INFLUENCE OF PRIMING HYPOGENOUS SEEDS OF *PHASEOLUS COCCINEUS* IN CUCURBITACIN-CONTAINING PHYTONEMATICIDES ON PLANT GROWTH AND NEMATODE SUPPRESSION

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DECLARATION

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

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DEDICATION

To the Lord, God of all mankind, my family and my friends.

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ABSTRACT

Runner beans are extremely sensitive to root-knot (*Meloidogyne* species) nematodes. Phytonematicides had been consistently used in managing population densities of *Meloidogyne* species in various crops, with the application technologies being restricted to the ground leaching technology (GLT) and botinemagation technology, each with its own advantages and disadvantages. The use of seeds as carriers of active ingredients of phytonematicides and then drying prior to sowing, is being considered as another potential application strategy in seeds with hypogeal germination. In such seeds, during seedling emergence the seed cover and the endosperm remain below the soil surface, just above the developing root system. As a result, in phytonematicide-primed seeds, the seed structures could serve as carriers for the active ingredients of phytonematicides. In cucurbitacin phytonematicides, Nemarioc-AL and Nemafric-BL phytonematicides contain cucurbitacin A and B, respectively as active ingredients. The objectives of the study were two-fold, namely, to determine whether runner bean (*Phaseolus coccineus* L.) seeds would (1) serve as carriers of active ingredients of cucurbitacin-containing phytonematicides without affecting seed germination under *in vitro* conditions, (2) serve as carriers of cucurbitacins intended for suppression of *M. incognita* population densities under greenhouse and microplot conditions. Two separate studies were conducted under laboratory conditions, with seven treatment solutions at 0, 2, 4, 8, 16, 32 and 64% Nemafric-BL or Nemarioc-AL phytonematicide. After exposure to separate solutions for 2 h, runner bean seeds were dried on laboratory benches for 72 h. Treatments were arranged in a completely randomised design (CRD), with 8 replications. Two layers of filter papers were placed inside each Petri dish seeded with 10 primed and dried seeds. Petri dishes were incubated inside LABCON growth

chamber at 25ºC and 75% relative humidity. Successful seed germination, viewed as emergence of radicle from the testa, was recorded daily for a period of 10 days, with each count being removed from Petri dish to avoid re-counting. Under greenhouse and microplot conditions, primed-and dried seeds were sown in plastic pots containing 2 700 ml steam-pasteurised sandy loam soil, arranged in a randomised complete block design, replicated six times and eight times, respectively. Each seedling was inoculated by distributing 5 000 eggs and second-stage juveniles (J2) of *M. incognita* race 4 using a 50 ml plastic syringe. Originally, pots were irrigated using 500 ml chlorine-free tapwater, which was reduced to half after seedling emergence at every other day. Plant variables were collected at 56 days after inoculation and data were subjected to the Curve-fitting Allelochemical Response Dose algorithm model. *In vitro*, germination percentage (R^2 = 0.96), radicle length (R^2 = 0.89) and plumule diameter (R^2 = 0.96) versus Nemarioc-AL phytonematicide exhibited positive quadratic relations. Similarly, the variables versus Nemafric-BL phytonematicide, exhibited positive quadratic relations. *In vitro*, Mean Concentration Stimulation Point (MCSP) value of Nemarioc-AL phytonematicide on runner bean seeds was 1.05%, whereas for Nemafric-BL phytonematicide MCSP value was 0.58%. Under greenhouse conditions, plant height (R^2 = 0.97), chlorophyll content (R^2 = 0.92), dry shoot mass (R^2 = 0.98), dead nodule number (R^2 = 0.90), total pod number (R^2 = 0.97) and active nodule number (R^2 = 0.93) versus Nemarioc-AL phytonematicide exhibited positive quadratic relations., Similarly, chlorophyll content (R^2 = 0.95), gall rating (R^2 = 0.82), dry shoot weight (R^2 = 0.69), stem diameter (R^2 = 0.85) and total nodule number (R^2 = 0.86) versus Nemafric-BL phytonematicide exhibited positive quadratic relations. Under greenhouse conditions, MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides were 4.18 and 3.69%,

respectively. Under microplot conditions, total number of nodules (R^2 = 0.88), number of nodules dead (R^2 = 0.99), number of nodules active (R^2 = 0.95), fresh root mass (R^2 = 0.99), and fresh pod mass (R^2 = 0.99) versus Nemarioc-AL phytonematicide, exhibited positive quadratic relations, whereas plant height (R^2 = 0.85), number of nodules dead (R^2 = 0.87), dry shoot mass (R^2 = 0.97), fresh root mass (R^2 = 0.97) and total number of nodules (R^2 = 0.63) versus Nemafric-BL phytonematicide exhibited positive quadratic relations. Under microplot conditions, MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides were 3.76 and 3.93%, respectively, each with $\sum k = 0$. All degrees of Nemarioc-AL and Nemafric-BL phytonematicides profoundly reduced nematode numbers under greenhouse and microplot trials. Based on the information obtained from this study, it was confirmed that runner bean (*P. coccineus*) is sensitive to Nemafric-BL and Nemarioc-AL phytonematicides supported by the Curve-fitting Allelochemical Response Dose (CARD) model results due to most plant variables that had sensitivity values of zero. In conclusion, the priming technology should be developed further since it has the potential of being successful in nematode management in seeds with hypogeal germination.

CHAPTER 1

RESEARCH PROBLEM

1.1 Background

1.1.1 Description of the research problem

Runner bean (*Phaseolus coccineus* L.) is a warm-season crop, which is extremely sensitive to frost. *P. coccineus*. is a common member of the legume family that is mainly characterized by its climbing growing habits and bush determinate growth. Mostly used in the African cropping systems due to its participation in nitrogen fixation in the soil. Smýkal *et al*. (2015), reported that runner bean comprises of 27% of the world crop production. However, the production level may be reduced due to low and unstable yields caused by biotic and abiotic stress. Certain pathogens, pests and nematodes are biotic stress factors that limit bean production. Root-knot nematodes especially *Meloidogyne* species are an economically significant group in plant parasitic nematodes in that they cause important crop losses in temperate, subtropical and tropical climates (Perry *et al.*, 2009). Runner beans have the hypogeal type of germination whereby the cotyledons remain underneath the soil surface during germination. Plants resistance to root-knot nematodes could be affected within the soil, resulting in early or later infection, with later infection the most frequent (Fassuliotis, 1979). The crop yield loss is usually proportional to initial nematode population densities (Pi) (Seinhorst, 1965). *Meloidogyne incognita* causes 63% yield losses in bean in Colombia (Mullin *et al.*, 1994).

1.1.2 Impact of the research problem

Worldwide, runner bean is the most commonly consumed legume (Porch *et al*., 2013). Most legumes are produced directly for human utilisation, with the marketable value being beyond that of all other leguminous crops combined (Porch *et al*., 2013). The legume family is the second most economically important after the grass family. Runner bean is a good source of vitamin A and C, potassium, fibre, folate and an outstanding source of protein, zinc and iron. Runner beans contain antioxidants such as catechins found in green tea, which can help prevent cancer and improve heart health. The plant parasitic nematodes cause significant crop losses in temperate, subtropical and tropical climates (Perry *et al.*, 2009). Root-knot nematodes cause severe quality damage resulting in yield loss (Shree and Schwartz, 2011).

1.1.3 Possible causes of the research problem

The crop is mainly affected by aphids (*Aphis fabae* Scopoli), thrips (*Thrips palmi* Karny) and whiteflies (*Trialeurodes vaporariorum* Westwood) but in the current study the focus was on plant nematodes since they cause enormous damage on runner beans (Mouden *et al*., 2017). The root-knot (*Meloidogyne* species) nematodes have over 2 000 hosts (Mashela *et al*., 2017), are aggressive and most susceptible crops cannot be successfully produced unless the nematode numbers are suppressed (Sikora and Fernandez, 2005). Runner beans are highly susceptible to *Meloidogyne* species, and since most synthetic nematicides for managing most nematodes had been withdrawn from the agrochemical markets (Mashela *et al*., 2015a); there had been limited options to manage nematode population densities. Phytonematicides were developed for the suppression of nematode population used through irrigation systems (Mashela *et al*., 2015b). The general focus of the study is how runner bean

seeds primed with cucurbitacin-containing phytonematicides affect the growth and yield.

1.1.4 Proposed solution

The study would result in a better understanding of priming seeds with cucurbitacin containing phytonematicides before planting, which could enhance the pre-emergent metabolic process responsible for rapid seed germination, seedling emergence and growth as well as final crop yield under normal and stressed conditions. Understanding management of nematode population with an environmentally friendly practice that enhances plant growth and improve yield without negative side-effects as compared to the use of synthetic chemicals.

1.1.5 General focus of the study

This study would focus on appropriate pre-emergent application technology of Nemarioc-AL and Nemafric-BL phytonematicides, which would be more effective in suppressing nematode population numbers. The study would provide smallholder and commercial farmers with the importance of using phytonematicides for priming seeds before planting. This research would lead to the improvement of using cucurbitacin-containing phytonematicides which are environmentally friendly for managing *Meloidogyne* species as opposed to synthetic nematicides.

1.2 Problem statement

Priming of seeds would place the active ingredients of phytonematicides adjacent to the rhizosphere where the initial nematode Pi should be managed. According to Seinhorst (1965), plant damage is directly proportional to Pi in the rhizosphere. In

addition, the success of priming would depend on whether the primed structure of the seed remains in the soil, a condition referred to as hypogeal germination, versus epigeal germination where the seed remains are pulled out of the soil surface during emergence. Examples of seeds with epigeal germination include bean, mustard (*Brassica juncea* L.) and castor (*Ricinus commucis* L.) whereas those with hypogeal germination include runner bean (*P. coccineus*), pea (*Pisum sativum* L.) coconut (*Cocos nucifera* L.) and mango (*Mangifera indica* L.) (Copeland and McDonald, 2001). Alternatively, in seeds with hypogeal germination, seeds could be covered with powdered products of cucurbitacin-containing phytonematicides mixed with a sticker and then dried.

1.3 Rationale of the study

Runner beans are extremely sensitive to *Meloidogyne* species. Cucurbitacin phytonematicides had been consistently used in management of *Meloidogyne* species in various crops (Shadung *et al*., 2017), with the placement technology being a challenge, but with the use of seeds as carriers of active ingredients being another potential option for the runner beans. The runner bean has hypogeal germination where during emergence the seed cover and endosperm remain below the soil surface, adjacent to the developing root system (Copeland and McDonald, 2001). As a result, the seed structure could be used as a carrier for the active ingredients of the cucurbitacin phytonematicides. Runner bean is a legume crop, economically friendly, rich in calcium, vitamin A and vitamin K which strengthen the bones. The available application methods of phytonematicides, namely, the ground leaching technology (GLT) (Mashela, 2002) and the botinemagation technology (Pelinganga, 2013), are labour-intensive and infrastructure-intensive, respectively. The use of seeds as

carriers of the active ingredients for the management of nematodes would be cost-effective and therefore a suitable placement method for different farming systems.

1.4 Purpose of the study

1.4.1 Aim

Development of placement technology of phytonematicides using the seed as a carrier of the active ingredients.

1.4.2 Objectives

The objective of the study are to:

1. Examine whether runner bean seeds would serve as carriers of active ingredients from cucurbitacin phytonematicides under *in-vitro* conditions.
2. Investigate whether runner bean seeds would serve as carriers of active ingredients from cucurbitacin phytonematicides without affecting plant growth but suppressing nematode numbers under greenhouse and microplot conditions.

1.4.3 Null hypotheses

1. Runner bean seeds would serve as carriers of active ingredients from cucurbitacin phytonematicides under *in-vitro* conditions.
2. Runner bean seeds would serve as carriers of active ingredients from cucurbitacin phytonematicides without affecting plant growth but suppressing nematode numbers under greenhouse and microplot conditions.

1.5 Reliability, validity and objectivity

In the current study, data reliability was based on statistical analysis at the levels of significance ($P \leq 0.05$ or R^2), whereas validity was attained through repetition of the experiments in time. Objectivity was ensured by discussing results based on empirical evidence for elimination of all form of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

In each experiment, experimental error was reduced by replication to ensure that bias was minimised from the study. Randomisation of treatments were also assigned within the selected experimental design to avoid bias (Leedy and Ormrod, 2005).

1.7 Scientific significance of the study

The study would provide useful information on the potential use of seeds with hypogeal germination as carriers of active ingredients from cucurbitacin phytonematicides, thereby reducing application costs as experienced in existing application methods. The phytonematicides are environment-friendly, provide an important part of climate-smart agriculture and less costly for the smallholder farmers.

1.8 Structure of the mini-dissertation

Subsequent to detailed description of the research problem in Chapter 1, work done and not yet done on the research problem was reviewed (Chapter 2). Thereafter each research chapter addressed Objective 1 and Objective 2. In the last chapter (Chapter 5), the findings were summarised, with an outline of the significance of the

findings, along with the future recommendations, with the mini-dissertation ending in a conclusion that tied together the whole study into a unitary unit. Citations and references were used following the Harvard style as prescribed by Senate of the University of Limpopo.

CHAPTER 2

LITERATURE REVIEW

2. Introduction

2.1 Runner beans

Runner beans (*P. coccineus*) are also known as “Scarlet runners”, a term that reflects the climbing growth habit of the crop. The crop is a member of the *Fabaceae* family and the third-most economically important agronomic crop (Porch *et al*., 2013). The Fabaceae family comprises 27% of the world crop production (Smýkal *et al*., 2015). Leguminous crops are cultivated annually for their mature dry seeds and immature green pods as source of protein, and their capacity to symbiotic nitrogen fixation and improved soil fertility in several parts of the world. These species can be cultivated productively on different soils, ranging from sand to heavy clay, provided the soil is well drained. Sandy-loam to loam soils are preferred with the ideal pH (KCl) being between 5.5 and 6.0 and pH (H_2O) 5.8-6.5.

This leguminous crop originated from Northern Mexico to Northern Argentina, where it is currently mainly produced by the smallholder farmers. This is a summer crop, which is very sensitive to frost, with optimum temperatures between 15°C and 27°C (Serra *et al.*, 2014). Temperatures lower than 5°C cause puffy, malformed pods of poor quality. Temperatures above 35°C may induce unnecessary shedding of immature pods, leading to poor yields particularly when complemented by dry, hot winds. Average yield of runner beans in East Africa is approximately 850 kg/ha but might be as high as approximately 1 500 kg/ha under best agricultural practices. In order to get good quality mature pods, runner beans are full-grown on support

structures such as fence lines, poles, trellises or other crops (Brink and Belay, 2006). Worldwide, runner beans are among the most consumed legumes due to its human utilisation value, and its saleable value beyond that of other leguminous crops (Porch *et al*., 2013). The crop is a good source of Vitamins (A and C), fibre, potassium (K) and has excellent source of protein, iron (Fe), and zinc (Zn). Runner beans also contain antioxidants such as catechins that occur in green tea, which can help prevent cancer and improve heart health. The main essential components contained by mature grains from leguminous species are nearly 63% carbohydrates, 20% protein, 5% fibre, 3.5% ash and 1.5% fat (Kay, 1979).

2.2 Work done on the problem statement

2.2.1 Effects of phytonematicides

Root-knot *(Meloidogyne* species) nematodes cause root galls that might result in total crop failure due to the destruction of nutrient supply to the entire plant. Galls can damage root systems enough to decrease water and mineral uptake, which could eventually inhibit shoot growth. Phytonematicides are used as alternatives to methyl bromide that had been since removed from the agrochemical markets because of its environment-unfriendliness (Mashela *et al*., 2011). According to Shadung *et al*. (2017), Nemarioc-AL and Nemafric-BL (L = liquid formulation) phytonematicides, whereas A and B are respectively active ingredients cucurbitacin A ($C_{32}H_{46}O_8$), which disintegrates to cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) and cucurbitacin B ($C_{32}H_{48}O_8$). The two products consistently suppress nematode population densities when applied through irrigation water, technically the botinemagation technology. The granular formulation, Nemarioc-AG and Nemafric-

BG phytonematicides, are being applied through the ground leaching technology (GLT) (Mashela, 2002).

2.2.2 Efficacy of phytonematicides

Meloidogyne species are an economically significant group in plant parasitic nematodes that causes a major crop loss in temperate, subtropical and tropical climates. According to Mashela *et al*. (2011), the genus remains the most problematic soil-borne pathogen in crop production. The use of phytonematicides is often limited by the phytotoxicity to the protected plants (Mashela *et al.,* 2015a) and their quality is reliant on the amount of active ingredients that are related to its performance. However, the active ingredients of phytonematicides are in a continuous state of change because of potential microbial degradation. Phytonematicides were researched and developed to upgrade the challenges related to conventional organic amendments under the auspices of the Cucurbitaceae Technologies (Mashela *et al*., 2015b), where the focus was exclusively on using materials from *Cucumis* species, indigenous to Limpopo Province, South Africa. However, Mafeo *et al*. (2010) demonstrated that in granular formulation (G), Nemafric-AG phytonematicide from *C. myriocarpus* (Naud.) with cucurbitacin A as an active ingredient, inhibited seed germination of various crops, including maize (*Zea mays* L.), which has hypogeal germination, where the seed remnants remain below soil surface during seedling emergence.

2.2.3 Priming of seeds with phytonematicides

Runner beans have hypogeal emergence characteristics, which imply that the emerging seeds do not bring the cotyledons above the soil surface (Copeland and McDonald, 2001), while seeds with epigeal germination characteristics bring the

seed remnants above the soil surface during seedling emergence. Seeds with hypogeal germination attributes are ideal for use in priming with phytonematicides since the primed seed remnants would be positioned above the growing root system. Priming of seeds is a pre-sowing treatment where nothing breaks the seed coat before germination (Farooq *et al*., 2005). Seed treatment is a pre-germination tactic, which enhances seed performance by significantly improving the rate as well as seed germination percentage and conquers the negative effects related to an exposure to stress. Primed seeds exhibit rapid and uniform germination because of different metabolic activities, enzyme activation, protein synthesis, biological and chemical processes of cell repair and enhancement of the antioxidant protection during Phase I of seed germination process, which is reversible (Waqas *et al*., 2019).

In hypogeal germination, remnants of primed seeds below soil surface would contain the active ingredients of phytonematicides adjacent to the rhizosphere where the initial nematode Pi should be suppressed, thereby enabling nematode management. Seed priming technique may lead to improvement of germination, homogeneous growth of seedlings and lead to high yielding even under unfavourable conditions as compared to non-primed seeds (Waqas *et al.*, 2019). Seeds with hypogeal germination have an advantage in that stored energy supply remains beneath the soil surface and can be used for regrowth if the shoots are damaged or cut by frost, insects or other factors. Other crops that also express hypogeal germination include field pea (*P. sativum*), Austrian winter pea (*Pisum sativum subsp. Arvense* L.) and lentil (*Lens culinaris* L.).

Priming of rice seeds with KCl has led to improved seedling growth as shown by increasing lengths of radicle and plumule (Farooq *et al*, 2005). Khan *et al*. (2009) indicated that pre-treatment of pepper (*Capsicum annuum* L.) with epigeal germination using salicylic acid and acetylsalicylic acid, resulted in more uniformity of germination and seedling emergence under high salinity. In contrast, Moradi and Younesi (2009) observed that hydro-priming grain sorghum seeds led to reduction rate of emergence time, but improved germination percentage, along with negative results during tests of accelerated ageing when compared to non-primed seeds.

