DEVELOPMENT OF NON-PHYTOTOXIC CONCENTRATION OF NEMARIOC-AL AND NEMAFRIC-BL PHYTONEMATICIDES ON BEETROOT(*BETA VULGARIS*)CULTIVAR 'DETROIT DARK RED'

BY

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DECLARATION

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

Mashitoa M.F. (Miss) Date

DEDICATION

To my exquisite mother, Mrs Mankete Angelina Mashitoa, the mother I have always wanted to be my mother and my beloved nephew Lethabo Eugene Mokautu Mashitoa.

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ABSTRACT

Phytonematicides, mainly due to their allelopathic nature, might be highly phytotoxic to crops protected against nematode damage. Phytotoxicity issues are compounded by the fact that the efficacy of plant extracts on nematode suppression depended much on their concentration and duration of exposure to the nematodes. Phytotoxicity could result in low crop yield and/or even in the eventual death of the protected crops. Concentrations that were suppressive to nematode numbers, but phytotoxic to the tested crop would not be useful when applied as a post-planting phytonematicides. The Mean Concentration Stimulation Point (MCSP) values were developed from the Curve-fitting Allelochemical Response Dosage (CARD)computer based model to ensure that a non-phytotoxic concentration was applied for each crop. The objective of this study was to determine whether a series of Nemarioc-AL and Nemafric-BL phytonematicide concentrations would provide the MCSP for beetroot (*Beta vulgaris*) under greenhouse, microplot and field conditions. The greenhouse treatments included 0, 2, 4, 8, 16 and 32% for each phytonematicide. The microplot treatments were 0, 0.8, 1.6, 3.2, 6.4 and 12.8% concentrations, whereas in the field trial treatments were 0, 2.4, 4.8, 9.6, 19.2 and 38.4%. Under each condition, treatments of Nemarioc-AL and Nemafric-BL phytonematicides were, in separate experiments arranged in a randomised complete block design, with the greenhouse, microplot and field trials having 15, 10 and 9 replications, respectively. In the greenhouse, seedlings were raised in 20cm diameter plastic pots, containing pasteurised river sand and commercial seedling growing medium Hygromix® at 3:1 (v/v) ratio. Each seeding was inoculated with 5000 eggs and second-stage juveniles (J2) of *Meloidogyne incognita.* Seedlings were irrigated with

chlorine free tapwater every other day using 250 ml/plant, with irrigation substituted by the treatment once weekly. On the microplot, the procedures were as in the greenhouse except that they growing mixture comprised pasteurised soil collected from the site. In the field, seedlings were directly transplanted into the soil. At 56 days after inoculation, in the greenhouse trial, the effects of Nemarioc-AL and Nemafric-BL phytonematicides were highly significant ($P \leq 0.01$) on root galls, contributing 77 and 72% in total treatment variation (TTV) of root galls, respectively. Relative to untreated control, the respective products reduced root galls by 28-72% and 43-67%. Nemarioc-AL and Nemafric-BL phytonematicides had MCSP values on beetroot of 18.1 and 6.4%, respectively, with overall sensitivity values of 0 and 1, respectively.There was no treatment effect on nematode since there was absence of nematode in untreated control.Under microplottrials,Nemafric-BL phytonematicide had significant ($P \leq 0.05$) effects on fresh root mass, dry root mass and root galls, contributing 20, 19 and 57% in TTV of the three variables, with relative increases for fresh root mass and dry root mass of 65-159% and 63-143%, respectively, whereas root galls were reduced by 82-100%. Nemafric-BL phytonematicide had MCSP value on beetroot of 10.2%, with overall sensitivity value of 4 units. There was no treatment effect on nematode since there was absence of nematode in untreated control. In microplots, Nemarioc-AL phytonematicide did not have significant effects on all plant variables. Under field conditions, the treatments did not have significant effects on plant variables. In conclusion, results of the current study suggested that under greenhouse and microplot conditions the MCSP values of the phytonematicides ranged from 6.4 to 18.1%, with a wide range of overall sensitivities of phytonematicides to the test of beetroot cultivar.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Introduction

1.1.1 Description of the research problem

Phytotoxicity in phytonematicides limits the widespread use of these products in research and development of alternative products for managing population densities of nematodes (Mashela *et al.,* 2015). The non-phytotoxic concentration of phytonematicides, referred to as the Mean Concentration Stimulation Point (MCSP), was developed using thebiological indices generated through the Curve-fitting Allelochemical Response Dosage (CARD) computer-based model (Liu *et al*., 2003).However, the MCSP of a phytonematicide is specific to a given plant species. TheMCSP was also used to empirically establish the application interval (Mashela *et al.,* 2015), which was essential in the development of the dosage model (Mashela *et al.,* 2015). In South Africa, Nemarioc-AL and Nemafric-BL phytonematicides, manufactured from fruits of wild cucumber (*Cucumis myriocarpus*) and wild watermelon (*Cucumis africanus*), respectively, had been successfully tested to manage nematodes on a wide range of crops(Mashela *et al*., 2015).

1.1.2 Impact of the research problem

Nemarioc-AL and Nemafric-BL phytonematicides were consistently effective in suppression of root-knot (*Meloidogyne* species) nematodes under different conditions (Mashela *et al*., 2015). At above 10% concentration, the products were phytotoxic to tomato (*Solanum lycopersicum*) plants (Pelinganga *et al*., 2012).In crop production, estimations of yield losses due to phytotoxicity induced by phytonematicides were from

24 to 50% (Mashela *et al*., 2015). Mafeo and Mashela (2010) demonstrated that phytotoxicity of Nemarioc-AG phytonematicide could prevent seedling emergence by 100%. Also, Nemarioc-AGphytonematicide was shown to be highly phytotoxic to monocotyledonous and dicotyledonous crops, with most crops failing to emerge when the phytonematicide was applied as a pre-emergent drench product (Mafeo, 2012; Mafeo and Mashela, 2010; Mafeo *et al*., 2011a).

1.1.3 Possible causes of the research problem

The phytotoxicities of phytonematicides could be traced from their active ingredients, namely, the allelochemicals (Mashela *et al*., 2013). Generally, allelochemicals are secondary metabolites, which are used by plants during defense against pests, as well as other plant speciesduring competition (Rice, 1984). The general causes of phytotoxicities in phytonematicides on crops being protected against nematode damage could be due to limited information on plant-phytonematicide interaction, which include the amount to be applied and the application interval.

1.1.4 Proposed solutions

The development of MCSP values of Nemarioc-AL and Nemafric-BL phytonematicides on beetroot (*Beta vulgaris*) could ensure that each of the two products was not phytotoxic when used to manage population densities of *Meloidogyne* species.

1.2Problem statement

Beetroot cultivars are susceptible to infection by *Meloidogyne* species, with yield reduction being in the range17-23% in other countries (Abad*et al*., 2008). Prior to the

withdrawal of fumigant nematicides, damage by *Meloidogyne* species in beetroot production was widely managed using methyl bromide. However, following the 2005 withdrawal of methyl bromide, there were limited choices for managing nematode numbers.Recently, Mashela and Pofu (2016)demonstrated thatcertain beetroot cultivars in South Africa had some evidence of nematode resistance to *Meloidogyne* species. The use of phytonematicides to compliment nematode resistance in beetroots could have limitations, especially phytotoxicities, with the previously described non-phytotoxic level, namely, the MCSP, being plant-specific. Thus, it was imperative that the MCSP for Nemarioc-AL and Nemafric-BL phytonematicides for beetroot cultivarsbe empirically developed.

1.3 Rationale

The MCSP for each crop would ensure that environmental mistakes committed during the use of synthetic pesticides were not repeated (Mashela *et al*., 2015). The MCSP values in*Pelargonium sidoides* for Nemarioc-AL and Nemafric-BL phytonematicides were6.18% and 2.87%, respectively (Sithole *et al*., 2016), whereas for *Citrus volkameriana* were 8.6% and 6.3%,respectively (Mathabatha *et al*., 2016). The CARD model uses variables which were significantly affected by a series of increasing concentrations of phytonematicides to generate biological indices used to compute MCSP, which was previously expressed as MCSP = $D_m + (R_h/2)$ (Mashela *et al.,* 2015). The generation of MCSP values for beetroot would enhance the eventual development of the application interval and then the dosage model for phytonematicides (Mashela *et al*., 2015).The model could also provide information on the degree of sensitivity of the protected crop to the phytonematicides (Mashela *et al*., 2015).

1.4 Purpose of the study

1.4.1 Aim

To developthe non-phytotoxic concentration and overall sensitivity of phytonematicides on beetroot under different growing conditions.

1.4.2 Objective

To determine MCSP and overall sensitivityvalues of Nemarioc-AL and Nemafric-BL phytonematicideson beetroot under greenhouse, microplot and field conditions.

