NON-PHYTOTOXIC CONCENTRATION OF NEMARIOC-AL AND NEMAFRIC-BL PHYTONEMATICIDES ON GREEN BEAN CULTIVAR 'TAHOE'

by

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MINI-DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN AGRICULTURE (HORTICULTURE), DEPARTMENT OF PLANT PRODUCTION, SOIL SCIENCE AND AGRICULTURAL ENGINEERING, FACULTY OF SCIENCE AND AGRICULTURE, UNIVERSITY OF LIMPOPO, SOUTH AFRICA

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2017

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DECLARATION

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

Chokoe F.M. (miss) Date

DEDICATION

To my late mother, Miss Kgabo Edina Chokoe.

ACKNOWLEDGEMENTS

To God the Almighty, I thank You for your protection throughout my studies; I would not have made it without You, my Father Who art in Heaven. Thanks to the University of Limpopo for giving me an opportunity to study for my Master of Science degree, the National Research Foundation of South Africa for the bursary and the Agricultural Research Council-Universities Collaboration Centre for research grants. I would like to acknowledge my supervisor, Professor P.W. Mashela, because of your unfailing love for research; I am what I am today. Your principles of uncompromising hard work will remain the good part of my life. Thank you for not giving up on me whenever I fumbled! I do not want to forget the Green Technologies Research Centre team, Dr Zakheleni Dube, Ms Mosima Seshweni, Dr Kagiso Shadung, Mrs Vivian Mathabatha, Mr M.K. Ralefatana, Mr L.T. Letswalo, Mr E.M. Letswalo, Ms M.A. Mawasha, Ms S.R. Mawasha, Ms S.M. Seabela and all the postgraduate students for the technical support and for making the Centre to be home away from home. My unconditional love goes to my family and friends, especially my lovely Husband, Mr Pitse Oregan Phitlhela, thank you all for all your encouragement, your love and support. My kids, Kgabo, Lehlogonolo and Thabang, I know mommy was not there to cook for you and sing for you the lullabies, but I know you still love me and one day you are going to be proud of calling me your mother. To my special grandmother, Mrs Mmakgabo Raisibe Chokoe, thank you for taking care of my kids when I was away studying and for believing in me. You have been the grandmother that I have always wanted you to be.

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ABSTRACT

Green bean (*Phaseolus vulgaris*) is highly susceptible to *Meloidogyne* species with worldwide annual yield losses of about 11%. The use of Nemarioc-AL and Nemafric-BL phytonematicide to manage nematode population densities in this cultigen is restricted by the phytotoxicity they cause on the test crops. Development of Mean Concentration Stimulation Point (MCSP) values of Nemarioc-AL and Nemafric-BL phytonematicides on green bean would allow for the empirical determination of the application interval and eventually, the dosage model. The objective of this study was to determine whether the MCSP of Nemarioc-AL and Nemafric-BL phytonematicides on green bean cultivar 'Tahoe' inoculated with *M. javanica* would be established under the greenhouse, microplot and field conditions. In greenhouse and microplot experiments cv. 'Tahoe' green bean seeds were sown in 25 cm and 30 cm diameter pots, respectively. Pots were filled with pasteurised loam, sand soil and Hygromix-T, in 2:1:1 (v/v) ratio. In the field, seeds of cv. 'Tahoe' were sown in 5-cm deep holes, with four seeds per drip irrigation hole. Plants were inoculated with 5000 eggs and second-stage juveniles (J2) of *M. javanica*. Treatments for the greenhouse study were 0, 2, 4, 8, 16 and 32%, for the microplot 0, 0.8, 1.6, 3.2, 6.4 and 12.8% and for the field 0, 2.4, 4.8, 9.6, 19.2 and 38.4%, concentrations of Nemarioc-AL and Nemafric-BL phytonematicides each. Under all conditions treatments were arranged in a randomised complete block design, with ten replications. Nemarioc-AL phytonematicide significantly affected the number of galls and chlorophyll content under greenhouse conditions, contributing 68 and 95% in total treatment variation (TTV) of variables. Relative to the untreated control, Nemarioc-AL phytonematicide reduced gall number and increased chlorophyll content from 2 to 42%

and from 13 to 43%, respectively. Nemafric-BL phytonematicide also had significant effects on gall number, with treatments contributing 68% in TTV of the variable. Gall numbers were reduced from 7 to 35% by Nemafric-BL phytonematicide. In microplot trials Nemarioc-AL phytonematicide had significant effect on dry shoot mass, contributing 41% in TTV of the variable. Relative to the control, Nemarioc-AL phytonematicide increased dry shoots mass by 8 to 25%. Nemafric-BL phytonematicide had significant effects on gall numbers, contributing 82% in TTV of number of galls under field conditions. Increasing Nemafric-BL phytonematicide concentrations reduced gall numbers were reduced from 0 to 89%. In all experiments, nematode variables were reduced to as high as 100%. The MCSP value for Nemarioc-AL phytonematicide on green bean cv. 'Tahoe' was 2.11% and 2.67% under greenhouse and microplot conditions, respectively. In contrast, the MCSP of Nemafric-BL phytonematicide on green bean was 0.27% and 0.5% under greenhouse and field conditions, respectively. The overall sensitivity of green bean to Nemarioc-AL phytonematicide was 1 and 20 units under greenhouse and microplot, respectively. In contrast, the overall sensitivity of green bean to Nemafric-BL phytonematicide was 0 and 6 units under greenhouse and field conditions, respectively. In conclusion, both Nemarioc-AL and Nemafric-BL phytonematicides could be used for managing population densities of *Meloidogyne species* on green bean production.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

1.1.1 Description of research problem

The root-knot (*Meloidogyne* species) nematodes have over 2000 hosts (Mashela *et al*., 2011), are aggressive and most susceptible crops cannot be successfully produced unless the nematode numbers are suppressed (Sikora *et al*., 2005). Green beans (*Phaseolus vulgaris*) are highly susceptible to *Meloidogyne species*, with synthetic nematicides for managing most nematodes having been withdrawn from the agrochemical markets (Mashela *et al*., 2015); there are limited options to manage this pest. The withdrawal of fumigant nematicides from the agrochemical markets resulted in research and development of Nemarioc-AL and Nemafric-BL phytonematicides, which had been in the forefront of managing *Meloidogyne* species in South Africa (Mashela *et al*., 2015). However, phytotoxicity is one of the major limiting factors in the successful adoption of the two phytonematicides in managing nematode population densities of (Mahmood *et al*., 1979). Non-phytotoxic concentrations of phytonematicide are plant-specific and are empirically established for each plant species (Mashela *et al*., 2015).

1.1.2 Impact of the research problem

Nematode damage in crops results in yield losses as much as 10-fold, with global crop losses estimated at 12% (Ferraz and Brown, 2002), whereas the South African estimate is 14% (Pelinganga *et al*., 2013). Crop losses following the withdrawal of methyl bromide from the agrochemical markets in 2005 were estimated at US$125 billion

(Chitwood, 2003). In contrast, phytotoxicity of phytonematicides can reduce crop yield from 50% to complete crop failure (Mashela *et al*., 2015). Therefore, it is imperative that the non-phytotoxic concentration for each crop be empirically-established.

1.1.3 Possible causes of research problem

Phytonematicides such as Nemarioc-AL and Nemafric-BL have allelochemicals as active ingredients, which comprise cucurbitacins (Rice, 1984). Nemarioc-AL phytonematicide, derived from fermented ground fruits of wild cucumber (*Cucumis myriocarpus*), contains cucurbitacin A, which breaks down to cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Chen *et al*., 2005). In contrast, Nemafric-BL phytonematicide from wild watermelon (*Cucumis africanus*) fruit contains cucurbitacin B ($C_{32}H_{48}O_8$) as its active ingredient (Chen *et al*., 2005). Generally, cucurbitacins- as allelochemicals are highly phytotoxic to plant species outside the cucurbitaceae family.

1.1.4 Possible solutions of research problem

Liu *et al*. (2003) developed the Curve-fitting Allelochemical Response Dosage (CARD) computer-based model, which was adapted to develop the non-phytotoxic concentration of phytonematicides (Mashela *et al*., 2015). The latter was referred to as the Mean Concentration Stimulation Point (MCSP), which was the concentration that should consistently suppress nematode numbers without being phytotoxic to the protected plant species (Mashela *et al*., 2015). The CARD model has three phases, namely, stimulation, neutral and inhibition phases (Liu *et al*., 2003). Biological indices for the stimulation phase (D_m, R_h) had been used to generate the MCSP of phytonematicides

on various commercial crop cultivars, and was computed as MCSP = D_m + (R_h/2) (Mashela *et al*., 2015). The D_m and R_h are the threshold stimulation and the threshold saturation points, respectively, within the stimulation phase (Liu *et al*., 2003). The CARD model produces several biological indices which include the sensitivity index (Liu *et al*., 2003), which measures the degree of overall sensitivity of the plant to the test phytonematicide (Mashela *et al*., 2015).

1.2 Problem statement

The available phytonematicides, with their active ingredients being allelochemicals, could be highly phytotoxic to crops being protected against nematode damage. Phytonematicides could prevent seed germination, seedling emergence and normal plant growth (Mafeo and Mashela, 2010; Mafeo *et al*., 2011a) .The MCSP, derived from the CARD computer-based model, was viewed as the phytonematicide concentration which was not phytotoxic to a given crop, but could suppress nematode population densities consistently (Mashela *et* al., 2015). The MCSP is crop-specific and, therefore, should be developed through empirical studies for each cultigen. The MCSP values of Nemarioc-AL and Nemafric-BL phytonematicides on tomato (*Solanum lycopersicum*) were derived as 2.64 and 2.99%, respectively (Pelinganga, 2013), whereas for *Citrus volkameriana* were 8.6 and 6.3% respectively (Mathabatha *et al*., 2016). Also, those of Nemarioc-AL and Nemafric-BL phytonematicides for African ginger (*Pelargonium sidoides*) were 6.18 and 2.67%, respectively (Sithole *et al*., 2016). The MCSP values of Nemarioc-AL and Nemafric-BL phytonematicides on *P. vulgaris* cultivars had not been documented.

1.3 Rationale of the study

Green beans are highly susceptible to *Meloidogyne species* (Di Vito *et al*., 2005), with worldwide annual yield losses being tagged at 11% (Sasser and Freckman, 1987). In South Africa, the major *Meloidogyne species* include *M. incognita* races 2 and 4 and *M. javanica* (Kleyinhans *et al*., 1996). Green beans are nationally produced on more than 39 750 ha in South Africa (DAFF, 2012). However, the green beans are highly susceptible to damage by *Meloidogyne species* (Di Vito *et al*., 2005), with limited management options. Currently, in South Africa phytonematicides remain the most viable nematode management strategy, with phytotoxicity challenges. The developed MCSP for both Nemarioc-AL and Nemafric-BL phytonematicides on green bean would allow for the empirical determination of the application interval and eventually, the dosage model (Mashela *et al*., 2015).

1.4 Purpose of the study

1.4.1 Aim

The aim of the study was to develop the non-phytotoxic concentrations of Nemarioc-AL and Nemafric-BL phytonematicides for green bean productions under various conditions.

1.4.2 Objective

To determine whether the MCSP of Nemarioc-AL and Nemafric-BL phytonematicides on green bean cultivar 'Tahoe' infected with *M. javanica* could be established under greenhouse, microplot and field conditions.

1.4.3 Hypothesis

The MCSP of Nemarioc-AL and Nemafric-BL phytonematicides on green bean cultivar 'Tahoe' infected with *M. javanica* could be established under greenhouse, microplot and field conditions.

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, and while objectivity was achieved by ensuring that the findings were discussed on the basis of empirical evidence, in order to eliminate all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

Bias was reduced through minimising the experimental error by increasing the number of replications. The treatments were also randomised within the selected experimental design (Leedy and Ormrod, 2005).

1.7 Structure of mini-dissertation

The research problem of the study was outlined (Chapter 1), followed by the review of work done and not yet done on the research problem (Chapter 2). Chapter 3 addressed the objective of the study, whereas Chapter 4 provided the summary, significance of the findings, recommendations for future research and overall conclusions. The citations

and references followed the Harvard style as prescribed by the Senate of the University of Limpopo.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

The management of plant-parasitic nematodes in cropping systems is necessary if crop enterprises are to be profitable and improve food security on a global scale (Chitwood 2002; Okwute, 2012). Due to the withdrawal of synthetic chemical nematicides from the agrochemical markets, plant-derived phytonematicides had since attracted attention for use as alternatives (Mashela *et al*., 2015).

