PEA SEED PRIMING IN CUCURBITACIN-CONTAINING PHYTONEMATICIDES FOR GENERATING MEAN CONCENTRATION STIMULATION POINT

BY

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

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DECLARATION

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

Candidate: Vafana Attraction Ntuli   Signature   Date

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DEDICATION

I dedicate this research to the Almighty God and to my father Mr. Gezani Sam Ntuli.

ACKNOWLEDGEMENTS

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ABSTRACT

In use of phytonematicides as an alternative to synthetic chemical nematicides, the major challenge had been the development of appropriate application technologies, which are currently limited to the ground leaching technology (GLT) and botinemagation (BNT) systems. The former is labour-intensive, whereas the latter requires infrastructure that could be costly for smallholder farmers. The priming of seeds with hypogenous germination properties in phytonematicide solutions could serve as an alternative method of the application of phytonematicides, where the cotyledons would serve as carriers of the active ingredients that are leached into the rhizosphere for suppression of nematode numbers. However, since germination is a chemical process, it is not known whether the active ingredients in cucurbitacin-containing phytonematicides would interfere with germination and the subsequent emergence of the seedlings through the incidence of phytotoxicity as observed in the use of the products in crop production. The objectives of the study, therefore, were (1) to investigate the sensitivity and overall sensitivity of pea (*Pisum sativum* L.) plants to Nemarioc-AL and Nemafric-BL phytonematicides, and (2) to determine the mean concentration point (MCSP) for pea-inoculated with *Meloidogyne incognita* under greenhouse and microplot conditions, where seeds were previously primed in phytonematicide solutions. Two separate trials were conducted with seven treatments, namely, 0, 2, 4, 8, 16, 32 and 64% Nemarioc-AL or Nemafric-BL phytonematicide, arranged in completely randomised design (CRD), with 8 replications each. Pea seeds were primed in Nemarioc-AL and Nemafric-BL phytonematicide solutions for two hours and shade dried prior to sowing. In *vitro trial*, 10 seeds were spread uniformly on a moistened filter paper in sterilised petri-dishes with lids and placed in an incubator at 25°C. In vivo trials were under greenhouse and micro-plot conditions, pea seeds were

sown in 25-cm and 30-cm diameter plastic pots, respectively. Pots were filled with pasteurised loam soil. Seedlings were inoculated with 5 000 eggs + second-stage juveniles (J2) of *M. incognita*. Treatments in each case included priming seeds as explained earlier, arranged in a randomised complete block design (RCBD), with 6 replications under greenhouse conditions and 8 replications under micro-plot conditions. In all cases, plant growth variables were assessed using the Curve-fitting Allelochemical Response Dose (CARD) model to generate biological indices which were used to calculate MCSP and the overall sensitivity (Σk). Nematode variables in inoculated trials were assessed using the regression model. *In vitro* trials, germination variables had positive quadratic relation versus Nemafric-BL phytonematicide, with MCSP= 0.62 % and ∑k = 34 units. In contrast, tested germination variables exhibited negative quadratic relations versus Nemarioc-AL phytonematicide. In greenhouse trials, MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides were 0.62 and 2.18 %, respectively, with ∑k = 0. Plant height (R^2 = 0.86), stem diameter (R^2 = 0.93) and chlorophyll content (R^2 = 0.85), exhibited positive quadratic relationship against Nemarioc-AL phytonematicide, whereas, plant height (R^2 = 0.95), stem diameter (R^2 = 0.92), chlorophyll content (R^2 = 0.89), number of flowers (R^2 = 0.93) and dry shoot mass (R^2 = 0.94), exhibited positive quadratic relationship against Nemafric-BL phytonematicide. In micro-plot trials, MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides were 0.71 and 2.45 %, respectively, with ∑k = 0. Plant height (R^2 = 0.95), stem diameter (R^2 = 0.98), chlorophyll content (R^2 = 0.98), and gall ratings (R^2 = 0.98), exhibited positive quadratic relationships against Nemarioc-AL phytonematicide, while chlorophyll content (R^2 = 0.97) and gall ratings (R^2 = 0.96) exhibited positive quadratic relationships against Nemafric-BL phytonematicide. All degrees of Nemarioc-AL and Nemafric-BL phytonematicides profoundly reduced

nematode numbers under greenhouse and micro-plot trials. In conclusion, both Nemarioc-AL and Nemafric-BL phytonematicides could be applied through the priming technology on pea seeds which have hypogenous germination properties in suppression of nematode population densities.

CHAPTER 1

RESEARCH PROBLEM

1.1 Background

1.1.1 Description of the research problem

Following the universal withdrawal of synthetic methyl-bromide nematicides from agrochemical markets because of their environment-unfriendliness, alternatives for managing nematode population densities were broadly researched and developed (Mashela *et al.*, 2015). In Limpopo Province of South Africa, cucurbitacin-A and cucurbitacin-B containing phytonematicides were used to develop Nemarioc-AL and Nemafric-BL phytonematicide, respectively (Mashela *et al.,* 2015). However, the major challenge in the phytonematicide systems had been the development of appropriate application technologies for the products. The tested technologies had been limited to the ground leaching technology (Mashela, 2002) and the botinemagation systems (Pelinganga, 2013). The ground leaching technology (GLT) is labour-intensive and is limited for use in smallholder farming systems, whereas the botinemagation (BNT) system is infrastructure-intensive. Thus, the development of appropriate application technologies of the phytonematicides for various farming systems remain an important gap in the successful use of these products in the management of nematodes as an alternative to synthetic nematicides, which had since 2005 been suspended from the agrochemical markets.

1.1.2 Impact of the research problem

The major limiting factor in the use of Nemafric-BL and Nemarioc-AL phytonematicides had been the application technologies and phytotoxicity of the products to the crops

being protected against nematodes and the high cost of the equipment, particularly for fruits drying (Mashela *et al.*, 2011). The limitation of the application technologies of cucurbitacin-containing phytonematicides restrict the wide-spread application of the products, which lowers the adoption of the phytonematicides in the management of nematodes. Whereas the phytotoxicity of the products could reduce plant growth from as high as 50% to complete crop failure (Mashela *et al.,* 2011; Pofu *et al.*, 2010). However, using the Curve-fitting Allelochemical Response Dosage (CARD) computer-based model, other researchers (Liu *et al.,* 2003; Pelinganga *et al.,* 2012) adapted two of the seven biological indices to develop stimulatory concentrations to plant growth, with nematotoxic properties. These stimulatory concentrations had been referred to as mean concentration stimulation range (MCSR), which had since been used widely in botinemagation technology (Pelinganga, 2013; Pelinganga and Mashela, 2012; Pelinganga *et al.,* 2011; Pelinganga *et al.,* 2012).

1.1.3 Possible causes of the research problem

International yield loss as result of root-knot nematode damage prior to withdrawal of synthetic nematicides had been estimated at US$126 billion per annum (Chitwood, 2003), with the percentage of yield losses ranging between 6 to 20% (Sithole, 2016). Cucurbitacin-containing phytonematicides have been successfully developed and used in the suppression of *Meloidogyne species* in vegetable cultivation (Mashela *et al.*, 2015). However, the major challenge in the phytonematicide system had been the application technologies for the products. The application technologies used in the placement of phytonematicides are limited to the ground leaching technology and botinemagation systems, which require high inputs and prerequisite equipment that could be costly for marginal farming communities in South Africa.

1.1.4 Proposed solution

Currently, the use of cucurbitacin-containing phytonematicide is receiving greater attention as one of the possible sustainable alternatives for the management of plant-parasitic nematodes after the worldwide withdrawal of methyl-bromide in 2005 from the agrochemical markets (Mashela, 2007). The adoption of bio-nematicides such as cucurbitacin-containing phytonematicides are environment-friendly management interventions, which may be sustainable in the long-term (Chitwood, 2002). Therefore, priming of seeds with hypogenous germination characteristics in solutions of cucurbitacin-containing phytonematicides could serve as carriers of cucurbitacin active ingredients in nematode management. This would add to the two existing methodologies for the application of the phytonematicides, which would further help to broaden the accessibility of the phytonematicides.

1.1.5 General focus of the study

This study would provide both smallholder and commercial farmers with a suitable pre-emergent application technology of Nemarioc-AL and Nemafric-BL phytonematicides, which would effectively suppress nematode without requiring high inputs into the growing medium. This research would lead to an improvement in the application technologies of phytonematicides in pea production system in South Africa, with the use of cucurbitacin-containing phytonematicides as an alternative to synthetic nematicides, which are environmentally unfriendly for management of *Meloidogyne* species. The aim of this research study is to develop a pre-emergence application method of Nemarioc-AL and Nemafric- BL phytonematicides, which would require low inputs that can be affordable to marginal farming communities in Limpopo Province, South Africa, while being able to suppress *M. incognita* and stimulate pea growth.

1.2 Problem statement

Generally, in phytonematicide systems, the major challenge had been the application technologies for the products. The methods used in application of phytonematicides are limited to the ground leaching technology and botinemagation systems, which have advantages and disadvantages. The application of phytonematicides through the GLT system and BNT require high inputs that could be costly for marginal farming communities in South Africa. Recently, potato tubers were used as carriers of active ingredients of the cucurbitacin-containing phytonematicides, but the potato industry indicated that the technology was not suitable for use in potato husbandry since priming technology resulted in loss of quality in the tubers after harvest (Pofu, 2018: Personal communication). Pea seeds have hypogenous germination characteristics, which imply that during emergence they do not bring the cotyledons above the soil surface (Parolin *et al.,* 2003). Therefore, pea seeds primed in solutions of cucurbitacin-containing phytonematicides could serve as carriers of cucurbitacin active ingredients for the management of nematode population densities.

1.3 Rationale of the study

The majority of marginal farming communities in South Africa barely afford the use of conventional organic amendments and Indigenous Cucurbitaceae Technologies (ICT) in suppression of root-knot nematodes (Mashela *et al.,* 2011). The application of phytonematicides through the GLT system and Botinemagation requires high inputs that could be costly for marginal farming communities in South Africa (Mashela *et al.,* 2011). Therefore, priming seeds with hypogenous germination properties in phytonematicide solutions could serve as an alternative method of the application of phytonematicides. Generally, in plant-parasitic nematodes, plant damage is directly

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proportional to nematode numbers in the rhizosphere of the germinating seeds (Seinhorst, 1967).

1.4 Purpose of the study

1.4.1 Aim

Development of use-friendly and cost-effective technology for placing cucurbitacin-containing phytonematicides in the rhizosphere of seedlings.

1.4.2 Objectives

1. To investigate whether priming pea seeds in Nemarioc-AL and Nemafric-BL phytonematicide solutions under laboratory conditions would provide suitable sensitivity and overall sensitivity for pea plants.

2. To determine whether pea seed priming in Nemarioc-AL and Nemafric-BL phytonematicide solutions under greenhouse and micro-plot conditions would provide MCSP for pea-inoculated with *M. incognita*.

1.4.3 Null hypotheses

1. Priming pea seeds in Nemarioc-AL and Nemafric-BL phytonematicide solutions under laboratory conditions would not provide suitable sensitivity and overall sensitivity for pea plants.

2. Pea seed priming in Nemarioc-AL and Nemafric-BL phytonematicide solutions under greenhouse and micro-plot conditions would not provide MCSP for pea-inoculated with *M. incognita*.

1.5 Reliability, validity and objectivity

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

1.6 Bias

Bias was minimised by making sure that the experimental error in each experiment was reduced through adequate replications. Treatments were also assigned randomly within the selected research designs to avoid bias (Leedy and Ormrod, 2005).

1.7 Scientific significance of the study

The application of phytonematicides using seeds as carriers would add to the two existing methodologies for the application of the phytonematicides. This would help to broaden the accessibility of the phytonematicides. Furthermore, this would also reduce the phytonematicides in-puts, which could be affordable to marginal farming communities to suppress root-knot nematodes thereby stimulating plant growth in agricultural crops.

1.8 Structure of the mini-dissertation

Following the description and detailed outlining of the research problem (Chapter 1), the work done, and work not done on the research problem were outlined as Literature Review (Chapter 2). Then, the three objectives of the study each constituted a

research chapter, with the final chapter (Chapter 5), summarising the findings and their

significance, potential future recommendations, along with the conclusions.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In use of phytonematicides as alternatives to synthetic chemical nematicides, the major challenge is primarily the fit-for-purpose and user-friendly application technology. Mashela (2002) developed the ground leaching technology (GLT) system, which is successful in the application of cucurbitacin-containing phytonematicides. However, the main disadvantage of GLT system had been cited as its cost since it is labour-intensive (Mashela *et al.*, 2011). In this system, the product is applied around the seedling during transplanting, with the active being released into the rhizosphere through irrigation water. The other placement system of phytonematicides is the botinemagation system, where plant materials have to be dried and ground prior to subjection to fermentation in air-tight containers (Mashela *et al.,* 2011). In this system, the major disadvantage had been cited as the cost for the requisite equipment (Mashela *et al.*, 2011). The placement technologies for cucurbitacin-containing phytonematicides are currently limiting the widespread application of the phytonematicides.

2.2 Work done in the research problem statement

2.2.1 Sensitivity of germinating primed seeds on phytonematicides

Nemarioc-A and Nemafric-B phytonematicides are the two phytonematicides being developed and researched as alternatives to the withdrawn methyl bromide in South Africa under research niche called ICT (Mashela *et al.*, 2017). These phytonematicides are produced from two *Cucumis* species, namely *C. myriocarpus* and *C. africanus*,

and are available in liquid formulation as Nemarioc-AL and Nemafric-BL phytonematicides (Pelinganga *et al.,* 2012) and in granular formulation as Nemarioc-AG and Nemafric-BG phytonematicides (Mashela *et al.,* 2011). Liu *et al.* (2003) indicated that phytonematicide derived from crude extracts of *C. myriocarpus* fruit may stimulate and/or inhibit germination on various crops. In recent studies, germination and seedling emergence of monocotyledonous and dicotyledonous crops against Nemarioc-AG phytonematicide had negative quadratic relationships, which implied that the concentration of the product was already beyond the saturation point for germination and seedling emergence (Mafeo and Mashela, 2009; 2010).

Researchers at the University of Limpopo adopted the use of computer-based Curve-fitting Allelochemical Response Dose (CARD) model (Liu *et al.*, 2003), to generate mean concentration stimulation point (MCSP), which is the suitable phytonematicide concentration that is not phytotoxic to crops protected against nematodes (Mashela *et al.*, 2015). The CARD model quantifies the three DDG patterns using biological indices (Liu *et al.*, 2003). The biological indices include threshold stimulation (D_m), saturation point (R_h), 0% inhibition (D_0), 50% inhibition (D_{50}) and 100% inhibition (D_{100}). In the development of MCSP, two biological indices, D_m and R_h, are used through the relation: MCSP = D_m + (R_h/2) (Liu *et al.*, 2003). Along with the biological indices, the CARD model also provides the sensitivity index (k), which provides information about the sensitivity of the crop towards the product being used to protect it against nematode attack. Generally, the lower the k value, the higher is the sensitivity of the plant to the product and vice-verse (Liu *et al.*, 2003). Other researchers reported the k values which ranged from 0 to 1 (Pelinganga *et al.*, 2012) and 0 to 2 (Tseke *et al.*, 2013) for tomato plants when exposed to Nemarioc-AL phytonematicide,

2.2.2 Phytonematicides on inoculated plants

In several studies, exposure of plants to an increasing concentration of cucurbitacin-containing phytonematicide has been reported to have stimulatory effect on growth of various vegetable crops when applied as pre-emergent treatment (Mashela, 2007; Mashela *et al.,* 2011, 2015, 2017). Liu *et al.* (2003) observed that various types of allelochemicals in different plant species promote plant growth when applied at low quantities. Nemarioc-AL and Nemafric-BL phytonematicides had stimulatory effects on various plant growth when applied at low concentrations, while at high concentrations they inhibited plant growth (Mafeo and Mashela, 2010). According to Mafeo *et al.* (2011) exposure of maize (*Zea mays* L.), millet (*Eleusine coracana* L.), sorghum (*Sorghum bicolor* L.), chive (*Allium schoenoprasum* L.), leek (*Allium porrum* L.) and onion (*Allium cepa* L.) to increasing concentrations of Nemarioc-AG phytonematicide stimulated plant growth when used as pre-emergent phytonematicide. Similar findings were also observed on tomatoes (*Solanum lycopersicum* L.) (Mashela *et al.*, 2011) when exposed to several concentrations of phytonematicides. Furthermore, the crude extracts of *C. myriocarpus* fruits stimulated nodulation in cowpea crops of *Bradyrhizobium japonicum* K. (Shakwane *et al.*, 2004). However, the use of phytonematicides is often limited by the phytotoxicity to the protected plants (Mashela *et al.,* 2015). According to Okwute (2012), phytonematicides contain allelochemicals that are naturally phytotoxic to several plant species during interference interactions and this allelochemicals can lead to germination inhibition, suppression of seedling growth and increased seedling mortalities.

