MEAN CONCENTRATION STIMULATION POINT OF NEMARIOC-AL AND NEMAFRIC-BL PHYTONEMATICIDES ON *CUCURBITA PEPO* CULTIVAR 'CASERTA'

BY

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DECLARATION

I declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree Master of Science in Agriculture (Horticulture) has not previously been submitted by me for a degree at this or any other university or anybody 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 has been duly acknowledged.

Lebea, M.P. (Miss) Date

DEDICATION

To my ever supportive parents, Mr M.S. Lebea and Mrs M.E. Lebea, and my siblings.

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ABSTRACT

Butternut squash (*Cucurbita pepo*) is highly susceptible to root-knot (*Meloidogyne* species) nematodes. Nemafric-BL and Nemarioc-AL phytonematicides were being researched and developed for use in various crop farming systems for managing nematode numbers. However, the two products when not properly quantified are highly phytotoxic to crops. The Curve-fitting Allelochemical Response Dosage (CARD) computer based model was adopted to compute the Mean Concentration Stimulation Point (MCSP), which is a non-phytotoxic concentration. The objective of the study, therefore, was to determine whether the MCSP values of Nemarioc-AL and Nemafric-BL phytonematicides on squash under greenhouse, microplot and field conditions exist. Seedling were raised in 25-cm plastic bags filled with loam, pasteurised sand and Hygromix 2:1:1 (v/v) in the greenhouse , raised in 25-cm pots with pasteurised sand and loam 3:1 (v/v) on the microplot, and raised under field with Hutton sandy loam (65% sand, 30% clay and 5% silt). After establishment each plant was inoculated with 5 000 eggs and second-stage juveniles (J2) of *M. incognita*. Treatments comprised 0, 2, 4, 8, 16 and 32% concentration of Nemarioc-AL and Nemafric-BL phytonematicides with ten replicates. For greenhouse, treatments comprised 0.0, 0.8, 1.6, 3.2, 6.4 and 12.8% concentration of both Nemarioc-AL and Nemafric-BL phytonematicide with 10 replicates. For micro-plot and for field experiment treatments comprised 2.4, 4.8, 9.6, 19.2 and 38.4% of both Nemarioc-AL and Nemafric-BL with nine replicates. In all experiments, treatments were arranged in a randomised complete block design with ten replicates. In the greenhouse, Nemafric-BL phytonematicide had highly significant effects on dry fruit mass and significant on fruit number, but had no effect other plant variables recorded.

Treatments contributed 51 to 71% in total treatment variation (TTV) of dry fruit mass and fruit number, respectively. However, at higher concentrations the same phytonematicide decreased fruit number by 66 to 137% and dry fruit mass by 6 to 14%. In the greenhouse, MCSP value for Nemafric-BL phytonematicide was 2.83% of which the overall ∑k was 3 units. Plant variables and increasing concentration of phytonematicide exhibited quadratic relations. In microplot, Nemarioc-AL was highly significant for dry shoot mass and dry fruit mass with treatment contribution of 15 to 63% in TTV. At lower concentrations Nemarioc-AL phytonematicide increased dry shoot mass by 5%. However, with increasing concentrations dry shoot mass decreased from 7 to 30%. Phytonematicide increased dry shoot mass from 41 to 81% and decreased root galls from 3 to 73%. In microplot, MCSP value was 11.85%, with the ∑k zero. Plant variables and increasing concentration of phytonematicide exhibited quadratic relations. In field experiment, Nemarioc-AL and Nemafric-BL phytonematicide treatment effect were not significant on any plant variables. In conclusion, the MCSP and ∑k values appear to be location-specific since they were not similar in various locations.

CHAPTER 1
RESEARCH PROBLEM

1.1 Background

Rising withdrawal of synthetic chemical nematicides which happen to be environment-unfriendly, from the agro-chemical markets had undesired effects of the root-knot nematodes (*Meloidogyne* species) in butternut squash production. Globally, the *Meloidogyne* species remain the most troublesome soil-borne pathogen in crop production as a whole (Mashela *et al*., 2011). Following the withdrawal, the Green Technologies Research Centre researched and developed two phytonematicides, Nemarioc-AL and Nemafric-BL, to serve as substitutes for methyl bromide, which was a common synthetic fumigant nematicide that was used in various crop farming systems (Mashela *et al*., 2015). However, the successful utilisation of allellochemicals in management of plant-parasitic nematodes depended on the degree of phytotoxicity on the crops protected against nematodes. Moreover, conventional methods for determining phytotoxicity are tedious, sometimes resulting in inconsistent results in nematode suppression (Mashela *et al*., 2015). According to Mashela *et al*. (2015), the success of the phytonematicides depends upon allellochemicals as active ingredients which are naturally phytotoxic to plants within different plant species (Mashela *et al*., 2015). Therefore, the Curve-fitting Allellochemical Response Dosage (CARD) model was adopted for use in the development of two phytonematicides, Nemarioc-AL and Nemafric-BL, referred to as Mean Concentration Stimulation Point (MCSP). The MCSP is defined as the non-phytotoxic concentration that should consistently suppress nematodes numbers while stimulating plant growth and it is crop specific (Mashela *et al*., 2011).

Phytotoxicity limits the use of phytonematicides since it may unintentionally induce high yield losses (Mahmood *et al*., 1979). Phytonematicides have resulted in the death of the plant being protected from the pathogen in some cases, which turns out to be very uneconomic in terms of profit making for the commercial producers (Setia *et al*., 2007). This is mainly because there is zero tolerance to phytotoxicity in products used for the protection of crops towards pest in most countries. Phytotoxicity induced imbalances manifested as deficiencies and toxicities of nutrient elements in many crops.

Allellochemicals are said to be the main active ingredients on which the phytonematicides rely. The main problem with having allellochemicals as the active ingredients for the phytonematicides is that they are declared to be naturally phytotoxic to plant species during interference (Okwute, 2012). This happens because, in order to hamper plant growth, allellochemicals after accumulating they persist at phytotoxic levels in the rhizosphere soil. This may results into the compounds with modified biological properties (Ahmad *et al*., 2007). Nevertheless, most phytonematicides lose their nematode suppression capabilities and are accompanied by terrible high phytotoxicity levels on crops being protected against nematodes (Okwute, 2012).

The allellochemicals compounds as the main ingredients to the phytonematicides, determine the effectiveness, quality and performance of phytonematicides (Rice, 1984; Shadung *et al*., 2016). Active ingredients in phytonematicides are secondary metabolites and are in a state of continuous change (Luckner, 1984) due to microbial degradation and auto-oxidation (Gunatilaka, 2006). In plants, allellochemicals are classified to avoid

toxicity complex to the present cells (Rice, 1984). Allellochemicals have been used as potent pesticides, and have been widely used in medicine (Rice, 1984), with an outcome that save patients in medical area (Fujii and Hiradate, 2007). Generally, most allellochemicals affect biological systems through density-dependent growth (DDG) patterns, which have three phases, stimulation, neutral and inhibition phases (Liu *et al*., 2003). The stimulation phase of these materials had been used to generate non-phytotoxic concentrations of phytonematicides on various commercial crop cultivars (Mafeo *et al*., 2011; Pelinganga and Mashela, 2012). The MCSP was shown to be useful in generating non-phytotoxic concentrations of environment-friendly phytonematicides (Mashela *et al*., 2015). The current study focused on developing the MCSP for Nemarioc-AL and Nemafric-BL phytonematicides on squash. Developed MCSR values would be such that they reduce population densities of *Meloidogyne* species on squash without inducing any phytotoxicity.

1.2 Problem statement

Mitigation of phytotoxicities in the successful use of Nemarioc-AL and Nemafric-BL phytonematicides on commercial squash production in the management of *Meloidogyne* species can be active. However, the two phytonematicides are derived from plant species which are different from *C. pepo* and, therefore, their active ingredients could be phytotoxic. The developed MCSP, which is plant species-specific, would help in resolving the challenge of phytotoxicity of phytonematicides in squash production.

1.3 Rationale of the study

Development of MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides on squash would allow for the concentrations of each product to be non-phytotoxic and also consistently suppress nematode numbers (Mashela *et al*., 2015). The MCSP values are the concentrations to be used at each irrigation, which would in future be required to determine the application interval and the dosage model (Mashela *et al*., 2015) of the two phytonematicides on squash production. Eventually, this would allow for the registration of the two products on squash for management of *Meloidogyne* species and thereby ensuring that the South African squash industry remains competitive in job and wealth creation, with improved environmental and human health benefits.

1.4 Purpose

1.4.1 Aim

The aim of the study was to develop the non-phytotoxic concentration values for Nemarioc-AL and Nemafric-BL phytonematicides on *C. pepo*.

1.4.2 Objective

To determine the non-phytotoxic concentrations of Nemarioc-AL and Nemafric-BL phytonematicides on squash under greenhouse, microplot and field using MCSP.

1.4.3 Hypothesis

The MCSP values for Nemarioc-AL and Nemafric-BL concentrations on squash under greenhouse, microplot and field conditions do not exist.

1.5 Reliability, validity and objectivity

The reliability of data was based on statistical analysis of data at the probability level of 5 %; validity was achieved through repeating the experiments in time, whereas the objectivity was achieved by ensuring that the findings are discussed on the basis of empirical evidence, in order to eliminate 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 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 outline of the research problem (Chapter 1), the work done and not yet done on the problem statement was reviewed (Chapter 2). Then the subsequent chapter (Chapter 3) addressed the single objective of this report. In the final chapter (Chapter 4), findings were summarized and integrated to provide the significance of the findings and the recommendations with respect to future research, culminating in a conclusion which tied together the entire study. Citations and references were used following the Harvard style as prescribed by Senate of the University of Limpopo.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Worldwide, root-knot (*Meloidogyne* species) nematodes continued to be one of the most devastating soil-borne pathogens in crop production (Cancino *et al*., 2009). Infestation is frequently reported from areas lacking resources and is a major constraint in household food security in South Africa (Khosa, 2013; Ntidi *et al*., 2012). Following the withdrawal of synthetic fumigant nematicides from agrochemical markets, there has been a call for enough research and development of strategies that would serve as alternatives for the synthetic chemicals. For this reason, plant extracts have been used widely as an alternative and economically feasible option (Malungane, 2014). Thus, this review focuses on what has been written already on the research problem and existing gaps.