Most plant species had been reported to have organs that contain bioactive chemical compound (Van Wyk and Wink, 2004). Phytonematicides are among the preferred alternative nematode management strategies and consist of a class of plant-based bioactive chemical compounds, which could be used as aqueous plant extracts, methanol plant extracts, ethanol plant extracts, oilcakes (Muller and Gooch, 1982), essential oils (Meyer *et al*., 2008), fermented crude plant extracts, powders and granules (Mashela *et al*., 2015). The review on the research problem in this study focused on two aspects: (i) what had already been done on the research problem and (ii) what had not yet been done on the research problem.

2.2 Work done on problem statement

2.2.1 Phytotoxicity in phytonematicides

Fruits of wild cucumber (*Cucumis myriocarpus*) and wild watermelon (*Cucumis africanus*) had been used to produce Nemarioc-AL or AG and Nemafric-BL or BG

phytonematicide, respectively (Mashela *et al*., 2015). The suffix A and B represent the active ingredients, cucurbitacin A and B, whereas L (liquid) and G (granular) represent the formulations (Mashela *et al*., 2015). The two phytonematicides, despite their capability to manage nematode population densities consistently, could also results in incidence of phytotoxicities on the crops protected against nematode damage. Phytotoxicity of the materials could results in yield losses as high as 50% to total crop failures (Mashela *et al*., 2015). Mafeo and Mashela (2009a, 2010) reported high phytotoxicity of Nemarioc-AG phytonematicide to eight monocotyledonous and ten dicotyledonous crops, with most crops failing to emerge when 5 g Nemarioc-AL phytonematicide was applied as a pre-emergent drench. Similarly, when both Nemarioc-AL and Nemafric-BL phytonematicides were applied on tomato seedlings at above 10% concentration after transplanting growth was inhibited (Pelinganga and Mashela 2012; Pelinganga *et al*., 2013).

Generally, at high concentrations, phytonematicide inhibit crop growth. The increasing concentration of Nemafric-BL phytonematicide from 10 to 60% was highly toxic on tomato (*Solanum lycopersicum*) plant, with CARD model suggesting that the dilution should be below 10% (Pelinganga *et al.,* 2013). Nemarioc-AL phytonematicide inhibited growth of leek, onion and chive under greenhouse conditions (Mafeo *et al*., 2010), the material also inhibited germination of maize (*Zea mays*), finger millet (*Eleusine coracana*), sorghum (*Sorghum bicolor*) and onion (*Alliums cepa*) when tested on crops *ex vitro* (Mafeo and Mashela, 2009).

2.2.2 Non-phytotoxic concentrations of phytonematicides

Mashela *et al*. (2015) introduced the concept of the dosage model intended for management of phytotoxicity and consistent suppression of nematode numbers in crop production. In the model, the mean concentration stimulation point (MCSP) is the concentration of a phytonematicide which would stimulate plant growth and consistently suppress nematode numbers (Mashela *et al*., 2015). The MCSP values of phytonematicides are being empirically developed for each crop because they are plant-specific (Mashela *et al*., 2015).

Mathabatha *et al*. (2016) developed the MCSP values for two phytonematicides on *Citrus volkameriana* under greenhouse conditions as being 8.6 and 6.3% for Nemarioc-AL and Nemafric-BL phytonematicides, respectively. In contrast, Pelinganga (2013) reported lower concentrations of 2.64 and 2.99% for the two respective products on tomato. The MCSP values on African ginger (*Pelargonium sidoides*) were 6.18 and 2.67% for the two phytonematicides, respectively, under micro-plot conditions (Sithole, 2016).

The non-phytotoxicity of MCSP values depends on the application frequency, which is referred to as the number of times the product is applied per the growing season (Mashela *et al*., 2015). In the model, dosage (%) = MCSP (%) × application frequency (Mashela *et al*., 2015). According to Mashela *et al*. (2015), the MCSP values should be related to the overall sensitivity values and concentration that reduced nematode population densities. In other words, since nematode numbers were reduced at low

concentrations, when MCSP values were high, the MCSP values could be adjusted to the minimum concentration that suppressed nematodes.

2.2.3 Overall sensitivity of crops to phytonematicides

The Curve-fitting Allelochemical Response Dosage (CARD) model, which was used to develop the MCSP values of phytonematicides, also provided the sensitivity indices (k values) of variables, which could be used to determine the overall sensitivity index (Σk) of the whole plant to the test material (Liu *et al*., 2003). Generally, the closer the Σk is to zero, the higher the sensitivity of the crop to the material (Liu *et al*., 2003).

The sensitivity of crops to Nemarioc-AL (L= liquid formulation) or AG (G = granular formulation) and Nemafric-BL or BG phytonematicides had been established on several studies. The Σk for *C. volkameriana* seedlings to Nemarioc-AL and Nemafric-BL phytonematicides were 2 and 4 units, respectively (Mathabatha *et al*., 2016). Tomato plants were highly sensitive to Nemarioc-AL and Nemafric-BL phytonematicides, with the overall sensitivities of 0 and 3 units, respectively (Pelinganga, 2013). Similarly, African ginger was highly sensitive to Nemarioc-AL and Nemafric-BL phytonematicides, with the overall sensitivity of 3 units for the two materials (Sithole, 2016). Mafeo *et al.* (2011a) observed that sorghum was most sensitive to Nemarioc-AL phytonematicide with overall sensitivity of 9 units and millet was the least sensitive to the material with the overall sensitivity of 18 units.

2.2.4 Efficacy of phytonematicides

The use of phytonematicides in nematode suppression under *in vitro* trials, have had in excess of 90% suppression of nematode numbers (Okwute, 2012). Nemarioc-AL phytonematicide reduced nematode population densities on African ginger under microplot conditions to as high as 81% reduction (Sithole *et al*., 2016). Under greenhouse conditions, Nemarioc-AG phytonematicide reduced nematodes from 73-83% in roots and 49-68% of nematodes in soil (Mashela, 2002). Similarly, final nematode population were reduced when Pelinganga *et al*. (2013) and Pelinganga and Mashela (2012) exposed tomato plants to Nemarioc-AL phytonematicide under field and greenhouse conditions. Mashela and Mphosi (2002) used Nemarioc-AG phytonematicide to suppress population levels of *Meloidogyne* species and the citrus nematode (*Tylenchulus semipenetrans*) in pot trials with nematodes in both trials reduced by at least 90%.

2.2.5 Density-dependent growth (DDG) patterns in phytonematicides

Plants and microbes respond to increasing concentrations of allelochemicals in phytonematicides through DDG patterns, which comprise three phases, namely, stimulation, neutral and inhibition phases (Mashela *et al*., 2015). Liu *et al*. (2003) quantified responses of entities to concentrations of allelochemicals which lead to three phases that characterise the DDG patterns. An important feature of the DDG patterns is that the variable (y-axis) and the concentration of allelochemicals (x-axis) exhibit quadratic relationships, with the coefficient of determination (R^2) determining the strength of the model (Pelinganga and Mashela, 2012; Pelinganga *et al*., 2013).

In the study by Dube and Mashela (2016), juvenile hatch responded to increasing cucurbitacin A and B concentrations through DDG patterns. Similar results were observed in several other studies on different plant variables (Chuwuka *et al*., 2014; Mafeo *et al*., 2011; Mashela *et al*., 2015; Pelinganga *et al*., 2013; Sithole, 2016). In the DDG patterns, the material can stimulate, neutralise or inhibit plant growth. Generally the no-effect results in the use of phytonematicides are related to the neutral zone of the DDG pattern (Mashela *et al*., 2015).

Nemarioc-AL and Nemafric-BL phytonematicides had no effect on plant variables of African ginger under microplot conditions (Sithole, 2016). In contrast, Nemarioc-AL and Nemafric-BL phytonematicides had effects on plant variables in tomato plants (Pelinganga and Mashela, 2012). The no-effect results was also observed by Ghaferbi *et al*. (2012) when exposing eight selected plant species to seed extracts of wheat (*Triticum aestivum*). Similarly, at 2% crude extracts of yellow nutsedge (*Cyperus esculentus*) had no effect on germination of lettuce (*Lactuca sativa*).

2.3 Work not done on problem statement

The MCSP is phytonematicide- and plant species-specific. Therefore, the MCSP values of Nemarioc-AL and Nemafric-BL phytonematicides on green beans had to be empirically-developed if the products had to be successfully used in nematode suppression on green beans.

CHAPTER 3

RESPONSE OF GREEN BEAN TO NEMARIOC-AL AND NEMAFRIC-BL PHYTONEMATICIDES

3.1 Introduction

The Mean Concentration Stimulation Point (MCSP) is the concentration of phytonematicides capable of managing nematode population densities without inducing phytotoxicity to the test crop (Mashela *et al*., 2015). The MCSP is empirically-developed for each crop because it is plant-specific (Mashela *et al*., 2015). The MCSP differs within phytonematicides; therefore, MCSP values for Nemarioc-AL phytonematicide would not necessarily be similar to that for Nemafric-BL phytonematicide on similar crops (Mashela *et al*., 2015). The objective of this study was to determine the MCSP of Nemarioc-AL and Nemafric-BL phytonematicides on green bean cultivar 'Tahoe' infected with *M. javanica* under greenhouse, microplot and field conditions.

3.2 Materials and methods

3.2.1 Plant growth conditions

The study was conducted under greenhouse, microplot and field conditions at the Green Technologies Research Centre (GTRC), University of Limpopo, South Africa (23°53'10"S, 29°44'15"E). The first set of trials ran concurrently during autumn (January-March) in 2016 and repeated in late spring (July-September) to early summer (October-December).

Greenhouse conditions: The trial was conducted under the greenhouse that was 20-m wide × 100-m long, thus conditions inside were not homogenous. Thus, the experiments, depending on experimental size, had to be designed appropriately. The end of the greenhouse had fans which were thermostatically-activated to extract warm air, with the wet wall being on the other end, to moderate relative humidity. Ambient day/night temperatures averaged 27/18°C. The top of the greenhouse was covered with a 35% green net.

Legend 3.1 Effect of Nemarioc-AL and Nemafric-BL phytonematicides on *Phaseolus vulgaris* cv. 'Tahoe' under greenhouse conditions.

Microplot conditions: Micro-plots where created by placing plastic pots on lids. The crop was exposed to Hot and dry summers had maximum temperatures ranging from 28°C to 38°C, with summer rainfall being less than 500 mm.

Legend 3.2 Effect of Nemarioc-AL and Nemafric-BL phytonematicides on *Phaseolus vulgaris* cv. 'Tahoe' under micro-plot conditions.

Field conditions: The field experiment was conducted at the GTRC outside the greenhouse. The field had Hutton soil (65% sand, 30% clay, 5% silt), with organic C at 1.6%, electrical conductivity 0.148 dS/m and pH (H_2O) 6.5. The climatic conditions were the same as in microplot experiment.

Legend 3.3 Effect of Nemarioc-AL and Nemafric-BL phytonematicides on *Phaseolus vulgaris* cv. 'Tahoe' under field conditions.

3.2.2 Experimental design, inoculation and cultural practices

The greenhouse experiment had six treatments, namely, 0, 2, 4, 8, 16 and 32% concentrations of each phytonematicides, microplot experiment had six treatments, namely, 0, 0.8, 1.6, 3.2, 6.4 and 12.8% for each phytonematicide, whereas the field experiment also had six treatments, but comprised 0, 2.4, 4.8, 9.6, 19.2 and 38.4% concentrations for each product. The experiments were arranged in a randomised complete block design, with 10 replications.

In the greenhouse and microplot experiments, *P. vulgaris* cv. 'Tahoe' green bean seeds were primed with tap-water for 45 minutes and then sown in 25-cm and 30-cm diameter plastic pots, respectively. Each pot was filled with pasteurised loam, sand soil and Hygromix-T (Hygrotech, Pretoria North) at 2:1:1 (v/v) ratio. The pots were arranged at 0.45 m × 0.45 m intra- and inter-row spacing. In the field experiment, cv. 'Tahoe' green bean seeds were sown in a sandy-loam soil in 5-cm holes prepared using a special gadget referred to as 3S planter, which allowed the sowing of four seeds in a squared area per hole of drip irrigation.

