DETERMINING THE OVERALL SENSITIVITIES OF SWISS CHARD TO CUCURBITACIN-CONTAINING PHYTONEMATICIDES UNDER DIFFERENT CONDITIONS

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DECLARATION

I, Tshepo Segwadi Mashela, declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree Master of Science in Plant Protection 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.

Candidate: Tshepo Segwadi Mashela _____ Signature _____ Date

DEDICATION

I dedicate this to my loving parents, Prof P.W. Mashela and Mrs M. Mashela, and my supportive siblings, Tebelelo, William Jnr and Khensani.

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ABSTRACT

The unavailability of environment-friendly nematicides for managing root-knot (*Meloidogyne* species) nematodes in crop husbandry have led to various alternative methods being sort which includes the development of cucurbitacin-containing phytonematicides. The cited phytonematicides consistently suppressed nematode numbers on different crops under greenhouse, microplot and field conditions, although there is lack of information on how the products would affect susceptible Swiss chard infected by root-knot nematodes. Swiss chard is one of most nutritious vegetables, grown throughout the year and is well adapted to different soil types. However, these products have the potential to induce phytotoxicity on various crops, if applied improperly. Phytotoxicity of phytonematicides on different crops, has been resolved by deriving Mean Concentration Stimulation Point (MCSP). The MCSP, developed using the Curve-fitting Allelochemical Response Data (CARD) computer-based model, is crop-specific, hence it should be developed for every crop. The objectives of this study were to investigate (1) whether population densities of *Meloidogyne* species, growth and accumulation of selected nutrient elements in Swiss chard would respond to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides under greenhouse and microplot conditions and (2) whether the nemarioc-group and nemafric-group phytonematicides in liquid and granular formulations would affect population densities of *Meloidogyne* species and the productivity of Swiss chard with related accumulation of nutrient elements in leaf tissues under field conditions. Parallel experiments for Nemarioc-AL and Nemafric-BL phytonematicides were conducted concurrently under greenhouse and microplot conditions. Greenhouse experiment was prepared by arranging 25-cm-diameter plastic

pods on greenhouse benches, whereas microplot experiment was prepared by digging holes and inserting 30-cm-diameter plastic pots in the field. The four-week-old Swiss chard seedlings were transplanted into the pots, filled with steam-pasteurised loam, sand and Hygromix-T at 3:1:1 (v/v) ratio. Treatments comprised 0, 2, 4, 8, 16, 32 and 64% phytonematicides arranged in randomised complete block design (RCBD), with six replications. Treatments were applied seven days after inoculation, with 3000 eggs and J2 of *M. incognita* race 4 under greenhouse conditions, whereas under microplot conditions were inoculated with 6000 eggs and J2 of *M. javanica.* Under field conditions, treatments comprised untreated control (0), 2 g Nemarioc-AG and 3% Nemarioc-AL phytonematicides (nemarioc-group) or 0, 2 g Nemafric-BG and 3% Nemafric-BL phytonematicides (nemafric-group), arranged in RCBD, each experiment with 8 replications. At 56 days after initiation of treatments, eggs in roots, J2 in roots and Pf exhibited negative quadratic relations under both greenhouse and microplot conditions. Under greenhouse conditions, dry shoot mass (R^2 = 0.81), dry root mass (R^2 = 0.87) and leaf number (R^2 = 0.91) over Nemarioc-AL phytonematicide exhibited positive quadratic relations. In contrast, dry shoot mass (R^2 = 0.78), dry root mass (R^2 = 0.93) and leaf number (R^2 = 0.70) over Nemafric-BL phytonematicide exhibited positive quadratic relations. Under microplot conditions, dry shoot mass (R^2 = 0.95) and gall rating (R^2 = 0.96) over Nemarioc-AL phytonematicide, exhibited positive quadratic relations. Dry shoot mass (R^2 = 0.84) and gall rating (R^2 = 0.97) versus Nemafric-BL phytonematicide exhibited positive quadratic relations. Selected nutrient elements under greenhouse conditions K (R^2 = 0.96), Ca (R^2 = 0.79), Mg (R^2 = 0.64), Fe (R^2 = 0.78) and Zn (R^2 = 0.77) over Nemarioc-AL phytonematicide exhibited positive quadratic relations. In contrast,

only Ca (R^2 = 0.90), Mg (R^2 = 0.68) and Zn (R^2 = 0.84) over Nemafric-BL phytonematicide exhibited positive quadratic relations, whereas K (R^2 = 0.72) and Fe (R^2 = 0.63) over the product exhibited negative quadratic relations. Under microplot conditions, K (R^2 = 0.82), Ca (R^2 = 0.90) and Mg (R^2 = 0.98) over Nemarioc-AL phytonematicide exhibited positive quadratic relations, whereas Fe (R^2 = 0.91) and Zn (R^2 = 0.79) over the product exhibited negative quadratic relations. In contrast, K (R^2 = 0.60), Ca (R^2 = 0.68) and Zn (R^2 = 0.95) over Nemafric-BL phytonematicide exhibited positive quadratic relation, whereas Mg and Fe over the product did not have significant relationships. Under greenhouse conditions, MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides on Swiss chard were 3.03 and 2.36%, whereas overall sensitivity (∑k) values of the crop to the product were 3 and 0 units, respectively. In contrast, MCSP values of Nemarioc-AL and Nemafric-BL phytonematicides on Swiss chard under microplot conditions was successfully established at 3.71 and 3.33%, whereas the ∑k values were 2 and 1 units, respectively. Under field conditions, at 64 days after initiating the treatments, the nemarioc-group phytonematicides had highly significant effects on eggs in roots and reproductive potential (RP), contributing 79 and 77% in total treatment variation (TTV) of the respective variables. In contrast, the nemafric-group phytonematicides had highly significant effects on eggs in roots and RP, contributing 67 and 76% in TTV of the respective variables. Under field conditions, all plant growth variables were not significantly affected by the treatments. The nemarioc-group phytonematicides had significant effects on K and Mg in leaf tissues of Swiss chard, contributing nemafric-group phytonematicides had significant effects on Mg, contributing 62% in TTV of the variable. In conclusion, the products could be used on Swiss chard for managing population densities of *Meloidogyne* species.

However, due to the sensitivity of Swiss chard to the products, it would be necessary to use the derived MCSP values to determine the application intervals of the products on the test cultigen.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

1.1.1 Description of the research problem

Root-knot (*Meloidogyne* species) nematodes pose a serious threat to Swiss chard (*Beta vulgaris* L. cicla) production due to the unavailability of registered synthetic chemical nematicides for leafy vegetable crops. Damage by nematodes on Swiss chard invariably results in delayed maturity, incipient wilting despite adequate moisture, reduced yields and poor quality produce (Onkendi *et al*., 2014). All these responses translate into high production costs and therefore, loss of income to producers (Onkendi *et al.*, 2014). Additionally, infection by *Meloidogyne* species reduces the ability of Swiss chard to extract available soil water and mineral nutrient elements due to the formation of root galls, which increase the root/shoot ratio (Trudgill, 1992). However, there has been a void created by withdrawal of synthetic chemical nematicides from the agrochemical markets in the management of nematodes.

The withdrawal of long used fumigant nematicides from agrochemical markets resulted in research and other alternatives for nematode management (Mashela *et al*., 2015). At Green Biotechnology Research Centre of Excellence, University of Limpopo, South Africa, Nemarioc-AG, Nemafric-BG, Nemarioc-AL and Nemafric-BL phytonematicides were developed as alternatives to synthetic chemical nematicides (Mashela *et al*., 2017; Pelinganga, 2013). The efficacy of the cited phytonematicides on nematode suppression where comparable to that one of nemacur and aldicarb which was a remarkable success for the products (Mashela *et al*., 2008). However,

the successful use of phytonematicides in crop husbandry depends on the amount of concentration that is applied.

1.1.2 Impact of the research problem

Estimated yield losses due to nematode damage three years prior to the withdrawal of methyl-bromide in 2005, were at US$126 billion globally (Chitwood, 2003). Following the withdrawal of methyl bromide in 2005, relative to 8 years after the withdrawal, yield losses ascribed to nematode damage on the global scale had increased by 37% (Mashela *et al*., 2016). The use of plant-based products in nematode management has been leading other alternative nematode management options, with cucurbitacin-containing phytonematicides leading the pack (Mashela *et al*., 2017). However, the cited cucurbitacin-containing phytonematicides have had challenges of phytotoxicity, which is common in botanicals since they rely on allelochemicals as active ingredients (Sithole, 2016).

1.1.3 Possible causes of the research problem

Globally, phytotoxicity caused by agricultural inputs used in plant protection is not allowed on the protected crop (EPPO, 2010). Phytotoxicity on cucurbitacin-containing phytonematicides have been observed on eight monocotyledonous and ten dicotyledonous crops (Mafeo and Mashela, 2010; Mafeo and Mashela, 2009b). Also, at higher concentration phytonematicides have shown phytotoxicity by inhibition phase observed on tomato plants (*Solanum lycopersicum* L.) (Pelinganga, 2013; Tseke, 2013), butternut squash (*Cucurbita pepo* L.) (Lebea, 2017), green bean (*Phaseolus vulgaris* L.*)* (Chokoe, 2017) and wild geranium (*Pelargonium sidoides* DC.) (Sithole, 2016). Phytotoxicity can result in decrease of yield from 50% to complete crop failure (Mashela *et al*., 2015).

1.1.4 Proposed solutions

The challenges of phytotoxicity have been resolved by using the Curve-fitting Allelochemical Response Data (CARD) model, developed in Australia (Liu *et al*., 2003). Mashela *et al*. (2017) used the biological indices from the CARD model to develop the concept 'Mean Concentration Stimulation Point (MCSP)'. The MCSP and k provide information on whether the phytonematicide would be phytotoxic to the plant protected against nematodes. Generally, MCSP is the concentration of the phytonematicide that consistently suppress nematode population densities, while inducing no deleterious effects on plants protected against nematodes damage (Mashela *et al*., 2017). The MCSP is concentration-specific and plant-specific; consequently, empirically based trials must be conducted for each plant species.

1.1.5 General focus of the study

The study would provide both sustainable and commercial farmers with non-phytotoxic concentration of cucurbitacin-containing phytonematicides that will consistently suppress root-knot nematodes without being phytotoxic to Swiss chard. This study would also be compatible with the context of climate-smart agriculture and serve as alternative to synthetic chemical nematicides. Additionally, the study would provide the information on how phytonematicides affect the accumulation of nutrient elements in leaf tissue of Swiss chard under different conditions. Moreover, this study would result on eventual registration of cucurbitacin-containing phytonematicides in agrochemical market in accordance with South African Act 36 of 1947.

1.2 Problem statement

The cucurbitacin-containing phytonematicides produced from crude extracts of wild cucumber (*Cucumis myriocarpus* Naude.) and wild watermelon (*Cucumis africanus* L.) fruits were developed and used as alternative to synthetic chemical nematicides (Pelinganga, 2013). The potential of cucurbitacin-containing phytonematicides in suppressing root-knot (*Meloidogyne* species) nematodes population densities on cultigens is well-documented (Mashela *et al*., 2015; Mashela *et al*., 2017). Nemarioc-AG and Nemarioc-AL phytonematicides contain active ingredient cucurbitacin A ($C_{32}H_{46}O_9$), whereas Nemafric-BG and Nemafric-BL phytonematicides contain cucurbitacin B ($C_{22}H_{48}O_8$). As allelochemicals, these active ingredients have the potential to induce phytotoxicity on cultigens being protected against nematodes (Sithole, 2016). In order to avoid phytotoxicity of cucurbitacin-containing phytonematicides on cultigens, a CARD computer-based algorithm model was used to develop the MCSP, along with k and overall sensitivity (∑k) values (Mashela *et al*., 2017; Pelinganga, 2013), which are important in the successful use of phytonematicides.

1.3 Rationale of the study

Swiss chard is one of most nutritional vegetables that is well-adapted to different soil types and seasons, although it is known to be highly susceptible to *Meloidogyne* species due to limited availability of resistant genotypes to this nematode genus (Mashela *et al*., 2011). The cucurbitacin-containing phytonematicides are low-cost inputs that are suitable for climate-smart agriculture following the withdrawal of synthetic chemical nematicides from the agrochemical markets. However, the successful use of these phytonematicides is based on their capability to suppress

nematodes without inducing phytotoxicity that could interfere with foliar chemical composition. Previous studies reported that, cucurbitacin-containing phytonematicides consistently reduced nematode population densities (Mashela *et al*., 2015; Pelinganga *et al*., 2012). However, the suitability of the products had been limited by their phytotoxicity (Mashela *et al*., 2015), which was solved by using MCSP, derived from biological indices generated through CARD computer-based algorithm model (Liu *et al*., 2003). The MCSP is plant-specific and it should be established for each crop, as already developed for green bean (*Phaseolus vulgaris* L.*)* (Chokoe, 2017), butternut squash (*Cucurbita pepo* L.) (Lebea, 2017), tomato (*Solanum lycopersicum* L.) (Pelinganga, 2013) and wild geranium (*Pelargonium sidoides* DC.) (Sithole, 2016), each with different overall sensitivities.

1.4 Purpose of the study

1.4.1 Aim

Development of non-phytotoxic concentration in cucurbitacin-containing phytonematicides on Swiss chard for managing root-knot nematodes.

1.4.2 Objectives

1. To investigate whether population densities of *Meloidogyne* species, growth and accumulation of selected nutrient elements in Swiss chard would respond to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides under greenhouse and microplot conditions.
2. To determine whether nemarioc-group and nemafric-group phytonematicides in liquid and granular formulations would affect population densities of

Meloidogyne species and the productivity of Swiss chard with related accumulation of nutrient elements in leaf tissues under field conditions.

1.4.3 Hypotheses

1. Population densities of *Meloidogyne* species, growth and accumulation of selected nutrient elements in Swiss chard would respond to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides under greenhouse and microplot conditions.
2. Nemarioc-group and nemafric-group phytonematicides in liquid and granular formulations would affect population densities of *Meloidogyne* species and the productivity of Swiss chard with related accumulation of nutrient elements in leaf tissues under field conditions.

1.5 Reliability, validity and objectivity

The reliability in the current study was based on statistical analysis of data at the probability level of 5%. Validity was achieved in time, whereas objectivity was achieved by ensuring that the findings were discussed on the basis of empirical-based evidence in order to eliminate subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

Bias was minimised by ensuring that the experimental error in each trial was reduced through replications. The treatments were randomised within the selected experimental design (Leedy and Ormrod, 2005).

1.7 Scientific significance of the study

The CARD-computer based model would resolve the problem of phytotoxicity on cucurbitacin-containing phytonematicides by providing the MCSP and k for Swiss chard. The findings would make cucurbitacin-containing phytonematicides to be registered in the agrochemical market according to South African Act 36 of 1947 (as amended) and the international specifications authorities (EPPO, 2010).

1.8 Structure of the mini-dissertation

Following the detailed outline of the research problem (Chapter 1), work done on the research problem was reviewed and work not done was clearly stated (Chapter 2). Then, Chapters 3 addressed objective 1, whereas Chapter 4 addressed objective 2. In the last chapter (Chapter 5), the findings from all Chapter were summarised and integrated to provide the significance of the findings, with recommendations regarding to future research, culminating in conclusions that tied the entire study together. This mini-dissertation followed the Harvard style using author-alphabets as approved by University of Limpopo Senate.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Globally, the use of plant extracts for managing notorious root-knot (*Meloidogyne* species) nematodes became more popular since 2005, due to crop losses caused by restricted use of synthetic chemical nematicides (Chitwood, 2003; Mashela *et al*., 2011; Oka *et al*., 2012). The cucurbitacin-containing phytonematicides as an alternative to synthetic chemical nematicides have been developed from the fruits of wild cucumber (*Cucumis myriocarpus* Naude.) and wild watermelon (*Cucumis africanus* L.) plants indigenous to Limpopo Province (Mashela, 2002; Mashela *et al*., 2017; Pelinganga, 2013). The products are in granular (G) and liquid (L) formulations as Nemarioc-AG, Nemafric-BG, Nemarioc-AL and Nemafric-BL phytonematicides, whereas the letter as depicted by A and B are active ingredients namely, cucurbitacin A ($C_{32}H_{46}O_9$) and cucurbitacin B ($C_{32}H_{46}O_8$), respectively (Chen *et al.,* 2005).

The successful utilisation of cucurbitacin-containing phytonematicides in plant protection is determined by non-phytotoxic concentration known as Mean Concentration Stimulation Point (MCSP) (Mashela *et al*., 2017). The MCSP is a concentration that will stimulate plant growth and suppress nematode population densities without inducing phytotoxicity on protected cultigens (Sithole, 2016). However, MCSP is plant- and concentration specific (Chapter 1), therefore must be developed on Swiss chard. This review focuses on work done and not yet done on the research problem.

2.2 Work done on the problem statement

2.2.1 Phytotoxicity

Phytotoxicity is a common limiting factor for most plant extract to be registered in agrochemical market for the management of nematodes (Mashela *et al*., 2015). Most plant extracts contain allelochemicals as active ingredients, which can induce phytotoxicity to plants being protected (Okwute, 2012). Nemarioc-AG phytonematicide when applied at high concentrations induced phytotoxicity on eight monocotyledonous and ten dicotyledonous crops (Mafeo and Mashela, 2009a, b; Mafeo and Mashela, 2010). Similarly, phytotoxicity was detected on tomato (*Solanum lycopersicum* L.) plants when Nemarioc-AL and Nemafric-BL phytonematicides were applied at 10% (Pelinganga *et al*., 2012). Plant extracts from holy basil (*Ocimum tenuiflorum* L.) as phytonematicide had phytotoxic effects on two dicotyledonous plants, namely, cress (*Lepidium sativum* L.) and alfalfa (*Medicago sativa* L.), as well as two monocotyledonous plants, namely, Italian ryegrass (*Lolium multiflorum* Lam.) and timothy (*Phleum pratense* L.) (Islam and Kato-Noguchi, 2014). In all cases, the product reduced germination and early seedling growth of test plants under controlled environmental conditions (Islam and Kato-Noguchi, 2014).