2.2 Work done on the problem statement

2.2.1 Success in phytonematicides

Four phytonematicides had been produced in South Africa (Mashela *et al*., 2015), two in granular formulation and the other two in liquid formulation. Their success is better reusing their reviewed using their mode of application of the phytonematicides, bioactivities and quality protocols.

2.2.1.1 Ground leaching technology

The ground leaching technology (GLT) involves the application of ground materials from selected plant organs in small quantities (Mashela, 2002; Mashela *et al*., 2011). Nemarioc-AG phytonematicide was assessed as a soil amendment (Mashela, 2002), to

develop the GLT systems. Mature fruits of *Cucumis myriocarpus* or *Cucumis africanus* are cut into small pieces, dried at 52% (Makkar,1999) for 72 h and ground in Wiley mill to pass through a 1-mm-pore sieve (Mashela, 2002). Nemarioc-AG phytonematicide was spread in small quantities of 2-5 g/plant in a shallow hole at the base of the stem of the transplant (Mashela, 2002). According to Mashela and Mphosi (2002), the materials were applied at transplanting without allowing them to undergo any microbial degradation. Mashela *et al*. (2011) demonstrated that the materials used in GLT could be applied in small quantities of 0.20 to 0.71 t/ha (Mashela and Mphosi, 2002), which prevented the high costs which could have been incurred when transporting the materials to fields.

Post-emergent application: Nemarioc-AG phytonematicide suppressed plant-parasitic nematodes in greenhouse trials by over 90% (Mashela, 2002), in microplot trials by over 90% (Mofokeng *et al*., 2004) and field trials by over 80% (Mashela *et al*., 2011). Nemarioc-AG phytonematicide was shown to have no effect on soil pH, but increased soil electrical conductivity (EC) by 95 to 160% (Mashela *et al*., 2011). The phytonematicide improved tomato fruit yield and plant growth, in what was referred to as having fertiliser effect (Mashela, 2007; 2002). However, detailed analysis of nutrient elements did not result in substantial differences in tissue nutrient elements of treated and untreated plants (Mashela, 2002). Fruit tissues of *C. myriocarpus* were shown to have high quantities of Fe (Mashela, 2002).

In GLT systems, microbial decomposition played a role in the efficacy of Nemarioc-AG phytonematicide it. The independence of GLT system from microbial degradation was

demonstrated trough elimination of *Bacillus* species when using crude extracts of castor (*Ricinus communis*) bean (Mphosi *et al*., 2004) and fever tea (*Lippia javanica*) leaves (Mashela *et al*., 2010). The results, therefore, demonstrated that the efficacy of crude extracts was dependent on leaching through irrigation water, which was analogous to the use of granular synthetic nematicides.

Pre-emergent application: Crop yield losses are generally proportional to the initial population densities (Pi) of nematodes (Seinhorst, 1967). The use of materials in GLT systems is as a pre-emergent bio-nematicide and they keep the initial population densities (Pi) of nematodes at the lowest level as possible. In a seed germination study, Mafeo and Mashela (2009a) observed that at 5 g Nemarioc-AG phytonematicide was highly phytotoxic to dicotyledonous seedlings of tomato, watermelon and butternut squash, with the same findings noted in monocotyledonous seedlings such as maize, finger millet and sorghum and onion (Mafeo and Mashela, 2009b).

Cucumin, from Nemarioc-AG phytonematicide has capabilities to suppress the division of cancer cells in animals at high concentrations – where cytotoxicity was observed, whereas at low concentrations the material stimulated cell division (Van Wyk *et al*., 1997). Concentrations of Nemarioc-AG phytonematicide *ex vitro* were reduced from 0 to 2.25 g\plant due to stimulatory effects observed *in vitro* on germination of tomato, watermelon and butternut squash, which resulted in stimulation of plant growth, following positive quadratic relationships (Mafeo and Mashela, 2009b). Findings in all trials, however, suggested that Nemarioc-AG phytonematicide have had allelopathic effects on seed

germination of the test plants and it was concluded that the material was not suitable for use as pre-emergent bio-nematicide. Studies were, therefore, further initiated using the Curve-fitting Allelochemical Response Dosage (CARD) computer based model (Liu *et al*., 2003) to develop concentrations where Nemarioc-AG phytonematicide could stimulate plant growth, had no effect or inhibited germination of various crops, with the view of establishing the pre-emergent quantities (Mafeo and Mashela, 2010; Mafeo *et al*., 2010). The CARD model demonstrated that the 18 test crops had various sensitivity (k) values to Nemarioc-AG phytonematicide, (Mafeo and Mashela, 2010; Mafeo *et al*., 2011).

Malungane (2014) tested crude extracts of *Tubaghia violacea* (wild garlic) on growth of tomato and observed that the product increased plant growth when compared with untreated plants that were infected with *Meloidogyne* species. Plants infected with *Meloidogyne* species exhibited stunted shoot growth and increased root mass – probably due to root galls. The product, however, did not have any effect on soil pH and EC, but reduced population densities of *M. incognita* race 2 at all treatment levels. Generally, on the product reduced nematode population densities more in roots than in soil samples, suggesting that the active ingredients had the ability to penetrate roots (Malungane, 2014).

Khosa (2013) applied phytonematicides from selected wild plant species that are indigenous to Limpopo Province and observed that all used plant species had nematicidal activity to *M. incognita* on tomato under both greenhouse and microplot conditions. Khosa (2013) also confirmed the incidence of phytotoxicity from some products, whereas others

resulted in stimulatory effects as initially observed by Mashela (2002). In all the studies, Khosa (2013) used Nemarioc-AG phytonematicide as a standard, with the product asserting its superiority.

Neem (*Azadirachta indica*) had been widely used as plant extracts with nematoxic properties in a number of studies in Asia, particularly in India, where the tree has the centre of biodiversity (Akhtar and Malik, 2000; Oka 2010). Most original neem studies on nematode management were conducted between 1971 and 1981, with nematicidal activities against nematode numbers (Muller and Gooch, 1982). Neem extracts also enhanced the performance of other organic amendments when used in combination (Oka, 2010). Zasada *et al*. (2006) reported that *M. incognita* eggs were less sensitive to crude aqueous extracts of velvet bean than J2.

2.2.1.2 Botinemagation

Botinemagation is defined as the use of phytonematicides through irrigation systems (Mashela *et al*., 2011). This technology was developed to mitigate the drawbacks associated with the GLT system, primarily its labour-intensiveness (Pelinganga, 2013). Nemarioc-AG phytonematicide was successfully used in suppression of plant-parasitic nematodes when leached out through irrigation water in GLT system (Mashela, 2002). The GLT system was only suitable for smallscale farming communities (Pelinganga *et al*., 2013). In the botinemagation technique, the ground fruits were first fermented (Mashela *et al*., 2015), with the products applied through irrigation system, thereby overcoming the challenge of labour costs associated with the GLT system in botinemagation,

phytonematicides derived from fermented crude extracts of *C. africanus* and *C. myriocarpus* fruits were referred to Nemafric-BL (L = liquid formulation) and Nemarioc-AL (L = liquid formulation) phytonematicides. The two products reduced nematode population densities by 89% and 69%, respectively (Pelinganga *et al*., 2011), with recent results suggesting that population reductions could go as high as 100% (Seshweni, 2017; Sithole, 2016).

At low dilutions both products had consistently stimulation effects on growth of different crops, whereas at high dilutions each was consistently phytotoxic (Mashela *et al*., 2015). Results of the study (Pelinganga *et al*., 2011) demonstrated that the two products could serve as potent bio-nematicides at low concentrations. Similarly, Pelinganga *et al*. (2012) tested the MCSP values of the two phytonematicides on tomato plants infected with nematodes, resulting in final nematode population densities for *M. incognita* being reduced by at least 90% when compared with untreated controls.

Sithole *et al*. (2016) tested Nemarioc-AL and Nemafric-BL phytonematicides on geranium (*Pelargonium* species), with the products reducing nematode numbers by as high as 100% when compared with untreated control. The two products were also tested on *Citrus volkameriana* seedlings, with the results suggesting that citrus was highly sensitive to both (Mathabatha *et al*., 2016).

2.2.1.3 Bioactivities of phytonematicides

Generally, phytonematicides have multiple active ingredients, with complementary mode of actions (Mashela *et al*., 2015). Egress, which is a physical process, in most plant-parasitic nematodes, is stimulated by external chemical cues from roots (Prot, 1980). The body of a nematode, mainly the frontal and cervical regions, is covered with multiple chemo-receptors (Ferraz and Brown, 2002). The presence and concentration of chemicals in soil solutions determine the success of egress (McSorley, 2003). Nemafric-BL phytonematicide induced juvenile hatch inhibition in *M. incognita*, when using a series of water-diluted phytonematicide solutions (Dube and Mashela, 2016). Similarly, the dynamics of juvenile hatch inhibition in the overall reduction of *M .incognita* population densities by Nemarioc-AL and Nemafric-BL phytonematicides were investigated using pure cucurbitacin A and B at 24-, 48- and 72-h incubation periods (Dube *et al*., 2016). Regardless of the incubation period, juvenile hatch responded positively to increasing concentrations of cucurbitacin and provided significant evidence of the existence of the density dependent growth patterns. At low concentrations the two cucurbitacins inhibited juvenile hatch, whereas, at high concentrations the activity was stimulated (Dube, 2016).