1.5 Reliability, validity and objectivity

Reliability of data would be based on statistical analysis of data at the probability level of 5%, validity would be achieved through repeating the experiments in time, whereas objectivity would be achieved by ensuring that the findings are discussed on the basis of empirical evidence, in order to eliminate all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

Bias would be minimised by ensuring that the experimental error in each experiment was reduced through replications. Also, randomly assigning treatments within an appropriate research designs would reduce bias (Leedy and Ormrod, 2005).

1.7Scientific contributions

The MCSP values of the two phytonematicides and the overall sensitivities would allow the empirical determination of the application interval of the two products on beetroot

production, and then the dosage model, which would be essential in environmental impact studies. Findings in the study would improve the use of both phytonematicides for management of population densities of*M. incognita* by smallholderand commercial farmers who intend to grow*B. vulgaris* as a vegetable crop.

1.8 Format of mini-dissertation

Following the description and detailed outlining of the research problem (Chapter 1), the work done and not done on the research problem would be reviewed (Chapter 2). Then, the objectivewould constitute a separate chapter,separated into greenhouse, microplot and field trials (Chapters 3). In the final chapter (Chapter 4), findings from the greenhouse, microplot and field trials would be summarised and integrated to provide the significance of the findings and recommendations with respect to future research,ending with a conclusion that would intend to provide a take home message regarding the entire study.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Phytonematicides are being researched and developed from various plant organs that containallelochemicals. The two *Cucumis* species which are indigenous to Botlokwa in Limpopo Province, South Africa, namely, wild cucumber (*Cucumis myriocarpus*) and wild watermelon (*Cucumis africanus*),contain allelochemicals in fruits which had been used as phytonematicides (Mashela *et al*., 2015). Due to their active ingredients,the cucurbitacins, phytonematicides could be highly phytotoxic to the protected crops and had been viewed in certain cases as having inconsistent results in crop production systems (Mashela *et al*., 2015). However, the inconsistency had since been clarified in terms of the density-dependent growth (DDG) patterns (Mashela *et al*., 2015), which comprise three phases, namely, stimulation, neutral and inhibition phases (Liu *et al*., 2003; Mashela *et al*., 2015; Salisbury and Ross, 1992)

2.2 Work done on problem statement

2.1.1 Phytonematicides from *Cucumis* fruits

Effects of phytonematicides on plant growth and nematode suppression: Nemarioc-AG and Nemafric-BG phytonematicides inhibited seedling emergence of the test plants regardless of whether they were dicotyledonous or monocotyledonous crops (Mafeo 2012). Mafeo *et al*. (2011a) observed that in chive (*Allium schoenoprasum*), leek (*Allium ampeloprasum*) and onion (*Allium cepa*) there were strong allelopathic effects from Nemarioc-AG phytonematicide. Generally, at low concentration, Nemarioc-AG

phytonematicide stimulated growth, whereas at high dosage the material inhibited growth of various seedlings. Mafeo *et al*. (2011b) reported similar effects on Nemarioc-AG phytonematicide on maize (*Zea mays*), millet (*Pennisetum glaucum*) and sorghum (*Sorghum bicolor*). Similarly, others (Pelinganga and Mashela, 2012; Pelinganga *et al*., 2012; Pelinganga, 2013; Tseke *et al*., 2013) reported that Nemarioc-AL phytonematicide at low concentrations stimulated growth of tomato (*Solanum lycorpersicum*), but inhibited growth at high under various conditions. However, throughout increasing concentrations, the product reduced population densities of *Meloidogyne* species. The stimulation of growth by phytonematicides was first observed by Mashela (2002), who suggested that the product used, Nemarioc-AG phytonematicide, had a 'fertiliser effect'.

In phytonematicides, due to the presence of a wide range of active ingredients, no single chemical can be pointed out to be responsible for suppressing population densities of plant-parasitic nematodes (Pelinganga *et al*., 2012). However, phytonematicides from fruits of *Cucumis* species appear to have specific active ingredients, which were highly effective in nematode suppression, regardless of the concentration. The active ingredients in Nemarioc-AG or AL and Nemafric-BG or BL phytonematicides were cucurbitacin A and cucurbitacin B, respectively (Mashela *et al*., 2015). The two products, in granular (G) or liquid (L) formulation, the products consistently suppressed nematode numbers to as high as from 80% to 100% (Mafeo, 2012; Mashela and Pofu 2016; Pelinganga, 2013). In some cases, from low to high concentrations, phytonematicides effects were not different from each other, whereas the effects were significantly different to those of the untreated control. Maile (2013) observed that phytonematicides increased population densities of the citrus nematode (*Tylenchulus semipenetrans*) on citrus seedlings, thereby confirming the

much discussed concept of inconsistent results in nematode suppression (McSorley, 2000). Mashela *et al*. (2015) demonstrated that the observed inconsistent results in *T. semipenetrans* (Maile, 2013), was primarily due to application interval, which in most phytonematicides was far less than that used in for *T. semipenetrans*. In tomato production, the application intervals for Nemarioc-AL and Nemafric-BL phytonematicides were16 and 18 days, respectively (Pelinganga, 2013).

Mechanism of nematode suppression: Dube and Mashela (2016) demonstrated pure cucurbitacins A and B were each having suppressive effects on nematode numbers, also exhibiting the DDG patterns. Dube *et al*., (2016; Personal com) observed that Nemafric-BL phytonematicide had bioactivity on *M. incognita* J2 hatch and the effects were irreversible when eggs were exposed to the product for 24, 48 and 72 h. Suppression of *M. incognita*second-stage juveniles (J2)hatch inhibition by Nemafric-BL phytonematicide suggested that J2 hatch inhibition was one of the mechanisms involved in suppression of nematode population densities (Dube *et al*., 2016; Personal com). Similar responses were observed when Nemarioc-AL phytonematicide and pure cucurbitacin A and B were tested on various stages of *Meloidogyne* species (Dube and Mashela, 2016).

Phytotoxicity of phytonematicides: Among the three Graminae crops, maize and millet had more or less similar overall sensitivities for seedling height, whereas millet and sorghum had similar overall sensitivities for coleoptile diameter (Mafeo *et al*., 2011b). The radicle length in maize was the most sensitive to the Nemarioc-AG phytonematicide, whereas the coleoptile length in millet was the least sensitive (Mafeo *et al*., 2011b). Overall, sorghum was the most sensitive to the product, whereas millet was the least sensitive (Mafeo *et al*.,

2011b). The cited studies demonstrated that the overall sensitivities of plants to Nemarioc-AG phytonematicide were plant-specific and with a plant species, the overall sensitivities were organ-specific. Dry root mass of tomato crop had 0 k value, whereas plant height had k value of 3 units, with the overall sensitivity being equivalent to 3 units. Individual organs of *C. volkameriana* were highly sensitive to each of the phytonematicide, with zero or unity k values(Mathabatha *et al*., 2016).

The overall sensitivity of egress inhibition was higher in cucurbitacin A than in cucurbitacin B (Mashela *et al.,* 2016). The ∑k of *C. volkameriana* seedlings to Nemarioc-AL and Nemafric-BL phytonematicides were 2 and 4 units, respectively (Mathabatha *et al*., 2016). Mafeo *et al*. (2011a) reported that plant height of onion seedlings were the most sensitive to Nemarioc-AG phytonematicide, whereas the radicle length of leeks was the least sensitive to the product. Overall, onion was the most sensitive to the product, whereas leek was the least sensitive. Mafeo and Mphosi (2012) reported that Nemarioc-AG phytonematicide inhibited emergence of all test monocotyledonous seedlings under greenhouse conditions. The emergence of all test seedlings had strong negative quadratic relationships when exposed to Nemarioc-AG concentrations ranging from 0 to 15 g (Mafeo *et al*., 2011a).

Managing phytotoxicity: The MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides were empirically determined as being 2.64 and 2.99% on tomato (Pelinganga, 2013). Pelinganga and Mashela (2012) reported that MCSP of Nemafric-BL phytonematicide, computed from the CARD biological indices, was 2.64% for tomato plants. Mashela *et al*. (2015) introduced the concept of the dosage model in the

management of phytotoxicity and consistent suppression of nematode numbers. In the model, MCSP was the concentration of a phytonematicide which would stimulate plant growth, while suppressing nematode numbers (Mashela *et al*., 2015). Non-phytotoxicity at the MCSP values was depended on the number of times the product was applied per growing season, which was referred to as the application frequency (Pelinganga *et al*., 2012). In the model, dosage (%) could be expressed as MCSP (%) × application frequency (Mashela *et al*., 2015). At the MCSP values, Nemarioc-AL and Nemafric-BL phytonematicides would not induce phytotoxicity to tomato plants, but would be consistent in suppression of nematode numbers (Mashela *et al*., 2015).

Quality protocols of phytonematicides:Shadung *et al.* (2016) observed that the quality of phytonematicides was dependent upon the concentration of active ingredient, which is directly associated with their performance. Furthermore, the storage period showed that cucurbitacin B in Nemafric-BL phytonematicides increased during the first three months of storage and decreased in the fifth month (Shadung *et al.*, 2016).