2.2.2 Resolving phytotoxicity of phytonematicides

Phytotoxicity on botanicals has been resolved by using CARD model (Liu *et al*., 2003), which was adapted to develop the Mean Concentration Stimulation Point (Mashela *et al.,* 2017). CARD model generates the Biological indices, which are used in the computation of MCSP. Biological indices are namely: (a) threshold stimulation (D_m); the allelochemical concentration where stimulation phase begin, (b) saturation point (R_h); the concentration

at which stimulation ends or where the neutral phase start; (c) 0% inhibition (D_0); the concentration at which neutral phase ends, (d) 50% inhibition (D_{50}); the concentration at half the distance of the inhibition phase, (e) 100% inhibition (D_{100}); the concentration that terminates the inhibition phase. In addition, CARD model also provides sensitivity of the crop to the phytonematicides through the sensitivity index (k) to compute overall sensitivity (∑k). The Σk value for a test crop which approaches zero, indicates that the plant organ is more sensitive to phytonematicides, whereas away from zero shows the plant organ is tolerant to the product (Liu *et al*., 2003).

Mean Concentration Stimulation Point is the concentration that would not induce phytotoxicity to a protected cultigen, while suppressing nematode population densities (Mashela *et al*., 2017). The two biological indices, D_m and R_h, are used to calculate MCSP. The MCSP value was derived using the relation:

$$MCSP = D_m + (R_h/2)$$

Mean Concentration Stimulation Point is plant- and location-specific (Mashela *et al*., 2017), as observed on different crops. The MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides on dry bean (*Phaseolus vulgaris* L.) *cv.* 'Tahoe' under greenhouse conditions were 2.11 and 0.27%, whereas ∑k) values were 1 and 0 unit, respectively (Chokoe, 2017). In butternut squash (*Cucurbita pepo* L.) cv. 'Caserta', Nemafric-BL phytonematicide in the greenhouse pot trial had MCSP of 2.83% with ∑k value being 3 units (Lebea, 2017), whereas that on tomato plants was 2.64%, with ∑k value of 4 (Pelinganga, 2013).

Nemarioc-AL and Nemafric-BL phytonematicides under micro-plot conditions on wild geranium (*Pelargonium sidoides* DC.) had MCSP values of 6.18 and 2.87%, respectively, with ∑k value for both products equal to 3 units (Sithole, 2016). Nemarioc-AL phytonematicide on butternut squash had MCSP value of 11.85%, with ∑k value of 0 unit (Lebea, 2017). Nemarioc-AL phytonematicide on green bean (*Phaseolus vulgaris* L.*)* under microplot conditions had MCSP value of 2.67%, with ∑k value of 20 units (Chokoe, 2017). All the results confirm that the product could be used in nematodes management within these accepted values without being phytotoxic to the tested crops.

Nematodes, plant growth and nutrient elements variables over cucurbitacin-containing phytonematicides exhibited positive or negative quadratic relations (Liu *et al.,* 2003), characterised by density-dependent growth (DDG) patterns (Mashela *et al.,* 2017). The DDG patterns have three phases known as stimulation, neutral and inhibition phase (Liu *et al.,* 2003). Therefore, the response of entities to cucurbitacin-containing phytonematicides depends on its initial and subsequent concentration, which starts from stimulation through the neutral to the inhibition phase (Mashela and Pofu, 2017). The DDG patterns of plant variables were evident on green bean (Chokoe, 2017), tomato (Malatji, 2017), beetroot (*Beta vulgaris* L.) (Mashitoa, 2017) and wild geranium (Sithole, 2016). Similarly, nematode variables followed DDG patterns as observed on tomato plants (Tseke and Mashela, 2018). Additionally, nutrient variables also ascribed to similar trends as observed on tomato plants (Pelinganga, 2013) and green bean (Mashela and Pofu, 2017).

In cucurbitacin-containing phytonematicides, many studies have been conducted with the intention of finding the alternative method on nematode management and developing non-phytotoxic concentrations of the products to the crop (Mashela *et al*., 2016). However, more attention is required on how these products influence the accumulation of nutrient elements in leaf tissue of protected cultigens. Mashela and Pofu (2017), observed the response of nutrients elements over increasing concentration of cucurbitacin-containing phytonematicides exhibited a positive and negative quadratic relation. In various studies, response of essential nutrient element in leaf tissues against increasing concentrations of cucurbitacin-containing phytonematicides ascribed to DDG pattern (Liu *et al*., 2003; Mashela and Pofu, 2017).

2.2.3 Efficacy of granular phytonematicides

The cucurbitacin-containing phytonematicides regardless of formulation consistently suppressed nematode population densities under field conditions. Nemarioc-AG and Nemafric-BG phytonematicides are in granular (G) formulations. In granular formulation, Nemafric-BG phytonematicide was applied on sweet stem sorghum (*Sorghum bicolor* L.) and it reduced final nematode population densities (Pf) in roots and soil by 76-85% and 24-65%, respectively (Mabuka, 2013). Under microplot conditions ground wild cucumber fruits has stimulated plant growth of tomato plants and reduced total nematodes in roots of tomato plants and soil by 73-83% and 49-68%, respectively (Mashela, 2002). Nemarioc-AG phytonematicide also showed nematicidal properties on tomato plants, reducing Pf of *Meloidogyne incognita* by 93% under similar conditions (Mashela, 2017).

Crude extract of pomegranate (*Punica granatum* L.) in granular formulation at 6 g exhibited nematicidal properties by reducing root galls, egg masses and reproduction factor of *Meloidogyne javanica* on tomato plants under greenhouse conditions (Regaieg *et al*., 2017). Bitter bush (*Chromolaena odorata* L.) and wild custard apple (*Annona Senegalensis* Pers.) in granular formulation reduced nematode numbers of *M. incognita* on pepper plants when applied at 80 kg/ha under field conditions (Agaba and Fawole, 2015). Castor bean (*Ricinus communis* L.) and dieffenbachia (*Dieffenbachia maculata* Schott.) when applied on tomato plants at 10 and 15 g, they also reduced *M. incognita* in roots under greenhouse conditions (Dura *et al*., 2018).

2.2.4 Efficacy of liquid phytonematicides

The cucurbitacin-containing phytonematicides in liquid formulation suppressed root-knot nematodes under field conditions (Mabuka, 2013). Nemarioc-AL phytonematicide have shown nematicidal properties on butternut squash by reducing eggs from 91 to 100% and J2 from 77 to 100% (Lebea, 2017). Similarly, Nemafric-BL phytonematicide also reduced both eggs and J2 by 100% under similar conditions (Lebea, 2017). Additionally, Nemarioc-AL phytonematicides reduced final nematode population densities (Pf) on green bean by 49 to 100%, whereas Nemafric-BL phytonematicide reduced Pf by 22 to 89% under field conditions (Chokoe, 2017). Moreover, Nemarioc-AL phytonematicide reduced Pf of *Meloidogyne* species in roots by 75 to 80% and soil by 26 to 68% on tomato plants under field conditions (Pelinganga, 2013). Also, Nemafric-BL phytonematicide suppressed nematode numbers in roots by 79 to 90% and in soil by 4 to 53% under similar conditions (Pelinganga, 2013).

The cucurbitacin-free phytonematicides in liquid formulation suppressed nematodes population densities of plant parasitic nematodes. Oil extracts from sesame (*Sesamum orientale* L.) and neem (*Azadirachta indica* L.) at 3% exhibited nematicidal properties by suppressing *M. javanica* in soil by 52 and 70%, respectively, and also reduced gall rating by 63 and 73%, respectively, on French beans under field conditions (Ogumo *et al*., 2019). Similarly, the crude extract of pignut (*Hyptis suaveolens* L.) in liquid formulations reduced nematodes numbers in soil and root galls on cowpea varieties and increased plant height, leaf numbers and pod numbers (Izuogu *et al*., 2016). Additionally, crude extract of lantana (*Lantana camara* L.) and marigold (*Tagetes minuta* L.) leaves in liquid formulation, were both effective when applied at 5% on tomato plants hence significantly reduced nematode population density and increased plant growth under field conditions (Taye *et al*., 2012).

2.2.5 Impact of phytonematicides on mineral elements

The cucurbitacin-containing phytonematicides can either increase, decrease or have non-significant effects on accumulation of nutrient elements in leaf tissues (Liu *et al.,* 2003; Mashela *et al*., 2017). Nemarioc-AL phytonematicide when applied at 3% on tomato plants under field conditions increased accumulation of Mg, Na, P and Ca in leaf tissues by 28, 38, 27 and 25%, respectively, whereas it decreased the accumulation of Fe by 26% (Shadung, 2016). Similarly, Nemafric-BL phytonematicide at 3% had an increase in Mg, Na, P and Ca of tomato plants by 18, 54, 22 and 25%, respectively, but also decreased Fe by 62% (Shadung, 2016). In addition, Nemarioc-AL and Nemafric-BL

phytonematicides increased S accumulation in tomato plants by 26 and 28%, also Mn by 64 and 79%, respectively (Maake, 2018).

Conversely, Nemarioc-AL and Nemafric-BL phytonematicides reduced accumulation of Mg in leaf tissues of tomato plants by 14%, but reduced K by 21 and 22%, respectively (Maake, 2018). Additionally, the interaction of Nemarioc-AL and Nemafric-BL phytonematicides increased accumulation of K in leaf tissues by 8%, but reduced Mg, S and Mn by 14, 1 and 82%, respectively (Maake, 2018). The mixed extract of neem leaf and wood ash when applied at 3 litre /36 m^2 plot increased K, Ca and Mg in soil by 31, 95 and 94%, respectively (Moyin-Jesu, 2014). Additionally, fly ash at lower concentration (< 25%) stimulated accumulation of macro elements, microelements and plant biomass (Yu *et al*., 2019).

2.3 Work not done on the problem statement

The MCSP values for both cucurbitacin-containing phytonematicides on Swiss chard under different conditions remain undocumented. In addition to MCSP values, subjecting Swiss chard to a series of high concentrations of the two phytonematicides could provide an opportunity to assess the sensitivities of Swiss chard to test products in relation to interference with the nutrient elements in leaf tissues.

CHAPTER 3
EFFECTS OF TWO PHYTONEMATICIDES ON GROWTH AND NUTRIENT ELEMENTS OF NEMATODE-INFESTED SWISS CHARD UNDER GREENHOUSE AND MICROPLOT CONDITIONS

3.1 Introduction

Nemarioc-AL and Nemafric-BL phytonematicides have been developed and used successfully to manage plant parasitic nematodes (Mashela *et al.,* 2017; Pelinganga, 2013). The products are currently gaining more interest with their success on nematodes management on various crops (Mashela *et al*., 2011). Just like any other Botanicals, Nemarioc-AL phytonematicide has allelochemicals as active ingredients; cucurbitacin A ($C_{32}H_{46}O_9$), which is partially soluble in water and breaks down into cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Chen *et al*., 2005). In contrast, Nemafric-BL phytonematicide has cucurbitacin B ($C_{32}H_{46}O_8$) as active ingredient, which is non-soluble in water (Chen *et al*., 2005). These products depend on their active ingredients for suppressing nematode population densities. However, the phytonematicides have the potential to induce phytotoxicity on protected crops.

Globally, in most recognised legal entities such as European and Mediterranean Plant Protection Organization, phytotoxicity is not allowed on agricultural inputs that are used for plant protection purposes (EPPO, 2010). Most botanicals have phytotoxicity challenges for instance, when powdered neem kernels (*Azadirachta indica* L.) was used on banana (*Musa acuminate* L.) plants to suppress nematode numbers, phytotoxicity was detected (Musabyimana *et al*., 2000). Also, on eight monocotyledonous and ten dicotyledonous crops, when the cucurbitacin-containing phytonematicides were applied

at 2.5, 5, 7.5, 10, 12.5 and 15 g/plant as pre-emergent drenches, phytotoxicity was detected by inhibiting germination indices (Mafeo, 2012). Since conventional methods of detecting phytotoxicity on botanicals are tedious (Mashela *et al*., 2015), phytotoxicity has been resolved by empirically derived Mean Concentration Stimulation Point (MCSP) values generated through Curve-fitting Allelochemical Response Dosage (CARD) data (Mashela *et al*., 2017). The objective of the study was to investigate whether population densities of *Meloidogyne* species, growth and accumulation of selected nutrient elements in Swiss chard would respond to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides under greenhouse and microplot conditions.

3.2 Materials and methods

3.2.1 Description of the study site

Parallel experiments for Nemarioc-AL and Nemafric-BL phytonematicides were conducted concurrently under greenhouse (Figure 3.1) and microplot (Figure 3.2) conditions. These experiments were conducted at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, South Africa (23º53"10'S, 29º44"15'E) during autumn (February – April) 2017 and validated in 2018.

Greenhouse experiments: The greenhouse structure had an area of 2000 m^2 (100 m × 20 m) in size, with thermostatically-activated fans on the north-facing wall and the wet wall on the south-facing side for moderating inside temperatures. Day/night ambient temperature range from 20/25°C, with the top part of the structure covered with a 35% radiation-allowing green net.

Figure 3.1 Swiss chard cv 'Fordhoek giant' treated with Nemarioc-AL and Nemafric-BL phytonematicides under greenhouse conditions.

Microplot experiment: The location has an average annual rainfall of less than 500 mm, with the highest distribution being during summer (November – January) and maximum/minimum temperatures of 38/25°C.

Figure 3.2 Swiss chard cv 'Fordhoek giant' treated with Nemarioc-AL and Nemafric-BL phytonematicides under microplot conditions.

3.2.2 Treatments and experimental design

Treatments comprised 0, 2, 4, 8, 16, 32 and 64% Nemarioc-AL or Nemafric-BL phytonematicides arranged in a randomised complete block design (RCBD), with 12 replications for both greenhouse and microplot experiments.

3.2.3 Procedures and cultural practices

Nemarioc-AL and Nemafric-BL phytonematicides were prepared using a locally developed method (Mashela *et al*., 2017). Briefly, Nemarioc-AL and Nemafric-BL phytonematicides method comprised by filling a 20 L-hermetically sealed plastic container with 16 L chlorine-free tapwater. Approximately, 80 and 40 g dried and ground fruit from *Cucumis myriocarpus* and *Cucumis africanus,* with 300 ml effective microorganisms

(EM), 300 ml molasses and 100 g sugar each added into the containers. After adding the listed ingredients, the containers were shaken to mix the materials. The containers had an outlet, airtight 5-mm-diameter and 50-cm-long pipe with one end glued to a hole on the lid of the 20 L containers, with the other end dangling into a 1 L bottle half-filled with chlorine-free tapwater to provide the generated CO_2 an escape route from the containers during fermentation process. The air-tight system was placed at room temperature for 14 days to allow for the fermentation-induced pH to drop approximately 3.7 units (Kyan *et al*., 1999).

Greenhouse experiment: The seeds of Swiss chard cv. 'Fordhook Giant' were planted and raised in a 200-hole seedling trays filled with Hygromix-T (Hygrotech, Pretoria North) under greenhouse conditions. At two-leaf stage, seedlings were hardened-off for a week outside the greenhouse prior planting. Twenty-five-cm-diameter plastic pots were arranged on greenhouse benches at intra- and inter- row spacing of 0.2 m. Uniform Swiss chard seedlings were transplanted directly into 25-cm-diameter plastic pots each containing approximately 3 375 ml growing mixture of steam-pasteurised loam (300˚C for 1 h), sand and Hygromix-T at the ratio of 3:1:1 (v/v). Each pot contained two plants (per drip hole). Irrigation was achieved by using 300 ml of chlorine-free water and seedlings were fertilised using 5 g 2:3:2 (26) NPK + 0.5% Zn + 5% S + 5% Ca and 2 g 2:1:2 (43) Multifeed fertiliser. Seven days after transplanting, seedlings were inoculated with 3 000 eggs + J2 of *Meloidogyne incognita* race 4, which were previously cultured on susceptible tomato (*Solanum lycopersicum* L.) cv. 'Floradade'. Treatments were applied at seven days after inoculation and thereafter once on weekly basis. Scouting and monitoring for

insect pests and diseases inside greenhouse were carried out on daily basis and powdery mildew was controlled by using Funginex.

Microplot experiments: All procedures were similar to those under greenhouse experiment, except that planting was prepared by digging holes in the field and inserting 30-cm-diameter plastic pots at intra- and inter-row space of 0.6 m. Seedlings were inoculated with 6 000 eggs and J2 of *Meloidogyne javanica* to ensure crop damage and irrigation was done by using 500 ml of chlorine-free water. A spraying programme was developed to manage orthoptera insect pest grasshoppers by using Malathion.

3.2.4 Data collection

At 56 days after initiating the treatments, plant height and chlorophyll content were measured and leaf number were counted. Shoots were detached from the roots above the ground and weighed for fresh shoot mass, thereafter shoots were oven-dried at 52°C for 72 h and weighed to obtain dried shoot mass, and later ground in a Wiley mill. Root systems from the soil were immersed in water to remove soil particles, and then pressed between paper towel to remove excess water. Root galls were assessed using the North Carolina Differential Rating Scale (Taylor and Sasser, 1978).

Nematodes were extracted from 10 g roots/plant by maceration and blending for 30 seconds in 1% NaOCl solution (Hussey and Barker, 1973). The material then passed through 75- and 25-µm nested sieves, with eggs and J2 collected from the 25-µm mesh sieve. Soil per station was thoroughly mixed and 250 cm^3 soil samples collected from

each station, with nematodes extracted from soil samples using the sugar-floatation and centrifugation methods (Jenkins, 1964). Eggs and J2 were counted from a 50 ml aliquot using 5 ml under a stereomicroscope. Eggs and J2 were expressed as reproductive potential (RP = eggs + J2/g root).