2.2.3 Phytonematicides on inoculated soil

The application of phytonematicide to *M. incognita* infested soil reduced the number of nematodes in tomato roots by 79-92 % thereby increasing plant height, stem diameter fruit yield and dry shot mass of tomato plants (Mashela *et al.,* 2010). According to Chokoe (2017), Nemarioc-AL phytonematicide reduced final *M. javanica* population densities on green beans by 40-71%. Sithole *et al.* (2016) confirmed that the increasing concentration levels of Nemarioc-AL phytonematicide on geranium plants reduced J2 in roots by 81% and J2 in soil by 100% of *M. javanica*. Dube (2016) reported that the two phytonematicides significantly affected distribution of population densities of *M. incognita* across the tested soil types, with Nemafric-BL phytonematicide reducing population densities of *M. incognita* relative to Nemarioc-AL phytonematicide. Furthermore, Maile (2013) reported that crude extracts of *C. myriocarpus* fruit reduced nematode populations by 22% in roots relative to untreated control. Tseke and Mashela (2017) reported that the increasing concentrations of Nemafric-BL phytonematicide reduced eggs in roots, J2 in roots and J2 in soil of *Meloidogyne* species by 67, 80 and 80%, respectively. Similar trends were observed on Pf in roots and soil, after exposing sweet stem sorghum var. ndendane-X1 to increasing concentrations of Nemafric-BG phytonematicide for management of *Meloidogyne* species under field conditions, where Pf in roots and soil were reduced by 77-85% and 24-65%, respectively (Mabuka, 2015). Nemafric-BL phytonematicide reduced J2 in roots by as high as 70 and 96% in Experiment 1 and 2, respectively (Sithole, 2016). Dube (2016) reported that the two phytonematicides significantly affected distribution of population densities of *M. incognita* across the tested soil types, with Nemafric-BL phytonematicide reducing population densities of *M. incognita* relative to Nemarioc-AL phytonematicide.

2.3 Work not yet done on the problem statement

Seeds with hypogenous germination characteristics, where the cotyledons are left in the soil during emergence, could be ideal candidates for serving as carriers of active ingredients of phytonematicides. Due to the phytotoxicity of cucurbitacin-containing phytonematicides, it is imperative that empirically-based trials be conducted to investigate the feasibility of priming technology.

2.4 Addressing the identical gaps

Normally, the biological systems react to both intrinsic and extrinsic factors in correspondence to the density-dependent growth (DDG) pattern, which is characterised by stimulation, saturation of growth and inhibition phases (Mashela *et al.,* 2015). In order to positively examine whether both Nemarioc-AL and Nemafric-BL phytonematicides could be used as pre-emergent bio-nematicides in seed priming technology, a series of trials need to be carried out to establish suitable concentrations for both phytonematicides on various crops with hypogenous germination characteristics in relation to the responses of the DDG pattern. Furthermore, to determine the suitable application concentrations for Nemarioc-AL and Nemafric-BL phytonematicides, one need to first establish the normal concentration used in priming technology in relation to the density dependent growth responses (Salisbury and Ross, 1992), which then need the use of CARD model (Liu *et al.,* 2003).

CHAPTER 3

EFFECT OF NEMARIOC-AL AND NEMAFRIC-BL PHYTONEMATICIDES ON *IN VITRO* GERMINATION OF PRIMED PEA SEEDS

3.1 Introduction

Seed germination is defined as a chemical process that takes place within the seed, starting from imbibition of water and ending the rapture of the seed coat by the radicle (Bewley, 1997; Campbell, 1990). After the seed has imbibed water, the embryo releases gibberellic acid (GA), which moves through the endosperm to the aleurone layer, which is triggered to synthesis and secrete alpha-amylase and other hydrolytic enzymes that digest the starch and other products in the endosperm. The produced soluble food is absorbed by the embryonic leaves – the cotyledons (Starr and Taggart, 1987). Following absorption, the embryo begins to develop and grow, ending with the radicle emerging through the seed coat, with the entire process from imbibition to the rapture of the seed coat being referred to as germination. Generally, germination is a chemical process as opposed to emergence which is a physical process.

The movement of GA is under the Brownian principles, where chemicals move through diffusion from higher concentration in the embryo to lower concentration in the aleurone layer (Campbell, 1990). Any factor that can counter the arrival of GA at the aleurone layer or prevent the synthesis and secretion of alpha-amylase and other hydrolytic enzymes or the subsequent digestion of starch and related products in the endosperm or absorption of digested materials by the radicle, would inhibit seed germination (Campbell, 1990). Some of the hydrolysed products are fats, which are hydrolysed into lipids, which are fats in liquid phase.

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The cucurbitacin-containing phytonematicides are being researched and developed for use as alternative for managing population densities of plant-parasitic nematodes (Mashela *et al.*, 2017). These products have cucurbitacins as active ingredients. Investigations are currently under way to assess user-friendly and cost-effective methods of applying the active ingredients through seed treatment, especially those seeds where the entire seed remains below soil-surface during seedling emergence. Such plants are technically referred as to having hypogeal germination (Parolin *et al.,* 2003). Most seeds contain fats and during hydrolysis most become lipids. Also, membranes around organs of seeds such as embryo consist of layers that contain lipids (Campbell, 1990). Cucurbitacins are known to be lipophilic (Bartalis and Halaweish, 2005) and could therefore interfere with germination. Mafeo (2012) demonstrated that Nemarioc-AG phytonematicide significantly reduced seedling emergence in both dicotyledonous and monocotyledonous plants. However, the influence of cucurbitacin-containing phytonematicides on seed germination had not been documented. The objective of this study was to investigate whether in vitro priming of pea (*Pisum sativum* L.) seeds, with hypogeal germination attributes, in solutions of Nemarioc-AL and Nemafric-BL phytonematicides, would provide suitable sensitivity and overall sensitivity for pea plants. The null hypothesis of this study was priming pea seeds in Nemarioc-AL and Nemafric-BL phytonematicide solutions under laboratory conditions would not provide suitable sensitivity and overall sensitivity for pea plants.

3.2 Materials and methods

3.2.1 Study site, treatments and research design

Two separate *in vitro* experiments were conducted in the laboratory at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, South Africa (23°53′10″S, 29°44′15″E) during autumn (February-April) 2018. The seven treatments, namely, 0, 2, 4, 8, 16, 32 and 64% Nemarioc-AL or Nemafric-BL phytonematicides, were arranged in completely randomised design, with eight replications. Each experiment was repeated once.

3.2.3 Procedures

Fruits of *C. myriocarpus* and *C. africanus* were harvested from the field-grown plants, washed, cut into pieces, oven-dried at 52°C and ground in a Wiley Mill (Mafeo and Mashela, 2009). Approximately 80 g ground fruit of *C. myriocarpus* and 40 g ground fruit of *C. africanus* were fermented using effective micro-organisms (EM) in 16 L chlorine-free tapwater in 20 L container for 14 days at room temperature (Pelinganga, 2013) to produce Nemarioc-AL and Nemafric-BL phytonematicides, respectively. The EM culture comprised South African strains of yeast, lactic acid bacteria, photosynthetic bacteria, actinomycete bacteria and minor strains of fungi (Higa and Parr, 1994). During fermentation process, carbon dioxide was allowed to escape from the container through an airtight 5-mm-diameter tube, with one end glued to a hole on the lid of the 20 L container, with the outlet dangling into a 2-L bottle half-filled with chlorine-free tapwater.

Pea seeds were primed in treatment dilutions for 2 h and shade-dried for 8 h before germination tests in Petri-dishes. Treated seeds were placed on top of a filter paper in

sterilised Petri-dishes with lids. An appropriate amount of distilled water was added to completely moisten the paper without soaking it. Ten seeds were, therefore, spread uniformly on the moistened filter paper, ensuring that none of the seeds touched each other. The Petri-dishes were closed with lids and placed in a germination chamber at 25°C (Figure 3.1).

Figure 3.1. Effects of cucurbitacin-containing phytonematicides on germination of peas cv. 'Green Feast' under *in vitro* conditions.

3.2.4 Data collection

Successful germinated seeds were recorded and removed every day until 100% germination. Successful seed germination was observed as seed coat ruptured by the radicle. Radicles and plumules were each measured on day 7 after 100% germination.

3.2.5 Data analysis

The geometric series was transformed into an exponential series 2^0, 2^1, 2^2, 2^3, 2^4, 2^5 and 2^6 prior to log-transformation (Mashela *et al*., 2020). Using $\log_2 2^x = x \times (\log_2 2) = x \times (1) = x$, the log-transformed series became 0, 1, 2, 3, 4, 5 and 6%. Data were

subjected to the Curve-fitting Allelochemical Response Dose (CARD) algorithm model to generate curves and biological indices (Liu *et al.,* 2003), with those of interest being the first, namely, the stimulation threshold point (D_m) and the last, the saturation point (R_h), within the stimulation phase. The two biological indices, D_m and R_h, are used for computation of the Mean Concentration Stimulation Point [MCSP = D_m + ($R_h/2$)] (Mashela *et al.,* 2017). The MCSP, is technically, referred to as the first law of phytonematicides since at this concentration, phytonematicides do not induce phytotoxicity.

3.3 Results

Germination percentage (Figure 3.2), radicle (Figure 3.3) and plumule (Figure 3.4) over increasing concentration of Nemarioc-AL phytonematicide each exhibited negative quadratic relation, with the models explained by 90, 79 and 97% associations, respectively (Figure 3.2 to Figure 3.4). In contrast, germination percentage (Figure 3.5), radicle (Figure 3.6) and plumule (Figure 3.7) over increasing Nemafric-BL phytonematicide each exhibited positive quadratic relations, with the model explained by 99, 95 and 82% associations, respectively (Figure 3.5 to Figure 3.7). Biological indices for germination percentage, radicle and plumule were generated using the CARD model, with the model explained each sensitivity by 0, 14 and 20 respectively, and the overall sensitivities of 34 against Nemafric-BL phytonematicide (Table 3.1). The MCSP value of Nemafric-BL phytonematicide on germination of pea seeds was 0.17 % (Table 3.1).
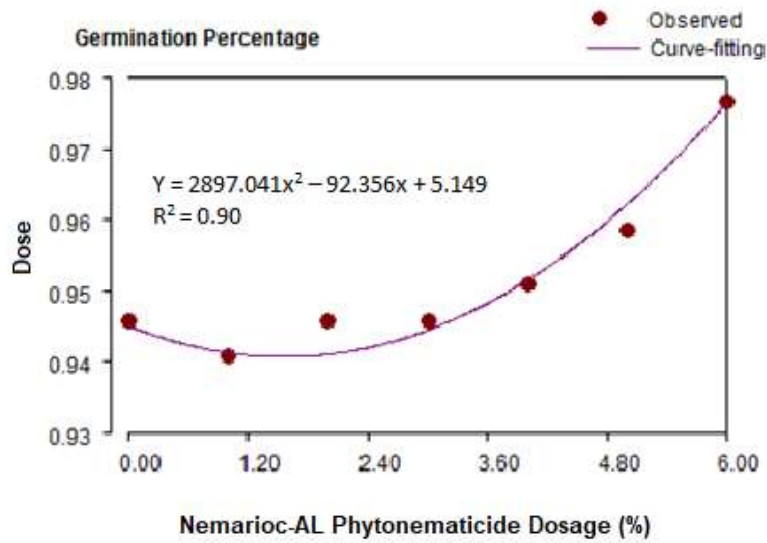
Figure 3.2. Quadratic relationship between germination of peas and Nemarioc-AL phytonematicide at 5 days after treatment.
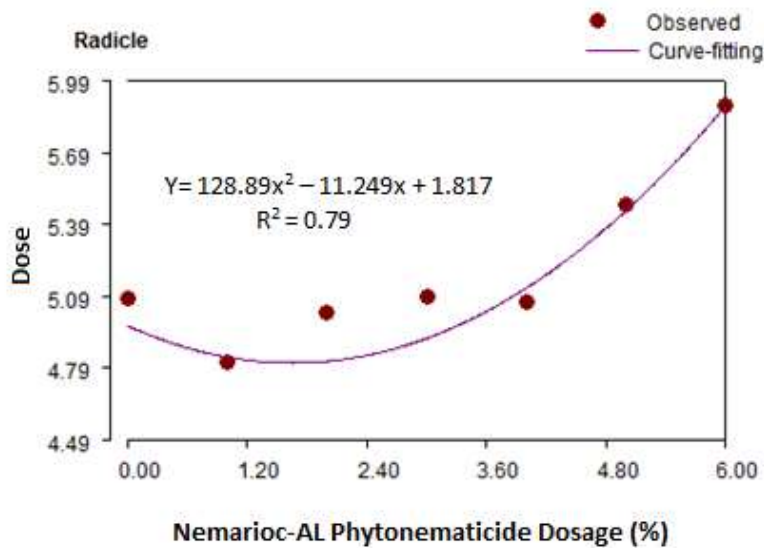
Figure 3.3. Quadratic relationship between radicle of pea seeds and Nemarioc-AL phytonematicide at 5 days after treatment.

