PRE- AND POST-EMERGENT APPLICATION EFFECTS OF NEMARIOC-AG PHYTONEMATICIDE ON GROWTH OF POTATO AND SUPPRESSION OF *MELOIDOGYNE INCOGNITA*

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

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DECLARATION

I, Selaleo Khutso Sefefe, declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree Master of Agriculture 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 in execution, and related materials contained herein had been duly acknowledged.

Candidate: Selaelo Khutso Sefefe	Signature	Date
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DEDICATION

To my late grandmother, Mashao Sefefe.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to my Heavenly Father for knowledge and wisdom You have given me throughout this research project. I am really grateful for Your love. To my supervisory team, Professor P.W. Mashela and Dr K.M. Pofu for their guidance, patience and constant supervision as well as providing the necessary consumables for the project to be a success. I would also like to express my appreciation to Dr Dube and Mr Tseke for their inputs, encouragement and for believing that I could make the research project a success. I have learnt a lot from you, that through dedication, one could achieve a lot. To my beloved mother, Ms Melita Sefefe, and my uncle, Mr Ezekiel Sefefe, I am really thankful for your support and love throughout my studying journey. You played a special role in the journey; your support and sacrifices made my academic journey easier. I am glad to have you by my side. For that I, will forever be thankful. I am also grateful to my brother, Thabo Sefefe, for his emotional and financial support. I could not have made it without you. You have always been there for me when I needed you the most. To my postgraduate colleagues, for all the assistance from establishing and harvesting the experiments, data analysis and writing, I could not appreciate all of you more and more! The service-workers at the Green Biotechnologies Research Centre of Excellence, Ms S.M. Seabela, Ms M.A. Mawasha, Ms M.R. Mawasha, Mr M.K. Ralefatana, Mr L.T. Letsoalo and Mr E.M. Letsoalo, I appreciate all your manual assistance throughout this project. My dearest sisters Kholofelo Sefefe, Asnath Boloka and Mashao Boloka, I am thankful for all your enormous support, words of encouragement and foremost, your prayers. Your support kept me going until the finishing line.

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ABSTRACT

Damage and significant losses of potato cultivar due to *Meloidogyne incognita* has become a serious challenge, after the withdrawal of synthetic chemical nematicides due to their environment-unfriendliness. Various alternatives have been investigated each with a wide range of drawbacks. Most phytonematicides were highly phytotoxic to crops, while their effects on nematode suppression were highly variable. The use of Nemarioc-AG phytonematicide at pre- and post-emergence would help in determining the level that is effective in supressing *M. incognita* without being phytotoxic. The objective of this study was to determine whether Nemarioc-AG phytonematicide could serve as pre- and post-emergent phytonematicide without inducing phytotoxicity while suppressing population densities of *M. incognita.* For achieving this objective, treatments, namely, 0, 2, 4, 8 and 16 g of Nemarioc-AG phytonematicide, were arranged in a randomised complete block design (RCBD), with 7 replicates. Potato seed tubers were sown into 20 cm pots, Nemarioc-AG phytonematicide placed above the tubers and covered with soil, after initiation of treatments 5 000 eggs and second stage juveniles (J2) of *M. incognita* per plant were inoculated. For post-emergent, treatments, replications and design were the same as in pre-emergent. Potato seed tubers were sown and inoculated with 5000 eggs and second-stage juveniles (J2) of *M. incognita* per plant after 100% emergence. Nemarioc-AG phytonematicide were applied 7 days after inoculation. Trials were conducted in autumn (February-April) 2017 (Experiment 1) and repeated in autumn 2018 (Experiment 2). Plant growth variables and selected nutrient elements were collected and analysed using the Curve Fitting Allelochemical Response Data (CARD) model and lines of best fit, respectively. In pre-emergent application, Experiment 1, MCSP was established at 1.95 g, with the overall

sensitivity (∑k) being equal to zero. Therefore, in Experiment 1 and Experiment 2, all nutrient elements to increasing concentration of Nemarioc-AG phytonematicide exhibited negative quadratic relations. In both Experiments, nematode variables over increasing concentration of Nemarioc-AG phytonematicide on potato exhibited negative quadratic relations, except in Experiment 1, where J2 in roots exhibited positive quadratic relations, with models ranging between 72 to 99%. In post-emergent, Experiment 1, MCSP was established at 1.57 g, with the overall sensitivity (∑k) being equal to 2. In Experiment 1 and Experiment 2, nutrient elements over increasing concentration of Nemarioc-AG phytonematicide exhibited positive and negative quadratic relations, with models ranging from 89 to 97%. In Experiment 1, nematode variables over increasing concentration of Nemarioc-AG phytonematicide exhibited negative quadratic relations, with models ranging between 92 and 98%. Positive and negative relations suggested that the product stimulated and inhibited plant growth or accumulation of selected essential nutrient elements, respectively. Increasing concentration of Nemarioc-AG phytonematicide had stimulated certain plant variables and inhibited population densities of *M. incognita* in pre- and post-emergent application; therefore, this product was suitable for use as pre- and post-emergent in management of nematodes on the test crop.

