibits growth by 50%, (5) 100% inhibition ($D_{100}$) - the dosage where the allelochemical inhibits growth by 100%, (6) k - the number of In(D + 1) transformations that serve as a biological indicator of the degree of sensitivity with relation to stimulation or inhibition to allelochemicals and (7) $R^2$ - the coefficient of determination (Liu *et al*., 2003). In the adaptation of the model for use in computing the stimulating concentrations, $D_m$, $R_h$ and k biological indices are of great importance. The distance between $D_m$ and $R_h$ had been referred to as the stimulation range (Mafeo, 2012; Mafeo

*et al*., 2011a,b,c), which for the first time explained the previously observed fertiliser effect on crops when using crude extracts of *C. myriocarpus* fruit (Mashela, 2002). Generally, the limiting factor in developing any pesticide intended for post-plant use is its degree of phytotoxicity. The k value in the CARD model is important in establishing whether a material is phytotoxic or non-phytotoxic (Mafeo *et al*., 2011a,b,c). Usually, k values start from zero and increased as discrete numbers when the sensitivity of the plant to the test material decreased (Liu *et al*., 2003). In other words, the sensitivity of the test plant to the bio-nematicide is inversely proportional to the k values. Thus, this biological index serves as an indicator of whether selected dosages of a particular botanical were suitable or not for use as a post-planting phytonematicide.

Incidentally, the CARD model quantifies all stages in the density-dependent growth patterns of biological entities to increasing concentrations of allelochemicals and was used in phytonematicides to determine non-phytotoxic dosages (Mafeo, 2012). In short, the CARD model provided the biological index - total sum of transformations (k), which expressed the sensitivities of the test plants to the phytonematicide (Liu *et al*., 2003). Generally, k is inversely proportional to the degree of sensitivity of the test plant to the material (Liu *et al*., 2003). Initially, various studies at the University of Limpopo involved 18 different plant cultivars in greenhouse trials, where each, with 10 dosages from 0 to 2.25 g. At harvest, 18 days after initiating the treatment, seedling height, radicle length, coleoptile length and coleoptile diameter were each subjected to ANOVA and then the CARD model (Mafeo and Mashela, 2010; Mafeo *et al*., 2011a,b,c). The CARD models indicated that the 18 crops had different overall sensitivities (∑k values) to crude

extracts of *C. myriocarpus* fruit, with clear stimulatory, neutral and inhibitory dosages to seedling emergence within the quadratic curves (Mafeo and Mashela, 2010; Mafeo *et al*., 2011a,b,c). Pre-emergent quantities for applying crude extracts of *C. myriocarpus* fruit using GLT were eventually established and validated, where germination of the selected crops was not affected but nematode numbers were significantly reduced (Mafeo, 2012).

2.5 *Meloidogyne* species in tomato production

The genus *Meloidogyne*, with over 90 described species (Sikora and Fernandez, 2005), parasitises over 3 000 host-plants (De Waele and Elsen, 2007; Rizvi and Rizvi, 1992). Due to the existence of numerous biological races within the genus (Robertson and Diez-Rojo, 2008), it is almost impossible to import nematode-resistant genotypes from one country to another. Prior to the suspension of methyl bromide in 2005, the estimated global annual crop yield losses due to all plant-parasitic nematodes were US$125 billion (Chitwood, 2003). In particular, the southern root-knot nematode *(Meloidogyne incognita)* causes substantial economic yield losses in various crops (Koening *et al*., 1999), with the degree of damage being substantial in sandy soils (Sikora and Fernández, 2005).

A female lays approximately 350 eggs in a gelatinous matrix over a life cycle of 19-43 days – depending on soil temperature (Sikora and Fernandez, 2005). Infection by *Meloidogyne* species induces formation of root galls, causing stunted growth, decreased water uptake, imbalances of essential nutrient elements, low evapo-

transpiration and increased root exudation of amino acids, which invariably reduce soil pH (Maqbool *et al*., 1987; Mashela, 2002). The availability of most essential nutrient elements in soil to plants is sensitive to slight changes in soil pH (Bohn *et al*., 1985), which may partly provide some explanation on how *Meloidogyne* species affect nutrient uptake, thus, limiting responses to fertiliser utilisation (Stirling, 1991), all of which translate into reduced crop yield and profit.

## 2.6 Clarification of concepts

In ICT, subniche botinemagation technology, the main focus is to use fermented crude extracts through irrigation system to manage plant-parasitic nematodes (Mashela *et al*., 2011). Generally, the concepts are similar to those of conventional pesticides, where the used material could either be phytotoxic or non-phytotoxic. In various studies (Meyer *et al*., 2008; Musabyimana *et al*., 2000; Ramazan and Yarba, 2010; Setia *et al*., 2007), it was empirically demonstrated that botanicals could be highly phytotoxic. This is not surprising since the materials originated from allelochemicals, which are responsible for allelopathy (Inderjit, 2001; Inderjit and Malik, 2002; Inderjit *et al*., 1995).

Mafeo (2006) demonstrated that *C. myriocarpus* seeds have auto-allelopathy. Also, Mafeo (2006) demonstrated that the observed auto-allelopathy could be ameliorated through leaching in tapwater or exposure to 45˚C. Later, Mafeo (2012) demonstrated that crude extracts of *C. myriocarpus* fruits were allelopathic to a wide range of commercially available crops in agriculture. The inhibition of seed germination was density-dependent as described by Salisbury and Ross (1992). These observations call

for clarification of the two concepts, dosage and concentration. Lieu *et al*. (2003) developed the CARD model on the basis of the concept dosage, while in actual fact the model was based on increasing concentrations of allelochemicals. Similarly, Mafeo (2012) adopted the use of dosage, when in actual fact the study was based on concentration. In order to proceed without causing confusion, two concepts, namely, dose and dosage, need to be clarified.

Van Gundy and McKenry (1975) defined dose as the amount of the active ingredient received and which would induce the desired effect in an individual nematode's behaviour, while dosage is the amount of toxicant (active ingredient + carriers) placed in the environment of the nematode for a known number of application times per crop cycle. In short, dosage = concentration × time (Van Gundy and McKenry, 1975), which had been adopted for use in this study.

## 2.7 Work not yet done on the research problem

The GLT system is labour-intensive and could therefore, be costly for large-scale commercial farming systems (Mashela *et al*., 2011). Development of a bio-nematicide using fermented crude extracts of *C. africanus* or *C. myriocarpus* fruits would, therefore, enhance the application of GLT through irrigation water in large-scale commercial farming agriculture – a conceptual technology which had since been referred to as 'botinemagation', which is the use of botanicals through irrigation water for managing plant-parasitic nematodes (Mashela *et al*., 2011). However, since only cucurbitacin A in *C. myriocarpus* fruit is water-soluble (Chen *et al*., 2005), it remains uncertain whether

crude extracts of *C. africanus* fruit could also serve as fermented crude extracts in suppression of nematodes since cucurbitacin B is insoluble in water (Chen *et al*., 2005). The major challenge in using fermented crude extracts would be the phytotoxicity of the materials as previously shown that potent chemicals in *Cucumis* species were highly phytotoxic to most commercial crops (Mafeo, 2012). Thus, the CARD computer-based model, along with the principles of density-dependent growth patterns and dosage, would be used in the research and development of the envisaged botinemagation technology, using products from the two *Cucumis* species.

# CHAPTER 3
# POTENTIAL USE OF FRESH FRUITS FROM INDIGENOUS *CUCUMIS* SPECIES AS BIO-NEMATICIDES IN TOMATO PRODUCTION

## 3.1 Introduction

Increased withdrawal of synthetic chemical nematicides from agrochemical markets exacerbated effects of the root-knot nematodes (*Meloidogyne* species) in tomato (*Solanum lycopersicon* L.) production (Mashela *et al*., 2011). Worldwide, all the four races of *M. incognita* and the two of *M. arenarea,* including *M. javanica* and *M. hapla*, induce damage to tomato cv. 'Rutgers', which was originally used in the development of the differential host test classification system (Hartman and Sasser, 1985). The relationship between initial population densities of *M. incognita* and yield of susceptible and resistant tomato cultivars suggested a tolerance limit of 0.55 eggs and juveniles/cm$^3$ soil for both types of tomatoes (Di Vito *et al*., 1991a,b). In the tropics and subtropics, estimations of yield losses on tomato were 24-38% (Sasser, 1979b).

Withdrawal of methyl bromide from agrochemical markets in 2005 resulted in more products and technologies being researched, developed and tested as alternatives to methyl bromide technology for managing numbers of plant-parasitic nematodes. However, just like synthetic nematicides (Van Gundy and McKenry, 1975), introduced alternative technologies have had their unique drawbacks (Chapter 2), which are mainly due to lack of empirically-based knowledge systems in evolved technologies. Among the alternatives to methyl bromide, Indigenous Cucurbitaceae Technologies (ICT), are being researched and developed in South Africa for the management of plant-parasitic nematodes (Chapter 2; Mashela *et al*., 2011). Investigations under this study fall in one

of the five subniches of ICT – botinemagation, which comprises the use of fermented crude extracts using effective micro-organims (EM) to mine-out active ingredients in fruits of *Cucumis* species and then applied through drip irrigation in diluted form for nematode suppression. The technology is already used in open field tomato systems using fermented crude extracts of *Lantana camara* L at 2% concentration (Nzanza *et al*., 2013). Generally, as in the use of *L. camara* in tomato production, the plant used and the plant protected against nematodes are from different plant species, with the potential risk of allelopathy, therefore, inducing phytotoxicity (Inderjit *et al*., 1995).

Liu *et al*. (2003) introduced the curve-fitting allelochemical response dosage (CARD) computer-based model for quantifying density-dependent growth patterns in response to increasing concentration of allelochemicals. Mafeo *et al*. (2011a,b,c) adapted the CARD model to determine the sensitivities of various crops to allelochemicals from wild cucumber (*Cucumis myriocarpus* Naude.) fruit in order to develop the concentration that would not be phytotoxic but suppressive to nematode numbers as reviewed earlier (Chapter 2). The objective of this study was to investigate whether EM-fermented crude extracts from fresh fruits of wild watermelon (*Cucumis africanus* L.F.) and *C. myriocarpus* would suppress population densities of *M. incognita* race 2, and to determine the non-phytotoxic concentration for these materials on tomato plants under greenhouse conditions.

## 3.2 Materials and methods

### 3.2.1 Location of study and preparation

Separate experiments for *C. africanus* and *C. myriocarpus* were conducted at the greenhouse of the Plant Protection Skills Centre, University of Limpopo, South Africa (23°53''10'S, 29°44''15'E) in autumn (August to October) 2010. Ambient day/night temperatures averaged 28/21°C, with maximum temperatures controlled using thermostatically-activated fans. Other greenhouse variables such as relative humidity, photosynthetically active radiation and solar radiation were not measured. *Cucumis africanus* and *C. myriocarpus* fruits were separately collected from locally cultivated plants after fruit maturity (Mafeo and Mashela, 2009a,b) and cut into pieces. Approximately 500 g fresh materials of each *Cucumis* species were separately fermented in 20 L-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 EM was added into each container. After a 14-day incubation period, when pH was at 3.7 or less (Kyan *et al*., 1999), FCE were passed through a 2 mm-opening mesh sieve into a 20 L container for subsequent preparations of the required concentrations on irrigation days. Consequently, for each weekly application, new concentrations were prepared from freshly fermented crude extracts. When required, nematode inoculums were prepared by extracting eggs and juveniles of *M. incognita* race 2 from roots of greenhouse-grown

nematode-susceptible kenaf (*Hibiscus cannabinus* L.) in 1% NaOCl solution (Hussey and Barker, 1973).

### 3.2.2 Experimental design and cultural practices

Twenty-cm-diameter plastic pots, at 0.3 m inter-row spacing and 0.25 m intra-row spacing, were each filled with 1 800 ml steam-pasteurised sand and Hygromix (Hygrotech, Pretoria North, South Africa) at 3:1 (v/v). Uniform four-week old tomato seedlings cv. 'Floradade' were transplanted and inoculated with 1 130 eggs and juveniles of *M. incognita* race 2. Seven treatments, viz. 0, 10, 20, 30, 40, 50 and 60% concentrations of each *Cucumis* species were arranged in a randomised complete block design, with 5 replicates for each experiment (Figure 3.1). Three days after transplanting, each plant was fertilised with 3 g 2:3:2 (22) to provide a total of 186 mg N, 126 mg K and 156 mg P per ml water and 2 g 2:1:2 (43) – providing 0.35 mg N, 0.32 mg K and 0.32 mg P, 0.9 mg Mg, 0.75 mg Fe, 0.075 mg Cu, 0.35 mg Zn, 1.0 mg B, 3.0 mg Mn and 0.07 mg Mo per 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 300 ml chlorine-free tapwater as soon as 50% of the moisture meters had readings below 2 units. Once a week, irrigation amounts were substituted for treatments using appropriate concentrations for each product. Plants were scouted for the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood 1856*)* and sprayed with 1.33 ml Lebaycid (fenthion 50% ml)/L water when population densities increased above 10 whiteflies per five randomly selected plants.

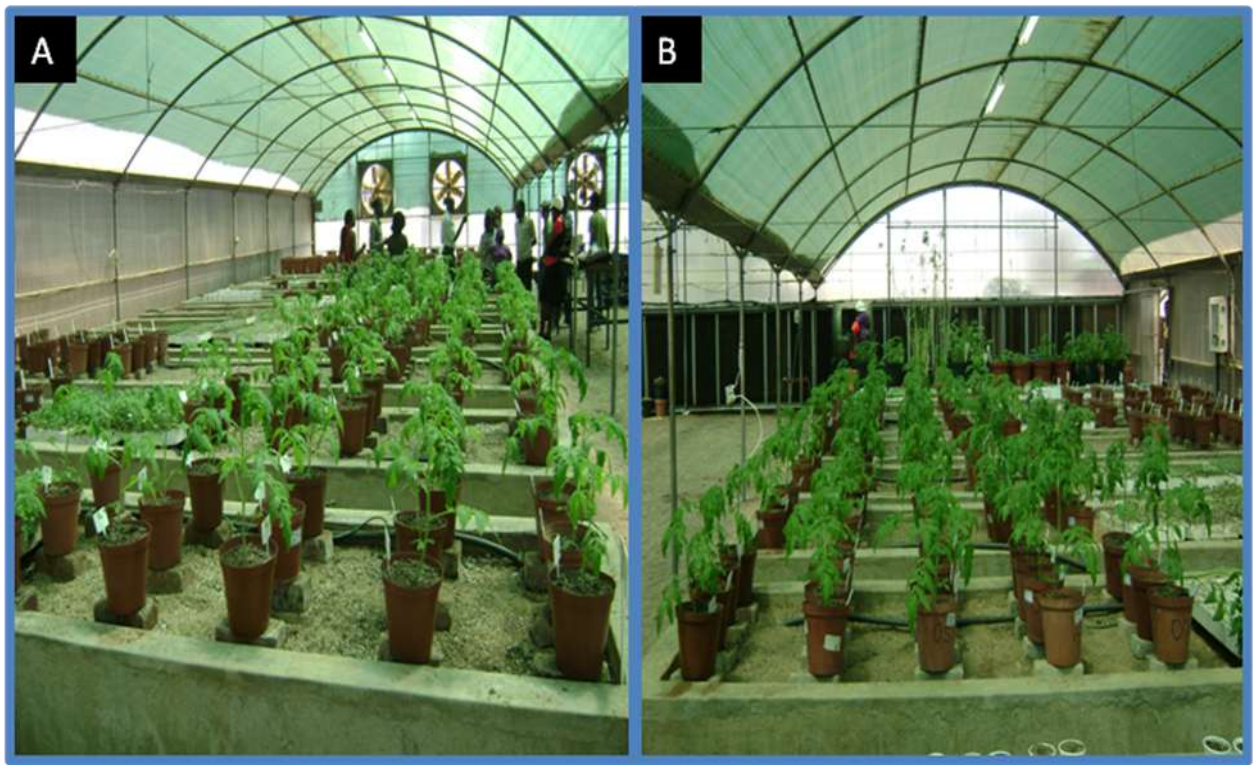


Figure 3.1 Greenhouse experiments using concentrations from fermented crude extracts of fresh fruits of **(A)** *Cucumis africanus* and **(B)** *Cucumis myriocarpus.*

3.2.3 Data collection

At 56 days after inoculation, fruit/plant were collected and plant height measured from the soil surface to the tip of the flag leaf. Stems were cut off at the soil surface and the stem diameters measured at 5 cm above the severed ends using a digital vernier caliper. Fresh fruit were weighed, shoots were oven-dried at 70°C for 72 h and weighed. 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 were extracted from total root system/plant by maceration and blending for 30 sec in 1% NaOCl (Hussey and Barker, 1973). The material was passed through nested 61- and 38-µm mesh sieves. The contents of the 38-µm mesh sieve

were collected for further separation of nematodes from debris using the sugar-flotation and centrifugation method (Jenkins, 1964). Soil in each pot was thoroughly 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 samples were each counted using a stereomicroscope and converted to total root system per plant and total soil per pot, respectively. Final nematode population density (Pf) allowed for calculation of reproductive factor (RF = Pf/Pi) values, where Pi was initial nematode population density.

### 3.2.4 Data analysis

Data were subjected to analysis of variance (ANOVA) through the 2008 SAS software (SAS Institute, Inc., Cary, NC., U.S.A.). Nematode numbers were transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984), but untransformed means were reported. Treatment mean separation was achieved using Waller-Duncan multiple range test at the probability level of 5%. Mean plant variables were subjected to the CARD model generating the regression curve estimations using the quadratic equation: $Y = b_2x + b_1x + a$, where Y = plant variable value and x computed from $x = -b_1/2b_2$, where x = the optimum concentration level, which is a concentration value where saturation sets in (Salisbury and Ross, 1992), along with the biological indices, viz. $D_m$, $R_h$, $D_0$, $D_{50}$, $D_{100}$, k and $R^2$ (Liu *et al*., 2003). Unless otherwise stated, only treatments that were significant at the probability level of 5% are discussed (Appendices 3.1-3.8).

## 3.3 Results

Relative to Pi, Pf at the same concentrations was reduced, whereas increasing concentrations from 10 to 60% had no effect on Pf (Table 3.1). The impact of concentrations on Pf at the same level ranged from 80 to 92% in *C. africanus* and 50 to 90% in *C. myriocarpus.* Threshold stimulation ($D_m$) for various organs differed from 1 unit in dry shoot mass to 22 units in fruit yield mass for *C. africanus* and from 4 units in dry root mass to 12 units in plant height for *C. myriocarpus* with saturation points ($R_h$) being attained within almost a fraction of the respective $D_m$ values (Table 3.2). Using the fruit yield mass to describe the observed $D_m$ values, $D_m$ occurred at 21.845 units and from $D_m$ to $R_h$ additional 0.323 units were required for a total concentration of 22.168 (21.845 + 0.323) units from the untreated control. Similarly, from untreated control to $D_{100}$ a total concentration of 81.845 (21.845 + 60.000) units were required.

Graphic presentation of plant variables and FCE concentrations for *C. africanus* and *C. myriocarpus* demonstrated that at low concentrations the material stimulated growth, while at high concentrations inhibition occurred (Figures 3.2-3.5). In *C. africanus* transformation levels for fruit yield decreased from k = 1 ($R^2$ = 0.93), with the model ceasing to run at k = 0 ($R^2$ = 0.90), whereas in *C. myriocarpus* transformation levels for dry root mass decreased from k = 2 ($R^2$ = 0.99) to k = 1 ($R^2$ = 0.98). In dry shoot mass, plant height and stem diameter, the best fits to the CARD model for *C. africanus* were achieved at k = 0 for each variable, contrary to *C. myriocarpus* that had the best fits to the CARD model at k = 1 for dry root mass, dry shoot mass and stem diameter, except plant height which had k = 0 (Table 3.2). Sensitivity ranking (Σk) of tomato exposed to

the test materials was equivalent to zero and three for *C. africanus* and *C. myriocarpus*, respectively. Computed mean concentration stimulation range (MCSR) for *C. africanus* was at 10.84%, while for *C. myriocarpus* it was at 7.35% (Table 3.3). The $R^2$ values for all the models ranged from 88 to 97% in *C. africanus* and 96 to 98% in *C. myriocarpus*. Computed optimum response concentration values (Table 3.4), derived from the quadratic relationships for plant variables, were more or less equivalent to those derived from the CARD model.

Table 3.1 Influence of diluted fermented crude extracts of fresh *Cucumis africanus* and *Cucumis myriocarpus* fruits on final nematode population density (Pf) and percentage impact of *Meloidogyne incognita* race 2 (n = 30).

| **Concentration (%)** | Fresh *Cucumis africanus* fruit | | | | | Fresh *Cucumis myriocarpus* fruit | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **(Pi)** | **$Pf_{root}$** | **$Pf_{soil}$** | **Pf** | **Impact (%)[y]** | **(Pi)** | **$Pf_{root}$** | **$Pf_{soil}$** | **Pf** | **Impact (%)[y]** |
| 10 | 1130 | 28 | 65 | 93 | -92** | 3000 | 36 | 627 | 663 | -78** |
| 20 | 1130 | 69 | 151 | 220 | -80** | 3000 | 68 | 396 | 464 | -84** |
| 30 | 1130 | 69 | 65 | 134 | -88** | 3000 | 90 | 828 | 918 | -69** |
| 40 | 1130 | 66 | 43 | 109 | -90** | 3000 | 104 | 1242 | 1346 | -55** |
| 50 | 1130 | 37 | 130 | 167 | -85** | 3000 | 107 | 1404 | 1511 | -50** |
| 60 | 1130 | 58 | 65 | 123 | -89** | 3000 | 79 | 216 | 295 | -90** |

[y]Impact (%) = [(Treatment/Pi) ─ 1) × 100] at same level of inoculation, where ** implied that Pi and Pf were significantly different at 5% level of probability according to student t-test.

Table 3.2 Biological indices of fruit yield mass (FYM), dry shoot mass (DSM), dry root mass (DRM), plant height (PHT) and stem diameter (SDR) of tomato exposed to diluted fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits at 56 days after treatments (n = 35).

| | Fresh *Cucumis africanus* fruit | | | | Fresh *Cucumis myriocarpus* fruit | | | |
|---|---|---|---|---|---|---|---|---|
| **Biological index** | **FYM** | **DSM** | **PHT** | **SDR** | **DRM** | **DSM** | **PHT** | **SDR** |
| Threshold stimulation ($D_m$) | 21.845 | 1.012 | 1.470 | 18.639 | 4.510 | 5.802 | 12.123 | 4.543 |
| Saturation point ($R_h$) | 0.323 | 0.002 | 0.025 | 0.459 | 1.085 | 2.188 | 1.113 | 0.481 |
| 0% inhibition ($D_0$) | 43.690 | 0.005 | 2.939 | 37.278 | 29.360 | 45.271 | 24.245 | 29.730 |
| 50% inhibition ($D_{50}$) | 52.941 | 51.414 | 65.204 | 69.351 | 63.944 | 344.334 | 83.327 | 293.325 |
| 100% inhibition ($D_{100}$) | 60.000 | 73.100 | 91.600 | 87.900 | 114.500 | 1247.700 | 107.572 | 1166.400 |
| $R^2$ | 0.90 | 0.92 | 0.88 | 0.97 | 0.98 | 0.96 | 0.97 | 0.98 |
| Sensitivity index (k) | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 |
| | Sensitivity ranking: ∑k = 0 | | | | Sensitivity ranking: ∑k = 3 | | | |

Table 3.3 Mean concentration stimulation range (MCSR) for fruit yield mass (FYM), dry shoot mass (DSM), dry root mass (DRM), plant height (PHT) and stem diameter (SDR) of tomato exposed to diluted fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits at 56 days after treatments (n = 35).

| | Fresh *Cucumis africanus* fruit | | | | | Fresh *Cucumis myriocarpus* fruit | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Biological index** | **FYM** | **DSM** | **PHT** | **SDR** | Mean | **DRM** | **DSM** | **PHT** | **SDR** | **Mean** |
| Threshold stimulation ($D_m$) | 21.845 | 1.012 | 1.470 | 18.639 | 10.742 | 4.510 | 5.802 | 12.123 | 4.543 | 6.745 |
| Adjusted saturation point ($R_h$)[y] | 22.168 | 1.014 | 1.495 | 19.098 | 10.944 | 5.595 | 7.990 | 13.236 | 5.024 | 7.960 |
| | | | | MCSR | 10.843 | | | | MCSR | 7.353 |

[y]Adjusted $R_h = D_m + R_h$, while MCSR = $(D_m + \text{Adjusted } R_h)/2$

Table 3.4 Quadratic relationship, coefficient of determination and computed optimum response concentration for variables of tomato from the Curve-fitting Allelochemical Response Concentration against diluted fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits at 56 days after treatments (n = 35).

| | Fresh *Cucumis africanus* fruit | | | | Fresh *Cucumis myriocarpus* fruit | | | |
|---|---|---|---|---|---|---|---|---|
| **Plant variable** | **Quadratic relationship** | **$R^2$** | **$(x)^z$** | **P ≤** | **Quadratic relationship** | **$R^2$** | **$(x)^z$** | **P ≤** |
| Fruit yield mass (g) | $-0.003x^2 + 0.131x + 5.911$ | 0.90 | 21.833 | 0.01 | $-0.372x^2 + 1.271x + 2.364$ | 0.98 | 1.708 | 0.01 |
| Dry shoot mass (g) | $-0.002x^2 + 0.005x + 12.880$ | 0.92 | 0.357 | 0.01 | $-0.595x^2 + 2.283x + 13.988$ | 0.96 | 1.918 | 0.01 |
| Plant height (cm) | $-0.011x^2 + 0.033x + 92.183$ | 0.88 | 1.500 | 0.01 | $-0.008x^2 + 0.184x + 74.550$ | 0.97 | 11.500 | 0.01 |
| Stem diameter (mm) | $-0.001x^2 + 0.049x + 5.877$ | 0.97 | 24.500 | 0.01 | $-0.164x^2 + 0.562x + 4.214$ | 0.98 | 1.713 | 0.01 |

[z]Calculated optimum response concentration (x) = $-b_1/2b_2$

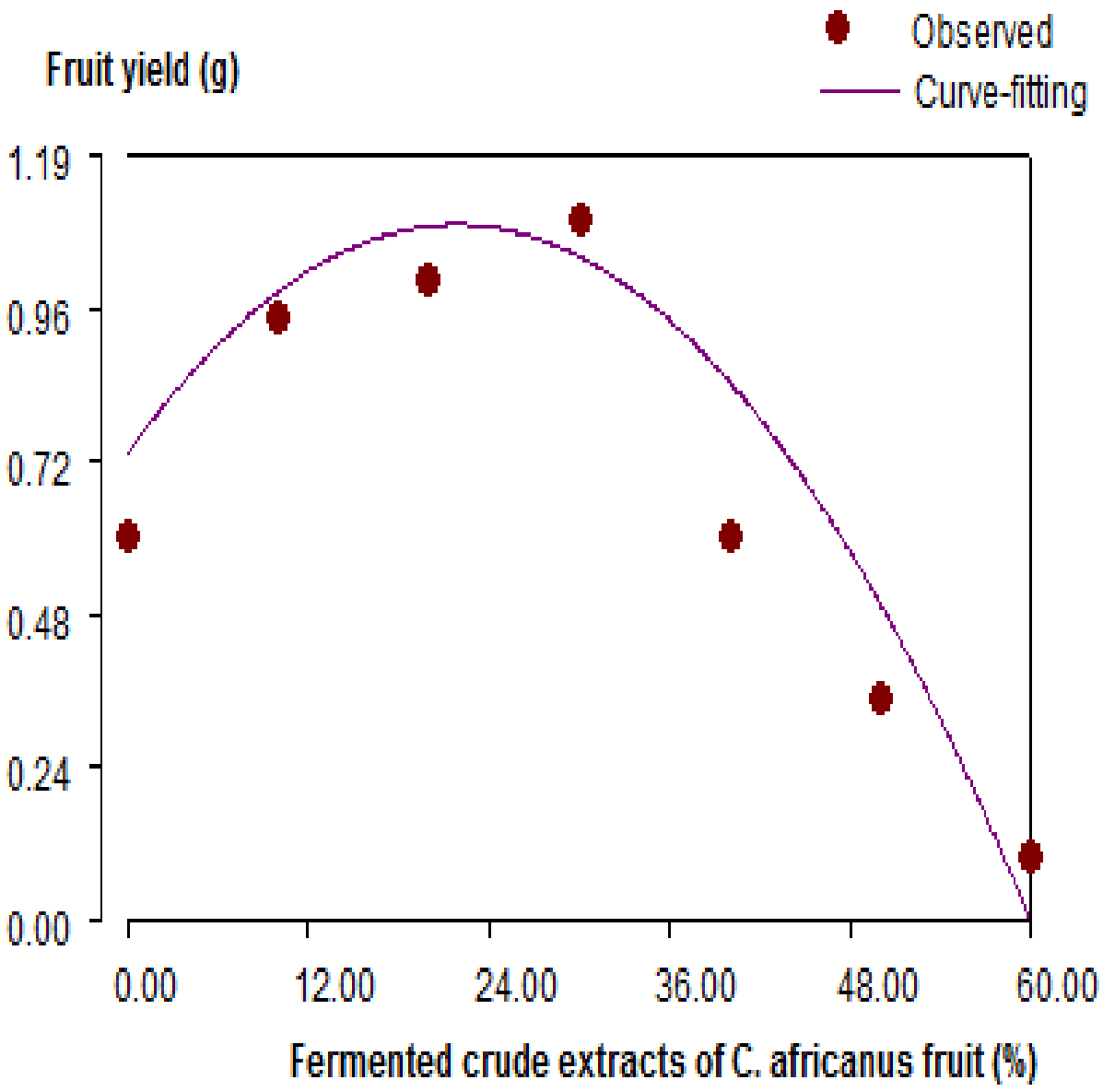


Figure 3.2 Response of fruit yield to concentrations of fermented crude extracts of *Cucumis africanus* fruit at 56 days after initiating treatments (n = 35).