Once the MCSP values had been established, these are used to determine the application intervals in days. Mashela *et al*. (2016) used the MCSP values and the duration of the life cycle of *Meloidogyne* species to determine the application interval of phytonematicides, which were 2.99 and 2.63% for Nemafric-BL and Nemarioc-AL phytonematicides, respectively. All the reviewed work confirmed that the two products

have potent nematicidal properties, and could be used as alternative to methyl bromide when the MCSP values had been empirically established.

2.2.1.4 Quality protocols

Nemafric-BL phytonematicide, produced from fermented fruit of *C. africanus*, had been widely used to suppress root-knot nematode population densities (Shadung, 2016; Shadung *et al*., 2016). The quality of the material, however, can be compromised with increasing storage period, due to the biological nature of the phytonematicide (Shadung *et al*., 2016). Shadung *et al*. (2015) reported that when *C. africanus* and *C. myriocarpus* fruits were oven-dried at 52 °C and stored over six months prior to developing the products, the concentrations of cucurbitacin A and B in the phytoinventories exhibited positive quadratic relations. The behaviour of cucurbitacin A and B in the stimulation phase was elaborated on the basis of the thermo-stable enzyme-driven precursors, which were clearly spelt out by Chen *et al*. (2014). In contrast, in the inhibition phase the decreases were explained in terms of auto-oxidation and possibly some degradation by microbes. The changes in the concentrations of cucurbitacin were shown to have the potential effects on the quality of the final products (Shadung, 2016; Shadung *et al*., 2016). Cucurbitacin A and B concentrations at the end of storage periods, were still more than three-hundred times those at T_0, suggesting that the products were still suitable for use in managing nematode numbers (Shadung, 2016; Shadung *et al*., 2016).

2.2.2 Phytotoxicity

Nemarioc-AL and Nemafric-BL phytonematicides were shown to be highly phytotoxic to tomato seedlings when applied at above 10% concentration after transplanting (Pelinganga and Mashela, 2012). Similarly, Mafeo and Mashela (2010) demonstrated that Nemarioc-AG phytonematicide was highly phytotoxic to seedlings of both dicotyledonous and monocotyledonous seedlings, with emergence prevented from as high as 60% to complete failure.

2.2.3 Managing phytotoxicity

Curve-fitting Allelochemical Response Dosage model: The major limiting factor in the development of any phytonematicide intended for post-planting use is its degree of phytotoxicity. Mashela *et al*. (2015) adapted the Curve-fitting Allelochemical Response Dosage (CARD) computer-based model (Liu *et al*., 2003) to develop the concept of the Mean Concentration Stimulation Point (MCSP). The CARD model quantifies the three DDG patterns, namely, stimulation, saturation and inhibition phases, using the biological indices (Liu *et al*., 2003). Two biological indices, the threshold stimulation (D_m) and the saturation point (R_h) were used on the development the MCSP relation: MCSP = D_m + (R_h\2) (Mashela *et al*., 2015). The MCSP is the concentration at which a given phytonematicide would not be phytotoxic to the crop being protected from nematode damage, whereas nematode population densities would be constantly suppressed (Mashela et al., 2015). The MCSP had since its introduction been validated for use in various crops (Mathabatha *et al*., 2016; Sithole, 2016).

Dosage model and application frequency: The concept of the dosage model in phytonematicides in managing phytotoxicity and consistent suppression of nematode numbers was introduced by Mashela *et al*. (2015). The application frequency is the unit-less factor which is empirically derived. After empirically-deriving the MCSP, the result is then used to derive the application interval (T_a), where the concept of day-week-month in relation to the nematode life cycle was introduced (Mashela *et al*., 2015). Once the application interval was 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)], was computed (Mashela *et al*., 2015). The application frequency is unit-less, with the result that: Dosage model = MCSP (%) × T_f, (Mashela *et al*., 2015). Dosage was then defined as the amount of the total active ingredient that would have been put into a given soil by the end of the crop cycle (Mashela *et al*., 2015).

2.3 Work not yet done

The use of the two phytonematicides, Nemarioc-AL and Nemafric-BL to develop MCSP values on squash constitutes most part of the work not yet been established. The MCSP values will be the concentration to be used at every irrigation, which would in future be required to determine the irrigation interval and the dosage model of the two phytonematicides on squash production.

CHAPTER 3
RESPONSES OF SQUASH TO NEMARIOC-AL AND NEMAFRIC-BL PHYTONEMATICIDES

3.1 Introduction

Yield loss in crops due to plant-parasitic nematodes is proportional to the initial population density of nematodes at planting (Seinhorst, 1965). Nemarioc-AL and Nemafric-BL phytonematicides were researched and developed at the Green Technologies Research Centre, University of Limpopo, South Africa, for use against plant-parasitic nematodes (Mashela *et al*., 2015) in order to develop the Mean Concentration Stimulation Point (MCSP). Therefore, the objective of this study was to determine if MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides for butternut squash (*Cucurbita pepo*) under greenhouse, microplot and field conditions existed.

3.2 Materials and methods

3.2.1 Description of the study area

Greenhouse conditions: The available greenhouse was 100 m × 20 m in size, with thermostatically-activated fans on one end and the wet wall on the other end, for moderating inside temperatures. In summer (October to December) the greenhouse maximum/minimum temperatures averaged 28/21°C, whereas in winter (April to June) the maximum/minimum temperatures averaged 18/5°C. The top of the greenhouse was covered with a 35% radiation-allowing green net, whereas the sides were covered with black nets. Due to the size of the greenhouse and the wind-blown generated currents, conditions inside the greenhouse were not homogeneous, thereby dictating that experiments, depending on their size, be appropriately designed. The experiments under

all three conditions were conducted during autumn (January-March) 2016 and repeated in late spring (July-September).

Microplot and field conditions: Microplots were conducted outside the greenhouse using the pasteurised soil derived from digging single holes where the plastic pots were inserted. The soil mixture comprised Hutton form, with soil structure comprising 65% sand, 30% clay and 5% silt. The location had average rainfall less than 500 mm, which occurred mostly during summer.

3.2.2 Research design

Under all three conditions the treatments were arranged in randomised complete block design, with five replications. In the greenhouse, microplot and field trials, treatments comprised 0, 2, 4, 8, 16 and 32%, 0.0, 0.8, 1.6, 3.2, 6.4 and 12.8% and 0.0, 2.4, 4.8, 9.6, 19.2 and 38.4%, respectively.

Legend 3.1 Showing Nemarioc-AL and Nemafric-BL phytonematicides experiments under greenhouse condition.

Legend 3.2 Showing Nemarioc-AL and Nemafric-BL phytonematicides experiments under microplot condition.

Legend 3.3 Showing Nemarioc-AL and Nemafric-BL phytonematicides experiments under field condition.

3.2.3 Procedures

Nemarioc-AL and Nemafric-BL phytonematicides were prepared using the locally-developed method (Mashela *et al*., 2015). Briefly, the method comprised filling 20 litre containers with 16 litre chlorine-free tapwater, with 80 g dried and ground fruit from one *Cucumis* species, 300 ml effective microorganisms (EM), 300 ml molasses and 100 g sugar each added into the container. After adding the listed ingredients, the container was shaken to mix the materials. The container had an outlet dangling into a bottle half-filled with water in order to provide for the escape route of gasses generated during the fermentation process. The airtight system was placed at room temperature for 14 days to allow for the fermentation-induced pH to drop to ca. 3.7 units (Kyan *et al*., 1999). The phytonematicides were each applied once a week as substitute for irrigation.

The greenhouse trials were established by putting 25-cm-diameter plastic bags on greenhouse benches and filling them with the growing mixture comprising pasteurised (300ºC for 1 h) loam, pasteurised river sand and Hygromix-T (Hygrotech, Pretoria North) at 2:1:1 (v\v) ratio. The microplot trials were established by filling 25-cm-diameter plastic pots with 10 litre steam-pasteurised river sand and loam soil at 3:1 (v/v) ratio and placed at 1.0 m intra-row and 1.0 m inter-row spacing outside the greenhouse. Field trials were established on Hutton sandy loam (65% sand, 30 % clay, 5% silt), containing 1.6% organic C, with EC at 0.148 DS/m and pH (H_2O) at 6.5. The two experiments, one for each phytonematicide, were situated at 10 m away from each other. In all cases, seeds for squash cv. 'Caserta' were sown at two seeds per planting station and thinned to two-leaf stage.

Meloidogyne incognita race 2 inocula were prepared by extracting eggs and second-stage juveniles (J2) from roots of greenhouse-grown nematode-susceptible kenaf (*Hibiscus cannabinus*) in 1% NaOCl (Hussey and Barker, 1973). After emergence, when the seedlings had secondary leaves, each plant was inoculated with ca. 5 000 *M. incognita* eggs and J2 using a 20-ml plastic syringe by placing into 3-cm-deep holes on the cardinal points of the seedling. Plants were then fertilised with 2.5 g N:P:K 2:3:2 (22) per plant, which provided a total of 155 mg N, 105 mg P and 130 mg per ml water and 1 g N:P:K 2:1:2 (43) to provide 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 of water (Mashela, 2002). Every other day, each plant was irrigated with 250 ml chlorine-free tapwater. Weekly sprays for disease management comprised alternating

Mycoguard, Bravo, Funginex and Dithane M45, whereas insect pests were scouted and monitored on daily basis.

