MEAN CONCENTRATION STIMULATION POINT OF NEMARIOC-AL AND NEMAFRIC-BL PHYTONEMATICIDES ON *PELARGONIUM SIDOIDES*: AN INDIGENOUS FUTURE CULTIGEN

by

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DECLARATION

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

Candidate: Nokuthula Thulisile Sithole (Ms) Date

DEDICATION

To my beloved siblings (Mos, Sai, Lucia and Khensy), much love the Nkomos.

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ABSTRACT

Pelargonium sidoides has numerous medicinal applications, with economic potential to serve as a future cultigen in smallholder farming systems. However, it is highly susceptible to the root-knot (*Meloidogyne* species) nematodes, without any identifiable nematode resistant genotypes. Nemarioc-AL and Nemafric-BL phytonematicides, with cucurbitacin A and cucurbitacin B active ingredients, respectively, are being researched and developed as an alternative to synthetic nematicides at the University of Limpopo. However, since active ingredients in phytonematicides are allelochemicals, the two phytonematicides have the potential of inducing phytotoxicity on crops protected against nematode damage. The objectives of the study, therefore, were (1) to determine the non-phytotoxic concentration of Nemarioc-AL phytonematicide on plant growth of *P. sidoides,* and (2) to determine the non-phytotoxic concentration of Nemafric-BL phytonematicide in plant growth of *P. sidoides*. Cuttings were raised in 30-cm-diameter plastic pots containing 10 000 ml steam-pasteurised river sand and Hygromix-T at 3:1 (v/v) under microplot conditions in autumn (March-May) and repeated in spring (August-October) 2015. After establishment each plant was inoculated with 5 000 eggs and second-stage juveniles (J2s) of *M. javanica*. Six treatments, namely, 0, 2, 4, 6, 8 and 10% concentrations of each phytonematicide on separate trials were arranged in a randomised complete block design, with seven replicates. At 56 days after inoculation, in Experiment 1, Nemarioc-AL phytonematicide, treatment significantly ($P \leq 0.05$) affected plant height, dry root mass and root galls, contributing 62, 69 and 70% to total treatment variation of the three variables, respectively. Relative to untreated control Nemarioc-AL phytonematicide increased plant height and dry root mass by 34 to 61%

and 20 to 76%, respectively, with a slight decrease by 5% in plant height at the highest concentration. However, the material decreased root galls by 5 to 50%. Significant ($P \leq 0.05$) plant variables were subjected to Curve fitting-allelochemical respond dosage model, to generate biological indices which were used to compute the mean concentration stimulation point (MCSP) using the relation: $MCSP = D_m + R_h/2$ and the overall sensitivity value ($\sum k$). In Experiment 1, MCSP = 6.18% and $\sum k = 3$. Plant variables and increasing concentration of phytonematicide exhibited quadratic relations. Treatments reduced nematode variables, at all levels including at the lowest, but the effect were not different. In Experiment 2, Nemarioc-AL phytonematicide treatment effects were not significant on plant variables except for root galls, but were significant for root nematodes except for eggs. Data for plant variables in Experiment 2 were not subjected to Curve fitting-allelochemical respond dosage model because they were not significant ($P \leq 0.05$). In Experiment 1, Nemafric-BL phytonematicide treatment significantly ($P \leq 0.05$) affected plant height and root galls, contributing 63 and 67% to total treatment variation of the two variables, respectively. Relatively to untreated control, plant height was increased by 10 to 36%, while root galls was reduced by 2.43 to 60%. In Experiment 1, MCSP = 2.87% and $\sum k = 3$. Concentrations of Nemafric-BL phytonematicide significantly ($P \leq 0.05$) reduced eggs, juveniles and Pf at all levels including at the lowest, but the effect were not significant different, with treatments contributing 78, 72 and 90% to the total treatment variation. In Experiment 2, Nemafric-BL phytonematicide treatment effects were not significant on plant variables except for root galls, but were significant for root. In conclusion, Nemarioc-AL and Nemafric-BL

phytonematicides could be applied at the lowest concentration of 2% where it was shown to be effective in suppressing population densities of *M. javanica.*

Figure 1 *Pelargonium sidoides* at harvest.

Figure 2 Experimental layout.

CHAPTER 1
RESEARCH PROBLEM

1.1 Background

Wild geranium (*Pelargonium sidoides* DC.) had been identified as a potential economic future cultigen (van Wyk and Wink, 2004), with medicinal uses for respiratory infections, acute/chronic infections, ear infections, nose infections, throat infections and analgesic effects (Brendler and van Wyk, 2008). However, *P. sidoides* is highly susceptible to root-knot (*Meloidogyne* species) nematodes (Mofokeng *et al*., 2013), without any nematode resistant genotypes. Historically, unmanaged various plant-parasitic nematodes resulted in the collapse of wheat (*Triticum aestivum* L.), onion (*Allium cepa* L.) and beetroot (*Beta vulgaris* L.) industries prior to the discovery of synthetic nematicides (Mashela, 2007). In crops without nematode resistant genotypes like watermelon (*Citrullus lanatus* Thunb.), nematodes reduced yield by as much as 50% to complete crop failure (Lamberti, 1979). Fumigant nematicides, which were relied upon for managing nematode population densities in specialty crops such as *P. sidoides* and *C. lanatus*, had since been withdrawn from agrochemical markets due to their environment-unfriendliness (Speth, 2004). Globally, most of the available environment-friendly phytonematicides are still at the research and developmental stages (Mashela *et al*., 2011), with phytotoxicity limiting the successful registration of most tested phytonematicides (Mashela *et al*., 2015).

Worldwide, yield loss due to nematode damage prior to the withdrawal of synthetic nematicides had been estimated at US$126 billion per annum (Chitwood, 2003), with percentage yield losses ranging from 6 to 20% (Ferraz and Brown, 2002). Nemarioc-AG and Nemafric-BG phytonematicides (G = granular formulation) had been

successfully used in suppression of *Meloidogyne* species in vegetable cultivation (Mashela *et al*., 2015), with the efficacy of the product being comparable to that of aldicarb and fenamiphos synthetic nematicides (Mashela *et al*., 2008). Nemarioc-AL and Nemafric-BL phytonematicides (the liquid formulations), with bioactive chemical compounds cucurbitacin A ($C_{32}H_{46}O_9$) and cucurbitacin B ($C_{22}H_{48}O_8$), respectively, are produced from fermented crude extracts of fruits from wild cucumber (*Cucumis myriocarpus* Naud.) and wild watermelon (*Cucumis africanus* L.), respectively (Mashela *et al*., 2015). Therefore, active ingredients in phytonematicides are allelochemicals. Nemarioc-AL and Nemafric-BL phytonematicides have the potential challenge associated with phytotoxicity (Pelinganga and Mashela, 2012). Allelopathy from certain phytonematicides could reduce plant growth of different plant species by as high as 50% to complete crop failure (Mashela *et al*., 2015). The use of agricultural inputs to protect crops against pests has, internationally, zero tolerance towards phytotoxicity (EPPO, 2010).

Generally, allelochemicals are produced as secondary metabolites (Bertin *et al*., 2003), without any physiological roles in plants (Rice, 1984). In plants, allelochemicals are compartmentalised to avoid toxicity to cells (Rice, 1984). Allelochemicals are potent pesticides and have been widely used in medicine (Rice, 1984). Most allelochemicals affect biological systems through density-dependent growth (DDG) patterns (Liu *et al*., 2003), which have three phases, stimulation, neutral and inhibition phases (Salisbury and Ross, 1992). The stimulation phase of these materials had been used to generate non-phytotoxic concentrations of phytonematicides on various commercial crop cultivars (Mafeo *et al*., 2011a,b; Pelinganga and Mashela, 2012). The computer-generated concept of mean

concentration stimulation point (MCSP) was conceptualized as a useful tool in generating non-phytotoxic concentrations of environment-friendly phytonematicides (Mashela *et al*., 2015).

The MCSP is defined as the concentration that would not induce phytotoxicity in the protected plant species, while suppressing population densities of nematodes (Mashela *et al*., 2015). In addition to providing D_m and R_h, CARD model also provides the sensitivity (k) of the organs to the product used, with the overall sensitivities being the summation of all k values (Liu *et al*., 2003). The higher the k value, the less is the target organ to the product, *vice versa* (Liu *et al*., 2003). In tomato (*Solanum lycopersicum* L.) plants, MCSP for Nemarioc-AL and Nemafric-BL phytonematicides were 2.98% and 2.64%, respectively (Pelinganga *et al*., 2012), with the application interval of 16 and 18 days, respectively. The respective k values ranged from 1 to 4 and 0 to 1 (Pelinganga *et al*., 2012, 2013a). In seedlings the k-values for chive (*Allium schoenoprasum* L.), leek (*Allium ampeloprasum* L.) and onion (*Allium cepa* L.) were 5-8, 7-20 and 3-7, respectively (Mafeo *et al*., 2011a; Mafeo, 2012). Factors that affect MCSP had not been determined, but it is probably that this variable is plant-specific. Apparently, phytotoxicity trials of Nemarioc-AL and Nemafric-BL phytonematicides should be conducted for each crop prior to use.

1.2 Problem statement

Active ingredients of Nemarioc-AL and Nemafric-BL phytonematicides are allelochemical compounds, with the potential of inducing phytotoxicity on the highly nematode susceptible *P. sidoides*, which is being viewed as a potential future cultigen inland South Africa. The researcher intends to develop MCSP and overall

sensitivity values of the two phytonematicides on *P. sidoides*, which would allow for suppression of nematodes without having detrimental effects on growth of the cultigen.

1.3 Rationale of the study

Pelargonium sidoides is potential future cultigen, with desirable socio-economic attributes (van Wyk and Wink, 2004). However, due to lack of nematode-resistant genotypes in *Pelargonium* species (Mofokeng *et al*., 2013), it would not be feasible to successfully produce this cultigens without using alternative strategies to manage nematodes. However, due to the allelopathic nature of phytonematicides (Mashela *et al*., 2015) it would be prudent to develop MCSP for each of the two phytonematicides. Development of MCSP and overall sensitivity values for Nemarioc-AL and Nemafric-BL phytonematicides would allow for the eventual registration of the two products on *P. sidoides* for management of *Meloidogyne* species in accordance with ACT No. 36 of 1947.

1.4 Purpose

1.4.1 Aim

To develop the MCSP values of Nemarioc-AL and Nemafric-BL phytonematicides which would not be phytotoxic to *P. sidoides*, but would suppress population densities of *Meloidogyne* species.