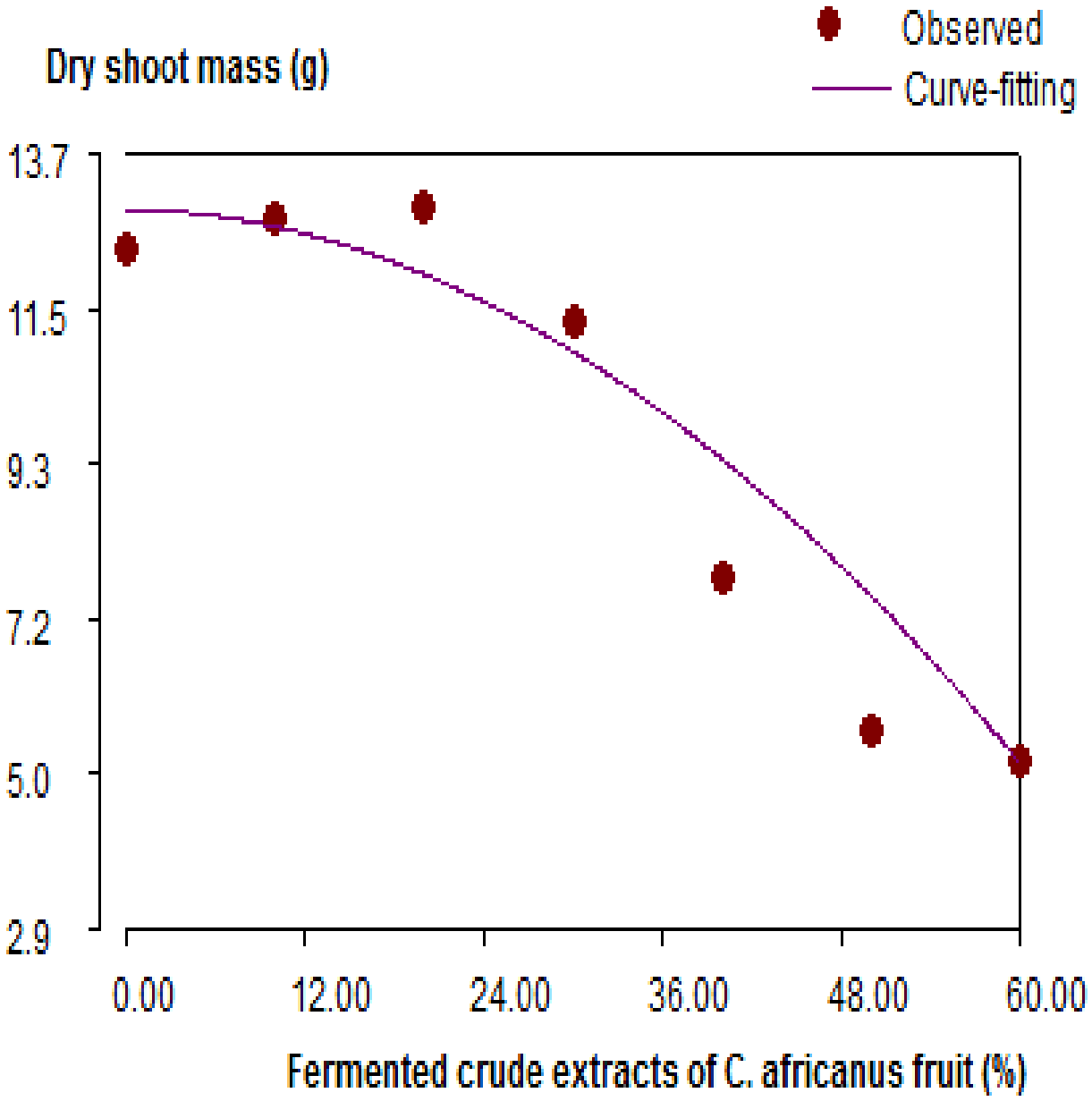


Figure 3.3 Response of dry shoot mass to concentrations of fermented crude extracts of *Cucumis africanus* fruit at 56 days after initiating treatments (n = 35).

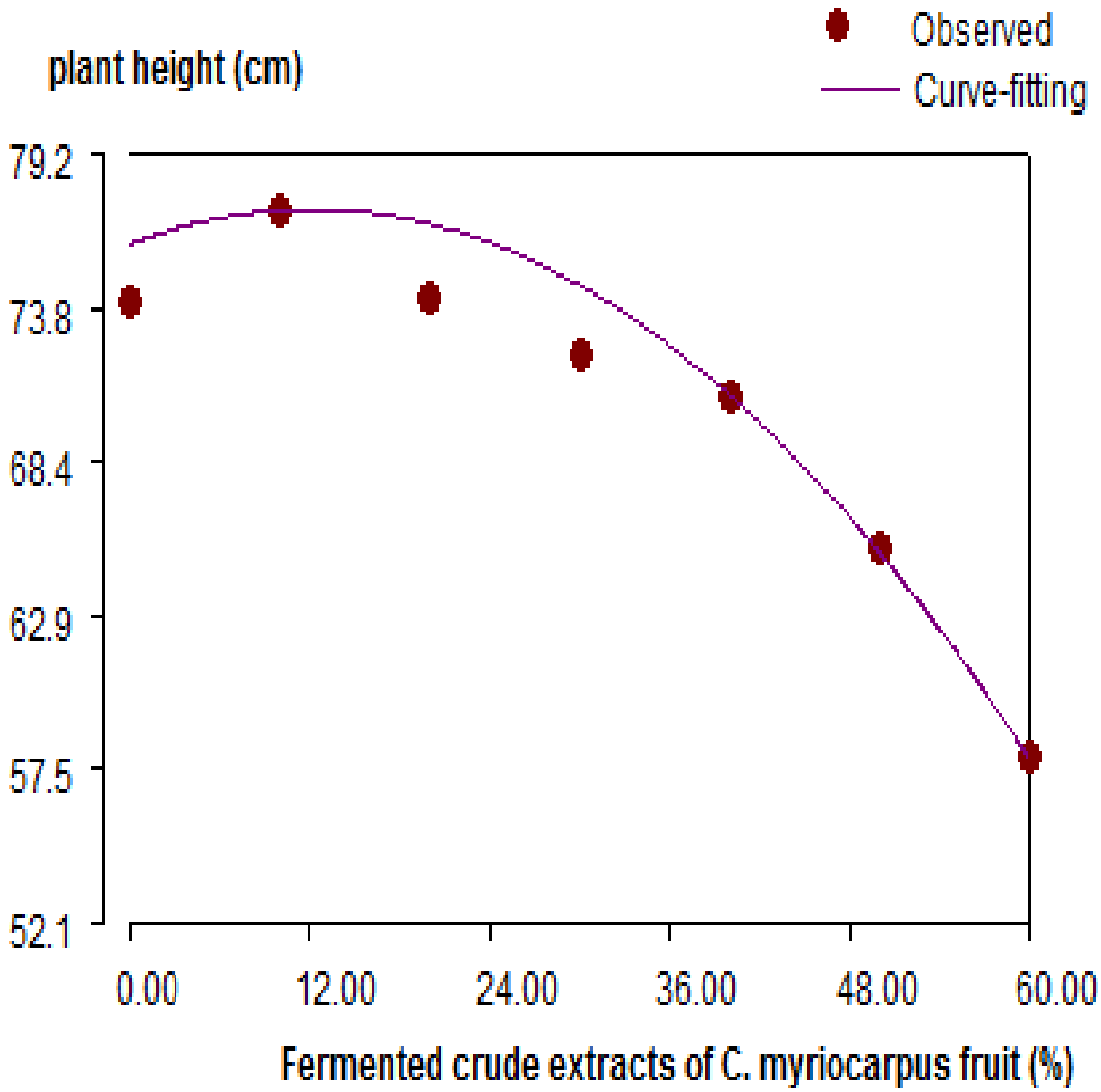


Figure 3.4 Response of plant height to concentrations of fermented crude extracts of *Cucumis myriocarpus* fruit at 56 days after initiating treatments (n = 35).

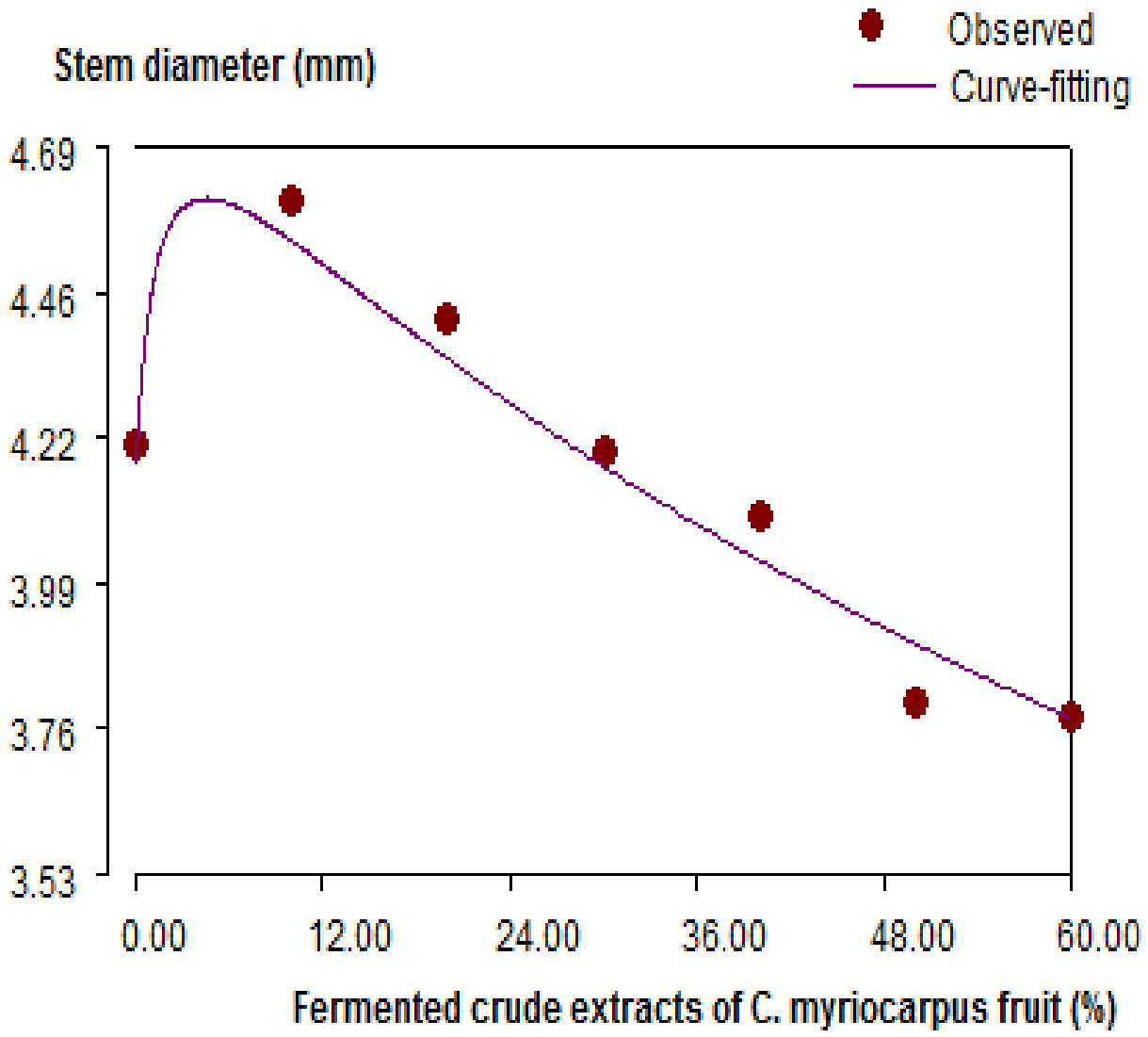


Figure 3.5 Response of stem diameter to concentrations of fermented crude extracts of *Cucumis myriocarpus* fruit at 56 days after initiating treatments (n = 35).

## 3.4 Discussion

Increasing FPE concentrations of *C. africanus* and *C. myriocarpus* fruits suppressed population densities of *M. incognita* race 2 on tomato plants equally at all levels, which confirmed the efficacy of these materials when used as dried ground crude extracts in the ground leaching technology systems (Mashela, 2002; Mphosi, 2004) and other plant materials (Mashela and Nthangeni, 2002; Mashela *et al*., 2010). Fermenting the materials did not interfere with potent chemicals that confer nematicidal properties in fruits of the two *Cucumis* species. Another product, Nemlab – produced from EM-fermented plant extracts of *L. camara* is used in managing nematode numbers in open field systems (Nzanza *et al*., 2013; Taurayi, 2011), although the product had not been empirically-quantified.

Potent chemicals in *C. africanus* fruit had been identified as cucurbitacin B ($C_{32}H_{46}O_8$), which is insoluble in water, while that in *C. myriocarpus* fruit – cucurbitacin A, is soluble in water, with two potent chemicals, namely, cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Chen *et al*., 2005; Jeffrey, 1978). In FPE, one cannot guarantee that the identified potent chemicals remain intact and pure, since fruits of the two *Cucumis* species are associated with an acid-loving fungus *Penicillium simplicissimum* (Mphahlele and Mashela, 2012), which induces heavy post-harvest fruit decay. Nzanza and Mashela (2012) also observed that FPE materials were highly associated with various fungi, possibly from the EM.

Incidentally, concentrations that are suppressive to nematode numbers, but phytotoxic to the protected plant, would not be useful, particularly when applied as a post-planting bio-nematicide. Generally, at low concentrations, FPE of *Cucumis* fruits stimulated growth of tomato plants, while at high concentrations the material inhibited plant growth, which agreed with the primary tenets of density-dependent growth patterns in biological systems exposed to increasing concentrations of allelochemicals (Liu *et al*., 2003). The CARD model in this study confirmed density-dependent growth patterns of various crops, including tomato, when exposed to unfermented crude extracts of *C. myriocarpus* fruit (Mafeo, 2012). However, crude extracts of *C. myriocarpus* fruit were suitable for use as pre-emergent bio-nematicide when the concentration was equivalent to the mean of the stimulation range [$(D_m + R_h)/2$], referred to as the 'mean concentration stimulation range' (MCSR) (Mafeo, 2012). When used at MCSR values, Mafeo (2012) demonstrated that crude extracts of *C. myriocarpus* fruit suppressed Pf from 88 to 99%, while growth of various crops was promoted.

In the CARD model, as k increased from zero, generally $R^2$ initially increased to a peak, where k = i and then decreased from i + 1 transformations until the model ceased running (Liu *et al*., 2003). In *C. africanus* experiment, as concentrations increased from 10 to 60%, k remained at zero for all variables, whereas in *C. myriocarpus* k remained at one except for plant height where the model started and ceased running at k equalled 1 and 0, respectively, but with the highest $R^2$ being at k = 0. The k = 0 value agreed with that observed for the radicle length in maize seedlings exposed to crude extracts of *C. myriocarpus* fruit over 18 days (Mafeo *et al*., 2011a,b,c). In other words, with the

exception for the dry root mass, dry shoot mass and stem diameter in *C. myriocarpus* experiment, sensitivities of tomato organs as concentrations increased were quite high and organ non-specific. However, in tomato seedlings exposed to increasing concentrations of crude extracts of *C. myriocarpus* fruit over 18 days, k differed from organ to organ, with k for hypocotyl diameter, hypocotyl length, epicotyl length and seedling height being equal to 15, 7, 20 and 9 units, respectively (Mafeo, 2012). In the 18 day study (Mafeo, 2012), sensitivities of organs to the test material in emerging tomato seedlings were organ-specific, with the hypocotyl length being the most sensitive and the epicotyl length the least sensitive. The ∑k of zero in *C. africanus* study, suggested that the tomato plant was highly sensitive to FCE of *C. africanus* fruit when compared to ∑k of three in FCE of *C. myriocarpus* and unfermented crude extracts of *C. myriocarpus* fruit, where ∑k in tomato was 51 (Mafeo, 2012). In addition to different *Cucumis* species having different potent chemicals, the exposure time and age of the test plants could also play a role, since in the other study (Mafeo, 2012) seedlings were exposed for 18 days when compared to the 56-day exposure time which started with four-week old seedlings. In the 18 days study (Mafeo, 2012), tomato seedlings were the least sensitive (∑k = 51) to crude extracts of *C. myriocarpus* fruit, while eggplant (*Solanum melongena*) seedlings were the most sensitive (∑k = 9).

On the whole, results of this study suggested that increasing FPE concentrations of *C. africanus* fruit from 10 to 60% was highly phytotoxic to tomato plants as shown by the overall sensitivity ranking of zero, while the overall sensitivity ranking of three for *C. myriocarpus* fruit suggested that this material was less phytotoxic to tomato plants.

Using the CARD model, MCSR values for FPE of *C. africanus* and *C. myriocarpus* fruits for tomato plants were at 10.84% and 7.35% concentration, which agreed with concentrations used in this study. The CARD model demonstrated the importance of choosing appropriate concentrations in phytotoxicity studies as described elsewhere (Mamphiswana *et al*., 2010). For instance, when concentrations are already above the saturation point ($R_h$), the relation between plant growth and increasing concentrations of FPE would be depicted by a negative linear relationship, while that before $R_h$ would be described by positive linear relationships (Mamphiswana *et al*., 2010). In contrast, if the relation is neutral, it would imply that growth was occurring at either the saturation point or below the threshold stimulation point ($D_m$).

## 3.5 Conclusions

In the current studies, most of the selected concentrations were already on the $R_h$ point as shown by the high levels of phytotoxicity as denoted by the low sensitivity values. Due to the robust iterative nature of the CARD model, it became evident that appropriate FPE concentrations of fresh fruits from *C. africanus* and *myriocarpus* would be below 10.84% and 7.35% concentration, respectively. Consequently, in order to avoid phytotoxicity, while the primary objective of suppressing nematode numbers is achieved, concentrations in the range of 0, 2, 4, 6 and 8% concentrations should be tested for phytotoxicity and nematode suppression.

# CHAPTER 4
# CONCENTRATION OF DRIED FRUITS FROM INDIGENOUS *CUCUMIS* SPECIES AS BIO-NEMATICIDES IN TOMATO PRODUCTION UNDER GREENHOUSE CONDITIONS

## 4.1 Introduction

A baseline study was conducted (Chapter 3) using fresh fruits from wild watermelon (*Cucumis africanus*) and wild cucumber (*Cucumis myriocarpus*) through botinemagation in order to ameliorate the cost-ineffectiveness of the ground leaching technology (GLT) system in large-scale tomato (*Solanum lycorpersicum*) production (Mashela *et al*., 2011). Generally, use of allelochemicals from botanicals is limited by their high levels of phytotoxicity (Inderjit *et al*., 1995). Using a Curve-fitting Allelochemical Response Dosage (CARD) computer-based model, fresh fruits of *C. africanus* and *C. myriocarpus* had mean dosage stimulation range (MCSR) of 7% and 11% concentrations, respectively, which suppressed the southern root-knot nematode (*Meloidogyne incognita* race 2) numbers by 87% and 71%, respectively (Chapter 3). The MCSR is the dosage that is expected to be the least non-phytotoxic and/or stimulate plant growth, while it is suppressive to nematode numbers (Mashela *et al*., 2011).

Incidentally, botanicals in fresh form are less effective in nematode suppression than in dried form (Rodriguez-Kabana, 1986). In GLT system, since fruits of *C. africanus* and *C. myriocarpus* had to be ground, they were used in dried form, which ameliorated a wide range of challenges that emerged with the development of this new technology (Mashela, 2002; Mashela and Nthangeni, 2002; Mashela *et al*., 2011). In fresh form, fruit of *C. africanus* and *C. myriocarpus* have high incidence of post-harvest decay from

*Penicillium simplicissimum* (Oudem.) Thom infection (Mphahlele *et al*., 2012), with unavailability of the materials being a challenge in areas where tomatoes are produced all-year-round. Successful use of crude extracts of dried *C. africanus* and *C. myriocarpus* fruits in fermented form would invariably prolong the shelf-life and therefore, mitigate unavailability. In this and subsequent studies, the primary focus was in avoiding phytotoxicity, while nematode suppression was secondary. The objective of this study was to determine whether fermented crude extracts from dried fruits of *C. africanus* and *C. myriocarpus* would be suitable for use in tomato production for the management of *M. incognita* race 2.

## 4.2 Materials and methods

### 4.2.1 Location and preparation of materials

Separate experiments, using *C. africanus* and *C. myriocarpus* fruits were conducted at the greenhouse of the Plant Protection Skills Centre, University of Limpopo, South Africa (23$^{o}$53”10’S, 29$^{o}$44”15’E) in spring (August – October) 2011 (Figure 4.1). Greenhouse conditions and preparation of plant materials were as described previously (Chapter 3), except that pieces of fruits were dried in air-forced ovens at 52ºC for 72 h (Mashela *et al*., 2011) and ground in a Wiley mill to pass through a 1-mm-opening sieve. Separate *C. africanus* and *C. myriocarpus* laboratory experiments were simultaneously fermented as previously explained (Chapter 3) using 0, 20, 40, 80, 160, 320 and 640 g with the objective to determine the ideal amount of plant material for each *Cucumis* species required to produce a fermented crude extract with a pH value ≤ 3.7 at 14 days under room temperature. At the end of 14 days, *C. africanus* 40 and *C.*

*myriocarpus* 80 g had a pH value ≤ 3.7. Approximately 40 g of crude extracts of *C. africanus* and 80 g of *C. myriocarpus* per 16 L tapwater in 20 L containers were each separately fermented for 14 days at room temperature for pH to decline from 6.8 to 3.7 for preparation of treatment concentrations (Chapter 3). When required, nematode inoculums were prepared as described previously (Chapter 3).

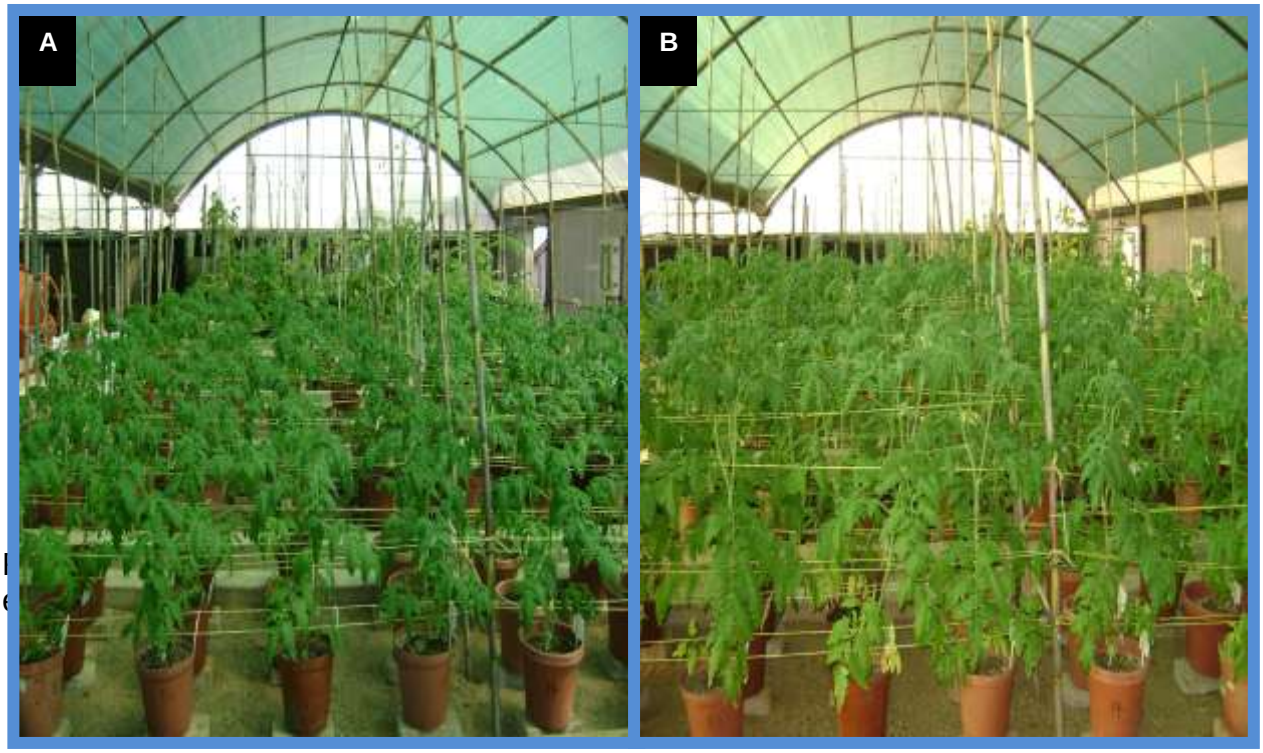


Figure 4.1 Greenhouse experiments using concentrations from fermented crude extracts of dried fruits of **(A)** *Cucumis africanus* and **(B)** *Cucumis myriocarpus*.

4.2.2 Experimental design and cultural practices

Twenty-cm-diameter plastic pots, at 0.3 m inter-row spacing and 0.25 m intra-row spacing, were each filled with 1 800 ml steam-pasteurised sand and Hygromix (Hygrotech, Pretoria North, South Africa) at 3:1 (v/v). Uniform four-week-old tomato

‘Floradade’ seedlings were transplanted and inoculated with 1 500 eggs and J2s of *M. Incognita* race 2. Seven treatments, namely, 0, 2, 4, 8, 16, 32 and 64% concentrations of each *Cucumis* species were arranged in a randomised complete block design, with 10 replicates for each experiment. Fertilisation at three days after transplanting, irrigation when necessary and pest management were done as described previously (Chapter 3). Once a week, irrigation amounts were substituted for treatments using appropriate concentrations for each product. In a 3% concentration a total of 36 L of undiluted material/ha of 4 000 tomato plants would be required for both *Cucumis* species under greenhouse application.

### 4.2.3 Data collection

Plant and nematode variables were collected and analysed as described previously (Chapter 3). Briefly, flowers were counted weekly with pedicels marked to avoid recounting. At 56 days after inoculation, fruit of all sizes were harvested, plant height, stem diameter, dry shoot mass, dry root mass and nematode numbers were recorded.

### 4.2.4 Data analysis

Data were subjected to analysis of variance (ANOVA) through the 2008 SAS software (SAS Institute, Inc., Cary, NC., USA) as described previously (Chapter 3). Discrete data were transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984), but untransformed means were reported. Sum of squares were partitioned, while treatment mean separation was achieved using Waller-Duncan multiple range test at the probability level of 5% and then subjected to CARD computer-

based model to generate appropriate biological indices (Liu *et al*., 2003). Then, after adjusting $R_h$ for $D_m$ in plant variables, MCSR was computed by halving the sum of $D_m$ and adjusted $R_h$. Unless otherwise stated, only treatment means significant at 5% level of probability are discussed (Appendices 4.1-4.8).

## 4.3 Results

Treatment effects were significant for dry shoot mass, dry root mass, plant height, stem diameter and nematode numbers in *C. africanus*, while in *C. myriocarpus*, treatment effects were only significant for dry shoot mass and dry root mass. Fermented crude extracts of dried *C. africanus* fruit contributed 42%, 38%, 28%, and 19% in dry shoot mass, dry root mass, plant height and stem diameter, while those of *C. myriocarpus* fermented crude extracts contributed 84%, and 44% in dry root mass and dry shoot mass to the total treatment variation (TTV), respectively (Table 4.1).

Treatments were significant for nematode numbers in roots, soil and final population in both *Cucumis* species. Dried *C. africanus* fruit contributed 84%, 44% and 82% in nematodes in root soil and final population to total treatment variation. Whereas, in dried *C. myriocarpus* fruit contributed 64%, 30% and 50% in nematode numbers in root, soil and final population to total treatment variation, respectively (Table 4.2). Dosage levels had no effect on number of flowers and fruit mass in *C. africanus*, while in *C. myriocarpus* dosage levels had no effect on number of flowers, plant height and stem diameter (data not shown).

Plant growth had density-dependent growth patterns as dosage levels increased in both *C. africanus* and *C. myriocarpus* fruits (Figures 4.2-4.7). Biological indices for dry shoot mass, dry root mass, plant height and stem diameter produced by the CARD model were strongly explained by dosage levels as shown by the coefficients of variation ($R^2$) at 96%, 97%, 99% and 90% as concentrations of fermented dried *C. africanus* fruit increased, while those in dried fruits of *C. myriocarpus* explained the model for dry shoot mass and dry root mass by 98% each (Table 4.3). Various plant organs had different biological indices except for k values, which were for each organ equivalent to unity. In *C. africanus* and *C. myriocarpus* the overall sensitivity of tomato plants was $\sum k = 4$ and $\sum k = 3$, respectively. Overall, MCSR values for concentration of fermented crude extracts from dried fruits of *C. africanus* and *C. myriocarpus* for tomato were at 2.64% and 2.99%, respectively (Table 4.3), which are equivalent to 3% for the purpose of this study. The application of these materials would result in 26 L/ha for each *Cucumis* species.

Relative to untreated control, dosages of crude extracts from *C. africanus* dried fruits reduced nematode numbers by 85-97%, 45-96% and 78-97%, while those of C. myriocarpus by 97-99%, 47-90% and 87-97% for root, soil and total nematodes, respectively (Table 4.4). By convention, 3% concentration of fermented crude extracts from dried fruits of *C. africanus* and *C. myriocarpus* reduced total nematode numbers by 87% and 96%, respectively. The impact of concentrations appeared to be inversely proportional to the dosage level in all measurement units. In untreated control, galling

was higher than in treated plots, while the latter were not different from one another (data not shown).

Table 4.1 Partitioning sum of squares for dry shoot mass (DSM), dry root mass (DRM), plant height (PHT) and stem diameter (SDR) of tomato 'Floradade' treated weekly with seven different concentrations of allelochemicals from fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits for 56 days from transplanting (n = 70).

| **Source of** | | Dried *Cucumis africanus* fruit | | | | | | | | Dried *Cucumis myriocarpus* fruit | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variation** | **DF** | **DSM** | | **DRM** | | **PHT** | | **SDR** | | **DRM** | | **DSM** | |
| | | SS | % | SS | % | SS | % | SS | % | SS | % | SS | % |
| Replication | 9 | 72.76 | $19^{ns}$ | 6.98 | $8^{ns}$ | 803.17 | $20^{ns}$ | 8.77 | $19^{ns}$ | 284430 | $2^{ns}$ | 192405 | $8^{ns}$ |
| Treatment | 6 | 164.35 | $42^{***}$ | 34.83 | $38^{***}$ | 1158.03 | $28^{***}$ | 8.75 | $19^{**}$ | 1.087E+07 | $84^{***}$ | 1034273 | $44^{***}$ |
| Error | 54 | 151.94 | 39 | 49.89 | 54 | 2151.03 | 52 | 28.41 | 62 | 185578 | 14 | 1144581 | 48 |
| Total | 69 | 389.04 | 100 | 91.71 | 100 | 4112.23 | 100 | 45.93 | 100 | 1.301E+07 | 100 | 2371259 | 100 |

$^{ns}$Means that the factor was not significant at $P \leq 0.05$; while $^{**}$ and $^{***}$ mean that the factor (s) was significant at $P \leq 0.10$, $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 4.2 Partitioning sum of squares for final *Meloidogyne incognita* population density in root and soil of tomato 'Floradade' treated weekly with seven different concentrations of allelochemicals from fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruit for 56 days from transplanting (n = 70).

| **Source of** | | Dried *Cucumis africanus* fruit | | | | | | Dried *Cucumis myriocarpus* fruit | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variation** | **DF** | **Total root** | | **Total soil** | | **Pf** | | **Total root** | | **Total soil** | | **Pf** | |
| | | SS | % | SS | % | SS | % | SS | % | SS | % | SS | % |
| Replication | 9 | 284430 | $2^{ns}$ | 192405 | $8^{ns}$ | 691719 | $3^{ns}$ | 2.093 | $3^{ns}$ | 8.321 | $13^{ns}$ | 5.562 | $10^{ns}$ |
| Treatment | 6 | 1.087E+07 | $84^{***}$ | 1034273 | $44^{***}$ | 1.781E+07 | $82^{***}$ | 50.988 | $64^{***}$ | 18.908 | $30^{***}$ | 29.606 | $50^{***}$ |
| Error | 54 | 1855780 | 14 | 1144581 | 48 | 3156889 | 15 | 26.573 | 33 | 35.194 | 57 | 23.558 | 40 |
| Total | 69 | 1.301E+07 | 100 | 2371259 | 100 | 2.166E+07 | 100 | 79.655 | 100 | 62.423 | 100 | 58.726 | 100 |

$^{ns}$Means that the factor (s) was not significant at $P \leq 0.05$; while $^{**}$ and $^{***}$ mean that the factor (s) was significant at $P \leq 0.10$, $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 4.3 Biological indices and mean dosage stimulation range of dry shoot mass (DSM), dry root mass (DRM), plant height (PHT) and stem diameter (SDR) of tomato exposed to diluted fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* dried fruits at 56 days after transplanting (n = 70).

| | Dried *Cucumis africanus* fruit | | | | | Dried *Cucumis myriocarpus* fruit | | |
|---|---|---|---|---|---|---|---|---|
| **Biological index** | **DSM[x]** | **DRM** | **PHT** | **SDR** | **Mean** | **DRM[x]** | **DSM** | **Mean** |
| Threshold stimulation ($D_m$) | 2.53 | 2.20 | 2.73 | 1.53 | 2.25 | 3.907 | 1.100 | 2.504 |
| Saturation point ($R_h$) | 0.71 | 0.32 | 1.98 | 0.08 | 0.77 | 0.511 | 1.456 | 0.984 |
| 0% inhibition ($D_0$) | 11.48 | 9.21 | 12.94 | 5.42 | 9.76 | 23.079 | 12.533 | 17.806 |
| 50% inhibition ($D_{50}$) | 164.90 | 59.00 | 2899.74 | 1603.20 | 1181.71 | 135.811 | 13.989 | 74.900 |
| 100% inhibition ($D_{100}$) | 703.50 | 170.30 | 2901.72 | 1603.28 | 1344.70 | 410.6 | 15.445 | 13.032 |
| $R^2$ | 0.96 | 0.97 | 0.99 | 0.90 | 0.96 | 0.98 | 0.980 | 0.98 |
| Sensitivity index (k) | 1 | 1 | 1 | 1 | | 1 | 2 | |
| Total plant sensitivity | | | ∑k = 4 | | | | ∑k = 3 | |
| | | | | **Mean dosage stimulation range (MCSR)** | | | | |
| Threshold stimulation ($D_m$) | 2.53 | 2.20 | 2.73 | 1.53 | 1.53 | 3.907 | 1.100 | 2.504 |
| Adjusted saturation point ($R_h$)[y] | 3.24 | 2.52 | 4.71 | 1.61 | 1.61 | 4.418 | 2.556 | 3.487 |
| | | | | MCSR | 2.640 | | MCSR | 2.995 |

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

Table 4.4 Impact of allelochemicals from diluted fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits to final nematode numbers of *Meloidogyne incognita* race 2 in roots of tomato alone, soil alone and in both roots and soil at 56 days after inoculating each plant with 1 500 eggs and second stage juveniles (n = 70).

| Concen-tration | Dried *Cucumis africanus* fruit | | | | | | Dried *Cucumis myriocarpus* fruit | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Nematode in root** | | **Nematode in Soil** | | **Total nematode** | | **Nematode in root** | | **Nematode in soil** | | **Total nematode** | |
| **(%)** | **Variable** | **%[z]** | **Variable** | **%[z]** | **Variable** | **%[z]** | **Variable** | **%[z]** | **Variable** | **%[z]** | **Variable** | **%[z]** |
| 0 | 1 224a[y] | – | 401a | – | 1 623a[x] | – | 1 305a | – | 367a | – | 1 672a | – |
| 2 | 120cd | –90 | 51de | –87 | 169bc | –90 | 8b | –99 | 40cd | –89 | 48c | –97 |
| 4 | 39d | –97 | 15e | –96 | 52c | –97 | 13b | –99 | 40bcd | –89 | 53c | –97 |
| 8 | 82cd | –93 | 75cde | –81 | 155bc | –90 | 15b | –99 | 36d | –90 | 51c | –97 |
| 16 | 184b | –85 | 177bc | –56 | 359b | –78 | 8b | –99 | 166bc | –55 | 174bc | –90 |
| 32 | 67bcd | –95 | 113bcd | –72 | 178bc | –89 | 25b | –98 | 196ab | –47 | 221b | –87 |
| 64 | 138bc | –89 | 221ab | –45 | 357b | –78 | 34b | –97 | 187abc | –49 | 221b | –87 |

[ns]Column mean (s) followed by the same letter are not different (P ≤ 0.05) according to Duncan multiple range test.