Nemarioc-AL and Nemafric-BL phytonematicides were prepared as described previously (Mashela *et al*., 2015). Nematode eggs and second-stage juveniles (J2) were extracted from roots of nematode-susceptible tomato cv. 'Floradade' in 1% NaOCl solution (Hussey and Barker, 1973). Seedlings were inoculated with 5000 eggs and J2 of *M. javanica*. At two-leaf stage, seedlings were fertilised with 5 g NPK of 2:3:2 (26) + 0.5% Zn + 5% S + 5% Ca, 5 g per plant and 1 g 2:1:2 (43) Multifeed to provide macro- and micro-nutrients except for Ca. Each seedling was irrigated with 500 ml chlorine-free tapwater every other day under the greenhouse and on microplot trials. Under field conditions, plants were irrigated with drip irrigation system that had an output of 2 L water per hole per hour. Once a week, irrigation was substituted with solutions of the respective treatments. A spray programme was developed to manage diseases, with Funginex, Bravo and Dithane being alternated weekly. The trials were monitored for insect pests, which were, however, not observed.

3.2.3 Data collection

At 56 days after inoculation, plant height was measured from the crown to the tip of the flag leaf, with leaf number and pod number per plant counted. Chlorophyll meter was used to measure chlorophyll content of three matured leaves per plant. Stem diameter was measured using a digital caliper. Pods and shoots were separately oven-dried at 70°C for 72 h and weighed. Root systems were removed from pots, immersed in water to remove soil particles, blotted dry and weighed to facilitate the calculation of nematode density 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). Nodules were rated using the nodulation rating scale of 0 = no nodules, 1 = 5 or one 1 large nodule, 2 = 10 or 2 large nodules, 3 = 15 or 3 large nodules, 4 = 20 or 4 large nodules, 5 = 25 or 5 large nodules, 6 = 25 or 5 large nodules and 7= ≥ 30 nodules.

Nematodes were extracted from 10 g roots per plant by maceration and blending for 30 seconds in 1% NaOCl (Hussey and Barker, 1973). The materials were passed through 150-, 45- and 25- nested sieves, with nematodes being collected from the 25-µm mesh sieve into 50 ml containers for sugar-floatation and centrifugation (Jenkins, 1964). Soil per pot was thoroughly mixed and a 250 ml soil sample was collected, with nematodes being extracted from soil samples using the sugar-floatation and centrifugation (Jenkins, 1964). Eggs and J2 from root samples were counted from a 10-ml aliquot with the use of a stereomicroscope. Nematode numbers from roots were converted to nematodes per total root system per plant, whereas J2 from soil samples were converted to total

soil per pot, which was 400 ml and 800 ml under greenhouse and microplot, respectively.

3.2.4 Data analysis

Data collected were subjected to analysis of variance (ANOVA) using SAS software (SAS Institute, 2008). The degrees of freedom and their associated mean sum of squares were partitioned to provide the total treatment variation (TTV) of different variables. Mean separation was achieved through the Waller-Duncan Multiple-Range test at 5% level of probability. Mean plant variables were subjected to the Curve-fitting Allelochemical Response Dosage (CARD) computer-based model (Liu *et al*., 2003) to generate biological indices used to calculate the MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides on green beans. Unless stated otherwise, treatment effects were discussed at 5% level of probability. Data were evaluated for seasonal effects and since the seasonal interactions were not significant, data were pooled and subjected to ANOVA (n = 60).

3.3 Results

3.3.1 Greenhouse trials

3.3.1.1 Plant variables

Treatment effects: Nemarioc-AL phytonematicide had highly significant effects on chlorophyll content and gall rating. The treatments contributed 68 and 95% in TTV of the two respective variables (Table 3.1). In contrast, Nemafric-BL phytonematicide had highly significant effect on gall rating, contributing 68% in TTV of the variable (Table

3.1). Effects of the two phytonematicides were not significant on other plant and nematode variables (Appendices 3.1- 3.30).

Relative treatment effects: Relative to untreated control, at low concentration Nemarioc-AL phytonematicide did not affect chlorophyll content, whereas at the two highest concentrations the product reduced the variable by 9% (Table 3.2). Nemarioc-AL phytonematicide also reduced gall rating by 42% at the highest concentration of 32%, whereas the reduction in soil J2 was from 22 to 100%. In contrast, Nemafric-BL phytonematicide reduced gall rating from 13 to 35% (Table 3.2).

Curve-fitting Allelochemical Response Dosage: The chlorophyll content and gall rating with increasing concentration each exhibited a quadratic relationship, with the models being explained by 75 and 70%, respectively (Figure 3.1, 3.2). Similar quadratic relationships were observed when gall number and increasing concentrations of Nemafric-BL phytonematicide were subjected to the CARD model (Figure 3.3), with the model being explained by 83% (Table 3.3). Using the relation $\times = -b_1/2b_2$, the optimum chlorophyll content and gall number were achieved at 1.8 and 1.2% concentration of Nemarioc-AL phytonematicide, respectively (Table 3.3). However, the optimum Nemafric-BL phytonematicide concentration for gall number was 1.7% (Table 3.3).

Biological indices: Using the biological indices D_m and R_h (Table 3.4), the MCSP value of Nemarioc-AL phytonematicide on green bean cultivar 'Tahoe' was 2.11%, whereas that for Nemafric-BL phytonematicide was 0.27%. The overall sensitivities of green

bean to Nemarioc-AL and Nemafric-BL phytonematicide were 1 and 0 units, respectively.

3.3.1.2 Nematode variable

Nemarioc-AL phytonematicide had significant effects on J2 in roots (Table 3.1), contributing 59% in TTV of the variable. In contrast, Nemarioc-AL phytonematicide did not have significant effects on the other nematode variables (Appendices 3.12, 3.13, 3.15). Nemafric-BL phytonematicide had no effects on all nematode variables (Appendices 3.27, 3.28, 3.29, 3.30).

Table 3.1 Sources of variation as affecting chlorophyll content (CPC), gall rating (GR), and nematode second-stage juveniles (J2) in soil at 56 days after initiation of treatments under greenhouse conditions (n = 60).

		Nemarioc-AL						Nemafric-BL	
		Chlorophyll		Gall rating		$J2_{roots}$		Gall rating	
Source	DF	MS	%	MS	%	MS	%	MS	%
Replication	9	20.576	15	0.002	2	0.301	18	0.015	24
Treatment	5	92.877	68***	0.104	95***	0.994	59**	0.043	68***
Error	45	22.562	17	0.004	3	0.391	23	0.005	8
Total	59	136.015	100	0.11	100	1.686	100	0.063	100

*** Highly significant at P ≤ 0.01.

Table 3.2 Relative impact (RI) of Nemarioc-AL and Nemafric-BL phytonematicides on chlorophyll content (CPC) and gall rating (GR) and nematode second-stage juveniles (J2) in soil at 56 days after initiation of treatments under greenhouse conditions (n = 60).

Treatment	Nemarioc-AL						Nemafric-BL	
	Chlorophyll	RI (%)	Gall rating	RI (%)	$J2_{soil}$	RI (%)	Gall rating	RI (%)
0	39.37^{ab}	–	0.59^{a}	–	0.999^{a}	–	0.49^{a}	–
2	41.37^{a}	5	0.58^{a}	–2	0.000^{c}	–100	0.42^{bc}	–13
4	42.68^{a}	8	0.57^{a}	–4	0.000^{c}	–100	0.45^{ab}	–7
8	42.05^{a}	7	0.51^{a}	–14	0.260^{bc}	–74	0.35^{d}	–28
16	35.74^{b}	–9	0.58^{a}	–2	0.781^{ab}	–22	0.37^{cd}	–25
32	36.01^{b}	–9	0.34^{b}	–42	0.000^{c}	–100	0.32^{d}	–35
P ≤	0.01		0.01		0.01		0.01	

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

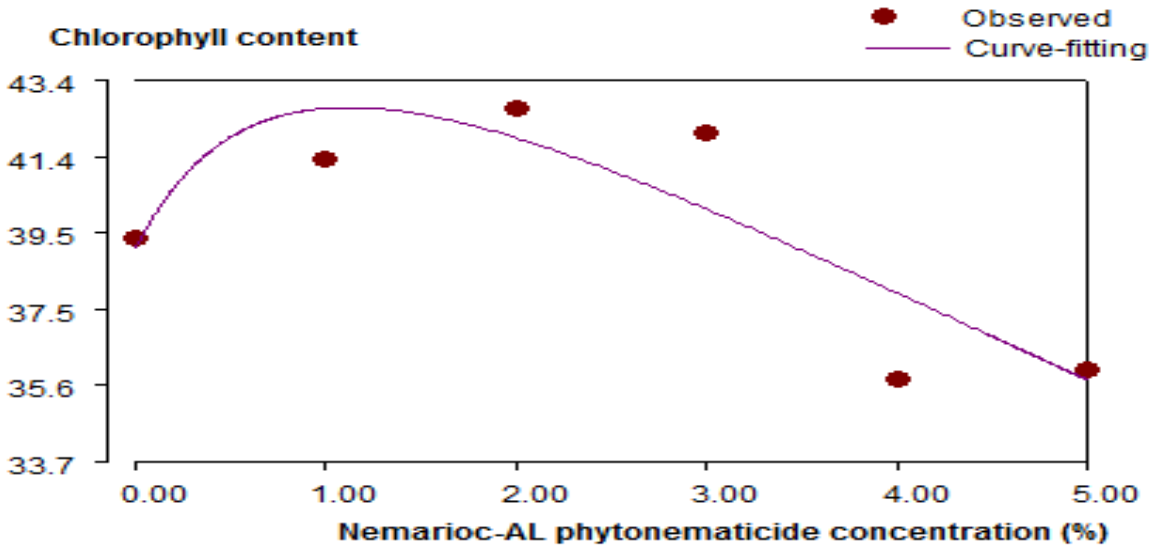

Figure 3.1 Responses of chlorophyll content to concentrations of Nemarioc-AL phytonematicide under greenhouse conditions.

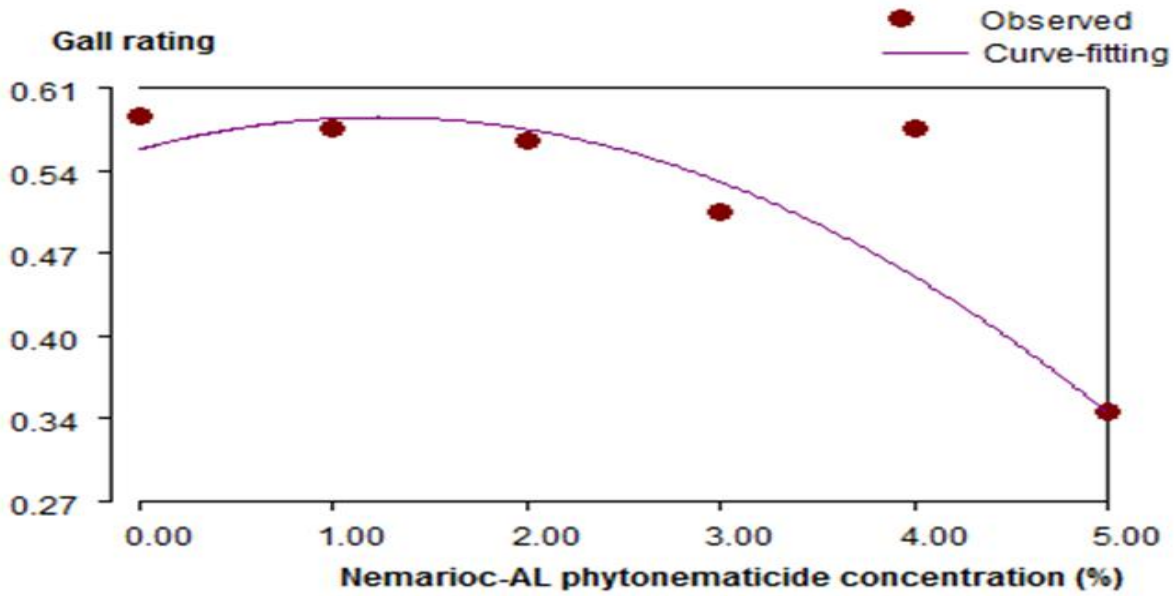

Figure 3.2 Responses of gall rating to concentrations of Nemarioc-AL phytonematicide under greenhouse conditions.