2.2.4 Runner bean management

Pests and diseases might have been partly liable for unstable runner bean production which has been encountered in the past. The crop is mainly affected by pests such as aphids (*A. fabae*), thrips (*T. palmi*) and whiteflies (*T. vaporariorum*) and nematode since they cause a significant damage and limit the production. Root-knot (*Meloidogyne* spp.) nematodes cause significant crop losses in runner bean production. The nematode genus has wide host range and high rate of reproduction, thereby making it difficult to manage its nematode population densities (Peters, 1996). Runner beans are highly susceptible to *M. incognita* race 4 which resulted in 63% yield losses in Colombia (Mullin *et al.*, 1994). The existences of races, which are morphologically similar within a species (Mashela *et al.,* 2011), makes it difficult to manage nematode population densities using nematode resistance to *Meloidogyne* species. Other *Meloidogyne* species were shown to have the potential to cause severe reduction (90%) in quality and yield loss to dry and green common beans (Shree and Schwartz, 2011). However, certain resistant bean cultivars can delay the development of the nematode, with the resultant higher crop yield.

Generally, chemical nematicides have been effective in management of root-knot nematodes (Mashela *et al*., 2011). Fumigant synthetic nematicides such as 1,3-Dichloropropene (1,3-D) and methyl bromide were having high volatility challenges, with biocidal and lengthy persistence, resulting in dead soils and assault of non-target organisms. However, due to their environment-unfriendliness, which resulted in global warming, fumigant nematicides were withdrawn from the agrochemical markets with the cut-off date of 2005. Currently, the focus had been redirected to the use of alternative nematode management strategies, including the cucurbitacin phytonematicides, Nemarioc-AL and Nemafric-BL phytonematicides, from fruits of wild cucumber (*Cucumis myriocarpus)* and wild watermelon (*Cucumis africanus* L.), respectively (Mashela *et al*., 2015a).

2.2.5 Suppression of nematodes

Meloidogyne species have over 2000 hosts (Mashela *et al*., 2017). Most susceptible crops cannot produce successfully except that nematode populations are suppressed (Sikora and Fernandez, 2005). The aggressiveness of this species, the degree of resistance in the crops, age of the crop, the initial nematode Pi at sowing together with the presence of the abiotic and biotic factors influences crop yield losses (Mashela *et al*., 1992). Yield reduction on crop is directly proportional to Pi at sowing (Seinhorst, 1965). Cucurbitacin-containing phytonematicides are used in plant protection against nematodes, insects and fungi (Mashela *et al*., 2017), with certain results demonstrating at least 90% nematode suppression under greenhouse and microplot conditions, with over 80% suppression occurring under field conditions (Mashela, 2007). Pelinganga *et al*. (2011) demonstrated that Nemafric-BL and

Nemarioc-AL phytonematicides could reduce population densities of *Meloidogyne* species on tomato by 89 and 69%, respectively.

The efficacy of the two phytonematicides were shown to be similar to those of synthetic chemical nematicides, aldicarb and phenamiphos (Mashela *et al*., 2008). However, the major challenge in the use of cucurbitacin phytonematicides in general had been phytotoxic (Pelinganga *et al*., 2013) and inconsistent results in nematode suppression. In contrast, the challenges of conventional organic material for use in managing nematode population densities included (1) unavailability of materials, (2) large quantities required to achieve adequate nematode suppression, (3) inconsistent results, (4) high transport costs, (5) negative period and (6) decreased soil pH which interfered with the availability of some essential nutrient elements for plant growth (Mashela, 2002).

2.2.6 Resolving phytonematicide challenges

Biological indices from the Curve-fitting Allelochemical Response Dose (CARD) algorithm computer model (Liu *et al*., 2003), were adapted to establish concentration of phytonematicides that improve plant growth to resolve phytotoxicity challenges (Mafeo and Mashela, 2010; Mafeo *et al*., 2010). In the CARD model, density-dependent growth (DDG) patterns are characterised by several biological indices, namely,

a) Threshold stimulation (D_m) - the concentration at which the allelochemical commences to have a measurable effect on plant growth,
b) Saturation point (R_h) - the concentration at which plant growth remains stable before declining,

c) 0% inhibition (D_0) - the endpoint concentration of R_h, where the allelochemical has no inhibition effect on plant growth,-50% inhibition (D_{50}) - the concentration where the allelochemical reduces plant growth by 50%,

d) 100% inhibition(D_{100}) - the concentration where the allelochemical reduces plant growth by 100%,

e) sensitivity value (k) - the number transformations that serve as a biological indicator of the degree of sensitivity with relation to stimulation or inhibition to allelochemicals, and

f) R^2 - the coefficient of determination (Liu *et al*., 2003).

The Mean Concentration Stimulation Point (MCSP), which is half the sum of two biological indices, namely D_m and the adjusted saturation point (aR_h) (Pelinganga and Mashela, 2012; Pelinganga *et al*., 2013). According to the adjusted aR_h, $aR_h = D_m + R_h$, with $MCSP = (D_m + aR_h)/2 = (D_m) + (D_m + R_h)/2 = (2D_m + R_h)/2 = D_m + R_h/2$. The practical importance of MCSP is that it is the concentration of the phytonematicide which stimulates plant growth, while at the same time suppressing population densities of nematodes (Pelinganga and Mashela, 2012; Pelinganga *et al*., 2012; 2013).

2.2.7 Application technologies of phytonematicides

Currently there are two application technologies for the cucurbitacin phytonematicides, for granular and liquid formulations:

Ground leaching technology (GLT): The technology involves using small quantities of powdered organs from *C. myriocarpus* and *C. africanus* to suppress plant-parasitic

nematodes (Mashela, 2002). In this intervention, the materials are applied after seedling emergence of the protected crops against nematodes (Mashela *et al.*, 2017). The GLT technology is user-friendly and affordable for use in small-scale farming system. Nemarioc-AG phytonematicide was successfully used in managing nematode population densities through the GLT system (Mashela, 2002). Active ingredients of phytonematicides suppressed plant-parasitic nematodes in microplot trials by 90% since they are placed on the rhizosphere. This method is labour-intensive and infrastructure-intensive, respectively. The use of seeds as carriers of active ingredients for the suppression of nematodes would be cost-effective and therefore suitable as a placement method for different farming systems. Availability of resources, expensive transportation costs, waiting period for bacterial degradation and reduction of soil pH are all often cited as drawbacks of conventional organic amendments (Mashela and Nthangeni, 2002).

Botinemagation technology: In botinemagation application technology (BAT), the phytonematicide in liquid formulation is applied through irrigation water (Mashela *et al*., 2011), and this was developed for ameliorating some of the drawbacks associated with the ground Leaching technology (GLT), where phytonematicides were applied in granular formulation. Botinemagation was tested on tomato plants where growth was stimulated at low concentration and inhibited at high concentration. Similarly, Tseke *et al*. (2013), observed plant height and dry root mass that of tomato plants exhibited strong positive quadratic relation when exposed to Nemarioc-AL phytonematicide, with the model explaining the relations by 84 and 98%, respectively. Additionally, Sithole *et al*. (2016) observed similar results on plant height and dry shoot mass of geranium (*Pelargonium sidoides* L.) plant when

exposed to increasing concentration of Nemafric-AL phytonematicide, where the relations were explained by 95 and 89%, respectively.

Priming Technology: Seed priming is a process of pre-sowing treatments in osmotic solution or water that allows seeds to absorb water to initiate germination (Subedi *et al.,* 2015). After sowing seeds in treatments, the cotyledons stay in the soil for some time to imbibe water and nutrients for its growth. Seed priming reduces the time seeds take to imbibe water and makes germination faster and uniformly. Seed priming also decreases the sensitivity of seed to environmental factors (Afzal *et al.,* 2016). Priming promotes seed germination under three stages such as imbibition, germination, and growth (Subedi *et al.,* 2015).

2.3 Work not yet done on the problem statement

Cucurbitacin containing phytonematicides had been researched and developed for application through GLT and botinemagation, but not for priming technology. The concept of using hypogeal germinated seeds, as carriers of active ingredients from the cucurbitacin-containing phytonematicides would improve the adoption of these products in the production of crops with hypogeal germination attributes. Currently, the technology has not been tested on runner bean seeds that have hypogeal germination.

2.3 Addressing the identified gaps

The cucurbitacin phytonematicides suppressed nematode populations under microplot and greenhouse experiments by at least 90% in roots (Mashela *et al*., 2011). The current study intends to address the gap by focusing on the influence of

cucurbitacin containing phytonematicides on plant growth and yield, all together with the suppression of nematode populations under greenhouse and microplot conditions. Additionally, the study would report on the effects of phytonematicides on the hypogeal germinated seeds of runner beans.

CHAPTER 3

RUNNER BEAN SEEDS *IN VITRO* AS CARRIERS OF ACTIVE INGREDIENTS OF PHYTONEMATICIDES

3.1 Introduction

The southern root-knot nematode (*Meloidogyne s*pecies*)* causes severe damages on crops, mainly in tropical areas with sandy soils (Sikora and Fernandez, 2005). This nematode species threatens food security in various countries, including South Africa (Ntidi *et al*., 2012). The damage caused by nematodes to crops is directly proportional to the initial nematode Pi at sowing or transplanting (Seinhorst, 1965). Therefore, Pi should be at its lowest at sowing or transplanting in order to optimise crop yield in various crops. In management of nematodes, it is vital to maintain the Pi below the damage threshold level at sowing if the economic crop yields are to be realised.

Germination of seeds and emergence of seedlings are chemical and physical processes, associations (Hartmann *et al*., 2002). Normally, biological activities of allelochemicals in germination of seeds is concentration-dependent with a response threshold being lower than that where growth is improved (Einhellig, 1986). Previously, fumigant nematicides were used to reduce Pi levels, however, due to their high levels of phytotoxicity, mammalian toxicity and eco-unfriendliness the product had been removed from the agrochemical markets (Chitwood, 2002). The withdrawal of fumigant nematicides with the 2005 deadline (Mashela *et al.,* 2015a), led to increased focus on other approaches for managing root-knot nematodes under climate smart agriculture (McSorley, 2011). Hence cucurbitacin containing phytonematicides were examined and established for use as alternative for

managing nematode population densities (Mashela *et al.,* 2017). Mafeo (2012) demonstrated that Nemarioc-AG phytonematicide, a granular formulation, significantly reduced seedling emergence in both dicotyledonous plants such as onion, leek and chive, and monocotyledonous plants such as maize, millet and sorghum. Ntuli (2021) demonstrated that in liquid formulation, priming of seeds in Nemarioc-AL and Nemafric-BL (L = liquid) phytonematicides did not affect seed germination for pea plants under *in vitro* conditions. Pea seeds have hypogeal germination attributes. Also, runner beans have such germination attributes, but their responses to priming in cucurbitacin phytonematicides on germination had not been documented. Therefore, the objective of this study was to examine whether runner bean seeds would serve as carriers of active ingredients when primed in cucurbitacin-containing phytonematicide solution under *in vitro* conditions.

3.2 Materials and methods

3.2.1 Description of the study site

Seed germination trials were conducted in petri dishes under laboratory conditions inside the LABCON (Model: L.T.G.C) growth chamber at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, Limpopo Province, South Africa (23°53′10″S, 29°44′15″E) during autumn (March to May 2018) and validated in 2020.

3.2.2 Treatments and research design

Parallel but separate trials were conducted for Nemarioc-AL and Nemafric-BL phytonematicides. Ten seeds of runner bean cv. 'Lazy housewife' were separately primed for 2 h, in 50 ml of each phytonematicide concentration of 0, 2, 4, 8, 16, 32,

and 64% (Figure 3.1), with solutions discarded and seeds for each treatment wiped dry using tissue paper and seeds dried at room temperature petri dishes were lined with Whatmann No. 1 filter paper, with 10 seeds placed in the petri dish and covered with another filter paper, which was moistened with tapwater and seven treatments arranged in a completely randomised design (CRD) in a growing chamber, with six replications.

Figure 3.1: Priming seeds of cv. 'Lazy housewife' runner been in Nemafric-BL and Nemarioc-AL phytonematicides.

3.2.3 Procedures of phytonematicides preparation and seed priming

Matured fruits of *C. myriocarpus* and *C. africanus* were collected locally, washed with chlorine-free tapwater, chopped into smaller parts, and dried at 52ºC for 72 h inside an oven (Mashela e*t al*., 2011). The material was ground with Wiley mill via 1-mm-mesh sieve and then finely powdered using A43 Monlinex coffee grinder. Ground materials were kept in hermitically airtight plastic bags at room temperature for future use. Approximately 80 g ground material of *C. myriocarpus* and 40 g for *C. africanus* were used for fermentation in 20 L-hermetically impenetrable plastic container with 16 L chlorine-free tapwater (Mashela *et al*., 2017). Carbon dioxide was allowed to outflow from the fermentation container via impermeable 5 mm diameter tube glued on one end to a hole on the container lid, whereas the other end was placed inside

half-filled 2 L bottle with tapwater. Nearly 100 g brown sugar, 300 ml molasses and 300 ml ZZ2 effective microorganisms (EM) were supplemented into the container (Pelinganga *et al*., 2012). After 14 days incubation period, once pH was at ±3.7 (Kyan *et al*., 1999), the phytonematicide was used to prime seeds for 2 h. Whatmann No. 1 filter papers were laid inside 90-mm plastic Petri dishes, with each Petri dishes seeded with primed 10 runner bean seeds. Petri dishes were placed inside LABCON (Model: L.T.G.C.) growth chamber in laboratory conditions at 25ºC and 75% relative humidity. Successful seed germination, viewed as emergence of radicle from the testa, was recorded everyday a period of 10 days. Counts were being removed from the petri dishes to avoid re-counting.

3.2.4 Data collection

Successful germinated seeds, observed as seed coat ruptured by the radicle, were recorded daily for a period of 10 days. Counts were removed from the Petri dishes to avoid re-counting. On day 10 after 100% germination, radicle length and plumule length were measured using a measuring ruler, whereas radicle diameter and plumule diameter was measured with a Vernier calliper.

3.2.5 Data analysis

The geometric concentration series was transformed into an exponential series 2^0, 2^1, 2^2, 2^3, 2^4, 2^5 and 2^6 prior to log-transformation (Causton, 1977; Mashela *et al*., 2020). Using $\log_2 2^x = x;(\log_2 2) = 1$ the log-transformed series became 0, 1, 2, 3, 4, 5 and 6%. Data were subjected to the Curve-fitting Allelochemical Response Dose (CARD) algorithm model to generate biological indices and curves (Liu *et al.,* 2003), with those of interest being ton the stimulation phase, namely, the stimulation

threshold point (D_m) at the beginning of the phase, the saturation point (R_h), at the end of the phase. The two biological indices, D_m and R_h, were used for computation of the Mean Concentration Stimulation Point [MCSP = D_m + (R_h/2)] (Mashela *et al.,* 2017). The MCSP is technically referred to as the first law of phytonematicides, which is the concentration of phytonematicides that does not induce phytotoxicity on crops being protected against nematodes (Mashela *et al*., 2017).

3.3 Results

Germination percentage (Figure 3.2), radicle length (Figure 3.3) and plumule diameter (Figure 3.4) over increasing concentration of Nemarioc-AL phytonematicide exhibited positive quadratic relations, with models explained by 96, 89 and 96%, respectively (Table 3.1). However, radicle diameter (Figure 3.5) against Nemarioc-AL phytonematicide exhibited negative quadratic relation, with the models explained by 82%. In contrast, germination percentage (Figure 3.6), radicle diameter (Figure 3.7) plumule diameter (Figure 3.8) and plumule length (Figure 3.9) over increasing Nemafric-BL phytonematicide exhibited positive quadratic relations, with the models explained by 97, 98, 97 and 94%, respectively (Table 3.1). Biological indices for germination percentage, radicle length, radicle diameter, plumule length and plumule diameter were generated using the CARD model (Table 3.1). The MCSP value of Nemarioc-AL phytonematicide on runner bean seeds was 1.05%, while for Nemafric-BL phytonematicide was 0.58% (Table 3.1).

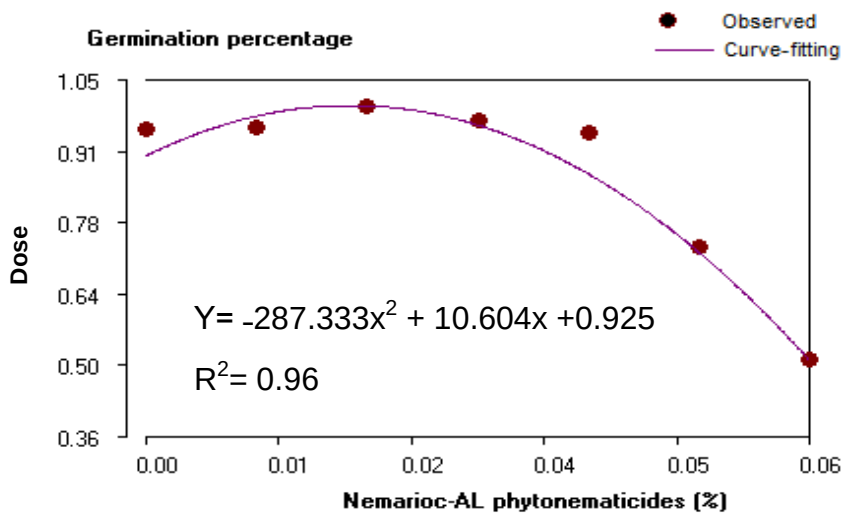


Figure 3.2: Quadratic relationship between germination of runner beans and Nemarioc-AL phytonematicide (%) at 7 days after treatment.

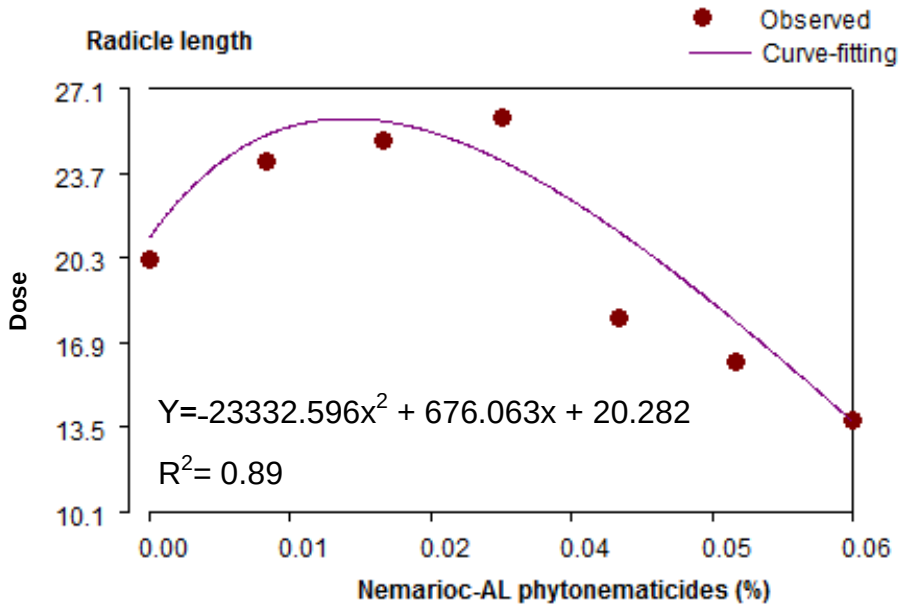


Figure 3.3: Quadratic relationship between radicle length of runner beans and Nemarioc-AL phytonematicide (%) at 7 days after treatment.