CHAPTER 1
RESEARCH PROBLEM

1.1 Background

Potato (*Solanum tuberosum* L.) has no genotypes that are resistant to root-knot (*Meloidogyne* species) nematodes (Brodie *et al*., 1993). Yield losses due to *Meloidogyne* species amount to as high as 50% to complete crop failure (Chitwood, 2003) Worldwide, due to the withdrawal of methyl bromide in 2005, alternatives for managing nematode population densities are being researched and developed (Mashela *et al*., 2017). In South Africa, cucurbitacin-containing phytonematicides have been in the forefront of the research agenda for alternatives for managing nematodes, with notable achievements in research and development of Nemarioc-AL (L = liquid formulation) or Nemarioc-AG (G = granular formulation) and Nemafric-BL or Nemafric-BG phytonematicides.

1.1.1 Description of the research problem

In granular and liquid formulations the cucurbitacin-containing phytonematicides are applied using the ground-leaching technology (GLT) and botinemagation technology, respectively (Mashela *et al*., 2017). In the GLT, phytonematicides are applied by hand around the stem of a seedling at transplanting (Mashela, 2002) and therefore, the technology is labour-intensive and not suitable for large commercial potato farming systems. In contrast, botinemagation is the application of phytonematicides through irrigation systems, which could be suitable for irrigated fields in potato production, whereas there could be affordability challenges for the smallholder farming systems. Additionally, in regions with high rainfall in South Africa, potato husbandry is under dryland farming. Consequently, a suitable application technology for managing nematode population densities in potato husbandry remains a challenge.

Yield loss in crops due to plant-parasitic nematodes is proportional to the initial population density of nematodes at planting (Seinhorst, 1967). Synthetic chemical nematicides that effectively control parasitic nematodes are being withdrawn due to their toxicity to the crop and environment. An increased withdrawal of synthetic chemical nematicides from agrochemical markets had since increased the detrimental effects of *Meloidogyne* species in various crop production systems (Mashela *et al*., 2011). Smallholder and largescale farmers were affected by limited options in the management of nematode population densities since yield losses due to nematode infection in crops without resistance were as high as 50%, to complete crop failure (Manju and Sankari, 2015). Therefore, the development of alternate phytonematicide application technologies at pre- and post-emergence of potato remains important.

1.1.2 Impact of the research problem

Crop losses prior to withdrawal of methyl bromide in 2005 were estimated at US$125 billion (Chitwood, 2003), with percentage yield losses ranging from 6 to 20%. Plant genotypes without nematode resistance such as watermelon, pepper and potato have yield losses as high as 50% due to infection by *Meloidogyne* species, and at times tipping towards a complete crop failure. During the heydays of synthetic chemical nematicides, global losses in potato due to nematode damage stood at approximately US$6 billion annually (Sasser and Freckman, 1987), whereas in South Africa the losses were at approximately 17% (Keetch, 1989). Eight years after the withdrawal of methyl bromide, relative yield losses in monetary terms have increased by as high as 37% (Mashela *et al*., 2016).

1.1.3 Possible causes of the research problem

The application challenges of phytonematicides remain the possible cause of the research problem for the current study. Attempts were made to use seed tubers as carriers of phytonematicides in liquid formulation, where the tubers were imbibed in solutions prior to sowing (Thopola *et al*., 2018). The trial was conducted for Potato South Africa, which later withdrew the project on the auspices that any imbibition of seed tubers in liquid solutions results in loss of quality in the harvested tubers. Additionally, precautionary measures in the use of phytonematicides are necessary since due to the allelopathic nature of the products that serve as active ingredients, high incidence of phytotoxicities had been reported (Mashela *et al*., 2015; 2017). The incidents of phytotoxicity in crops from the cucurbitacin-containing phytonematicides had been resolved using the concept of mean concentration stimulation point (MCSP) and the application interval in context of the dosage model (Mashela *et al*., 2017).

1.1.4 Proposed solutions

The MCSP is the amount of phytonematicide that should be applied to suppress nematode population densities without inducing phytotoxicity on the crop (Mashela *et al*., 2017). The MCSP is plant-specific, as is allelopathy in plants (Rice, 1984). Consequently, the MCSP for pre- and post-emergent application for phytonematicides had to be empirically-established. Additionally, in order to avoid phytotoxicity, empirical studies should be conducted to establish the time when repeated applications of MCSP should be made, technically referred to as application interval (Mashela *et al*., 2017).

1.2 Problem statement

Phytotoxicity could inherently limit the use of phytonematicides, since it might unintentionally result in high yield losses (Mashela *et al*., 2017). In granular formulation, Nemarioc-AG phytonematicide would require water to release the active ingredient cucurbitacin A ($C_{32}H_{46}O_9$) into soil solution if nematode population densities have to be successfully suppressed (Chen *et al*., 2005; Mashela *et al*., 2015). In addition to being unstable, due to its partial polarity, cucurbitacin A is slightly soluble in water, and it hydrolysis into cucumin and leptodermin, which were shown to have some insecticidal properties (Chen *et al*., 2005). Although, it is suspected that cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{40}O_8$) could also be having nematicidal properties, purified materials have not been derived to test the view. In addition to establishing the MCSP, it would be necessary to use the derived MCSP value to establish the application interval for the pre- and post-emergent application methods. However, in the current study, investigations will be limited to investigating the MCSP of Nemarioc-AG phytonematicide when the product was applied as pre- and post-emergent product on potato plants.

1.3 Rationale of the study

Damage and significant losses of potato cultivar due to *M. incognita* has become a serious challenge, after the withdrawal of synthetic chemical nematicides due to their environment-unfriendliness. Various alternatives have been investigated each with a wide range of drawbacks. Most phytonematicides were highly phytotoxic to crops, while their effects on nematode suppression were highly variable (Pofu *et al*., 2012). The use of Nemarioc-AG phytonematicide at pre- and post-emergence would help in determining the level that would be effective in supressing *M. incognita* without being phytotoxic.

