# NEMARIOC-AL AND NEMAFRIC-BL PHYTONEMATICIDES: BIOACTIVITIES IN *MELOIDOGYNE INCOGNITA*, TOMATO CROP, SOIL TYPE AND ORGANIC MATTER

by

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THESIS

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DECLARATION

I declare that the thesis hereby submitted to the University of Limpopo, for the degree Doctor of Philosophy in Agriculture (Plant Production) has not been submitted previously by me for a degree at this or any other University; that it is my work in design and in execution, and that all material contained herein had been duly acknowledged.

| | |
|---|---|
| Dube, Z.P. (Mr) | Date |

# DEDICATION

To my beloved parents, Vashee Palane and Christinah Dube, my lovely wife, Tsitsi Belinda Dube and son, Gabadzo Palane Dube.

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ABSTRACT

Nemarioc-AL and Nemafric-BL phytonematicides, had been researched and developed from indigenous plants at the University of Limpopo, Green Technologies Research Centre, under the auspices of the Indigenous Cucurbitaceae Technologies (ICT) Research Programme. After the international 2005 cut-off withdrawal date of the highly effective methyl bromide nematicide from the agrochemical markets, management options on nematode population densities shifted to more environment-friendly alternatives. Nemarioc-AL and Nemafric-BL phytonematicides as environment-friendly alternatives to synthetic chemical nematicides had been consistent in nematode suppression under diverse conditions. In order to avoid challenges similar to those experienced with the use of synthetic chemical nematicides, the South African Fertiliser, Farm Feeds, Agricultural Remedies and Stock Remedies Act No. 36 of 1947 (amended) require that the product to be used in agriculture must first be registered with the National Department of Agriculture, Forestry and Fisheries, after extensive efficacy and bioactivity tests. The information on bioactivity of the phytonematicides is also critical in the effective application of the product for efficient management of nematodes. Information on bioactivities of Nemarioc-AL and Nemafric-BL phytonematicides on nematodes, plant and soil was not available. This study comprised eight objectives: (1) to examine whether (i) increasing concentration of cucurbitacin A and B would have impact on second-stage juvenile (J2) hatch of *M. incognita*, (ii) the Curve-fitting Allelochemical Response Dosage (CARD) model would quantify the three phases of density-dependent growth (DDG) patterns on J2 hatch when exposed to increasing cucurbitacin concentrations, (iii) computed J2 hatch inhibition concentration (EHIC) and

CARD-generated D-values would be statistically similar, (iv) the CARD model would provide information on minimum inhibition concentration (MIC) and (v) J2 hatch inhibition would be reversible when cucurbitacins were diluted, (2) to determine whether (i) increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides would have impact on J2 hatch of *M. incognita*, (ii) the CARD model would quantify the three phases of DDG pattern on J2 hatch when compared to increasing phytonematicide concentrations, (iii) comparison of computed EHIC and CARD-generated D-values would be statistically comparable in magnitudes, (iv) the CARD model would provide information on MIC and (v) J2 hatch inhibition would be reversible when phytonematicides were diluted, (3) to establish whether (i) increasing concentration of cucurbitacin A and B would have impact on *M. incognita* J2 immobility, (ii) the CARD model would quantify the three phases of DDG pattern on J2 immobility when compared to increasing cucurbitacin concentration, (iii) comparison of computed J2 immobility concentration and CARD-generated D-values would be statistically comparable in magnitudes, (iv) the CARD model would provide information on MIC and (v) juvenile immobility would be reversible when cucurbitacins were diluted, (4) to test whether (i) increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides would have impact on *M. incognita* J2 immobility, (ii) the CARD model would quantify the three phases of DDG pattern on J2 immobility when compared to increasing phytonematicide concentrations, (iii) comparison of computed J2 immobility concentration and CARD-generated D-values would be statistically comparable in magnitudes, (iv) the CARD model would provide information on MIC and (v) juvenile immobility would be reversible when phytonematicides were diluted, (5) to determine whether (i) increasing

concentration of cucurbitacin A and B would have impact on *M. incognita* J2 mortality, (ii) the CARD model would quantify the three phases of DDG patterns on J2 mortality when compared to increasing cucurbitacin concentration, (iii) comparison of computed lethal concentration (LC) and CARD-generated D-values would be statistically comparable in magnitudes and (iv) the CARD model would provide information on minimum lethal concentration (MLC), (6) to investigate whether (i) increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides would have impact on *M. incognita* J2 mortality, (ii) the CARD model would quantify the three phases of DDG pattern on J2 mortality when compared to increasing phytonematicide concentrations, (iii) comparison of computed LC and CARD-generated D-values would be statistically comparable in magnitudes and (iv) the CARD model would provide information on MLC, (7) to test whether (i) increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides would impact on *M. incognita* J2 infectivity of susceptible tomato plant, (ii) the CARD model would quantify the three phases of DDG pattern (iii) generated inhibition concentration (IC) and CARD-generated D-values would be statistically comparable in magnitudes and (iv) the CARD model would provide information on MIC and (8) to determine whether nematodes can serve as bioindicators of Nemarioc-AL and Nemafric-BL phytonematicides in tomato plant roots/fruits, soil types and organic matter at different depths. To achieve these objectives, reliability of measured variables was ensured by using statistical levels of significance ($P \leq 0.05$) and coefficient of determination ($R^2$), with validity ensured by conducting three independent experiments over time. In Objective 1, pure cucurbitacin A and B concentration effects on J2 hatch were significant, with both exhibiting DDG patterns.

The DDG patterns demonstrated that J2 hatch was inhibited at low pure cucurbitacin concentrations and slightly stimulated at higher cucurbitacin concentrations. At 24-, 48- and 72-h exposure periods, cucurbitacin A reduced J2 hatch by 40–67, 34–66 and 34–45%, respectively, whereas cucurbitacin B reduced J2 hatch by 12–57, 3–36 and 9–54%, respectively. CARD model quantified the concentration ranges of the two pure cucurbitacins associated with the phases of DDG patterns. The J2 hatch was highly sensitive to cucurbitacin B and highly tolerant to cucurbitacin A, as shown by sensitivities values of 0–2 and 5–20 units, respectively. The CARD-generated MIC-values for cucurbitacin A and B were 1.75–2.88 and 1.31–1.88 $\mu g.mL^{-1}$, respectively. The conventionally generated J2 hatch inhibition concentrations were higher than CARD-generated D-values at all exposure periods for both pure cucurbitacins. The J2 hatch inhibition effect was not reversible for both pure cucurbitacins. In Objective 2, Nemarioc-AL and Nemafric-BL phytonematicide concentration effects on J2 hatch were highly significant ($P \leq 0.01$), with both exhibiting DDG patterns. The DDG patterns demonstrated that J2 hatch inhibition increased with increase in phytonematicide concentrations. Relative to water control, Nemarioc-AL phytonematicide significantly reduced J2 hatch at 48-, 72-h and 7-d by 22–92, 3–79 and 1–42%, respectively, whereas Nemafric-BL phytonematicide reduced it by 41–93, 1–80 and 12–84%, respectively. The J2 hatch inhibition was highly sensitive to Nemarioc-AL and Nemafric-BL phytonematicides, with sensitivity of 0–1 and 0–4 units, respectively. The conventionally generated J2 hatch inhibition concentrations at 50 and 100% were higher than CARD-generated D-values for both phytonematicides. The J2 hatch inhibition effect was not reversible for both phytonematicides. In Objective 3, pure cucurbitacin A

and B concentration effects on J2 immobility were significant, with both exhibiting DDG patterns. The J2 immobility over increasing concentrations of pure cucurbitacins had DDG patterns which were similar for conventional method and those from CARD model. The DDG patterns were characterised by stimulation of J2 immobility at low concentrations, followed by saturation at higher concentrations. The CARD model could not generate the D-values for comparison with JMC-values, but generated MIC-values for cucurbitacin A and B which were 0.5–0.6 and 0.5–0.7 $\mu g.mL^{-1}$, respectively. The J2 immobility was moderately sensitive to both cucurbitacins with sensitivity of 4 units and the inhibition effect of the two pure cucubitacins was not reversible. In Objective 4, Nemarioc-AL and Nemafric-BL phytonematicide concentration effects on J2 immobility were highly significant ($P \leq 0.01$), with both phytonematicides exhibiting DDG patterns. The DDG pattern had stimulation, saturation and inhibition effects for Nemarioc-AL phytonematicide, whereas for Nemafric-BL phytonematicide they had stimulation and saturation effects on J2 immobility as concentrations increased. The MIC-values for Nemarioc-AL and Nemafric-BL phytonematicides were 3.6–115.2 and 0.1–6.5%, respectively. The CARD generated D-values were comparable with computed JMC-values for Nemafric-BL phytonematicide unlike for Nemarioc-AL phytonematicide. The J2 immobility was highly sensitive to the two phytonematicides with sensitivity values of 0–4 and 0–2 units, respectively. The effects on J2 immobility of the two phytonematicides were not reversible. In Objective 5, pure cucurbitacin A and B concentration effects on J2 mortality were highly significant ($P \leq 0.01$), with both cucurbitacins exhibiting DDG patterns. The DDG pattern had stimulation, saturation and slight inhibition effects for both cucurbitacin A and B as concentrations increased. The

MIC-values for cucurbitacin A and B were 0.63 and 0.61 µg.mL$^{-1}$, respectively. The CARD-generated D-values were higher than the computed LC-values for both cucurbitacin A and B, with J2 mortality being highly sensitive to cucurbitacin A and B, with sensitivity of 4 units for both cucurbitacins. In Objective 6, Nemarioc-AL and Nemafric-BL phytonematicide effects on J2 mortality were highly significant ($P \leq 0.01$), with both phytonematicides exhibiting DDG patterns. The DDG pattern had stimulation effect at low phytonematicide concentrations and saturation effects at higher concentrations for both relative impact and CARD-generated graphs of J2 exposed to both phytonematicides. The MIC-values for Nemarioc-AL and Nemafric-BL phytonematicides were 1.12 and 0.67%, respectively. The CARD-generated D-values were higher than the computed LC-values for both phytonematicides and J2 mortalities were highly sensitive to Nemarioc-AL and Nemafric-BL phytonematicides with sensitivity value of 2 and 1 units, respectively. In Objective 7, Nemarioc-AL and Nemafric-BL phytonematicide concentrations had a highly significant effect on infectivity of *M. incognita* post-exposure on susceptible tomato seedlings. The relationship between infectivity and increasing concentrations of the two phytonematicides exhibited DDG patterns. The DDG patterns were characterised by stimulation effect at low Nemarioc-AL phytonematicide concentrations and saturation effects at higher phytonematicide concentrations, whereas for Nemafric-BL phytonematicide slight inhibition, saturation and stimulation effects were observed. The CARD-generated inhibition concentrations for Nemarioc-AL phytonematicide were comparable with computed inhibition concentrations, whereas for Nemafric-BL phytonematicides, the values were not comparable. The MIC-values for Nemarioc-AL and Nemafric-BL phytonematicides were

0.2 and 0.7%, respectively and J2 infectivity were highly sensitive to the two phytonematicides, with sensitivity value of 2 and 0 units, respectively. In Objective 8, *M. incognita* was an excellent bioindicator in response to the application of two phytonematicides. The two phytonematicides significantly affected distribution of population densities of *M. incognita* across the tested soil types, with Nemafric-BL phytonematicide reducing population densities of *M. incognita* relative to Nemarioc-AL phytonematicide. The active ingredient of Nemafric-BL phytonematicide, cucurbitacin B tended to remain in the top layers of soil, where more roots accumulated, thereby reducing a relatively higher population densities of *M. incognita* than did active ingredient of Nemarioc-AL phytonematicide, cucurbitacin A which moved with water beyond the effective root zone. Soil type alone and phytonematicide alone had no effect on nematode numbers, whereas the interaction of soil type, phytonematicides and depth, the nematode population densities were inversely proportional to soil depth. The interaction of clay with any of the two phytonematicides, reduced *M. incognita* population densities compared to sand and loam interactions. More than 62% tomato root systems occurred in the top 0–25 cm depth. The interactions between organic matter levels, phytonematicides and depth had no effect on the population densities of *M. incognita*. The two phytonematicides were able to reduce nematode population densities throughout the soil column in all four soil types and organic matter levels. Cucurbitacin residues were not detected in all tomato fruit samples. In conclusion, Nemarioc-AL and Nemafric-BL phytonematicides have bioactivities on J2 hatch, J2 immobility, J2 mortality and J2 infectivity. The CARD model quantified the three phases of DDG patterns for most of the variables. Even though CARD-generated inhibition

concentrations at 50 and 100% were not comparable with computed values for pure cucurbitacins they were for most phytonematicide variables, the model was able to generate excellent MIC-values for all variables. The inhibition effects of the two phytonematicides were irreversible. The major findings of this study were that the two phytonematicides exhibited DDG patterns for all variables tested and that the CARD model could be adopted for the *in vitro* evaluation of phytonematicides. *Meloidogyne incognita* was an excellent bioindicator on movement of two phytonematicides across soil types and organic matter levels at different depths. Nemarioc-AL and Nemafric-BL phytonematicides did not leave any cucurbitacin residues in tomato fruit. The information on bioactivities of the two phytonematicides generated in this study provides a much needed data for the registration of the products as required by the law. Proposed future research area includes, microscopy study of molecular effects of the phytonematicides on nematodes post-exposure.

PUBLICATIONS GENERATED FROM THE THESIS

1. Peer reviewed journals
    a. Dube, Z.P. and P.W. Mashela. 2016. Nemafric-BL phytonematicide induces egg hatch inhibition in Meloidogyne incognita. Acta Agriculturae Scandinavica, Section B - Soil & Plant Science: 66 (5): 384-386.
    b. Dube, Z.P. and P.W. Mashela. 2016. Response of *Meloidogyne incognita* egress and overall sensitivity to active ingredients of Nemarioc-AL and Nemafric-BL phytonematicides. Acta Agriculturae Scandinavica, Section B - Soil & Plant Science DOI. 10.1080/09064710. 2016.1155641.
    c. Dube, Z.P., Mashela, P.W. and D. De Waele. 2016. *In vitro* characterization of *Meloidogyne incognita* egg hatching to Nemarioc-AL phytonematicide concentrations: Using a computer-based model. Transylvanian Review 24(7):954-960.
    d. Dube, Z.P., Mashela, P.W. and D. De Waele. 2016. Nematode egg hatch dynamics in response to a series of cucurbitacins A and B concentrations at different exposure periods. Acta Agriculturae Scandinavica, Section B - Soil & Plant Science (in press).
    e. Dube, Z.P., Mashela, P.W. and D. De Waele. 2016. Density-dependent growth patterns of nematode egg hatch exposed to active ingredients of Nemarioc-AL and Nemafric-BL phytonematicides. Natural Product Research. (in press).
2. Oral Conference presentations
    a. Dube, Z.P., Mashela, P.W., Ndhlala, A.R. and Waele, D. 2016. Density-dependent growth patterns of nematode egress under increasing cucurbitacin

A and cucurbitacin B concentrations: Bioactive ingredients of Nemafric-AL and Nemafric-BL phytonematicides. Combined Congress 2016, 18-21 January, University of the Free State, Bloemfontein, Page, 40.

b. Dube, Z.P., Mashela, P.W., Ndhlala, A.R. and D. De Waele. 2015. Density-dependent growth patterns of nematode egress under increasing cucurbitacin B concentrations: An active ingredient of Nemafric-BL phytonematicide. Faculty of Science and Agriculture Research Day, 1-2 October 2015, Bolivia Lodge, Polokwane. Awarded the best PhD oral presentation-School of Agricultural and Environmental Sciences

3. Poster Conference presentations

a. Dube, Z.P., Shadung, K.G. and Mashela, P.W. 2016. Residual effect of Nemarioc-AL and Nemafric-BL phytonematicides in tomatoes. Page, 171, Combined Congress 2016, 18-21 January, University of the Free State, Bloemfontein, Poster no 10.

Two chapters were used from the thesis to generate the above stated research outputs, the remaining chapters have the potential to generate three journal articles each.

# CHAPTER 1
# RESEARCH PROBLEM

## 1.1 Introduction

International withdrawal of synthetic nematicides from agrochemical markets shifted control to management options on population densities of plant-parasitic nematodes (Mashela *et al*., 2015). Incidentally, both smallholder and large-scale farmers were affected by limited options in the management of nematode population densities since yield losses due to nematode infection in crops without resistance were as high as 50%, to complete crop failure (Manju and Sankari, 2015). Three years prior to the 2005 cut-off date, estimated global annual crop yield losses due to nematode damage were at US$126 billion (Chitwood, 2003). Three and eight years after the cut-off date, the estimated yield losses were at US$157 (Abad *et al*., 2008) and US$173 (Elling, 2013) billions, respectively — the relative increase in yield losses of 25 and 37%, respectively.

Following the withdrawal of the highly effective synthetic chemical nematicides, various environment-friendly strategies were intensively being researched and developed for management of nematode population densities. The strategies included nematode-resistance (Pofu *et al*., 2012), organic amendments (Thoden *et al*., 2011), phytonematicides (Mashela *et al*., 2015) and other biological control agents (Anastasiadis *et al*., 2008; Hashem and Abo-Elyousr, 2011; Kiewnick and Sikora, 2006). In Limpopo Province, South Africa, alternatives to synthetic chemical nematicides in managing nematodes has been focusing on using allelochemicals from crude extracts of selected indigenous plants under the auspices of the

Indigenous Cucurbitaceae Technologies (ICT) Research Programme (Mashela *et al*., 2015), which had been quite successful (Mafeo, 2006; Mashela, 2002; Mashela *et al*., 2015; Pelinganga *et al*., 2013a,b; Pofu, 2012). The ICT Research Programme had since produced two phytonematicide prototypes, which are undergoing final assessment stages for registration.

The two phytonematicides under the ICT Research Programme are Nemarioc-AG or Nemafric-BG and Nemarioc-AL or Nemafric-BL, in granular (G) and liquid (L) formulations. In either formulation, each phytonematicide relies on the same active ingredient, thus similarities in the suffix for Nemarioc-A phytonematicide in granular and Nemarioc-A in liquid formulations, where A represents an active ingredient cucurbitacin A, whereas B in Nemafric-B phytonematicide represents cucurbitacin B. Nemarioc-AL and Nemafric-BL phytonematicides are produced using effective microorganisms (EM) fermented crude extracts of wild cucumber (*Cucumis myriocarpus* Naudin) and wild watermelon (*C. africanus* L.) fruits, respectively (Mashela, 2002; Mashela and Mphosi, 2001). The efficacy of the two phytonematicides was shown to be similar to that of synthetic nematicides, aldicarb and phenamiphos (Mashela *et al*., 2008).

In order to guard against some of the previous oversights in the use of synthetic chemical nematicides, the South African Fertiliser, Farm Feeds, Agricultural Remedies and Stock Remedies Act No. 36 of 1947 (amended) requires that the products to be used in agriculture be first registered with the National Department of Agriculture, Forestry and Fisheries, after undergoing extensive efficacy, phytotoxicity

and bioactivity tests. The comprehensive scientific data must unequivocally demonstrate that the product is effective for the intended purposes and does not pose unacceptable risk to the crops and non-target organisms, animals, people and the environment (Anon., 2012). A wide range of trials had been conducted under the ICT Research Programme in order to comply with the specifications of the Act (Mashela *et al*., 2015). In the current study, the bioactivities of the two phytonematicides on root-knot (*Meloidogyne* species) nematodes were assessed in support of the previous efficacy trials (Maile *et al*., 2013; Pelinganga *et al*., 2013a).

## 1.2 Problem statement

Effectiveness for the intended purposes of products includes data that demonstrate the efficacy of the product in terms of two requirements: (1) Reducing population densities of the target pest and (2) Bioactivities in pest, plant and soil. The effectiveness of Nemarioc-A and Nemafric-B phytonematicides in reducing population densities of *Meloidogyne* species under various environments and cropping systems at the Green Technologies Research Centre, University of Limpopo, is well-documented (Mashela *et al*., 2015). However, the bioactivies of the two phytonematicides on *Meloidogyne* species and their mobility through different soil types, organic matter and plants have not been studied.

One of the characteristics of synthetic chemical nematicides that distinguishes them from the phytonematicides is their single active ingredients, with well-defined bioactivities (Mashela *et al*., 2015). The single active ingredients in synthetic pesticides resulted in high incidents of pest resistance, particularly in those pests

with high reproductive rates (Nzanza and Mashela, 2012). The phyto-pesticides, unlike the synthetic chemical pesticides, have multiple active ingredients, with multiple target sites of action (Mashela *et al*., 2015). The use therefore, of phyto-pesticides could provide a broad spectrum of active ingredients, with multiple modes of action. In insects, the multiple modes of action of phyto-insecticides were shown to include serving as antifeedants and repellents, delaying and preventing moulting, reduced growth, development and oviposition and in some instances, they even caused death (Nzanza and Mashela, 2012). However, the mode of action is poorly documented in nematology for phytonematicides, except that they had been limited to second-stage juvenile (J2) hatch, chemotaxis, J2 motility and J2 mortality (Mashela *et al*., 2015). Generally, modes of action had been conventionally assessed using logit (Haas *et al*., 1999), log-logistic (Wu *et al*., 2000) and probit analysis (Finney, 1952).

The logit and log-logit had been used in a number of dose-response toxicological studies, whereas the probit analysis had been ideally used in dose-response trials in a variety of fields mainly in crop protection (Azhagumurugan and Rajan, 2014, 2015; Ibrahim *et al*., 2006; Wuyts *et al*., 2006). The extensive uses of probit analysis also encompassed allelochemical-dose response trials (Wuyts *et al*., 2006). Liu *et al*. (2003) demonstrated that dose-response relationships in microorganism-allelochemical relations have an inverted U-shape, a phenomenon that is not captured by other methods of analysis (Liu *et al*., 2003). To address this challenge, Liu *et al*. (2003) introduced the Curve-fitting Allelochemical Response Dosage (CARD) computer-based model, which was adapted for the use in allelochemical-

dosage response trials (Mafeo, 2012; Mashela *et al*., 2015; Pelinganga, 2013). The model generated biological indices that quantify phases of density-dependent growth (DDG) patterns (Mashela *et al*., 2015). The CARD-generated biological indices (Liu *et al*., 2003) had been effectively explored in a wide range of conditions that included field, microplot and greenhouse trials (Mashela *et al*., 2015) in an effort to address the two major demerits of phytonematicides, namely, phytotoxicity and inconsistent results (Mashela *et al*., 2015). In general bioactivities against nematodes using the CARD model focused much on $D_{50}$, $D_{100}$ and $\sum k$, with the first two being viewed as equivalents to concentration inhibiting 50% and 100% of test organism ($L_{50}$ and $L_{100}$), respectively.

The study intended to investigate bioactivities of Nemarioc-AL and Nemafric-BL phytonematicides on the behavioural responses of *M. incognita*, their potential residues in tomato fruit and movements through different soil types and organic matter, through the aid of the CARD model.

1.3 Rationale

Nemarioc-AL and Nemafric-BL phytonematicides had been tested on *Meloidogyne* species and the citrus nematode (*Tylenchulus semipenetrans* Cobb) under various cropping systems with the results suggesting that the two phytonematicides were consistently effective on nematode suppression (Mathabatha *et al*., 2016; Pelinganga *et al*., 2013a,b). However, for effective management of nematodes, information on their mode of action is a prerequisite. Phytonematicides have been reported as safe and less persistent in the environment (Stirling, 2014), but the

presence of any phytochemical in the environment and/or produce would be highly undesirable, primarily because in small quantities, the cucurbitacins could be carsinogenic (Lee *et al*., 2010), as were most synthetic chemical nematicides (Pope, 2014).

## 1.4 Aim and objectives

### 1.4.1 Aim

The aim of this study was to establish the bioactivity protocols of Nemarioc-AL and Nemafric-BL phytonematicides on *M. incognita*, tomato fruit, four soil types (calcareous, clay, loam and sand) and organic matter levels.

### 1.4.2 Objectives

The study comprised eight objectives:

To examine whether (i) increasing concentrations of cucurbitacin A and B would have impact on J2 hatch of *M. incognita*, (ii) the CARD model would quantify the three phases of the DDG patterns on J2 hatch when compared to increasing cucurbitacin concentrations, (iii) computed J2 hatch inhibition concentration (EHIC) and CARD-generated inhibition dosage (D)-values would be statistically comparable in magnitudes, (iv) the CARD model would provide information on minimum inhibition concentration (MIC) and (v) J2 hatch inhibition would be reversible when cucurbitacins were diluted.

To determine whether (i) increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides would have impact on J2 hatch of *M. incognita*, (ii) the CARD model would quantify the three phases of DDG pattern on J2 hatch when compared

to increasing phytonematicide concentrations, (iii) computed EHIC and CARD-generated D-values would be statistically comparable in magnitudes, (iv) the CARD model would provide information on MIC and (v) J2 hatch inhibition would be reversible when phytonematicides were diluted.

To establish whether (i) increasing concentration of cucurbitacin A and B would have impact on *M. incognita* J2 immobility, (ii) the CARD model would quantify the three phases of DDG pattern on J2 immobility when compared to increasing cucurbitacin concentration, (iii) computed J2 immobility concentration and CARD-generated D-values would be statistically comparable in magnitudes, (iv) the CARD model would provide information on MIC and (v) J2 immobility would be reversible when cucurbitacins were diluted.

To test whether (i) increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides would have impact on *M. incognita* J2 immobility, (ii) the CARD model would quantify the three phases of DDG pattern on J2 immobility when compared to increasing phytonematicide concentrations, (iii) computed J2 immobility concentration and CARD-generated D-values would be statistically comparable in magnitudes, (iv) the CARD model would provide information on MIC and (v) J2 immobility inhibition would be reversible when phytonematicides were diluted.

To determine whether (i) increasing concentration of cucurbitacin A and B would have impact on *M. incognita* J2 mortality, (ii) the CARD model would quantify the three phases of DDG patterns on J2 mortality when compared to increasing cucurbitacin concentration, (iii) computed lethal concentration (LC)- and CARD-generated D-values would be statistically comparable in magnitudes and (iv) the CARD model would provide information on minimum lethal concentration (MLC).

To investigate whether (i) increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides would have impact on *M. incognita* J2 mortality, (ii) the CARD model would quantify the three phases of DDG pattern on J2 mortality when compared to increasing phytonematicide concentrations, (iii) computed LC and CARD-generated D-values would be statistically comparable in magnitudes and (iv) the CARD model would provide information on MLC.

To test whether (i) increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides would have an impact on *M. incognita* J2 infectivity of susceptible tomato plant, (ii) the CARD model would quantify the three phases of DDG pattern on *M. incognita* J2 infectivity when compared to increasing phytonematicide concentrations, (iii) computed infectivity inhibition concentration (IC) and CARD-generated D-values would be statistically comparable in magnitudes and (iv) the CARD model would provide information on MIC.

To determine whether nematodes can serve as bioindicators of Nemarioc-AL and Nemafric-BL phytonematicides in tomato plant roots/fruits, soil types and organic matter at different depths.

## 1.5 Hypotheses

Increasing concentration of cucurbitacin A and B would have impact on J2 hatch of *M. incognita* J2, (ii) the CARD model would quantify the three phases of DDG pattern on J2 hatch when exposed to increasing cucurbitacin concentrations, (iii) comparison J2 hatch inhibition concentration (EHIC) and CARD-generated D-values would be statistically comparable in magnitudes, (iv) the CARD model would provide

information on minimum inhibition concentration (MIC) and (v) J2 hatch inhibition would be reversible when cucurbitacins were diluted.

Increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides would have impact on J2 hatch of *M. incognita*, (ii) the CARD model would quantify the three phases of DDG pattern on J2 hatch when compared to increasing phytonematicide concentrations, (iii) comparison of computed EHIC and CARD-generated D-values would be statistically comparable in magnitudes, (iv) the CARD model would provide information on MIC and (v) J2 hatch inhibition would be reversible when phytonematicides were diluted.

Increasing concentration of cucurbitacin A and B would have impact on *M. incognita* J2 immobility, (ii) the CARD model would quantify the three phases of DDG pattern on J2 immobility when compared to increasing cucurbitacin concentration, (iii) comparison of computed J2 immobility concentration and CARD-generated D-values would be statistically comparable in magnitudes, (iv) the CARD model would provide information on MIC and (v) J2 immobility would be reversible when cucurbitacins were diluted.

Increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides would have impact on *M. incognita* J2 immobility, (ii) the CARD model would quantify the three phases of DDG pattern on J2 immobility when compared to increasing phytonematicide concentrations, (iii) comparison of computed J2 immobility concentration and CARD-generated D-values would be statistically comparable in magnitudes, (iv) the CARD model would provide information on MIC and (v) juvenile immobility would be reversible when phytonematicides were diluted.

Increasing concentration of cucurbitacin A and B would have impact on *M. incognita* J2 mortality, (ii) the CARD model would quantify the three phases of DDG patterns on J2 mortality when compared to increasing cucurbitacin concentration, (iii) comparison of computed lethal concentration (LC) and CARD-generated D-values would be statistically comparable in magnitudes and (iv) the CARD model would provide information on minimum lethal concentration (MLC).

Increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides would have impact on *M. incognita* J2 mortality, (ii) the CARD model would quantify the three phases of DDG pattern on J2 mortality when compared to increasing phytonematicide concentrations, (iii) comparison of computed LC and CARD-generated D-values would be statistically comparable in magnitudes and (iv) the CARD model would provide information on MLC.

Increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides would impact on *M. incognita* J2 infectivity of susceptible tomato plant, (ii) the CARD model would quantify the three phases of DDG pattern on *M. incognita* J2 infectivity when compared to increasing phytonematicide concentrations, (iii) computed infectivity inhibition concentration (IC) and CARD-generated D-values would be statistically comparable in magnitudes and (iv) the CARD model would provide information on MIC.

Nematodes can serve as bioindicators of Nemarioc-AL and Nemafric-BL phytonematicides in tomato plant roots/fruits, soil types and organic matter at different depths.

## 1.6 Reliability, validity and objectivity

Reliability is described previously 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). Validity was ensured by conducting the experiment at the same location over time (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 was achieved by discussing the findings on the basis of empirical evidence as shown by statistical analyses, with findings compared and contrasted with findings in other studies (Little and Hills, 1981).

## 1.7 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).

## 1.8 Ethical considerations

In this study, the commercial use of the indigenous plants to Limpopo Province, as initiated by the University of Limpopo, is envisioned. The researcher would ensure that moral or legal rights of any potential claimants by the University were respected. The University policies, appropriate legal framework and ethical considerations as outlined here would endure beyond the completion of the study.

## 1.9 Significance of the study

The study was intended to clarify bioactivities of Nemarioc-AL and Nemafric-BL phytonematicides on *M. incognita*, tomato plant, soil types and organic matter levels, thereby providing the required information to expedite the registration of Nemarioc-AL and Nemafric-BL phytonematicides in terms of Act No. 36 of 1947 (amended). Currently, there is minimal work done on the effects of cucurbitacins on phytoparasitic nematodes (Chitwood, pers. comm.).

## 1.10 Format of thesis

Following the description and detailed outlining of the research problem (Chapter 1), the work done and not yet done on the problem statement was reviewed (Chapter 2). Then, each of the subsequent chapters (Chapter 3–10) addressed each of the objectives in sequence. In the final chapter (Chapter 11), findings in all chapters were summarised and integrated to provide the significance of the findings and recommendations with respect to future research, culminating in a conclusion which tied the entire study together. In the text and references the Harvard style, along with

U.K. English, as approved by Senate of the University of Limpopo, were used. Also, each chapter would be a stand alone, with its own list of references.

## 1.11 References


ABAD, P., GOUZY, J., AURY, J.M., CASTAGNONE-SERENO, P., DANCHIN, E.G., DELEURY, E., PERFUS-BARBEOCH, L., ANTHOUARD, V., ARTIGUENAVE, F., BLOK, V.C., *et al*. 2008. Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. *Nature Biotechnology* 26:909–915.

ANASTASIADIS, I.A., GIANNAKOU, I.O., PROPHETOU-ATHANASIADOU, D.A. and S.R. GOWEN. 2008. The combined effect of the application of a biocontrol agent *Paecilomyces lilacinus*, with various practices for the control of root-knot nematodes. *Crop Protection* 27:352–361.

ANON., 2012. The US Tomato Industry, Seed-quest, Central Information. http://www.ers.usda.gov.

AZHAGUMURUGAN, C. and M.K. RAJAN. 2014. Effect of leaf extract of nilakumil, (*Gmelina asiatica*) against the root-knot nematode (*Meloidogyne incognita*). *Research Journal of Recent Sciences* 3:264–266.

AZHAGUMURUGAN, C. and M.K. RAJAN. 2015. Effect of leaf extracts of selected toxic plants on egg hatchability and larval mortality of root-knot nematode, *Meloidogyne incognita*. *Academic Journal of Entomology* 8:92–95.

BERENSON, M.L. and D.M. LEVINE. 1996. Basic Business Statistics: Concepts and Applications. Prentice-Hall, Englewood Cliffs, New Jersey.

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

ELLING, A.A. 2013. Major emerging problems with minor *Meloidogyne* species. *Phytopathology* 103:1092–1102.

FINNEY, D.J. 1952. Probit Analysis. Cambridge University Press, Cambridge.

HAAS, C.N., ROSE, J.B. and C.P. GERBA. 1999. Quantitative Microbial Risk Assessment. John Wiley and Sons, Toronto.

HASHEM, H. and K.A. ABO-ELYOUSR. 2011. Management of the root-knot nematode, *Meloidogyne incognita* on tomato with combinations of different biocontrol organisms. *Crop Protection* 30:285–292.

IBRAHIM, S.K., TRABOULSI, A.F. and S. EL-HAJ. 2006. Effect of essential oils and plant extracts on hatching, migration and mortality of *Meloidogyne incognita*. *Phytopathologia Mediterranea* 45:238–246.

KIEWNICK, S. and R.A. SIKORA. 2006. Biological control of the root-knot nematode *Meloidogyne incognita* by *Paecilomyces lilacinus* strain 251. *Biological Control* 38:179–187.

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

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

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

LIU, D.L., AN, M., JOHNSON, I.R. and J.V. LOVETT. 2003. Mathematical modeling of allellopathy. III. A model for curve-fitting allellochemical dose responses. *Non-linearity Biology, Toxicology and Medicine* 1:37–50.

MAFEO, T.P. 2006. Propagation, Fertilisation and Irrigation of *Cucumis myriocarpus.* MSc. Dissertation Submitted to the University of Limpopo. Sovenga, South Africa.

MAFEO, T.P. 2012. Responses of Economically Important Crops to Crude Extracts of *Cucumis* Fruit when Used as Pre-emergent Bio-nematicide. PhD Thesis, University of Limpopo. Sovenga, South Africa.

MAILE, K.D., MASHELA, P.W. and P.E. TSEKE. 2013. Responses of the citrus nematode to a phytonematicide nemarioc-AG with and without micro-organisms in citrus production. *African Crop Science Conference Proceedings* 11:333–337.

MANJU, P. and M.K. SANKARI. 2015. Antinemic properties of the botanicals. A review. *International Journal of Science and Nature* 6:125–134.

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

MASHELA, P.W. and M.S. MPHOSI. 2001. Wild cucumber fruit residue reduces population densities of *Meloidogyne incognita* in tomato production under greenhouse conditions. *Proceedings of Nematological Society of South Africa* 15:43.

MASHELA, P.W., SHIMELIS, H.A. and F.N. MUDAU. 2008. Comparison of the efficacy of ground wild cucumber fruits, aldicarb and fenamiphos on

suppression of the root-knot nematode in tomato. *Journal of Phytopathology* 156:264–267.

MASHELA, P.W., DUBE, Z.P. and K.M. POFU. 2015. Managing the Phytotoxicity and Inconsistent Nematode Suppression in Soil Amended with Phytonematicides. In: Meghvansi, M.K. and A. Vorma (eds.). Organic Amendments and Soil Suppressiveness in Plant Disease Management, Soil Biology 46. Springer International Publishers, Switzerland.

MATHABATHA, R.V., MASHELA, P.W. and N.M. MOKGALONG. 2016. Sensitivity of Nemarioc-AL and Nemafric-BL phytonematicides to *Citrus volkameriana* seedling rootstocks. *Translyvanian Review* (*in press*).

NZANZA, B. and P.W. MASHELA. 2012. Control of whiteflies and aphids in tomato by fermented plant extracts of neem leaf and wild garlic. *African Journal of Biotechnology* 11:16077–16082.

PELINGANGA, O.M. 2013. Developing Bio-nematicides Using Indigenous *Cucumis africanus* and *Cucumis myriocarpus* Fruits for Tomato Production System. PhD Thesis, University of Limpopo. Sovenga, South Africa.

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

PELINGANGA, O.M., MASHELA, P.W., MPHOSI, M.S., MAFEO, T.P. and Z.P. DUBE. 2013b. Using computer-based model to determine phytotoxicity concentration of Nemarioc-A phytonematicide in tomato production. *African Crop Science Conference Proceedings* 11:349–353.

POFU, K.M. 2012. Potential Uses of Indigenous *Cucumis africanus* and *Cucumis myriocarpus* As Root-knot Nematode-resistant Rootstocks in Watermelon (*Citrullus lanatus*) Husbandry. PhD Thesis Submitted to the University of Limpopo. Sovenga, South Africa.

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

POPE, C. 2014. Pesticides. *Encylopedia of Toxicology* 3:826–827.

STIRLING, G.R. 2014. Biological Control of Plant Parasitic Nematodes: Soil Ecosystem Management in Sustainable Agriculture. CAB International, Wallingford.

THODEN, T.C., KORTHALS, G.W. and A.J. TERMORSHUIZEN. 2011. Organic amendments and their influences on plant-parasitic and free-living nematodes: A promising method for nematode management. *Nematology* 13:133–153.

WU, H., PRATLEY, J., LEMERLE, D. and T. HAIG. 2000. Laboratory screening for allelopathic potential of wheat (*Triticum aestivum*) accessions against annual ryegrass (*Lolium rigidum*). *Australian Journal of Agricultural Research* 51:259–2660.

WUYTS, N., SWENNEN, R. and D. DE WAELE. 2006. Effects of plant phenylpropanoid pathway products and selected terpenoids and alkaloids on the behavior of the plant-parasitic nematodes *Radopholus similis*, *Pratylenchus penetrans* and *Meloidogyne incognita. Nematology* 8:89–101.

# CHAPTER 2
# LITERATURE REVIEW

## 2.1 Introduction

Management of plant-parasitic nematodes in cropping systems is indispensable if crop enterprises are to be profitable and thereby improving food security, job creation and wealth creation as envisaged in the National Development Plan of South Africa (Mashela *et al*., 2015). Following the withdrawal of highly effective synthetic chemical nematicides due to their environment-unfriendliness, various environment-friendly nematode management strategies have been tested for the suppression of nematode population densities, which included nematode-resistance (Pofu *et al*., 2012), organic amendments (Thoden *et al*., 2011), phytonematicides (Mashela *et al*., 2015) and other biological control agents. The major setbacks in the use of the four strategies had limited their large-scale commercial uses. This review focuses exclusively on phytonematicides.

Phytonematicides as an alternative management strategy in nematode suppression has had some successes (Chedekal, 2013; Mashela *et al*., 2015; Okwute, 2012; Pelinganga *et al*., 2013). Nemarioc-AL and Nemafric-BL phytonematicides are being researched and developed at the Green Technologies Research Centre, University of Limpopo, South Africa. The two phytonematicides are produced from fermented dried fruits of wild cucumber (*Cucumis myriocarpus* Naudin*)* and wild watermelon (*C. africanus* L.*)*, respectively (Pelinganga and Mashela, 2012). The two phytonematicides had since been tested on nematode population densities of root-knot (*Meloidogyne* species) nematode and the citrus nematode (*Tylenchulus*

*semipenetrans* Cobb) under various cropping systems (Maile, 2013; Pelinganga *et al*., 2012; Seshweni *et al*., 2016; Sithole *et al*., 2016). The major challenges in the use of phytonematicides in general had been phytotoxicities (Pelinganga *et al*., 2013) and inconsistent results in nematode suppression (McSorley, 2011). The Curve-fitting Allelochemical Response Dosage (CARD)-computer based model was adopted to enhance the management of the observed challenges, particularly the phytotoxicities (Pelinganga *et al*., 2013). The CARD model generates biological indices used in the explanation of the density-dependent growth (DDG) patterns that exist between organisms exposed to increasing concentration of phytonematicide (Liu *et al*., 2003; Mashela *et al*., 2015). Conversional methods for determining DDG patterns are laborious and at times are not repeatable (Inderjit, 2001). Mashela *et al*. (2015) provided the basis for successful uses of the two phytonematicides in nematode management, without any information on mode of action of the products on nematodes. The objective of this study was to review work done and not yet done on mode of action of plant-parasitic nematodes using phytonematicides.

## 2.2 Work done on the problem statement

### 2.2.1 Phytonematicides and organic amendments

Phytonematicides as an alternative comprise a wide range of forms, which include aqueous plant extracts (Chedekal, 2013; Rossner and Zebitz, 1987), methanol plant extracts (Usman, 2013), ethanol plant extracts (Khan *et al*., 2008), oil cakes (Muller and Gooch, 1982), essential oils (Meyer *et al*., 2008), fermented crude plant extracts (Pelinganga and Mashela, 2012; Pelinganga *et al*., 2013), powders (Ahmad *et al*., 2013) and granules (Mashela *et al*., 2011). Aqueous extracts of moringa (*Moringa*

*oleifera* Lam), african basil (*Ocimum gratissium* L.) and neem (*Azadirachta indica* A. Juss) (Cladius-Cole *et al*., 2010) and aqueous extracts of baker tree (*Milletia ferruginea* Hochst), bitter leaf (*Vernonia amygodalina* Delile), parthenium (*Parthenium hysterophorus* L.), lantana (*Lantana camara* L.), mexican marigold (*Tagetes minuta* L.), mexican tea (*Chenopodium ambrosioides* L.), *A. indica* and pyrethrum (*Chrysanthemum cinerariafolium* L.) had significantly reduced root-knot (*Meloidogyne incognita* Kofoid & White) nematode J2 hatching and enhanced J2 mortality (Taye *et al.,* 2013). Castor bean (*Ricinus communis* L.) and clove (Syzygium aromaticum L.) oils were observed to have immobilising effects on *M. incognita* J2 (Katooli *et al*., 2010; Meyer *et al*., 2008), whereas neem oils reduced nematode population densities in the soil (Javed *et al*., 2008). Dry leaf powders of rock fleabane (*Inula viscose* L.) (Oka *et al*., 2001) and dry neem leaves (Khan *et al*., 2012) had nematicidal effects on *M. incognita*.

In contrast, organic amendments include crop residues, manure, compost, agro-industrial wastes and sewage sludges (Castagnone-Sereno and Kermarrec, 1991; D'Addabbo, 1995; Stirling, 2014; Thoden *et al*., 2011). Neem cake and mustard (*Sinapis arvensis* L.) cake applied as organic amendments were effective in the suppression of root-knot nematodes. Powder of cocoa bean (*Theobroma cacao* L.) testa and palm fruit (*Elaeis guineensis* Jacq) fiber oil applied as mulch significantly reduced the damage caused by *M. incognita* (Ojo and Umar, 2013), whereas dry leaves of *A. indica,* king's crown (*Calotropis procera* Aiton*),* Angel's Trumpet (*Datura stramonium* L.)*,* sunn hemp (*Crotolarza juncea* L.) and chinese chastetree (*Vitex negundo* L.) were effective in reducing root lesion nematode (*Pratylenchus coffeae*

Goodey*)* infesting banana (Sundararaju *et al*., 2003). Root bark of peony (*Paeonia suffructicosa* Andrews) and stem barks of amur cork tree (*Phellodendron amurense* Rupr) and Chinese cinnamon (*Cinnamomum cassia* Nees & Nees) were able to reduce *M. incognita* population densities (Ferris and Zheng, 1999).

Phytonematicides were introduced by Mashela (2002) to mitigate the drawbacks of conventional organic amendments in suppression of nematodes. The latter included (1) inconsistent results, (2) large quantities required to achieve adequate suppression, (3) unavailability of materials, (4) high transport costs, (5) negative period and (6) decreased soil pH which interfered with availability of some essential nutrient elements for plant growth (Belair and Tremblay, 1995; Kimpinski *et al*., 2003; Mashela 2002; Stirling, 2014; Thoden *et al*., 2011). The phytonematicides are produced from locally collected indigenous plants (Mashela *et al*., 2011) which possess a complex allelochemical compounds (Chitwood, 2002; Okwute, 2012). The allelochemicals are produced by plants for protection against pests and to give the plant competitive advantage against other plants in the environment (Inderjit and Foy, 1999; Rice, 1984).

The major distinctions between phytonematicides and organic amendments are (1) in ground form the active ingredients of phytonematicides are gradually released into the rhizosphere through leaching by irrigation water or rainfall, whereas organic amendments are released through microbial degradation, (2) phytonematicides mimic synthetic chemical nematicides since they could be commercially packaged in relatively small containers, (3) phytonematicides just like non-fumigant nematicides

do not have negative periods and could therefore be applied as post-planting products (Mashela *et al*., 2015).

### 2.2.2 Plants with nematicidal properties

Many plants have been identified as being antagonistic against plant-parasitic nematodes (Manju and Sankari, 2015). The antagonistic properties of these plants stems from their ability to produce secondary volatile and non-volatile exudates from different parts. The physiological roles of these secondary metabolites are unknown but they are thought to contribute towards the defense of plants against various pests (Manju and Sankari, 2015). Various pathways are involved in the production of these chemicals, with the major ones being the shikimic acid pathway, malonic acid pathway and mevalonic acid pathway (Lai, 2008; Mashela *et al*., 2015).

Manju and Sankari (2015) identified 91 plant species as the most commonly used plant species from 32 families out of over 620 families in the plant kingdom. These families constitute only 5% of all plant families with the potential for use. Fabaceae, Asteraceae, Apocynaceae and Lamiaceae had the highest number of species used in the management of nematodes contributing 15, 13, 10 and 9%. Meliaceae family had few plant species with antagonistic properties to nematodes even though *A. indica* in this family is one of the most studied of all plant species in nematode management, with a wide range of commercial products registered not only as nematicides but also as insecticides, fungicides and miticides (Chitwood, 2002). In all the plants identified by Manju and Sankari (2015) the leaf extracts were the most used sources of phytonematicides followed by seeds, roots, flowers, bulbs, fruits,

stems and rhizomes at 70, 9, 7, 2, 1, 1 and 0.7%, respectively. *Azadirachta indica* is the only plant where all its parts have been tested against nematodes and found to possess bioactive properties. Eighty-nine percent of the plants were found to have bioactivities on *Meloidogyne* species (Manju and Sankari, 2015). Mashela *et al*. (2015) classified 372 South African medicinal plants into six groups using their degree of toxicity to humans and animals, with less than 10% tested for their nematicidal properties. Mashela *et al*. (2015) demonstrated that the toxicity to humans and animals had no bearing on the status of the plants to serve as a source of phytonematicides.

### 2.2.3 Mode of action in phytonematicides

One major distinction between synthetic nematicides and phytonematicides is on the mode of action. Most synthetic nematicides have a single active ingredient, with well-defined mode of action. A single active ingredient confers a single mode of action, but with high incidents of pest resistance, particularly in pests with high reproductive capabilities (Nzanza and Mashela, 2012). In contrast, phytonematicides have multiple action ingredients, with complementary modes of action, which had been limited to J2 hatch, J2 mobility, J2 chemotaxis and J2 mortality (Mashela *et al*., 2015; Wuyts *et al*., 2006), without any information on behavioural responses of adult nematodes.

J2 hatch: The J2 hatch in nematodes is mainly a physical process, involving increased J2 movements. As movements intensify, J2 continuously presses its stylet against the egg shell, tearing it in the process (Bohlmann, 2015; Curtis, 2008; Perry

and Moens, 2011). Even though J2 hatch is a physical process, in most plant-parasitic nematodes, it is stimulated by external cues in the environment (Mashela *et al*., 2015). The stimulation is made possible by a number of chemoreceptors, which cover the frontal and cervical regions (Matsuura *et al*., 2007; McSorley, 2003). A number of plant extracts have been shown to possess some bioactivities on nematode J2 hatch. Such plant species include, garlic *(Allium sativum* L.*),* chrysathemum (*Chrysathemum coronarium* L.) and fennel (*Foeniculum vulgare* Mill) (Ibrahim *et al*., 2006), mugwort (*Artemisia vulgaris* L.*)* (Costa *et al*., 2003), *A. indica* (Javed *et al*., 2008), *I. viscose* (Oka *et al*., 2001), white cedar *(Melia azedarach* L.*)* and elderberry (*Sambucus nigra* L.) (Akyazi, 2014) and *Tagetes* sp. (Kalaiselvam and Devaraj, 2011). Density-dependent growth responses have been observed in most studies of these plant extracts with majority of reports showing an inverse relationship between J2 hatch suppression and the increasing concentrations (Akyazi, 2014; Javed *et al*., 2008; Kalaiselvam and Devaraj, 2011). The nematode or plant responses to increasing concentration of phytonematicides have DDG patterns (Mashela *et al*., 2015). The mechanisms related to J2 hatch inhibition include interference with stylet development, disruption of lipid parts of cell membranes by lipophilic extracts, interference with cytokinesis without affecting karyokinesis resulting in multinucleated cells and may also induce cell cycle arrest (Lee *et al*., 2010).

J2 mobility: The effect of plant crude extracts on nematode J2 mobility has received less attention when compared with J2 hatch and mortality. Oka *et al*. (2000) working with 27 different essential oils observed that twelve could inhibit 80% *M. javanica* J2

mobility. Crude extracts of *A. sativum* and *A. indica* each exhibited a density-dependent response when J2 were exposed to different concentrations (Agbenin *et al*., 2005). Wuyts *et al*. (2006) observed that some extracts were not only concentration-dependent, but also different nematodes responded differently to the same chemical compound. Density-dependent responses in J2 mobility inhibition to increasing concentrations had been observed where multiple range of extract concentrations were used (Abdul, 2013; Azhagumurugan and Rajan, 2014). *Caenorhabditis elegens* and *Heterodera glycine* J2 mobility was inhibited at low concentrations of geldanamycin, whereas at higher concentrations J2 mobility was stimulated (Skantar *et al*., 2005). Javed *et al*. (2007) working with few concentrations of neem extracts on *M. javanica* observed only the J2 mobility inhibition.

Chemotaxis: Chemotaxis as described by Mashela *et al*. (2015) is the phenomenon where nematode movement is affected by the gradient of the chemical cues. Movement towards the chemical is referred to as positive chemotaxis and chemicals that induce it are called chemoattractants, whereas movement away from the chemical cue is called negative chemotaxis and the chemicals involved are called chemorepellents (Hida *et al*., 2015; Rasmann *et al*., 2012; Reynolds *et al*., 2010). In the rhizosphere the nematode is exposed to both liquid and airborne volatilised chemicals. The nematode is adapted to this environment through various chemoreceptors located mainly on the frontal and cervical regions (Hida *et al*., 2015; Matsuura *et al*., 2007; Rasmann *et al*., 2012). The nematode response to chemoattractants and chemorepellents play a critical role in the behaviour of the nematode helping them to adapt. Plants release numerous chemicals through

exudation, leaching, volatilisation and microbial degradation and these induce various responses on the nematode (Mashela *et al*., 2015). Phytonematicides release the potent chemicals through the same ways (Mashela *et al*., 2011). Wuyts *et al*. (2006) working with pure extracts of rain tree (*Philenoptera violacea* Klotzsch) in the Fabaceae family observed that chemotaxis effect was dependent on the nematode species. Among the tested chemicals, 26% had repellent effect on burrowing nematode (*Radopholus similis* Cobb), 2.6% had attractant effect, whereas 45% had no effect (Wuyts *et al*., 2006). Chemoattractant phytonematicides work by causing disorientation of the nematode, thereby delaying penetration and attack of host by the nematode (Mashela *et al*., 2015), whereas chemorepellents cause a number of behavioural changes including paralysis and death.

J2 mortality: A number of crude plant extracts and pure extracts had been found to be lethal to nematodes (Archana and Prasad, 2014; Manners, 2007; Ntalli and Caboni, 2012). *In vitro* studies of essential oil from true myrtle (*Myrtus communis* L.) showed 100% mortalities of *M. incognita* (Archana and Prasad, 2014). Extracts from *D. stramonium* and *A. indica* (Nelaballe and Mukkara, 2013), *Moringa* species (Claudius-Cole *et al*., 2010) and *A. vulgaris* and *A. sativum* (Ibrahim *et al*., 2006), all have displayed lethal properties to *Meloidogyne* species. Crude extracts of either cocoa bean testa or oil palm fibre resulted in high mortalities of *M. javanica* (Ojo and Umar, 2013).