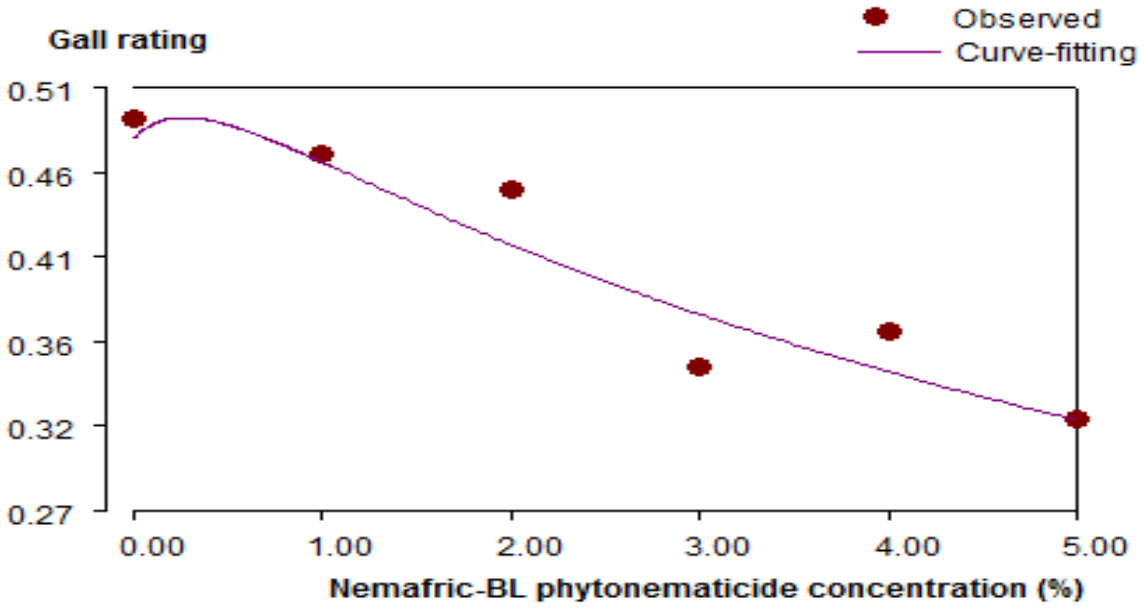

Figure 3.3 Responses of gall rating to concentrations of Nemafric-BL phytonematicide under greenhouse conditions.

Table 3.3 Quadratic relationship, coefficient of determination and computed optimum response concentration for chlorophyll content (CPC) and gall rating (GR) of green beans from the Curve-fitting Allelochemical Response Dosage against Nemarioc-AL and Nemafric-BL phytonematicide under greenhouse conditions.

Nemarioc-AL				
Organs	Quadratic relation	R^2	x^z	Y
CPC	$y = –0.6988x^2 + 2.5132x + 39.659$	0.75	1.8	42
GR	$y = –0.0148x^2 + 0.0367x + 0.5725$	0.70	1.2	0.60
Nemafric-BL				
Organ	Quadratic relation	R^2	x^z	Y
GR	$y = 0.0011x^2 – 0.0368x + 0.4821$	0.8331	1.7	0.45

$x^z = –b_1/2b_2$

Table 3.4 Biological indices for chlorophyll content (CPC) and gall rating (GR) of green beans to increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides under greenhouse conditions.

Biological index[z]	Nemarioc-AL			Nemafric-BL	
	CPC (%)	GR (%)	Mean (%)	GR (%)	Mean (%)
Threshold stimulation (D_m)	1.115	1.237	1.176	0.268	0.268
Saturation point (R_h)	3.721	0.023	1.872	0.012	0.012
0% inhibition (D_0)	3.474	2.475	2.975	0.703	0.703
50% inhibition (D_{50})	12.755	5.803	9.279	8.723	8.723
100% inhibition (D_{100})	25.8	7.6	16.7	44.5	44.5
R^2	0.761	0.701	0.731	0.912	0.912
K- value	1	0		0	
Overall sensitivity	Σk = 1			Σk = 0	
MCSP = D_m + (R_h/2) = 1.176+ (1.872/2) = 1.176 + 0.936 = 2.11%				MCSP = 0.27%	

3.3.2 Microplot trials

3.3.2.1 Plant variables

Treatment effects: Nemarioc-AL phytonematicide had significant effect on dry shoot mass, contributing 41% in TTV (Table 3.5). In contrast, the material had no effect on other plant variables (Appendices 3.31 – 3.42). Nemafric-BL phytonematicide also had no significant effects on plant variables (Appendices 3.48 – 3.59).

Relative treatment effects: Relative to untreated control, Nemarioc-AL phytonematicide reduced dry shoots mass by 7.8% at medium concentration and increased the variable by 25% at low concentration (Table 3.7).

Curve-fitting Allelochemical Response Dosage: Dry shoot mass over the increasing concentrations of Nemarioc-AL phytonematicide formed a quadratic relationship (Figure 3.9). The model explained the relationship of Nemarioc-AL phytonematicide and dry shoot mass by 98% (Table 3.9). Using the relation $x = -b_1/2b_2$, optimum dry shoot mass was obtained at 4.78% concentration of Nemarioc-AL phytonematicide (Table 3.9).

Biological indices: Using the relation MCSP = $D_m + (R_h/2)$, MCSP was 2.67% in experiment for Nemarioc-AL phytonematicide (Table 3.10). The MCSP of Nemafric-BL phytonematicide was not obtained under micro-plot conditions since all variables were not significant. Overall sensitivity value of the crop to Nemarioc-AL phytonematicide was 20 units (Table 3.10).

3.3.2.2 Nematode variables

Treatment effects: When exposing green bean to increasing concentration of Nemarioc-AL phytonematicide, the treatments significantly affected nematode juveniles in roots, nematode eggs in roots, nematode juveniles in soil and final nematode population contributing in TTV 80, 56, 74 and 87% of the respective variables (Table 3.6). Similarly, Nemafric-BL phytonematicide significantly affected nematode juveniles in roots, nematode eggs in roots and final nematode population with 31, 77 and 95% in TTV of the respective variables (Table 3.7).

Relative treatment effects: In relation to untreated control, juveniles in roots were reduced from 65 to 100%, eggs reduced from 4 to 100%, juveniles in soil were reduced

from 52 to 100% and final nematode population was reduced from 40 to 100% when exposing green bean to Nemarioc-AL phytonematicide (Table 3.10). Nemafric-BL phytonematicide reduced nematode juveniles in roots from 87 to 100%, nematode eggs in roots from 64 to 100% and nematode final population from 72 to 100% in relation to control (Table 3.11).

Table 3.5 Sources of variation as affecting dry shoot mass (DSM) at 56 days after initiation of Nemarioc-AL phytonematicide treatments under microplot conditions (n = 60).

Source	DF	MS	%
Replication	9	19.645	43
Treatment	5	18.319	41**
Error	45	7.199	16
Total	59	45.163	100

** Significant at P ≤ 0.01

Table 3.6 Sources of variation affecting nematode second-stage juveniles (J2), nematode eggs and final nematode (Pf) on green bean population at 56 days after initiation of treatments under micro-plot conditions.

Nemarioc-AL phytonematicide

		$J2_{roots}$		$Eggs_{roots}$		$J2s_{soil}$		Pf	
Source	DF	MS	%	MS	%	MS	%	MS	%
Rep	9	0.141	7	0.833	29	1.004	14	1.278	9
Trt	5	1.698	80***	1.577	56***	5.255	74***	12.366	87***
Error	45	0.275	13	0.426	15	0.845	12	0.572	4
Total	59	2.114	100	2.836	100	7.104	100	14.216	100

Nemafric-BL phytonematicide

		$J2_{roots}$		$Eggs_{roots}$		$J2_{soil}$		Pf	
Source	DF	MS	%	MS	%	MS	%	MS	%
Rep	9	0.139	40	0.107	8	0.328	25	0.134	2
Trt	5	0.107	31***	1.096	77***	0.590	44ns	7.224	95***
Error	45	0.102	29	0.214	15	0.403	31	0.286	3
Total	59	0.348	100	1.417	100	1.321	100	7.644	100

*** Highly significant at $P \leq 0.01$, ns Not significant at $P \leq 0.05$

Table 3.7 Relative impact (RI) of Nemarioc-AL phytonematicides on dry shoot mass of green beans at 56 days after initiation of treatments under microplot conditions.

Treatments	Dry shoot mass	RI (%)
0	12.3[b]	—
0.8	15.39[a]	25
1.6	12.57[b]	2
3.2	11.34[b]	–7.8
6.4	12.72[b]	3.4
12.8	12.67[b]	3

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

Table 3.8 Relative impact (RI) of Nemarioc-AL and Nemafric-BL phytonematicides on juveniles, nematode eggs and total nematode population at 56 days after initiation of treatments under micro-plot conditions.

Trt	Nemarioc-AL phytonematicide							
	$J2_{roots}$	RI (%)	Egg_{roots}	RI (%)	$J2s_{soil}$	RI (%)	Pf	RI (%)
0	1.05^{a}	–	0.57^{ab}	–	1.80^{a}	–	2.69^{a}	–
0.8	0.37^{b}	–65	0.91^{a}	61	0.87^{b}	–52	1.62^{b}	–40
1.6	0.18^{b}	–83	0.59^{ab}	4	0.29^{bc}	–84	0.79^{c}	–71
3.2	0.00^{b}	–100	0.00^{b}	–100	0.00^{c}	–100	0.00^{d}	–100
6.4	0.00^{b}	–100	0.00^{b}	–100	0.00^{c}	–100	0.00^{d}	–100
12.8	0.00^{b}	–100	0.00^{b}	–100	0.00^{c}	–100	0.00^{d}	–100

Nemafric-BL phytonematicide						
Treatment	$J2s_{roots}$	%	$Eggs_{roots}$	%	Pf	%
0	0.98^{a}	–	0.82^{a}	–	2.12^{a}	–
0.8	0.13^{b}	–87	0.29^{b}	–64	0.56^{b}	–72
1.6	0.00^{b}	–100	0.00^{b}	–100	0.00^{c}	–100
3.2	0.00^{b}	–100	0.00^{b}	–100	0.00^{c}	–100
6.4	0.00^{b}	–100	0.00^{b}	–100	0.00^{c}	–100
12.8	0.00^{b}	–100	0.00^{b}	–100	0.00^{c}	–100

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

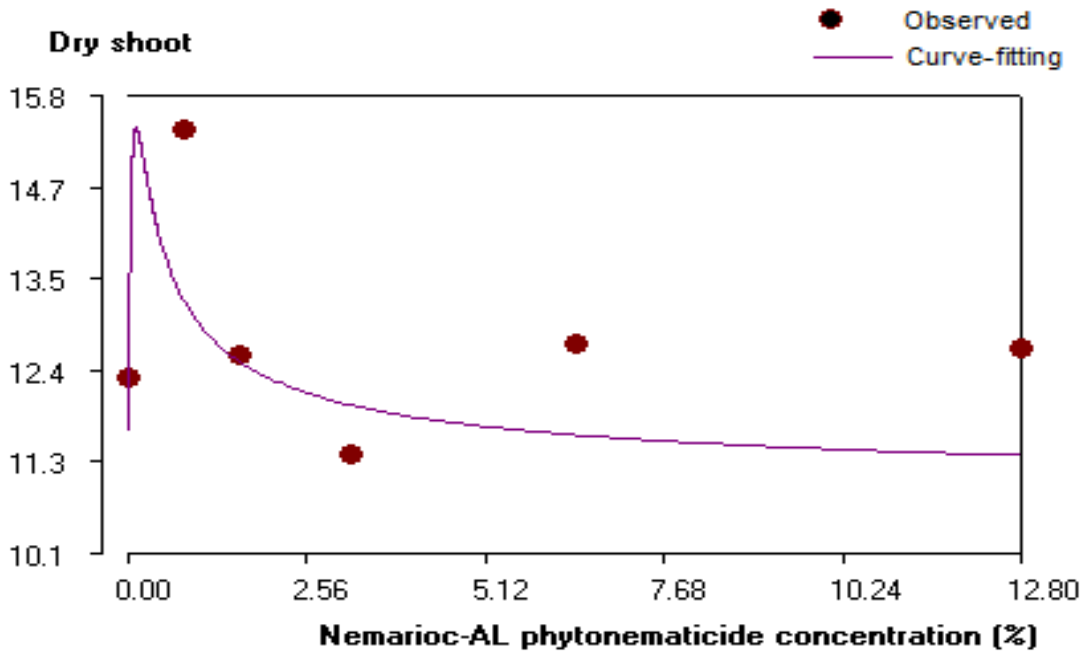

Figure 3.4 Responses of dry shoot mass to concentrations of Nemarioc-AL phytonematicide under microplot conditions.