Approximately, 0.4 g ground Swiss chard leaves tissue were mixed in 75 ml vessel with 5.0 ml nitric acid (HNO_3) and 3.0 ml hydrogen peroxide (H_2O_2). The mixture was vortexed for at least 2 minutes and samples allowed to cool for about 10 minutes before the vessels were closed. Vessels were then placed into a microwave digester (PerkinElmer, Titan MPS) and allowed to run for 46 min under temperature ranging up to 260°C. Thereafter, vessels were allowed to cool down at room temperature for 20 minutes to avoid foaming or splashing of the mixture. Samples were decanted into 50 ml tubes and stored in cold room to avoid evaporation prior to analytical process. Prepared samples were analysed for K, Ca, Mg, Fe and Zn using the inductively coupled plasma optical emission spectrometry (ICPE-9000).

3.2.5 Data analysis

Data for plant variables were subjected to CARD model to generate biological indices for calculating MCSP (D_m, R_h), development of curves, quadratic equations and the related biological indices (k) (Liu *et al*., 2003).

$$MCSP = D_m + (R_h/2)$$

Prior to CARD model, the geometric series of the phytonematicide concentration was expressed as an exponential series (2^0, 2^1, 2^2, 2^3, 2^4, 2^5 and 2^6) and since ($\log_2 2$) = x, the

log-transformed series was equivalent to 0, 1, 2, 3, 4, 5 and 6% (Tseke and Mashela, 2018). This was done to avoid observations being overcrowded at lower concentration and to ensure the correct equidistances between observations. The MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides were calculated from plant variables that exhibited positive quadratic relations, with $R^2 \geq 0.25$ (*i.e*. r = 0.5). Nematode variables and nutrient elements data were subjected to the line of best fit (Gomez and Gomez, 1984).

3.3 Results

Seasonal interactions were not significant on all variables and, therefore; the data for two seasons (experiment 1 and 2) were pooled (n = 84) and re-analysed (Gomez and Gomez, 1984).

3.3.1 Greenhouse experiment

Nematode variables: Eggs in root, J2 in roots and final nematode population (Pf) of *M. incognita* against Nemarioc-AL and Nemafric-BL phytonematicides exhibited negative quadratic relations (Figure 3.3). The models for nematode variables in the Nemarioc-AL phytonematicide experiment were explained by 91, 78 and 85% associations on eggs in roots, J2 in roots and final nematode population density (Pf), respectively, whereas for Nemafric-BL phytonematicide the models were explained by 96, 97 and 97% associations for the respective variables (Figure 3.3).

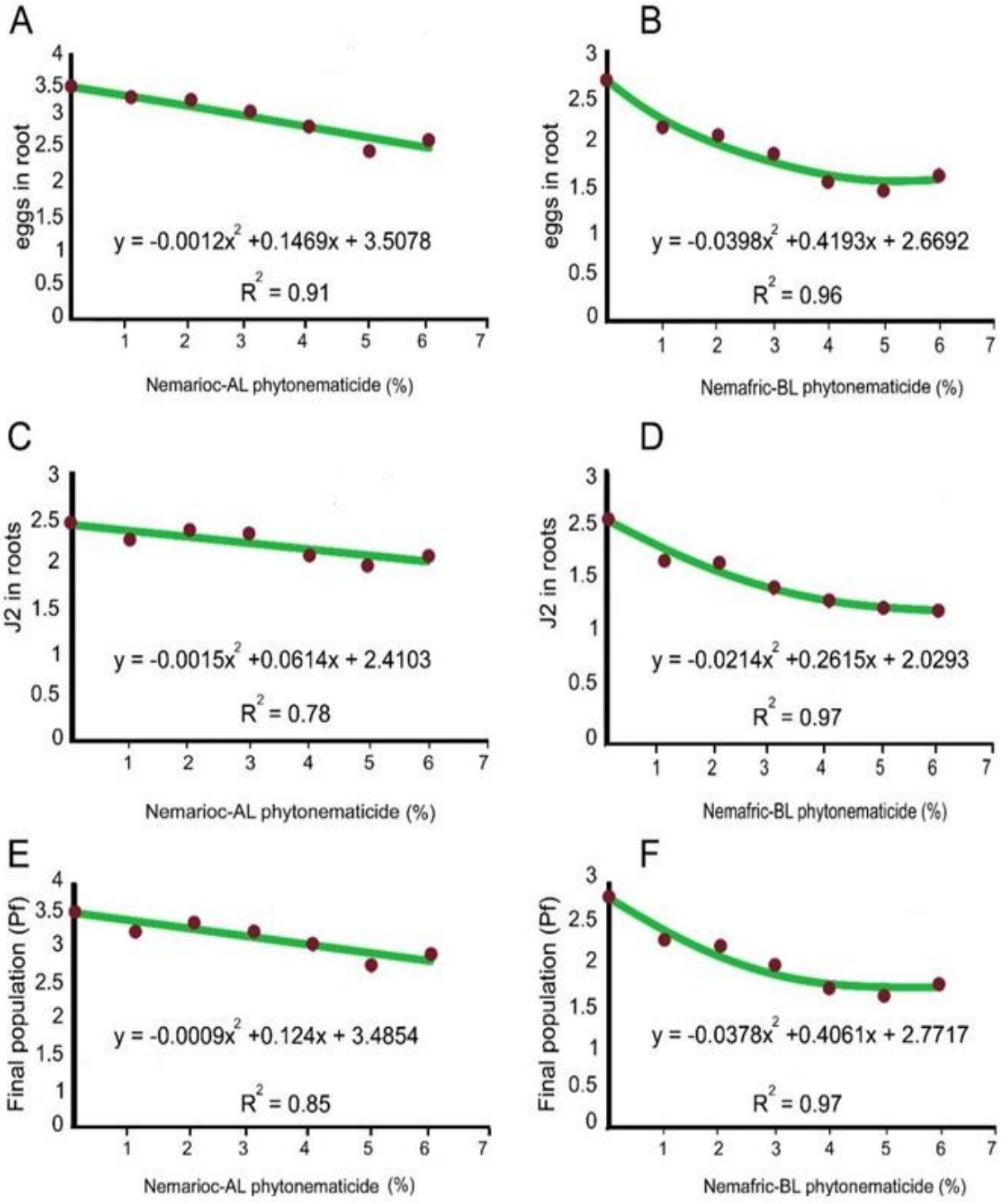

Figure 3.3 Response of *Meloidogyne incognita* eggs in root, J2 in roots and Pf to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides at 56 days after initiation of treatment under greenhouse conditions.

Plant variables: Dry shoot mass (Figure 3.4), dry root mass (Figure 3.5) and leaf number (Figure 3.6) over Nemarioc-AL phytonematicide exhibited positive quadratic relations, with the model explained by 81, 87 and 91% associations, respectively (Table 3.1). In contrast, dry shoot mass (Figure 3.7), dry root mass (Figure 3.8) and leaf number (Figure 3.9) over Nemafric-BL phytonematicide exhibited positive quadratic relations, with the model explained by 78, 93 and 70%, respectively (Table 3.1). In both Nemarioc-AL and Nemafric-BL phytonematicide experiments, chlorophyll content, plant height and gall rating were not significantly affected by the treatments.

Biological indices which exhibited positive quadratic relations, were used to calculate the average MCSP values. Using the biological indices D_m and R_h, the MCSP values of Nemarioc-AL and Nemafric-BL phytonematicides suitable for application on Swiss chard were 3.03 and 2.36%, respectively (Table 3.1). Dry shoot mass, dry root mass and leaf number when exposed to Nemarioc-AL phytonematicide had sensitivity (k) values of 1, 2 and 0, respectively, with the overall sensitivity (Σk) on Swiss chard being equal to 3 units (Table 3.1). In contrast, when exposing Swiss chard to Nemafric-BL phytonematicide, dry shoot mass, dry root mass and leaf number had each the sensitivity values of 0 unit, with Σk value of 0 unit (Table 3.1).

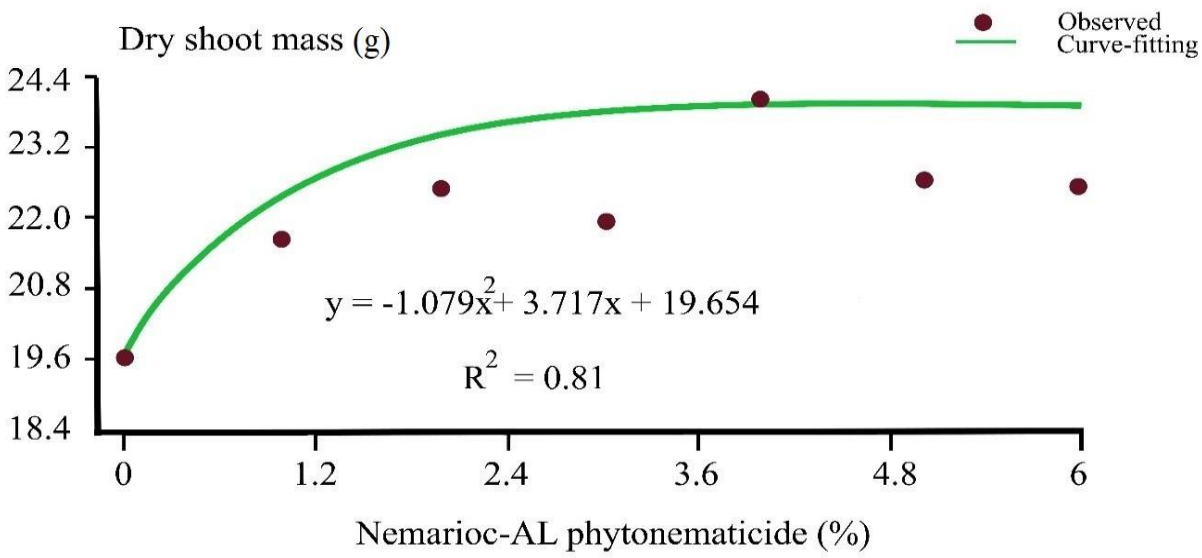

Figure 3.4 Response of dry shoot mass to increasing concentration of Nemarioc-AL phytonematicide under greenhouse conditions.

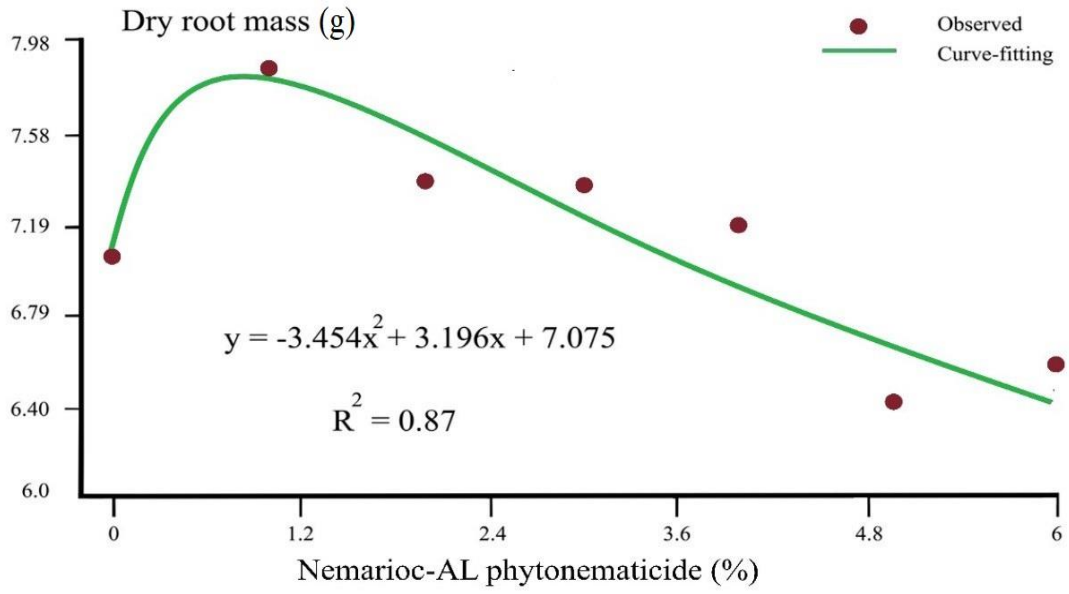

Figure 3.5 Response of dry root mass to increasing concentration of Nemarioc-AL phytonematicides under greenhouse conditions.

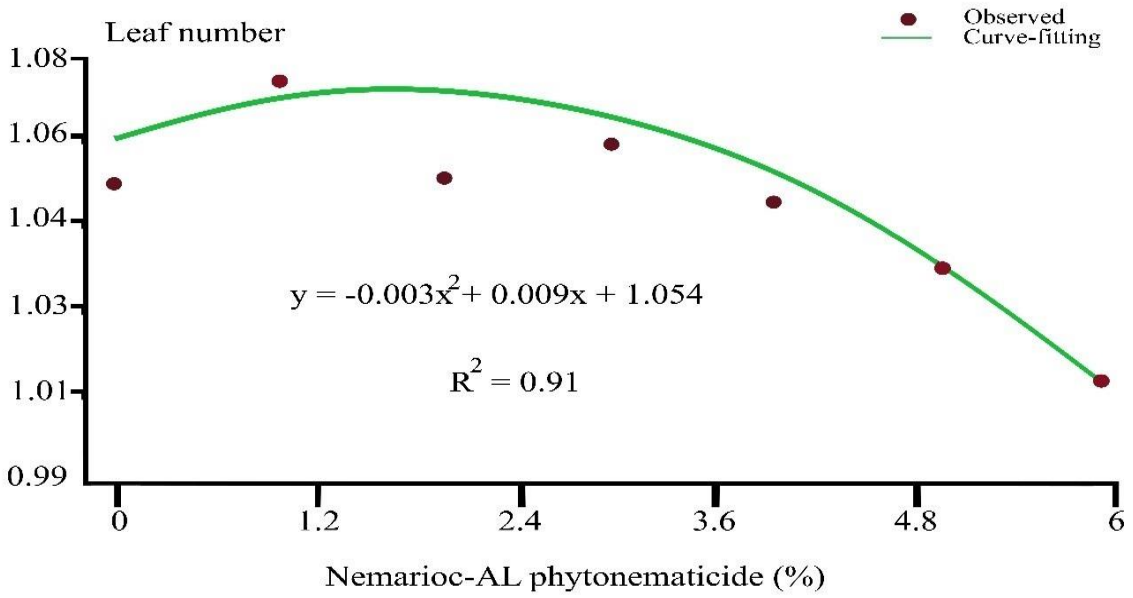

Figure 3.6 Responses of leaf number to increasing concentration of Nemarioc-AL phytonematicide under greenhouse conditions.

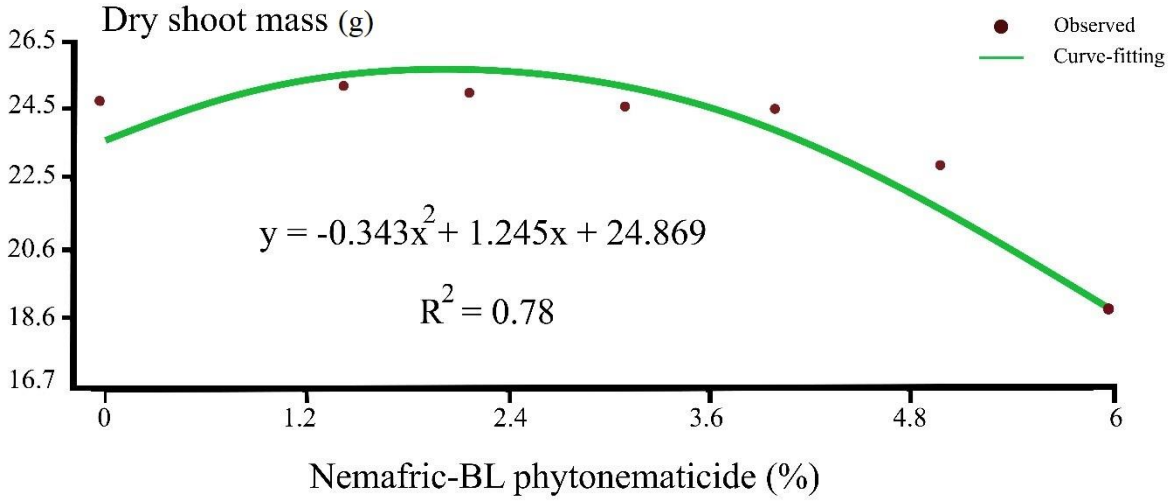

Figure 3.7 Response of dry shoot mass to increasing concentration of Nemafric-BL phytonematicide under greenhouse conditions.

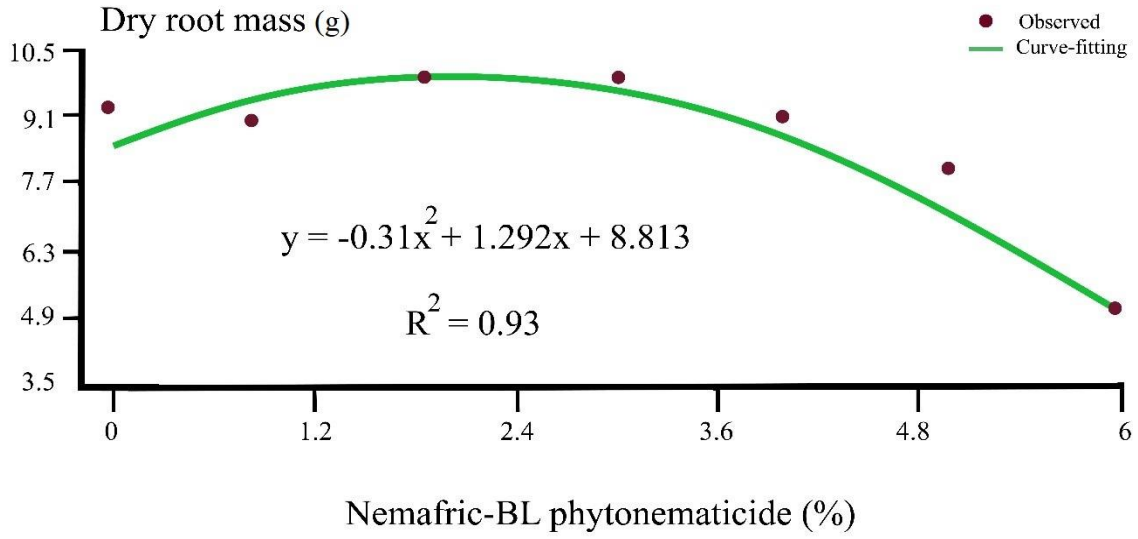

Figure 3.8 Response of dry root mass to increasing concentration of Nemafric-BL phytonematicide under greenhouse conditions.