Malungane (2014) observed that the crude extracts of *Tulbaghia violacea* significantly affected the final nematode population density (Pf) when applied at rates of 2, 4 and 8 per plot reduced the number of nematodes by 50, 64 and 73% in roots and by 21, 30 and 58 % in soil, respectively. Crude extracts of *T. violacea* significantly affected the measured tomato growth variables. Crude extracts of *T.violacea* stimulated growth of tomato (Malungane, 2014).Khosa (2013) reported that crude plant extracts significantly stimulated growth of tomato plants. Under glasshouse conditions, treatments significantly bettereffects on nematode managementthan control (Khosa, 2013). Under

similarconditions, root mass was significantly improved by non-crop plant species as compared to the control. Khosa (2013) reported that all the crude plant-meal soil amendments significantly reduced the numbers of *M. incognita* eggs and J2 relative to the control. All the plant materials tested compared favourably with the standard Nemarioc-AG phytonematicide, which reduced nematode numbers significantly below those in the untreated control (Khosa, 2013).

Thovhakhale*et al*. (2006) reported that extracts of chilli and tamboti significantly increased growth of tomato plant under greenhouse and microplot conditions.*Brassica* species couldbe used as a potential alternative to methyl bromide for management of root-knot nematodes in vegetable production (Monfort*et al*., 2006). *Brassica* species produce general biocides and were grown as cover crops and incorporated as green manures prior to transplanting of vegetable crops. Monfort*et al*. (2006) Incorporation of *Brassica* species reduced root-knot population and root damage caused by *Meloidogyne* species. Generally, increased growth and yield of corresponded with cover crop treatments that had lowest levels of root-knot nematode populations at planting of vegetable crops (Monfort*et al*., 2006).

2.1.2International trials on phytonematicides

Egg masses or juveniles of *M. incognita*were exposed to varying concentrations of neem (*Azadirachta indica*) leaf (fresh and dry), *Borrelia* species, groundnut leaf and garlic root. Neem leaf and garlic root extracts inhibited hatching of egg masses and were lethal to larva (Singh, 2014). These extracts significantly reduced root-knot infection on tomato when compared to the control. The aqueous extracts of neem leaf, neem seed kernel,

futuka (*Melastoma malabathricum*) leaf, bihlongoni (*Polygonum hydropiper*) leaf, germany bon (*Ageratum conyzoides*) leaf, all at 1:2 and 1:5 concentrations, were tested for their toxicity under laboratory conditions against *Meloidogyne* species (Singh, 2014). All the extracts were toxic to *M.graminicola*and its efficacy increased with the increase in the concentration of the extract and time of exposure. Singh (2014) reported that aqueous leaf extracts of *Argemonemaxicana*, and neem seed kernel suspension proved to be most effective causing complete inhibition of egg hatching and larval penetration of *M. incognita* in banana.

In purified formulation, most phytonematicides lose their nematode suppression abilities (Ntuli and Caboni, 2012), which isfollowed by high phytotoxicity levels on crops being protected against nematodes (Mian and Rodriguez-Kabana, 1982). At low concentration crude extracts of neem leaf stimulated growth of maize (*Zea mays*) and tomato seedlings, while at high concentration the opposite occurred (Egunjobi and Afolamin, 1976; Rossner and Zebitz, 1987). Inderjit *et al*. (1999) also noted that at low concentrations root leachates from golden crown beard (*Verbesina encelloides*) consistently simulated plant growth invarious plant species.

2.2.3*Meloidogyne* species in beetroot production

Root-knot nematode infestations have become an increasing source of concern in beetroot productiondue to recent restrictions on the use of chemical nematicides and the withdrawal of some of the most active compounds from the agrochemical markets (Djian-Caporalino, 2012). Test of pathogenicity reveals that increase in nematode inoculums was associated with progressive reduction in plant growth variables of beetroot crop which

gave conclusive evidence that *M. incognita* is a potential pathogen for beetroot (Anamika, 2015). Anamika (2015) reported that the development of root system of beetroot which was noted to be significantly reduced in plants receiving the highest population of nematodes per plant. Inhibition in growth of roots resulted in formation of profuse knots in roots and tubers (Anamika, 2015). *Meloidogyne* species in beetroot result in root galling which lead to the reduction of plant vigour, yellowing plants which wilt in hot weather (Martin, 2003).

2.2.4Current management strategies in nematode-beetroot interactions

Major factors that influence the seasonal fluctuations of nematode populations include: their biology, environmental parameters and especially, management practices. The temporal population dynamics of *M. incognita* are typical of many nematodes (Anamika, 2015). This pathogen increases to very high population densities during the growing season and then declines very shortly after harvest.Bioassays utilising suitable host plants have much to offer in managing certain nematodes. Different inoculum levels of *M. incognita* and *M. javanica* were used on two beetroot cultivars 'Detroit Red Dark' and 'Crimson Globe', Mashela and Pofu (2016) observed that at 56 days after inoculation, roots of both cultivars had small undeveloped root galls, showing resistance and tolerance abilities of the cultivars. Critically timed gall ratings of beetroots are very useful for identifying species and host races of *Meloidogyne* (Hartmann and Sasser, 1985). Carefully and properly timed compilations of root-gall and root-necrosis indices also are useful as a basis for obtaining beetroot yield-loss estimates from these pathogens (Barker, 1985).

2.3 Work not yet done on problem statement

The degree of phytotoxicity of Nemarioc-AL and Nemafric-BL phytonematicides on *B*. *vulgaris* had not been documented. Due to the economic and health potential qualities of *B. vulgaris* as a vegetable crop, MCSP values for the two phytonematicides would be established. In order to successfully investigate whether Nemarioc-AL and Nemafric-BL phytonematicides would be useful as phytonematicides in *B. vulgaris* production, a series of experiments would be conducted to determine the appropriate MCSP values on this vegetable crop.

CHAPTER 3
RESPONSES OF BEETROOT(*BETA VULGARIS*) GROWTHTO PHYTONEMATICIDES

3.1 Introduction

Plants respond to increasing concentration of phytonematicides through density-dependent growth (DDG) patterns (Liu *et al*., 2003; Mashela *et al*., 2015; Salisbury and Ross, 1992). The DDG patterns are characterised by three phases, namely, the stimulation, neutral and inhibition phases (Liu *et al*., 2003; Mashela *et al*., 2015). Generally, when plant growth responses are under stimulation and/or inhibition phases, the analysis of variance (ANOVA) on the affected variables was significant at theprobabilitylevel of 5% (Mashela*et al*., 2015), with the DDG patterns being characterised by quadratic relationships (Mashela *et al*., 2015). In contrast, when plant growth responses are under the neutral phase, as had been the case in various plant-phytonematicide interactions (Mathabatha *et al*., 2016; Sithole *et al*., 2016), ANOVA for the variables would not be significant at the probability of 5% (Mashela *et al*., 2015). Using restricted concentrations of phytonematicide, Mashela *et al*. (2015) provided a detailed explanation on observations with positive and/or negative linear relationships, when the tested concentration range did not conform to the DDG patterns. In order for the Mean Concentration Stimulation Point (MCSP) to be developed from the Curve-fitting Allelochemical Response Dosage (CARD) model (Liu *et al*., 2003),the concentrations ranges of the tested phytonematicides should comply with the dictates of the DDG patterns (Mashela *et al.,* 2015). The MCSP is the concentration that could be applied without inducing phytotoxicity on the crop being protected against nematode damage (Mashela *et al*., 2015).The MCSP values for Nemarioc-AL and Nemafric-BL

phytonematicides on beetroot (*Betavulgaris*) cv. 'Detroit Dark Red' had not been documented. The beetroot cv. 'Detroit Dark Red' was recently shown to be tolerant to *Meloidogyne* species (Mashela and Pofu, 2016). Thus, it is necessary to control nematodes in this cultivar.The objective of this study, therefore, was to determine the MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides on beetroot cv. 'Detroit Dark Red' under greenhouse, microplot and field conditions.

3.2 Materials and methods

3.2.1 Description of the study area

Greenhouse conditions: The available greenhouse was 20m × 100m, with thermostatically-activated fans on one end and the wet wall on the other end, for moderating inside temperatures. In summer (October-December), the greenhouse maximum/minimum temperatures average 28/21°C, whereas in winter (April-June), the maximum/minimum temperatures average 24/16°C. The top of the greenhouse was covered with a 35% green-net, whereas the long sides were covered with black nets. Due to the large size of the greenhouse and the wind-blown generated currents, conditions inside the greenhouse were not homogeneous, thereby, dectating that experiments, depending on experimental size, being appropriately designed.

Microplot and field conditions: Microplot trials were established outside the greenhouse using the pasteurised soils derived from digging single holes, in which the plastic pots were inserted. The steam-pasteurised growing mixture, collected from the microplot site, comprised Hutton soil (65% sand, 30% clay and 5% silt). The ambient

temperatures maximum/minimum temperatures averaged 32/20°C. The location had averaged rainfall less than 600mm. which mostly occurs in summer.