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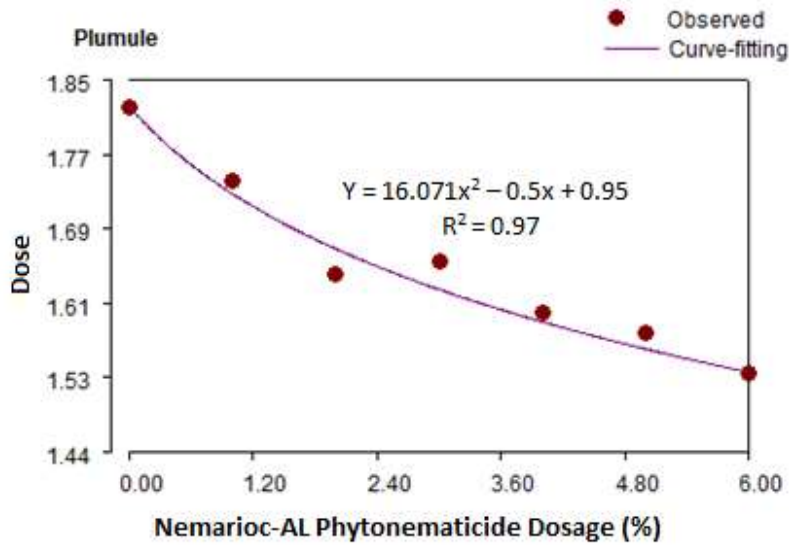
Figure 3.4. Quadratic relationship between plumule of peas and Nemarioc-AL phytonematicide at 5 days after treatment.
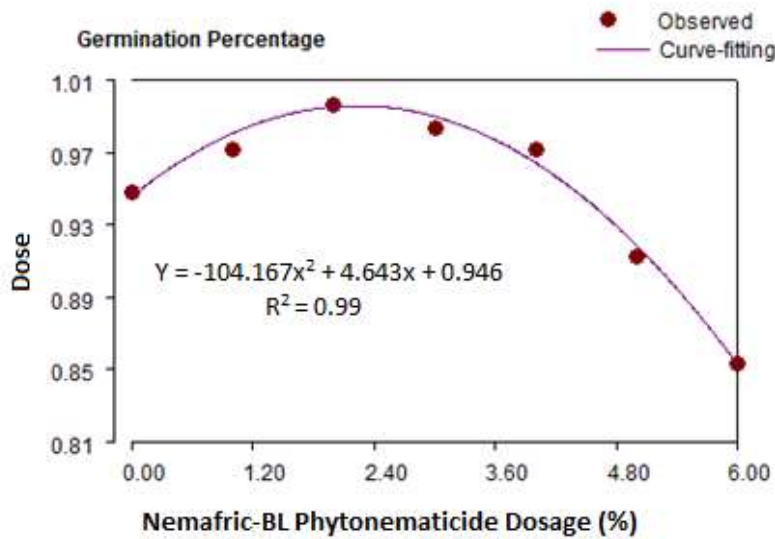
Figure 3.5. Quadratic relationship between germination percentage of peas and Nemafric-BL phytonematicide at 5 days after treatment.
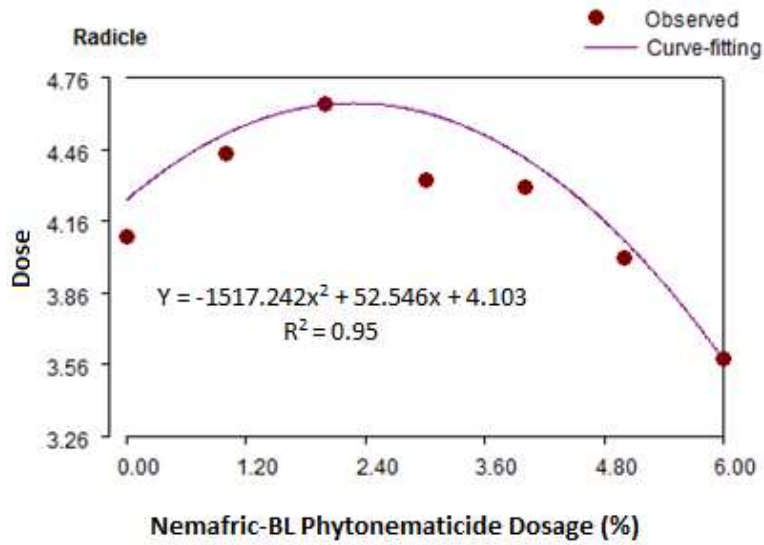
Figure 3.6. Quadratic relationship between radicle of peas and Nemafric-BL phytonematicide at 5 days after treatment.
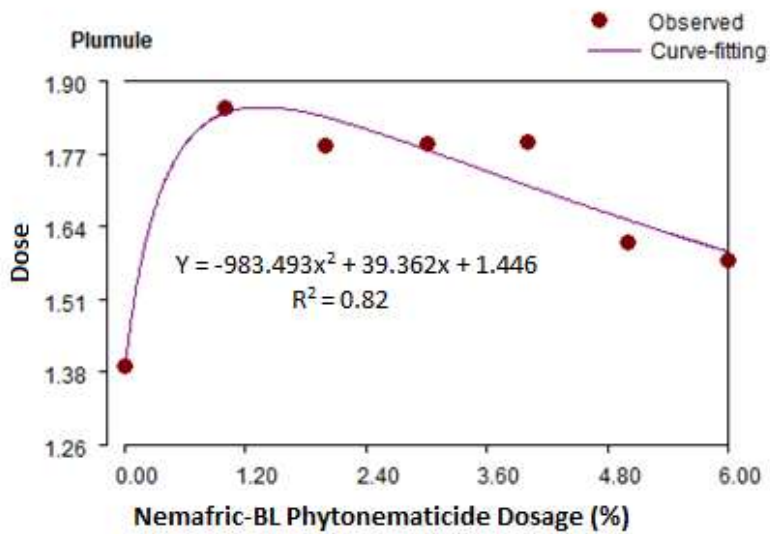
Figure 3.7. Quadratic relationship between plumule of peas and Nemafric-BL phytonematicide at 5 days after treatment.

Table 3.1: Biological indices for germination percentage, radicle and plumule of pea in response to increasing concentrations of Nemafric-BL phytonematicide.

Biological index[Z]	% Germination	Radicle	Plumule	Mean
Threshold stimulation (D_m)	0.022	0.02	0.025	0.022
Saturation point (R_h)	0.052	0.455	0.394	0.300
0% inhibition (D_0)	0.045	0.046	0.066	0.052
50% inhibition (D_{50})	0.093	0.097	0.114	0.101
100% inhibition (D_{100})	0.1	0.1	0.2	0.13
R^2	0.99	0.95	0.82	
Sensitive index (k)	0	14	20	
Overall sensitivity ($\sum k$)	34			
MCSP = D_m + ($R_h/2$)	0.17%			

3.4 Discussion

Seed germination versus Nemarioc-AL phytonematicide exhibited negative quadratic relationships, which could not be used to generate MSCP (Mashela *et al.,* 2017). In other words, the concentration of Nemarioc-AL phytonematicide used in the current study were already in the inhibition phase for use in priming pea seeds. Previously, it was shown that germination of seeds in various crops was highly sensitive to Nemarioc-AG phytonematicide, the granular counter part of Nemarioc-AL phytonematicide (Mafeo, 2012; Mafeo and Mashela, 2009; 2010; Mafeo *et al.,* 2011). Further studies would be necessary to establish the mechanism involved in inhibition of seed germination by the test product in seeds, which would suggest a general phenomenon.

The active ingredient in Nemarioc-AL phytonematicide is cucurbitacin A ($C_{32}H_{46}O_8$) and it is soluble in water since it is partially polar (Mashela *et al.,* 2017). Generally, cucurbitacin A is unstable it quickly breaks into cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) chemical compounds, which have strong bioactivities in living entities (Chen *et al.,* 2005). Other plant-based chemicals that inhibit germination include terpenoids and flavonoids phenolic chemical compounds (Marcias *et al.,* 2002). Flavonoids and terpenoids are hydroxylated phenolic compounds, which are contained in Compositae Family and inhabit a superior role amongst secondary metabolites (Sülsen *et al.,* 2017). These hydroxylated phenolic compounds have been reported to have defensive effects over numerous infectious diseases such as cancer (Kumar and Pandey, 2013; Mamadalieva *et al.*, 2011; Sülsen *et al.*, 2017)

The majority of plants in the family Cucurbitaceae, especially under the genus *Cucumis*, contain allelochemicals called cucurbitacins, which exhibit strong allelopathy attributes (Chen *et al.,* 2005), with powerful inhibition of germination (Martin and Blackburn, 2003). However, Maila (2015) showed that seed dormancy in wild *Cucumis* species was hardly due to allelochemicals, but Maila (2015) also failed to detect the existence of any dormancy due to hard seed coat since there were definitive channels for improved imbibition and radicle breakdown of seeds. However, Mafeo and Mashela (2009) suggested that seed dormancy in *C. myriocarpus* could primarily be broken by exposing seeds to high temperatures prior to sowing.

Although in the current study the mechanisms behind the inhibition of Nemarioc-AL phytonematicide on germination was not investigated, literature is replete with possible mechanisms involved from other plant extracts. Mazloom *et al.* (2009) demonstrated

that alkaloids from winter wheat (*Triticum aestivum* L.) grasses delayed the metabolism of stored food in the endosperm of germinating thorn-apple seeds (*Datura stramonium* L.). In linseed (*Linum utatissimum* L.), the radicle protrusion and elongation were inhibited by an allelochemical called benyl-amine, which is produced from leaf washings of camelina weed (Lovett *et al.,* 1989). Others (Einhellig, 1985; Inderjit and Duke, 2003; Jeronimo *et al.,* 2005; Martin and Blackburn, 2003) indicated that allelopathic chemicals from various plants prohibited cell division of embryos, although in other cases allelopathy inhibited hydrolytic enzyme activities or GA. Einhellig (1985) also reported that the primary action of ATP production in white mustard (*Sinapis alba* L.) germinating seeds was inhibited by juglone and sorgoleone which are powerful allelochemicals from crude extracts of black walnut (*Junglans nigra* L.) leaves, thereby affecting mitochondrial functions as well as preventing chloroplast-oxygen evolution in the cotyledons. Furthermore, extracts of *Raphanus sativus* L. prevented germination and rhizome growth of *Sorghum halepense* L. seeds, and inhibited growth of okra, carrot, corn and tomato (Kadioglu and Yanar, 2004). The residues of *Melia azederach* L. and *Nerium oleander* L. was reported to stimulated growth of maize (Uygur and Iskenderoglu, 1995). In some other studies, it was reported that crude extracts of *Chenopodium album* and *Amaranthus retroflexus* promoted growth of *Zea mays* and *Lepinus albus* (Uygur *et al.,* 1991).

Priming pea seeds in solutions of Nemafric-BL phytonematicide had opposite effects to those of Nemarioc-AL phytonematicide. The positive quadratic relationship suggested that the concentration of Nemafric-BL phytonematicide used in this study was within the stimulation phase to the saturation phase, thereby allowing the generation of biological indices required to generate the non-phytotoxic concentration,

technically referred to as Mean Concentration Stimulation Point (MCSP) (Mashela *et al.*, 2017). The generated MCSP of 0.17%, implies that 17 L (or ml) Nemafric-BL phytonematicide per 100 L (or ml) water would be suitable for priming peas seeds without inducing phytotoxicity. Furthermore, in the current study, the overall sensitivity of seed germination was 34 units, which implied that pea seeds were highly tolerant to Nemafric-BL phytonematicides. Generally, most seedlings have high overall sensitivity values to cucurbitacin-containing phytonematicides, as observed in various seedlings subjected to Nemarioc-AL phytonematicide (Mafeo *et al.*, 2011)

3.5 Conclusion

The results obtained from this study suggest that the quantities of Nemarioc-AL phytonematicide were not well-suited with germination and emergence of peas. However, due to the observed density-dependent relationships, there could be dosages of Nemafric-BL phytonematicide that could stimulate germination of pea seeds. The CARD model demonstrated that the response of peas when exposed onto a series of cucurbitacin-containing phytonematicides exhibited the density-dependent growth patterns, which are characterised by stimulation, saturation and inhibition. Using the integration of the responses of germination and emergence to Nemafric-BL phytonematicide within the stimulation range, the quantity of the material which could be used as pre-emergent seed treatment was estimated as 0.17 % material/ seed. Pea seeds priming in Nemarioc-AL phytonematicide solutions in this study did not provide suitable sensitivity and overall sensitivity for pea plants, which lead to the acceptation of the null hypothesis. In contrast, priming pea seeds in Nemafric-BL phytonematicide solutions under laboratory conditions provided suitable sensitivity and overall sensitivity for pea plants, which rejected the null hypothesis.

CHAPTER 4

RESPONSE OF PEA GROWTH, ACCUMULATION OF NUTRIENT ELEMENTS AND NEMATODE POPULATIONS TO PRIMING OF SEEDS IN PHYTONEMATICIDES

4.1 Introduction

Cucurbitacin-containing phytonematicides as granular formulations are applied using the ground leaching technology (GLT) system, whereas in liquid formulation the products are applied using botinemagation system (Mashela *et al*., 2017). Moreover, Maila (2015) demonstrated that seed dormancy in wild *Cucumis* species, from whose fruits cucurbitacin-containing phytonematicides are made, were not due to the cucurbitacins, but physical structures in the testa. In both purified and extract forms, various studies demonstrated that the products were highly effective in suppressing nematode population densities (Dube, 2016), with bioactivities that included inhibition of juvenile hatch and mobility, whereas the products improved juvenile motility. Also, it was recently (Mashela *et al*., 2020) shown that the products induced structural changes in morphometries of various nematodes and that the products were highly destructive to total proteins in cuticles of plant nematodes. Generally, second-stage juveniles (J2) could spend as much as 12 weeks in soil solutions searching for suitable penetration sites (Adam *et al.,* 2014). Consequently, seed dressing with cucurbitacin-containing phytonematicides could be highly suitable for use as a technology intended for managing nematode population densities.

In plant nematodes, the magnitude of the initial nematode population densities (Pi) is important in determining final crop loss (Seinhorst, 1965). Generally, during

emergence, seeds with hypogenous germination leave cotyledons below soil surface and such seeds are suitable for using the seed dressing technology. In an *in vitro* study (Chapter 3), it was shown that the cotyledons in seeds with hypogenous germination under laboratory conditions could absorb the cucurbitacins, as active ingredients of phytonematicides. Hypogenous germination is a case where cotyledons remain below soil surface, where the primed seeds could release cucurbitacins into the rhizosphere to manage nematodes. The objective of this study was, therefore, to investigate whether dressing pea seed in Nemarioc-AL and Nemafric-BL phytonematicide solutions under greenhouse and microplot conditions would provide MCSP for pea-inoculated with *M. incognita*. The null hypothesis of the study was pea seed priming in Nemarioc-AL and Nemafric-BL phytonematicide solutions under greenhouse and microplot conditions would not provide MCSP for pea-inoculated with *M. incognita*.

4.2 Materials and methods

4.2.1 Description of the study area

The study was conducted under greenhouse and micro-plot conditions at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, Limpopo Province, South Africa (23°53′10″S, 29°44′15″E). The trials were conducted during autumn (February-April) in 2018 and validated in autumn 2019.

Greenhouse conditions: Two separate trials, one for Nemarioc-AL phytonematicide (Trial 1: Experiment 1) and the other for Nemafric-BL phytonematicide (Trial 2: Experiment 1) were conducted in inside the greenhouse during autumn (February-April) in 2018 and repeated in autumn 2019. Due to the size of the greenhouse (100 m × 20 m), along with the wind that was created by the heat-extracting fans, conditions

inside the facility were heterogeneous. The greenhouse had an ambient day/night temperatures averaged 28/18°C, with high temperatures controlled using thermostatically-activated fans on one end and a wet wall on the opposite end, with relative humidity of 70-75%.

Figure 4.1. Effects of cucurbitacin-containing phytonematicides on growth of peas cv. 'Green Feast' under greenhouse conditions.

Micro-plot conditions: Two separate experiments were conducted at a location that receives a summer rainfall with mean annual rainfall of 500 mm. The experiments were established by inserting 30-cm-diameter plastic pots into 20-cm-deep holes at an intra-row spacing of 0.3 m and an inter-row spacing of 0.6 m, at a location that has hot dry summers, with daily maximum temperature (DMT) ranges of 28–38 °C.

Figure 4.2. Effects of cucurbitacin-containing phytonematicides on growth of peas cv. 'Green Feast' under micro-plot conditions.

4.2.2 Treatments and research design

Experiments for Nemarioc-AL phytonematicide (Experiment 1) and Namafric-BL phytonematicide (Experiment 2) were first conducted under greenhouse conditions and then validated. Similar experiments were conducted under micro-plot conditions. At each site, treatments, namely, 0, 2, 4, 8, 16, 32 and 64% concentration of each phytonematicide, were arranged in a randomised complete block design (RCBD), with 6 replications under greenhouse and 8 replications under micro-plot conditions.

4.3.3 Procedures

Nemarioc-AL and Nemafric-BL phytonematicides were prepared as described previously (Mashela *et al.,* 2017). Nematode inocula were prepared by extracting eggs and J2 of *M. incognita* race 2 from roots of the greenhouse-grown nematode-susceptible tomato cv. 'Floradade' in 1% NaOCl solution (Hussey and Barker, 1973).

Twenty-cm-diameter plastic pots, at 0.3 m × 0.25 m spacing, under greenhouse conditions, and 30-cm-diameter plastic pots at 0.6 × 0.3 m spacing under micro-plot conditions, were each filled with steam-pasteurised (300°C for 1 h) loamy soil.