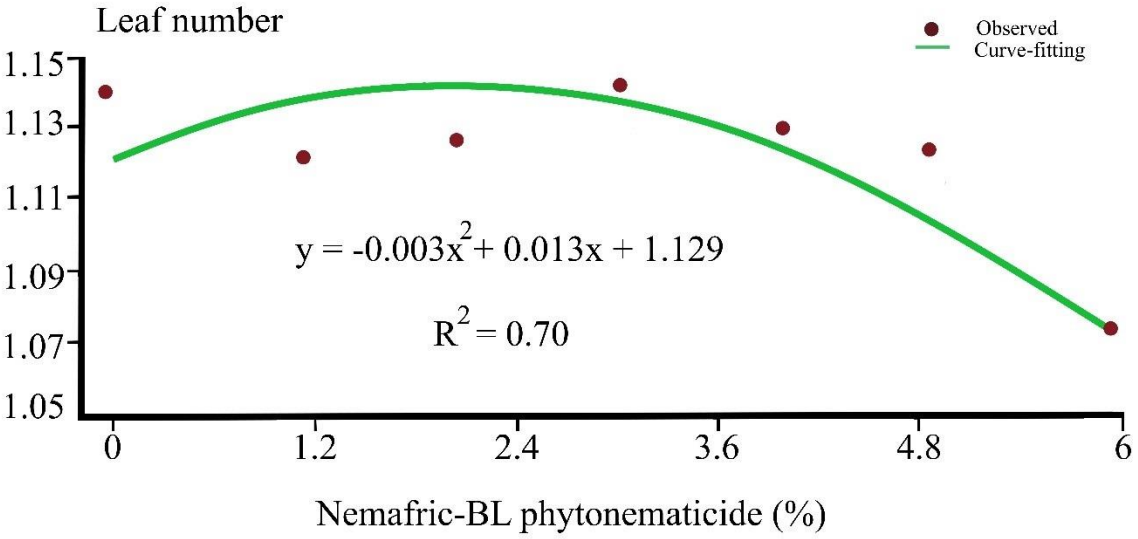

Figure 3.9 Response of leaf number to increasing concentration of Nemafric-BL phytonematicides under greenhouse conditions.

Table 3.1 Biological indices for dry shoot mass (DSM), dry root mass (DRM) and leaf number (LFN) of Swiss chard exposed to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides under greenhouse conditions.

Biological index	DSM	DRM	LFN	Average
	Nemarioc-AL phytonematicide			
Threshold stimulation (D_m)	4.595	0.801	1.717	2.371
Saturation point (R_h)	3.200	0.739	0.008	1.316
0% inhibition (D_0)	30.309	3.585	3.434	12.443
50% inhibition (D_{50})	179.595	45.200	15.882	80.226
100% inhibition (D_{100})	556.700	466.800	21.700	348.400
R^2	0.81	0.87	0.91	
Sensitivity (k)	1	2	0	
Overall sensitivity ($\sum k$) = 3				
	Nemafric-BL phytonematicide			
D_m	1.813	2.087	1.932	1.944
R_h	1.129	1.348	0.013	0.830
D_0	3.627	4.173	3.864	3.888
D_{50}	8.098	6.398	14.891	9.796
D_{100}	10.500	7.800	20.200	12.833
R^2	0.78	0.93	0.70	
Sensitivity (k)	0	0	0	
Overall sensitivity ($\sum k$) = 0				

Nemarioc-AL phytonematicide: MCSP = $D_m + (R_h/2)$ = 2.371 + (1.316/2) = 3.03%

Nemafric-BL phytonematicide: MCSP = $D_m + (R_h/2)$ = 1.944 + (0.83/2) = 2.36%

Nutrient elements: Potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and zinc (Zn) against Nemarioc-AL phytonematicide exhibited positive quadratic equations (Figure 3.10; Figure 3.11). The quadratic models for the respective variables were explained by 96, 79, 64, 78 and 77% association. Also, Ca, Mg and Zn in leaf tissues of Swiss chard

over Nemafric-BL phytonematicide exhibited positive quadratic relations, whereas K and Fe exhibited negative quadratic relations (Figure 3.10; Figure 3.11). The models for the respective variables were explained by 90, 68, 84, 72 and 63% associations (Table 3.2). Using $x = -b_1/2b_2$ relations (Gomez and Gomez, 1984), K, Ca, Mg, Fe and Zn (Table 3.2) in leaf tissues under Nemarioc-AL phytonematicide were optimised at 0.48, 1.87, 14,45, 2.57 and 1.87%, respectively. Potassium, Ca, Mg, Fe and Zn under Nemafric-BL phytonematicide were optimised at 3.37, 2.18, 2.56, 2.16 and 2.19%, respectively (Table 3.2).

Table 3.2 Optimisation model of nutrient elements in leaf tissues of Swiss chard as affected by increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides under greenhouse conditions.

	Model	R^2	x^z
Element	Nemarioc-AL phytonematicide		
K	$Y = 0.0204x^2 - 0.0197x + 1.8015$	0.96	0.48
Ca	$Y = -0.0005x^2 + 0.0172x + 0.4486$	0.79	1.87
Mg	$Y = -0.001x^2 + 0.0289x + 0.7268$	0.64	14.45
Fe	$Y = 0.9961x^2 + 5.1254x + 627.26$	0.78	2.57
Zn	$Y = -0.4811x^2 + 1.7996x + 172.54$	0.77	1.87
	Nemafric-BL Phytonematicide		
K	$Y = 0.0441x^2 - 0.2977x + 2.7648$	0.72	3.37
Ca	$Y = -0.0058x^2 + 0.0253x + 0.5484$	0.90	2.18
Mg	$Y = -0.0114x^2 + 0.0584x + 0.8628$	0.68	2.56
Fe	$Y = 9.735x^2 - 42.069x + 734.11$	0.63	2.16
Zn	$Y = -1.842x^2 + 8.0968x + 172.05$	0.84	2.19

$X = -b_1/2b_2$.

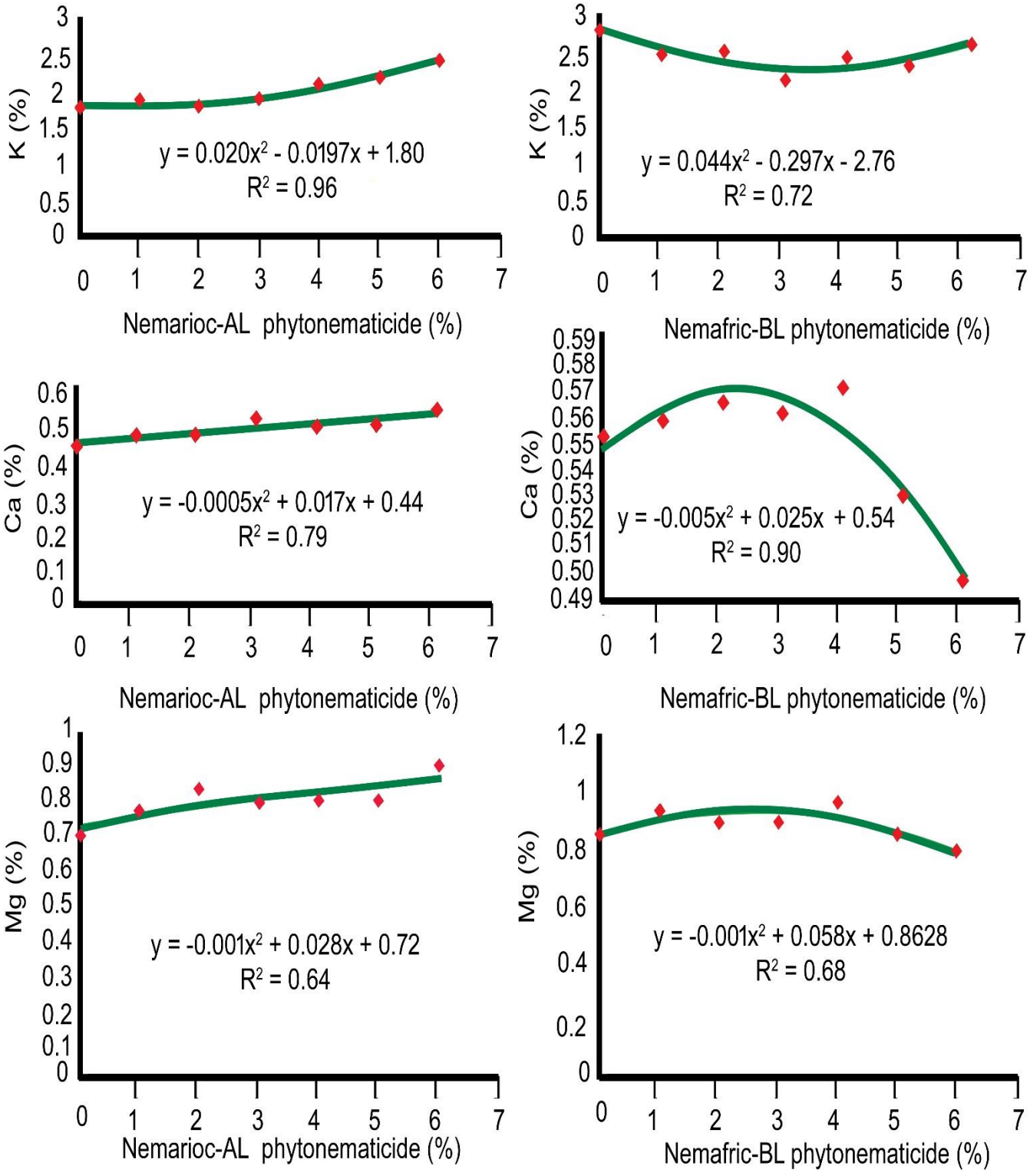

Figure 3.10 Response of potassium (K), calcium (Ca) and magnesium (Mg) in leaf tissues of Swiss chard to increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides at 56 days after initiation of treatments under greenhouse conditions.

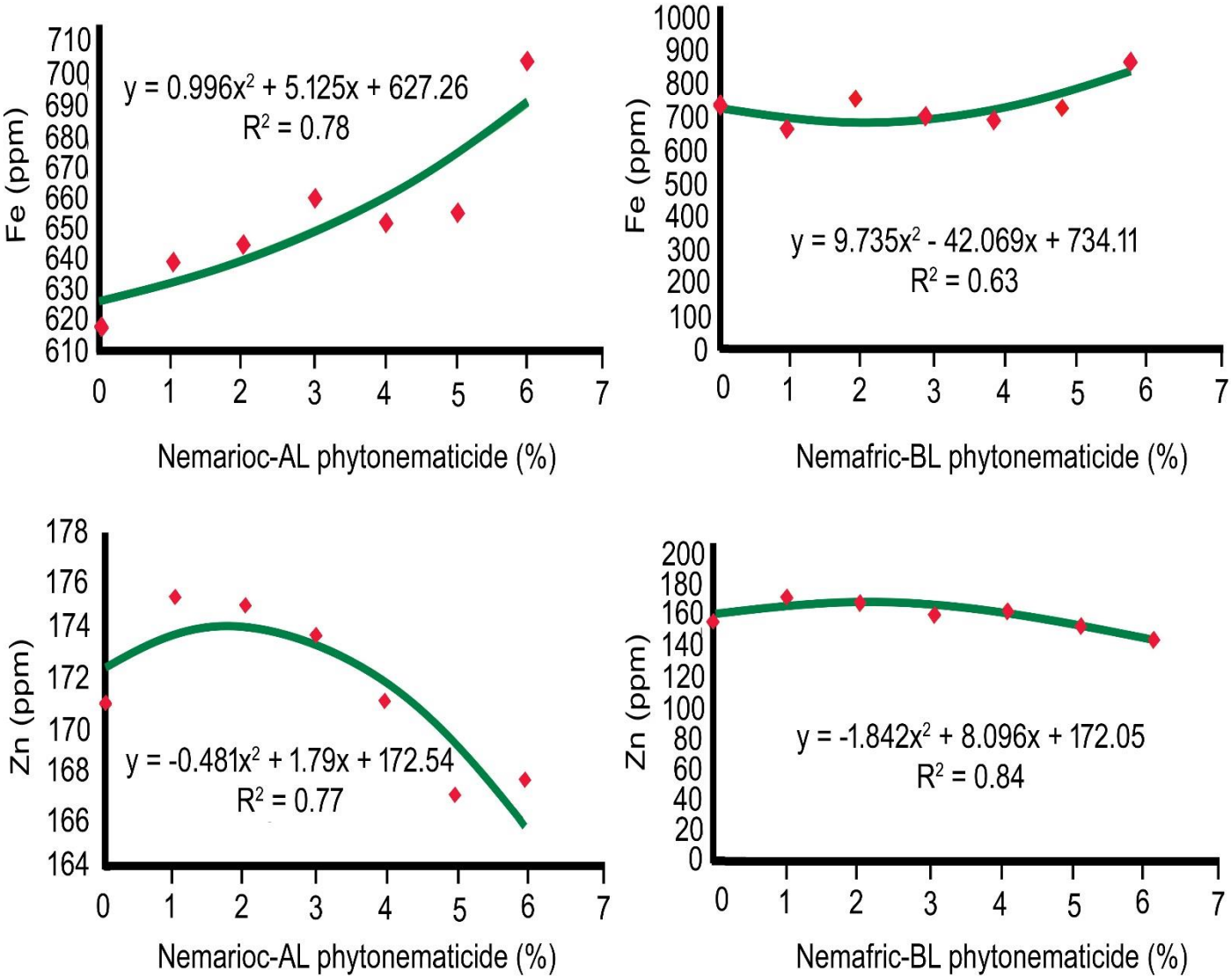

Figure 3.11 Response of iron (Fe) and zinc (Zn) in leaf tissues of Swiss chard to increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides at 56 days after initiation of treatments under greenhouse conditions.

3.3.2 Microplot experiment

Nematode variables: Eggs in root, J2 in roots and final nematode population (Pf) against increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides exhibited negative quadratic relations (Figure 3.12). The model for eggs in root, J2 in roots and Pf for Nemarioc-AL phytonematicide were explained by 84, 92 and 72%, whereas for Nemafric-BL phytonematicide were explained by 92, 82 and 91%, respectively (Figure 3.12).

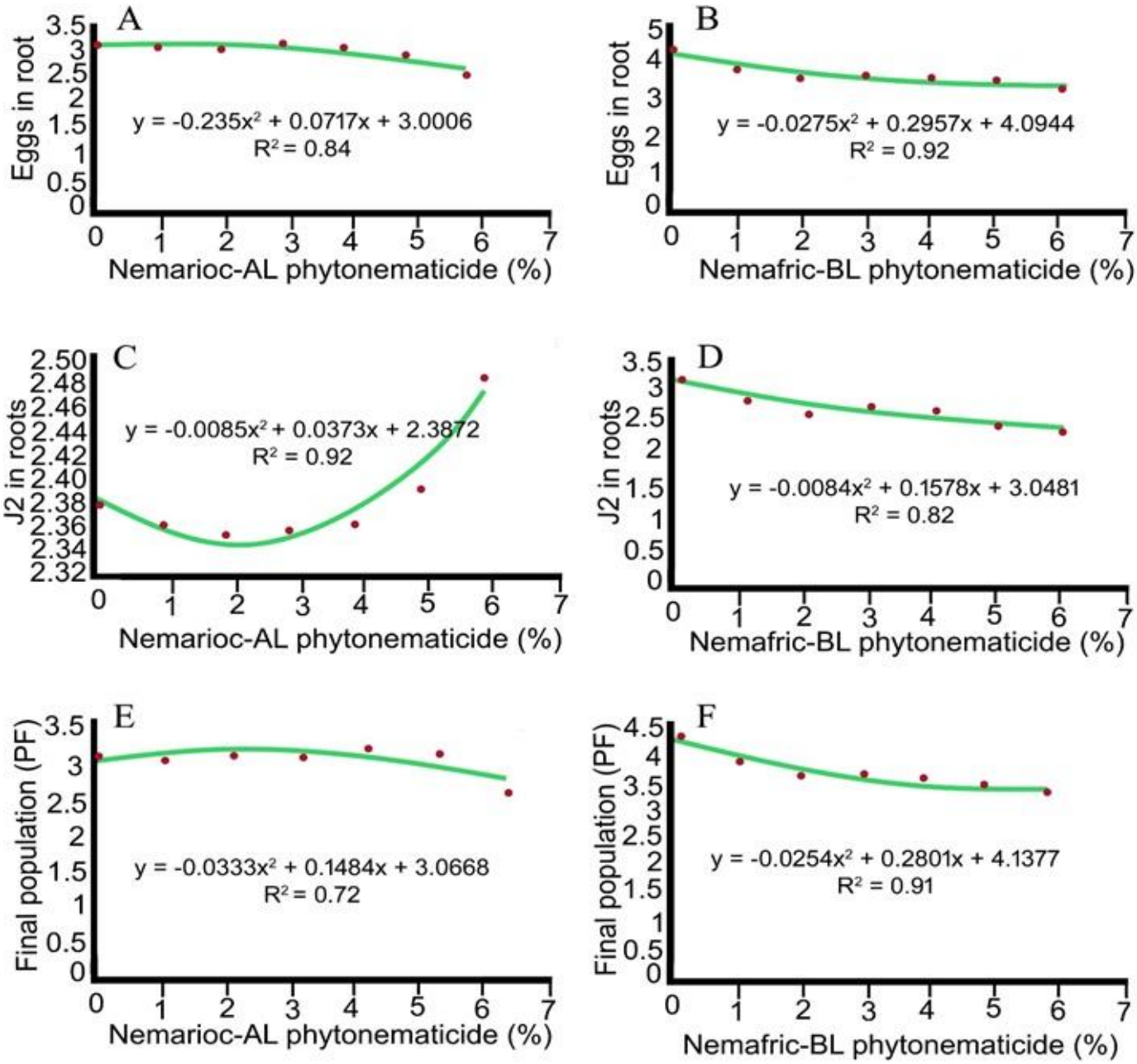

Figure 3.12 Response of *Meloidogyne javanica* eggs in root, J2 in roots and Pf to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides at 56 days after initiation of treatment under microplot conditions.