1.4 Purpose of the study

1.4.1 Aim

Development of pre- and post-emergent application MCSP of cucurbitacin-containing phytonematicide on potato cultivars for managing nematode population densities.

1.4.2 Objective

To determine whether Nemarioc-AG phytonematicide could serve as pre- and post-emergent phytonematicide without inducing phytotoxicity while suppressing population densities of *M. incognita.*

1.4.3 Hypothesis

Nemarioc-AG phytonematicide could serve as pre- and post-emergent phytonematicide without inducing phytotoxicity while suppressing population densities of *M. incognita.*

1.5 Reliability, validity and objectivity

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

1.6 Bias

Bias was, in this study, eliminated through adequate replications and randomisation of treatments (Leedy and Ormrod, 2005).

1.7 Scientific significance of the study

Both MCSP and application interval are plant-specific and should therefore be empirically established. The scientific significance of the current study was that it would give the MCSP for Nemarioc-AG phytonematicide when used as pre- and post-emergent applications. The MCSP would in future be used to establish the application interval and the dosage model for Nemarioc-AG phytonematicide in potato production.

1.8 Structure of mini-dissertation

Following the description and detailed outlining of the research problem (Chapter 1), the work done and not yet done on the research problem was reviewed (Chapter 2). Then, in Chapter 3, the components of the objective, pre- and post-emergent applications, were addressed sequentially. In the final chapter (Chapter 4), findings were summarised and integrated to provide their significance, which was followed by future recommendations that culminated in conclusions with respect to the two components of the objective of the study. Citations in text and their listing on the references followed the Harvard style (author-alphabet) as approved by the Senate of the University of Limpopo.

Chapter 2
LITERATURE REVIEW

2.1 Work done on problem statement

2.1.1 Biological responses to pre-emergent application of phytonematicides

Most plants in the cucurbitaceae family, such as bitter mutant Hawkesbury watermelon (*Citrullus vulgaries* S chad.) contain cucurbitacins, which previously inhibited seed germination of different crops (Martin and Blackburn, 2003). Wild cucumber (*Cucumis myriocurpus* Naudin.) fruit are known for their high levels of water soluble cucurbitacin A (Chen *et al*., 2005). Nemarioc-AG phytonematicide has an active ingredient which is called cucurbitacin A (Mashela *et al*., 2015). Mafeo and Mphosi (2012) reported that when Nemarioc-AG phytonematicide was applied as pre-emergent, the material was highly phytotoxic to eight monocotyledonous crops under greenhouse conditions. Nemarioc-AG phytonematicide had allelopathic effects on seed germination of onion (*Allium cepa* L.), leek (*Allium ampeloprasum* L.) and chive (*Allium schoenoprasum* L.), with stimulation and inhibition effects on chive, leek and onion (Mafeo *et al*., 2011a). Consequently, Mafeo *et al*. (2011a) concluded that the product was not suitable for use as pre-emergent phytonematicide. When neem (*Azandirachta indica* A. Juss) was applied as pre-emergent application, the material had inhibitory effects on emergence of lettuce, mustard (*Sinapis arvensis* L.), carrot (*Daucus carota* L.) and radish (*Raphonus sativa* L.) (Ashrafi *et al*., 2008). It was reported that germination rate and subsequent growth of eggplant, spinach and lettuce were inhibited by yuzu (*Citrus jonos* L.).

According to Gatti *et al*. (2010), leaf and stem extracts of *Aristolochia esperanzae* O. Kuntze did not cause any changes in germination percentage of sesame seeds at any concentrations tested. However, at 3% root extract caused significant reduction in the germination percentage. Regarding germination speed, all extracts caused delay in germination except for 1.5% stem extract. Mutlu and Atici (2009) conducted a study using roots and leaves of catmint (*Nepeta Meyiri* Benth.), the material inhibited growth of barley (*Hordium vulgare* L.*)* and sunflower. Mashela *et al*. (2015) provided an explanation for the possible outcomes when exposing plants to increasing concentration of phytonematicides, which were concentration-dependent. In concentrations within the stimulation range, growth would be stimulated, whereas within the inhibition range, growth would be inhibited. Wondimeneh *et al*. (2013) reported that plant crude extracts from baker tree (*Milletia ferruginea* Hochst.), bitter leaf (*Vernonia amygodalina* Del.), parthenium (*Parthenium hysterophorus* L.), lantana (*Lantana camara* L.), Mexican marigold (*Tagetes minuta* L.), Mexican tea (*Chenopodium ambrosioides* L.), neem (*Azadirachta indica* A. Juss) and pyrethrum (*Chrysanthemum cinerariafolium* Trevir.) were effective in reducing *M. incognita* eggs.