Pea seeds were primed in 0, 2, 4, 8, 16, 32 and 64% solutions of each phytonematicide for 2 h and shade-dried for 8 h before sowing. Two treated seeds were sown, with seedlings thinned into one plant per pot at two-leaf stage. Each plant was irrigated every other day with 250 ml chlorine-free tapwater under greenhouse conditions and 500 ml chlorine-free tapwater under micro-plot conditions. At five leaf-stage, each seedling was inoculated with 5 000 eggs + J2 of *M. incognita*. At inoculation, plants were fertilised with 5 g N:P:K 2:3:2 (22) per plant, which provided a total of 155 mg N, 105 mg P and 130 mg K per ml of water and 1 g N:P:K 2:1:2 (43) per plant to provide a total of 0.175 mg N, 0.16 mg K and 0.16 mg P, 0.45 mg, 0.378 mg Fe, 0.0375 mg Cu, 0.175 mg Zn, 0.5 mg B, 1.5 mg Mn and 0.035 mg Mo per ml chlorine-free tapwater (Mashela, 2002). Scouting for whiteflies was done on a daily basis with plants sprayed with recommended chemical when population densities detected go beyond 10 insects per plant.

4.2.4 Data collection

At 56 days after initiating the treatments, plant height was measured from the soil surface to the tip of the flag leaf and recorded. Stems were severed at the soil surface and the stem diameter measured at 5 cm above the severed end using a digital Vernier Calliper. Shoots were dried in air-forced ovens at 52°C for 72 h for dry shoot mass. For nutrient analysis, mature pea leaves were selected and ground using grading machine prior to digestion, and approximately 0.4 g ground leaf material was digested

in 75 ml vessel with 3 ml 30 % hydrogen peroxide (H_2O_2) and 5 ml 70 % nitric acid (HNO_3). The mixture was vortexed, and samples were allowed to cool down for at least 10 minutes prior to closing the vessel, which were then inserted into the microwave digester (PerkinElmer, Tatan MPS) to run for 46 minutes under temperatures ranging up to 260°C. Thereafter, the vessels were allowed to cool at room temperature for 20 minutes. The samples from the vessels were decanted into 50 ml tubes and stored in the cold room to avoid evaporation of samples prior to analytical work. Pea leaf samples were then analysed for K, Fe, Zn and Na using inductively coupled plasma spectrometry (Shimadzu, ICPE-9000).

Root systems were removed from pots, immersed in water to remove soil particles, blotted dry and weighed to facilitate the calculation of nematode density/total roots/plant. Root galls were assessed using the North Carolina Differential Rating Scale of 1 = 0 galls, 2 = 1-10 galls, 3 = 11-31 galls, 4 = 32-100 galls, 5 = >100 galls (Taylor and Sasser, 1978). Nematodes (eggs + juveniles) were extracted from total root system/plant by maceration and blending for 30 s in 1% NaOCl solution (Hussey and Barker, 1973). The material was passed through nested 75- and 25-μm-opening sieves. Contents of the 25-μm-opening-sieve were collected for separation of nematodes from debris using the sugar-flotation and centrifugation method (Jenkins, 1964). Soil in each pot was mixed and a 250 ml soil sample collected for nematode extraction using the sugar centrifugation and flotation method (Jenkins, 1964). Eggs and juveniles from root and juveniles from soil were each counted using a stereomicroscope and converted to total root system per plant and total soil per pot, respectively. Root and soil nematodes from samples were converted to final nematode population densities (Pf) in root and soil.

4.2.5 Data analysis

Concentration data were log-transformed to improve normality (Chapter 3). Plant variables were subjected to the Curve-fitting Allelochemical Response Data (CARD) model to generate appropriate biological indices (Liu *et al.,* 2003). All variables that had positive quadratic relations with the concentration of phytonematicides, had biological indices, namely, threshold stimulation (D_m) and saturation point (R_h), used in computing the average for estimating the Mean Concentration Stimulation Point [$MCSP = D_m + (R_h/2)$] (Mashela *et al.,* 2017). The CARD model also provides sensitivity values (k) and overall sensitivity values ($\sum k$) for the variables (Liu *et al.,* 2003). Nutrient elements (K, Fe, Zn and Na) and nematode variables were assessed using the regression models, with data subjected to lines of the best fit. Unless stated otherwise, treatment effects were discussed at the probability level of 5%.

4.3 Results

Seasonal interactions were not significant at the probability level of 5% and data for Experiment 1 and Experiment 2 for each phytonematicide under greenhouse and microplot conditions were pooled and re-analysed.

4.3.1 Nemarioc-AL phytonematicide under greenhouse conditions

Plant variables: Plant height, stem diameter and chlorophyll content versus Nemarioc-AL phytonematicide exhibited positive quadratic relations (Figure 4.3), with the model being explained by 86, 93 and 85% associations, respectively (Table 4.1). However, flower number and dry shoot mass versus Nemarioc-AL phytonematicide exhibited negative quadratic relations (Figure 4.4). The MCSP for Nemarioc-AL phytonematicide on peas was 0.62% (Table 4.1). The k value for plant height, stem

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diameter and chlorophyll content were zero for each variable, with the $\Sigma k = 0$ (Table 4.1).

Plant Height

Observed: ● ; Curve-fitting: —

$Y = -14833.334x^2 + 213.586x + 69.063$

$R^2 = 0.86$

Y-axis: Dose (79.6, 65.4, 51.3, 37.1, 23.0, 8.9)

X-axis: Nemarioc-AL Phytonematicide Dosage (%) (0.00, 1.20, 2.40, 3.60, 4.80, 6.00)

Stem Diameter

Observed: ● ; Curve-fitting: —

$Y = -771.739x^2 + 18.316x + 3.213$

$R^2 = 0.93$

Y-axis: Dose (3.63, 3.07, 2.50, 1.93, 1.37, 0.80)

X-axis: Nemarioc-AL Phytonematicide Dosage (%) (0.00, 1.20, 2.40, 3.60, 4.80, 6.00)

Chlorophyll Content

Observed: ● ; Curve-fitting: —

$Y = -10611.188x^2 + 343.107x + 38.892$

$R^2 = 0.85$

Y-axis: Dose (41.9, 35.9, 29.9, 24.0, 18.0, 12.0)

X-axis: Nemarioc-AL Phytonematicide Dosage (%) (0.00, 1.20, 2.40, 3.60, 4.80, 6.00)

Figure 4.3. Response of plant height, stem diameter and chlorophyll content of peas to Nemarioc-AL phytonematicide at 56 days after initiation of treatments.
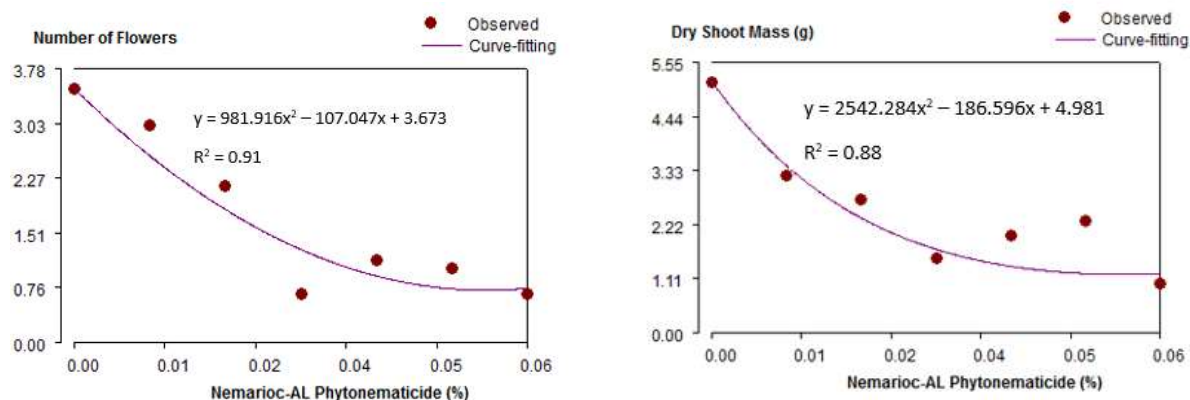
Figure 4.4. Responses of number of flowers and dry shoot mass of peas to increasing concentrations of Nemarioc-AL phytonematicide under greenhouse conditions at 56 days after initiation of treatments.

Table 4.1: Biological indices for plant height (PHT), stem diameter (SD) and chlorophyll content (CC) of pea cultivar 'Green Feast' exposed to increasing concentrations of Nemarioc-AL phytonematicide.

Biological indices	PHT	SD	CC	Mean
Threshold stimulation (D_m)	0.007	0.012	0.016	0.012
Saturation point (R_h)	0.769	0.109	2.774	1.217
0% inhibition(D_0)	0.014	0.024	0.032	0.023
50% inhibition (D_{50})	0.056	0.059	0.062	0.059
100% inhibition (D_{100})	0.1	0.1	0.1	0.1
R^2	0.86	0.93	0.85	
Sensitivity (k)	0	0	0	
Overall sensitivity ($\sum k$)	0			
MCSP = D_m + (R_h/2)	0.62 %			

Nutrient variables: Nemarioc-AL phytonematicide had significant effects on Na, Fe, K and Zn in leaf tissues of pea plants. The Na and K versus Nemarioc-AL phytonematicide exhibited positive quadratic relations, with the models explained by 78 and 87% associations, respectively (Figure 4.5). However, Fe and Zn versus Nemarioc-AL phytonematicide exhibited negative quadratic relations, with the models explained by 93 and 86% associations, respectively (Figure 4.6). When using the relation, $x = -b_1/2b_2$ (Gomez and Gomez, 1984), Na and K in leaf tissues of pea plants were optimised at 3.14 and 3.25% concentration of Nemarioc-AL phytonematicide, respectively (Table 4.2).

Table 4.2: Optimisation model of selected nutrient elements in leaf tissues of pea cultivar 'Green Feast' as affected by pre-emergent application concentrations of Nemarioc-AL phytonematicide

Element	Model	R^2	x (%)
	Experiment 1 (Nemarioc-AL Phytonematicide)		
Na	$Y = -2.2076x^2 + 13.847x + 106.99$	0.78	3.14
K	$Y = -2.4262x^2 + 15.776x + 125.42$	0.87	3.25

$x = -b_1/2b_2$.

Figure 4.5. Response of sodium (Na) and potassium (K) in leaf tissues of pea cv. 'Green Feast' to Nemarioc-AL phytonematicide at 56 days after initiation of treatments.

Figure 4.6. Response of iron (Fe) and zinc (Zn) in leaf tissues of pea cv. 'Green Feast' to Nemarioc-AL phytonematicide at 56 days after initiation of treatments.

Nematode variables: Gall ratings in roots, eggs in roots, J2 in roots and final nematode population of *M. incognita* versus Nemarioc-AL phytonematicide exhibited negative quadratic relations, with the models explained by 65, 71, 53 and 69%, respectively (Figure 4.7 - 4.8).

Figure 4.7. Response of gall ratings in roots and eggs in roots of *Meloidogyne incognita* race 2 to Nemarioc-AL phytonematicide at 56 days after initiation of treatments.

Figure 4.8. Response of second-stage juveniles in roots and final nematode population of *Meloidogyne incognita* race 2 to Nemarioc-AL phytonematicide at 56 days after initiation of treatments.

4.3.2 Nemafric-BL phytonematicide under greenhouse conditions

<u>Plant variables</u>: Plant height, stem diameter, chlorophyll content, number of flowers and dry shoot mass versus Nemafric-BL phytonematicide exhibited positive quadratic relations (Figure 4.9), with the model being explained by 95, 92, 89, 94 and 93%,

respectively (Figure 4.9). The MCSP for Nemafric-BL phytonematicide on peas was 2.18% (Table 4.3). The k value for plant height, stem diameter, chlorophyll content, number of flowers and dry shoot mass was zero for each variable, with the $\Sigma k = 0$ (Table 4.3).
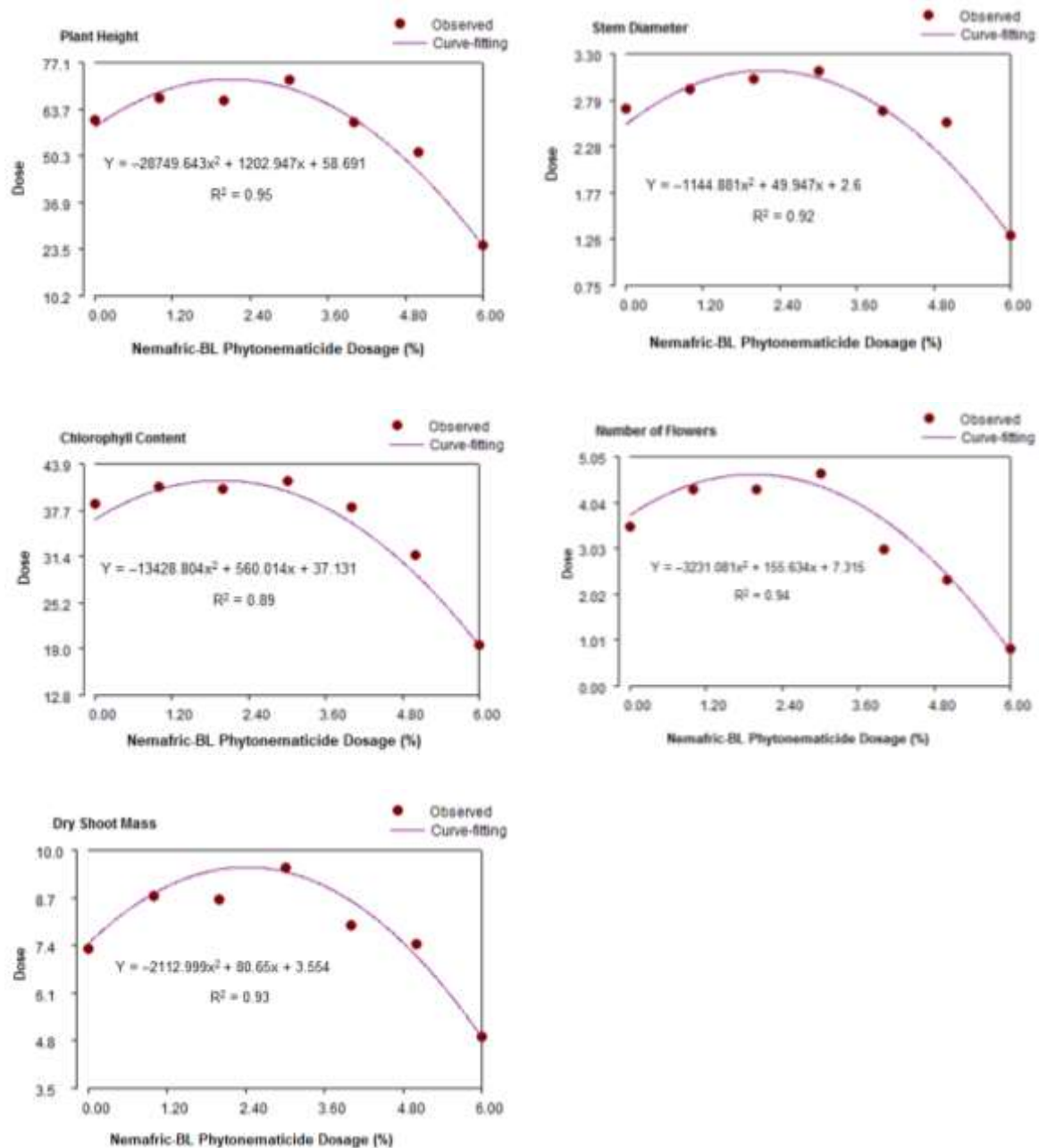
Figure 4.9. Response of plant height, stem diameter, chlorophyll content, number of flowers and dry shoot mass of peas to Nemafric-BL phytonematicide at 56 days after initiation of treatments.