Plant variables: Dry shoot mass (Figure 3.13) and gall rating (Figure 3.14) over Nemarioc-AL phytonematicide exhibited positive quadratic relation, with models explained by 95 and 96% associations, respectively. Similarly, dry shoot mass (Figure 3.15) and gall rating (Figure 3.16) over Nemafric-BL phytonematicide exhibited positive quadratic relations, with models being explained by 84 and 97% associations, respectively.

Using the relation MCSP = D_m + (R_h/2) (Mashela *et al*., 2017), MCSP of Nemarioc-AL and Nemafric-BL phytonematicides for application on Swiss chard was 3.71 and 3.33%, respectively (Table 3.3). Nemarioc-AL phytonematicide had k values of 2 and 0 units on dry shoot mass and gall rating, respectively, with the Σk values of 2 units on Swiss chard (Table 3.3). Similarly, Nemafric-BL phytonematicide had k values of 1 and 0 units on dry shoot mass and gall rating, respectively, with the Σk of 1 unit (Table 3.3).

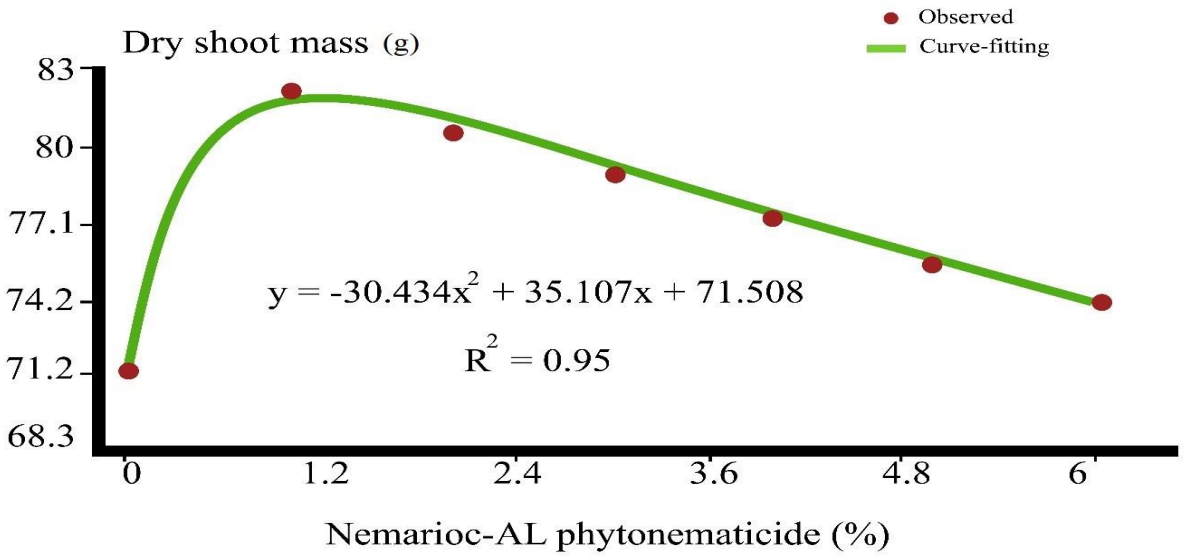

Figure 3.13 Response of dry shoot mass to increasing concentration of Nemarioc-AL phytonematicide under microplot conditions.

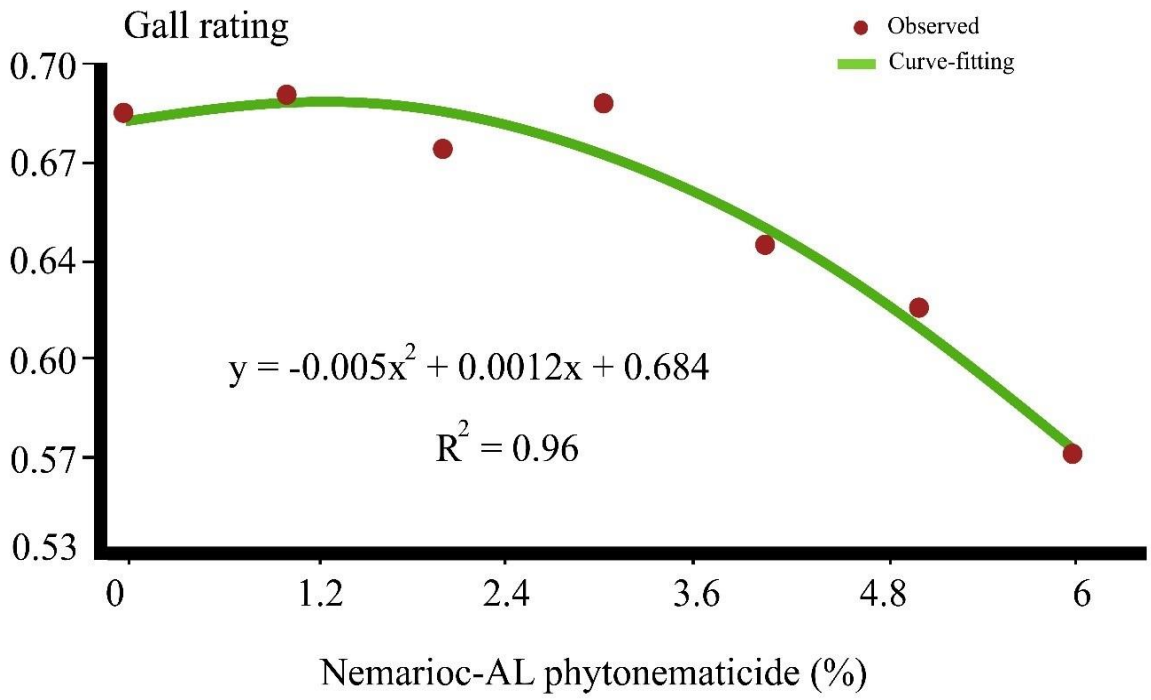

Figure 3.14 Response of gall rating to increasing concentration of Nemarioc-AL phytonematicide under microplot conditions.

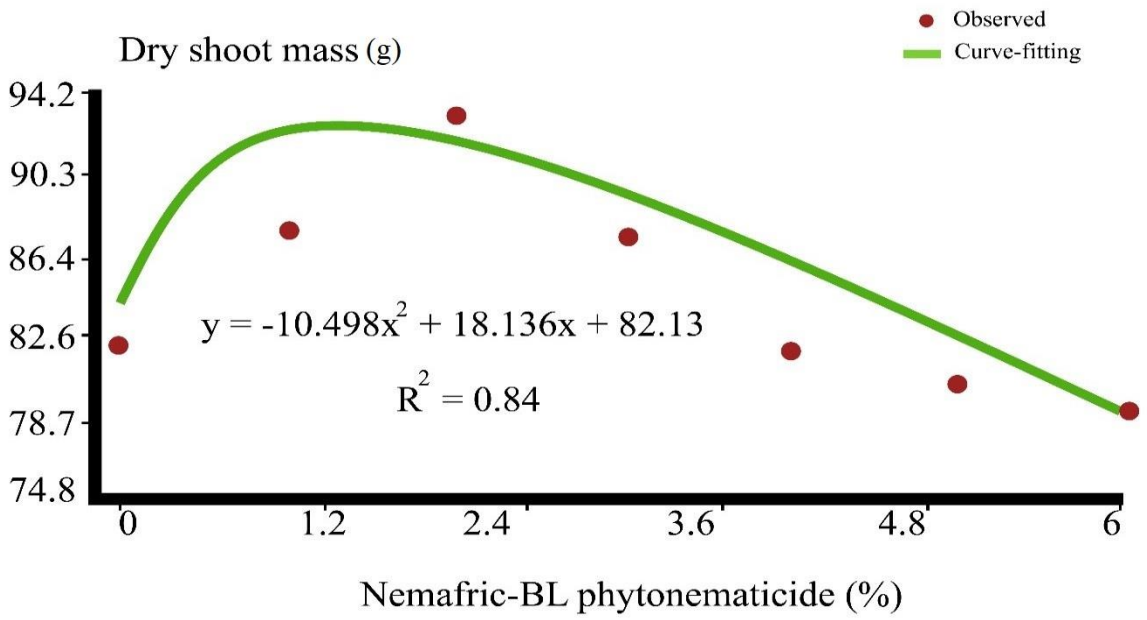

Figure 3.15 Response of dry shoot mass to increasing concentration of Nemafric-BL phytonematicide under microplot conditions.

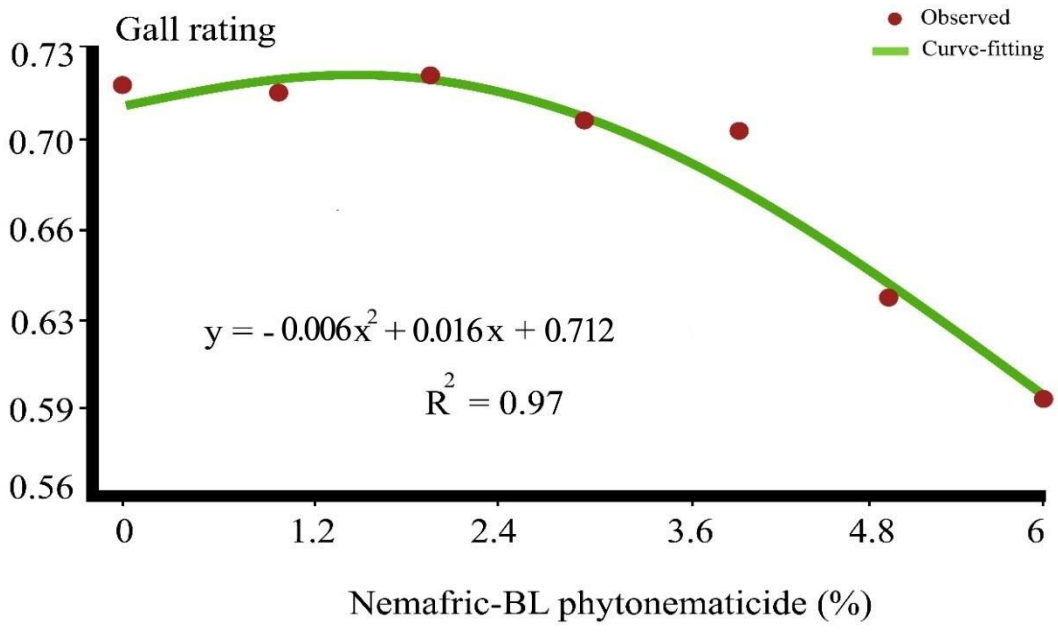

Figure 3.16 Response of gall rating to increasing concentration of Nemafric-BL phytonematicide under microplot conditions.

Table 3.3 Biological indices for dry shoot mass (DSM) and gall rating (GR) of Swiss chard exposed to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicides under micro-plot conditions.

	DSM	GR	Average
Biological index	Nemarioc-AL phytonematicide		
Threshold stimulation (D_m)	1.182	1.179	1.181
Saturation point (R_h)	10.125	0.007	5.066
0% inhibition (D_0)	7.754	2.359	5.057
50% inhibition (D_{50})	159.355	9.448	84.402
100% inhibition (D_{100})	-	12.8	6.4
R^2	0.95	0.96	
Sensitivity (k)	2	0	
Overall sensitivity (∑k) = 2			
	Nemafric-BL phytonematicide		
D_m	1.372	1.367	1.371
R_h	7.833	0.011	3.922
D_o	4.627	2.734	3.681
D_{50}	19.533	9.196	14.365
D_{100}	43.300	12.400	27.850
R^2	0.84	0.97	
Sensitivity (k)	1	0	
Overall sensitivity (∑k) = 1			

Nemarioc-AL phytonematicide: MCSP = $D_m + (R_h/2)$ = 1.181+ (5.066/2) = 3.71%

Nemafric-BL phytonematicide: MCSP = $D_m + (R_h/2)$ = 1.371+ (3.922/2) =3.33%

Nutrient elements: Potassium, Ca and Mg over Nemarioc-AL phytonematicide exhibited positive quadratic relations, whereas Fe and Zn exhibited negative quadratic relations (Figure 3.17; Figure 3.18). The models for K, Ca, Mg, Fe and Zn were explained by 82, 90, 98, 91 and 79% associations, respectively (Table 3.4). Similarly, K, Ca and Zn against Nemafric-BL phytonematicide exhibited positive quadratic relations (Figure 3.17; Figure 3.18), with the respective models explained by 60, 68 and 95% associations, respectively (Table 3.4). Using $x = -b_1/2b_2$ relations (Gomez and Gomez, 1984), K, Ca, Mg, Fe and Zn in leaf tissues over Nemarioc-AL phytonematicide were optimised at the x-values 6.82, 54, 3.59, 4.44 and 11.26%, respectively (Table 3.4). Similarly, under Nemafric-BL phytonematicide, K, Ca and Zn in leaf tissues were optimised at 0.49, 15.13 and 2.56 %, respectively (Table 3.4).

Table 3.4 Optimisation model of nutrient elements in leaf tissues of Swiss chard as affected by increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides under microplot conditions.

	Model	R^2	x^z
Element	Nemarioc-AL phytonematicide		
K	$Y = -0.0138x^2 + 0.1881x + 1.0263$	0.82	6.82
Ca	$Y = -0.0002x^2 + 0.0216x + 0.5063$	0.90	54
Mg	$Y = -0.0067x^2 + 0.0482x + 0.8272$	0.98	3.59
Fe	$Y = 9.6142x^2 - 85.424x + 857.4$	0.91	4.44
Zn	$Y = 0.3476x^2 - 7.8307x + 213.7$	0.79	11.26
	Nemafric-BL phytonematicide		
K	$Y = 0.0099x^2 - 0.0098x + 1.4414$	0.60	0.49
Ca	$Y = 0.0074x^2 - 0.0224x + 0.5586$	0.68	15.13
Zn	$Y = -2.1939x^2 + 11.228x + 188.38$	0.95	2.56

$X = -b_1/2b_2$.

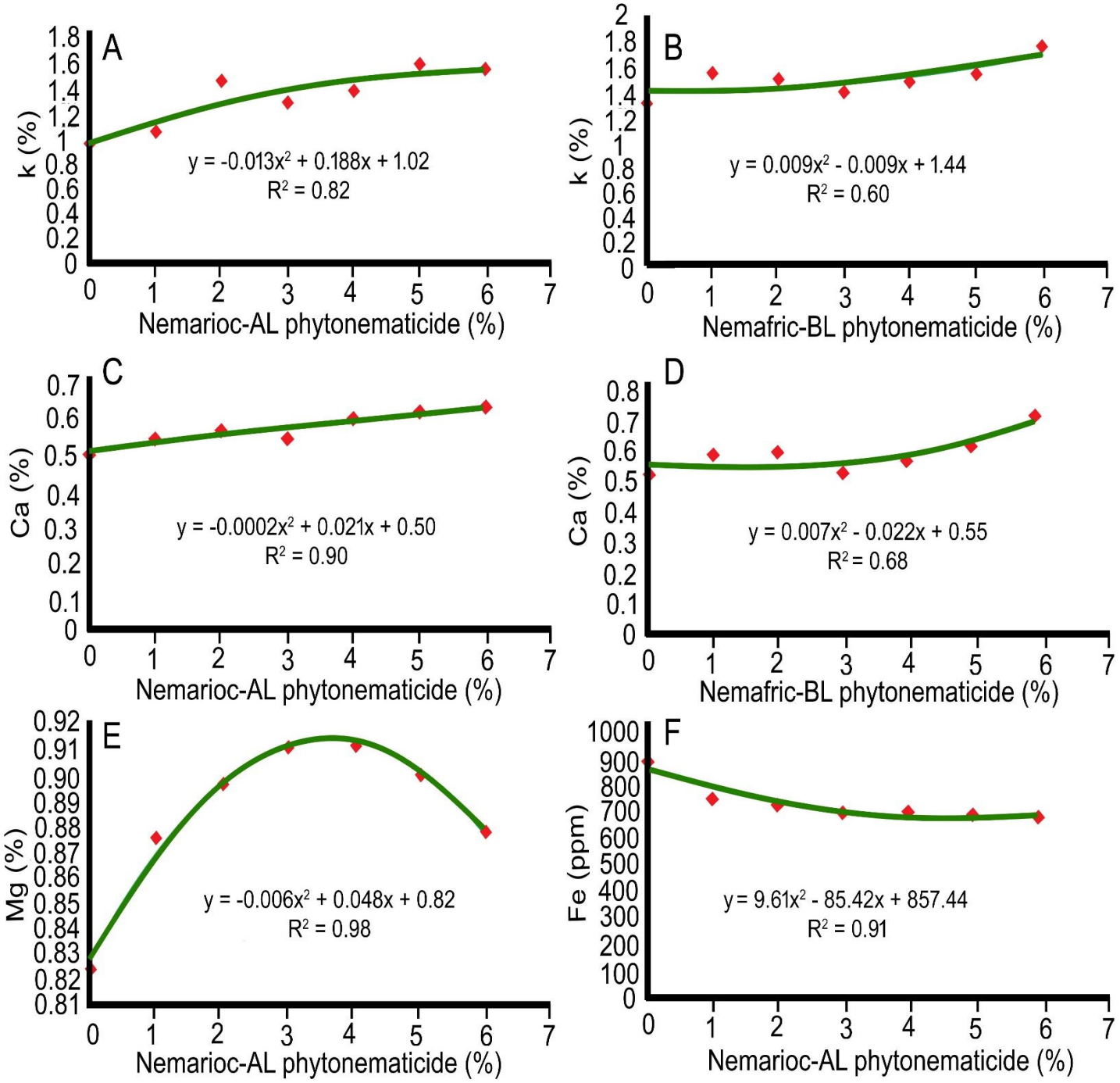

Figure 3.17 Response of potassium (K), calcium (Ca), magnesium (Mg) and iron (Fe) in leaf tissues of Swiss chard to increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides at 56 days after initiation of treatments under microplot conditions.