3.2.2 Research design

In the greenhouse, the treatments included 0, 2, 4, 8, 16 and 32% Nemarioc-AL or Nemafric-BL phytonematicide, which were arranged in a randomised complete block design (RCBD), due to the heterogeneity of the greenhouse. The treatments had 15 replications. On microplots, the treatments were 0, 0.8, 1.6, 3.2, 6.4 and 12.8% Nemarioc-AL or Nemafric-BL phytonematicide, which were arranged in a randomised complete block design with 10 replications. In the field trial the treatments were 0, 2.4, 4.8, 9.6, 19.2 and 38.4% of Nemarioc-AL and Nemafric-BL phytonematicides in separate trials arranged in a randomised complete block design with 9 replications.

Legend 3.1 Trial layout of Nemarioc-AL phytonematicide on beetroot under greenhouse conditions.

Legend 3.2 Trial layout of Nemarioc-AL and Nemafric-BL phytonematicides on beetroot under microplot conditions.

Legend 3.3 Trial layout of Nemarioc-AL and Nemafric-BL phytonematicides on beetroot under field conditions.

3.2.3 Procedures

Nemarioc-AL and Nemafric-BL phytonematicides were prepared using the ZZ2 method (Pelinganga *et al*., 2012). Briefly, the method consisted of filling 20 L containers with 16 L chlorine-free tapwater, 40 g and 80 g dried and ground fruits from wild watermelon (*Cucumisafricanus*) and wild cucumber (*Cucumismyriocarpus*),respectively, 300 ml effective microorganisms (EM), mixed with 300 ml molasses, 10 g brown sugar and 16 L chlorine free water in 20 L plastic container. The whole system was air-tight. The container had an outlet which dangled into a bottle with water in order to provide for escape of gases generated during fermentation. The system was stored at room temperature for 14 days, to allow for a decline of pH to 3.7. Greenhouse experiments were established by putting 25-cm pots on the greenhouse benches, while artificial microplots were established by putting 25-cm diameter plastic pots onto 25-cm lids at 0.25 m intra-row and 0.25 m inter-row spacing. Each pot was filled with 5 L steam-pasteurised loam and sand at 3:1 (v/v) ratio. Beetroot cv. 'Detroit Dark Red'seedlings raised using seedling trays under greenhouse conditions were transplanted into pots.

A day after transplanting, seedlings were fertilised with 5 g NPK 2:3:2 (26), 5% Ca, 0.5% Zn and 5% S per plant. Plants were fertilised once at inoculation using 2 g 2:1:2 (43) Multifeed® fertiliser to provide a total of 0.70 mg N, 0.64 mg K and 0.64 mg P, 1.8 mg Mg, 1.5 mg Fe, 0.15 mg Cu, 0.7 mg Zn, 2 mg B, 6 mg Mn and 0.14 mg Mo/ml tapwater.A week after transplanting, each pot was infested with 5 000 *Meloidogyne incognita* eggs andsecond-stage juveniles(J2). *M. incognita* race 2 inoculum was prepared by extracting eggs and J2 from roots of greenhouse-raised nematode-susceptible kenaf (*Hibiscus cannabinus*) in 1% NaOCl (Hussey and Barker, 1973).

Seven days after transplanting, beetrootseedlings were inoculated with 5 000 *M. incognita* eggs and J2. A week after transplanting, each pot was infested with 5 000 *M. incognita* J2 and eggs using a 5ml plastic syringe by infesting into approximately 3cm deep holes on the cardinal points of the stem of the plants. Each plant was irrigated with 250 ml chlorine-free tapwater every other day. Once a week, irrigation was substituted for treatments using appropriate concentrations for each product.

3.2.4 Data collection

At 56 days after inoculation, plant height was measured from the crown to the tip of the flag leaf and the number of leaves per plant was counted. Shoots were cut at the crown and oven-dried at 70°C for 72 h and weighed for dry mass. Root systems were removed from the pots, immersed in water to remove soil particles, separated from the roots, 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). Nematodes were extracted from 10 g roots per plant by maceration and blending for 30 seconds in 1% NaOCl (Hussey and Barker, 1973). The material was passed through 150 µm, 45 µm and 25 µm nested sieves, with nematode eggs and J2 collected from the 25 µm mesh sieve. Soil per pot was thoroughly mixed and a 250 cm^3 soil sample collected, J2 extracted from soil samples using the sugar-floatation and centrifugation method (Jenkins, 1964). Eggs and J2 from root samples and J2 from soil samples were counted from a 5 ml aliquot under a stereomicroscope. Nematode numbers for greenhouse and microplot trials were converted to nematodes per total root system per plant, whereas soil nematode numbers were converted to

volume growing mixture per pot, all to allow for the determination of the final nematode population density (Pf).

3.2.5 Data analysis

Data were subjected to analysis of variance using SAS (SAS Institute Inc., Cary, NC, USA). The degrees of freedom and their associated sum of squares were partitioned to provide the total treatment variation (TTV) for different sources of variation. Mean separation was achieved through Fisher's Least Significant Difference test at 5% level of probability. Significant mean plant variables were further subjected to the CARD model to generate biological indices (Liu *et al*., 2003) which allowed for the calculation of the MCSP for Nemarioc-AL and Nemafric-BL phytonematicides. Unless stated otherwise, treatment effects were reported at the probability of 5%.

3.3 Results

3.3.1 Greenhouse trials

Treatment effects: In both Nemarioc-AL and Nemafric-BL phytonematicides, effects of phytonematicides were highly significant ($P \leq 0.01$) on root galls, but had no effect on, fresh root mass, dry shoot mass, dry root mass and leaf number. Nemarioc-AL and Nemafric-BL phytonematicides contributed, 77%and 72% in TTV of root galls, respectively (Table 3.1). Relative to untreated control for Nemarioc-AL and Nemafric-BL phytonematicides, root galls were reduced by 28-72% and 43-67%, respectively (Table 3.2). In both Nemarioc-AL and Nemafric-BL phytonematicides, treatments had no effects on all other plant variables (Table 3.3).

Table 3.1 Sources of variation as affecting gall rating at 56 days after initiation of treatments for Nemafric-BL and Nemarioc-AL phytonematicides under greenhouse conditions.

		Nemarioc-AL phytonematicide					
		Fresh root mass		Dry root mass		Gall rating	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Rep	14	198.252	50	5.86567	69	0.04878	19
Treatment	5	117.044	30^{ns}	1.25134	15^{ns}	0.18626	72***
Error	70	80.003	20	1.37514	16	0.02264	9
Total	89	395.299	100	8.49215	100	0.25768	100
		Nemafric-BL phytonematicide					
		Fresh root mass		Dry root mass		Gall rating	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Rep	14	226.665	68	4.98292	82	0.02208	11
Treatment	5	58.150	17^{ns}	0.48651	8^{ns}	0.14973	77***
Error	70	49.954	15	0.63470	10	0.02377	12
Total	89	334.769	100	6.10413	100	0.19558	100

***Highly significant at $P \leq 0.01$, ^{ns}Notsignificant at $P \leq 0.05$.

Curve-fitting Allelochemical Response Dosage: In Nemarioc-AL and Nemafric-BL phytonematicides, root galls and treatments exhibited quadratic relations (Figure 3.1), with the model explaining the relationships by 77% and 97% (Table 3.4). In Nemarioc-AL and Nemafric-BL phytonematicides, MCSP of *B. vulgaris* was 6.4% and 18.1% with

root galls having k values of 1 and 0 and overall sensitivity (∑k) of beetrootbeing equivalent to 1 and 0 units, respectively (Table 3.5). For plant variables in Nemafric-BL and Nemarioc-AL phytonematicides, treatment effects were not significant, therefore, treatment means were not subjected to the CARD model and overall sensitivity (∑k) of *B. vulgaris* was not determined.

Table 3.2 Effect of fermented crude extracts of Nemafric-BL and Nemarioc-AL phytonematicides on root galls of *Beta vulgaris* at 56 days after initiation of treatments under greenhouse conditions.

	Nemarioc-AL phytonematicide		Nemafric-BL phytonematicide	
Concentration	Variable[y]	[z]RI (%)[z]	Variable[y]	[z]RI (%)[z]
0	0.4665[a]	−	0.4302[a]	−
2	0.2560[bc]	−45	0.2844[b]	−34
4	0.1723[bc]	−63	0.3079[b]	−28
8	0.2677[b]	−43	0.2844[b]	−34
16	0.2526[bc]	−46	0.2677[b]	−38
32	0.1522[c]	−67	0.1204[c]	−72

[y]Column means followed by the same letter were not different (P≤ 0.05) according to Fisher's Least Significant Difference test.