3.2.4 Data collection

At 56 days after inoculation, plant height was measured from the crown to the tip of the flag leaf and number of leaf per plant was recorded. Chlorophyll was measured using a chlorophyll meter. Fruit were harvested and weighed, shoots were separated from roots and oven-dried at 70°C for 72 h and weighed. Root systems were removed from pots, immersed in water to remove soil particles, blotted dry and weighed to facilitate the calculation of nematode densities per total roots 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 10 g roots per plant using the maceration and blending method for 30 seconds in 1% NaOCl (Hussey and Barker, 1973). The aliquot was passed through 150-, 45- and 25-µm nested sieves, with nematodes being collected from the 25-µm mesh sieve. Soil per pot was thoroughly mixed and a 250-cm^3 soil sample was collected, with J2 extracted from soil samples using the sugar-floatation and centrifugation (Jenkins, 1964). Eggs and J2 from root samples and soil samples were counted from a 5-ml aliquot under a stereomicroscope. Nematode numbers for greenhouse and microplot trials were converted to nematodes per total root system per plant, whereas soil nematode numbers were converted to volume growing mixture per pot, all to allow for the determination of the final nematode population density (Pf) and the calculation of the reproductive factor

(RF = Pf/Pi). The reproductive potential (eggs + J2/g root) was calculated using a proportion of nematode counts in roots to total root mass.

3.2.5 Data analysis

Data were subjected to analysis of variance using SAS (SAS Institute Inc., 2008). The degrees of freedom and their associated sum of squares were partitioned to provide the total treatment variation (TTV) for different sources of variation. Mean separation was achieved through the Waller-Duncan Multiple Range Test at 5% level of probability. Significant treatment mean plant variables were further subjected to the CARD model to generate the biological indices D_m and R_h (Liu *et al*., 2003), which allowed for the calculation of MCSP values for the two phytonematicides (Mashela *et al*., 2015).

3.3 Results

The seasonal effects for the variables measured were not significant and therefore, the data were pooled for the greenhouse (n = 60), microplot (n = 60) and field (n = 60).

3.3.1 Greenhouse experiments

Plant variables

Treatment effects: Nemafric-BL phytonematicide had highly significant effects on dry fruit mass, but significant on fruit number, with no significant effects on dry shoot mass, plant height, stem diameter, chlorophyll content and leaf number. Treatments contributed from 51 to 71% in TTV of dry fruit mass and fruit number, respectively (Table 3.1). At lower

concentrations Nemafric-BL phytonematicide increased the fruit number from 10 to 29%. However, at higher concentrations Nemafric-BL phytonematicide decreased fruit number from 84-137% and dry fruit mass by 6-14% (Table3.2).

Table 3.1 Sources of variation in affecting dry fruit mass (DFM) and fruit number (FN) of squash under the effect of Nemafric-BL phytonematicide at 56 days after initial treatment (n = 60) in greenhouse experiments.

		Dry fruit mass		Fruit number	
Source	DF	MS	TTV (%)	MS	TTV (%)
Replication	9	4.5827	12	0.10241	31
Treatment	5	26.9521	71***	0.17101	51**
Error	4	6.2220	17	0.6056	18
Total	59	37.7568	100	0.33398	100

***Highly significant at P ≤ 0.01; **Significant at P ≤ 0.05.

Table 3.2 Effect of Nemafric-BL phytonematicide on dry fruit mass (DFM) and number of fruit (NOF) of squash at 56 days after initiation of treatments in greenhouse experiments.

Concentration (%)	Dry fruit mass (g)	RI (%)	Fruit number	RI (%)
0.0	9.135[a]	–	0.2806[b]	–
2	9.700[a]	6	0.5158[a]	84
4	9.876[b]	8	0.5677[a]	102
8	10.052[a]	10	0.6657[a]	137
16	9.561[a]	5	0.5687[a]	103
32	10.397[a]	14	0.4669[ab]	66

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

Curve-fitting Allelochemical Response Dosage: In Nemafric-BL phytonematicide, dry fruit mass and fruit number with increasing concentrations of the phytonematicide each exhibited positive quadratic relations (Figure 3.1). The models explained the relationship by 75 and 58%, respectively (Table 3.3). The concentrations for the optimum dry fruit mass and fruit number were at 28.42 and 17.72%, respectively, which were derived using the $x = -b_1/2b_2$ relation (Table 3.3). Using the MCSP = $D_m + (R_h/2)$ relation (Mashela *et al*., 2016), the MCSP value of Nemafric-BL phytonematicide on squash was 2.83% (Table 3.4).

Dry fruit mass had the k value of 2 units, whereas the fruit number had the k value of 1, resulting in 3 units of the overall sensitivity (∑k) of Nemafric-BL phytonematicide on squash. In Nemarioc-AL phytonematicide, plant variables were not subjected to the CARD model since the treatment effects were not significant under all trial conditions.

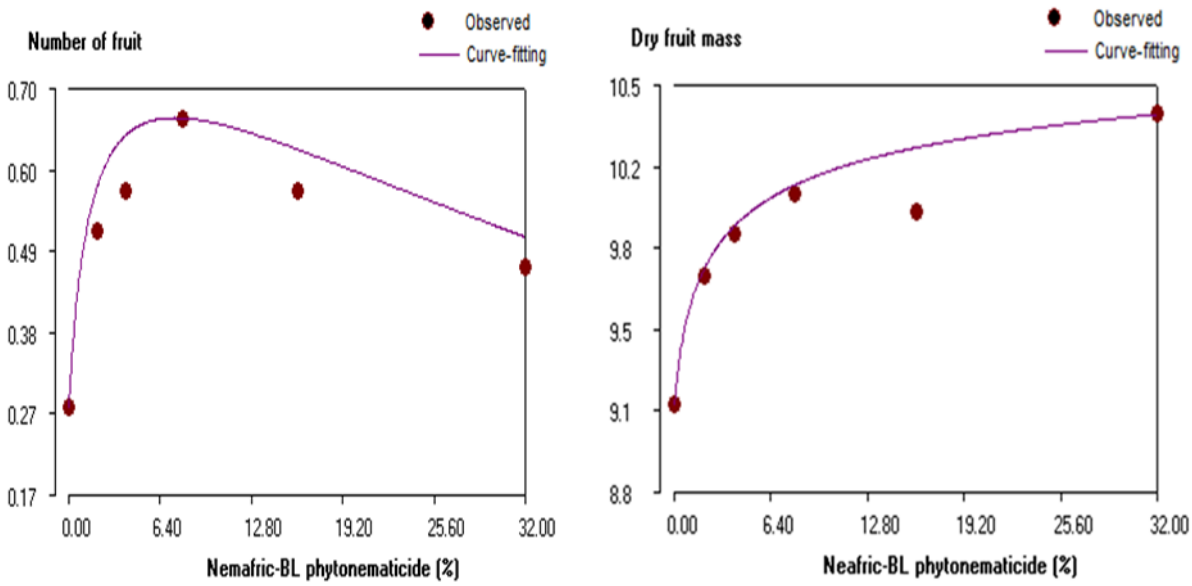

Figure 3.1 Responses of dry fruit mass (DFM) and number of fruit (NOF) for squash to concentrations of Nemafric-BL phytonematicide at 56 days after inoculation in greenhouse experiments.

Table 3.3 Quadratic relationship, coefficient of determination and computed optimum response concentration for dry fruit mass (DFM) and number of fruit (NOF) of squash from Curve-fitting Allelochemical Response Dosage against Nemafric-BL phytonematicide at 56 days after treatments in greenhouse experiments.

Organs	Quadratic relation	R^2	x^z	Y
DFM	$Y= -0.0012x^2 + 0.0663x + 9.4354$	0.75	28.42	10.40
NOF	$Y = -0.0009x^2 + 0.0319x + 0.3962$	0.58	17.72	0.68

$^z x = -b_1/2b_2$

Table 3.4 Biological indices for dry fruit mass and number of fruit for squash to increasing concentrations of Nemafric-BL phytonematicide at 56 days after initiation of treatment in greenhouse experiments.

Biological index[z]	Dry fruit mass	Fruit number	Mean
Threshold stimulation (D_m)	–0.632	7.426	3.397
Saturation point (R_h)	2.614	0.344	–1.135
0% inhibition (D_0)	–0.632	69.993	34.6805
50% inhibition (D_{50})	–0.374	103.327	51.4765
100% inhibition (D_{100})	–0.3	144.6	72.15
R^2	0.95	0.96	
k-value	2	1	
Overall sensitivity ($\sum k$) = 3			
MCSP = $D_m + (R_h/2)$ = 3.397 + (-1.135/2) = 2.83%.			

Nematode variables

In Nemarioc-AL phytonematicide, increasing concentrations of the phytonematicide had high significant effects on eggs, J2 in roots, J2 in soil and total nematode, contributing 75, 87, 88 and 96%, respectively, in TTV of the respective variables (Table 3.5). In Nemafric-BL phytonematicide, increasing concentrations of the phytonematicide had significant effects on eggs, J2 in roots, J2 in soil and total nematode, contributing 75, 92, 66 and 92%, respectively in TTV of the respective variables (Table 3.5). In Nemarioc-AL

phytonematicide, relative to untreated control, eggs were reduced by 50 - 100%, J2 in roots by 95-100%, J2 in soil by 98-100% and total nematode by 98-100% (Table 3.6). In Nemafric-BL phytonematicide, eggs were reduced by 50-100%, J2 in roots by 92-100% and total nematode by 94-100% (Table 3.6).

Table 3.5 Sources of variation among nematode variables at 56 days after initiation of treatments under greenhouse condition.

		Nemarioc-AL phytonematicide							
		Eggs		J2 in roots		J2 in soil		Pf	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Replication	9	0.09	10	0.01	6	0.31	6	0.19	2
Treatment	5	0.76	75***	0.19	87***	4.22	88***	7.99	96***
Error	4	0.52	15	0.01	6	0.31	6	0.13	2
Total	59	1.45	100	0.22	100	4.85	100	8.32	100
		Nemafric-BL phytonematicide							
		Eggs		J2 in roots		J2 in soil		Pf	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Replication	9	0.09	10	0.20	5	0.26	17	0.32	5
Treatment	5	0.76	75***	3.85	92***	1.02	66***	5.76	92***
Error	4	0.15	15	0.13	3	0.26	17	0.20	3
Total	59	1.01	100	4.19	100	1.54	100	6.28	100

*** Highly significant at P ≤ 0.01.

Table 3.6 Influence of increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides on nematode eggs, juveniles (J2) in roots and in final population (Pf) at 56 days after inoculation of treatments under greenhouse condition.