1.4.2 Objectives

1. To determine whether a series of Nemarioc-AL concentrations applied on nematode-infested *P. sidoides* would generate non-phytotoxic MCSP value for the phytonematicide on *P. sidoides*.
2. To investigate whether increasing concentrations of Nemafric-BL phytonematicide applied on nematode-infested *P. sidoides* would generate MCSP for the phytonematicide on the test crop.

1.4.3 Hypotheses

1. A series of Nemarioc-AL concentrations applied on nematode-infested *P. sidoides* would generate non-phytotoxic MCSP value for the phytonematicide on *P. sidoides*.
2. Increasing concentrations of Nemafric-BL phytonematicide applied on nematode-infested *P. sidoides* would generate MCSP for the phytonematicide on the test crop.

1.5 Reliability, validity and objectivity

In this study, reliability of data were based on statistical analysis of data at the probability level of 5%. Validity was achieved through repeating the experiments in time. Objectivity was achieved by ensuring that the findings are discussed on the basis of empirical evidence, thereby eliminating all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

Bias was minimised by ensuring that the experimental error in each experiment was reduced through adequate replications. Also, treatments were assigned randomly within the selected research designs (Leedy and Ormrod, 2005).

1.7 Structure of mini-dissertation

Following the description and detailed outlining of the research problem (Chapter 1), the work done and not yet done on the research problem was reviewed (Chapter 2). Then, each of the two subsequent chapters (Chapters 3 and 4) addressed each of the two objectives, sequentially. In the final chapter (Chapter 5), 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 together the entire study. The citation and references used the Harvard style as prescribed by the University of Limpopo.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Phytonematicides, as an alternative management strategy in nematode management, comprise a class of botanicals, are available as aqueous plant extracts (Egunjobi and Afolami, 1976) and granules (Mashela, 2002). Phytonematicides had been introduced as a mitigation strategy to drawbacks of conventional organic amendments in suppression of nematodes in climate smart agriculture (Mashela, 2002). The most common drawbacks include: (a) inconsistent results in nematode suppression (McSorley, 2011), (b) large quantities (10-500 ton/ha) required to achieve nematode suppression (McSorley and Gallaher, 1995; Stirling, 2014), (c) unavailability of the material, (d) high transport costs for moving the products to the production sites (Mashela, 2002), (e) negative period, which leads to time-lag for allowing microbial decomposition to avoid effects of mineralization (Stirling, 2014) and (f) decrease in soil pH, which could lead to imbalances of available essential nutrient elements (Mashela and Nthangani, 2002).

Inputs for most bio-nematicides are collected locally from indigenous plants (Mashela *et al*., 2011), with complex allelochemical compounds that suppress nematode population densities (Chitwood, 2003; Okwute, 2012). The successful use of allelochemicals in managing plant-parasitic nematodes relies on the degree of non-phytotoxicity to the protected crops. Conventional methods of determining non-phytotoxicity of botanicals are tedious, since allelopathy is plant-specific, chemical-specific and concentration-specific (Pelinganga and Mashela, 2012). The literature

review in this study was intended to review work done in the research problem and work not yet done.

2.2 Work done on the research problem

2.2.1 Efficacy of phytonematicides

Several *in vitro* trials have had in excess of 90% suppression of nematode numbers from phytonematicides (Okwute, 2012). Crude extracts of wild cucumber (*Cucumis myriocarpus* Naud.) fruit in the Ground Leaching Technology (GLT) system consistently suppressed *M*. *incognita* race 2 on tomato (*Lycopersicon esculentum* L.) (Mashela, 2002; Mashela *et al*., 2009). In the GLT system fruits of *C. myriocarpus* and *Cucumis africanus* L. consistently suppressed nematode (*Meloidogyne* species) population densities in greenhouse trials by over 90% (Mashela, 2002) and in field trials by over 80% (Mashela, 2007). Pelinganga *et al*. (2011) observed similar results when using fruits of *C. myriocarpus* and *C. africanus* in the fermented crude extract technology. The two *Cucumis* products reduced nematode population densities by over 69% and by over 89%, respectively, through the process termed as “botinemagation” (Pelinganga *et al*., 2011). Wondimeneh *et al*. (2013) reported that inhibitory effect of botanical extract on juveniles of *M. incognita* were concentration dependent. The plant crude extracts from baker tree (*Milletia ferruginea* Hochst.), bitter leaf (*Vernonia amygodalina* Del.), parthenium (*Parthenium hysterophorus* L.), lantana (*Lantana camara* L.), Mexican marigold (*Tagetes minuta* L.), Mexican tea (*Chenopodium ambrosioides* L.), neem (*Azadirachta indica* A. Juss.) and pyrethrum (*Chrysanthemum cinerariafolium* Trevir.) were effective in reducing *M. incognita* egg (Wondimeneh *et al*., 2013).

The application of fever tea (*Lippia javanica* F. Burm.) leaves to *M. incognita* infested soil reduced the number of nematode in tomato roots by 79-92% thereby increasing fruit yield, dry shot mass, plant height and stem diameter of tomato plants (Mashela *et al*., 2010). Ground *C. myriocarpus* and castor bean (*Ricinus communis* L.) fruits consistently reduced densities of *M. incognita* in tomato roots and soil (Mashela *et al*., 2010). Olabiyi (2008) reported that aqueous extracts from roots of marigold (*Tagetes erecta* L.), nitta (*Hyptis suaveolens* L.) and basil (*Ocimum gratissium* L.) plants reduced root-knot nematode populations in the soil. Also, ethanol extracts of four plant species, neem (*Azadirachta indica*), ashwagandha (*Withania somnifera* Linnaeus.), marigold (*Tagetes erecta* L.) and eucalyptus (*Eucalyptus citriodora* E.) reduced gall index, number of juveniles and egg masses of *M. incognita* on papaya (*Carica papaya* L.) (Khan *et al*., 2008). Pelinganga *et al*. (2013a) reported that Nemarioc-AL phytonematicide reduced nematode population densities in roots and soil by 94% and 48%, respectively. Tseke *et al*. (2013) observed similar results when using Nemarioc-AL phytonematicide in the management of root-knot nematode. The product consistently reduced population densities of *M. incognita* race 2 from 46 to 92% and 74 to 96% in roots and soil, respectively. Pelinganga *et al*. (2012) reported that Nemafric-BL phytonematicide reduced nematode population densities by 85-97%, 45-95% and 78-97% in roots, soil and total nematodes, respectively.

2.2.2 Phytotoxicity in phytonematicides

Allelochemicals as active ingredients in phytonematicides are naturally phytotoxic to other plant species during interference interactions (Okwute, 2012). The various negative effects of allelochemicals studied on plants include germination inhibition (Mafeo *et al*., 2011a,b), suppression of seedling growth (Bhatt and Todoria, 1990)

and increased seedling mortalities (Smith, 1990). Allelochemicals may regulate plant growth and development processes in terms of respiration, transpiration, photosynthesis, as well as nucleic acid and protein synthesis (Chou, 2006). The mode of allelochemical action include stimulation, neutral, or inhibitory, depending on the level of concentrations and the sensitivity of the receiving target plant organs (Liu *et al*., 2003; Mashela *et al*., 2015; Rice, 1979).

The effects of allelochemicals on plant phytotoxicity and nematode suppression are concentration-specific (Mashela *et al*., 2015). Crude extracts of garlic bulb at 50% concentration reduced nematode densities, but were phytotoxic to tomato seedlings (Sukul *et al*., 1974). However, at 20% concentration, there were no noticeable effects on tomato plant growth, whereas *M. incognita* population densities were suppressed (Agbenin *et al*., 2005). Nemarioc-AL and Nemafric-BL phytonematicides were shown to be highly phytotoxic to tomato (*Solanum lycopersicum* L.) seedlings at above 10% concentration after transplanting (Pelinganga and Mashela, 2012; Pelinganga *et al*., 2012). Similarly, when 5 g crude extracts Nemarioc-AG (G = granular formulation) phytonematicide were applied as pre-emergent drenches, the material was highly phytotoxic to eight monocotyledonous and ten dicotyledonous crops (Mafeo, 2012).

At low concentration crude extracts of neem (*Azadirachta indica*) leaf stimulated growth of maize (*Zea mays* L.) and tomato seedlings, while at high concentrations the opposite was observed (Egunjobi and Afolamin, 1976; Rossner and Zebitz, 1987). Inderjit *et al*. (1999) also noted that at low concentrations root leachates from golden crown beard (*Verbesina encelloides* Cav.) consistently simulated plant growth of various plant species. Similarly, at low concentration, Nemarioc-BL

stimulated growth of tomato seedlings, with the products being viewed as having a fertiliser effect (Mashela, 2002). However, detailed subsequent nutrient analysis in various plant organs could not link the observed stimulated growth with essential nutrient elements (Mashela and Nthangeni, 2002). Mashela *et al*. (2015) reviewed the phases which could be induced by allelochemicals in phytonematicides, namely, stimulation, neutral and inhibition phases. The phases were quantified using concept of biological indices (Liu *et al*., 2003; Mashela *et al*., 2015).

2.2.3 Curve-fitting allelochemical dosage model

Mashela *et al*. (2015) adapted the Curve-fitting Allelochemical Response Dosages (CARD) computer-based model (Liu *et al*., 2003) to develop the concepts of mean concentration stimulation point (MCSP). The CARD model quantifies the three DDG patterns using biological indices (Liu *et al*., 2003). The biological indices include: (a) threshold stimulation (D_m) the allelochemical concentration where stimulation phase begin, (b) saturation point (R_h) the concentration at which stimulation ends or where the neutral phase start, (c) 0% inhibition (D_0) the concentration at which neutral phase ends, (d) 50% inhibition (D_{50}) the concentration at half the distance of the inhibition phase, (e) 100% inhibition (D_{100}) the concentration that terminates the inhibition phase), (f) the sensitivity index (k) provides the level of sensitivity of an organism to the test product and (g) the coefficient of determination (R^2) provides the degree of the strength of the CARD model. In the development of MCSP, two biological indices, D_m and R_h, are used through the relation: $MCSP = D_m + (R_h/2)$ (Mashela *et al*., 2015) is the concentration at which a given phytonematicide would not be phytotoxic to the crop being protected against nematode damage (Mashela *et al*., 2015), whereas nematode population densities would be consistently suppressed

(Mashela *et al*., 2015). MCSP is generated from the first two (D_m, R_h) biological indices from the CARD model using the relation MCSP = D_m + (R_h/2) (Mashela *et al*., 2015). Along with the biological indices, the CARD model provide the sensitivity index (k), which provides information of the sensitivity of the crop to the product being used to protect it against pest. Generally, the lower the k value, the higher is the sensitivity of the plant to the material (Liu *et al*., 2003). Mafeo (2012) reported k values which ranged from 5 to 8, 7 to 20, 3 to 7, 0 to 7, 2 to 8 and 1 to 4 on chive, leek, onion, maize, millet and sorghum seedlings, respectively, when exposed to crude extracts of *C. myriocarpus*. Tseke *et al*. (2013) reported k values of 2, 1, 0 and 2 for dry root mass, dry shoot mass, plant height and stem diameter for tomato plant, respectively. Pelinganga *et al*. (2012) reported k values which ranged from 0 to 1 for tomato plant variables when exposed to Nemafric-BL phytonematicide.