Table 3.9 Quadratic relationship, coefficient of determination and computed optimum response concentration for dry shoot mass (DSM) and gall rating (GR) of green beans from the CARD model against Nemarioc-AL phytonematicide under microplot.

Organ	Quadratic relation	R^2	x^z	Y
DSM	$y = 0.0081x^2 – 0.0775x+12.337$	0.9822	4.78	12.15

Table 3.10 Biological indices for dry shoot mass of green beans to increasing concentrations of Nemarioc-AL phytonematicide under microplot conditions.

Biological index[z]	DSM (g)	Mean
Threshold stimulation (D_m)	0.108	0.108
Saturation point (R_h)	5.135	5.135
0% inhibition (D_0)	5.354	5.354
50% inhibition (D_{50})	–	–
100% inhibition (D_{100})	–	–
R^2	0.522	0.522
K- value	20	
Overall sensitivity	Σk = 20	
MCSP = D_m + (R_h/2) = 0.108 + (5.135/2) = 2.67%		

3.3.3 Field trials

3.3.3.1 Plant variables

Treatment effects: All plant variables were not significant when exposed to increasing concentration of Nemarioc-AL phytonematicide (Appendices 3.65-3.76). Nemafric-BL phytonematicide had significant effect on number of galls (Table 3.11) and other plant variables were not significantly affected by increasing concentration of the material. The treatments contributed in 82% in TTV of gall number (Table 3.11).

Relative treatment effects: In relation to untreated control, Nemafric-BL phytonematicide had no effect on number of galls; however the material reduced the variable by 89% at the highest concentration of 12.8% (Table 3.12).

Curve-fitting allelochemical response dosage: Number of galls over the increasing concentrations of Nemafric-BL phytonematicide also formed a quadratic relationship

(Figure 3.5). The relationship between Nemafric-BL phytonematicide and gall rating was 99% (Table 3.13). In the use of the relation × = $–b_1/2b_2$, minimum gall numbers were achieved at 30.36% Nemafric-BL phytonematicide (Table 3.13).

Biological indices: The MCSP value was 0.5% of Nemafric-BL phytonematicide on green bean under field experiment, with the overall sensitivity of 6 units (Table 3.14).

3.3.3.2 Nematode variables

Treatment effects: Nemarioc-AL phytonematicide had significant effects on the final nematode population contributing 72% in TTV of the variable (Table 3.11).

Relative treatment effects: Relative to untreated control, the final nematode population densities were reduced from 49 to 100% (Table 3.12).

Table 3.11 Sources of variation affecting gall rating (GR) and final nematode population (Pf) at 56 days after initiation phytonematicide treatments under field conditions (n = 60).

		Nemafric-BL phytonematicide		Nemarioc-AL phytonematicide	
		GR		Pf	
Source	DF	MS	%	MS	%
Replication	8	0.015	10	0.286	13
Treatment	5	0.120	82***	1.557	72***
Error	40	0.011	8	0.323	15
Total	53	0.146	100	2.166	100

*** Highly significant P ≤ 0.01.

Table 3.12 Relative impact (RI) of Nemarioc-AL and Nemafric-BL phytonematicides on final nematode population (Pf) and gall rating (GR) on green bean at 56 days after initiation of treatments under field conditions.

Treatment	Nemarioc-AL		Nemafric-BL	
	Pf	RI (%)	GR	RI (%)
0	1.144[a]	–	0.30	–
2.4	0.588[b]	– 49	0.30	0
4.8	0.345[bc]	– 70	0.23	– 22
9.6	0.179[bc]	– 84	0.17	– 44
19.2	0.147[bc]	– 87	0.07	– 78
38.4	0.000[c]	– 100	0.03	– 89
P ≤	0.01		0.01	

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

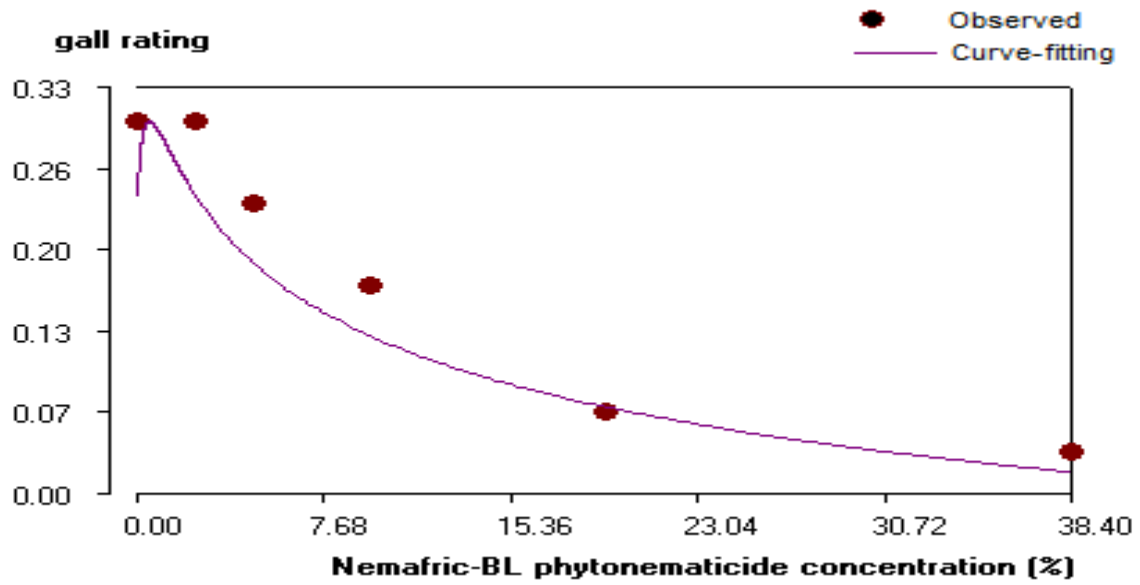

Figure 3.5 Responses of gall rating to concentrations of Nemafric-BL phytonematicide under field conditions.

Table 3.13 Quadratic relationship, coefficient of determination and computed optimum response concentration for gall rating of green beans from the Curve-fitting Allelochemical Response Dosage against Nemafric-BL phytonematicide under field conditions

Organ	Quadratic relationship	R^2	x^z	Y
GR	$y = 0.0003x^2 – 0.0184x + 0.3189$	0.9857	– 30.667	1.165

$x^z = – b_1/2b_2$

Table 3.14 Biological indices for gall rating of green bean to increasing concentrations of Nemafric-BL phytonematicide under field conditions

Biological index^z	Gall rating	Mean
Threshold stimulation (D_m)	0.502	0.502
Saturation point (R_h)	0.075	0.075
0% inhibition (D_0)	2.397	2.397
50% inhibition (D_{50})	10.41	10.41
100% inhibition (D_{100})	4.91	4.91
R^2	0.99	0.99
K- value	3	
Overall sensitivity	Σk = 3	
MCSP = $D_m + (R_h/2)$ = 0.502 + (0.075/2) = 0.5%		

3.4 Discussion

3.4.1 Plant variables

Treatment effects: In greenhouse experiments, Nemarioc-AL phytonematicide had significant effect on chlorophyll content and number of galls. Similarly, the material reduced gall numbers on the roots of African ginger (Sithole, 2016). Nemarioc-AL phytonematicide had no effect on plant variables of green bean. The non-effect or neutral phase of DDG patterns imply that the material affected plant growth at the

saturation point of the CARD model were growth was neither stimulated nor inhibited. The results are also in line with observations of Sithole, (2016), but, however, contradict to the findings of Pelinganga, (2013). Nemafric-BL phytonematicide also had reduced number of galls, supporting the findings of Sithole, (2016).

Under microplot conditions, increasing concentrations of Nemarioc-AL phytonematicide significantly increased dry shoot mass on green bean at low concentration. The same results were observed by Pelinganga, (2013) on tomato trial using 3% Nemarioc-AL phytonematicide. Under field trials, number of galls were reduced when managing nematode population densities with Nemafric-BL phytonematicide on green beans, however, plant variables were not significant.

In the same trial, no significant results were obtained when managing nematodes with Nemarioc-AL phytonematicide. The same results were obtained in all the three experiment further stipulating that the materials had no effect on the growth of the crop but are able to manage nematodes numbers. Similar results were reported when Ghaferbi *et al*. (2012) exposed eight selected plant species to seed extracts from wheat (*Triticum aestivum* L.). Generally, the efficacy of phytonematicides depends on the concentration of allelochemicals in the organ used for processing the intended products. The accumulation of secondary metabolites in organs varies with seasons with high inconsistent results in nematode suppression and high phytotoxicities during certain seasons (Mashela *et al*., 2015).

Curve-fitting Allelochemical Response Dosage: The MCSP value of Nemarioc-AL phytonematicide on green bean under greenhouse conditions was 2.11%, which was similar to the 2.64% on tomato as reported by Pelinganga, (2013) but lower than the 6.18% of African ginger (Sithole, 2016). In greenhouse trial the overall sensitivity of the crop to the material was Σk = 1. The value is in line with the Σk of 3 on tomato by Pelinganga, (2013), suggesting that green bean is highly sensitive to the material as tomato. This is supported by the hypothesis postulated by Mashela *et al*. (2011), that, the closer the value of "k" was to zero, the higher the sensitivity of the crop to the material. The value of MCSP for Nemafric-BL phytonematicide on green bean under greenhouse conditions was developed at 0.27%, which contradicts with the findings of Pelinganga, (2013) which was 2.99% on tomato. The crop is also sensitive to Nemafric-BL phytonematicide under greenhouse conditions with Σk = 1.

The relationship between Nemarioc-AL and Nemafric-BL phytonematicides and chlorophyll content and gall numbers were quadratic relations, and are an illustration of density-dependent growth patterns (Mashela *et al*., 2015). In general, density-dependent growth patterns suggest that, depending on the concentration, there is stimulation, neutral and inhibition growth phase (Mashela *et al*., 2015). Chlorophyll content was stimulated at lowest concentration and inhibited at highest concentration, gall numbers were also reduced from lowest concentration to the highest concentration.

Under microplot conditions, the relationship between Nemarioc-AL phytonematicide and dry shoot mass was also quadratic. The developed MCSP of Nemarioc-AL

phytonematicide for this experiment was 2.67% on green bean. The concentration is the same as the one derived for tomato plant to manage the notorious *M. javanica* which was 2.63% (Pelinganga, 2013). Mathabatha *et al*. (2016) reported the MCSP value of 8.6% of the same material on *Citrus volkameriana*. The crop had shown sensitivity of Σk = 20, suggesting that green bean is not sensitive to Nemarioc-AL phytonematicide, However, the degree of sensitivity in plants to allelochemicals is plant specific (Rice, 1984), the developed sensitivity of green bean to Nemarioc-AL phytonematicide could not be similar to other crops. Pelinganga, (2013), observed that tomato was not sensitive to the dried material at Σk of 4 units.

The allelochemicals in most phytonematicides affect the biological systems of crops through (DDG) patterns (Liu *et al*., 2003). The DDG patterns have three phases: stimulation, neutral and inhibition phases (Liu *et al*., 2003; Salisbury and Ross, 1992). This was supported by the quadratic relations between dry shoot mass in the current study. Plant variables such and number of leaves, number of pods, stem diameter, chlorophyll content, plant height, fresh shoot mass, fresh root mass, fresh pods mass, gall rating, nodule rating and dry pods mass were not significant. No significant effects were attained on increasing concentrations of Nemafric-BL phytonematicide in the experiment. However, the study conducted on tomato crop by Pelinganga and Mashela (2012) had shown significant results on dry shoot, dry root mass, plant height and stem diameter, when exposing the crop to increasing concentrations of Nemafric-BL phytonematicide concentrations. The results of this study also contradict to that of Mashela *et al*., (2013), where Nemafric-BL phytonematicide was tested on cowpea and significant results were observed on nodule number and cowpea yield. Green bean crop

might be less sensitive to the product than tomato and cowpea, and might show effects at high concentrations since phytotoxicity differ between crops.