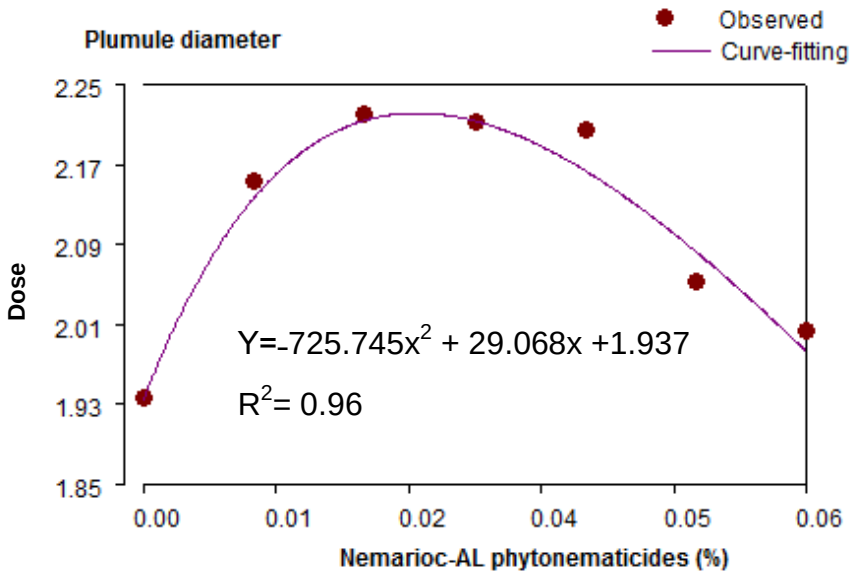


Figure 3.4: Quadratic relationship between plumule diameter of runner beans and Nemarioc-AL phytonematicide (%) at 7 days after treatment.

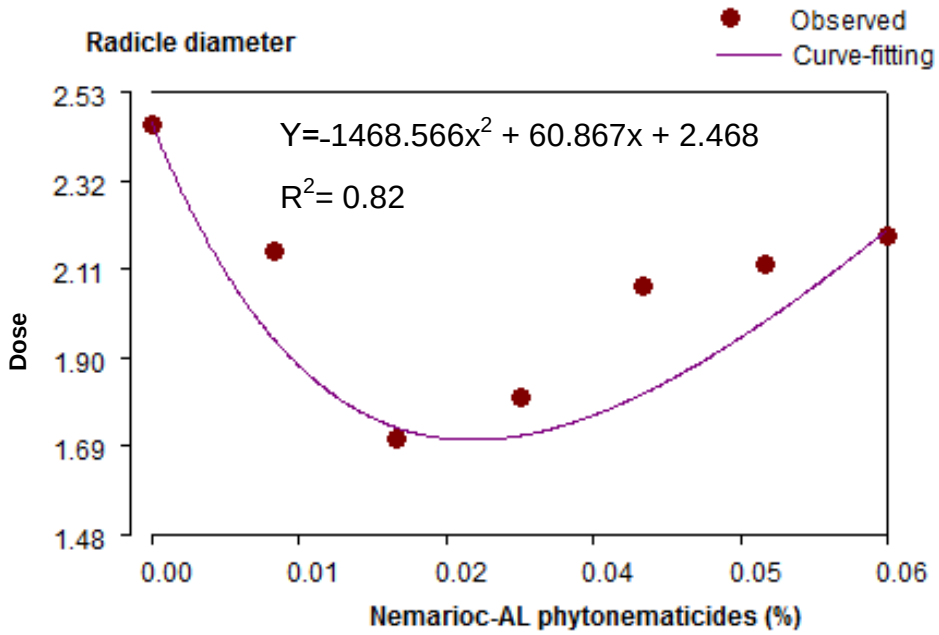


Figure 3.5: Quadratic relationship between radicle diameter of runner beans and Nemarioc-AL phytonematicide (%) at 7 days after treatment.

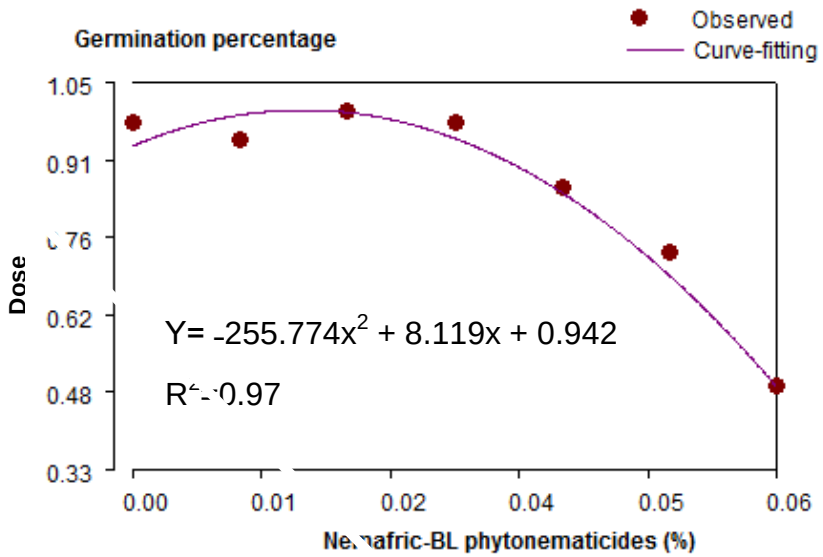


Figure 3.6: Quadratic relationship between germination percentage of runner beans and Nemafric-BL phytonematicide (%) at 7 days after treatment.

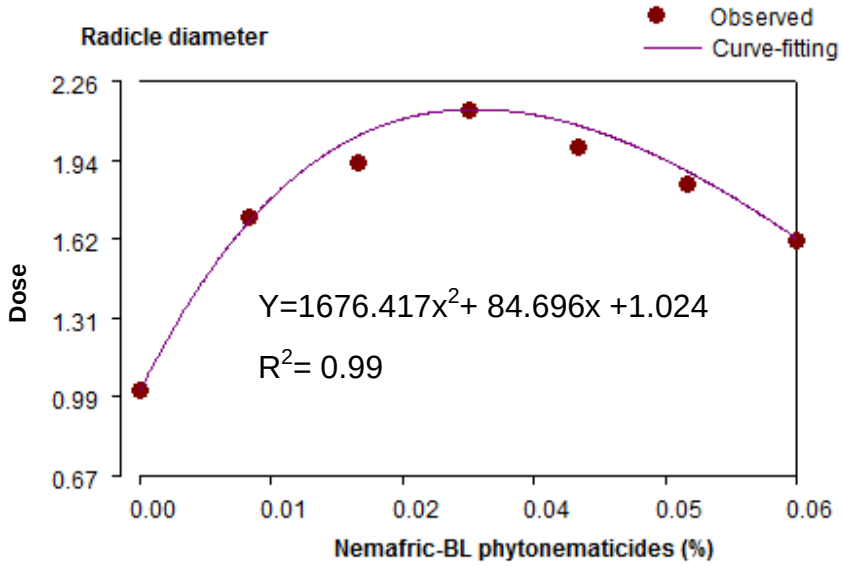


Figure 3.7: Quadratic relationship between radicle diameter of runner beans and Nemafric-BL phytonematicide (%) at 7 days after treatment.

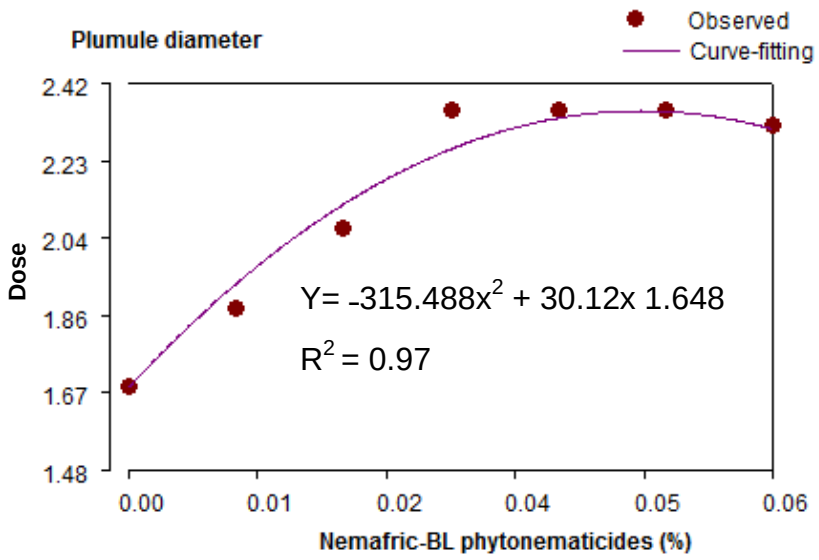


Figure 3.8: Quadratic relationship between plumule diameter of runner beans and Nemafric-BL phytonematicide (%) at 7 days after treatment.

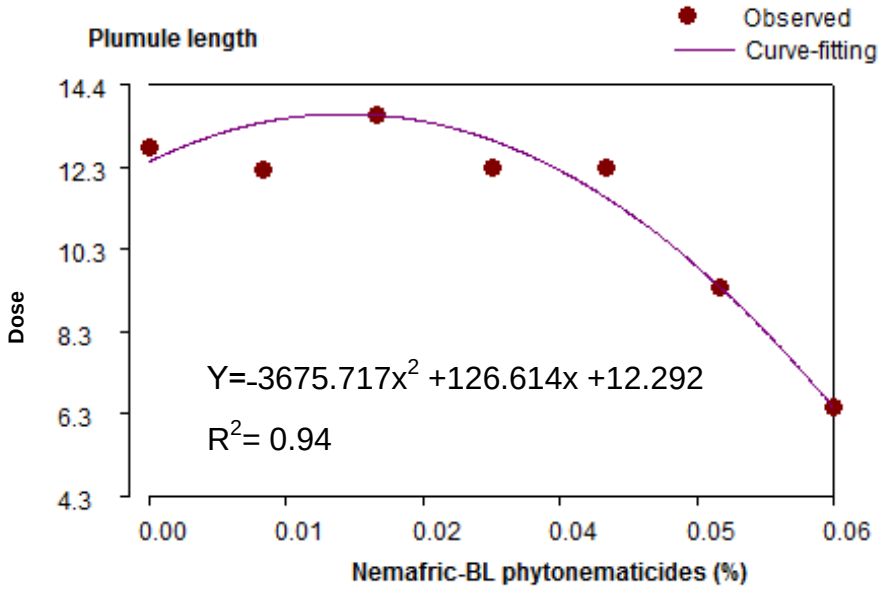


Figure 3.9: Quadratic relationship between plumule length of runner beans and Nemafric-BL phytonematicide (%) at 7 days after treatment

Table 3.1 Biological indices for germination percentage, radicle and plumule of runner bean in response to increasing concentration of Nemafric-BL phytonematicide (%).

Biological index[Z]	Nemarioc-AL phytonematicide				Nemafric-BL phytonematicide				
	Germination percentage	Radicle length	Plumule diameter	Mean	Germination percentage	Radicle diameter	Plumule diameter	Plumule length	Mean
Threshold stimulation (D_m)	0.018	0.017	0.025	0.020	0.016	0.031	0.480	0.017	0.136
Saturation point (R_h)	0.98	4.897	0.291	2.056	0.064	1.07	0.719	1.09	0.880
0% inhibition (D_0)	0.037	0.041	0.064	0.047	0.032	0.078	0.095	0.034	0.060
50% inhibition (D_{50})	0.063	0.066	0.146	0.092	0.062	0.091	0.118	0.062	0.083
100% inhibition (D_{100})	0.1	0.1	0.3	0.167	0.1	0.1	0.1	0.1	0.01
R^2	0.96	0.89	0.96		0.97	0.99	0.97	94	
Sensitive index (k)	0	20	19		0	14		0	
Overall sensitivity (∑k)		39					14		
MCSP = D_m + (R_h/2)		1.05%					0.58%		

3.4 Discussion

Exposure of runner bean seeds to an increasing concentration of Nemarioc-AL phytonematicide, seed germination, exhibited positive quadratic relationships, which contradicted other findings (Mafeo, 2012). The positive quadratic relationships could suggest that the concentration of Nemarioc-AL phytonematicide used in this study were within the saturation point needed to induce stimulation responses. Similarly, seed germination, radicle diameter and plumule length against Nemafric-BL exhibited positive quadratic relationships, which contradicted other observations (Mafeo, 2012). In contrast, radicle diameter versus Nemarioc-AL phytonematicide exhibited negative quadratic relations, which could mean that the concentration of Nemarioc-AL phytonematicide used in the study were already above the saturation point essential to induce stimulation responses.

The focus of this study was to investigate whether priming hypogeal seeds such as runner bean seeds in Nemarioc-AL and Nemafric-BL phytonematicides could reduce seed germination. Seed germination is a chemical process, which occurs within a seed, starting from water uptake and ending immediately when the radicle raptures the seed coat (Bewley, 1997). Germination of seeds start with an instigation of complicated chemical processes which comprise of sugars and minerals absorption, hormone synthesis, membrane permeability, cell division, and enzyme activity (Campbell, 1990). However, allelopathic chemicals from both Nemafric-BL and Nemarioc-AL phytonematicides can affect any of the activities mentioned above, and therefore, restrict or stimulate germination.

Apart from cucurbitacin-containing phytonematicides, other researchers indicated that the majority of plant extracts can inhibit or stimulate seed germination in various agricultural crops. Chon *et al.* (2005) indicated that allelopathic chemicals from plant extracts may affect plants, either positively or negatively, which support the findings obtained from this study. Lee *et al.* (2010) reported that cucurbitacins stimulate cell division of animals at low concentration. The observed quadratic relationships between germination and concentration of Nemafric-BL phytonematicide indicated that there may have been some concentration which stimulated seed germination and seedling emergence. The detected relationships suggest to conditions of the DDG patterns as expressed for mainly biological systems (Salisbury and Ross, 1992; Liu *et al.,* 2003).

In contrast, Mazloom *et al*. (2009) demonstrated that alkaloids found in winter wheat (*Triticum aestivum* L.) grasses delayed the reserve food metabolism in the endosperm of germinating seeds of thorn-apple (*Datura stramonium* L.). In linseed (*Linum utatissimum* L.), the radicle protrusion and elongation were inhibited by an allelochemical called benyl-amine, which is produced from leaf washings of camelina weed (*Camelina sativa* L.) (Lovett *et al.,* 1989). Moreover, Inderjit and Duke (2003) indicated that allelopathic chemicals from other sources prohibited division of embryo cells (Einhellig, 1985; Martin and Blackburn, 2003), although in other cases allelopathy inhibited hydrolytic enzyme activities or GA (Jerônimo *et al.,* 2005). Einhellig (1985) also reported that juglone and sorgoleone, which are powerful allelochemicals from *Junglans nigra* (L.) leaves, prevented the first action for the production of ATP in germinating seeds of white mustard (*Sinapis alba* L.) by

preventing chloroplast oxygen evolution in the cotyledons and disturbing mitochondrial functions.

The results observed in this current study, proposed that processes involved in the prevention of radicle diameter growth of runner beans were having similar pathways, as outlined in germination of seeds and seedling emergence (Campbell, 1990). Chen *et al*. (2005) indicated that numerous plants in the family Cucurbitaceae under the genus *Cucumis* contain cucurbitacins, which are allelochemicals that have an auto-allelopathy with powerful inhibition of germination. Marcias *et al.* (2002) indicated that some of the allelopathic chemicals that have been reported in inhibiting germination as well as seedling emergence involve flavonoids, phenolic and terpenoids compounds, with the latter including cucurbitacins.

In the biological systems, the quadratic relationships are indicative of the existence of DDG patterns (Pofu *et al.,* 2010). The DDG patterns are characterised by the existence of three phases, namely, stimulation phase, neutral phase and inhibition phase, each with distinct concentration ranges (Liu *et al.,* 2003). The quadratic relationships for runner beans were straddling the stimulation phase, neutral phase and inhibition phase for germination when exposed to increasing concentration of Nemarioc-AL and Nemafric-BL phytonematicide prior to germination.

The MCSP obtained in this study for Nemarioc-AL and Nemafric-BL phytonematicides were derived at 1.05% and 0.58%, respectively. The overall sensitivity index of runner beans to against Nemarioc-AL and Nemafric-BL phytonematicides were 39 and 14 units respectively, thereby suggesting that the

tested crops are less sensitive to the product when used as a pre-emergent phytonematicide under laboratory condition (Mashela *et al.,* 2015b). Others demonstrated that green bean (Chokoe, 2017), geranium (Sithole, 2016) and tomato (Pelinganga and Mashela, 2012) plants exhibited moderate sensitivity to both phytonematicides. Crop sensitivity to cucurbitacin-containing phytonematicide is inversely proportional to k values, with high k values indicating that the crop was less sensitivity to the product used, while zero or low values suggested high sensitivities (Liu *et al.,* 2003).

3.5 Conclusions

The results obtained from this study propose that the quantities of both phytonematicides were well-suited with germination and emergence of runner bean seeds. In respect to the observed density-dependent relationships in the current study, there could be concentration of both phytonematicides that could stimulate seed germination of runner beans. The CARD model illustrated that the response of runner beans when exposed onto crude extracts of *Cucumis* fruits exhibited the DDG patterns. Using the combination of the responses of germination and emergence to phytonematicides within the stimulation range, the amount of the product which could be used as pre-emergent seed treatment were estimated as 1.05% (Nemarioc-AL phytonematicide) and 0.58% (Nemafric-BL phytonematicide) material/ seed.

CHAPTER 4

RUNNER BEAN SEEDS AS CARRIERS OF ACTIVE INGREDIENTS OF PHYTONEMATICIDES UNDER *EX VITRO* CONDITIONS

4.1 Introduction

The cucurbitacin-containing phytonematicides had been consistent in supressing population densities of root-knot (*Meloidogyne species)* nematodes (Mashela *et al*., 2015b). Currently, there are two empirically based application technologies, namely, the ground leaching technology (GLT) and the botinemagation technology (Mashela *et al*., 2011). In GLT, active ingredients of granular formulations are leached from phytonematicides through irrigation water (Mashela, 2002), whereas in botinemagation phytonematicides in liquid formulation active ingredients are applied through irrigation water (Mashela *et al*., 2011). Each of the application technologies have advantages and disadvantages. The GLT was established for use in improving the drawbacks of conventional organic amendments in suppressing root-knot nematodes, which included: (1) unavailability of organic resources in adequate amounts, (2) large amounts needed for nematode management, (3) inconsistent results in nematode suppression, (4) high transportation costs when more amounts were available distant from the site (5) decline of soil pH, which consistently improved the unavailability of certain nutrient elements from the soil, and (6) the waiting period to boost microbial decay and, therefore, to evade negative period (Mankau, 1968; Mashela, 2002; 2007; Rodriguez-Kabana, 1986; Stirling, 1991). One other advantage of using GLT is that nematode suppression is reliably attained, irrespective of the environment where the experiment was carried out (Mashela *et*

al., 2017). However, the system is labour-intensive and therefore, suitable for smallholder farming systems (Pelinganga *et al*., 2013).