### 2.2.4 Nemarioc-AL and Nemafric-BL phytonematicides

*Cucumis* species are used as raw materials in the production of Nemarioc-AL and Nemafric-BL phytonematicides (Pelinganga and Mashela, 2012). Work done in South Africa in the management of plant-parasitic nematodes using *C. myriocarpus* and *C. africanus* resulted in the development of a research niche called Indigenous Cucurbitaceae Technologies (ICT). Cucurbitaceae family of which the two *Cucumis* species belong, has 115 genera (Schaefer and Renner, 2011), most of which have been widely used for centuries in African traditional medicine (Mashela *et al*., 2015). South Africa has been identified as the center of biodiversity for the two *Cucumis* species where they have been widely used as food and traditional medicine (Kristkova *et al*., 2003; Mashela *et al*., 2011). Bioactive compounds in *Cucumis* species have been isolated and identified as cucurbitacins (Jeffrey, 1978). Plants in the Cucurbitaceae family contain a total of 12 cucurbitacins (Chen *et al*., 2005).

Nemarioc-A and Nemafric-B phytonematicides are two phytonematicides being researched and developed under ICT niche as alternatives to methyl bromide in South Africa. The two are produced from fruits of *Cucumis* species and are available in granular formulation as Nemarioc-AG and Nemafric-BG phytonematicides (Mashela *et al*., 2011), and in liquid formulation as Nemarioc-AL and Nemafric-BL phytonematicides (Pelinganga *et al.,* 2013). The ground formulation was developed mainly for small scale farmers because it is labour-intensive and hence, not cost effective for large-scale commercial farmers. The liquid formulation was therefore, produced to serve the large-scale farmers through its compatibility with irrigation in a

technology called botinemagation used in tomato (*Solanum lycopersicum* L.) production (Pelinganga and Mashela, 2012).

Nemarioc-AL and Nemafric-BL phytonematicides are produced from effective microbe fermented mature fruits of *C. myriocarpus* and *C. africanus*, respectively. The active ingredients in the two phytonematicides are cucurbitacin A ($C_{32}H_{46}O_8$) and cucurbitacin B ($C_{32}H_{46}O_9$), respectively. The two cucurbitacins are oxygenated tetracyclic triterpenes with glycosides and originate from the mevalonic acid pathway (Mashela *et al*., 2015). The nonpolar cucurbitacin B is insoluble in water (Chen *et al*., 2005), whereas the slightly polar cucurbitacin A is partially soluble in water and rapidly oxidising to cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Chen *et al*., 2005).

Efficacy of Nemarioc-AG phytonematicide: The suppressive potential of Nemarioc-AG phytonematicide on population densities of *M. incognita* have been done extensively (Mashela, 2002; Mashela and Mphosi, 2001; Mashela *et al*., 2008; Muedi *et al*., 2005). The product suppressed population densities of *Meloidogyne* species in roots by 78–92% and in soil by 81–98% (Mashela, 2002, 2007; Mashela and Mphosi, 2001; Mashela and Mphosi, 2002; Mashela and Pofu, 2012). In comparative trials, Nemarioc-AG, aldicarb and phenamiphos reduced population densities of *M. incognita* by 83–99% in roots, but had no significant differences (Mashela *et al*., 2008).

Efficacy of Nemarioc-AL phytonematicide: The product from fresh fruits reduced population densities of *M. incognita* in roots by 46–99% and in soil by 53–96% (Pelinganga, 2013; Pelinganga and Mashela, 2012; Pelinganga *et al*., 2011). Nemarioc-AL phytonematicide reduced nematode numbers in roots by 78–99% and in soil by 7–90% (Pelinganga *et al*., 2013).

Efficacy of Nemafric-BL phytonematicide: Under various conditions, Nemafric-BL phytonematicide from fresh fruit reduced *M. incognita* in roots by 64–99% and soil by 38–97% (Pelinganga, 2013). The same product from dried fruits also reduced *M. incognita* in roots by 85–97% and in the soil by 45–96% (Pelinganga, 2013; Pelinganga *et al*., 2012).

Efficacy on *Tylenchulus semipenetrans*: Generally, the efficacy of the two phytonematicides on population densities of *T. semipenetrans* is limited to the materials in granular formulations. In an *in vitro* trial, Nemarioc-AG phytonematicide resulted in 83–96% *T. semipenetrans* J2 mortalities (Muedi *et al*., 2005). When assessed at 56 days after application, Nemarioc-AG phytonematicide reduced *T. semipenetrans* population densities by at least 90% in both roots and soil (Mashela, 2007). However, when assessed at 150 days after application, Nemarioc-AG phytonematicide reduced *T. semipenetrans* population densities by 22% in roots, but increased the numbers in soil by 93% (Maile, 2013). Similarly, when assessed at 150 days after application, Nemafric-BG phytonematicide reduced *T. semipenetrans* population densities by 80% in roots, but increased the nematode population densities in soil by 178% (Maile, 2013). Observations where the two products

appeared to increase population densities were explained on the basis of the cyclic growth of nematode densities (Maile, 2013; Pofu and Mashela, 2014). Generally, soon after application the products reduced nematode population densities, whereas under untreated controls the nematode population densities increased, resulting in a situation where growth of the population densities from the two treatments remained permanently opposed (Mashela *et al*., 2015).

### 2.2.5 Curve-fitting Response Dosage

Generally, biological systems respond to extrinsic and intrinsic factors in a DDG patterns, which are characterised by three growth phases, namely, stimulation, saturation (neutral) and inhibition phases (Mashela *et al*., 2015). Conversional methods of determining DDG patterns are tedious and usually result in inconsistent results (Mashela *et al*., 2015). The model was developed to quantify the DDG response patterns of biological entities to increasing concentration of allelochemicals (Liu *et al*., 2003).

The CARD model quantifies DDG patterns using seven biological indices, namely, (1) threshold stimulation ($D_m$) — the dosage at which the allelochemicals begins to have a measurable stimulation effect, (2) saturation point ($R_h$) — the dosage at which response is neutral before decreasing, (3) 0% inhibition ($D_0$) — the end-point dosage of $R_h$ where the allelochemical has zero effect, (4) 50% inhibition ($D_{50}$) — the dosage where the allelochemical inhibits 50%, (5) 100% inhibition ($D_{100}$) — the dosage where the allelochemical inhibits by 100%, (6) k — the number of In(D+1)

transformation that serves as a biological indicator of the degree of sensitivity in relation to stimulation or inhibition by allelochemical (Figure 2.1).

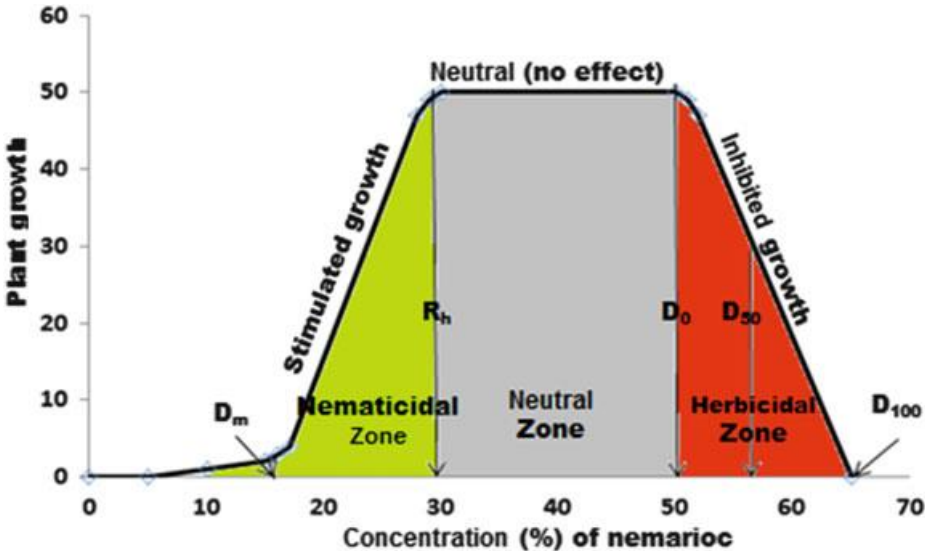


Figure 2.1 Indices of Curve-fitting Response Dosage model. (adopted from Mashela *et al*., 2015).

Usually the k-value starts from zero and increase as discrete numbers, the sensitivity of the test entity to the allelochemical is inversely proportional to the k-values (Mashela *et al*., 2015) and (7) $R^2$ — the coefficient of determination (Liu *et al*., 2003). Pelinganga (2013) and Mafeo (2012) adopted the CARD computer-based model and they successfully quantified all stages of the DDG pattern of different plant growths to increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides and determination of MCSP — a concentration of a phytonematicide which stimulates plant growth, while suppressing population densities of the target pest (Mashela *et al*., 2015, Pelinganga *et al*., 2013).

### 2.2.6 CARD model versus other biological models

There are basically two models that describe the dose-responses which have been extensively used in pest management, namely, probit and logic models. Basically, the two give the same conclusion hence reports state that the preference is a matter of taste (Fahrmeir and Tutz, 2001; Gill *et al*., 2001; Hardin and Hilbe, 2001). The two methods are very complex both at preparing the data for analysis and in the interpretation of the analysed data. Liu *et al*. (2003) developed a highly flexible and simple model for describing the DDG patterns, as described earlier. Unlike the other two models, the CARD model is able to describe the stimulation-inhibition phenomenon reported when allelochemicals are used (Liu *et al*., 2003). The stimulation-inhibition phenomenon describes the relationship between the increasing concentration of allelochemical and response of an organism in a DDG response with three phases, stimulation, neutral and inhibition phases (Mashela *et al*., 2015). Together with the DDG responses, the CARD model also gives the level of sensitivity of the organism or part of it to the allelochemical as a biological index k (Liu *et al*., 2003). The CARD model, when using increasing concentration of allelochemicals, is a fairly easy model to run and interprete.

## 2.3 Movement of phytonematicides in soil

The use of pesticides in agricultural practices has often been linked with improved yields. However, along side improved yields there is the occurrence and the persistence of pesticide residues in the environment (Dem *et al.,* 2007). Globally, around 2.5 million tons of synthetic chemical pesticides had previously been applied each year (FAO, 2002). Synthetic chemical pesticides are highly toxic and persistent

causing several problems such as disrupting natural enemy complex, development of pest resistance, environmental pollution and human health hazard (Adnan *et al*., 2014). The main advantages in the use of botanical pesticides lie in their rapid degradation and lack of persistence and bioaccumulation. A number of studies have reported high levels of synthetic pesticide persistence in agricultural produce and environment when compared with phytopesticides (Arkbar *et al*., 2010; Hassan *et al*., 2005; Sial *et al*., 2009). Arkbar *et al*. (2010) observed that a neem extract, azadirachtin, can be applied up to harvest of cabbage without leaving residues on the leaves and also in the soil. Sial *et al*. (2009) reported that synthetic pesticides could be nearly 1000 times more toxic to non-target organisms and persistent in the environment than phytonematicides. Adnan *et al*. (2014) observed that neem products were less persistent and even less toxic to natural enemies than synthetic pesticides, the same was observed by others (Naqvi *et al*., 2002). Because of these reasons, official organisations such as UN, US Environmental Protection Agency (USEPA) and EU, had been regulating the presence of organic contaminants in soil, but these regulations do not include phytopesticides. Soils are active filters where chemical compounds are degraded by physical, chemical and biological processes (Cavoski *et al*., 2008). The fate of pesticides in soil environment is influenced by the physico-chemical properties of both soil and pesticide (Cavoski *et al*., 2008; Dem *et al*., 2007). Currently, most studies have focused on the effects of synthetic pesticides, whereas the effects of phytopesticides have been overlooked, because they have been considered safer and less damaging than synthetic chemical pesticides (Romero-Gonzalez *et al*., 2015). However, the presence of phytopesticides in the soil is a fact that can have negative effects on the

environment. In order to evaluate the environmental impact of Nemarioc-AL and Nemafric-BL phytonematicides, studies that monitor their residues in the soil matrix and crop produce are necessary to avoid them finding their way into the food, ground and surface water reservoirs.

## 2.4 Work not done on the research problem

The information on bioactivities of Nemarioc-AL and Nemafric-BL phytonematicides in nematodes, crops and soil is not available. Nematode type, plant type and soil type are known to have influence on bioactivities of synthetic nematicides (McKenry, 1994). The CARD model has been adopted in the description of plant growth to increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides but no work is documented on the use of the model in explaining the impacts of phytonematicides on J2 hatch, J2 mobility and J2 mortality. Also the use of CARD model to provide information on minimum inhibition concentrations, overall sensitivity on nematodes to the two phytonematicides is not available. The infectivity of nematodes post-exposure is also critical in the understanding of the variability that occurs at soil-nematode level. Even though phytonematicides are considered safe and less persistent in the environment their presence in the soil is a fact that can have negative effects hence there is a need to determine the movement and distribution of the two phytonematicides in the soil and produce of crop being protected.

## 2.5 References

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

ADNAN, M., BIBI, R., MUSSARAT, S., TARIQ, A. and Z.K. SHINWARI. 2014. Ethnomedicinal and phytochemical review of Pakistani medicinal plants used as antibacterial agents against *Escherichia coli*. *Annals of Clinical Microbiology and Antimicrobials* 13:1.

AGBENIN, N.O., EMECHE, A.M., MARLEY, P.S. and A.D. AKPA. 2005. Evaluation of nematicidal action of some botanicals on *Meloidogyne incognita in vitro*. *Journal of Agriculture and Rural Development in the Tropics and Subtropics* 1:29–39.

AHMAD, F., SIDDIQUI, M.A. and O.O. BABALOLA. 2013. Characterisation of nematicidal activity of plant residues and their application with moisture approach against *Meloidogyne incognita* in tomato. *African Journal of Agricultural Research* 8:93–101.

AKYAZI, F. 2014. Effect of some plant methanol extracts on egg hatching and juvenile mortality of root-knot nematode *Meloidogyne* incognita. *American Journal of Experimental Agriculture* 4:1471–1479.

ARCHANA, U.S. and D. PRASAD. 2014. Management of plant-parasitic nematodes by the use of botanicals. A review. *Journal of Plant Physiology and Pathology* 2:9–16.

ARKBAR, M.F., HAQ, M.A., PARVEEN, F., YASMIN, N. and S.A. SAYEED. 2010. Determination of synthetic and bio-insecticides residues during aphid, *Myzus*

*persicae* (Sulzer) control on cabbage crop through high performance liquid chromatography. *Pakistan Entomologist* 32:155–162.

AZHAGUMURUGAN, C. and M.K. RAJAN. 2014. Effect of leaf extract of nilakumil, (*Gmelina asiatica*) against the root-knot nematode, (*Meloidogyne incognita*). *Research Journal of Recent Sciences* 3:264–266.

BELAIR, G. and N. TREMBLAY. 1995. The influence of chitin-urea amendments applied to an organic soil on a *Meloidogyne hapla* population and on the growth of greenhouse tomato. *Phytoprotection* 76:75–80.

BOHLMANN, H. 2015. Introductory chapter on the basic biology of cyst nematodes. *Advances in Botanical Research* 73:33–59

CASTAGNONE-SERENO, P. and A. KERMARREC. 1991. Invasion of tomato roots and reproduction of *Meloidogyne incognita* as affected by raw sewage sludge. *Supplementary Journal of Nematology* 23:724–728.

CAVOSKI, I., CABONI, P., SARAIS, G. and T. MIANO. 2008. Degradation and persistence of rotenone in soils and influence of temperature variations. *Journal of Agricultural and Food Chemistry* 56:8066–8073.

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

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

CHITWOOD, D.J. 2002. Phytochemical based strategies for nematode control. *Annual Review of Phytopathology Journal* 40:221–249.

CLAUDIUS-COLE, A.O., AMINU, A.E. and B. FAWOLE. 2010. Evaluation of plant extracts in the management of root-knot nematode *Meloidogyne incognita* on cowpea (*Vigna unguiculata* (L) Walp). *Mycopathology* 8:53–60.

COSTA, S.S.R., SANTOS, M.S.N.A. and M.F. RYAN. 2003. Effect of *Artemisia vulgaris* rhizome extracts on hatching, mortality, and plant infectivity of *Meloidogyne megadora*. *Journal of Nematology* 35:437–442.

CURTIS, R.H.C. 2008. Plant-nematode interactions: Environmental signals detected by the nematode's chemosensory organs control changes in the surface cuticle and behavior. *Parasite* 15:310–316.

D'ADDABBO, T. 1995. The nematicidal effect of organic amendments: Review of the literature 1982–1994. *Nematologia Mediterranea* 23:299–305.

DEM, S.B., COBB, J.M. and D.E. MULLINS. 2007. Pesticide Residues in Soil and Water from Four Cotton Growing Areas of Mali, West Africa. *Journal of Agricultural, Food and Environmental Sciences* 1:16–28.

FAHRMEIR, L. and G. TUTZ. 2001. Multivariate Statistical Modelling Based on Generalized Linear Models. Springer, New York.

FERRIS, H. and L. ZHENG. 1999. Plant sources of Chinese herbal remedies: Effects on *Pratylenchus vulnus* and *Meloidogyne javanica*. *Journal of Nematology* 31:241–263.

FOOD AND AGRICULTURAL ORGANIZATION (FAO). 2002. FAO/WHO global forum of food safety regulators. [http://www.fao.org/DOCREP/MEETING/004/ AB428E.HTM Agenda Item 4.2a, GF/ CRD Iran-1].

GILL, K., MEHTA, S.K., MALIK, M.S., MALIK, O.P. and R.K. WALIA. 2001. Toxicity of methanolic leaf extracts and essential oils from various plants to the root-

knot nematode *Meloidogyne incognita*. *Nematologia Mediterranea* 29:219–222.

HARDIN, J. and J. HILBE. 2001. Generalized Linear Models and Extensions. College Station, Stata Press, TX.

HASSAN, M., AHMAD, F., SAGHEER, M., IQBAL, M.F. and M. TARIQ. 2005. Residual persistence of chlorpyrifos, imidacloprid and acephate in brinjal fruit. *Pakistan Entomologist* 27:53–55.

HIDA, H., NISHIYAMAD, H., SAWAD, S., HIGASHIYAMA, T. and H. ARATA. 2015. Chemotaxis assay of plant-parasitic nematodes on a gel-filled microchannel device. *Sensors and Actuators B* 221:1483–1491.

IBRAHIM, S.K., TRABOULSI A.F. and S. EL-HAJ. 2006. Effect of essential oils and plant extracts on hatching, migration and mortality of *Meloidogyne incognita*. *Phytopathologia Mediterranea* 45:238–246.

INDERJIT, K. 2001. Soils: Environmental effect on allelochemical activity. *Agronomy Journal* 93:79–84.

INDERJIT, K. and C.L. FOY. 1999. Nature of the interference mechanism of mugwort (*Artimisia vulgaris*). *Weed Technology* 13:176–182.

JAVED, N., GOWEN, S.R., INAM-UL-HAQ, M., ABDULLAH, K. and F. SHAHINA. 2007. Systemic and persistent effect of neem (*Azadirachta indica*) formulations against root-knot nematodes, *Meloidogyne javanica* and their storage life. *Crop Protection* 26:911–916.

JAVED, N., GOWEN, S.R., EL-HASSAN, S.A., INAM-UL-HAQ, M., SHAHINA, F. and B. PEMBROKE. 2008. Efficacy of neem (*Azadirachta indica*) formulations

on biology of root-knot nematodes (*Meloidogyne javanica*) on tomato. *Crop Protection* 27:36–43.

JEFFREY, C. 1978. Cucurbitaceae. In: Launert, E. (ed.). Flora Zambesiaca Managing Committee, London.

KALAISELVAM, I. and A. DEVARAJ. 2011. Effect of root exudates of *Tagetes* sp. on egg hatching behaviour of *Meloidogyne incognita*. *International Research Journal of Pharmacy* 2:93–96.

KATOOLI, N., MOGHADAM, E.M., TAHERI, A. and S. NASROLLAHNEJAD. 2010. Management of root knot nematode on cucumber with the extract and oil of nematicidal plants. *International Journal of Agricultural Research* 5:582–586.

KHAN, A., SAYED, M., SHAUKAT, S.S. and Z.A. HANDOO. 2008. Efficacy of four plant extracts on nematodes associated with papaya in Sindh, Pakistan. *Nematologia Mediterranea* 36:93–98.

KHAN, M.R., MOHIDDIN, F.A., EJAZ, M.N. and M.M. KHAN. 2012. Management of root-knot disease in eggplant through the application of biocontrol fungi and dry neem leaves. *Turkish Journal of Biology* 36:161–169.

KIMPINSKI, J., GALLANT, C.F., HENRY, R., MACLEOD, J.A., SANDERSON, J.B. and A.V. STURZ. 2003. Effect of compost and manure soil amendments on nematodes and yields of potato and barley: A 7-year study. *Journal of Nematology* 35:289–293.

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

LAI, E. 2008. Secondary Metabolites and Plant Defense. In: Taiz, L. and E. Zeiger (eds.). A Companion to Plant Physiology. I.K. International, New Delhi.

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

LIU, D.L., AN, M., JOHNSON, I.R. and J.V. LOVETT. 2003. Mathematical modeling of allellopathy. III. A model for curve-fitting allellochemical dose responses. *Non-linearity Biology, Toxicology and Medicine* 1:37–50.

MAFEO, T.P. 2012. Responses of Economically Important Crops to Crude Extracts of *Cucumis* Fruit when Used as Pre-emergent Bio-nematicide. PhD Thesis, University of Limpopo. Sovenga, South Africa.

MAILE, K.D. 2013. Responses of *Tylenchulus semipenetrans* to Crude Extracts of Indigenous *Cucumis* Fruits With and Without Effective Micro-organisms in Citrus Production. MSc. Dissertation, University of Limpopo. Sovenga, South Africa.

MANJU, P. and M.K. SANKARI. 2015. Antinemic properties of the botanicals. A review. *International Journal of Science and Nature* 6:125–134.

MANNERS, G.D. 2007. Citrus limonoids: Analysis, Bioactivity and Biomedical prospects. *Journal of Agricultural and Food Chemistry* 55:8285−8294.

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

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

MASHELA, P.W. and M.S. MPHOSI. 2001. Wild cucumber fruit residue reduces population densities of *Meloidogyne incognita* in tomato production under greenhouse conditions. *Proceedings of Nematological Society of South Africa* 15:43.

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

MASHELA, P.W. and K.M. POFU. 2012. Interactive effects of *Meloidogyne incognita* race 2, *Bradyrhizobium japonicum* and crude extracts of *Cucumis myriocarpus* fruit on *Vigna unguiculata*. *Crop Protection* 40:69–72.

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

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

MASHELA, P.W., DUBE, Z.P. and K.M. POFU. 2015. Managing the Phytotoxicity and Inconsistent Nematode Suppression in Soil Amended with Phytonematicides. In: Meghvansi, M.K. and A. Vorma (eds.). Organic Amendments and Soil Suppressiveness in Plant Disease Management, Soil Biology 46. Springer International Publishers, Switzerland.

MATSUURA, T., ENDO, S., IWAMOTO, R., TAKAHASHI, H. and M. ICHINOSE. 2007. Developmental changes in chemotactic response and choice of two attractants, sodium acetate and diacetyl, in the nematode *Caenorhabditis elegans*. *Comparative Biochemistry and Physiology* 147:920–927.

McKENRY, M. 1994. Nematicides. *Encyclopedia of Agricultural Science* 3:87–95.

McSORLEY, R. 2003. Adaptations of nematodes to environmental extremes. *Florida Entomologist* 86:138–142.

McSORLEY, R. 2011. Overview of organic amendments for management of plant-parasitic nematodes, with case studies from Florida. *Journal of Nematology* 43:69–81.

MEYER, S.L.F., LAKSHMAN, D.K., ZASADA, I.A., VINYARD, B.T. and D.J. CHITWOOD 2008. Phytotoxicity of clove oil to vegetable crop seedlings and nematotoxicity to root-knot nematodes. *HortTechnology* 18:631–638.

MUEDI, H.T.H., MASHELA, P.W. and M.C. MATHABE. 2005. Bioactivity of wild cucumber fruit extracts on plant-parasitic nematodes. *Proceedings of Nematological Society of Southern Africa* 17:27.

MULLER, R. and P.S. GOOCH. 1982. Organic amendments in nematode control: An examination of the literature. *Nematropica* 12:319–326.

NAQVI, S.S.M., MUMTAZ, S. SHEREEN A. and M.A. KHAN. 2002. Comparative performance of two methods for proline estimation. *Pakistan Journal of Botany* 34:355–358.

NELABALLE, V.K. and L.D. MUKKARA. 2013. A preliminary study on the nematicidal effect of some local flora on *Meloidogyne incognita* Chitwood

infesting Mulberry. *International Journal of Chemical, Environmental and Biological Sciences* 1:475–477.

NTALLI, N.G. and P. CABONI. 2012. Botanical nematicides: A review. *Journal of Agricultural and Food Chemistry* 60:9929–9940.

NZANZA, B. and P.W. MASHELA. 2012. Control of whiteflies and aphids in tomato by fermented plant extracts of neem leaf and wild garlic. *African Journal of Biotechnology* 11:16077–16082.

OJO, G.T. and I. UMAR. 2013. Evaluation of some botanicals on root-knot nematode (*Meloidogyne javanica*) in tomato (*Lycopersicum esculentum* Mill) in Yola Adamawa State, Nigeria. *Biological Forum – An International Journal* 5:31–36.

OKA, Y., BEN-DANIEL, B. and Y. COHEN. 2001. Nematicidal activity of powder and extracts of *Inula viscose*. *Nematologica* 3:735–742.

OKA, Y., NACAR, S., PUTIEVSKY, E., RAVID, U., YANIV, Z. and Y. SPIEGEL. 2000. Nematicidal activity of essential oils and their components against the root-knot nematode. *Phytopathology* 90:710–715.

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

PELINGANGA, O.M. 2013. Developing Bio-nematicides Using Indigenous *Cucumis africanus* and *Cucumis myriocarpus* Fruits for Tomato Production System. PhD Thesis, University of Limpopo. Sovenga, South Africa.

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

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

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

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

PERRY, R.N. and M. MOENS. 2011. Introduction to Plant-Parasitic Nematodes: Modes of Parasitism. In: Jones, J., Gheysen, G. and C. Fenoll (eds.). Genomics and Molecular Genetics of Plant-Nematode Interactions. Springer International Publishers, Netherlands.

POFU, K.M. and P.W. MASHELA. 2014. Density-dependent growth patterns of *Meloidogyne javanica* on Hemp cultivars: Establishing nematode-sampling timeframes in host-status trials. *American Journal of Experimental Agriculture* 4:639–650.

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

RASMANN, S., ALI, J.G., HELDER, J. and W.H. VAN DER PUTTEN. 2012. Ecology and evolution of soil nematode chemotaxis. *Journal of Chemical Ecology* 38:615–28.

REYNOLDS, A.M., DUTTA, T.K., CURTIS, R.H.C., POWERS, S.J., GAUR, H.S. and B.R. KERRY. 2010. Chemotaxis can take plant-parasitic nematodes to the source of a chemo-attractant via the shortest possible routes. *Journal of the Royal Society Interface* 8:568–577.

RICE, E.L. 1984. Allelopathy. Academic, New York.

ROMERO-GONZALEZ, R., FRENICH, A.G. and J.L. MARTINEZ-VIDAL. 2015. Biopesticide Residues in Soil. In: Nollet, L.M.L. and H.S. Rathone (eds.). Biopesticide Handbook. Taylor and Francis Group, Florida.

ROSSNER, J. and C.P.W. ZEBITZ, 1987. Effect of Neem Products on Nematodes and Growth of Tomato (*Lycopersicon esculentum*) Plants. *Proceedings of the International Neem Conference* 3:611–621.

SCHAEFER, H. and S.S. RENNER. 2011. Phylogenetic relationships in the order Cucurbitales and a new classification of the gourd family (Cucurbitaceae). *Taxon* 60:122–138.

SESHWENI, M.D., POFU, K.M., OELOFSE, D., MASHELA, P.W. and Z.P. DUBE. 2016. Interactive effects of Nemacur, Biocult mycorrhizae and Nemarioc-AL phytonematicide on *Meloidogyne javanica* population densities and growth of potato cv. ‘Mondial G3’. *Combined Congress.* Abstract No. 46.

SIAL, I.M., KAZMI, M.A., KAZMI, Q.B. and S.N. NAQVI. 2009. Toxicity of biosal (phytopesticide) and permethrin (pyrethroid) against common carp, *Cyprinus carpio*. *Pakistan Journal of Zoology* 41:235–238.

SITHOLE, N.T., POFU, K.M., MASHELA, P.W. and Z.P. DUBE. 2016. Influence of Nemafric-BL phytonematicide on *Meloidogyne javanica* and growth of *Pelargonium sidoides*. *Combined Congress*. Abstract No. 68.

SKANTAR, A.M., AGAMA, K., MEYER. S.L.F., CARTA, L.K. and B.T. VINYARD. 2005. Effects of geldanamycin on hatching and juvenile motility in *Caenorhabdit elegans* and *Heterodera glycines*. *Journal of Chemical Ecology* 31:2481–2491.

STIRLING, G.R. 2014. Biological Control of Plant Parasitic Nematodes: Soil Ecosystem Management in Sustainable Agriculture. CAB International, Wallingford.

SUNDARARAJU, P., PADMANABAN, B. and S. SATHIAMOORTHY. 2003. Efficacy of certain botanicals against root-lesion nematode, *Pratylenchus coffeae* in banana. *Nematologia Mediterranea* 31:201–205.

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

THODEN, T.C., KORTHALS, G.W. and A.J. TERMORSHUIZEN. 2011. Organic amendments and their influences on plant-parasitic and free-living nematodes: a promising method for nematode management? *Nematology* 13:133–153.

USMAN, A. 2013. Studies on the Integrated Management of Phytonematodes Attacking Some Vegetable Crops. PhD thesis, Aligarh Muslim University, Aligarh.

WUYTS, N., SWENNEN, R. and D. DE WAELE. 2006. Effects of plant phenylpropanoid pathway products and selected terpenoids and alkaloids on the behavior of the plant-parasitic nematodes *Radopholus similis, Pratylenchus penetrans* and *Meloidogyne incognita*. *Nematology* 8:89–101.

# CHAPTER 3
# RESPONSES OF NEMATODE TO PURE CUCURBITACINS A AND B: JUVENILE HATCH TRIALS

## 3.1 Introduction

Active ingredients in botanical pesticides developed using fermentation of crude plant extracts occur in multiple forms, with multiple modes of action, which are relatively well-documented for phyto-insecticides (Nzanza and Mashela, 2012), with scant information for phytonematicides. Nemarioc-AL and Nemafric-BL phytonematicides produced from fermentation of crude extracts of wild cucumber (*Cucumis myriocarpus* Naudin*)* and wild watermelon (*C. africanus* L.) dried fruits, respectively (Pelinganga and Mashela, 2012), consistently suppressed root-knot (*Meloidogyne* species) nematodes (Mashela *et al*., 2015; Pelinganga and Mashela, 2012). The active ingredients in the two phytonematicides are cucurbitacin A ($C_{32}H_{46}O_8$) and B ($C_{32}H_{46}O_9$), respectively, which are tetracyclic triterpenoids (Mashela *et al*., 2015). The nonpolar cucurbitacin B is insoluble in water, whereas the slightly polar cucurbitacin A is partially water-soluble and oxidises rapidly to cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Jeffrey, 1978). In insects, cucumin and leptodermin chemical compounds are bioactive (Damalas, 2011), but their respective bioactivities on nematodes are not documented.

Generally, in pure form, most phytonematicides are not bioactive to nematode, whereas evidence of the bioactivity of active ingredients is one of the requirements for the registration of phytonematicides (Act 36 of 1947). Another challenge in testing pure active ingredients is that nematode eggs and second-stage juveniles (J2), when exposed to low concentrations have the ability to enter cryptobiosis which can be

confounded with mortality (Mashela *et al*., 2015). During cryptobiosis, control tactics and environmental factors have negligent effects on the physiology of nematodes (Zheng and Ferris, 1991). Cryptobiosis in eggs and J2 are referred to as diapause and dauer stages, respectively (McSorley, 2003). The condition could render phytonematicide efficacy and bioactivity results difficult to interpret. The objective of this study was fivefold, namely, to examine whether (i) increasing concentrations of cucurbitacin A and B would have impact on J2 hatch of *M. incognita*, (ii) the Curve-fitting Allelochemical Response Dosage (CARD) model would quantify the three phases of the density-dependent growth (DDG) patterns on J2 hatch when exposed to increasing cucurbitacin concentrations, (iii) computed J2 hatch inhibition concentration (EHIC) and CARD-generated inhibition dosage (D)-values would be statistically similar, (iv) the CARD model would provide information on minimum inhibition concentration (MIC) and (v) J2 hatch inhibition would be reversible when cucurbitacin concentrations were diluted.

## 3.2 Materials and methods

The *in vitro* trials were conducted at the Green Technologies Research Centre, University of Limpopo, South Africa (23°53′10″S, 29°44′15″E). Purified cucurbitacin A and B were procured from ChemFaces (Wuhan, China).

### 3.2.1 Preparation of material

Purified cucurbitacin A and B (1000 µg each), were dissolved in 5 µL methanol (ca. 99% purity) to enhance solubility. In each, 1-mL distilled water was added to make stock solutions. When required, dark brown coloured egg masses of *M. incognita*

were obtained from 2-month-old tomato (*Solanum lycopersicum* L.) cv. 'Floradade' plants raised under greenhouse conditions. Roots were rinsed in 1% NaOCl solution, egg masses dislodged using a tooth pick and placed in a petri dish containing 5 mL distilled water.

### 3.2.2 Second-stage juvenile hatch bioassay

In two parallel trials, stock solutions of cucurbitacin A and B were each diluted in distilled water and pipetted into a 96 well-plate making cucurbitacin concentrations of 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25 and 2.5 $\mu g.mL^{-1}$ distilled water. Distilled water and methanol concentration at 0.005% (equivalent to the percentage in the highest cucurbitacin concentration) were used as controls. In all trials, treatments were replicated three times and arranged in a completely randomised design in an incubator at 25 $\pm$ 3 °C. Hatched J2 were counted under a stereomicroscope after incubation periods 24-, 48- and 72-h, 7 and 10-d (Wuyts *et al*., 2006). The 7- and 10-d exposure was to establish whether the saturation phase could be attained. Ten days after the initial incubation, all treatments were diluted 5 times using distilled water and eggs and J2 incubated to assess the reversibility of J2 hatch inhibition. Three sequential experiments were conducted at monthly interval for each cucurbitacin.

### 3.2.3 Statistical analysis

Cumulative J2 counts were made per treatment after each incubation period, but statistical analysis was performed on number hatched between the incubation periods. Data were transformed using $\log_{10}(x + 1)$ prior to analysis of variance (SAS

Institute, 2008). Treatment means were separated using Waller-Duncan multiple range test and the relative impact computed using the relation [(treatment/control) – 1] x 100. The EHIC values at 50 and 100% were computed from the quadratic equations ($y = ax^2 + bx + c$), generated from the relative impact values, where x-values were equal to $EHIC_{50}$ and $EHIC_{100}$ for the y-values at 50 and 100%, respectively, using the quadratic formula (Qu *et al*., 2000):

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

Lines of the best fit between relative impact values and increasing concentrations of cucurbitacins were established. Mean exposure period values were subjected to the CARD model (Liu *et al*., 2003) to generate the J2 hatch curves using the quadratic equation $Y = b_2x^2 + b_1x + c$, where Y = J2 hatch inhibition mean value and x = exposure period mean value. The relation $x = -b_1/2b_2$ was used to establish the MIC for J2 hatch inhibition. Additionally, the CARD-generated biological indices, *viz*., threshold stimulation ($D_m$), saturation point ($R_h$), 0% inhibition concentration ($D_0$), 50% inhibition concentration ($D_{50}$), 100% inhibition concentration ($D_{100}$), sensitivity index (k) and coefficient of determination ($R^2$) (Liu *et al*., 2003), were summarised. The CARD-generated indices were adjusted to get the actual indices; adjusted $R_h$ = (CARD-generated $R_h$ + $D_m$), adjusted $D_0$ = (CARD-generated $D_0$ + adjusted $R_h$), adjusted $D_{50}$ = (CARD-generated $D_{50}$ + adjusted $D_0$) and adjusted $D_{100}$ = (CARD-generated $D_{100}$ + adjusted $D_{50}$) (Mashela *et al*., 2015). Unless otherwise stated, only treatments that were significant at the probability level of 5% were discussed.

## 3.3 Results

In both cucurbitacin A and B trials, mean values in methanol and distilled water were not statistically different. The distilled water control therefore was used throughout the study. The monthly interval interactions were not significant and therefore the data were pooled (n = 108) and re-analysed for each sampling period.

### 3.3.1 Cucurbitacin A

Relative impact: Treatment effects of cucurbitacin A on J2 hatch were significant for 24-, 48- and 72-h exposure periods (Appendix 3.1-3.3)with high treatment contributions to total treatment variations (TTV) of 67, 66 and 60%, respectively (Table 3.1). In contrast, treatment effects for extended incubation periods (7- and 10-d) were not significant (Appendix 3.4-3.5). Relative impact values of J2 hatch over increasing cucurbitacin A concentration exhibited DDG patterns, which had an inhibition and slight stimulation effects at low and high concentrations, respectively (Figure 3.1A). Increasing cucurbitacin A concentrations from 0.25 to 1.25 $\mu g.mL^{-1}$ distilled water, resulted in a decrease in the number of *M. incognita* J2 hatching, whereas a further increase in concentrations resulted in a steady increase in J2 hatch (Table 3.3). The DDG patterns were explained at 24-, 48- and 72-h exposure periods by 79, 86 and 69%, respectively (Figure 3.1 A, B).

Table 3.1 Partitioning mean sum of squares for second-stage juvenile hatch in pure cucurbitacin A at 24-, 48-, 72-h and 7- and 10-d exposure periods.

| | | Exposure period | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 24 h | | 48 h | | 72 h | | 7 d | | 10 d | |
| Source | DF | MS | % | MS | % | MS | % | MS | % | MS | % |
| Treatment | 11 | 1.0 | 67* | 1.2 | 66* | 1.2 | 60* | 0.3 | 50ns | 0.2 | 50ns |
| Error | 96 | 0.5 | 33 | 0.6 | 34 | 0.8 | 40 | 0.3 | 50 | 0.2 | 50 |
| Total | 107 | 1.5 | 100 | 1.8 | 100 | 1.9 | 100 | 0.5 | 100 | 0.5 | 100 |

*Significant at P ≤ 0.05; nsNot significant (P ≤ 0.05).

Table 3.2 Relative impact of pure cucurbitacin A on second-stage juvenile hatch of *Meloidogyne incognita* at 24-, 48- and 72-h exposure periods.

| | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|
| Concentration (µg.mL$^{-1}$) | Mean[x] | RI (%)[y] | Mean | RI (%) | Mean | RI (%) |
| 0.00 | 1.50a | – | 1.81a | – | 2.11a | – |
| 0.25 | 0.48c | –40 | 0.61c | –38 | 0.69c | –35 |
| 0.50 | 0.67bc | –56 | 0.84bc | –54 | 1.36abc | –36 |
| 0.75 | 0.62bc | –59 | 0.83bc | –54 | 1.08bc | –45 |
| 1.00 | 0.37c | –67 | 0.64c | –65 | 0.92bc | –43 |
| 1.25 | 0.40c | –67 | 0.62c | –66 | 1.22bc | –42 |
| 1.50 | 0.59c | –61 | 0.82bc | –55 | 1.24bc | –41 |
| 1.75 | 0.69bc | –54 | 0.82bc | –55 | 1.28bc | –39 |
| 2.00 | 0.71bc | –53 | 1.13abc | –52 | 1.312abc | –38 |
| 2.25 | 0.73bc | –52 | 1.07bc | –41 | 1.20bc | –38 |
| 2.50 | 0.79bc | –48 | 1.19abc | –34 | 1.37abc | –34 |

[x]Column means followed by the same letter are not significantly different at P ≤ 0.05 according to Waller-Duncan Multiple Range test.
[y]Relative impact (%) = [(treatment/control) – 1] x 100.

Curve-fitting Allelochemical Response Dosage model: The CARD model quantified concentration ranges that could stimulate ($D_m$–$R_h$), saturate ($R_h$–$D_0$) and inhibit ($D_0$–$D_{100}$), J2 hatch (Table 3.3). The stimulation phase concentration range was characterised by positive to negative values, whereas at all three exposure periods, increasing cucurbitacin A concentration resulted in a shift from saturation phase to inhibition phase. The sensitivity of J2 hatch to increasing concentration of cucurbitacin A decreased with increase in exposure periods (Table 3.3), with lower value at 72-h exposure period than at the other two. The CARD-generated DDG patterns demonstrated that at low concentrations cucurbitacin A inhibited J2 hatch, whereas at high concentrations J2 hatch was stimulated (Figure 3.2). The DDG patterns were explained by 86, 86 and 81% of the derived models at 24-, 48- and 72-h exposure periods, respectively (Table 3.3). The sensitivity of J2 hatch in cucurbitacin A was ranged from 5–20 units (Table 3.3).

Table 3.3 Biological indices of *Meloidogyne incognita* second-stage juvenile hatch to pure cucurbitacin A.

| | Exposure period (h) | | |
|---|---|---|---|
| Biological index | 24 | 48 | 72 |
| Threshold stimulation ($D_m$) | 0.61 | 0.52 | 0.16 |
| Saturation point ($R_h$) | –0.41 | –0.64 | –1.12 |
| 0% inhibition ($D_0$) | –0.41 | –0.64 | –1.12 |
| 50% inhibition ($D_{50}$) | –0.23 | –0.47 | –1.07 |
| 100% inhibition ($D_{100}$) | 0.07 | –0.17 | –0.97 |
| $R^2$ | 0.86 | 0.86 | 0.81 |
| Sensitivity index (k) | 5 | 5 | 20 |

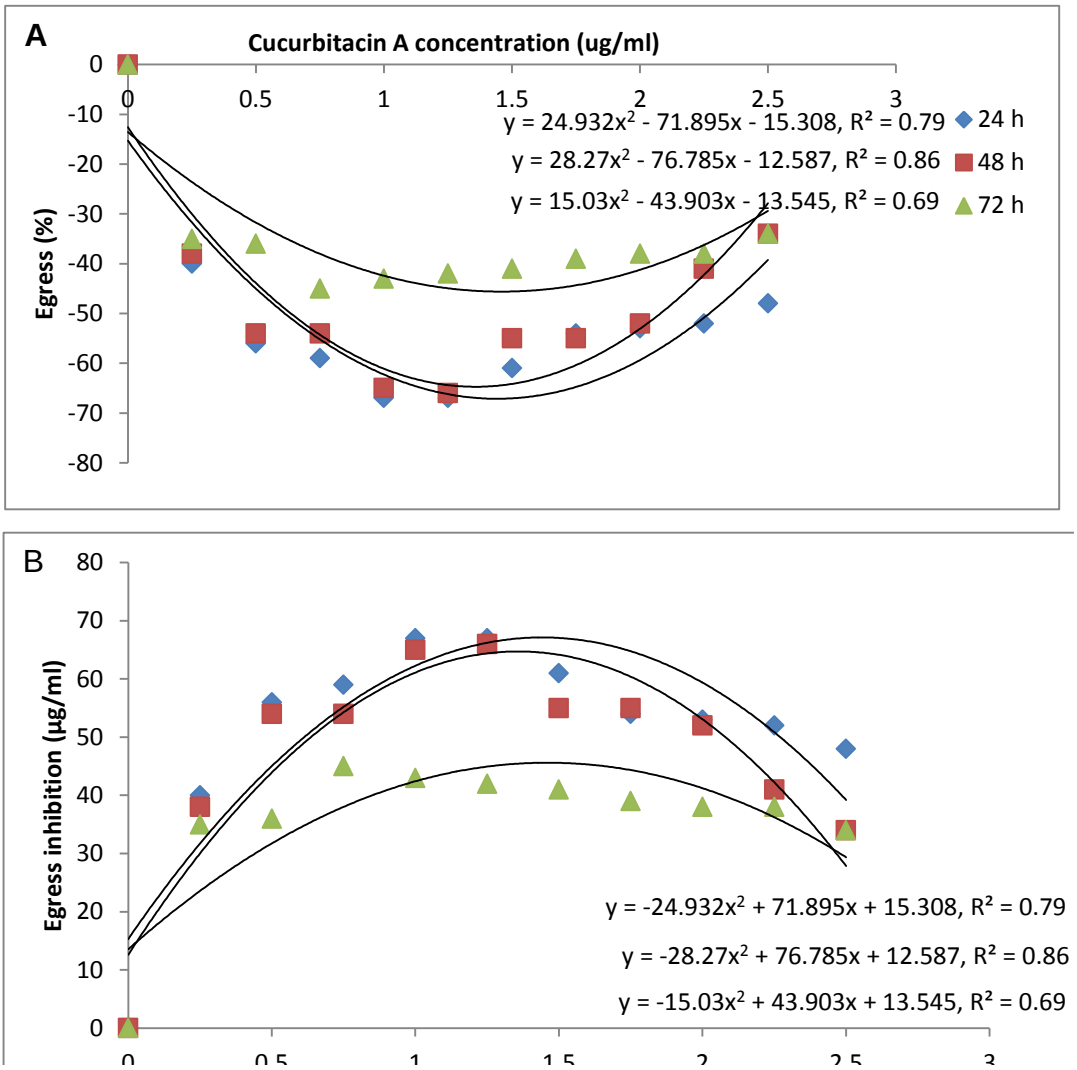


Figure 3.1 Relative impact of pure cucurbitacin A on second-stage juvenile hatch (A) and second-stage juvenile hatch inhibition (B) of *Meloidogyne incognita.*

Comparison of second-stage juvenile hatch Inhibition Concentration (EHIC) and inhibition dosage (D)-values: Generally, EHIC at 50 and 100% were lower than the CARD-generated D-values at 50 and 100% (Table 3.4). At all exposure periods, EHIC at 50 and 100% had negative values with lower $EHIC_{100}$ values than $EHIC_{50}$

values. The D-values for cucurbitacin A decreased with increase in exposure periods.

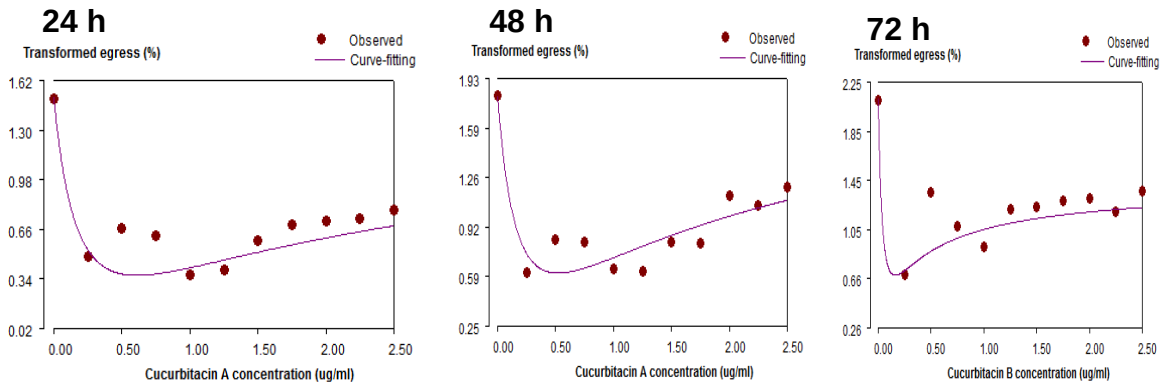


Figure 3.2 Curve-fitting Allelochemical Response Dosage (CARD)-generated density-dependent growth responses of *Meloidogyne incognita* second-stage juvenile hatch to increasing concentrations of pure cucurbitacin A at 24-, 48- and 72-h exposure periods.

Table 3.4 Comparison of pure cucurbitacin A second-stage juvenile hatch inhibition concentration (EHIC) and inhibition dosage (D)-values.

| Biological index | Exposure period (h) | | | | | |
|---|---|---|---|---|---|---|
| | 24 | | 48 | | 72 | |
| $EHIC_{50}$ | –0.726 | | –0.656 | | –1.062 | |
| $D_{50}$ | –0.234 | (0.171)[x] | –0.471 | (0.173) | –1.067 | (0.055) |
| $EHIC_{100}$ | –1.147 | | –1.056 | | –1.652 | |
| $D_{100}$ | 0.066 | (0.300) | –0.171 | (0.300) | –0.967 | (0.100) |

[x]Values in brackets are adjusted index values.

Minimum inhibition concentration (MIC): MIC values of J2 hatch in *M. incognita* increased with increasing exposure periods from 1.75 to 2.88 $\mu g.mL^{-1}$ (Table 3.5).

Table 3.5 Minimum inhibition concentration of pure cucurbitacins A on second-stage juvenile hatch of *Meloidogyne incognita* from quadratic curves generated by Curve-fitting Allelochemical Response Dosage (CARD) model.

| Incubation period (h) | Model | $x\ (\mu g.mL^{-1})^z$ |
|---|---|---|
| 24 | $y = 0.3043x^2 – 1.0671x + 1.3939$ | 1.75 |
| 48 | $y = 0.2834x^2 – 1.1155x + 1.7069$ | 1.97 |
| 72 | $y = 0.1359x^2 – 0.7827x + 1.9594$ | 2.88 |

$^z x = –b_1/2b_2$, where $y = b_2x^2 + b_1x + c$.

Reversibility of second-stage juvenile hatch inhibition: The J2 hatch inhibition effects of cucurbitacin A on *M. incognita* eggs were not reversible, as demonstrated by non-significant treatment means ($P > 0.05$) in ANOVA (Table 3.6, Appendix 3.6).

Table 3.6 Partitioning mean sum of squares for reversibility of *Meloidogyne incognita* second-stage juvenile hatch inhibition in pure cucurbitacin A.

| Source | DF | MS | % |
|---|---|---|---|
| Treatment | 11 | 0.15761 | $43^{ns}$ |
| Error | 96 | 0.21255 | 57 |
| Total | 107 | 0.37016 | 100 |

$^{ns}$Not significant ($P \leq 0.05$).

### 3.3.2 Cucurbitacin B

Relative impact: Treatment effects of cucurbitacin B at the first three incubation periods were significant (Appendix 3.7-3.9), contributing 70, 67 and 66% to TTV (Table 3.7). Relative impact values of J2 hatch plotted against increasing cucurbitacin B concentrations exhibited quadratic relations (Figure 3.4). The quadratic model explained the relations between J2 hatch and concentrations at 24-, 48- and 72-h exposure by 91, 92 and 74%, respectively (Figure 3.4). In contrast, treatment effects for the extended incubation periods and reversal trials were not significant (Table 3.7, Appendix 3.10-3.11). As with cucurbitacin A, cucurbitacin B inhibited and stimulated J2 hatch at low and high concentrations, respectively (Figure 3.4), whereas relative to the 24-h exposure period, fewer eggs hatched at all cucurbitacin B concentrations compared to the other two exposure periods.

Curve-fitting Allelochemical Response Dosage model: The CARD-generated cucurbitacin B stimulation phase concentration ranges were characterised by positive to negative values at all three incubation periods, whereas saturation and inhibition concentration ranges were similar, with a value of zero at 24- and 48-h exposure periods (Table 3.9, 3.10). At 72-h exposure period an increase in concentrations shifted the saturation phase range towards the inhibition phase concentration range (Table 3.9). The sensitivity of J2 hatch to increasing concentration of cucurbitacin B was 0–2 units (Table 3.9). The J2 hatch and increasing concentration of cucurbitacin B exhibited negative curvilinear quadratic relations, irrespective of the exposure period, with inhibition being at low cucurbitacin B and stimulation at higher concentrations (Figure 3.3).

Table 3.7 Partitioning mean sum of squares for *Meloidogyne incognita* second-stage juvenile hatch in pure cucurbitacin B at 24-, 48-, 72-h and 7- and 10-d exposure periods.

| | | Exposure period | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 24 h | | 48 h | | 72 h | | 7 d | | 10 d | |
| Source | DF | MS | % | MS | % | MS | % | MS | % | MS | % |
| Treatment | 11 | 0.5 | 70* | 0.6 | 67* | 0.6 | 66* | 1.0 | 71ns | 0.6 | 66ns |
| Error | 96 | 0.2 | 30 | 0.3 | 33 | 0.3 | 34 | 0.4 | 29 | 0.3 | 34 |
| Total | 107 | 0.7 | 100 | 0.9 | 100 | 0.9 | 100 | 1.4 | 100 | 0.9 | 100 |

*Significant at P ≤ 0.05, nsNot significant (P ≤ 0.05).