Under field conditions, the MCSP developed was 0.5% and sensitivity level of Σk = 3, still illustrating that the test crop is sensitive to Nemafric-BL phytonematicide. The observations of Mathabatha *et al*. (2016) were not in agreement with the findings of the current study since the MCSP value for Nemafric-BL phytonematicide on citrus was 6.3% with overall sensitivity of 4 units. Pelinganga *et al*. (2013) illustrated that the increasing concentrations of Nemafric-BL phytonematicide from 10 to 60% were highly phytotoxic to tomato plants, with the CARD model suggesting that the dilution should be below 10% (Pelinganga and Mashela, 2012). In most studies, depending on the level of phytonematicide concentration, stimulation effects were observed (Mashela, 2002; Pelinganga, 2013).

3.4.2 Nematode variables

Treatment effects: in greenhouse trials, Nematodes in the soil were reduced by 100% from the lowest concentration to the highest concentration. The findings of this study were in agreement with reports of Pelinganga and Mashela, (2012), Mashela *et al*. (2015) and Sithole, (2016).

Nematodes variable were significantly affected when the green bean plants were exposed to increasing concentration of both Nemarioc-AL and Nemafric-BL phytonematicides under micro-plot conditions. Nemarioc-AL phytonematicide had an

effect on J2 in roots, eggs in roots, J2 in soil and final nematode population, reducing the variables to 100%. Nemafric-BL phytonematicide also had an effect on J2 on roots, eggs on roots and final nematode population. Tseke *et al*. (2013) reported that Nemarioc-AL phytonematicide reduced J2s and eggs by 46 to 92% in tomato roots and J2 by 74 to 96% in soil. Findings of this study are also supported by several studies where Nemarioc-AL and Nemafric-BL phytonematicides were shown to be highly effective in nematode suppression (Mashela *et al.*, 2015; Pelinganga and Mashela, 2012; Pelinganga *et al*., 2012). Undoubtedly, the products are highly effective in nematode suppression in microplot conditions.

The final nematode population was reduced when exposed to Nemarioc-AL phytonematicide under field conditions. This is in line with observations of Sithole *et al*. 2016 were the material reduced Pf by 88-94% was observed on African ginger. Phytonematicides have multiple-site activities which restrict nematodes from reproducing on a test crop (Tseke *et al*., 2013). Normally, after egg-hatch, J2 of *Meloidogyne* species migrate into the soil to infect newly developed roots at the elongation region (Ferraz and Brown, 2002). Apparently, during migrations, active ingredients from phytonematicides came into direct contact with J2 and limited their chemotaxis and mobilities (Wuyts *et al*., 2006).

In all the greenhouse, micro-plot and field trials, non-significant results dominated the significant results. However, there are contradictions, where a lot of studies stimulation is observed. These contradictions were, however, in agreement with the hypothesis stated by Mashela *et al*. (2015) and Rice (1984), that allelopathy was concentration-

specific, organ-specific and plant-specific. Mafeo *et al.* (2011) also noted that biological entities differ in their degree of sensitivity to allelochemicals which is indirectly proportional to the developmental stage, with embryonic and seedling stages, for instance, being highly sensitive to allelochemicals.

3.5 Conclusion

The developed MCSP values of Nemarioc-AL phytonematicide on green beans under greenhouse and microplot conditions were 2.11% and 2.67%, respectively. In contrast, the MCSP values of Nemafric-BL under greenhouse and field conditions were 0.27% and 0.5%, respectively. These MCSP values for management of *M*. *javanica* on green beans were within the accepted values that would not be detrimental to other microorganisms. However, the derived MCSP values could further be used to determine the application intervals and then the dosage model for each product on green bean production.

CHAPTER 4
SUMMARY, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

4.1 Summary

The use of phytonematicides to manage root knot (*Meloidogyne species*) nematode population densities, as an alternative to synthetic nematicides which were withdrawn from agrochemical markets, is limited by phytotoxicity they induce on protected crops. This study was conducted to develop the non-phytotoxic concentrations of Nemarioc-AL and Nemafric-BL phytonematicides on green bean under different conditions. In greenhouse trials, numbers of galls were reduced. Chlorophyll content was increased at low concentration and started to decline at high concentrations, and J2 in soil were reduced when exposing the crop to increasing concentrations of Nemarioc-AL phytonematicide. In contrast, Nemafric-BL phytonematicide only had significant effects on number of galls, were reduction in galls was observed. Under microplot conditions, Nemarioc-AL phytonematicide significantly increased dry shoot mass, however, J2 in roots, eggs in roots, J2 in soil and final nematode population were reduced. Nemafric-BL phytonematicide also reduced J2 in roots, eggs in roots and final nematode population. Under field trials, Nemafric-BL phytonematicide had significant effects on number of galls and final nematode population, postulated by reduction of the variables. Under all the conditions, plant variables were not significant. The MCSP values were developed at 2.11% for Nemarioc-AL phytonematicide and 0.27% for Nemafric-BL phytonematicide under greenhouse conditions, 2.67% for Nemarioc-AL phytonematicide under micro-plot conditions and 0.5% for Nemafric-BL phytonematicide in field

experiment. The ‘k’ values were used to determine the overall sensitivities of green bean to the two products. The overall sensitivity of green bean to Nemarioc-AL phytonematicide was 1 and 20 units under greenhouse and microplot, respectively. In contrast, the overall sensitivity of green bean to Nemafric-BL phytonematicide was 0 and 6 units under greenhouse and field conditions, respectively.

4.2 Significance of the findings

The findings of this study will close the gap on the use of phytonematicides to manage *Meloidogyne species* in production of green bean cultivar ‘Tahoe’. The derived MCSP values for managing of *M. javanica* without causing phytotoxicity on green beans were within the accepted values that would not be detrimental to other microorganisms and the environment.

4.3 Recommended future research

Since Nemarioc-AL and Nemafric-BL phytonematicides has shown the capabilities of managing the *Meloidogyne species* in the production of green bean, it is recommended that through empirical studies, the application interval and dosage model should be developed to avoid the incidence of phytotoxicities.

4.4 Conclusions

The MCSP values were developed at 2.11% for Nemarioc-AL phytonematicide and 0.27% for Nemafric-BL phytonematicide under greenhouse conditions, 2.67% for Nemarioc-AL phytonematicide under micro-plot conditions and 0.5% for Nemafric-BL

phytonematicide in field experiment. The lowest MCSP values of such as 2.11% of Nemarioc-AL phytonematicide and 0.5% of Nemafric-BL phytonematicide could be applied to manage *Meloidogyne species* in these cultigens as an alternative to synthetic nematicides.

REFERENCES

Chen, J.C., Chiu, M.H., Nie, R.L., Cordell, G.A. and S.X. Qiu. 2005. Cucurbitacins and cucurbitane glycosides: Structures and biological activities. Natural Products Report 22, 386-399.

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

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

Chukwuka, K.S., Obiakara, M.C. and I.A. Ogunsumi. 2014. Effects of aqueous plant extracts and inorganic fertilizer on germination, growth and development of maize (*Zea mays* L.). *Journal of Agricultural Sciences* 59:243–254.

Department of Agriculture, Forestry and Fisheries. 2012. South Africa year book. Pretoria, South Africa.

Di Vito, M., Parisi, B., Carboni, A. and F. Catalano. 2005. Breeding of common bean for resistance to *Meloidogyne* spp. *Nematropica* 35:70.

Dube, Z.P. and P.W. Mashela. 2016. Response of *Meloidogyne incognita* egress and overall sensitivity to active ingredients of Nemarioc-AL and Nemafric-BL phytonematicides. *Transylvanian Review* 24:954–960.

Ferraz, L.C.C.B. and D.J.F. Brown. 2002. An introduction to nematodes: Plant *nematology*. Pensoft: Sofia.

Ghafarbi, S.P., Hassanejad, S. and R. Lofti. 2012. Allelophathic effects of wheat seed extracts on seed and seedling growth of eight selected weed species. *International Journal of Agriculture and Crop Sciences* 4:1452–1457.

Hussey, R.S. and K.R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter* 57:1025–1028.

Jenkins, W.R. 1964. A rapid centrifugal-floatation technique for separating nematodes from soil. *Plant Disease Reporter* 48:692.

Kleynhans, K.P.N., Van der Berg, E., Swart, A., Marais, M. and N.H. Buckley. 1996. Plant Nematodes in South Africa. Handbook No. 8. Plant Protection Research Institute: Pretoria.

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

Liu, D.L., An, M., Johnson, I.R. and J.V. Lovett. 2003. Mathematical modeling of allelopathy. III. A model for curve-fitting allelochemical dose responses. *Nonlinear Biology Toxicology and Medicine* 1:37–50.

Mafeo, T.P., Mashela, P.W., Mphosi, M.S. and K.M. Pofu. 2011a. Modelling responses of maize, millet and sorghum seedlings to crude extracts of *Cucumis myriocarpus* fruit as pre-emergent bio-nematicide. *African Journal of Agricultural Research* 6:3678–3684.

Mafeo, T.P., Mashela P.W. and M.S. Mphosi. 2011b. Allelopathic responses of various seeds to crude extracts of *Cucumis myriocarpus* fruits when used as a pre-emergent bio-nematicide. *Acta Horticulturae* 938:409–414.

Mafeo, T.P. and P.W. Mashela. 2009a. Responses of germination in tomato, watermelon and butternut squash to a *Cucumis* bio-nematicide. *Journal of Agriculture and Environmental Sciences* 6:215–219.

Mafeo, T.P. and P.W. Mashela. 2009b. Responses of monocotyledonous crops to crude extracts of *Cucumis* myriocarpus fruit as a pre-emergent bionematicide. *African Crop Science Proceedings* 9:631–634.

Mafeo, T.P. and P.W. Mashela. 2010. Allelopathic inhibition of seedling emergence in dicotyledonous crops by *Cucumis* bio-nematicide. *African Journal of Biotechnology* 9:8349–8354.

Mahmood, I., Masood, A., Saxena, S.K. and S.I. Hussain. 1979. Effect of some plant extracts on the mortality of *Meloidogyne incognita* and *Rotylenchulus reniformis*. *Acta Botanica Indica* 7:129–132.

Mashela, P.W. 2002. Ground wild cucumber fruit suppress numbers of *Meloidogyne incognita* on tomato in micro plots*. Nematropica* 32*:*13–19*.*

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

Mashela, P.W, De Waele, D. and K.M. Pofu. 2011. Use of indigenous Cucumis technologies as alternative to synthetic nematicides in management of root- knot nematodes in low-input agricultural farming systems: A review. *Science Research Essay* 6:6762–6768.

Mashela, P.W., Dirk De Waele, D. and K.M Pofu. 2013. Use of indigenous *Cucumis* technologies as alternative to synthetic nematicides in management of root-knot nematodes in low-input agricultural farming systems. *International Journal of Agrochemicals and Plant Protection* 1:1-7.

Mashela, P.W., Dube, Z.P. and K.M. Pofu. 2015. Managing the phytotoxicity and inconsistent nematode suppression in soil amended with phytonematicides. In: Meghvansi, M.K. and A. Vorma (eds.). Organic amendments and soil suppressiveness in plant disease management. Soil Biology 46. Springer International Publishers: Heidelberg, Switzerland.

Mathabatha, R.V., Mashela, P.W. and N.M. Mokgalong. 2016. Sensitivity of Nemarioc-AL and Nemafric-B phytonematicides to *Citrus volkameriana* seedling rootstock. Transylvanian Review 24:969–972.

Meyer, S.L.F., Lakshman, D.K., Zasada, I.A., Vinyard, B.T. and D.J. Chitwood. 2008. Phytotoxicity of clove oil to vegetable crop seedlings and nematotoxicity to root-knot nematodes. *Horticultural Technology* 18:631–638.

Muller, R. and P.S. Gooch. 1982. Organic amendments in nematode control: an examination of the literature. *Nematropica* 12:319–326.

Okwute, S.K. 2012. Plants as potential sources of pesticidal agents: A review. In: Soundararajan, R.P. (ed.). Pesticides: Advances in chemical and botanical pesticides.