In some cases, seeds are dressed using synthetic chemical pesticides, especially in cases where the pesticides are not phytotoxic (Mancini and Romanazzi, 2014). In phytonematicides, which could be highly phytotoxic, Mafeo (2012) demonstrated that seedlings of certain crops were highly sensitive to cucurbitacin phytonematicides in granular formulation, suggesting that seed dressing using such materials could hardly be feasible. Priming seeds in liquid formulation of phytonematicides for short periods would probably allow various parts of the seed to imbibe the cucurbitacin active ingredients, thereby using remnants in plants with hypogeal germination attributes as carriers of active ingredients. However, due to the existence of epigeal and hypogeal germination attributes in seeds, the priming technology might not be a panacea for all plant seeds. Apparently, the technology would be suitable for plants with hypogeal germination, where the seed remnants below the soil surface could serve as a source for releasing cucurbitacins into the rhizosphere as the radicle develops into the root system. In a preliminary trial under *in vitro* conditions, it was shown that seeds of runner bean (*P. coccineus*) were able to germinate after being soaked in solutions of cucurbitacins and then germinated under *in vitro* conditions (Chapter 3). However, growth of such seeds under *ex vitro* conditions had not been documented. The objective of this study was to investigate whether seeds of runner bean, with hypogeal germination properties, would be amenable to serve as carriers of cucurbitacin intended for suppression of plant-parasitic nematodes under greenhouse and microplot conditions.

4.2 Materials and methods

4.2.1 Description of the study site

The study was conducted at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, South Africa (23°53′10″S, 29°44′15″E).

Greenhouse studies: Nemafric-BL phytonematicide and Nemarioc-AL phytonematicide parallel studies were conducted inside the greenhouse during autumn (March to May) in 2018 and validated in autumn 2020. The greenhouse temperature and humidity were controlled by thermostatically activated fans and a wet wall for moderating relative humidity with ambient temperatures (day/night) during each season averaged 28/21°C. Based on the size of the greenhouse and wind streams created by heat extracting fans, conditions inside the greenhouse environment were not homogeneous, therefore, necessitating that treatments be properly arranged.

Figure 4.1: Effects of cucurbitacin-containing phytonematicides on growth of runner beans under greenhouse conditions.

Microplot conditions: Two separate studies were conducted at a site that receives a summer rainfall with an annual mean of 500 mm. The study was conducted during autumn (March to May) in 2018 and validated in 2020. The two experiments were initiated by positioning 30-cm-diameter plastic pots into 20-cm deep holes at an intra-row spacing of 0.3 m and an inter row spacing of 0.6 m, at a site that has dry hot summers, with daily extreme temperatures fluctuating between 28 to 38°C. Windbreak shading in the afternoon necessitated properly designed experiments.

Figure 4.2: Effects of cucurbitacin-containing phytonematicides on growth of runner beans under microplot conditions.

4.2.2 Treatments and research design

Separate trials were conducted for Nemafric-BL and Nemarioc-AL phytonematicides. In each trial, treatments comprised 0, 2, 4, 8, 16, 32 and 64% phytonematicide solutions. Treatments were arranged in a randomised complete block design (RCBD) since greenhouse and microplot conditions were not homogeneous, with six replications.

4.2.3 Procedures of phytonematicides preparation and seed priming

Nemafric-BL and Nemarioc-AL phytonematicides were prepared as described previously (Mashela *et al.,* 2017; Pelinganga, 2013). Nematode inocula from tomato cv. 'Floradade' were prepared as described by Hussey and Barker (1973). Pasteurised loam soil (65% sand, 30% silt and 5% clay) was used to fill 20-cm and 30-cm diameter plastic pots on greenhouse benches and on microplots, respectively. Runner bean seeds were primed in solutions of the respective phytonematicides at 0, 2, 4, 8, 16, 32 and 64% for 2 h and then dried at room temperature to arrest Phase I of the germination process (Lechowska, *et al*., 2019). Two seeds were then sown per pot.

At 5 days after 100% seedling emergence, 2 g of 2:2:2 (43) fertiliser was used to fertilise each seedling, which provided a total of 0.175 mg N, 0.16 mg K and 0.16 mg P, 0.45 mg, 0.378 mg Fe, 0.0375 mg Cu, 0.175 mg Zn, 0.5 mg B, 1.5 mg Mn and 0.035 mg Mo per ml chlorine-free tapwater (Mashela, 2002). Each seedling was irrigated to full capacity using 500 ml using a measuring beaker with chlorine free water immediately 50% moisture meter readings were less than 2 units.

Inocula were prepared by obtaining and eggs and second-stage juveniles (J2) of *M. incognita* race 4 from the root systems of highly nematode-susceptible tomato cv. 'Floradade' grown under greenhouse conditions. Each plant was inoculated by dispensing 5 000 eggs + J2 of *M. incognita* race 4 using a 50 ml plastic syringe by inserting into 5-cm deep holes around cardinal points on the stem of seedlings. At two leaf-stage, seedlings were thinned to one per pot. Seedlings were irrigated every

other day using 250 ml tapwater. Weekly scouting for whiteflies (*T. vaporariorum*) inside the greenhouse was conducted, with plants being sprayed with 1.33-ml Cypermethrin per litre of water when the number of whiteflies (*T. vaporariorum*) spotted were more than 10 insects per five randomly selected crops.

4.2.4 Data collection

At 56 days after inoculation, a meter stich was used to measure plant height from the crown to the tip of the flag leaf, with pod number per plant counted. Chlorophyll meter was used to measure chlorophyll content of three mature and healthy-looking leaves per plant. Shoots were severed at ground level and Vernier calliper used to measure stem diameter at 5-cm above the severed end. Shoots and pods were separately oven-dried for 72 h at 70°C and weighed. Root systems were immersed in water to dislodge soil particles, with excess moisture removed by wrapping roots in laboratory paper towel and then weigh fresh root mass to enhance nematode calculation per entire root system. North Carolina Differential Rating Scale was used to determine the root galls at 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, 5 = > 100 (Taylor and Sasser, 1978). Nodules were rated using the nodulation rating scale of 0 = no nodules, 1 = 5 or 1 large nodule, 2 = 10 or 2 large nodules, 3 = 15 or 3 large nodules, 4 = 20 or 4 large nodules, 5 = 25 or 5 large nodules (Truchet *et al*., 1989). Nematode extraction was achieved from 10 g fresh root per plant through blending and maceration in 1% NaOCl solution for 30 seconds (Hussey and Barker, 1973). The materials were passed through 75-µm and 25-µm nested sieves, with contents of 25-µm sieve, further subjected to sugar floatation and centrifugation method (Jenkins, 1964). Soil inside each pot was mixed thoroughly and a 250 ml sample was collected for nematode extraction using the

sugar floatation and centrifugation method (Jenkins, 1964). Eggs and J2 from root samples and J2 from soil samples were counted using the stereomicroscope from a 10-ml aliquot. Nematodes from root systems were transformed to total number of nematodes per entire root system for each plant, whereas J2 from soil samples were transformed to total soil per pot, which for greenhouse and microplots were 2700 and 5000 ml, respectively.

Nutrient analysis were achieved from selected mature runner bean leaves and ground using grading machine prior to digestion. Approximately 0.4 g ground leaf material was digested in 75 ml vessel with 3 ml 30% hydrogen peroxide (H_2O_2) and 5 ml 70% nitric acid (HNO_3). The mixture was vortexed, and samples were allowed to cool down for at least 10 minutes prior to closing the vessel, which were then inserted into the microwave digester (PerkinElmer, Tatan MPS) to run for 46 minutes under temperatures ranging up to 260°C. Thereafter, the vessels were allowed to cool at room temperature for 20 minutes. The samples from the vessels were decanted into 50 ml tubes and stored in the cold room to avoid evaporation of samples prior to analytical work. Runner bean leaf samples were then analysed for Ca, Fe, K, Mg and Zn using inductively coupled plasma spectrometry (Shimadzu, ICPE-9000).

4.2.5 Data analysis

Data for plant variables were subjected to the Curve-fitting Allelochemical Response Dose (CARD) algorithm model (Liu *et al*., 2003) to generate biological indices used to calculate Mean Concentration Stimulation Point (MCSP) values for both Nemafric-BL and Nemarioc-AL phytonematicides on runner beans under greenhouse and

microplot conditions. All plant variables that had positive quadratic relation with phytonematicides were averaged using their indices. The MCSP was derived using (Mashela *et al*., 2015a):

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

The CARD also provided the sensitivity (k) and overall sensitivity ($\sum_k$) of the variables (Liu *et al*., 2003).

Calcium, Fe, K, Mg and Zn in leaf tissues was first subjected to analysis of variance, with Mean Sum of Squares partitioned to generate the total treatment variation (TTV) (Appendix 4.1- 4.4), with treatment means then subjected to lines of the best fit. Unless stated otherwise, treatment effects were discussed at the probability level of 5%.

4.3 Results

4.3.1 Greenhouse studies

Nemafric-BL phytonematicide: Treatment had significant effects on chlorophyll content, gall rating, dry shoot weight, stem diameter and total number of nodules of runner bean (Table 4.1). Chlorophyll content, gall rating, dry shoot weight, stem diameter and total nodule number versus Nemafric-BL phytonematicide exhibited positive quadratic relationships, with the model explained by 95, 82, 69, 85 and 86% associations (R^2), respectively (Figure 4:3–4.4). The MCSP was 3.69% Nemafric-BL phytonematicide, with the $\Sigma k = 5$ (Table 4.1).

Nemarioc-AL phytonematicide: Plant height, chlorophyll content, dry shoot mass, number of nodules dead, total number of pods and number of nodules active, versus Nemarioc-AL phytonematicide exhibited positive quadratic relation (Figure 4.5 – 4.6), with the model explained by 97, 92, 98, 90, 97 and 93% associations, respectively (Figure 4.5 – 4.6). The MCSP for Nemarioc-AL phytonematicide was 4.18%, with k value of zero (Σk = 0) in all variables (Table 4.1).

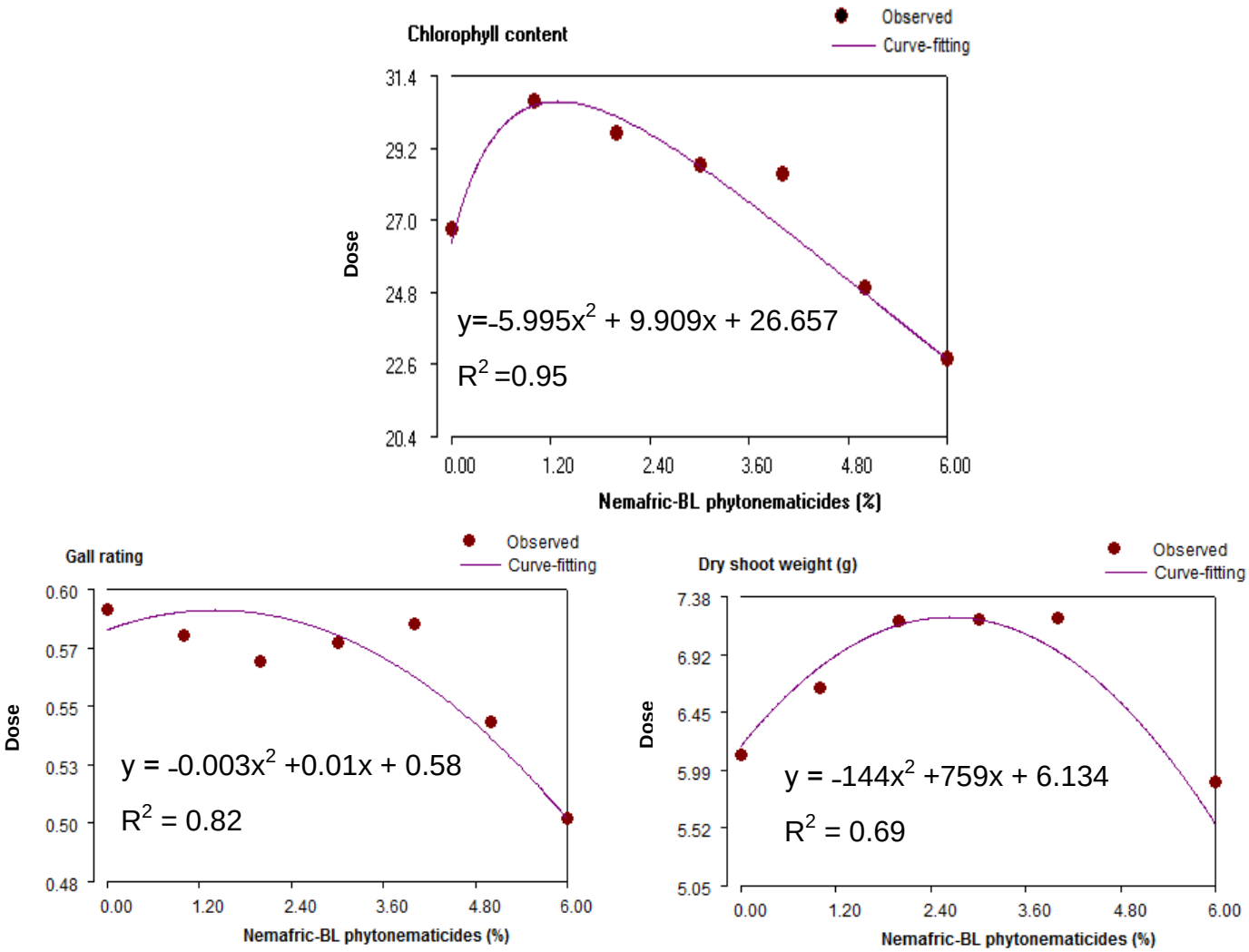
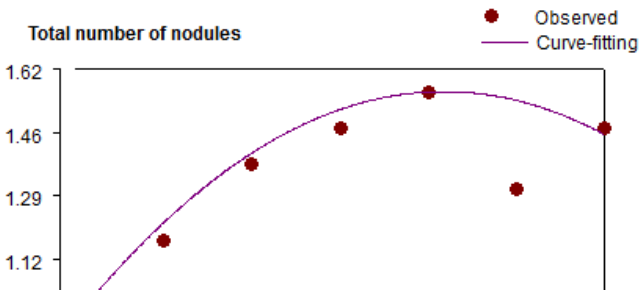


Figure 4.3: Responses of chlorophyll content (CC), gall rating (GR), dry shoot mass (DSM) of runner bean primed with Nemafric-BL phytonematicide (%) at 56 days after initiation of treatments.



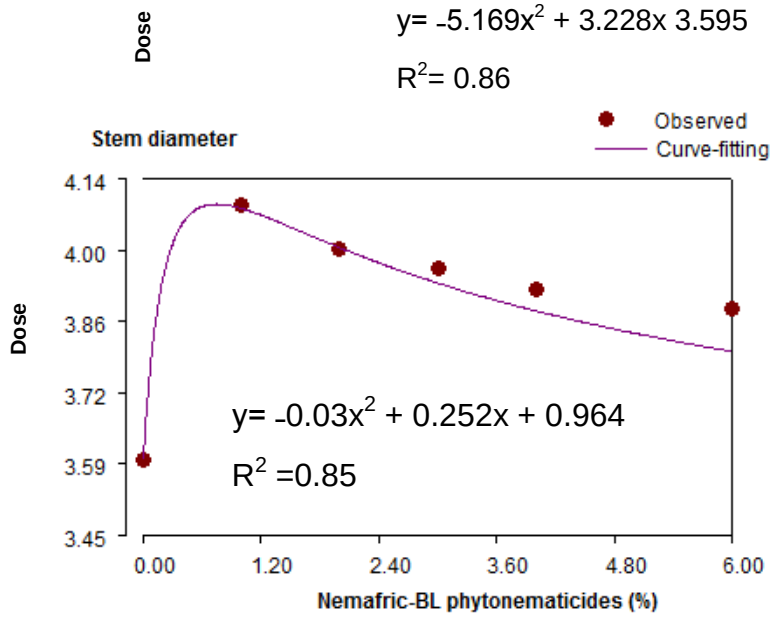


Figure 4.4: Responses of total number of nodules (TNN) and stem diameter (SD) of runner beans primed with Nemafric-BL phytonematicide (%) at 56 days after initiation of treatments.

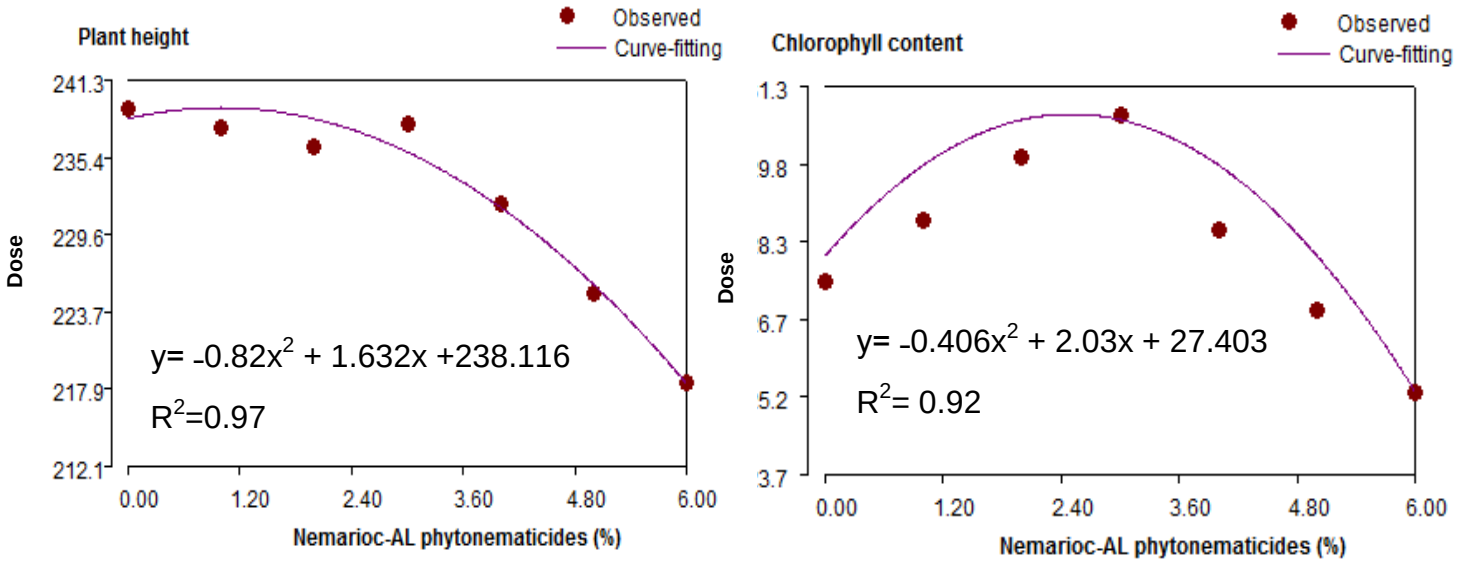
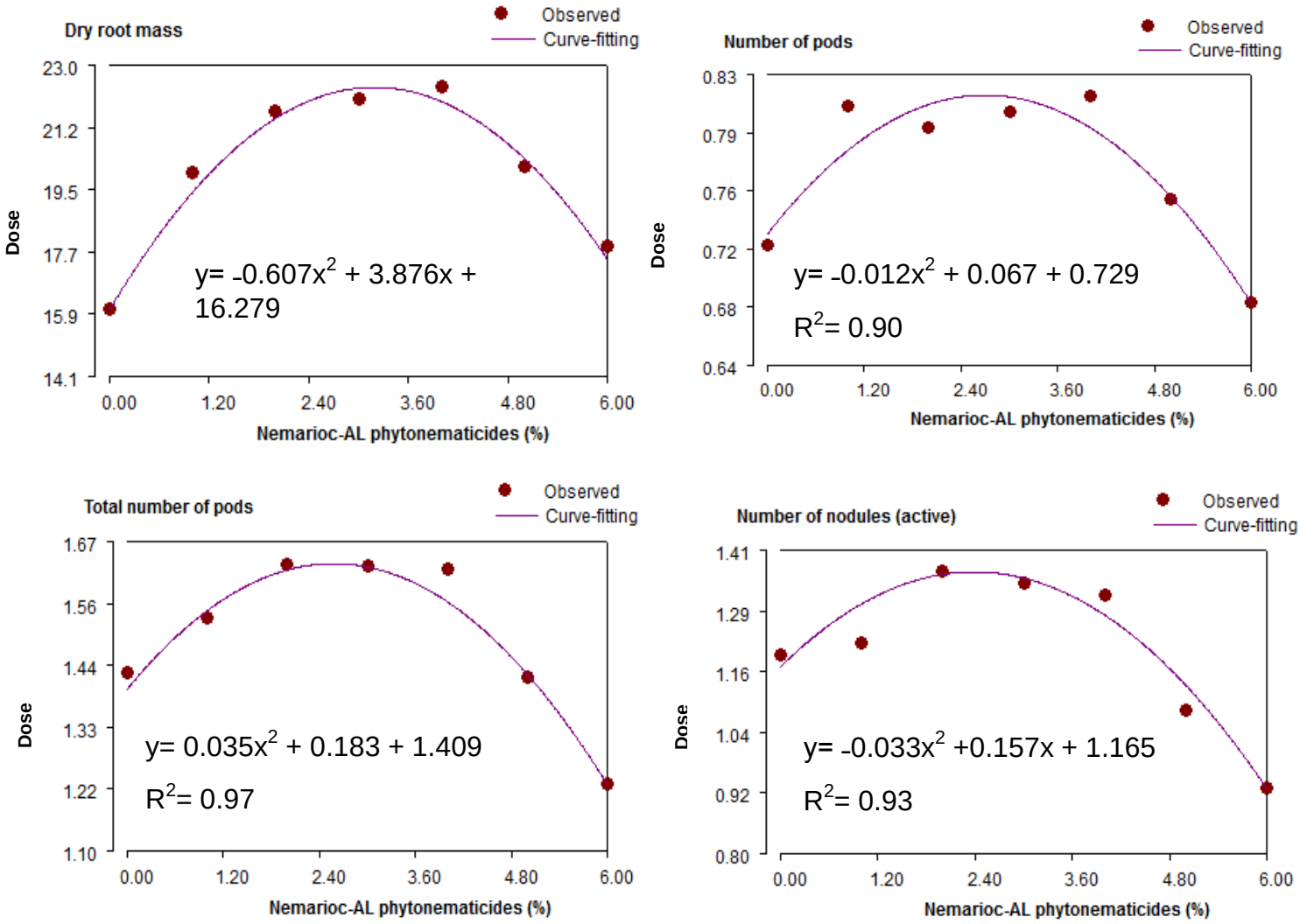