2.2.4 Dosage model in phytonematicides

Mashela *et al*. (2015) introduced the concept of the dosage model in the management of phytotoxicity and consistent suppression of nematode numbers. The MCSP is derived using the biological indices D_m and R_h from CARD model as MCSP = D_m + (R_h/2) (Mashela *et al*., 2015). The application frequency is a unit less factor, which is also empirically derived (Mashela *et al*., 2015). After empirically-deriving the MCSP, the result is used to derive the application interval (Ta), where the concept of day-week-month for a nematode life cycle (Mashela *et al*., 2015). For instance, *Meloidogyne* species have a life cycle period of 30 days. Therefore, to determine the application interval, the concept of 30 days × week (month) for life cycle of *Meloidogyne* was used (Mashela *et al*., 2015). Using the concept, the previously derived MCSP was applied at 0, 7.5, 15, 22.5 and 30 days for *Meloidogyne* species.

In the citrus nematode (*Tylenchulus semipenetrans* Cobb.), with the life cycle of 42 days, the empirically derived MCSP would be applied at 0, 10.5, 21, 31.5 and 42 days (Mashela *et al*., 2015). Once the application interval is derived, the application frequency (T_f) which is the proportion of the crop cycle to the application interval, [(T_f = crop cycle (days) / application interval (days)] is computed (Mashela *et al*., 2015). The application frequency is unit-less, with the result that:

Dosage model = MCSP (%) × T_f (Mashela *et al*., 2015).

The dosage is the amount of the total active ingredient that would have been put into the soil by the end of life cycle of the crop (Mashela *et al*., 2015). Due to oxidation microbial degradation and environment-induced degradation, it is believed that when applying a given phytonematicide for a given crop and nematode species within the confines of the dosage model, the phytonematicide would not induce environmental imbalances (Mashela *et al*., 2015).

2.3 Work not yet done on the research problem

The degree of phytotoxicity of Nemarioc-AL and Nemafric-BL phytonematicides on *P. sidoides* remains undocumented. Due to the economic potential attributes of *P. sidoides* as a future crop, MCSP values for the two phytonematicides would be established. In order to successfully investigate whether Nemarioc-AL and Nemafric-BL phytonematicides would be useful as phytonematicides in *P. sidoides* production, a series of experiments would be conducted to determine the appropriate MCSP values on this future crop.

CHAPTER 3
NEMARIOC-AL PHYTONEMATICIDE ON *PELARGONIUM SIDOIDES*

3.1 Introduction

The 2005 cut-off date for phasing out of methyl bromide and most other synthetic nematicides, increased the global focus on alternatives such as botanicals in managing nematode population densities through a process termed botinemagation (Mashela *et al*., 2015). Nemarioc-AL and Nemafric-BL phytonematicides were produced from fruits of wild cucumber (*Cucumis myriocarpus* Naud.) and wild watermelon (*Cucumis africanus* L.), respectively (Mafeo *et al*., 2011a,b; Mashela, 2002; Mashela *et al*., 2011; Pelinganga and Mashela, 2012). The products were shown to consistently suppress nematode population densities (Mashela *et al*., 2015), with occasional incidents of stimulating plant growth (Mashela, 2002). The objective of this study was to determine whether a series of Nemarioc-AL concentrations applied on nematode-infested *P. sidoides* would generate non-phytotoxic MCSP value for the phytonematicide on *P. sidoides*.

3.2 Materials and methods

3.2.1 Plant growth conditions and preparation of materials

The study was conducted under microplot conditions at the Green Technologies Research Centre (GTRC), University of Limpopo, South Africa (23°53'10"S, 29°44'15"E). Experiment 1 was conducted in autumn (March-May) and Experiment 2 in spring (August-October) 2015. Artificial micro-plots were established by inserting 30-cm-diameter plastic pots into 20-cm-deep holes at 1.0 m intra-row and 1.0 m inter-row spacing. Each pot was filled with 10 000 ml steam-pasteurised river sand

and Hygromix at 3:1 (v/v). One-month-old *P. sidoides* cuttings were transplanted into pots.

Matured fruit of *C. myriocarpus*, were collected locally, washed in tap water, chopped into small pieces and dried in air-forced oven at 52ºC for 72 h (Mashela *et al*., 2011). The material was ground in a Wiley mill through a 1-mm-mesh sieve and then finely powdered using A43 Monlinex coffee grinder. Ground material was stored at room temperature in hermitically sealed plastic bags for future use. Approximately 80 g ground material of *C. myriocarpus* was fermented in 20 L-hermetically sealed plastic container with 16 L chlorine-free tapwater. Allowance for released CO_2 to escape from the container was provided through an airtight 5 mm diameter tube with one end glued to a hole on the lid of the 20 L container, while the outlet end dangled into a litre bottle half-filled with tapwater. Approximately 300 ml molasses, 100 g brown sugar and 300 ml ZZ2 effective microorganisms (EM) was added into the container (Pelinganga *et al*., 2012). After a 14-day incubation period, when pH was at least ±3.7 (Kyan *et al*., 1999), the phytonematicide was applied once a week as substitute to irrigation.

3.2.2 Experimental design, inoculation and cultural practices

Six treatments, namely, 0, 2, 4, 6, 8 and 10% concentrations of Nemarioc-AL phytonematicide were arranged in a randomised complete block design, with seven replicates. Root-knot nematode (*Meloidogyne javanica* Treub.) inoculum was prepared by extracting eggs and second-stage juveniles (J2s) from roots of greenhouse-grown nematode-susceptible kenaf (*Hibiscus cannabinus* L.) in 1% NaOCl (Hussey and Barker, 1973). A day after transplanting, each pot was infested

with 5 000 *M. javanica* eggs and J2s using a 20 ml plastic syringe by placing into ca. 3-cm-deep holes on the cardinal points. Plants were fertilised a day after transplanting with 2.5 g of 2:3:2 (22) fertilizer mixture per plant to provide a total of 155 mg N, 105 mg P, and 130 mg k per ml water and 1 g 2:1:2 (43) Multifeed (Nulandies, Johannesburg) which provided a total of 0.175 mg N, 0.16 mg K and 0.16 mg P, 0.45 mg Mg, 0.378 mg Fe, 0.0375 mg Cu, 0.175 mg Zn, 0.5 mg B, 1.5 mg Mn and 0.035 mg Mo per ml water (Mashela, 2002). Every other day each plant was irrigated with 500 ml chlorine-free tapwater.

3.2.3 Data collection

At 56 days after inoculation, in Experiment 1, plant height was measured from the crown to the tip of the flag leaf and number of leaves per plant were counted. Chlorophyll content on two matured leaves per plant was measured using chlorophyll meter (Minolta Spad-502). Shoots were severed from roots and stem diameter measured at 5 cm from the distal end of stem prior to oven-drying at 70°C for 72 h and weighed. Root systems per pot were removed from pots, immersed in water to remove soil particles, blotted dry and weighed to facilitate the calculation of nematode density per total root system per plant. Root galls were assessed using the North Carolina Differential Rating Scale of 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, 5 = >100 (Taylor and Sasser, 1978).

Nematodes were extracted from whole root system per plant using maceration and blending method for 30 seconds in 1% NaOCl (Hussey and Barker, 1973). The material was passed through 75 and 25-µm nested sieves, with nematodes being collected from the 25-µm mesh sieve. Soil per pot was thoroughly mixed and a 250

ml soil sample was collected, with nematodes extracted using the sugar-floatation and centrifugation method (Jenkins, 1964). Eggs and J2s from root samples and J2s from soil samples were counted from a 10 ml aliquot of each sample with the use of a stereomicroscope. Nematode numbers from soil were converted to 10 000 ml soil per pot and were used to determine the final nematode population densities (Pf). The latter was used to compute the reproductive factor (RP = Pf/Pi), which is a proportion of Pf and the initial nematode population densities (Pi). In Experiment 2, data collection for plant and nematode variables were done as described in Experiment 1.

3.2.4 Data analysis

Data for plant and nematode variables were subjected to analysis of variance using SAS (SAS Institute Inc., Cary, NC, USA). Discrete nematode data were transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984), but untransformed means were reported. The degrees of freedom and their associated mean sum of squares were partitioned to provide the total treatment variation (TTV) for different sources of variation. Mean separation were achieved through the Waller-Duncan Multiple Range test at 5% level of probability. Means for significant plant variables were subjected to CARD model to generate appropriate biological indices (Liu *et al*., 2003; Mashela *et al*., 2015). Unless otherwise stated, treatments discussed were significant at 5% level of probability. In Experiment 2, data for plant and nematode variables were analysed as described in Experiment 1. Data for plant variables were not subjected to CARD model because there were no significant differences among the treatments.

3.3 Results

3.3.1 Treatment effects

In Experiment 1, effects of Nemarioc-AL phytonematicide were highly significant (P ≤ 0.01) on plant height, dry root mass and gall rating, but had no effects on chlorophyll content, dry shoot mass and dry tuber mass (Appendix 3.2 - 3.5). Treatments contributed 62, 69 and 70% in TTV of plant height, dry root mass and gall rating, respectively in Experiment 1, while in Experiment 2, treatments contributed 97% in TTV of gall rating (Table 3.1). Relative to untreated control, concentrations of Nemarioc-AL phytonematicide increased plant height and dry root mass by 34 to 61% and 20 to 76%, respectively, with a slight decrease by 5% in plant height at the highest concentration, however, the material decreased root galls by 5 to 50% (Table 3.2). In Experiment 2, treatments significantly reduced root galls by 93 to 100% (Table 3.2), but had no significant effects on other plant variables (Appendix 3.12 - 3.18).