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

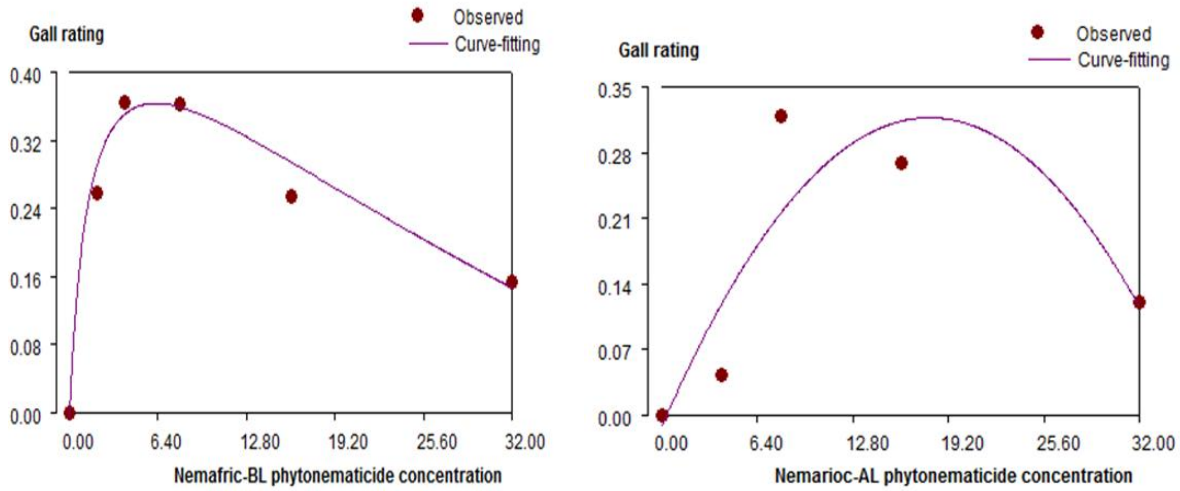

Figure 3.1 Response of gall rating of beetrootto concentrations of Nemarioc-AL and Nemafric-BL phytonematicides at 56 days after inoculation under greenhouse conditions.

Table 3.3 Quadratic relationships, coefficient of determination and computed optimum response concentration for root galls of beetrootfrom the Curve-fitting Allelochemical Response Dosage against Nemafric-BL and Nemarioc-AL phytonematicides at 56 days after treatments under greenhouse conditions.

	Nemarioc-AL phytonematicide			
Variable	Quadratic relation	R^2	x^z	Y
Root galls	$Y = -0.001x^2 + 0.0363x + 0.011$	0.76	181.5	39.51
	Nemafric-BL phytonematicide			
Variable	Quadratic relation	R^2	x^z	Y
Root galls	$Y = -0.0009x^2 + 0.027x + 0.147$	0.43	15	0.35

$^z x = -b_1/2b_2$, where x is optimum concentration.

Table 3.4 Biological indices for root galls of beetrootto increasing concentrations of Nemafric-BL and Nemarioc-AL phytonematicides at 56 days after initiation of treatments under greenhouse conditions.

Biological index[z]	Nemarioc-AL phytonematicide	Nemafric-BL phytonematicide
Threshold stimulation (D_m)	17.941	6.221
Saturation point (R_h)	0.326	0.352
0% inhibition (D_0)	35.882	51.145
50% inhibition (D_{50})	35.73	50.93
100% inhibition (D_{100})	35.6	50.7
R^2	0.77	0.97
k-value	0	1
[y]Overall sensitivity	$\sum k = 0$	$\sum = 1$
MCSP	18.1%	6.4%

Mean Concentration Stimulation Point (MCSP) = $D_m + (R_h/2)$.

Table 3.5 Responses of *Meloidogyne incognita* to Nemarioc-AL and Nemafric-BL phytonematicides under greenhouse conditions.

Concentration (%)	J2	Eggs	Total	RF
0	0	0	0	0
2.4	0	0	0	0
4.8	0	0	0	0
9.6	0	0	0	0
19.2	0	0	0	0
38.4	0	0	0	0

RF = Reproductive factor.

J2 = Second-stage juveniles.

3.3.2 Microplot trials

Treatment effects: Effects in Nemafric-BL phytonematicide were significant (P≤ 0.05) on fresh root mass, dry root mass and gall rating but had no effect on, fresh root mass, dry shoot mass and number of leaves. Nemafric-BL phytonematicide contributed 20.4, 18.5 and 57.2% in total treatment variation (TTV) of fresh root mass, dry root mass and root gall, respectively with Nemarioc-AL phytonematicide, treatments having no effects on all plant variables(Table 3.7). Relative to untreated control, fresh root mass was increased by 65.1 to 236.8%, dry root mass was increased by 62.8 to 228.5% and root galls were reduced by 82.3 to 100% (Table 3.8).

Curve-fitting Allelochemical Response Dosage: In Nemafric-BL phytonematicide, treatments exhibited quadratic relations on fresh root mass, dry root mass and root galls (Figure 3.2), with the model explaining the relationships by, 87, 76 and 52 % (Table

3.10). In Nemafric-BL phytonematicide, MCSP of *B. vulgaris* was 10.2% with root galls having k values of k = 2, whereas dry root and fresh root had k value of k = 1, with overall sensitivity (∑k) of *B. vulgaris* being equivalent to 4 units (Table 3.11). In Nemarioc-AL phytonematicide, treatment effects were not significant, therefore, treatment means were not subjected to the CARD model and overall sensitivity (∑k) of *B. vulgaris* was not determined.

Nematode variables: Nematodes were not detected in both roots and soil samples in microplot trials.

Table 3.6 Responses of *Meloidogyne incognita* to Nemarioc-AL and Nemafric-AL phytonematicides under microplot conditions.

Concentration	J2	Eggs	Total	RF
0	0	0	0	0
2.4	0	0	0	0
4.8	0	0	0	0
9.6	0	0	0	0
19.2	0	0	0	0
38.4	0	0	0	0

RF = Reproductive factor.

J2 = Second-stage juveniles.

Table 3.7 Sources of variation as affecting gall rating at 56 days after initiation of treatments for Nemarioc-AL and Nemafric-BL phytonematicides under microplot conditions.

		Nemarioc-AL phytonematicide					
		Fresh root mass		Dry root mass		Gall rating	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Rep	9	70.4204	30	0.52023	13	0.60926	36
Treatment	5	71.2639	30^{ns}	1.56835	39^{ns}	0.65667	38^{ns}
Error	45	95.7721	40	1.9096	48	0.4492	26
Total	59	237.456	100	3.9982	100	1.7151	100
		Nemafric-BL phytonematicide					
		Fresh root mass		Dry root mass		Gall rating	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Rep	9	8466.81	72	186.65	74	0.18	18
Treatment	5	2395.81	20**	46.26	19**	0.55	57**
Error	45	871.09	8	17.81	7	0.24	25
Total	59	11733.7	100	250.73	100	0.96	100

***Highly significant, ^{ns}Notsignificant at $P \leq 0.05$.

Table 3.8 Effect of fermented crude extracts of Nemafric-BL phytonematicide on dry root mass (DRM), fresh root mass (FRM), and gall rating (GR) of beetrootat 56 days after initiation of treatments under microplot conditions.

	DBM		FBM		GR	
Concentration	Variable[y]	[z]RI (%)	Variable[y]	[z]RI (%)	Variable[y]	[z]RI (%)
0	2.53[c]	–	17.87[c]	–	0.60[a]	–
0.8	4.12[bc]	62.8	29.50[bc]	65.1	0.10[b]	−82.3
1.6	8.31[a]	228.5	60.18[a]	236.8	0.00[b]	−100
3.2	7.48[ab]	195.7	51.43[ab]	187.8	0.00[b]	−100
6.4	5.01[abc]	98	35.47[abc]	98.5	0.00[b]	−100
12.8	6.14[abc]	142.7	46.29[ab]	159.0	0.10[b]	−82.3

[y]Column means followed by the same letter were not different ($P \leq 0.05$) according to Fisher's Least Significant Difference test.