Figure 4.5: Responses of plant height (PH) and chlorophyll content (CC) of runner bean seeds primed with Nemarioc-AL phytonematicide (%) at 56 days after initiation of treatments.



nodules active (NONA) of runner bean seeds primed

Figure 4.6: Responses of dry root mass (DRM), Number of pods (NP), Total number of pods (TNP) and number of nodules active (NONA) of runner bean seeds primed with Nemarioc-AL phytonematicide (%) at 56 days after initiation of treatments.

Table 4.1: Biological indices of chlorophyll content (CC), dry shoot mass (DSM), gall rating (GR), stem diameter (SD), total number of nodules (TTN), dry root mass (DRM), number of pods (NP) and number of nodules active (NONA) of runner bean seeds primed with increasing concentration of Nemafric-BL and Nemarioc-AL phytonematicides (%) (Experiment 1) at 56 days after initiating treatments.

Biological indices	Nemafric-BL					Nemarioc-AL					
	CC	DSM	GR	SD	TNN	PH	CC	DRM	NP	TNN	NONA
Threshold stimulation (D_m)	1.285	2.645	1.409	0.745	4.177	0.995	2.5	3.192	2.697	2.578	2.388
Saturation point (R_h)	4.095	1.004	0.007	0.504	0.526	0.812	2.537	6.187	0.09	0.236	0.187
0% inhibition (D_0)	4.223	5.289	2.817	18.641	8.355	1.99	5	6.385	5.395	5.155	4.776
50% inhibition (D_{50})	11.571	7.97	10.7	-	9.958	13.085	8.824	8.05	8.754	7.727	7.23
100% inhibition (D_{100})	21	9.7	14.5	-	11.2	18.1	11.1	9.3	10.8	9.4	8.8
R^2	0.95	0.69	0.82	0.86	0.85	0.97	0.92	0.98	0.90	0.97	0.93
Sensitivity index (k)	1	0	0	4	0	0	0	0	0	0	0
Overall sensitivity ($\sum_k$)		5						0			

4.3.2 Microplot studies

Nemafric-BL phytonematicide: Plant height, number of nodules, dry shoot mass, J2 in roots, fresh root mass, and total number of nodules versus Nemafric-BL phytonematicide exhibited positive quadratic relation (Figure 4.7 – Figure 4.8), with the model explained by 85, 87, 97, 71, 97 and 63%, associations, respectively (Figure 4.7 – Figure 4.8). The MCSP for Nemafric-BL phytonematicides was 3.93% (Table 4.3) with the k value of zero (Σk =0) in all the variables (Table 4.2).

Nemarioc-AL phytonematicide: Total number of nodules, number of nodules (dead), number of nodules (fresh), fresh root mass, and fresh pod mass versus Nemarioc-AL phytonematicide demonstrated positive quadratic relation (Figure 4.9), with the model explained by 88, 99, 95, 99 and 99% associations, respectively (Figure 4.7). The MCSP for Nemarioc-AL phytonematicide on runner bean was 3.76%, only total number of nodules had the k value of one (k = 1) and the rest were zero (Σk = 0) (Table 4.2).

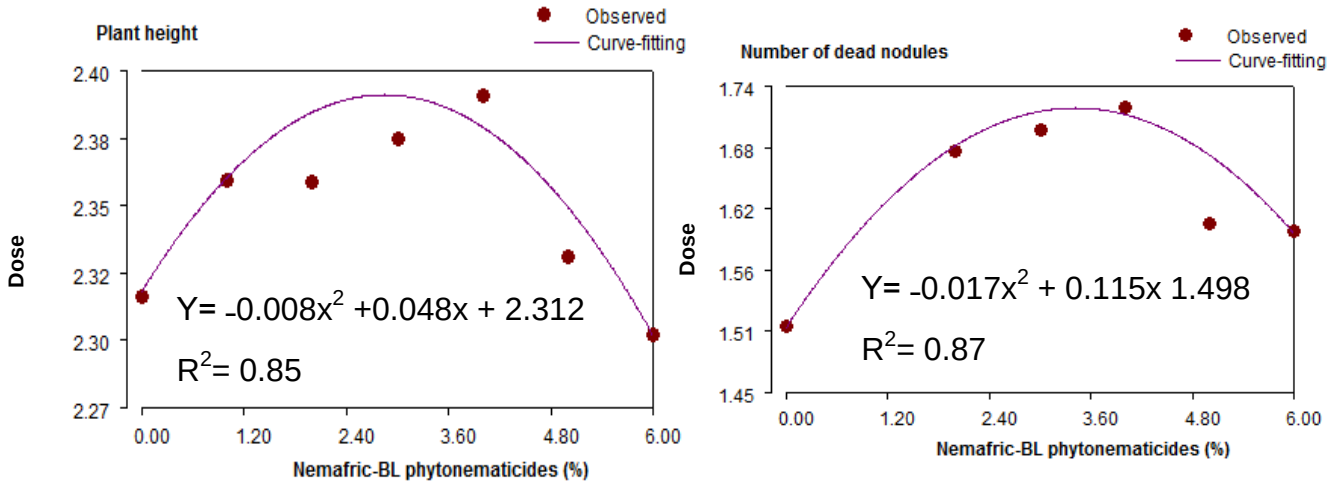


Figure 4.7: Response of plant height (PH) and number of nodules dead (NND) of runner bean to Nemafric-BL phytonematicide (%) at 56 days after initiation of treatments.

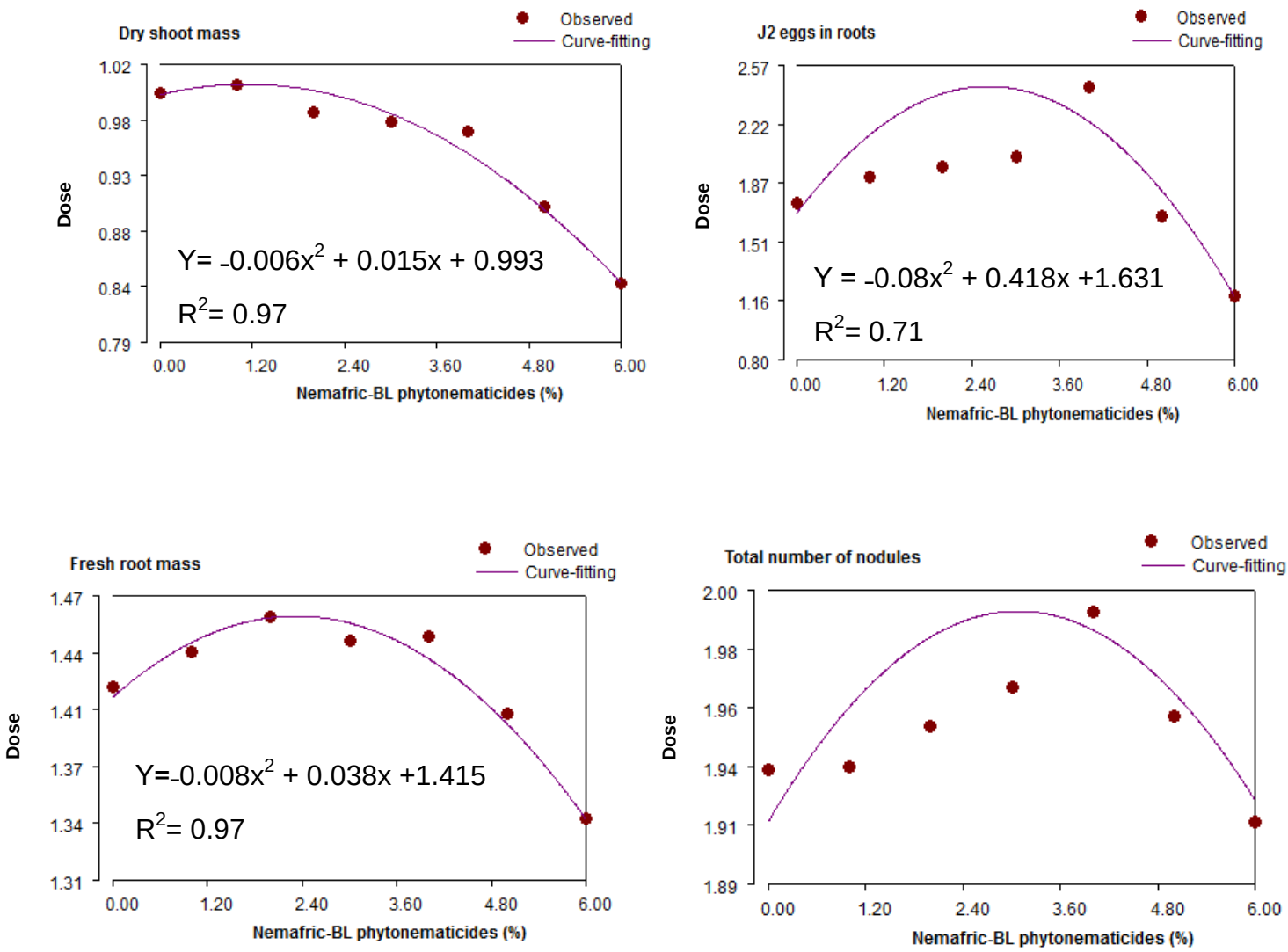


Figure 4.8. Response of dry shoot mass (DSM), J2 in roots, fresh root mass (FRM), and total number of nodules (TNN) of runner bean to Nemafric-BL phytonematicide (%) at 56 days after initiation of treatments.

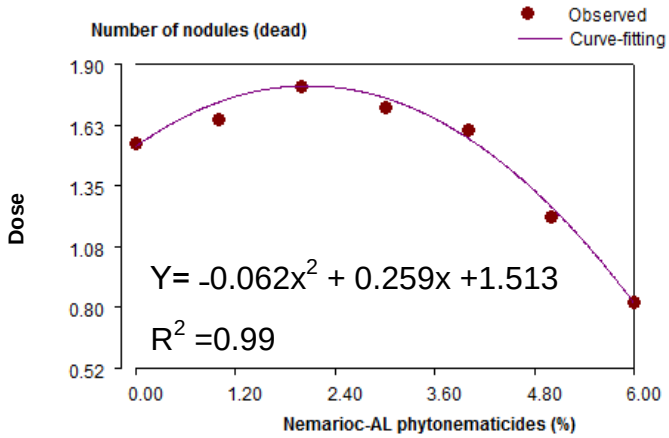
$Y= -0.006x^2 + 0.034x +1.92$

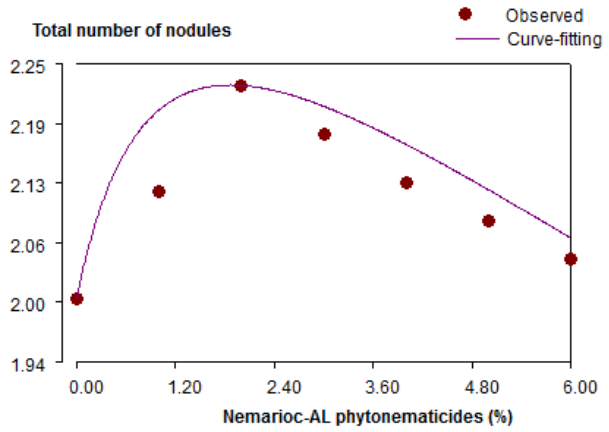
$R^2= 0.63$

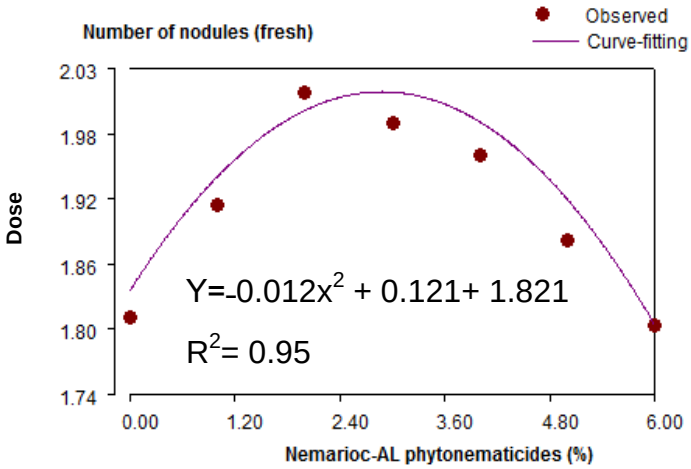
Dose

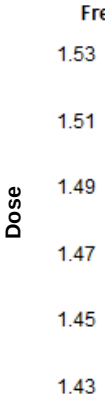
$Y=-0.176x^2+0.37x+1.993$

$R^2 = 0.88$









$Y= -0.006x^2 + 0.044x + 1.451$

$R^2 =0.99$

Figure 4.9: Response of total number

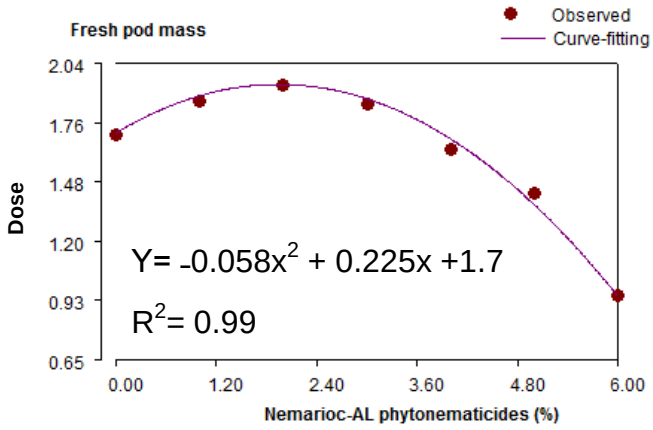


Figure 4.9: Response of total number of nodules (TNN), number of nodules dead (NND), number of nodules active (NONA), fresh root mass (FRM), and fresh pod mass (FPM) of runner bean to Nemarioc-AL phytonematicide (%) at 56 days after initiation of treatment

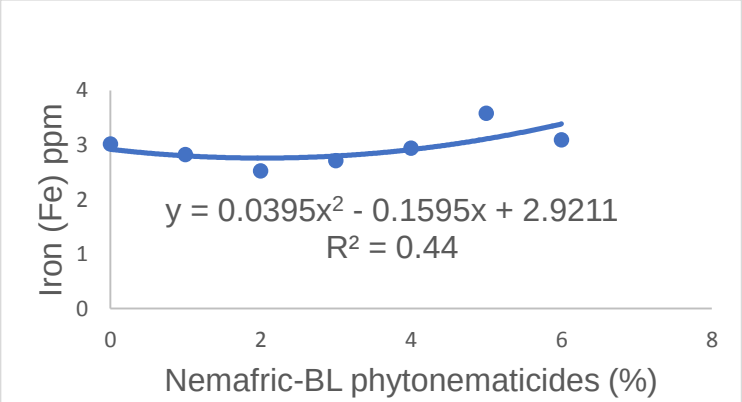
Table 4.2: Biological indices of plant height (PH), number of nodules dead (NND), dry shoot mass (DSM), second-stage juvenile (J2) in roots , fresh root mass (FRM), total number of nodules (TNN), fresh number of nodule (FNN) and fresh pod mass (FPM) of runner bean seeds primed with Nemafric-BL and Nemarioc-AL phytonematicides (%) at 56 days after treatments.

	Nemafric-BL						Nemarioc-AL				
Biological indices	PH	NND	DSM	J2 roots	FRM	TNN	TNN	NND	FNN	FPM	FRM
Threshold stimulation (D_m)	2.847	3.416	1.122	2.621	2.322	3.079	1.867	2.072	2.881	1.942	3.456
Stimulation point (R_h)	0.068	0.197	0.008	0.548	0.044	0.052	0.195	0.268	0.174	0.218	0.076
0% inhibition (D_0)	5.693	6.833	2.244	5.242	4.643	6.157	7.217	4.144	5.761	3.884	6.912
50% inhibition (D_{50})	14.899	10.901	9.95	6.756	11.925	16.639	37.73	6.123	10.079	6.236	14.67
100% inhibition (D_{100})	19.7	13.4	13.6	7.8	15.7	22	96.6	7.4	12.6	7.7	18.9
R^2	0.85	087	0.97	0.71	0.97	0.63	0.88	0.99	0.95	0.99	0.99
Sensitivity index (k)	0	0	0	0	0	0	1	0	0	0	0
Overall sensitivity ($\sum_k$)	0						1				

4.3.3 Selected nutrient elements

Greenhouse studies:

Calcium, K, Mg and Zn in leaf tissues of runner bean plants versus Nemafric-BL phytonematicide exhibited positive quadratic relation with the model explained by 87, 30, 84 and 64%, associations, respectively (Figure 4.11). However, Fe exhibited negative quadratic relation against Nemafric-BL phytonematicide with the model explained by 44% association (Figure 4.10). Similarly, Nemarioc-AL phytonematicide had significant effects on Ca, Fe, K, Mg and Zn in leaf tissues of runner bean plants. The Ca, Fe, K, Mg and Zn versus Nemarioc-AL phytonematicide exhibited negative quadratic relations, with the models explained by 88, 85, 88, 96 and 70% associations, respectively (Figure 4.12 – 4.13). Generally, when using the relation, $x = -b_1/2b_2$ (Gomez and Gomez, 1984), Ca, Fe, K, Mg and Zn in leaf tissues of runner bean plants were optimised at 3.3, 2, 3.7, 3.4 and 3.4% Nemafric-BL phytonematicides, respectively (Table 4.3). Only Ca, Mg, Zn and K exhibited quadratic relation with the



increasing concentration of Nemafric-BL phytonematicide.