Table 3.1 Sources of variation as affecting plant height (PHT), dry root mass (DRM) and gall rating (GR) at 56 days after initiation of treatments.

		Exp. 1						Exp. 2	
		PHT		DRM		GR		GR	
Source	DF	MS	%	MS	%	MS	%	MS	%
Rep	6	1701.84	30	1.07	10	0.01	16	0.10	2
Treatment	5	3548.50	62***	7.48	69***	0.05	70***	5.24	97***
Error	30	507.11	8	2.05	21	0.01	14	0.04	1
Total	40	5757.15	100	10.79	100	0.07	100	5.37	100

*** = highly significance at P ≤ 0.01.

Table 3.2 Effect of fermented crude extracts of Nemarioc-AL on dry root mass (DRM), plant height (PHT) and gall rating (GR) in *Pelargonium sidoides* at 56 days after initiation of treatments.

	Experiment 1						Experiment 2	
Treat	DRM (g)[y]	%[z]	PHT (cm)	%	GR	%	GR	%
0	2.70[a]	−	105.51[c]	−	3.29[a]	−	2.14[a]	−93
2	3.24[ab]	20	170.14[a]	61	3.14[ab]	−5	0.14[b]	−100
4	4.74[abc]	76	156.71[ab]	49	2.99[ab]	−9	0.00[b]	−100
6	3.93[bc]	26	150.14[ab]	42	2.57[ab]	−22	0.00[b]	−100
8	4.06[bc]	50	157.74[ab]	50	3.00[b]	−9	0.00[b]	−100
10	2.57[c]	−5	141.14[b]	34	1.57[c]	−52	0.00[b]	−100
P ≤	0.01		0.01		0.01		0.00	

[y]column means followed by same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

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

In Experiment 1, treatments had a highly significant effects on eggs and J2s, contributing 81 and 45%, respectively, in TTV of the two variables (Appendix 3.18 - 3.19), whereas in Experiment 2, treatments significantly affected J2s in roots and Pf, contributing 48 and 67%, respectively, in TTV of the two variables (Appendix 3.18 - 3.19). In Experiment 1, relative to untreated control, eggs were reduced by 92 to 97%, J2s by 51 to 81% and Pf by 88 to 94% (Table 3.5), whereas in Experiment 2, eggs and J2s in soil *of M. javanica* were affected by treatments (Appendix 3.21 - 3.22), but J2s in roots were reduced by 42 to 88% and Pf by 1 to 88% (Table 3.5).

3.3.2 Curve-fitting allelochemical response dosage

Mean concentration stimulation point: In Experiment 1, dry root mass and plant height over increasing concentrations of Nemarioc-AL phytonematicide exhibited quadratic relations (Figure 3.1). The model explained the relationship by 81 and 97% in dry root mass and plant height, respectively (Table 3.3). Using the relation $X = -b_1/2b_2$, concentrations for optimum dry root mass and plant height were 5.1 and 5.7, the respectively (Table 3.3). In Experiment 1, using the relation $MCSP = D_m + (R_h/2)$ relation, MCSP was equal to 6.18% (Table 3.4), with the compute validation results in the same value.

Sensitivity: Dry root mass had k value of k = 0, whereas plant height had k value of k = 3, with the overall sensitivity (∑k) of *P. sidoides* being equivalent to three. In Experiment 2, the plant variables were not subjected to CARD model since the treatment effects were not significant.

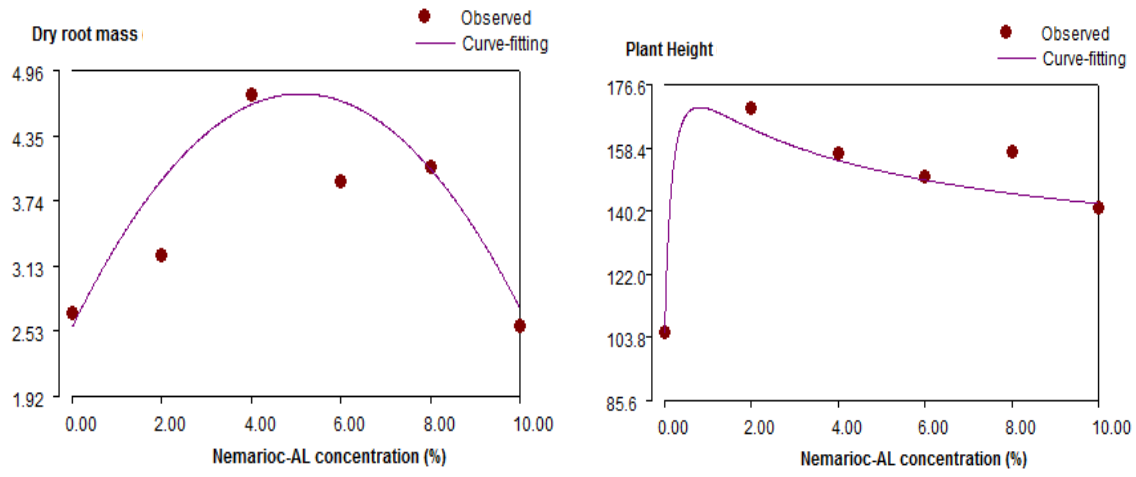

Figure 3.1 Responses of dry root mass and plant height of *P. sidoides* to concentrations of Nemarioc-AL phytonematicide in Experiment 1 at 56 days after inoculation.

Table 3.3 Quadratic relationship, coefficient of determination and computed optimum response concentration for dry root mass (DRM) and plant height (PHT) of *Pelargonium sidoides* from the Curve-fitting Allelochemical Response Dosage against Nemarioc-AL phytonematicide at 56 days after treatments in Experiment 1.

Organ	Quadratic relation	R^2	x^z	Y
DRM	$y = -0.0698x^2 + 0.7121x + 2.5382$	0.812	5.101	4.354
PHT	$y = -1.4376x^2 + 16.296x + 118.13$	0.971	5.667	164.311

$^z x = -b_1/2b_2$.

Table 3.4 Biological indices for dry root mass and plant height of *Pelargonium sidoides* to increasing concentrations of Nemarioc-AL phytonematicide at 56 days after initiation of treatments in Experiment 1.

Biological index[Z]	Dry root mass	Plant height	Mean
Threshold stimulation (D_m)	5.105	0.588	2.847
Saturation point (R_h)	1.817	11.505	6.661
0% inhibition (D_0)	10.208	3.352	6.780
50% inhibition (D_{50})	11.589	−	11.589
100% inhibition (D_{100})	13	−	13
R^2	0.812	0.971	
K- value	0	3	
Overall sensitivity		∑k= 3	

Average of $D_m + R_h = (2.847 + 9.507)/2 = 6.18$ %.

MCSP = $D_m + (R_h/2) = (2.847 + (6.661/2) = 2.847 + 3.330 = 6.18$ %.

Table 3.5 Influence of increasing concentrations of Nemarioc-AL phytonematicide on nematode juveniles (J2s) in roots, eggs in roots and final population (Pf) at 56 days after initiation of treatments.

Concentration	Experiment 1						Experiment 2							
	Eggs[y]	%[z]	J2s	%	Pf	%	Eggs	%	J2s	%	J2s	%	Pf	
0	373[a]	–	53[a]	–	426[a]	–	94	–	296	–	171[a]	–	562[b]	–
2	27[b]	−93	26[b]	−51	53[b]	−88	195	107	91	−69	57[a]	−67	341[a]	−39
4	10[b]	−97	26[b]	−51	36[b]	−92	406	330	90	−70	57[a]	−67	554[a]	−1
6	31[b]	−92	14[b]	−72	46[b]	−89	176	87	70	−76	0[ab]	−100	247[a]	−56
8	20[b]	−95	10[b]	−81	30[b]	−93	72	−23	25	−42	171b	0	369[a]	−34
10	12[b]	−97	14[b]	−72	27[b]	−94	30	−68	35	−88	0.01	−100	65[a]	−88
P value ≤	0.01		0.01		0.01		0.24		0.61		0.01		0.01	

[y]column means followed by same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

[z]Impact = [treatment/(control − 1)] × 100.

3.4 Discussion

Increasing concentrations of Nemarioc-AL phytonematicide had significant effects on plant height, dry root mass and root-knot gall rating in *P. sidoides*. Similar observations were made, when maize (*Zea mays* L.), millet (*Eleusine coracana* L.) and sorghum (*Sorghum bicolor* L.) (Mafeo *et al*., 2011a), tomato seedlings (Ghazalbash and Abdollahi, 2013; Mashela *et al*., 2011; Pelinganga *et al.*, 2012; Tseke *et al*., 2013) and other plant species (Inderjit *et al*., 1999) were exposed to various concentrations of allelochemicals. Similar results were observed when exposing maize (*Zea mays* L.), millet (*Eleusine coracana*), sorghum (*Sorghum bicolor*), chive (*Allium schoenoprasum* L.), leek (*Allium porrum* L.) and onion (*Allium cepa* L.) to increasing concentrations of Nemarioc-AG phytonematicide (Mafeo *et al*., 2011a,b). When lettuce (*Lactuca sativa* L.) seeds were exposed to increasing concentrations of banana (*Musa acuminate* L.) plant extracts, germination and seedling growth were inhibited (Roy *et al*., 2006).

Mafeo *et al*. (2011a) reported the stimulation and inhibition effects on chive, leek and onion when exposed to increasing concentration of Nemarioc-AG (G = granulation formulation) phytonematicide. At low concentrations the phytonematicide stimulated coleoptiles length, radical length, seedlings height and coleoptiles diameter. However, at higher concentrations the material inhibited the listed plant variables. Also, Mafeo *et al*. (2011b) observed similar effects on maize, millet and sorghum. Pelinganga *et al*. (2013a) reported similar results on tomato plant when exposed to Nemarioc-AL (L = liquid formulation) phytonematicide. Chukwuka *et al*. (2014) noted that the stimulation and inhibition of crude extracts from bitterleaf (*Vernonis amygdalina* Del.) on maize seedlings were dosage-dependent. In all cited studies,

the plant variables over increasing phytonematicide concentrations were characterised by quadratic relations. Collectively, the responses had been described as having density-dependent growth (DDG) patterns (Liu *et al*., 2003; Salisbury and Ross, 1992), which had been developed to quantify MCSP (Mashela *et al*., 2015).