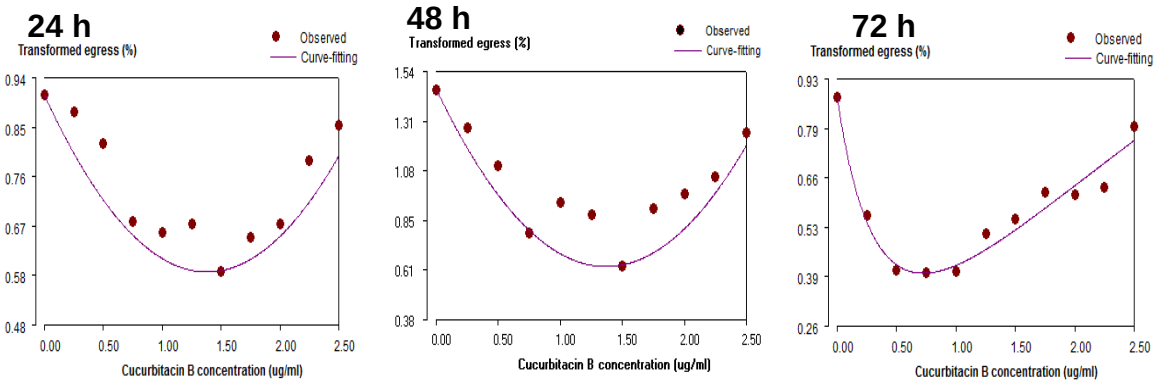


Figure 3.3 Curve-fitting Allelochemical Response Dosage (CARD)-generated responses of *Meloidogyne incognita* J2 hatch to increasing concentrations of pure cucurbitacin B at 24-, 48- and 72-h exposure periods.

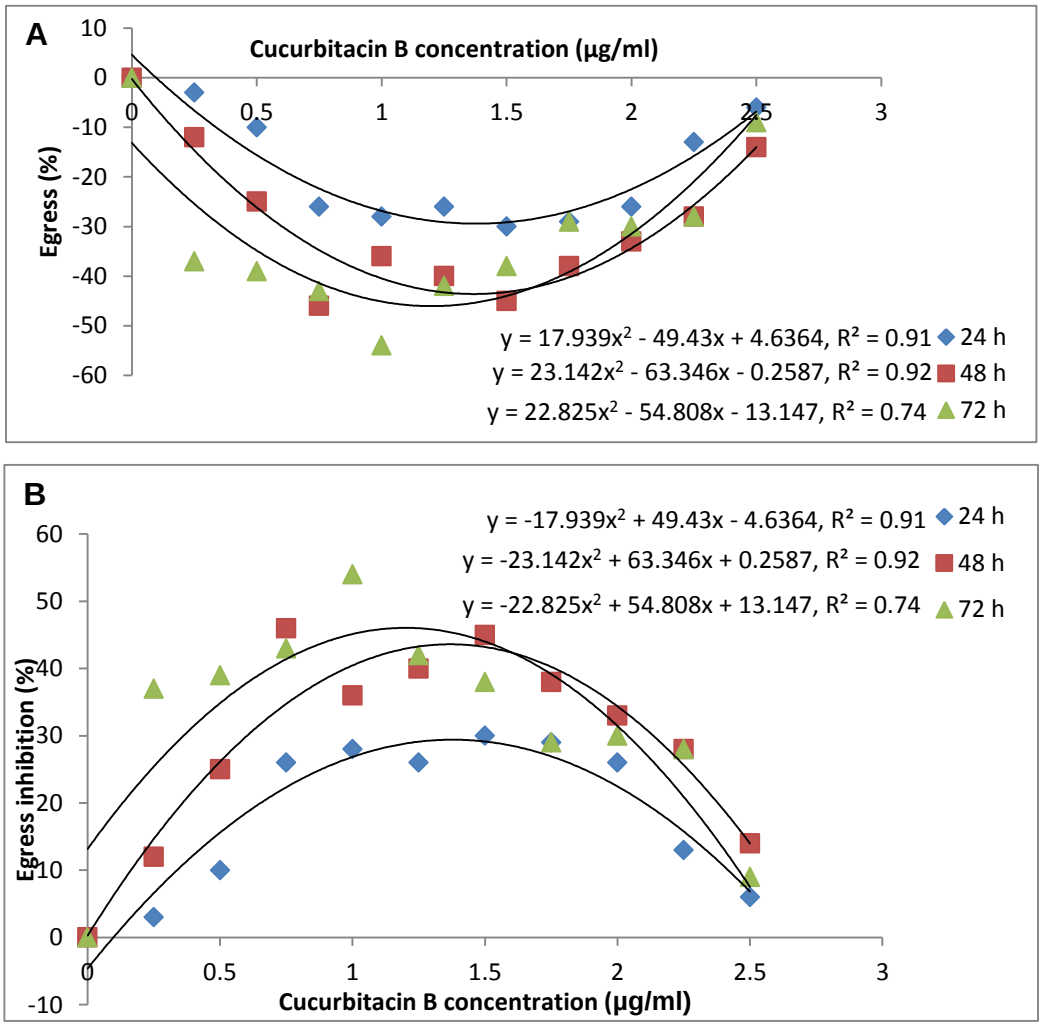


Figure 3.4 Relative impact of pure cucurbitacin B on second-stage juvenile hatch (A) and second-stage juvenile hatch inhibition (B) of *Meloidogyne incognita.*

Comparison of second-stage juvenile hatch inhibition concentration (EHIC) and inhibition dosage (D)-values: All computed EHIC values were negative, whereas the CARD-generated D-values were all positive (Table 3.10). As in cucurbitacin A, cucurbitacin B, $EHIC_{100}$ values were lower than those of $EHIC_{50}$ values at all exposure periods. At 72-h exposure period, $D_{50}$ values were lower than $D_{100}$ values,

whereas at the other two exposure periods there was no difference between $D_{50}$ and $D_{100}$.

Table 3.8 Responses of *Meloidogyne incognita* second-stage juvenile hatch to pure cucurbitacin B at 24-, 48- and 72-h exposure periods.

| Concentration. ($\mu g.mL^{-1}$) | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|
| | Mean[y] | RI (%)[z] | Mean | RI (%) | Mean | RI (%) |
| 0.00 | 1.46a | – | 0.91a | – | 0.88a | – |
| 0.25 | 1.28ab | –12 | 0.89a | –3 | 0.56bc | –37 |
| 0.50 | 1.10abc | –25 | 0.82ab | –10 | 0.41d | –53 |
| 0.75 | 0.79bcd | –46 | 0.68c | –26 | 0.40d | –54 |
| 1.00 | 0.93abcd | –36 | 0.66c | –28 | 0.41d | –54 |
| 1.25 | 1.17abc | –40 | 0.67c | –26 | 0.51c | –42 |
| 1.50 | 0.63cd | –57 | 0.58cd | –36 | 0.55bc | –38 |
| 1.75 | 1.35a | –38 | 0.65c | –29 | 0.62b | –29 |
| 2.00 | 0.55d | –33 | 0.67c | –26 | 0.62b | –30 |
| 2.25 | 1.05abcd | –28 | 0.79ab | –13 | 0.64b | –28 |
| 2.50 | 1.26ab | –14 | 0.85b | –6 | 0.80a | –9 |

[y]Column means followed by the same letter are not significantly different at $P \leq 0.05$ according to Waller-Duncan Multiple Range test.

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

Table 3.9 Biological indices of *Meloidogyne incognita* second-stage juvenile hatch to increasing concentrations of pure cucurbitacin B.

| Biological index | Exposure period (h) | | |
|---|---|---|---|
| | 24 | 48 | 72 |
| Threshold stimulation ($D_m$) | 1.38 | 1.37 | 0.73 |
| Saturation point ($R_h$) | 1.06 | 0.68 | 0.26 |
| 0% inhibition ($D_0$) | 1.06 | 0.68 | 0.26 |
| 50% inhibition ($D_{50}$) | 1.06 | 0.68 | 0.73 |
| 100% inhibition ($D_{100}$) | 1.06 | 0.68 | 1.53 |
| $R^2$ | 0.90 | 0.88 | 0.95 |
| Sensitivity index (k) | 0 | 0 | 2 |

Table 3.10 Comparison of pure cucurbitacin B second-stage juvenile hatch inhibition concentration (EHIC) and inhibition dosage (D)-values.

| Biological index | Exposure period (h) | | | | | |
|---|---|---|---|---|---|---|
| | 24 | | 48 | | 72 | |
| $EHIC_{50}$ | –0.73 | | –0.64 | | –0.85 | |
| $D_{50}$ | 0.00 | (1.06)[x] | 0.00 | (0.68) | 0.00 | (0.73) |
| $EHIC_{100}$ | –1.31 | | –1.22 | | –1.33 | |
| $D_{100}$ | 0.00 | (1.06) | 0.00 | (0.68) | 1.53 | (0.80) |

[x]Values in brackets are adjusted indices.

Minimum inhibition concentration: Generally, MIC values of J2 hatch increased with increasing exposure periods from 24- to 72-h (Table 3.11).

Table 3.11 Minimum inhibition concentration (MIC) of pure cucurbitacins B on second-stage juvenile hatch of *Meloidogyne incognita* from quadratic curves generated by Curve-fitting Allelochemical Response Dosage (CARD) model.

| Incubation Period (h) | Model | x ($\mu g.mL^{-1}$)$^z$ |
|---|---|---|
| 24 | $y = 0.3337x^2 - 0.9045x + 1.4535$ | 1.36 |
| 48 | $y = 0.1819x^2 - 0.4774x + 0.9398$ | 1.31 |
| 72 | $y = 0.1117x^2 - 0.4191x + 0.8768$ | 1.88 |

$^z x = -b_1/2b_2$, where $y = b_2x^2 + b_1x + c$.

Reversibility of second-stage juvenile hatch inhibition: The J2 hatch inhibition effects of cucurbitacin B on *M. incognita* eggs were not reversible as shown by non-significant treatment effects in ANOVA (Table 3.12, Appendix 3.12).

Table 3.12 Partitioning mean sum of squares for reversibility of *Meloidogyne incognita* second-stage juvenile hatch inhibition in pure cucurbitacin B.

| Source | DF | MS | % |
|---|---|---|---|
| Treatment | 11 | 0.33162 | 38[ns] |
| Error | 96 | 0.53853 | 62 |
| Total | 107 | 0.87015 | 100 |

[ns]Not significant (P ≤ 0.05).

## 3.4 Discussion

### 3.4.1 Inhibition of J2 hatch

The major observation in DDG patterns generated by the current study was that at low concentration ranges the products gradually reduced J2 hatch (inhibition), followed by neutral and then stimulated J2 hatch at higher concentrations of cucurbitacin A and B. The observations depicted the reverse of what were observed in phytotoxicity trials, where at low concentrations plant growth was stimulated, followed by neutral and then inhibition responses (Mashela *et al*., 2015). The phytotoxicity observations were in agreement with observations of responses in various organisms to increasing concentrations of various allelochemicals (Liu *et al*., 2003). Due to limited concentrations used in nematode J2 hatch-phytonematicide trials, others (Giannakou, 2011; Odeyemi and Adewale, 2011) depicted inhibition as negative linear models (Mashela *et al*., 2015). Inhibition phases in the current trials were followed by concentration ranges where J2 hatch levelled off (neutral), depicted

in other studies (Oka *et al*., 2000; Payan *et al*., 1987) as no effect on J2 hatch (Mashela *et al*., 2015). The neutral effects were possible due to the ability of eggs to enter diapause survival stage (McSorley, 2003). Finally, high concentration ranges, where J2 hatch was stimulated in the current trials were depicted by others (Meyer *et al*., 2008; Skantar *et al*., 2005) as positive linear models. In agreement with observations in the current trials, Wuyts *et al*. (2006) demostrated that at least 14 purified active ingredients of wide range of plant extracts had no effect on *M. incognita* J2 hatch, whereas salicyclic acid and caffeic acid inhibited the activity.

Second-stage juvenile hatch is generally a physical process (Prot, 1980), with the first-stage juvenile (J1s) relying on external chemical cues from roots to grow and develop, and then moulting into J2, which initiate the hatch process. The body of a nematode, particularly the frontal and cervical regions, is covered with an extensive network of chemoreceptors (Troemel *et al*., 1995). Chemoattractant and chemorepellent chemical compounds play indispensable roles in behavioural activities of nematodes (McSorley, 2003; Perry and Gaur, 1996). In plant-parasitic nematodes, successful J2 hatch depends on the traceability of chemical concentrations in soil solutions by J2 inside eggs (McSorley, 2003). The J1 and J2 in nematode eggs use chemical cues in soil solutions for behavioural activities such as growth and development, moulting, J2 hatch and/or entering adaptation stages (McSorley, 2003). The common adaptation stage in J1 within the eggs is diapause, with that for J2 prior to infection of roots being the dauer stage (McSorley, 2003). Depending on the developmental stage, plants release various chemical compounds through exudation, leaching, volatilisation and/or microbial degradation for various

reasons (Stirling, 2014), which play various roles in the behavioural activities of plant-parasitic nematodes. Nematode chemoreceptors are able to detect water-soluble chemoattractant chemicals at micromolar (µM) concentrations, whereas volatile chemoattractants could be detected at picomolar (pM) concentrations (Troemel *et al*., 1995).

Phytonematicides release potent biochemicals either through leaching, volatilisation or microbial degradation (Mashela *et al*., 2011). These biochemicals are intercepted and interpreted variously by nematode J2. In the current study, at low concentrations J2 might have interpreted the concentrations as being evidence of waning root exudates with the onset of plant senescence, thereby entering the dauer stage, which is a form of survival strategy (McSorley, 2003). In contrast, as cucurbitacin concentrations increased, J2 in this study were tricked into perceiving the situation as being analogous to increased root exudates as in trap crops (Wuyts *et al*., 2006) and increasingly hatched, thereby exposing their bodies to unfavourable conditions induced by cucurbitacins in solutions. Similar increases in J2 hatch in response to increasing concentration of allelochemicals were observed by Qi *et al*. (2015).

### 3.4.2 Curve-fitting Allelochemical Response Dosage model

The CARD-generated quadratic trends observed in this study were similar to those of relative impacts described above. Cucurbitacin A, which is soluble in water (Chen *et al*., 2005), oxidises readily to cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Jeffrey, 1978), which could to some extent explain the higher k values on J2 hatch inhibition. The lower the k value, the higher is the sensitivity of the microorganism to

the allelochemical tested, *vice versa* (Liu *et al*., 2003). Apparently, the two chemical compounds, cucumin and leptodermin could be less effective in J2 hatch inhibition.

### 3.4.3 Minimum inhibition concentration

During the three exposure periods, MIC of cucurbitacin A and B for J2 hatch inhibition at 24-, 48- and 72-h was fairly low at 1.75, 1.97 and 2.88 $\mu g.mL^{-1}$, respectively. Low MIC indicates high level of toxicity to J2 hatch as confirmed by k-values of CARD model. Siam (*Chromolaena odorata* L.) weed and neem (*Azadirachta indica* A. Juss) active ingredients had MIC values of 0% each on J2 hatch inhibition in *Meloidogyne* species (Nimbalkar and Rajurkar, 2009). In contrast, that of fervenulin, an isolate from *Streptomyces* species, was at 30 $\mu g.mL^{-1}$ distilled water (Ruanpunun *et al*., 2011). Currently, there is limited information on MIC values for phytonematicides on nematodes. However, the ease with which this information can be derived from the CARD-generated quadratic equations should improve this area in plant-parasitic nematology.

### 3.4.4 Overall sensitivity ∑k of second-stage juvenile hatch to cucurbitacins

Generally, the higher the sensitivity value, the higher the tolerance to allelochemicals, *vice versa* (Liu *et al*., 2003). In the current trials, J2 hatch was highly tolerant to cucurbitacin A with sensitivity of 5–20 units, but highly sensitive to cucurbitacin B with sensitivity of 0–2 units. These findings could be due to the rapid breakdown of cucurbitacin A, which could also suggest that J2 hatch was not sensitive to the resulting cucumin and leptodermin chemical compounds. In contrast, due to its stability, J2 hatch remained highly sensitive to cucurbitacin B. The k-values

in this study could not be compared with those in other studies where nematode eggs were subjected to phytonematicides since nematode variables were not subjected to the CARD model (Mashela *et al*., 2015; Pelinganga and Mashela, 2012; Pelinganga *et al*., 2012).

### 3.4.5 Reversibility of J2 hatch inhibition

Incubation for 7- and 10-d, regardless of the cucurbitacins, resulted in saturation of J2 hatch, with treatment effects in ANOVA tables not being significant. Observations at the two incubation periods across all the concentrations in the two cucurbitacins were important since they added an empirically-based observation on the neutral phase in the DDG patterns (Liu *et al*., 2003). The CARD-computer based model clarified the three phases of the DDG patterns, with the neutral phases being at the top of the convex quadratic curves, between the stimulation and the inhibition phases (Liu *et al*., 2003). Findings in the study, during 24-, 48- and 72-h incubation periods suggested that neutral phases can also start from the inhibition to the stimulation phases, which is biologically sound due to the existence of the survival strategies in nematodes. Post-extended incubation periods, J2 hatch inhibition was irreversible at all levels of cucurbitacin. The observation agreed with empirically-based extended period, where treatment effects were not significant since eggs were saturated with cucurbitacins. Others observed that J2 hatch inhibition was reversed for certain active ingredients and nematode species (Wuyts *et al*., 2006).

## 3.5 Conclusion

The J2 hatch inhibition over increasing concentrations of pure cucurbitacins had DDG patterns with different trends to those originally generated by the CARD model. At low concentrations, cucurbitacins inhibited J2 hatch, whereas at high concentrations the material stimulated J2 hatch. The CARD model provided excellent MIC values, when compared to using conventional methods. The J2 hatch inhibition concentration ($EHIC_{50}$, $EHIC_{100}$) and the CARD-generated 50 and 100% inhibition values ($D_{50}$, $D_{100}$) were not comparable, although the CARD model demonstrated that J2 hatch was highly sensitive to cucurbitacin B, but more tolerant to cucurbitacin A. Three stages in DDG patterns addressed the view that phytonematicides had “inconsistent results” in nematode suppression. Results demonstrated that J2 hatch responses to cucurbitacins were a function of concentration and incubation period. At limited incubation periods, low and high cucurbitacin concentrations inhibited and stimulated J2 hatch, respectively. Under extended incubation periods, J2 could possibly not exit cryptobiosis, which could be interpreted as being due to paralysis, where J2 died.

## 3.6 References

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

DAMALAS, C.A. 2011. Potential uses of turmeric (*Curcuma longa*) products as alternative means of pest management in crop production. A review. *Plant Omics Journal* 4:136–141.

GIANNAKOU, I.O. 2011. Efficacy of a formulated product containing *Quillaja saponaria* plant extracts for the control of root-knot nematodes. *European Journal of Plant Pathology* 130:587–596.

JEFFREY, C. 1978. Cucurbitaceae. In: Launert, E. (ed.). Flora Zambesiaca Managing Committee, London.

LIU, D.L., AN, M., JOHNSON, I.R. and J.V. LOVETT. 2003. Mathematical modeling of allellopathy. III. A model for curve-fitting allellochemical dose responses. *Non-linearity Biology, Toxicology and Medicine* 1:37–50.

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

MASHELA, P.W., DUBE, Z.P. and K.M. POFU. 2015. Managing the Phytotoxicity and Inconsistent Nematode Suppression in Soil Amended with Phytonematicides. In: Meghvansi, M.K. and A. Vorma (eds.). Organic Amendments and Soil Suppressiveness in Plant Disease Management, Soil Biology 46. Springer International Publishers, Switzerland.

McSORLEY, R. 2003. Adaptations of nematodes to environmental extremes. *Florida Entomologist* 86:138–142.

MEYER, S.L.F., LAKSHMAN, D.K., ZASADA, I.A., VINYARD, B.T. and D.J. CHITWOOD. 2008. Phytotoxicity of clove oil to vegetable crop seedings and nematotoxicity to root-knot nematodes. *HortTechnology* 18:631–638.

NIMBALKAR, R.K. and S.K. RAJURKAR. 2009. Effect of plant root extracts to control root-knot nematode (*Meloidogyne* spp.) of soybean (*Glycine max*). *Biological Forum – An International Journal* 1:65–68.

NZANZA, B. and P.W. MASHELA. 2012. Control of whiteflies and aphids in tomato by fermented plant extracts of neem leaf and wild garlic. *African Journal of Biotechnology* 11:16077–16082.

ODEYEMI, I.S. and K.A. ADEWALE. 2011. Phythonematoxic properties and nematicidal potential of *Tithonia diversifolia* extract and residue on *Meloidogyne incognita* infecting yam (*Discoria rotundata*). *Archives of Phytopathology and Plant Protection* 44:1745−1753.

OKA, Y., NACAR, S., PUTIEVSKY, E., RAVID, U., YANIV, Z. and Y. SPIEGEL. 2000. Nematicidal activity of essential oils and their components against the root-knot nematode. *Phytopathology* 90:710–715.

PAYAN, L.A., JOHNSON, A.W. and R.H. LITTRELL. 1987. Effects of nematicides and herbicides alone or combined on *Meloidogyne incognita* egg hatch and development. *Annals of Applied Nematology* 1:67–70.

PERRY, R.N. and H.S. GAUR. 1996. Host plant influences on the hatching of cyst nematodes*. Fundamental and Applied Nematology* 19:505–510.

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

PELINGANGA, O.M., MASHELA, P.W., NZANZA, B. and M.S. MPHOSI. 2012. Baseline information on using fermented crude extracts from *Cucumis*

*africanus* fruit for suppression of *Meloidogyne incognita* and improving growth of tomato plant. *African Journal of Biotechnology* 11:11407–11413.

PROT, J.C. 1980. Migration of plant-parasitic nematodes towards plant roots. *Revue de Nematologie* 3:305–318.

QI, Y., HU, G., CAO, S., YE, D. and S. CHEN. 2015. Effects of Hyoscyamus alkaloids on egg masses, eggs hatching and 2nd instar larvae survival of *Meloidogyne incognita*. *Acta Agriculturae Boreali-Sinica* 30:272–277.

QU, A., LINDSAY, B.G., and B. LI. 2000. Improving generalized estimating equations using quadratic inference functions. *Biometrika* 87:823–836.

RUANPUNUM, P., LAATSCH, H., TANGCHITESOMKID, N. and S. LUMYONG. 2011. Nematicidal activity of fervenulim isolated from a nematicidal actinomycete, *Streptomyces* sp. CMU-MH21, on *Meloidogyne incognita*. *World Journal of Microbiology Biotechnology* 1373–1380.

SAS INSTITUTE. 2008. SAS/STAT 9.2 Qualification Tools User's Guide. SAS Institute, Cary, NC.

SKANTAR, A.M., AGAMA, K., MEYER, S.L.F., CARTA, L.K. and B.T. VINYARD. 2005. Effects of geldanamycin on hatching and juvenile motility in *Caenorhabditis elegans* and *Heterodera glycines*. *Journal of Chemical Ecology* 31:2481–2491.

STIRLING, G.R. 2014. Biological control of plant parasitic nematodes: soil ecosystem management in sustainable agriculture. CAB International, Wallingford.

TROEMEL, E.R., CHOU, J.H., DWYER, N.D., COLBERT, H.A. and C.I. BARGMANN.1995. Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. *Cell* 83:207–218.

WUYTS, N., SWENNEN, R. and D. DE WAELE. 2006. Effects of plant phenylpropanoid pathway products and selected terpenoids and alkaloids on the behavior of the plant-parasitic nematodes *Radopholus similis, Pratylenchus penetrans* and *Meloidogyne incognita*. *Nematology* 8:89–101.

ZHENG, L. and H. FERRIS. 1991. Four types of dormancy exhibited by eggs of *Heterodera schachtii*. *Revue de Nematologie* 14:419–426.

# CHAPTER 4
# RESPONSES OF NEMATODE TO PHYTONEMATICIDES: JUVENILE HATCH TRIALS

## 4.1 Introduction

In pure form, active ingredients of Nemarioc-AL and Nemafric-BL phytonematicides, cucurbitacin A and B, respectively, reduced second-stage juveniles (J2) hatch in root-knot (*Meloidoygne* species) nematodes in density-dependent growth (DDG) patterns (Chapter 3). The J2 hatch inhibition concentration ($EHIC_{50}$, $EHIC_{100}$) and the Curve-fitting Allelochemical Response Dosage (CARD) — generated 50 and 100% inhibition values ($D_{50}$, $D_{100}$) were not comparable. However, the CARD model provided better estimates of overall sensitivity ($\sum k$) and minimum inhibition concentration (MIC) of J2 hatch to the two active ingredients (Chapter 3). Generally, J2 hatch was highly sensitive to cucurbitacin B and highly tolerant to cucurbitacin A, with MIC increasing with incubation period of eggs in cucurbitacins. Additionally, the concentration range used in pure cucurbitacins ranged from 2.5 to 0.25 $\mu g.mL^{-1}$.

In crude form, Nemarioc-AL and Nemafric-BL phytonematicides are used at 3% (Pelinganga *et al*., 2012, 2013). However, information on how J2 hatch at concentration ranges below and above 3% respond had not been established. The objective of this study was to determine whether (i) increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides would have impact on J2 hatch of *M. incognita*, (ii) the CARD model would quantify the three phases of DDG pattern on J2 hatch when compared to increasing phytonematicide concentrations, (iii) computed EHIC and CARD-generated D-values would be statistically comparable in

magnitudes, (iv) the CARD model would provide information on MIC and (v) J2 hatch inhibition would be reversible when phytonematicides were diluted.

## 4.2 Materials and methods

### 4.2.1 Preparation of phytonematicides

Nemarioc-AL and Nemafric-BL phytonematicides were prepared by effective microorganism (EM) fermentation of oven-dried ground fruits from *Cucumis myriocarpus* and *C. africanus*, respectively (Pelinganga *et al*., 2013). The two Cucumis species were produced as discussed previously (Shadung, 2016). Ten concentrations, 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0% for each phytonematicide were made in distilled water, with distilled water containing EM used as control.

### 4.2.2 Collection of eggs

Egg masses of *Meloidogyne incognita* Kofoid & White were obtained from two month-old tomato (*Solanum lycopersicum* L.) cv. 'Floradade' seedlings raised in the greenhouse at the Green Technologies Research Centre, University of Limpopo, South Africa (23°53′10″S, 29°44′15″E) as described previously (Chapter 3).

### 4.2.3 J2 hatch inhibition assay

Effects of different concentrations were tested in 96-well plates. A 200-µl concentration of each phytonematicide was pipetted into each well and 107 eggs in small amount distilled water placed into each well. The 10 concentrations were arranged in a completely randomised design, with three replications. Three

independent experiments were conducted in an incubator set at 25 ± 3 °C for each phytonematicide. The number of J2 hatched were counted after 24-, 48- and 72-h and 7-d. The 10-d incubation period was left out because of fungal contamination on the contents. After 7-d, contents of each well were diluted five times in pasteurised distilled water and incubated for 5-d to assess the reversibility of J2 hatch inhibition.

### 4.2.4 Statistical analysis

The J2 out of the egg shell were considered hatched. Counts on J2 hatch were first transformed through $\log_{10}(x + 1)$ (Gomez and Gomez, 1984) prior to analysis of variance (ANOVA) through the SAS software (SAS Institute, 2008). Mean separation was achieved using Waller-Duncan multiple range test at the probability level of 5%, with variables subjected to the CARD model (Mashela *et al*., 2015). Unless otherwise stated, treatment effects were significant at 5% level of probability.

## 4.3 Results

In trials of both Nemarioc-AL and Nemafric-BL phytonematicides, there were no statistically significant differences between the effective microorganism and distilled water controls. The distilled water control therefore was used throughout the study. There were also no statistically significant differences between the three independent experiments, hence the data were pooled.

### 4.3.1 Nemarioc-AL phytonematicide

Relative impact: Treatment effects on J2 hatch were highly significant ($P \leq 0.01$) for all exposure periods except for 24-h exposure (Table 4.1, Appendix 4.1-4.4).

Increasing phytonematicide concentration at 48-, 72-h and 7-d exposure contributed 89, 88 and 75% in total treatment variation (TTV) of J2 hatch, respectively. Relative to untreated control, J2 hatch at 48-, 72-h and 7-d was reduced by 22–92, 3–79 and 1–42%, respectively (Table 4.2). Relative impact values of J2 hatch plotted against increasing phytonematicide concentrations exhibited DDG patterns, with J2 hatch inhibition increasing with increasing concentrations of Nemarioc-AL phytonematicide (Mashela *et al*., 2015). Relative impacts on J2 hatch of Nemarioc-AL phytonematicide decreased with increases in exposure periods (Figure 4.1A).

Table 4.1 Partitioning mean sum of squares for *Meloidogyne incognita* second-stage juvenile hatch in Nemarioc-AL phytonematicide after 48-, 72-h and 7-d exposure periods.

| | | 48 h | | 72 h | | 7 d | |
|---|---|---|---|---|---|---|---|
| Source | DF | MS | % | MS | % | MS | % |
| Treatment | 11 | 0.51908 | 89** | 0.79844 | 88** | 0.33552 | 75** |
| Error | 96 | 0.06508 | 11 | 0.10849 | 12 | 0.11037 | 25 |
| Total | 107 | 0.58416 | 100 | 0.90693 | 100 | 0.44589 | 100 |

**Significant at $P \leq 0.01$.

Curve-fitting Allelochemical Response Dosage: The threshold stimulation ($D_m$) values decreased with increase in exposure period (Table 4.3). In contrast, all other biological indices increased with increase in exposure period. Sensitivity of J2 hatch to Nemarioc-AL phytonematicide was 0–1 unit. Second-stage juvenile hatch

decreased with increases in concentration of Nemarioc-AL phytonematicide, with the relations being explained by 96, 96 and 95% at 48-h, 72-h and 7-d exposure periods, respectively (Figure 4.2).

Table 4.2 Influence of Nemarioc-AL phytonematicide on *Meloidogyne incognita* second-stage juvenile hatch after 48-, 72-h and 7-d exposure periods.

| | 48 h | | 72 h | | 7 d | |
|---|---|---|---|---|---|---|
| Concentration (%) | Mean[y] | RI (%)[z] | Mean | RI (%) | Mean | RI (%) |
| 0.0 | 0.82a | – | 1.03ab | – | 1.11a | – |
| 0.5 | 0.49bc | –22 | 0.76bc | –11 | 1.09a | –1 |
| 1.0 | 0.38cd | –34 | 1.01ab | –3 | 1.00ab | –10 |
| 1.5 | 0.50bc | –38 | 0.85bc | –18 | 1.01a | –9 |
| 2.0 | 0.39cd | –53 | 0.73bc | –15 | 1.02b | –8 |
| 2.5 | 0.46bc | –44 | 0.75bc | –27 | 0.80bc | –25 |
| 3.0 | 0.36cd | –56 | 0.87abc | –28 | 0.86bc | –22 |
| 3.5 | 0.12e | –74 | 0.80bc | –35 | 0.81bc | –27 |
| 4.0 | 0.19de | –77 | 0.58c | –44 | 0.63c | –34 |
| 4.5 | 0.00e | –88 | 0.19d | –65 | 0.56c | –42 |
| 5.0 | 0.07e | –92 | 0.22d | –79 | 0.72bc | –35 |

[y]Column means followed by the same letter were not different at P ≤ 0.05, according to Waller-Duncan multiple range test.

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

Table 4.3 Biological indices produced by the Curve-fitting Allelochemical Response Dosage (CARD) model at 48-, 72-h and 7-d exposure of *Meloidogyne incognita* eggs to Nemarioc-AL phytonematicide.

| Biological index | 48 h | 72 h | 7 d |
|---|---|---|---|
| Threshold stimulation ($D_m$) | 13.87 | 0.01 | 0.08 |
| Saturation point ($R_h$) | 12.70 | 0.01 | 0.08 |
| 0% inhibition ($D_0$) | 12.70 | 0.02 | 0.25 |
| 50% Inhibition ($D_{50}$) | 15.17 | 4.12 | 6.89 |
| 100% Inhibition ($D_{100}$) | 20.77 | 9.92 | 23.09 |
| $R^2$ | 0.96 | 0.96 | 0.95 |
| Sensitivity index (k) | 0 | 0 | 1 |

Comparison of second-stage juvenile hatch (EHIC) and inhibition dosage (D)-values: The CARD model computed $D_{50}$ and $D_{100}$ corresponded with $EHIC_{50}$ and $EHIC_{100}$, respectively, calculated from regression equations (Table 4.4). When the CARD-generated values were adjusted to compute the actual values a great increase in $D_{50}$ at 24-h exposure period and $D_{100}$ at all exposure periods was observed which differed greatly from the $EHIC_{50}$ and $EHIC_{100}$.

Table 4.4 Comparison of Nemarioc-AL phytonematicide second-stage juvenile hatch inhibition concentration (EHIC) and inhibition dosage (D)-values.

| Biological index | 48 h | | 72 h | | 7 d | |
|---|---|---|---|---|---|---|
| $EHIC_{50}$ | 2.20 | | 4.00 | | 6.10 | |
| $D_{50}$ | 2.40 | (15.17)[x] | 4.10 | (4.12) | 6.60 | (6.89) |
| $EHIC_{100}$ | 5.60 | | 5.80 | | 12.00 | |
| $D_{100}$ | 5.60 | (20.77) | 5.80 | (9.92) | 16.20 | (22.09) |

[x]Values in brackets are adjusted CARD-generated values.

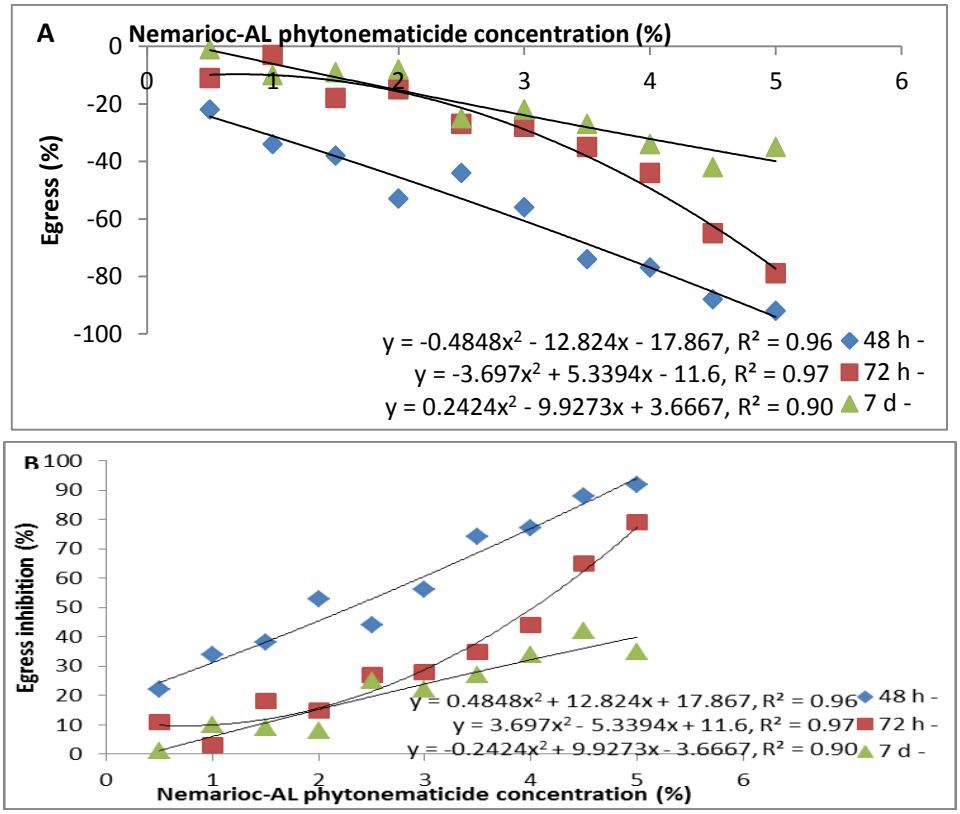


Figure 4.1 Relative impact of Nemarioc-AL phytonematicide on second-stage juvenile hatch (A) and second-stage juvenile hatch inhibition (B) of *Meloidogyne incognita.*

Reversibility of J2 hatch inhibition: Effects of Nemarioc-AL phytonematicide on *M. incognita* J2 hatch inhibition were irreversible (Table 4.5, Appendix 4.5).

Table 4.5 Partitioning mean sum of squares for reversibility of *Meloidogyne incognita* second-stage juvenile hatch inhibition in Nemarioc-AL phytonematicide.

| Source | DF | MS | % |
|---|---|---|---|
| Treatment | 11 | 0.46851 | 44$^{ns}$ |
| Error | 96 | 0.59612 | 56 |
| Total | 107 | 1.06463 | 100 |

$^{ns}$Not significant (P > 0.05).

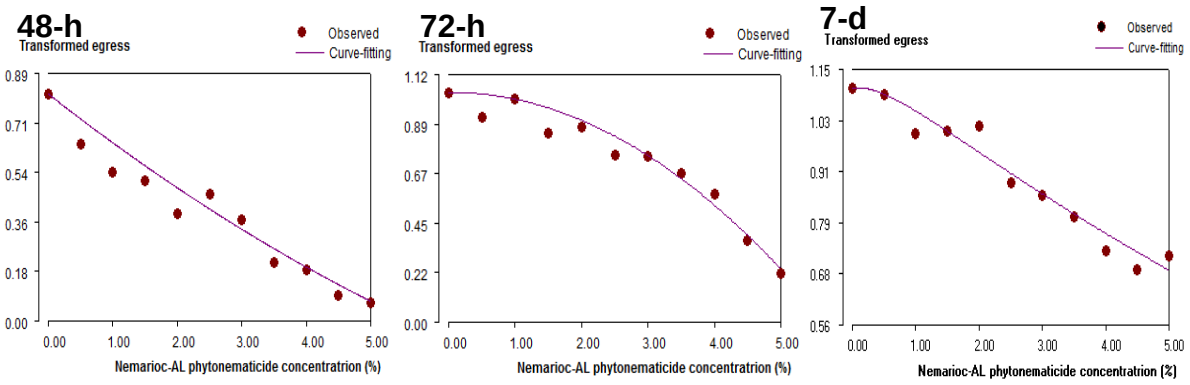


Figure 4.2 Curve-fitting Allelochemical Response Dosage (CARD)-generated density-dependent growth responses of *Meloidogyne incognita* second-stage juvenile hatch to increasing concentrations of Nemarioc-AL phytonematicide.

4.3.2 Nemafric-BL phytonematicide

Relative impact: Treatment effects on J2 hatch were highly significant (P ≤ 0.01) for all exposure periods except for 24-h exposure period (Table 4.6, Appendix 4.6-4.9). Relative impact values of J2 hatch over increasing concentrations of Nemafric-BL phytonematicide exhibited quadratic relations (Figure 4.3), which signified the existence of density-dependent growth (DDG) patterns (Mashela *et al*., 2015). The model was explained at 48-, 72-h and 7-day exposure periods by 95, 94 and 98%, respectively. Relative to untreated control, J2 hatch at 48-, 72-h and 7-d were reduced by 41–93, 1–80 and 12–84%, respectively (Table 4.7).

Table 4.6 Partitioning mean sum of squares for *Meloidogyne incognita* second-stage juvenile hatch in Nemafric-BL phytonematicide after 48-, 72-h and 7-d exposure periods.

| | | 24 h | | 48 h | | 72 h | | 7 d | |
|---|---|---|---|---|---|---|---|---|---|
| Source | DF | MS | % | MS | % | MS | % | MS | % |
| Treatment | 11 | 0.0254 | 27$^{ns}$ | 0.2178 | 81$^{**}$ | 0.6182 | 94$^{**}$ | 0.8949 | 94$^{**}$ |
| Error | 96 | 0.0674 | 73 | 0.0524 | 19 | 0.0407 | 6 | 0.0564 | 6 |
| Total | 107 | 0.0928 | 100 | 0.2702 | 100 | 0.6590 | 100 | 0.9513 | 100 |

$^{**}$Significant at P ≤ 0.01; $^{ns}$Not significant (P ≤ 0.05).

Table 4.7 Influence of Nemafric-BL phytonematicide on *Meloidogyne incognita* second-stage juvenile hatch after 48-, 72-h and 7-d exposure periods.

| Concentration (%) | 48 h | | 72 h | | 7 d | |
|---|---|---|---|---|---|---|
| | Mean[y] | RI (%)[z] | Mean | RI (%) | Mean | RI (%) |
| 0.0 | 0.81a | – | 0.80a | – | 1.48a | – |
| 0.5 | 0.48bc | –41 | 0.79ab | –1 | 1.31ab | –12 |
| 1.0 | 0.36cd | –56 | 0.74ab | –7 | 1.26ab | –15 |
| 1.5 | 0.30cd | –63 | 0.61bc | –24 | 1.22ab | –18 |
| 2.0 | 0.16de | –80 | 0.44c | –45 | 1.04bc | –30 |
| 2.5 | 0.11e | –86 | 0.39c | –51 | 0.98bc | –34 |
| 3.0 | 0.10e | –88 | 0.39c | –52 | 0.90bc | –39 |
| 3.5 | 0.16de | –80 | 0.35c | –56 | 0.59d | –60 |
| 4.0 | 0.06e | –93 | 0.29c | –64 | 0.62d | –58 |
| 4.5 | 0.09e | –89 | 0.16d | –80 | 0.51d | –65 |
| 5.0 | 0.16de | –80 | 0.28c | –65 | 0.23e | –84 |

[y]Column means followed by the same letter were not different at $P \leq 0.05$, according to Waller-Duncan multiple range test.

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

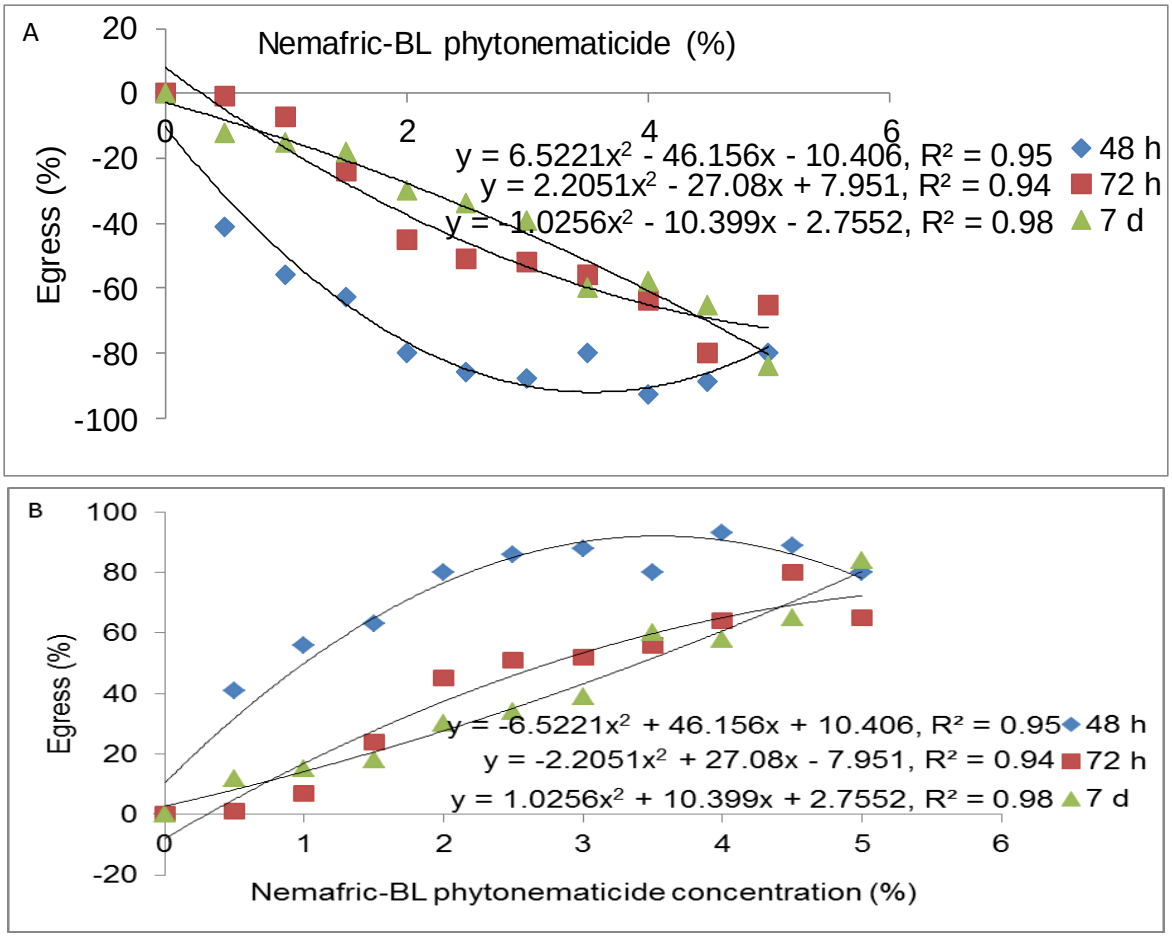


Figure 4.3 Relative impact of Nemafric-BL phytonematicide on second-stage juvenile hatch (A) and J2 hatch inhibition (B) of *Meloidogyne incognita.*

Curve-fitting Allelochemical Response Dosage: The $D_m$ values decreased with increase in exposure period for Nemafric-BL phytonematicide (Table 4.8), a trend also observed for Nemarioc-AL phytonematicide. In contrast, $R_h$ increased with increase in exposure period. Sensitivity of J2 hatch to Nemafric-BL phytonematicide was high low as shown by the sensitivity ranking of 0–4 units. The CARD-generated DDG patterns demonstrated a decrease in *M. incognita* J2 hatch with an increase in concentrations of Nemafric-BL phytonematicide, with the pattern being explained by 97, 96 and 98% at 48-, 72-h and 7-d, respectively (Figure 4.7). The trends were

similar to those of Nemarioc-AL phytonematicide and relative impact observed above.

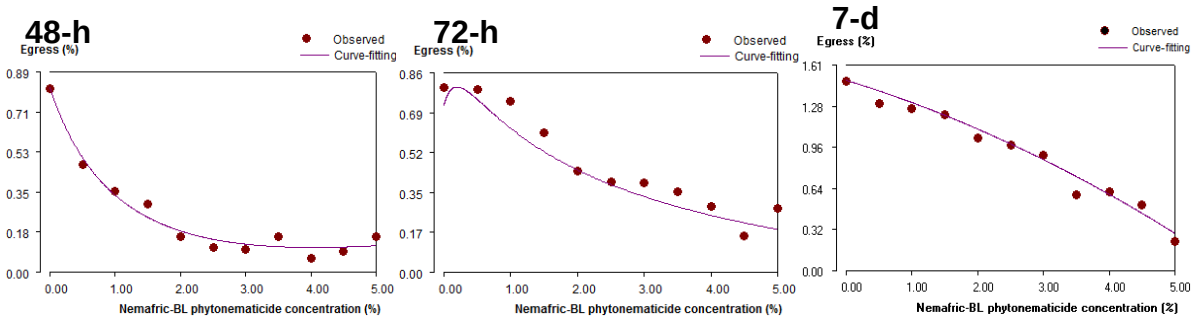


Figure 4.4 Curve-fitting Allelochemical Response Dosage (CARD)-generated responses of *Meloidogyne incognita* second-stage juvenile hatch to increasing concentrations of Nemafric-BL phytonematicide.

Table 4.8 Biological indices produced by the Curve-fitting Allelochemical Response Dosage (CARD) model at 48-, 72-h and 7-d exposure of *Meloidogyne incognita* eggs to Nemafric-BL phytonematicide.

| Biological index | 48 h | 72 h | 7 d |
|---|---|---|---|
| Threshold stimulation ($D_m$) | 4.05 | 0.19 | –4.89 |
| Saturation point ($R_h$) | –0.70 | 0.09 | 0.37 |
| 0% inhibition ($D_0$) | 0.00 | 0.60 | 0.00 |
| 50% Inhibition ($D_{50}$) | 0.76 | 2.71 | 3.47 |
| 100% Inhibition ($D_{100}$) | 1.10 | 10.90 | 5.90 |
| $R^2$ | 0.97 | 0.96 | 0.98 |
| Sensitivity index (k) | 1 | 4 | 0 |

Comparison of EHIC and D values: The $EHIC_{50}$ and $EHIC_{100}$ were higher than the CARD-generated $D_{50}$ and $D_{100}$ at all exposure periods (Table 4.9). At 48-h exposure the $EHIC_{50}$ and $EHIC_{100}$ corresponded with $D_{50}$ and $D_{100}$. They differed when adjustments were made to get the actual values.

Table 4.9 Comparison of Nemafric-BL phytonematicide second-stage juvenile hatch (EHIC) and inhibition dosage (D)-values.

| Biological index | 48 h | | 72 h | | 7 d | |
|---|---|---|---|---|---|---|
| $EHIC_{50}$ | 1.13 | | 13.68 | | 13.54 | |
| $D_{50}$ | 0.76 | (4.11)[x] | 2.71 | (3.59) | 3.47 | (-1.04) |
| $EHIC_{100}$ | 1.89 | | 15.05 | | 16.05 | |
| $D_{100}$ | 1.10 | (5.21) | 10.90 | (14.49) | 5.90 | (4.86) |

[x]Values in brackets are adjusted indices.

Reversibility of J2 hatch inhibition: Effects of Nemafric-BL phytonematicide on *M. incognita* J2 hatch inhibition were irreversible (Table 4.10, Appendix 4.10).

Table 4.10 Partitioning mean sum of squares for reversibility of *Meloidogyne incognita* second-stage hatch inhibition in Nemafric-BL phytonematicide.

| Source | DF | MS | % |
|---|---|---|---|
| Treatment | 11 | 0.24668 | 48[ns] |
| Error | 96 | 0.26435 | 52 |
| Total | 107 | 0.51103 | 100 |

[ns]Not significant ($P \leq 0.05$).

## 4.4 Discussion

### 4.4.1 Effects of phytonematicides on nematode second-stage juvenile hatch

Inhibition of J2 hatch in this study substantiate the nematicidal properties of Nemarioc-AL and Nemafric-BL phytonematicides when the same material were used in greenhouse and field studies (Mashela *et al*., 2015; Pelinganga and Mashela, 2012; Pelinganga *et al*., 2012).

### 4.4.2 Relative impact

Second-stage juvenile hatch in nematodes has been observed to be mainly a physical process, involving increased J2 movement towards hatching, with J2 continuously pressing its stylet against the egg shell, tearing it in the process (Bird, 1959; Doncaster and Shepherd, 1967; Wallace, 1968). Any materal that interfers with J2 behaviour and/or anatomy would therefore, affect hatching. Hough and Thomason (1975) observed that low concentrations of aldicarb stimulated J2 hatch through increased activity of J2 inside the eggs. According to DDG patterns (Liu *et al*., 2003), effects of phytonematicides on nematode J2 hatch can be stimulative, neutral or inhibitive. Wuyts *et al*. (2006) reported that some phytochemical compounds were neutral towards J2 hatch, whereas others where inhibitive, with one flavanone having both stimulative and inhibitive effects on J2 hatch of burrowing nematode (*Radopholus similis* Cobb).

In the current study, Nemarioc-AL and Nemafric-BL phytonematicides had consistent inhibitive effects on *M. incognita* J2 hatch during three different exposure periods. Similar findings have been made with other plant extracts, mugwort (*Artemisia*

*vulgaris* L.) (Costa *et al*., 2003), garlic (*Allium sativum* L.) and fennel (*Foeniculum vulgare* Mill) (Ibrahim *et al*., 2006), *Tagetes sp*. (Kalaiselvam and Devaraj, 2011), cedar (*Melia azedarach* L.) and elderberry (*Sambucus nigra* L.) (Akyazi, 2014) and neem (*Azadirachta indica* A. Juss) (Javed *et al*., 2008). Chrysathemum (*Chrysathemum coronarium* L.) on the other hand stimulated hatching of *M. incognita* (Ibrahim *et al*., 2006), whereas, rock fleabane (*Inula viscose* L.) had no effect on stem and bulb nematode (*Ditylenchus dipsaci* Kuhn) (Oka *et al*., 2001).

### 4.4.3 Density-dependent growth patterns

Density-dependent growth patterns between nematode counts and increasing concentrations of Nemarioc-AL and/or Nemafric-BL phytonematicides have been reported under greenhouse and field studies (Pelinganga and Mashela, 2012; Pelinganga *et al*., 2012). Density-dependent growth patterns had been observed in quite a number of studies where nematode eggs where exposed to varying concentrations of plant extracts (Abdul, 2013; Azhagumurugan and Rajan, 2014; Costa *et al*., 2003; Ibrahim *et al*., 2006). All these studies confirmed the theory postulated by Liu *et al*. (2003) that most biological entities would display a density-dependent response when exposed to increasing concentrations of allelochemicals.

In most cases, the limited ranges used in these studies are such that one stage is observed. For instance, Ibrahim *et al*. (2006) observed that *C. coronarium* and French marigold (*Tagetes patula* L.) had no effects on J2 hatching of *M. incognita,* whereas Perez *et al*. (2003) reported nematicidal activities of *C. coronarium*. Kalaiselvam and Devaraj (2011) observed that *T. patula* inhibited J2 hatching of the

same nematode. The contradictions had been fully explainded through the dosage model (Mashela *et al*., 2015), which showed that response were concentration specific. In the current study, the decreases in J2 hatch with increasing concentrations of the two phytonematicides suggested that the concentrations used were at the inhibition phases of DDG patterns.