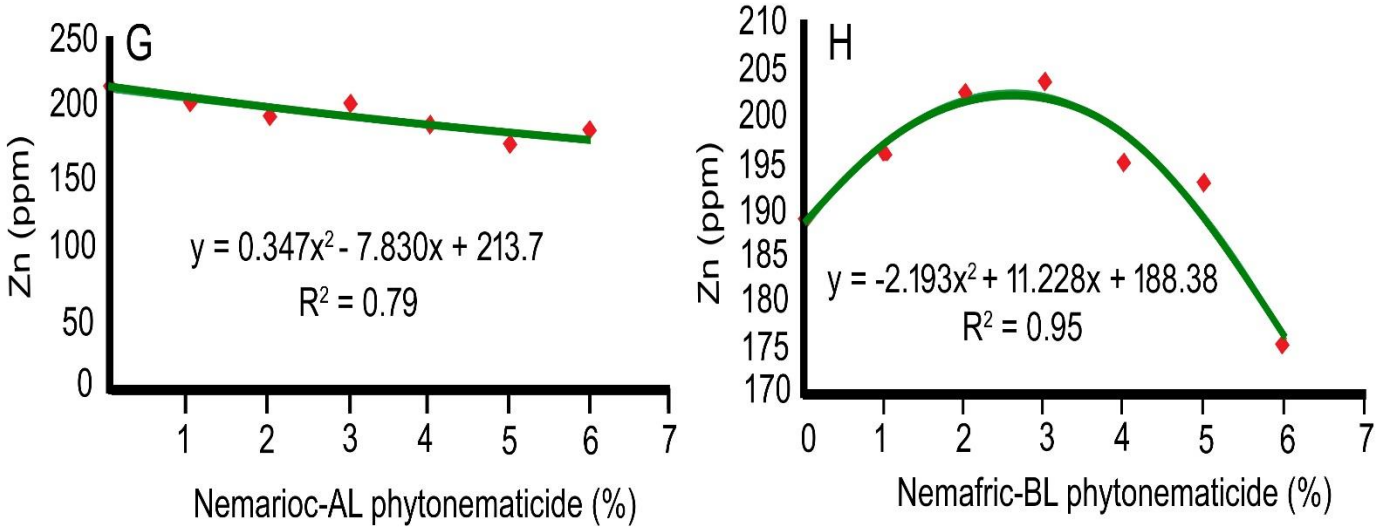

Figure 3.18 Response of zinc (Zn) in leaf tissues of Swiss chard to increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides at 56 days after initiation of treatments under microplot conditions.

3.4 Discussion

3.4.1 Nematode variables

Greenhouse experiment: Eggs in roots, J2 in roots and Pf against each phytonematicide exhibited negative quadratic relations, characterised by having three phases, namely, stimulation, neutral and inhibition phases (Mashela *et al.*, 2017). The observed results agree with the findings on tomato plants, where eggs in roots, J2 in roots and Pf against Nemarioc-AL phytonematicide exhibited negative quadratic relations (Tseke and Mashela, 2018). Nemarioc-AL and Nemafric-BL phytonematicides, as shown in this and other studies (Pelinganga, 2013; Tseke and Mashela, 2017), consistently reduced nematode numbers when used under greenhouse conditions. Additionally, the suppression of nematode population densities by the test, the cucurbitacin-containing phytonematicides in the current study confirmed observations on green bean (*Phaseolus vulgaris* L.*)* (Chokoe, 2017), butternut squash (*Cucurbita pepo* L.) (Lebea, 2017) and

beetroot (*Beta vulgaris.* L.) (Mashitoa, 2017). Additionally, J2 hatch of *M. incognita in vitro* at 24-, 48- and 72-h exposure periods to purified active ingredients, cucurbitacin A and B, of the two phytonematicides exhibited negative quadratic relations (Dube, 2016).

Generally, the response of nematode variables to the two cucurbitacin-containing phytonematicides exhibited negative quadratic relations, characterised by the DDG patterns as conceptualised by Mashela *et al*. (2017). The DDG patterns have three phases, namely; stimulation, neutral and inhibition phases (Liu *et al*., 2003). In the current study, the inhibition phase, as shown on nematode variables against Nemarioc-AL and Nemafric-BL phytonematicides, confirmed reports that the products effectively and consistently suppressed nematode population densities (Mashela *et al*., 2017). A decrease in all nematode variables suggest that cucurbitacin A and cucurbitacin B as active ingredients (Chen *et al*., 2005) in the respective phytonematicides were nematicidal as opposed to being nematostatic.

Microplot experiment: Eggs in roots, J2 in roots and Pf of *M. javanica* over Nemarioc-AL and Nemafric-BL phytonematicides exhibited negative quadratic relations, suggesting their subscription to the DDG pattern. The observed results agreed with findings on different crops, where the test phytonematicides had nematicidal effects on nematodes in both roots and soils under microplot conditions (Chokoe, 2017; Lebea, 2017; Mashitoa, 2017; Sithole, 2016). Eggs in roots depicted inhibition patterns, suggesting that the active ingredients for both phytonematicides penetrated female nematode bodies and actually killed them, with eggs failing to be released into the egg masses (Agbenin *et al*., 2005;

Mashela *et al*., 2015), thereby reducing the egg number. Generally, bioactivity studies (Dube, 2016) demonstrated that the two phytonematicides have multiple-modes of action, which suppress nematode population numbers through increased mortalities, inhibited J2 hatch and disoriented mobility. In the life cycle of *Meloidogyne* species, J2 hatch, with J2 moving through soil solutions searching for penetration sites in the elongation region (Ferraz and Brown, 2002). During J2 migration, J2 become exposed to active ingredients in soil solutions, resulting in increased mortality as confirmed by inhibition phase on J2 in roots when exposed to cucurbitacin-containing phytonematicides (Mashela *et al*., 2017). Inhibition patterns in the current study confirmed that nematode variables were more sensitive to the active ingredients cucurbitacin A and B, as observed under *in vitro* conditions on J2 hatch, motility and viability of eggs and J2 of *Meloidogyne* species (Dube, 2016; Dube *et al*., 2019). Consequently, Pf in roots was reduced, in agreement with the conceptualised inhibition phase (Mashela *et al*., 2017).

3.4.2 Plant variables

Greenhouse experiment: Dry shoot mass, dry root mass and leaf number over Nemarioc-AL phytonematicide exhibited positive quadratic relations, with such relations being preferred in the use of the CARD model for establishing the MCSP value. Similar results were observed on tomato plants when dry shoot mass and dry root mass were subjected to Nemarioc-AL phytonematicide under greenhouse conditions (Pelinganga, 2013; Tseke, 2013). In contrast, only dry shoot mass and dry root mass over Nemafric-BL phytonematicide displayed positive quadratic relations under greenhouse conditions. The observed results confirm findings on green bean (Chokoe, 2017), butternut squash

(Lebea, 2017) and beetroot (Mashitoa, 2017) under greenhouse conditions, where plant variables and Nemafric-BL phytonematicide also exhibited positive quadratic relations, characterised by the DDG patterns. The results indicated that at high concentration the two phytonematicides would be phytotoxic to Swiss chard, resulting in reduced plant growth. Conversely, the DDG patterns also displayed the stimulation phase when Swiss chard was exposed to Nemarioc-AL and Nemafric-BL phytonematicides at lower concentration (Liu *et al*., 2003), with the neutral phase lying between the two mentioned phases. Results in the current study supported observation on tomato and other plants exposed to different concentration of the two products (Mashela *et al.*, 2017; Pelinganga, 2013; Tseke, 2013).

The MCSP values of Nemarioc-AL and Nemafric-BL phytonematicides on Swiss chard under greenhouse conditions were at 3.03 and 2.36%, respectively, which was equivalent to that on tomato plants (2.64 and 2.99%) under similar conditions (Pelinganga, 2013). However, the 2.11% MCSP value for Nemarioc-AL phytonematicide on green bean cultivar 'Tahoe' under greenhouse conditions was comparatively lower than that on Swiss chard (Chokoe, 2017). The MCSP value for Nemafric-BL phytonematicide on Swiss chard was 2.36%, which was equivalent to that of tomato and butternut squash plants at 2.64% and 2.83%, respectively, under greenhouse conditions (Lebea, 2017; Pelinganga, 2013). Findings of the current study confirmed observations (Mashela *et al*., 2017) that MCSP values were plant-specific. In general, MCSP values which are on the higher side, for example, above 3%, could be adjusted downward (e.g. below 3%) since such values were still suppressing Pf for *Meloidogyne* species.

Another most important attribute of the CARD model was its ability to provide the overall sensitivity (Σk) value of the test crop to the phytonematicide. Generally, as Σk approaches zero, the test plant is increasingly sensitive to the test phytonematicide, whereas the opposite suggests tolerance (Liu *et al*., 2003). In Nemarioc-AL phytonematicide study, Swiss chard had Σk value of 3 units, suggesting a higher degree of tolerance. This Σk value of Swiss chard was similar to that of tomato plants (Pelinganga, 2013), but was rather higher than that of 1 unit on green bean (Chokoe, 2017) and 0 unit on beetroot (Mashitoa, 2017). However, Σk of Nemafric-BL phytonematicide on Swiss chard was 0 unit, suggesting that the test crop was sensitive to the product. The finding was similar to that on green bean (Chokoe, 2017), but slightly less than the 1 unit on beetroot (Mashitoa, 2017). In contrast, the Σk values on tomato plants for Nemafric-BL phytonematicide were at 3 units (Tseke, 2013) or at 5 units (Pelinganga, 2013), suggesting tolerance of the test plant to the test product. Apparently, the degree of sensitivity of plants to cucurbitacin-containing phytonematicides is product-, plant- and concentration-specific.

Microplot experiment: Dry shoot mass and gall rating over Nemarioc-AL phytonematicide exhibited positive quadratic relations, with models explained by high associations. The results confirm findings on green bean (Chokoe, 2017), wild geranium (*Pelargonium sidoides* DC.) (Sithole, 2016) and beetroot (Mashitoa, 2017) under microplot conditions, where plant variables against Nemarioc-AL phytonematicide exhibited positive quadratic relations. Additionally, other phytonematicides such as nemalan from lantana (*Lantana camara* L.) plants, stimulated plant growth in tomato plants under microplot conditions (Malatji, 2017). Nemarioc-AL and Nemafric-BL phytonematicides also stimulated growth

on tomato plants during interaction trials under microplot conditions (Maake, 2018). In granular formulation, cucurbitacin-containing phytonematicides increased growth of tomato plants under microplot conditions (Khosa, 2013; Mashela *et al*., 2017).

Dry shoot mass and gall rating over Nemafric-BL phytonematicide exhibited positive quadratic relations, as observed on wild geranium (Sithole, 2016) and beetroot (Mashitoa, 2017) under similar conditions. These relations were observed when tomato plants were exposed to Nemalan phytonematicide (Malatji, 2017). Under microplot conditions, as observed under the greenhouse conditions, responses of Swiss chard variables to the two phytonematicides had attributes of the DDG patterns as articulated for plant variables versus allelochemical-containing products (Liu *et al*., 2003; Mafeo, 2012).

On the microplot, generated MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides appeared to support most other reports, which suggested that such an environment was highly variable to produce consistent MCSP data. For example, on microplot the MCSP values for the two phytonematicdes on Swiss chard were 3.71 and 3.33%, respectively. In contrast, MCSP value for Nemarioc-AL phytonematicide under similar conditions on wild geranium was 6.18% (Sithole, 2016) and on butternut squash was 11.85% (Lebea, 2017), both of which were rather high since nematode population densities could be reduced at much lower MCSP values (Mashela *et al*., 2017). However, the MCSP for Nemarioc-AL phytonematicide on green bean under microplot conditions was at 2.67% (Chokoe, 2017), which was equivalent to the usual values for most crops under greenhouse conditions (Mashela *et al*., 2017), which was also close to that for

Nemafric-BL phytonematicide on *P. sidoides* at 2.87% (Sithole, 2016). Conversely, the MCSP value of Nemafric-BL phytonematicide on beetroot at 10.2% (Mashitoa, 2017) supported the view of the unsuitability of this environment for the generation of MCSP data.

Under microplot conditions, the Σk values of Nemarioc-AL and Nemafric-BL phytonematicides on Swiss chard were 2 units and 1 unit, respectively. In other microplot studies, the Σk values of Nemarioc-AL phytonematicide on green bean was 20 units (Chokoe, 2017) and butternut squash at 0 unit (Lebea, 2017). In contrast, the Σk values of Nemafric-BL phytonematicide on beetroot (Mashitoa, 2017) and wild geranium (Sithole, 2016) were 3 and 4 units, respectively. Although the MCSP values of the two phytonematicides on crops were inconsistent, with the exception of the value on butter squash, empirically-based values suggested that most crops tolerant to the two test phytonematicides when exposed to such products under microplot conditions.

3.4.3 Nutrient elements

Greenhouse experiment: The accumulation of K, Ca, Mg, Fe and Zn in leaf tissue of Swiss chard exposed to Nemarioc-AL phytonematicide displayed positive quadratic relations, with attributes that depicted the DDG patterns (Mashela *et al*., 2017) as described for other variables in the study. The optimum values for accumulation nutrient elements in tissues of Swiss chard in the Nemarioc-AL and Nemafric-BL phytonematicides had 3.59-54% and 0.49-15.13% ranges, with most being outside of the empirically-derived MCSP values. Most importantly, it should be noted that the MCSP values and the optima values could not be compared since the data were analysed using different statistical

instruments, namely, the CARD (Liu *et al*., 2003) and the post-hoc test for ANOVA, namely, lines of the best fit.

Microplot experiment: Effects of the two phytonematicides had limited significant effects on nutrient elements in Swiss chard tissues under microplot conditions. For examples, K, Ca and Mg versus Nemarioc-AL phytonematicide exhibited positive quadratic relations, whereas Fe and Zn exhibited negative quadratic relations. The increase in K, Ca and Mg in leaf tissue of Swiss chard when exposed to Nemarioc-AL phytonematicide should not be confounded with the “fertiliser effects” which was conceptualised during the origin of cucurbitacin-plant interaction (Mashela, 2002). During the origination of the concept, there was no evidence of the accumulation of nutrient elements in leaf tissues of tomato plants (Mashela, 2002). Actually, what was observed then (Mashela, 2002), was the stimulation phase on plant growth, which had since been observed on numerous studies as outlined in the current study.

In leafy vegetables, K is required for photosynthesis, enzyme activation and protein synthesis (Prajapati and Modi, 2012), whereas Ca regulates cell wall construction and a component of plant cell walls (McCauley *et al*., 2009). Also, Mg is an important constituent of the chlorophyll molecule and component of the middle lamella that is essential in gluing together the adjacent cell walls (Huber and Jones, 2013). The decrease of Fe and Zn in leaf tissues of Swiss chard could be a disadvantage since the two are important in both plant growth and human nutrition. Lack of Fe could result in poor plant yield and reduced subsequent nutritional quality (Rout and Sahoo, 2015), whereas Zn deficiency results in

stunted growth, shortened petioles and small malformed leaves, along with nutritional quality (Hafeez *et al*., 2013). The optimum values for K, Ca and Mg falls within stimulation range, suggesting non-lethal activities on its accumulation in leaf tissue, whereas Fe and Zn were within inhibition phase indicating it was phytotoxic to the product.

In the current study, Nemafric-BL phytonematicide stimulated the accumulation of K, Ca and Zn in leaf tissues of Swiss chard, which confirmed observations when most plants are subjected to increasing concentration of this product (Mashela *et al*., 2017). Since in the current study the products were applied at one level, comparisons with those at different levels using the CARD algorithm computer model are appropriate (Liu *et al*., 2003; Mashela *et al*., 2017). Notwithstanding, the stimulation of the three elements was an indication of tolerance to Nemafric-BL phytonematicides.

3.5 Conclusion

The non-phytotoxic concentration of Nemarioc-AL and Nemafric-BL phytonematicides were successfully developed at 3.03 and 2.36% under greenhouse conditions, wheareas microplot conditions had 3.71 and 3.33%, respectively. Nematode variables, plant variables and selected nutrient elements followed a density-dependent growth (DDG) patterns as affected by increasing Nemarioc-AL and Nemafric-BL phytonematicides concentrations. However, in order to avoid challenges of phytotoxicity caused by phytonematicides on Swiss chard, the derived MCSP must be utilised on nematode infested Swiss chard.