Lack of significant effects on plant variables to increasing levels of Nemarioc-AL phytonematicide (Experiment 2) confirmed those of Ghafarbi *et al*. (2012), who observed that exposing eight selected plant species to seed extracts from wheat (*Triticum aestivum* L.) did not have significant effects on plant variables. Similarly, Kohli *et al*. (2001) observed that at 2% crude extracts of yellow nutsedge (*Cyperus esculentus* L.), had no effect on germination of lettuce, whereas at 5% the extracts inhibited germination. Mashela *et al*. (2015) provided a detailed explanation of possible outcomes when exposing plants to increasing concentrations of phytonematicides, which were concentration-dependent. In most cases, when concentrations are within the stimulation range, growth would be stimulated, whereas within the inhibition range, growth would be inhibited. In contrast, when growth was within the neutral range, growth would level off, with growth of untreated control plants “catching up” so that the treatment effects would not result in significant differences (Mashela *et al*., 2015). This explanation, which views growth responses in terms of stimulation, neutral and inhibition phases, had since laid to rest what had been perceived as ‘inconsistent results’ of organic amendments in plant-parasitic nematology (McSorley, 2011).

In this study, the MCSP value for Nemarioc-AL phytonematicide on *P. sidoides* was empirically derived as 6.18%, which appeared to have been high when compared

with that derived for tomato (*Solanum lycopersicum* L.) plants, which was at 2.63% (Pelinganga and Mashela, 2012). Because the overall sensitivity index of *P. sidoides* to Nemarioc-AL phytonematicide was low ($\sum k = 3$), the plant would be sensitive to the product. Mafeo *et al.* (2011a,b) demonstrated that 18 seedlings from different crops had different overall sensitivity values to Nemarioc-AG phytonematicide and the values were rather high as compared to the one for *P. sidoides*. Generally, the lower the overall sensitivity index, the higher is the sensitivity of the plant to the tested phytonematicide (Mashela *et al*., 2015). Rice (1984) noted that the degree of sensitivity in plants to allelochemicals was plant specific, with seedlings being highly tolerant than other stages in the life of a given plant species (Mafeo *et al.* 2011a,b).

In both experiments, the product reduced nematode stages with high magnitudes, which confirmed Tseke *et al*. (2013) observed that this product reduced J2s and eggs from 46 to 92% in roots and J2s from 74 to 96% in roots of tomato plants. The high effects of Nemarioc-AL phytonematicide on various stages of *Meloidogyne* species confirmed those observed by others (Mashela *et al*., 2015; Pelinganga *et al*., 2013a,b; 2012).

Generally, MCSP values should not be viewed in isolation, but should be viewed in relation to the overall sensitivity values, the concentration that was effective in reducing nematodes population densities and the application interval (Mashela *et al*., 2015). The application interval for effectively managing *Meloidogyne* population densities is 16 days (Pelinganga, 2013). Also, it is important to remember that phytonematicide are not fertilisers, but nematicides for suppressing nematode population densities. Using this view, since Nemarioc-AL phytonematicide

consistently reduced various stages of *M. javanica* in both soil and root samples at low concentrations, the tested low concentration should be adopted as the MCSP of product on *P. sidoides*, namely, 2% MCSP. This view is important since the product is not intended for use as fertiliser, but a nematicide (Mashela *et al*., 2015).

3.5 Conclusion

The non-phytotoxic concentration of Nemarioc-AL phytonematicide on *P. sidoides* was 6.18%. However, since *M. javanica* population densities were reduced at low MCSP values, it would be ideal to adopt the lowest concentration, such as 2% for Nemarioc-AL phytonematicide in the management of nematodes on *P. sidoides*. In this way, the initial non-phytotoxic concentration (D_0), which would occur at 6.78%, would not be attained due to accumulation in soil solution. However, it is important that application interval of Nemarioc-AL phytonematicide on *P. sidoides* be established.

CHAPTER 4
NEMAFRIC-BL PHYTONEMATICIDE ON *PELARGONIUM SIDOIDES*

4.1 Introduction

Mean concentration stimulation point (MCSP) is phytonematicide-specific (Mashela *et al*., 2015). Therefore, MCSP for Nemarioc-AL phytonematicide as developed previously (Chapter 3) should not be viewed as being similar to that of Nemafric-BL phytonematicide. Raw material for this phytonematicide is derived from mature fruits of an indigenous wild watermelon (*Cucumis africanus* L.) plant, which contain large quantities of cucurbitacin B ($C_{22}H_{48}O_8$) as an active ingredient in all organs (Chen *et al*., 2005). Since phytotoxicity is also plant-specific, MCSP value for Nemafric-BL phytonematicide should be developed for African geranium (*Pelargonium sidoides* DC.). The objective of this study was, therefore, to investigate whether the increasing concentrations of Nemafric-BL phytonematicide applied on nematode-infested *P. sidoides* would generate MCSP for the phytonematicide on the test crop.

4.2 Materials and methods

4.2.1 Location and plant growth conditions

The study was conducted under microplot conditions at the Green Technologies Research Centre (GTRC), University of Limpopo, South Africa (23°53'10"S, 29°44'15"E). Trials were conducted in autumn (March-May) and spring (August-October) 2015. The establishment of micro-plots, pot size, growing mixture, transplanting of *Pelargonium* seedlings and preparation of inoculum were as described previously (Chapter 3). Approximately 40 g ground material *C. africanus* fruit were fermented in 20 L-plastic container with 16 L chlorine-free tapwater as described previously (Chapter 3).

4.2.2 Experimental design, inoculation and cultural practices

Six treatments, namely, 0, 2, 4, 6, 8 and 10% Nemafric-BL phytonematicide concentrations were arranged in a randomised complete block design (RCBD), with seven replicates. Each pot was infested with 5000 *M. javanica* eggs and second-stage juveniles (J2s) as described previously (Chapter 3). Plants were fertilised with 2.5 g 2:3:2 (22) NPK and 1 g 2:1:2 (43) Multifeed as described previously (Chapter 3). Plants were irrigated every other day with 500 ml chlorine-free tapwater. The treatments were applied once a week as substitute to irrigation.

4.2.3 Data collection and analysis

At 56 days after inoculation, in Experiment 1 and 2, data for plant and nematode variables were collected and analysed (Chapter 3). Significant means of plant variables were subjected to the Curve-fitting Allelochemical Response Dosage (CARD) model as described previously (Chapter 3).

4.3 Results

4.3.1 Treatment effects

In Experiment 1, effects of Nemafric-BL phytonematicide were highly significant ($P \leq 0.01$) on plant height and gall rating, but had no effects on chlorophyll content, dry shoot mass, dry tuber mass and dry root mass (Appendix 4.1 - 4.7). Nemafric-BL phytonematicide contributed 63 and 67% in total treatment variation (TTV) of plant height and root galls, respectively (Table 4.1). Relative to untreated control, plant height was increased by 10 to 36%, whereas root galls were reduced by 2.43 to 60% (Table 4.2). In Experiment 2, treatments had no significant effects on all plant variables, except for root galls (Appendix 4.11 - 4.17), with treatments contributing

95% in TTV of root galls (Table 4.1). Root galls were reduced by 0 to 99% with increasing concentration of the phytonematicide (Table 4.5).

In Experiment 1, increasing concentrations of Nemafric-BL phytonematicide had significant effects on eggs and J2s, contributing 78 and 72%, respectively in TTV of the two respective variables (Table 4.6). In Experiment 2, increasing concentrations of Nemafric-BL phytonematicide significantly affected eggs and J2s in roots, contributing 63 and 71%, respectively, in TTV (Appendix 4.8 - 4.10). In Experiment 1, relative to untreated control, eggs were reduced by 96 to 99%, J2s by 52 to 70% and Pf by 95 to 97% (Table 4.7). In Experiment 2, relative to untreated control, eggs were reduced by 97 to 98%, J2s in roots by 66 to 96%, J2s in soil by 57 to 100% and Pf by 91 to 97% (Table 4.7).

4.3.2 Curve-fitting allelochemical response dosage

In Experiment 1, treatments exhibited quadratic relations on plant height and root galls (Figure 4.1), with the model explaining the relationship by 87 and 97% (Table 4.3), which were more or less equivalent to those generated from the CARD model (Table 4.4). Using the relation $X = -b_1/2b_2$, concentrations for optimum plant height and root galls were 5.06 and 108, respectively (Table 4.3). In Experiment 1, MCSP of *P. sidoides* is 2.87% with plant height having k value of k = 1, whereas root galls had k value of k = 2, with overall sensitivity (∑k) of *P. sidoides* being equivalent to three (Table 4.4). In Experiment 2, treatment means of plant variables were not significant at $P \leq 0.05$, therefore, treatment means were not subjected to the CARD model and overall sensitivity (∑k) of *P. sidoides* was not determined.

Table 4.1 Sources of variation as affecting plant height and gall rating at 56 days after initiation of treatments.

		Experiment 1				Experiment 2	
		Plant height		Gall rating		Gall rating	
Source	DF	MS	%	MS	%	MS	%
Rep	6	7.44	20	0.03	16	0.01	1
Trt	5	22.43	63***	0.16	67***	0.31	95***
Error	30	6.01	17	0.03	15	0.01	4
Total	41	35.89	100	0.17	100	0.33	100

*** = highly significance at P ≤ 0.01.

Table 4.2 Effect of fermented crude extracts of Nemafric-BL phytonematicide on chlorophyll content (CC), dry shoot mass (DSM), dry root mass (DRM), number of leaves (NOL), plant height (PHT), dry tuber mass (DTM) and gall rating (GR) of *Pelargonium sidoides* at 56 days after initiation of treatments in Experiment 1.