### 4.4.4 J2 hatch inhibition concentration (EHIC) and inhibition dosage (D)-values

The current study reports, the first similarities between EHIC values and CARD-generated D-values at all exposure times for Nemarioc-AL phytonematicides and at 48 h exposure for Nemafric-BL phytonematicide. The corresponding values of EHIC values with CARD-generated values expand the possible uses of the CARD model as a valuable tool in the evaluation of phytonematicides.

### 4.4.5 Overall sensitivity of second-stage juvenile hatch

Second-stage juvenile hatch was highly sensitive to Nemarioc-AL and Nemafric-BL phytonematicides as shown by low k-values across all incubation periods. Sensitivity of an entity to an allelochemical is inversely proportional to k-values, with smaller values suggesting high sensitivities, whereas large values connote the opposite (Liu *et al*., 2003). Apparently, this is the first report of empirically-derived evidence of direct sensitivity of a phytonematicide on nematode J2 hatch. Sensitivity index can be a valuable tool for comparison of different plant extracts when used in the management of nematodes.

### 4.4.6 Reversibility of second-stage juvenile hatch inhibition

The irreversibility of nematode J2 hatch inhibition exposed to the two products in the current study confirmed effects of phytochemicals from both cannonball tree (*Couroupita guianensis* Aubl) and catnip (*Nepeta cataria* L.) (Pavaraj *et al*., 2012) and phloretin (Wuyts *et al*., 2006) which had irreversible effects on J2 hatch inhibition of *M. incognita* and *R. similis*, respectively. Similarly, phytochemicals from *C. guianensis* and *N. cataria* on *M. incognita* (Pavaraj *et al*., 2012) and phloretin on *R. similis* (Wuyts *et al*., 2006) had irreversible effects on J2 hatch inhibition. Cucurbitacins are members of the lipophilic triterpene chemical compounds that inherently disrupt permeability of membranes, leading to uncontrolled efflux of ions and metabolites or even cell leakage (van Wyk and Wink, 2014). Interference with permeability of membranes causes paralysis and eventually death, with the effects being irreversible (Pavaraj *et al*., 2012). The latter could explain the irreversibility of J2 hatch inhibition after extended incubation periods to Nemarioc-AL and Nemafric-BL phytonematicides.

## 4.5 Conclusion

Second-stage juvenile hatch over increasing concencentrations of Nemarioc-AL and Nemafric-BL phtyonematicides had a DDG patterns with similar trends to those originally generated by the CARD model. At low phytonematicide concentrations J2 hatch inhibition was low and at high concentrations J2 hatch inhibition was also high. The CARD model also provided excellent MIC values. The EHIC-values were comparable to CARD-generated D-values at all exposure times for Nemarioc-AL phytonematicide and at 48 h exposure for Nemafric-BL phytonematicide. Also, the

CARD model demostrated that J2 hatch was highly sensitive to both, Nemarioc-AL and Nemafric-BL phytonematicides.

## 4.6 References


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

AKYAZI, F. 2014. Effect of some plant methanol extracts on egg hatching and juvenile mortality of root-knot nematode *Meloidogyne* incognita. *American Journal of Experimental Agriculture* 4:1471–1479.

AZHAGUMURUGAN, C. and M.K. RAJAN. 2014. Effect of leaf extract of nilakumil, (*Gmelina asiatica*) against the root-knot nematode, (*Meloidogyne incognita*). *Research Journal of Recent Sciences* 3:264–266.

BIRD, A.F. 1959. The attractiveness of roots to the plant-parasitic nematodes *Meloidogyne javanica* and *Meloidogyne hapla*. *Nematologica* 4:322–335.

COSTA, S.S.R., SANTOS, M.S.N.A. and M.F. RYAN. 2003. Effect of *Artemisia vulgaris* rhizome extracts on hatching, mortality, and plant infectivity of *Meloidogyne megadora*. *Journal of Nematology* 35:437–442.

DONCASTER, C.C. and A.M. SHEPHERD. 1967. The behavior of second-stage *Heterodera rostochiensis* larvae leading to their emergence from the egg. *Nematologica* 13:476–478.

GOMEZ, K.A. and A.A. GOMEZ. 1984. Statistical Procedures for Agricultural Research. Wiley, New York.

HOUGH, A. and I.J. THOMASON. 1975. Effects of aldicarb on the behavior of *Heterodera schachtii* and *Meloidogyne javani*. *Journal of Nematology* 7:221–229.

IBRAHIM, S.K., TRABOULSI A.F. and S. EL-HAJ. 2006. Effect of essential oils and plant extracts on hatching, migration and mortality of *Meloidogyne incognita*. *Phytopathologia Mediterranea* 45:238–246.

JAVED, N., GOWENA, S.R., EL-HASSAN, S.A., INAM-UL-HAQ, M., SHAHINA, F. and B. PEMBROKE. 2008. Efficacy of neem (*Azadirachta indica*) formulations on biology of root-knot nematodes (*Meloidogyne javanica*) on tomato. *Crop Protection* 27:36–43.

KALAISELVAM, I. and A. DEVARAJ. 2011. Effect of root exudates of *Tagetes* sp. on egg hatching behaviour of *Meloidogyne incognita*. *International Research Journal of Pharmacy* 2:93–96.

LIU, D.L., AN, M., JOHNSON, I.R. and J.V. LOVETT. 2003. Mathematical modeling of allellopathy. III. A model for curve-fitting allellochemical dose responses. *Non-linearity Biology, Toxicology and Medicine* 1:37–50.

MASHELA, P.W., DUBE, Z.P. and K.M. POFU. 2015. Managing the Phytotoxicity and Inconsistent Nematode Suppression in Soil Amended with Phytonematicides. In: Meghvansi, M.K. and A. Vorma (eds.). Organic Amendments and Soil Suppressiveness in Plant Disease Management, Soil Biology 46. Springer International Publishers, Switzerland.

OKA, Y., BEN-DANIEL, B. and Y. COHEN. 2001. Nematicidal activity of powder and extracts of *Inula viscose*. *Nematologica* 3:735–742.

PAVARAJ, M., BAKAVATHIAPPAN, G. and S. BASKARAN. 2012. Evaluation of some plant extracts for their nematicidal properties against root-knot nematode, *Meloidogyne incognita*. *Journal of Biopesticides* 5:106–110.

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

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

PELINGANGA, O.M., MASHELA, P.W., MPHOSI, M.S., MAFEO, T.P. and Z.P. DUBE. 2013. Using computer-based model to determine phytotoxicity concentration of nemarioc-A phytonematicide in tomato production. *African Crop Science Conference Proceedings* 11:349–353.

PEREZ, M.P., NAVAS-CORTÉS, J.A., PASCUAL-VILLALOBOS, M.J. and P. CASTILLO. 2003. Nematicidal activity of essential oils and organic amendments from Asteraceae against root knot nematodes. *Plant Pathology* 52:395–401.

SAS INSTITUTE. 2008. SAS/STAT 9.2 Qualification tools user's guide. SAS Institute, Cary, NC.

SHADUNG, K.G. 2016. Quality Protocols for Nemarioc-AL and Nemafric-BL phytonematicides and their Respective Chemical Residues in Tomato Fruits. PhD Thesis, University of Limpopo. Sovenga, South Africa.

VAN WYK, B.E. and M. WINK. 2014. Medicinal Plants of the World. Briza Publications, Pretoria.

WALLACE, H.R. 1968. Nematode Ecology and Plant Disease. Edward Arnold, London.

WUYTS, N., SWENNEN, R. and D. DE WAELE. 2006. Effects of plant phenylpropanoid pathway products and selected terpenoids and alkaloids on the behavior of the plant-parasitic nematodes *Radopholus similis, Pratylenchus penetrans* and *Meloidogyne incognita*. *Nematology* 8:89–101.

# CHAPTER 5
# RESPONSES OF NEMATODE TO PURE CUCURBITACIN A AND B: JUVENILE MOBILITY TRIALS

## 5.1 Introduction

The influence of various plant extracts on nematode mobility has had less attention when compared to other mechanisms of nematode suppression, such as second-stage juveniles (J2) hatch and J2 mortality, with information on the pure active ingredients being even more scant (Mashela *et al*., 2015). Crude extracts of garlic (*Allium sativum* L.) and neem (*Azadirachta indica* A. Juss) at various concentrations each inhibited J2 motility (Agbenin *et al*., 2005). Pure carvacrol ($C_6H_3CH_3(OH)(C_3H_7)$), linalool ($C_{10}H_{18}O$), thymol ($C_{10}H_{14}O$), menthone ($C_{10}H_{18}O$) and glucosinolate [$RC(S\text{-}C_6H_{12}O_6)NOSO_3$] degradation products were found to immobilise *Meloidogyne incognita* J2 (Ibrahim *et al.*, 2006; Lazzeri *et al*., 2004). Oka *et al*. (2000) showed that essential oils from 12 different plants immobilised more than 80% *M. javanica* J2, with observed immobilisation being amenable to density-dependent growth (DDG) patterns.

According to the DDG principles (Liu *et al*., 2003), different concentrations of phytonematicides might have no effect (neutral), stimulate and/or inhibit the behaviour of nematodes. Several workers (Skantar *et al*., 2005; Wuyts *et al*. 2006; Zasada and Ferris, 2003) demonstrated that in addition to DDG pattern responses of nematodes to phytonematicides was nematode species-specific. Wuyts *et al*. (2006) observed that a chemical compound which had one effect on one nematode species could have a different effect on another nematode species, *vice versa*. Exposure of roundworm (*Caenorhabditis elegans* Maupas) and soyabean cyst nematode

(*Heterodera glycine* Ichinohe) to a series of geldanamycin ($C_{29}H_{40}N_2O_9$) concentrations exhibited contrasting DDG patterns (Skantar *et al*., 2005). Skantar *et al*. (2005) provided supporting evidence for phytonematicide-nematode species-specificity when low concentrations of geldanamycin inhibited the mobility of *C. elegans* but stimulated mobility of *H. glycine*, higher concentrations had contrasting effects on the two nematodes with *C. elegans* being stimulated whereas *H. glycine* was inhibited. The objective of this study was five-fold, namely, to establish whether (i) increasing concentration of cucurbitacin A and B would have impact on *M. incognita* J2 immobility, (ii) the Curve-fitting Allelochemical Response Dosage (CARD) model would quantify the three phases of density-dependent growth (DDG) pattern on J2 immobility when compared to increasing cucurbitacin concentration, (iii) computed J2 immobility concentration and CARD-generated inhibition dosage (D)-values would be statistically comparable in magnitudes, (iv) the CARD model would provide information on minimum inhibition concentration (MIC) and (v) J2 immobility would be reversible when cucurbitacins were diluted.

## 5.2 Materials and methods

*In vitro* trials were conducted in the location described previously (Chapter 3). Purified cucurbitacin A and B (1000 µg each), were prepared as explained previously (Chapter 3). Egg masses of *M. incognita* were obtained from two month-old. The egg masses were then placed in distilled water in an incubator set at 25 ± 2 ˚C. Juveniles that hatched in the first 24 h were discarded and those that hatched in the subsequent 48 h were used in the bioassay.

### 5.2.1 Mobility bioassay

Pure cucurbitacin A and B concentrations were tested for inhibition of nematode motility using modified method of Wuyts *et al*. (2006) in two parallel trials. The assessment was carried out using pure cucurbitacin in 9-cm-diameter petri dishes containing 10 mL of 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, and 2.50 $\mu g.mL^{-1}$ distilled water. Distilled water and methanol concentration 0.005% were used as control. Approximately, 450 freshly hatched J2 were added to each concentration. In all trials, treatments were replicated three times and arranged in a completely randomised block design in an incubator at 25 ± 2 $^{\circ}C$ for 12-, 24-, 48- and 72-h. After the pre-allotted time intervals each dish was emptied into a counting chamber, the mobile and immobile nematodes were counted using a stereomicroscope. Nematodes were considered immobile when no movement is observed during two seconds even after mechanical prodding with a bristle. Concentrations were considered motile inhibitive when significantly more nematodes became immobilised than in the control. In all trials, three independent experiments with treatments replicated three times were conducted.

### 5.2.2 Statistical analysis

Bioassay data were subjected to analysis of variance (ANOVA) through the SAS software (SAS Institute, 2008). Data were transformed using $\log_{10}(x + 1)$ prior to ANOVA. Mean separation was achieved using Waller-Duncan multiple range tests at the probability level of 5%, with the means further subjected to the CARD computer-based model to generate appropriate biological indices (Liu *et al*., 2003). Relative impact values were computed using [(treatment/control) – 1] x 100, relation. The

EHIC values at 50 and 100% were computed from relative impact quadratic equations, where x-values were equal to $EHIC_{50}$ and $EHIC_{100}$ for y-values equal to 50 and 100, respectively, using the quadratic formula as explained previously (Chapter 3)

Lines of the best fit between relative impact values and increasing concentrations of cucurbitacins were established. Mean exposure period values were subjected to the CARD model (Liu *et al*., 2003) to generate the regression curve estimations using the quadratic equation: $Y = b_2x^2 + b_1x + c$, where Y = J2 hatch inhibition mean value and x = exposure period mean value. The relation $x = -b_1/2b_2$ was used to determine the minimum inhibition concentration (MIC) for J2 hatch inhibition. Additionally, CARD-generated biological indices, *viz*., threshold stimulation ($D_m$), saturation point ($R_h$), 0% inhibition concentration ($D_0$), 50% inhibition concentration ($D_{50}$), 100% inhibition concentration ($D_{100}$), sensitivity index (k) and coefficient of determination ($R^2$) (Liu *et al*., 2003) were summarised. Unless otherwise stated, only treatments that were significant at the probability level of 5% were discussed.

## 5.3 Results

In both cucurbitacin A and B trials, there were no statistically significant differences between the methanol and distilled water controls. The distilled water control, therefore, was used throughout the study. There were also no statistically significant differences between the three independent experiments, hence the data were pooled.

5.3.1 Pure cucurbitacin A

Relative impact: Pure cucurbitacin A concentration effects on J2 immobility of *M. incognita* were highly significant (P ≤ 0.01) for all exposure times (Table 5.1, Appendix 5.1-5.4). At 12-, 24-, 48- and 72-h the cucurbitacin A contributed 84 99, 99 and 99% in total treatment variation (TTV) of J2 immobility, respectively (Table 5.1). Relative to untreated control, J2 immobility increased with increase in cucurbitacin A concentration and exposure time (Table 5.2). Relative impact values of J2 immobility when plotted against cucurbitacin A concentrations showed DDG patterns (Figure 5.1). In cucurbitacin A, the DDG patterns had stimulation and neutral effects on J2 immobility as concentrations increased (Figure 5.1).

Table 5.1 Partitioning mean sum of squares for *Meloidogyne incognita* second-stage juvenile immobility in pure cucurbitacin A after 12-, 24-, 48- and 72-h exposure periods.

| Source | DF | 12 h | | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|---|---|---|
| | | MS | % | MS | % | MS | % | MS | % |
| Trt | 11 | 3.163 | 84** | 3.014 | 99** | 3.366 | 99** | 3.197 | 99** |
| Error | 96 | 0.013 | 16 | 0.011 | 1 | 0.016 | 1 | 0.015 | 1 |
| Total | 107 | 3.767 | 100 | 3.025 | 100 | 3.382 | 100 | 3.212 | 100 |

**Significant at P ≤ 0.01.

Table 5.2 Influence of pure cucurbitacin A on *Meloidogyne incognita* second-stage juvenile immobility in Nemarioc-AL phytonematicide after 12-, 24-, 48- and 72-h exposure periods.

| Concentration ($\mu g.mL^{-1}$) | 12 h | | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|---|---|
| | Mean[y] | Rl (%)[z] | Mean | Rl (%) | Mean | Rl (%) | Mean | Rl (%) |
| 0.00 | 0.82a | – | 0.91a | – | 0.87a | – | 0.90a | – |
| 0.25 | 1.68b | 105 | 1.67b | 84 | 1.66b | 91 | 1.71b | 90 |
| 0.50 | 2.06c | 151 | 2.09c | 130 | 2.11c | 143 | 2.17c | 141 |
| 0.75 | 2.20d | 168 | 2.19d | 141 | 2.26d | 160 | 2.28d | 153 |
| 1.00 | 2.23d | 172 | 2.24de | 146 | 2.30de | 164 | 2.33de | 159 |
| 1.25 | 2.28de | 178 | 2.26de | 148 | 2.32de | 167 | 2.34de | 160 |
| 1.50 | 2.31def | 182 | 2.33ef | 156 | 2.38ef | 174 | 2.40ef | 167 |
| 1.75 | 2.38efg | 190 | 2.42fg | 166 | 2.44fg | 180 | 2.46fg | 173 |
| 2.00 | 2.41fg | 194 | 2.45gh | 169 | 2.47fg | 184 | 2.49fg | 177 |
| 2.25 | 2.44g | 198 | 2.48gh | 173 | 2.51g | 189 | 2.52g | 180 |
| 2.50 | 2.49g | 204 | 2.52h | 177 | 2.55g | 193 | 2.56g | 184 |

[y]Column means followed by the same letter were not different at $P \leq 0.05$, according to Waller-Duncan multiple range test.

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

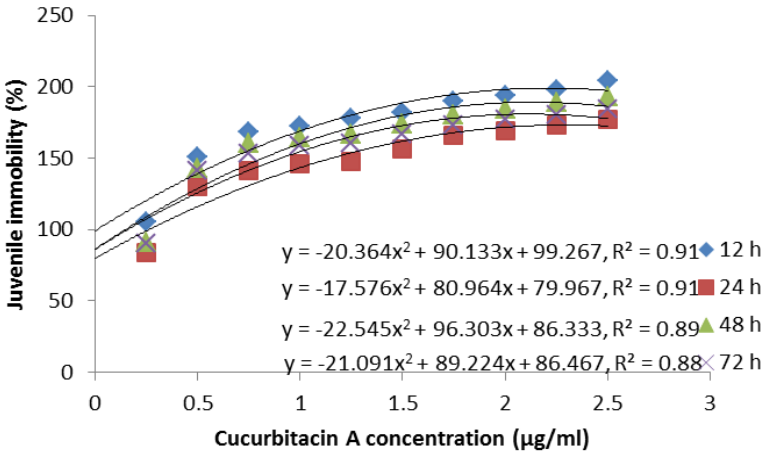


Figure 5.1 Relative impact of pure cucurbitacin A on second-stage juvenile immobility of *Meloidogyne incognita*.

Table 5.3 Biological indices of *Meloidogyne incognita* second-stage juvenile immobility to increasing concentrations of pure cucurbitacin A after 12-, 24-, 48- and 72-h exposure periods.

| Biological index | 12 h | 24 h | 48 h | 72 h |
|---|---|---|---|---|
| Threshold stimulation ($D_m$) | 5.76 | 22.41 | 9.29 | 6.18 |
| Saturation point ($R_h$) | 1.64 | 1.68 | 1.71 | 1.66 |
| 0% inhibition ($D_0$) | – | – | – | – |
| 50% inhibition ($D_{50}$) | – | – | – | – |
| 100% inhibition ($D_{100}$) | – | – | – | – |
| $R^2$ | 0.99 | 0.99 | 0.99 | 0.99 |
| Sensitivity index (k) | 4 | 4 | 4 | 4 |

Curve-fitting Allelochemical Response Dosage: The CARD model quantified concentration ranges that could stimulate ($D_m$-$R_h$) J2 immobility only (Table 5.3). The stimulation phase concentration range was characterised by positive values for J2 immobility at all exposure periods (Table 5.3). The CARD-generated DDG patterns demonstrated that at low cucurbitacin concentrations J2 immobility was stimulated whereas at high concentrations J2 immobility was neutral (Figure 5.2). The sensitivity of J2 to increasing concentrations of cucurbitacin A was very high for J2 immobility (Table 5.3) with sensitivity values of 4 units for all exposure periods. The sensitivity of J2 immobility to cucurbitacin A was 4 units (Table 5.3).

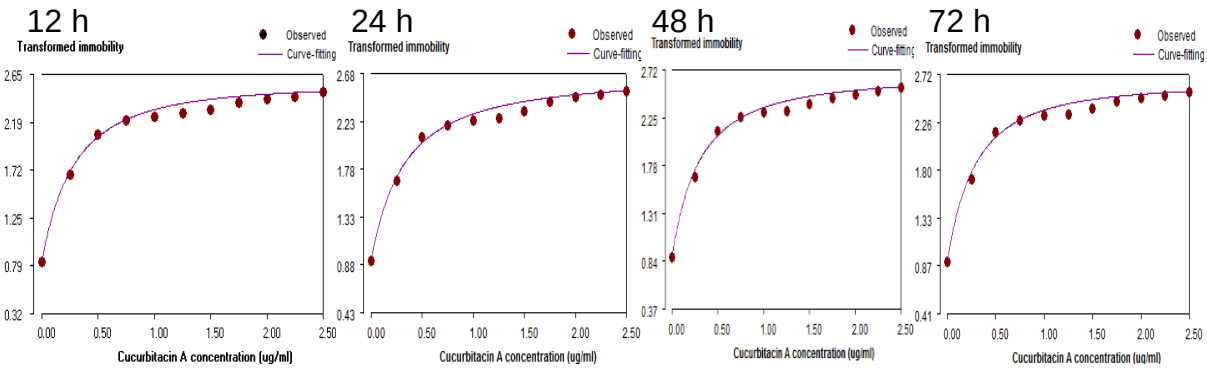


Figure 5.2 Curve-fitting Allelochemical Response Dosage (CARD)-generated density-dependent growth responses of *Meloidogyne incognita* second-stage juvenile immobility to increasing concentrations of pure cucurbitacin A at 12-, 24-, 48- and 72-h exposure periods.

Comparison of juvenile immobility (JIC) to CARD-generated D-values: The CARD model was unable to generate $D_{50}$ and $D_{100}$ values for J2 immobility (Table 5.4). Computed JIC-values decreased with increase in the exposure periods (Table 5.4).

Table 5.4 Comparison of pure cucurbitacin A second-stage juvenile immobility concentration (JIC) and inhibition dosage (D)-values.

| Biological index | Exposure period (h) | | | |
|---|---|---|---|---|
| | 12 | 24 | 48 | 72 |
| $JIC_{50}$ | –0.5 | –0.3 | –0.4 | 0.4 |
| $D_{50}$ | – | – | – | – |
| $JIC_{100}$ | 0.0 | 0.3 | 0.2 | 0.2 |
| $D_{100}$ | – | – | – | – |

Minimum inhibition concentration (MIC): The MIC values of J2 immobility in *M. incognita* were increasing with increase in exposure periods for 12- to 24-h exposure periods, thereafter it remained constant (Table 5.5).

Table 5.5 Minimum inhibition concentration of pure cucurbitacin A on second-stage juvenile immobility of *Meloidogyne incognita* after 12-, 24-, 48- and 72-h exposure periods.

| Incubation period (h) | Model | x (%)[z] |
|---|---|---|
| 12 | $y = –5.502x^2 + 6.011x + 0.828$ | 0.5 |
| 24 | $y = –4.184x^2 + 5.306x + 0.917$ | 0.6 |
| 48 | $y = –5.041x^2 + 5.87x + 0.868$ | 0.6 |
| 72 | $y = –5.432x^2 + 5.996x + 0.901$ | 0.6 |

[z] $x = –b_1/2b_2$, where $y = b_2x^2 + b_1x + c$.

Reversibility of juvenile immobility: The juvenile immobility effects of pure cucurbitacin A were not reversible, as demonstrated by non-significant treatment means ($P > 0.05$) in ANOVA (Table 5.6, Appendix 5.5).

Table 5.6 Partitioning mean sum of squares for reversibility of *Meloidogyne incognita* second-stage juvenile immobility in pure cucurbitacin A.

| Source | DF | MS | % |
|---|---|---|---|
| Treatment | 11 | 0.33162 | 49$^{ns}$ |
| Error | 96 | 0.33853 | 51 |
| Total | 107 | 0.67015 | 100 |

$^{ns}$Not significant ($P > 0.05$).

5.3.2 Pure cucurbitacin B

Relative impact: Treatment effects of cucurbitacin B at all exposure periods were highly significant on J2 immobility (Table 5.7, Appendix 5.6-5.9). Treatment effects at 12-, 24-, 48- and 72-h exposure periods contributed 99, 99, 99 and 99% in TTV on J2 immobility, respectively (Table 5.7). Relative to untreated control, J2 immobility increased with increase in cucurbitacin B concentrations and exposure period (Table 5.8). Relative impact values of J2 immobility over increasing concentrations of cucurbitacin B exhibited a DDG pattern, which had a neutral and inhibition effect on J2 immobility at low and high concentrations, respectively (Figure 5.4).

Table 5.7 Partitioning mean sum of squares for *Meloidogyne incognita* second-stage juvenile immobility in pure cucurbitacin B after 12-, 24-, 48- and 72-h exposure periods.

| | | 12 h | | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|---|---|---|
| Source | DF | MS | % | MS | % | MS | % | MS | % |
| Trt | 11 | 3.470 | 99** | 3.406 | 99** | 0.0989 | 99** | 3.059 | 99** |
| Error | 96 | 0.018 | 1 | 0.015 | 1 | 0.0007 | 1 | 0.022 | 1 |
| Total | 107 | 3.449 | 100 | 3.422 | 100 | 0.099 | 100 | 3.082 | 100 |

**Significant at P ≤ 0.01.

Curve-fitting Allelochemical Response Dosage: The CARD-generated biological indices showed only cucurbitacin B concentration range that stimulates J2 immobility (Table 5.9). The stimulation phase concentration range was characterised by negative to positive values at all exposure times for J2 immobility. J2 immobility was relatively sensitive to increasing concentrations of cucurbitacin B at all exposure periods (Table 5.9). The sensitivity of J2 immobility in cucurbitacin B concentrations was 3–5 units (Table 5.9). The CARD-generated DDG patterns demonstrated that low concentration of cucurbitacin B stimulated J2 immobility at all exposure periods and as concentration increased J2 immobility became neutral (Figure 5.7).

Table 5.8 Influence of pure cucurbitacin B on *Meloidogyne incognita* second-stage juvenile immobility in after 12-, 24-, 48- and 72-h exposure periods.

| Concentration ($\mu g.mL^{-1}$) | 12 h | | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|---|---|
| | Mean[y] | RI (%)[z] | Mean | RI (%) | Mean | RI (%) | Mean | RI (%) |
| 0.00 | 0.78a | - | 0.83a | - | 0.26a | - | 0.92a | - |
| 0.25 | 1.62b | 108 | 1.59b | 92 | 0.43b | 65 | 1.69b | 84 |
| 0.50 | 2.06c | 164 | 2.05c | 147 | 0.49c | 88 | 2.09c | 127 |
| 0.75 | 2.17cd | 178 | 2.19d | 164 | 0.51cd | 96 | 2.21cd | 140 |
| 1.00 | 2.24d | 187 | 2.25d | 171 | 0.52de | 100 | 2.27d | 147 |
| 1.25 | 2.28de | 192 | 2.30de | 177 | 0.52def | 100 | 2.31de | 151 |
| 1.50 | 2.39ef | 206 | 2.41ef | 190 | 0.54efg | 108 | 2.42ef | 163 |
| 1.75 | 2.41f | 209 | 2.43fg | 193 | 0.54efg | 108 | 2.44ef | 165 |
| 2.00 | 2.45f | 214 | 2.47fg | 198 | 0.54fg | 108 | 2.47f | 168 |
| 2.25 | 2.48f | 218 | 2.49fg | 200 | 0.55g | 112 | 2.49f | 171 |
| 2.50 | 2.52f | 223 | 2.53g | 205 | 0.55g | 112 | 2.52f | 174 |

[y]Column means followed by the same letter were not different at $P \leq 0.05$, according to Waller-Duncan multiple range test.

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

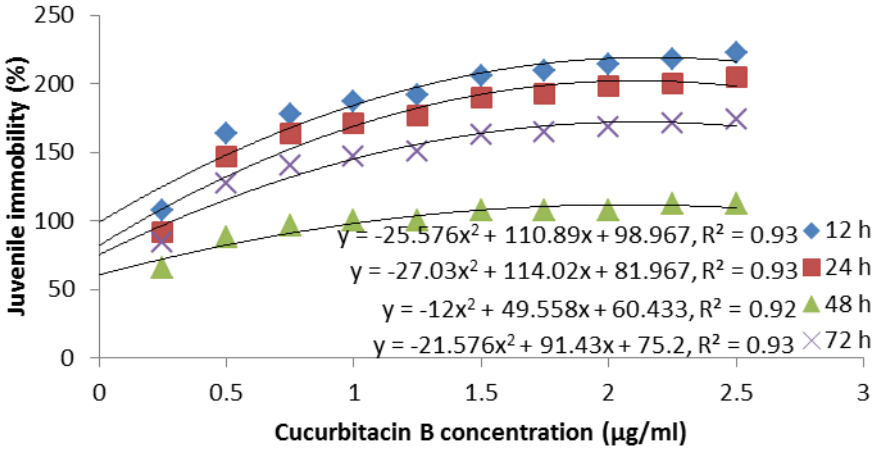


Figure 5.3 Relative impact of pure cucurbitacin B increasing concentrations on *Meloidogyne incognita* second-stage juvenile immobility at 12-, 24-, 48- and 72-h exposure periods.

Table 5.9 Biological indices of *Meloidogyne incognita* second-stage juvenile immobility to increasing concentrations of pure cucurbitacin B after 12-, 24-, 48- and 72-h exposure periods.

| Biological index | 12 h | 24 h | 48 h | 72 h |
|---|---|---|---|---|
| Threshold stimulation ($D_m$) | 12.20 | 4.32 | 9.64 | 13.57 |
| Saturation point ($R_h$) | 1.80 | 1.70 | 0.30 | 1.68 |
| 0% inhibition ($D_0$) | – | – | – | – |
| 50% inhibition ($D_{50}$) | – | – | – | – |
| 100% inhibition ($D_{100}$) | – | – | – | – |
| $R^2$ | 0.99 | 0.99 | 0.99 | 0.99 |
| Sensitivity index (k) | 4 | 3 | 5 | 4 |

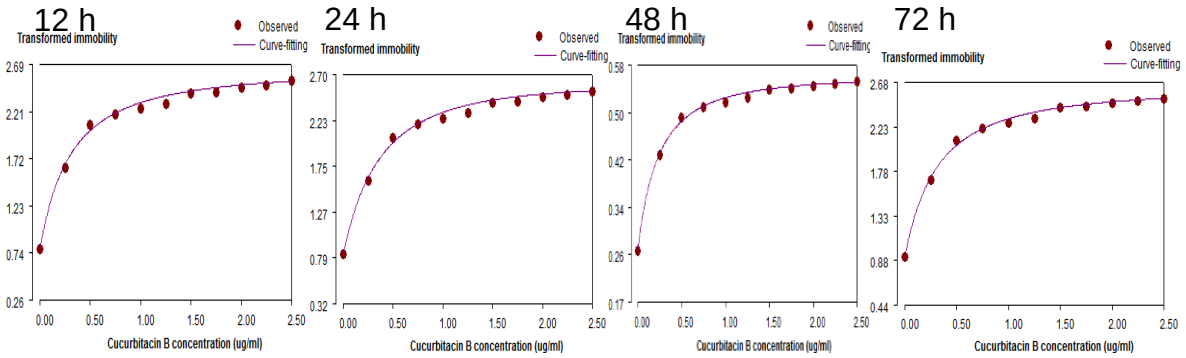


Figure 5.4 Curve-fitting Allelochemical Response Dosage (CARD)-generated density-dependent growth responses of *Meloidogyne incognita* second-stage juvenile immobility to increasing concentrations of pure cucurbitacin B at 12-, 24-, 48- and 72-h exposure periods.

Comparison of juvenile immobility (JIC) to CARD-generated D-values: The CARD model was unable to generate $D_{50}$ and $D_{100}$ values for cucurbitacin B on J2 immobility (Table 5.10).

Table 5.10 Comparison of cucurbitacin B second-stage juvenile immobility concentration and inhibition dosage (D)-values.

| Biological index | 12 h | 24 h | 48 h | 72 h |
|---|---|---|---|---|
| $JIC_{50}$ | –46.9 | –10.4 | –2.9 | –2.0 |
| $D_{50}$ | – | – | – | – |
| $JIC_{100}$ | –50.0 | –12.6 | –4.1 | –3.1 |
| $D_{100}$ | – | – | – | – |

Minimum inhibition concentration (MIC): The MIC values of J2 immobility in *M. incognita* had no defined single trends, increasing between exposure periods 12- to 48-h, then a sharp drop at 72-h exposure period (Table 5.11).

Table 5.11 Minimum inhibition concentration of pure cucurbitacin B on second-stage juvenile immobility of *Meloidogyne incognita* from quadratic curves generated by Curve-fitting Allelochemical Response Dosage (CARD) model.

| Incubation period (h) | Model | x (%)[z] |
|---|---|---|
| 12 | $y = -4.986x^2 + 5.984x + 0.780$ | 0.6 |
| 24 | $y = -3.623x^2 + 4.960x + 0.832$ | 0.7 |
| 48 | $y = -1.388x^2 + 1.278x + 0.262$ | 0.5 |
| 72 | $y = -4.557x^2 + 5.528x + 0.915$ | 0.6 |

[z] $x = -b_1/2b_2$, where $y = b_2x^2 + b_1x + c$.

Reversibility of juvenile immobility: The juvenile immobility effects of pure cucurbitacin B was not reversible, as demonstrated by non-significant treatment means in ANOVA (Table 5.12, Appendix 5.10).

Table 5.12 Partitioning mean sum of squares for reversibility of *Meloidogyne incognita* second-stage juvenile immobility in pure cucurbitacin B.

| Source | DF | MS | % |
|---|---|---|---|
| Treatment | 11 | 0.41359 | 42$^{ns}$ |
| Error | 96 | 0.56071 | 58 |
| Total | 107 | 0.97430 | 100 |

$^{ns}$Not significant (P > 0.05).

## 5.4 Discussion

### 5.4.1 Relative impact

The *M. incognita* J2 immobility induced by cucurbitacins observed in this study supports other findings where pure chemical compounds derived from plants (Ibrahim *et al*., 2007; Lazzeri *et al*., 2004; Wuyts *et al*., 2006), crude plant extracts (Ibrahim *et al*., 2007; Javed *et al*., 2007; Skantar *et al*., 2005) and other natural chemical compounds (Al-Azzeh and Abu-Gharbieh, 2004; Hough and Thomason, 1975) had effects on J2 mobility. The major finding in the current study was the DDG patterns of J2 immobility to increasing cucurbitacin concentrations. At low concentrations J2 immobility was stimulated, but as cucurbitacin concentrations were increased neutral effects were observed. Ibrahim *et al*. (2006), using three concentrations, namely, 1-, 2- and 4-mg.L$^{-1}$ water, observed the inhibitive effects of pure components, carvacrol, linalool, thymol and menthone, on *M. incognita* J2.

Three concentrations of glucosinolate degradation products, ranging from 0.0025 to 25 mM had also showed an inhibition effect on *M. incognita* (Lazzeri *et al*., 2004). Javed *et al*. (2007), using three concentrations of 10, 5 and 2.5% neem crude extracts observed an increase in *M. javanica* J2 mobility inhibition with increase in concentrations. When multiple concentrations of geldanamycin (GA) were used on *C*. *elegans* and *H*. *glycine* J2, multiple effects were observed (Skantar *et al*., 2005). At low concentrations (below 25 GA $\mu g.mL^{-1}$), *C. elegans* J2 mobility was inhibited but higher concentrations (30-100 $\mu g.mL^{-1}$) stimulated mobility of the nematode, opposite trend was witnessed for *H. gylcine* (Skantar *et al*., 2005).

Skantar *et al*. (2005) used the principle of hormesis to explain the observed DDG patterns. Hormesis is an adaptative response where there is induction of beneficial effects when the organism is exposed to low dosages of harmful chemical or physical agent (Zhao and Wang, 2012). In hormesis, after a small stress, special proteins responsible for the removal of damage produced from stressor are over-produced resulting in not only the removal of damage produced by the current stress, but also removal of the pre-existing damage, this produces a stimulative effect (Butov *et al*., 2001). *Caenorhabditis elegans* had been used as a model nematode in a number of studies where the phenomenon of hormesis has been observed and a number of proteins responsible for removal of damage observed (Helmcke and Aschner, 2010; Wang and Xing, 2009; Yanase *et al*., 1999). Attempts to explain DDG pattern using hormesis could explain well the stimulative effect of GA on *H. glycine* at low concentrations, but fails to explain the inhibition effect of GA on

*C. elegans* at low concentrations. Therefore the theory could not be used to explain the findings in current study.

The inhibition effects at low concentrations (Skantar *et al*., 2005) of cucurbitacins could be explained by another adaptation behaviour of nematodes called the dauer stage (McSorley, 2003). At low concentration ranges J2 might have interpreted the condition as being due to waning root exudates with the onset of plant senescence, thereby entering the dauer stage, high concentration of cucurbitacins in this study could have triggered the response analogous to increased root exudates in trap crops (Wuyts *et al*., 2006), resulting in increased mobility of *M. incognita* J2.

### 5.4.2 Curve-fitting Allelochemical Response Dosage model

The CARD-generated DDG patterns observed in this study were similar to those of relative impacts described above and also to those when eggs were exposed to similar products (Chapter 3). This is the first report of use of CARD model in generation of DDG patterns for J2 immobility of nematodes. The similarity of the CARD-generated DDG patterns with those of impact values provide more evidence that the CARD model can be adopted for these kind of studies.

### 5.4.3 Juvenile immobility concentration (JIC) and inhibition dosage (D) -values

In the current study, the CARD model could not generate the D-values for J2 immobility, hence the comparison between JIC and D-values could not be made.

### 5.4.4 Minimum inhibition concentration

The J2 MIC values exposed to pure cucurbitacins in this study displayed a similar trend to those observed when eggs were exposed to the same concentrations (Chapter 3), but were relatively lower. The minimum J2 immobility inhibition concentrations of between 0.5 to 0.7 $\mu g.mL^{-1}$ in distiled water were higher than those of *H. schachtii* and *M. javanica* J2 exposed to aldicarb at 1 to 5 $\mu g.mL^{-1}$ water (Hough and Thomason, 1975).

### 5.4.5 Overall sensitivity of juvenile immobility

Juvenile immobility was highly sensitive to cucurbitacin A and B as shown by low k values across all incubation periods. *Meloidogyne incognita* J2 were highly sensitive to cucurbitacin A across all concentrations when compared with eggs of the same nematode (Chapter 3), whereas the opposite was observed for cucurbitacin B. Previous studies on plant phytotoxicity of Nemarioc-AL and Nemafric-BL phytonematicides had observed that different plant organs had different k-values, with those that come in direct contact with the phytonematicides, such as the roots having higher sensitivity than shoots that do not come in direct contact with the phytonematicide (Pelinganga *et al*., 2012). In the current study this could explain high sensitivity of J2 to cucurbitacin A when compared to eggs (Chapter 3), but could not explain the higher sensitivity of J2 when compared to eggs when exposed to cucurbitacin B. Apparently, this is the first report of empirically-derived evidence of direct sensitivity of nematode J2 mobility to pure cucurbitacins using the CARD model. The observation provided evidence that the CARD model could be a valuable

tool for comparison of different plant extracts when used in the management of plant-parasitic nematodes.

## 5.5 Conclusion

Juvenile immobility over increasing concentrations of pure cucurbitacins had DDG patterns which were similar for conventional method and those from CARD model. At low concentrations, cucurbitacins inhibited J2 mobility, whereas at high concentrations the material was neutral both for relative impact value graphs and CARD-generated graphs. CARD-generated $D_{50}$ and $D_{100}$ were comparative to the computed $JMC_{50}$ and $JMC_{100}$. The CARD model was able to generate MIC values and demostrate that J2 immobility was relatively sensitive to both cucurbitacin A and cucurbitacin B. There was no reversibility of the J2 immobility effects in both pure cucurbitacins.

## 5.6 References

AGBENIN, N.O., EMECHE, A.M., MARLEY, P.S. and A.D. AKPA. 2005. Evaluation of nematicidal action of some botanicals on *Meloidogyne incognita in vitro*. *Journal of Agriculture and Rural Development in the Tropics and Subtropics* 1:29–39.

AL-AZZEH, T.K. and W.I. ABU-GHARBIEH. 2004. Effect of oxamyl and fenamiphos on egg hatching, motility, and root penetration of *Tylenchulus semipenetrans*. *Nematologia Mediterranea* 32:19–23.

BUTOV, A., JOHNSON, T., CYPSER, J., SANNIKOV, I., VOLKOV, M., SEHL, M. and A. YASHIN. 2001. Hormesis and debilitation effects in stress experiments

using the nematode worm *Caenorhabditis elegans*: the model of balance between cell damage and hsp levels. *Experimental Gerontology* 37:57–66.

HELMCKE, K.J. and M. ASCHNER. 2010. Hormetic effect of methyl-mercury on *Caenorhabditis elegans*. *Toxicology and Applied Pharmacology* 248:156–164.

HOUGH, A. and I.J. THOMASON. 1975. Effects of aldicarb on the behavior of *Heterodera schachtii* and *Meloidogyne javanica*. *Journal of Nematology* 7:221–229.

IBRAHIM, S.K., TRABOULSI, A.F. and S. EL-HAJ. 2006. Effect of essential oils and plant extracts on hatching, migration and mortality of *Meloidogyne incognita*. *Phytopathologia Mediterranea* 45:238–246.

IBRAHIM, I.K.A., EL-SAEDY, M.A.M. and A.A. MOKBEL. 2007. Control of the root-knot nematode *Meloidogyne incognita* on sunflower plants with certain organic plant materials and biocontrol agents. *Egyptian Journal of Phytopathology* 35:13–24.

JAVED, N., GOWEN, S.R., INAM-UL-HAQ, M. and S.A. ANWAR. 2007. Protective and curative effect of neem (*Azadirachta indica*) formulations on the development of root-knot nematode, *Meloidogyne javanica* in roots of tomato plants. *Crop Protection* 26:530–534.

LAZZERI, L., CURTO, G., LEONI, O. and E. DALLAVALLE. 2004. Effects of glucosinolates and their enzymatic hydrolysis products via myrosinase on the root-knot nematode *Meloidogyne incognita* Kofoid and White Chitw. *Journal of Agricultural and Food Chemistry* 52:6703–6707.

LIU, D.L., AN, M., JOHNSON, I.R. and J.V. LOVETT. 2003. Mathematical modeling of allellopathy. III. A model for curve-fitting allellochemical dose responses. *Non-linearity Biology, Toxicology and Medicine* 1:37–50.

MASHELA, P.W., DUBE, Z.P. and K.M. POFU. 2015. Managing the Phytotoxicity and Inconsistent Nematode Suppression in Soil Amended with Phytonematicides. In: Meghvansi, M.K. and A. Vorma (eds.). Organic Amendments and Soil Suppressiveness in Plant Disease Management, Soil Biology 46. Springer International Publishers, Switzerland.

McSORLEY, R. 2003. Adaptations of nematodes to environmental extremes. *Florida Entomologist* 86:138–142.

OKA, Y., NACAR, S., PUTIEVSKY, E., RAVID, U., YANIV, Z. and Y. SPIEGEL. 2000. Nematicidal activity of essential oils and their components against the root-knot nematode. *Phytopathology* 90:710–715.

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

SAS INSTITUTE. 2008. SAS/STAT 9.2 Qualification tools user's guide. SAS Institute, Cary, NC.

SKANTAR, A.M., AGAMA, K., MEYER, S.L.F., CARTA, L.K. and B.T. VINYARD. 2005. Effects of geldanamycin on hatching and juvenile motility in *Caenorhabditis elegans* and *Heterodera glycines*. *Journal of Chemical Ecology* 31:2481–2491.

WANG, D. and X. XING. 2009. Pre-treatment with mild metal exposure suppresses the neurotoxicity on locomotion behavior induced by the subsequent severe metal exposure in *Caenorhabditis elegans*. *Environmental Toxicology and Pharmacology* 28:459–464.

WUYTS, N., SWENNEN, R. and D. DE WAELE. 2006. Effects of plant phenylpropanoid pathway products and selected terpenoids and alkaloids on the behavior of the plant-parasitic nematodes *Radopholus similis, Pratylenchus penetrans* and *Meloidogyne incognita*. *Nematology* 8:89–101.

YANASE, S., HARTMAN, P.S., ITO, A. and N. ISHII. 1999. Oxidative stress pretreatment increases the X-radiation resistance of the nematode *Caenorhabditis elegans. Mutation Research* 426:31–39.

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

ZHAO, Y.L. and D.Y. WANG. 2012. Formation and Regulation of Adaptive Response in Nematode *Caenorhabditis elegans*. *Oxidative Medicine and Cellular Longevity* 8:38–78.

# CHAPTER 6
# RESPONSES OF NEMATODE TO PHYTONEMATICIDES: JUVENILE MOBILITY TRIALS

## 6.1 Introduction

In pure form, active ingredients of Nemarioc-AL and Nemafric-BL phytonematicides, cucurbitacin A and B, respectively, affected *M. incognita* second-stage juveniles (J2) immobility in a density-dependent growth (DDG) pattern (Chapter 5). The Curve-fitting Allelochemical Response Dosage (CARD) model could not generate J2 inhibition values at 50 and 100% ($D_{50}$, $D_{100}$), but was able to provide good estimates of sensitivity values and minimum inhibition concentration of J2 immobility, for the two active ingredients (Chapter 5). Generally, J2 immobility was relatively sensitive to both cucurbitacin A and B. In crude form, Nemarioc-AL and Nemafric-BL phytonematicides had been reported to suppress plant-parasitic nematodes in the greenhouse trials by over 90% (Mashela, 2002), in microplot trials by 90% (Pelinganga, 2013) and in field trials by over 80% (Mashela, 2007). However, information on how J2 mobility would respond to the two phytonematicides had not been established. The objective of this study was fivefold, namely, to test whether (i) increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides would have impact on *M. incognita* J2 immobility, (ii) the CARD model would quantify the three phases of DDG patterns on J2 immobility when compared to increasing phytonematicide concentrations, (iii) computed J2 immobility concentration and CARD-generated D-values would be statistically comparable in magnitudes, (iv) the CARD model would provide information on minimum inhibition concentration (MIC) and (v) J2 immobility inhibition would be reversible when phytonematicides were diluted.

## 6.2 Materials and methods

The *in vitro* trials were conducted at the Green Technologies Research Centre (GTRC), University of Limpopo, South Africa (23°53′10″S, 29°44′15″E).

### 6.2.1 Phytonematicide preparations

Nemarioc-AL and Nemafric-BL phytonematicides were prepared by fermenting oven-dried fruits of wild cucumber (*Cucumis myriocarpus* Naudin) and wild watermelon (*C. africanus* L.), respectively (Mashela *et al*., 2015; Pelinganga *et al*., 2013). Ten concentrations, namely, 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0% for each phytonematicide were made in 9 cm-diameter petri dish. Two controls were chosen, distilled water alone and distilled water containing effective microorganisms to assess whether the effective microorganisms used in the preparation of the phytonematicide had an added effect. Eggs were collected and prepared as described previously (Chapter 5).

### 6.2.2 Mobility bioassay

Nemarioc-AL and Nemafric-BL phytonematicides were tested for inhibition of nematode motility using the modified methods of Wuyts *et al*. (2006) in two parallel trials. The assessment was carried out in 9-cm-diameter petri dishes containing 10 mL of different extract concentrations. Freshly hatched second-stage juveniles were added to each concentration. The petri dishes were then incubated at 25 ± 2 °C for 12-, 24-, 48- and 72-h. After the pre-allotted time intervals each dish was emptied into a counting chamber, using a stereo dissecting microscope at magnification 40 X, the immobile nematodes were counted. Nematodes were considered immobile when

no movement is observed during two seconds even after mechanical prodding with a bristle (Wuyts *et al*., 2006). Concentrations were considered mobile inhibitive when significantly more nematodes became immobilised than in the control. In all trials, three independent experiments with treatments replicated three times were conducted (Wuyts *et al*., 2006).

### 6.2.3 Statistical analysis

Bioassay data were subjected to analysis of variance (ANOVA) through the SAS software (SAS Institute, 2008). Separation of means was achieved using Waller-Duncan multiple range tests at a probability level of 5%, with the means further subjected to the Curve-fitting Allelochemical Response Dosage (CARD) computer-based model to generate appropriate biological indices (Liu *et al*., 2003). The concentration that causes 50 and 100% nematode J2 immobility ($JIC_{50}$- 12-, 24-, 48- and 72-h) was determined as previously described (Chapter 5). Unless otherwise stated, treatment effects were discussed at 5% level of probability.

## 6.3 Results

In both Nemarioc-AL and Nemafric-BL phytonematicide trials, there were no statistically significant differences between the effective microorganism and distilled water controls. The distilled water control therefore was used throughout the study. There were also no statistically significant differences between the three independent experiments, hence the data were pooled.

6.3.1 Nemarioc-AL phytonematicide

Relative impact: Nemarioc-AL phytonematicide concentration effects on J2 immobility of *M. incognita* were highly significant (P ≤ 0.01) for all exposure times (Table 6.1, Appendix 6.1-6.4). Nemarioc-AL phytonematicide concentrations at 12-, 24-, 48- and 72-h contributed 97, 98, 98 and 99% in TTV of J2 immobility, respectively (Table 6.1). Relative to untreated control, J2 immobility increased with increase in phytonematicide concentration and exposure time (Table 6.2). Relative impact values of J2 immobility when plotted against phytonematicide concentrations showed DDG patterns (Figure 6.1). The DDG patterns had stimulation, neutral and inhibition effect on J2 immobility as phytonematicide concentration increased (Figure 6.1).

Table 6.1 Partitioning mean sum of squares for *Meloidogyne incognita* second-stage juvenile immobility in Nemarioc-AL phytonematicide after 12-, 24-, 48- and 72-h exposure periods.

| | | 12 h | | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|---|---|---|
| Source | DF | MS | % | MS | % | MS | % | MS | % |
| Trt | 11 | 2.1428 | 97** | 2.409 | 98** | 2.601 | 98** | 2.962 | 99** |
| Error | 96 | 0.0680 | 3 | 0.055 | 2 | 0.055 | 2 | 0.042 | 1 |
| Total | 107 | 2.211 | 100 | 2.464 | 100 | 2.656 | 100 | 3.004 | 100 |

**Significant at P ≤ 0.01.

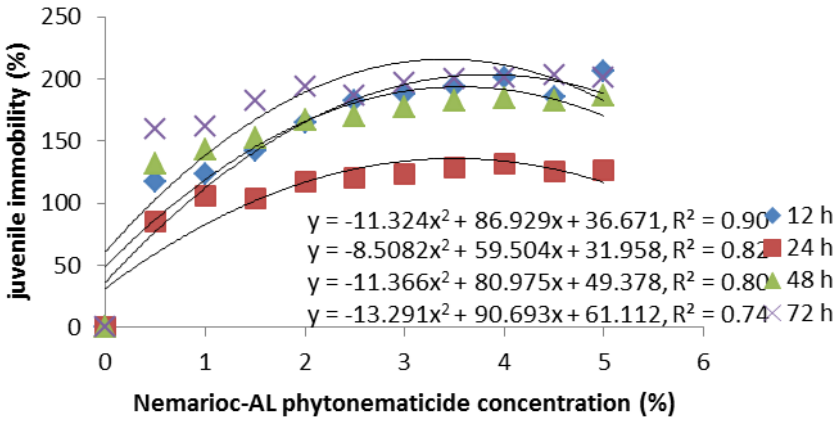


Figure 6.1 Relative impact of Nemarioc-AL phytonematicide on second-stage juvenile immobility of *Meloidogyne incognita*.

Curve-fitting Allelochemical Response Dosage: The CARD model quantified concentration ranges that could stimulate ($D_m$–$R_h$), saturate ($R_h$–$D_0$) and inhibit ($D_0$–$D_{100}$), J2 immobility, for 12- and 72-h exposure periods and only concentration range that stimulate for 24-h exposure period (Table 6.3). The CARD-generated J2 immobility DDG patterns demonstrated a stimulation effect on J2 immobility at low concentrations and saturation effect at higher concentrations (Figure 6.2). Generally, J2 immobility was highly sensitive to concentrations of Nemarioc-AL phytonematicide, with sensitivity values of 0–4 units (Table 6.3). Sensitivity of J2 immobility to Nemarioc-AL phytonematicide decreased with increase in exposure period (Table 3).