Nemarioc-AL phytonematicide								
Concentration (%)	Eggs	RI (%)	$J2_{roots}$	RI (%)	$J2_{soil}$	RI (%)	Pf	RI (%)
0	12^{a}	_	38^{a}	_	280^{a}	_	330^{a}	_
2	0^{b}	–100	2^{b}	–95	0^{b}	–100	2^{b}	–99
4	6^{ab}	–50	0^{b}	–100	0^{b}	–100	6^{b}	–98
8	0^{b}	–100	0^{b}	–100	0^{b}	–100	0^{b}	–100
16	0^{b}	–100	0^{b}	–100	0^{b}	–100	0^{b}	–100
32	0^{b}	–100	0^{b}	–100	0^{b}	–100	0^{b}	–100

Nemarioc-BL phytonematicide						
Concentration (%)	Eggs	%	$J2_{roots}$	%	Pf	%
0.0	12^{a}	_	50^{a}	_	182^{a}	_
0.8	0^{b}	–100	0^{b}	–100	0^{b}	–100
1.6	6^{ab}	–50	4^{b}	–92	10^{b}	–94
3.2	0^{b}	–100	2^{b}	–96	2^{b}	–99
6.4	0^{b}	–100	0^{b}	–100	0^{b}	–100
12.8	0^{b}	–100	4^{b}	–92	4^{b}	-98

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

3.3.2 Microplot experiments

Plant variables

Treatment effects: Effects of Nemarioc-AL phytonematicide were significant on dry shoot mass, but highly significant on dry fruit mass (Table 3.7), with treatments contributing 15 and 63% in TTV of the respective variables (Table 3.7), whereas there were no treatment effects on plant height, chlorophyll content, stem diameter and fresh fruit mass. Although the effect of Nemarioc-AL phytonematicide on dry shoot mass was not consistent (Table 3.8), it tended towards being phytotoxic, whereas on dry fruit mass it had stimulation effects.

Table 3.7 Sources of variation in dry shoot mass (DSM) and dry fruit mass (DFM) of squash under the effect of fermented crude extracts at 56 days after initial treatments in microplot experiments.

		Dry shoot mass		Dry fruit mass	
Source	DF	MS	TTV (%)	MS	TTV (%)
Replication	9	174.263	79	21.8552	20
Treatment	5	34.013	15**	68.5283	63***
Error	4	13.091	6	17.9460	17
Total	59	221.367	100	108.3295	100

***Highly significant at P ≤ 0.01, **Significant at P ≤ 0.05.

Table 3.8 Effect of fermented crude extracts of Nemarioc-AL and Nemafric-BL phytonematicide on dry shoot mass(DSM) and dry fruit mass (DFM) in squash at 56 days after initiation of treatments (n=60) in microplot experiments.

Concentration (%)	Dry shoot mass (g)	RI (%)	Dry fruit mass (g)	RI (%)
0.0	25.76^{ab}	–	7.86^{b}	–
0.8	27.14^{a}	5	14.43^{a}	83
1.6	23.83^{bc}	–7	14.77^{a}	88
3.2	22.27^{c}	–13	11.11^{ab}	41
6.4	26.05^{ab}	1	12.68^{a}	61
12.8	23.83^{bc}	–7	13.88^{a}	76

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

Curve-fitting Allelochemical Response Dosage: In Nemarioc-AL phytonematicide, dry shoot mass and dry fruit mass each with concentrations of the phytonematicide exhibited positive quadratic relations (Figure 3.2). The models explained the relationships by 72 and 91% in dry shoot mass and dry fruit mass, respectively (Table 3.9). Concentrations for optimum dry shoot mass and dry fruit mass were 44.36 and 3.59%, respectively, using the $x = x^z = -b_1/2b_2$ relation (Table 3.9). Using the $MCSP = D_m + (R_h/2)$ relation, MCSP of Nemarioc-AL phytonematicide on squash was 8.81% (Table 3.10).

Both dry shoot mass and fruit mass each had k value of zero, with the overall sensitivity (∑k) of squash to Nemarioc-AL phytonematicide being zero. In contrast, Nemafric-BL phytonematicide effects on plant variables were not significant and therefore were not assessed using the CARD model.

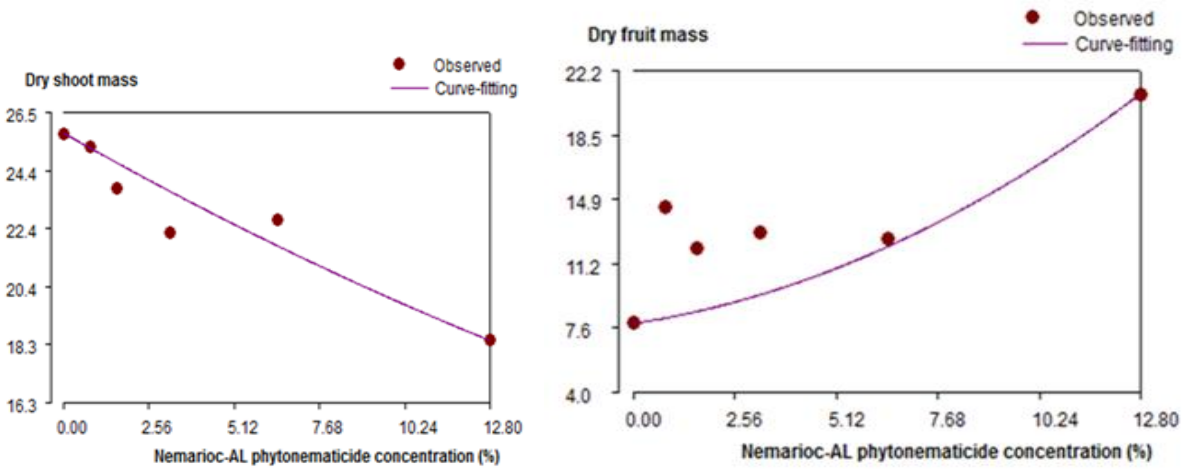

Figure 3.2 Responses of dry shoot mass (DSM) and dry fruit mass (DFM) of squash to concentrations of Nemarioc-AL phytonematicide at 56 days after inoculation in microplot experiments.

Table 3.9 Quadratic relationship, coefficient of determination and computed optimum response concentration dry shoot mass (DSM) and dry fruit mass (DFM) of squash from Curve-fitting Allelochemical Response Dosage against Nemarioc-AL phytonematicide at 56 days after treatments in micro-plot experiment.

Organ	Quadratic relation	R^2	x^z	Y
DSM	$y = 0.0069x^2 – 0.6122x + 25.334$	0.91	44.36	66.071
DFM	$Y = –0.0363x^2 + 0.261x + 110.75$	0.73	3.59	111.22

$^z x = –b_1/2b_2$.

Table 3.10 Biological indices for dry shoot mass (DSM) and dry fruit mass (DFM) of squash to increasing concentrations of Nemarioc-AL phytonematicide at 56 days after initiation of treatments in microplot experiment.

Biological index[z]	DSM	DFM	Mean
Threshold stimulation (D_m)	44.581	–3.226	20.677
Saturation point (R_h)	–13.646	–4	–17.646
0% inhibition (D_0)	0	–6.452	–3.226
50% inhibition (D_{50})	32.642	–6.452	–19.547
100% inhibition (D_{100})	32.6	–6.5	13.05
R^2	0.911	0.74	0.824
k-value	0	0	
Overall sensitivity		∑k = 0	

MCSP = $D_m + (R_h/2)$ = 20.667 + (–17.646/2) = 11.85%

Nematode variables

In Nemarioc-AL phytonematicide, increasing concentrations had high significant effects on eggs, J2 in roots, J2 in soil and total nematode, contributing 86, 89, 45 and 78% in TTV of the respective variables (Table 3.11). In Nemafric-BL phytonematicide, increasing concentrations had high significant effects on eggs, J2 in roots and total nematode, contributing 81, 86 and 69% in TTV of the respective variables (Table 3.11), but had no effect on J2 in soil. In Nemarioc-AL phytonematicide, relative to the untreated control, J2 in roots were reduced from 88 to 100%, J2 in soil from 25 to 100% (Table 3.12), whereas, in Nemafric-BL phytonematicide J2 were reduced from 49 to 99% (Table 3.12).

Table 3.11 Sources of variation among nematode variables at 56 days after initiation of treatments under microplot condition.

		Nemarioc-AL phytonematicide							
		Eggs		J2 in roots		J2 in soil		Pf	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Replication	9	0.09	7	0.32	7	1.41	34	1.04	13
Treatment	5	1.26	86***	3.76	89***	1.88	45***	6.20	78***
Error	4	0.09	7	0.16	4	0.88	21	0.67	9
Total	59	1.45	100	4.24	100	4.18	100	7.91	100
		Nemafric-BL phytonematicide							
		Eggs		J2 in roots		J2 in soil		Pf	
Source	DF	MS	%	MS	%	MS	%	MS	%
Replication	9	0.11	9	0.04	2	0.82	29	0.85	16
Treatment	5	0.95	81***	1.81	86***	1.11	39ns	3.72	69***
Error	4	0.11	9	0.25	12	0.90	32	0.81	15
Total	59	1.17	100	2.10	100	2.84	100	5.38	100

***Highly significant at P ≤ 0.01, nsNot significant at P ≤ 0.05.

Table 3.12 Influence of increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides on nematode eggs, juveniles (J2) in roots and in final population (Pf) at 56 days after inoculation of treatments under microplot condition.