2.1.2 Biological responses to post-emergent application of phytonematicides

The effects of allelochemicals on plant phytotoxicity and nematode suppression are concentration-specific (Mashela *et al*., 2015). At low concentration crude extracts of neem (*Azadirachta indica* A. Juss) leaf stimulated growth of maize (*Zea mays* var.) and tomato seedlings, whereas at high concentrations the opposite was observed (Rossner and Zebitz, 1987). Masrie *et al*. (2015), when exposing potato plants to increasing concentrations of crude extracts of dill weed (*Anethum graveolens* L.), spearmint (*Mentha spicata* L.), black cucumin (*Nigella sativa* L.) and eucalyptus spp, observed stimulation/inhibition effects on plant growth. Increasing the concentrations of Nemarioc-AG phytonematicide explained 91, 97 and 91% of the total treatment variation in stimulation of seed germination of tomato, watermelon and butternut squash plants, respectively (Mafeo and Mashela, 2009). At the highest concentration, the product constantly had allelopathic effects on seed germination of test plants. Also, when Maile (2013) exposed rough lemon seedlings to increasing concentration of Nemarioc-AG phytonematicide, observed significant effect of measured plant variables. Kohli *et al*. (2001) observed that crude extracts of yellow nutsedge (*Cyperus esculentus* L.) at 2% had no effect on germination of lettuce. The root and stem extracts of *Aristolocia espanzae* caused abnormalities and inhibited root growth of *Lactuca sativa* and *R. sativus* seedlings (Gatti *et al*., 2004).

Crude extracts of *C. myriocarpus* fruit in the Ground Leaching Technology (GLT) system consistently suppressed *M. incognita* race 2 on tomato (Mashela, 2002). In the GLT system, *C. myriocarpus* fruit suppressed nematode (*Meloidogyne* species) population densities in field trials by over 80% (Mashela *et al*., 2007). *Cucumis myriocarpus* appeared to have specific active ingredients, which were highly effective in nematode suppression, regardless of the concentration. The product consistently suppressed nematode numbers to as high as from 80 to 100% (Mafeo, 2012). In some cases, from low to high concentrations, phytonematicide effects were not different from each other, whereas the effects were significantly different to those of the untreated control. Tseke *et al*. (2013) observed similar effects when using Nemarioc-AL phytonematicide in the management of root-knot nematodes. The product consistently reduced population densities of *M. incognita* race 2 from 46 to 92% and 74 to 96% in roots and soil, respectively. Maile (2013) observed that phytonematicides increased population densities of the citrus nematode

(*Tylenchulus semipenetrans* Cobb) on citrus seedlings. Using the conceptual framework of density-dependent growth (DDG) patterns, Mashela *et al*. (2017) argued that there were no such thing as inconsistent results as observed in *T. semipenetrans* previously (Maile, 2013). Later, Mashela *et al*. (2017), using various products with nematicidal properties, demonstrated that the sampling time for nematodes was very important relative to the time the products were applied and the nematode samples collected.

2.2 Work not yet done on the problem statement

The influence of pre- and post-application of Nemarioc-AG phytonematicide on growth of potato and nematode population densities had not been documented. Such an investigation would provide information as to whether cucurbitacin-containing phytonematicides could be applied as pre- or post-emergent products in potato husbandry.

2.3 Addressing the identified gaps

Nemarioc-AG phytonematicide has potential of inducing phytotoxicity to crops. Phytotoxicity was also observed in this study in both pre- and post-emergence application, where MCSP was obtained with values that would not be phytotoxic to potato plant and would also be able to reduce nematode population densities.

CHAPTER 3

RESPONSES OF POTATO AND *MELOIDOGYNE INCOGNITA* TO PRE- AND POST-EMERGENT APPLICATION OF NEMARIOC-AG PHYTONEMATICIDE

3.1 Introduction

Worldwide, the root-knot (*Meloidogyne* species) nematodes had been the most damaging soil-borne pest (Brodie *et al*., 1993). Potato (*Solanum tuberosum* L.) cultivars do not have genotypes that are resistant to *Meloidogyne* species (Brodie *et al*., 1993). Following the international withdrawal of methyl bromide from the agrochemical markets, cucurbitacin-containing phytonematicides were widely tested on *Meloidogyne* species in tomato production (Mashela *et al*., 2016). The application of cucurbitacin-containing phytonematicides in smallholder farming systems was primarily through the ground leaching technology (GLT), which was consistently shown to be highly effective on suppression of population densities of nematodes (Mashela, 2002). The pellet form of the product derived from the fruit of wild cucumber (*Cucumis myriocarpus* Naude.) is technically referred to as Nemarioc-AG phytonematicide. The GLT involves spot application of ground materials as pellets in a shallow hole around the seedlings at transplanting (Mashela, 2002). However, since potato plants are not transplanted, various placement methods would be necessary for the successful use of this phytonematicide. The objective of this study was to determine whether Nemarioc-AG phytonematicide could serve as pre- and post-emergent phytonematicide without inducing phytotoxicity while suppressing population densities of *M. incognita*

3.2 Materials and methods

3.2.1 Description of the study site

The two trials (pre-emergent trial and post-emergent trial) were conducted separately but during the same period under greenhouse conditions at the Green Biotechnologies Research Centre (GBRC), University of Limpopo, South Africa (23°53'10"S, 29°44'15"E). The greenhouse had an area of 2 000 m^2 (100 m × 20 m) with a wet wall on the south-facing wall. The structure was partitioned into two equal area in order to reduce the heterogeneity, which dictated that treatments, depending on their size, be blocked for wind-streams. The top of the facility was covered with a photosynthetically active radiation-allowing green net, whereas eastern and western sides were covered with black nets. Trials were conducted in autumn (February-April) 2017 (Experiment 1) and repeated in autumn 2018 (Experiment 2).

3.2.2 Treatments and research design

Treatments, namely, 0, 2, 4, 8 and 16 g Nemarioc-AG phytonematicide, were arranged in a randomised complete block design (RCBD), with seven replicates (Figure 3.1). In pre-emergent trial, the seed tuber was placed in a pot half-filled with the growing medium, covered with the growing medium, and then the phytonematicide placed on top, with the remaining pot volume filled using the medium to the mark. In contrast, in post-emergent application, similar treatments were applied on the surface of the growing medium after 100% emergence of the seed tubers and slightly covered with the growing medium.