Figure 4.10: Quadratic relationship between Nemafric-BL phytonematicide (%) and Iron (Fe) ppm of runner bean seeds at 56 days.

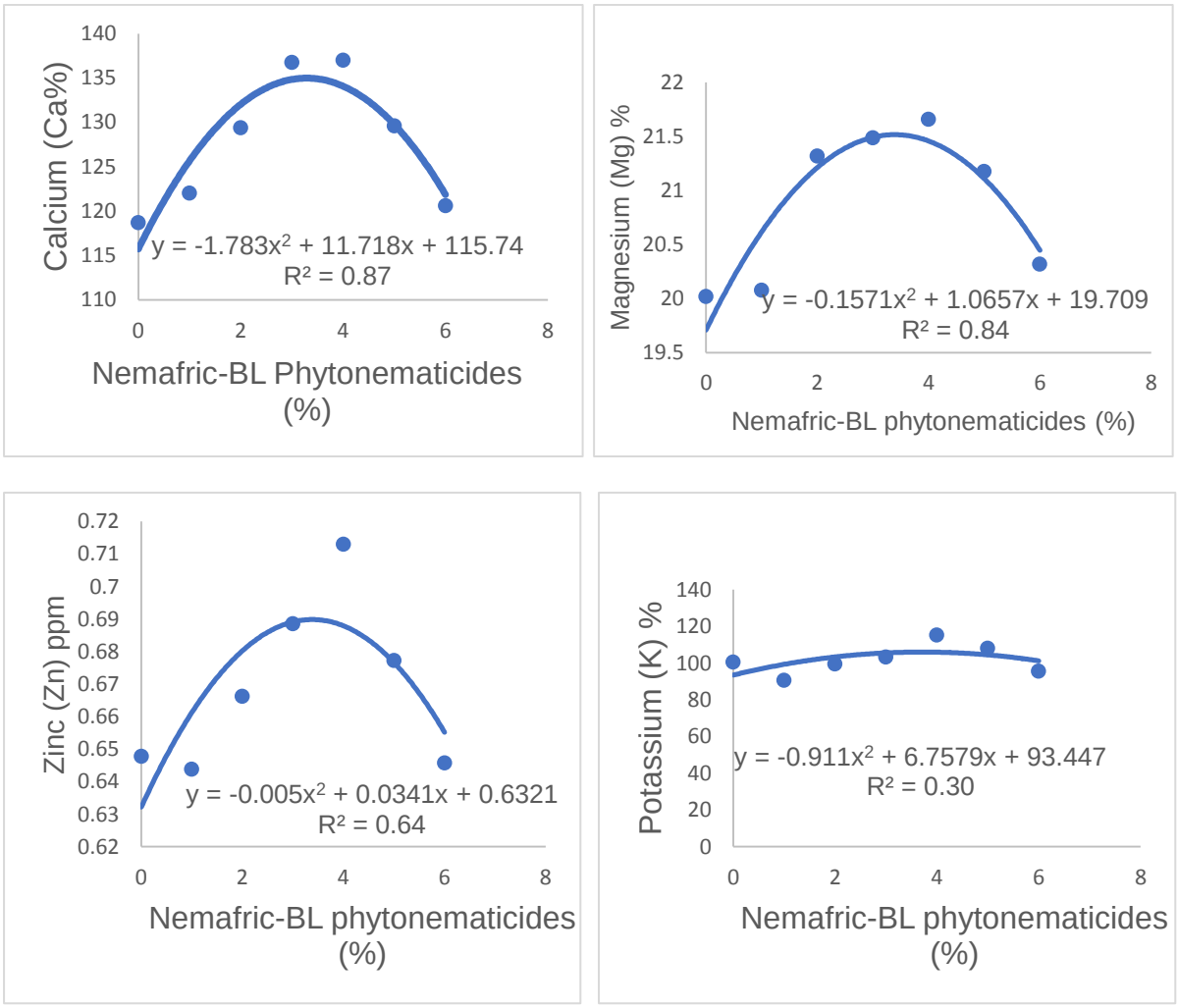


Figure 4.11: Quadratic relationship of calcium (Ca), magnesium (Mg), zinc (Zn) and potassium (K) versus Nemafric-BL phytonematicide (%) on runner bean at 56 days after initiation of treatments.

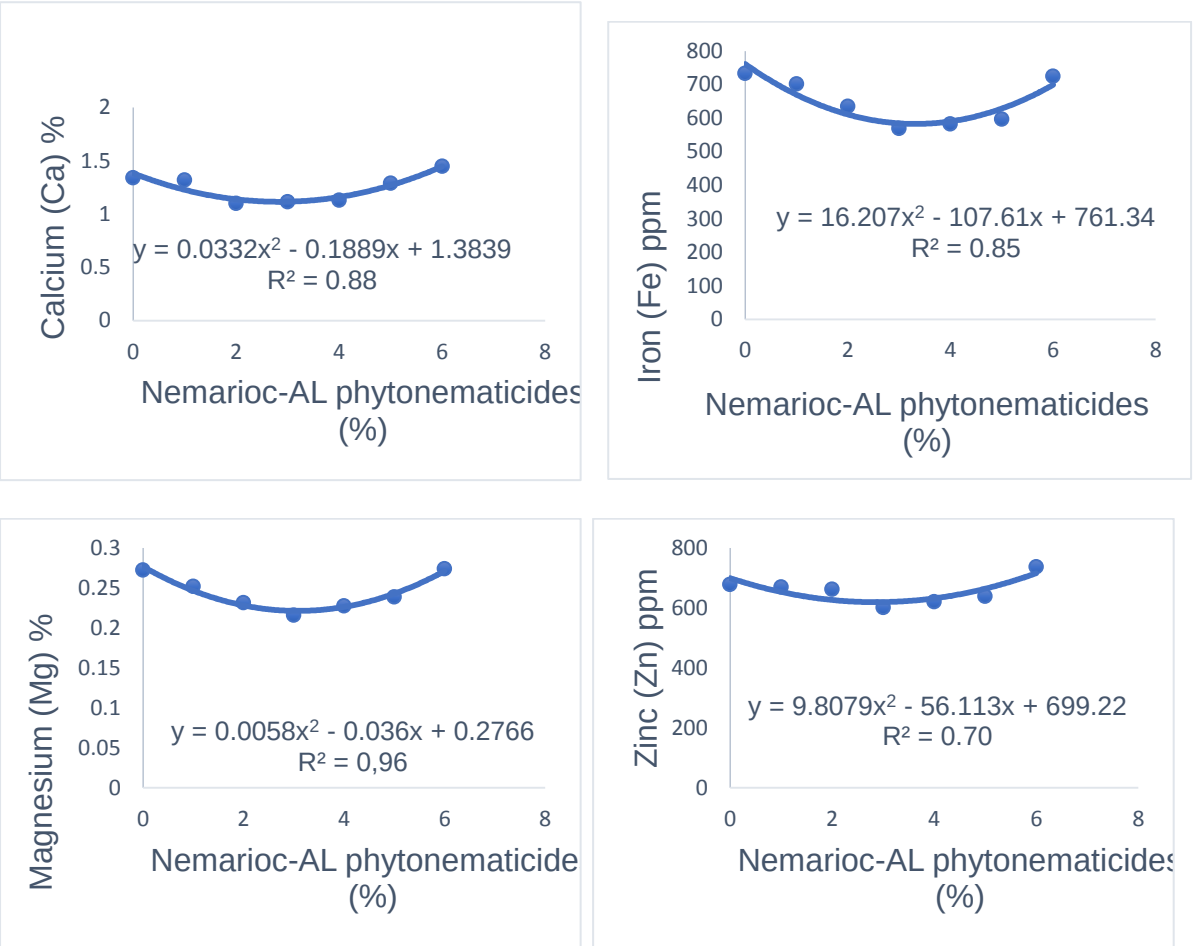


Figure 4.12: Quadratic relationship between calcium (Ca), iron (Fe), magnesium (Mg) and zinc (Zn) versus Nemarioc-AL phytonematicide (%) on runner bean seeds at 56 days.

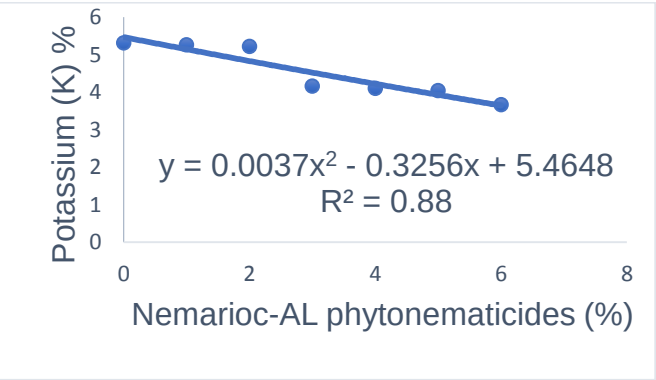


Figure 4.13: Quadratic relationship between Nemarioc-AL phytonematicide (%) and Potassium (K) of runner bean seeds at 56 days.

Table 4.3: Optimisation model of selected plant variables of runner bean as affected by concentration of Nemafric-BL and Nemarioc-AL phytonematicides (%).

	Model	R^2 (%)	x^x
Treatment	Nemafric-BL phytonematicide		
Ca	$y = -1.783x^2 + 11.718x + 115.74$	87	3.3
Fe	$y = 0.0395x^2 - 0.1595x + 2.9211$	44	2
K	$y = -0.911x^2 + 6.7579x + 93.447$	30	3.7
Mg	$y = -0.1571x^2 + 1.0657x + 19.709$	84	3.4
Zn	$y = -0.005x^2 + 0.0341x + 0.6321$	64	3.4
	Nemarioc-AL phytonematicide		
Ca	$y = 0.0332x^2 - 0.1889x + 1.3839$	88	2.8
Fe	$y = 16.207x^2 - 107.61x + 761.34$	85	3.3
K	$y = 0.0037x^2 - 0.3256x + 5.4648$	88	4.4
Mg	$y = 0.0058x^2 - 0.036x + 0.2766$	96	3.1
Zn	$y = 9.8079x^2 - 56.113x + 699.22$	70	2.9

$x=-b_1/2b_2$

Microplot study: Potassium, Fe and Mg in leaf tissues of runner bean plants versus Nemafric-BL phytonematicide exhibited positive quadratic relation with the model explained by 98, 92 and 88% associations, respectively (Figure 4.14). However, Ca and Zn exhibited negative quadratic relation against Nemafric-BL phytonematicide with the model explained by 94 and 99% associations, respectively (Figure 4.15). Similarly, Nemarioc-AL phytonematicide had significant effects on Ca, Fe, K, Mg and Zn in leaf tissues of runner bean plants. Iron, Zn and Mg versus Nemarioc-AL

phytonematicide exhibited positive quadratic relations, with the models explained by 87, 44 and 17% associations, respectively (Figure 4.17). In contrast, Ca and K versus Nemarioc-AL phytonematicide exhibited negative quadratic relationship with the model explained by 86 and 54% respectively (Figure 4.16).

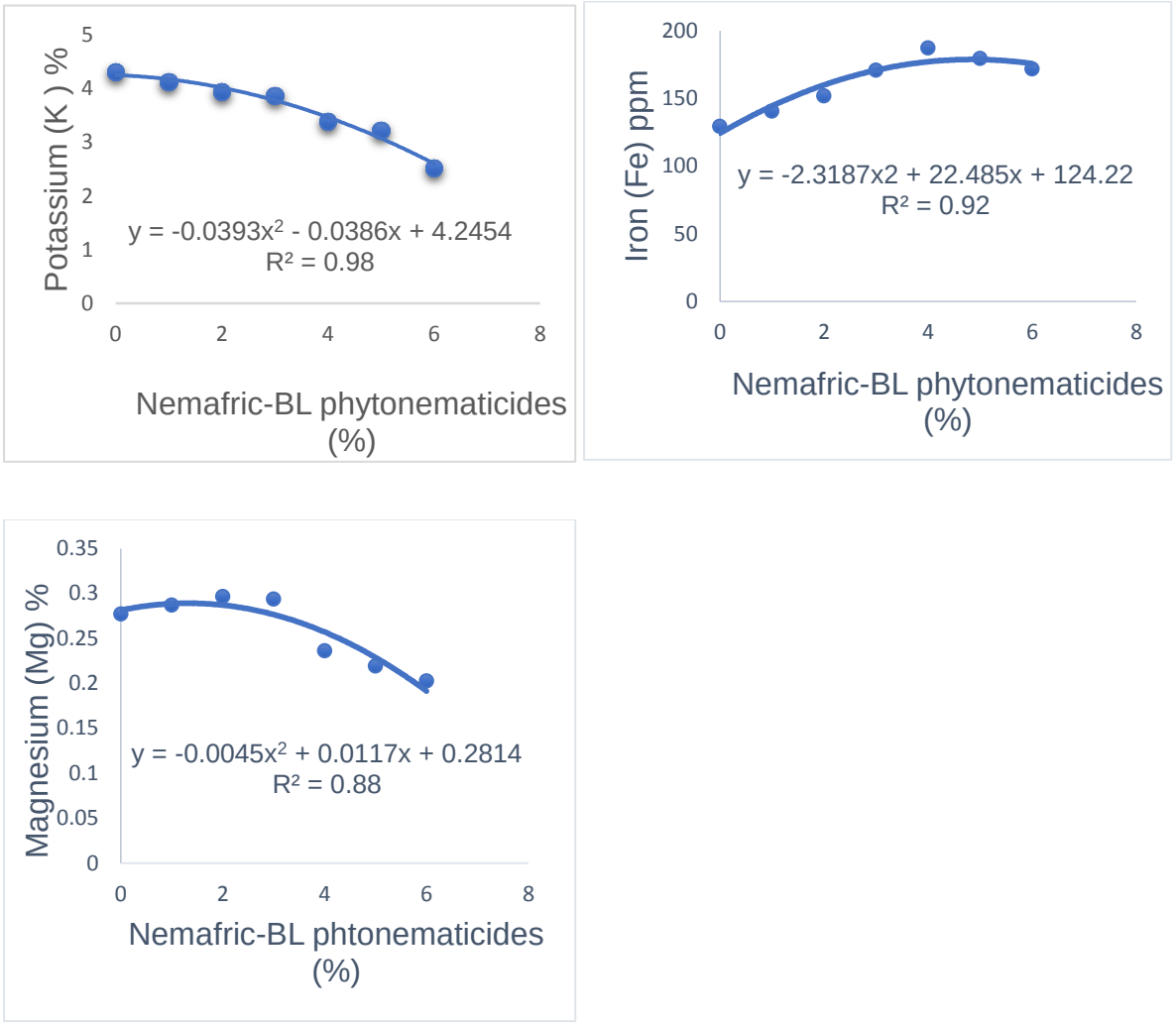


Figure 4.14: Quadratic relationship of potassium (K), iron (Fe) and magnesium (Mg) against Nemafric-BL phytonematicide (%) on runner bean at 56 days after initiation of treatments.

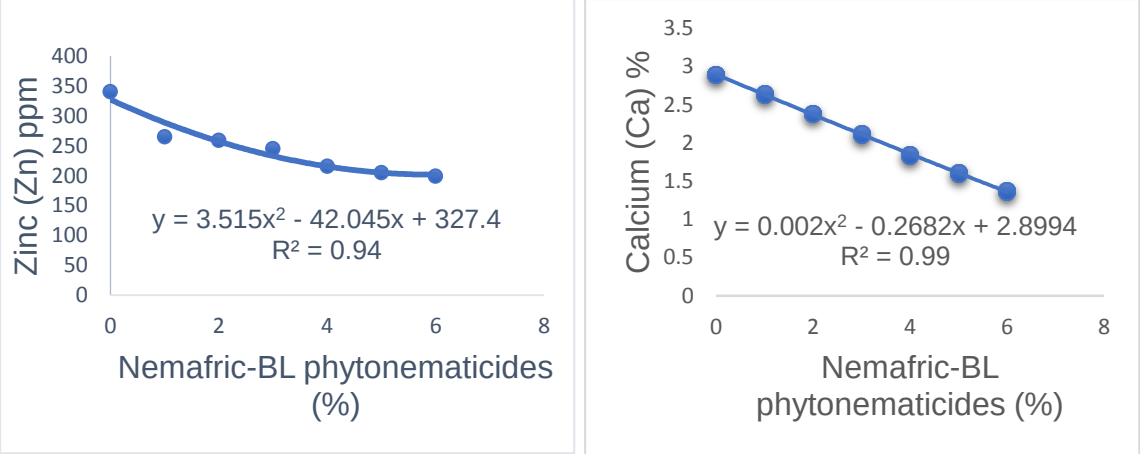


Figure 4.15: Quadratic relationship of zinc (Zn) and calcium (Ca) against Nemafric-BL phytonematicide (%) of runner bean seeds at 56 days.

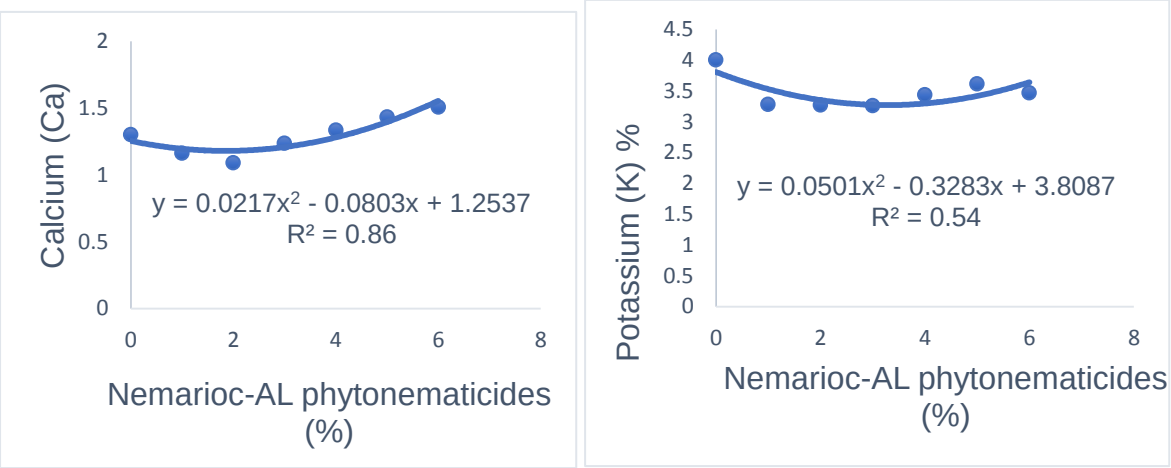


Figure 4.16: Quadratic relationship between calcium (Ca) and potassium (K) against Nemarioc-AL phytonematicide (%) on runner bean at 56 days after initiation of treatments.