Nemarioc-AL phytonematicide								
Concentration (%)	eggs	%	$J2_{roots}$	%	$J2_{soil}$	%	Pf	%
0.0	18^{a}	_	50^{a}	_	160^{ab}	_	224^{a}	_
0.8	0^{b}	-100	6^{b}	-88	120^{ab}	-25	126^{ab}	-44
1.6	0^{b}	-100	4^{b}	-92	80^{ab}	-50	84^{ab}	-63
3.2	0^{b}	-100	0^{b}	-100	0^{b}	-100	0^{b}	-100
6.4	0^{b}	-100	6^{b}	-88	200^{a}	-25	206^{a}	-8
12.8	0^{b}	-100	0^{b}	-100	0	-100	0^{b}	-100

Nemafric-BL phytonematicide						
Concentration (%)	Eggs	%	$J2_{roots}$	%	Pf	
0.0	18^{a}	_	24^{a}	_	242^{a}	_
0.8	0^{b}	-100	4^{b}	-83	124^{ab}	-49
1.6	0^{b}	-100	4^{b}	-83	84^{ab}	-65
3.2	0^{b}	-100	2^{b}	-92	2^{b}	-99
6.4	0^{b}	-100	0^{b}	-100	120^{ab}	-50
12.8	0^{b}	-100	2^{b}	-92	2	-99

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

3.3.3 Field experiments

Plant variables

After subjecting the data for plant variables to ANOVA, variables were not subjected to the CARD model because there were no significant differences among the treatments.

Nematode variables

In Nemarioc-AL phytonematicide, increasing concentrations of the phytonematicide had significant effects on eggs and J2, contributing 82 and 83% in TTV of eggs and J2, respectively (Table 3.13). In Nemafric-BL phytonematicide, increasing concentrations of phytonematicide had significant effects on egg and J2, contributing 82 and 96%, respectively, in TTV of eggs and J2 of the respective variables (Table 3.13). Relative to untreated control, in Nemarioc-AL phytonematicide, eggs were reduced from 91 to 100% and J2 from 77 to 100% (Table 3.14). In Nemafric-BL phytonematicide, relative to untreated control, both eggs and J2 were reduced by 100% each (Table 3.14).

Table 3. 13 Sources of variation among nematode variables at 56 days after initiation of treatments under field condition.

		Nemarioc-AL phytonematicide						Nemafric-BL phytonematicide					
		Eggs		J2 in roots		Pf		Eggs		J2 in roots		Pf	
Source	DF	MS	%	MS	%	MS	%	MS	%	MS	%	MS	%
Replication	8	83.3	9	0.36	11	901.85	10	0.11	9	0.06	2	0.01	0.5
Treatment	5	781.1	82***	2.77	83***	7527.41	83***	1.04	82***	3.24	96***	4.51	99***
Error	3	87.8	9	0.20	6	657.47	7	0.11	9	0.06	2	0.2	0.5
Total	53	952.2	100	3.33	100	9086.67	100	1.26	100	3.37	100	4.54	100

***Highly significant at P ≤ 0.01.

Table 3.14 Influence of increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides on nematode eggs, juveniles (J2) in roots and final population (Pf) at 56 days after initiation of treatments under field condition.

	Nemarioc-AL						Nemafric-BL					
Concentration	Eggs	RI (%)	$J2s_{roots}$	RI (%)	Pf	RI (%)	Eggs	RI (%)	$J2_{roots}$	RI (%)	Pf	RI (%)
0.0	24.44^{a}	_	48.88^{a}	_	73.33^{a}	_	20^{a}	_	48.89^{a}	_	68.89^{a}	_
2.4	0^{b}	–100	0^{b}	–100	0^{b}	–100	0^{b}	–100	0^{b}	–100	0^{b}	–100
4.8	0^{b}	–100	2.22^{b}	–95	2.22^{b}	–97	0^{b}	–100	0^{b}	–100	0^{b}	–100
9.6	0^{b}	–100	0^{b}	–100	0^{b}	–100	0^{b}	–100	0^{b}	–100	0^{b}	–100
19.2	2.22^{b}	–91	11.11^{b}	–77	13.33^{b}	–81	0^{b}	–100	0^{b}	–100	0^{b}	–100
38.4	0^{b}	–100	0^{b}	–100	2.22^{b}	–97	0^{b}	–100	0^{b}	–100	0^{b}	–100

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

3.4 Discussion

3.4.1 Plant variables

Greenhouse: Increasing concentrations of Nemafric-BL phytonematicide had significant effects on dry fruit mass and number of fruits. Similar stimulation effects were observed when tomato plant cv. 'Floradade' was exposed to 3% Nemarioc-AL phytonematicide (Pelinganga *et al*., 2013) or Nemarioc-AG phytonematicide (Mashela, 2002). Mashela *et al.* (2013) also had similar observations when increasing concentrations of Nemafric-BL phytonematicide were applied on cowpea, with highly significant effects on cowpea pod yield. Similar results were observed when maize (*Zea mays*), millet (*Eleusine coracana*), sorghum (*Sorghum bicolor*), chive (*Allium schoenoprasam*), leek (*Allium porrum*) and onion (*Allium cepa*) seedlings were exposed to increasing concentrations Nemarioc-AG phytonematicide (Mafeo *et al*., 2011).

In contrast, Mathabatha *et al*. (2016) also observed negative quadratic relations of certain variables of *Citrus volkameriana* seedling rootstocks exposed to increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides. The latter confirmed some of the observations on dicotyledonous or monocotyledonous crops exposed to increasing concentrations of Nemarioc-AG phytonematicide (Mafeo, 2012).

Overall, observations on squash plant variables exposed to increasing concentrations of the two phytonematicides confirmed the concept of density-dependent growth (DDG) patterns (Liu *et al*., 2003), which had been described in plant-phytonematicide relations in detail (Mashela *et al*., 2016). The DDG patterns are characterised by the stimulation,

neutral and inhibition concentration ranges, with the stimulation being used in phytonematicides, whereas the inhibition concentrations are suitable for use in the development of herbicides. In cases where plant variables and increasing concentration of phytonematicide exhibited quadratic relations as observed in squash and Nemafric-BL phytonematicide, the MCSP could be computed using the CARD computer-based model as shown in the current study. The use of the DDG patterns is unique in the sense that four scenarios, depending on the concentration ranges used, could occur (Mashela *et al*., 2015). Relation could be (a) positive linear if stimulation concentrations were involved, (b) neutral (ANOVA not significant at $P \leq 0.05$), (c) negative linear if inhibition concentrations are involved and (d) quadratic relations when the stimulation, neutral and inhibition concentrations are involved.

On the whole, results of this study suggested that squash was moderately sensitive to increasing concentrations of Nemafric-BL phytonematicide, supported by the overall sensitivity ranking of 3 units. Mafeo (2012), using 18 different plant species, demonstrated that the overall sensitivity values in Nemarioc-AG phytonematicide were plant-specific, with seedlings being more tolerant to phytonematicides than mature plant species (Pelinganga, 2013). Such varying overall sensitivities were also observed in various nematode stages (Dube and Mashela, 2016), with egress in *M. incognita* being more sensitive to cucurbitacin B than cucurbitacin A.

The MCSP computed for this study under greenhouse was 2.83% for Nemafric-BL phytonematicide on squash, which was comparable to the MCSP values of 2.64 and

2.98% for Nemarioc-AL and Nemafric-BL phytonematicides, respectively, on tomato under greenhouse conditions (Mashela *et al*., 2015, 2013; Pelinganga, 2013)..

Microplot: Increasing concentrations of Nemarioc-AL phytonematicide had significant effect on dry shoot mass and dry fruit mass in squash. Similar results were observed on tomato plant when exposed to Nemarioc-AL phytonematicide (Pelinganga *et al*., 2013; Tseke *et al*., 2013). The fact that chlorophyll content, leaf number, plant height and stem diameter were not affected by increasing levels of the phytonematicide in this study, suggested that the organs at harvest time were still on the saturation phase (Mashela *et al*., 2015), which is a common phenomenon in phytonematicides (Dube and Mashela, 2016).

In the CARD model, generally k values remained constant for all the variables with k value of k = 0. Similarly, Sithole *et al*. (2016) observed the k value of zero for dry root mass when Nemarioc-AL phytonematicide was tested on geranium plants under microplot conditions. However, the overall sensitivity ∑k generated in the current study disagreed with the findings of Sithole *et al*. (2016), whereby, the ∑k was 3 units. The lower the overall sensitivity, the higher the sensitivity of the plant to the phytonematicide (Liu *et al*., 2003). Generally, sensitivities of squash to Nemarioc-AL phytonematicide were high.

In the Nemafric-BL phytonematicide experiment, which was barely mentioned, all the plant variables were not affected by increasing levels of the phytonematicide. Similar results often occur, where the two phytonematicides have no effect since the test organs

were saturated by the active ingredients at harvest (Dube, 2016; Mafeo, 2012; Pelinganga, 2013). Saturation is not unique to the two phytonematicides, since the similar results were observed when Ghaferbi *et al*. (2012) exposed eight plant species to seed extracts of wheat (*Triticum aestivum*).

The MCSP value for Nemarioc-AL phytonematicide of squash was 11.85%, which was relatively high when compared with that of Nemafric-BL phytonematicide under greenhouse conditions. Additionally, the observed MCSP was higher than that of the same phytonematicide on geranium plants, namely, 6.18% under microplot conditions (Sithole *et* al., 2016), but close to that on *C. volkameriana* of 9% under greenhouse conditions (Mathabatha *et al*., 2016). Apparently, the observations confirmed the insistence that the MCSP was plant-specific (Mashela *et al*., 2015), with the current observations suggesting that the environment under which plants were being raised could also play a role on the magnitude of MCSP values.