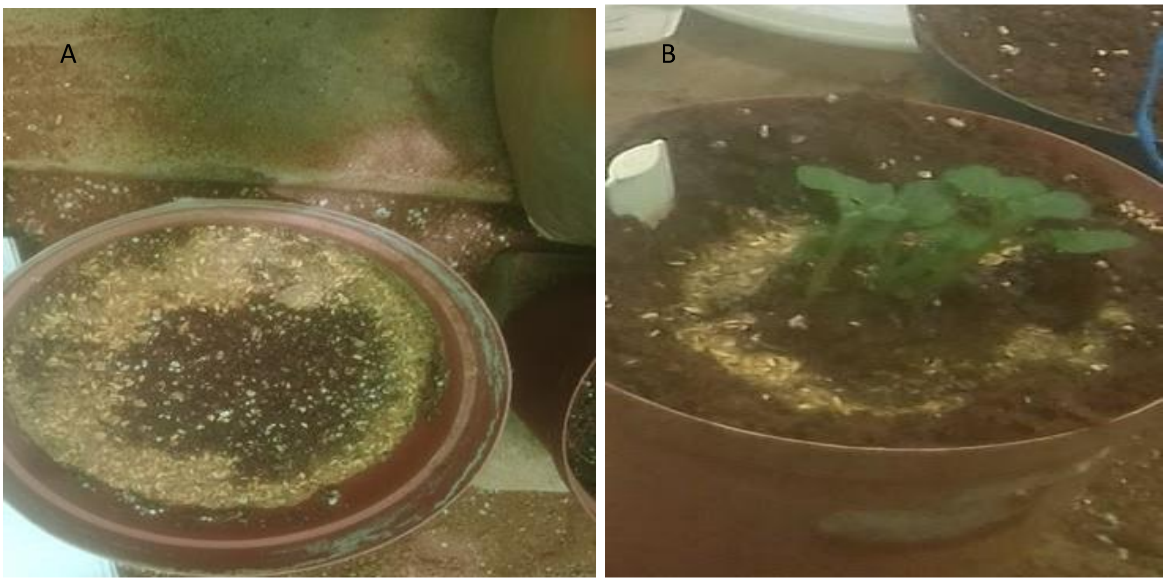


Figure 3.1 Pre- and post-emergent application of Nemarioc-AG phytonematicide on potato cv. 'Mondial G3'.

Figure 3.2 Experimental layout at post-emergent.

3.2.3 Procedures

The 20-cm-diameter plastic pots were each filled with 2.7 L steam-pasteurised (300ºC for 1 hour) river sand and Hygromix at 3:1 (v/v) ratio. Inoculum was prepared by extracting eggs and second-stage juveniles (J2) from roots of greenhouse-grown nematode-susceptible kenaf (*Hibiscus cannabinus* L.) in 1% NaOCl solution (Hussey and Barker, 1973). Potato seed tubers were sown into pots, Nemarioc-AG phytonematicide placed above the tubers and covered with soil, after initiation of treatments 5 000 eggs and J2 of *M. incognita* per pot were inoculated. Plants were irrigated with 250 ml chlorine-free tapwater. At planting each plant was fertilised with 5 g NPK 2:3:2 (22) + 0.5% S + 5% Zn + 5% Ca fertiliser and 2 g NPK 2:3:2 (43) Multifeed (Nulandies, Johannesburg) to provide 0.47 N, 0.43 K, 0.43 P, 121 Mg, 1 Fe, 0.10 Cu, 0.47 Zn, 1.34 B, 4.02 Mn and 0.09 mg Mo per ml tapwater without Ca (Mashela *et al*., 2017). The whiteflies (*Aleyrodes proletella*) were managed on weekly basis using alternative sprays, SK ECO OIL and Kombat with a G-49 wetter containing active ingredient Isotridecanol. Insect pests were monitored daily, with *Tuta absoluta* (Meyrick) being controlled once using Indoxacarb.

3.2.4 Data collection

At 56 days after inoculation, plant height were measured from the crown to the terminal end of the flag leaf, leaves were cut at the crown and the stem diameters measured at 3 cm above the severed ends using a digital Vernier caliper. Shoots were oven-dried at 52°C for 72 h and weighed. Chlorophyll content was measured using a chlorophyll meter. Root systems were removed from pots, immersed in water to remove soil particles, blotted dry, weighed to facilitate the calculation of nematode density per total root system per plant. Root galls were assessed using the North Carolina Differential Rating Scale (Taylor and Sasser, 1978).

Dried materials of 0.4 g were digested in 40 ml 5% nitric acid (HNO_3) solution, followed by placing the container on a vortex to allow for complete wetting of the mixture. The materials were magnetically stirred, thereafter incubated in a 95°C water-bath for 60 minutes, allowed to cool down at room temperature, filtered, decanted into 50 ml tubes which were covered with a foil and then K, Fe, Zn and Na in leaf tissues were analysed using the Inductively Coupled Plasma Optical Emission Spectrometry (ICPE-9000).