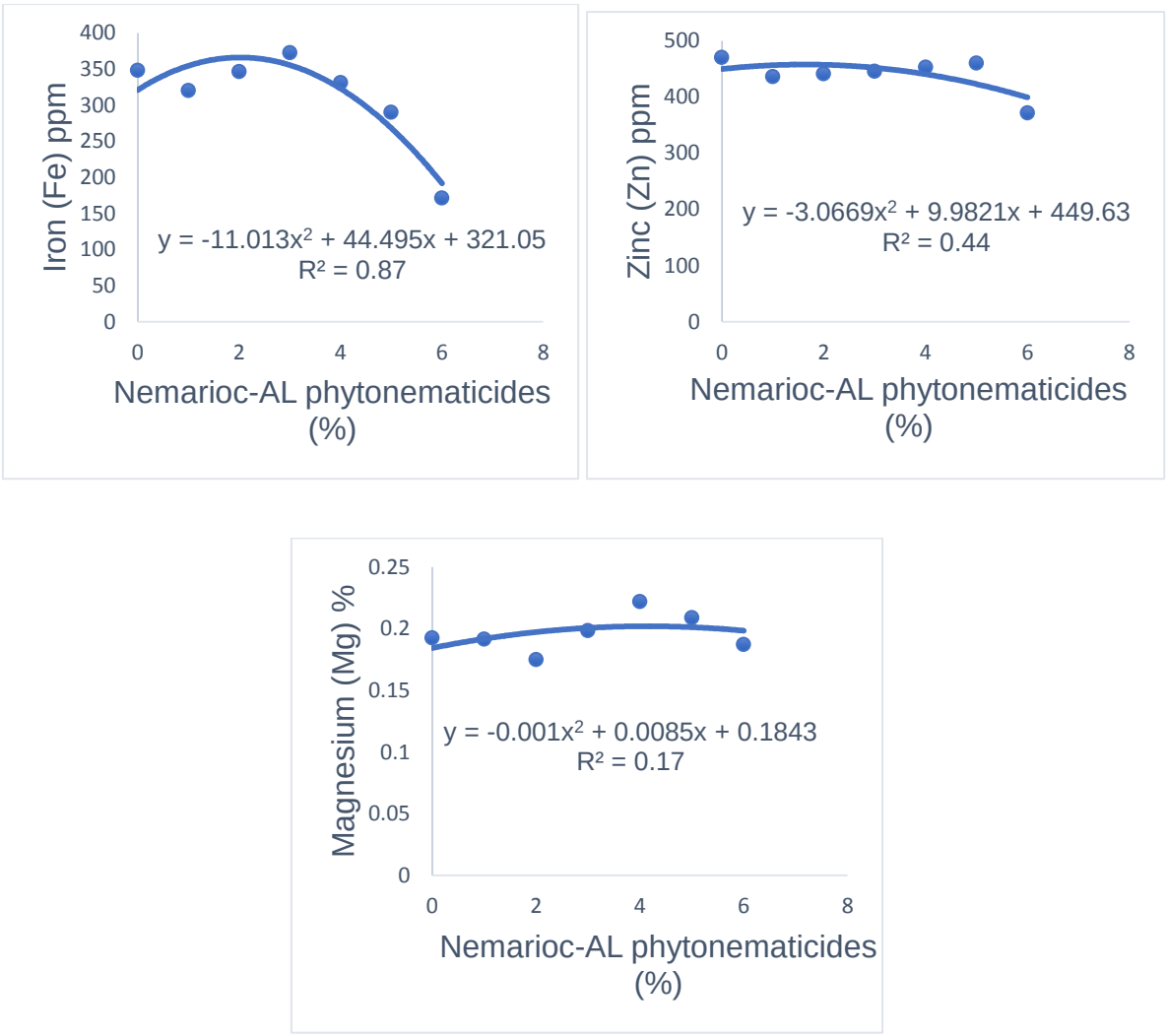


Figure 4.17: Quadratic relationship of iron (Fe), zinc (Zn) and magnesium (Mg) against Nemarioc-AL phytonematicide (%) on runner beans at 56 days after initiation of treatments.

Table 4.4: Quadratic relationship, coefficient of determination and computed

optimum response concentration for various nutrients from Curve-fitting Allelochemical Response Dose against Nemafric-BL phytonematicide (%) at 56 days after treatments in microplot conditions.

Nutrients	Quadratic relation	R^2	x^x
Ca	$y = 0.002x^2 - 0.2682x + 2.8994$	99	67.05
Fe	$y = -2.3187x2 + 22.485x + 124.22$	92	4.8
K	$y = -0.0393x^2 - 0.0386x + 4.2454$	98	-0.49
Mg	$y = -0.0045x^2 + 0.0117x + 0.2814$	88	1.3
Zn	$y = 3.515x^2 - 42.045x + 327.4$	94	5.98

Calculated optimum treatment level of Nemafric-BL phytonematicides (%),

$x=-b_1/2b_2$

Where b_1 = coefficient of x and b_2 = coefficient of x^2 on the quadratic equation.

Then x was the optimum level.

Table 4.5: Quadratic relationship, coefficient of determination and computed optimum response concentration for various nutrients from Curve-fitting

Allelochemical Response Dose against Nemarioc-AL phytonematicide (%) at 56 days after treatments in microplots conditions.

Nutrients	Quadratic relations	R^2	x^x
Ca	$y = 0.0217x^2 - 0.0803x + 1.2537$	86	1.9
Fe	$y = -11.013x^2 + 44.495x + 321.05$	87	2
K	$y = 0.0501x^2 - 0.3283x + 3.8087$	54	3.3
Mg	$y = -0.001x^2 + 0.0085x + 0.1843$	17	4.3
Zn	$y = -3.0669x^2 + 9.9821x + 449.63$	44	1.6

Calculated optimum treatment level of Nemafric-AL phytonematicides (%),

$x=-b_1/2b_2$

Where b_1 = coefficient of x and b_2 = coefficient of x^2 on the quadratic equation.

Then x was the optimum level.

4.3.4 Nematode variables

Greenhouse studies: Nemafric-BL phytonematicide had significant effects on eggs in roots, J2 in roots, J2 in soil and final population of *M. incognita.* Second-stage juveniles in soil and final population of *M. incognita* versus Nemafric-BL phytonematicide exhibited negative quadratic relations, with models explained by 80 and 71%, associations, respectively (Figure 4.18). In contrast, eggs in roots and J2 in roots versus Nemafric-BL phytonematicide exhibited positive quadratic relations, with models explained by 42 and 62% respectively (Figure 4.19).

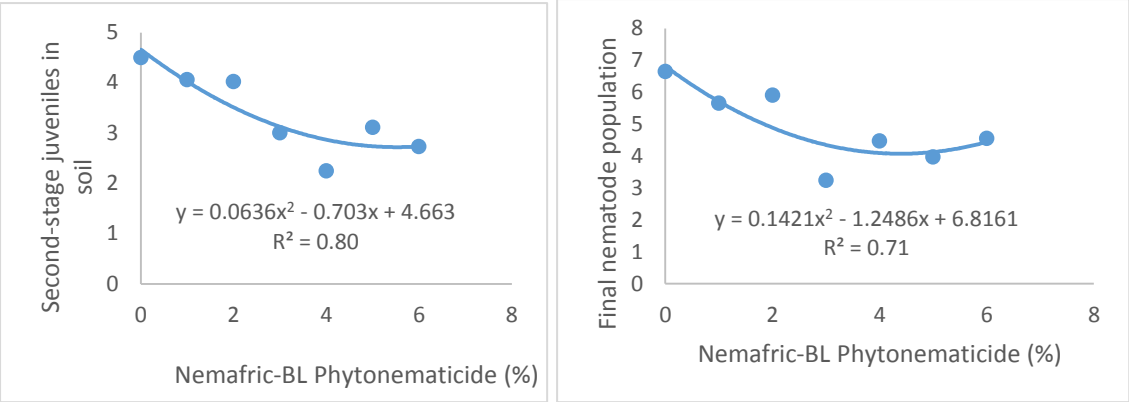


Figure 4.18: Response of second-stage juveniles (J2) in soil and final nematode population of *Meloidogyne incognita* to Nemafric-BL phytonematicide (%) at 56 days after initiation of treatments.

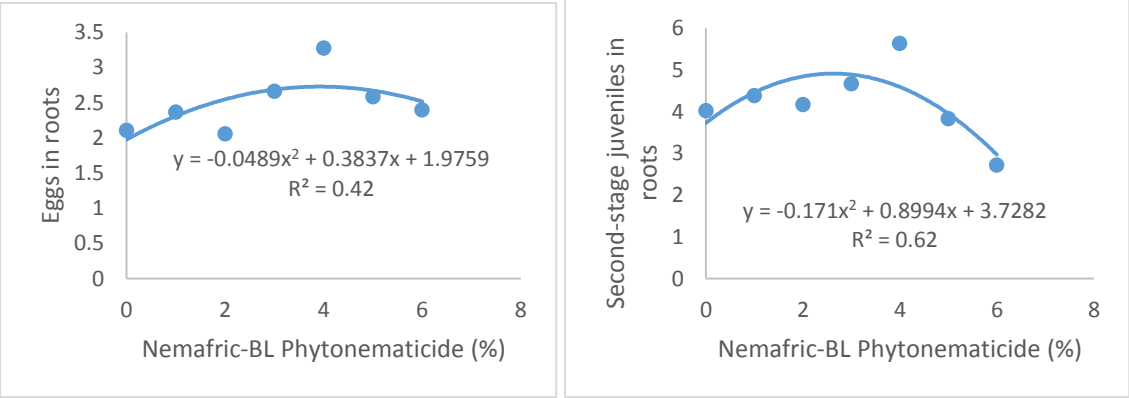


Figure 4.19: Response of eggs in roots and second-stage juveniles (J2) in roots of *Meloidogyne incognita* to Nemafric-BL phytonematicide (%) at 56 days after initiation of treatments.

Nemarioc-AL phytonematicide had significant effects on eggs in roots, J2 in roots, J2 in soil and final population of *M. incognita.* Eggs in roots, J2 in soil and final population of *M. incognita* versus Nemarioc-AL phytonematicide exhibited negative quadratic relations, with models explained by 76, 61 and 90%, associations, respectively (Figure 4.20). In contrast, J2 in roots versus Nemarioc-AL

phytonematicide exhibited positive quadratic relations, with models explained by 46%, association (Figure 4.21).

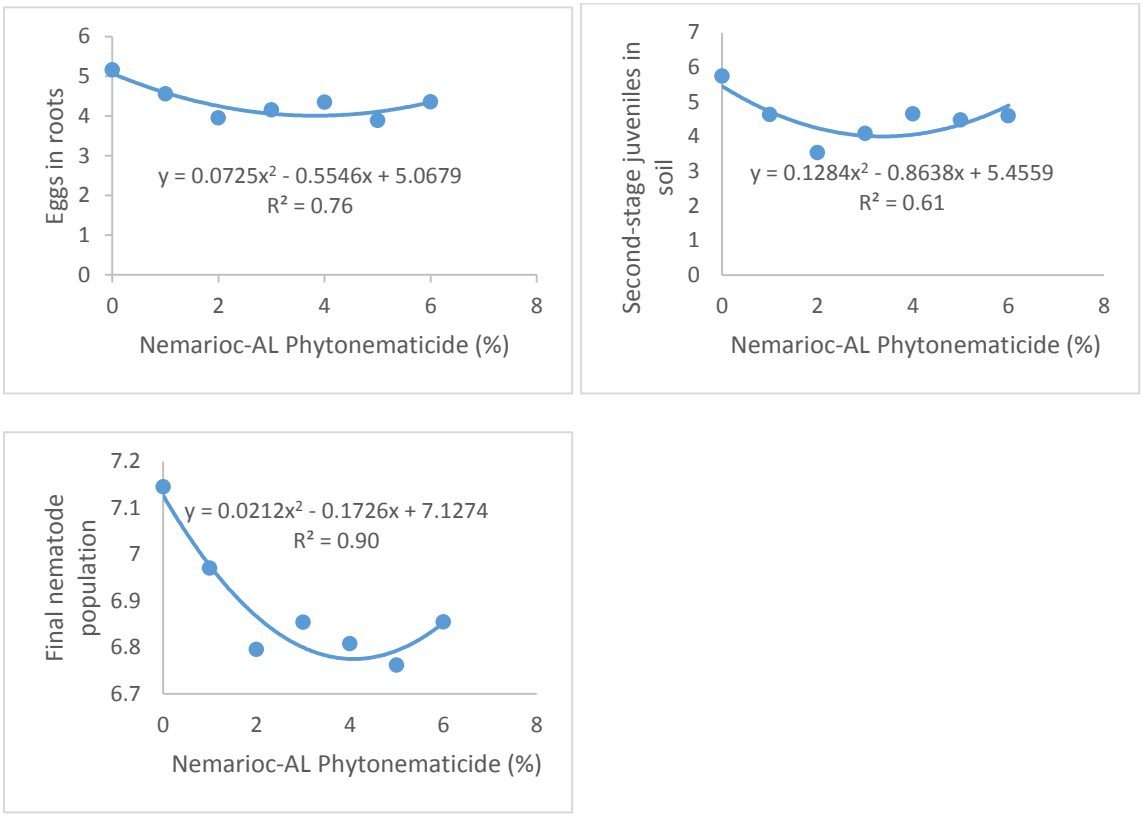


Figure 4.20: Response of eggs in roots, second-stage juveniles (J2) in soil and final nematode population of *Meloidogyne incognita* to Nemarioc-AL phytonematicide (%) at 56 days after initiation of treatments.

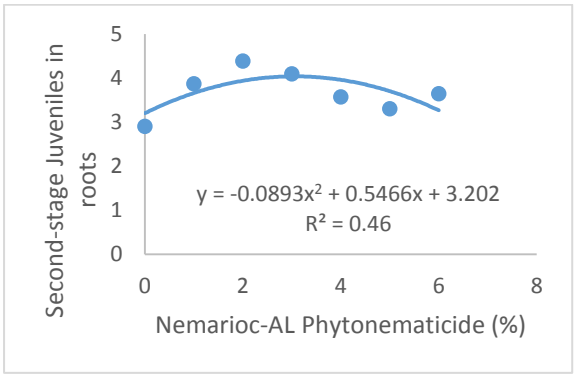


Figure 4.21: Response of second-stage juveniles (J2) in roots of *Meloidogyne incognita* to Nemarioc-AL phytonematicide (%) at 56 days after initiation of treatments.

Microplot studies: Nemafric-BL phytonematicide had significant effects on eggs in roots, J2 in roots, J2 in soil and final population of *M. incognita.* Second-stage juveniles in soil and J2 in roots of *M. incognita* versus Nemafric-BL phytonematicide exhibited negative quadratic relations, with models explained by 79 and 63% associations, respectively (Figure 4.23). In contrast, eggs in roots and final nematode population versus Nemafric-BL phytonematicide exhibited positive quadratic relations, with models explained by 82 and 45%, associations, respectively (Figure 4.23).

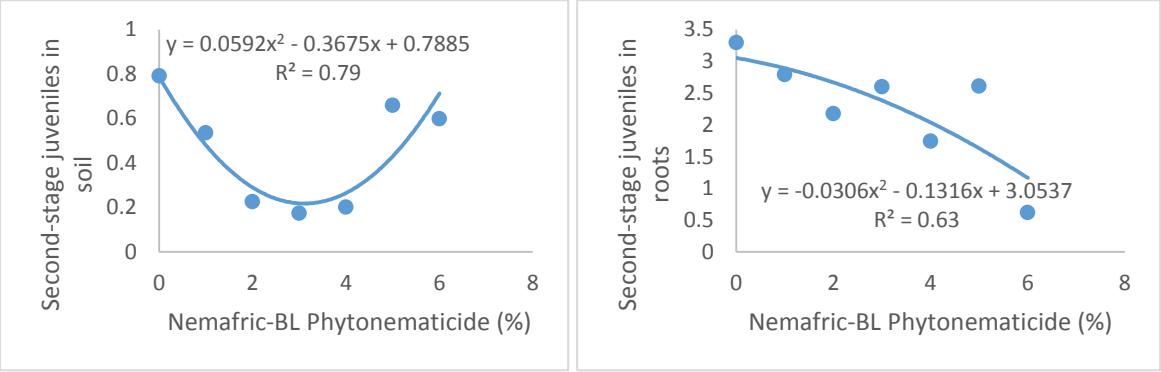
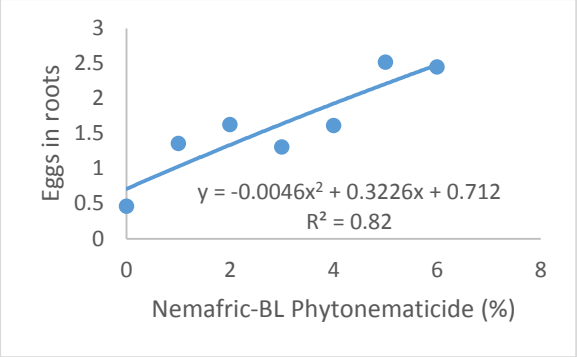


Figure 4.22: Response of second-stage juveniles (J2) in soil and second-stage juveniles in roots of *Meloidogyne incognita* to Nemafric-BL phytonematicide (%) at 56 days after initiation of treatments.



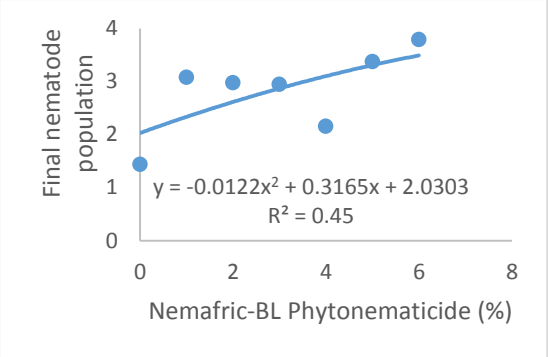
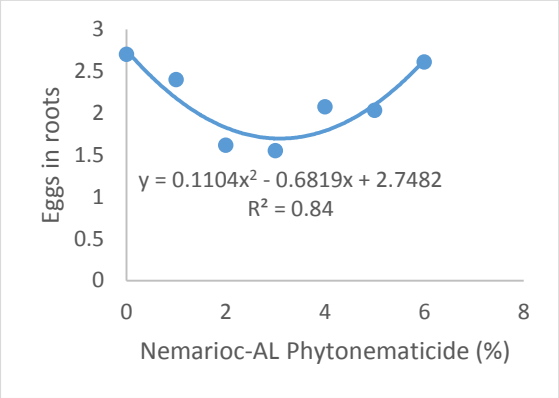
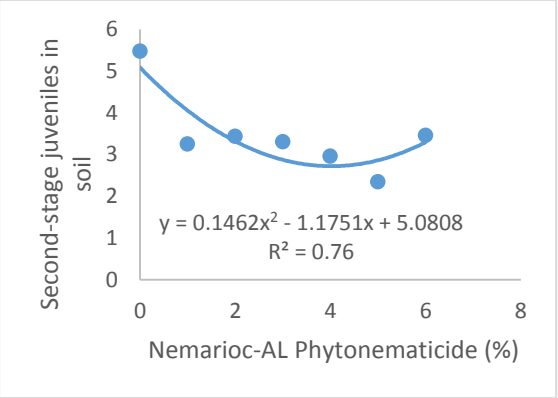


Figure 4.23: Response of eggs in roots and final nematode population of *Meloidogyne incognita* to Nemafric-BL phytonematicide (%) at 56 days after initiation of treatments.