[z]Impact (%) = [(treatment/control) ─ 1)] × 100

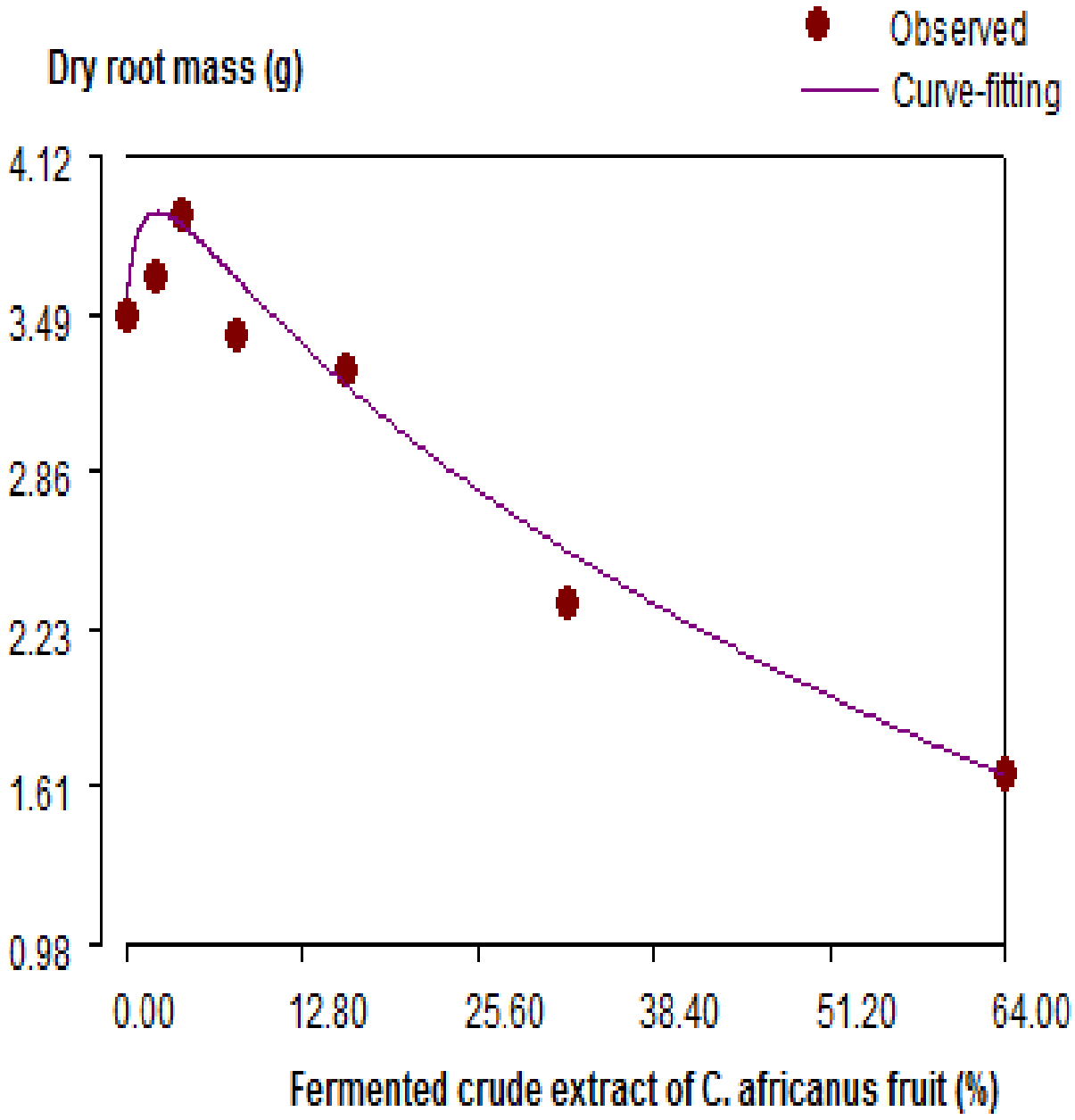


Figure 4.2 Response dry root mass to dosages of fermented crude extracts of *Cucumis africanus* fruit at 56 days after initiating treatments (n = 70).

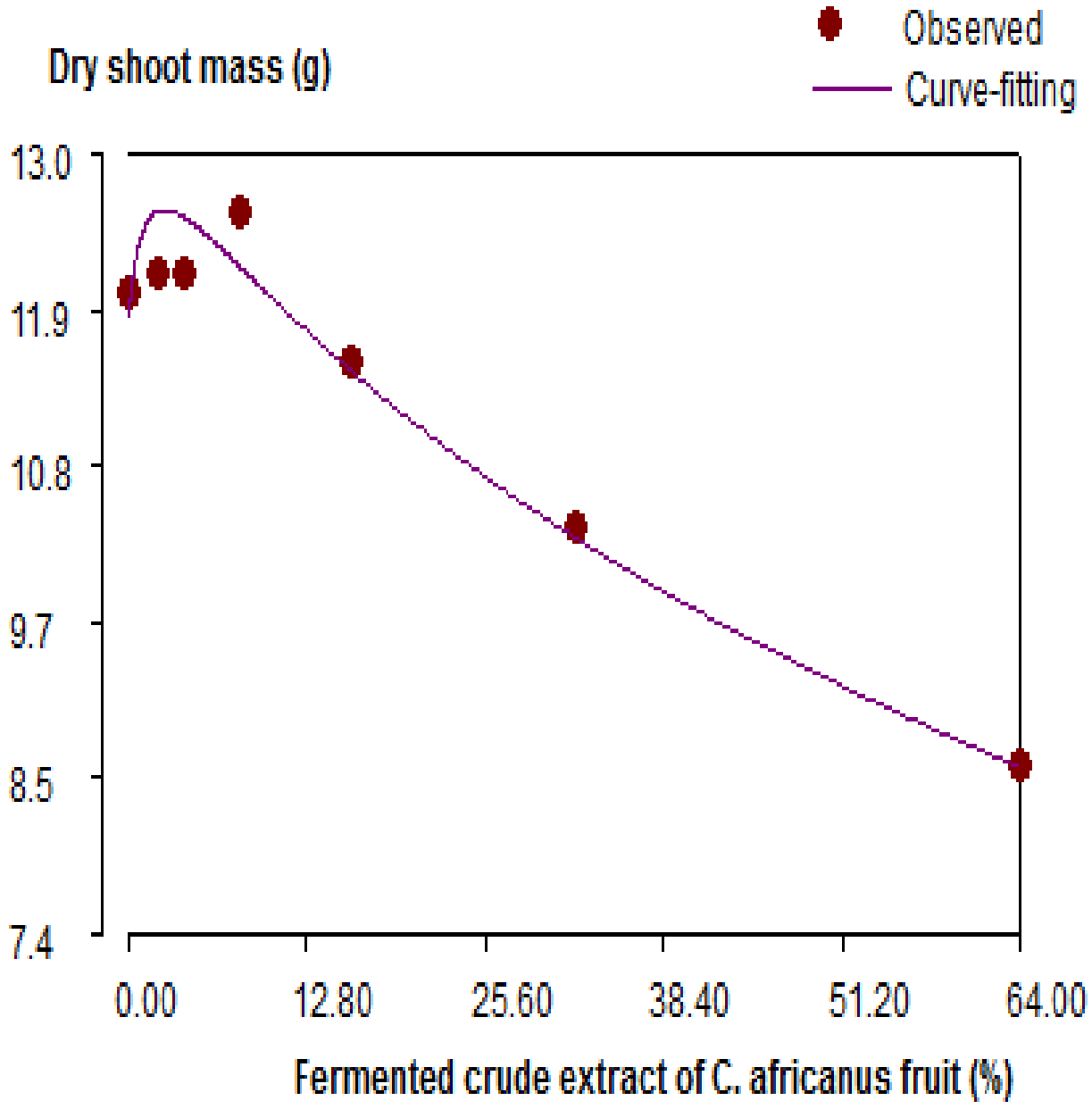


Figure 4.3 Response of dry shoot mass to dosages of fermented crude extracts of *Cucumis africanus* fruit at 56 days after initiating treatments (n = 70).

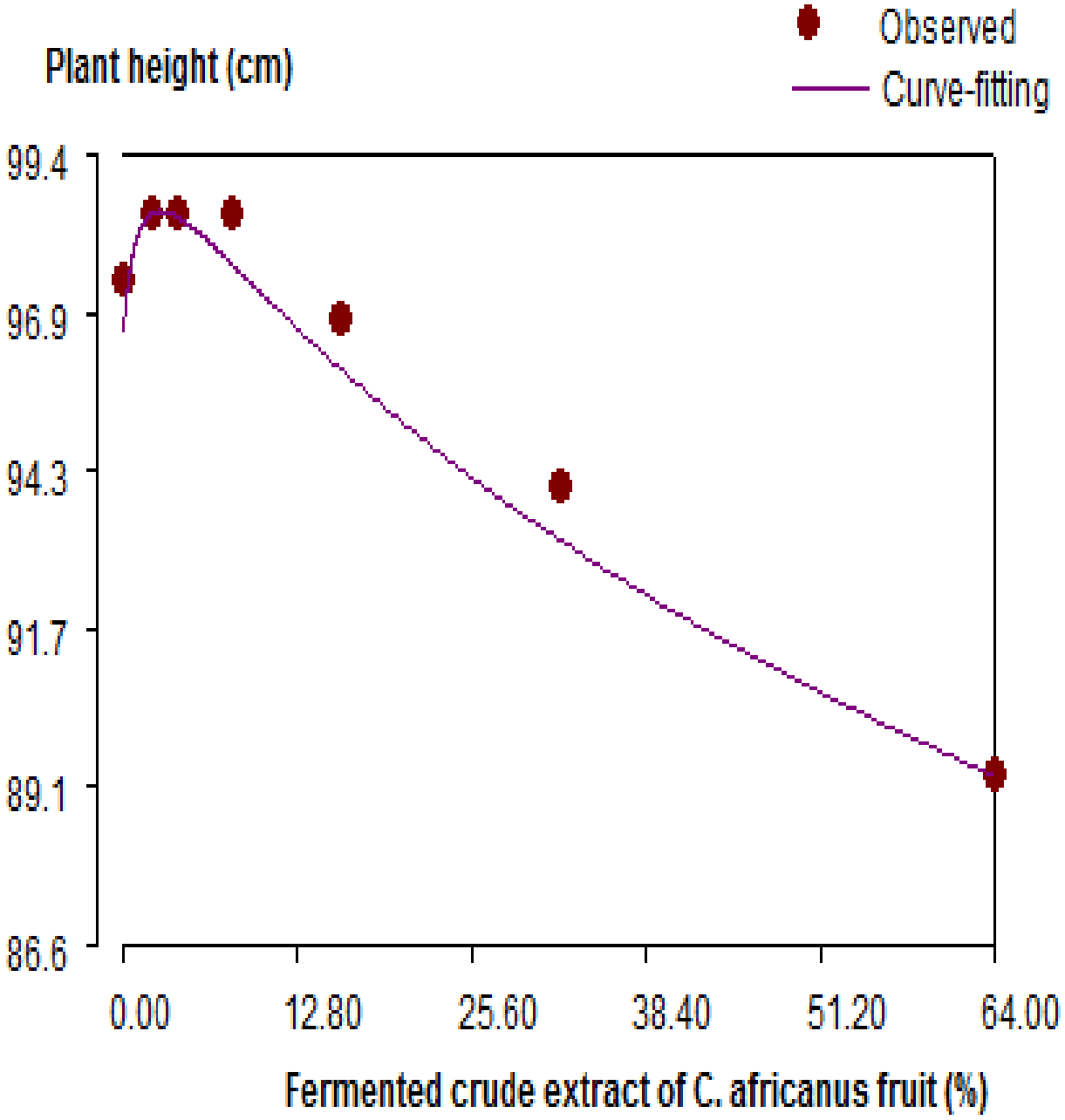


Figure 4.4 Response of plant height to dosages of fermented crude extracts of *Cucumis africanus* fruit at 56 days after initiating treatments (n = 70).

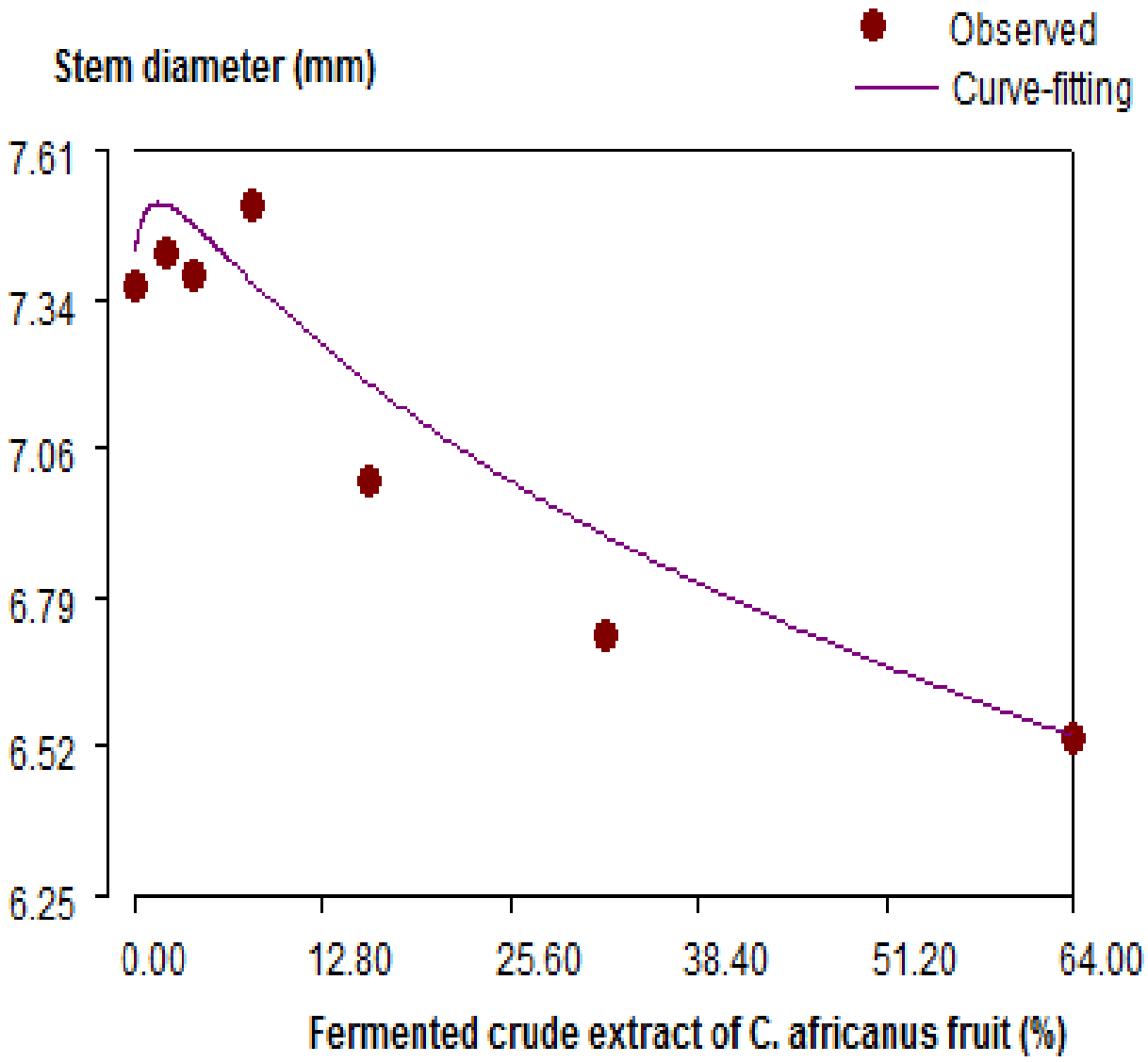


Figure 4.5 Response of stem diameter to dosages of fermented crude extracts of *Cucumis africanus* fruit at 56 days after initiating treatments (n = 70).

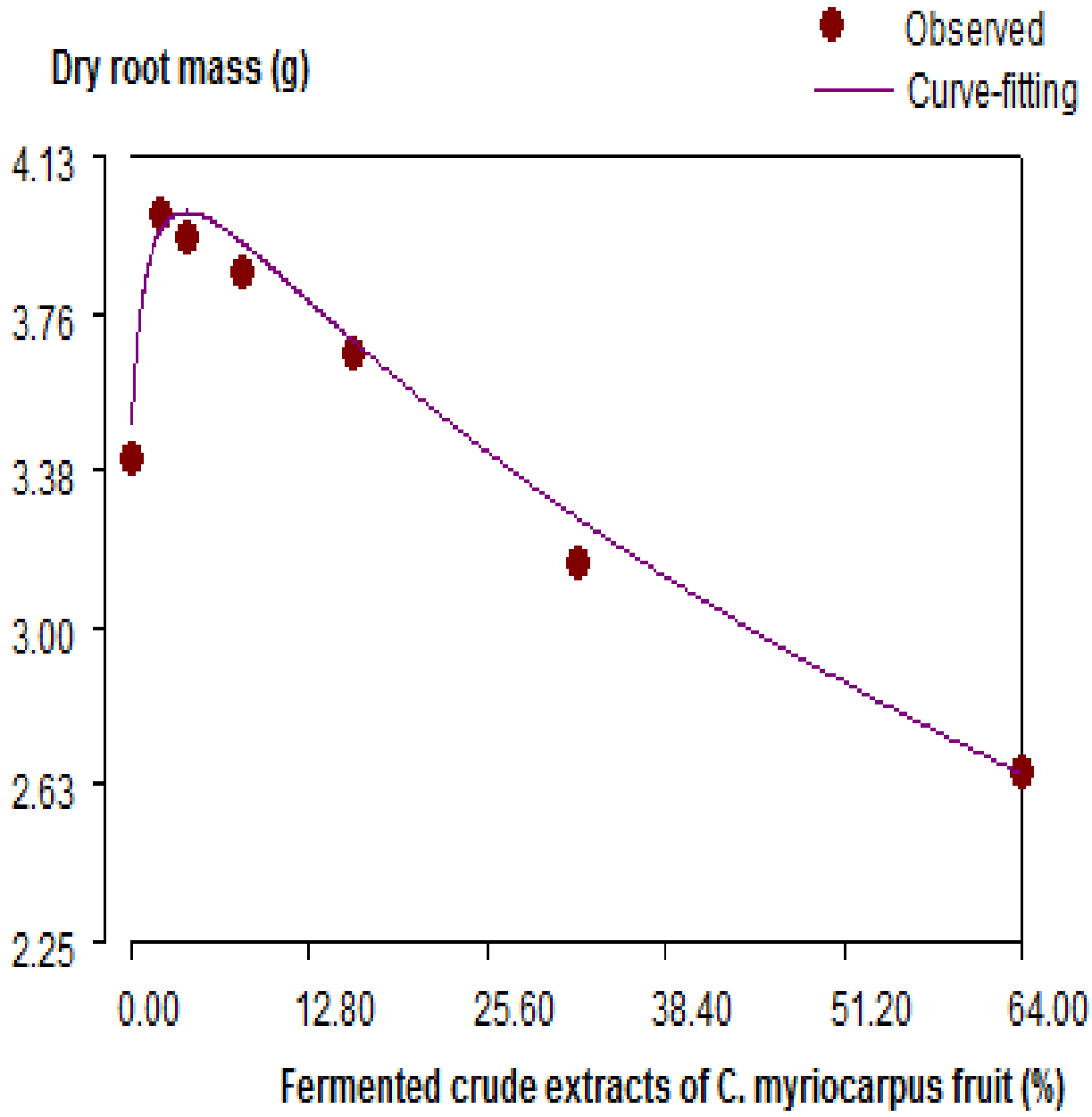


Figure 4.6 Response of dry root mass to dosages of fermented crude extracts of *Cucumis myriocarpus* fruit at 56 days after initiating treatments (n = 70).

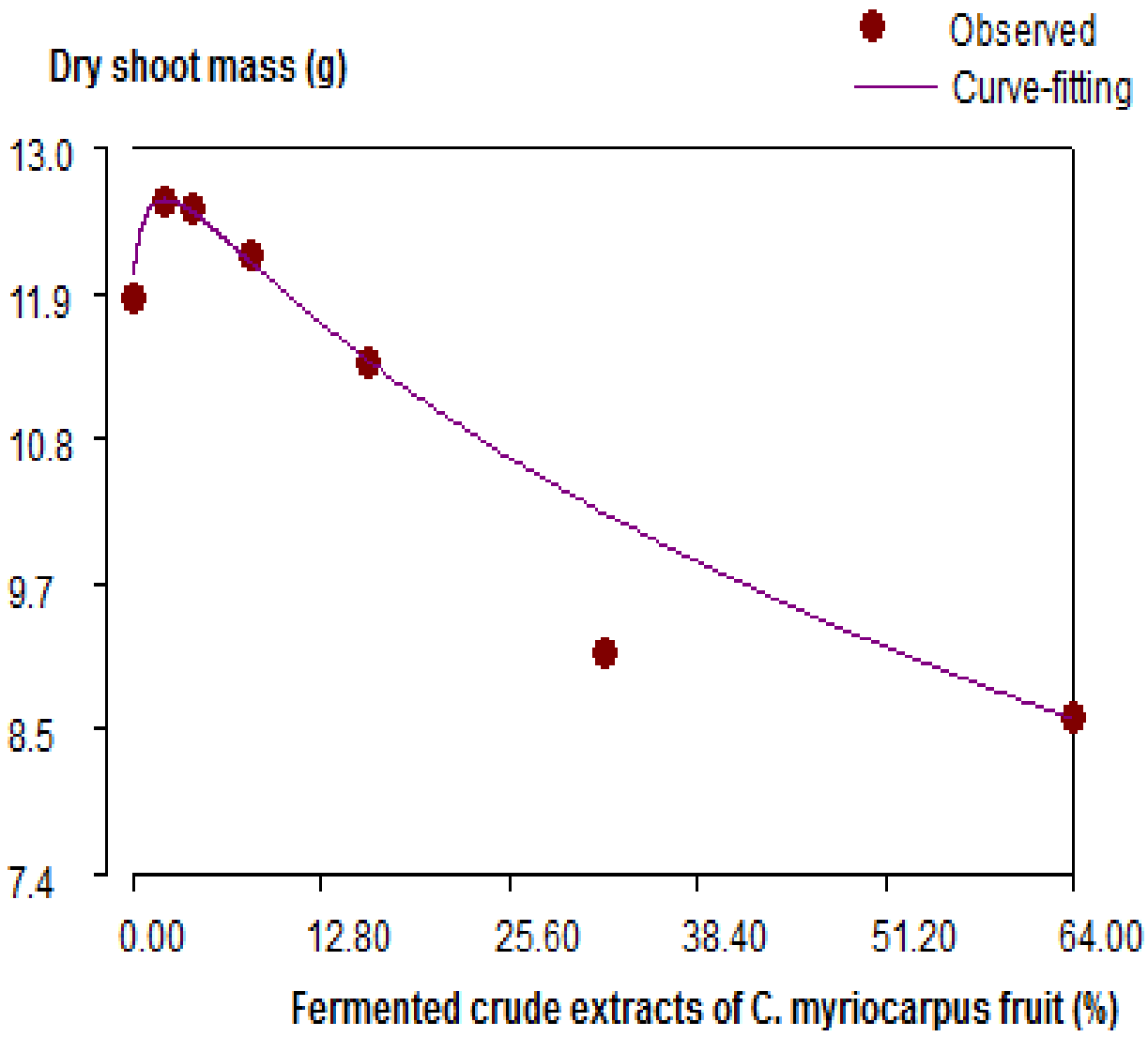


Figure 4.7 Response of dry shoot mass to dosages of fermented crude extracts of *Cucumis myriocarpus* fruit at 56 days after initiating treatments (n = 70).

## 4.4 Discussion

High coefficients of determination ($R^2$) for the CARD models in four plant variables suggested strong density-dependent relationships between growth of tomato and increasing concentrations of crude extracts of *C. africanus* and *C. myriocarpus* fruits. Generally, k values of unity in all organs suggested that in tomato plant, the assessed organs had similar sensitivities to concentrations of allelochemicals from fermented crude extracts of *C. africanus* and *C. myriocarpus* fruits. Plant sensitivity is indirectly proportional to k values, with zero suggesting the highest sensitivity to allelochemicals used, while high k values suggested decreased sensitivities (Liu *et al*., 2003). Observed k values on tomato in these studies were different from zero values observed for dry shoot mass, plant height and stem diameter under concentrations from fresh fermented *C. africanus* fruit (Chapter 3). In GLT, Mafeo (2012) showed that k values of tomato seedlings ranged from 9 to 20 depending on the investigated organ. Apparently, k values are affected by various factors, which may include fermentation of dried versus fresh materials, fermented versus unfermented, age of the test plant and/or organ of the test plant.

Overall, for the two materials in dried form ∑k values for whole tomato plant were 4 and 3, while in fresh form the values were zero and three for *C. africanus* and *C. myriocarpus*, respectively (Chapter 3). Consequently, in fresh form, fermented crude extracts of *C. africanus* fruit are highly toxic to tomato plants, when compared to the dried form, which are less phytotoxic. In contrast, the sensitivity of tomato to fermented crude extracts of *C. myriocarpus* fruits was not affected by whether the material was in

dried or fresh form. Previously, Pofu (2012) demonstrated that the two *Cucumis* species had strong differences in their resistance to various *Meloidogyne* species, which was attributed to the differences in their active ingredients, namely, cucurbitacin A and cucurbitacin B in *C. myriocarpus* and *C. africanus*, respectively (Chen *et al*., 2005). This observation constitutes the first evidence to suggest that fermented fruit of *C. africanus* are less phytotoxic in dried form, while those of *C. myriocarpus* are not affected by form.

Stimulation of plant growth at low dosages of crude extracts of *Cucumis* fruits appears to be universal, as shown in various biological entities when using various types of allelochemicals (Liu *et al*., 2003). The phenomenon was previously observed in eight and ten monocotyledonous and dicotyledonous plants, respectively (Mafeo, 2012). Cucumin ($C_{27}H_{40}O_9$), which is one of the two active ingredients in cucurbitacin A, was shown to have anticancer activities at high concentrations, which however, had nonspecific cytotoxicity properties, while at low dosages the materials stimulated cell division (Chen *et al*., 2005). Mean dosage stimulation range of equivalent to 3% concentration from fermented crude extracts of dried fruits in both *Cucumis* species was much lower than those derived from fermented fresh fruits (Chapter 3) of *C. africanus* (11% concentration) and *C. myriocarpus* (7% concentration). Disparities in MCSR values from dried and fresh fruits could explain why fermented fresh fruit of *C. africanus* had much lower k values (Chapter 3) than those of fermented dried fruit. Another point to note is that the recommended MCSR values are much lower than $D_0$ and $D_{50}$ inhibition biological indices, which, in dried form were 10% and 1182% in *C. africanus,* while for *C. myriocarpus* they were 18% and 75%, respectively. Consequently, MCSR

values as recommended from the CARD model ensure that a phytotoxic dosage level is not incorrectly recommended.

Suppression of nematodes in all test dosages confirmed the nematicidal properties of allelochemicals in *C. africanus* and *C. myriocarpus* (Mashela *et al*., 2011). Observed nematode suppression in both root and soil were quite high. The decline in the efficacy of increasing concentrations in nematode suppression could also be attributed to the degree of phytotoxicity of the materials, which inadvertently affect infection sites and therefore, nematode numbers. Nematode suppression using fermented crude extracts from dried fruits in this study was comparable to that when using fermented crude extracts from fresh fruits (Chapter 3). In agreement with Makkar (1999), drying at 52ºC for 72 h had no significant effect on cucurbitacins A and B, which had been identified as potent allelochemicals that confer nematicidal properties to *C. africanus* and *C. myriocarpus* fruits.

In a simple proportion using the MCSR of 3%, dried *C. africanus* fruit would reduce nematode numbers in root, soil and root + soil by 80 J2s (120 × 2/3), 34 J2s (51 × 2/3) and 128 J2s (169 × 2/2.64), respectively, while using the MCSR of 3% from dried *C. myriocarpus* fruit nematode numbers would be reduced by 5 (8 × 2/3), 27 J2s (40 × 2/3) and 32 J2s (48 × 2/3) in root, soil and root + soil, respectively. In terms of percentage reduction of nematodes in root, soil and root + soil, using both *Cucumis* species in relation to untreated control, the impact would be 93%, 90% and 92% in *C. africanus* at MCSR of 3% and 99%, 93% and 98% in *C. myriocarpus* at MCSR of 3%. Generally, the

highest percentages on reduction of nematode numbers were at low dosages in both *Cucumis* species.

## 4.5 Conclusions

In dried form, MCSR values from fermented crude extracts of dried fruits of *C. africanus* and *C. myriocarpus* fruits was equivalent to 3% concentrations, which was therefore, much less than those from fermented crude extracts of fresh fruits. Also, the 3% concentrations from dried fruits were less phytotoxic to tomato plants, but highly suppressive to nematode numbers. Therefore, the dried fruits of the two *Cucumis* species were more efficient and effective in serving as phytonematicides than their fresh counterparts.