Table 6.2 Influence of Nemarioc-AL phytonematicide on *Meloidogyne incognita* second-stage juvenile immobility in Nemarioc-AL phytonematicide after 12-, 24-, 48- and 72-h exposure periods.

| | 12 h | | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|---|---|
| Conc. (%) | Mean[y] | Rl (%)[z] | Mean | Rl (%) | Mean | Rl (%) | Mean | Rl (%) |
| 0.0 | 0.76a | – | 1.01b | – | 0.82a | – | 0.77a | – |
| 0.5 | 1.66b | 117 | 1.88b | 85 | 1.91b | 132 | 2.00b | 160 |
| 1.0 | 1.71b | 123 | 2.01bc | 106 | 1.99bc | 143 | 2.02bc | 162 |
| 1.5 | 1.85bc | 142 | 2.07bcd | 104 | 2.07bcd | 152 | 2.17bcd | 182 |
| 2.0 | 2.03cd | 165 | 2.20cde | 117 | 2.19cde | 167 | 2.26cd | 194 |
| 2.5 | 2.15de | 182 | 2.23cde | 120 | 2.21de | 170 | 2.21d | 187 |
| 3.0 | 2.20de | 188 | 2.26de | 123 | 2.27de | 177 | 2.29d | 197 |
| 3.5 | 2.25de | 194 | 2.32e | 129 | 2.31e | 182 | 2.31d | 200 |
| 4.0 | 2.30de | 201 | 2.35e | 132 | 2.33e | 184 | 2.32d | 201 |
| 4.5 | 2.18e | 186 | 2.29e | 126 | 2.31e | 182 | 2.33d | 203 |
| 5.0 | 2.34e | 206 | 2.30e | 127 | 2.35e | 187 | 2.32d | 201 |

[y]Column means followed by the same letter were not different at $P \leq 0.05$, according to Waller-Duncan multiple range test.

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

J2 immobility concentration (JIC) and inhibition dosage (D)-values: The CARD model generated the D-values for J2 immobility for Nemarioc-AL phytonematicide

concentrations at 12- and 72-h exposure periods (Table 6.4), but did not generate the D-values at 24- and 48-h exposure periods (Table 6.4). At 12-h exposure periods JIC-values were almost half the unadjusted D-values (Table 6.4). At 72-h exposure period all D-values were negative, whereas the JIC-values were positive (Table 6.4).

Table 6.3 Biological indices of *Meloidogyne incognita* second-stage juvenile immobility to increasing concentrations of Nemarioc-AL phytonematicide after 12-, 24-, 48- and 72-h exposure periods.

| Biological index | 12 | 24 | 48 | 72 |
|---|---|---|---|---|
| Threshold stimulation ($D_m$) | 6.97 | 34.13 | – | –0.37 |
| Saturation point ($R_h$) | 241.56 | 291.67 | 587.00 | –22462.14 |
| 0% inhibition ($D_0$) | 255.49 | – | – | –22462.51 |
| 50% inhibition ($D_{50}$) | 269.47 | – | – | –22462.88 |
| 100% inhibition ($D_{100}$) | 283.47 | – | – | –22463.28 |
| $R^2$ | 0.97 | 0.97 | 0.97 | 0.97 |
| Sensitivity index (k) | 0 | 1 | 2 | 4 |

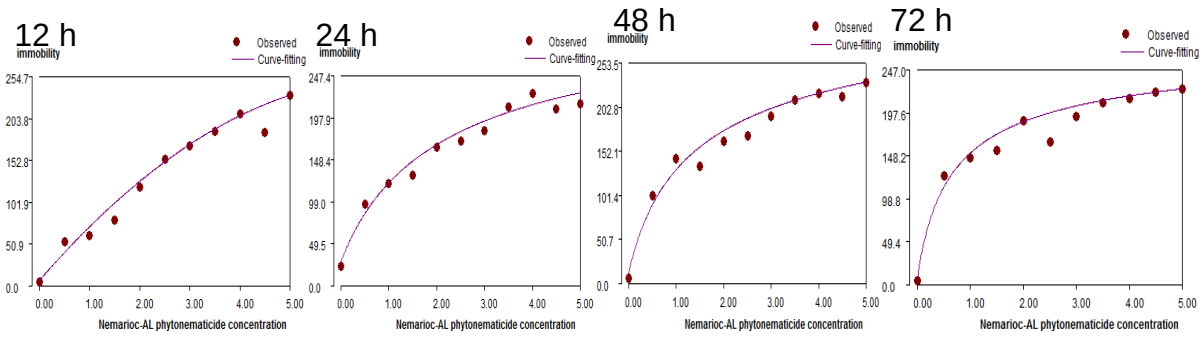


Figure 6.2 Curve-fitting Allelochemical Response Dosage (CARD)-generated density-dependent growth responses of *Meloidogyne incognita* second-stage juvenile immobility to increasing concentrations of Nemarioc-AL phytonematicide at 12-, 24-, 48- and 72-h exposure periods.

Table 6.4 Comparison of Nemarioc-AL phytonematicide second-stage juvenile immobility concentration and inhibition dosage (D)-values.

| Biological index | Exposure period (h) | | | | | |
|---|---|---|---|---|---|---|
| | 12 | | 24 | 48 | 72 | |
| $JIC_{50}$ | 6.86 | | 5.55 | 6.43 | 6.94 | |
| $D_{50}$ | 13.98 | (269.47)[x] | – | – | –0.37 | (–22462.88) |
| $JIC_{100}$ | 7.50 | | 6.68 | 7.13 | 12.22 | |
| $D_{100}$ | 14.00 | (283.47) | – | – | –0.40 | (–22463.28) |

[x]Values in brackets are adjusted indices.

Minimum inhibition concentration (MIC): The MIC values of J2 immobility in *M. incognita* increased with increase in exposure periods from 24- to 72-h exposure period, whereas, the opposite was observed for J2 immobility (Table 6.5).

Table 6.5 Minimum inhibition concentration of Nemarioc-AL phytonematicide on second-stage juvenile immobility of *Meloidogyne incognita* after 12-, 24-, 48- and 72-h exposure periods.

| Incubation period (h) | Model | x (%)[y] |
|---|---|---|
| 12 | $y = –4.835x^2 + 67.354x + 6.825$ | 6.965 |
| 24 | $y = –20.331x^2 + 144.719x + 27.964$ | 3.559 |
| 48 | $y = –22.358x^2 + 229.122x + 12.378$ | 5.124 |
| 72 | $y = 1.694x^2 – 390.166x + 8.416$ | 115.161 |

[y]$x = –b_1/2b_2$, where $y=b_2x^2 + b_1x+ c$.

Reversibility of juvenile immobility: The J2 immobility effects of Nemarioc-AL phytonematicide on *M. incognita* were not reversible, as demonstrated by non-significant treatment means in ANOVA (Table 6.6, Appendix 6.5).

Table 6.6 Partitioning mean sum of squares for reversibility of *Meloidogyne incognita* second-stage juvenile immobility in Nemarioc-AL phytonematicide.

| Source | DF | MS | % |
|---|---|---|---|
| Treatment | 11 | 0.36720 | 51$^{ns}$ |
| Error | 96 | 0.35826 | 49 |
| Total | 107 | 0.72546 | 100 |

$^{ns}$Not significant (P > 0.05).

6.3.2 Nemafric-BL phytonematicide

Relative impact: Treatment effects of Nemafric-BL phytonematicide at all exposure periods where highly significant on J2 mobility and J2 immobility (Table 6.7, Appendix 6.6-6.9). Treatment effects at 12-, 24-, 48- and 72-h exposure periods contributed 93, 91, 96 and 97% to TTV on J2 immobility, respectively (Table 6.7). Relative to untreated control, Nemafric-BL phytonematicide reduced J2 mobility by between 2–29, 6–39, 3–75 and 1–80% after 12-, 24-, 48- and 72-h exposure periods, respectively, whereas J2 immobility was increased by between 67–117, 159–219, 109–213 and 81–181%, respectively. Generally, J2 immobility decreased with increase in Nemafric-BL phytonematicide concentrations and increase in exposure period (Table 6.8). Relative impact values of J2 immobility over increasing

concentrations of Nemafric-BL phytonematicide exhibited a DDG pattern, which had a stimulation and saturation effect at low and high concentrations, respectively (Figure 6.4). The DDG patterns were explained at 12-, 24-, 48- and 72-h exposure periods by 95, 98, 98 and 93% for J2 immobility, respectively (Figure 6.4).

Table 6.7 Partitioning sum of squares for *Meloidogyne incognita* second-stage juvenile immobility in Nemafric-BL phytonematicide after 12-, 24-, 48- and 72-h exposure periods.

| Source | DF | 12 h | | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|---|---|---|
| | | MS | % | MS | % | MS | % | MS | % |
| Treatment | 11 | 0.0627 | 99* | 3.512 | 99* | 3.079 | 99* | 2.979 | 99* |
| Error | 96 | 0.0005 | 1 | 0.039 | 1 | 0.013 | 1 | 0.012 | 1 |
| Total | 107 | 0.0632 | 100 | 3.552 | 100 | 3.092 | 100 | 2.980 | 100 |

*Significant at P ≤ 0.01.

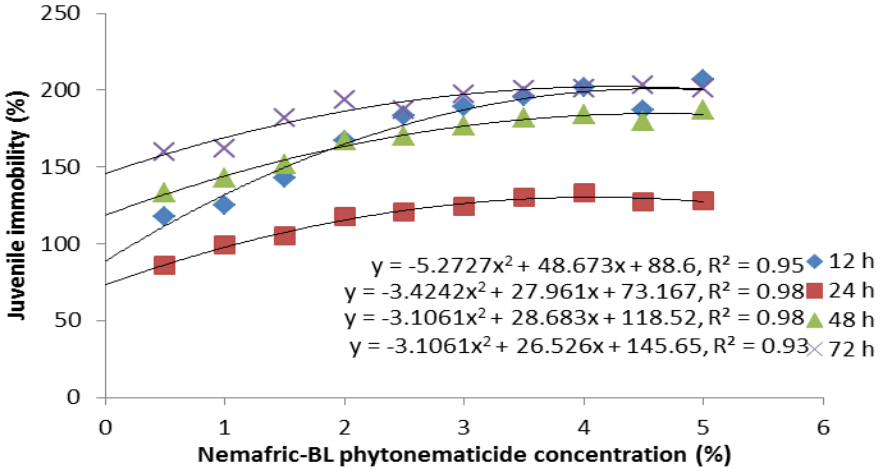


Figure 6.3 Relative impact of Nemafric-BL phytonematicide increasing concentrations on *Meloidogyne incognita* second-stage juvenile immobility at 12-, 24-, 48- and 72 h exposure periods.

Table 6.8 Influence of Nemafric-BL phytonematicide on *Meloidogyne incognita* second-stage juvenile immobility in after 12-, 24-, 48- and 72-h exposure periods.

| | Exposure period (h) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 12 | | 24 | | 48 | | 72 | |
| Concentration (%) | Mean[y] | RI (%)[z] | Mean | RI (%) | Mean | RI (%) | Mean | Rl (%) |
| 0.0 | 0.24a | – | 0.63a | – | 0.76a | – | 0.84a | – |
| 0.5 | 0.40b | 67 | 1.63b | 159 | 1.59b | 109 | 1.52b | 81 |
| 1.0 | 0.43c | 79 | 1.82c | 189 | 1.68b | 121 | 1.91c | 127 |
| 1.5 | 0.45d | 88 | 1.99cd | 216 | 1.89c | 149 | 2.00c | 138 |
| 2.0 | 0.47d | 96 | 2.08d | 230 | 2.09d | 175 | 2.17d | 158 |
| 2.5 | 0.50e | 108 | 2.28e | 262 | 2.15d | 183 | 2.28e | 171 |
| 3.0 | 0.50ef | 108 | 2.29e | 263 | 2.29e | 201 | 2.31ef | 175 |
| 3.5 | 0.51ef | 113 | 2.37e | 276 | 2.38e | 213 | 2.39ef | 184 |
| 4.0 | 0.51ef | 113 | 2.36e | 275 | 2.34e | 208 | 2.36ef | 181 |
| 4.5 | 0.50ef | 108 | 2.34e | 271 | 2.37e | 212 | 2.38f | 183 |
| 5.0 | 0.52f | 117 | 2.01e | 219 | 2.38e | 213 | 2.36f | 181 |

[y]Column means followed by the same letter were not different at $P \leq 0.05$, according to Waller-Duncan multiple range test.

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

Curve-fitting Allelochemical Response Dosage: The CARD model quantified concentration ranges that could stimulate ($D_m$–$R_h$), saturate ($R_h$–$D_0$) and inhibit

($D_0$–$D_{100}$), J2 immobility (Table 6.9). The stimulation phase concentration range was characterised by positive to negative values at 48-h exposure period, whereas all other exposure periods and phases had positive values. Juvenile immobility was very sensitive at all exposure periods with sensitivity values of 0–2 units (Table 6.9). The the highest sensitivity value of J2 immobility to Nemafric-BL phytonematicide was 2 units (Table 6.9). The CARD-generated DDG patterns demonstrated that low concentration of Nemafric-BL phytonematicide J2 immobility was stimulated reaching saturation point at higher concentration for all exposure periods (Figure 6.7).

Table 6.9 Biological indices of *Meloidogyne incognita* second-stage juvenile immobility to increasing concentrations of Nemafric-BL phytonematicide after 12-, 24-, 48- and 72-h exposure periods.

| | Exposure period (h) | | | |
|---|---|---|---|---|
| Biological index | 12 | 24 | 48 | 72 |
| Threshold stimulation ($D_m$) | 6.52 | 5.91 | 0.131 | 5.05 |
| Saturation point ($R_h$) | 227.38 | 285.84 | –4.13 | 263.53 |
| 0% inhibition ($D_0$) | 240.41 | 297.66 | –4.13 | 273.63 |
| 50% inhibition ($D_{50}$) | 253.41 | 309.46 | –4.03 | 283.69 |
| 100% inhibition ($D_{100}$) | 266.41 | 321.26 | –3.93 | 293.69 |
| $R^2$ | 0.95 | 0.96 | 0.96 | 0.98 |
| Sensitivity index (k) | 0 | 0 | 2 | 0 |

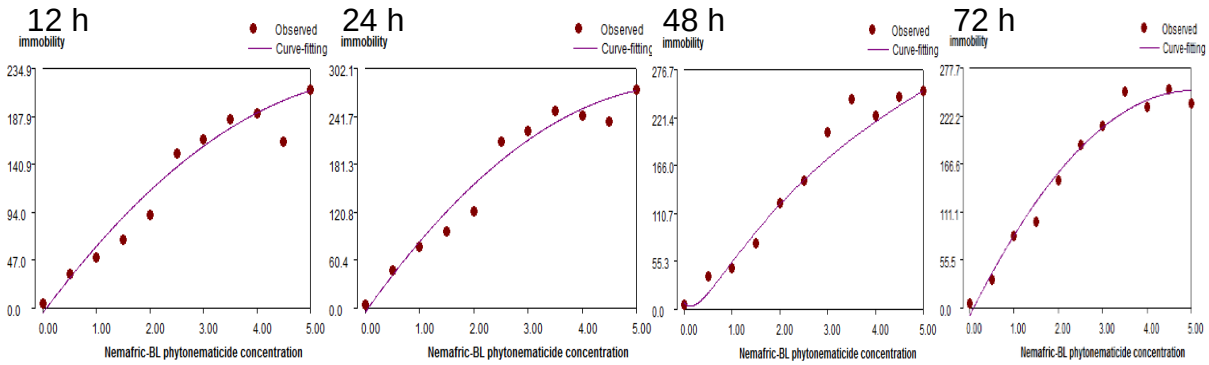


Figure 6.4 Curve-fitting Allelochemical Response Dosage (CARD)-generated density-dependent growth responses of *Meloidogyne incognita* second-stage juvenile immobility to increasing concentrations of Nemafric-BL phytonematicide at 12-, 24-, 48- and 72-h exposure periods.

Comparison of Juvenile Immobility Concentration (JIC) and inhibition dosage (D)-values: The CARD model generated D-values ($D_{50}$, $D_{100}$) for J2 immobility at all exposure periods (Table 6.10). The unadjusted D-values were comparable to computed JIC-values at 12-, 24- and 72-h exposure periods (Table 6.10).

Table 6.10 Comparison of Nemafric-BL phytonematicide second-stage juvenile immobility concentration and inhibition dosage (D)-values.

| | Exposure period (h) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Biological index | 12 | | 24 | | 48 | | 72 | |
| $JIC_{50}$ | 8.99 | | 7.05 | | 9.84 | | 11.27 | |
| $D_{50}$ | 13.00 | (253.41)[x] | 11.80 | (309.46) | 0.10 | (–4.03) | 10.06 | (283.69) |
| $JIC_{100}$ | 9.97 | | 8.92 | | 11.20 | | 10.01 | |
| $D_{100}$ | 13.00 | (266.41) | 11.80 | (321.26) | 0.10 | (–3.93) | 10.00 | (293.69) |

[x]Values in brackets are adjusted indices.

Minimum inhibition concentration (MIC): The MIC values of *M. incognita* J2 immobility decreased with increase in exposure period from 12- to 48-h, with an increase at 72-h exposure period (Table 6.11).

Table 6.11 Minimum inhibition concentration of Nemafric-BL phytonematicide on second-stage juvenile immobility of *Meloidogyne incognita* from quadratic curves generated by Curve-fitting Allelochemical Response Dosage (CARD) model.

| Incubation period (h) | Model | x (%)[z] |
|---|---|---|
| 12 | $y = –5.203x^2 + 67.796x – 4.3290$ | 6.515 |
| 24 | $y = –8.009x^2 + 94.701x – 4.7620$ | 5.912 |
| 48 | $y = 317.901x^2 – 73.613x + 8.240$ | 0.116 |
| 72 | $y = –10.131x^2 + 102.344x – 8.762$ | 5.051 |

[z] $x = –b_1/2b_2$, where $y = b_2x^2 + b_1x + c$.

Reversibility of juvenile immobility: The juvenile immobility effects of Nemarioc-AL and Nemafric-BL phytonematicides was not reversible, as demonstrated by non-significant treatment means in ANOVA (Table 6.12, Appendix 6.10).

Table 6.12 Partitioning mean sum of squares for reversibility of *Meloidogyne incognita* second-stage juvenile immobility in Nemafric-BL phytonematicide.

| Source | DF | MS | % |
|---|---|---|---|
| Treatment | 11 | 0.22557 | 50$^{ns}$ |
| Error | 96 | 0.22227 | 50 |
| Total | 107 | 0.44784 | 100 |

$^{ns}$Not significant (P > 0.05).

## 6.4 Discussion

### 6.4.1 Relative impact

Juvenile immobility in this study further substantiate the nematicidal properties of Nemarioc-AL and Nemafric-BL phytonematicides observed when *M. incognita* eggs were exposed to the same material (Chapter 4). Similar findings had been made with other plant extracts (Ibrahim *et al*., 2006; Ojo and Umar, 2013). Unlike observations of continous inhibition of J2 hatch by the two phytonematicides (Chapter 4), the influence of the two phytonematicides on J2 immobility had stimulation effect at low phytonematicide concentrations followed by neutral effect and lastly the inhibition effect at highest concentrations. The difference in effects of the same products on *M. incognita* eggs and J2 can be explained in terms of the role played by the layers of the eggs that could have provide some kind of restrictions to the movement of

chemicals in reducing the concentrations to which J1 are exposed. Limited DDG pattern phases have been obsereved where limited plant extract concentrations have been used (Giannakou, 2011; Odeyemi and Adewale, 2011; Oka *et al*., 2000). The reduced penetration could have resulted in only one phase of DDG pattern being observed when eggs where exposed to phytonematicides as compared to when J2 where exposed to the same phytonematicide concentrations. The hightened sensitivity of J2 to plant extracts as compared to eggs has been reported in a number of other studies (Akyazi, 2014; Javed *et al*., 2008).

### 6.4.2 Curve-fitting Allelochemical Response Dosage model

The CARD-generated DDG patterns observed in this study were similar to those of relative impacts described above and also to those when eggs were exposed to similar products (Chapter 4). This provides further evidence to support the theory postulated by Liu *et al*. (2003) that biological entities would display a density-dependent response when exposed to increasing concentrations of allelochemicals. Density-dependent growth patterns had been observed in quite a number of studies where nematodes where exposed to varying concentrations of plant extracts (Abdul, 2013; Azhagumurugan and Rajan, 2014; Costa *et al*., 2003). This is the first report of the CARD model use in generating DDG patterns for J2 immobility of nematodes.

### 6.4.3 Minimim inhibition concentration

The MIC values in this study displayed a similar trend to those observed when eggs where exposed to concentrations of pure cucurbitacins, increasing with increase in concentration of phytonematicide (Chapter 3), but they were faily higher.

### 6.4.4 J2 immobility concentration (JIC) and inhibition concentration (D)-values

The JIC and D-values were not comparable for Nemarioc-AL phytonemeticides but were comparable for Nemafric-BL phytonematicide. This provides evidence that the CARD model can be used in J2 mobility studies.

### 6.4.5 Overall sensitivity of juvenile immobility

Juvenile immobility was highly sensitive to Nemarioc-AL and Nemafric-BL phytonematicides as shown by low k-values across all incubation periods. The high sensitivity to the two phytonematicides supports observations made when eggs were exposed to similar phytonematicides (Chapter 4). Previous studies on plant phytotoxicity of the two phytonematicides had observed that different plant organs had different k-values, with those that come in direct contact with the phytonematicides, such as the roots having higher sensitivity than shoots, which do not come in direct contact with the phytonematicide (Pelinganga *et al*., 2013). Apparently, this is the first report of empirically-derived evidence of direct sensitivity of a phytonematicide on nematode J2 immobility further providing evidence that sensitivity index can be a valuable tool for comparison of different plant extracts when used in the management of nematodes.

### 6.4.6 Reversibility of J2 immobility

In the current study, both Nemarioc-AL and Nemafric-BL phytonematicides inhibition was irreversible. Extracts of neem leaves and cake were observed to cause 83 and 85% mobility inhibition of *M. incognita* J2 at a concentration of 10% (Javed *et al*., 2008) and the inhibition was temporaly. Wuyts *et al*. (2006) tested 36 pure plant

extracts on *R. similis* and *M. incognita* and observed that 12 of those could inhibite the mobility of the two nematodes, unlike in the current study, the inhibition of *R. similis* and *M. incognita* was reversible.

## 6.5 Conclusion

Juvenile immobility over increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides had DDG patterns which had different trends to those of same products when eggs were exposed to them. At low concentrations, the phytonematicides stimulated J2 immobility, whereas at high concentrations the material inhibited J2 immobility. The CARD model generated excellent MIC-values and demostrated that J2 immobility was highly sensitive to Nemarioc-AL and Nemafric-BL phytonematicides. The suppressiveness of the two phytonematicides on J2 immobility was not reversible after dilution of the phytonematicides.

## 6.6 References

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

AKYAZI, F. 2014. Effect of some plant methanol extracts on egg hatching and juvenile mortality of root-knot nematode *Meloidogyne* incognita. *American Journal of Experimental Agriculture* 4:1471–1479.

AZHAGUMURUGAN, C. and M.K. RAJAN. 2014. Effect of leaf extract of nilakumil, (*Gmelina asiatica*) against the root-knot nematode (*Meloidogyne incognita*). *Research Journal of Recent Sciences* 3:264–266.

COSTA, S.S.R., SANTOS, M.S.N.A. and M.F. RYAN. 2003. Effect of *Artemisia vulgaris* rhizome extracts on hatching, mortality, and plant infectivity of *Meloidogyne megadora*. *Journal of Nematology* 35:437–442.

GIANNAKOU, I.O. 2011. Efficacy of a formulated product containing *Quillaja saponaria* plant extracts for the control of root-knot nematodes. *European Journal of Plant Pathology* 130:587–596.

IBRAHIM, S.K., TRABOULSI, A.F. and S. EL-HAJ. 2006. Effect of essential oils and plant extracts on hatching, migration and mortality of *Meloidogyne incognita*. *Phytopathologia Mediterranea* 45:238–246.

JAVED, N., GOWEN, S.R., EL-HASSAN, S.A., INAM-UL-HAQ, M., SHAHINA, F. and B. PEMBROKE. 2008. Efficacy of neem (*Azadirachta indica*) formulations on biology of root-knot nematodes (*Meloidogyne javanica*) on tomato. *Crop Protection* 27:36–43.

LIU, D.L., AN, M., JOHNSON, I.R. and J.V. LOVETT. 2003. Mathematical modeling of allellopathy. III. A model for curve-fitting allellochemical dose responses. *Non-linearity Biology, Toxicology and Medicine* 1:37–50.

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

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

MASHELA, P.W., DUBE, Z.P. and K.M. POFU. 2015. Managing the Phytotoxicity and Inconsistent Nematode Suppression in Soil Amended with Phytonematicides. In: Meghvansi, M.K. and A. Vorma (eds.). Organic

Amendments and Soil Suppressiveness in Plant Disease Management, Soil Biology 46. Springer International Publishers, Switzerland.

ODEYEMI, I.S. and K.A. ADEWALE. 2011. Phythonematoxic properties and nematicidal potential of *Tithonia diversifolia* extract and residue on *Meloidogyne incognita* infecting yam (*Discoria rotundata*). *Archives of Phytopathology and Plant Protection* 44:1745−1753.

OJO, G.T. and I. UMAR. 2013. Evaluation of some botanicals on root-knot nematode (*Meloidogyne javanica*) in tomato (*Lycopersicum esculentum* Mill) in Yola Adamawa State, Nigeria. *Biological Forum – An International Journal* 5:31–36.

OKA, Y., NACAR, S., PUTIEVSKY, E., RAVID, U., YANIV, Z. and Y. SPIEGEL. 2000. Nematicidal activity of essential oils and their components against the root-knot nematode. *Phytopathology* 90:710–715.

PELINGANGA, O.M. 2013. Developing Bio-nematicides Using Indigenous *Cucumis africanus* and *Cucumis myriocarpus* Fruits for Tomato Production System. PhD Thesis, University of Limpopo. Sovenga, South Africa.

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

SAS INSTITUTE, 2008. SAS/STAT 9.2 Qualification tools user's guide. SAS Institute, Cary, NC.

WUYTS, N., SWENNEN, R. and D. DE WAELE. 2006. Effects of plant phenylpropanoid pathway products and selected terpenoids and alkaloids on the behavior of the plant-parasitic nematodes *Radopholus similis, Pratylenchus penetrans* and *Meloidogyne incognita*. *Nematology* 8:89–101.

# CHAPTER 7
# RESPONSES OF NEMATODE TO PURE CUCURBITACIN A AND B: JUVENILE MORTALITY TRIALS

## 7.1 Introduction

In order to identify the precise mode of action induced by the active ingredients in botanical pesticides, bio-assays should be carried out first with purified ingredients. Many compounds, including alkaloids, diterpenes, fatty acids, glucosinolates, isothiocyanates, phenols, polyacetylenes, sesquiterpenes and thienyls have been extracted from plants and purified for test on their bioactivities on nematodes (Ntalli and Caboni, 2012). Nematicidal activities of steroidal saponins and asparanins against J2 of the southern root-knot (*Meloidogyne incognita* Kofoid & White) nematode had been reported (Manners, 2007; Roy and Saraf, 2006). Mortalities of roundworm (*Caenorhabditis elegans* Maupas) when exposed to medicarpin ($C_{16}H_{14}O_4$) and 4-hydroxymedicarpin ($C_{16}H_{14}O_5$) from *Medicago* species plants had been observed (Archana and Prasad, 2014). Nematicidal effects of colchicines, cyclocurcumin, curcuminoides and diphynylesheptanoides, active ingredients of plants in the Zingiberaceae family against *M. incognita* J2 had been observed, whereas azadirachtin and triazophos effects against reniform nematode (*Rotylenchulus reniformis* Linford & Oliveira) had been noticed (Archana and Prasad, 2014).

Ardakani *et al*. (2013) reported that essential oil from dried leaves of true myrtle (*Myrtus communis* L.) at rates of 4000 and 8000 $mg.L^{-1}$ caused 100% mortality of *M. incognita* second-stage juveniles (J2), whereas below 250 $mg.L^{-1}$ there was no activity. Aromatic aldehyde benzaldehyde from almond (*Prunus dulcis* Mill) plants

was found to reduce 50% of *M. javanica* at concentration of 9 $\mu g.mL^{-1}$ (Ntalli *et al*., 2010), whereas tests of 1,2 Dehydropyrrolizidine alkaloids at concentrations of 70-350 $\mu g.L^{-1}$ exhibited nematicidal activities on *M. incognita* (Thoden and Boppre, 2010). Isothiocyanate from horse radish (*Armoracia rusticana* Gaertn) plants however had irreversible nematicidal activities against *M. javanica*, J2, following a 72-h exposure, at concentrations as low as 5 $\mu g.mL^{-1}$ (Wu *et al*., 2011).

*In vitro* studies of pure cucurbitacin A and B had shown a density-dependent growth (DDG) patterns on *M. incognita* J2 hatching when using Curve-fitting Allelochmical Response Dosage (CARD) model, which effectively generated the mimimun inhibition concentration (Dube and Mashela, 2016). The J2 hatching and J2 mobility were highly sensitive to cucurbitacin A and B (Chapters 3, 5), but information on mortality of J2 in response to increasing concentration of cucurbitacins is not documented. The objective of this study therefore was to determine whether (i) increasing concentration of pure cucurbitacin A and B would have impact on *M. incognita* J2 mortality, (ii) the Curve-fitting Allelochemical Response Dosage (CARD) model would quantify the three phases of density-dependent growth (DDG) pattern on J2 mortality when compared to increasing cucurbitacin concentrations, (iii) computed lethal concentration (LC)- and CARD-generated D-values would be statistically comparable in magnitudes and (iv) the CARD model would provide information on minimum lethal concentration (MLC).

## 7.2 Materials and methods

*In vitro* trials were conducted at the Green Technologies Research Centre, University of Limpopo, South Africa (23°53′10″S, 29°44′15″E). Purified cucurbitacin A and cucurbitacin B (1000 µg each), obtained from ChemFaces (Wuhan, China), were prepared as explained previously (Chapter 3). Hatching of *M. incognita* eggs to provide freshly hatched J2 was as described previously (Chapter 5).

### 7.2.1 Mortality bioassay and data analysis

Pure cucurbitacin A and B concentrations were tested for J2 mortality using modified methods of Wuyts *et al*. (2006) in two parallel trials. The assessment was carried out in 9 cm petri dishes containing 10 mL of different extract concentrations. Freshly hatched second-stage J2 were added to each concentration. The petri dishes were then incubated at 25 ± 2 °C for 72-h. After 72-h, nematodes were stained in 0.015% methylene blue for 1-h. All stained dark blue nematodes were considered dead (Saifullah, 2002). The concentration in which 50% of the nematodes were killed was calculated ($LC_{50}$-72 h incubation). In all trials, three independent experiments with treatments replicated three times in a CRD were conducted. Data were analysed as described previously (Chapter 5).

## 7.3 Results

### 7.3.1 Pure cucurbitacin A

Relative impact: Pure cucurbitacin A concentration effects on J2 mortality of *M. incognita* were highly significant ($P \leq 0.01$)(Appendix 7.1) contributing 95% in total treatment variation (TTV) (Table 7.2). Relative to untreated control, J2 mortality

increased with increase in pure cucurbitacin A concentration (Table 7.1). When relative impact values were plotted against J2 mortality, a density-dependent growth (DDG) pattern was observed (Figure 7.1). The DDG patterns had stimulation, neutral and slight inhibition effects as cucurbitacin A concentrations were increased (Figure 7.1).

Table 7.1 Influence of pure cucurbitacin A on *Meloidogyne incognita* second-stage juvenile mortality after 72-h exposure.

| Concentration (µg.mL$^{-1}$) | Mean[y] | RI (%)[z] |
|---|---|---|
| 0.00 | 0.88a | – |
| 0.25 | 1.62b | –84 |
| 0.50 | 2.04c | –132 |
| 0.75 | 2.19d | –149 |
| 1.00 | 2.24de | –155 |
| 1.25 | 2.26de | –157 |
| 1.50 | 2.31ef | –163 |
| 1.75 | 2.38fg | –170 |
| 2.00 | 2.42fgh | –175 |
| 2.25 | 2.46fgh | –180 |
| 2.50 | 2.50h | –184 |

[y]Column means followed by the same letter were not different at P ≤ 0.05, according to Waller-Duncan multiple range test.
[z]Relative impact % = [(treatment/control) – 1] x 100.

Curve-fitting Allelochemical Response Dosage: The CARD model quantified concentration ranges of pure cucurbitacin A that could stimulate ($D_m$–$R_h$), saturate ($R_h$–$D_0$) and inhibit ($D_0$–$D_{100}$), mortality (Table 7.3). The CARD-generated similar pure cucurbitacin A values for saturation and inhibition ranges. The CARD-generated DDG patterns demonstrated only two phases, stimulation and neutral phase (Figure 7.2). Juvenile mortality was highly sensitive to pure cucurbitacin A concentrations with sensitivity (k) value of 4 units (Table 7.3).

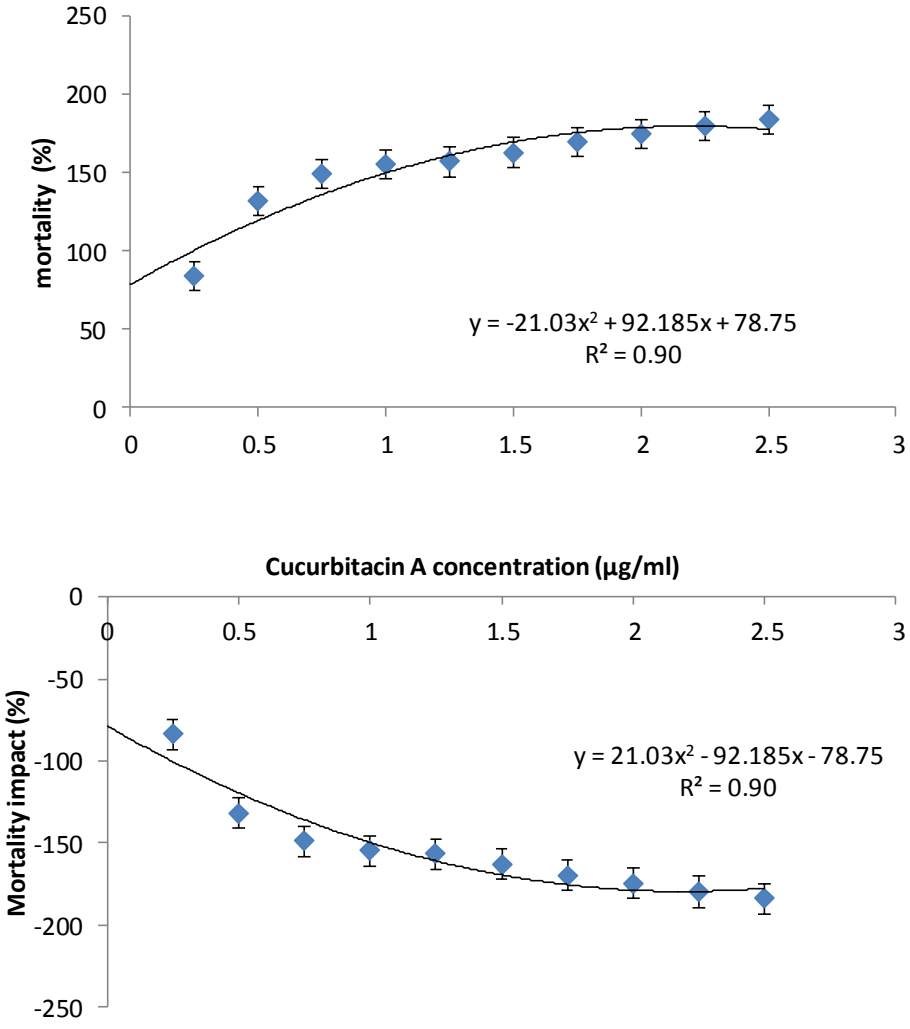


Figure 7.1 Relative impact of pure cucurbitacin A on second-stage juvenile mortality of *Meloidogyne incognita.*

Table 7.2 Partitioning sum of squares for *Meloidogyne incognita* second-stage juvenile mortality after 72-h exposure to pure cucurbitacin A.

| Source | DF | SS | % |
|---|---|---|---|
| Treatment | 11 | 3.011 | 99** |
| Error | 96 | 0.016 | 1 |
| Total | 107 | 3.027 | 100 |

**Significant at P ≤ 0.01.

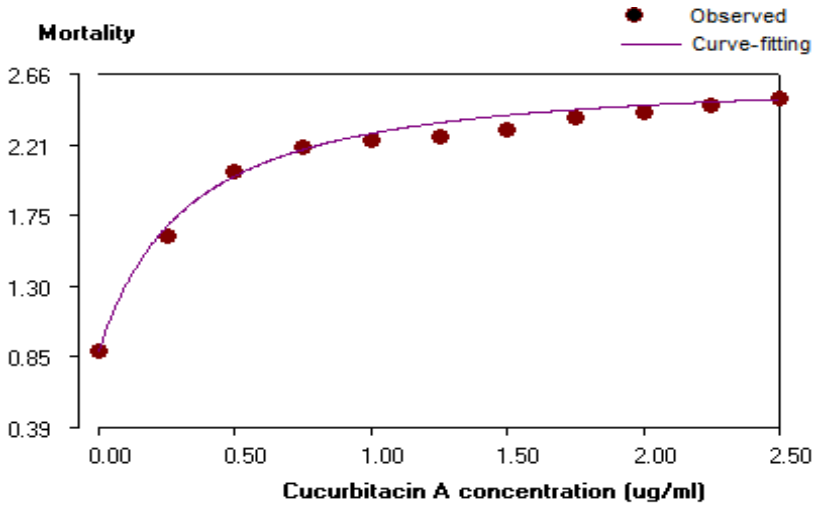


Figure 7.2 Curve-fitting Allelochemical Response Dosage (CARD)-generated density-dependent growth responses of *Meloidogyne incognita* second-stage juvenile mortality to increasing concentrations of pure cucurbitacin A.

Table 7.3 Biological indices of *Meloidogyne incognita* second-stage juvenile mortality to increasing concentrations of pure cucurbitacin A.

| Biological index | J2 mortality |
|---|---|
| Threshold stimulation ($D_m$) | 20.70 |
| Saturation point ($R_h$) | 22.39 |
| 0% inhibition ($D_0$) | 22.39- |
| 50% inhibition ($D_{50}$) | 22.39 |
| 100% inhibition ($D_{100}$) | 22.39 |
| $R^2$ | 0.99 |
| Sensitivity index (k) | 4. |

Minimum Lethal Concentration (MLC): The MLC value for pure cucurbitacin A from CARD-generated quadratic equation was very low at 0.63 $\mu g.mL^{-1}$ of distilled water (Table 7.4).

Table 7.4 Minimum lethal concentration (MLC) of pure cucurbitacin A on second-stage juvenile mortality of *Meloidogyne incognita* from Curve-fitting Allelochemical Response Dosage (CARD)-generated quadratic equations.

| Model | x ($\mu g.mL^{-1}$)$^z$ |
|---|---|
| $y = -4.276x^2 + 5.388x + 0.877$ | 0.63 |

$^z x = -b_1/2b_2$, where $y = b_2x^2 + b_1x + c$.

Comparison of lethal concentration (LC) and D-value: Generally, the LC-values were lower than the CARD-generated D-values (Table 7.5). The $D_{50}$ and $D_{100}$ values were the same, whereas the $LC_{50}$ was smaller than the $LC_{100}$ and negative (Table 7.5).

Table 7.5 Comparison of cucurbitacin A lethal concentration (LC) and inhibition dosage (D)-values.

| Biological index | Cucurbitacin | |
|---|---|---|
| $LC_{50}$ | –0.29 | |
| $^{y}D_{50}$ | 0.00 | (22.39)[x] |
| $LC_{100}$ | 0.24 | |
| $^{z}D_{100}$ | 0.00 | (22.39) |

[x]Values in brackets are adjusted indices.

7.3.2 Pure cucurbitacin B

Relative impact: Treatment effects on J2 mortality of *M. incognita* were highly significant ($P \leq 0.01$)(Appendix 7.2) contributing 99% in TTV (Table 7.6). Relative to untreated control, J2 mortality increased with increase in pure cucurbitacin B concentration (Table 7.7). When relative impact values were plotted against J2 mortality, a density-dependent growth (DDG) pattern was observed (Figure 7.5). The DDG patterns had stimulation, neutral and slight inhibition effects as cucurbitacin B concentrations were increased (Figure 7.5).

Table 7.6 Partitioning sum of squares for *Meloidogyne incognita* second-stage juvenile mortality after 72-h exposure to pure cucurbitacin B.

| Source | DF | SS | % |
|---|---|---|---|
| Treatment | 11 | 3.016 | 99** |
| Error | 96 | 0.021 | 1 |
| Total | 107 | 3.037 | 100 |

**Significant at $P \leq 0.01$.

Curve-fitting Allelochemical Response Dosage: The CARD model quantified the concentration ranges of pure cucurbitacin A that could stimulate ($D_m$–$R_h$), saturate ($R_h$–$D_0$) and inhibit ($D_0$–$D_{100}$), mortality (Table 7.8). As with pure cucurbitacin A, concentration ranges of pure cucurbitacin B values that saturate and inhibit were similar. The CARD-generated DDG patterns demonstrated two phases of DDG patterns, stimulation at low pure cucurbitacin B concentrations and saturation at higher pure cucurbitacin B concentrations (Figure 7.4). Juvenile mortality was also highly sensitive to pure cucurbitacin A concentrations with sensitivity (k) value of 4 units (Table 7.8).

Table 7.7 Influence of pure cucurbitacin B on *Meloidogyne incognita* second-stage juvenile mortality after 72-h exposure.

| Concentration (µg.mL$^{-1}$) | Mean[y] | Rel. impact (%)[z] |
|---|---|---|
| 0.00 | 0.88a | – |
| 0.25 | 1.65d | –88 |
| 0.50 | 2.05c | –133 |
| 0.75 | 2.17d | –147 |
| 1.00 | 2.23de | –153 |
| 1.25 | 2.26de | –157 |
| 1.50 | 2.37ef | –169 |
| 1.75 | 2.39fg | –172 |
| 2.00 | 2.43fgh | –176 |
| 2.25 | 2.45gh | –178 |
| 2.50 | 2.47h | –181 |

[y]Column means followed by the same letter were not different at P ≤ 0.05, according to Waller-Duncan multiple range test.

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

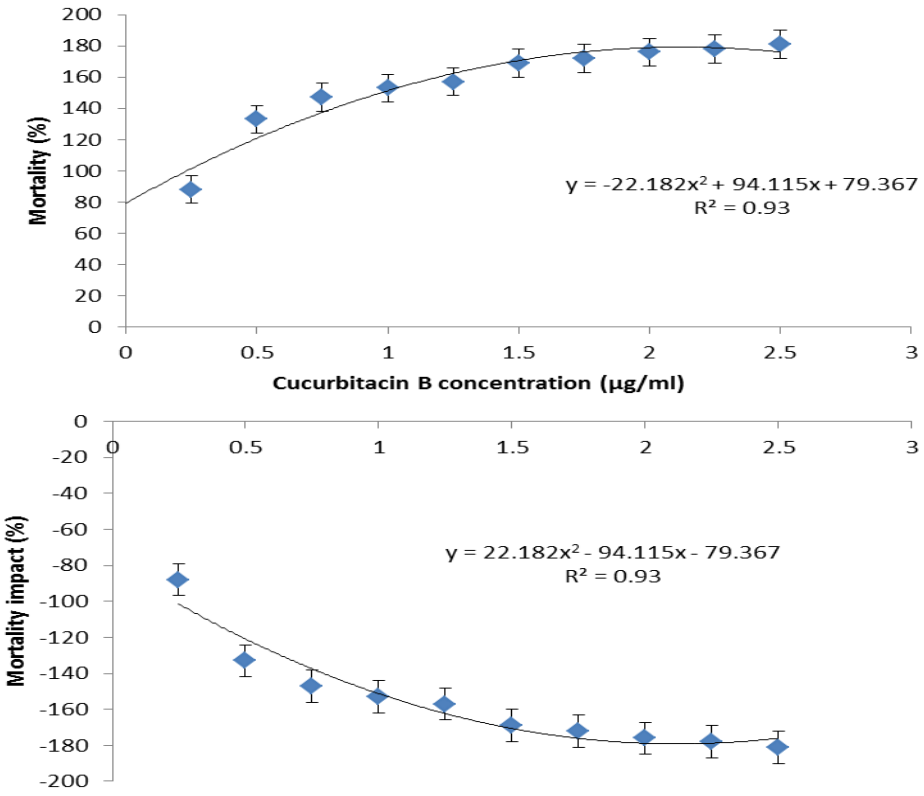


Figure 7.3 Relative impact of pure cucurbitacin B on second-stage juvenile mortality of *Meloidogyne incognita.*

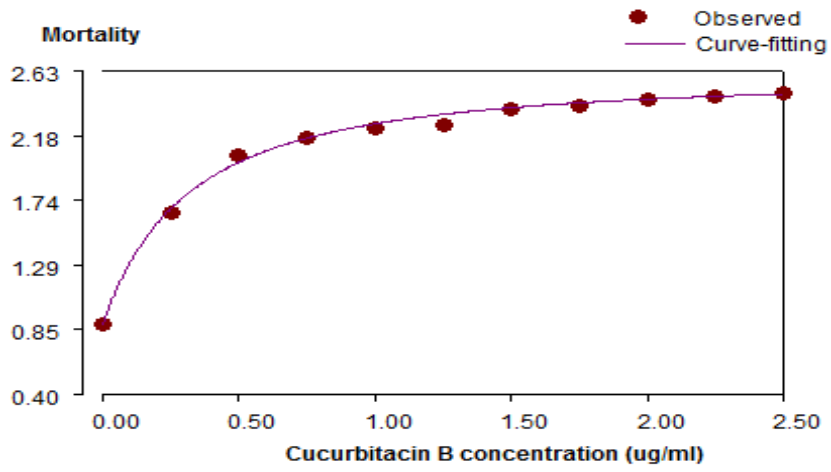


Figure 7.4 Curve-fitting Allelochemical Response Dosage (CARD)-generated density-dependent growth responses of *Meloidogyne incognita* second-stage juvenile mortality to increasing concentrations of pure cucurbitacin B.

Table 7.8 Biological indices of *Meloidogyne incognita* second-stage juvenile mortality to increasing concentrations of pure cucurbitacin B.

| Biological index | Cucurbitacin |
|---|---|
| Threshold stimulation ($D_m$) | 13.662 |
| Saturation point ($R_h$) | 15.328 |
| 0% inhibition ($D_0$) | 15.328 |
| 50% inhibition ($D_{50}$) | 15.238 |
| 100% inhibition ($D_{100}$) | 15.238 |
| $R^2$ | 0.990 |
| Sensitivity index (k) | 4. |

Minimum Lethal concentration (MLC): The minimum pure cucurbitacin B concentration that could cause mortality was established at 0.61 $\mu g.mL^{-1}$ of distilled water, using a quadratic equation generated by CARD model (Table 7.9).

Table 7.9 Minimum lethal concentration (MLC) of pure cucurbitacins B on *Meloidogyne incognita* second-stage juvenile mortality from Curve-fitting Allelochemical Response Dosage (CARD)-generated quadratic equations.

| Model | $x\ (\mu g.mL^{-1})^z$ |
|---|---|
| $y = -4.522x^2 + 5.488x + 0.88$ | 0.61 |

$^z x = -b_1/2b_2$, where $y = b_2x^2 + b_1x + c$.

Comparison of Lethal concentration (LC) and D-values: The CARD-generated $D_{50}$ and $D_{100}$ values of pure cucurbitacin B on J2 mortality were similar at 15.33 µg.mL$^{-1}$ distilled water (Table 7.10). The $LC_{50}$ and $LC_{100}$-values were lower than the $D_{50}$ and $D_{100}$-values (Table 7.10).

Table 7.10 Comparison of pure cucurbitacin B lethal concentration (LC) and inhibition dosage (D)-values.

| Biological index | Cucurbitacin (µg.mL$^{-1}$) |
|---|---|
| $LC_{50}$ | –0.30 |
| $D_{50}$ | 0.00 (15.328)[x] |
| $LC_{100}$ | 0.23 |
| $D_{100}$ | 0.00 (15.328) |

[x]Values in brackets are adjusted indices.

## 7.4 Discussion

### 7.4.1 Relative impact

The nematicidal activity of pure cucurbitacins in this study is the first such report and it is one of the few reports of DDG patterns of pure compounds on nematodes. Many plant derived compounds with nematicidal activity have been found including alkaloids, diterpenes, fatty acids, glucosinolates, isothiocyanates, phenols, polyacetylenes, sequiterpenes and thienyls (Ntalli and Caboni, 2012). Most of these studies report observation of only one phase of DDG pattern, mostly the phase that

cause mortality, mainly because of limited number of concentrations used and bias towards nematode control rather than management (Mashela *et al*., 2015). The three phases of DDG patterns are stimulation, neutral and inhibition (Mashela *et al*., 2015). Reports of inhibition phase, include nematicidal effects of colchicines, cyclocurcumin, curcuminoides and diphynylesheptanoides against *M. incognita* (Kiuchi *et al*., 1993; Sharma, 2000; Prasad and Mittal, 2004). Other pure compounds that had the inhibition effect (nematicidal) include ketones (Ntalli *et al*., 2010; Oka, 2001), flavone-C-glycosides (Du *et al*., 2011), saponins (Saha *et al*., 2010), salycylic acid (Faizi *et al*., 2011) and ascaridole (Chuan *et al*., 2011). Al-Banna *et al*. (2003) reported neutral (no effect) of cineole, menthol and pinene on *M. javanica*. In this study mortality increased with increase in concentration at low cucurbitacin concentrations (stimulation phase), flattened out (neutral phase) and slight decrease at high concentrations (inhibition phase). Shakil *et al*. (2008) had similar observations when he exposed J2 of *M. incognita* and *R. reniformis* to prenylated flavanones, compound isolated from stonebreaker (*Phyllathus niruri* L.) plant.

### 7.4.2 Curve-fitting Allelochemical Response Dosage model

The CARD model generated concentration ranges for pure cucurbitacin A were higher than those of cucurbitacin B, whereas the sensitivity value for the two cucurbitacins were the same. The higher cucurbitacin A than B mortality concentration ranges are in sharp contrast to what was observed for J2 hatching and J2 immobility, where cucurbitacin B gave higher concentration ranges than cucurbitacin A (Chapter 3, 5). The J2 hatching was highly sensitive to cucurbitacin B than A, whereas J2 mobility and mortality were equally sensitive to the two

cucurbitacins (Chapter 3, 5). The CARD-generated DDG patterns were similar to the relative impact graphs. This is the first report on the use of the CARD model to explain the relationship between increasing concetrations of cucurbitacins and mortality of *M. incognita* J2.

### 7.4.3 Minimum lethal concentration

The minimum lethal concentration due to cucurbitacins observed in this study was very low for both cucurbitacins at 0.6 $\mu g.mL^{-1}$ distilled water. Essential oils at concentrations as high as 250 $mg.mL^{-1}$ could not cause mortality of *M. incognita* J2 (Ardakani *et al*., 2013). *Meloidogyne incognita* J2 mortality was observed at concentrations of 5 $\mu g.mL^{-1}$, 15 and 13 $\mu l.L^{-1}$ for isothiocyanate, aldehydes and ketones, respectively (Ntalli and Caboni, 2012). The low minimum concentration and high sensitivity value observed in this study provides evidence of high potency of the cucurbitacins when compared to other plant based compound.

### 7.4.4 Lethal concentrations and D-values

Generally the CARD-generated D-values were higher than the LC-values for both cucurbitacin A and B. Cucurbitacin LC-values and D-values were very low when compared with the LC-values of other studies. When *R. reniformis* J2 were exposed to saponins, $LC_{50}$ ranged from 68.8 to 181.9 $\mu g.mL^{-1}$ (Ntalli and Caboni, 2012), whereas L-carvone and pulegone had higher $LC_{50}$ of 115 and 150 $\mu g.L^{-1}$, respectively (Ntalli *et al*., 2010, 2011). Even higher $LC_{50}$ of 114.66 and 323.09 $\mu g.mL^{-1}$, respectively, were observed for schaftoside and isoschaftoside, two flavone-C-glycoside, extracted from cobra lily (*Arisaema erubescens* Wall) tubers

(Du *et al*., 2011). Comparatively small $LC_{50}$ (21 μg.mL$^{-1}$ and 0.17 μg.mL$^{-1}$) were observed from non-essential amino acid, L-3,4-dihydroxyphenylalanine when tested against *M. incognita* and soyabean cyst (*Heterodera glycines* Ichinole) nematode, respectively (Barbarosa *et al*., 1999). Ascaridole, extracted from velame (*Croton regelianus* Muell) and mexican tea (*Chenopodium ambrosioides* L.), also had relatively low $LC_{50}$ of 32.79 and 49.55 μg.mL$^{-1}$, respectively (Chuan *et al*., 2011). This is also the first report of LC-values and D-values of cucurbitacin A and B on nematodes.