	CC		DSM (g)		DRM (g)		NOL		PHT (cm)		DTM (g)		GR	
Concentration	Variable	%[z]	Variable	%	Variable	%	Variable	%	Variable[y]	%	Variable	%	Variable	%
0	67.73	−	12.40	−	3.60	−	102.14	−	12.49[c]	−	13.90	−	3.71[a]	−
2	62.13	−8	9.36	−20	2.87	−20	66.57	−35	16.31[ab]	31	13.33	−4	3.62[a]	−2.43
4	62.99	−7	11.06	−11	2.90	−19	91.71	−10	16.99[a]	36	16.24	16	2.71[ab]	−27
6	64.99	−4	10.07	−19	2.49	−31	81.86	−20	16.49[a]	32	13.18	−2	2.30[ab]	−38
8	60.40	−11	9.74	−19	2.33	−35	68.29	−33	14.79ab[c]	18	14.97	11	1.78[bc]	−52
10	65.01	−4	10.27	−17	2.80	−22	89.43	−12	13.74b[c]	10	12.34	−9	1.48[c]	−60
P-value ≤	0.53		0.86		0.77		0.25		0.01		0.74		0.01	

[y]column means followed by same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

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

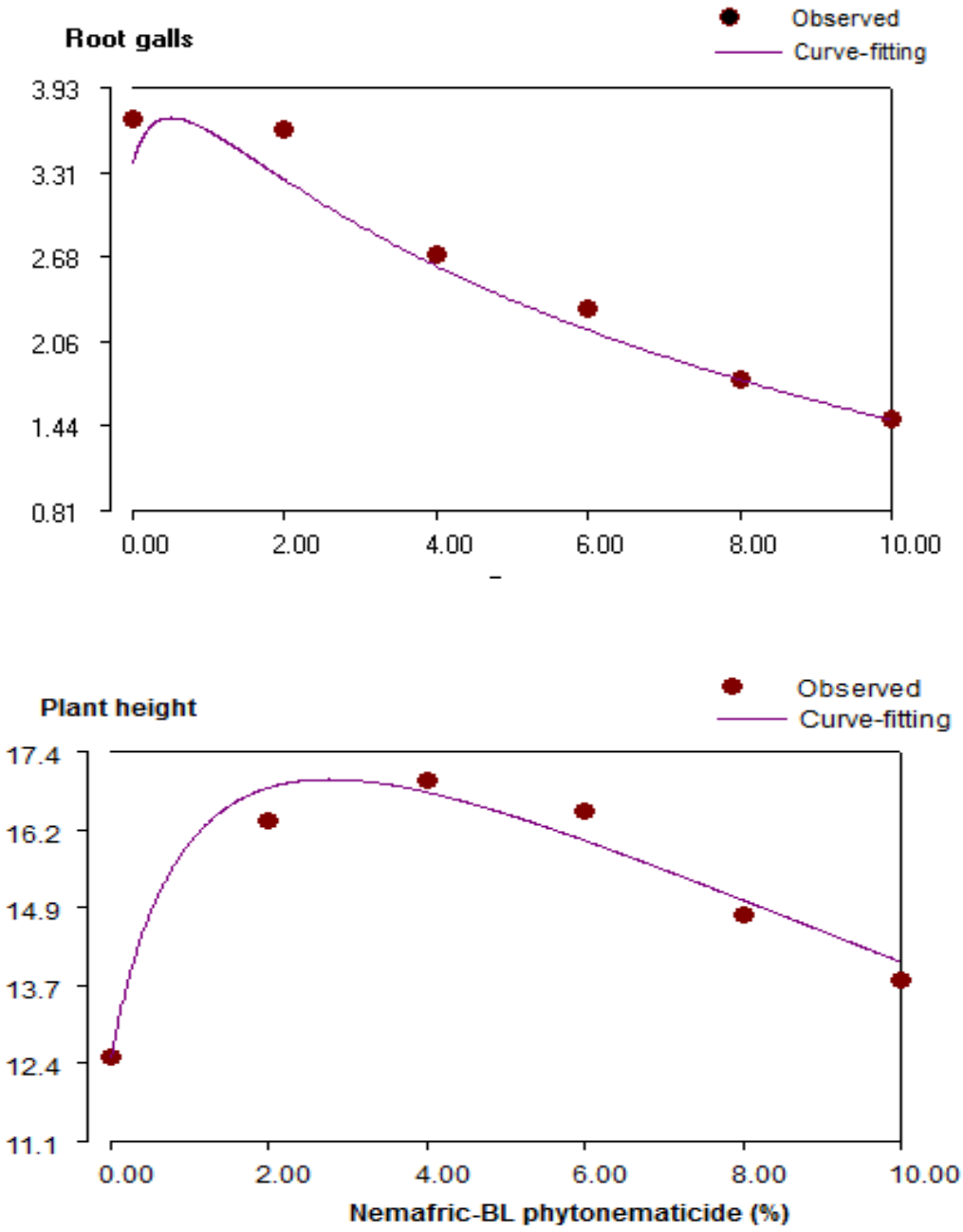

Figure 4.1 Responses of plant height and dry root mass of *P. sidoides* to concentrations of Nemafric-BL phytonematicide in Experiment 1 at 56 days after inoculation.

Table 4.3 Quadratic relationship, coefficient of determination and computed optimum response concentration for variables of *Pelargonium sidoides* from the Curve-fitting Allelochemical Response Dosage against Nemafric-BL phytonematicide at 56 days after treatments in Experiment 1.

Organ	Quadratic relation	R^2	x^z	Y
Root galls	$y = 0.0012x^2 - 0.2592x + 3.845$	0.97	108	−10.2
Plant height	$y = -0.1512x^2 + 1.5291x + 13.034$	0.87	5.056	16.9

$^z x = -b_1/2b_2$.

Table 4.4 Biological indices for dry root mass (DRM) and plant height (PHT) of *Pelargonium sidoides* to increasing concentrations of Nemafric-BL phytonematicide at 56 days after initiation of treatments in Experiment 1.

Biological index^Z	Root galls	Plant height	Mean
Threshold stimulation (D_m)	0.503	2.761	1.632
Saturation point (R_h)	0.4	4.56	2.48
0% inhibition (D_0)	1.666	13.148	7.407
50% inhibition (D_{50})	7.757	27.766	17.6705
100% inhibition (D_{100})	23.8	47.4	35.6
R^2	0.99	0.96	
k-value	2	1	
Overall sensitivity	$\sum k = 3$		

MCSP = $D_m + (R_h/2)$ = 1.632 + 2.48/2= 2.872%.

Table 4.5 Effect of fermented crude extracts of Nemafric-BL phytonematicide on chlorophyll content (CC), dry shoot mass (DSM), dry root mass (DRM), number of leaves (NOL), plant height (PHT), dry tuber mass (DTM) and gall rating (GR) of *Pelargonium sidoides* at 56 days after initiation of treatments in Experiment 2.

	CC		DSM (g)		DRM (g)		NOL		PHT (cm)		DTM (g)		GR	
Concentration	Variable	%	Variable	%	Variable	%	Variable	%	Variable	%	Variable	%	Variable[y]	%[z]
0	67.27	−	9.11	−	2.44	−	197.80	−	19.00	−	8.33	−	2.71[a]	−
2	67.31	0.1	9.95	9	3.79	55	205.78	4	16.29	−14	6.42	−23	0.14[b]	−95
4	68.13	1	9.12	0.1	2.66	9	204.34	3	18.07	−5	5.59	−33	0.14[b]	−95
6	67.31	0.1	9.98	10	3.04	25	206.71	6	16.79	−12	7.37	−12	0.29[b]	−89
8	68.94	2	9.40	3	2.87	18	178.53	−9	17.93	−6	6.50	−22	0.00[b]	0
10	66.00	−2	7.07	−22	2.41	−1	186.44	−5	19.14	1	4.61	−45	0.00[b]	0
P ≤	0.99		0.55		0.70		0.11		0.57		0.38		0.01	

[y]column means followed by same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

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

Table 4.6 Sources of variation as affecting nematode variables at 56 days after initiation of treatments.

		Experiment 1						Experiment 2							
		Eggs		J2s in roots		Pf		Eggs		J2s in roots		J2s in soil		Pf	
Source	DF	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%
Rep	6	0.83	16	0.04	16	0.17	7	0.71	15	0.80	14	0.70	36	0.13	3
Treatment	5	4.10	78***	0.16	72***	2.23	90***	3.02	63**	4.21	71***	0.65	34^{ns}	3.17	70**
Error	30	0.35	6	0.03	12	0.08	3	1.11	22	0.85	15	0.57	30	1.25	27
Total	41	5.28	100	0.23	100	2.52	100	4.84	100	5.86	100	1.91	100	4.55	100

*** = highly significance at P ≤ 0.01, ** = significance at P ≤ 0.05, ^{ns} = not significant at P ≤ 0.05.

Table 4.7 Influence of increasing concentrations of Nemafric-BL phytonematicide on nematode eggs, juveniles (J2s) in roots and final population (Pf) at 56 days after initiation of treatments.

	Experiment 1						Experiment 2							
Concentration	Eggs^{y}	%^{z}	$J2s_{roots}$	%	Pf	%	Eggs	%	$J2s_{roots}$	%	$J2s_{soil}$	%	Pf	%
0	1087^{a}	−	33^{a}	−	1120^{a}	−	2214^{a}	−	352^{a}	−	114	−	2680^{a}	−
2	29^{b}	−97	16^{b}	−52	44^{b}	−96	56a^{b}	−97	72^{b}	−80	0	−100	128^{ab}	−95
4	24^{b}	−98	11^{b}	−70	34^{b}	−97	34^{abc}	−98	38^{b}	−89	0	−100	72^{ab}	−97
6	16^{b}	−99	16^{b}	−52	31^{b}	−97	128^{abc}	−94	120^{b}	−66	0	−100	249^{b}	−91
8	39^{b}	−96	11^{b}	−65	50^{b}	−95	47^{bc}	−98	15^{b}	−96	57	−50	119^{b}	−96
10	26^{b}	−98	14^{b}	−57	40^{b}	−96	77^{c}	−97	13^{b}	−96	57	−50	147^{b}	−95
P ≤	0.01		0.01		0.01		0.04		0.01		0.37		0.05	

^{y}column means followed by same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

^{z}Impact (%) = [(treatment/control) −1] × 100.

4.4 Discussion

In this study significant effects were observed on plant height and root galls of *P. sidoides* when exposed to increasing concentrations of Nemafric-BL phytonematicide. Pelinganga and Mashela (2012) observed significant results on dry shoot, dry root mass, plant height and stem diameter of tomato plant when exposed to increasing concentrations of Nemafric-BL phytonematicide. Similar significant results were observed by Pelinganga *et al*. (2012) when using concentration levels which were above 10%. At higher or lower concentrations of Nemafric-BL phytonematicide plant height seem to respond fast as compared to other plant variables. Aladejimokun *et al*. (2014) observed similar results when exposing cowpea (*Vigna unguiculata* L.) and maize (*Zea mays* L.) to increasing concentrations of *T*. *diversifolia*, the product had no significant effects. Sayed *et al*. (2011) reported similar findings when using *A*. *indica* extracts from 10 to 20 µl on she-oak (*Casuarina equisetifolia* L.). Moringa (*Moringa oleifera* Lam.) leaf extracts was reported to have no significant effect on germination of sorghum (*Sorghum bicolor* L.), maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) by Phiri (2010). In the current study non-significant effects were observed for dry root mass, dry shoot mass, number of leaves and dry tuber mass of *P*. *sidoides*.