# CHAPTER 5
# APPLICATION TIME INTERVALS FOR FERMENTED CRUDE EXTRACTS FROM INDIGENOUS *CUCUMIS* SPECIES IN TOMATO PRODUCTION UNDER MICROPLOT CONDITIONS

## 5.1 Introduction

Botanicals, mainly due to their allelopathic nature, may be highly phytotoxic to crops (Meyer *et al*., 2008; Musabyimana *et al*., 2000; Ramazan and Yarba, 2010; Setia *et al*., 2007). In some cases, the materials are applied weekly and/or more (Nzanza and Mashela, 2012). Phytotoxicity may result in low crop yield and/or even in the eventual death of the protected crops (Musabyimana *et al*., 2000). Phytotoxicity issues are compounded by the fact that the efficacy of plant extracts on nematode suppression depends much on their concentration and duration of exposure of the nematode (Kali and Gupta, 1980; Mahmood *et al*., 1979; Setia *et al*., 2007). In operations, the duration of exposure of the nematode to a particular dosage is a function of the application time interval of the material.

Empirical results from using crude extracts in dried fruits from wild watermelon (*Cucumis africanus L.f.*) and wild cucumber (*Cucumis myriocarpus* Naude.) in management of the southern root-knot nematode (*Meloidogyne incognita*) under greenhouse conditions suggested that the mean dosage stimulation range was 3% concentration (Chapter 4). Mean dosage stimulation range is a concentration that is suitable for suppressing nematode numbers without being phytotoxic to the protected plants, which was in this case tomato (*Solanum lycopersicum* L.). However, the application time interval of 3% and 6% concentrations from materials of both species is

not documented. The objective of this study, therefore, was to develop optimum application time intervals for fermented crude extracts from dried fruits of *C. africanus* and *C. myriocarpus* at 3% and 6% concentrations for suppression of *M. incognita* race 2 in tomato production under microplot conditions.

## 5.2 Materials and methods

### 5.2.1 Location and initiation of study

Separate experiments for *C. africanus* and *C. myriocarpus* were conducted concurrently on microplots at the Plant Protection Skills Centre, University of Limpopo, South Africa (23$^{o}$53"10'S, 29$^{o}$44"15'E) under microplot conditions (Figure 5.1). The location has summer rainfall with mean annual rainfall of 600 mm, while maximum/minimum temperatures average 28$^{o}$C/19$^{o}$C. Trials were conducted in summer (October-December) 2011 and were not repeated in space or time since they were factorial experiments (Gomez and Gomez, 1984). Artificial micro-plots were established by inserting 30-cm-diameter plastic pots into 20-cm-deep holes at 1.0 m intra-row and 1.0 m inter-row spacing. Each pot was filled with 10 L steam-pasteurised sand and Hygromix at 3:1 (v/v). Sources and preparation of *Cucumis* fruits and nematode inoculum, along with transplanting were as described previously (Chapter 4). Uniform four-week-old tomato cv. 'Floradade' seedlings were transplanted and inoculated with 5 000 eggs and J2s (Chapter 3).


Figure 5.1 Microplot experiments using concentrations from fermented crude extracts of dried fruits of **(A)** *Cucumis africanus* and **(B)** *Cucumis myriocarpus.*

5.2.2 Experimental design and cultural practices

Treatments, namely, application time interval at zero, one, two, three and four intervals per 30-day month period were arranged in randomised complete block design, with 12 replications for each *Cucumis* species. All treatments were initiated five days after transplanting and thereafter 3% and 6% concentrations were separately applied during appropriate times. Fertilisation and irrigation were as described previously. Three days thereafter, each plant was fertilised with 3 g 2:3:2 (22) plus 2 g 2:1:2 (43) to provide adequate quantities of nutrient elements (Chapter 3) and irrigated as explained earlier

(Chapter 4). Each drip discharged 1 L/hour. In a 3% concentration and 6% concentration a total of 60 L and 120 L of undiluted material/ha of 4 000 tomato plants would be required for both *Cucumis* species under microplots. Plants were scouted weekly for insect pests which were managed using commercially-recommended materials in tomato production, while plants were sprayed weekly with Dithane M-45 and copper oxide for disease management or soon after rainfall. Weeds were removed among pots using hand-hoes when necessary.

### 5.2.3 Data collection

At 56 days after inoculation, plant and nematode variables were collected, prepared and recorded as described previously (Chapter 3).

### 5.2.4 Data analysis

Nematode and plant data were subjected to analysis of variance (ANOVA) through the 2008 SAS software as described previously (Chapter 3). Plant variables with significant ($P \leq 0.05$) treatment means were also subjected to lines of the best fit using plant growth responses to increasing application time interval and modelled by the regression curve estimations resulting in a quadratic equation (Mamphiswana *et al*., 2010):

$$Y = b_2x^2 + b_1x + a$$

Where Y = Plant growth response; x = application time interval with $-b_1/2b_2$ = x value for the optimum application time interval. Treatment mean separation for nematodes was achieved using Fisher's least significant different test at the probability level of 5%.

Unless otherwise stated, only treatment means significant at the probability level of 5% are discussed (Appendices 5.1-5.10).

## 5.3 Results

### 5.3.1 Application time interval for 3% concentration

Density-dependent growth patterns, as shown by quadratic relationships, were observed as the materials were being applied at 3% over increasing time intervals (Figures 5.2 – 5.10). The models accounted for 98%, 83%, 99%, 77% and 63% in total treatment variation of dry fruit mass, dry root mass, dry shoot mass, plant height and stem diameter in *C. africanus* and 65%, 92%, 64%, 88% and 65% in *C. myriocarpus* for the same plant variables. Various plant organs were optimised at different time intervals (Table 5.1), with the integrated mean time interval of 2.40 and 2.20 weeks/month in *C. africanus* and *C. myriocarpus*, which translated to 18 days (2.4/4 × 30) and 16 days (2.2/4 × 30), respectively. The 3% concentration accounted for 72% and 77% in *C. africanus* and 61% and 52% in *C. myriocarpus* for total treatment variation of final nematode numbers in roots and both roots plus soil, respectively (data not shown). The lowest final nematode population densities were observed at 22½ days (¾ × 30) and 30 days after initiating the treatment (Table 5.2).

Table 5.1 Quadratic relationship, coefficient of determination and computed optimum application time of 3% concentration from fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits on dry root mass, dry fruit mass, dry shoot mass, plant height and stem diameter of tomato plant 'Floradade' for 56 days under microplot conditions (n = 60).

| | Dried *Cucumis africanus* fruit | | | Dried *Cucumis myriocarpus* fruit | | |
|---|---|---|---|---|---|---|
| **Plant variable** | **Formula** | **$R^2$** | **$x^z$** | **Formula** | **$R^2$** | **$x^z$** |
| Dry root mass (g) | $y = -0.4475x^2 + 2.2505x + 4.3475$ | 0.83 | 2.51 | $y = -0.3838x^2 + 1.5878x + 6.901$ | 0.92 | 2.07 |
| Dry fruit mass (g) | $y = -4.165x^2 + 20.623x - 0.545$ | 0.98 | 2.45 | $y = -0.8833x^2 + 4.9781x + 17.914$ | 0.65 | 2.82 |
| Dry shoot mass (g) | $y = -3.61x^2 + 15.734x + 27.875$ | 0.99 | 2.18 | $y = -1.3405x^2 + 5.0903x + 44.374$ | 0.64 | 1.90 |
| Plant height (cm) | $y = -2.1275x^2 + 11.173x + 45.762$ | 0.77 | 2.63 | $y = -0.7202x^2 + 3.6208x + 57.275$ | 0.88 | 2.51 |
| Stem diameter (mm) | $y = -0.4275x^2 + 1.8805x + 11.003$ | 0.63 | 2.20 | $y = -0.3427x^2 + 1.1775x + 12.945$ | 0.65 | 1.72 |
| | Mean integrated application time interval | | 2.40 | Mean integrated application time interval | | 2.20 |

$^z$Calculated optimum application time (x) = $-b_1/2b_2$, where $-b_1 = 20.623$ and $b_2 = -4.165$ for dry fruit mass.

Table 5.2 Influence of application time of 3% concentration from fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits on final population density of *Meloidogyne incognita* on the roots, soil and final population numbers (roots and soil) on tomato plant 'Floradade' for 56 days under microplot conditions (n = 60).

| | *Cucumis afrcicanus* | | | | | | *Cucumis myriocarpus* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $Nematode_{root}$ | | $Nematode_{soil}$ | | $Total_{nematode}$ | | $Nematode_{root}$ | | $Nematode_{soil}$ | | $Total_{nematode}$ | |
| Time (weeks/month) | Variable | Impact (%) | Variable | Impact (%) | Variable | Impact (%) | Variable | Impact (%) | Variable | Impact (%) | Variable | Impact (%) |
| 0 | 22 551a | - | 7 375a | - | 29 926a | - | 22 511a | - | 5 208a | - | 27 719a | - |
| 1 | 8 107b | -64 | 4 333b | -41 | 12 441b | -58 | 4 567b | -80 | 4 833a | -7 | 9 400b | -66 |
| 2 | 5 631bc | -75 | 2 500bc | -66 | 8 131bc | -73 | 4 598b | -80 | 2 750ab | -47 | 7 348bc | -73 |
| 3 | 797c | -96 | 1 917bc | -74 | 2 714c | -91 | 866b | -96 | 1 333b | -74 | 2 199bc | -92 |
| 4 | 236c | -99 | 667c | -91 | 903c | -97 | 525b | -98 | 725b | -86 | 1 250c | -96 |

[ns]Column means followed by the same letter are not different (P ≤ 0.05) according to Duncan's multiple range test.

[z]Impact (%) = [(treatment/control) ─ 1) × 100

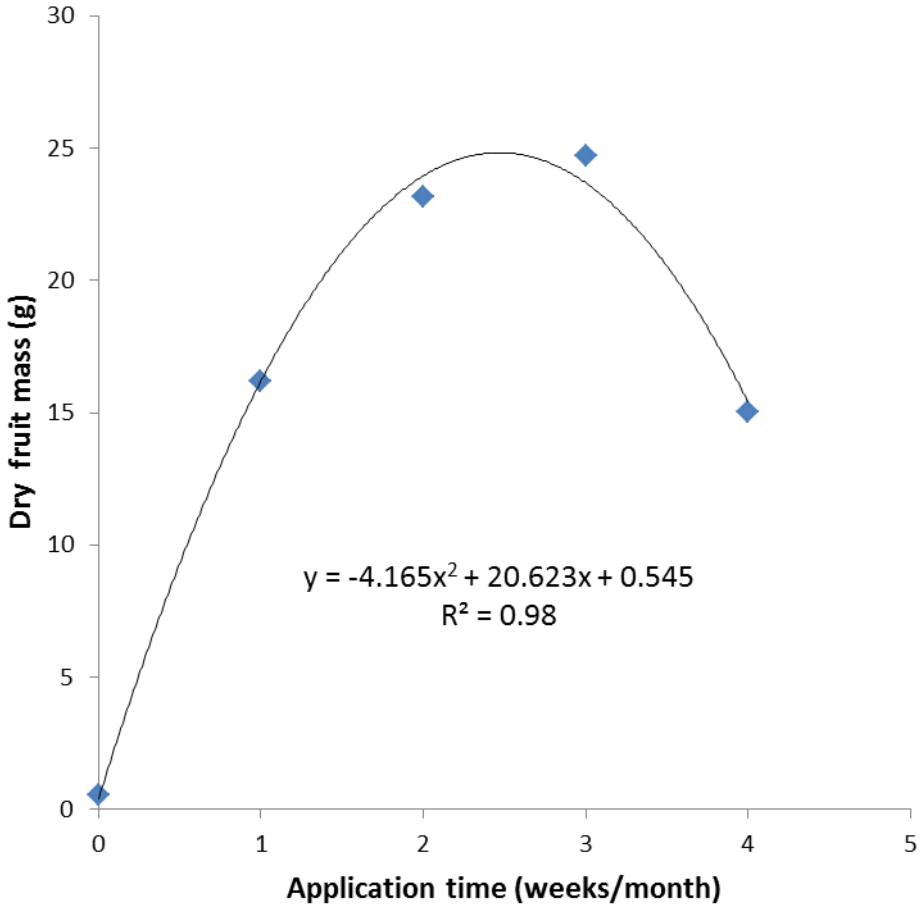


Figure 5.2 Influence of application time of 3% concentration from fermented crude extracts of *Cucumis africanus* fruit on dry fruit mass of tomato plant 'Floradade' for 56 days under microplot conditions (n = 60).

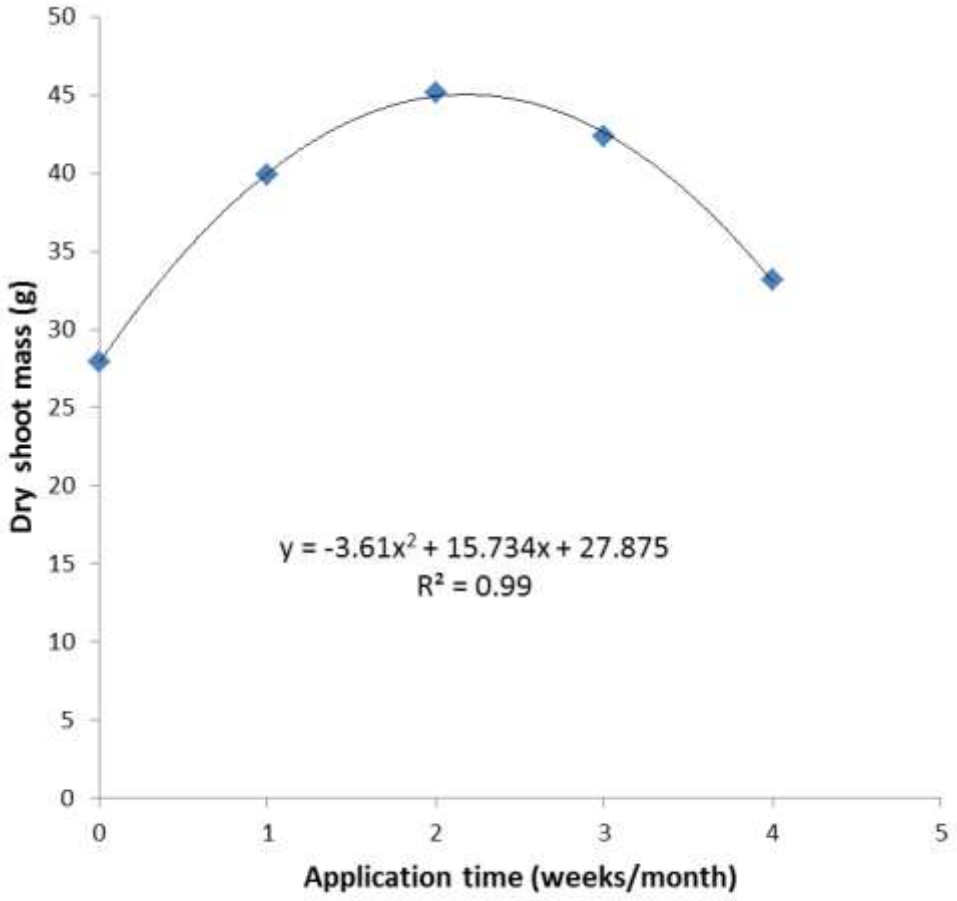


Figure 5.3 Influence of application time of 3% concentration from fermented crude extracts of *Cucumis africanus* fruit on dry shoot mass of tomato plant 'Floradade' for 56 days under microplot conditions (n = 60).

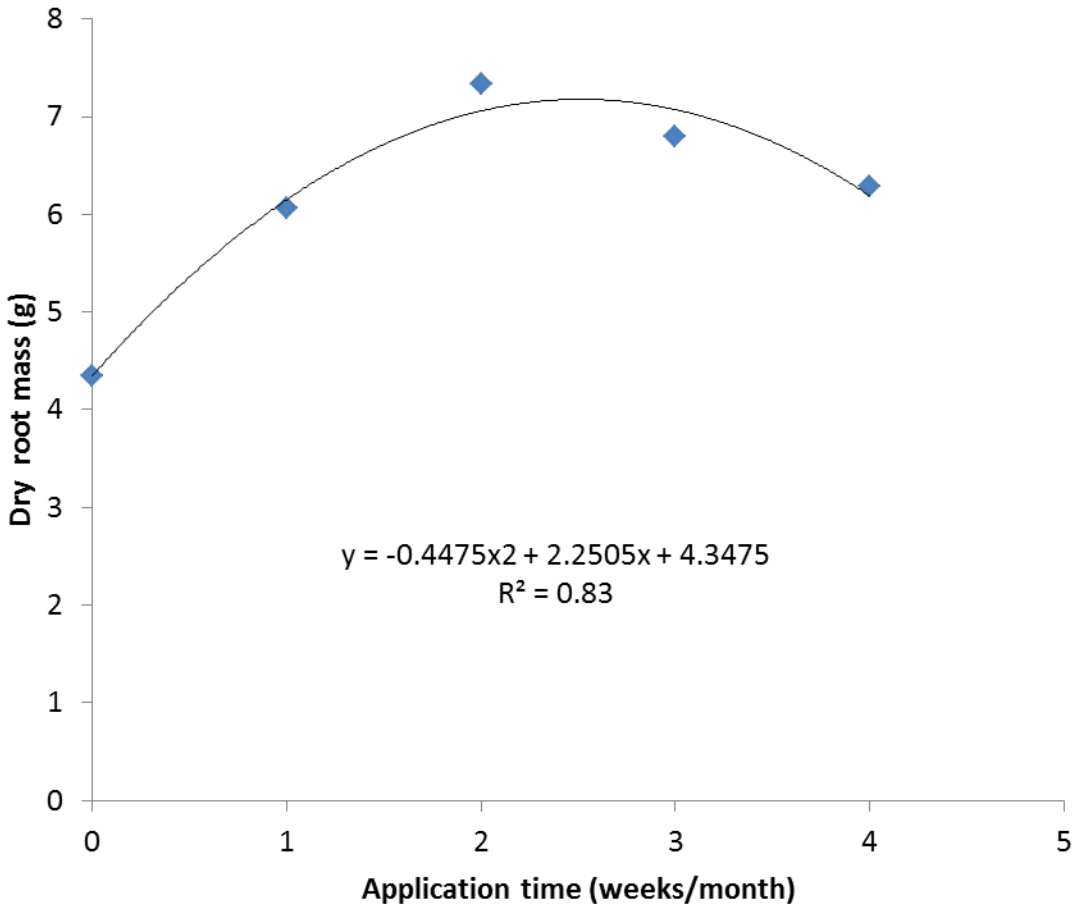


Figure 5.4 Influence of application time of 3% concentration from fermented crude extracts of *Cucumis africanus* fruit on dry root mass of tomato plant 'Floradade' for 56 days under microplot conditions (n = 60).

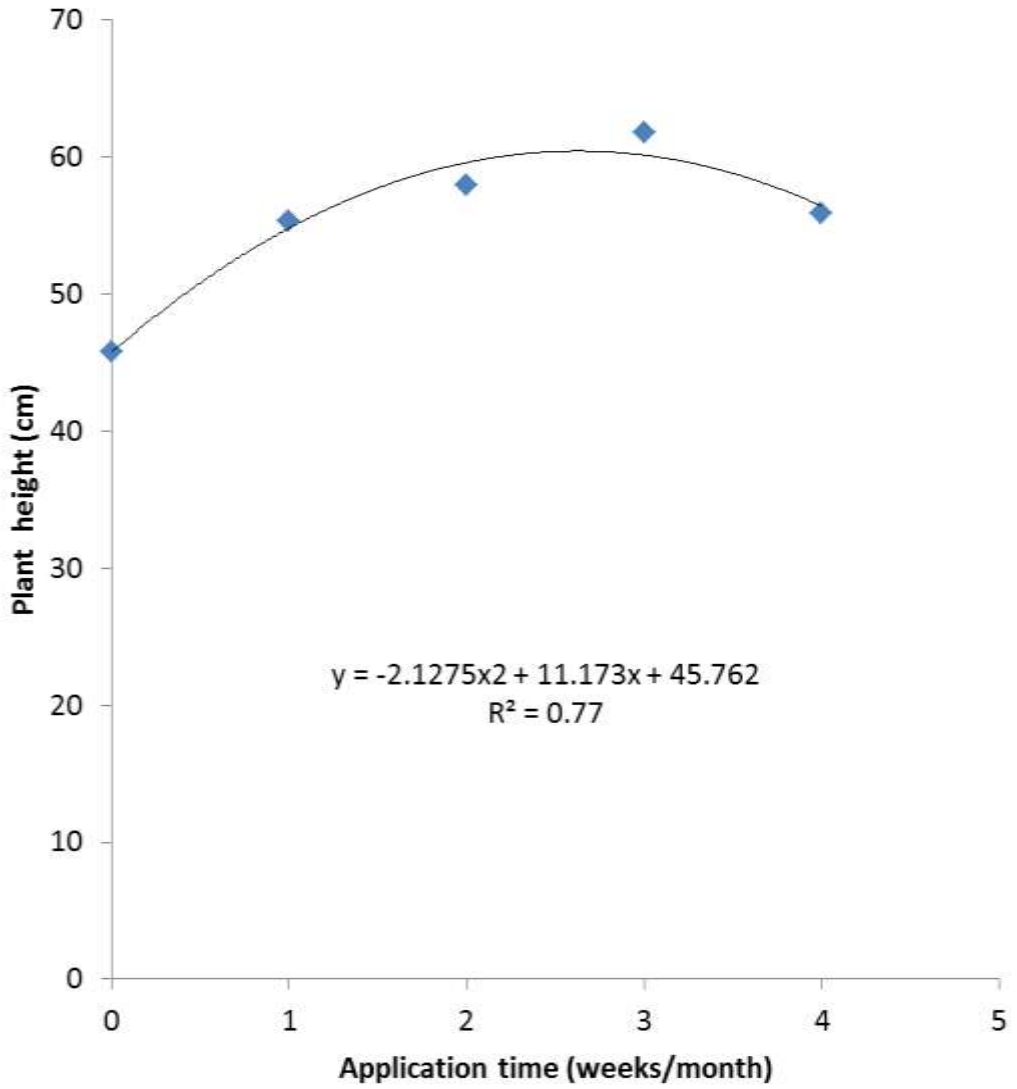


Figure 5.5 Influence of application time of 3% concentration from fermented crude extracts of *Cucumis africanus* fruit on plant height of tomato plant 'Floradade' for 56 days under microplot conditions (n = 60).

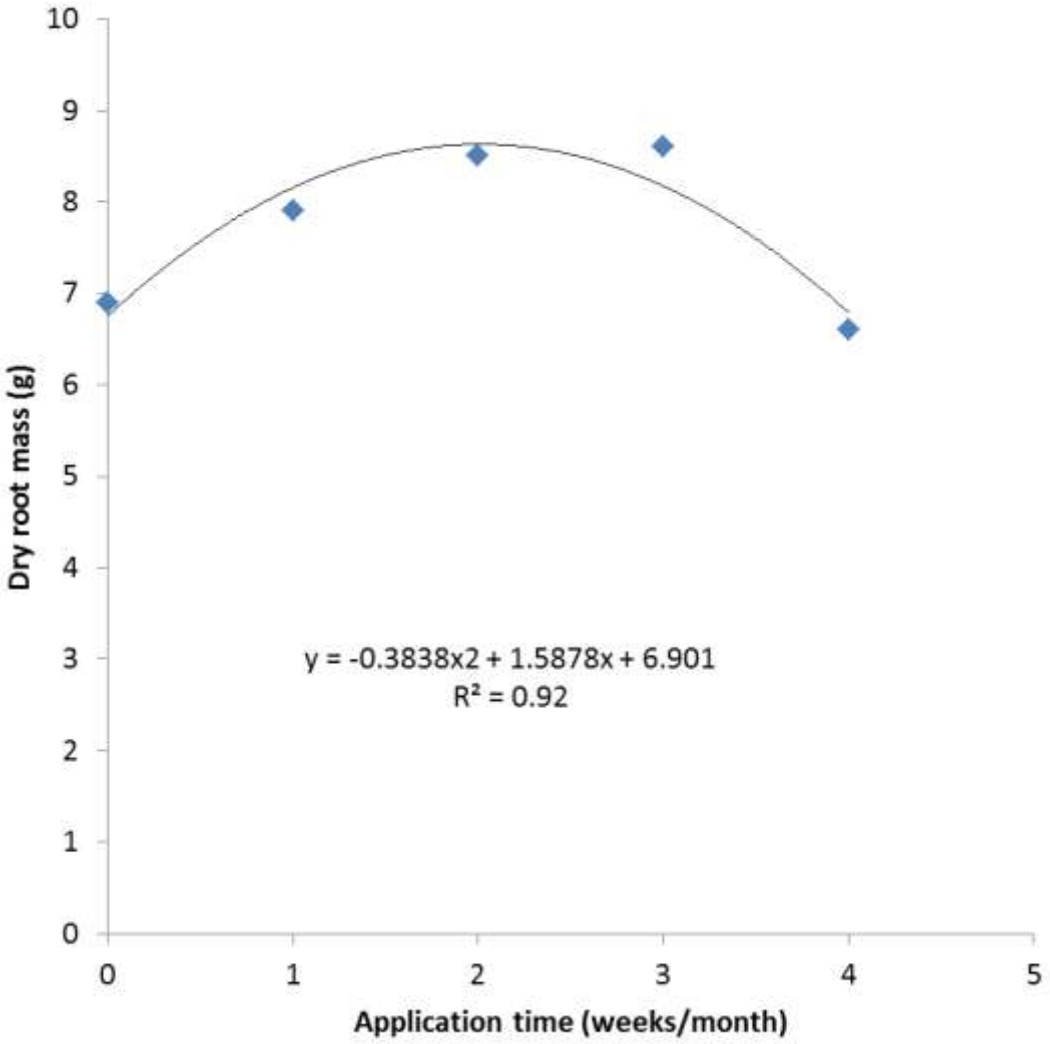


Figure 5.6 Influence of application time of 3% concentration from fermented crude extracts of *Cucumis myriocarpus* fruit on dry root mass of tomato plant 'Floradade' for 56 days under microplot conditions (n = 60).

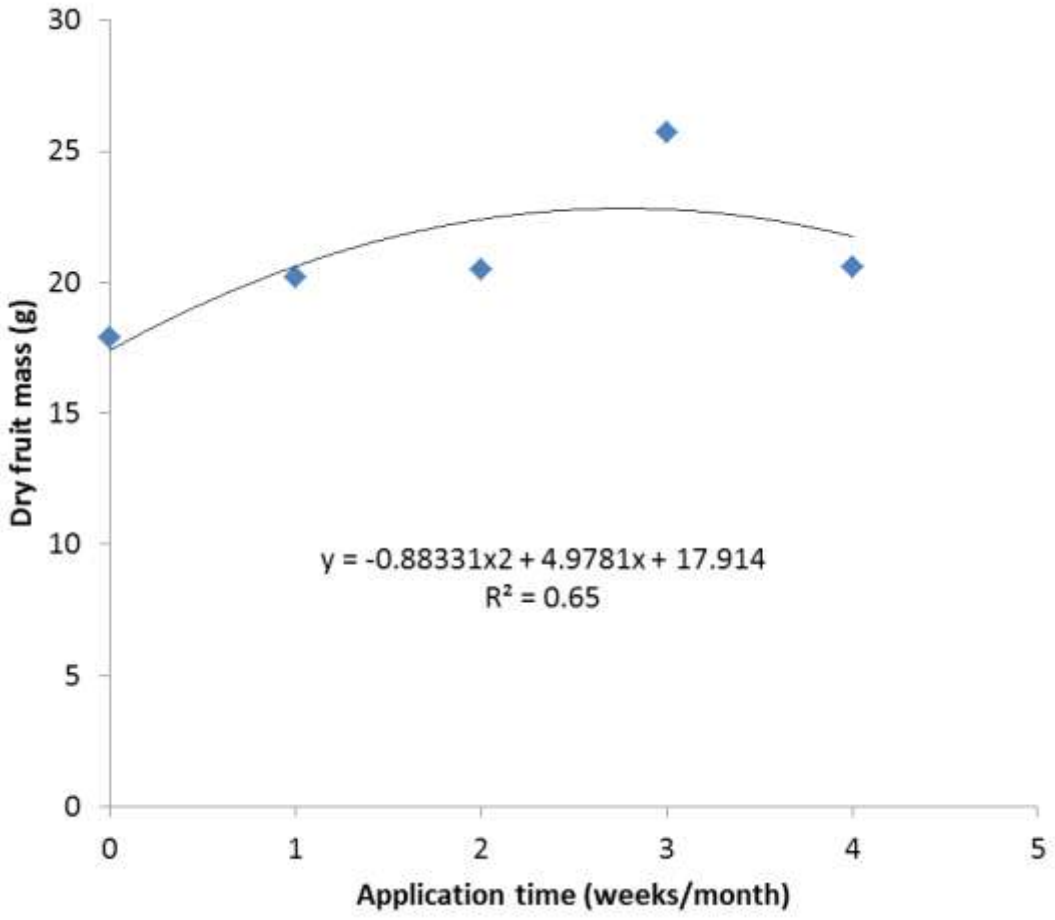


Figure 5.7 Influence of application time of 3% concentration from fermented crude extracts of *Cucumis myriocarpus* fruit on dry fruit mass of tomato plant 'Floradade' for 56 days under microplot conditions (n = 60).

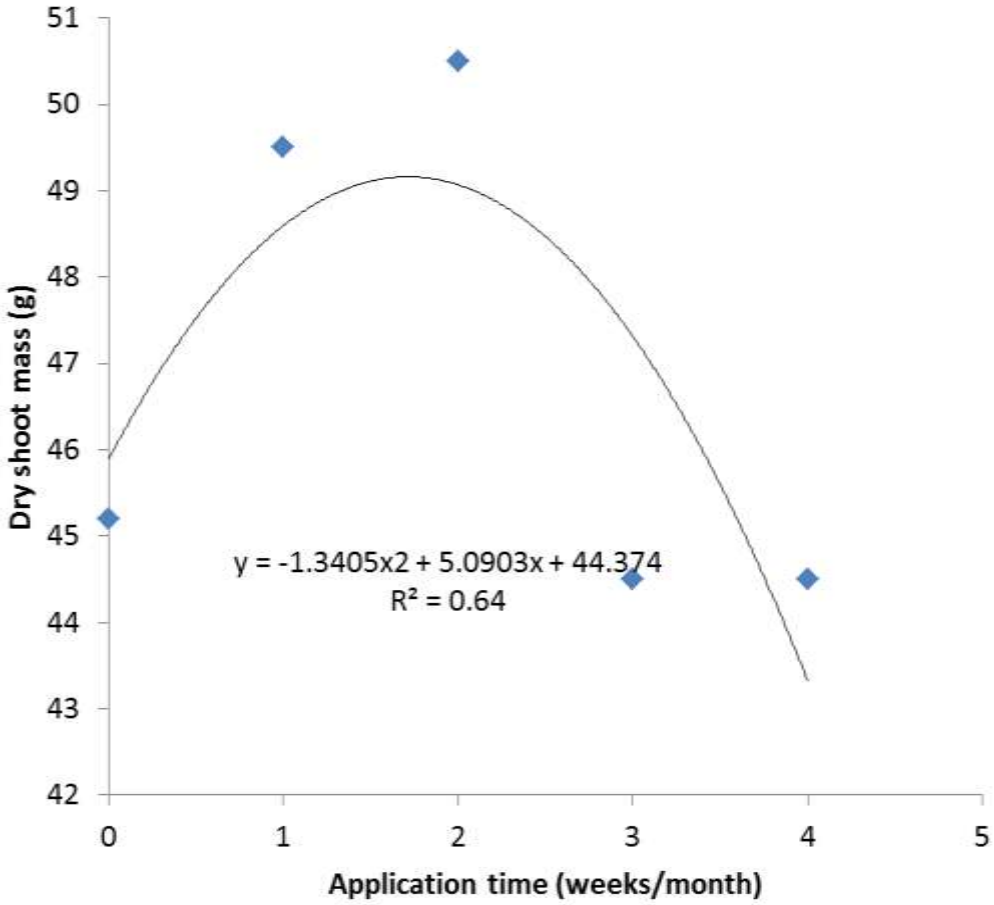


Figure 5.8 Influence of application time of 3% concentration from fermented crude extracts of *Cucumis myriocarpus* fruit on dry shoot mass of tomato plant 'Floradade' for 56 days under microplot conditions (n = 60).