## 7.5 Conclusion

*Meloidogyne incognita* J2 mortality over increasing concentrations of pure cucurbitacins had similar trends of DDG patterns for relative impact values and those generated by CARD model. At low concentrations mortality increased and became neutral at higher cucurbitacin concentrations. The CARD model provided excellent MLC-values, whereas the LC-values were generally smaller than the CARD-generated D-values. Also, the CARD model demonstrated that J2 mortality was highly sensitive to both cucurbitacin A and B. Toxicities of cucurbitacin A and B to *M. incognita* J2 when compared to those of pure plant extracts from other plants were relatively high.

## 7.6 References

Al-BANNA, L., DARWISH, R. and T. ABURJAL. 2003. Effect of plant extracts and essential oils on root-knot nematode. *Phytopathologia Mediterranea* 42:123−128.

ARCHANA, U.S. and D. PRASAD. 2014. Management of plant-parasitic nematodes by the use of botanicals. A review. *Journal of Plant Physiology and Pathology* 2:9−16.

ARDAKANI, A.S., HOSYNINEJ, S.A. and A. POURSHIRZ. 2013. Killing effects of *Myrtus communis* L. essential oil on *Meloidogyne incognita. International Journal of Agriculture and Crop Sciences* 5:806−810.

BARBAROSA, L.C.A., BARCELOS, F.F., DEMUNER, A.J. and M.A. SANTOS. 1999. Chemical constituents from *Mucuna aterrima* with activity against *Meloidogyne incognita* and *Heterodera glycines*. *Nematropica* 29:81−88.

CHUAN, Q.B., ZHI, L.L. and Z.L. QI. 2011. Nematicidal constituents from the essential oil of *Chenopodium ambrosioides* aerial parts. *E-Journal of Chemistry* 8:43–48.

Du, S.S., ZHANG, H.M., BAI, C.Q. and Z.W. DENG. 2011. Nematocidal flavone-C-glycosides against the root-knot nematode (*Meloidogyne incognita*) from *Arisaema erubescens* tubers. *Molecules* 16:5079−86.

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

FAIZI, S., FAYYAZ, S., BANO, S., IQBAL, Y.E., LUBNA, L., SIDDIQI, H. and A. NAZ. 2011. Isolation of nematicidal compounds from *Tagetes patula* L. yellow flowers: Structure-activity relationship studies against cyst nematode *Heterodera zeae* infective stage larvae. *Journal of Agricultural and Food Chemistry* 59:9080−9093.

KIUCHI, F., GOTO, Y., SUGIMOTO, N., AKAO, N., KONDO, K., *et al*. 1993. Nematicidal activity of turmeric: Synergistic action of curcuminoids. *Chemical and Pharmaceutical Bulletin* 41:1640−1643.

MANNERS, G.D. 2007. Citrus limonoids: Analysis, bioactivity and biomedical prospects. *Journal of Agricultural and Food Chemistry* 55:8285−8294.

MASHELA, P.W., DUBE, Z.P. and K.M. POFU. 2015. Managing the Phytotoxicity and Inconsistent Nematode Suppression in Soil Amended with Phytonematicides. In: Meghvansi, M.K. and A. Vorma (eds.). Organic Amendments and Soil Suppressiveness in Plant Disease Management, Soil Biology 46. Springer International Publishers, Switzerland.

NTALLI, N.G. and P. CABONI. 2012. Botanical nematicides. A review. *Journal of Agricultural and Food Chemistry* 60:9929–9940.

NTALLI, N.G., FERRARI, F., GIANNAKOU, I. and U. MENKISSOGLU-SPIROUDI. 2010. Phytochemistry and nematicidal activity of the essential oils from 8 Greek Lamiaceae aromatic plants and 13 terpene components. *Journal of Agricultural and Food Chemistry* 58:7856−7863.

NTALLI, N.G., FERRARI, F., GIANNAKOU, I. and U. MENKISSOGLU-SPIROUDI. 2011. Synergistic and antagonistic interactions of terpenes against *Meloidogyne incognita* and the nematicidal activity of essential oils from seven plants indigenous to Greece. *Pest Management Science* 67:341−351.

OKA, Y. 2001. Nematicidal activity of essential oil components against the root-knot nematode *Meloidogyne javanica*. *Nematology* 3:159–164.

PRASAD, D. and A. MITTAL. 2004. Effect of *Calotropis*, oilcakes and phorate on growth of soybean and *Meloidogyne incognita* population. *Annals of Plant*

*Protection Sciences* 12:234−235.

ROY, A. and SARAF, S. 2006. Limonoids: Overview of significant bioactive triterpenes distributed in plants kingdom. *Biological and Pharmaceutical Bulletin* 29:191−201.

SAHA, S., WALIA, S., KUMAR, J., PARMER, B.S. and D. PRASAD. 2010 Synergistic/potentiation interaction between nematostatic constituents from *Azadirachta indica*, *Madhuca indica* and *Sapindus mukorossi*. *Archives of Phytopathology and Plant Protection* 43:357−367.

SAIFULLAH, A. 2002. New blue R: The best stain for finding out the life status of nematode eggs. *Journal of Biological Sciences* 2:63.

SHAKIL, N.A., PANKAJ, KUMAR, J., PANDEY, R.K. and D.B. SAXENA. 2008. Nematicidal prenylated flavanones from *Phyllanthus niruri*. *Phytochemistry* 69:759−764.

SHARMA, G.C. 2000. Efficacy of neem based formulations against the root-knot nematode *Meloidogyne incognita*. *Pesticide Research Journal* 12:183−187.

THODEN, T.C. and M. BOPPRE. 2010. Plants producing pyrrolizidine alkaloids: Sustainable tools for nematode management. *Nematology* 12:1–24.

WU, H., WANG, C.J., BIAN, X.W. and X. ZHANG. 2011. Nematicidal efficacy of isothiocyanates against root-knot nematode *Meloidogyne javanica* in cucumber. *Crop Protection* 30:33−37.

WUYTS, N., SWENNEN, R. and D. DE WAELE. 2006. Effects of plant phenylpropanoid pathway products and selected terpenoids and alkaloids on the behavior of the plant-parasitic nematodes *Radopholus similis,*

*Pratylenchus penetrans* and *Meloidogyne incognita*. *Nematology* 8:89–101.

# CHAPTER 8
# RESPONSES OF NEMATODE TO PHYTONEMATICIDES: JUVENILE MORTALITY TRIALS

## 8.1 Introduction

In pure form, active ingredients of Nemarioc-AL and Nemafric-BL phytonematicides, cucurbitacin A and B, respectively, affected second-stage juveniles (J2) mortality in root-knot (*Meloidogyne* species) nematodes in density-dependent growth (DDG) patterns (Chapter 7). The observation was similar to those of the two active ingredients on J2 hatch (Chapter 3) and J2 mobility (Chapter 5). The lethal concentrations ($LC_{50}$, $LC_{100}$) and the Curve-fitting Allelochemical Response Dosage (CARD)-generated 50 and 100% lethal concentrations ($D_{50}$, $D_{100}$) were not comparable. However, the CARD model provided good estimates of overall sensitivity ($\sum k$) and minimum lethal concentration (MLC) for the two active ingredients (Chapter 7). Generally, J2 mortality was highly sensitive to cucurbitacin A and B, with very low MLC. In crude form, Nemarioc-AL and Nemafric-BL phytonematicides are used at 3% (Mashela *et al*., 2015; Pelinganga *et al*., 2013a,b). However, information on how J2 mortality would respond to the two phytonematicides at concentration ranges below and above 3% had not been established. The objective of this study was to investigate whether (i) increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides would have impact on *M. incognita* J2 mortality, (ii) the CARD model would quantify the three phases of DDG pattern on J2 mortality when compared to increasing phytonematicide concentrations, (iii) computed LC- and CARD-generated D-values would be statistically comparable in magnitudes and (iv) the CARD model would provide information on MLC.

## 8.2 Materials and methods

*In vitro* trials were conducted at a location described previously (Chapter 3) Nemarioc-AL and Nemafric-BL phytonematicides were prepared as explained previously (Chapter 4). Eggs were collected and hatched as described previously (Chapter 6).

### 8.2.1 Mortality bioassay and data analysis

Nemarioc-AL and Nemafric-BL phytonematicides were tested for J2 mortality using modified method of Wuyts *et al*. (2006) in two parallel trials. The assessment was carried out in 9 cm petri dishes containing 10 mL of different extract concentrations. Freshly hatched second-stage J2 were added to each concentration. The petri dishes were then incubated at 25 ± 2 °C for 72-h. After 72-h, nematodes were first examined for motility, when no movement was observed in two seconds even after mechanical prodding with a bristle, nematodes were then stained in 0.015% methylene blue for 1-h. All stained dark blue nematodes were considered dead (Saifullah, 2002). The concentration in which 50% of the nematodes were killed was calculated ($LC_{50}$-72-h incubation). In all trials, three independent experiments with treatments replicated three times in a CRD were conducted. Data were analysed as described previously (Chapter 5). Treatment effects were, otherwise stated, discussed at 5% level of probability.

## 8.3 Results

In both Nemarioc-AL and Nemafric-BL phytonematicides there were no statistically significant differences between the effective microorganisms and distilled water controls. The distilled water control, therefore was used throughout the study.

### 8.3.1 Nemarioc-AL phytonematicide

Relative impact: Treatment effects of Nemarioc-AL phytonematicide on J2 mortality of *M*. *incognita* were highly significant (P ≤ 0.01) (Appendix 8.1) contributing 89% in total treatment variation (TTV) (Table 8.1). Relative to untreated control, J2 mortality increased with increasing Nemarioc-AL phytonematicide concentrations (Table 8.2). When J2 mortality relative impact values were plotted against Nemarioc-AL phytonematicide concentrations a density-dependent growth (DDG) pattern was observed (Figure 8.1). The DDG patterns had stimulation effect at low Nemarioc-AL phytonematicide concentrations and neutral effect at higher concentrations (Figure 8.1A).

Table 8.1 Partitioning mean sum of squares for *Meloidogyne incognita* second-stage juvenile mortality after 72-h exposure to Nemarioc-AL phytonematicide.

| Source | DF | MS | % |
|---|---|---|---|
| Treatment | 11 | 2.948 | 99** |
| Error | 96 | 0.042 | 1 |
| Total | 107 | 2.990 | 100 |

**Significant at P ≤ 0.01.

Table 8.2 Influence of Nemarioc-AL phytonematicide on *Meloidogyne incognita* second-stage juvenile mortality after 72-h exposure.

| Concentration (%) | Mean[y] | Rel. impact (%)[z] |
|---|---|---|
| 0.0 | 0.76a | – |
| 0.5 | 1.98b | –161 |
| 1.0 | 2.00bc | –163 |
| 1.5 | 2.15bcd | –183 |
| 2.0 | 2.24cd | –195 |
| 2.5 | 2.26d | –197 |
| 3.0 | 2.26d | –197 |
| 3.5 | 2.29d | –201 |
| 4.0 | 2.30d | –203 |
| 4.5 | 2.31d | –204 |
| 5.0 | 2.33d | –207 |

[y]Column means followed by the same letter were not different at $P \leq 0.05$, according to Waller-Duncan multiple range test.

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

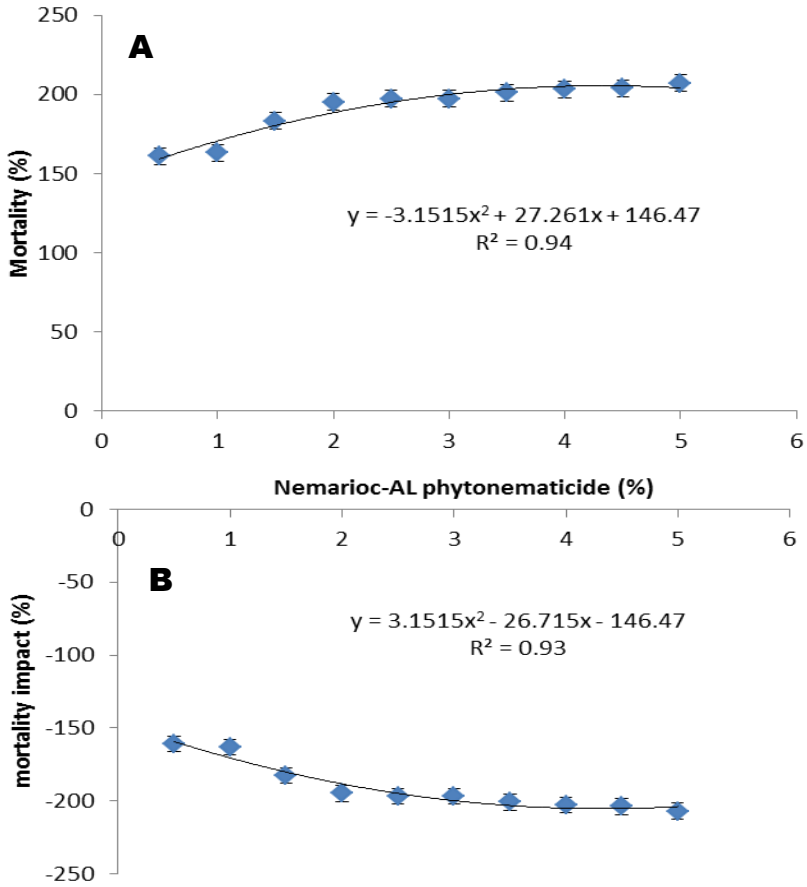


Figure 8.1 Relative impact of Nemarioc-AL phytonematicide on second-stage juvenile mortality of *Meloidogyne incognita.*

Curve-fitting Allelochemical Response Dosage (CARD): The CARD model quantified concentration ranges of Nemarioc-AL phytonematicide that could stimulate ($D_m$–$R_h$), saturate ($R_h$–$D_0$) and inhibit ($D_0$–$D_{100}$), mortality (Table 8.3). The CARD-generated DDG patterns demonstrated only two phases, stimulation and neutral phase for Nemarioc-AL phytonematicide (Figure 8.2). Juvenile mortality was highly sensitive to Nemarioc-AL phytonematicide concentrations with sensitivity (k) value of 2 units (Table 8.3).

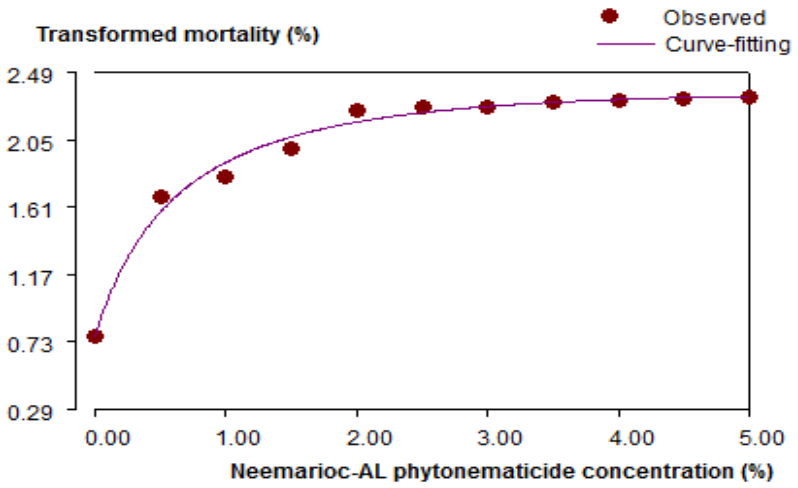


Figure 8.2 Curve-fitting Allelochemical Response Dosage (CARD)-generated density-dependent growth responses of *Meloidogyne incognita* second-stage juvenile mortality to increasing concentrations of Nemarioc-AL phytonematicide.

Table 8.3 Biological indices of *Meloidogyne incognita* second-stage juvenile mortality to increasing concentrations of Nemarioc-AL phytonematicide.

| Biological index | Phytonematicide |
|---|---|
| Threshold stimulation ($D_m$) | 6.77 |
| Saturation point ($R_h$) | 8.331 |
| 0% inhibition ($D_0$) | 8.331 |
| 50% inhibition ($D_{50}$) | 8.331 |
| 100% inhibition ($D_{100}$) | 8.331 |
| $R^2$ | 0.99 |
| Sensitivity index (k) | 2. |

Minimum Lethal Concentration (MLC): Minimum lethal concentration value from CARD-generated quadratic equation was at 1.12% Nemarioc-AL phytonematicide (Table 8.4).

Table 8.4 Minimum lethal concentration (MLC) of Nemarioc-AL phytonematicide on second-stage juvenile mortality of *Meloidogyne incognita* from Curve-fitting Allelochemical Response Dosage (CARD)-generated quadratic equations.

| Model | x (%)[z] |
|---|---|
| $y = -1.255x^2 + 2.8x + 0.77$ | 1.12 |

[z] $x = -b_1/2b_2$, where $y = b_2x^2 + b_1x + c$.

Comparison of lethal concentration (LC) and D-value: Generally, the LC-values were lower than the CARD-generated D-values (Table 8.5). The $LC_{100}$ and $D_{100}$ values were comparable, at 10.1 and 8.3%, respectively (Table 8.5).

Table 8.5 Comparison of Nemarioc-AL phytonematicide lethal concentration (LC) and inhibition dosage (D)-values.

| Biological index | Phytonematicide (%) | |
|---|---|---|
| $LC_{50}$ | 2.7 | |
| $D_{50}$ | 0.0 | (8.3)[x] |
| $LC_{100}$ | 10.1 | |
| $D_{100}$ | 0.0 | (8.3) |

[x] Values in brackets are adjusted indices.

8.3.2 Nemafric-BL phytonematicide

Relative impact: Nemafric-BL phytonematicide effects on J2 mortality of *M*. *incognita* were highly significant (P ≤ 0.01) (Appendix 8.2) contributing 97% in TTV (Table 8.6). Relative to untreated control, J2 mortality increased with increasing Nemafric-BL phytonematicide concentration (Table 8.7). When relative impact values of J2 were plotted against Nemafric-BL phytonematicide concentrations, a density-dependent growth (DDG) pattern was observed (Figure 8.3). The DDG patterns had stimulation, neutral and slight inhibition effects as Nemafric-BL phytonematicide concentrations were increased (Figure 8.3A).

Table 8.6 Partitioning mean sum of squares for *Meloidogyne incognita* second-stage juvenile mortality after 72-h exposure Nemafric-BL phytonematicide.

| Source | DF | MS | % |
|---|---|---|---|
| Treatment | 11 | 2.960 | 99** |
| Error | 96 | 0.012 | 1 |
| Total | 107 | 2.972 | 100 |

**Significant at P ≤ 0.01.

Curve-fitting Allelochemical Response Dosage: The CARD model managed to generate the concentration ranges of Nemafric-BL phytonematicide that could stimulate ($D_m$–$R_h$), saturate ($R_h$–$D_0$) and inhibit ($D_0$–$D_{100}$), mortality (Table 8.8). The CARD-generated DDG patterns demonstrated two phases of DDG patterns,

stimulation at low Nemafric-BL phytonematicide concentrations and saturation at higher Nemafric-BL phytonematicide concentrations (Figure 8.4). Juvenile mortality was also highly sensitive to Nemafric-BL phytonematicide with k-value of 1 unit (Table 8.8).

Table 8.7 Influence of Nemafric-BL phytonematicide on *Meloidogyne incognita* second-stage juvenile mortality after 72-h exposure.

| Concentration (%) | Mean[y] | Rel. impact (%)[z] |
|---|---|---|
| 0.0 | 0.81a | – |
| 0.5 | 1.49b | –84 |
| 1.0 | 1.89c | –133 |
| 1.5 | 1.97c | –143 |
| 2.0 | 2.15d | –165 |
| 2.5 | 2.25e | –178 |
| 3.0 | 2.29ef | –183 |
| 3.5 | 2.37ef | –193 |
| 4.0 | 2.33ef | –188 |
| 4.5 | 2.36f | –191 |
| 5.0 | 2.33f | –188 |

[y]Column means followed by the same letter were not different at P ≤ 0.05, according to Waller-Duncan multiple range test.

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

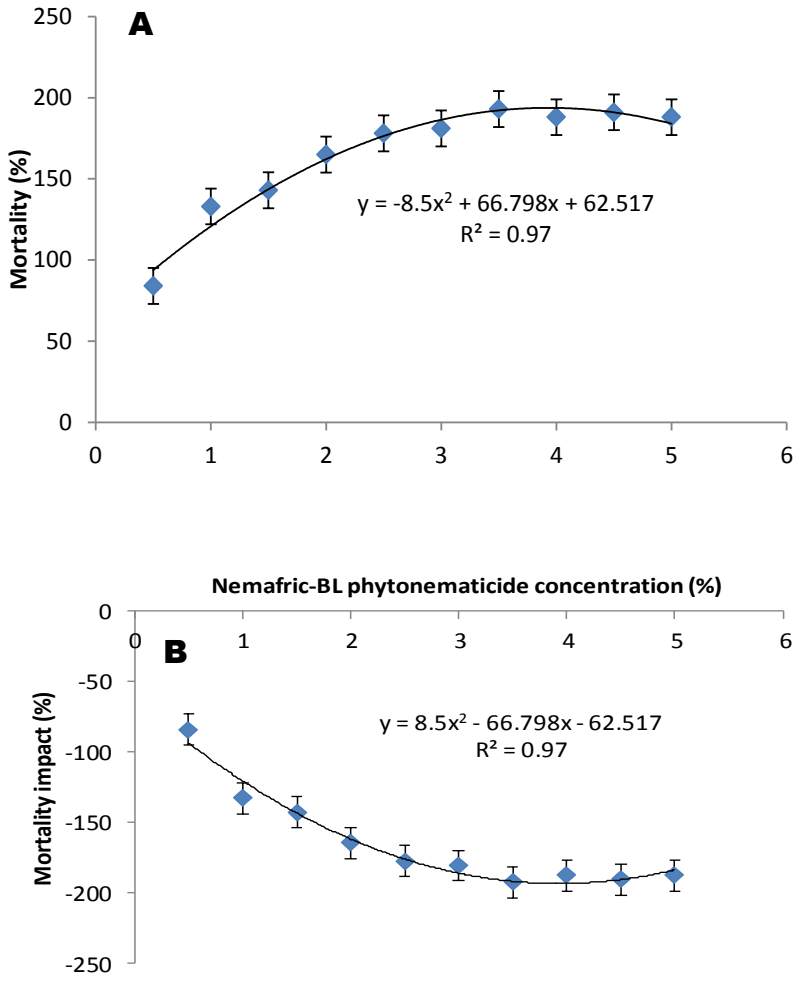


Figure 8.3 Relative impact of Nemafric-BL phytonematicide on second-stage juvenile mortality of *Meloidogyne incognita*.

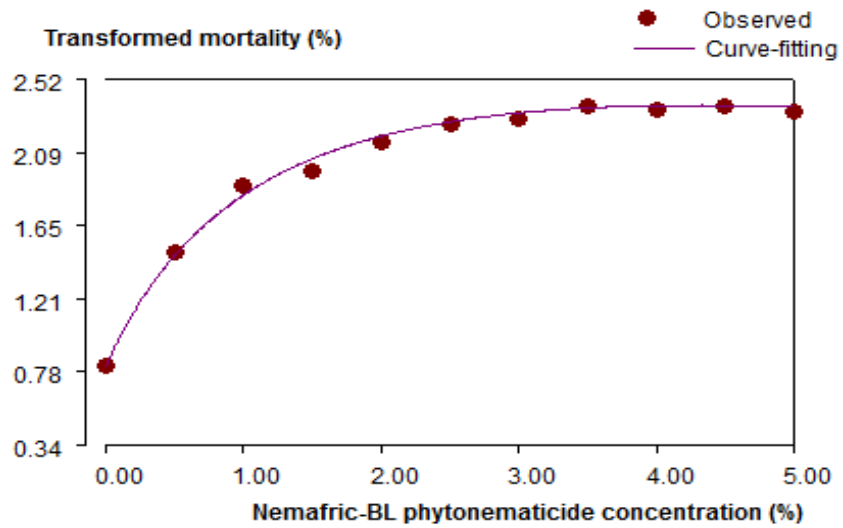


Figure 8.4 Curve-fitting Allelochemical Response Dosage (CARD)-generated density-dependent growth responses of *Meloidogyne incognita* second-stage juvenile mortality to increasing concentrations of Nemafric-BL phytonematicide.

Table 8.8 Biological indices of *Meloidogyne incognita* second-stage juvenile mortality to increasing concentrations of Nemafric-BL phytonematicide.

| Biological index | Phytonematicide |
|---|---|
| Threshold stimulation ($D_m$) | 1.33 |
| Saturation point ($R_h$) | 2.86 |
| 0% inhibition ($D_0$) | 30.29 |
| 50% inhibition ($D_{50}$) | 64.48 |
| 100% inhibition ($D_{100}$) | 106.01 |
| $R^2$ | 0.99 |
| Sensitivity index (k) | 1. |

Minimum Lethal Concentration (MLC): The minimum Nemafric-BL phytonematicide concentration that could cause mortality was established at 0.67%, using a quadratic equation generated by CARD model (Table 8.9).

Table 8.9 Minimum lethal concentration (MLC) of Nemafric-BL phytonematicide on *Meloidogyne incognita* second-stage juvenile mortality from Curve-fitting Allelochemical Response Dosage (CARD)-generated quadratic equations.

| Model | x (%)[z] |
|---|---|
| $y = -0.543x^2 + 1.819x + 0.824$ | 0.67 |

[z] $x = -b_1/2b_2$, where $y = b_2x^2 + b_1x + c$.

Comparison of Lethal Concentration (LC) and D-values: The CARD-generated D-values were higher than LC-values at both 50% and 100% (Table 8.10).

Table 8.10 Comparison of Nemafric-BL phytonematicide lethal concentration (LC) and inhibition dosage (D)-values.

| Biological index | Phytonematicide (%) | |
|---|---|---|
| $LC_{50}$ | 7.25 | |
| $D_{50}$ | 35.72 | $(64.48)^x$ |
| $LC_{100}$ | 8.04 | |
| $D_{100}$ | 70.29 | (106.01) |

[x] Values in brackets are adjusted indices.

## 8.4 Discussion

### 8.4.1. Relative impact

The *in vitro* mortalities of fermented crude extracts of *Cucumis myriocarpus* and *C. africanus*, namely Nemarioc-AL and Nemafric-BL phytonematicide, reported in this study is the first such report. A number of crude plant extracts have been found to have nematicidal effects on nematodes these include, Angel's Trumpet (*Datura stramonium* L.) and neem (*Azadirachta indica* A. Juss) (Nelaballe and Mukkara, 2013), *Moringa* species (Claudius-Cole *et al*., 2010), mugwort (*Artemisia vulgaris* L.) (Costa *et al*., 2003) and garlic (*Allium sativum* L.) (Ibrahim *et al*., 2006). The mortality displayed on *M. incognita* by both phytonematicides in this study had a DDG pattern an observation less common in a lot of studies due to limited number of concentration levels used (Chapter 3). The limited concentration ranges used in other studies result in observation of only one phase of the DDG pattern. Stimulation effect of mortality at low concentrations observed in this study is depicted in other studies as positive linear models (Azhagumurugan and Rajan, 2014; Pavaraj *et al*., 2012). Stimulation was followed by neutral effect at higher concentration ranges were mortality levelled off, depicted in other studies as no effect (Ardakani *et al*., 2013).

### 8.4.2 Curve-fitting Allelochemical Response Dosage model

The CARD model generated concentration ranges for Nemarioc-AL phytonematicide were much lower than those of Nemafric-BL phytonematicide, whereas the sensitivity values were comparable, 2 units and 1 unit, respectively. The CARD-generated DDG patterns were similar to the relative impact graphs. This is the first

report on the use of CARD model to explain the relationship between increasing concentrations of phytonematicides and nematode mortality.

### 8.4.3 Minimum lethal concentrations

The MLC observed in this study were very low for both phytonematicides and when compared with other extracts. Potency of clove (*Syzygium aromaticum* L.) against *M. incognita* and burrowing nematode (*Radopholus similes* Cobb) were reported to be at 1% concentration, a figure higher than observed for the two phytonematicides in this study (Mustika and Slamet, 1994). Taye *et al*. (2013) recorded mortalities of *M. incognita* at 5%, whereas Agbenin *et al*. (2005) observed even higher minimum mortalities of *M. incognita* J2 exposed to dry neem extract concentration of 10% after 3 hours. The low minimum concentration and high sensitivity value observed in this study provides evidence of high potency of the Nemarioc-AL and Nemafric-BL phytonematicides when compared to other plant extracts.

### 8.4.4 Lethal concentrations (LC) and inhibition dosage (D)-values

Generally, the CARD-generated D-values were higher than the LC-values for both phytonematicides. Nemarioc-AL phytonematicide LC-values and D-values were very low when compared to those of Nemafric-BL phytonematicide. Cucurbitacin A, an active ingredient of Nemarioc-AL phytonematicide is water soluble (Chen *et al*., 2005), oxidises readily to cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Jeffrey, 1978), which could to some extent explain the lower LC and D-values observed. Toxic effects of cucumin and leptodermin, have been observed in insects, hence probability that they could be having lethal effects on *M. incognita* J2 as well

resulting in lower LC- and D-values for Nemarioc-AL phytonematicide when compared with Nemafric-BL phytonematicide.

The LC and D-values provide a universal measure that can be used to compare the toxicity of a range of crude extracts across a variety of trials but the use of different forms of these extracts in different trials such as, parts per million, percentages and mass, make it impossible to do it across all trials. The $LC_{50}$ and $LC_{100}$ of two phytonematicides when compared with those of other plant extracts were generally low. Neem leaf extracts at 20 and 30% could cause 50 and 90% mortality of *M. incognita* J2 (Mukesh and Sobita, 2013), whereas castor bean (*Ricinus communis* L.) and lemongrass (*Cymbopogon citratrus* Stapf) could not cause 100% J2 mortality even when used at 100%. The $LC_{100}$ of African marigold (*Tagetes erecta* L.) at 10% (Kalaiselvan and Devaraj, 2011) was comparable with those of the two phytonematicides in this study. Akyazi (2014) showed that white cedar (*Melia azedarach* L.) and black elderberry (*Sambucus nigra* L.) had fairly lower $LC_{100}$ values against *M. incognita* J2 of 5 and 2.5%, respectively, when compared with Nemarioc-AL and Nemafric-BL phytonematicides. This is also the first report of LC- and D-values of Nemarioc-AL and Nemafric-BL phytonematicides.

## 8.5 Conclusion

*Meloidogyne incognita* J2 mortality over increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides had similar trends of DDG patterns for relative impact values and those generated by CARD model. At low concentrations mortality increased and became neutral at higher phytonematicide concentrations. The CARD

model provided excellent MLC-values, whereas the LC-values were generally smaller than the CARD-generated D-values. Also, the CARD model demonstrated that J2 mortality was highly sensitive to both Nemarioc-AL and Nemafric-BL phytonematicides. The toxicities of the two phytonematicides to *M. incognita* J2 were relatively higher when compared to a number of plant extracts.

## 8.6 References


AGBENIN, N.O., EMECHE, A.M., MARLEY, P.S. and A.D. AKPA. 2005. Evaluation of nematicidal action of some botanicals on *Meloidogyne incognita in vitro*. *Journal of Agriculture and Rural Development in the Tropics and Subtropics* 1:29–39.

AKYAZI, F. 2014. Effect of some plant methanol extracts on egg hatching and juvenile mortality of root-knot nematode *Meloidogyne* incognita. *American Journal of Experimental Agriculture* 4:1471–1479.

ARDAKANI, A.S., HOSYNINEJAD, S.A. and A. POURSHIRZAD, 2013. Killing effects of *Myrtus communis* L. essential oil on *Meloidogyne incognita. International Journal of Agriculture and Crop Sciences* 5:806–810.

AZHAGUMURUGAN, C. and M.K. RAJAN. 2014. Effect of leaf extract of Nilakumil, (*Gmelina asiatica*) against the root-knot nematode, (*Meloidogyne incognita*). *Research Journal of Recent Sciences* 3:264–266.

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

CLAUDIUS-COLE, A.O., AMINU, A.E. and B. FAWOLE. 2010. Evaluation of plant extracts in the management of root-knot nematode *Meloidogyne incognita* on cowpea (*Vigna unguiculata* (L) Walp). *Mycopathology* 8:53–60.

COSTA, S.S.R., SANTOS, M.S.N.A. and M.F. RYAN. 2003. Effect of *Artemisia vulgaris* rhizome extracts on hatching, mortality, and plant infectivity of *Meloidogyne megadora*. *Journal of Nematology* 35:437–442.

IBRAHIM, S.K., TRABOULSI, A.F. and S. EL-HAJ. 2006. Effect of essential oils and plant extracts on hatching, migration and mortality of *Meloidogyne incognita*. *Phytopathologia Mediterranea* 45:238–246.

JEFFREY, C. 1978. Cucurbitaceae. In: Launert, E. (ed.). Flora Zambesiaca Managing Committee, London.

KALAISELVAM, I. and A. DEVARAJ. 2011. Effect of root exudates of *Tagetes* sp. on egg hatching behaviour of *Meloidogyne incognita*. *International Research Journal of Pharmacy* 2:93–96.

MASHELA, P.W., DUBE, Z.P. and K.M. POFU. 2015. Managing the Phytotoxicity and Inconsistent Nematode Suppression in Soil Amended with Phytonematicides. In: Meghvansi, M.K. and A. Vorma (eds.). Organic Amendments and Soil Suppressiveness in Plant Disease Management, Soil Biology 46. Springer International Publishers, Switzerland.

MUKESH, D. and S. SOBITA. 2013. Efficacy of certain botanical extracts in the management of *Meloidogyne graminicola* of rice. *International Journal of Agricultural Science and Research* 3:91–98.

MUSTIKA, I. and A.R. SLAMET. 1994. In Efication of Clove Products and other Botanical Plants against Nematodes Attacking Black Pepper, conference on

results of the experiments in order to utilize botanical pesticides. (Indonesian). Research Institute for Spice and Medicinal Crops, Bogor, Indonesia.

NELABALLE, V.K. and L.D. MUKKARA. 2013. A preliminary study on the nematicidal effect of some local flora on *Meloidogyne incognita* Chitwood infesting Mulberry. *International Journal of Chemical, Environmental and Biological Sciences* 1:475–477.

PAVARAJ, M., BAKAVATHIAPPAN, G. and S. BASKARAN. 2012. Evaluation of some plant extracts for their nematicidal properties against root-knot nematode, *Meloidogyne incognita*. *Journal of Biopesticides* 5:106–110.

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

PELINGANGA, O.M., MASHELA, P.W., MPHOSI, M.S., MAFEO, T.P. and Z.P. DUBE. 2013b. Using computer-based model to determine phytotoxicity concentration of nemarioc-A phytonematicide in tomato production. *African Crop Science Conference Proceedings* 11:349–353.

SAIFULLAH, A. 2002. New blue R: The best stain for finding out the life status of nematode eggs. *Journal of Biological Sciences* 2:63.

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

WUYTS, N., SWENNEN, R. and D. DE WAELE. 2006. Effects of plant phenylpropanoid pathway products and selected terpenoids and alkaloids on the behavior of the plant-parasitic nematodes *Radopholus similis, Pratylenchus penetrans* and *Meloidogyne incognita*. *Nematology* 8:89–101.

# CHAPTER 9
# INFECTIVITY OF NEMATODE POST-EXPOSURE TO PHYTONEMATICIDE

## 9.1 Introduction

*In vitro* exposure of nematodes to phytonematicides is an initial approach in determining the influence of these compounds on nematode suppression (Payan *et al*., 1987). However, *in vitro* studies lack the ability to show the variability that occurs at soil-nematode interface. A few studies have attempted to show the infectivity of nematodes post-exposure to phytonematicides (Costa *et al*., 2003; Silva *et al*., 2008). Costa *et al*. (2003) observed that infectivity of root-knot (*Meloidogyne megadora* Whitehead) nematode second-stage juveniles (J2) on a susceptible, field bean (*Phaseolus vulgaris* L.) cultivar, decreased in a density-dependent growth (DDG) pattern with increase in concentration of mugwort (*Artemisia vulgaris* L.) extracts. Root galling on *P. vulgaris* was reduced by 50% when *M. megadora* J2 were exposed to *A. vulgaris* concentration of 32.36 $mg.mL^{-1}$ for 24-h (Costa *et al*., 2003). However, when soyabean cyst (*Heterodera glycines* Ichinole) nematode J2 were exposed to 41.6 $mg.L^{-1}$ aqueous extracts of neem (*Azadirachta indica* A. Juss) and 1000 $mg.L^{-1}$ of methanolic extracts of the same product, the number of nematodes developing to females were reduced by 84% (Silva *et al*., 2008).

*In vitro* bioactivities of Nemarioc-AL and Nemafric-BL phytonematicides had since been conducted (Chapter 4,6,8). The bioactivities of the two phytonematicides had been confirmed on root-knot (*Meloidogyne incognita*) nematode J2 hatching, J2 mobility and J2 mortality (Chapter 4, 6, 8). However, the infectivity of *M. incognita* J2 post-exposure to Nemarioc-AL and Nemafric-BL phytonematicides is not

documented. The objective of this study was to test whether (i) increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides would have an impact on *M. incognita* J2 infectivity of susceptible tomato plant, (ii) the Curve-fitting Allelochemical Response Dosage (CARD) model would quantify the three phases of DDG pattern on *M. incognita* J2 infectivity when compared to increasing phytonematicide concentrations, (iii) computed infectivity inhibition concentration (IC) and CARD-generated D-values would be statistically comparable in magnitudes and (iv) the CARD model would provide information on minimum infectivity concentration (MIC).

## 9.2 Materials and methods

Trials were conducted in greenhouse at the Green Technologies Research Centre, University of Limpopo, South Africa. Ambient day/night temperatures averaged 28/21 $^{\circ}$C, with maximum temperatures inside the greenhouse regulated at 25 °C using thermostatically-activated fans. Nemarioc-AL and Nemafric-BL phytonematicides were prepared by fermenting oven-dried fruits of *C. myriocarpus* and *C. africanus*, respectively (Mashela *et al*., 2015). Ten concentrations, namely, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0% for each phytonematicide were made in distilled water. Two controls were chosen, distilled water alone and distilled water with effective microorganisms to assess whether the effective microorganisms (EM) used in the preparation of the phytonematicide had an added effect. Eggs of *M. incognita* on tomato cv. ‘Floradade’ were collected and hatched as described previously (Chapter 6).

9.2.1 Infectivity trials

Four tomato cv. ‘Floradade’ seeds were placed in 15 cm-diameter pots containing steam pasteurised sand (300 °C for 1-h). After germination, seedlings were thinned by pulling out the whole root system from the soil leaving only one plant per pot of uniform seedlings (Figure 9.1). Freshly hatched *M. incognita* J2 were exposed for 10-d to concentrations of Nemarioc-AL and Nemafric-BL phytonematicides in 5 cm petri dishes. Thereafter, phytonematicide solutions were diluted 5 times and incubated for a further 5 d. At 3-weeks, in separate experiments, arranged in a randomised complete block design with 4 replications, each plant was inoculated with *M. incognita* J2 previously exposed to concentrations of Nemarioc-AL and Nemafric-BL phytonematicides.

Figure 9.1 Tomato plant seedlings used in the infectivity trial.

### 9.2.2 Data collection and analysis

Thirty-days after inoculation, stems were severed at the soil line and shoots dried at 52 °C for 72-h to obtain dry shoot mass. Root systems were removed from pots, immersed in water to remove soil particles, blotted dry and weighed. Root galls per root system were counted before oven drying the root system for dry root mass. Data were analysed as described previously (Chapter 5).

## 9.3 Results

### 9.3.1 Nemarioc-AL phytonematicide

Relative impact: Treatment effects of Nemarioc-AL phytonematicide concentrations on root gall inhibition post-exposure of *M*. *incognita* J2 to the phytonematicide were highly significant ($P \leq 0.01$) (Appendix 9.3), contributing 91% in total treatment variation (TTV) of the variable (Table 9.1). Relative to untreated control, the number of root galls decreased with increasing Nemarioc-AL phytonematicide concentrations (Table 8.2). When relative impact values were plotted against Nemarioc-AL phytonematicide concentrations a DDG patterns were observed with the relationship explained by 94% (Figure 9.1). The DDG patterns had stimulation effect at low Nemarioc-AL phytonematicide concentrations and neutral effect at higher phytonematicide concentrations (Figure 9.1A).

Table 9.1 Partitioning mean sum of squares for root gall inhibition post-exposure of *Meloidogyne incognita* second-stage juvenile to Nemarioc-AL phytonematicide.

| Source | DF | MS | % |
|---|---|---|---|
| Replication | 3 | 0.290 | 7 |
| Treatment | 11 | 3.656 | 91** |
| Error | 33 | 0.094 | 2 |
| Total | 47 | 4.040 | 100 |

**Significant at P ≤ 0.01.

Table 9.2 Root gall inhibition post-exposure of *Meloidogyne incognita* second-stage juvenile to Nemarioc-AL phytonematicide.

| Concentration (%) | Root gall[y] | Rel. impact (%)[z] |
|---|---|---|
| 0.0 | 2.20a | – |
| 0.5 | 2.24a | 2 |
| 1.0 | 2.13ab | –3 |
| 1.5 | 1.97ab | –10 |
| 2.0 | 1.37ab | –38 |
| 2.5 | 0.61ab | –72 |
| 3.0 | 0.38bc | –83 |
| 3.5 | 0.61c | –72 |
| 4.0 | 0.00d | –100 |
| 4.5 | 0.00e | –100 |
| 5.0 | 0.00e | –100 |

[y]Column means followed by the same letter were not different at P ≤ 0.05, according to Waller-Duncan multiple range test.
[z]Relative impact % = [(treatment/control) – 1] x 100.

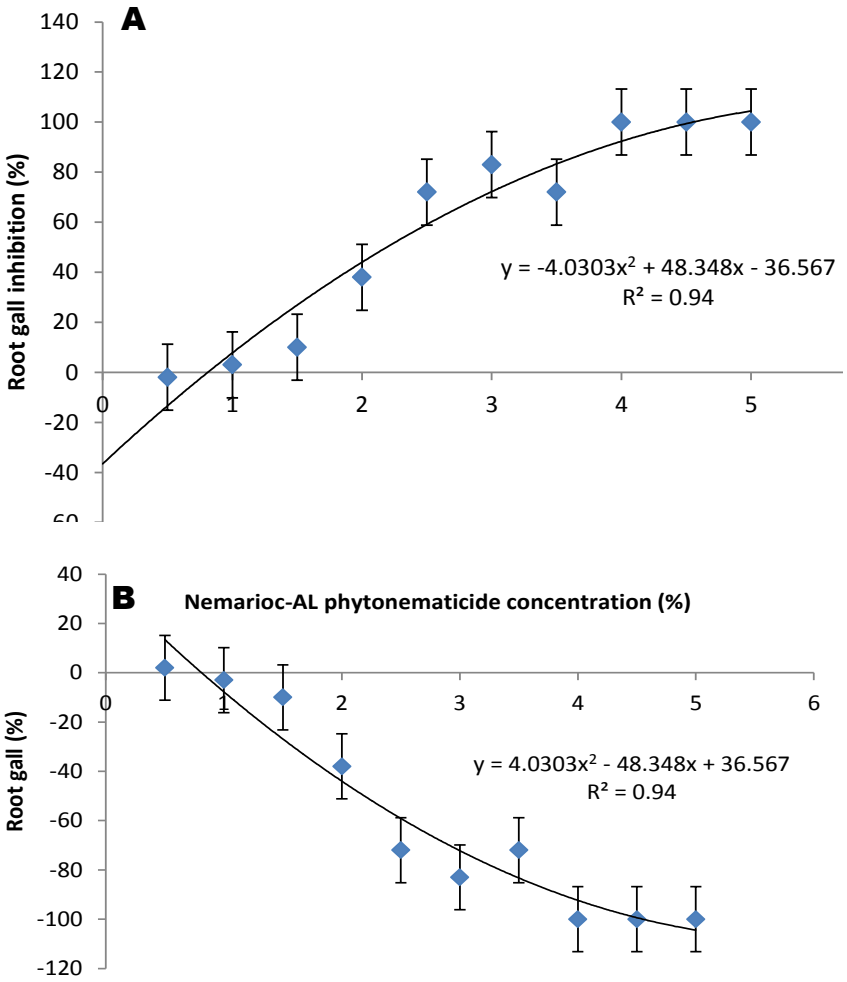


Figure 9.2 Root gall inhibition (A) and root gall (B) of Nemarioc-AL phytonematicide post-exposure of *Meloidogyne incognita* second-stage juvenile.

Curve-fitting Allelochemical Response Dosage: The CARD model quantified concentration ranges of Nemarioc-AL phytonematicide that could stimulate ($D_m$–$R_h$), saturate ($R_h$–$D_0$) and inhibit ($D_0$–$D_{100}$), root galling (Table 9.3). The CARD-generated DDG patterns demonstrated slight stimulation effects at low concentrations, neutral

and inhibition effects as concentrations of Nemarioc-AL phytonematicide increased (Figure 9.3). Root gall inhibition was highly sensitive to Nemarioc-AL phytonematicide concentrations with sensitivity (k) value of 2 units (Table 9.3).

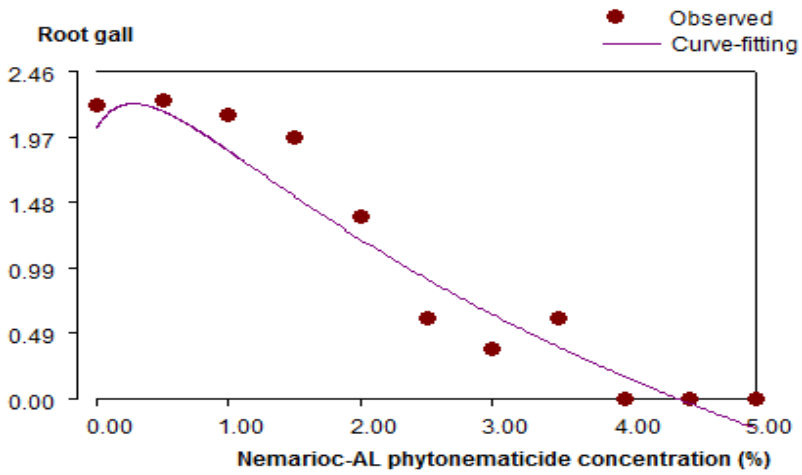


Figure 9.3 Curve-fitting Allelochemical Response Dosage (CARD)-generated density-dependent growth responses of root gall inhibition post-exposure of *Meloidogyne incognita* second-stage juvenile to increasing concentrations of Nemarioc-AL phytonematicide.

Minimum Inhibition Concentration (MIC): Minimum inhibition concentration value computed from CARD-generated quadratic equation was at 0.2% Nemarioc-AL phytonematicide (Table 9.4).

Table 9.3 Biological indices of root gall inhibition post-exposure of *Meloidogyne incognita* second-stage juvenile to increasing concentrations of Nemarioc-AL phytonematicide.

| Biological index | Phytonematicide |
|---|---|
| Threshold stimulation ($D_m$) | 0.28 |
| Saturation point ($R_h$) | 0.47 |
| 0% inhibition ($D_0$) | 1.20 |
| 50% inhibition ($D_{50}$) | 3.50 |
| 100% inhibition ($D_{100}$) | 7.90 |
| $R^2$ | 0.95 |
| Sensitivity index (k) | 2. |

Table 9.4 Minimum root gall inhibition concentration (MIC) of Nemarioc-AL phytonematicide post-exposure of *Meloidogyne incognita* second-stage juvenile computed from Curve-fitting Allelochemical Response Dosage (CARD)-generated quadratic equations.

| Model | x (%)[z] |
|---|---|
| $y = –4.052x^2 + 1.772x + 2.210$ | 0.2 |

[z] $x = –b_1/2b_2$, where $y = b_2x^2 + b_1x + c$.

Comparison of root gall inhibition concentration (IC) and D-value: The IC-values were comparable to the CARD-generated D-values (Table 9.5).

Table 9.5 Comparison of Nemarioc-AL phytonematicide root gall inhibition concentration (IC) and inhibition dosage (D)-values.

| Biological index | Phytonematicide (%) | |
|---|---|---|
| $IC_{50}$ | 2.19 | |
| $D_{50}$ | 2.49 | (3.50)[x] |
| $IC_{100}$ | 7.44 | |
| $D_{100}$ | 5.41 | (7.90) |

[x]Values in brackets are adjusted indices.

9.3.2 Nemafric-BL phytonematicide

Relative impact: Nemafric-BL phytonematicide effects on root gall inhibition were highly significant ($P \leq 0.01$) (Appendix 9.6), contributing 92% in TTV (Table 9.6). Relative to untreated control, root gall inhibition increased with increase in Nemafric-BL phytonematicide concentrations (Table 9.7). When relative impact values were plotted against Nemafric-BL phytonematicide concentrations, a density-dependent growth (DDG) pattern was observed (Figure 9.3). The DDG patterns had slight inhibition, neutral and stimulation effects as Nemafric-BL phytonematicide concentrations were increased (Figure 9.3A).

Curve-fitting Allelochemical Response Dosage (CARD): The CARD model managed to generate the concentration ranges of Nemafric-BL phytonematicide that could stimulate ($D_m$–$R_h$), saturate ($R_h$–$D_0$) and inhibit ($D_0$–$D_{100}$), root galling (Table 9.8). The CARD-generated DDG patterns demonstrated two phases of DDG patterns, stimulation at low Nemafric-BL phytonematicide concentrations and saturation at higher Nemafric-BL phytonematicide concentrations (Figure 9.4). Juvenile infectivity inhibition was also highly sensitive to Nemafric-BL phytonematicide with k-value of 1 unit (Table 9.8).

Table 9.6 Partitioning mean sum of squares for root gall inhibition post-exposure of *Meloidogyne incognita* second-stage juvenile to Nemafric-BL phytonematicide.

| Source | DF | MS | % |
|---|---|---|---|
| Replication | 3 | 0.241 | 6 |
| Treatment | 11 | 3.716 | 92** |
| Error | 33 | 0.080 | 2 |
| Total | 47 | 4.037 | 100 |

**Significant at P ≤ 0.01.

Table 9.7 Influence of Nemafric-BL phytonematicide on root gall inhibition post-exposure of *Meloidogyne incognita* second-stage juvenile.

| Concentration (%) | Mean[y] | Rel. impact (%)[z] |
|---|---|---|
| 0.0 | 2.44a | – |
| 0.5 | 2.44a | 0 |
| 1.0 | 2.36a | –3 |
| 1.5 | 2.28a | –7 |
| 2.0 | 2.12b | –13 |
| 2.5 | 2.06c | –16 |
| 3.0 | 1.94cd | –20 |
| 3.5 | 1.59c | –35 |
| 4.0 | 0.47d | –81 |
| 4.5 | 0.00d | –100 |
| 5.0 | 0.00d | –100 |

[y]Column means followed by the same letter were not different at P ≤ 0.05, according to Waller-Duncan multiple range test.

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

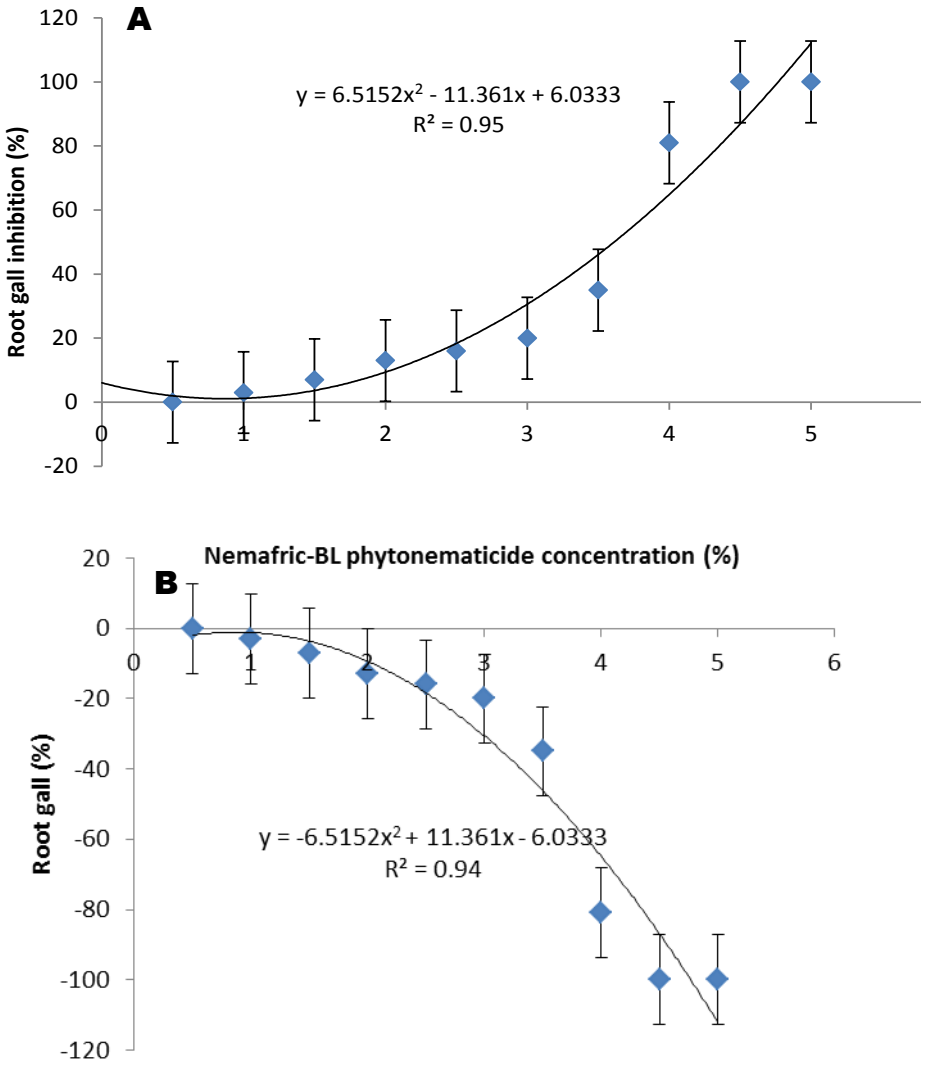


Figure 9.4 Root gall inhibition (A) and root gall (B) of Nemafric-BL phytonematicide post-exposure of *Meloidogyne incognita* second-stage juvenile.