Table 4.3: Biological indices for plant height (PHT), stem diameter (SD), chlorophyll content (CC), number of flowers (NF) and dry shoot mass (DSM) of pea cultivar 'Green Feast' exposed to increasing concentrations of Nemafric-BL phytonematicide.

Biological indices	PHT	SD	CC	NF	DSM	Mean
Threshold stimulation (D_m)	0.021	0.022	0.021	0.019	0.024	0.021
Saturation point (R_h)	12.583	0.545	5.838	0.77	1.874	4.322
0% inhibition(D_0)	0.042	0.044	0.042	0.038	0.048	0.042
50% inhibition (D_{50})	0.059	0.062	0.063	0.054	0.065	0.061
100% inhibition (D_{100})	0.1	0.1	0.1	0.1	0.1	0.1
R^2	0.95	0.92	0.89	0.93	0.94	
Sensitivity (k)	0	0	0	0	0	
Overall sensitivity ($\sum k$)	0					
MCSP = D_m + (R_h/2)	2.18 %					

Nutrient variables: Nemafric-BL phytonematicide had significant effect on Na, Fe, K and Zn in leaf tissues of pea plants. The Na and Zn in leaf tissues of pea plants exhibited negative quadratic relation over the increasing concentration of Nemafric-BL phytonematicide with the model explained by 99 and 89% respectively (Figure 4.10). Iron and K exhibited positive linear-quadratic relation with the models explained by 94 and 96%, respectively (Figure 4.11).
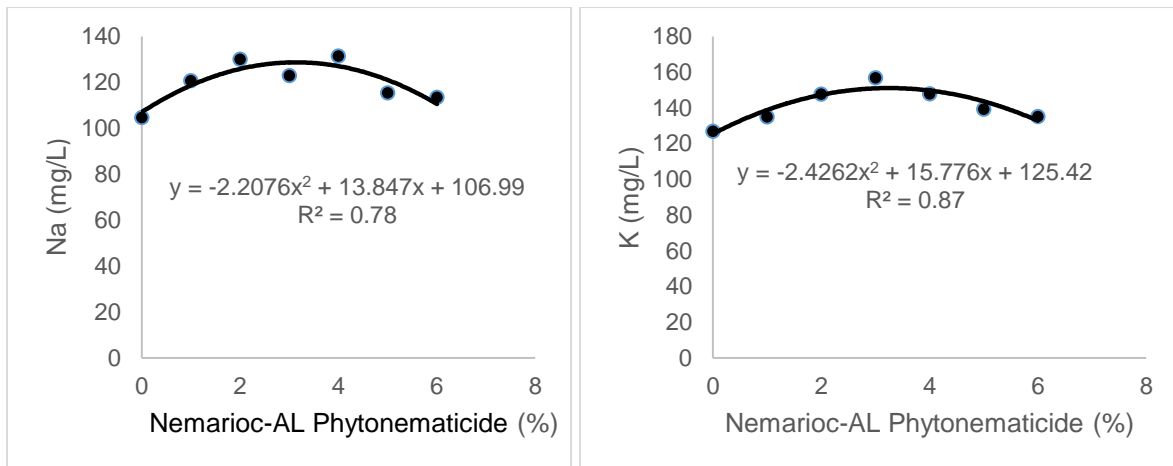
Figure 4.10. Response of sodium (Na) and zinc (Zn) in leaf tissues of pea cv. 'Green Feast' to Nemafric-BL phytonematicide at 56 days after initiation of treatments.

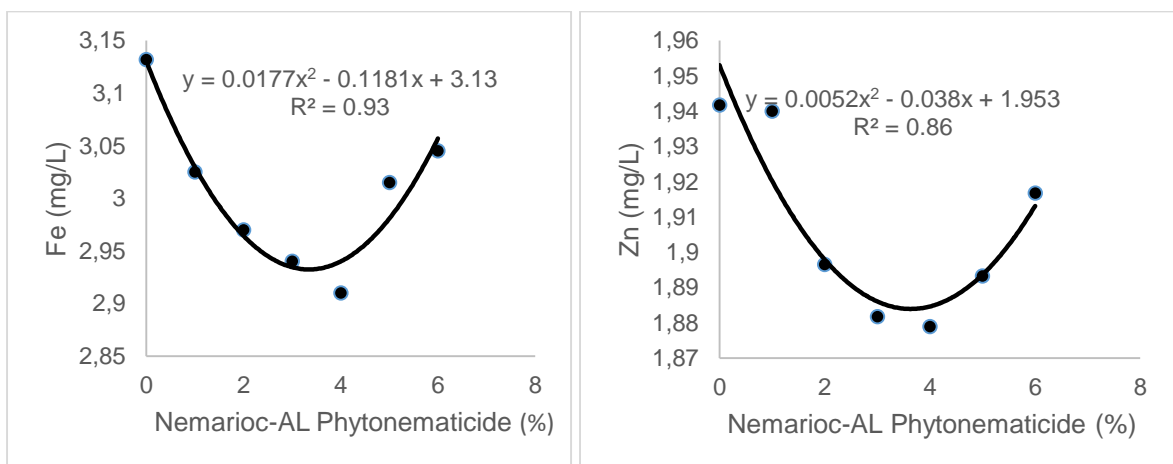Figure 4.11. Response of potassium (K) and iron (Fe) in leaf tissues of pea cv. 'Green Feast' to Nemafric-BL phytonematicide at 56 days after initiation of treatments.

Nematode variables: Gall ratings in roots, eggs in roots, J2 in roots and final nematode population of *M. incognita* versus increasing concentrations of Nemafric-BL phytonematicide exhibited quadratic relations, with the models explained by 83, 82, 80 and 83%, respectively (Figure 4.12 – 4.13).
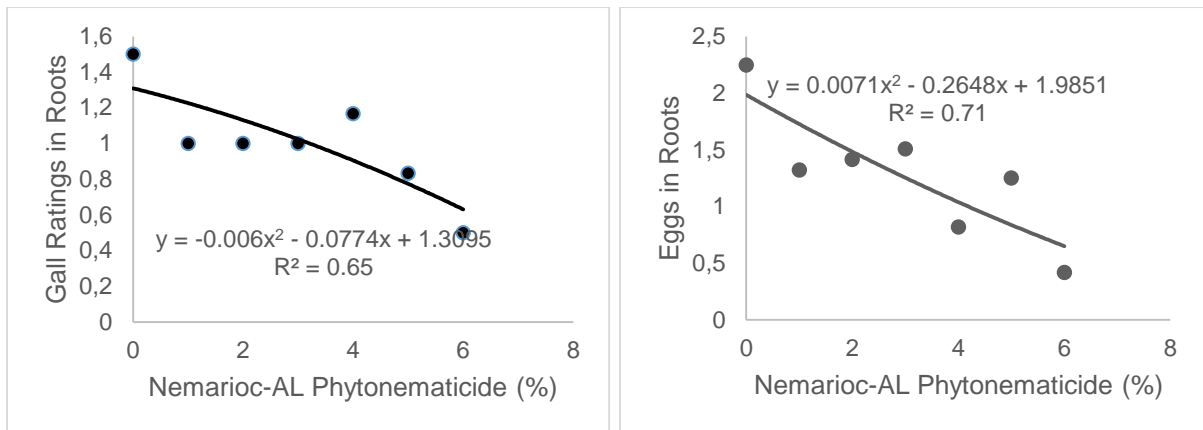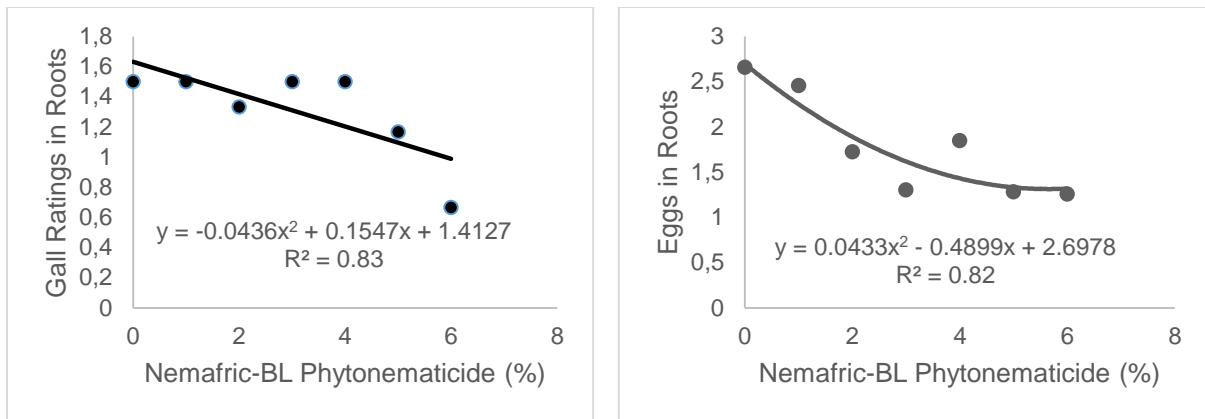
Figure 4.12. Response of gall ratings in roots and eggs in roots of *Meloidogyne incognita* race 2 to Nemafric-BL phytonematicide at 56 days after initiation of treatments.
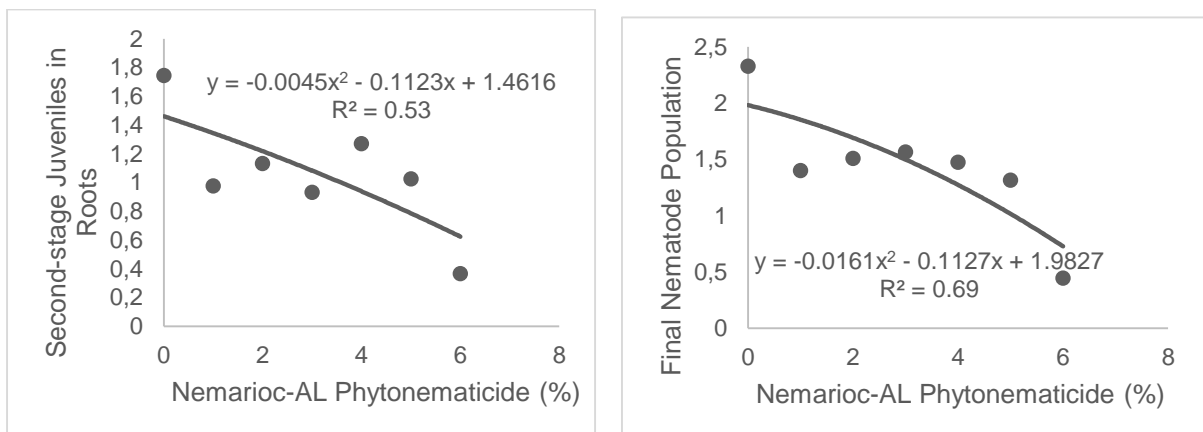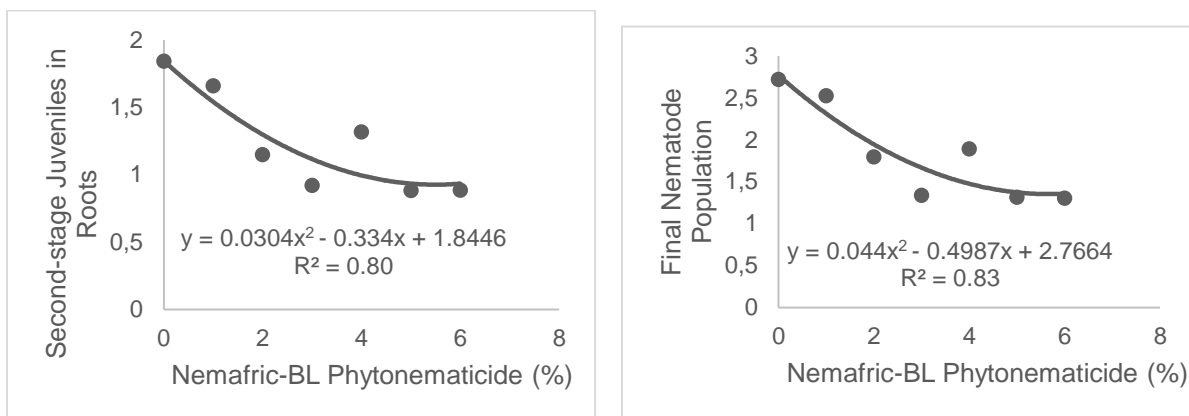

Figure 4.13. Response of second-stage juveniles in roots and final nematode population of *Meloidogyne incognita* race 2 to Nemafric-BL phytonematicide at 56 days after initiation of treatments.

4.3.3 Nemarioc-AL phytonematicide under micro-plot conditions

Plant variables: Plant height, stem diameter, chlorophyll content and gall ratings versus Nemarioc-AL phytonematicide exhibited positive quadratic relations (Figure 4.14), with the model explained by 95, 98, 98 and 98%, respectively (Figure 4.14). However, number of flowers and dry shoot mass versus Nemarioc-AL

phytonematicide exhibited negative quadratic relations (Figure 4.15). Using the

relation $x = -b_1/2b_2$, concentration for optimum plant height, stem diameter, chlorophyll

content and gall ratings were 0.86, 0.32, 0.81 and 0.29%, respectively (Table 4.4). The

MCSP for Nemarioc-AL phytonematicide on peas was 0.71% (Table 4.4). the k value

for plant height, stem diameter, chlorophyll content and gall ratings versus Nemarioc-

AL phytonematicide was zero, with the $\Sigma k = 0$ (Table 4.4).

Figure 4.14. Response of plant height, stem diameter, chlorophyll content and gall

ratings of peas to Nemarioc-AL phytonematicide at 56 days after initiation of
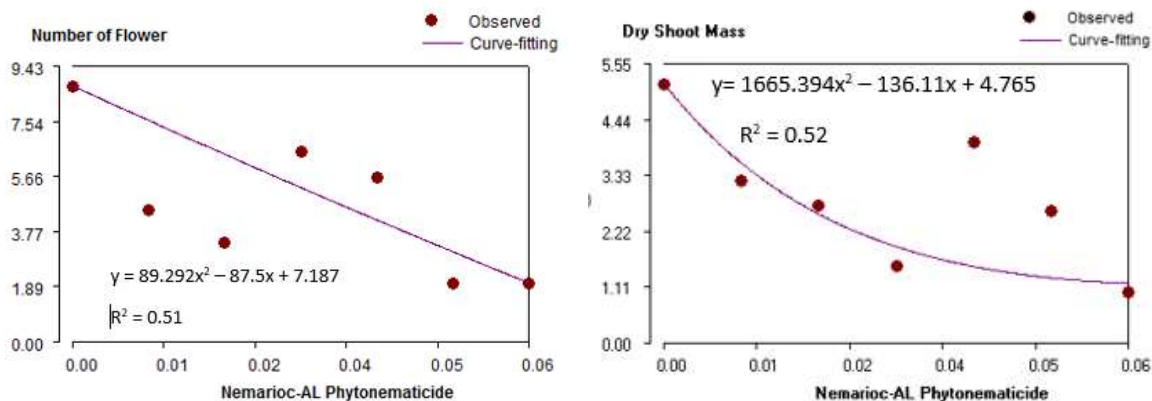
treatments.

Figure 4.15. Responses of number of flowers and dry shoot mass of peas to increasing concentration of Nemarioc-AL phytonematicide under microplot conditions at 56 days after initiation of treatments.

Table 4.4: Biological indices for plant height (PHT), stem diameter (SD), chlorophyll content (CC) and gall ratings (GR) of pea cultivar 'Green Feast' exposed to increasing concentrations of Nemarioc-AL phytonematicide.