Nematodes were extracted from whole root system per plant using maceration and blending method for 30 seconds in 1% NaOCl solution (Hussey and Barker, 1973). The material was passed through 75 and 25-µm nested sieves, with nematodes being collected from the 25-µm mesh sieve. Soils per pot were thoroughly mixed and 250 ml soil sample were collected, with nematodes extracted using the sugar-floatation and centrifugation method (Jenkins, 1964). Nematode numbers were converted to nematodes per total root system per plant, whereas soil nematode numbers were converted to volume of soil per pot, all to allow for the determination of the final nematode population density (Pf) and the calculation of the reproductive factor (RF = Pf/Pi). In Experiment 2, data collection for plant variables, nutrient analysis and nematode variables were done as described in Experiment 1.

3.2.5 Data analysis

Plant growth variables for pre- and post-emergent application of Nemarioc-AG phytonematicide were subjected to the CARD computer-based model (Liu and An, 2005) to generate the curves, quadratic equations and the related biological indices

(Liu *et al*., 2003). Nutrient element and nematode variables were subjected to regression. The MCSP values were calculated from the biological indices of variables that, along with increasing concentration of Nemarioc-AG phytonematicide, exhibited positive quadratic relations, with $R^2 \geq 0.25$.

3.3 Results

3.3.1 Pre-emergent application of Nemarioc-AG phytonematicide

Plant responses to pre-emergent application of Nemarioc-AG phytonematicide: In Experiment 1, chlorophyll content, dry shoot mass and gall rating exhibited density-dependent growth (DDG) pattern over increasing concentrations of Nemarioc-AG phytonematicide (Figure 3.3). The model explained the relationship by 63, 98 and 91% in chlorophyll content, dry root mass and gall rating, respectively (Table 3.1). The MCSP was established at 1.95 g (Table 3.1). Biological indices where negative quadratic relations were exhibited, were excluded in the calculation of the MCSP, because they did not have stimulation phase. Chlorophyll content, dry shoot mass and gall rating had sensitivity (k value) of k = 0, respectively, with the overall sensitivity (∑k) being equal to zero. In Experiment 2, all plant variables were excluded in the MCSP, because they did not have stimulation phase.

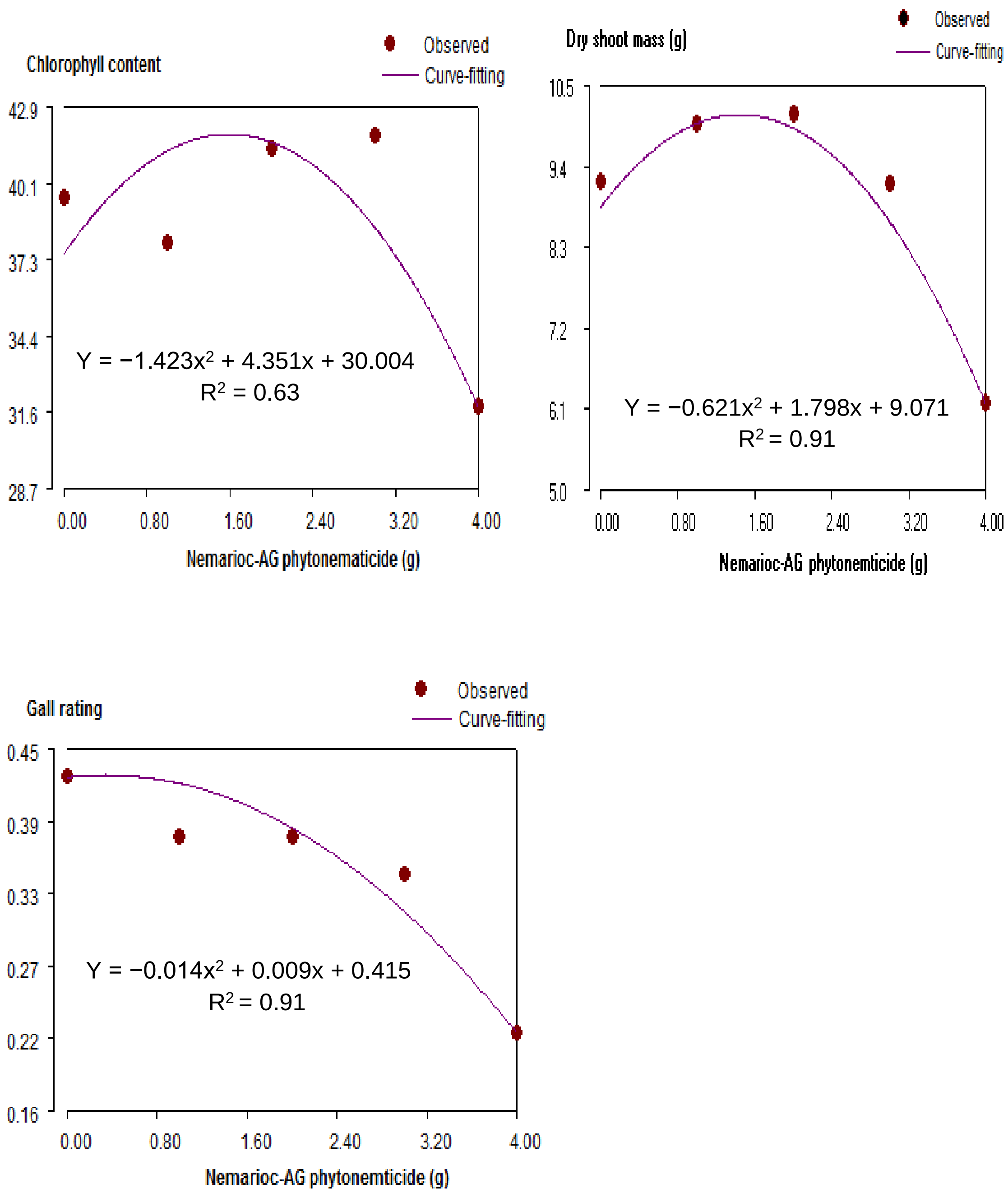


Figure 3.3 Responses of chlorophyll content, dry shoot mass and gall rating of potato to increasing concentrations of Nemarioc-AG phytonematicide in Experiment 1 at 56 days after inoculation.