Lack of significant effects of Nemafric-BL on *P. sidoides* were not consistent with findings in Pelinganga *et al*. (2012), who reported a general improvement in crop performance in response to the application of Nemafric-BL phytonematicide on tomato plants. These contradictions were in agreement with the hypothesis postulated by others (Mashela *et al*., 2015; Pelinganga and Mashela, 2012; Rice, 1984) that allelopathy was concentration-specific, organ-specific and plant-specific.

Additionally, the neutral effects of Nemafric-BL on *P. sidoides* were consistent with those with of Nemarioc-AL phytonematicide on certain variables of this plant species. Nemafric-BL phytonematicide had stimulatory, neutral and inhibition effects on various plant variables of *P. sidoides*. The stimulation, neutral and inhibition of Nemafric-BL phytonematicide on *P. sidoides* observed in the study were in agreement with the characteristics of density-dependent growth (DDG) patterns in biological systems (Liu *et al*., 2003; Mafeo *et al*., 2011a,b; Mashela *et al*., 2015; Pofu *et al*., 2010). In the current study, the observations were in line with hypothesis postulated by Liu *et al*. (2003), that all biological entities would display a DDG response when exposed to increasing concentrations of allelochemicals. Generally, DDG patterns advocate that there was, depending on the concentration, stimulation, followed by saturation/neutral and then inhibition growth range (Liu *et al*., 2003; Mashela *et al*., 2015; Rice, 1979; Salisbury and Ross, 1992). Neelamegan (2011) reported stimulatory effect of leaf extract of jungle geranium (*Ixora coccinea* L.) on seed germination and seedling growth of paddy (*Oryza sativa* L.). Nasir *et al*. (2005) also reported stimulatory effects of sweet leaf (*Stevia rebaundiana* Hemsl.) on lettuce (*Lactuca sativa* L.) and cucumber (*Cucumis sativus* L.). Bano *et al*. (2012), Musyimi *et al*. (2012) and Tongma *et al*. (1997) observed stimulatory effect of neem (*Azadirachta indica* A. juss.) leaf extracts and Mexican sunflower (*Tithonia diversifolia* Hemsi.) shoot extracts on growth of wild oats (*Avena fatua* L.), spider plant (*Cleome gynandra* L.) and paddy (*Oryza sativa L*.), respectively.

Pelinganga *et al*. (2013b) observed that the increasing concentrations of Nemafric-BL phytonematicide from 10 to 60% was highly phytotoxic to tomato (*Solanum lycopersicum*) plants, with the CARD model suggesting that the dilution should be

below 10% (Pelinganga, 2013). Depending on the concentration range of phytonematicides used, some studies reported stimulation effects (Mashela, 2002; Pelinganga *et al*., 2012, 2013b), whereas some studies reported no significant effects (Aladejimakun *et al*., 2014; Phiri, 2010; Sayed *et al*., 2011) and other reported inhibition effects (Mafeo *et al*., 2011a; Pelinganga and Mashela, 2012).

In the current study, the MCSP value for Nemafric-BL phytonematicide on *P. sidoides* was empirically derived as 2.87%, which appeared to have been similar to that derived for tomato (*Solanum lycopersicum* L.) plants at 2.64% (Pelinganga *et al*., 2012). Because the overall sensitivity index (∑k) of *P. sidoides* to Nemafric-BL phytonematicide was at 3 units, the plant would be moderately sensitive to the product (Mashela *et al*., 2015). Pelinganga and Mashela (2012) demonstrated that tomato plants were moderate sensitive Nemafric-BL phytonematicide, which was off-set by developing the application interval of 18 days for *Meloidogyne* species on tomato plants (Pelinganga, 2013).

All levels of Nemafric-BL phytonematicide were highly effective in suppression of *Meloidogyne* population densities, which confirmed observations of Nemarioc-AL phytonematicide on *P. sidoides* (Chapter 3). The J2s in soil were the most affected stages, with 100% reductions at some concentrations, which confirmed other findings (Mashela *et al*., 2015). Results in this study were in agreement with those reported on tomato plants (Pelinganga and Mashela, 2012). Aqueous extracts of moringa (*Moringa oleifera*), African basil (*Ocimum gratissium*) and neem (*Azadirachta indica*) leaves reduced population densities of *M. incognita* race 2 on infested cowpea (*Vigna unguiculata*) by from 40 to 63.7% (Cladius-Cole *et al*., 2010).

4.5 Conclusion

Nemafric-BL phytonematicide exhibited stimulatory effects on growth of *P. sidoides* with moderate sensitivity, and was able to suppress the population densities of *M. javanica* at times by as high as 100%. However 2.87% of dilution would be recommended in order to avoid any initiation of phytotoxicity to *P. sidoides*.

CHAPTER 5 SUMMARY, SIGNIFICANCE, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary

Phytotoxicity is a major challenge in the implementation of phytonematicides as an alternative to methyl bromide for managing nematode population densities. The study was initiated to determine non-phytotoxic concentrations of Nemarioc-AL and Nemafric-BL phytonematicides using the Curve-fitting Allelochemical Response Dosage (CARD) computer-based model, which provided seven biological indices (Liu *et al*., 2003; Mashela *et al*., 2015). The first two biological indices (D_m and R_h) were used in computation of the Mean Concentration Stimulation Point (MCSP) for African geranium (*Pelargonium sidoides* DC.) when exposed to increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides in the management of root-knot nematode (*Meloidogyne javanica* Treub.), whereas the k-values were used to determine the overall sensitivity of *P. sidoides* to the two phytonematicides.

5.2 Significance of findings

In Experiment 1, Nemarioc-AL phytonematicide had significant effects on plant height, dry root mass and root galls of *P. sidoides*, whereas the product had no effect on other plant variables. In Experiment 2, plant variables were not significantly affected by this material. In Experiment 1, plant variables and concentrations of Nemarioc-AL phytonematicide had density-dependent growth (DDG) patterns, which had three phases, stimulation, saturation/neutral and inhibition. The CARD model was used to compute the biological indices for stimulation, saturation and inhibition

phases, with Mean Concentration Stimulation Point [MCSP = D_m + (R_h/2)] for Nemarioc-AL being 6.18%, whereas the overall sensitivity was ∑k = 3 (Liu *et al*., 2003; Mashela *et al*., 2015). The product reduced population densities of *M. javanica* in Experiment 1 and 2 by 88 - 94% and 1 - 88%, respectively. In Experiment 1, *P. sidoides was* sensitive to Nemafric-BL phytonematicide, with overall sensitivity of 3 and MCSP of 2.87%. In this Experiment Nemafric-BL phytonematicide significantly reduced final population densities of *M. javanica* by 95 - 97% and in Experiment 2 by 91 - 97%. Notably, in some cases J2s in soil were reduced by 100%.

5.3 Recommendations

Nemarioc-AL and Nemafric-BL phytonematicides could be applied at MCSP = 2%, which should be validated under various conditions. The 2% should be used to establish the application interval and eventually the dosage model as described elsewhere (Mashela *et al*., 2015). Because the tuber is the edible part of the plant, residues of the two phytonematicides should be established, since at low concentrations, cucurbitacins could be cancerous to human beings (Lee *et al*., 2010).

5.4 Conclusions

Nemarioc-AL and Nemafric-BL phytonematicides could, upon validation and determination of dosage model, be suitable for use through botinemagation on *P. sidoides* to manage nematodes. The two products could each be used at 2%, provided the active ingredients do not accumulate in tubers.

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APPENDICES

Appendix 3.1 Sources of variation as affecting nematode variables at 56 days after initiation of treatments.

		Experiment 1						Experiment 2							
		Eggs		J2s roots (g)		Pf		Eggs		J2s roots (g)		J2s soil (ml)		Pf	
Source	DF	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%
Rep	6	0.29824	6	0.62108	31	0.04134	21	1.55178	33	1.84510	39	0.80419	35	1.28642	21
Treatment	5	4.13628	81***	0.89271	45**	0.11204	57***	1.81957	40^{ns}	2.27768	48***	0.61871	27^{ns}	4.04239	67
Error	30	0.66701	13	0.47457	24	0.04472	22	1.20291	27	0.66544	14	0.85985	38	0.69270	12
Total	40	5.10153	100	1.98836	100	0.1981	100	4.64045	100	4.78802	100	2.28985	100	6.02151	100

*** = highly significance at P ≤ 0.01, ** = significance at P ≤ 0.05, ^{ns} = not significant at P ≤ 0.05.

Appendix 3.2 Analysis of variance for chlorophyll content of *Pelargonium sidoides* to Nemarioc-AL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	313.11	52.18		
Treatment	5	428.90	85.78	1.00	0.43
Error	30	2584.37	86.14		
Total	41	3326.38			

Appendix 3.3 Analysis of variance for dry shoot mass (g) of *Pelargonium sidoides* to Nemarioc-AL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	102.68	17.11		
Treatment	5	70.65	14.13	0.85	0.52
Error	30	498.46	16.62		
Total	41	671.78			

Appendix 3.4 Analysis of variance for dry root mass (g) of *Pelargonium sidoides* to Nemarioc-AL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	6.45	1.07		
Treatment	5	37.08	7.42	3.22	0.02
Error	30	69.14	2.30		
Total	41	112.67			

Appendix 3.5 Analysis of variance for number of leaves of *Pelargonium sidoides* to Nemarioc-AL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	0.22	0.04		
Treatment	5	0.16	0.03	0.72	0.61
Error	30	1.36	0.05		
Total	41	1.74			

Appendix 3.6 Analysis of variance for plant height (cm) of *Pelargonium sidoides* to Nemarioc-AL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	102.09	17.02		
Treatment	5	177.42	35.49	7.00	0.01
Error	30	152.13	5.07		
Total	41	413.65			

Appendix 3.7 Analysis of variance for dry tuber mass (g) of *Pelargonium sidoides* to Nemarioc-AL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	143.43	23.90		
Treatment	5	269.11	53.82	0.64	0.67
Error	30	2526.48	48.23		
Total	41	2939.02			

Appendix 3.8 Analysis of variance for gall rating of *Pelargonium sidoides* to Nemarioc-AL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	0.06	0.01		
Treatment	5	0.24	0.05	5.02	0.01
Error	30	0.29	0.01		
Total	41	0.59			

Appendix 3.9 Analysis of variance for juveniles of *Meloidogyne javanica* at 56 days after inoculation and initiation of treatments of Nemarioc-AL phytonematicide.