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

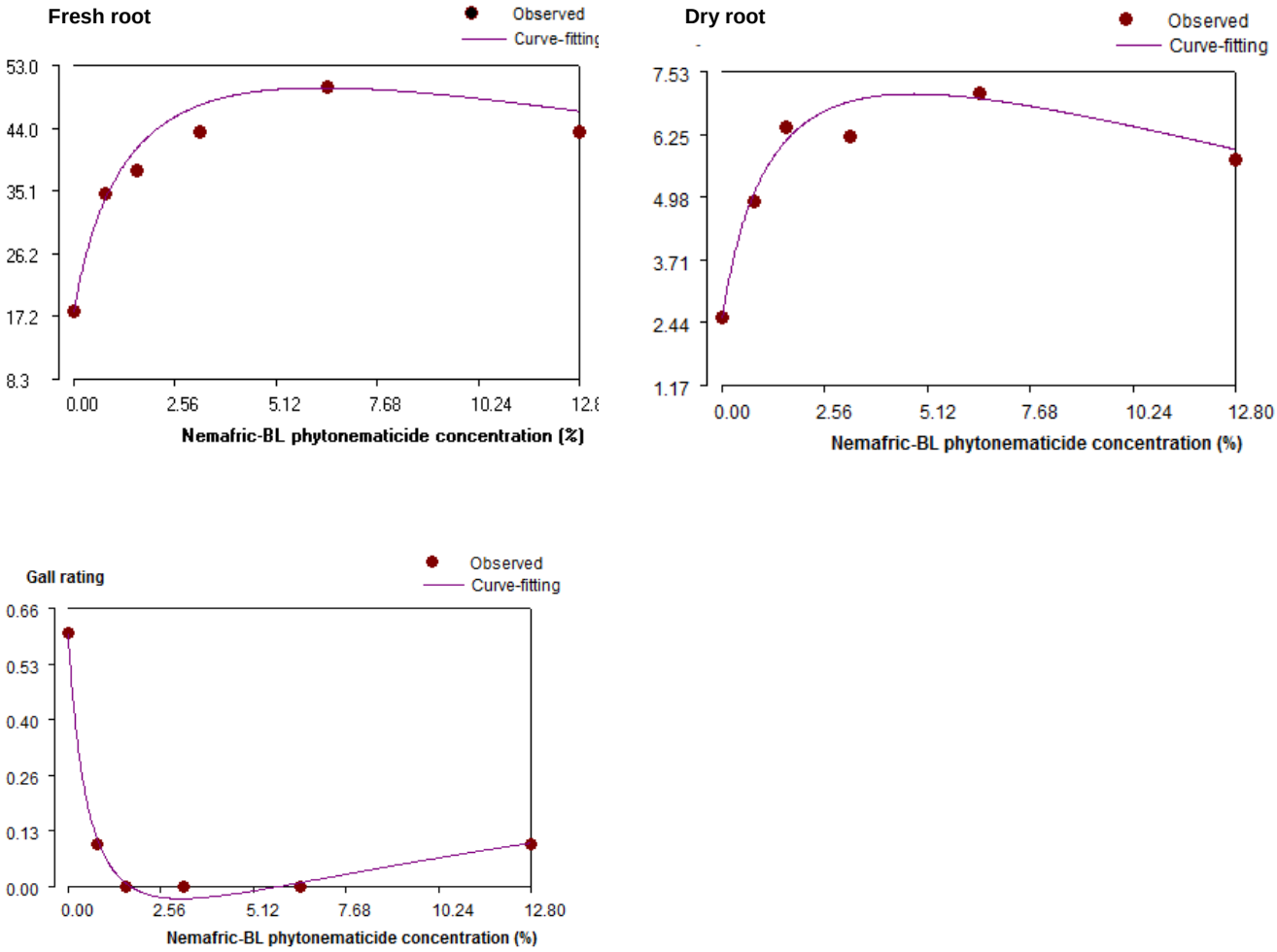

Figure 3.2 Response of fresh root mass, dry root mass and gall rating of *B. vulgaris* to concentrations of Nemafric-BL phytonematicide at 56 days after inoculation under microplot conditions.

Table 3.9 Quadratic relationships, coefficient of determination and computed optimum response concentration for variables of beetrootfrom the Curve-fitting Allelochemical Response Dosage against Nemafric-BL phytonematicide at 56 days after treatments under microplot conditions.

Variable	Quadratic relation	R^2	x^z	Y
Fresh root	$Y = -0.4656x^2 + 7.4249x + 24.157$	0.87	7.97	53.68
Dry root	$Y = -0.0724x^2 + 1.0824x + 3.6277$	0.76	7.48	7.67
Root galls	$Y = -0.009x^2 + 0.1326x + 0.3553$	0.53	7.36	1.12

$^z x = -b_1/2b_2$, where x is optimum concentration.

Table 3.10 Biological indices for fresh root mass, dry root mass and root galls of beetrootto increasing concentrations of Nemafric-BL phytonematicide at 56 days after initiation of treatments under microplot conditions.

Biological index^z	Fresh root (g)	Dry root (g)	Root galls (g)	Mean
Threshold stimulation (D_m)	6.23	4.77	3.13	4.71
Saturation point (R_h)	29.22	4.32	-0.62	10.97
0% inhibition (D_0)	51.81	32.29	0	28.03
50% inhibition (D_{50})	69.39	41.42	0.32	37.04
100% inhibition (D_{100})	89.8	51.6	1.7	47.7
R^2	0.98	0.96	0.99	
k-value	1	1	2	
^{y}Overall sensitivity		$\sum k = 4$		

MCSP = $D_m + (R_h/2)$ = 4.71+ 10.97/2 = 10.2%.

Table 3.11 Sources of variation as affecting gall rating at 56 days after initiation of treatments for Nemafric-BL and Nemarioc-AL phytonematicide under field conditions.

		Nemarioc-AL phytonematicide					
		Fresh root mass		Dry root mass		Gall rating	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Rep	9	71.1513	43.97	0.04966	38.85	121.930	28.68
Treatment	5	40.9082	25.28^{ns}	0.03410	26.68^{ns}	106.578	25.07^{ns}
Error	40	49.7748	30.75	0.04406	34.47	196.577	46.25
Total	53	161.8343	100	0.12782	100	425.085	100

		Nemafric-BL phytonematicide					
		Fresh root mass		Dry root mass		Gall rating	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Rep	8	111.856	47	2.16535	41	50.0216	56
Treatment	5	70.465	30^{ns}	1.21617	23^{ns}	17.0582	19^{ns}
Error	40	53.252	23	1.89335	36	22.9459	25
Total	53	235.573	100	5.27487	100	90.0257	100

^{ns}Notsignificant at $P \leq 0.05$.

3.3.3 Field trials

Treatment effects: Both Nemafric-BL and Nemarioc-AL phytonematicides had no treatment effect on all plant variables and gall rating. Therefore, the plant variables and gall rating were not further subjected to the CARD model.

Table 3.12 Responses of *Meloidogyne incognita* to Nemarioc-AL and Nemafric-BL phytonematicides under field conditions.

Concentration	J2	Eggs	Total	RF
0	0	0	0	0
2.4	0	0	0	0
4.8	0	0	0	0
9.6	0	0	0	0
19.2	0	0	0	0
38.4	0	0	0	0

RF = Reproductive factor.

J2 = Second-stage juveniles.

3.4 Discussion

3.4.1 Greenhouse conditions: In the current study, Nemarioc-AL and Nemafric-BL phytonematicides did not have significant effects on variables of beetroot cv. 'Detroit Dark Red'. This observation confirmed those of Nemafric-BL phytonematicide on tomato (Pelinganga *et al*., 2012; Pelinganga, 2013)and citrus(Mathabatha *et al*., 2016) forcrops grown under the greenhouse conditions. Similar observations were made where Nemarioc-AL phytonematicide in tomato roots (Tseke *et al*., 2013) and citrus roots (Mathabatha *et al*., 2016)crops under greenhouse conditions. In relation to DDG patterns, the observations implied that at harvest the concentrations of the phytonematicides for the variables measured were between R_h and D_0, which is referred

to as the neutral phase (Liu *et al*., 2003; Mashela *et al*., 2015). Should the plants have stayed longer, the inhibition phase would have been entered.

The variable which was consistently affected by the two phytonematicides was the gall rating, which confirmed observations in tomato and citrus(Pelinganga *et al*., 2012; Mathabatha *et al*., 2016). It should be remembered that cv. 'Detroit Dark Red' hadpreviously, shown to be tolerant to *M*. *javanica* (Mashela and Pofu, 2016). The intended objective in the current study was to establish the MCSP for the two phytonematicides on beetroot cv. 'Detroit Dark Red', but could not be done since the treatment effects were not significant on plant variables. The observed MCSP values on the gall rating were used to provide estimates of the MCSP of the two products on the tested cultivar. The MCSP values were 18.1 and 6.4% for Nemarioc-AL and Nemafric-BL phytonematicides, respectively. In Nemarioc-AL phytonematicide on tomatoand citrus crops the MCSP values were 2.6and 8.6%, respectively, under greenhouse conditions (Tseke *et al.,* 2013; Pelinganga, 2013;Mathabatha *et al.,* 2016). In contrast, the MCSP values for Nemafric-BL phytonematicide on tomato and citrus, crops had been 2.9 and 6.3%, respectively (Pelinganga, 2013; Mathabatha *et al*., 2016). The observed MCSP values on beetroot in the current study, it should be borne in mind, were for root galls, while those of other crops were mainly averages for various variables. The latter could explain why in beetroot the MCSP values were rather high when compared to other crops. The overall sensitivities of Nemarioc-AL and Nemafric-BL phytonematicides were 0 and 1 units on beetroot, for the root galls. These values were not different from those observed on tomato and citrus crops of5, 3and 2 units for Nemarioc-AL phytonematicide (Tseke *et al.,* 2013; Pelinganga, 2013; Mathabatha *et al*.,

2016), on 1,4 and4 units for Nemafric-BL phytonematicide under greenhouse conditions (Pelinganga *et al*., 2012 ; Pelinganga, 2013; Mathabatha *et al*., 2016).