Table 3.1 Biological indices for dry chlorophyll content, dry shoot mass and gall rating of potato to increasing concentrations of Nemarioc-AG phytonematicide at 56 days after inoculation.

Biological index	Chlorophyll content	Dry shoot mass	Gall rating	Mean
Threshold stimulation (D_m)	1.592	1.446	0.342	1.127
Saturation point (R_h)	3.608	1.3	0.002	1.637
0% inhibition (D_0)	3.185	2.893	0.684	2.254
50% inhibition (D_{50})	5.579	4.511	4.267	4.786
100% inhibition (D_{100})	7.000	5.11	5.9	6.003
R^2	0.63	0.98	0.91	
k value	0	0	0	
Overall sensitivity $\sum k = 0$				
MCSP = $D_m + (R_h/2) = 1.127 + (1.637/2) = 1.127+0.8185 = 1.95$ g				

Nutrient element responses to pre-emergent application of Nemarioc-AG phytonematicide: Osmoticum ions, in Experiment 1 and Experiment 2, K over increasing concentration of Nemarioc-AG phytonematicide on potato exhibited negative quadratic relations, with models being explained by 99 and 99%, respectively (Figure 3.4). In both experiments, Na over increasing concentrations of Nemarioc AG phytonematicide exhibited negative quadratic relations, with models being explained by 84 and 95%, respectively (Figure 3.4).

Micronutrient elements: In Experiment 1 and Experiment 2, Fe over increasing concentration of Nemarioc-AG phytonematicide on potato exhibited negative quadratic relations, with models being explained by 96 and 98%, respectively (Figure 3.5). In both experiments, Zn over increasing concentrations of Nemarioc-AG phytonematicide exhibited negative quadratic relations, with models being explained by 94 and 97% (Figure 3.5).