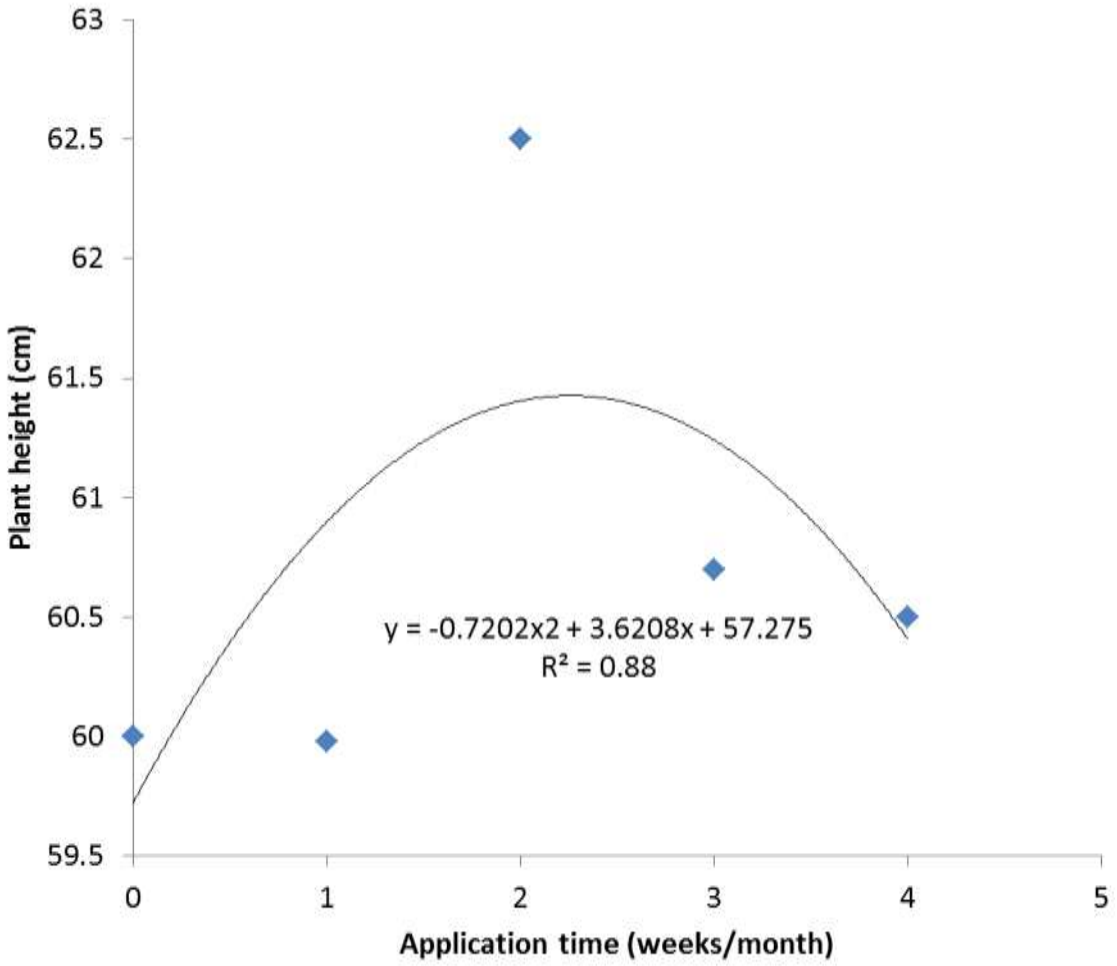


Figure 5.9 Influence of application time of 3% concentration from fermented crude extracts of *Cucumis myriocarpus* fruit on plant height of tomato plant 'Floradade' for 56 days under microplot conditions (n = 60).

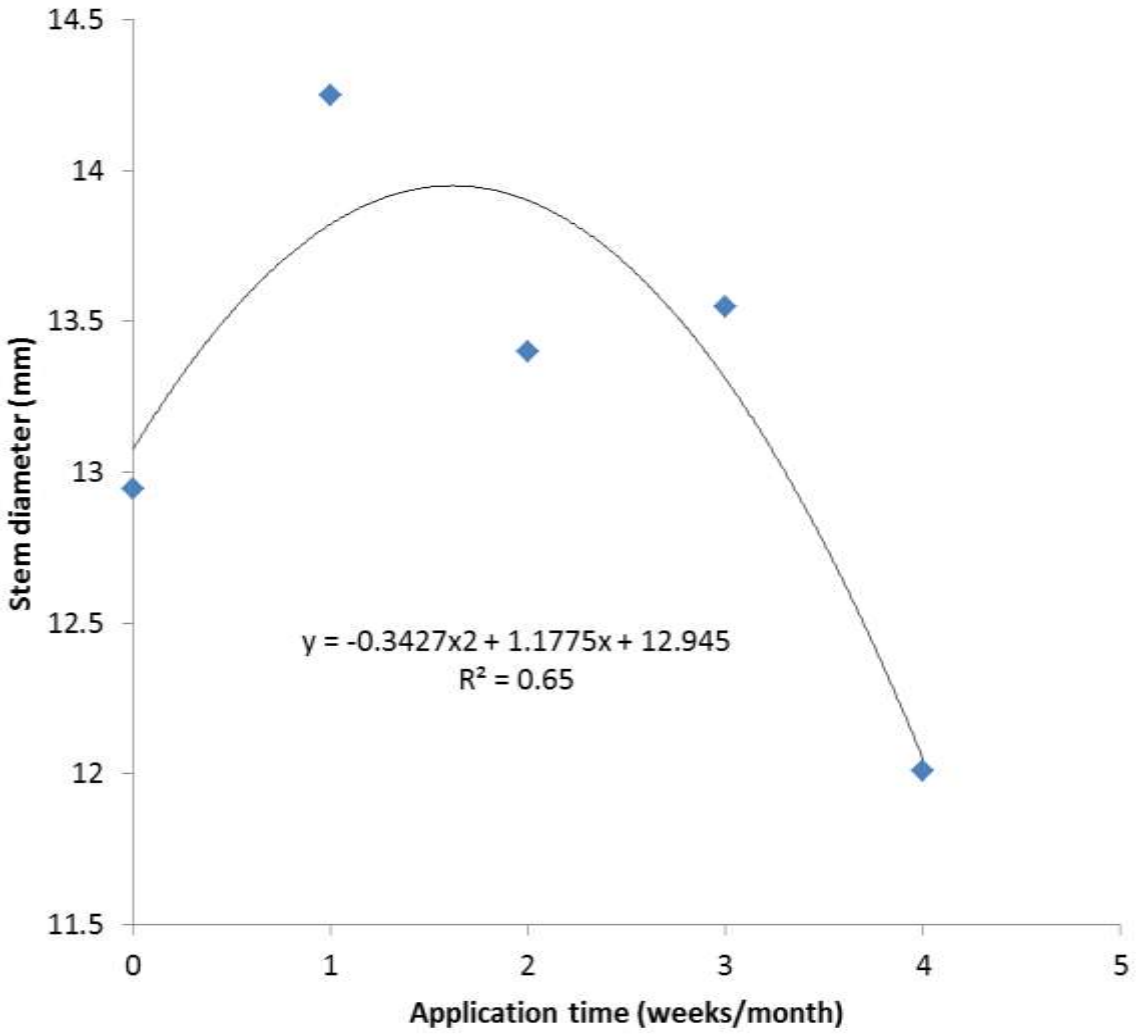


Figure 5.10 Influence of application time of 3% concentration from fermented crude extracts of *Cucumis myriocarpus* fruit on stem diameter of tomato plant 'Floradade' for 56 days under microplot conditions (n = 60).

5.3.2 Application time interval at 6% concentration

Density-dependent growth patterns, as shown by quadratic curves, were observed as the botanical nematicides were being applied at 6% over increasing application frequencies (Figures 5.11-5.14). At lower and higher application time intervals, the material stimulated and inhibited growth of tomato plants for both *Cucumis* species, respectively. The models accounted for 97%, 88% and 79% in total treatment variation of dry fruit mass, dry shoot mass and plant height for *C. africanus* and 98%, 99% and 89% in *C. myriocarpus* for dry fruit mass, dry shoot mass and fruit number, respectively. Various plant organs were optimised at different time intervals (Table 5.3), with integrated mean time interval being 2.614 weeks/30-day month period for *C. africanus*, which translated to 20-day (2.614/4 × 30) application time interval, while in *C. myriocarpus*, the integrated mean time being at 2.139 weeks/30-day month period translated to 16-day (2.139/4 x 30) application time interval.

The sum of squares suggested that 6% concentration accounted for 57%, 26% and 58% in total treatment variation of Pf in roots, soil and root + soil in *C. africanus* and 72%, 10% and 64% in *C. myriocarpus* for Pf in roots, soil and root + soil (data not shown). Nematode suppression was proportional to application time interval as the material suppressed more nematodes at higher application frequencies with a weekly application of 6% concentration of *C. africanus* and *C. myriocarpus* fruit-FPE reducing nematode numbers in root, soil and root + soil by 98%, 92% and 97% in *C. africanus* and 98%, 90% and 96% in *C. myriocarpus* (Table 5.4).

Table 5.3 Quadratic relationship, coefficient of determination and computed optimum application time of 6% concentration from fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits on dry fruit mass (DFM), dry root mass (DRM), dry shoot mass (DSM), fruit number (FNB) and plant height (PHT) of tomato plant 'Floradade' for 56 days under microplot conditions (n = 60).

| *Cucumis africanus* | | | | *Cucumis myriocarpus* | | | |
|---|---|---|---|---|---|---|---|
| **Variable** | **Formula** | **$R^2$** | **$x^z$** | **Variable** | **Formula** | **$R^2$** | **$x^z$** |
| DFM (g) | $y = -1.2062x^2 + 5.5856x + 15.527$ | 0.97 | 2.315 | DFM (g) | $y = -0.3438x^2 + 0.5831x + 21.564$ | 0.98 | 0.848 |
| DRM (g) | $y = -1.5957x^2 + 7.0304x + 37.85$ | 0.88 | 2.203 | DSM (g) | $y = -1.1042x^2 + 3.9914x + 41.762$ | 0.99 | 1.807 |
| PHT (cm) | $y = -0.7293x^2 + 4.8496x + 66.138$ | 0.79 | 3.325 | FNB | $y = -0.1875x^2 + 1.4125x + 10.02$ | 0.89 | 3.767 |
| | Mean integrated application time interval | | 2.614 | | Mean integrated application time interval | | 2.139 |

[z]Calculated optimum application time (x) = $-b_1/2b_2$, where $-b_1 = 5.5856$ and $b_2 = -1.2062$ for dry fruit mass.

Table 5.4 Influence of application time of 6% concentration from fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits on final population density of *Meloidogyne incognita* on the roots, soil and final population numbers (roots and soil) on tomato plant 'Floradade' for 56 days under microplot conditions (n = 60).

| | *Cucumis africanus* | | | | | | *Cucumis myriocarpus* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nematode root | | Nematode soil | | Total nematode | | Nematode root | | Nematode soil | | Total nematode | |
| Time (weeks/month) | Variable | Impact (%) | Variable | Impact (%) | Variable | Impact (%) | Variable | Impact (%) | Variable | Impact (%) | Variable | Impact (%) |
| 0 | 22 551a | - | 7 375a | - | 29 926a | - | 22 511a | - | 5 208a | - | 27 719a | - |
| 1 | 4 498b | -80 | 4 583ab | -38 | 9 081b | -70 | 4 966b | -78 | 1 667b | -68 | 6 633b | -76 |
| 2 | 2 031b | -91 | 4 167ab | -44 | 6 198bc | -79 | 1 663c | -93 | 2 500ab | -52 | 4 163bc | -85 |
| 3 | 843b | -96 | 1 833bc | -75 | 2 676bc | -91 | 850cd | -96 | 1 583ab | -70 | 2 433c | -91 |
| 4 | 389b | -98 | 583c | -92 | 972c | -97 | 549d | -98 | 500b | -90 | 1 049d | -96 |

[y]Means followed by the same letter are not different (P ≤ 0.05) according to Fisher's least significant difference test. [z]Impact (%) = [(treatment/control) ─ 1) × 100.

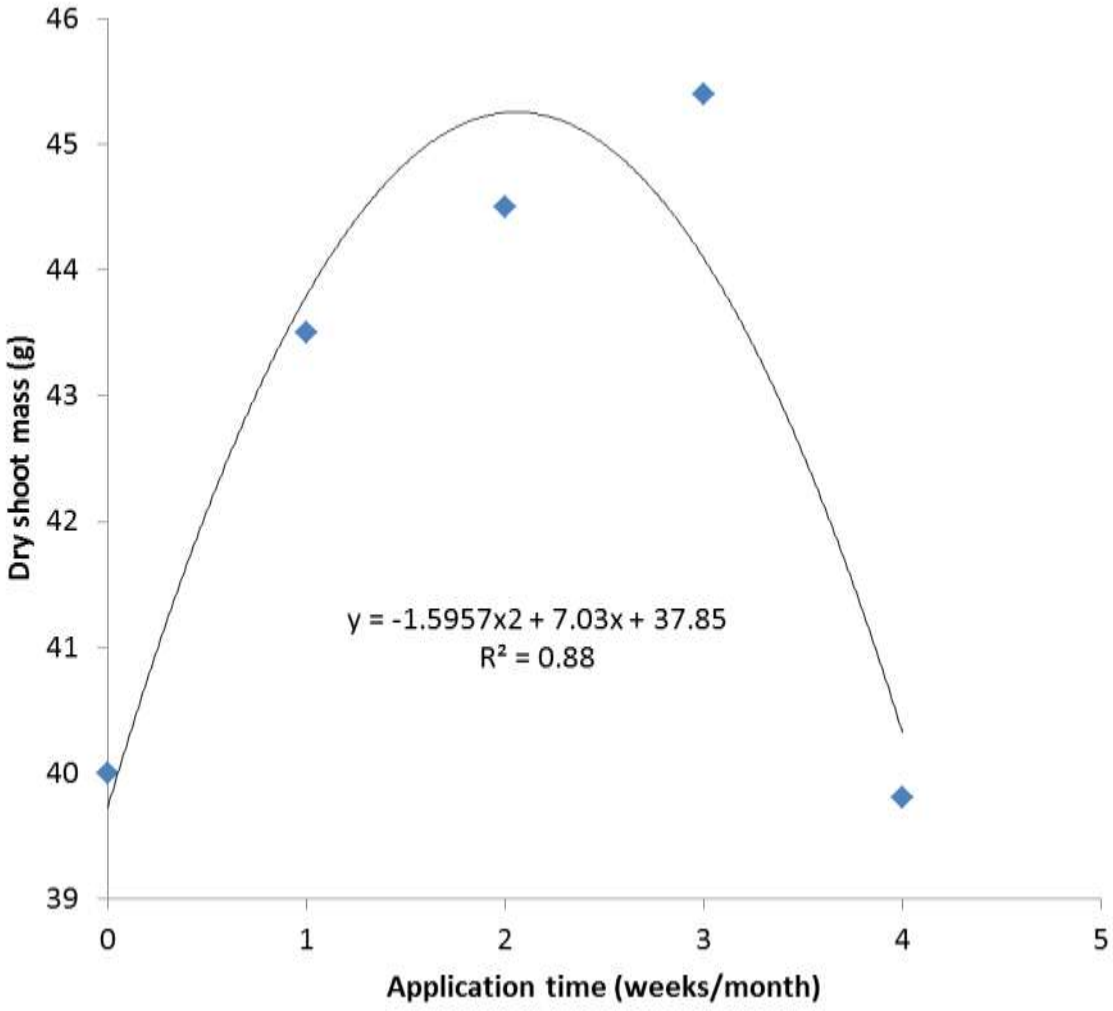


Figure 5.11 Influence of application time of 6% concentration from fermented crude extracts of *Cucumis africanus* fruit on dry shoot mass of tomato plant 'Floradade' for 56 days under microplot conditions (n = 60).

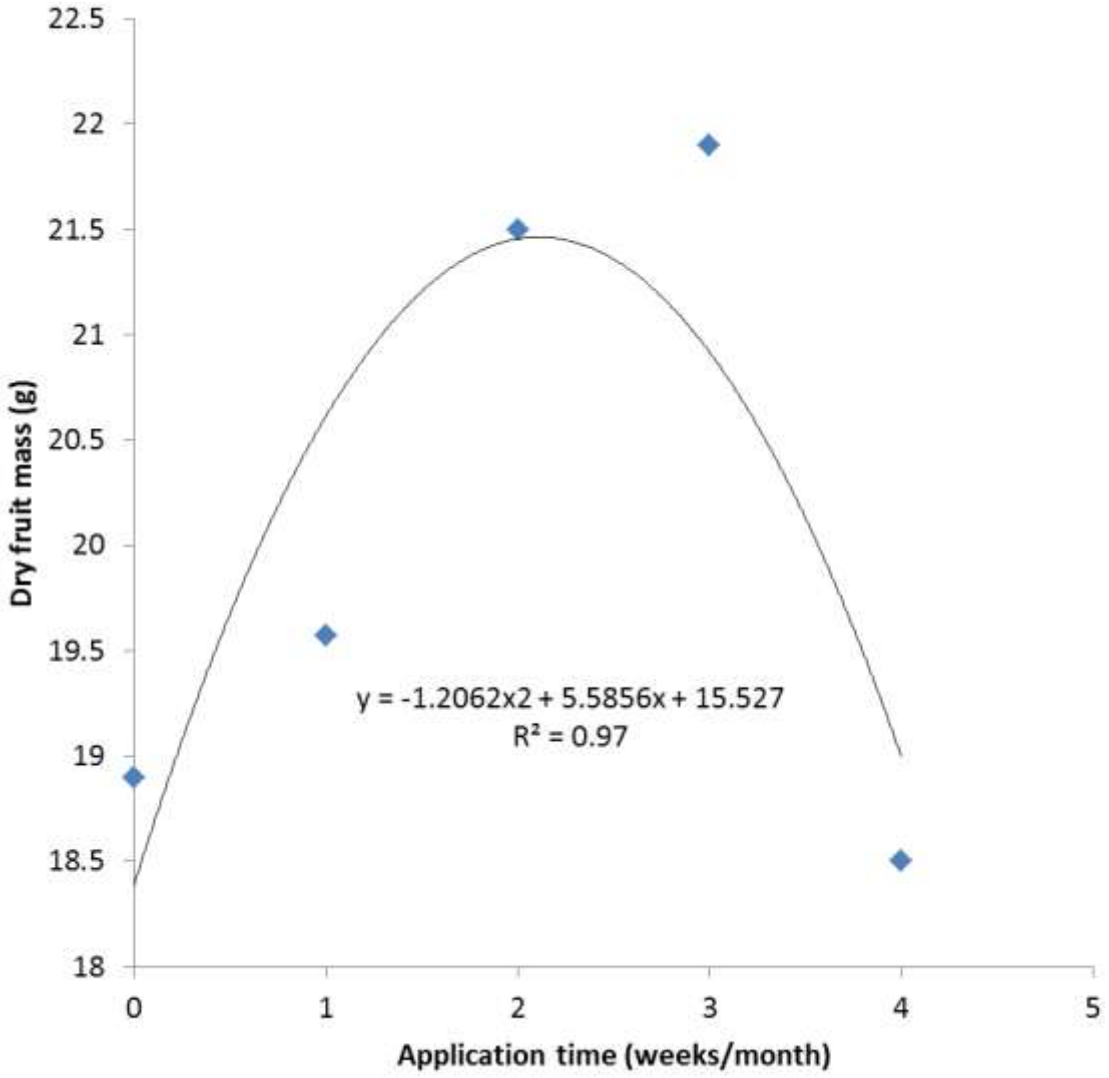


Figure 5.12 Influence of application time of 6% concentration from fermented crude extracts of *Cucumis africanus* fruit on dry fruit mass of tomato plant 'Floradade' for 56 days under microplot conditions (n = 60).

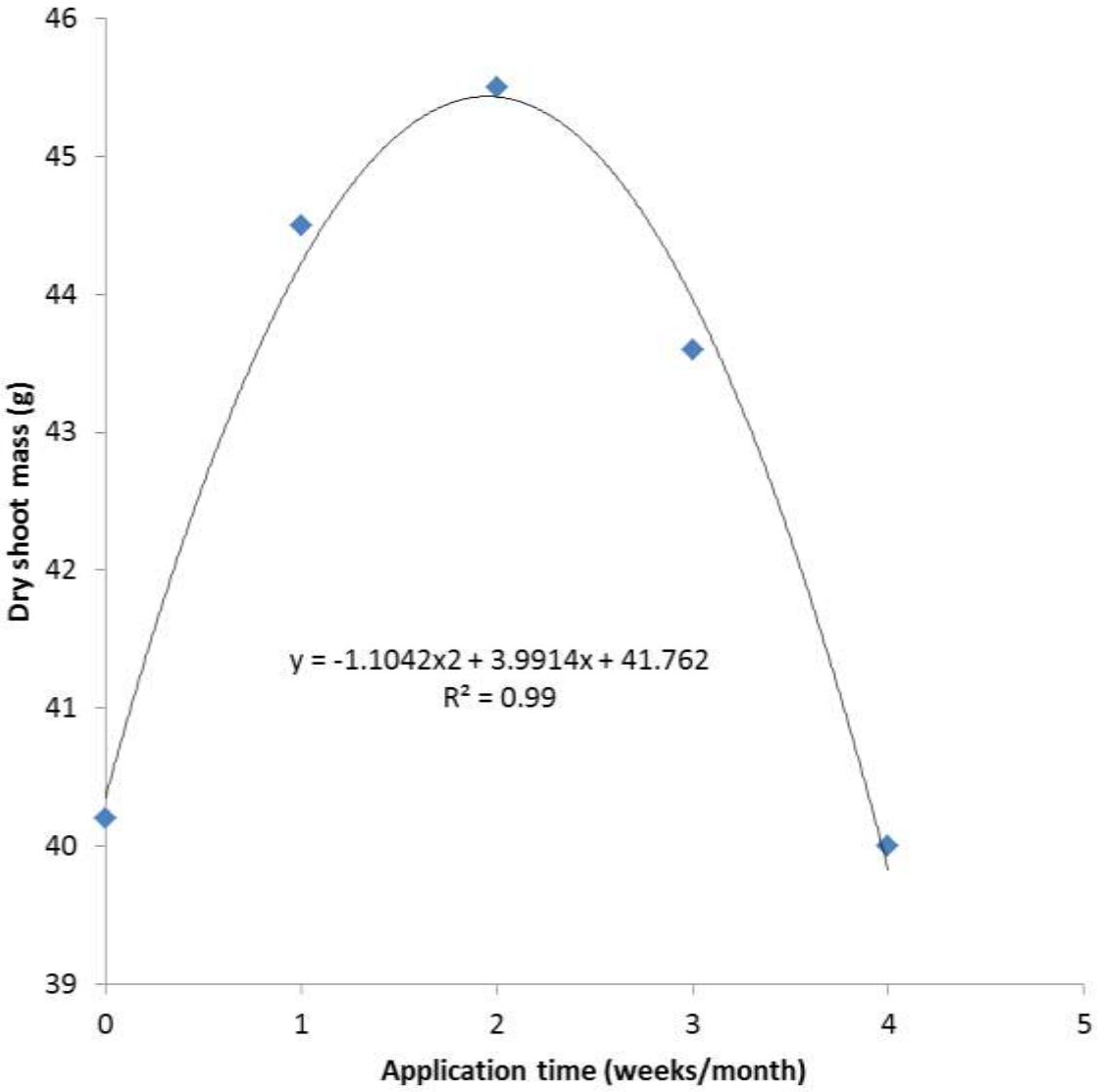


Figure 5.13 Influence of application time of 6% concentration from fermented crude extracts of *Cucumis myriocarpus* fruit on dry shoot mass of tomato plant 'Floradade' for 56 days under microplot conditions (n = 60).

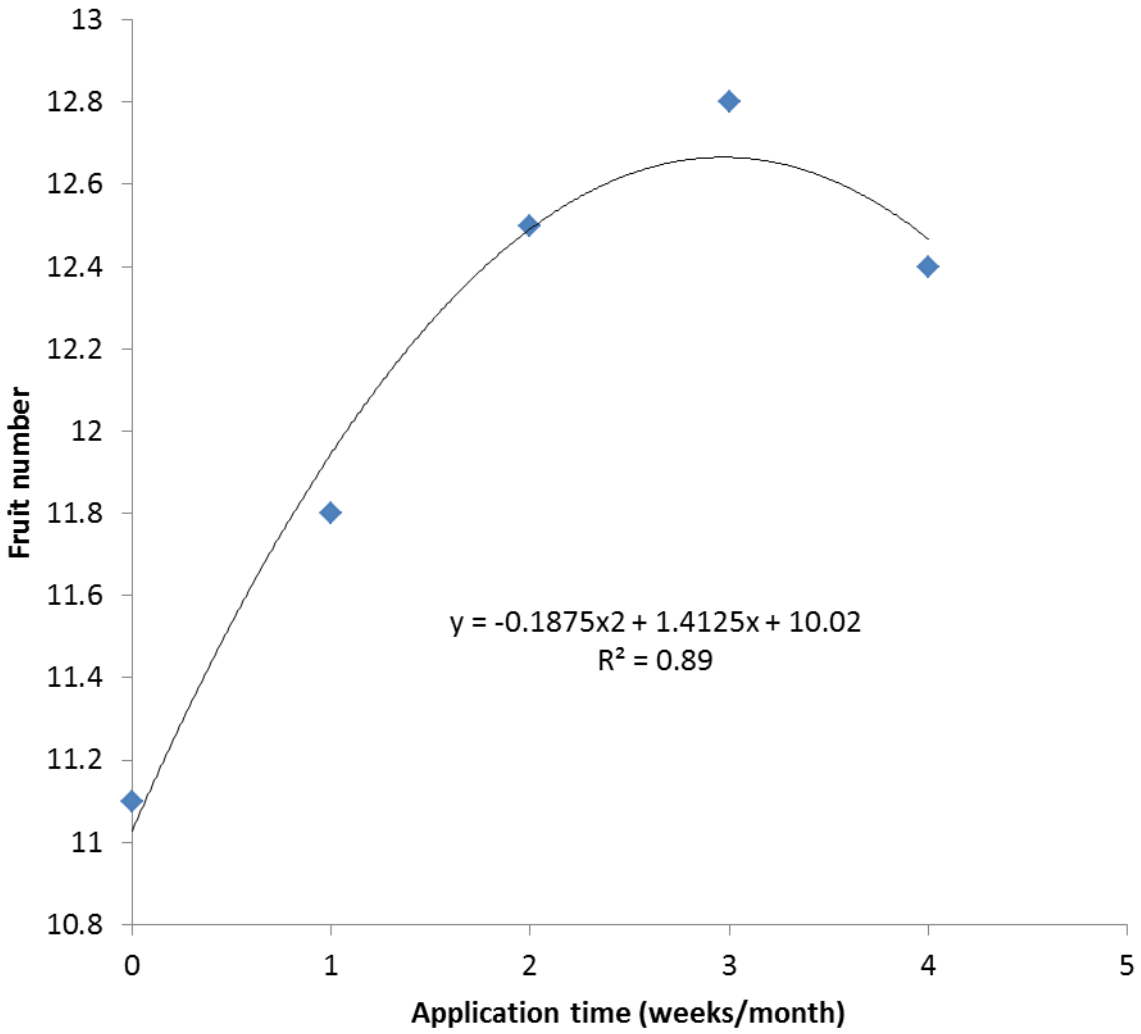


Figure 5.14 Influence of application time of 6% concentration from fermented crude extracts of *Cucumis myriocarpus* fruit on fruit number on tomato plant 'Floradade' for 56 days under microplot conditions (n = 60).

## 5.4 Discussion

The 18-day and 20-day intervals for fermented crude extracts of dried fruits from *C. africanus* at 3% and 6% concentrations, respectively, as well as the 16-day interval for 3% and 6% concentrations of crude extracts from *C. myriocarpus* fruit, form an integral part in research and development of bio-nematicides from the two plant species. Apparently, this is the first report where application time interval of fermented plant extracts was empirically quantified. In other plant species, there was actually no empirically-based evidence why the materials were being applied at certain frequencies. For instance, for nematode management fermented crude extracts of fresh leaves from lantana (*Lantana camara*) were applied weekly in tomato production (Taurayi, 2011; Nzanza *et al*., 2013). Similarly, fermented plant extracts of neem (*Azadirachta indica* A. Juss.) leaf and wild garlic (*Tulbaghia violacea*) in management of whitefly and aphid population densities were applied weekly in tomato production under open field conditions (Nzanza and Mashela, 2012).

Most of the time, the application time interval is based on whether at the applied interval and concentration the material is suppressive to nematode numbers, while phytotoxicity is secondary (Agbenin *et al*., 2005). In plants with nematicidal properties, the efficacy of plant extracts is a function of the concentration and the exposure period of nematodes to the extracts (Kali and Gupta, 1980). In this study, especially under crude extracts of *C. africanus* fruit, there was tendency of the concentration to interact with the application time interval to reduce the dry root mass, while this effect was not observed in *C. myriocarpus*. Additional studies under various conditions would be necessary to

substantiate this observation. In most botanicals, the materials are applied once in large quantities or weekly in small concentrations (Higa and Wididana, 1991; Nzanza *et al.*, 2013; Sivakumar and Gunasekaran, 2011), with phytotoxicity being a limiting factor.

In this study, the 18-20 day and 16 day application time intervals for *C. africanus* and *C. myriocarpus*, respectively, would probably minimise phytotoxicity since they were determined on the basis of crop productivity rather than the degree of nematode suppression. The latter was due to the fact that the products used in this study from two *Cucumis* species consistently suppressed population densities of *Meloidogyne* species at high percentages (Chapters 3, 4). Primarily, at 18-20 day and 16 day application time intervals for *C. africanus* and *C. myriocarpus*, respectively, at the two empirically-derived concentrations (3%, 6%), phytotoxicity would be avoided while nematode numbers would be suppressed. Since, the former is initiated at adjusted 0% inhibition ($D_0$) concentrations 48% and 41% for the two respective plant species as expounded previously for 3% concentration (Chapter 4).

Generally, the short application time intervals in fermented crude extracts from plant organs could be explained in terms of the small quantities used, along with the limited lifespans of these materials in the soil. In contrast, long application time intervals of synthetic fumigant nematicides were primarily due to the degree of the toxicities of the active ingredients, large dosages (500-2000 kg/ha) and their long lifespans. Some active residues lasted in the soil from no less than 2 to 8 years (Van Gundy and McKenry, 1975). Consequently, most fumigant nematicides, with their high

phytotoxicities and nematicidal properties, were, in most cases, applied once as pre-plant materials. In comparison to botanicals, dosages of non-fumigant nematicides are relatively small (10-15 kg/ha), are not phytotoxic and last in the plants or the soil for no longer than 30-60 days (Van Gundy and McKenry, 1975), which explains their relatively short application frequencies.