3.4.2 Microplot conditions: In this study, increasing concentrations of Nemafric-BL phytonematicide resulted in significant effect on fresh root mass, dry root mass and root galls of beetroot but was not significant on other plant variables; whereas Nemarioc-AL phytonematicide had no significant effect on all plant variables of beetroot. The results showed that the plant growth was stimulated and root galls were inhibited. The results contradicted the findings of Sithole *et al*. (2016) and Pelinganga (2013), where Nemarioc-AL and Nemafric-BL phytonematicides significantly affected plant growth of*P*. *sidoides*and tomato*,* respectively.

The variables which were significantly affected by Nemafric-BL phytonematicide were fresh root mass, dry root mass and gall rating. The observed MCSP value on fresh root mass, dry root mass and the gall rating was 10.2% and was used to provide estimates of the MCSP of the Nemafric-BL phytonematicide on the tested cultivar. In Nemarioc-AL phytonematicide on*P.sidoides,*the MCSP value was 6.18% under microplot conditions (Sithole *et al.,* 2016). The observed MCSP value on beetroot in the current study was for fresh root mass, dry root mass and gall rating while those of other crops were mainly averages for various variables. The latter could explain why in beetroot the MCSP value was rather high when compared tothose ofother crops.The overall sensitivity of 4 units in beetroot when nematodes were managed using Nemafric-BL phytonematicide was similar to that on tomato seedlings (Pelinganga, 2013), but higher than that in *P. sidoides* ($\sum k = 3$) as observed by(Sithole *et al*., 2016). Generally, plants with overall

sensitivity values of less than 5 units are viewed as being moderately sensitive to the phytonematicides, whereas values above 5 units are highly tolerant (Mashela *et al*., 2015). Generally, the closer ∑k is to zero, the more sensitive is the plant to the phytonematicide, *vice versa* (Mashela *et al*., 2015).

3.4.3 Field conditions: Nemarioc-AL and Nemafric-BL phytonematicides had no significant effect on all plant variables. Therefore, the two products at low and high concentrations had saturatedeffects on plant growth of beetroot. The fact that plant variables of beetroot were not stimulated nor inhibited by increasing levels of the phytonematicides in this study, suggested that the organs were, by harvest time at saturation point (Mashela *et al*., 2015). Mashela *et al*. (2015) postulated thatwhen the concentrations are within the neutral range for plant variables, treatment effects are not significant,whereas within stimulation or inhibition range, treatment effects are significant (Mashela *et al*., 2015).

The absence of nematodes in roots of beetroot under field conditions, confirms the observations byMashela and Pofu (2016) that beetroot cv. 'Detroit Dark Red' was tolerant to various *Meloidogyne* species. Sithole *et al*. (2016) observed that effects of Nemarioc-AL phytonematicide on all nematode stages were not different, with the exception to the density in untreated controls. Thus, suggesting that the efficacy was not density dependent.In this study, observations contradicted with the results of Pelinganga *et al*. (2013), where Nemarioc-AL and Nemafric-BL phytonematicides reduced eggs and J2 of root-knot nematodes under greenhouse and microplot conditions. Maile (2013) reported that Nemafric-BL phytonematicidereduced eggs and juvenilesin citrus roots by 80%, but increased juveniles in soil and final total citrus

(*Tylenchulus semipenetrans*) nematode by 178% and 70%, respectively. The observations were clarified in terms of differences in application period to nematode sampling (Maile, 2013).

3.5 Conclusion

The MCSP value of Nemafric-BL phytonematicide on beetrootseedlings under greenhouse and microplot conditions was relatively higher than that of tomato, citrus and geranium. The values for beetroot under the two conditions must be reduced to 3% since the product is not intended for use as fertilisers but as management of *Meloidogyne* species. Under all three conditions, beetroot has shown to have some degree of tolerance towards*M. incognita*. Therefore, this attribute in beetroot cv. 'Detroit Dark Red' can be used alongside with phytonematicides to successfully manage nematode densities in beetroot production.

CHAPTER 4
SUMMARY, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

4.1 Summary

The study was carried out to determine the Mean Concentration Stimulation Point (MCSP) of Nemarioc-AL and Nemafric-BL phytonematicides on beetroot (*Beta vulgaris*) using the Curve-fitting Allelochemical Response Dosage (CARD) model (Mashela *et al*., 2015). Two biological indices (D_m, R_h) from the CARD model were used to establish the MCSP of the two phytonematicides on beetroot cv. 'Detroit Dark Red'. At the identified MCSP values, namely, 18.1% and 6.4% for Nemarioc-AL and Nemafric-BL phytonematicides, respectively, the products would suppress nematode numbers without inducing phytotoxicity to the beetroot cultivar. Additionally, the CARD model provided the sensitivity index of the crop to the product used. Relative to untreated control, Nemarioc-AL and Nemafric-BL phytonematicides reduced root galls by 28-72% and 43-67%, respectively. Under microplot conditions, Nemafric-BL phytonematicide, effects were significant on fresh root mass, dry root mass and root galls, but had no effect on other plant variables. In Nemafric-BL phytonematicide, variables and treatments exhibited quadratic relations on fresh root mass, dry root mass and root galls, with the model explaining the relationships by 87, 76 and 52%. In Nemafric-BL phytonematicide, the MCSP for the phytonematicide on *B. vulgaris* was 10.2%, with the overall sensitivity (∑k) of *B. vulgaris* to the phytonematicide being equivalent to 4 units. In Nemarioc-AL phytonematicide,the treatment effects were not significant, therefore, treatment means were not subjected to the CARD model and the overall sensitivity of this phytonematicide to *B. vulgaris* was not determined. Under field conditions, all the

plant variables and root galls were not significant and were therefore, not subjected to the CARD model. Generally, the absence of significant treatment effects in phytonematicides suggested that the variables were, at harvest, at the neutral phase, which signifies concentration ranges prior to the set-in of the inhibition concentrations.

4.2 Significance of findings

The findings demonstrated that the two phytonematicides showed two phases of DDG patterns on beetroot (*B. vulgaris*) cv. 'Detroit Dark Red' which were stimulation phase and neutral phase under greenhouse, microplot and field conditions. It was also observed that beetroot is tolerant to *M. incognita* since there was an absence of nematodes in root and soil samples.

4.3 Recommendations

Unlike in tomato plants, the edible produce in beetroot comes into direct contact with cucurbitacins whenever the phytonematicides are applied. Consequently, it is recommended that the cucurbitacin residues for the two phytonematicides be investigated at various withholding periods, as well as after preparation of beetroot dishes. This is important since at low concentrations, cucurbitacins are carcinogenic (Lee *et al*., 2010), an admirable phenomenon in crops, which is simply referred to as stimulation.

4.4 Conclusions

Under greenhouse and microplot conditions, the MCSP for Nemarioc-AL and Nemafric-BL phytonematicides on *B. vulgaris* was rather high when compared with those in

tomato plants (Mashela *et al.,* 2015). Since nematodes were not detected in root and soil samples, beetroot cv. 'Detroit Dark Red' was tolerant to root-knot nematodes and could be planted in areas with high population densities of *M. incognita.* Therefore, the MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides should be reduced to 3% as the two products were used for nematode suppression not as fertilisers. The potential existence of cucurbitacin residues in beetroot roots should also be prioritized since the roots are in direct contact with the phytonematicides during the treatments.

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APPENDICES

Appendix 3.1 Analysis of variance of six different concentrations of fermented plant extracts of Nemarioc-AL and Nemafric-BL phytonematicides on number of leaves for beetroot crop.

	Nemarioc-AL phytonematicide				
Source	DF	SS	MS	F	P ≤
Replication	14	0.20080	0.01434		
Treatment	5	0.01884	0.00377	0.45	0.8102
Error	70	0.58303	0.00833		
Total	89	0.80267	0.02644		
	Nemafric-BL phytonematicide				
Source	DF	SS	MS	F	P ≤
Replication	14	0.12105	0.00865		
Treatment	5	0.06494	0.01299	0.94	0.4604
Error	70	0.96669	0.01381		
Total	89	1.15267	0.03545		

Appendix 3.2 Analysis of variance of six different concentrations of fermented plant extracts of Nemarioc-AL and Nemafric-BL phytonematicides on root mass of beetroot crop.