Source	DF	SS	MS	F	P
Rep	6	7590.5	1265.08		
Treatment	5	8561.9	1712.38	2.88	0.05
Error	30	17838.1	594.6		
Total	41	33990.5			

Appendix 3.10 Analysis of variance for eggs of *Meloidogyne javanica* at 56 days after inoculation and initiation of treatments of Nemarioc-AL phytonematicide.

Source	DF	SS	MS	F	P
Rep	6	14429	2405		
Treatment	5	727448	145490	45.81	0.01
Error	30	95286	3176		
Total	41	837162			

Appendix 3.11 Analysis of variance for the final population densities of *Meloidogyne javanica* at 56 days after inoculation and initiation of treatments of Nemarioc-AL phytonematicide.

Source	DF	SS	MS	F	P
Rep	6	24424	4071		
Treatment	5	878857	175771	38.78	0.01
Error	30	135976	4533		
Total	41	1039257			

Appendix 3.12 Analysis of variance for chlorophyll content of *Pelargonium sidoides* to Nemarioc-AL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Reps	6	311.86	51.98		
Treatment	5	266.18	53.28	0.98	0.44
Error	30	1626.77	54.23		
Total	41	2204.81			

Appendix 3.13 Analysis of variance for dry shoot mass (g) of *Pelargonium sidoides* to Nemarioc-AL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	88.61	14.78		
Treatment	5	24.61	4.92	0.50	0.77
Error	30	295.40	9.85		
Total	41	408.61			

Appendix 3.14 Analysis of variance for dry root mass (g) of *Pelargonium sidoides* to Nemarioc-AL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	13.77	2.29		
Treatment	5	8.74	1.75	0.61	0.69
Error	30	85.42	2.85		
Total	41	107.93			

Appendix 3.15 Analysis of variance for number of leaves of *Pelargonium sidoides* to Nemarioc-AL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	0.27	0.05		
Treatment	5	0.16	0.03	0.54	0.74
Error	30	1.74	0.06		
Total	41	2.17			

Appendix 3.16 Analysis of variance for plant height of *Pelargonium sidoides* to Nemarioc-AL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	82.82	13.80		
Treatment	5	24.64	4.93	0.53	0.75
Error	30	280.11	9.34		
Total	41	387.57			

Appendix 3.17 Analysis of variance for dry tuber mass (g) of *Pelargonium sidoides* to Nemarioc-AL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	24801	4133.58		
Treatment	5	21385	4277.06	0.99	0.44
Error	30	12043	4301.42		
Total	41	17529			

Appendix 3.18 Analysis of variance for gall rating of *Pelargonium sidoides* to Nemarioc-AL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	0.03	0.00		
Treatment	5	1.39	0.28	128.07	0.01
Error	30	0.07	0.00		
Total	41	1.48			

Appendix 3.19 Analysis of variance for juveniles in roots of *Meloidogyne javanica* at 56 days after inoculation and initiation of treatments of Nemarioc-AL phytonematicide.

Source	DF	SS	MS	F	P
Rep	6	11.07	1.85		
Treatment	5	11.39	2.28	0.42	0.1
Error	30	19.96	0.67		
Total	41	42.42			

Appendix 3.20 Analysis of variance for eggs of *Meloidogyne javanica* at 56 days after inoculation and initiation of treatments of Nemarioc-AL phytonematicide.

Source	DF	SS	MS	F	P
Rep	6	9.31	1.55		
Treatment	5	9.09	1.82	1.44	0.24
Error	30	37.89	1.26		
Total	41	56.30			

Appendix 3.21 Analysis of variance for juveniles in soil of *Meloidogyne javanica* at 56 days after inoculation and initiation of treatments of Nemarioc-AL phytonematicide.

Source	DF	SS	MS	F	P
Rep	6	4.83	0.80		
Treatment	5	3.09	0.62	0.72	0.61
Error	30	25.80	0.86		
Total	41	33.71			

Appendix 3.22 Analysis of variance for final population densities of *Meloidogyne javanica* at 56 days after inoculation and initiation of treatments of Nemarioc-AL phytonematicide.

Source	DF	SS	MS	F	P
Rep	6	7.72	1.29		
Treatment	5	20.21	4.04	5.84	0.01
Error	30	20.78	0.69		
Total	41	48.71			

Appendices for Chapter 4

Appendix 4.1. Analysis of variance for chlorophyll content of *Pelargonium sidoides* to Nemafric-BL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	346.10	57.6827		
Treatment	5	233.09	46.6174	0.83	0.54
Error	30	1684.04	56.1346		
Total	41	2263.22			

Appendix 4.2. Analysis of variance for dry shoot mass (g) of *Pelargonium sidoides* to Nemafric-BL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	104.150	17.3583		
Treatment	5	42.238	8.4477	0.38	0.86
Error	30	670.170	22.3390		
Total	41	816.558			

Appendix 4.3. Analysis of variance for dry root mass (g) of *Pelargonium sidoides* to Nemafric-BL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	26.358	4.39302		
Treatment	5	6.793	1.35852	0.50	0.77
Error	30	81.159	2.70530		
Total	41	114.310			

Appendix 4.4. Analysis of variance for number of leaves of *Pelargonium sidoides* to Nemafric-BL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	0.24527	0.04088		
Treatment	5	0.26603	0.05321	1.42	0.25
Error	30	1.2420	0.03747		
Total	41	1.63551			

Appendix 4.5. Analysis of variance for plant height (cm) of *Pelargonium sidoides* to Nemafric-BL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	44.660	7.4433		
Treatment	5	112.170	22.4341	3.73	0.01
Error	30	180.346	6.0115		
Total	41	337.176			

Appendix 4.6. Analysis of variance for dry tuber mass (g) of *Pelargonium sidoides* to Nemafric-BL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	449.24	74.8728		
Treatment	5	68.79	13.7573	0.54	0.74
Error	30	762.94	25.4313		
Total	41	1280.96			

Appendix 4.7. Analysis of variance for dry shoot mass (g) of *Pelargonium sidoides* to Nemafric-BL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	0.16066	0.02678		
Treatment	5	0.57779	0.11556	4.39	0.01
Error	30	0.78946	0.02632		
Total	41	1.52791			

Appendix 4.8. Analysis of variance for juveniles of *Meloidogyne javanica* at 56 days after inoculation and initiation of treatments of Nemarioc-AL phytonematicide.

Source	DF	SS	MS	F	P
Rep	6	7590.5	1265.08		
Treatment	5	8561.9	1712.38	2.88	0.05
Error	30	17838.1	594.6		
Total	41	33990.5			

Appendix 4.9. Analysis of variance for eggs of *Meloidogyne javanica* at 56 days after inoculation and initiation of treatments of Nemarioc-AL phytonematicide.

Source	DF	SS	MS	F	P
Rep	6	14429	2405		
Treatment	5	727448	145490	45.81	0.01
Error	30	95286	3176		
Total	41	837162			

Appendix 4.10. Analysis of variance for final population densities of *Meloidogyne javanica* at 56 days after inoculation and initiation of treatments of Nemarioc-AL phytonematicide.

Source	DF	SS	MS	F	P
Rep	6	24424	4071		
Treatment	5	878857	175771	38.78	0.01
Error	30	135976	4533		
Total	41	1039257			

Appendix 4.11. Analysis of variance for chlorophyll content of *Pelargonium sidoides* to Nemafric-BL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Reps	6	336.11	56.0176		
Treatment	5	33.94	6.7872	0.08	0.99
Error	30	2669.32	88.9772		
Total	41	3039.36			

Appendix 4.12 Analysis of variance for dry shoot mass (g) of *Pelargonium sidoides* to Nemafric-BL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	150.880	25.1466		
Treatment	5	39.905	7.9810	0.81	0.55
Error	30	293.923	9.7974		
Total	41	484.708			

Appendix 4.13. Analysis of variance for dry root mass (g) of *Pelargonium sidoides* to Nemafric-BL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	11.566	1.92769		
Treatment	5	9.260	1.85192	0.60	0.70
Error	30	92.840	3.09466		
Total	41	113.666			

Appendix 4.14. Analysis of variance for number of leaves of *Pelargonium sidoides* to Nemafric-BL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	0.09999	0.01666		
Treatment	5	0.20169	0.04034	1.99	0.11
Error	30	0.60930	0.02031		
Total	41	0.91097			

Appendix 4.15. Analysis of variance for plant height of *Pelargonium sidoides* to Nemafric-BL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	629.82	104.97		
Treatment	5	46.39	9.277	0.78	0.57
Error	30	357.32	11.911		
Total	41	1033.53			

Appendix 4.16. Analysis of variance for dry tuber mass (g) of *Pelargonium sidoides* to Nemafric-BL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	29.537	4.9229		
Treatment	5	59.399	11.8798	1.10	0.38
Error	30	324.039	10.8013		
Total	41	412.975			

Appendix 4.17. Analysis of variance for gall rating of *Pelargonium sidoides* to Nemafric-BL phytonematicide under microplot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	6	0.02677	0.00446		
Treatment	5	1.556217	0.31043	29.98	0.01
Error	30	0.31068	0.01036		
Total	41	1.88962			

Appendix 4.18. Analysis of variance for juveniles of *Meloidogyne javanica* at 56 days after inoculation and initiation of treatments of Nemafric-BL phytonematicide.

Source	DF	SS	MS	F	P
Rep	6	7590.5	1265.08		
Treatment	5	8561.9	1712.38	2.88	0.05
Error	30	17838.1	594.6		
Total	41	33990.5			

Appendix 4.19. Analysis of variance for eggs of *Meloidogyne javanica* at 56 days after inoculation and initiation of treatments of Nemafric-BL phytonematicide.

Source	DF	SS	MS	F	P
Rep	6	14429	2405		
Treatment	5	727448	145490	45.81	0.01
Error	30	95286	3176		
Total	41	837162			

Appendix 4.20. Analysis of variance for juveniles in soil of *Meloidogyne javanica* at 56 days after inoculation and initiation of treatments of Nemafric-BL phytonematicide.

Source	DF	SS	MS	F	P
Rep	6	7590.5	1265.08		
Treatment	5	8561.9	1712.38	2.88	0.05
Error	30	17838.1	594.6		
Total	41	33990.5			

Appendix 4.21. Analysis of variance for final population densities of *Meloidogyne javanica* at 56 days after inoculation and initiation of treatments of Nemafric-BL phytonematicide.

Source	DF	SS	MS	F	P
Rep	6	24424	4071		
Treatment	5	878857	175771	38.78	0.01
Error	30	135976	4533		
Total	41	1039257			