CHAPTER 4
EFFECTS OF TWO PHYTONEMATICIDES ON GROWTH AND NUTRIENT ELEMENTS OF NEMATODE-INFESTED SWISS CHARD UNDER FIELD CONDITIONS

4.1 Introduction

The cucurbitacin-containing phytonematicides reduced population densities of root-knot (*Meloidogyne* species) nematodes and improved growth and accumulation of selected nutrient elements in Swiss chard (*Beta vulgaris* L. cicla) leaf tissues raised in plastic pot containers under greenhouse and microplot conditions (Chapter 3). Greenhouse and microplot conditions constitute controlled and semi-controlled conditions, respectively, with the common attribute being the pasteurised growing media in pots. In addition to restricted microbes which could interact with the phytonematicides, such containers restrict root growth as well as the percolation of the active ingredients throughout the soil, thereby increasing root contact with the active ingredients (Salisu *et al*., 2018). In contrast, under field conditions most of the factors which were previously controlled (Chapter 3), are purely in the form in which the farmers naturally use the land. Under such conditions, root growth and active ingredients are not restricted by physical barriers. Additionally, *Meloidogyne* species co-habit the soil with other plant-parasitic nematodes such as *Pratylenchus neglectus*, *Nanidorus minor* and *Scutylenchus rugosus* (Shokoohi *et al*., 2019), free-living nematodes like *Acrobeles* (Shokoohi *et al*., 2007a), *Chiloplacu*s (Shokoohi *et al*., 2007b) and *Cephalobus* (Amirzadi *et al*., 2013) and soil microbes. All these could interact with the phytonematicides and *Meloidogyne* species, to give a different result from what was observed in the previous study (Chapter 3). For example, the ring (*Helicotylenchus* species) nematodes, which occur in high numbers at the

location of the study, are known to displace *Meloidogyne* species when occurring in large numbers. Consequently, results from studies under controlled conditions could be different from those under uncontrolled conditions, although the latter are ideal since the conditions emulate those under which the farmers operate. The effects of nemarioc-group [Nemarioc-AL (L = liquid formulation), Nemarioc-AG (G = granular formulation)] and nemafric-group [Nemafric-BL, Nemafric-BG] phytonematicides on suppression of *Meloidogyne* species and the productivity and accumulation of nutrient elements on Swiss chard under field conditions had not been documented. The objective of this study, therefore, was to determine whether nemarioc-group and nemafric-group phytonematicides in liquid and granular formulations would affect population densities of *Meloidogyne* species and the productivity of Swiss chard with related accumulation of nutrient elements in leaf tissues under field conditions.

4.2 Materials and methods

4.2.1 Description of the study site

The study was conducted on an open field at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, South Africa (23º53''10'S, 29º44''15'E) during autumn (February – April) 2018 and validated during 2019. The location has summer (October – December) mean annual rainfall of less than 500 mm and average summer maximum/minimum temperatures of 38/19˚C, with Hutton loam soil (65% sand, 30% clay, 5% silt), containing 1.6% organic C, EC 0.148 dS/m and pH (H_2O) 6.5.

4.2.2 Treatments and experimental design

Two parallel experiments were initiated with experiment 1 and experiment 2 comprising the nemarioc-group and nemafric-group phytonematicides, respectively. Experiment 1, with treatments 0, 2 g Nemarioc-AG phytonematicide and 3% Nemarioc-AL phytonematicide arranged in a randomised complete block design (RCBD) was replicated 8 times. In contrast, Experiment 2, with treatments 0, 2 g Nemafric-BG phytonematicide and 3% Nemafric-BL phytonematicide, arranged in RCBD was replicated 7 times. In both experiments, blocking was done for shading from windbreaks trees and a slight slope (Figure 4.1).

Figure 4.1 Swiss chard cv. 'Fordhoek giant' treated with Nemarioc-AG and Nemarioc-AL phytonematicides or Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

4.2.3 Procedures and cultural practices

Procedures and cultural practices were as described previously (Chapter 3), except that a portion of each product was not fermented to provide a granular formulation. Also, plants were not inoculated because the field was already infested with nematodes. Uniform seedlings were transplanted directly into the field at 0.60 m × 0.30 m spacing, where each hole of drip irrigation system had four Swiss chard seedlings at similar distances from the drip hole. Soon after transplanting, cutworm bait was applied at 5 g per drip hole and mulched using maize stalks to suppress weeds and conserve soil moisture. Nemarioc-AG and Nemafric-BG phytonematicides at 2 g each, were applied once 7 days after transplanting, with subsequent repeats. In contrast, although Nemarioc-AL and Nemafric-BL phytonematicides were applied at 3% each soon after transplanting, the products were re-applied at every 17 days. Plants were irrigated with chlorine-free tapwater every other day for 2 hours using drip irrigation system with an output 1 litre water per hour.

4.2.4 Data collection

At 64 days after initiating the treatments, plant and nematode data were collected as described previously (Chapter 3), except that nematodes were not extracted from soil samples. Eggs from roots and second-stage juveniles (J2) were further expressed as the reproductive potential (RP = Eggs + J2/ g fresh roots).

4.2.5 Data analysis

Data were subjected to analysis of variance (ANOVA) using SAS software (SAS Institute Inc, 2008). The degrees of freedom and their associated mean of squares were partitioned for significant variables at the probability level of 5%, to determine the

percentage contribution of sources of variation in the total treatment variation (TTV) of the variables. Mean separation was achieved using Fisher's Least Significant Difference test at the probability level of 5%. Unless otherwise stated, only treatment effects significant at the probability of 5% were discussed.

4.3 Results

The assessed seasonal interactions were not significant on all variables, therefore data within each experiments (experiment 1 and experiment 2) were pooled and re-analysed (Gomez and Gomez, 1984).

4.3.1 Nematode variables

Nemarioc-AG and Nemarioc-AL phytonematicides were highly significant ($P \leq 0.01$) on eggs in roots and reproductive potential (RP), contributing 79 and 77% in TTV of the respective variables (Appendix 4.1). Although, the two phytonematicides were not significant on second-stage juveniles (J2) in root (Appendix 4.1). In contrast, Nemafric-BG and Nemafric-BL phytonematicides were highly significant on eggs in roots and the RP, contributing 67 and 76% in TTV of the respective variables (Appendix 4.1). However, the products did not have significant effects on J2 in roots.

Relative to untreated control, Nemarioc-AG and Nemarioc-AL phytonematicides significantly reduced eggs in roots by 14 and 15%, respectively, whereas the effects of the two products did not differ from each other (Table 4.1). Similarly, the respective products reduced RP by 59 and 61%. In contrast, Nemafric-BL phytonematicide significantly increased eggs in roots and RP by 13 and 173%, respectively, whereas the

effects of Nemafric-BG phytonematicide on the two variables were not different to those of the untreated control (Table 4.1).

4.3.2 Plant variables

Both the nemarioc-group phytonematicides (Appendix 4.12 – 4.13) and nemafric-group phytonematicides (Appendix 4.14 – 4.15) did not have significant effects on all plant variables.

4.3.3 Nutrient elements

The nemarioc-group phytonematicides were significant on K and Mg, contributing 56 and 71% in TTV of the respective variables, but did not have significant effects on Na, Ca, P and S (Appendix 4.2). The nemafric-group phytonematicides were significant on Mg, contributing 62% in TTV of the variable, but did not affect Na, K, Ca, P and S (Appendix 4.3). The nemarioc-group (Appendix 4.4) and nemafric-group (Appendix 4.5) phytonematicides did not have significant effects on micronutrient elements B, Cu, Fe, Mn, Mo, Ni, Se and Zn.

Relative to the untreated control, Nemarioc-AG phytonematicide had no significant difference on K and Mg in leaf tissues of Swiss chard, but Nemarioc-AL phytonematicide reduced K and Mg by 17%, respectively, although the effects of Nemarioc-AL phytonematicides on K and Mg were similar (Table 4.2). Relative to untreated control, Nemafric-BG phytonematicide reduced Mg in Swiss chard by 10%, but Nemafric-BG and

Nemafric-BL phytonematicides did not have significant effects on the nutrient element, as were the untreated control and Nemafric-BL phytonematicide (Table 4.2).

Table 4.1 Response of eggs in roots and reproductive potential (RP) to cucurbitacin-containing phytonematicides on Swiss chard under field conditions at 64 days (n = 45).

	Eggs in roots		RP	
	Nemarioc-group phytonematicides			
Treatment	Mean[y]	R.I. (%)[z]	Mean	R.I. (%)
Control	3.25[a]	−	28.52[a]	−
Nemarioc-AG	2.79[b]	−14	11.67[b]	−59
Nemarioc-AL	2.74[b]	−15	11.15[b]	−61
	Nemafric-group phytonematicides			
Control	3.03[b]	−	24.86[b]	−
Nemafric-BG	3.19[b]	5	29.54[b]	19
Nemafric-BL	3.42[a]	13	67.93[a]	173

[y]Column means with 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].

Table 4.2 Response of potassium (K) and magnesium (Mg) in leaf tissues of Swiss chard to Nemarioc-AG/AL and Nemafric-BG/BL phytonematicides under field conditions at 64 days (n = 45).

Nemarioc-AG/AL phytonematicides					Nemafric-BG/BL phytonematicides		
	K		Mg			Mg	
Treatment	Mean	R.I. (%)	Mean	R.I. (%)	Treatment	Mean	R.I. (%)
Control	187.30[a]	−	66.69[a]	−	Control	74.60[a]	−
Nemarioc-AG	188.07[a]	0.41	63.15[ab]	−5.31	Nemafric-BG	67.34[b]	−9.73
Nemarioc-AL	155.24[b]	−17	55.30[b]	−17	Nemafric-BL	70.11[ab]	−6.02

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

4.4 Discussion

4.4.1 Nematode variables

The nemarioc-group phytonematicides were highly significant on nematode variables under Swiss chard production, confirming with other observations on butternut squash (*Cucurbita pepo* L.) (Lebea, 2017), beetroot (*Beta vulgaris.* L.) (Mashitoa, 2017) and tomato plants (*Solanum lycopersicum* L.) (Maake, 2018). Additionally, the results were comparable to another phytonematicide, pomegranate (*Punica granatum* L.) peel in granular formulation which significantly reduced nematode variables under greenhouse conditions (Regaieg *et al*., 2017).

In this study, Nemarioc-AL phytonematicide had significant effects on nematode variables under field conditions, which confirmed findings on nematodes in tomato (Maake, 2018; Seshweni, 2016), green bean (Chokoe, 2017) and butternut squash (Lebea, 2017) production under field conditions. Liquid formulations of neem (Abo-Elyousr *et al*., 2009), myrtle (*Myrtus communis* L.*)* (Oka *et al*., 2012) and pignut (*Hyptis suaveolens* L.) (Izuogu *et al*., 2016) also reduced nematode population densities on tomato and cowpea cultivars under field conditions. Additionally, liquid formulation of yellow fleabane (*Inula viscosa* L.*)* plant extract suppressed *Meloidogyne javanica* on lettuce plants under field conditions (Oka *et al.,* 2006). Similarly, liquid formulation of pignut plant extract significantly reduced nematode population density under field conditions (Izuogu *et al*., 2016).

In granular formulation, as observed under various conditions (Mashela *et al*., 2017), the cucurbitacin-containing phytonematicides in the current study suppressed various stages

of nematodes. Similarly, jimson weed (*Datura stramonium* L.) in granular formulation reduced root-knot nematodes on sweet melon under field conditions (Umar and Ngwamdai, 2015).

Nematodes are exposed to phytonematicides either by granular, liquid or air-borne volatilised chemicals in the rhizosphere (Mashela *et al*., 2015). Recent studies demonstrated that cucurbitacin-containing phytonematicides were nematicidal to nematodes, in other words, the products killed nematodes by affecting various morphometrics and breaking down proteins in the cuticles (Mashela *et al*., 2020a; 2020b). In contrast, nematostatic products serve as chemoattractants and chemo-repellents which disorientate nematodes, thereby inhibiting mating and delaying root location and penetration (Bargmann and Mori, 1997; Mashela *et al*., 2015; Wuyts *et al*., 2006). The cucurbitacin-containing phytonematicides are therefore, potent products that could be relied upon for managing nematode population densities under field conditions.

The sampling time after application of the products is very important. In context of DDG patterns, phytonematicides can also stimulate nematode population densities (Mashela *et al*., 2015). Actually, Dube (2016) demonstrated that at low concentration cucurbitacin-containing phytonematicides stimulated J2 hatch and thereby increasing nematode population densities. Similarly, when samples from granular formulations were collected further from the life span (42 days) of the citrus nematode (*Tylenchulus semipenetrans* Cobb.), namely; at 112 days Nemafric-BG phytonematicide had significantly increased J2 in soil and final nematode population densities.

Nemafric-BL phytonematicide significantly increased total eggs in roots and RP, which contracted findings by Lebea (2017) where the product on butternut squash reduced nematode variables. Apparently, the observed disparities were due to differences in harvesting times, as observed in other studies (Maile, 2013; Mashela *et al*., 2015). The observed increase in nematode population densities in the current study can be attributed to the degradation of the phytonematicides, which is compounded by the cyclic growth nature in nematode population densities. Pofu (2008), previously described using the concept of an equilibrium (E) point (Seinhorst, 1967). In the E point, nematode population densities oscillate around the E point, with the minima depending on external factors such as nematicides, as shown in a recent study (Mashela *et al*., 2015). Findings in the current study indicated that Nemafric-BG and Nemafric-BL phytonematicides could be operating differently in various formulations, with related reasons not being clear yet since the products have each cucurbitacin B as active ingredient. Should the experiment be terminated at 56 days, Nemafric-BG and Nemafric-BL phytonematicides would have lower nematode population density whereas that of the untreated control would increase. In the current study, the experiment was terminated at 64 days after initiating the treatments, where population densities under untreated control were lower and higher in Nemafric-BG and Nemafric-BL phytonematicides.

4.4.2 Plant variables

Nemarioc-AG and Nemarioc-AL phytonematicides were not significant on dry shoot mass of Swiss chard and gall rating under field conditions, which contradicted findings in Nemarioc-AG phytonematicide where effects were significant on all tomato plant variables under field conditions (Khosa, 2013). Crude extracts of bead-bean (*Maerua angolensis* DC.) and toad tree (*Tabernaemontana elegans* Stapf*.)* on tomato plants had significant effects on plant height alone (Khosa, 2013). Under microplot conditions, Nemarioc-AG phytonematicide had significant effects on plant height, dry shoot mass and fresh fruit mass on tomato plants (Mashela, 2002).

In the current study, Nemarioc-AL phytonematicide did not have significant effects on plant variables, which confirmed findings on butternut squash (Lebea, 2017), beetroot (Mashitoa, 2017) and green bean (Chokoe, 2017) subjected to Nemarioc-AL phytonematicide under field conditions. Although in some instances the Mean Concentration Stimulation Point (MCSP) stimulated growth of certain plant variables (Pelinganga, 2013; Seshweni, 2016), it should be noted that MCSP is not intended to stimulate plant growth, but to avoid phytotoxicity (Mashela *et al*., 2017). Generally, of critical importance, the MCSP, in addition to avoiding phytotoxicity, it must reduce nematode population densities.

Nemafric-BG and Nemafric-BL phytonematicides were not significant on dry shoot mass and gall rating under field conditions, nor was Nemafric-BL phytonematicide in all plant variables. The observations confirmed other findings on sweet stem sorghum (*Sorghum*

bicolor L.) under field conditions (Mabuka, 2013). In other granular formulations, Umar and Ngwamdai (2015) observed that jimson weed was also not having significant effects on plant variables of sweet melon (*Cucumis melon* L.) under field conditions. In contrast, when fine leaf fumitory (*Fumaria parviflora* Lam.) was applied as granules on tomato plants at 10, 20 and 30 g, plant variables were significantly increased (Naz *et al*., 2015). Apparently, the plant from which the product originates, along with the amount applied are important in inducing a measurable response. The latter agrees with observed responses in the Curve-fitting Allelochemical Response Dose (CARD) algorithm model, where prior to the D_m biological index and within the neutral phase (R_h-D_0) there is no measurable response (Liu *et al*., 2003). Conversely, plant extracts in liquid formulation from eucalyptus (*Eucalyptus chamadulonsis* Dehnh.), garlic (*Allium sativium* L.), marigold (*Tagetes erecta* L.) and neem (*Azadirachta indica* L.) plants had significant effects on tomato plant variables (Abo-Elyousr *et al*., 2009). Nemafric-BL phytonematicide also had significant effects on tomato plant variables (Maake, 2018).

Generally, plant variables when subjected to different phytonematicides concentrations displayed quadratic relations, which illustrates the existence of density-dependent growth (DDG) patterns (Mashela *et al*., 2017; Pelinganga, 2013; Sithole, 2016). The DDG patterns have three phases, namely, stimulation phase, neutral phase and inhibition phase (Liu *et al*., 2003). Neutral phase occurs when the concentration of the test product in plant organs operate within a neutral range and the treatment effects are not significant, whereas within the stimulation or the inhibition phase, treatments are characterised by having significant effects (Mashela *et al*., 2017). Effects of the cucurbitacin-containing

phytonematicides on dry shoot mass and gall rating were not significant, indicating that at lower concentration, treatments had saturated the plant variables. Apparently, the reason the cucurbitacin-containing phytonematicides did not have significant effects on Swiss chard plant variables under field conditions, suggested that at 64 days, the organs were saturated with the active ingredients of the products as explained in other plants (Mashela *et al*., 2015).