The proposed application time intervals of 16-20 days for the two test products are suitable for interrupting the life cycle of *M. incognita*, which varies from 19 to 43 days, depending on prevailing soil temperature. Generally, at high soil temperatures (30.6$^{o}$C) the life cycle of this nematode is completed in 19 days, while at low soil temperature (21.8$^{o}$C) it is completed in up to 43 days (Sikora and Fernandez, 2005). Naturally, after egress, J2s of this nematode species migrate into the soil for probing and invading newly developed roots at the elongation region (Ferraz and Brown, 2002; Wyss *et al*., 1992). During migration, active ingredients from the products come into contact with the juveniles and limit their chemotaxis and mobility (Wuyts *et al*., 2006). However, most other botanical nematicides were shown to have the capability to permeate through the egg mass and incapacitate the first-stage juveniles (J1s) prior to egress (Hirschmann, 1985). In such cases, the stylets of the exposed J1s are incapacitated so that they fail to pierce through the egg-shell, resulting in failure to hatch (Hirschmann, 1985; Parmar, 1987). Apparently, due to the presence of various active ingredients in botanicals, not a single chemical can be attributed to a decrease in population densities of plant-parasitic nematodes (Haseeb *et al*., 1980; Mukherjee and Sukul, 1978; Toida and Moriyama, 1978; Wuyts *et al.*, 2006). Crude extracts of *C. myriocarpus* fruit were shown to result in

high juvenile mortalities of *M. incognita* race 2 and the citrus nematode (*Tylenchulus semipenetrans* Cobb, 1913) at $LC_{50}$ of 7 µg/mL distilled water (Muedi, 2005). Due to the multiplicity of biological activities of botanicals, the 16 days and 18-20 days for applying concentrations of fermented crude extracts from the two *Cucumis* species would sufficiently be suitable to reduce population of *Meloidogyne* species in tomato production.

The unique survival strategies of nematodes, particularly during J1s and J2s, suggest that, as was the case with synthetic nematicides (Mashela, 2007), it would not be possible to wipe-out plant-parasitic nematodes from plant production systems. It had been established that when J1s and J2s are gradually being exposed to adversarial environmental conditions like those of non-fumigant nematicides and botanicals, J1s and J2s enter the dauer and cryptobiotic stages, respectively (Mashela, 2007; Wuyts *et al.*, 2006). During these stages, nematode juveniles have minimum metabolic activities and can survive extended periods of exposure, but when the stress is ameliorated, the juveniles exit the survival stages and assume normal activities. Using a total of 38 chemical compounds from botanicals, Wuyts *et al*. (2006) demonstrated that nematode responses such as egress, motility and mortality, were a function of the chemical compound, the concentration, the exposure time and the nematode species as expounded by the curve-fitting allelochemical response dosage model (Liu *et al*., 2003) and the density-dependent growth patterns (Salisbury and Ross, 1992).

High efficacies of most synthetic fumigants, along with their extended application time intervals, should also be viewed from the fact that the materials were biocidal, which implied that they indiscriminately killed all forms of life in soil, while non-fumigants were nemastatic (Van Gundy and McKenry, 1975). The latter means that synthetic non-fumigant nematicides did not kill nematodes, but modified behaviours such as failure to detect chemical cues required in nematode infection of new roots, disruption of development and/or restriction of reproduction capabilities, as observed for botanicals. Incidentally, all nemastatic properties appear to have the same end result of slowing down increases in final nematode population densities, and therefore initial population densities for subsequent crops, which is all that matters in plant protection (Seinhorst, 1967).

## 5.5 Conclusions

The optimum application time intervals of fermented crude extracts from *C. africanus* and *C. myriocarpus* fruits were at 18-20 and 16 days, respectively. At these intervals, the materials would be able to disrupt the life cycle of *M. incognita* race 2 in tomato production, without reducing growth of tomato plants.

# CHAPTER 6
# INFLUENCE OF DOSAGE OF DRIED FERMENTED FRUITS FROM INDIGENOUS *CUCUMIS* SPECIES ON SUPPRESSION OF *MELOIDOGYNE* SPECIES, GROWTH AND ACCUMULATION OF NUTRIENT ELEMENTS IN TOMATO UNDER FIELD CONDITIONS

## 6.1 Introduction

Hithertofore (Chapters 3, 4 and 5), the focus was on the development of optimum concentrations and application time intervals of fermented crude extracts from wild watermelon (*Cucumis africanus* L.) and wild cucumber (*Cucumis myriocarpus* Naude.) fruits. In (Chapter 5) fermented crude extracts from fruits of *C. africanus* and *C. myriocarpus* each had concentrations of 3% and 6%, which consistently suppressed population densities of root-knot (*Meloidogyne incognita)* nematodes under greenhouse and microplot conditions. Efficacies of the concentrations from *C. africanus* and *C. myriocarpus* fruits were optimised at different application time intervals, namely, 18-20 days and 16 days, respectively (Chapter 5).

Van Gundy and McKenry (1975) indicated that concentration and application interval are associated to each other through the concept of dosage, where dosage = concentration x application time. The efficacy of dosages of the two products from empirically-derived concentrations and application frequencies is not documented. The objective of this study, therefore, was to compare the efficacy of dosages of individual products in suppression of population densities of *M*. *incognita,* growth of tomato plants and accumulation of nutrient elements in tomato leaves under field conditions.

## 6.2 Materials and methods

### 6.2.1 Location of study and preparation

Separate experiments for *C. africanus* and *C. myriocarpus* were conducted concurrently on an open field system at the Plant Protection Skills Centre, University of Limpopo, South Africa (23°53''10'S, 29°44''15'E) (Figure 6.1). The location has summer rainfall with mean annual rainfall of 600 mm, while maximum/minimum temperatures average 28°C/19°C. Trials were conducted in summer (October-December) 2011, but were not repeated in space or time since they were factorial experiments (Gomez and Gomez, 1984). The site contained Hutton soil (65% sand, 30% clay, 5% silt; 1.6% organic C, $EC_e$ 0.148 dS/m and pH ($H_2O$) 6.5).

A plot comprised 20 $m^2$ with 20-cm-deep holes dug in the centre of 1 $m^2$ subplot in order to allow for 1.0 m intra-row and 1.0 m inter-row spacing. Sources and preparation of *Cucumis* fruits and nematode inoculum, along with transplanting, were as described previously (Chapter 4). Prior to transplanting, soil samples were collected for the determination of initial population density (Pi), extracted and counted as described previously (Chapter 4), with mean Pi = 3 700 second-stage juveniles/250 ml soil. Uniform four-week-old nematode-free tomato cv. 'Floradade' seedlings were transplanted in 500 ml chlorine-free tapwater. *Cucumis africanus* and *C. myriocarpus* fruits were obtained and then dried and later prepared as fermented crude extracts as described previously (Chapter 4).

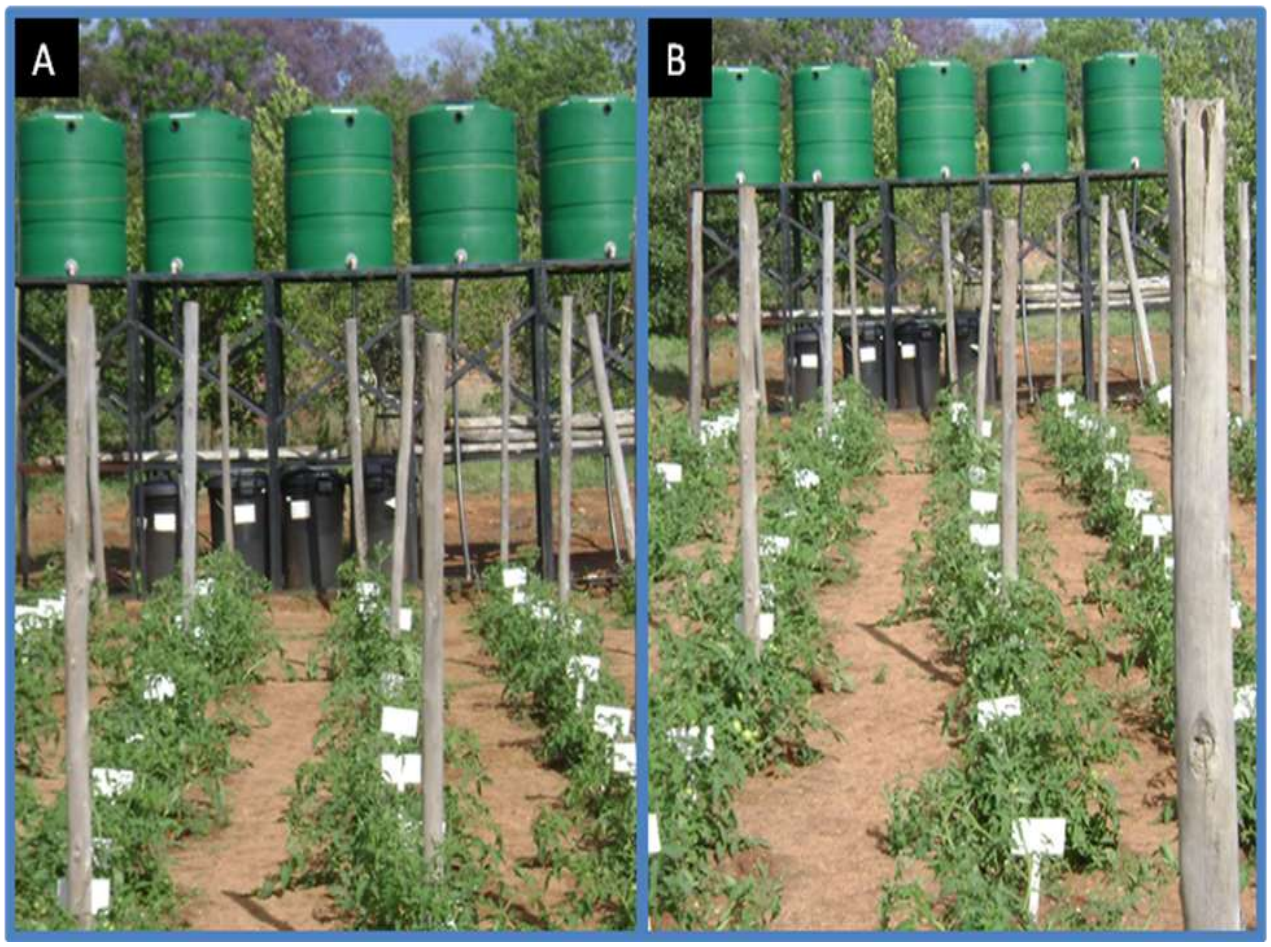


Figure 6.1 Open field experiments using concentrations from fermented crude extracts of dried fruits of **(A)** *Cucumis africanus* and **(B)** *Cucumis myriocarpus*.

### 6.2.2 Experimental design and cultural practices

Two separate experiments, one for *C. africanus* and the other for *C. myriocarpus*, were conducted concurrently. Each experiment was designed as a 5 × 4 factorial treatment, arranged in a randomised complete block with six replications. The first factor comprised concentration levels of fermented crude extracts from each plant species at 0, 2, 4, 6 and 8%, while the second was application time intervals at 1, 2, 3 and 4 weeks per 30-day month period. Weeks per 30-day month period were devised to allow for

converting weeks in application time interval into days. All treatments were initiated at transplanting. Fertilisation with 3 g 2:3:2 (22) and 2 g 2:1:2 (43) was achieved as described previously (Chapter 4). Irrigation was achieved through a drip irrigation system, with 2 h and 1 h in the morning and in the afternoon, respectively, on days when 50% moisture meter readings were below 2 units (Chapter 3). At irrigation interval, irrigation was substituted for by 1000 ml of appropriate concentrations through manual application of treatments. Each drip discharged 1 L per hour. In a 3% concentration and 6% concentration a total of 120 L and 240 L of undiluted material/ha of 4 000 tomato plants would be required for both *Cucumis* species in open field application.

### 6.2.3 Data collection

At 56 days after initiating the treatment, plant variables were collected and nematodes extracted from 5 g roots and 250 ml soil as described previously (Chapter 3). Mature leaves were collected from *C. africanus* per plant, dried at 80$^{o}$C for 24 h and finely ground. Approximately 0.10 g dried materials were digested in 40 ml 4% nitric acid ($HNO_3$), followed by placing the container on a vortex to allow for complete wetting of the mixture. The materials were magnetically stirred, thereafter incubated in a 95$^{o}$C water-bath for 90 minutes, allowed to cool down at room temperature, filtered, decanted into 50 ml tubes which were covered with a foil and then selected nutrient elements (Ca, Mg, P, K, Mn, Na, S, Zn) analysed using the inductively coupled plasma optical emission spectrometry (ICPE-9000).

### 6.2.4 Data analysis

Data were subjected to analysis of variance (ANOVA) through the 2008 SAS software (SAS Institute, Inc., Cary, NC., USA). Nematode data were transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984), but untransformed means were reported. Sum of squares were partitioned to determine the contribution of sources of variation to the total treatment variation in plant and nematode variables (Gomez and Gomez, 1984). When interactions were not significant, treatment mean separation was achieved using Fisher's least significant test at the probability level of 5%. In the event interactions were not significant for a particular variable, for the main factor of concentrations where treatment effects were significant at 5% level of probability, means were subjected to the curve-fitting allelochemical response dosage model to generate related biological indices as described previously (Chapter 4). Unless otherwise stated, only treatments significant at the probability level of 5% are discussed (Appendices 6.1-6.14).

## 6.3 Results

The interaction had no influence on nematode population density in any of the three units used under both *Cucumis* species (Table 6.1). Under *C. africanus*, concentration was the only main factor that contributed 36% and 2%, respectively, to the total treatment variation on final nematode population density in roots and soil. Similarly, under *C. myriocarpus*, concentration was the only main factor that contributed 30% and 4% to total treatment variation in roots and soil, respectively. Relative to untreated control, concentrations of crude extracts from *C. africanus* reduced nematode numbers

in roots and soil from 86-90% and 4-53%, respectively (Table 6.2). Similarly, concentrations from *C. myriocarpus* crude extracts reduced the two variables by 75-80% and 26-68%, respectively.

Concentrations x application time interaction under fermented crude extracts of *C. africanus* fruit on dry root mass was significant, contributing 16% to the total treatment variation in this variable, while the interaction had no effect on dry fruit mass, dry shoot mass, fruit number, plant height and stem diameter (Table 6.3). In contrast, under *C. myriocarpus* fruit the interaction affected dry shoot mass and fruit number, contributing 19% and 18% to total treatment variation, respectively, without having any effect on dry fruit mass, dry shoot mass and plant height. Relative to mean 0% dosage, subsequent dosages of crude extracts of *C. africanus* fruit on dry root mass of tomato had 50% frequency of reducing this variable (Table 6.4). In contrast, under *C. myriocarpus* fruit, dosages had a tendency of increasing dry shoot mass and fruit numbers, with a 12% reduction frequency. Fruit mass versus increasing concentration of fermented crude extracts of *C. myriocarpus* had a quadratic relation (Table 6.5), with the model explaining 84% of the total treatment variation in fruit mass (Figure 2).

Concentration x time per 30-day month period interactions of fermented crude extracts of *C. africanus* dried fruit and application frequency was significant on foliar Ca, Mg, Mn, Na, P, S and Zn, contributing approximately 33%, 19%, 19%, 17%, 16% and 23% to the total treatment variation (TTV), respectively (Table 6.6). However, the interaction had no significant effect on foliar K (Table 6.6). The relative percentage reduction frequencies

of the interaction for Ca and Mg were 6% and 25%, respectively (Table 6.7), while for Mn, Na, S and Zn they were 19%, 94%, 69% and 12%, respectively (Table 6.8). With the exception of 4% concentration of *C. africanus* extracts, relative to the untreated control, concentrations increased foliar K by 32-81% (Table 6.9).

Table 6.1 Sum of squares for five concentration levels of fermented crude extracts of dried fruits of *Cucumis africanus* or *Cucumis myriocarpus* (concentration) and four levels of application frequency (time) and their interactions on nematode in root of tomato 'Floradade', and nematode in soil at 56 days after the treatment (n = 120).

| Source of variation | DF | *Cucumis africanus* | | | | *Cucumis myriocarpus* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $Nematode_{root}$ | | $Nematode_{soil}$ | | $Nematode_{root}$ | | $Nematode_{soil}$ | |
| | | SS | % | SS | % | SS | % | SS | % |
| Replication | 5 | 2.177 | $3^{ns}$ | 3.989 | $6^{ns}$ | 4.935 | $7^{ns}$ | 13.666 | $16^{ns}$ |
| Concentration | 4 | 27.499 | $36^{***}$ | 1.075 | $2^{ns}$ | 22.059 | $30^{***}$ | 3.720 | $4^{ns}$ |
| Time | 3 | 2.593 | $3^{ns}$ | 1.239 | $2^{ns}$ | 0.175 | $0^{ns}$ | 1.789 | $2^{ns}$ |
| C × T | 12 | 3.547 | $5^{ns}$ | 6.071 | $10^{ns}$ | 4.122 | $6^{ns}$ | 9.302 | $11^{ns}$ |
| Error | 95 | 40.428 | 53 | 49.661 | 80 | 43.357 | 58 | 54.523 | 66 |
| Total | 119 | 76.245 | 100 | 62.035 | 100 | 74.649 | 100 | 83.001 | 100 |

$^{ns}$Means that the factor (s) was not significant at P ≤ 0.05; while $^{**}$ and $^{***}$ mean that the factor (s) was significant at P ≤ 0.05 and P ≤ 0.01, respectively.

Table 6.2 Response of *Meloidogyne incognita* race 2 numbers in roots (5 g) and soil (250 ml) to concentrations of fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits under tomato production at 56 days from transplanting (n = 120).

| Concentration (%) | *Cucumis africanus* | | | | *Cucumis myriocarpus* | | | |
|---|---|---|---|---|---|---|---|---|
| | $Nematode_{root}$ | | $Nematode_{soil}$ | | $Nematode_{root}$ | | $Nematode_{soil}$ | |
| | Variable | % | Variable | % | Variable | % | Variable | % |
| 0 | 1 346a | - | 100a | - | 1 100a | - | 145a | - |
| 2 | 185b | -86 | 75a | -25 | 227b | -79 | 79a | -45 |
| 4 | 283b | -79 | 80a | -20 | 270b | -75 | 81a | -44 |
| 6 | 128b | -90 | 96a | -4 | 224b | -80 | 107a | -26 |
| 8 | 188b | -86 | 47a | -53 | 220b | -80 | 46a | -68 |

[ns]Means with the same letter were not statistically different according to Fisher's least significant difference test.

Relative effect (%) = [(Treatment/0% concentration) – 1)] × 100.

Table 6.3 Sum of squares for five concentration levels of fermented crude extracts of dried fruits of *Cucumis africanus* and *Cucumis myriocarpus* and four levels of application time interval and their interactions on dry fruit mass, dry root mass, dry shoot mass, fruit number, plant height and stem diameter of tomato 'Floradade' at 56 days after the treatment under field conditions (n = 120).

| Source of Variation | DF | Dry fruit mass | | Dry root mass | | Dry shoot mass | | Fruit number | | Plant height | | Stem diameter | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SS | % | SS | % | SS | % | SS | % | SS | % | SS | % |
| *Cucumis africanus* | | | | | | | | | | | | | |
| Replication | 5 | 1035.20 | $3^{ns}$ | 13.38 | $4^{ns}$ | 418.70 | $1^{ns}$ | 0.43 | $7^{ns}$ | 2613.69 | $26^{ns}$ | 3.83 | $2^{ns}$ |
| Concentration | 4 | 1064.80 | $3^{ns}$ | 3.83 | $1^{ns}$ | 387.10 | $1^{ns}$ | 0.12 | $2^{ns}$ | 387.99 | $4^{ns}$ | 5.33 | $3^{ns}$ |
| Time | 3 | 1638.50 | $5^{ns}$ | 9.12 | $3^{ns}$ | 606.40 | $2^{ns}$ | 0.10 | $2^{ns}$ | 259.04 | $3^{ns}$ | 10.95 | $5^{ns}$ |
| D × T | 12 | 2973.90 | $9^{ns}$ | 50.86 | $16^{**}$ | 2324.40 | $8^{ns}$ | 0.73 | $12^{ns}$ | 719.46 | $7^{ns}$ | 10.95 | $5^{ns}$ |
| Error | 95 | 27295.10 | 80 | 241.73 | 76 | 26417.00 | 88 | 4.80 | 78 | 5937.63 | 60 | 172.04 | 85 |
| Total | 119 | 34007.50 | 100 | 318.93 | 100 | 30153.70 | 100 | 6.19 | 100 | 9917.80 | 100 | 203.100 | 100 |
| *Cucumis myriocarpus* | | | | | | | | | | | | | |
| Replication | 5 | 486.30 | $1^{ns}$ | 2.93 | $1^{ns}$ | 2472.30 | $7^{ns}$ | 0.18 | $2^{ns}$ | 461.76 | $5^{ns}$ | 9.10 | $3^{ns}$ |
| Concentration | 4 | 3638.50 | $11^{**}$ | 2.84 | $1^{ns}$ | 2208.60 | $6^{**}$ | 0.59 | $8^{***}$ | 397.04 | $5^{ns}$ | 7.48 | $3^{ns}$ |
| Time | 3 | 1169.40 | $4^{ns}$ | 5.64 | $1^{ns}$ | 664.20 | $2^{ns}$ | 0.04 | $0^{ns}$ | 184.01 | $2^{ns}$ | 4.71 | $2^{ns}$ |
| D × T | 12 | 4079.70 | $12^{ns}$ | 24.10 | $6^{ns}$ | 6892.60 | $19^{***}$ | 1.40 | $18^{***}$ | 869.44 | $10^{ns}$ | 37.22 | $13^{ns}$ |
| Error | 95 | 23710.40 | 72 | 399.54 | 92 | 24268.70 | 66 | 5.54 | 72 | 6883.37 | 78 | 236.14 | 80 |
| Total | 119 | 33084.40 | 100 | 435.04 | 100 | 36506.50 | 100 | 7.74 | 100 | 8795.62 | 100 | 294.64 | 100 |

$^{ns}$Means that the factor(s) was not significant at $P \leq 0.05$; while $^{**}$ and $^{***}$ mean that the factor(s) was significant at $P \leq 0.10$, $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 6.4 Two-way table of first order interaction of concentrations from crude extracts of dried *Cucumis africanus* and *Cucumis myriocarpus* fruits and application interval (time) within a 30-day month period for dry root mass, dry shoot mass and fruit number (n = 120).

| Dosage | | | *C. africanus* | | *C. myriocarpus* | | | |
|---|---|---|---|---|---|---|---|---|
| Concentration | | Application | Dry root mass | | Dry shoot mass | | Number of fruit | |
| (%) | | interval[w] | Variable | %[z] | Variable | % | Variable | % |
| 0 | × | 1 | 4.10 | - | 43.28 | - | 0.79 | - |
| 0 | × | 2 | 3.72 | - | 50.42 | - | 1.00 | - |
| 0 | × | 3 | 6.05 | - | 52.68 | - | 1.00 | - |
| 0 | × | 4 | 5.50 | - | 57.53 | - | 1.14 | - |
| | | | $\overline{x}$= 4.84 | - | $\overline{x}$ = 50.98 | - | $\overline{x}$ = 0.98 | - |
| 2 | × | 1 | 4.55 | -6 | 63.08 | 24 | 1.22 | 24 |
| 2 | × | 2 | 5.45 | 13 | 48.02 | -6 | 0.96 | -2 |
| 2 | × | 3 | 5.15 | 6 | 62.50 | 23 | 1.13 | 15 |
| 2 | × | 4 | 4.92 | 2 | 62.18 | 22 | 1.17 | 19 |
| 4 | × | 1 | 4.22 | -13 | 60.27 | 18 | 1.21 | 23 |
| 4 | × | 2 | 4.95 | 2 | 63.73 | 25 | 0.94 | -4 |
| 4 | × | 3 | 4.85 | 0 | 65. 5 | 28 | 1.18 | 20 |
| 4 | × | 4 | 4.63 | -4 | 48.33 | -5 | 1.05 | 7 |
| 6 | × | 1 | 5.80 | 20 | 66.68 | 31 | 1.26 | 29 |
| 6 | × | 2 | 3.43 | -29 | 74.20 | 46 | 1.29 | 32 |
| 6 | × | 3 | 4.38 | -10 | 68.68 | 35 | 1.15 | 17 |
| 6 | × | 4 | 4.37 | -10 | 47.92 | -6 | 1.00 | 2 |
| 8 | × | 1 | 3.93 | -19 | 55.10 | 8 | 1.17 | 19 |
| 8 | × | 2 | 5.58 | 15 | 73.35 | 44 | 1.27 | 30 |
| 8 | × | 3 | 5.75 | 19 | 44.78 | -12 | 1.00 | 2 |
| 8 | × | 4 | 4.15 | -14 | 61.08 | 20 | 1.17 | 19 |
| Reduction frequency (%) | | | | 50% | | 25% | | 12% |
| | | | Std error = 0.921 | | Std error = 9.228 | | Std error = 0.140 | |

[z]Relative impact (%) = [(treatment/Mean 0% dosage) – 1)] × 100

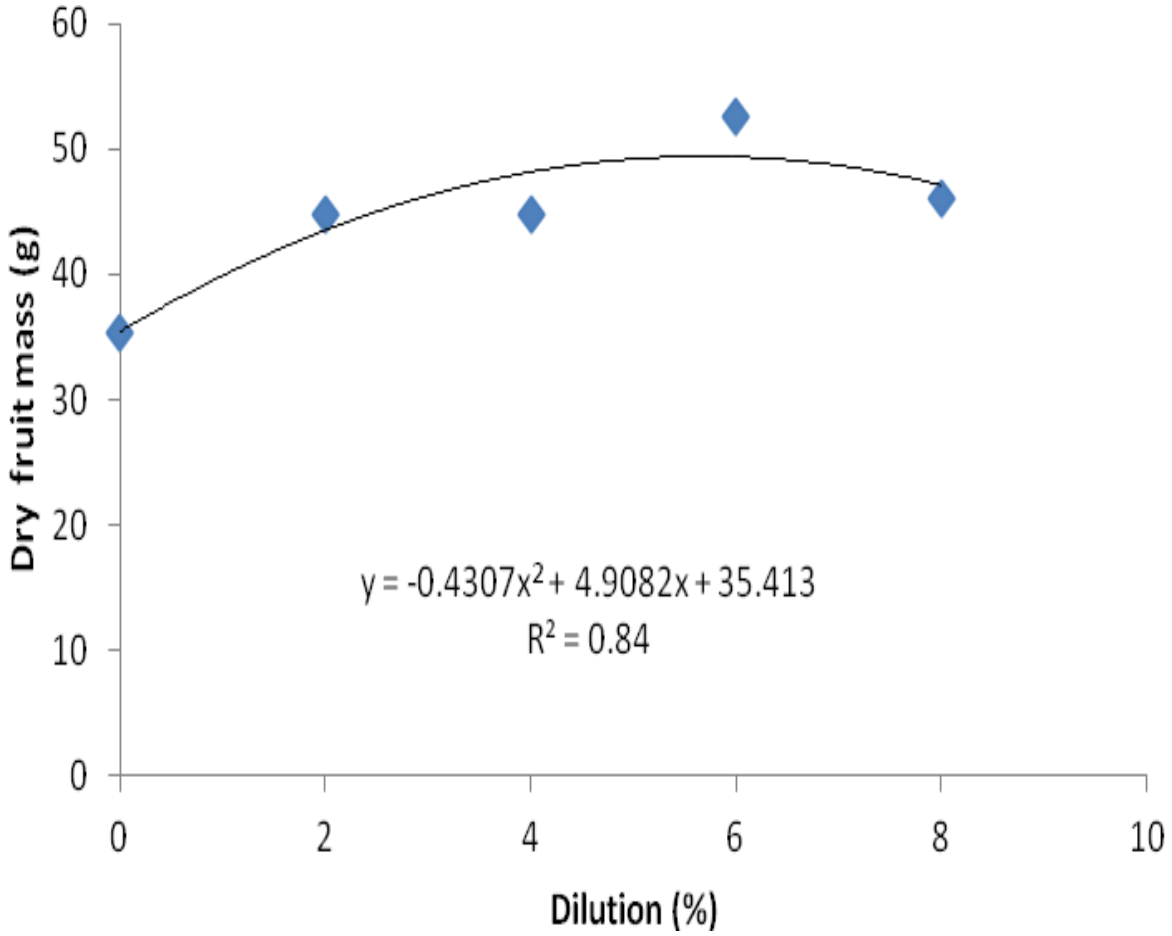


Figure 6.2 Response of dry fruit mass to concentrations of fermented crude extracts from *Cucumis myriocarpus* fruit at 56 days after the treatment under field conditions (n = 120).

Table 6.5 Response of dry fruit mass of tomato plant 'Floradade' at eight concentration levels of fermented crude extracts of *Cucumis myriocarpus* fruit and associated quadratic relationship, coefficient of determination and computed optimum concentration at 56 days after initiating the treatment (n = 120).

| Concentration (%) | | | | |
|---|---|---|---|---|
| 0 | 2 | 4 | 6 | 8 |
| 35.33b | 44.80ab | 44.78ab | 52.57a | 46.08ab |

| Determination of optimum concentration | | | | |
|---|---|---|---|---|
| Plant variable | Quadratic equation | $R^2$ | $x^z$ | P ≤ |
| DFM (g) | $y = -0.4307x^2 + 4.9082x + 35.413$ | 0.84 | 5.70 | 0.01 |

**Fruit yield is optimum at x = 5.70, where y = 49.40**

[ns]Means with the same letter were not statistically different according to Duncan's multiple range test. DFM = dry fruit mass.

[z]Calculated optimum response dosage (x) = $-b_1/2b_2$, where for dry fruit mass $b_1 = 4.9082$ and $b_2 = -0.4307$.