Biological indices	PHT	SD	CC	GR	Mean
Threshold stimulation (D_m)	0.858	0.322	0.815	0.275	0.568
Saturation point (R_h)	0.817	0.008	0.695	0.001	0.280
0% inhibition(D_0)	1.717	0	1.629	0.55	0.974
50% inhibition (D_{50})	5.805	5.865	5.619	5.629	5.723
100% inhibition (D_{100})	7.8	8.4	7.6	7.8	7.9
R^2	0.95	0.98	0.98	0.98	
Sensitivity (k)	0	0	0	0	
Overall sensitivity ($\sum k$)	0				
MCSP = D_m + (R_h/2)	0.71%				

Table 4.5: Optimisation model of selected plant variables of pea cultivar 'Green Feast' as affected by concentrations of Nemarioc-AL phytonematicide.

	Model	R^2	x (%)
Treatment	Experiment 1: Nemarioc-AL		
PHT	$Y = -1.109x^2 + 1.903x + 52.613$	0.95	0.86
SD	$Y = -0.082x^2 + 0.053x + 6.237$	0.98	0.32
CC	$Y = -1.047x^2 + 1.706x + 46.954$	0.98	0.81
GR	$Y = -0.019x^2 + 0.011x + 1.114$	0.98	0.29

$X = -b_1/2b_2$.

Nutrient variables: Nemarioc-AL phytonematicide had significant effects on Na, Fe, K and Zn in leaf tissues of pea plants. Sodium and K versus Nemarioc-AL phytonematicide exhibited negative quadratic relation, with the model explained by 82 and 91% association (Figure 4.16). In contrast, Fe and Zn content in leaf tissues of pea plants versus Nemarioc-AL phytonematicide exhibited positive quadratic relations, with the models explained by 90 and 86% associations, respectively (Figure 4.17). The Fe and Zn were optimised at 7.64 and 0.08% concentration of Nemarioc-AL phytonematicide, respectively (Table 4.6).

Table 4.6: Optimisation model of selected nutrient elements in leaf tissues of pea cultivar 'Green Feast' as affected by pre-emergent application concentrations of Nemarioc-AL phytonematicide.

Element	Model	R^2	x (%)
Fe	$Y = -0.0053x^2 + 0.0815x + 2.7388$	0.90	7.64
Zn	$Y = -0.0024x^2 + 0.0004x + 1.5943$	0.86	0.08
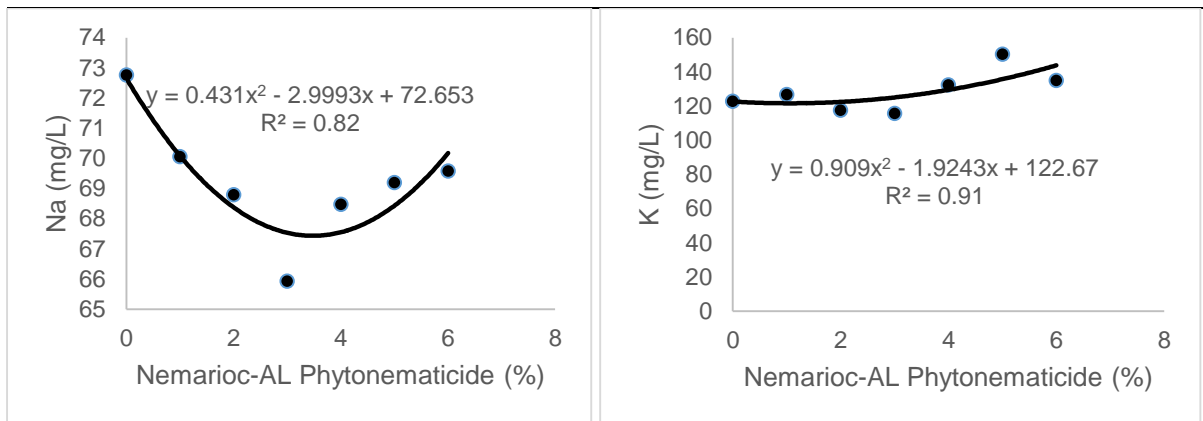
Figure 4.16. Response of sodium (Na) and potassium (K) in leaf tissues of pea cv. 'Green Feast' to Nemarioc-AL phytonematicide at 56 days after initiation of treatments.
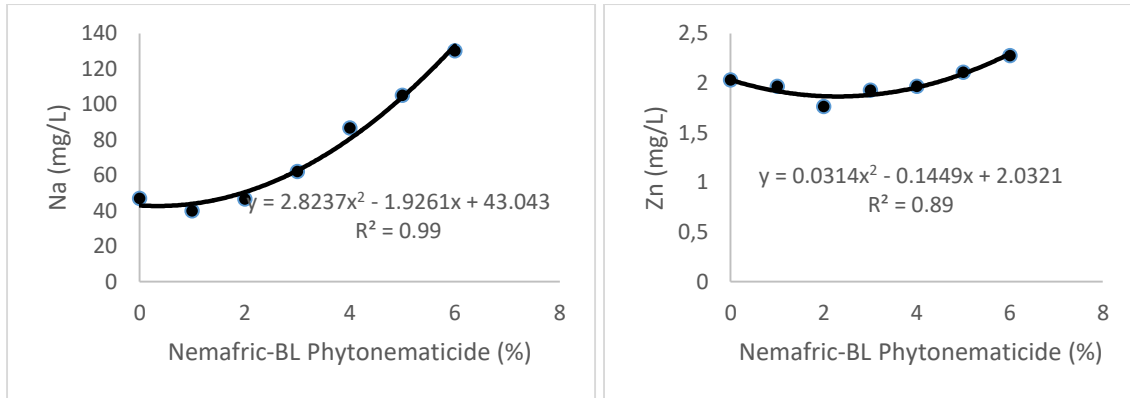

Figure 4.17. Response of iron (Fe) and zinc (Zn) in leaf tissues of pea cv. 'Green Feast' to Nemarioc-AL phytonematicide at 56 days after initiation of treatments.
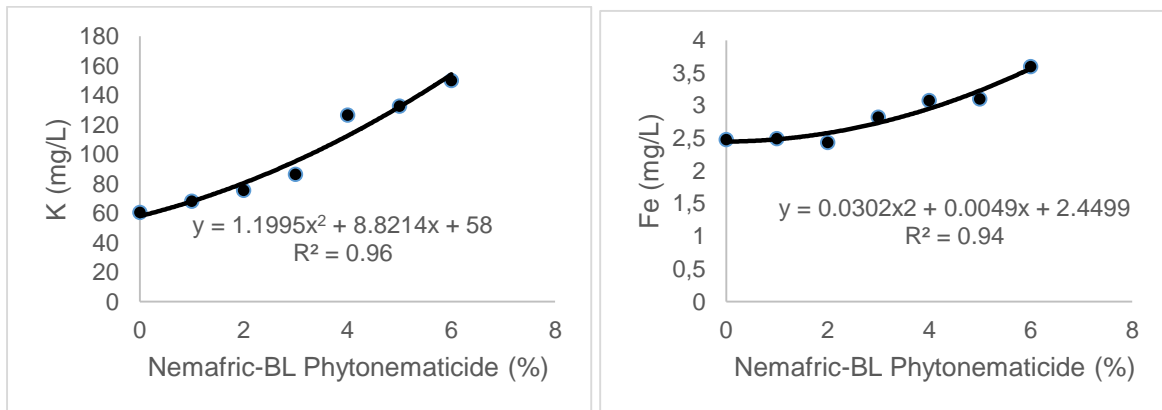
Nematode variables: Eggs in roots, J2 in roots, J2 in soil and final population of *M. incognita* versus Nemarioc-AL phytonematicide exhibited negative quadratic relations (Figure 4.18 – 4.19). The quadratic models for the respective nematode variables against increasing concentrations of Nemarioc-AL phytonematicide were explained by 87, 92, 90 and 81%, respectively (Figure 4.18 – 4.19).

Figure 4.18. Response of eggs in roots and second-stage juveniles in roots of *Meloidogyne incognita* race 2 to Nemarioc-AL phytonematicide at 56 days after initiation of treatments.

Figure 4.19. Response of second-stage juveniles in soil and final nematode population of *Meloidogyne incognita* race 2 to Nemarioc-AL phytonematicide at 56 days after initiation of treatments.

4.3.4 Nemafric-BL phytonematicide under micro-plot conditions

<u>Plant variables</u>: Nemafric-BL phytonematicide had significant effects on chlorophyll content and gall ratings. Chlorophyll content and gall ratings versus Nemafric-BL phytonematicide exhibited positive quadratic relations (Figure 4.20), with the model explained by 97 and 96%, respectively (Figure 4.20). Chlorophyll content and gall

ratings were optimised at 7.64 and 0.08% concentration of Nemafric-BL phytonematicide, respectively (Table 4.8). The MCSP for Nemafric-BL phytonematicide on chlorophyll content and gall ratings of peas was 2.45% (Table 4.7). The k value for chlorophyll content and gall ratings versus Nemafric-BL phytonematicide was zero, with the $\Sigma k = 0$ (Table 4.7).
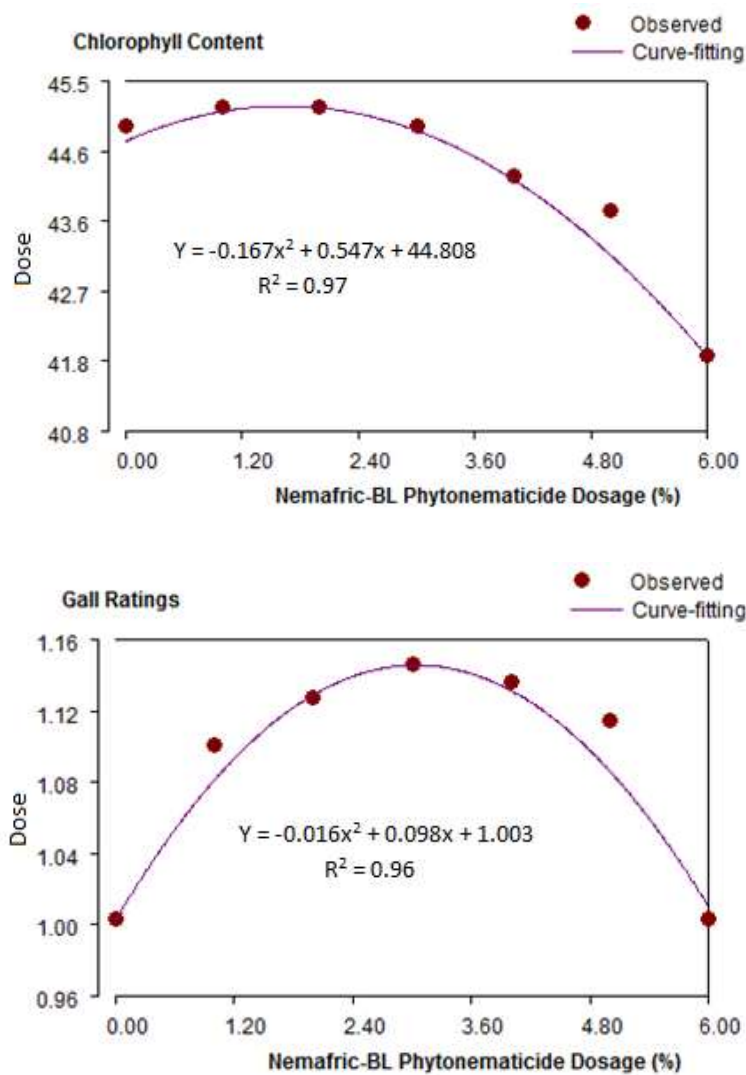
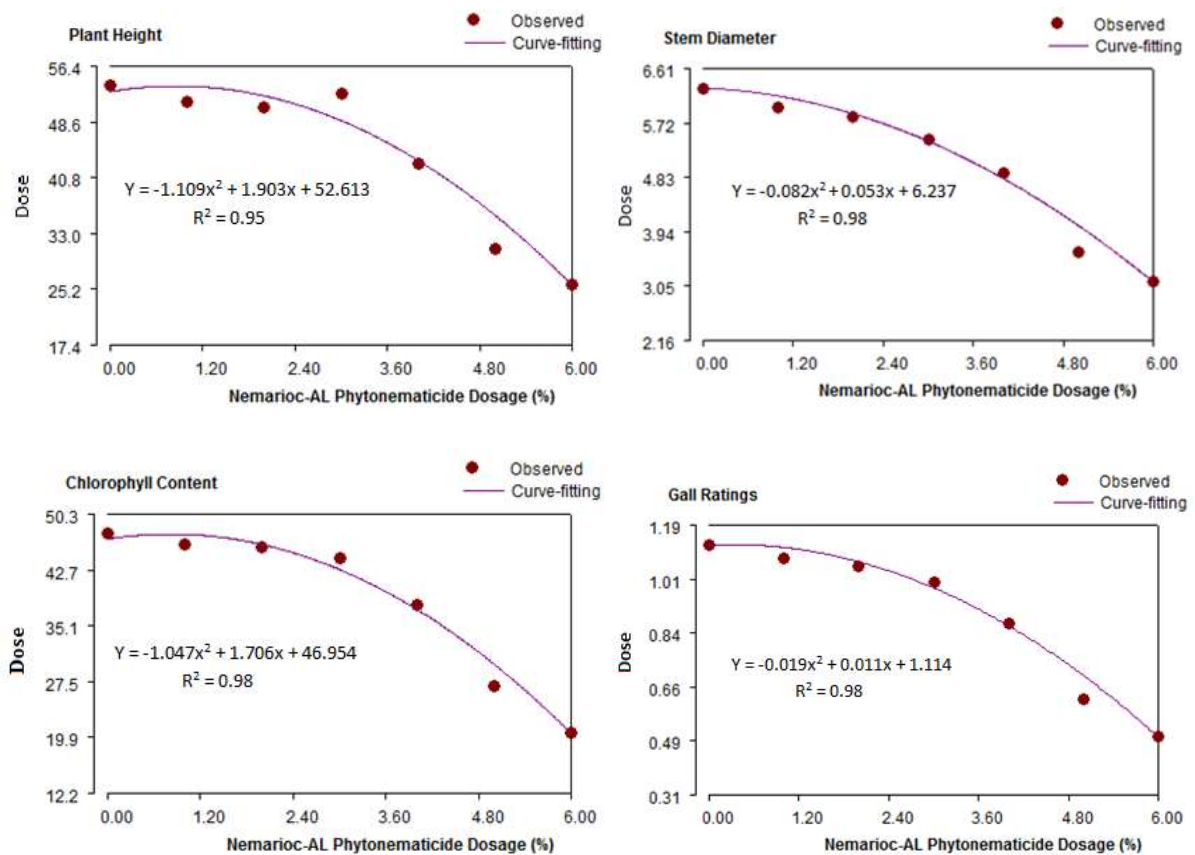Figure 4.20. Response of chlorophyll content and gall ratings of peas to Nemafric-BL phytonematicide at 56 days after initiation of treatments.

Table 4.7: Biological indices for chlorophyll content (CC) and gall ratings (GR) of pea cultivar 'Green Feast' exposed to increasing concentrations of Nemafric-BL phytonematicide.

Biological indices	CC	GR	Mean
Threshold stimulation (D_m)	1.634	3.04	2.337
Saturation point (R_h)	0.447	0.148	0.298
0% inhibition(D_0)	3.267	6.08	4.674
50% inhibition (D_{50})	13.32	9.403	11.362
100% inhibition (D_{100})	18.1	11.5	14.80
R^2	0.97	0.96	
Sensitivity (k)	0	0	
Overall sensitivity ($\sum k$)		0	
MCSP = D_m + (R_h/2)	2.45%		

Table 4.8: Optimisation model of selected plant variables of pea cultivar 'Green Feast' as affected by concentrations of Nemafric-BL phytonematicide.

	Model	R^2	X (%)
Treatment	Experiment 1: Nemarioc-AL		
CC	$Y = -0.167x^2 + 0.547x + 44.808$	0.97	1.64
GR	$Y = -0.016x^2 + 0.098x + 1.003$	0.96	3.06

$X = - b_1/2b_2$.