4.4.3 Nutrient elements

The effects of Nemarioc-AG phytonematicide on K in leaf tissues of Swiss chard agreed with those and the product in tomato leaf tissue under microplot conditions. Stimulation of tomato plant growth by Nemarioc-AG phytonematicide was not referred as “fertiliser effect” since the accumulation of nutrient elements was negligent (Mashela, 2002). Similarly, Khosa (2013) observed stimulation of tomato plant growth by Nemarioc-AG phytonematicide under field conditions was not related to the accumulation of nutrient elements. In the current study, Nemarioc-AG phytonematicide significantly reduced the accumulation of Mg in leaf tissue on Swiss chard, which is not desirable. The observation suggested that at harvest, Mg within the leaf tissues was in the inhibition phase of the DDG pattern of the phytonematicide as explained by the innovators of the CARD algorithm model (Liu *et al*., 2003). In the current study, Swiss chard leaves were harvested once at 64 days after inoculation with nematodes. Repeated harvesting as is the norm in Swiss chard production, might result in a different response in the accumulation of Mg in leaf tissues.

In the current study, Nemarioc-AL phytonematicide had shown significant decrease on K and Mg in leaf tissues as observed in tomato plant leaf tissues under field conditions (Maake, 2018). However, the interaction of Nemarioc-AL and Nemafric-BL phytonematicides increased K accumulation in tomato leaf tissues (Maake, 2018). In another study (Shadung, 2016), it was observed that Nemarioc-AL phytonematicide increased the accumulation of Mg, P and Ca in tomato leaf tissues, but had no significant effects on K. Nemafric-BG and Nemafric-BL phytonematicides each decreased Mg in Swiss chard leaf tissues, suggesting that for the nutrient element the concentration of the phytonematicides were at harvest in the inhibition phase as articulated by the DDG patterns (Liu *et al*., 2003). Since Mg is an essential nutrient element that forms an important constituent of the chlorophyll molecule and the middle lamella, deficiencies of Mg may not be desirable (Huber and Jones, 2013).

Generally, the successful use of cucurbitacin-containing phytonematicides on management of nematode population densities is dependent on whether the effective concentration was not phytotoxic to the test plant. This is difficult since plant growth and most physiological activities have different DDG phases within the concentration ranges of phytonematicides. Generally, for plant growth and its related physiological activities, the concentration should be within the range of stimulation or neutral phase (Liu *et al*., 2003).

4.5 Conclusion

Under field conditions, to a large extent, the response of nematodes to the cucurbitacin-containing phytonematicides in liquid and granular formulations consistently suppressed nematode numbers, thereby confirming observations under greenhouse and microplot conditions at different levels of inoculation. In contrast, due to the nature of application levels of the products, for plant variables there were limited responses under field conditions. However, it should be remembered that these products are intended for nematode suppression and not for stimulating plant growth nor improving the accumulation of nutrient elements.

CHAPTER 5
SUMMARY OF FINDINGS, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary of findings

The study assessed the relation between cucurbitacin-containing phytonematicides and responses of Swiss chard and population densities of root-knot (*Meloidogyne* species) nematodes. The Curve-fitting Allelochemical Response Dose (CARD) algorithm model demonstrated that most plant variables versus phytonematicides had positive quadratic relations, whereas limited variables versus phytonematicides had negative quadratic relations. Using the CARD biological indices for positive quadratic relations, the Mean Concentration Stimulation Point (MCSP) values were 3.03 and 2.36% for Nemarioc-AL and Nemafric-BL phytonematicides, respectively. Potassium, Ca, Mg, Fe and Zn versus Nemarioc-AL phytonematicide had positive quadratic relations, whereas K and Fe versus Nemafric-BL phytonematicide had negative quadratic relations. In all cases, the CARD overall sensitivity values demonstrated that Swiss chard was susceptible to phytonematicides in liquid formulations, but tolerant to the products in granular formulations. In all experiments, the phytonematicides consistently suppressed population densities of *Meloidogyne* species.

Under field conditions, the phytonematicides regardless of their formulation, have shown similar potential on plant variables and nematode variables. The phytonematicides in granular and liquid formulations did not have any significant effects on plant variables, suggesting that at harvest, the concentrations of the products was within the neutral phase. Nemarioc-AG and Nemarioc-AL phytonematicides reduced nematode numbers at

64 days after the application of the products, with incidents of increased nematode numbers explained using cyclic growth patterns in nematode population densities.

5.2 Significance of findings

The findings in the current study demonstrated that the cucurbitacin-containing phytonematicides could be used in managing nematode population densities in Swiss chard. This study also provided farmers with suitable concentrations of cucurbitacin-containing phytonematicides that would be ideal for managing nematodes, without inducing phytotoxicity on Swiss chard. The findings demonstrated that the crop was highly sensitive to the products and should therefore be used with caution. Also, when using the cucurbitacin-containing phytonematicides, the produce could benefit on certain nutrient elements, whereas for others the produce would have deficiencies. Most importantly, the findings of the current study are adding Swiss chard as one of the crops that could be added to the increasing list of cultigens where the four cucurbitacin-containing phytonematicides could be used for managing nematode population densities.

5.3 Recommendations

The MCSP is only one factor in the determination of non-phytotoxicity and it would be imperative that the application interval for both phytonematicides be established. In the current study, the responses of nutrient elements were restricted to macro and micro elements alone. In future studies, the MCSP values should be used to establish the application intervals for the products. Additionally, the scope of the studies could be expanded to other nutrient elements and substances such as essential amino acids and

PF vitamins in order to have a comprehensive view of the effects of the products on Swiss chard production. Such a comprehensive view would enhance the development of appropriate fertilisation of the crop when the products were used to manage nematode population densities.

5.4 Conclusions

The nemarioc-group and nemafric-group phytonematicides were shown to be suitable for managing nematode population densities in Swiss chard production. Due to the sensitivity of various organs to the products, it would be necessary to use the derived MCSP values to determine the application intervals of the four products on the test cultigen.

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APPENDICES

Appendix 4.1 Partitioning mean sum of squares for eggs in root, second-stage juveniles (J2) in roots and reproductive potential (RP) for *Meloidogyne* species to cucurbitacin-containing phytonematicides on Swiss chard under field conditions at 64 days (n = 45).

Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
		Eggs in root		J2		RP	
		Nemarioc-group phytonematicides					
Block	14	0.13387	9	0.19144	24	215.16	11
Treatment	2	1.18340	79***	0.39494	49ns	1464.90	77***
Error	28	0.17187	12	0.22149	27	237.92	12
Total	44	1.48914	100	0.80787	100	1917.98	100
		Nemafric-group phytonematicides					
Block	14	0.19502	22	0.10002	20	85.26	14
Treatment	2	0.59263	67***	0.23655	47ns	8378.81	76***
Error	28	0.09502	11	0.16343	23	1071.75	10
Total	44	0.88267	100	0.5	100	10964.09	100

ns Not significant at P ≤ 0.05, **Significant at P ≤ 0.05, ***Highly significant at P ≤ 0.01.

Appendix 4.2 Partitioning mean sum of squares for sodium (Na), potassium (K), calcium (Ca), phosphorus (P), sulphur (S) and magnesium (Mg) in leaf tissues of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides at 64 days after initiation of treatment (n = 45).

	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Source		Na		K		Ca	
Block	14	133.28	32	2689.89	29	1308.50	37
Treatment	2	191.55	47^{ns}	5265.49	56**	1572.81	44^{ns}
Error	28	87.21	21	1430.77	15	691.77	19
Total	44	412.04	100	9386.15	100	3573.08	100
		P		S		Mg	
Block	14	161.88	44	39.45	40	85.26	12
Treatment	2	149.55	41^{ns}	30.99	31^{ns}	509.41	71**
Error	28	55.03	15	28.17	29	123.79	17
Total	44	366.46	100	98.61	100	718.46	100

^{ns} Not significant at P ≤ 0.05, **Significant at P ≤ 0.05.

Appendix 4.3 Partitioning mean sum of squares for sodium (Na), potassium (K), calcium (Ca), phosphorus (P), Sulphur (S) and magnesium (Mg) in leaf tissues of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides at 64 days after initiation of treatment (n = 45).

	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Source		Na		K		Ca	
Block	14	135.99	27	2827.33	47	2719.55	37
Treatment	2	186.77	37^{ns}	492.46	8^{ns}	3102.27	43^{ns}
Error	28	179.42	36	2699.27	45	1464.20	20
Total	44	502.18	100	6019.06	100	7286.02	100
		P		S		Mg	
Block	14	187.13	43	59.01	42	59.96	19
Treatment	2	84.86	20^{ns}	33.74	24^{ns}	200.99	62**
Error	28	158.58	37	47.26	34	62.76	19
Total	44	430.57	100	140.01	100	323.71	100

^{ns} Not significant at P ≤ 0.05, **Significant at P ≤ 0.05.

Appendix 4.4 Partitioning mean sum of squares for boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) in leaf tissues of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides at 64 days after treatment initiation (n = 45).

	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Source		B		Cu		Fe		Mn	
Block	14	4.75	30	1249.27	33	119.77	34	97.29	35
Treatment	2	5.77	36^{ns}	1244.94	33^{ns}	106.37	30^{ns}	81.75	29^{ns}
Error	28	5.47	34	1249.91	34	125.56	36	102.55	36
Total	44	15.99	100	3744.12	100	351.7	100	281.59	100
		Mo		Ni		Se		Zn	
Block	14	1.18	25	26.60	33	0.18	33	0.10	59
Treatment	2	2.28	49^{ns}	25.93	33^{ns}	0.25	46^{ns}	0.02	6^{ns}
Error	28	1.23	26	26.69	34	0.11	21	0.06	35
Total	44	4.69	100	79.22	100	0.54	100	0.18	100

^{ns} Not significant at P ≤ 0.05, **Significant at P ≤ 0.05.

Appendix 4.5 Partitioning mean sum of squares for boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) in leaf tissues of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides at 64 days after treatment initiation (n = 45).

	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Source		B		Cu		Fe		Mn	
Block	14	1.28	17	3.40	25	5.95	63	0.65	33
Treatment	2	4.44	60^{ns}	2.22	16^{ns}	0.18	2^{ns}	0.68	35^{ns}
Error	28	1.67	23	8.25	59	3.28	35	0.62	32
Total	44	7.39	100	13.87	100	9.41	100	1.95	100
		Mo		Ni		Se		Zn	
Block	14	5.85	33	9.76	50	0.10	36	0.38	76
Treatment	2	2.96	17ns	2.16	11^{ns}	0.02	7^{ns}	0.02	4^{ns}
Error	28	8.96	5 0	7.75	39	0.16	57	0.10	20
Total	44	17.77	100	19.67	100	0.28	100	0.5	100

^{ns} Not significant at P ≤ 0.05, **Significant at P ≤ 0.05.

Appendix 4.6 Analysis of variance for total eggs in roots of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	0.13387	6.89	0.0037
Treatment	2	1.18340		
Error	28	0.17187		
Total	44	1.4885		

Appendix 4.7 Analysis of variance for second-stage juvenile in roots of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	0.19502	6.24	0.0058
Treatment	2	0.59263		
Error	28	0.09502		
Total	44	0.88267		

Appendix 4.8 Analysis of variance for reproductive potential (RP) in roots of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	215.16	6.15	0.0061
Treatment	2	1464.90		
Error	28	237.92		
Total	44	1917.98		

Appendix 4.9 Analysis of variance for total eggs in root of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	0.19502	6.24	0.0058
Treatment	2	0.59263		
Error	28	0.09502		
Total	44	0.88267		

Appendix 4.10 Analysis of variance for second-stage juvenile in roots Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	0.10002	1.45	0.2522
Treatment	2	0.23655		
Error	28	0.16343		
Total	44	0.5		

Appendix 4.11 Analysis of variance for reproductive potential (RP) in roots of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	1513.53	7.82	0.0020
Treatment	2	8378.81		
Error	28	1071.75		
Total	44	10964.09		

Appendix 4.12 Analysis of variance for dry shoot mass (DSM) of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	169.428	0.65	0.5304
Treatment	2	39.328		
Error	28	60.633		
Total	44	269.389		

Appendix 4.13 Analysis of variance for gall rating (GR) of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	0.18413	0.65	0.5292
Treatment	2	0.08889		
Error	28	0.13651		
Total	44	0.40953		

Appendix 4.14 Analysis of variance for dry shoot mass (DSM) of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	194.192	1.02	0.3727
Treatment	2	278.147		
Error	28	272.029		
Total	44	744.368		

Appendix 4.15 Analysis of variance for gall rating (GR) in Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	0.51746	0.42	0.6607
Treatment	2	0.15556		
Error	28	0.36984		
Total	44	1.04286		

Appendix 4.16 Analysis of variance for sodium (Na) leaf tissue of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	133.28	2.20	0.1300
Treatment	2	191.55		
Error	28	87.21		
Total	44	412.04		

Appendix 4.17 Analysis of variance for potassium (K) in leaf tissue of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	2689.89	3.68	0.0381
Treatment	2	5265.49		
Error	28	1430.77		
Total	44	9386.15		

Appendix 4.18 Analysis of variance for calcium (Ca) in leaf tissue of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	1308.50	2.27	0.1216
Treatment	2	1572.81		
Error	28	691.77		
Total	44	3573.08		

Appendix 4.19 Analysis of variance for phosphorus (P) in leaf tissue of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	161.88	2.72	0.0834
Treatment	2	149.55		
Error	28	55.03		
Total	44	366.46		

Appendix 4.20 Analysis of variance for sulphur (S) in leaf tissues of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	39.45	1.10	0.3468
Treatment	2	30.99		
Error	28	28.17		
Total	44	98.61		

Appendix 4.21 Analysis of variance for magnesium (Mg) in leaf tissue of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	85.264	4.12	0.0271
Treatment	2	509.410		
Error	28	123.791		
Total	44	718.465		

Appendix 4.22 Analysis of variance for sodium (Na) in leaf tissues of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	135.99	1.04	0.3664
Treatment	2	186.77		
Error	28	179.42		
Total	44	502.18		

Appendix 4.23 Analysis of variance for potassium (K) in leaf tissues of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	2827.33	0.18	0.8342
Treatment	2	492.46		
Error	28	2699.27		
Total	44	6019.06		

Appendix 4.24 Analysis of variance for calcium (Ca) in leaf tissues of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	2719.55	2.12	0.1390
Treatment	2	3102.27		
Error	28	1464.20		
Total	44	7286.02		

Appendix 4.25 Analysis of variance for phosphorus (P) in leaf tissues of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	187.13	0.54	0.5915
Treatment	2	84.86		
Error	28	158.58		
Total	44	430.57		

Appendix 4.26 Analysis of variance for sulphur (S) in leaf tissues of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	59.01	0.71	0.4984
Treatment	2	33.74		
Error	28	47.26		
Total	44	140.01		

Appendix 4.27 Analysis of variance for magnesium (Mg) in leaf tissue of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	59.96	3.20	0.0559
Treatment	2	200.99		
Error	28	62.76		
Total	44	323.71		

Appendix 4.28 Analysis of variance for boron (B) in leaf tissues of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	4.75	1.05	0.3617
Treatment	2	5.77		
Error	28	5.47		
Total	44	15.99		

Appendix 4.29 Analysis of variance for copper (Cu) in leaf tissues of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	1249.27	1.00	0.3821
Treatment	2	1244.94		
Error	28	1249.91		
Total	44	3744.12		

Appendix 4.30 Analysis of variance for iron (Fe) in leaf tissues of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	119.77	0.85	0.4401
Treatment	2	106.37		
Error	28	125.56		
Total	44	351.7		

Appendix 4.31 Analysis of variance for manganese (Mn) in leaf tissues of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	97.29	0.80	0.4606
Treatment	2	81.75		
Error	28	102.55		
Total	44	281.59		

Appendix 4.32 Analysis of variance for molybdenum (Mo) in leaf tissues of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	1.18	0.19	0.8305
Treatment	2	2.28		
Error	28	1.23		
Total	44	4.69		

Appendix 4.33 Analysis of variance for nickel (Ni) in leaf tissues of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	26.60	0.97	0.3909
Treatment	2	25.93		
Error	28	26.69		
Total	44	79.22		

Appendix 4.34 Analysis of variance for selenium (Se) in leaf tissues of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	0.18	2.37	0.1122
Treatment	2	0.25		
Error	28	0.11		
Total	44	0.54		

Appendix 4.35 Analysis of variance for zinc (Zn) in leaf tissues of Swiss chard to Nemarioc-AG and Nemarioc-AL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	0.10	0.29	0.7516
Treatment	2	0.02		
Error	28	0.06		
Total	44	1.18		

Appendix 4.36 Analysis of variance for boron (B) in leaf tissues of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	1.28	0.21	0.7684
Treatment	2	4.44		
Error	28	1.67		
Total	44	7.39		

Appendix 4.37 Analysis of variance for copper (Cu) in leaf tissues of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	3.40	0.27	0.7657
Treatment	2	2.22		
Error	28	8.25		
Total	44	13.87		

Appendix 4.38 Analysis of variance for iron (Fe) in leaf tissues of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	5.95	0.06	0.9447
Treatment	2	0.18		
Error	28	3.28		
Total	44	9.41		

Appendix 4.39 Analysis of variance for manganese (Mn) in leaf tissues of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	0.65	1.10	0.3452
Treatment	2	0.68		
Error	28	0.62		
Total	44	1.95		

Appendix 4.40 Analysis of variance for molybdenum (Mo) in leaf tissues of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	0.13387	6.89	0.0037
Treatment	2	1.18340		
Error	28	0.17187		
Total	44	1.4885		

Appendix 4.41 Analysis of variance for nickel (Ni) in leaf tissues of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	9.76	0.28	0.7593
Treatment	2	2.16		
Error	28	7.75		
Total	44	19.67		

Appendix 4.42 Analysis of variance for selenium (Se) in leaf tissues of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	0.10	0.12	0.8869
Treatment	2	0.02		
Error	28	0.16		
Total	44	0.28		

Appendix 4.43 Analysis of variance for zinc (Zn) in leaf tissues of Swiss chard to Nemafric-BG and Nemafric-BL phytonematicides under field conditions.

Source	DF	MS	F	P
Replications	14	0.38	0.20	0.8207
Treatment	2	0.02		
Error	28	0.10		
Total	44	0.5		