Field: In the study, there were no significant effects observed on plant variables when exposed to increasing concentrations of both phytonematicides. The observation was a further support to the view that MCSP values were condition-specific. Apparently, the three diverse conditions, greenhouse, microplot and field, affected squash plants in different ways. Generally, plant responses to phytonematicides could depend on factors such as soil type, climate and other environmental factors, as observed in organic amendment studies (McSorley, 2011).

3.4.2 Nematode variables

All levels of Nemarioc-AL and Nemafric-BL phytonematicides were highly effective in relation to the suppression of the nematode population densities as observed in various greenhouse studies (Mafeo, 2012; Mashela *et al*., 2015; Pelinganga, 2013; Pelinganga and Mashela, 2012). Similarly, in microplot and field trials both Nemarioc-AL and Nemafric-BL phytonematicides reduced nematode numbers with high magnitudes, which also confirmed other studies under similar conditions (Mashela *et al*., 2015; Pelinganga, 2013). Generally, the MCSP values are not exclusively based on the results of the CARD model, but also on the concentrations of the phytonematicides that reduced nematode numbers (Mashela *et al*., 2015). The concept of choosing the value for MCSP much lower than the ones derived from the CARD model had been discussed in detail in other studies (Mathabatha *et al*., 2016; Sithole *et al*., 2016). The advantage of choosing such lower concentrations is that the products would still reduce nematode number, without causing phytotoxicity to the plant being protected against nematode damage.

3.5 Conclusions

The MCSP values and overall sensitivities of crops to Nemarioc-AL and Nemafric-BL phytonematicides were condition-specific, which could imply that the rate of plant growth played a role in the two physiological activities. In the current study, for all conditions, the MCSP value for both phytonematicides could be adopted as 2.83%, which was derived empirically as for Nemafric-BL phytonematicide under greenhouse conditions. At 2.83% phytonematicide, nematode numbers would be reduced without inducing phytotoxicity on squash plants.

CHAPTER 4
SUMMARY, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSSIONS

4.1 Summary

In greenhouse trials, Nemafric-BL phytonematicide stimulated growth of squash (*Cucurbita* pepo) plants by 51-71% on dry fruit mass and fruit number. In Nemarioc-AL phytonematicide, eggs were reduced by 50-100%, J2 in roots by 95-100%, J2 in soil by 98-100% and total nematode by 98-100%. In Nemafric-BL phytonematicide, eggs were reduced by 50-100%, J2 in roots by 92–100% and total nematode by 94-100%. Squash plants were highly sensitive to products reflected by the overall sensitivity (∑k) of 3 units. The MCSP generated for Nemafric-BL phytonematicide was 2.83%.

In microplot trials, Nemarioc-AL phytonematicide stimulated growth of squash plants by 15-63% on dry shoot mass and dry fruit mass, respectively. In Nemarioc-AL phytonematicide, relative to untreated control, J2 in roots were reduced by 88–100%, J2 in soil were reduced by 25-100%. In Nemarioc-BL phytonematicide, J2 were reduced by 49-99%. The squash were highly sensitive to the product as shown by the overall sensitivity (∑k) zero. The MCSP computed for Nemarioc-AL phytonematicide was 11.85%.

For field trials, Nemarioc-AL and Nemafric-BL phytonematicides did not have any effect on plant variables and data were not subjected to analysis of variance. However, the two phytonematicides reduced *M. incognita* populations. In Nemarioc-AL phytonematicide,

eggs were reduced by 91 – 100% and J2 by 77 – 100%. In Nemafric-BL phytonematicide, eggs and J2 were each reduced by 100%.

4.2 Significance of findings

Phytotoxicity remained a greater hindrance on the implementation of phytonematicides as alternatives to methyl bromide in the management of nematode population densities. The study conducted, however, determined the 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). The first two biological indices (D_m and R_h) were used to compute the Mean Concentration Stimulation Point (MCSP) for squash (*Cucurbita pepo*) when exposed to increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides in the management of root-knot nematodes (*Meloidogyne incognita*), whereas the k-values were used to determine the overall sensitivity of squash for both phytonematicides. The non-phytotoxic concentrations of Nemarioc-AL and Nemafric-BL phytonematicides were 11.85 and 2.83%, respectively, with the overall sensitivities ($\sum k$) of the respective phytonematicides being zero and three

4.3 Recommendations

Nemarioc-AL and Nemafric-BL phytonematicides could be applied at 2.83% under all conditions. The derived MCSP value of 2.83% could be used to establish the application interval and eventually the dosage model for the two phytonematicides on squash (Mashela *et al*., 2015). Further, it would be imperative to use the derived MCSP value

and the future application interval to investigate the potential cucurbitacin A and B chemical residues in squash fruit where Nemarioc-AL and Nemafric-BL phytonematicides, respectively, were used to manage nematodes. Additionally, after deriving the dosage model, it would be imperative to assess the environmental impact of the two products in terms of the persistence of cucurbitacin A and B in the soil.

4.4 Conclusions

Nemarioc-AL and Nemafric-BL phytonematicides could be suitable for use in managing *Meloidogyne* population densities in squash production provided the products are used at the MCSP value of 2.83%. At this value, the two products would each be expected to consistently suppress population densities of nematodes, without causing phytotoxicity to squash plants.

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APPENDICES

Appendix 3.1 Analysis of variance for stem diameter of squash to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	147.4822	14.4247		
Treatment	5	46.994	9.3988	1.00	0.4285
Error	3	422.810	9.3958		
Total	53	617.626			

Appendix 3.2 Analysis of variance for plant height of squash to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	2056.29	228.477		
Treatment	5	161.45	32.291	1.31	0.2777
Error	3	1111.06	24.698		
Total	53	3328.81			

Appendix 3.3 Analysis of variance for chlorophyll content of squash to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	955.46	106.385		
Treatment	5	184.43	36.885	0.82	0.5440
Error	3	2031.84	45.152		
	53	3173.73			

Appendix 3.4 Analysis of variance for number of leaves of squash to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	0.11698	14.4247		
Treatment	5	0.04619	9.3988	1.13	00.3602
Error	3	0.36904	9.3958		
Total	53	0.53221			

Appendix 3.5 Analysis of variance for fresh shoot mass of squash to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	53784.1	5976.01		
Treatment	5	7123.9	1424.78	2.86	0.0251
Error	3	22412.9	498.07		
Total	53	83320.9			

Appendix 3.6 Analysis of variance for fresh root mass of squash to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	20765.3	2307.26		
Treatment	5	955.9	191.19	0.57	0.7213
Error	3	15052.5	334.50		
Total	53	36773.7			

Appendix 3.7 Analysis of variance for dry shoot mass of squash to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	1568.37	174.263		
Treatment	5	170.06	34.013	2.60	0.0376
Error	3	589.08	13.091		
Total	53	2327.51			

Appendix 3.8 Analysis of variance for dry fruit mass of squash to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	196.70	21.8552		
Treatment	5	342.64	68.5283	3.82	0.0057
Error	3	807.57	17.9460		
Total	53	1346.91			

Appendix 3.9 Analysis of variance for number of fruits of squash to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	0.46833	0.05204		
Treatment	5	0.19588	0.03918	1.55	0.1925
Error	3	1.13425	0.02521		
Total	53	1.79845			

Appendix 3.10 Analysis of variance for fruit mass of squash to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	20228.0	2247.56		
Treatment	5	11338.8	2267.76	2.04	0.0911
Error	3	50036.8	1111.93		
Total	53	81603.6			

Appendix 3.11 Analysis of variance for gall rating of squash to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	1.25322	0.13925		
Treatment	5	0.53445	0.10689	1.39	0.2458
Error	3	3.45846	0.07685		
Total	53	5.24614			

Appendix 3.12 Analysis of variance for stem diameter of squash to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	237.823	26.4248		
Treatment	5	9.861	1.8723	1.60	0.1800
Error	3	52.709	1.1713		
Total	53	299.893			

Appendix 3.13 Analysis of variance for plant height of squash to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	5031.7	559.074		
Treatment	5	310.5	62.094	0.58	0.7144
Error	3	4810.8	106.907		
Total	53	1015.9			

Appendix 3.14 Analysis of variance for chlorophyll content of squash to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	1774.55	197.172		
Treatment	5	265.86	53.172	1.72	0.1484
Error	3	1387.23	30.827		
Total	53	3427.64			

Appendix 3.15 Analysis of variance for number of leaves of squash to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	0.50001	0.05556		
Treatment	5	0.04963	0.00993	1.30	0.2824
Error	3	0.34457	0.00766		
Total	53	0.89421			

Appendix 3.16 Analysis of variance for fresh root mass of squash to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	11678.2	1297.58		
Treatment	5	1508.6	301.72	1.44	0.2271
Error	3	9402.6	208.95		
Total	53	22589.4			

Appendix 3.17 Analysis of variance for fresh shoot mass of squash to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	76046	8449.56		
Treatment	5	6073	1214.58	1.35	0.2610
Error	3	40481	899.58		
Total	53	122600			

Appendix 3.18 Analysis of variance for dry shoot mass of squash to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	1446.36	160.707		
Treatment	5	112.15	22.429	1.20	0.3237
Error	3	839.80	18.662		
Total	53	2398.31			

Appendix 3.19 Analysis of variance for dry fruit mass of squash to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	1763.62	195.957		
Treatment	5	76.06	15.213	1.43	0.2336
Error	3	480.38	10.675		
Total	53	2320.06			

Appendix 3.20 Analysis of variance for number of fruit of squash to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	1.01818	0.11313		
Treatment	5	0.10521	0.02104	0.44	0.8167
Error	3	2.14224	0.04761		
Total	53	3.265663			