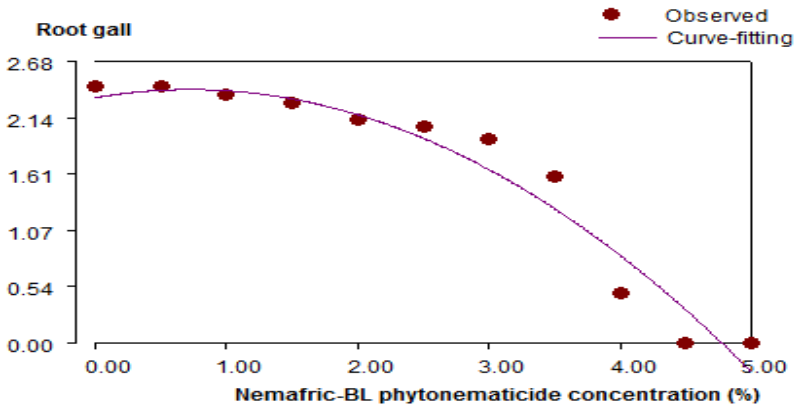


Figure 9.5 Curve-fitting Allelochemical Response Dosage (CARD)-generated density-dependent growth responses of root gall inhibition post-exposure of *Meloidogyne incognita* second-stage juvenile to increasing concentrations of Nemafric-BL phytonematicide.

Table 9.8 Biological indices of root gall inhibition post-exposure of *Meloidogyne incognita* second-stage juvenile to increasing concentrations of Nemafric-BL phytonematicide.

| Biological index | Phytonematicide |
|---|---|
| Threshold stimulation ($D_m$) | 0.72 |
| Saturation point ($R_h$) | 0.80 |
| 0% inhibition ($D_0$) | 2.25 |
| 50% inhibition ($D_{50}$) | 5.89 |
| 100% inhibition ($D_{100}$) | 10.69 |
| $R^2$ | 0.95 |
| Sensitivity index (k) | 0. |

Minimum inhibition concentration (MIC): The minimum Nemafric-BL phytonematicide concentration that could cause root gall inhibition was established at 0.7%, using a quadratic equation generated by CARD model (Table 9.9).

Table 9.9 Minimum root gall inhibition concentration (MLC) of Nemafric-BL phytonematicide post-exposure of *Meloidogyne incognita* second-stage juvenile computed from Curve-fitting Allelochemical Response Dosage (CARD)-generated quadratic equations.

| Model | x (%)[z] |
|---|---|
| $y = –0.149x^2 + 0.216x + 2.374$ | 0.7 |

[z] $x = –b_1/2b_2$, where $y = b_2x^2 + b_1x + c$.

Comparison of root gall inhibition concentration (IC) and D-values: The CARD-generated D-values were higher than IC-values at both 50% and 100% (Table 9.10).

Table 9.10 Comparison of Nemafric-BL phytonematicide root gall inhibition concentration (IC) and inhibition dosage (D)-values.

| Biological index | Phytonematicide (%) | |
|---|---|---|
| $IC_{50}$ | 3.61 | |
| $D_{50}$ | 1.45 | $(5.89)^x$ |
| $IC_{100}$ | 4.77 | |
| $D_{100}$ | 4.80 | (10.69) |

[x] Values in brackets are adjusted indices.

## 9.4 Discussion

### 9.4.1 Relative impact

The *in vitro* bioassays in the study of nematode number suppression by phytonematicides forms the initial approach towards evaluating the potential of the product in the overall nematode management program (Payan *et al*., 1987). The knowledge of infectivity of the exposed nematodes to a susceptible host provides crucial information on the actual impact of the nematode at soil-nematode interface in the development of a disease. Inhibition of *M. incognita* J2 infectivity on susceptible tomato cultivar by Nemarioc-AL and Nemafric-BL phytonematicides observed in this study is the first of such a report. Aqueous extracts of neem at a concentration of 41.6 $mg.L^{-1}$ and 1000 $mg.L^{-1}$ methanol extracts of the same product were observed to reduce the number of *H. glycines* females by 84% in roots of susceptible soyabean plants (Silva *et al*., 2008). In this study, inhibition of *M. incognita* J2 infectivity had the DDG patterns for both phytonematicides.

The two phytonematicides exhibited two phases of DDG patterns, namely inhibition and neutral phases on root galling but the order differed between them. Nemarioc-AL phytonematicide had root gall inhibition at low concentrations and neutral effect at high concentrations, whereas Nemafric-BL phytonematicide neutral effect was observed at low phytonematicide concentrations with inhibition occurring at higher concentrations. The identified active ingredient of Nemarioc-AL phytonematicide, cucurbitacin A is partially soluble in water due to the partial polarity of the chemical compound (Chen *et al*., 2005), oxidises readily to cucumin and leptodermin (Jeffrey, 1978), whereas the active ingredient of Nemafric-BL phytonematicide, cucurbitacin B

is not soluble in water and is also stable. These properties of active ingredients of the two phytonematicides could to some extent explain the varying trends between them. A few studies have examined the penetration and development of nematodes to their susceptible hosts after exposing them to plant extracts (Silva *et al*., 2008), with few no observations suggesting DDG pattern response. Costa *et al*. (2003) observed a DDG pattern when *M. megadora* J2 were exposed to concentrations of *A. vulgaris* which reduced the root galling on susceptible *P. vulgaris* cultivar (Costa *et al*., 2003).

### 9.4.2 Curve-fitting Allelochemical Response Dosage

The CARD-generated biological indices for Nemarioc-AL phytonematicide were much lower than those of Nemafric-BL phytonematicide, whereas the overall sensitivity of root galling to both phytonematicides was high as shown by low values of 2 units and 0 units, respectively. The CARD model DDG patterns were similar to the relative impact graphs described above. This is the first report on the use of the CARD model to explain the relationship between increasing concentrations of phytonematicides and nematode J2 infectivity post-exposure.

### 9.4.3 Minimum inhibition concentration

The MIC observed in this study was very low for both phytonematicides, when the two are compared a higher MIC-value was observed for Nemafric-BL phytonematicide than for Nemarioc-AL phytonematicide. When compared with other extracts the MIC-values for Nemarioc-AL and Nemafric-BL phytonematicides were generally very low.

At 100% concentration, Shadung *et al*. (2016) determining the quality protocols for Nemarioc-AL and Nemafric-BL phytonematicides quantified concentration of cucurbitacins in the two phytonematicides to be between 2 and 14 $\mu g.mL^{-1}$ depending on storage duration, hence a minimum inhibition concentration of 0.2 and 0.7% for Nemarioc-AL and Nemafric-BL phytonematicides, respectively, observed in this study will be very low when compared to those of other studies. Aqueous extracts of neem at concentrations as high as 4.16 $mg.L^{-1}$ could not reduce infectivity of *H. glycines* on susceptible soyabean plants, this is very high when compared to inhibitive concentrations in this study (Silva *et al*., 2008). Apparently, Nemarioc-AL and Nemafric-BL phytonematicides had inhibitive effects, whereas the synthetic nematicide carbofuran did not have effects on *M. incognita* infectivity on a susceptible tomato cultivar at concentration of 6 $\mu g.mL^{-1}$ post-exposure (Payan *et al*., 1987). The low MIC and high overall sensitivity values observed in this study provide further evidence of the high potency of Nemarioc-AL and Nemafric-BL phytonematicides.

### 9.4.4 Inhibition concentration (IC) and D-values

Generally, the CARD-generated D-values were higher than the LC-values for both phytonematicides. Nemarioc-AL phytonematicide LC-values and D-values were comparable but lower than those of Nemafric-BL phytonematicide. Cucurbitacin A, an active ingredient of Nemarioc-AL phytonematicide could explain the lower concentration required for inhibition of J2 infectivity. Toxic effects of cucumin and leptodermin, have been observed in insects, hence probability that they could be having some effects on *M. incognita* J2 as well resulting in lower LC- and D-values

for Nemarioc-AL phytonematicide when compared with Nemafric-BL phytonematicide. Similar observations between Nemarioc-AL and Nemafric-BL phytonematicides were made when the two phytonematicides where used in *M. incognita* mobility and mortality *in vitro* studies (Chapters 6, 8). *Artemisia vulgaris* had $IC_{50}$ of 32.36 $mg.mL^{-1}$ when *M. megadora* J2 were exposed to the plant extract (Costa *et al*., 2003). The two phyonematicides were even more effective than oxamyl and fenamiphos which showed reduced the citrus nematode (*Tylenchulus semipenetrans* Cobb) root penetration by only 3.4 and 2.4%, respectively, each at 100 $\mu g.mL^{-1}$ water (Al-Azzeh and Abu-Gharbieh, 2004). This is also the first report of LC- and D-values of Nemarioc-AL and Nemafric-BL phytonematicides on *M. incognita* J2 infectivity post-exposure to the two phytonematicides.

## 9.5 Conclusion

*Meloidogyne incognita* J2 root infectivity over increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides had similar trends of DDG patterns for relative impact values and those generated by CARD model. At low concentrations J2 infectivity inhibition increased and became neutral at higher Nemarioc-AL phytonematicide concentrations, whereas at low Nemafric-BL phytonematicide concentrations J2 infectivity inhibition had a neutral effect and increased at higher phytonematicide concentrations. The CARD model provided excellent MIC-values, whereas the IC- values were generally smaller than the CARD-generated D-values for both phytonematicides. Nemarioc-AL phytonematicide had IC and D-values that were comparable. Using the $R^2$ to compare between IC and D-values, the D-values are recommended since they have a higher $R^2$. Also, the CARD model demonstrated

that J2 infectivity was highly sensitive to both Nemarioc-AL and Nemafric-BL phytonematicides. The toxicity of the two phytonematicides to *M. incognita* J2 were relatively very high when compared to a number of plant extracts and some synthetic nematicides building a strong case for use of the two phytonematicides in nematode number suppression.

## 9.6 References


AL-AZZEH, T.K. and W.I. ABU-GHARBIEH. 2004. Effect of oxamyl and fenamiphos on egg hatching, motility, and root penetration of *Tylenchulus semipenetrans*. *Nematologia Mediterranea* 32:19–23.

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

COSTA, S.S.R., SANTOS, M.S.N.A. and M.F. RYAN. 2003. Effect of *Artemisia vulgaris* rhizome extracts on hatching, mortality, and plant infectivity of *Meloidogyne megadora*. *Journal of Nematology* 35:437–442.

JEFFREY, C. 1978. Cucurbitaceae. In: Launert, E. (ed.). Flora Zambesiaca Managing Committee, London.

MASHELA, P.W., DUBE, Z.P. and K.M. POFU. 2015. Managing the Phytotoxicity and Inconsistent Nematode Suppression in Soil Amended with Phytonematicides. In: Meghvansi, M.K. and A. Vorma (eds.). Organic Amendments and Soil Suppressiveness in Plant Disease Management, Soil Biology 46. Springer International Publishers, Switzerland.

PAYAN, L.A., JOHNSON, A.W. and R.H. LITTRELL. 1987. Effects of nematicides and herbicides alone or combined on *Meloidogyne incognita* egg hatch and development. *Annals of Applied Nematology* 1:67–70.

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

SILVA, J.C.T., OLIVEIRA, R.D.L., JHAM, G.N. and N.D.C. AGUIAR. 2008. Effect of neem seed extracts on the development of the soybean cysts nematode. *Tropical Plant Pathology* 33:171–179.

# CHAPTER 10
# NEMATODES AS BIOINDICATORS OF PHYTONEMATICIDE MOBILITY IN PLANTS, SOIL AND ORGANIC MATTER

## 10.1 Introduction

The use of phytonematicides is gradually becoming an integral part of good agricultural practices since the products are favourably viewed as environment-friendly alternatives to synthetic chemical nematicides in managing nematodes (Mashela *et al*., 2015). Indiscriminate use of synthetic chemical nematicides such as methyl bromide over decades has had indescribable harmful effects on human health and the ecosystems (Mashela, 2007; Soomro *et al*., 2008; Zarins *et al*., 2009). Fumigant nematicides in the atmosphere, water resources, soil and produce caused extensive harm to non-target organisms and the environment (Akbar *et al*., 2010; Carson, 1962). Current views are, therefore, to ensure that whenever a pesticide is introduced, empirical-based evidence to ensure that it is an ecologically-sound alternative, is important in terms of various legislation such as the South African Act No. 37 of 1947 as amended in 2012. Phytonematicides could be viewed as a promising alternative to the withdrawn environment-unfriendly systemic and fumigant chemical nematicides since the former have been observed to degrade rapidly, with limited persistence and bioaccumulation in the environment (Mashela and Dube, 2014; Naqvi *et al*., 2007), when compared to the fumigant nematicides (Carson, 1962).

Even though the phytonematicides have been classified as safe, certain studies have shown that the products could also harm non-target organisms and the environment (Kreuntzweiser *et al*., 2000; Mashela and Dube, 2014; McKenry, 1994;

Punzo and Parker, 2005; Scott and Kaushik, 2000). McKenry (1994) reported that the cucurbitacins, which serve as active ingredients in Nemarioc-AL and Nemafric-BL phytonematicides (Mashela *et al*., 2015), are cancerous at low concentrations. Cucurbitacins from Nemarioc-AL and Nemafric-BL phytonematicides accumulated in the soil and resulted in reduction of nematode numbers and affected either negatively or positively the successor cowpea and sweet stem sorghum crops (Mashela, 2014; Mashela and Dube, 2014). Punzo and Parker (2005) observed that neem (*Azadirachta indica* A. Juss) extracts could affect survival capacity, reproductive fertility and swimming speed in larval stages of cane toad (*Bufo marinus* L.). Scott and Kaushik (2000) also noted that neem extracts could be toxic to aquatic life when they find themselves in water resources as had been the case with most fumigant nematicides (Carson, 1962). The presence of any pesticide in the soil or food produce continues, therefore, to be of great concern as shown by various ISO standards on chemical residues in many countries.

Nematodes have been used as bioindicators of ecological health conditions in a number of studies (Hoss *et al*., 2011; Park *et al*., 2011; Rodriguez-Martin *et al*., 2014). The latter could be attributed to their high sensitivities to environmental changes (Gutierrez *et al*., 2016). Sochova *et al*. (2006) reported that besides nematodes being appropriate bioindicators of soil condition, they are also suitable organisms for *in vitro* cytotoxicity testing. Nemarioc-AL and Nemafric-BL phytonematicides had been consistent in supressing nematode population densities under various conditions (Mashela *et al*., 2015). The sensitivity of root-knot (*Meloidogyne* species) nematode eggs and second-stage juveniles (J2) to the two

phytonematicides and their pure active ingredients had been adequately addressed previously (Chapters 3–9). However, there is no information on the use of nematodes as bioindicators of the two phytonematicides in plants, soil and organic matter. The objective of this study was therefore, to determine whether nematodes can serve as bioindicators of Nemarioc-AL and Nemafric-BL phytonematicides in tomato plant roots/fruits, soil types and organic matter at different depths.

## 10.2 Materials and methods

Experiments for use of nematodes as bioindicators of phytonematicide mobility through plants, soil type and organic matter were carried-out in a special-designed structure (Figure 10.1) at the Green Technologies Research Centre, University of Limpopo, South Africa (23°53′10″S, 29°44′15″E). Standard agronomic practices for growing tomatoes were followed as described previously (Pelinganga, 2013)

### 10.2.1 Preparation of phytonematicides

Nemarioc-AL and Nemafric-BL phytonematicides were prepared using effective microorganisms (EM) fermentation of oven-dried ground fruits from *Cucumis myriocarpus* and *C. africanus*, respectively, using materials and infrastructure described previously (Mashela *et al*., 2015).

### 10.2.2 Procedure

Soil type experiments: Four pasteurised soil types, namely, loam (22% clay, 40% silt, 38% sand), sand (89% sand, 6% clay, 5% silt), calcareous and clay (65% clay, 20% sand, 15% silt) soils were arranged in a split-split plot design, with six replications.

Soil type was assigned to the main plot, the two products, Nemarioc-AL and Nemafric-BL phytonematicides, constituted the subplot factor, whereas depth was the sub-subplot factor. Loam soil, which is predominantly viewed as the high potential soil, was used as control. The trial was carried-out in 15-cm-diameter plastic cylinders, with 100-cm depth, lined with polyethylene foil (Figure 10.1). Uniform four-week-old tomato cv. 'Floradade' seedlings were transplanted into each soil column before being inoculated each with 5 000 eggs and juveniles of *M. incognita*. Nemarioc-AL and Nemafric-BL phytonematicides at 3% each were applied at 17 day intervals (Pelinganga, 2013).

Organic matter experiments: Organic matter was obtained from the ZZ2 Boerdery Pty (Mooketsi, South Africa). Pasteurised sand soil was mixed with different organic matter level to make 2, 4, 8, 16, 32 and 64% organic matter treatments. Sandy soil without organic matter was used as a control. A split-split-plot design was used with 4 replications. Organic matter levels were assigned to main plots, phytonematicides to subplots and depth to sub-subplots. The trial was carried-out in plastic cylinders as described above. Uniform four-week-old tomato 'Floradade' seedlings were transplanted into each medium column before being inoculated each with 5 000 eggs and J2 *M. incognita*. The concentration and application interval was as described for soil type.

Figure 10.1 Plastic cylinder pipes filled with different soil types and organic matter levels.

10.2.3 Data collection

Fifty-six days after inoculation, plant variables such as stem diameter, plant height and chlorophyll content were determined. Plant height was measured from the soil surface to the tip of the flag leaf. Stems were then severed at the soil line and the stem diameter measured at 5 cm above the severed end using a digital vernier caliper. Fresh fruit and shoots were weighed, oven-dried at 52 °C for 72 h and weighed. Phytonematicide residues were measured from dried fruits using isocratic elution Shimadzu High Performance Liquid Chromatography (HPLC) Prominance, with detection using Shimadzu CTO-20A diode array detector, cucurbitacin A and B were used as standards for Nemarioc-AL and Nemafric-BL phytonematicides, respectively (Shadung, 2016).

The plastic cylinders were cut into four sections of 25 cm each using an angle grinder, with each section constituting a depth. Soil column from each section were

then transferred into labelled plastic bags with root system removed from each section, immersed in water to remove soil particles, blotted dry and weighed. The four soil section depths were 0–25, 26–50, 51–75 and 76–100 cm, from the top to the bottom of the plastic cylinder. Nematodes were extracted from root system/section by maceration and blending for 30 s in 1% NaOCl (Hussey and Barker, 1973). The materials were passed through nested 75- and 25-µm mesh sieves. The contents of the 25-µm mesh sieve were collected for further separation of nematodes from debris using the sugar-flotation method (Jenkins, 1964). Soil in each section 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.

### 10.2.4 Data analysis

Data were subjected to analysis of variance (ANOVA) through the SAS software (SAS Institute, 2008). Nematode numbers were transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984). Three-way and two-way tables were constructed for variables showing statistically significant interactions. For variables where interactions were not statistically significant, treatment means were separated using Waller-Duncan multiple range test at the probability level of 5%. Unless otherwise stated, only treatments that were significant at the probability level of 5% are discussed. The total number of nematodes in each section was used as bioindicator of movement and distribution of phytonematicides in different soil type

and organic matter experiments. Unless otherwise stated treatment effects were discussed at the probability level of 5%.

## 10.3 Results

### 10.3.1 Interactions on nematode variables

The second order interaction, soil type (S) ˣ phytonematicide (P) ˣ depth (D), had significant effects on J2 in root and total nematode (Appendix 10.2, 10.4), contributing 6 and 9% in total treatment variation (TTV) of the two variables, respectively (Table 10.1). This interaction had no effect on eggs in root and J2 in soil (Appendix 10.1, 10.3). Additionally, the first order interaction, S ˣ D, and depth each had highly significant effects on eggs in root and total nematode, each contribute 9–27% and 12–39% in TTV of the two variables, respectively. The other first order interactions (S ˣ P or P ˣ D) and soil type or phytonematicide main factors had no effect on nematode variables.

The pairwise comparison of the effects of the second order interaction, S ˣ P ˣ D, on eggs in root and total nematode had similar trends (Table 10.2, 10.3). Due to loam soil being viewed as an ideal soil, solubility of cucurbitacin A in water and over 80% of roots accumulating in the 0–25 cm soil depth, the pairwise comparison of loam, Nemarioc-AL phytonematicides and 0–25 cm depth was arbitrarily assigned as the standard for comparison purposes (Table 10.2, 10.3). Relative to the arbitrary standard, all pairwise comparisons except for calcareous, Nemafric-BL phytonematicide and 0–25 cm depth reduced total nematode number (Table 10.2, 10.3). Notably, in the two deepest soil depths, 51–75 cm and 76–100 cm, almost all pairwise comparisons reduced eggs and total nematode numbers by 100%.

Table 10.1 Partitioning mean sum of squares for eggs in root, J2 in root, J2 in soil and total *Meloidogyne incognita* under different soil types, phytonematicides and depth.

| | | Eggs in root | | J2 in root | | J2 in soil | | Total nematode | |
|---|---|---|---|---|---|---|---|---|---|
| Source | DF | MS | % | MS | % | MS | % | MS | % |
| Replication | 5 | 0.01 | 9 | 0.036 | 2 | 0.001 | 10 | 0.045 | 3 |
| Soil type (S) | 3 | 0.01 | $9^{ns}$ | 0.060 | $3^{ns}$ | 0.001 | $10^{ns}$ | 0.083 | $6^{ns}$ |
| Error | 15 | 0.01 | 9 | 0.056 | 3 | 0.001 | 10 | 0.066 | 4 |
| Phytonematicide (P) | 1 | 0.01 | $9^{ns}$ | 0.035 | $2^{ns}$ | 0000 | $0^{ns}$ | 0.044 | $3^{ns}$ |
| S×P | 3 | 0.01 | $9^{ns}$ | 0.205 | $10^{ns}$ | 0.001 | $10^{ns}$ | 0.210 | $14^{ns}$ |
| Error | 20 | 0.01 | 9 | 0.084 | 4 | 0.002 | 20 | 0.088 | 6 |
| Depth (D) | 3 | 0.01 | $9^{ns}$ | 0.536 | $27^{**}$ | 0.001 | $10^{ns}$ | 0.582 | $39^{**}$ |
| S×D | 9 | 0.01 | $9^{ns}$ | 0.179 | $9^{**}$ | 0.001 | $10^{ns}$ | 0.187 | $12^{**}$ |
| P×D | 3 | 0.01 | $9^{ns}$ | 0.116 | $6^{ns}$ | 0000 | $0^{ns}$ | 0.136 | $9^{ns}$ |
| S×P×D | 9 | 0.01 | $9^{ns}$ | 0.120 | $6^{*}$ | 0.001 | $10^{ns}$ | 0.136 | $9^{*}$ |
| Error | 120 | 0.01 | 9 | 0.563 | 28 | 0.001 | 10 | 0.060 | 4 |
| Total | 191 | 0.11 | 100 | 1.990 | 100 | 0.010 | 100 | 1.501 | 100 |

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

Table 10. 2 A three-way matrix of second order interaction among the factors soil type, phytonematicide and depth on eggs of *Meloidogyne incognita* in root of tomato plants.

| | | Depth (cm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0–25 | | 26–50 | | 51–75 | | 76–100 | |
| Soil type | Phytonematicide | Egg | %[y] | Egg | % | Egg | % | Egg | % |
| Loam | Nemarioc-AL | 0.50 | –[x] | 0.00 | –100 | 0.00 | –100 | 0.00 | –100 |
| Loam | Nemafric-BL | 0.44 | –12 | 0.00 | –100 | 0.00 | –100 | 0.00 | –100 |
| Sand | Nemarioc-AL | 0.00 | –100 | 0.39 | –22 | 0.12 | –76 | 0.28 | –44 |
| Sand | Nemafric-BL | 0.16 | –68 | 0.08 | –84 | 0.00 | –100 | 0.00 | –100 |
| Calcareous | Nemarioc-AL | 0.00 | –100 | 0.00 | –100 | 0.00 | –100 | 0.00 | –100 |
| Calcareous | Nemafric-BL | 0.61 | 22 | 0.00 | –100 | 0.00 | –100 | 0.00 | –100 |
| Clay | Nemarioc-AL | 0.27 | –46 | 0.16 | –68 | 0.00 | –100 | 0.00 | –100 |
| Clay | Nemafric-BL | 0.00 | –100 | 0.00 | –100 | 0.00 | –100 | 0.00 | –100 |

[x]The standard Loam-Nemarioc-AL phytonematicide-0 to 25 cm depth was based on loam soil is an ideal soil type, the water-soluble cucurbitacin A of the phytonematicide and the accumulation of roots within the 0–25 cm depth.

[y]Relative impact = [(treatment/Standard) – 1] x 100.

Table 10. 3 A three-way matrix of second order interaction among the factors soil type, phytonematicide and depth on total *Meloidogyne incognita* (TMi) in root of tomato plants.

| | | Depth (cm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0–25 | | 26–50 | | 51–75 | | 76–100 | |
| Soil type | Phytonematicide | TMi | %[y] | TMi | % | TMi | % | TMi | % |
| Loam | Nemarioc-AL | 0.53 | –[x] | 0.00 | –100 | 0.00 | –100 | 0.00 | –100 |
| Loam | Nemafric-BL | 0.44 | –17 | 0.00 | –100 | 0.00 | –100 | 0.00 | –100 |
| Sand | Nemarioc-AL | 0.00 | –100 | 0.47 | –11 | 0.12 | –77 | 0.28 | –47 |
| Sand | Nemafric-BL | 0.21 | –60 | 0.08 | –85 | 0.00 | –100 | 0.00 | –100 |
| Calcareous | Nemarioc-AL | 0.00 | –100 | 0.00 | –100 | 0.00 | –100 | 0.00 | –100 |
| Calcareous | Nemafric-BL | 0.61 | 15 | 0.00 | –100 | 0.00 | –100 | 0.00 | –100 |
| Clay | Nemarioc-AL | 0.27 | –99 | 0.16 | –70 | 0.00 | –100 | 0.00 | –100 |
| Clay | Nemafric-BL | 0.00 | –100 | 0.00 | –100 | 0.00 | –100 | 0.00 | –100 |

[x]The standard Loam-Nemarioc-AL phytonematicide-0 to 25 cm depth was based on loam soil is an ideal soil type, the water-soluble cucurbitacin A of the phytonematicide and the accumulation of roots within the 0–25 cm depth.

[y]Relative impact = [(treatment/Standard) – 1] x 100.

The second order interaction, organic matter (O) x phytonematicide (P) x depth (D), along with the associated first order interactions and organic matter and depth main factor effects, did not have any effects on any component of nematode final population densities of *M. incognita* (Table 10.4, Appendix 10.11,10.12). However, the phytonematicide main factor had significant effects on J2 in root and total nematodes, each contributing 51% in TTV of the two variables (Table 10.4).

Table 10.4 Partitioning mean sum of squares for eggs in root, J2 in root and total nematodes under different organic matter levels, phytonematicides and depth.

| | | Eggs in root | | J2 in roots | | Total nematode | |
|---|---|---|---|---|---|---|---|
| Source | DF | MS | % | MS | % | MS | % |
| Replication | 3 | 0.0022 | 9 | 0.063 | 6 | 0.064 | 6 |
| Organic matter (O) | 6 | 0.0022 | $9^{ns}$ | 0.046 | $4^{ns}$ | 0.046 | $4^{ns}$ |
| Error | 18 | 0.0022 | 9 | 0.097 | 9 | 0.098 | 10 |
| Phytonematicide (P) | 1 | 0.0022 | $9^{ns}$ | 0.556 | $51^{*}$ | 0.562 | $51^{*}$ |
| O×P | 6 | 0.0022 | $9^{ns}$ | 0.051 | $5^{ns}$ | 0.052 | $5^{ns}$ |
| Error | 21 | 0.0022 | 9 | 0.051 | 5 | 0.052 | 5 |
| Depth (D) | 3 | 0.0022 | $9^{ns}$ | 0.043 | $4^{ns}$ | 0.042 | $4^{ns}$ |
| O×D | 18 | 0.0022 | $9^{ns}$ | 0.042 | $4^{ns}$ | 0.043 | $4^{ns}$ |
| P×D | 3 | 0.0022 | $9^{ns}$ | 0.012 | $1^{ns}$ | 0.011 | $1^{ns}$ |
| O×P×D | 18 | 0.0022 | $9^{ns}$ | 0.064 | $6^{ns}$ | 0.064 | $6^{ns}$ |
| Error | 126 | 0.0022 | 9 | 0.061 | 6 | 0.062 | 6 |
| Total | 223 | 0.0242 | 100 | 1.086 | 100 | 1.096 | 100 |

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

Relative to Nemarioc-AL phytonematicide, Nemafric-BL phytonematicide reduced J2 in root and total nematode each by 83% (Table 10.5).

Table 10.5 Effect of phytonematicide on J2 in root and total *Meloidogyne incognita* under different organic matter, phytonematicide and depth trial.

| | J2 in root | | Total nematode | |
|---|---|---|---|---|
| Phytonematicide | Mean[y] | Rel. impact %[z] | Mean | Rel. impact % |
| Nemarioc-AL | 0.1206a | – | 0.1211a | – |
| Nemafric-BL | 0.0210b | –83 | 0.0210b | –83 |

[y]Column means followed by the same letter were not different (P ≤ 0.05) according to two sample t-test.

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

10.3.2 Interactions on plant variables

The second order interaction, S x P x D, and its associated first order interaction and main factors, except for S x D, depth and soil type, had no effect on dry root mass (Table 10.6) (Appendix 10.5). The S x D interaction and depth had highly significant effects on dry root mass, contributing 3 and 92% in TTV of the variables, respectively (Table 10.6). In contrast, soil type had significant effects, contributing 4% in TTV of the variable.

The S x P interaction had no effect on all plant variables, whereas soil type had highly significant effects on chlorophyll content(Appendix 10.6-10.10), contributing 44, 56 and 76% in TTV of the variables, respectively (Table 10.7). In contrast, soil type had significant effect on tomato plant height, contributing 46% in TTV of the variable. Phytonematicide had significant effects on dry shoot mass and stem diameter, contributing 54 and 20% in TTV of the variables, respectively (Table 10.7).

Table 10.6 Partitioning mean sum of squares for dry root mass under different soil types, phytonematicides and depth.

| Source | DF | MS | % |
|---|---|---|---|
| Replication | 5 | 1.57 | 0 |
| Soil type (S) | 3 | 105.51 | 4* |
| Error | 15 | 11.05 | 0 |
| Phytonematicide (P) | 1 | 16.04 | 1ns |
| S×P | 3 | 2.78 | 0ns |
| Error | 20 | 6.86 | 0 |
| Depth (D) | 3 | 2301.09 | 92** |
| S×D | 9 | 64.35 | 3** |
| P×D | 3 | 2.60 | 0ns |
| S×P×D | 9 | 2.72 | 0ns |
| Error | 120 | 6.95 | 0 |
| Total | 191 | 2521.52 | 100 |

**Significant at P ≤ 0.01, *Significant at P ≤ 0.05.

nsNot significant at P ≤ 0.05.

The O x P interaction had no effect on any variable (Appendix 10.13-10.18), whereas organic matter had significant effect on plant height contributing 21% in TTV of the variable, but had highly significant effect on chlorophyll content, contributing 26% in TTV of the variable (Table 10.8). In contrast, phytonematicide had highly significant effect on stem diameter, contributing 63% in TTV of the variable.

The O x P x D interaction had no effect on all plant variables, whereas the main factor depth had highly significant effects on dry root mass, contributing 93% in TTV of the variable (Table 10.9). Relative to the top soil 0–25 cm depth, depth reduced dry root mass from 77 to 82% (Table 10.10).

Table 10.7 Partitioning mean sum of squares for fruit mass (FM), dry shoot mass (DSM), stem diameter (SD), plant height (PHT) and chlorophyll content (CC) under different soil types and phytonematicides.

| | | FM | | DSM | | SD | | PHT | | CC | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | DF | MS | % | MS | % | MS | % | MS | % | MS | % |
| Replication | 5 | 2461.12 | 12 | 3.12 | 2 | 0.34 | 4 | 30.19 | 7 | 33.07 | 6 |
| Soil type (S) | 3 | 9108.38 | 44$^{**}$ | 22.49 | 16$^{ns}$ | 4.33 | 56$^{**}$ | 195.89 | 46$^{*}$ | 436.61 | 72$^{**}$ |
| Error | 15 | 1189.16 | 6 | 8.87 | 6 | 0.50 | 6 | 53.64 | 13 | 40.64 | 7 |
| Phytonematicide (P) | 1 | 5429.38 | 26$^{ns}$ | 74.25 | 54$^{*}$ | 1.59 | 20$^{*}$ | 27.91 | 7$^{ns}$ | 27.91 | 5$^{ns}$ |
| S×P | 3 | 629.41 | 3$^{ns}$ | 14.21 | 10$^{ns}$ | 0.72 | 9$^{ns}$ | 16.44 | 4$^{ns}$ | 41.66 | 7$^{ns}$ |
| Error | 20 | 1758.58 | 9 | 13.66 | 10 | 0.28 | 5 | 95.83 | 23 | 20.29 | 3 |
| Total | 47 | 20576.03 | 100 | 136.60 | 100 | 7.76 | 100 | 419.90 | 100 | 600.18 | 100 |

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

Table 10.8 Partitioning mean sum of squares for fruit mass (FM), dry shoot mass (DSM), stem diameter (SD), plant height (PHT) and chlorophyll content (CC) under different organic matter levels and phytonematicides.

| | | FM | | DSM | | SD | | PHT | | CC | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | DF | MS | % | MS | % | MS | % | MS | % | MS | % |
| Replication | 3 | 4193.71 | 26 | 35.09 | 38 | 0.09 | 1 | 16.49 | 8 | 17.99 | 11 |
| Organic matter (O) | 6 | 5408.82 | $33^{ns}$ | 13.34 | $14^{ns}$ | 2.06 | $18^{ns}$ | 44.80 | $21^{*}$ | 43.52 | $26^{**}$ |
| Error | 18 | 2430.02 | 15 | 6.69 | 7 | 0.92 | 8 | 62.01 | 30 | 14.98 | 39 |
| Phytonematicide (P) | 1 | 1166.63 | $8^{ns}$ | 13.41 | $14^{ns}$ | 7.24 | $63^{**}$ | 2.93 | $1^{ns}$ | 65.79 | $40^{ns}$ |
| O×P | 6 | 1249.65 | $8^{ns}$ | 6.33 | $7^{ns}$ | 0.64 | $6^{ns}$ | 43.49 | $21^{ns}$ | 11.43 | $7^{ns}$ |
| Error | 21 | 1923.03 | 10 | 18.48 | 20 | 0.42 | 4 | 38.84 | 19 | 12.81 | 8 |
| Total | 55 | 16371.86 | 100 | 93.34 | 100 | 11.37 | 100 | 208.56 | 100 | 166.52 | 100 |

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

Table 10.9 Partitioning mean sum of squares for dry root mass under different organic matter levels, phytonematicides and depth.

| Source | DF | MS | % |
|---|---|---|---|
| Replication | 3 | 51.12 | 1 |
| Organic matter (O) | 6 | 31.09 | 1$^{ns}$ |
| Error | 18 | 28.99 | 1 |
| Phytonematicide (P) | 1 | 8.36 | 0 |
| O×P | 6 | 26.21 | 1$^{ns}$ |
| Error | 21 | 37.61 | 1 |
| Depth (D) | 3 | 4298.78 | 93$^{**}$ |
| O×D | 18 | 39.23 | 1$^{ns}$ |
| P×D | 3 | 2.40 | 0$^{ns}$ |
| O×P×D | 18 | 17.49 | 0$^{ns}$ |
| Error | 126 | 23.94 | 1 |
| Total | 223 | 4564.99 | 100 |

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

Table 10.10 Effect of depth on dry root mass under different organic matter, phytonematicide and depth trial.

| Depth (cm) | Dry root mass | |
|---|---|---|
| | Mean[y] | Rel. impact %[z] |
| 0–25 | 43.90a | – |
| 26–50 | 9.88b | –77 |
| 51–5 | 9.07b | –79 |
| 76–100 | 7.91b | –82 |

[y]Column means followed by the same letter were not different at P ≤ 0.05 according to Fisher's least significant difference.
[z]Relative impact = [(treatment/control) – 1] x 100.

10.3.3 Cucurbitacin residues in fruit

Cucurbitacin A and B residues in fruit of tomato plants protected against nematodes with Nemarioc-AL and Nemafric-BL phytonematicides were not detected (Figure 10.2, 10.3). The peaks for cucurbitacin A and B standards occurred at 21.003 and 35.257 minutes, respectively.

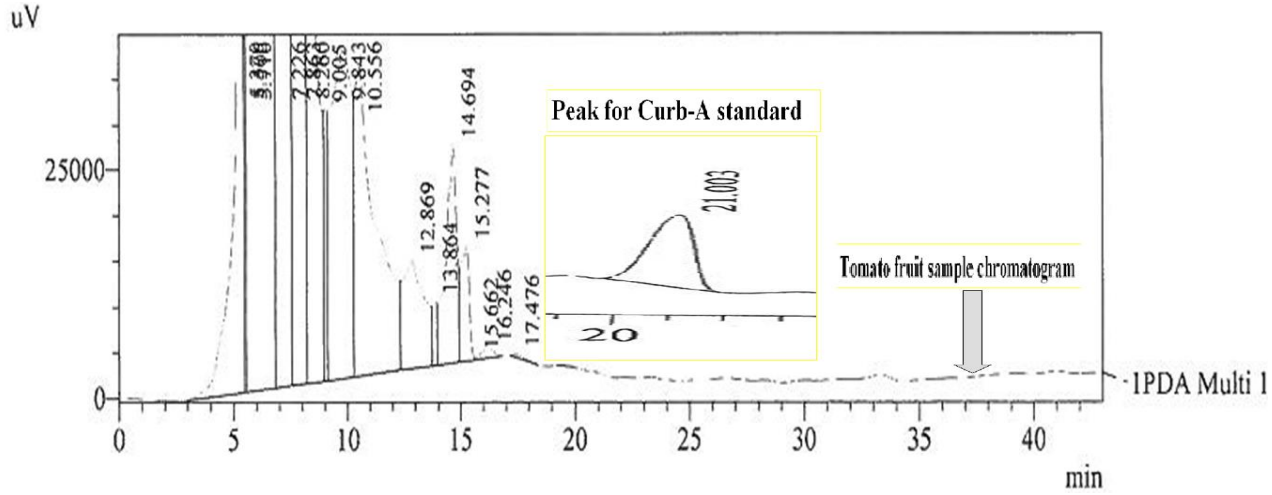


Figure 10.2 Chromatogram of tomato fruit sample exposed to Nemarioc-AL phytonematicide and that of cucurbitacin A standard.

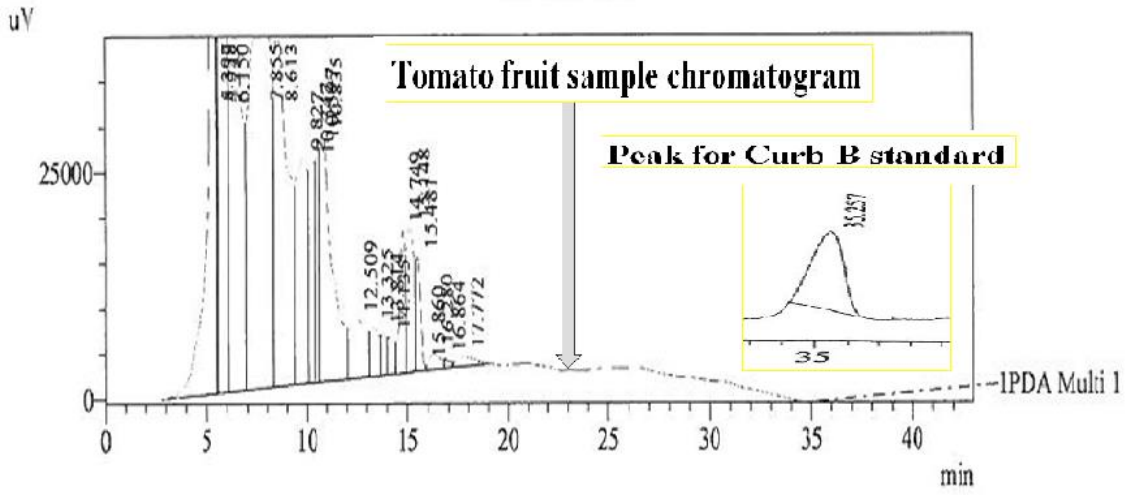


Figure 10.3 Chromatogram of tomato fruit sample exposed to Nemafric-BL phytonematicide and that of cucurbitacin B standard.

## 10.4 Discussion

### 10.4.1 Nematode variables

*Meloidogyne incognita* was an excellent bioindicator in response to the application of two phytonematicides as reported elsewhere (Mashela *et al*., 2015). In the interaction of soil type, phytonematicides and depth, the nematode population densities were inversely proportional to soil depth. This should not, however, be viewed as implying that more phytonematicides accumulated at the lower than in the upper depths. Higher nematode population densities in the upper soil depths might have been due to the fact that *Meloidogyne* species are obligate plant-parasitic nematodes (Davies, 2009). Generally, sedentary nematodes like *Meloidogyne* species occur where the highest root densities are situated, referred to as the effective root zone. In the current study, more than 62% tomato root systems occurred in the top 0–25 cm depth, confirming reports which showed that the highest effective root zones accumulate in the top 0–40 cm for most plant species (Cai *et al*., 2014; Jiang *et al*., 2013; Jiang *et al*., 2016; Song *et al*., 2003).

Another complicating factor was the fact that water, together with the dissolved solutes, first move laterally to wet the top layer of soil surface prior to percolating to the underlying soil layers (Anon., n.d.). Unlike under natural conditions, the walls of the pipe restricted the lateral flow of water, forcing water to move downwards faster than under normal conditions, resulting in phytonematicides occurring uniformly at all depth levels. The movement of both phytonematicides throughout the 1-m depth of the used pipe could therefore, not fully explain the movement of phytonematicides under field conditions.

A number of studies separately reported on the performance of soil type, phytonematicides and depth as they influenced nematode population densities (Ebrahimi *et al*., 2016; Hegazi, 2015; Mashela, 2016; Mashela *et al*., 2015). In the current study the factors were individually not significant except for depth, a contradiction with most other reports (Forge *et al*., 2016; Olabiyi *et al*., 2009; Pelinganga *et al*., 2013; Timper, 2014), but supported Koppenhofer and Fuzy (2006), who observed that soil type had no effect on nematodes. Olabiyi *et al*. (2009) observed that sandy soils harbour large population densities of plant-parasitic nematodes when compared with the finer textured soils. The difference was primarily explained on the basis of efficient aeration in sandy soil, fewer competitors/predators and the ability of nematodes to move with ease through the effective root zones in pores of coarse than in fine textured soils (Robinson, 2005; Wang and McSorley, 2005). In the current study, soil type alone and phytonematicide alone had no effect on nematode numbers, whereas the interactions had significant effects. The interaction of clay with any of the two phytonematicides reduced *M. incognita* population densities compared to sand and loam interactions.

The two phytonematicides significantly affected distribution of population densities of *M. incognita* across the tested soil types, with Nemafric-BL phytonematicide reducing population densities of *M. incognita* relative to Nemarioc-AL phytonematicides by a high magnitude. The disparity between the two phytonematicides on the efficacy of nematode suppression had been explained previously on the basis of the differences in active ingredient molecular structures (Mashela *et al*., 2015). The active ingredient of Nemarioc-AL phytonematicide, cucurbitacin A ($C_{32}H_{46}O_8$), is partially polar and soluble in water (Chen *et al*., 2005), whereas cucurbitacin B ($C_{32}H_{46}O_9$) in Nemafric-BL phytonematicide is non-polar and insoluble in water (Chen *et al*., 2005). On this basis, the active ingredient of Nemafric-BL phytonematicide tended to remain in the top layers of soil, where more roots accumulated, thereby reducing relatively higher population densities of *M. incognita* than did Nemarioc-AL phytonematicide which moved as solutes beyond the effective root zones.

The interactions between organic matter levels, phytonematicides and depth had no effect on the population densities of *M. incognita*. Also, when viewed alone organic matter levels had no effect on nematode population densities. The findings in the current study contradicted those where organic matter had effects on nematode numbers (Ebrahimi *et al*., 2016; Forge *et al*., 2016; Oka, 2010; Thoden *et al*., 2011). Ebrahimi *et al*. (2016) observed a reduction in the number of viable potato cyst nematodes, namely, *Globodera rostochiensis* Wollenweber and *G. pallida* Stone, under organic amendments. The suppressive nature of organic matter on nematode population densities was explained in terms of four mechanisms: (i) they improve the physical and chemical properties of the soil which might have adverse influence on hatching, mobility and survival of J2, (ii) release of nematicidal compounds by the

organic material, for example organic acids, phenolic compounds and ammonium, (iii) improvement of plant growth and (iv) the production of antibiotics or chitineses (Stirling, 2014). Although the credibility of organic matter in nematode suppression was castigated due to the inconsistent results in nematode suppression (McSorley, 2011), the riddle had since been resolved through the Curve-fitting Allelochemical Response Dosage (CARD) computer-based model (Liu et al., 2003; Mashela et al., 2015). In the current study, nematicidal compounds could not have any effects because mature organic materials were used and its known that activities of ammonium are short-lived in the soil (Tenuta and Lazarovits, 2002) and also composting transforms ammonium, volatile fatty acids and other compounds to more stable ones (Forge *et al*., 2016).

Liu *et al*. (2003) reported that when organisms are exposed to increasing concentrations of allelochemical they respond in a density-dependent growth (DDG) patterns, characterised by stimulation, no effect and inhibition (Mashela *et al*., 2015). Apparently, in the organic matter levels used in the current study, the concentrations of the produced allelochemicals might have been within the neutral phase of the DDG patterns. The argument is strengthened by observations from other studies that reported either no effect or stimulation of nematodes when organic amendments were used (Thoden *et al*., 2011). The significant effect of phytonematicides in the study supported a lot of observations that had been made when the two phytonematicides were used in the management of nematodes at the empirically-derived mean concentration stimulation point (Maile *et al*., 2013; Mashela *et al*., 2015; Pelinganga, 2013).

The major observation in the current study was that the two phytonematicides were able to reduce nematode population densities throughout the soil column in all four soil types and organic matter levels. This should, however, not be viewed as an indication that the phytonematicides were persistent enough to reduce the nematodes at all soil columns in all soil types and organic matter levels since repeated applications were carried out at every 17 days. Basically, the findings confirmed that the application interval of 17 days (Pelinganga, 2013) was suitable for various soil types and organic matter.

Studies on movement of pesticides have focused on synthetic pesticides, whereas phytonematicides as alternatives could be overlooked because they have been considered to be safe and less damaging to the environment (Romero-Gonzalez *et al*., 2015). Kumar and Poehling (2006) confirmed that neem product, NeemAzal, degraded rapidly in the soil environments as reported earlier by others (Barrek *et al*., 2004; Johnson *et al*., 2003; Scott and Kaushik, 2000). More work is required to substantiate the movement and distribution of Nemarioc-AL and Nemafric-BL phytonematicides in the soil under open-field agricultural systems.

### 10.4.2 Plant variables

The effect of depth observed on plant variables in the current study is well-documented and the phenomenon had been associated with all plants (Cai *et al*., 2014; Jiang *et al*., 2013; Jiang *et al*., 2016; Song *et al*., 2003). Machado and Oliveira (2005) reported that most of the tomato root system is found in the top 40 cm of the soil profile, whereas in the current study 67% effective roots accumulated in the top 25 cm depth. The 3% phytonematicide concentration used in this study was

developed for tomato as the mean concentration stimulation point — a concentration that stimulates plant growth, while at the same time suppresses nematode population densities (Pelinganga *et al*., 2013). In the current study, the stimulation of growth by the two phytonematicides was a confirmation of various observations in tomato production (Mashela *et al*., 2015). findings (Pelinganga, 2013).

### 10.4.3 Cucurbitacin residues in fruits

In the current study, cucurbitacin residues were not detected in all tomato fruit samples, which confirmed other similar studies under field conditions (Shadung, 2016). The complete absence of cucurbitacin residues in the current study contradicted with observations made in other studies where phytopesticides were used (Adnan *et al*., 2014, Akbar *et al*., 2010; Baig *et al*., 2009; Naqvi *et al*., 2007). Adnan *et al*. (2014) observed some chemical residues of azadirachtin in cabbage leaves a week after application even though it was at levels that were safe for consumption of the produce. Botanicals such as piperamines and alpha terthienyl readily degrades in the environment hours or days after application. The non-detection could also be due to low concentrations used and rapid rate of degradation associated with organic compounds (Arias-Estevez *et al*., 2008). Shadung (2016) suggested that the non-detectability of cucurbitacins in the fruit of tomato plants could also be due to the non-polar nature of cucurbitacins. Generally, non-polar molecules cannot be translocated through the symplastic pathways in the endodermis of the root systems (Shadung, 2016).

## 10.5 Conclusion

The efficacy of Nemarioc-AL and Nemafric-BL phytonematicides in suppression of nematode population densities is a function of soil type, organic matter level, soil depth, the effective root zone and the phytonematicide type. In the current study, *M. incognita* served as a strong bioindicator of the movement and distribution of active ingredients of phytonematicides under different soil types. The non-detection of cucurbitacin residues in tomato fruit observed in this trial was important. An ideal pesticide should be highly specific and not cause any adverse effects on non-target organisms. It should also be biodegradable and have no residues in the produce. Nemarioc-AL and Nemafric-BL phytonematicides have the characteristics of ideal pesticides, hence they can be incorporated into management strategies of nematodes in cropping systems. More work still needs to be done to completely understand the movement of the two phytonematicides under open-field conditions.

## 10.6 References

ADNAN, M., BIBI, R., MUSSARAT, S., TARIQ, A. and Z.K. SHINWARI. 2014. Ethnomedicinal and phytochemical review of Pakistani medicinal plants used as antibacterial agents against *Escherichia coli*. *Annals of Clinical Microbiology and Antimicrobials* 13:1.

AKBAR, M.F., HAQ, M.A., PARVEEN, F., YASMIN, N. and S.A. SAYEED. 2010. Determination of synthetic and bio-insecticides residues during aphid, *Myzus persicae* (Sulzer) control on cabbage crop through high performance liquid chromatography. *Pakistan Entomologist* 32:155–162.

ANON., n.d. Irrigation management. Plant and Science eLibrary. Retrieved from htt://croptechnology.ul.edu.

ARIAS-ESTEVEZ, M., PERIAGO, J.E., MARTINEZ-CARBALLO, E. and A. GARCIA-RIO. 2008. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. *Agriculture Ecosystems and Environment* 123:247–260.

BAIG, S.A., AKHTER, N.A., ASHFAQ, M. and M.R. ASI. 2009. Determination of the organophosphorus insecticide in vegetables by high-performance liquid chromatography. *American-Eurasian Journal of Agricultural & Environmental* 6:513–519.

BARREK, S., PAISSE, O. and M.F. GRENIER-LOUSTALOT. 2004. Analysis of neem oils by LC-MS and degradation kinetics of azadirachtin-A in a controlled environment – Characterization of degradation products by HPLC-MS-MS. *Analytical and Bioanalytical Chemistry* 378:753–763.

CAI, H., MA, W., ZHANG, X., PING, J., YAN, X., LIU, J., YUAN, J., WANG, L. and J. REN. 2014. Effect of subsoil tillage depth on nutrient accumulation, root distribution, and grain yield in spring maize. *The Crop Journal* 2:297–307.