Nemarioc-AL phytonematicide had significant effects on eggs in roots, J2 in roots, J2 in soil and final population of *M. incognita.* Eggs in roots, J2 in soil and final population of *M. incognita* versus Nemarioc-AL phytonematicide exhibited negative quadratic relations, with models explained by 84, 76 and 65% associations, respectively (Figure 4.24). In contrast, J2 in roots versus Nemarioc-AL phytonematicide exhibited positive quadratic relations, with model explained by 32% association (Figure 4.25).





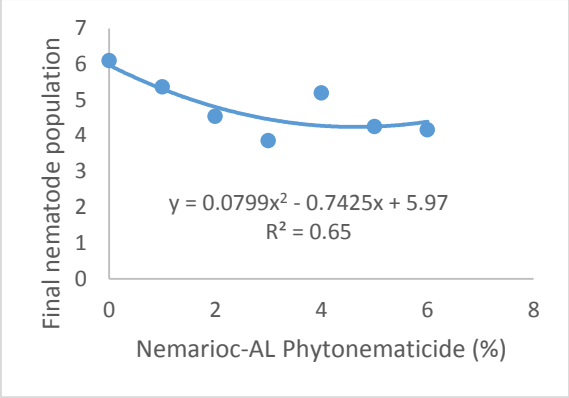


Figure 4.24: Response of eggs in roots, second-stage juveniles (J2) in soil and final nematode population of *M. incognita* to Nemarioc-AL phytonematicide (%) at 56 days after initiation of treatments.

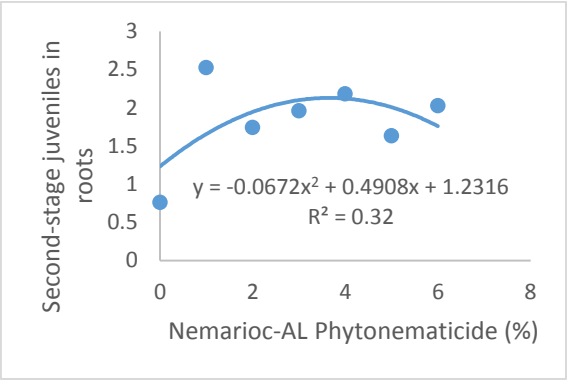


Figure 4.25: Response of second-stage juveniles (J2) in roots of *Meloidogyne incognita* to Nemarioc-AL phytonematicide (%) at 56 days after initiation of treatments.

4.4 Discussion

Plant variables: Cucurbitacin-containing phytonematicides in liquid formulations versus plant and nutrient variables had DDG patterns. The DDG patterns characterise most biological responses when exposed to increasing concentration of allelochemicals (Liu *et al*., 2003). Chlorophyll content, dry shoot mass and total number of nodules had DDG patterns with increasing concentration of Nemafric-BL phytonematicide in both greenhouse and microplot experiments. The above-

mentioned plant variables exhibited positive quadratic relationships against Nemafric-BL phytonematicides, which could suggest that the concentration of phytonematicides used in this study were within the saturation point needed to induce stimulation responses (Mashela *et al*., 2011; Pelinganga *et al*., 2012; Salisbury and Ross, 1992). Liu *et al.* (2003) indicated that various types of allelochemicals in various plant species stimulate plant growth when applied at low concentration.

Similar trend was observed when chlorophyll content, dry shoot mass and total number of nodules were exposed to Nemarioc-AL phytonematicide. Sithole *et al*. (2016) observed similar results on plant height and dry shoot mass of geranium plant when exposed to increasing concentration of Nemafric-AL phytonematicide, where the relations were explained by 95 and 89% associations, respectively. Similar observations were made on tomato plants where plant height and dry root mass exhibited strong positive quadratic relation when exposed to Nemarioc-AL phytonematicide, with the model explaining the relations by 84 and 98% associations, respectively (Tseke *et al*., 2013). Moreover, growth of African geranium (*Pelargonium sidoides* DC.) seedlings (Sithole *et al*., 2016), tomato plants (Pelinganga and Mashela, 2012; Tseke *et al*., 2013) and *Citrus volkameriana* seedling rootstocks (Mathabatha *et al*., 2017) had similar DDG patterns when exposed to increasing concentration of Nemarioc-AL phytonematicide. When using other cucurbitacin-containing phytonematicides, similar results were observed when exposing maize, millets and sorghum plants to increasing pre-emergent application concentration of Nemarioc-AG phytonematicide (Mafeo *et al*., 2011b).

Pelinganga (2013) also observed similar trend on plant height of tomato plants when exposed to increasing concentration of fermented crude extracts of *C. myriocarpus* with the relation explained by 97% association. Similarly, Mafeo *et al*. (2011a) when exposing chive, leek and onion seedlings to different levels of crude extracts of *C. myriocarpus* fruits (Nemafric-AG phytonematicide), seedling height, radicle length, coleoptile length and coleoptile diameter exhibited quadratic relations.

The fact that increasing concentration of Nemafric-BL phytonematicide had no effect on stem diameter under both greenhouse and microplot conditions, as well as Nemarioc-AL phytonematicide on gall ratings and stem diameter in the current study, could be suggesting that the organs were exposed to saturation concentration ranges by harvest time (Mashela *et al.*, 2015b). The non-significant effects of cucurbitacin-containing phytonematicides on plant variables implies that the material affected plant growth at saturation point of the CARD model where growth was neither stimulated nor inhibited (Mashela *et al.,* 2011, 2015). Mashela *et al.* (2015) indicated that lack of significant effect on certain plant variables towards the increasing concentration of phytonematicide suggested that the organs were, by harvest time at saturation concentration. Absence of significant effects on certain plant variables of runner beans against Nemarioc-AL and Nemafric-BL phytonematicides support the observations of Kohli *et al*. (2001), who reported that 2% crude extracts of yellow nutsedge (*Cyperus esculentus* L.) had no effect on germination of lettuce (*Lactuca sativa* L.), whereas at 5% the extracts inhibited germination. Ghafarbi *et al*. (2012) also observed similar trend when eight selected plant species were exposed to seed extracts from wheat (*T. aestivum* L.), but the extracts had no effects on plant variables.

Priming technology: Seed priming is an environmental-friendly and effective method for applying treatment, which improves germination, resulting in early flowering and maturity, and boosts protected crops to be highly resilient to abiotic stresses (Rhaman *et al.,* 2020). Apart from using phytonematicides, Tania *et al.* (2020) indicated that priming improves seed germination and yield of okra. Bastia *et al.* (1999) reported that priming of safflower (*Carthamus tinctorius* L.) seed for resulted in a higher grain yield and oil content compared to untreated seed. Moreover, primed maize seeds showed improved plant height and dry shoot mass (Matthews and Hosseini, 2007). Matthews and Hosseini (2007) also observed that primed maize seeds produced consistently and longer shoots after 5 days, than the untreated crops. Similar findings were observed in rice, maize, chickpea (Harris *et al.,* 1999), and pearl millet (Kumar *et al.,* 2002) grown under dry-land conditions. Rhaman *et al.* (2020) also reported that seed priming not only promotes germination and seedling emergence, but also stimulates plant growth, thereby improving crop yield.

In the current study when using the two biological indices (D_m and R_h), the MCSP for pre-emergent application of Nemafric-BL phytonematicide was 3.69% under greenhouse, whereas MCSP was 3.93% under microplot conditions. Moreover, the MCSP for pre-emergent application of Nemafric-BL phytonematicide was 4.18% under greenhouse, whereas MCSP was 3.76% under microplot conditions. The generated MCSP under greenhouse and microplot trials for both cucurbitacin-containing phytonematicides, were higher than the MCSP generated for tomatoes and green bean against Nemarioc-AL phytonematicide, which were 2.64% (Pelinganga, 2013) and 2.67% (Chokoe, 2017), green bean at 0.27% and 0.5%

under greenhouse and field conditions, respectively (Chokoe, 2017) and African geranium plants 2.87% (Sithole, 2016). Pelinganga and Mashela (2012) indicated that the vital significance of MCSP is to establish the concentration level of the phytonematicide, which could stimulate plant growth along with nematode suppression, without inducing phytotoxicity to the protected crop.

In this study, the overall sensitivity index (Σk) of runner beans (*P. coccineus*) to increasing concentration of Nemafric-BL phytonematicide was at 5 and 0 units under greenhouse and microplot trials, respectively, therefore it is suggested that runner beans were less sensitive to the product when used as a pre-emergent phytonematicide (Mashela *et al*., 2015a). In contrast, the Σk of runner beans to Nemarioc-AL phytonematicide ranged between 0 and 1 units under greenhouse and microplot trials, respectively, thereby suggesting that runner beans were highly sensitive to the product when used as a pre-emergent phytonematicide (Mashela *et al*., 2015a). When using other cucurbitacin-containing phytonematicides, Mafeo (2012) showed that 18 different crops had different sensitivities (k) values towards Nemarioc-AG phytonematicide, with vibrant stimulatory and inhibitory concentration. At low k values, the product is highly phytotoxic to the test plant, while the opposite is true at high values (Liu *et al*., 2003; Mafeo *et al*., 2011b). Similar results were observed on tomato (Tseke *et al*., 2013) and *P. sidoides* plants when treated with Nemarioc-AL phytonematicide (Sithole *et al*., 2016). Generally, the degree of crops sensitivity against cucurbitacins is plant-stage-specific, with seedlings being highly tolerant than other stages in the life of a given plant species (Mashela *et al*., 2015b).

Nutrient elements: The evaluated nutrient elements and increasing Nemafric-BG phytonematicide concentration exhibited quadratic relations, which are the main features of DDG patterns (Mashela *et al*., 2017). The DDG patterns, are grouped into three stages, *viz*., neutral, stimulation, and inhibition phases (Liu *et al*., 2003), which provided much perception into how phytonematicides affect plant growth, nematode suppression (Mashela *et al*., 2016; Shadung, 2016) and nutrient elements (Mashela and Pofu, 2017). Depending on the initial and subsequent concentration, the response of entities as confirmed by nutrient elements, start from stimulation through the neutral to the inhibition phases or vice versa (Mashela *et al*., 2016; Mashela and Pofu, 2017). Calcium, Iron, Potassium, Magnesium and Zinc in leaf tissues of runner bean each with increasing Nemarioc-AL phytonematicide concentration exhibited negative quadratic relations. The assessed nutrient elements exhibited inhibition phase which is one of the features in DDG patterns observed in entity allelochemical relations (Liu *et al*., 2003; Mashela *et al*., 2016). Similarly, Ca, Fe, K, Mg and Zn in leaf tissues of runner bean each exhibited negative quadratic relations against Nemafric-BL phytonematicide. Calcium, Fe, K, Mg and Zn in leaf tissues were optimised at 3.3, 2, 3.7, 3.4 and 3.4% Nemafric-BL phytonematicide, were all nearer to the concentration used in nematode management in various crops, including the test crop. Mashela and Pofu (2017), observed similar results when using Nemafric-BL and Nemarioc-AL phytonematicides on green beans under greenhouse conditions, where the optimum concentration for Ca, K, Na and Fe when using Nemafric-BL phytonematicide, and when using Nemarioc-AL phytonematicide the optimum for K and Fe, were all further to the concentration used in nematode management in various crops, except for Fe in leaf tissues of green beans (Mashela and Pofu, 2017).

Nematode variables: In the current study, J2 in soil and final nematode population under greenhouse conditions, and J2 in soil and J2 in roots under microplot conditions of *M. incognita* versus Nemafric-BL phytonematicides exhibited negative quadratic relations, which confirmed that at small quantities of crude extracts from *Cucumis* fruits, the materials constantly reduced nematode numbers (Mafeo, 2012; Pelinganga, 2013), thereby confirming the existence of the inhibition phase in the DDG patterns in response to lower concentration of allelochemicals (Liu *et al.,* 2003). Similarly, under both conditions, eggs in roots, J2 in soil and final nematode population of *M. incognita* versus Nemarioc-AL phytonematicides exhibited negative quadratic relations. All degrees of Nemafric-BL and Nemarioc-AL phytonematicides were profoundly effective in comparable to the suppression of nematode numbers as observed in different greenhouse and microplot trials, where both products reduced nematode population densities with high magnitudes (Mashela *et al*., 2017; Pelinganga and Mashela, 2012). The suppression of *M. incognita* with by both Nemafric-BL and Nemarioc-AL phytonematicides were consistent with the observations made in other crops where nematodes were managed using the same materials (Mashela *et al*., 2015a; Pelinganga and Mashela, 2012; Pelinganga *et al.,* 2012). Mashela *et al.* (2015) demonstrated that the products are highly effective in suppression of root-knot nematodes under different conditions, as it had been shown that the increasing concentration of cucurbitacin-containing phytonematicides, which are allelochemicals, affect nematode population densities through the DDG patterns, which was adapted from the CARD model (Liu *et al*., 2003).

4.5 Conclusion

Results of this Chapter demonstrated that the quantities of both Nemarioc-AL and Nemafric-BL phytonematicides when applied through priming were sensitive to the products supported by the CARD model results due to most plant variables that had sensitivity values of zero. However, due to the observed density-dependent relationships, Nemarioc-AL and Nemafric-BL phytonematicides were observed to promote runner bean plant growth when applied separately. The average between the initial point of stimulation and saturation on the DDG patterns, could be the point where the products would be suitable for use as a pre-emergent phytonematicide. In conclusion, the priming technology should be developed further since it has the potential of being successful in nematode management in crops with hypogeal seeds.

CHAPTER 5

SUMMARY OF FINDINGS, SIGNIFICANCE, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary of findings

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

In vitro, the CARD model demonstrated that the response of runner beans when exposed to phytonematicides through priming technology. The responses exhibited the density-dependent growth patterns, which were characterised by stimulation, neutral and inhibition phases. Using the integration of the responses of germination and emergence to Nemarioc-AL and Nemafric-BL phytonematicides within the stimulation range, the amount of the products estimated for use as pre-emergent seed treatment were estimated as 1.05% (Nemarioc-AL phytonematicide) and 0.58% (Nemafric-BL phytonematicide) material/ seed.

Under greenhouse and microplot conditions, Nemarioc-AL and Nemafric-BL phytonematicides when applied through priming technology, were observed to promote runner bean plant growth when applied separately. Nemafric-BL phytonematicide had a high sensitivity rank as compared to Nemarioc-AL phytonematicide. The CARD model showed the responses of runner bean when exposed to cucurbitacin-A and cucurbitacin-B that displayed the DDG patterns. However, some plant variables demonstrated stimulation which means the dose amount was effective in increasing growth while suppressing the nematode population, and not phytotoxic which is the inhibition phase that reduces growth.

5.2 Significance

The study provided useful information on the potential use of hypogeal germinated seeds as carriers of active ingredients from cucurbitacin-containing phytonematicides, thereby reducing application costs of the phytonematicides as depicted in existing application technologies. Additionally, the proposed technology had the potential of reducing the amount of cucurbitacins that are placed in the environment when compared with those of the existing technologies.

5.3 Recommendations

The current study addressed the influence of cucurbitacin containing phytonematicides on plant growth and yield, all together with the suppression of nematode populations under *in vitro* and *ex vitro* conditions. Based on the information obtained from this study, it was confirmed that runner bean is sensitive to Nemarioc-AL and Nemafric-BL phytonematicides supported by the CARD model results due to most plant variables that had sensitivity values of zero. The suitable

level of sensitivity and overall sensitivity of runner bean when primed with Nemarioc-AL and Nemafric-BL phytonematicides should be established. The study reported on the effects of phytonematicides on the hypogeal germinated seeds of runner beans, therefore, values suitable to prime the seeds should be established.

5.4 Conclusions

The results obtained from this study suggest that the quantities of Nemarioc-AL and Nemafric-BL phytonematicides were well-suited with germination and emergence of runner bean seeds. Using the integration of the responses of germination and emergence to Nemarioc-AL and Nemafric-BL phytonematicides within the stimulation range, the amount of the products estimated for use as pre-emergent seed treatment were 1.05% (Nemarioc-AL phytonematicide) and 0.58% (Nemafric-BL phytonematicide) material/ seed. Under greenhouse and microplot conditions Nemarioc-AL and Nemafric-BL phytonematicides were observed to promote runner bean plant growth when applied separately. Nemafric-BL phytonematicide had a high sensitivity rank as compared to Nemarioc-AL phytonematicide. The CARD model showed the responses of runner bean when exposed to cucurbitacin-A and cucurbitacin-B which displayed the DDG patterns.

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APPENDICES

Appendix 4.1: Analysis of variances for calcium (Ca), iron (Fe), potassium (K), magnesium (Mg) and zinc (Zn) in runner bean to Nemarioc-AL phytonematicides (%) under greenhouse conditions after 56 days of inoculation.

		Ca (%)		Mg (%)		K (%)		Fe (ppm)		Zn (ppm)	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	5	00006.645	45	0009.939	41	23.1645	51	833047	76	683485	70
Treatment	6	0001.061	7^{ns}	0007.739	33^{ns}	12.5260	27^{ns}	25032	2^{ns}	151095	15^{ns}
Error	30	00006.998	48	0006.316	26	9.9507	22	235345	22	142753	15
Total	41	14.704	100	23.994	100	45.6412	100	1093424	100	977333	100

^{ns} not significant.

Appendix 4.2: Analysis of variances for calcium (Ca), iron (Fe), potassium (K), magnesium (Mg) and zinc (Zn) in runner bean to Nemafric-BL phytonematicides (%) under greenhouse conditions after 56 days of inoculation.

		Ca (%)		Mg (%)		K (%)		Fe (ppm)		Zn (ppm)	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	7	1.51934	28	0.00547	20	1.87805	24	24388.8	65	24684.0	46
Treatment	6	2.28715	43^{ns}	0.01217	44^{ns}	2.96805	38^{ns}	6479.6	17^{ns}	18985.3	35^{ns}
Error	42	1.53597	29	0.01009	36	2.99566	38	6891.8	18	10342.9	19
Total	55	5.34246	100	0.02773	100	7.84176	100	37760.2	100	54012.2	100

^{ns} not significant.

Appendix 4.3: Analysis of variances for calcium (Ca), iron (Fe), potassium (K), magnesium (Mg) and zinc (Zn) in runner bean to Nemarioc-AL phytonematicides (%) under microplot conditions after 56 days of inoculation.

		Ca (%)		Mg (%)		K (%)		Fe (ppm)		Zn (ppm)	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	7	0.70834	47	0008.290	44	9.09503	47	161987	47	118601	56
Treatment	6	0.39485	26^{ns}	0005.917	31^{ns}	4.72721	25^{ns}	97313	28^{ns}	44283	21^{ns}
Error	42	0.39486	27	0004.712	25	5.36148	28	88998	26	47849	23
Total	55	1.49805	100	18.919	100	19.18372	100	348298	100	210733	100

^{ns} not significant.

Appendix 4.4: Analysis of variances for calcium (Ca), iron (Fe), potassium (K), magnesium (Mg) and zinc (Zn) in runner bean to Nemafric-BL phytonematicides (%) under microplot conditions after 56 days of inoculation.

		Ca (%)		Mg (%)		K (%)		Fe (ppm)		Zn (ppm)	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	7	4533.38	48	52.1198	50	66148.2	67	10.2200	47	5.48153	52
Treatment	6	2527.03	26^{ns}	29.0367	28^{ns}	14655.9	15^{ns}	6.0115	27^{ns}	2.53064	24^{ns}
Error	42	2527.74	26	24.1130	22	18637.2	18	5.7170	26	2.57107	24
Total	55	9588.15	100	105.2695	100	99441.3	100	21.9485	100	10.58324	100

^{ns} not significant.