Table 6.6 Sum of squares for calcium, magnesium, phosphorus, potassium, manganese, sodium, sulfur and zinc in tomato 'Floradade' leaves at 56 days after treatment under field conditions (n = 120).

| Source of | | Ca | | Mg | | P | | K | | Mn | | Na | | S | | Zn | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variation | Df | SS | % | SS | % | SS | % | SS | % | SS | % | SS | % | SS | % | SS | % |
| Replication | 5 | 0.010 | $0^{ns}$ | 0.906 | $6^{ns}$ | 0.494 | $40^{ns}$ | 2.176 | $22^{ns}$ | 0.906 | $6^{ns}$ | 33.630 | $3^{ns}$ | 0.067 | $3^{ns}$ | 4.686 | $5^{ns}$ |
| Concent | 4 | 0.011 | $33^{***}$ | 3.306 | $21^{**}$ | 0.376 | $30^{***}$ | 2.211 | $22^{***}$ | 3.306 | $21^{***}$ | 149.420 | $12^{***}$ | 0.847 | $35^{***}$ | 11.274 | $12^{***}$ |
| Time | 3 | 0.010 | $0^{**}$ | 1.461 | $9^{ns}$ | 0.376 | $30^{***}$ | 0.051 | $1^{ns}$ | 1.461 | $9^{***}$ | 27.290 | $2^{ns}$ | 0.177 | $7^{***}$ | 17.621 | $19^{***}$ |
| C x T | 12 | 0.011 | $33^{***}$ | 3.048 | $19^{**}$ | 0.000 | $0^{***}$ | 0.835 | $8^{ns}$ | 3.048 | $19^{***}$ | 217.390 | $17^{**}$ | 0.373 | $16^{***}$ | 21.062 | $23^{***}$ |
| Error | 95 | 0.011 | 33 | 7.312 | 46 | 0.000 | 0 | 4.711 | 47 | 7.312 | 46 | 859.540 | 67 | 0.933 | 39 | 38.931 | 42 |
| Total | 119 | 0.053 | 100 | 16.034 | 100 | 1.246 | 100 | 9.983 | 100 | 16.034 | 100 | 1287.940 | 100 | 2.397 | 100 | 93.574 | 100 |

$^{ns}$Means that the factor (s) was not significant at $P \leq 0.05$; while $^{**}$ and $^{***}$ mean that the factor (s) was significant at $P \leq 0.10$, $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 6.7 Influence of dosages from fermented crude extracts of *Cucumis africanus* fruit on calcium and magnesium in tomato 'Floradade' leaves at 56 days after treatment under field conditions (n = 120).

| Concentration (%) | Application interval | Ca (%)[z] | Impact (%) | Mg (%) | Impact (%) |
|---|---|---|---|---|---|
| 0 × | 1 | 1.10 | - | 1.00 | - |
| 0 × | 2 | 1.00 | - | 1.00 | - |
| 0 × | 3 | 1.00 | - | 0.70 | - |
| 0 × | 4 | 1.10 | - | 1.00 | - |
| | | $\overline{x}$ =1.10 | | $\overline{x}$ = 0.90 | |
| 2 × | 1 | 1.40 | 27 | 0.20 | -81 |
| 2 × | 2 | 1.70 | 55 | 1.00 | 9 |
| 2 × | 3 | 1.80 | 64 | 1.00 | 9 |
| 2 × | 4 | 1.10 | 0 | 0.80 | -9 |
| 4 × | 1 | 1.10 | 0 | 1.20 | 26 |
| 4 × | 2 | 2.00 | 82 | 1.00 | 9 |
| 4 × | 3 | 1.40 | 27 | 1.20 | 26 |
| 4 × | 4 | 1.00 | -9 | 1.20 | 26 |
| 6 × | 1 | 1.10 | 0 | 1.00 | 9 |
| 6 × | 2 | 1.60 | 46 | 1.00 | 9 |
| 6 × | 3 | 1.30 | 18 | 1.20 | 26 |
| 6 × | 4 | 2.70 | 146 | 1.00 | 9 |
| 8 × | 1 | 1.70 | 55 | 1.00 | 9 |
| 8 × | 2 | 1.4 | 27 | 0.70 | -27 |
| 8 × | 3 | 1.4 | 27 | 0.70 | -27 |
| 8 × | 4 | 1.4 | 27 | 1.00 | 9 |
| Reduction frequency (%) | | | 6 | | 25 |

[z]Relative impact (%) = [(Treatment/Mean 0% dosage) – 1)] × 100

Table 6.8 Influence of dosages from fermented crude extracts of *Cucumis africanus* fruit on manganese, sodium, sulfur and zinc of tomato 'Floradade' leaves at 56 days after treatment under field conditions (n = 120).

| Concentration (%) | Application interval | Mn (ppm) | Mn Imp %[z] | Na (ppm) | Na Imp % | S (ppm) | S Imp % | Zn (ppm) | Zn Imp % |
|---|---|---|---|---|---|---|---|---|---|
| 0 × | 1 | 15.74 | - | 67.85 | - | 10.00 | - | 26.54 | - |
| 0 × | 2 | 15.73 | - | 76.17 | - | 10.00 | - | 26.07 | - |
| 0 × | 3 | 15.73 | - | 75.67 | - | 10.00 | - | 26.07 | - |
| 0 × | 4 | 13.73 | - | 36.86 | - | 11.60 | - | 39.99 | - |
| | | $\bar{x}$ = 15.23 | | $\bar{x}$ = 64.14 | - | $\bar{x}$= 10.40 | - | $\bar{x}$ = 29.67 | - |
| 2 × | 1 | 13.71 | -10 | 32.06 | -50 | 10.00 | -4 | 28.45 | -4 |
| 2 × | 2 | 16.89 | 11 | 91.77 | 43 | 10.00 | -4 | 36.62 | 23 |
| 2 × | 3 | 13.32 | -13 | 41.81 | -35 | 10.00 | -4 | 30.22 | 2 |
| 2 × | 4 | 18.20 | 20 | 45.48 | -29 | 10.00 | -4 | 39.46 | 33 |
| 4 × | 1 | 16.70 | 10 | 44.98 | -30 | 10.00 | -4 | 39.06 | 32 |
| 4 × | 2 | 16.35 | 7 | 33.85 | -47 | 10.00 | -4 | 40.01 | 35 |
| 4 × | 3 | 16.07 | 6 | 27.75 | -57 | 11.60 | 12 | 29.70 | 0 |
| 4 × | 4 | 16.11 | 6 | 35.29 | -45 | 10.00 | -4 | 30.67 | 3 |
| 6 × | 1 | 16.11 | 6 | 34.66 | -46 | 10.00 | -4 | 30.69 | 3 |
| 6 × | 2 | 18.88 | 24 | 25.58 | -60 | 10.00 | -4 | 38.94 | 31 |
| 6 × | 3 | 15.10 | -1 | 52.31 | -18 | 15.00 | 44 | 27.03 | -9 |
| 6 × | 4 | 24.05 | 58 | 40.90 | -36 | 10.00 | -4 | 49.24 | 66 |
| 8 × | 1 | 18.41 | 21 | 38.47 | -40 | 18.30 | 76 | 33.48 | 13 |
| 8 × | 2 | 20.78 | 36 | 2.383 | -63 | 10.00 | -4 | 39.85 | 34 |
| 8 × | 3 | 18.24 | 20 | 51.77 | -19 | 20.00 | 92 | 40.37 | 36 |
| 8 × | 4 | 20.32 | 33 | 34.35 | -46 | 20.00 | 92 | 41.82 | 41 |
| Reduction freq. (%) | | | 19 | | 94 | | 69 | | 12 |

[z]Relative impact (%) = [(treatment/Mean 0% dosage) – 1)] × 100, where Imp. = Impact.

Table 6.9 Influence of various concentrations of *Cucumis africanus* dried fruit on potassium content in tomato 'Floradade' leaves at 56 days after treatment under field conditions (n = 120).

| Concentration (%) | K (%) | Impact (%) |
|---|---|---|
| 0 | 1.31c | - |
| 2 | 2.38a | 81 |
| 4 | 1.30c | -1 |
| 6 | 1.73b | 32 |
| 8 | 1.96b | 50 |

[ns]Means with the same letter were not statistically different according to Fisher's least significant difference test.

Impact = [(Treated/untreated) – 1] × 100.

## 6.4 Discussion

The observed reduction of *M. incognita* juveniles in roots of tomato under field conditions and the related soil samples by fermented crude extracts from fruits of the two *Cucumis* species under field conditions confirmed previous observations under greenhouse and microplot conditions (Chapters 3 and 4). Similarly, the reduction of nematode juveniles in both root and soil samples agreed with those of the two *Cucumis* species when ground fruits were used in the ground leaching technology (Mashela, 2002; Mashela *et al*., 2011). Consequently, whether the materials from fruits of *C. africanus* and *C. myriocarpus* are used in botinemagation or ground leaching

technology, the materials retained their nematicidal properties, regardless of whether they were used under greenhouse, microplot or field conditions.

Efficacies of plant extracts depend on their concentration and duration of exposure to the nematode (Kali and Gupta, 1980; Mahmood *et al*., 1979). The product of the two concepts comprises a dosage, as outlined earlier in the Introduction of this trial. In the current trial, frequency of application within the 30-day month period had no significant effect on nematode numbers in both experimental units, which may suggest that the effect may be situational, since this differed with observations under microplot and greenhouse conditions. Under field conditions, the aqueous solutions containing the nematicidal chemical compounds from the two *Cucumis* species have unrestricted downward and sideward movements, which incidentally, reduce concentrations of the materials in the rhizosphere, while in plastic containers used under greenhouse and microplot trials the plastic walls may restrict these movements, particularly the horizontal movements, and therefore, increasing the concentrations. In this trial, confirming observations under the ground leaching technology systems (Mashela *et al*., 2011), the concentration was important in suppressing populations of *M. incognita* juveniles in tomato production.

In *C. africanus*, the first order interactions were not significant for all variables except for dry fruit mass, while in *C. myriocarpus* the interaction was significant for both dry shoot mass and dry fruit numbers. Under both *Cucumis* species, interactions were not significant for nematode numbers. Generally, non-significant interactions suggested that

for the affected variables the main factors had either additive or multiplicative effects (Salisbury and Ross, 1992). In additive responses the main factors act on different sites or organelles to elicit a greater response, while in multiplicative responses they act on different steps of a process in a casual sequence, so that the effect of one is always a fraction of the other (Salisbury and Ross, 1992). In contrast, when the interactions are significant, the main factors have synergistic effects, which implies that the factors act on the same site and therefore, resulting in a much greater response than when the reaction towards their action is the sum of individual reactions (Salisbury and Ross, 1992).

In this trial, synergistic effects were apparent in the three variables where concentration x application time interactions were significant as shown by increases of variables relative to the untreated controls. Further, the frequencies of reductions for a particular variable were arbitrarily aggregated using the concept of percentage reduction frequencies, where the value above 50% suggested the tendency towards non-synergestic effects. Using this arbitrary aggregation, the interactions had synergestic effects on dry root mass and fruit number since the percentage reduction frequencies was less than 50%. Similarly, the interactions had synergestic effects on Ca, Mg, Mn and Zn since the percentage reduction frequencies were less than 50%, while for Na and S the interactions had non-synergestic effects as shown by percentage reduction frequencies above 50%. Generally, various organs in tomato have different sensitivities to crude extracts of *C. africanus* and *C. myriocarpus* (Mafeo, 2012), while nutrient elements also respond differently to various interactions (Mashela, 1992). The dosage

from crude extracts of *C. africanus* tendered to be phytotoxic to roots, which are in direct contact with the products in soil solutions, while the opposite was true for *C. myricarpus* crude extracts, which had no effect on dry root mass. On the basis of percentage reduction frequencies, the product had synergestic effects on dry shoot mass and fruit number.

The different responses of tomato plants to crude extracts of the two *Cucumis* species are consistent with previous observations in nematode-resistant studies (Pofu, 2012). This could possibly be due to their chemical composition. The active chemical in *C. africanus* is cucurbitacin B ($C_{32}H_{46}O_8$) which is insoluble in water, while in *C. myriocarpus* the active chemical is cucurbitacin A which is soluble in water comprising of cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Chen *et al*., 2005; Jeffrey, 1978). The non-phytotoxicity of crude extracts from *C. myriocarpus* on tomato plants under field conditions enhances its potential candidacy in botinemagation. Previously it was shown that the $LC_{50}$ mortality of *C. myriocarpus* for *M. incognita* and the citrus nematode (*Tylenchulus semipenetrans* Cobb 1913) juveniles was 7 µL/ml water (Muedi *et al*., 2005), while for other plant-parasitic nematodes the $LC_{50}$ had not been documented. In crude extracts from plants, pests are affected by multiple active ingredients (Wyut *et al*., 2006). Most active ingredients from crude extracts of plants have the capability to permeate the gelatinous matrix, with the result that the first stage juveniles become exposed to the aqueous solutions and interfere with stylet development which fails to pierce through the egg-shell with the result that eggress fails (Hirschmann, 1985; Parmar, 1987). In crude extracts of three plants from India, namely,

*Fleurya in-errupta*, *Peritrophe bicalyculata* and *Andrographis paniculata*, 100% mortality of *Meloidogyne* species juveniles upon exposure was reported (Mukherjee and Sukul, 1978). Similar high mortalities were observed from crude extracts of marigold (*Tagetes* species), *Emblica officinalis* and *Carrissa curandas* (Haseeb *et al*., 1980; Toida and Moriyama, 1978). Obviously, evidence is abundant to suggest that crude extracts from various plant species suppress population densities of various plant-parasitic nematodes.

## 6.5 Conclusions

Crude extracts of *C. africanus* fruit had no effect on nutrient elements, except that the dosage played a role for certain nutrient elements, particularly Ca, Mg, Mn, Zn, Na and S, which confirms observations in leaves of tomato under crude extracts of *C. myriocarpus* fruits in the ground leaching technology systems (Mashela, 2002). In the current trial, concentration levels affected K, which is a non-structural element in plants (Salisbury and Ross, 1992). However, when applied over an extended period in commercial tomato production systems, the cumulative effects of the nutrient elements in the rhizosphere may result in observable accumulation of nutrient elements in leaves. The non-structural status of this elements accord it the status of being leached into the rhizosphere during irrigation. The reduction of Na in leaves of tomato may be undesirable since this element is required for improvement of fruit quality in tomato (Salisbury and Ross, 1992).

# CHAPTER 7
# VALIDATION OF COMPUTER-GENERATED DOSAGES FROM DRIED FRUITS OF *CUCUMIS* SPECIES ON GROWTH OF TOMATO PLANTS AND SUPPRESSION OF *MELOIDOGYNE INCOGNITA* NUMBERS UNDER FIELD CONDITIONS

## 7.1 Introduction

The mean dosage stimulation range (MCSR) is generated using two biological indices from the Curve-fitting Allelochemical Response Dosage (CARD), namely, stimulation threshold ($D_m$) and saturation point ($R_h$) (Chapters 4 and 5; Mafeo, 2012). Under greenhouse and microplot conditions, the MCSR values for both wild watermelon (*Cucumis africanus* L.) and wild cucumber (*Cucumis myriocarpus* Naude.) were 3% and 6%, respectively (Chapters 5). At both concentrations, crude extracts from the two *Cucumis* species significantly reduced the southern root-knot (*Meloidogyne incognita*) nematode. Optimum application intervals, determined under greenhouse conditions, for the 3% and 6% concentrations of fermented crude extracts (FCE) from fruit with respect to growth of tomato plants were 18 and 20 days, respectively (Chapter 5). In contrast, the optimum application time interval for 3% and 6% concentrations of FCE from *C. myriocarpus* fruit was at 16 days (Chapter 5).

In order to avoid confusion, two concepts, namely, dose and dosage, need to be defined. Van Gundy and McKenry (1975) defined dose as the amount of the toxicant received by the individual nematode, while dosage is the amount of toxicant placed in the environment of the nematode for a known length of exposure time (concentration × time). By definition Mean concentration stimulation range (MCSR) should stimulate plant growth, while suppressing nematode numbers. Due to the allelopathic nature of

chemical compounds in crude extracts of the materials used in this study, phytotoxicity could be a limiting factor in the use of these materials (Mafeo, 2012). The derived MCSR had not been validated. The objective of this study therefore, was to validate the two MCSR values for *C. africanus* and *C. myriocarpus* at their respective optimum application time intervals for growth of tomato plants and suppression of *M. incognita* numbers under field conditions.

## 7.2 Materials and methods

### 7.2.1 Location of study and preparation

Separate experiments for *C. africanus* and *C. myriocarpus* were conducted concurrently in open field systems at the Plant Protection Skills Centre, University of Limpopo, South Africa ($23^{o}$53''10'S, $29^{o}$44''15'E) (Figure 7.1). The location has summer rainfall with mean annual rainfall of 600 mm, while maximum/minimum temperatures average $28^{o}$C/$19^{o}$C. Trials were conducted in spring (August-November) 2012 and were not repeated in space or time since they were validation studies (Gomez and Gomez, 1984). The site contained Hutton soil (65% sand, 30% clay, 5% silt; 1.6% organic C, $EC_e$ 0.148 dS/m and pH ($H_2O$) 6.5. A plot comprised 20 $m^2$ with 20-cm-deep holes dug in the centre of 1 $m^2$ subplot in order to allow for 1.0 m intra-row and 1.0 m inter-row spacing. Sources and preparation of *Cucumis* fruits and nematode inoculum were as described previously (Chapter 4). Prior to transplanting, soil samples were collected for the determination of initial population density (Pi), extracted and counted as described previously for nematode samples (Chapter 4), with mean Pi = 3700 second stage juveniles/250 ml soil. Uniform four-week-old nematode-free tomato cv. ‘Floradade’

seedlings were transplanted in 500 ml chlorine-free tapwater. *Cucumis africanus* and *C. myriocarpus* fruits were obtained and prepared as fermented crude extracts as described previously (Chapter 4).

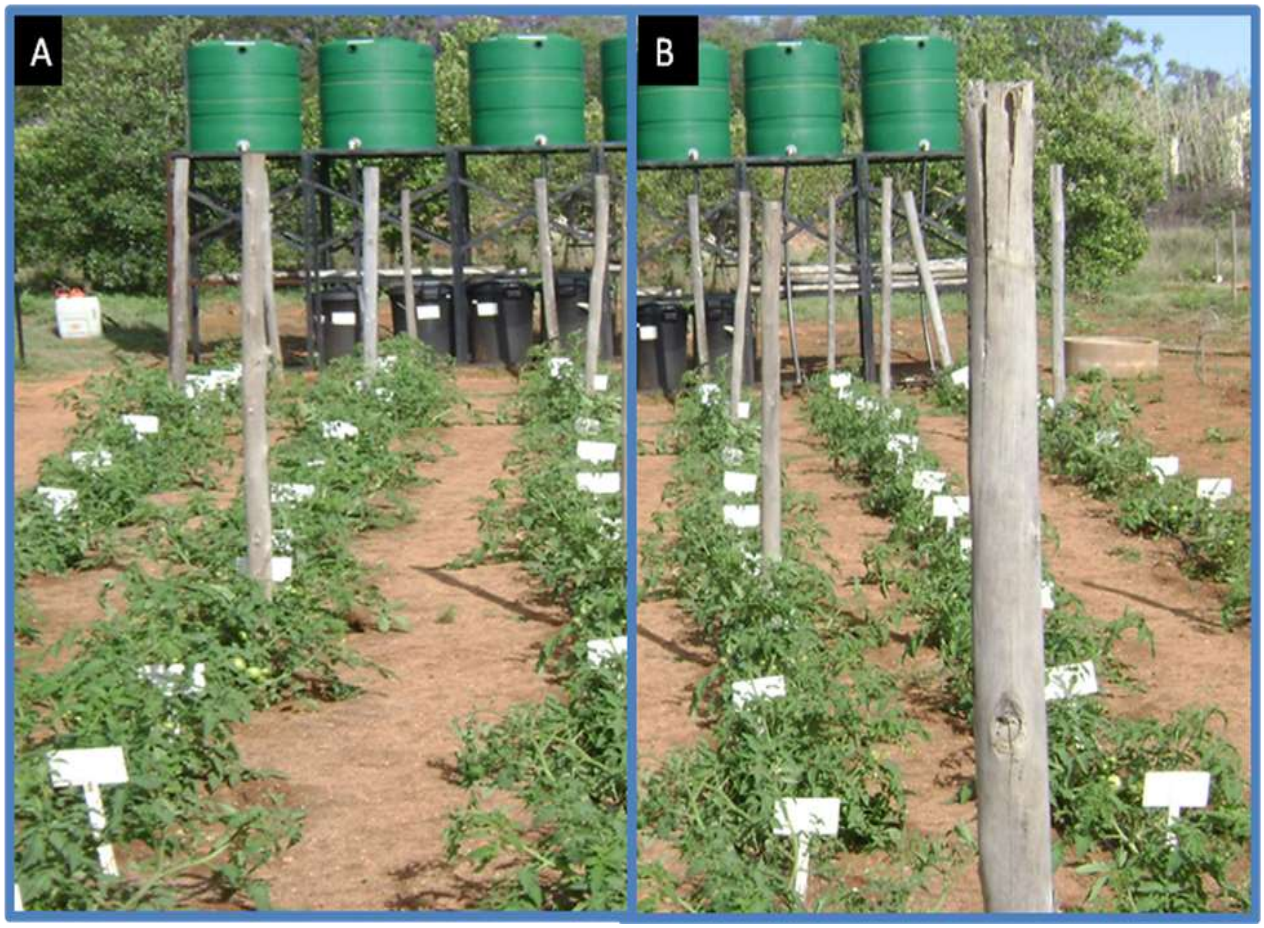


Figure 7.1 Open field validation experiments using concentrations from fermented crude extracts of dried fruits of **(A)** *Cucumis africanus* and **(B)** *Cucumis myriocarpus*.

### 7.2.2 Experimental design and cultural practices

In both experiments, the dosage (concentration × time) levels were arranged in randomised complete block design, with 14 replications. Treatments were initiated 5 days after transplanting and thereafter 3% and 6% concentrations from FCE of *C. africanus* fruit applied at 18 and 20 days, respectively, while the two concentrations for *C. myriocarpus* fruit were each applied at 16-day intervals. Fertilisation with 3 g 2:3:2 (22) plus 2 g 2:1:2 (43) was achieved as described previously (Chapter 4). Irrigation was achieved through drip irrigation system, with 2 h and 1 h in the morning and in the afternoon, respectively, only on days where 50% moisture meter readings were below 2 units (Chapter 3).

### 7.2.3 Data collection

At 75 days after inoculation, plant and nematode variables were collected as described previously (Chapter 3).

### 7.2.4 Data analysis

Data were subjected to simple analysis of variance (ANOVA) through the 2008 SAS software (SAS Institute, Inc., Cary, NC., USA). Nematode data were transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984), but untransformed means were reported. Sum of squares were 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 Fisher's least significant test at the probability level of 5%. Unless otherwise stated, only treatments significant at the probability level of 5% are discussed (Appendices 7.1-7.4).

## 7.3 Results

Under *C. africanus*, treatment effects were significant for plant height only and contributed 15% to total treatment variation in this variable (Table 7.1). In contrast, under *C. myriocarpus*, treatment effects contributed 29%, 18% and 34% to total treatment variation in dry shoot mass, dry root mass and plant height, respectively.

Relative to untreated control, dosage consistently increased plant height under *C. africanus* by 3-15%, while it had no effect on dry shoot mass and dry root mass. Under *C. myriocarpus*, the dosage reduced and increased dry shoot mass by 16% and 38%, respectively, but consistently increased dry root mass and plant height by 17-45% and 2-22%, respectively (Table 7.2). In all cases under both *Cucumis* species, the effect of dosage was significantly higher than that of the untreated control.

Under *C. africanus*, treatment effects each contributed 62% to total treatment variation of nematode juveniles in roots and in soil (Table 7.3). Similarly, *C. myriocarpus* treatment contributed 50% and 49% to total treatment variation of nematode juveniles in the three respective units. Under *C. africanus*, relative to untreated control, dosage reduced nematode juveniles by 79-85% and 79-85% in roots and soil samples, respectively (Table 7.4). Similarly, under *C. myriocarpus*, dosage reduced nematode juveniles by 79-85% and 79-85% in the two respective samples.

Table 7.1 Partitioning sum of squares for fourteen replications, three concentration levels from fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits and the error over dry shoot mass (DSM), dry fruit mass (DFM), dry root mass (DRM), fruit number (FNB), plant height (PHT) and stem diameter (SDR) of tomato plant 'Floradade' at 75 days after treatment (n = 42).

| Source of variation | DF | DSM | | DFM | | DRM | | FNB | | PHT | | SDR | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SS | % | SS | % | SS | % | SS | % | SS | % | SS | % |
| *Cucumis africanus* fruit | | | | | | | | | | | | | |
| Replication | 13 | 25975.600 | $29^{ns}$ | 23508.800 | $40^{ns}$ | 90.797 | $29^{ns}$ | 0.586 | $41^{ns}$ | 2134.490 | $24^{ns}$ | 203.096 | $47^{ns}$ |
| Treatment | 2 | 1313.600 | $1^{ns}$ | 4975.300 | $9^{ns}$ | 34.821 | $11^{ns}$ | 0.132 | $9^{ns}$ | 1349.430 | $15^{***}$ | 5.019 | $1^{ns}$ |
| Error | 26 | 61583.800 | 69 | 29867.000 | 51 | 192.123 | 60 | 0.700 | 49 | 5328.570 | 60 | 225.761 | 52 |
| Total | 41 | 88873.000 | 100 | 58351.100 | 100 | 317.740 | 100 | 1.417 | 100 | 8812.490 | 100 | 433.875 | 100 |
| *Cucumis myriocarpus* fruit | | | | | | | | | | | | | |
| Replication | 13 | 22501.000 | $18^{ns}$ | 11284.100 | $28^{ns}$ | 126.472 | $34^{ns}$ | 0.493 | $30^{ns}$ | 833.650 | $9^{ns}$ | 94.627 | $40^{ns}$ |
| Treatment | 2 | 35127.000 | $29^{***}$ | 3518.700 | $9^{ns}$ | 67.955 | $18^{***}$ | 0.019 | $1^{ns}$ | 3107.530 | $34^{***}$ | 6.150 | $3^{ns}$ |
| Error | 26 | 65023.000 | 53 | 25559.600 | 63 | 177.938 | 48 | 1.150 | 69 | 5230.230 | 57 | 136.423 | 58 |
| Total | 41 | 122651.00 | 100 | 40362.400 | 100 | 372.366 | 100 | 1.661 | 100 | 9171.410 | 100 | 237.200 | 100 |

$^{ns}$Means that the factor (s) was not significant at P ≥ 0.05; while $^{**}$ and $^{***}$ mean that the factor was significant at P ≤ 0.05 and P ≤ 0.01, respectively.

Table 7.2 Influence of application time of 0%, 3% and 6% concentration from fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits on dry shoot mass, dry root mass and plant height in tomato plant 'Floradade' for 75 days under field conditions (n = 42).

| Plant | Dosage | | Dry shoot mass (g) | | Dry root mass (g) | | Plant height (cm) | |
|---|---|---|---|---|---|---|---|---|
| crude extracts | Concentration (%) | Application frequency | Variable | % | Variable | % | Variable | % |
| *Cucumis africanus* fruit | 0 | - | 128.93 | - | 6.84 | - | 87.16b | - |
| | 3 | × 4.17 | 115.26 | -11 | 8.88 | 30 | 90.03ab | 3 |
| | 6 | × 3.75 | 121.26 | -6 | 8.63 | 26 | 100.36a | 15 |
| *Cucumis myriocarpus* fruit | 0 | - | 128.93b | - | 6.84b | - | 87.16b | - |
| | 3 | × 4.69 | 108.26b | -16 | 7.98ab | 17 | 89.07b | 2 |
| | 6 | × 4.69 | 177.27a | 38 | 9.92a | 45 | 106.29a | 22 |

$^{ns}$Means that the factor (s) was not significant at P ≥ 0.05; while $^{**}$ and $^{***}$ mean that the factor (s) was significant at P ≤ 0.05 and P ≤ 0.01, respectively.

Impact (%) = [(treatment/control) - 1] x 100.

Application frequency = Crop cycle (days)/Application interval (days).

Table 7.3 Partitioning sum of squares for fourteen replications, three concentration levels from fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits and the error over nematodes in root and soil in tomato plant 'Floradade' at 75 days after treatment (n = 42).

| Source of variation | DF | Nematode root | | Nematode soil | |
|---|---|---|---|---|---|
| | | SS | % | SS | % |
| Dried *Cucumis africanus* fruit | | | | | |
| Replication | 13 | 1.627 | 16$^{ns}$ | 1.641 | 15$^{ns}$ |
| Treatment | 2 | 5.544 | 54$^{***}$ | 6.649 | 62$^{***}$ |
| Error | 26 | 3.025 | 30 | 2.435 | 23 |
| Total | 41 | 10.196 | 100 | 10.725 | 100 |
| Dried *Cucumis myriocarpus* fruit | | | | | |
| Replication | 13 | 1.575 | 20$^{ns}$ | 1.335 | 15$^{ns}$ |
| Treatment | 2 | 3.380 | 42$^{***}$ | 4.351 | 50$^{***}$ |
| Error | 26 | 2.999 | 38 | 3.074 | 35 |
| Total | 41 | 7.954 | 100 | 8.760 | 100 |

$^{ns}$Means that the factor (s) was not significant at P ≥ 0.05; while, $^{***}$ means that the factor (s) was significant at P ≤ 0.01.

Table 7.4 Effects of three dosages from fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits on juveniles of *Meloidogyne* species in roots and soil of tomato plant 'Floradade' at 75 days after treatment under field conditions (n = 42).

| Plant crude Extracts | Dosage | | Nematode root | | Nematode soil | |
|---|---|---|---|---|---|---|
| | Concentration (%) | Application frequency | Variable | % | Variable | % |
| *C. africanus* fruit | 0 | - | 43a[z] | - | 270a | - |
| | 3 × | 4.17 | 10b | -78 | 57b | -79 |
| | 6 × | 3.75 | 7b | -84 | 41b | -85 |
| *C. myriocarpus* fruit | 0 | - | 43a | - | 270a | - |
| | 3 × | 4.69 | 15b | -65 | 100b | -63 |
| | 6 × | 4.69 | 10b | -77 | 51b | -81 |

[z]Means with the same letter were not different (P ≤ 0.05) according to Fisher's least significant difference test.

Impact (%) = [(treatment/control) - 1] x 100.

Application frequency = Crop cycle (days)/Application time (days)

## 7.4 Discussion

Dosage from fermented crude extracts of *C. africanus* fruit did not have much stimulation effect on growth of tomato plants except for improving plant height. In contrast, dosage of fermented crude extracts of *C. myriocarpus* fruit had substantial stimulation effect on growth of tomato plants as shown by its effects on dry shoot mass, dry root mass and plant height. Unfortunately, validation data in this study with respect to stimulation of plant growth cannot be compared with those in other studies since the emphasis had been mainly on either neutral or inhibitory effects of crude extract concentrations on plant growth (Ramazan and Yarba, 2010). Most crude plant extracts have been tested against the suppression of plant-parasitic nematodes

*in vitro* (Ramazan and Yarba, 2010), but could not continue beyond the greenhouse trials due to their phytotoxicity (Meyer *et al*., 2008; Musabyimana *et al*., 2000; Ramazan and Yarba, 2010; Setia *et al*., 2007).

In the current study, the use of biological indices generated by the CARD computer-based model improved the understanding of the biological effects of fermented crude extracts, namely, stimulation, neutral and inhibition (Liu *et al*., 2003). Adaptation of the model for use to generate plant stimulatory concentrations in suppression of plant-parasitic nematodes (Mafeo, 2012), is increasingly opening innovative frontiers in the use of botanical nematicides in managing nematodes. The observed differences in dosage responses of tomato plant growth to biological effects of fermented crude extracts of *C. africanus* and *C. myriocarpus* fruits in this study confirmed differences in nematode resistance in living host plants (Pofu *et al*., 2010a,b) and application time intervals (Chapter 6) when the materials were used as fermented crude extracts in nematode management (Chapter 6). These differences have been attributed to different potent chemicals in the two *Cucumis* species, namely, cucurbitacin A and cucurbitacin B in *C. myriocarpus* and *C. africanus* fruits, respectively, along with differences in the structural chemical properties, which are reflected by their solubility and insolubility in water, respectively (Chen *et al*., 2005).

The stimulatory effect is not unique to botanical nematicides, since it was also observed in various crops treated with synthetic nematicides (Altman, 1970). The conversion of EDB fumigant nematicides to ethylene by soil organisms might account for some unexplained growth responses with that fumigant (Castro and Belser, 1968). Oftentimes, applications of synthetic nematicides improved crop yield

even if nematodes were not present in the treated soil (Van Gundy and McKenry, 1975). Later, Milne *et al.* (1977) proposed that increased accumulation of indole-acetic acid molecules in tissues of pineapple [*Ananas comosus* (L.) Merr.] plants were responsible for stimulating plant growth in the absence of nematode infection. Generally, infection by plant-parasitic nematodes below the damage threshold also results in stimulation of plant growth (Wallace, 1973). In summary, biological systems respond to increasing intrinsic or extrinsic factors through a density-dependent growth pattern, characterized by three phases: stimulation, saturation and inhibition phases (Salisbury and Ross, 1992); which were quantified for allelochemicals through six biological indices by Liu *et al*. (2003).

In both materials, dosages within either *C. africanus* or *C. myriocarpus* fruits had no significant effect on nematode numbers, while, relative to untreated controls, the materials reduced this variable. The observation in this study was consistent with observations in the previous trials (Chapters 3, 4, 5, 6) and in other studies (Mashela, 2002; Mphosi, 2004). Apparently, nematode suppression was independent of dosage after a certain concentration. The concept of dosage (concentration × time) as described earlier in this study, allows for varying the concentration or time without affecting the biological responses of nematodes (McKenry and Thomason, 1974). Generally, organisms have a threshold limit below which death is not obtained regardless of the exposure time. Similarly, applying the material above a recommended dosage would not necessarily result in a higher death rate than at the recommended dosage.