Nutrient variables: The Na, K and Zn contents in leaf tissues of pea versus Nemafric-BL phytonematicide exhibited negative quadratic relations (Figure 4.21), with the models explained by 90, 82 and 72%, respectively (Figure 4.21). In contrast, Fe versus Nemafric-BL phytonematicide exhibited positive quadratic relation, with the model

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explained by 86% (Figure 4.22). Iron was optimised at 2.32 % concentration of Nemafric-BL phytonematicide.

Table 4.9: Optimisation model of iron (Fe) nutrient element in leaf tissues of pea cultivar 'Green Feast' as affected by pre-emergent application concentrations of Nemafric-BL phytonematicides.

Element	Model	R^2	X (%)
Fe	$Y = -0.0082x^2 + 0.0365x + 2.7091$	0.86	2.23

$X = -b_1/2b_2$.
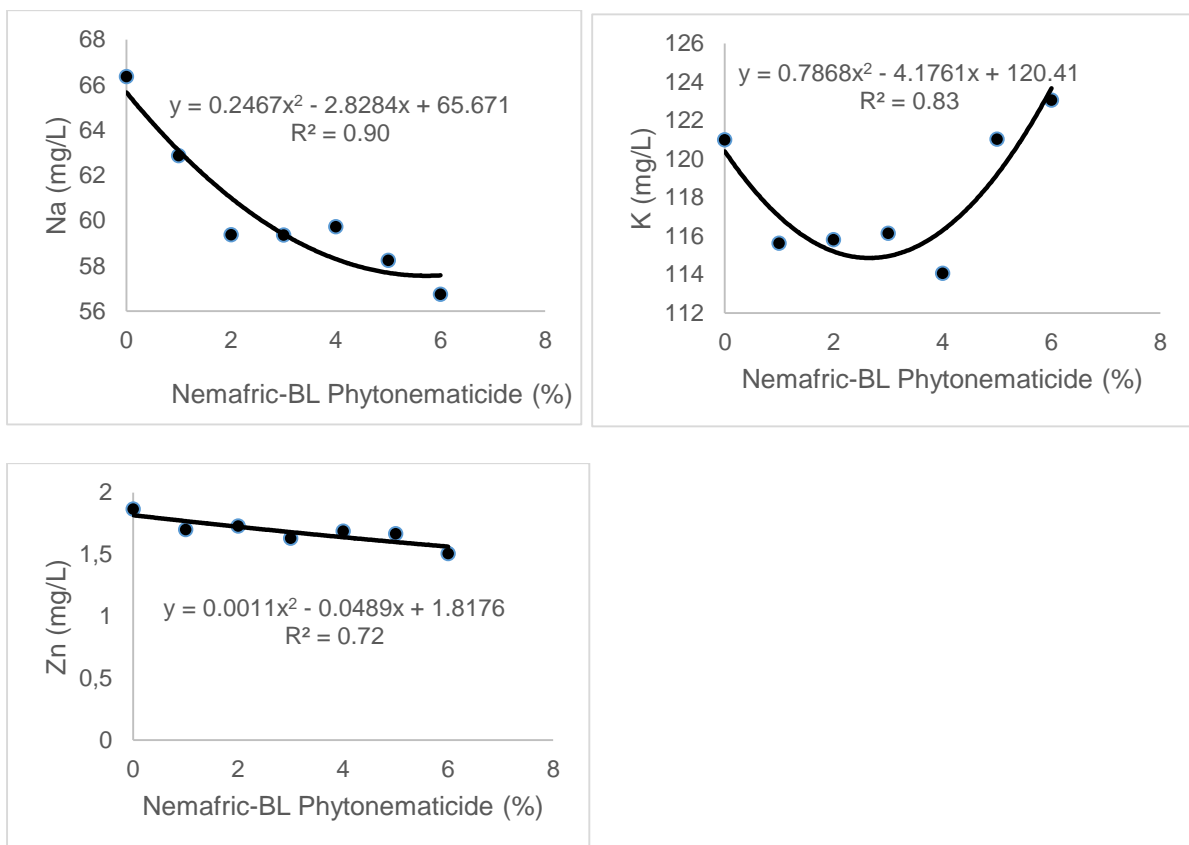
Figure 4.21. Response of sodium (Na), potassium (K) and zinc (Zn) in leaf tissues of pea cv. 'Green Feast' to Nemafric-BL phytonematicide at 56 days after initiation of treatments.
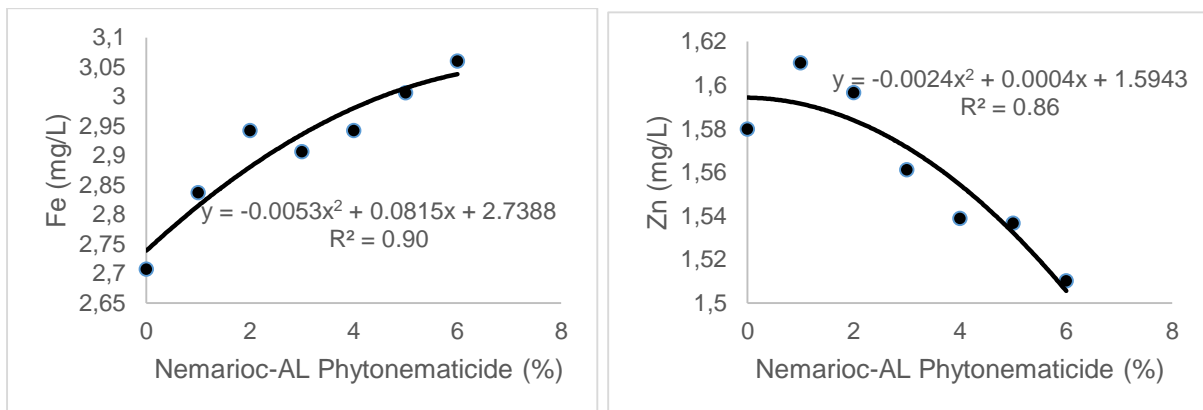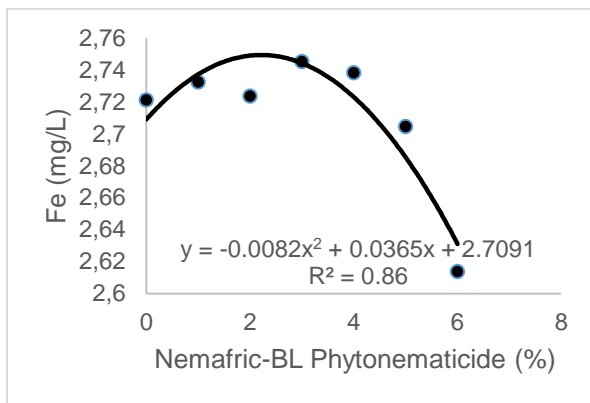
Figure 4.22. Response of iron (Fe) in leaf tissues of pea cv. 'Green Feast' to Nemafric-BL phytonematicide at 56 days after initiation of treatments.

Nematode variables: Nemafric-BL phytonematicide had significant effects on eggs in roots, J2 in roots, J2 in soil and final population of *M. incognita.* Eggs in roots, J2 in roots, J2 in soil and final population of *M. incognita* versus Nemafric-BL phytonematicide exhibited negative quadratic relations, with models explained by 92, 72, 28 and 91% respectively (Figure 4.23 – Figure 4.24).
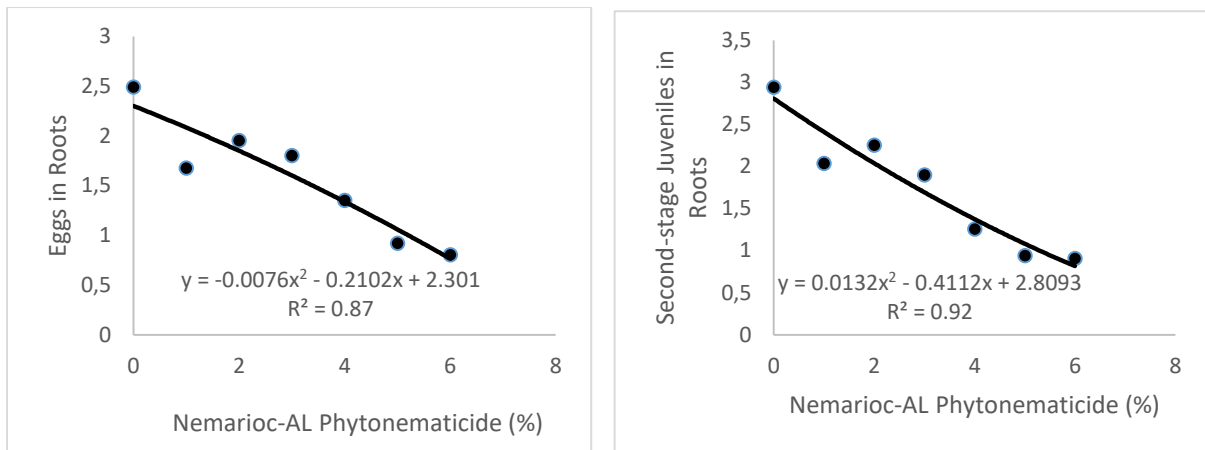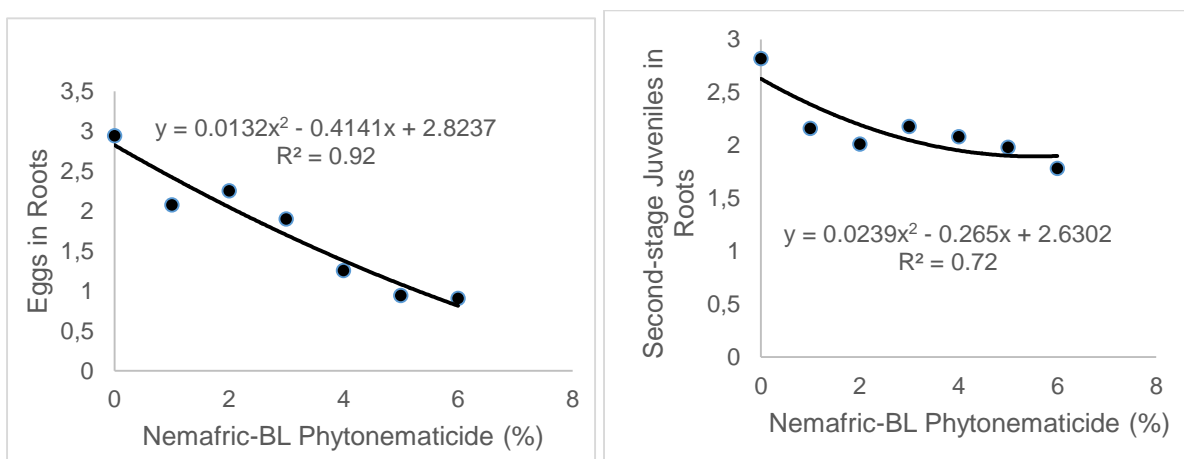

Figure 4.23. Response of eggs on roots and second-stage juveniles in roots of *M. incognita* to Nemafric-BL phytonematicide at 56 days after initiation of treatments.
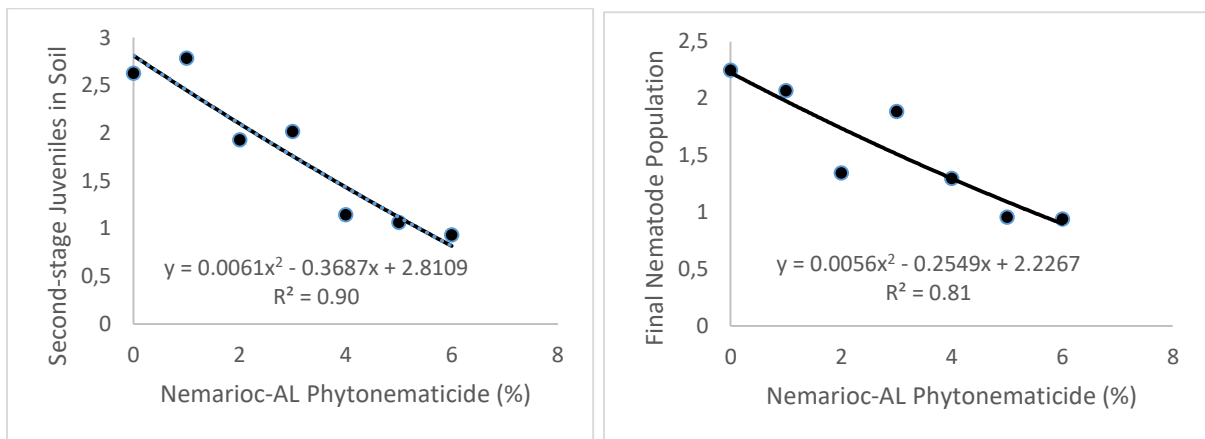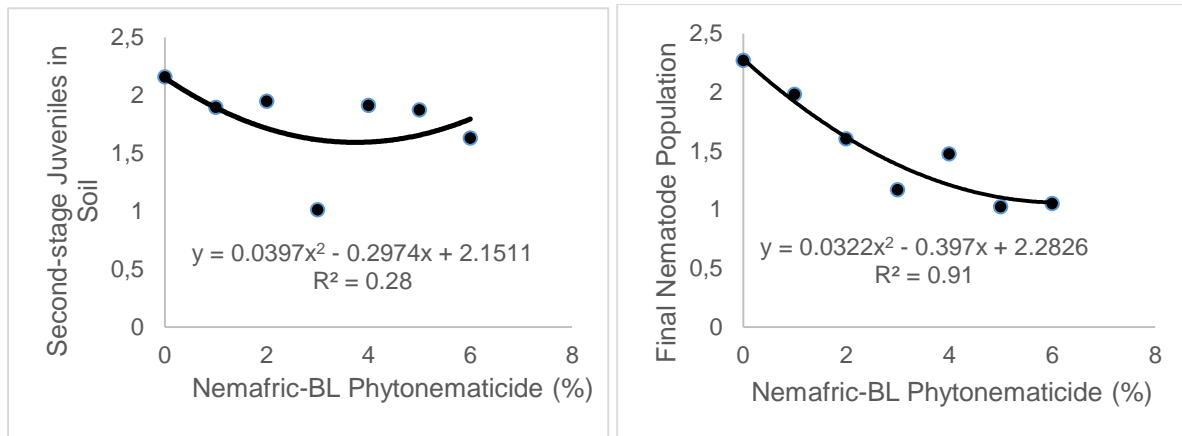
Figure 4.24. Response of second-stage juveniles in soil and final nematode population of *M. incognita* to Nemafric-BL phytonematicide at 56 days after initiation of treatments.

4.4 Discussion

4.4.1 Plant variables

<u>Positive quadratic relation</u>: Nemarioc-AL and Nemafric-BL phytonematicides had significant effects on numerous plant variables of peas under greenhouse trials. Plant height, stem diameter and chlorophyll content of pea plants versus Nemarioc-AL phytonematicide exhibited positive quadratic relationships, which were used to generate MSCP (Mashela *et al.,* 2017). Similarly, plant height, stem diameter, chlorophyll content, number of flowers and dry shoot mass exhibited positive quadratic relationships against Nemafric-BL phytonematicides, which could suggest that the dosages of phytonematicides used in this study were within the saturation point needed to induce stimulation responses (Mashela *et al.*, 2011; Pelinganga *et al.*, 2012; Salisbury and Ross, 1992). Liu *et al.* (2003) indicated that various types of allelochemicals in various plant species stimulate plant growth when applied at low concentration. Nemarioc-AL and Nemafric-BL phytonematicides had stimulatory

effects on various plant growth when applied at low dosages, while at high dosages they inhibited plant growth (Pelinganga, 2013).