Appendix 3.21 Analysis of variance for fruit mass of squash to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	47803.4	5311.48		
Treatment	5	2172.8	434.56	1.03	0.4092
Error	3	18906.6	420.15		
Total	53	68882.8			

Appendix 3.22 Analysis of variance for gall rating of squash to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	0.34539	0.03838		
Treatment	5	0.34599	0.06920	0.76	0.5803
Error	3	4.07416	0.09054		
Total	53	4.76554			

FIELD EXPERIMENT

Appendix 3.23 Analysis of variance for plant height of squash to Nemarioc-AL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	321.17	40.1457		
Treatment	5	109.95	21.9891	0.91	0.4850
Error	3	967.79	24.1947		
Total	53	1398.90			

Appendix 3.24 Analysis of variance for stem diameter of squash to Nemarioc-AL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	19.187	2.39833		
Treatment	5	6.217	1.24344	0.53	0.7515
Error	3	93.691	2.34228		
Total	53	119.095			

Appendix 3.25 Analysis of variance for fresh root mass of squash to Nemarioc-AL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	20.768	2.59600		
Treatment	5	34.431	6.88613	1.62	0.1760
Error	3	169.645	4.42112		
Total	53	224.843			

Appendix 3.2.26 Analysis of variance for fresh shoot mass of squash to Nemarioc-AL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	26416	3302.05		
Treatment	5	13223	2644.62	0.91	0.4824
Error	3	115853	2896.33		
Total	53	155493			

Appendix 3.27 Analysis of variance for dry fruit mass of squash to Nemarioc-AL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	3016.8	377.100		
Treatment	5	1677.9	335.577	0.85	0.5246
Error	3	15841.5	398.037		
Total	53	20536.2			

Appendix 3.28 Analysis of variance for number of fruit of squash to Nemarioc-AL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	0.20777	0.02597		
Treatment	5	0.05501	0.01100	0.39	0.8512
Error	3	1.12184	0.02805		
Total	53	1.38463			

Appendix 3.29 Analysis of variance for chlorophyll content of squash to Nemarioc-AL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	387.16	48.3951		
Treatment	5	232.60	46.5200	0.17	0.3395
Error	3	1586.77	39.6692		
Total	53	2206.53			

Appendix 3.30 Analysis of variance for dry fruit mass of squash to Nemarioc-AL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	17.896	2.23694		
Treatment	5	12.227	2.44539	0.37	0.2573
Error	3	71.583	1.78958		
Total	53	101.706			

Appendix 3.31 Analysis of variance for dry shoot mass of squash to Nemarioc-AL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	398.96	49.8702		
Treatment	5	94.23	18.8466	0.41	0.8368
Error	3	1824.81	45.6202		
Total	53	2318.00			

Appendix 3.32 Analysis of variance for gall rating of squash to Nemarioc-AL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	0.93762	0.11720		
Treatment	5	0.21739	0.10348	1.78	0.1400
Error	3	2.33101	0.05828		
Total	53	3.78602			

Appendix 3.33 Analysis of variance for plant height of squash to Nemafric-BL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	386.96	48.3695		
Treatment	5	40.04	8.0074	0.32	0.8955
Error	3	988.03	24.7008		
Total	53	1415.03			

Appendix 3.34 Analysis of variance for stem diameter of squash to Nemafric-BL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	8.460	1.05750		
Treatment	5	3.713	0.74256	0.25	0.9385
Error	3	119.936	2.99839		
Total	53	132.108			

Appendix 3.35 Analysis of variance for fresh root mass of squash to Nemafric-BL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	35.684	4.46044		
Treatment	5	19.719	3.94375	0.50	0.7713
Error	3	312.883	7.82208		
Total	53	368.285			

Appendix 3.36 Analysis of variance for fresh shoot mass of squash to Nemafric-BL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	1045.2	1306.28		
Treatment	5	7435.7	1487.15	0.81	0.5528
Error	3	73884.0	1847.10		
Total	53	91770.0			

Appendix 3.37 Analysis of variance for fruit mass of squash to Nemafric-BL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	2034.5	254.311		
Treatment	5	1346.3	269.256	0.67	0.6490
Error	3	16092.1	402.303		
Total	53	19472.9			

Appendix 3.38 Analysis of variance for number of fruit of squash to Nemafric-BL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	0.11889	0.01486		
Treatment	5	0.11247	0.02249	0.75	0.5888
Error	3	1.19478	0.02987		
Total	53	1.42614			

Appendix 3.39 Analysis of variance for chlorophyll content of squash to Nemafric-BL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	3969.5	496.193		
Treatment	5	3374.8	674.961	1.09	0.3808
Error	3	24741.3	618.531		
Total	53	32085.6			

Appendix 3.40 Analysis of variance for fresh root mass of squash to Nemafric-BL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	414.20	51.7752		
Treatment	5	100.82	20.1634	0.60	0.7028
Error	3	1352.43	33.8109		
Total	53	1867.45			

Appendix 3.41 Analysis of variance for dry fruit mass of squash to Nemafric-BL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	11.4403	1.43004		
Treatment	5	6.6427	1.32855	0.93	0.4706
Error	3	57.0150	1.42538		
Total	53	75.0981			

Appendix 3.42 Analysis of variance for gall rating of squash to Nemafric-BL phytonematicide under field conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	8	0.84728	0.10591		
Treatment	5	0.13351	0.02670	0.93	0.4709
Error	3	1.14857	0.02871		
Total	53	2.12936			

GREENHOUSE EXPERIMENTS

Appendix 3.43 Analysis of variance for dry shoot mass of squash to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	116.69	12.9650		
Treatment	5	50.76	10.1516	0.28	0.9222
Error	45	1636.94	36.3766		
Total	59	1804.39			

Appendix 3.44 Analysis of variance for dry fruit mass of squash to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	41.244	4.5827		
Treatment	5	134.761	26.9521	4.33	0.0027
Error	45	279.990	6.2220		
Total	59	455.995			

Appendix 3.45 Analysis of variance for plant height of squash to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	4370.7	485.635		
Treatment	5	1326.7	265.341	0.49	0.7789
Error	45	24165.7	537.017		
Total	59	29863.2			

Appendix 3.46 Analysis of variance for chlorophyll content of squash to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	138.068	15.3409		
Treatment	5	49.741	9.9483	0.77	0.5768
Error	45	581.907	12.9313		
Total	59	769.716			

Appendix 3.47 Analysis of variance for stem diameter of squash to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	6.0101	0.66779		
Treatment	5	4.1769	0.83538	0.30	0.0761
Error	45	32.8537	0.73008		
Total	59	43.0407			

Appendix 3.48 Analysis of variance for number of leaves of squash to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	0.05054	5.61603		
Treatment	5	0.01572	3.14303	0.45	0.8146
Error	45	0.31785	7.06303		
Total	59	0.38411			

Appendix 3.49 Analysis of variance for number of fruit of squash to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	0.92173	0.10241		
Treatment	5	0.85505	0.17101	2.82	0.0266
Error	45	2.72511	0.06056		
Total	59	4.50190			

Appendix 3.50 Analysis of variance for fruit mass of squash to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	5470.2	607.803		
Treatment	5	3999.2	799.836	1.10	0.3730
Error	45	32674.9	726.108		
Total	59	42144.3			

Appendix 3.51 Analysis of variance for gall rating of squash to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	0.22304	0.02478		
Treatment	5	0.22019	0.04404	0.66	0.4219
Error	45	2.22484	0.04944		
Total	59	2.66808			

Appendix 3.52 Analysis of variance for fresh root mass of squash to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	676.43	75.1584		
Treatment	5	49.37	9.8744	0.14	0.7818
Error	45	3156.19	70.1375		
Total	59	388.98			

Appendix 3.53 Analysis of variance for dry shoot mass of squash to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	500.48	55.6092		
Treatment	5	89.36	17.8722	0.51	0.7656
Error	45	1570.39	34.8976		
Total	59	2160.24			

Appendix 3.54 Analysis of variance for dry fruit mass of squash to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	158.39	17.5988		
Treatment	5	51.29	10.2580	0.37	0.8691
Error	45	1260.80	28.0178		
Total	59	1470.48			

Appendix 3.55 Analysis of variance for plant height of squash to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	142465	1582.4		
Treatment	5	47217	9443.4	0.65	0.6604
Error	45	650395	14453.2		
Total	59	840077			

Appendix 3.56 Analysis of variance for chlorophyll content of squash to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	366.04	40.6709		
Treatment	5	30.30	6.0606	0.42	0.8310
Error	45	646.47	14.3660		
Total	59	1042.81			

Appendix 3.57 Analysis of variance for stem diameter of squash to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	15.3079	1.70088		
Treatment	5	1.3278	0.26555	0.59	0.7044
Error	45	20.1088	0.44686		
Total	59	36.7445			

Appendix 3.58 Analysis of variance for number of fruit of squash to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	0.57933	0.06439		
Treatment	5	0.15674	0.03135	0.42	0.8301
Error	45	3.34296	0.07429		
Total	59	4.07923			

Appendix 3.59 Analysis of variance for fruit mass of squash to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	1808.7	200.971		
Treatment	5	791.8	158.366	0.37	0.8641
Error	45	19071.0	423.801		
Total	59	21671.6			

Appendix 3.60 Analysis of variance for gall rating of squash to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	0.30510	0.03399		
Treatment	5	0.33714	0.06743	1.37	0.2522
Error	45	2.20916	0.04909		
Total	59	2.85140			

Appendix 3.61 Analysis of variance for fresh root mass of squash to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after initiation and treatments and inoculation.

Source	DF	SS	MS	F	P
Replication	9	010.74	112.304		
Treatment	5	113.88	22.776	0.27	0.9278
Error	45	3813.71	84.749		
Total	59	4938.33			