Nematode results in this and other studies (Mashela, 2002; Mphosi, 2004) suggested that similar biological behaviours of *Meloidogyne* species may be affected by potent chemicals from the two *Cucumis* species. Generally, biological behaviours affected by botanicals are similar to those of non-fumigant synthetic nematicides, namely, chemotaxis, mortality, mobility and egress inhibition (Adegbite and Adesiyan, 2005; Agbenin *et al*., 2005; Haseeb *et al*., 1980; Toida and Moriyama, 1978; Wuyts *et al*., 2006). Under chemotaxis, the effect of a phytonematicide can be either repellent or attractive to the nematode (Hewlett *et al*., 1997), while mobility inhibition allows the host for the induction and expression of more powerful defense mechanisms, while egress inhibition and mortality have the potential ability of reducing nematode population densities in roots and soil (Agbenin *et al*., 2005; Wuyts *et al*., 2006). Nematode juveniles get into contact with phytonematicides in soil soon after egress since they have to migrate into the soil for re-infection of new roots (Wyss *et al*., 1992), where chemotaxis and mobility takes place (Wuyts *et al*., 2006). Certain potent chemicals enter the egg mass, where they interfere with the development of stylets of J1s and therefore inhibiting egg hatch since the stylets are required for this process (Hirschmann, 1985; Parmar, 1987).

The unique feature of plant-parasitic nematodes is that they have strong survival strategies, referred to as dauer stage and cryptobiosis, whereby certain stages are able to survive adverse conditions which advance gradually (Mashela, 2007). Generally, the dauer stage occurs in J1s prior to egress, while cryptobiosis occurs in J2s prior to moulting into J3s (Mashela, 2007). During the two survival stages, juveniles are in the lowest metabolic stage and can easily survive the vagaries of the toxicants. Generally, when the survival stages are removed from an adverse

environment to a suitable one, appropriate metabolic processes are restored with the result that the juveniles assume growth and development. In reality, use of botinemagation may be highly successful in management of plant-parasitic nematodes since application results in various behavioural changes of nematodes, with gradual induction of cryptobiosis within at least 72 h (Wuyts *et al*., 2005). As irrigation is applied within the irrigation intervals, the concentration of the phyto-nematicide is reduced, until a level where there is no longer any biological effect, with nematode juveniles – depending on whether they are at J1s or J2s, assuming normal activities, which coincide with the subsequent application of the phyto-nematicide. Thus, the efficacy of plant extracts depends on their concentration, duration of exposure of nematodes, stage of nematode and metabolic state of nematodes (Kali and Gupta, 1980; Mahmood *et al*., 1979; Wuyts *et al*., 2005), along with the chemical constituents of plant extracts and the nematode species (Wuyts *et al*., 2005). Incidentally, various direct and indirect forces may be at play in determining the efficacy of plant extracts in suppression of population densities of plant-parasitic nematodes.

## 7.5 Conclusions

In conclusion, the efficacy of the two *Cucumis* species appears to play a minor role in the selection of the appropriate dosage, since at the two tested dosages there were no differences in the reduction of population densities of *Meloidogyne* species. However, especially in *C. myriocarpus*, yield was improved at the 6% concentration. This suggested that the appropriate dosage for plant extracts of *C. myriocarpus* fruit for nematode suppression and improvement of tomato growth is 6% concentration at 16-day application time interval through irrigation system. Since *C. africanus* had no

significant differences from the two dosages (12.5% and 22.5%), it is recommended that the lower dosage (12.5%) be used since the material previously reduced dry root mass (Chapter 6).

# CHAPTER 8
# SUMMARY, SIGNIFICANCE OF FINDINGS, FUTURE RESEARCH AND CONCLUSIONS

## 8.1 Summary

Fermented plant extracts (FPE) from fresh fruits of wild watermelon (*Cucumis africanus*) and wild cucumber (*Cucumis myriocarpus*) consistently reduced population densities of *Meloidogyne* species by 80-92% and 50-90%, respectively. Tomato plants were highly sensitive to the two products when fresh fruits were used as reflected by the low total degree of sensitivities (∑k) of 0 and 3 for *C. africanus* and *C. myriocarpus*, respectively. Also, the mean concentration stimulation range (MCSR) of 11% and 7% concentrations for *C. africanus* and *C. myriocarpus*, respectively agreed with the concentrations used in this study.

Fermented crude extracts (FCE) of dried fruits from *C. africanus* and *C. myriocarpus* reduced population densities of *Meloidogyne* species by 78-97% and 87-97%, respectively. Tomato plants were highly tolerant to the two products in dried form as shown by the total degree of sensitivities (∑k) and biological index of 4 and 3, respectively. MCSR values for *C. africanus* and *C. myriocarpus* dried fruits on tomato were 2.64% and 2.99%, respectively, which for the purpose of this study were individually adjusted to 3%.

The MCSR values were empirically-derived as 3% and 6% concentrations from crude extracts of both *Cucumis* species using the CARD model. These were used to optimise the application time interval using the innovative concept of weeks (0, 1, 2, 3 and 4) in a 30-day month period. Application time interval for 3% and 6% concentrations of *C. africanus* fruits was optimised at 2.40 and 2.61 weeks in 30-day

month period, respectively, which translated to 18 days [(2.4 weeks/4 weeks) × 30 days] and 20 days [(2.6 weeks/4 weeks) × 30 days], respectively. In contrast, for both concentrations from fermented crude extracts of *C. myriocarpus* fruits, application time interval was optimised at 16 days for 2.2 and 2.1 weeks, respectively. During optimisation of application frequencies, fermented crude extracts from *C. africanus* and *C. myriocarpus* reduced final population densities of *M. incognita* race 2 by 70-97% and 76-96%, respectively. The derivation of optimum application frequencies, allowed the computation of dosage, which is a product of concentration and application frequency.

Empirically-derived dosages were validated under field conditions. At 6% every 16 days, application of crude extracts from *C. myriocarpus* fruit significantly ($P \leq 0.05$) improved growth of tomato plants when compared with those of either untreated control or 3% every 16 days. In contrast, dosages for *C. africanus* fruit at two application time intervals had no effect on growth of tomato plant. During the validation trials, the materials also reduced nematode numbers. In conclusion, crude extracts of the two *Cucumis* species have potentially similar reductive effects on population densities of *Meloidogyne* species and could be used as phyto-nematicides. However, since plant responses to the products differed in terms of their respective dosages, it implied that for further improvement of the two products, the overriding effect should be on their interaction with the protected plants. Ideally, future research should include environmental impact studies, especially on the influence of the products in fruit quality of tomato, earthworms and bees.

## 8.2 Significance of findings

Use of botanical nematicides in crop production could be the most eco-friendly tactic for managing plant-parasitic nematodes. The study developed protocols for quantifying botanical nematicides, which would be used in various plant materials to minimize phytotoxicity and/or promote phytotoxicity where the materials are intended to serve as phyto-herbicides. Also, the development of Indigenous Cucurbitaceae Technologies (ICT) to the current level through botinemagation has the potential of the latter having widespread adoption, particularly in commercial farming systems, with the resultant emergence of down- and up-stream industries, which would create the much desired jobs and wealth, in addition to improving soil, plant, workforce, consumer and environmental health systems as advocated in *natuurboerdery* (nature farming) (Nzanza *et al*., 2013).

## 8.3 Future research

Cucurbitacins accumulate in roots and fruits, particularly in seeds (Rimington, 1938). Therefore, it is imperative that the possibility that cucurbitacins – particularly the highly water-soluble cucurbitacin A, could accumulate in tomato fruits to the detriment of fruit quality, be investigated as a matter of urgency. Secondly, the shelf-life of the inputs and the products should be tested to facilitate the commercialisation of these phyto-nematicides. Thirdly, since the study demonstrated that the two products have similar capabilities for the reduction of *Meloidogyne* species, attempts should be made to understand the basic biological behaviours of nematodes in response to exposure to the two products. Additionally, since biological behaviours of tomato plants towards the two products were de-similar, this needs to be investigated further in order to unravel the underlining plant-product interactions.

Fourthly, environmental impact studies, particularly towards non-target organisms like earthworms and bees, should be undertaken. Fifthly and finally, since the products were efficient and effective in reducing population densities of *Meloidogyne* species, efficacy tests using botinemagation protocols developed in this study could be expanded to other nematode species and economic crops.

## 8.4 Conclusions

The results of this study, particularly the validation dosages, provide the ICT group with very powerful information to use when testing the material in various environments in South Africa, when developing basic data required for the registration of the products in terms of the Agricultural Act No. 36 of 1947.

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# APPENDICES

Appendix 3.1 Analysis of variance (ANOVA) of seven different dilutions of fermented plant extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits on nematode numbers in roots of tomato plant.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 4 | 2.508 | 9 | 1.56 | 0.22 |
| Treatment | 5 | 16.705 | 58 | 6.92 | 0.00 |
| Error | 20 | 9.653 | 33 | | |
| Total | 29 | 28.866 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 4 | 0.955 | 3 | 0.32 | 0.86 |
| Treatment | 5 | 11.770 | 39 | 2.64 | 0.04 |
| Error | 20 | 17.840 | 58 | | |
| Total | 29 | 30.565 | 100 | | |

Appendix 3.2 Analysis of variance (ANOVA) of seven different dilutions of fermented plant extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits on nematode numbers in soil of tomato plant.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 4 | 4.953 | 8 | 0.81 | 0.53 |
| Treatment | 5 | 20.766 | 33 | 2.26 | 0.05 |
| Error | 20 | 36.827 | 59 | | |
| Total | 29 | 62.546 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 4 | 4.817 | 10 | 1.10 | 0.38 |
| Treatment | 5 | 17.641 | 36 | 2.68 | 0.04 |
| Error | 20 | 26.315 | 54 | | |
| Total | 29 | 48.774 | 100 | | |

Appendix 3.3 Analysis of variance (ANOVA) of seven different dilutions of fermented plant extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits on total nematode numbers in root and soil of tomato plant.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 4 | 0.621 | 3 | 0.26 | 0.90 |
| Treatment | 5 | 10.419 | 41 | 2.97 | 0.03 |
| Error | 20 | 14.053 | 56 | | |
| Total | 29 | 25.092 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 4 | 2.846 | 11 | 1.19 | 0.34 |
| Treatment | 5 | 9.645 | 36 | 2.70 | 0.04 |
| Error | 20 | 14.302 | 53 | | |
| Total | 29 | 26.792 | 100 | | |

Appendix 3.4 Analysis of variance (ANOVA) of seven different dilutions of fermented crude extracts of *Cucumis africanus* fruit on fruit yield of tomato plant.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 4 | 0.125 | 5 | 0.67 | 0.62 |
| Treatment | 6 | 1.330 | 52 | 4.78 | 0.00 |
| Error | 24 | 1.113 | 43 | | |
| Total | 34 | 2.568 | 100 | | |

Appendix 3.5 Analysis of variance (ANOVA) of seven different dilutions of fermented plant extracts of *Cucumis myriocarpus* fruit on dry root mass of tomato plant.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 4 | 9.1168 | 21 | 4.07 | 0.01 |
| Treatment | 6 | 21.9096 | 49 | 6.52 | 0.00 |
| Error | 24 | 13.4326 | 30 | | |
| Total | 34 | 44.4591 | 100 | | |

Appendix 3.6 Analysis of variance (ANOVA) of seven different dilutions of fermented plant extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits on dry shoot mass of tomato plant.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 4 | 215.410 | 7.55 | 1.06 | 0.40 |
| Treatment | 6 | 1419.220 | 49.74 | 4.66 | 0.00 |
| Error | 24 | 1218.750 | 42.71 | | |
| Total | 34 | 2853.380 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 4 | 22.612 | 3 | 0.56 | 0.70 |
| Treatment | 6 | 496.833 | 65 | 8.24 | 0.00 |
| Error | 24 | 241.061 | 32 | | |
| Total | 34 | 760.506 | 100 | | |

Appendix 3.7 Analysis of variance (ANOVA) of seven different dilutions of fermented plant extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits on plant height of tomato plant.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 4 | 1062.200 | 8 | 1.14 | 0.34 |
| Treatment | 6 | 6326.300 | 49 | 4.52 | 0.00 |
| Error | 24 | 5594.000 | 43 | | |
| Total | 34 | 12982.500 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 4 | 441.010 | 5 | 0.91 | 0.47 |
| Treatment | 6 | 4554.460 | 58 | 6.26 | 0.00 |
| Error | 24 | 2910.830 | 37 | | |
| Total | 34 | 7906.30 | 100 | | |

Appendix 3.8 Analysis of variance (ANOVA) of seven different dilutions of fermented plant extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits on stem diameter of tomato plant.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 4 | 6.920 | 11 | 1.19 | 0.34 |
| Treatment | 6 | 22.930 | 35 | 2.63 | 0.04 |
| Error | 24 | 34.884 | 54 | | |
| Total | 34 | 64.733 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 4 | 3.668 | 23 | 2.85 | 0.05 |
| Treatment | 6 | 4.651 | 29 | 2.41 | 0.05 |
| Error | 24 | 7.735 | 48 | | |
| Total | 34 | 16.054 | 100 | | |

Appendix 4.1 Analysis of variance (ANOVA) of seven different dilutions of fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits on dry root mass of tomato plant.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 9 | 6.981 | 8 | 0.84 | 0.54 |
| Treatment | 6 | 34.826 | 38 | 6.28 | 0.00 |
| Error | 54 | 49.899 | 54 | | |
| Total | 69 | 91.707 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 9 | 3.110 | 5 | 0.43 | 0.91 |
| Treatment | 6 | 16.607 | 26 | 3.45 | 0.01 |
| Error | 54 | 43.297 | 69 | | |
| Total | 69 | 63.014 | 100 | | |

Appendix 4.2 Analysis of variance (ANOVA) of seven different dilutions of fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits on dry shoot mass of tomato plant.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 9 | 72.758 | 19 | 2.87 | 0.01 |
| Treatment | 6 | 164.345 | 42 | 9.73 | 0.00 |
| Error | 54 | 151.940 | 39 | | |
| Total | 69 | 389.043 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 9 | 30.512 | 11 | 0.96 | 0.48 |
| Treatment | 6 | 49.480 | 18 | 2.34 | 0.04 |
| Error | 54 | 190.197 | 71 | | |
| Total | 69 | 270.189 | 100 | | |

Appendix 4.3 Analysis of variance (ANOVA) of seven different dilutions of fermented crude extracts of *Cucumis africanus* fruit on plant height of tomato plant.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 9 | 803.170 | 20 | 2.24 | 0.03 |
| Treatment | 6 | 1158.030 | 28 | 4.85 | 0.00 |
| Error | 54 | 2151.030 | 52 | | |
| Total | 69 | 4112.230 | 100 | | |

Appendix 4.4 Analysis of variance (ANOVA) of seven different dilutions of fermented crude extracts of *Cucumis africanus* fruit on stem diameter of tomato plant.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 9 | 8.771 | 19 | 1.85 | 0.08 |
| Treatment | 6 | 8.752 | 19 | 2.77 | 0.02 |
| Error | 54 | 28.411 | 62 | | |
| Total | 69 | 45.934 | 100 | | |

Appendix 4.5 Analysis of variance (ANOVA) of seven different dilutions of fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits on nematode numbers in roots of tomato plant.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 9 | 0.028 | 2 | 0.94 | 0.49 |
| Treatment | 6 | 1.087 | 84 | 52.72 | 0.00 |
| Error | 54 | 0.185 | 14 | | |
| Total | 69 | 1.301 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 9 | 2.093 | 3 | 0.49 | 0.88 |
| Treatment | 6 | 50.988 | 64 | 16.04 | 0.00 |
| Error | 54 | 26.573 | 33 | | |
| Total | 69 | 79.655 | 100 | | |

Appendix 4.6 Analysis of variance (ANOVA) of seven different dilutions of fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits on nematode numbers in soil of tomato plant.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 9 | 192405 | 8 | 0.96 | 0.94 |
| Treatment | 6 | 1034273 | 44 | 19.77 | 0.00 |
| Error | 54 | 1144581 | 48 | | |
| Total | 69 | 2371259 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 9 | 8.321 | 13 | 1.43 | 0.20 |
| Treatment | 6 | 18.908 | 30 | 4.64 | 0.00 |
| Error | 54 | 35.194 | 57 | | |
| Total | 69 | 62.423 | 100 | | |

Appendix 4.7 Analysis of variance (ANOVA) of seven different dilutions of fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits on total nematode numbers in root and soil of tomato plant.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 9 | 0.069 | 3 | 1.08 | 0.39 |
| Treatment | 6 | 1.781 | 82 | 50.76 | 0.00 |
| Error | 54 | 0.316 | 15 | | |
| Total | 69 | 2.166 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 9 | 5.562 | 10 | 1.52 | 0.16 |
| Treatment | 6 | 29.606 | 50 | 16.04 | 0.00 |
| Error | 54 | 23.558 | 40 | | |
| Total | 69 | 58.726 | 100 | | |

Appendix 5.1 Analysis of variance (ANOVA) of dry fruit mass of tomato treated with 3% dilution of fermented crude extracts of *Cucumis africanus* fruit applied at 0, 2, 3 and 4 weeks in a 30-day month period.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 11 | 994.160 | 18 | 1.13 | 0.36 |
| Treatment | 4 | 862.390 | 16 | 2.69 | 0.04 |
| Error | 44 | 3520.520 | 66 | | |
| Total | 59 | 5377.070 | 100 | | |

Appendix 5.2 Analysis of variance (ANOVA) of stem diameter of tomato treated with 3% dilution of fermented crude extracts of *Cucumis africanus* fruit applied at 0, 2, 3 and 4 weeks in a 30-day month period.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 11 | 65.742 | 31 | 2.31 | 0.02 |
| Treatment | 4 | 28.566 | 14 | 2.75 | 0.04 |
| Error | 44 | 114.074 | 55 | | |
| Total | 59 | 208.382 | 100 | | |

Appendix 5.3 Analysis of variance (ANOVA) of nematode numbers in root of tomato, treated with 3% dilution of fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits applied at 0, 2, 3 and 4 weeks in a 30-day month period.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 11 | 1.762 | 4 | 0.61 | 0.81 |
| Treatment | 4 | 34.656 | 72 | 33.12 | 0.00 |
| Error | 44 | 11.511 | 24 | | |
| Total | 59 | 47.929 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 11 | 2.876 | 10 | 1.40 | 0.20 |
| Treatment | 4 | 17.218 | 61 | 23.12 | 0.00 |
| Error | 44 | 8.190 | 29 | | |
| Total | 59 | 28.284 | 100 | | |

Appendix 5.4 Analysis of variance (ANOVA) of total nematode numbers in root + soil of tomato, treated with 3% dilution of fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits applied at 0, 2, 3 and 4 weeks in a 30-day month period.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 11 | 0.741 | 3 | 0.66 | 0.77 |
| Treatment | 4 | 17.280 | 77 | 42.11 | 0.00 |
| Error | 44 | 4.514 | 20 | | |
| Total | 59 | 22.534 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 11 | 5.111 | 21 | 3.14 | 0.00 |
| Treatment | 4 | 12.749 | 52 | 21.53 | 0.00 |
| Error | 44 | 6.514 | 27 | | |
| Total | 59 | 24.373 | 100 | | |

Appendix 5.5 Analysis of variance (ANOVA) of nematode numbers in tomato roots, treated with 6% dilution of fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits applied at 0, 2, 3 and 4 weeks in a 30-day month period.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 11 | 0.621 | 8 | 0.91 | 0.54 |
| Treatment | 4 | 4.200 | 56 | 16.88 | 0.00 |
| Error | 44 | 2.737 | 36 | | |
| Total | 59 | 7.557 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | DF | SS | Percent | F | P ≤ |
| Replication | 11 | 0.561 | 2 | 0.36 | 0.96 |
| Treatment | 4 | 17.721 | 72 | 31.30 | 0.00 |
| Error | 44 | 6.227 | 26 | | |
| Total | 59 | 24.509 | 100 | | |

Appendix 5.6 Analysis of variance (ANOVA) of nematode numbers in soil of tomato, treated with 6% dilution of fermented crude extracts of *Cucumis africanus* fruit applied at 0, 2, 3 and 4 weeks in a 30-day month period.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 11 | 0.215 | 16 | 1.07 | 0.41 |
| Treatment | 4 | 0.332 | 24 | 4.53 | 0.00 |
| Error | 44 | 0.808 | 60 | | |
| Total | 59 | 1.355 | 100 | | |

Appendix 5.7 Analysis of variance (ANOVA) of total nematode numbers in root + soil of tomato, treated with 6% dilution of fermented crude extracts of *Cucumis africanus* and *Cucumis myriocarpus* fruits applied at 0, 2, 3 and 4 weeks in a 30-day month period.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 11 | 0.043 | 4 | 0.87 | 0.57 |
| Treatment | 4 | 0.657 | 63 | 21.04 | 0.00 |
| Error | 44 | 0.343 | 33 | | |
| Total | 59 | 1.043 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 11 | 2.561 | 12 | 2.66 | 0.04 |
| Treatment | 4 | 13.554 | 64 | 29.99 | 0.00 |
| Error | 44 | 4.971 | 24 | | |
| Total | 59 | 21.086 | 100 | | |

Appendix 6.1 Analysis of variance (ANOVA) of dry fruit mass of tomato plant treated with five levels of dried fruit of *Cucumis myriocarpus* and four levels of application time interval.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 5 | 486.30 | 1 | 1.42 | 0.23 |
| Concentration | 4 | 3638.50 | 11 | 3.64 | 0.01 |
| Time | 3 | 1169.40 | 4 | 1.56 | 0.20 |
| C x T | 12 | 4079.70 | 12 | 1.36 | 0.20 |
| Error | 95 | 23710.40 | 72 | | |
| Total | 119 | 33084.40 | 100 | | |

Appendix 6.2 Analysis of variance (ANOVA) of dry root mass of tomato plant treated with five levels of dried fruit of *Cucumis africanus* and four levels of application time interval.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 5 | 13.88 | 4 | 0.95 | 0.45 |
| Concentration | 4 | 3.83 | 1 | 0.38 | 0.82 |
| Time | 3 | 9.12 | 3 | 1.19 | 0.32 |
| C x T | 12 | 50.86 | 16 | 1.67 | 0.09 |
| Error | 95 | 241.73 | 76 | | |
| Total | 119 | 318.93 | 100 | | |

Appendix 6.3 Analysis of variance (ANOVA) of dry shoot mass of tomato plant treated with five levels of dried fruit of *Cucumis myriocarpus* and four levels of application time interval.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 5 | 2472.30 | 7 | 0.73 | 0.54 |
| Concentration | 4 | 2208.60 | 6 | 2.16 | 0.08 |
| Time | 3 | 664.20 | 2 | 0.87 | 0.46 |
| C x T | 12 | 6892.60 | 19 | 2.25 | 0.01 |
| Error | 95 | 24268.70 | 66 | | |
| Total | 119 | 36506.50 | 100 | | |

Appendix 6.4 Analysis of variance (ANOVA) of fruit number of tomato plant treated with five levels of dried fruit of *Cucumis myriocarpus* and four levels of application time interval.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 5 | 0.18 | 2 | 0.18 | 0.91 |
| Concentration | 4 | 0.59 | 8 | 2.50 | 0.05 |
| Time | 3 | 0.04 | 0 | 0.21 | 0.89 |
| C x T | 12 | 1.40 | 18 | 2.00 | 0.03 |
| Error | 95 | 5.54 | 72 | | |
| Total | 119 | 7.74 | 100 | | |

Appendix 6.5 Analysis of variance (ANOVA) of nematode numbers in roots of tomato plant treated with five levels of dried fruit of *Cucumis africanus* and *Cucumis myriocarpus* and four levels of application time interval.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 5 | 2.177 | 3 | 1.00 | 0.42 |
| Concentration | 4 | 27.499 | 36 | 16.15 | 0.00 |
| Time | 3 | 2.593 | 3 | 2.03 | 0.11 |
| C x T | 12 | 3.547 | 5 | 0.69 | 0.75 |
| Error | 95 | 40.428 | 53 | | |
| Total | 119 | 76.245 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 5 | 4.935 | 7 | 2.12 | 0.07 |
| Concentration | 4 | 22.059 | 30 | 12.08 | 0.00 |
| Time | 3 | 0.175 | 0 | 0.13 | 0.94 |
| C x T | 12 | 4.122 | 6 | 0.75 | 0.70 |
| Error | 95 | 43.357 | 58 | | |
| Total | 119 | 74.649 | 100 | | |

Appendix 6.6 Analysis of variance (ANOVA) of calcium (Ca) in leaves of tomato plant treated with five levels of dried fruit of *Cucumis africanus* and four levels of application time interval.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 5 | 0.010 | 0 | 1.37 | 0.26 |
| Concentration | 4 | 0.011 | 33 | 8.47 | 0.00 |
| Time | 3 | 0.010 | 0 | 2.63 | 0.06 |
| C x T | 12 | 0.011 | 33 | 7.58 | 0.00 |
| Error | 95 | 0.011 | 33 | | |
| Total | 119 | 0.053 | 100 | | |

Appendix 6.7 Analysis of variance (ANOVA) of magnesium (Mg) in leaves of tomato plant treated with five levels of dried fruit of *Cucumis africanus* and four levels of application time interval.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 5 | 0.067 | 6 | 0.48 | 0.70 |
| Concentration | 4 | 0.217 | 21 | 3.67 | 0.01 |
| Time | 3 | 0.067 | 9 | 0.59 | 0.62 |
| C x T | 12 | 0.398 | 19 | 2.20 | 0.02 |
| Error | 95 | 1.433 | 46 | | |
| Total | 119 | 2.147 | 100 | | |

Appendix 6.8 Analysis of variance (ANOVA) of phosphorus (P) in leaves of tomato plant treated with five levels of dried fruit of *Cucumis africanus* and four levels of application time interval.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 5 | 0.494 | 40 | 3.50 | 0.00 |
| Concentration | 4 | 0.376 | 30 | 3.00 | 0.00 |
| Time | 3 | 0.376 | 30 | 4.20 | 0.00 |
| C x T | 12 | 0.000 | 0 | 10.02 | 0.00 |
| Error | 95 | 0.000 | 0 | | |
| Total | 119 | 1.246 | 100 | | |

Appendix 6.9 Analysis of variance (ANOVA) of potassium (K) in leaves of tomato plant treated with five levels of dried fruit of *Cucumis africanus* and four levels of application time interval.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 5 | 2.176 | 22 | 0.32 | 0.81 |
| Concentration | 4 | 2.211 | 22 | 11.15 | 0.00 |
| Time | 3 | 0.051 | 1 | 0.34 | 0.79 |
| C x T | 12 | 0.835 | 8 | 1.40 | 0.18 |
| Error | 95 | 4.711 | 47 | | |
| Total | 119 | 9.984 | 100 | | |

Appendix 6.10 Analysis of variance (ANOVA) of manganese (Mn) in leaves of tomato plant treated with five levels of dried fruit of *Cucumis africanus* and four levels of application time interval.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 5 | 0.906 | 6 | 5.03 | 0.00 |
| Concentration | 4 | 3.306 | 21 | 10.74 | 0.00 |
| Time | 3 | 1.461 | 9 | 6.33 | 0.00 |
| C x T | 12 | 3.048 | 19 | 3.30 | 0.00 |
| Error | 95 | 7.312 | 46 | | |
| Total | 119 | 16.034 | 100 | | |

Appendix 6.11 Analysis of variance (ANOVA) of sodium (Na) in leaves of tomato plant treated with five levels of dried fruit of *Cucumis africanus* and four levels of application time interval.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 5 | 33.630 | 3 | 0.94 | 0.43 |
| Concentration | 4 | 149.420 | 12 | 4.13 | 0.00 |
| Time | 3 | 27.920 | 2 | 1.03 | 0.38 |
| C x T | 12 | 217.390 | 17 | 2.00 | 0.03 |
| Error | 95 | 859.540 | 67 | | |
| Total | 119 | 1287.90 | 100 | | |

Appendix 6.12 Analysis of variance (ANOVA) of sulfur (S) in leaves of tomato plant treated with five levels of dried fruit of *Cucumis africanus* and four levels of application time interval.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 5 | 0.067 | 3 | 5.05 | 0.00 |
| Concentration | 4 | 0.847 | 35 | 21.54 | 0.00 |
| Time | 3 | 0.177 | 7 | 5.99 | 0.00 |
| C x T | 12 | 0.373 | 16 | 3.17 | 0.00 |
| Error | 95 | 0.933 | 39 | | |
| Total | 119 | 2.397 | 100 | | |

Appendix 6.13 Analysis of variance (ANOVA) of zinc (Zn) in leaves of tomato plant treated with five levels of dried fruit of *Cucumis africanus* and four levels of application time interval.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 5 | 4.686 | 5 | 10.17 | 0.00 |
| Concentration | 4 | 11.274 | 12 | 6.88 | 0.00 |
| Time | 3 | 17.621 | 19 | 14.33 | 0.00 |
| C x T | 12 | 21.062 | 23 | 4.28 | 0.00 |
| Error | 95 | 38.931 | 42 | | |
| Total | 119 | 93.574 | 100 | | |

Appendix 7.1 Analysis of variance (ANOVA) of plant height of tomato plant treated with dosages of *Cucumis africanus* and *Cucumis myriocarpus* fruits.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 13 | 2134.490 | 24 | 0.80 | 0.65 |
| Treatment | 2 | 1349.430 | 15 | 3.29 | 0.05 |
| Error | 26 | 5328.570 | 60 | | |
| Total | 41 | 8812.490 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 13 | 833.650 | 9 | 0.32 | 0.98 |
| Treatment | 2 | 3107.530 | 34 | 7.72 | 0.00 |
| Error | 26 | 5230.230 | 57 | | |
| Total | 41 | 9171.410 | 100 | | |

Appendix 7.2 Analysis of variance (ANOVA) of dry shoot mass of tomato plant treated with dosages of *Cucumis myriocarpus* fruit.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 13 | 22501.000 | 18 | 0.69 | 0.75 |
| Treatment | 2 | 35127.000 | 29 | 7.02 | 0.00 |
| Error | 26 | 65023.000 | 53 | | |
| Total | 41 | 122651.000 | 100 | | |

Appendix 7.3 Analysis of variance (ANOVA) of dry root mass of tomato plant treated with dosages of *Cucumis myriocarpus* fruit.

| SOURCE | Df | SS | Percent | F | P ≤ |
|---|---|---|---|---|---|
| Replication | 13 | 126.472 | 34 | 1.42 | 0.21 |
| Treatment | 2 | 67.955 | 18 | 4.96 | 0.01 |
| Error | 26 | 177.938 | 48 | | |
| Total | 41 | 372.366 | 100 | | |

Appendix 7.4 Analysis of variance (ANOVA) of nematode numbers in roots of tomato plant treated with dosages of *Cucumis africanus* and *Cucumis myriocarpus* fruits.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 13 | 1.627 | 16 | 1.80 | 0.42 |
| Treatment | 2 | 5.544 | 54 | 23.83 | 0.00 |
| Error | 26 | 3.025 | 30 | | |
| Total | 41 | 10.196 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 13 | 1.575 | 20 | 1.05 | 0.44 |
| Treatment | 2 | 3.380 | 42 | 14.65 | 0.00 |
| Error | 26 | 2.999 | 38 | | |
| Total | 41 | 7.954 | 100 | | |

Appendix 7.5 Analysis of variance (ANOVA) of nematode numbers in soil of tomato plant treated with dosages of *Cucumis africanus* and *Cucumis myriocarpus* fruits.

| | | *Cucumis africanus* | | | |
|---|---|---|---|---|---|
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 13 | 1.641 | 15 | 1.35 | 0.25 |
| Treatment | 2 | 6.649 | 62 | 35.49 | 0.00 |
| Error | 26 | 2.435 | 23 | | |
| Total | 41 | 10.725 | 100 | | |
| | | *Cucumis myriocarpus* | | | |
| SOURCE | Df | SS | Percent | F | P ≤ |
| Replication | 13 | 1.335 | 15 | 0.68 | 0.42 |
| Treatment | 2 | 4.351 | 50 | 18.40 | 0.00 |
| Error | 26 | 3.074 | 35 | | |
| Total | 41 | 8.760 | 100 | | |