		Nemarioc-AL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	14	136.101	9.72150		
Treatment	5	18.850	3.77008	1.58	0.1779
Error	70	167.362	2.39089		
Total	89	322.314	15.88247		
		Nemafric-BL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	14	79.178	5.65555		
Treatment	5	11.412	2.28231	1.42	0.2263
Error	70	112.164	1.60234		
Total	89	202.753	9.5402		

Appendix 3.3 Analysis of variance of six different concentrations of fermented plant extracts of Nemarioc-AL and Nemafric-BL phytonematicides on fresh root mass of beetroot crop.

		Nemarioc-AL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	14	2775.53	198.252		
Treatment	5	585.22	117.044	1.46	0.2130
Error	70	5600.21	80.003		
Total	89	8960.96	395.299		
		Nemafric-BL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	14	3173.31	226.665		
Treatment	5	290.75	58.150	1.16	0.3356
Error	70	3496.76	49.954		
Total	89	6960.82	334.759		

Appendix 3.4 Analysis of variance of six different concentrations of fermented plant extracts of Nemarioc-AL and Nemafric-BL phytonematicides on gall rating of beetroot crop.

		Nemarioc-AL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	14	0.68298	0.04878		
Treatment	5	0.93131	0.18626	8.23	0.0000
Error	70	1.58509	0.02264		
Total	89	3.19938	0.25768		
		Nemafric-BL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	14	0.30917	0.02208		
Treatment	5	0.74863	0.14973	6.30	0.0001
Error	70	1.66422	0.02377		
Total	89	2.72202	0.19558		

Appendix 3.5 Analysis of variance of six different concentrations of fermented plant extracts of Nemarioc-AL and Nemafric-BL phytonematicides on dry root of beetroot crop.

		Nemarioc-AL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	14	82.119	5.86567		
Treatment	5	6.257	1.25134	0.91	0.4797
Error	70	96.259	1.37514		
Total	89	184.636	8.49215		
		Nemafric-BL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	14	69.761	4.98292		
Treatment	5	2.433	0.48651	0.77	0.77
Error	70	44.429	0.63470		
Total	89	116.622	6.10413		

Appendix 3.6 Analysis of variance of six different concentrations of fermented plant extracts of Nemarioc-AL and Nemafric-BL phytonematicides on dry shoot mass of beetroot crop.

		Nemarioc-AL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	14	44.643	3.18880		
Treatment	5	5.926	1.18519	0.51	0.7714
Error	70	164.237	2.34624		
Total	89	214.806	6.72023		
		Nemafric-BL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	14	69.761	4.98292		
Treatment	5	2.433	0.48651	0.77	0.77
Error	70	44.429	0.63470		
Total	89	116.622	6.10413		

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Appendix 3.7 Analysis of variance of six different concentrations of fermented plant extracts of Nemarioc-AL and Nemafric-BL phytonematicides on number of leaves for beetroot crop.

	Nemarioc-AL phytonematicide				
Source	DF	SS	MS	F	P ≤
Replication	9	0.003	6.849^{-04}		
Treatment	5	0.001	3.639^{-04}	0.39	0.8524
Error	45	0.019	9.427^{-04}		
Total	59	0.023	6.284×10^{-3}		
	Nemafric-BL phytonematicide				
Source	DF	SS	MS	F	P ≤
Replication	9	0.139	0.035		
Treatment	5	0.221	0.044	1.55	0.2208
Error	45	0.571	0.029		
Total	59	0.931	0.108		

Appendix 3.9 Analysis of variance of six different concentrations of fermented plant extracts of Nemarioc-AL and Nemafric-BL phytonematicides on root mass of beetroot crop.

		Nemarioc-AL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	9	323.740	80.934		
Treatment	5	101.650	20.330	0.48	0.7853
Error	45	843.030	42.152		
Total	59	1268.420	143.416		
		Nemafric-BL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	9	167.169	41.792		
Treatment	5	155.136	31.027	1.29	0.3054
Error	45	479.267	23.963		
Total	59	801.572	96.782		

Appendix 3.10 Analysis of variance of six different concentrations of fermented plant extracts of Nemarioc-AL and Nemafric-BL phytonematicides on fresh root mass of beetroot crop.

		Nemarioc-AL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	49	4512.900	1128.230		
Treatment	5	2894.200	578.850	0.22	0.9484
Error	45	51944.500	2597.230		
Total	59	59351.700	4304.310		
		Nemafric-BL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	9	24101.600	6025.390		
Treatment	5	21192.600	4238.530	3.06	0.0328
Error	45	27714.200	1385.710		
Total	59	73008.400	11649.63		

Appendix 3.11 Analysis of variance of six different concentrations of fermented plant extracts of Nemarioc-AL and Nemafric-BL phytonematicides on dry root mass of beetroot crop.

		Nemarioc-AL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	9	1040.02	115.558		
Treatment	5	32.29	6.457	0.20	0.9602
Error	45	1442.09	32.047		
Total	59	2514.40	154.062		
		Nemafric-BL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	9	1679.89	186.655		
Treatment	5	231.33	46.267	2.60	0.0380
Error	45	801.50	17.811		
Total	59	2712.73	250.733		

Appendix 3.12 Analysis of variance of six different concentrations of fermented plant extracts of Nemarioc-AL and Nemafric-BL phytonematicides on dry shoot mass of beetroot.

		Nemarioc-AL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	9	80.270	20.068		
Treatment	5	99.330	19.866	0.38	0.8547
Error	45	1038.410	51.920		
Total	59	1218.010	91.854		
		Nemafric-BL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	9	8.775	2.194		
Treatment	5	5.863	1.173	1.07	0.4054
Error	45	21.885	1.094		
Total	59	36.523	5.061		

Appendix 3.13 Analysis of variance of six different concentrations of fermented plant extracts of Nemarioc-AL and Nemafric-BL phytonematicides on gall rating of beetroot.

		Nemarioc-AL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	9	5.4833	0.60926		
Treatment	5	3.2833	0.65667	1.46	0.2212
Error	45	20.2167	0.44926		
Total	59	28.9833	1.71519		
		Nemafric-BL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	9	1.6000	0.17778		
Treatment	5	2.7333	0.54667	2.32	0.0586
Error	45	10.6000	0.23556		
Total	59	14.9333	0.96001		

Appendix 3.14 Analysis of variance of six different concentrations of fermented plant extracts of Nemarioc-AL and Nemafric-BL phytonematicides on root mass of beetroot.

		Nemarioc-AL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	8	120.872	15.1090		
Treatment	5	92.394	18.4787	1.22	0.3180
Error	40	606.306	15.1576		
Total	53	819.571	48.7453		
		Nemafric-BL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	8	196.16	24.5196		
Treatment	5	291.86	58.3723	2.17	0.0762
Error	40	1073.60	26.8400		
Total	53	1561.62	109.7319		

Appendix 3.15 Analysis of variance of six different concentrations of fermented plant extracts of Nemarioc-AL and Nemafric-BL phytonematicides on fresh root mass of beetroot.

		Nemarioc-AL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	8	4840.2	605.020		
Treatment	5	958.0	191.591	0.27	0.9247
Error	40	27980.9	699.523		
Total	53	33779.0	1496.134		
		Nemafric-BL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	8	8219.3	1027.41		
Treatment	5	4246.3	849.26	1.03	0.4142
Error	40	33035.5	825.89		
Total	53	45501.1	2702.56		

Appendix 3.16 Analysis of variance of six different concentrations of fermented plant extracts of Nemarioc-AL and Nemafric-BL phytonematicides on dry shoot mass of beetroot.

		Nemarioc-AL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	8	62.943	7.86782		
Treatment	5	21.098	4.21955	0.66	0.6564
Error	40	256.079	6.40196		
Total	53	340.119	18.48933		
		Nemafric-BL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	8	136.13	17.0157		
Treatment	5	80.28	16.0559	0.67	0.6505
Error	40	962.63	24.0658		
Total	53	1179.04	57.1374		

Appendix 3.17 Analysis of variance of six different concentrations of fermented plant extracts of Nemarioc-AL and Nemafric-BL phytonematicides on dry root mass of beetroot.

		Nemarioc-AL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	8	114.250	14.2813		
Treatment	5	23.244	4.6487	0.29	0.9170
Error	40	646.358	16.1589		
Total	53	783.851	35.0889		
		Nemafric-BL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	8	193.08	24.1354		
Treatment	5	89.35	17.8693	0.95	0.4610
Error	40	754.14	18.8536		
Total	53	1036.57	60.8583		

Appendix 3.18 Analysis of variance of six different concentrations of fermented plant extracts of Nemarioc-AL and Nemafric-BL phytonematicides on gall rating of beetroot.

		Nemarioc-AL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	8	0.03373	4.216E-03		
Treatment	5	0.02108	4.216E-03	1.00	0.4302
Error	40	0.16863	4.216E-03		
Total	53	0.22343	12.648E-03		
		Nemafric-BL phytonematicide			
Source	DF	SS	MS	F	P ≤
Replication	8	0.27908	0.03489		
Treatment	5	0.48742	0.09748	1.86	0.1237
Error	40	2.09982	0.05250		
Total	53	2.86632	0.18487		