Pelinganga, O.M. 2013. Developing bio-pesticides using indigenous *Cucumis africanus* and *Cucumis myriocarpus* fruits for tomato production systems. PhD thesis, University of Limpopo, Sovenga.

Pelinganga, O.M. and P.W. Mashela. 2012. Mean dosage stimulation range of allelochemicals from crude extracts of *Cucumis africanus* fruit for improving growth of tomato plant and suppressing *Meloidogyne incognita* numbers. *Journal of Agricultural Science* 4:8–12.

Pelinganga, O.M., Mashela, P.W., Nzanza, B. and M.S. Mphosi. 2012. Baseline information on using fermented crude extracts from *Cucumis africanus* fruit for suppression of *Meloidogyne incognita* and improving growth of tomato plant. *African Journal Biotechnology* 11:1407–1413.

Pelinganga, O.M., Mashela, P.W., Mphosi, M.S. and T.P Mafeo. 2013. Using computer-based model to determine phytotoxicity concentration of Nemarioc-A phytonematicide in tomato production. *African Crop Science Society Conference* (Abstract) 11:168.

Rice, E.L. 1984. Allelopathy. Academic Press: New York.

Rizvi, S.J.H. and V. Rizvi. 1992. Allelopathy: Basic and applied aspects. Chapman and Hall: London.

Salisbury, F.B. and Ross, C.W. 1992. Plant physiology, second edition. Wadsworth, Belmont.

SAS Institute, 2008. SAS/STAT® 9.2 Qualification Tools User's Guide. Cary, North Carolina: SAS Institute Inc.

Sasser, J. N. and D.W. Freckman. 1987. A world perspective on nematology: The role of the society. In: Veech, J.A. and D.W. Dickson (eds.). Vistas on nematology: A commemoration of the 25th anniversary of the Society of Nematologists. Sociey of Nematologists: Hyattsville, Maryland.

Sikora, R.A., Greco, N. and J.F.V. Silva. 2005. Nematode parasites of food legume. In Luc, M., Sikora R.A. and J. Bridge (eds.). Plant-parasitic nematodes in subtropical and tropical regions. CAB International: Wallingford, U.K.

Sithole, N.T. 2016. Mean Concentration Stimulation Point of Nemarioc-AL and Nemafric-BL phytonematicide on *Pelargonium sidoides*: An indigenous future cultigen. Master of science Mini-dissertation, University of Limpopo, Sovenga.

Sithole, N.T., Pofu, K.M., Mashela, P.W., Dube, Z.P. and H. Araya. 2016. Overall sensitivity of *Pelargonium sidoides* and root-knot nematodes to Nemarioc-AL phytonematicide. Transylvanian Review 24:2996–3001.

Taylor, A.L. and J.N. Sasser. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). Cooperation Publication of Department of Plant Pathology. North Carolina State University and United States Agency of International Development. Raleigh, North Carolina.

Tseke, P.E., Mashela, P.W. and N.M. Mokgalong. 2013. Responses of tomato plant growth and root-knot nematodes to Nemarioc-AL phytonematicide. *African Crop Science Conference Proceedings* 11:367–370.

Wuyts, N., Swennen, R. and D. De Waele. 2006. Effects of plant phenylpropanoid pathway products and selected terpenoids and alkaloids on the behaviour of the plant parasitic nematodes *Radopholus similis*, *Pratylenchus penetrans* and *Meloidogyne incognita*. *Nematology* 8:89–101.

Van Wyk, B.E. and M. Wink. 2004. Medicinal plants of the world. Timber Press: Portland, Oregon.

APPENDICES

Appendix 3.1 Analysis of variance for leaf numbers of green bean to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	0.06	6.81		
Treatment	5	0.03	6.27	1.06	0.30
Error	45	0.27	5.93		
Total	59	0.36			

Appendix 3.2 Analysis of variance for pod numbers of green bean to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	0.199	0.221		
Treatment	5	0.007	0.001	0.09	0.99
Error	45	0.637	0.014		
Total	59	0.843			

Appendix 3.3 Analysis of variance for stem diameter of green bean to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	1.42	0.16		
Treatment	5	3.33	0.66	0.99	0.43
Error	45	30.28	0.67		
Total	59	35.02			

Appendix 3.4 Analysis of variance for chlorophyll content of green bean to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	185.19	20.576		
Treatment	5	464.38	92.877	4.12	0.00
Error	45	1015.29	22.562		
Total	59	1664.86			

Appendix 3.5 Analysis of variance for plant height of green bean to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	959.61	106.62		
Treatment	5	188.65	37.73	0.68	0.64
Error	45	2507.43	55.72		
Total	59	3655.70			

Appendix 3.6 Analysis of variance for gall rating of green bean to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	0.014	0.002		
Treatment	5	0.521	0.104	29.35	0.00
Error	45	0.160	0.004		
Total	59	0.696			

Appendix 3.7 Analysis of variance for dry pod mass of green bean to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	360.70	40.078		
Treatment	5	152.44	30.488	0.95	0.46
Error	45	1447.43	32.165		
Total	59	1960.58			

Appendix 3.8 Analysis of variance for fresh pod mass of green bean to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	3392.9	376.99		
Treatment	5	504.2	100.84	0.53	0.75
Error	45	8567.4	190.39		
Total	59	12464.5			

Appendix 3.9 Analysis of variance for fresh root mass of green bean to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	10545.8	1171.76		
Treatment	5	2101.2	420.24	0.58	0.72
Error	45	32866.5	730.37		
Total	59	45513.6			

Appendix 3.10 Analysis of variance for nodule rating of green bean to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	0.648	0.072		
Treatment	5	0.383	0.077	1.51	0.21
Error	45	2.281	0.051		
Total	59	3.311			

Appendix 3.11 Analysis of variance for dry shoot mass of green bean to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	123.089	13.677		
Treatment	5	23.700	4.740	0.63	0.68
Error	45	338.207	7.516		
Total	59	484.995			

Appendix 3.12 Analysis of variance for juveniles in roots of green bean to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	3.760	0.418		
Treatment	5	0.420	0.084	0.30	0.91
Error	45	12.534	0.279s		
Total	59	16.714			

Appendix 3.13 Analysis of variance for eggs in roots of green bean to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	0.581	0.065		
Treatment	5	0.718	0.144	2.22	0.07
Error	45	2.907	0.065		
Total	59	4.206			

Appendix 3.14 Analysis of variance for juveniles in soil of green bean to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	2.711	0.301		
Treatment	5	4.969	0.994	2.54	0.04
Error	45	17.619	0.392		
Total	59	25.298			

Appendix 3.15 Analysis of variance for final nematode population on green bean to Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	5.169	0.574		
Treatment	5	2.506	0.501	0.70	0.63
Error	45	32.425	0.721		
Total	59	40.100			

Appendix 3.16 Analysis of variance for leaf numbers of green bean to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	0.050	5.577		
Treatment	5	0.012	2.309	0.48	0.79
Error	45	0.217	4.816		
Total	59	0.278			

Appendix 3.17 Analysis of variance for pod numbers of green bean to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	0.156	0.017		
Treatment	5	0.038	0.008	0.42	0.83
Error	45	0.803	0.018		
Total	59	0.997			

Appendix 3.18 Analysis of variance for stem diameter of green bean to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	8.418	0.935		
Treatment	5	1.120	0.224	0.51	0.77
Error	45	19.657	0.437		
Total	59	29.195			

Appendix 3.19 Analysis of variance for chlorophyll content of green bean to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	80.517	8.946		
Treatment	5	70.848	14.170	1.71	0.15
Error	45	373.035	8.290		
Total	59	524.400			

Appendix 3.20 Analysis of variance for gall rating of green bean to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	0.131	0.015		
Treatment	5	0.215	0.043	8.63	0.00
Error	45	0.224	0.005		
Total	59	0.569			

Appendix 3.21 Analysis of variance for dry pod mass of green bean to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	12.358	1.373		
Treatment	5	3.811	0.762	0.69	0.64
Error	45	50.007	1.111		
Total	59	66.176			

Appendix 3.22 Analysis of variance for nodule rating of green bean to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	0.684	0.076		
Treatment	5	0.285	0.057	2.00	0.09
Error	45	1.280	0.028		
Total	59	2.248			

Appendix 3.23 Analysis of variance for fresh pod mass of green bean to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	2446.4	271.818		
Treatment	5	565.9	113.181	0.61	0.69
Error	45	8351.2	185.583		
Total	59	11363.5			

Appendix 3.24 Analysis of variance for fresh root mass of green bean to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	9798.5	1088.73		
Treatment	5	1906.8	381.36	0.44	0.82
Error	45	39054.3	867.87		
Total	59	50759.6			

Appendix 3.25 Analysis of variance for plant height of green bean to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	324.80	36.089		
Treatment	5	340.25	68.050	1.58	0.19
Error	45	1940.03	43.112		
Total	59	2605.08			

Appendix 3.26 Analysis of variance for dry shoot mass of green bean to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	93.115	10.346		
Treatment	5	33.036	6.607	0.88	0.50
Error	45	338.666	7.526		
Total	59	464.817			

Appendix 3.27 Analysis of variance for nematode juveniles in roots of green bean to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	2.573	0.286		
Treatment	5	1.385	0.277	1.77	0.14
Error	45	7.029	0.156		
Total	59	10.987			

Appendix 3.28 Analysis of variance for nematode eggs in roots of green bean to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	1.427	0.159		
Treatment	5	0.368	0.074	0.66	0.65
Error	45	4.998	0.111		
Total	59	6.793			

Appendix 3.29 Analysis of variance for nematode juveniles in soil of green bean to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	2.372	0.264		
Treatment	5	1.017	0.203	0.57	0.72
Error	45	15.924	0.354		
Total	59	19.313			

Appendix 3.30 Analysis of variance for final nematode population on green bean to Nemafric-BL phytonematicide under greenhouse conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	3.334	0.370		
Treatment	5	1.888	0.377	0.63	0.68
Error	45	27.178	0.604		
Total	59	32.399			

Appendix 3.31 Analysis of variance for leaf numbers of green bean to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	3.126	0.347		
Treatment	5	0.046	0.009	1.42	0.23
Error	45	0.291	0.006		
Total	59	3.463			

Appendix 3.32 Analysis of variance for pod numbers of green bean to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	0.926	0.103		
Treatment	5	0.464	0.093	0.99	0.44
Error	45	4.221	0.094		
Total	59	5.610			

Appendix 3.33 Analysis of variance for stem diameter of green bean to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	15.639	1.738		
Treatment	5	5.937	1.188	1.19	0.33
Error	45	45.086	1.002		
Total	59	66.663			

Appendix 3.34 Analysis of variance for chlorophyll content of green bean to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	1078.40	119.822		
Treatment	5	192.29	38.459	1.41	0.24
Error	45	1224.60	27.213		
Total	59	2495.29			

Appendix 3.35 Analysis of variance for plant height of green bean to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	163.37	18.152		
Treatment	5	112.61	22.522	0.86	0.51
Error	45	1173.09	26.069		
Total	59	1449.07			

Appendix 3.36 Analysis of variance for fresh shoot mass of green bean to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	2014.9	223.882		
Treatment	5	859.7	171.933	0.76	0.59
Error	45	10246.6	227.701		
Total	59	13121.1			

Appendix 3.37 Analysis of variance for fresh root mass of green bean to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	7246.1	805.127		
Treatment	5	1428.1	285.617	0.61	0.70
Error	45	21187.8	470.839		
Total	59	29862.0			

Appendix 3.38 Analysis of variance for fresh pod mass of green bean to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	16528.4	1836.49		
Treatment	5	3078.2	615.63	0.63	0.67
Error	45	43675.7	970.57		
Total	59	63282.3			

Appendix 3.39 Analysis of variance for gall rating of green bean to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	0.068	0.008		
Treatment	5	0.061	0.012	0.71	0.62
Error	45	0.781	0.017		
Total	59	0.909			

Appendix 3.40 Analysis of variance for nodule rating of green bean to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	0.856	0.095		
Treatment	5	0.080	0.016	0.35	0.88
Error	45	2.032	0.045		
Total	59	2.968			

Appendix 3.41 Analysis of variance for dry shoot mass of green bean to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	176.885	19.645		
Treatment	5	91.599	18.319	2.54	0.04
Error	45	323.986	7.199		
Total	59	592.470			