Similar observations were reported on tomatoes (*Solanum lycopersicum* L.) (Mashela *et al.*, 2011; Pelinganga *et al.*, 2012), geranium (*Pelargonium sidoides* L.) (Sithole *et al.*, 2016) and other plant species (Inderjit *et al.*, 1999) when exposed to several concentrations of phytonematicides. Mafeo *et al.* (2011) reported that when maize (*Zea mays* L.), millet (*Eleusine coracana* L.), chive (*Allium schoenoprasum* L.), sorghum (*Sorghum bicolor* L.), onion (*Allium cepa* L.) and leek (*Allium porrum* L.) were exposed to the increasing concentration of Nemarioc-AG phytonematicide, stimulated their plant growth when used as pre-emergent phytonematicide.

Priming pea seeds in solutions of Nemarioc-AL and Nemafric-BL phytonematicide under micro-plot trials had similar effects on plant growth to those observed under greenhouse trials. Nemarioc-AL phytonematicide significantly increased plant height, stem diameter, chlorophyll content and gall ratings, while Nemafric-BL phytonematicide increased chlorophyll content and gall ratings on pea plants. Stimulated growth of selected plant variables in this study also confirmed the observations reported in various studies where cucurbitacin-containing phytonematicides were used (Chokoe, 2017; Mabuka, 2015; Maile, 2013; Mashela *et al.*, 2015; Pelinganga and Mashela, 2012; Pelinganga *et al.*, 2012; Sithole, 2016; Tseke *et al.,* 2013).

Negative quadratic relation: Nemarioc-AL phytonematicide had no significant effects on number of flowers and dry shoot mass of pea plants under both greenhouse and

micro-plot trials, whereas Nemafric-BL phytonematicide did not have any significant effects on plant height, stem diameter, number of flowers and dry shoot mass under micro-plot trials. The non-significant effects of cucurbitacin-containing phytonematicides on plant variables of peas implies that the material affected plant growth at saturation point of the CARD model where growth was neither stimulated nor inhibited (Mashela *et al.,* 2011, 2015). Mashela *et al.* (2015) indicated that lack of significant effect on certain plant variables towards the increasing concentrations of phytonematicide suggested that the organs were, by harvest time at saturation concentration.

Absence of significant effects on certain plant variables of peas against Nemarioc-AL and Nemafric-BL phytonematicides support observations by Kohli *et al.* (2001), who reported that 2% crude extracts of yellow nuts edge (*Cyperus esculentus* L.) had no effect on germination of lettuce (*Lactuca sativa* L.), whereas at 5% the extracts inhibited germination. Ghafarbi *et al.* (2012) also observed similar results after exposing eight selected plant species to seed extracts from wheat (*Triticum aestivum* L.), but the extracts had no effects on plant variables.

Curve-fitting Allelochemical Response Dosage: All plant variables exhibited positive quadratic relationships against Nemarioc-AL and Nemafric-BL phytonematicides after being subjected to CARD models had high coefficients of determination (R^2), which support the observations of several researchers (Pelinganga, 2013)*,* where plant variables had high R^2, when exposed to increasing concentration of Nemafric-BL and Nemarioc-AL phytonematicides. High coefficients of determination generated by CARD models for plant variables suggested strong density-dependent relationships

between growth of pea and increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides when used as a pre-emergent phytonematicide (Liu *et al.*, 2003). The relationship between Nemarioc-AL and Nemafric-BL phytonematicides and selected plant variables were quadratic relations and are an illustration of density-dependent growth patterns (Mashela *et al.,* 2015).

Mean Concentration Stimulation Point (MCSP): The positive quadratic relationship suggested that the concentration of Nemarioc-AL and Nemafric-BL phytonematicide used in this study was within the stimulation phase to the saturation phase, thereby allowing the generation of biological indices required to generate MCSP (Mashela *et al.*, 2017). The generated MCSP of 0.62% under greenhouse and 0.71% under micro-plot trials, were lower than the MCSP generated for tomatoes and green bean against Nemarioc-AL phytonematicide, which were 2.64% (Pelinganga, 2013) and 2.67% (Chokoe, 2017). In contrast, the MCSP generated for pea plants where Nemarioc-BL phytonematicide were used as pre-emergent bio-nematicide appeared to be higher than those generated for green bean at 0.27% and 0.5% under greenhouse and field conditions, respectively (Chokoe, 2017) but lower than 2.87% generated for African geranium plants (Sithole, 2016). Pelinganga and Mashela (2012) indicated that the vital significance of MCSP is to establish the concentration level of the phytonematicide which could stimulates plant growth along with nematode suppression, without inducing phytotoxicity to the protected crop.

Furthermore, in the current study, the overall sensitivity of peas was zero under both greenhouse and micro-plot trials, which implied that pea seeds were highly sensitive to Nemarioc-AL and Nemafric-BL phytonematicides when used as pre-emergent bio-

nematicide (Mashela *et al.,* 2015). The developed pea sensitivity to Nemarioc-AL and Nemafric-BL phytonematicide could not be similar to other crops. Other researchers demonstrated that green bean (Chokoe, 2017), geranium (Sithole *et al.*, 2016) and tomato (Pelinganga and Mashela, 2012) plants exhibited moderate sensitivity to cucurbitain-containing phytonematicides. Generally, the degree of sensitivity in crop species to cucurbitacins are plant-stage-specific, with seedlings being highly tolerant than other stages in the life of a given plant species (Mashela *et al.,* 2015). Plant sensitivity to cucurbitacin-containing phytonematicide is inversely proportional to k values, with zero suggesting the highest sensitivity to allelochemicals used, while high k values suggested decreased sensitivities (Liu *et al.*, 2003).

4.4.2 Nutrient elements

The evaluated nutrient elements versus Nemarioc-AL and Nemafric-BL phytonematicide concentrations exhibited quadratic relations, which are the main features of the DDG patterns (Liu *et al.*, 2003; Mashela *et al.*, 2017). The DDG patterns are grouped into three phases, that is stimulation, neutral and inhibition phases (Liu *et al.,* 2003), which had provided more awareness on how cucurbitacin-containing phytonematicides reduce nematode population (Dube, 2016), nutrient elements (Mashela and Pofu, 2017) and plant growth (Mashela *et al.,* 2015; Shadung, 2016). Depending on the primary and succeeding concentration, the response of entities as confirmed by nutrient elements accumulation, begin from stimulation through the neutral to the inhibition phases or vice versa (Mashela and Pofu, 2017; Mokoele, 2018; Shadung, 2016). Similar results were observed on various bioactivities of phytonematicides on nematode stages (Dube, 2016).

Greenhouse: Nemarioc-AL and Nemafric-BL phytonematicides significantly increased Na, K and Fe in the leaf tissues of pea plants. The stimulation concentration range for accumulation of selected nutrient elements in leaf tissues of pea cv. 'Green Feast' was approximately 0.62% concentration of Nemarioc-AL phytonematicide, which are empirically generated concentrations for nematode management in pea production, in this study. The observed quadratic models also provided optimum concentrations at which the selected nutrient elements would be at optimum contents.

The increase in Na content in the leaf tissues could be undesirable to plants, because it is typically viewed as a non-essential nutrient element or waste ions that C3 plants. Mashela and Pofu (2017) observed similar trend in accumulation of Na in leaf tissues of green beans when using Nemafric-BL phytonematicide under greenhouse conditions. Shadung (2016) also observed accumulation of Na in leaf tissues of tomato plants when using Nemafric-BL and Nemarioc-AL phytonematicides, where the two products increased Na in leaf tissues by 54 and 38%, respectively. Furthermore, research has shown that Na is not an essential element for plants, but it can be utilised in small quantities, similar to micronutrients, to help in metabolism and synthesis of chlorophyll. In some crops, it can be utilised as a partial replacement for K and aids in the opening and closing of stomata, which helps regulate internal water balance (Zalesny *et al.*, 2007). Generally, increasing Na content in leaves of certain plants such as citrus can be highly phytotoxic in leaves, with physiological phytotoxicity content being as low as 0.10% Na in leaf tissues (Robinson, 1981). The increase in Na content is ideal in C4 crops, where it plays an essential role in activities of phosphoenolpyruvate carboxylase (Shomer-Ilan and Waisel, 1973).

Micro-plot: Sodium and K in leaf tissues of pea plants against Nemarioc-AL phytonematicide, along with Na and K against Nemafric-BL phytonematicide, started from inhibition through the neutral to the stimulation phases. In contrast, Fe and Zn versus Nemarioc-AL phytonematicide, along with Fe versus Nemafric-BL phytonematicide, started from stimulation through the neutral to the inhibition phases. The decrease in Na content in both trials could be desirable to plants, because it is typically viewed as a non-essential nutrient element or waste ions that C3 plants, such as peas do not need. The findings observed in this study contradict with the observation reported by other researchers (Mashela and Pofu, 2017; Shadung, 2016), where there was an increase in the accumulation of Na in leaf tissues of green beans and tomato plants when cucurbitacin-containing phytonematicides were used under greenhouse conditions.

The decrease in K contents against Nemarioc-AL phytonematicide, and K and Zn contents against Nemafric-BL phytonematicide, could be undesirable to plants, because they are typically viewed as macronutrients and micronutrients that C3 plants, such as peas need for proper growth and yielding. Research has shown that K and Zn in plants helps with the activation of enzymes responsible for carbohydrate metabolism. Potassium is also essential for chlorophyll development and catalyses normal carbohydrate break down during respiration (Welch and Shuman, 1995).

4.4.3 Nematode variables

In the current study, all nematode variables of *M. incognita* versus Nemarioc-AL and Nemafric-BL phytonematicides exhibited negative quadratic relations, which confirmed that at small quantities of crude extracts from *Cucumis* fruits, the materials

constantly reduced nematode numbers (Mafeo, 2012; Pelinganga, 2013), thereby confirming the existence of the inhibition phase in the DDG patterns in response to lower concentrations of allelochemicals (Lui *et al.,* 2003). All degrees of Nemarioc-AL and Nemafric-BL phytonematicides were profoundly effective in comparable to the suppression of nematode numbers as observed in different greenhouse and micro-plot studies, where both products decreased nematode population densities with high magnitudes (Mashela *et al.*, 2015; 2017; Pelinganga, 2013; Pelinganga and Mashela, 2012). The suppression of *M. incognita* with increasing concentration of both Nemarioc-AL and Nemafric-BL phytonematicide were consistent with the observations made in other crops where nematodes were managed using the same materials (Mashela *et al.*, 2015; Pelinganga and Mashela, 2012; Pelinganga *et al.,* 2012). Moreover, in comparison with the observation made in GLT system where similar materials were used in granular formulation in the management of root-knot nematodes, the *Cucumis* bio-nematicide reduced *M.* incognita regularly (Mafeo, 2012; Mofokeng *et al.,* 2004; Mafeo and Mashela, 2012). Unquestionably, the products are highly effective in suppression of root-knot nematodes under different conditions, as it had been shown that the increasing concentrations of cucurbitacin-containing phytonematicides, which are allelochemicals, affect nematode population densities through the DDG patterns (Mashela *et al.,* 2015), which was adapted from the CARD model (Liu *et al.*, 2003).

4.5 Conclusion

According to the evidence gathered from this study, it is confirmed that pea cultivar 'Green Feast' is sensitive to cucurbitacin-A and cucurbitacin-B, which was supported by the results from CARD model, where all plant variables had sensitivity values of

zero under greenhouse and micro-plot trials. Using the integration of the responses of plant variables to Nemarioc-AL and Nemafric-BL phytonematicides within the stimulation range, the quantity of the material which could be used as seed dressing under greenhouse were estimated as 0.62 and 2.18 % material/ pea seed whereas under micro-plot trails were estimated as 0.71 and 2.45 % material/ pea seed, respectively. Furthermore, when both Nemarioc-AL and Nemafric-BL phytonematicides used as pre-application treatments for managing plant-parasitic nematodes under greenhouse and micro-plot environments, were able to suppress population numbers of *M. incognita* race 2. In conclusion, Nemarioc-AL and Nemafric-BL phytonematicides could be applied at the lowest concentration of 2% where it was shown to be effective in suppressing population densities of *M. incognita* without inducing phytotoxicity on pea plants. The results obtained in this study rejected the null hypothesis since priming pea seeds in Nemarioc-AL and Nemafric-BL phytonematicide solutions under greenhouse and micro-plot conditions provided MCSP values for pea-inoculated with *M. incognita*.

CHAPTER 5

SUMMARY OF FINDINGS, SIGNIFICANCE, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary of findings

Nemarioc-AL and Nemafric-BL phytonematicides, which are cucurbitacin-containing phytonematicides, consistently suppressed nematode population densities in various cropping systems using the ground leaching technology (GLT) and the botinemagation technology (Mashela *et al.,* 2017). In the current study, priming of seeds with hypogenous germination characteristics in Nemarioc-AL and Nemafric-BL phytonematicides was investigated as to whether the technology would stimulate or inhibit seed germination, seedling emergence and plant growth and accumulation of nutrient elements in leaf tissues of peas (*Pisum sativum* L.), along with suppressing nematode population densities.

In vitro, Nemarioc-AL phytonematicide did not have significant effects on seed germination and seedling emergence of peas. In contrast, Nemafric-BL phytonematicide as pre-application treatment did not have significant effects on seedling emergence but had significant effect ($P \leq 0.05$) on germination percentage. The observed positive quadratic relation between germination percentage and Nemafric-BL phytonematicide suggested that the product stimulated germination of pea seeds. The mean concentration stimulation point (MCSP) of Nemafric-BL phytonematicide on germination of peas was 0.17%.

Nemarioc-AL and Nemafric-BL phytonematicides promoted growth of pea plants and the accumulation of certain nutrient elements in leaf tissues of pea plants when applied separately under greenhouse and micro-plot trials. Findings confirmed that pea plants were sensitive to cucurbitacin-containing phytonematicides, as shown by the overall sensitivity values of zero unit. Using the integration of the responses of plant variables to Nemarioc-AL and Nemafric-BL phytonematicides within the stimulation range, the quantity of the material, which could be used as pre-emergent seed treatments were estimated as 0.62 and 2.18 %, and 0.71 and 2.45 % material/ pea seed under greenhouse and micro-plot trials, respectively. Nemarioc-AL and Nemafric-BL phytonematicides were significantly effective in suppression of nematode population densities under both greenhouse and micro-plot studies, where both products decreased nematode population densities with high magnitudes.

5.2 Significance

The significance of the findings in the study indicated that when Nemarioc-AL and Nemafric-BL phytonematicides used separately as pre-application treatments negatively and positively affected nematode and plant growth variables, respectively. Evidence from the study demonstrated that seeds with hypogeal germination properties could be used as carriers of the active ingredient of phytonematicides when applied through priming technology in the management of nematodes. In addition, the MCSP values for Nemarioc-AL (0.62% and 0.71%) and Nemafric-BL (2.18% and 2.45%) phytonematicides were more or less similar under greenhouse and micro-plot conditions. However, the magnitudes were quite different to those used under *in vitro* conditions.

5.3 Recommendations

The derived MCSP values for *in vitro* and *ex vitro* for each product should be compared to establish which values are suitable for use in priming pea seeds. This was the first report under laboratory, greenhouse and micro-plot conditions where pea seeds were primed in Nemarioc-AL and Nemafric-BL phytonematicide solutions to generate MCSP as well as suitable sensitivity and overall sensitivity for pea plants.

5.4 Conclusions

Nemarioc-AL and Nemafric-BL phytonematicides could be suitable for use through priming on *P. sativum* in the management of root-knot nematode population densities when used as pre-emergent bionematicide treatments. Nemarioc-AL phytonematicide could be used at 0.62 and 0.71 % under greenhouse and micro-plot conditions, respectively. However, the MCSP values for Nemafric-BL phytonematicide that could be used under *in-vitro*, greenhouse and micro-plot conditions were estimated as 0.17, 2.18 and 2.45 %, respectively, provided that the active ingredient do not have any detrimental effects in accumulation of nutrient elements or temper with nutritional value of peas, when applied as pre-emergent treatment through priming application technology.

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