CARSON, R. 1962. Silent Spring. Houghton Mifflin, Boston.

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

DAVIES, K. 2009. Understanding the interaction between an obligate hyperparasitic bacterium, *Pasteuria penetrans* and its obligate plant-parasitic nematode host, *Meloidogyne* spp. *Advances in Parasitology* 68:211–245.

EBRAHIMI, N., VIAENE, N., VANDECASTEELE, B., D'HOSE, T., DEBODE, J., CREMELIE, P., DE TENDER, C.A. and M. MOENS. 2016. Traditional and

new soil amendments reduce survival and reproduction of potato cyst nematodes, except for biochar. *Applied Soil Ecology* 107:191–204.

FORGE, T., KENNEY, E., HASHIMOTO, N., NEILSEN, D. and B. ZEBARTH. 2016. Compost and poultry manure as preplant soil amendments for red raspberry: Comparative effects on root lesion nematodes, soil quality and risk of nitrate leaching. *Agriculture, Ecosystems and Environment* 223:48–58.

GOMEZ, K.A. and A.A. GOMEZ. 1984. Statistical Procedures for Agricultural Research. Wiley, New York.

GUTIERREZ, C., FERNANDEZ, C., ESCUER, M., CAMPOS-HERRERA, R., RODRÍGUEZ, M.E.B., CARBONELL, G. and J.A. RODRÍGUEZ-MARTÍN. 2016. Effect of soil properties, heavy metals and emerging contaminants in the soil nematodes diversity. *Environmental Pollution* 213:184–194.

HEGAZI, A.M. 2015. Influence of soil type, sowing date and diluted seawater irrigation on seed germination, vegetation and chemical constitution of *Moringa oleifera*. *Journal of Agricultural Science* 7:138–147.

HOSS, S., CLAUS, E., VON DER OHE, P.C., BRINKE, M., GUDE, H., HEININGER, P. and W. TRAUNSPURGER. 2011. Nematode species at risk – a metric to assess pollution in soft sediments of freshwaters. *Environment International* 37:940–949.

HUSSEY, R.S. and K.R. BARKER. 1973. A comparison of methods of collecting inocula of *Meloidogyne* species, including a new technique. *Plant Disease Reporter* 42:865–872.

JENKINS, W.R. 1964. A rapid centrifugal-floatation technique for separating nematodes from soil. *Plant Disease Reporter* 48:692.

JIANG, W., WANG, K., WU, Q., DONG, S., LIU, P. and J. ZHANG. 2013. Effects of narrow plant spacing on root distribution and physiological nitrogen use efficiency in summer maize. *The Crop Journal* 1:77–83.

JIANG, C.H., BAI, Y., DU, H., HU, Y., RAO, Y., CHEN, C. and Y. CAI. 2016. The spatial and seasonal variation characteristics of fine roots indifferent plant configuration modes in new reclamation saline soil of humid climate in China. *Ecological Engineering* 86:231–238.

JOHNSON, S., DUREJA, P. and S. DHINGRA. 2003. Photostabilizers for Azadirachtin-A (A Neem-Based Pesticide). J*ournal of Environmental Science and Health Part B* 38:451–562.

KOPPENHOFER, A.M. and E.M. FUZY. 2006. Effect of soil type on infectivity and persistence of the entomopathogenic nematodes *Steinernema scarabaei*, *Steinernema glaseri*, *Heterorhabditis zealandica*, and *Heterorhabditis bacteriophora*. *Journal of Invertebrate Pathology* 92:11–22.

KREUTZWEISER, D.P., CAPELL, S.S. and T.A. SCARR. 2000. Community-level responses by stream insects to neem products containing azadirachtin. *Environmental Toxicology and Chemistry* 19:855–861.

KUMAR, P. and H.M. POEHLING. 2006. Persistence of soil and foliar azadirachtin treatments to control sweet potatoe whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) on tomatoes under controlled (laboratory) and field (netted greenhouse) conditions in the humid tropics. *Journal of Pesticide Science* 79:189–199.

LIU, D.L., AN, M., JOHNSON, I.R. and J.V. LOVETT. 2003. Mathematical modeling of allellopathy. III. A model for curve-fitting allellochemical dose responses. *Non-linearity Biology, Toxicology and Medicine* 1:37–50.

MACHADO, R.M.A. and M.R.G. OLIVEIRA. 2005. Tomato root distribution, yield and fruit quality under different subsurface drip irrigation regimes and depth. *Irrigation Science* 24:15–24.

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

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

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

MASHELA, P.W. 2016. Growth of *Moringa oleifera* on calcareous, clay and sandy soils relative to loam soil. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science* (in press).

MASHELA, P.W. and Z.P. DUBE. 2014. Soil allelochemical residue effects of Nemafric-BL and Nemarioc-AL phytonematicides on soil health, growth of sweet sorghum and Meloidogyne species. *Acta Agriculturae Scandinavica, Section B-Soil and Plant Science* 64:79–84.

MASHELA, P.W., DUBE, Z.P. and K.M. POFU. 2015. Managing the Phytotoxicity and Inconsistent Nematode Suppression in Soil Amended with Phytonematicides. In: Meghvansi, M.K. and A. Vorma (eds.). Organic Amendments and Soil Suppressiveness in Plant Disease Management, Soil Biology 46. Springer International Publishers, Switzerland.

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

McKENRY, M. 1994. Nematicides. *Encyclopedia of Agricultural Science* 3:87–95.

McSORLEY, R. 2011. Overview of organic amendments for management of plant-parasitic nematodes, with case studies from Florida. *Journal of Nematology* 43:69–81.

NAQVI, S.N.H., TABASSUM, R., KHAN, M.F., YASMIN, N., NURULAIN, S.M. and A.A. BURNEY. 2007. Toxic, residual, and teratomorphic effect of a neem extract (N-9) in comparison to Coopex 25 WP (Permethrin + Bioallethrin) against *Musca domestica* L. (Holland Strain). *Turkish Journal of Zoology* 31: 127–130.

OKA, Y. 2010. Mechanisms of nematode suppression by organic soil amendments–A review. *Applied Soil Ecology* 44:101–115.

OLABIYI, T.I., OLAYIWOLA, A.O. and G.O. OYEDIRAN. 2009. Influence of soil textures on distribution of phytonematodes in the South Western Nigeria. *World Journal of Agricultural Sciences* 5:557–560.

PARK, B.Y., LEE, J.K., RO, H.M. and Y.H. KIM. 2011. Effects of heavy metal contamination from an abandoned mine on nematode community structure as an indicator of soil ecosystem health. *Applied Soil Ecology* 51:17–24.

PELINGANGA, O.M. 2013. Developing Bio-nematicides Using Indigenous *Cucumis africanus* and *Cucumis myriocarpus* Fruits for Tomato Production System. PhD Thesis, University of Limpopo. Sovenga, South Africa.

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

PUNZO, F. and M. PARKER. 2005. Effects of azadirachtin on mortality, fertilization, and swimming speed in larvae of the cane toad, *Bufo marinus* (Anura: Bufonidae). *Journal of Environmental Biology* 26:687–691.

ROBINSON, E. 2005. Soil type guides VR nematodes applications. Farm press (http:// www.deltafarmpress.com/mag/farming_soil_type_guides/ index.html).

RODRÍGUEZ-MARTÍN, J.A., GUTIERREZ, C., ESCUER, M., GARCÍA-GONZALEZ, M.T., CAMPOS-HERRERA, R. and N. AGUILA. 2014. Effect of mine tailing on the spatial variability of soil nematodes from lead pollution in La Union (Spain). *Science of the Total Environment* 4:518–529.

ROMERO-GONZALEZ, R., FRENICH, A.G. and J.L. MARTINEZ-VIDAL. 2015. Biopesticide Residues in Soil. In: Nollet, L.M.L. and H.S. Rathone (eds.). Biopesticide Handbook. Taylor and Francis Group, Florida.

SCOTT, M. and N.K. KAUSHIK. 2000. The toxicity of a neem insecticide to populations of Culicidae and other aquatic invertebrates as assessed in in situ microcosms. *Archives of Environmental Contamination and Toxicology* 39: 329–336.

SHADUNG, K.G. 2016. Quality Protocols for Nemarioc-AL and Nemafric-BL phytonematicides and their Respective Chemical Residues in Tomato Fruits. PhD Thesis, University of Limpopo. Sovenga, South Africa.

SOCHOVA, I., HOFMAN, J. and I. HOLOUBEK. 2006. Using nematodes in soil ecotoxicology. *Environment International* 32:374–383.

SONG, R., WU, C.S., MA, Y.L. GUO, J.X. and F. XING. 2013. Comparison of roots distribution in different maize plant type cultivars in the Songnen Plain. *Chinese Journal of Applied Ecology* 11:1911–1913.

SOOMRO, A.M., SEEHAR, G.M., BHANGAR, M.I. and N.A. CHANNA. 2008. Pesticides in the blood samples of spray-workers at agriculture environment: The toxicological evaluation. *Pakistan Journal of Analytical & Environmental Chemistry* 9:32–37.

STIRLING, G.R. 2014. Biological Control of Plant Parasitic Nematodes: Soil Ecosystem Management in Sustainable Agriculture. CAB International, Wallingford.

TENUTA, M. and G. LAZAROVITS. 2002. Ammonia and nitrous acid from nitrogenous amendments kill the microsclerotia of *Verticillium dahliae*. *Phytopathology* 92:255–264.

THODEN, T.C., KORTHALS, G. and A. TERMORSHUIZEN. 2011. Organic amendments and their influences on plant-parasitic and free-living nematodes: A promising method for nematode management. *Nematology* 13:133–153.

TIMPER, P. 2014. Conserving and enhancing biological control of nematodes. *Journal of Nematology* 46:75–89.

WANG, K.H. and R. McSORLEY, 2005. Effects of soil ecosystem management on nematode pests, nutrient cycling and plant health. *APS net* 1-16.

ZARINS, I., DAUGAVIETIS, M. and J. HALIMONA. 2009. Biological activity of plant extracts and their application as ecologically harmless biopesticide. *Sodininkystė ir daržininkystė* 28:269–280.

# CHAPTER 11
# SUMMARY, SIGNIFICANCE OF FINDINGS, FUTURE RESEARCH AND CONCLUSIONS

## 11.1 Summary

The study investigated the influence of Nemarioc-AL and Nemafric-BL phytonematicides, along with their pure active ingredients, on their mode of action on second-stage juvenile (J2) hatch, J2 mobility and J2 mortality. The J2 hatch over increasing concentrations of Nemarioc-AL phytonematicide and its active ingredient, pure cucurbitacin A, had a density-dependent growth (DDG) patterns for the relative impact values and those generated through the Curve-fitting Allelochemical Response Dosage (CARD) model. The cucurbitacin A concentrations displayed all the three phases of the DDG pattern, namely, stimulation, neutral and inhibition phases. At low cucurbitacin A concentrations, J2 hatch inhibition was at a stimulation phase of the DDG pattern, followed by the neutral and then the inhibition phases as concentrations increased. In contrast, under increasing concentration of Nemarioc-AL phytonematicide, J2 hatch responded through the neutral and inhibition phases at low and high concentrations, respectively, without the stimulation responses. The J2 hatch inhibition was highly sensitive to Nemarioc-AL phytonematicide and moderately sensitive to pure cucurbitacin A, with the overall sensitivity values of 1 and 30 units, respectively.

Using the overall sensitivity values to denote the toxicity of the products, Nemarioc-AL phytonematicide was highly toxic to J2 hatch when compared with pure cucurbitacin A. This supported other observations which showed that in purified formulations (Ntalli and Caboni 2012; Oka 2010; Okwute, 2012; Wuyts *et al*. 2006), most phytonematicides lose some of their potency in nematode suppression. The J2

hatch inhibition concentration ($EHIC_{50}$, $EHIC_{100}$) and the CARD-generated 50 and 100% inhibition values ($D_{50}$, $D_{100}$) were not comparable for pure cucurbitacin A, but were comparable for Nemarioc-AL phytonematicide. The J2 hatch inhibition effects for pure cucurbitacin A and Nemarioc-AL phytonematicide were irreversible, an indication that a common mode of action might be involved. The J2 hatch inhibition over increasing concentrations of pure cucurbitacin B and Nemafric-BL phytonematicide had DDG patterns for relative impact values and those generated through the CARD model.

*Meloidogyne incognita* J2 hatch displayed all three DDG patterns when exposed to pure cucurbitacin B concentrations. At low cucurbitacin B concentrations J2 hatch inhibition was at a stimulation phase of DDG pattern, followed by neutral and then inhibition phases as concentrations increased, whereas Nemarioc-AL phytonematicide demonstrated only neutral and inhibition phases at low and high concentrations, respectively. The J2 hatch $EHIC_{50}$ and $EHIC_{100}$ and the CARD-generated $D_{50}$ and $D_{100}$ values were not comparable for pure cucurbitacin B, but were comparable for Nemafric-BL phytonematicide. The J2 hatch inhibition was highly sensitive to pure cucurbitacin B and Nemafric-BL phytonematicide, with overall sensitivity values of 2 and 5, respectively. The J2 hatch inhibition effects of pure cucurbitacin B and Nemafric-BL phytonematicide were each irreversible.

The J2 immobility over increasing concentrations of pure cucurbitacin A and Nemarioc-AL phytonematicide had DDG patterns, with similar trends for both materials. Also, similar trends were observed when relative impact values were compared with those generated through the CARD model. At low pure cucurbitacin A

and Nemarioc-AL phytonematicide concentrations, J2 immobility was at stimulation phase of the DDG patterns, whereas high concentrations resulted in neutral phase responses. The CARD model could, however, not generate the $D_{50}$ and $D_{100}$ values for both products. The J2 immobility was highly sensitive to Nemarioc-AL phytonematicide when compared with pure cucurbitacin A, with overall sensitivity values of 7 and 16, respectively. The J2 immobility effects of pure cucurbitacin A and Nemarioc-AL phytonematicide were each irreversible.

Juvenile immobility over increasing concentrations of pure cucurbitacin B and Nemafric-BL phytonematicide exhibited the DDG patterns, which were also similar for both products, with low concentrations stimulating J2 immobility, whereas high concentrations were neutral at all exposure periods. The trends were also similar between the relative impact values and those generated through the CARD model. The toxicity values as measured by the CARD-generated sensitivity biological index were higher for Nemafric-BL phytonematicide than those of pure cucurbitacin B, with overall sensitivity values of 2 and 16 units, respectively. The J2 immobility concentrations and the CARD-generated biological indices $D_{50}$ and $D_{100}$ were not comparable for pure cucurbitacin B, but were comparable for Nemafric-BL phytonematicide. The J2 immobility effects of pure cucurbitacin B and Nemafric-BL phytonematicide were also irreversible.

*Meloidogyne incognita* J2 mortality over increasing concentrations of pure cucurbitacin A and Nemarioc-AL phytonematicide had similar DDG patterns for relative impact values and those generated through the CARD model. At low concentrations J2 mortality was at stimulation phase and tended towards neutrality

at higher concentrations. The computed J2 mortality inhibition concentrations at $LC_{50}$ and $LC_{100}$ and the CARD-generated at $D_{50}$ and $D_{100}$, respectively, were not comparable for pure cucurbitacin A, but were comparable for Nemarioc-AL phytonematicide. The toxicity of the two products on J2 mortality was high for Nemarioc-AL phytonematicide and pure cucurbitacin A, with the overall sensitivity values being at 2 and 4, respectively. The J2 mortality effects of pure cucurbitacin A and Nemarioc-AL phytonematicide were irreversible.

*Meloidogyne incognita* J2 mortality over increasing concentrations of pure cucurbitacin B and Nemafric-BL phytonematicide had similar DDG patterns for relative impact values and those generated using the CARD model. The J2 mortality for pure cucurbitacin B and Nemafric-BL phytonematicide concentrations had stimulation and neutral responses at low and high concentrations. The computed J2 mortality $LC_{50}$ and $LC_{100}$ and the CARD-generated $D_{50}$ and $D_{100}$ values were not comparable for pure cucurbitacin B and Nemafric-BL phytonematicide. The toxicity of pure cucurbitacin B and that of Nemafric-BL phytonematicide were at 4 and 1 units, respectively, which implied that the toxicity of Nemafric-BL phytonematicide in inducing mortality to J2 was higher than that of pure cucurbitacin B. The J2 mortality effects of the two materials were not reversible.

*Meloidogyne incognita* J2 infectivity over increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides had similar DDG patterns for relative impact values and those generated through the CARD model. At low phytonematicide concentrations, J2 infectivity inhibitions were at stimulation phase of the DDG patterns and it became neutral at higher concentrations. The computed J2 infectivity

inhibition values and the CARD-generated $D_{50}$ and $D_{100}$ values were comparable for Nemarioc-AL phytonematicide, but not for Nemafric-BL phytonematicide. The CARD model showed that J2 infectivity post-exposure to Nemarioc-AL and Nemafric-BL phytonematicides were highly sensitive to the residual effects.

The interactions experiments inside the pipes provided important information with respect to the use of *Meloidogyne* species as bioindicators to the two phytonematicides. Most of the nematodes accumulated in the effective root zones which were restricted to the top 25-cm depth. Within this zone, Nemafric-BL phytonematicides was more effective in reducing nematode population densities than Nemafric-BL phytonematicides due to its insolubility in water. The organic matter used in a separate interaction had no effects on nematode population densities, probably due to its maturation.

## 11.2 Significance of findings

The findings in the study demonstrated that in purified form the phytonematicides were still active, but less effective than the crude extracts, in suppression of various stages of *M. incognita*. In both pure and crude extract phytonematicides, the mode of action was complete paralysis at the plant-stimulating concentrations (3%) recommended for tomato plants under field conditions, but was highly concentration- and exposure period-dependent. The major finding of this study was the demonstration that for the two phytonematicides, the concentrations that stimulated plant growth coincided with concentrations that inhibited various nematode stages (Figure 11.1). The absence of phytonematicide residues in tomato fruit is also valuable in dissipating any health concerns that might be raised when the products

are recommended for use and declaring the phytonematicides safe for use even in smallscale farming communities.

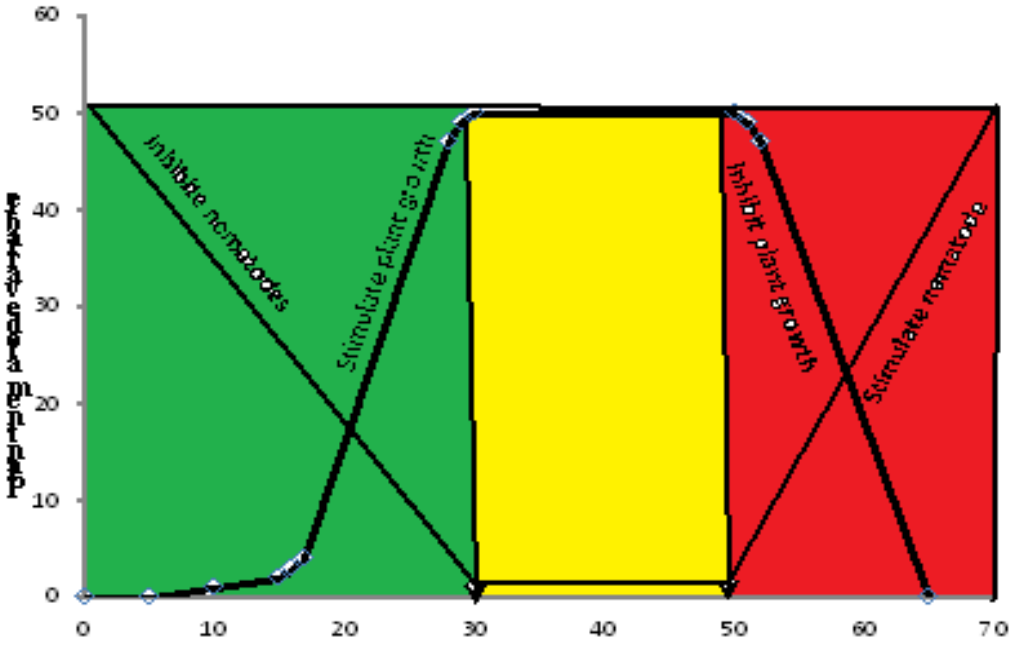


Figure 11.1 Nematode density-dependent growth (DDG) graph superimposed on the plant variable DDG graph.

## 11.3 Future research

Firstly, the two phytonematicides have been shown to induce paralysis in *M. incognita* as a function of concentration and exposure period. However, the current findings were not intended to demonstrate the potential damage of the two phytonematicides on nematodes at the cellular level. The latter could be important in understanding how the products induce damage on nematodes as part of mode of action at molecular level. Secondly, since the current study focused on bioactivity of the two phytonematicides on one nematodes species, it would be necessary to expand the mode of action testing to other economically important plant-parasitic

nematodes in order to broaden the scope of knowledge in this area. Thirdly, since the two phytonematicides are applied in the soil, their impact on other soil-borne organisms, especially other biocontrol agents of nematodes such as *Pasteuria penetrans* and *Trichoderma harzianum*, would be important in the determination of the compatibility of the products with other strategies used in integrated pest management (IPM) programmes. Fourthly, the movement and persistence of the active ingredients from the two phytonematicides should be investigated in detail under different field conditions. Fifthly and finally, necessary toxicology studies should be undertaken in order to finalise the achieving the requirements for registration of the two phytonematicides as articulated in Act Number 36 of 1947 as amended in 2012.

## 11.4 Conclusions

Nemarioc-AL and Nemafric-BL phytonematicides induced DDG patterns on J2 hatch, immobility and mortality, indicating that the control of nematodes by the two phytonematicides is concentration- and exposure period-specific. Also, as shown by high toxicity biological indices against different stages of *M. incognita*, the phytonematicides can be used at very low concentrations in the management of nematodes which is important in addressing the potential environment–unfriendly concerns. The CARD model, used in this study, generated important biological indices for the two phytonematicides, which might form an important set of information in research and development of phytonematicides. Hence, the tool was amenable for use under both *in vitro* and *ex vitro* conditions in phytonematicide trials. *Meloidogyne incognita* served as a strong bioindicator of the movement of active ingredients of phytonematicides under different soil types. Cucurbitacin residues

were not detected in all tomato fruit samples. Results in the current study have demonstrated that Nemarioc-AL and Nemafric-BL phytonematicides have the potential for use as commercial products in the management of *Meloidogyne* species in various cropping systems with no health concerns.

## 11.5 References


NTALLI, N.G. and P. CABONI. 2012. Botanical nematicides: A review. *Journal of Agricultural and Food Chemistry* 60:9929–9940.

OKA, Y. 2010. Mechanisms of nematode suppression by organic soil amendments–A review. *Applied Soil Ecology* 44:101–115.

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

WUYTS, N., SWENNEN, R. and D. DE WAELE. 2006. Effects of plant phenylpropanoid pathway products and selected terpenoids and alkaloids on the behavior of the plant-parasitic nematodes *Radopholus similis*, *Pratylenchus penetrans* and *Meloidogyne incognita. Nematology* 8:89–101.

# APPENDICES

Appendix 3.1 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile hatch in cucurbitacin A at 24-h exposure period.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 11.1033 | 1.00939 | 2.05 | 0.0318 |
| Error | 96 | 47.3136 | 0.49285 | | |
| Total | 107 | 58.4168 | | | |

Appendix 3.2 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile hatch in pure cucurbitacin A at 48-h exposure period.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 13.1338 | 1.19398 | 1.94 | 0.0431 |
| Error | 96 | 59.0289 | 0.61488 | | |
| Total | 107 | 72.1627 | | | |

Appendix 3.3 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile hatch in pure cucurbitacin A at 72-h exposure period.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 12.6856 | 1.15324 | 1.54 | 0.0129 |
| Error | 96 | 71.7800 | 0.74771 | | |
| Total | 107 | 84.4656 | | | |

Appendix 3.4 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile hatch in pure cucurbitacin A at 7-d exposure period.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 2.7688 | 0.25171 | 1.00 | 0.4530 |
| Error | 96 | 24.1831 | 0.25191 | | |
| Total | 107 | 26.9519 | | | |

Appendix 3.5 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile hatch in pure cucurbitacin A at 10-d exposure period.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 2.4768 | 0.22516 | 0.98 | 0.4656 |
| Error | 96 | 21.9471 | 0.22862 | | |
| Total | 107 | 24.4238 | | | |

Appendix 3.6 Analysis of variance (ANOVA) for reversal of *Meloidogyne incognita* second-stage juvenile hatch inhibition after removal of pure cucurbitacin A effect.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 1.7337 | 0.15761 | 0.74 | 0.6962 |
| Error | 96 | 20.4046 | 0.21255 | | |
| Total | 107 | 22.1383 | | | |

Appendix 3.7 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile hatch inhibition at 24-h exposure period to pure cucurbitacin B.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 5.81966 | 0.52906 | 2.32 | 0.0232 |
| Error | 96 | 21.88032 | 0.22792 | | |
| Total | 107 | 27.69998 | | | |

Appendix 3.8 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile hatch inhibition at 48-h exposure period to pure cucurbitacin B.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 6.19124 | 0.56284 | 2.06 | 0.0442 |
| Error | 96 | 26.26368 | 0.27358 | | |
| Total | 107 | 32.45492 | | | |

Appendix 3.9 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile hatch inhibition at 72-h exposure period to pure cucurbitacin B.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 6.55171 | 0.59561 | 1.98 | 0.0528 |
| Error | 96 | 28.82016 | 0.30021 | | |
| Total | 107 | 35.37187 | | | |

Appendix 3.10 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile hatch inhibition at 7-d exposure period to pure cucurbitacin B.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 11.34991 | 1.03181 | 2.46 | 0.0165 |
| Error | 96 | 40.27680 | 0.41955 | | |
| Total | 107 | 51.62671 | | | |

Appendix 3.11 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile hatch inhibition at 10-d exposure period to pure cucurbitacin B.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 6.30773 | 0.57343 | 1.98 | 0.0527 |
| Error | 96 | 27.73824 | 0.28894 | | |
| Total | 107 | 34.04597 | | | |

Appendix 3.12 Analysis of variance (ANOVA) for reversal of *Meloidogyne incognita* second-stage juvenile hatch inhibition after removal of pure cucurbitacin B effect.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 3.6478 | 0.33162 | 0.62 | 0.8113 |
| Error | 96 | 51.6986 | 0.53853 | | |
| Total | 107 | 55.3464 | | | |

Appendix 4.1 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile hatch inhibition at 24-h exposure period to Nemarioc-AL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 0.86884 | 0.07899 | 1.53 | 0.1325 |
| Error | 96 | 4.94848 | 0.05155 | | |
| Total | 107 | 5.81733 | | | |

Appendix 4.2 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile hatch inhibition at 48-h exposure period to Nemarioc-AL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 5.7099 | 0.51908 | 7.98 | 0.0000 |
| Error | 96 | 6.2480 | 0.06508 | | |
| Total | 107 | 11.9579 | | | |

Appendix 4.3 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile hatch inhibition at 72-h exposure period to Nemarioc-AL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 8.7829 | 0.79844 | 7.36 | 0.0000 |
| Error | 96 | 10.4150 | 0.10849 | | |
| Total | 107 | 19.1978 | | | |

Appendix 4.4 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile hatch inhibition at 7-d exposure period to Nemarioc-AL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 3.6908 | 0.33552 | 3.04 | 0.0016 |
| Error | 96 | 10.3744 | 0.11037 | | |
| Total | 107 | 14.0652 | | | |

Appendix 4.5 Analysis of variance (ANOVA) for reversal of *Meloidogyne incognita* second-stage juvenile hatch inhibition after exposure to Nemarioc-AL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 5.1536 | 0.46851 | 0.79 | 0.6534 |
| Error | 96 | 57.2280 | 0.59612 | | |
| Total | 107 | 62.3815 | | | |

Appendix 4.6 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile hatch inhibition at 24-h exposure period to Nemafric-BL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 0.27901 | 0.02536 | 0.38 | 0.9624 |
| Error | 96 | 6.47257 | 0.06742 | | |
| Total | 107 | 6.75159 | | | |

Appendix 4.7 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile hatch inhibition at 48-h exposure period to Nemafric-BL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 2.39596 | 0.21781 | 4.16 | 0.0001 |
| Error | 96 | 5.02702 | 0.05236 | | |
| Total | 107 | 7.42298 | | | |

Appendix 4.8 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile hatch inhibition at 72-h exposure period to Nemafric-BL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 6.8007 | 0.61824 | 15.18 | 0.0000 |
| Error | 96 | 3.9108 | 0.04074 | | |
| Total | 107 | 10.7115 | | | |

Appendix 4.9 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile hatch inhibition at 7-d exposure period to Nemafric-BL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 9.8440 | 0.89491 | 15.87 | 0.0000 |
| Error | 96 | 5.4145 | 0.05640 | | |
| Total | 107 | 15.2585 | | | |

Appendix 4.10 Analysis of variance (ANOVA) for reversal of *Meloidogyne incognita* second-stage juvenile hatch inhibition after exposure to Nemafric-BL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 2.7135 | 0.24668 | 0.93 | 0.5125 |
| Error | 96 | 25.3778 | 0.26435 | | |
| Total | 107 | 28.0912 | | | |

Appendix 5.1 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile immobility at 12-h exposure period to pure cucurbitacin A.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 34.7979 | 3.16345 | 239.02 | 0.0000 |
| Error | 96 | 1.2706 | 0.01323 | | |
| Total | 107 | 36.0700 | | | |

Appendix 5.2 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile immobility at 24-h exposure period to pure cucurbitacin A.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 33.1543 | 3.01403 | 266.02 | 0.0000 |
| Error | 96 | 1.0877 | 0.01133 | | |
| Total | 107 | 34.2420 | | | |

Appendix 5.3 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile immobility at 48-h exposure period to pure cucurbitacin A.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 37.0310 | 3.36646 | 214.40 | 0.0000 |
| Error | 96 | 1.5074 | 0.01570 | | |
| Total | 107 | 38.5384 | | | |

Appendix 5.4 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile immobility at 72-h exposure period to pure cucurbitacin A.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 35.1693 | 3.19721 | 212.04 | 0.0000 |
| Error | 96 | 1.4475 | 0.01508 | | |
| Total | 107 | 36.6168 | | | |

Appendix 5.5 Analysis of variance (ANOVA) of reversal of *Meloidogyne incognita* second-stage juvenile immobility to pure cucurbitacin A.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 3.6478 | 0.33162 | 0.98 | 0.8113 |
| Error | 96 | 32.4768 | 0.33853 | | |
| Total | 107 | 36.1246 | | | |

Appendix 5.6 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile immobility at 12-h exposure period to pure cucurbitacin B.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 38.1751 | 3.47047 | 188.32 | 0.0000 |
| Error | 96 | 1.7692 | 0.01843 | | |
| Total | 107 | 39.9443 | | | |

Appendix 5.7 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile immobility at 24-h exposure period to pure cucurbitacin B.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 37.4710 | 3.40645 | 222.94 | 0.0000 |
| Error | 96 | 1.4669 | 0.01528 | | |
| Total | 107 | 38.9378 | | | |

Appendix 5.8 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile immobility at 48-h exposure period to pure cucurbitacin B.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 1.08812 | 0.09892 | 145.34 | 0.0000 |
| Error | 96 | 0.06534 | 0.00068 | | |
| Total | 107 | 1.15346 | | | |

Appendix 5.9 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile immobility at 72-h exposure period to pure cucurbitacin B.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 33.6567 | 3.05970 | 137.50 | 0.0000 |
| Error | 96 | 2.1363 | 0.02225 | | |
| Total | 107 | 35.7929 | | | |

Appendix 5.10 Analysis of variance (ANOVA) for reversal of *Meloidogyne incognita* second-stage juvenile immobility after exposure to pure cucurbitacin B.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 4.5494 | 0.41359 | 0.74 | 0.7000 |
| Error | 96 | 53.8283 | 0.56071 | | |
| Total | 107 | 58.3777 | | | |

Appendix 6.1 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile immobility at 12-h exposure period to Nemarioc-AL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 23.5705 | 2.14278 | 31.53 | 0.0000 |
| Error | 96 | 6.5234 | 0.06795 | | |
| Total | 107 | 30.0940 | | | |

Appendix 6.2 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile immobility at 24-h exposure period to Nemarioc-AL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 26.5044 | 2.40949 | 44.04 | 0.0000 |
| Error | 96 | 5.2521 | 0.05471 | | |
| Total | 107 | 31.7565 | | | |

Appendix 6.3 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile immobility at 48-h exposure period to Nemarioc-AL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 28.6122 | 2.60111 | 47.57 | 0.0000 |
| Error | 96 | 5.2487 | 0.05467 | | |
| Total | 107 | 33.8609 | | | |

Appendix 6.4 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile immobility at 72-h exposure period to Nemarioc-AL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 32.5869 | 2.96244 | 69.94 | 0.0000 |
| Error | 96 | 4.0662 | 0.04236 | | |
| Total | 107 | 36.6531 | | | |

Appendix 6.5 Analysis of variance (ANOVA) for reversal of *Meloidogyne incognita* second-stage juvenile immobility after exposure to Nemarioc-AL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 4.0392 | 0.36720 | 1.02 | 0.4308 |
| Error | 96 | 34.3932 | 0.35826 | | |
| Total | 107 | 38.4324 | | | |

Appendix 6.6 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile immobility at 12-h exposure period to Nemafric-BL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 0.68944 | 0.06268 | 126.99 | 0.0000 |
| Error | 96 | 0.04738 | 0.00049 | | |
| Total | 107 | 0.73682 | | | |

Appendix 6.7 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile immobility at 24-h exposure period to Nemafric-BL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 38.6374 | 3.51249 | 88.92 | 0.0000 |
| Error | 96 | 3.7922 | 0.03950 | | |
| Total | 107 | 42.4296 | | | |

Appendix 6.8 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile immobility at 48-h exposure period to Nemafric-BL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 33.8647 | 3.07861 | 236.04 | 0.0000 |
| Error | 96 | 1.2521 | 0.01304 | | |
| Total | 107 | 35.1168 | | | |

Appendix 6.9 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile immobility at 72-h exposure period to Nemafric-BL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 32.7644 | 2.97858 | 254.09 | 0.0000 |
| Error | 96 | 1.1254 | 0.01172 | | |
| Total | 107 | 33.8897 | | | |

Appendix 6.10 Analysis of variance (ANOVA) for reversal of *Meloidogyne incognita* second-stage juvenile immobility after exposure to Nemafric-BL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 2.4813 | 0.22557 | 1.01 | 0.4394 |
| Error | 96 | 21.3375 | 0.22227 | | |
| Total | 107 | 23.8188 | | | |

Appendix 7.1 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile mortality after exposure to pure cucurbitacin A.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 33.1191 | 3.01082 | 186.00 | 0.0000 |
| Error | 96 | 1.5540 | 0.01619 | | |
| Total | 107 | 34.6730 | | | |

Appendix 7.2 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile mortality after exposure to pure cucurbitacin B.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 33.1730 | 3.01573 | 142.56 | 0.0000 |
| Error | 96 | 2.0308 | 0.02115 | | |
| Total | 107 | 35.2038 | | | |

Appendix 8.1 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile mortality after exposure to Nemarioc-AL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 32.4353 | 2.94759 | 69.23 | 0.0000 |
| Error | 96 | 4.0871 | 0.04257 | | |
| Total | 107 | 36.5106 | | | |

Appendix 8.2 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile mortality after exposure to Nemafric-BL phytonematicide.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 11 | 32.5634 | 2.96031 | 255.97 | 0.0000 |
| Error | 96 | 1.1103 | 0.01157 | | |
| Total | 107 | 33.6737 | | | |

Appendix 9.1 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile post-exposure to Nemarioc-AL phytonematicide on tomato plant dry root mass.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 3 | 128.791 | 42.9303 | | |
| Treatment | 11 | 61.502 | 5.5911 | 0.57 | 0.8366 |
| Error | 33 | 322.059 | 9.7594 | | |
| Total | 47 | 512.353 | | | |

Appendix 9.2 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile post-exposure to Nemarioc-AL phytonematicide on tomato plant dry shoot mass.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 3 | 699.67 | 233.224 | | |
| Treatment | 11 | 942.98 | 85.726 | 0.81 | 0.6289 |
| Error | 33 | 3486.47 | 105.651 | | |
| Total | 47 | 5129.12 | | | |

Appendix 9.3 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile post-exposure to Nemarioc-AL phytonematicide on tomato plant root galls.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 3 | 0.8691 | 0.28971 | | |
| Treatment | 11 | 40.2185 | 3.65623 | 38.97 | 0.0000 |
| Error | 33 | 3.0964 | 0.09383 | | |
| Total | 47 | 44.1841 | | | |

Appendix 9.4 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile post-exposure to Nemafric-BL phytonematicide on tomato plant dry root mass.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 3 | 110.084 | 36.6947 | | |
| Treatment | 11 | 41.544 | 3.7767 | 0.50 | 0.8925 |
| Error | 33 | 251.611 | 7.6246 | | |
| Total | 47 | 403.239 | | | |

Appendix 9.5 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile post-exposure to Nemafric-BL phytonematicide on tomato plant dry shoot mass.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 3 | 976.43 | 325.476 | | |
| Treatment | 11 | 213.42 | 19.402 | 0.60 | 0.8129 |
| Error | 33 | 1062.47 | 32.196 | | |
| Total | 47 | 2252.32 | | | |

Appendix 9.6 Analysis of variance (ANOVA) of *Meloidogyne incognita* second-stage juvenile post-exposure to Nemafric-BL phytonematicide on tomato plant root galls.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 3 | 0.7216 | 0.24053 | | |
| Treatment | 11 | 40.8776 | 3.71615 | 46.51 | 0.0000 |
| Error | 33 | 2.6367 | 0.07990 | | |
| Total | 47 | 44.2360 | | | |

Appendix 10.1 Analysis of variance (ANOVA) of split-split plot of soil type, phytonematicide and soil depth on *Meloidogyne incognita* eggs in roots.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 5 | 0.049609 | 0.009922 | 1.00 | |
| Soil type(S) | 3 | 0.029765 | 0.009922 | 1.00 | 0.420 |
| Error | 15 | 0.148827 | 0.009922 | 1.00 | |
| Phytonematicide (P) | 1 | 0.009922 | 0.009922 | 1.00 | 0.329 |
| S×P | 3 | 0.029765 | 0.009922 | 1.00 | 0.413 |
| Error | 20 | 0.198436 | 0.009922 | 1.00 | |
| Depth (D) | 3 | 0.029765 | 0.009922 | 1.00 | 0.395 |
| S×D | 9 | 0.089296 | 0.009922 | 1.00 | 0.444 |
| P×D | 3 | 0.029765 | 0.009922 | 1.00 | 0.395 |
| S×P×D | 9 | 0.089296 | 0.009922 | 1.00 | 0.444 |
| Error | 120 | 1.190614 | 0.009922 | | |
| Total | 191 | 1.895061 | | | |

Appendix 10.2 Analysis of variance (ANOVA) of split-split plot of soil type, phytonematicide and soil depth on *Meloidogyne incognita* second-stage juveniles in roots.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 5 | 0.18161 | 0.03632 | 0.64 | |
| Soil type(S) | 3 | 0.17970 | 0.05990 | 1.06 | 0.394 |
| Error | 15 | 0.84503 | 0.05634 | 0.67 | |
| Phytonematicide (P) | 1 | 0.03514 | 0.03514 | 0.42 | 0.525 |
| S×P | 3 | 0.61602 | 0.20534 | 2.44 | 0.094 |
| Error | 20 | 1.68054 | 0.08403 | 1.49 | |
| Depth (D) | 3 | 1.60913 | 0.53638 | 9.52 | <0.001 |
| S×D | 9 | 1.61186 | 0.17910 | 3.18 | 0.002 |
| P×D | 3 | 0.34831 | 0.11610 | 2.06 | 0.109 |
| S×P×D | 9 | 1.08097 | 0.12011 | 2.13 | 0.032 |
| Error | 120 | 6.75784 | 0.05632 | | |
| Total | 191 | 14.94616 | | | |

Appendix 10.3 Analysis of variance (ANOVA) of split-split plot of soil type, phytonematicide and soil depth on *Meloidogyne incognita* second-stage juveniles in soil.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 5 | 0.0037758 | 0.0007552 | 0.75 | |
| Soil type(S) | 3 | 0.0018879 | 0.0006293 | 0.62 | 0.610 |
| Error | 15 | 0.0151032 | 0.0010069 | 1.07 | |
| Phytonematicide (P) | 1 | 0.000000 | 0.000000 | 0.00 | 1.000 |
| S×P | 3 | 0.0037758 | 0.0012586 | 1.33 | 0.292 |
| Error | 20 | 0.0188790 | 0.0009439 | 1.00 | |
| Depth (D) | 3 | 0.0056637 | 0.0018879 | 2.00 | 0.118 |
| S×D | 9 | 0.0056637 | 0.0006293 | 0.67 | 0.738 |
| P×D | 3 | 0.000000 | 0.0000000 | 0.00 | 1.000 |
| S×P×D | 9 | 0.0113274 | 0.0012586 | 1.33 | 0.227 |
| Error | 120 | 0.1132738 | 0.0009439 | | |
| Total | 191 | 0.1793502 | | | |

Appendix 10.4 Analysis of variance (ANOVA) of split-split plot of soil type, phytonematicide and soil depth on total *Meloidogyne incognita* nematodes.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 5 | 0.22673 | 0.04535 | 0.68 | |
| Soil type(S) | 3 | 0.24757 | 0.08252 | 1.24 | 0.329 |
| Error | 15 | 0.99455 | 0.06630 | 0.76 | |
| Phytonematicide (P) | 1 | 0.04426 | 0.04426 | 0.50 | 0.486 |
| S×P | 3 | 0.63042 | 0.21014 | 2.39 | 0.099 |
| Error | 20 | 1.75623 | 0.08781 | 1.46 | |
| Depth (D) | 3 | 1.74666 | 0.58222 | 9.68 | <0.001 |
| S×D | 9 | 1.67887 | 0.18654 | 3.10 | 0.002 |
| P×D | 3 | 0.40979 | 0.13660 | 2.27 | 0.084 |
| S×P×D | 9 | 1.22120 | 0.13569 | 2.25 | 0.023 |
| Error | 120 | 7.22129 | 0.06018 | | |
| Total | 191 | 16.17757 | | | |

Appendix 10.5 Analysis of variance (ANOVA) of split-split plot of soil type, phytonematicide and soil depth on dry root mass of tomato plants.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 5 | 7.85 | 1.57 | | |
| Soil type (S) | 3 | 316.53 | 105.51 | 9.55 | 0.0009 |
| Error | 15 | 165.73 | 11.05 | | |
| Phytonematicide (P) | 1 | 16.04 | 16.04 | 2.34 | 0.1418 |
| S×P | 3 | 8.35 | 2.78 | 0.41 | 0.7503 |
| Error | 20 | 137.12 | 6.86 | | |
| Depth (D) | 3 | 6903.27 | 2301.09 | 330.87 | 0.0000 |
| S×D | 9 | 579.13 | 64.35 | 9.25 | 0.0000 |
| P×D | 3 | 7.79 | 2.60 | 0.37 | 0.7725 |
| S×P×D | 9 | 24.45 | 2.72 | 0.39 | 0.9376 |
| Error | 120 | 834.55 | 6.95 | | |
| Total | 191 | 9000.83 | | | |

Appendix 10.6 Analysis of variance (ANOVA) of split plot of soil type and phytonematicide on tomato fruit mass.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 5 | 12305.6 | 2461.12 | | |
| Soil type (S) | 3 | 27325.1 | 9108.38 | 7.66 | 0.0025 |
| Error | 15 | 17837.4 | 1189.16 | | |
| Phytonematicide (P) | 1 | 5429.4 | 5429.38 | 3.09 | 0.0942 |
| S×P | 3 | 1888.2 | 629.41 | 0.36 | 0.7840 |
| Error | 20 | 35171.6 | 1758.58 | | |
| Total | 47 | 99957.4 | | | |

Appendix 10.7 Analysis of variance (ANOVA) of split plot of soil type and phytonematicide on dry shoot mass of tomato plants.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 5 | 15.534 | 3.1067 | | |
| Soil type (S) | 3 | 67.477 | 22.4924 | 2.54 | 0.0959 |
| Error | 15 | 133.019 | 8.8679 | | |
| Phytonematicide (P) | 1 | 74.252 | 74.2519 | 5.44 | 0.0303 |
| S×P | 3 | 42.634 | 14.2113 | 1.04 | 0.3963 |
| Error | 20 | 273.229 | 13.6615 | | |
| Total | 47 | 606.145 | | | |

Appendix 10.8 Analysis of variance (ANOVA) of split plot of soil type and phytonematicide on stem diameter of tomato plants.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 5 | 1.7186 | 0.34373 | | |
| Soil type (S) | 3 | 12.9899 | 4.32995 | 8.68 | 0.0014 |
| Error | 15 | 7.4785 | 0.49857 | | |
| Phytonematicide (P) | 1 | 1.5878 | 1.58777 | 5.74 | 0.0265 |
| S×P | 3 | 2.1489 | 0.71631 | 2.59 | 0.0815 |
| Error | 20 | 5.5349 | 0.27675 | | |
| Total | 47 | 31.4586 | | | |

Appendix 10.9 Analysis of variance (ANOVA) of split plot of soil type and phytonematicide on plant height of tomato plants.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 5 | 150.95 | 30.189 | | |
| Soil type (S) | 3 | 587.65 | 195.885 | 3.65 | 0.0371 |
| Error | 15 | 804.64 | 53.642 | | |
| Phytonematicide (P) | 1 | 27.91 | 27.907 | 0.29 | 0.5954 |
| S×P | 3 | 49.33 | 16.444 | 0.17 | 0.9143 |
| Error | 20 | 1916.65 | 95.833 | | |
| Total | 47 | 3537.13 | | | |

Appendix 10.10 Analysis of variance (ANOVA) of split plot of soil type and phytonematicide on chlorophyll of tomato plants.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 5 | 165.33 | 33.066 | | |
| Soil type (S) | 3 | 1309.82 | 436.607 | 10.74 | 0.0005 |
| Error | 15 | 609.58 | 40.638 | | |
| Phytonematicide (P) | 1 | 27.91 | 27.908 | 1.38 | 0.2546 |
| S×P | 3 | 124.98 | 41.661 | 2.05 | 0.1387 |
| Error | 20 | 405.77 | 20.289 | | |
| Total | 47 | 2643.39 | | | |

Appendix 10.11 Analysis of variance (ANOVA) of split-split plot of organic matter levels, phytonematicide and soil depth on *Meloidogyne incognita* eggs in roots.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 3 | 0.006543 | 0.002181 | 1.00 | |
| Organic matter levels (O) | 6 | 0.013086 | 0.002181 | 1.00 | 0.455 |
| Error | 18 | 0.039259 | 0.002181 | 1.00 | |
| Phytonematicide (P) | 1 | 0.002181 | 0.002181 | 1.00 | 0.329 |
| O×P | 6 | 0.013086 | 0.002181 | 1.00 | 0.451 |
| Error | 21 | 0.045802 | 0.002181 | 1.00 | |
| Depth (D) | 3 | 0.006543 | 0.002181 | 1.00 | 0.395 |
| O×D | 18 | 0.039259 | 0.002181 | 1.00 | 0.464 |
| P×D | 3 | 0.006543 | 0.002181 | 1.00 | 0.395 |
| O×P×D | 18 | 0.039259 | 0.002181 | 1.00 | 0.464 |
| Error | 126 | 0.274814 | 0.002181 | | |
| Total | 223 | 0.486378 | | | |

Appendix 10.12 Analysis of variance (ANOVA) of split-split plot of organic matter, phytonematicide and soil depth on *Meloidogyne incognita* second-stage juveniles in roots.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 3 | 0.19170 | 0.06390 | 0.66 | |
| Organic matter levels (O) | 6 | 0.27430 | 0.04572 | 0.47 | 0.821 |
| Error | 18 | 1.74854 | 0.09714 | 1.89 | |
| Phytonematicide (P) | 1 | 0.55606 | 0.55606 | 10.82 | 0.003 |
| O×P | 6 | 0.30663 | 0.05111 | 0.99 | 0.454 |
| Error | 21 | 1.07903 | 0.05138 | 0.84 | |
| Depth (D) | 3 | 0.12959 | 0.04320 | 0.71 | 0.548 |
| O×D | 18 | 0.76264 | 0.04237 | 0.70 | 0.811 |
| P×D | 3 | 0.03512 | 0.01171 | 0.19 | 0.902 |
| O×P×D | 18 | 1.14808 | 0.06378 | 1.05 | 0.414 |
| Error | 126 | 7.67779 | 0.06093 | | |
| Total | 223 | 13.90948 | | | |

Appendix 10.13 Analysis of variance (ANOVA) of split plot of organic matter and phytonematicide on dry root mass of tomato plants.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 3 | 153.36 | 51.12 | 1.76 | |
| Organic matter levels (O) | 6 | 186.52 | 31.09 | 1.07 | 0.415 |
| Error | 18 | 521.74 | 28.99 | 0.77 | |
| Phytonematicide (P) | 1 | 8.36 | 8.36 | 0.22 | 0.642 |
| O×P | 6 | 157.27 | 26.21 | 0.70 | 0.655 |
| Error | 21 | 789.76 | 37.61 | 1.57 | |
| Depth (D) | 3 | 12896.33 | 4298.78 | 179.55 | <0.001 |
| O×D | 18 | 706.14 | 39.23 | 1.64 | 0.060 |
| P×D | 3 | 7.21 | 2.40 | 0.10 | 0.960 |
| O×P×D | 18 | 314.80 | 17.49 | 0.73 | 0.774 |
| Error | 126 | 3016.69 | 23.94 | | |
| Total | 223 | 18758.19 | | | |

Appendix 10.14 Analysis of variance (ANOVA) of split plot of organic matter and phytonematicide on fruit mass of tomato plants.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 3 | 12581 | 4193.71 | | |
| Organic matter levels (O) | 6 | 32453 | 5408.82 | 2.23 | 0.0880 |
| Error | 18 | 43740 | 2430.02 | | |
| Phytonematicide (P) | 1 | 1167 | 1166.63 | 0.61 | 0.4447 |
| O×P | 6 | 7498 | 1249.65 | 0.65 | 0.6899 |
| Error | 21 | 40384 | 1923.03 | | |
| Total | 55 | 137823 | | | |

Appendix 10.15 Analysis of variance (ANOVA) of split plot of organic matter and phytonematicide on dry shoot mass of tomato plants.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 3 | 105.282 | 35.0940 | | |
| Organic matter levels (O) | 6 | 80.047 | 13.3412 | 2.00 | 0.1197 |
| Error | 18 | 120.353 | 6.6863 | | |
| Phytonematicide (P) | 1 | 13.406 | 13.4064 | 0.73 | 0.4039 |
| O×P | 6 | 37.989 | 6.3314 | 0.34 | 0.9062 |
| Error | 21 | 388.005 | 18.4764 | | |
| Total | 55 | 745.082 | | | |

Appendix 10.16 Analysis of variance (ANOVA) of split plot of organic matter and phytonematicide on stem diameter of tomato plants.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 3 | 0.2833 | 0.09444 | | |
| Organic matter levels (O) | 6 | 12.3754 | 2.06257 | 2.25 | 0.0848 |
| Error | 18 | 16.4723 | 0.91513 | | |
| Phytonematicide (P) | 1 | 7.2432 | 7.24321 | 17.13 | 0.0005 |
| O×P | 6 | 3.8210 | 0.63684 | 1.51 | 0.2245 |
| Error | 21 | 8.8786 | 0.42279 | | |
| Total | 55 | 49.0739 | | | |

Appendix 10.17 Analysis of variance (ANOVA) of split plot of organic matter and phytonematicide on plant height of tomato plants.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 3 | 49.48 | 16.4938 | | |
| Organic matter levels (O) | 6 | 268.79 | 44.7979 | 0.72 | 0.6371 |
| Error | 18 | 1116.27 | 62.0149 | | |
| Phytonematicide (P) | 1 | 2.93 | 2.9257 | 0.08 | 0.7864 |
| O×P | 6 | 260.92 | 43.4874 | 1.12 | 0.3846 |
| Error | 21 | 815.57 | 38.8367 | | |
| Total | 55 | 2513.96 | | | |

Appendix 10.18 Analysis of variance (ANOVA) of split plot of organic matter and phytonematicide on chlorophyll of tomato plants.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replication | 3 | 53.961 | 17.9868 | | |
| Organic matter levels (O) | 6 | 261.149 | 43.5248 | 2.91 | 0.0367 |
| Error | 18 | 269.576 | 14.9764 | | |
| Phytonematicide (P) | 1 | 65.794 | 65.7945 | 5.13 | 0.0341 |
| O×P | 6 | 68.594 | 11.4324 | 0.89 | 0.5184 |
| Error | 21 | 269.106 | 12.8146 | | |
| Total | 55 | 988.180 | | | |