Appendix 3.42 Analysis of variance for dry pod mass of green bean to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	735.10	81.678		
Treatment	5	56.33	11.266	0.80	0.55
Error	45	632.08	14.406		
Total	59	1423.51			

Appendix 3.43 Analysis of variance for nematode juveniles in roots of green bean to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	1.271	0.141		
Treatment	5	8.490	1.698	6.17	0.00
Error	45	12.389	0.275		
Total	59	22.149			

Appendix 3.44 Analysis of variance for nematode eggs in roots of green bean to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	7.500	0.833		
Treatment	5	7.885	1.577	3.70	0.00
Error	45	19.188	0.426		
Total	59	34.573			

Appendix 3.45 Analysis of variance for nematode juveniles in soil of green bean to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	9.037	1.004		
Treatment	5	26.275	5.255	6.22	0.00
Error	45	38.03	0.846		
Total	59	73.345			

Appendix 3.46 Analysis of variance for nematode eggs in soil of green bean to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	3.168	0.352		
Treatment	5	3.220	0.644	1.49	0.21
Error	45	19.388	0.431		
Total	59	25.777			

Appendix 3.47 Analysis of variance for final nematode population on green bean to Nemarioc-AL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	11.519	1.279		
Treatment	5	61.831	12.367	21.63	0.00
Error	45	25.723	0.572		
Total	59	99.074			

Appendix 3.48 Analysis of variance for leaf numbers of green bean to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	4.231	0.470		
Treatment	5	0.050	0.009	1.35	0.26
Error	45	0.331	0.007		
Total	59	4.611			

Appendix 3.49 Analysis of variance for pod numbers of green bean to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	0.525	0.058		
Treatment	5	0.572	0.115	2.16	0.07
Error	45	2.380	0.053		
Total	59	3.478			

Appendix 3.50 Analysis of variance for stem diameter of green bean to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	6.672	0.741		
Treatment	5	2.847	0.570	0,71	0.62
Error	45	36.048	0.801		
Total	59	45.566			

Appendix 3.51 Analysis of variance for chlorophyll content of green bean to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	857.01	95.223		
Treatment	5	124.22	24.844	0.54	0.75
Error	45	2083.78	46.307		
Total	59	3065.01			

Appendix 3.52 Analysis of variance for plant height of green bean to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	153.48	17.054		
Treatment	5	195.69	39.138	1.77	0.14
Error	45	992.89	22.064		
Total	59	1342.06			

Appendix 3.53 Analysis of variance for fresh shoot mass of green bean to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	2826.8	314.089		
Treatment	5	1220.7	244.144	1.29	0.29
Error	45	8549.5	189.988		
Total	59	12597.0			

Appendix 3.54 Analysis of variance for fresh root mass of green bean to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	4323.9	480.430		
Treatment	5	417.0	83.394	0.20	0.96
Error	45	18973.1	421.624		
Total	59	23713.9			

Appendix 3.55 Analysis of variance for fresh pod mass of green bean to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	24033.2	2670.35		
Treatment	5	12568.9	2513.78	1.90	0.11
Error	45	59550.9	1323.35		
Total	59	96152.9			

Appendix 3.56 Analysis of variance for gall rating of green bean to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	0.298	0.033		
Treatment	5	0.066	0.013	0.53	0.75
Error	45	1.129	0.025		
Total	59	1.494			

Appendix 3.57 Analysis of variance for nodule rating of green bean to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	0.745	0.083		
Treatment	5	0.030	0.006	0.23	0.95
Error	45	1.169	0.026		
Total	59	1.944			

Appendix 3.58 Analysis of variance for dry shoot mass of green bean to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	125.931	13.992		
Treatment	5	33.137	6.627	0.69	0.63
Error	45	430.398	9.564		
Total	59	589.466			

Appendix 3.59 Analysis of variance for dry pod mass of green bean to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	1300.02	144.447		
Treatment	5	199.99	39.998	1.86	0.12
Error	45	966.95	21.488		
Total	59	2466.96			

Appendix 3.60 Analysis of variance for nematode juveniles in roots of green bean to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	1.251	0.139		
Treatment	5	7.776	1.555	15.25	0.00
Error	45	4.590	0.102		
Total	59	13.616			

Appendix 3.61 Analysis of variance for nematode eggs in roots of green bean to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	0.962	0.107		
Treatment	5	5.481	1.096	5.13	0.00
Error	45	9.608	0.214		
Total	59	16.051			

Appendix 3.62 Analysis of variance for nematode juveniles in soil of green bean to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	2.951	0.328		
Treatment	5	2.951	0.590	1.47	0.22
Error	45	18.127	0.403		
Total	59	24.028			

Appendix 3.63 Analysis of variance for nematode eggs in soil of green bean to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	2.248	0.249		
Treatment	5	2.810	0.562	2.25	0.07
Error	45	11.241	0.249		
Total	59	16.300			

Appendix 3.64 Analysis of variance for final nematode population eggs on green bean to Nemafric-BL phytonematicide under micro-plot conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	9	1.203	0.134		
Treatment	5	36.121	7.224	25.27	0.00
Error	45	12.866	0.286		
Total	59	50.189			

Appendix 3.65 Analysis of variance for leaf numbers of green bean to Nemarioc-AL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	0.046	5.766		
Treatment	5	0.006	1.283	0.14	0.98
Error	40	0.375	9.382		
Total	53	0.428			

Appendix 3.66 Analysis of variance for pod numbers of green bean to Nemarioc-AL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	0.628	0.079		
Treatment	5	0.208	0.042	0.75	0.59
Error	40	2.221	0.056		
Total	53	3.058			

Appendix 3.67 Analysis of variance for stem diameter of green bean to Nemarioc-AL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	0.063	0.008		
Treatment	5	0.052	0.010	1.04	0.41
Error	40	0.401	0.010		
Total	53	0.516			

Appendix 3.68 Analysis of variance for chlorophyll content of green bean to Nemarioc-AL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	261.00	32.624		
Treatment	5	102.37	20.474	0.89	0.50
Error	40	924.11	23.103		
Total	53	1287.48			

Appendix 3.69 Analysis of variance for plant height of green bean to Nemarioc-AL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	79.22	9.903		
Treatment	5	49.13	9.826	0.42	0.83
Error	40	940.66	23.517		
Total	53	1069.01			

Appendix 3.70 Analysis of variance for fresh shoot mass of green bean to Nemarioc-AL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	10381.5	1297.69		
Treatment	5	1862.0	372.40	0.55	0.74
Error	40	27184.7	679.62		
Total	53	39428.2			

Appendix 3.71 Analysis of variance for fresh root mass of green bean to Nemarioc-AL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	103.56	12.945		
Treatment	5	75.20	15.039	0.73	0.61
Error	40	828.84	20.721		
Total	53	1007.60			

Appendix 3.72 Analysis of variance for fresh pod mass of green bean to Nemarioc-AL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	233.92	29.240		
Treatment	5	64.97	12.994	0.44	0.82
Error	40	1183.78	29.595		
Total	53	1482.67			

Appendix 3.73 Analysis of variance for gall rating of green bean to Nemarioc-AL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	0.117	0.015		
Treatment	5	0.321	0.064	1.91	0.11
Error	40	1.345	0.034		
Total	53	1.783			

Appendix 3.74 Analysis of variance for nodule rating of green bean to Nemarioc-AL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	0.123	0.015		
Treatment	5	0.020	0.004	0.71	0.62
Error	40	0.224	0.006		
Total	53	0.368			

Appendix 3.75 Analysis of variance for dry shoot mass of green bean to Nemarioc-AL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	179.05	22.381		
Treatment	5	99.83	19.996	0.32	0.90
Error	40	2498.54	62.464		
Total	53	2777.42			

Appendix 3.76 Analysis of variance for dry pod mass of green bean to Nemarioc-AL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	2.784	0.348		
Treatment	5	1.324	0.265	0.79	0.56
Error	40	13.382	0.335		
Total	53	17.490			

Appendix 3.77 Analysis of variance for nematode juveniles in roots of green bean to Nemarioc-AL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	3.354	0.419		
Treatment	5	5.237	1.047	3.77	0.001
Error	40	11.125	0.278		
Total	53	19.716			

Appendix 3.78 Analysis of variance for nematode eggs in roots of green bean to Nemarioc-AL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	0.775	0.097		
Treatment	5	0.389	0.078	0.58	0.72
Error	40	5.403	0.135		
Total	53	6.567			

Appendix 3.79 Analysis of variance for final nematode population on green bean to Nemarioc-AL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	2.289	0.286		
Treatment	5	7.787	1.557	4.82	0.00
Error	40	12.919	0.323		
Total	53	22.996			

Appendix 3.80 Analysis of variance for leaf numbers of green bean to Nemafric-BL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	0.080	0.010		
Treatment	5	0.055	0.011	1.01	0.43
Error	40	0.439	0.011		
Total	53	0.574			

Appendix 3.81 Analysis of variance for pod numbers of green bean to Nemafric-BL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	1.452	0.182		
Treatment	5	0.346	0.069	0.98	0.44
Error	40	2.826	0.071		
Total	53	4.624			

Appendix 3.82 Analysis of variance for stem diameter of green bean to Nemafric-BL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	0.058	7.246		
Treatment	5	0.020	4.074	0.57	0.72
Error	40	0.286	7.137		
Total	53	0.364			

Appendix 3.83 Analysis of variance for chlorophyll content of green bean to Nemafric-BL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	299.17	37.396		
Treatment	5	18.19	3.638	0.20	0.96
Error	40	724.37	18.109		
Total	53	1041.72			

Appendix 3.84 Analysis of variance for plant height of green bean to Nemafric-BL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	161.04	20.130		
Treatment	5	120.14	24.028	0.86	0.52
Error	40	1117.26	27.932		
Total	53	1398.44			

Appendix 3.85 Analysis of variance for fresh shoot mass of green bean to Nemafric-BL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	11252.1	1406.52		
Treatment	5	1319.4	263.89	0.21	0.96
Error	40	51066.9	1276.67		
Total	53	63638.5			

Appendix 3.86 Analysis of variance for fresh root mass of green bean to Nemafric-BL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	34.581	4.323		
Treatment	5	22.531	4.506	0.33	0.89
Error	40	542.416	13.560		
Total	53	599.527			

Appendix 3.87 Analysis of variance for fresh pod mass of green bean to Nemafric-BL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	318.35	39.794		
Treatment	5	372.09	74.418	1.17	0.34
Error	40	2555.05	63.876		
Total	53	3245.49			

Appendix 3.88 Analysis of variance for gall rating of green bean to Nemafric-BL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	0.121	0.015		
Treatment	5	0.599	0.120	10.82	0.00
Error	40	0.443	0.011		
Total	53	1.163			

Appendix 3.89 Analysis of variance for nodule rating of green bean to Nemafric-BL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	0.183	0.023		
Treatment	5	0.062	0.012	0.99	0.44
Error	40	0.506	0.013		
Total	53	0.752			

Appendix 3.90 Analysis of variance for dry shoot mass of green bean to Nemafric-BL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	544.33	68.041		
Treatment	5	75.70	15.139	0.51	0.77
Error	40	1198.52	29.963		
Total	53	1818.54			

Appendix 3.91 Analysis of variance for dry pod mass of green bean to Nemafric-BL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	1.797	0.225		
Treatment	5	1.840	0.368	1.11	0.37
Error	40	13.298	0.332		
Total	53	16.935			

Appendix 3.92 Analysis of variance for nematode juveniles of green bean to Nemafric-BL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	3.858	0.482		
Treatment	5	2.895	0.579	1.83	0.13
Error	40	12.671	0.317		
Total	53	19.425			

Appendix 3.93 Analysis of variance for nematode eggs in roots of green bean to Nemafric-BL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	0.453	0.057		
Treatment	5	0.259	0.052	0.78	0.57
Error	40	2.655	0.066		
Total	53	3.367			

Appendix 3.94 Analysis of variance for final nematode population in roots of green bean to Nemafric-BL phytonematicide under field conditions at 56 days after inoculation and initiation of treatments.

Source	DF	SS	MS	F	P
Rep	8	3.893	0.487		
Treatment	5	3.467	0.693	2.00	0.09
Error	40	13.848	0.346		
Total	53	21.208			