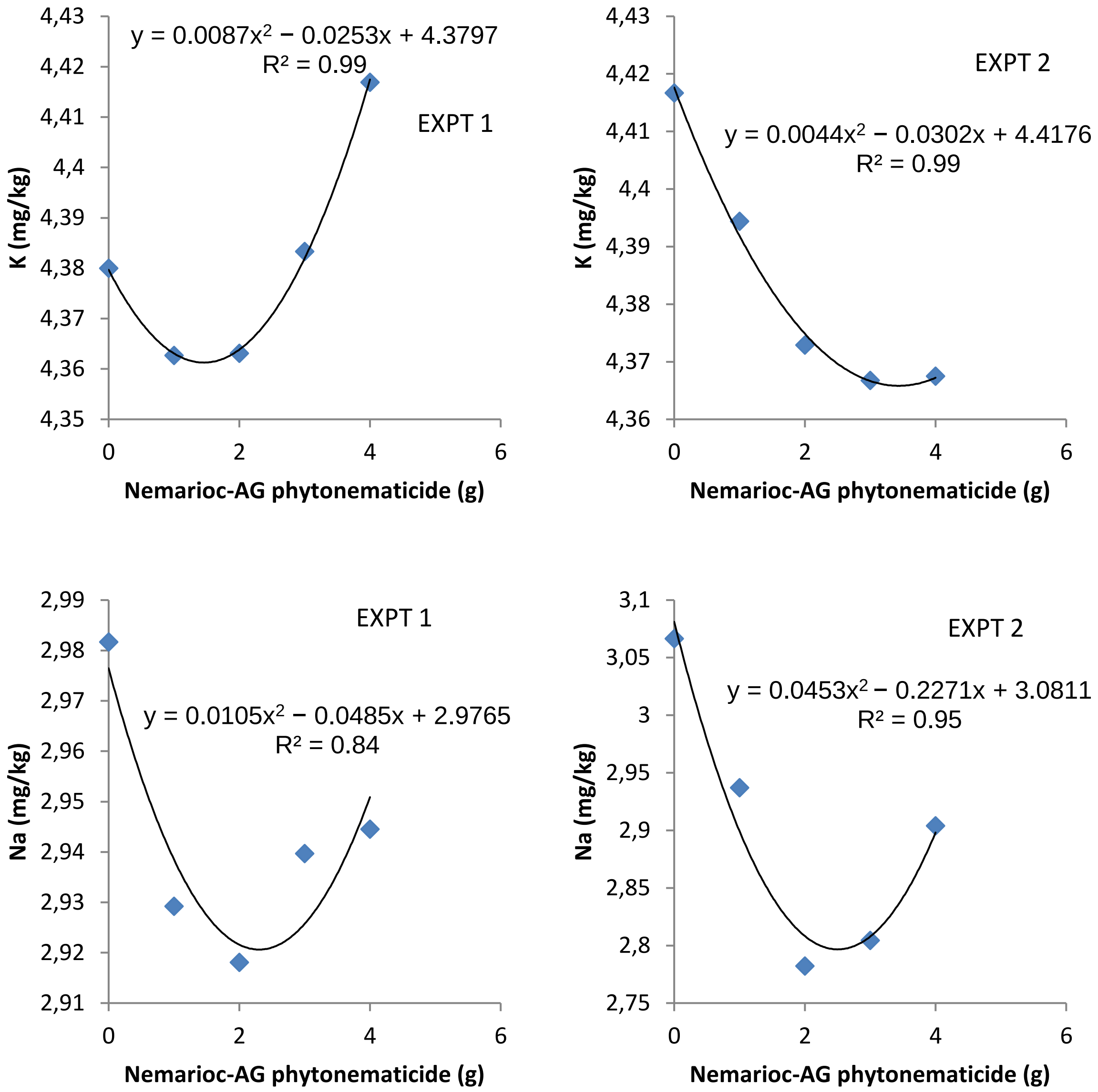


Figure 3.4 Responses of K and Na in leaf tissues of potato to increasing concentrations of Nemarioc-AG phytonematicide at 56 days after inoculation.

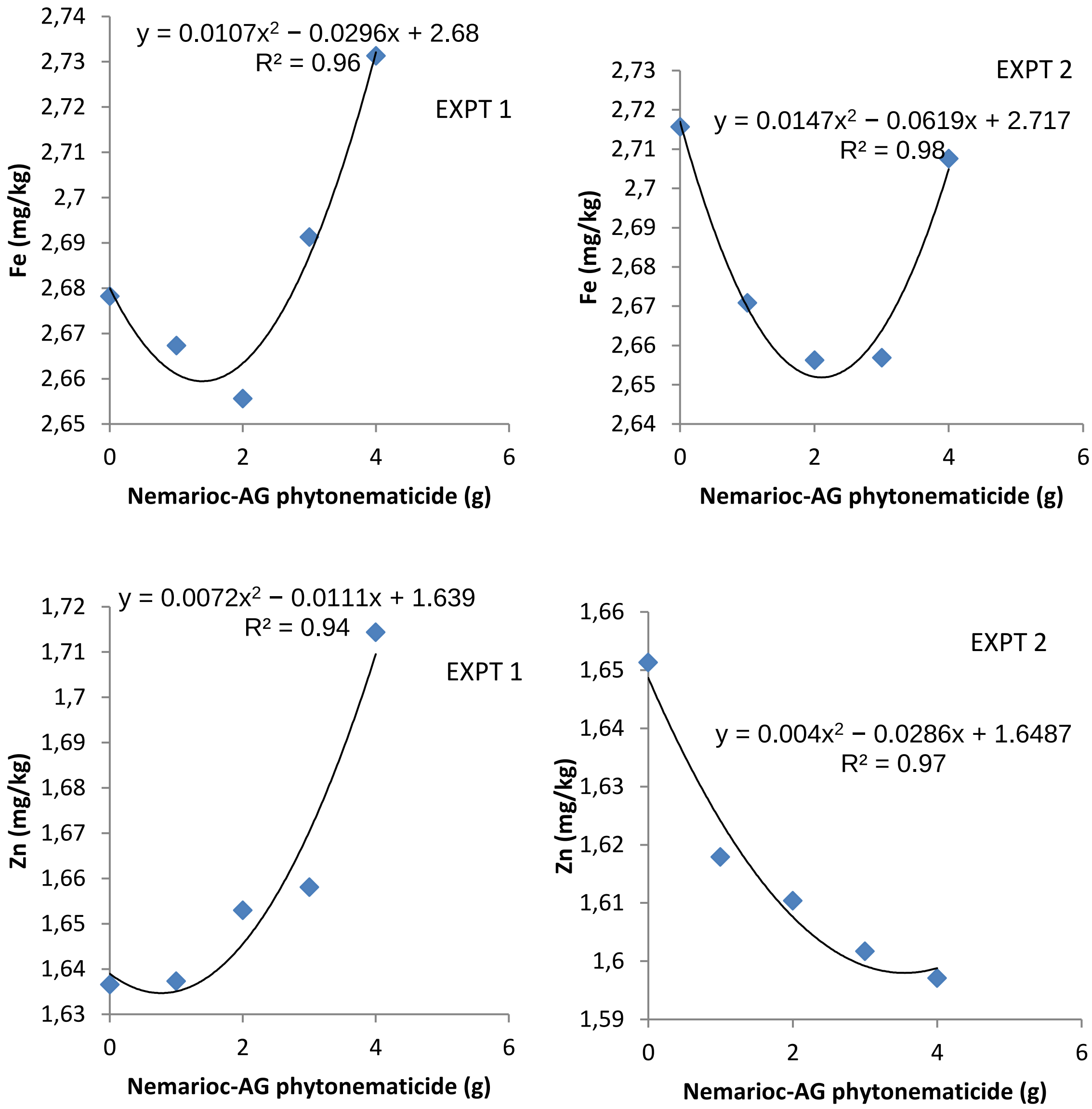


Figure 3.5 Responses of Fe and Zn in leaf tissues of potato to increasing concentration of Nemarioc-AG phytonematicide at 56 days after inoculation.

Nematode responses to pre-emergent application of Nemarioc-AG phytonematicide: In Experiment 1 and Experiment 2, J2 in roots, eggs in roots, Pf and J2 in soil over increasing concentration of Nemarioc-AG phytonematicide on potato exhibited negative quadratic relations, respectively, except in Experiment 1, where J2 in roots exhibited positive quadratic relations, with models being explained by 99, 72, 95, 97, 98, 99 and 81% respectively (Figure 3.6 and Figure 3.7).

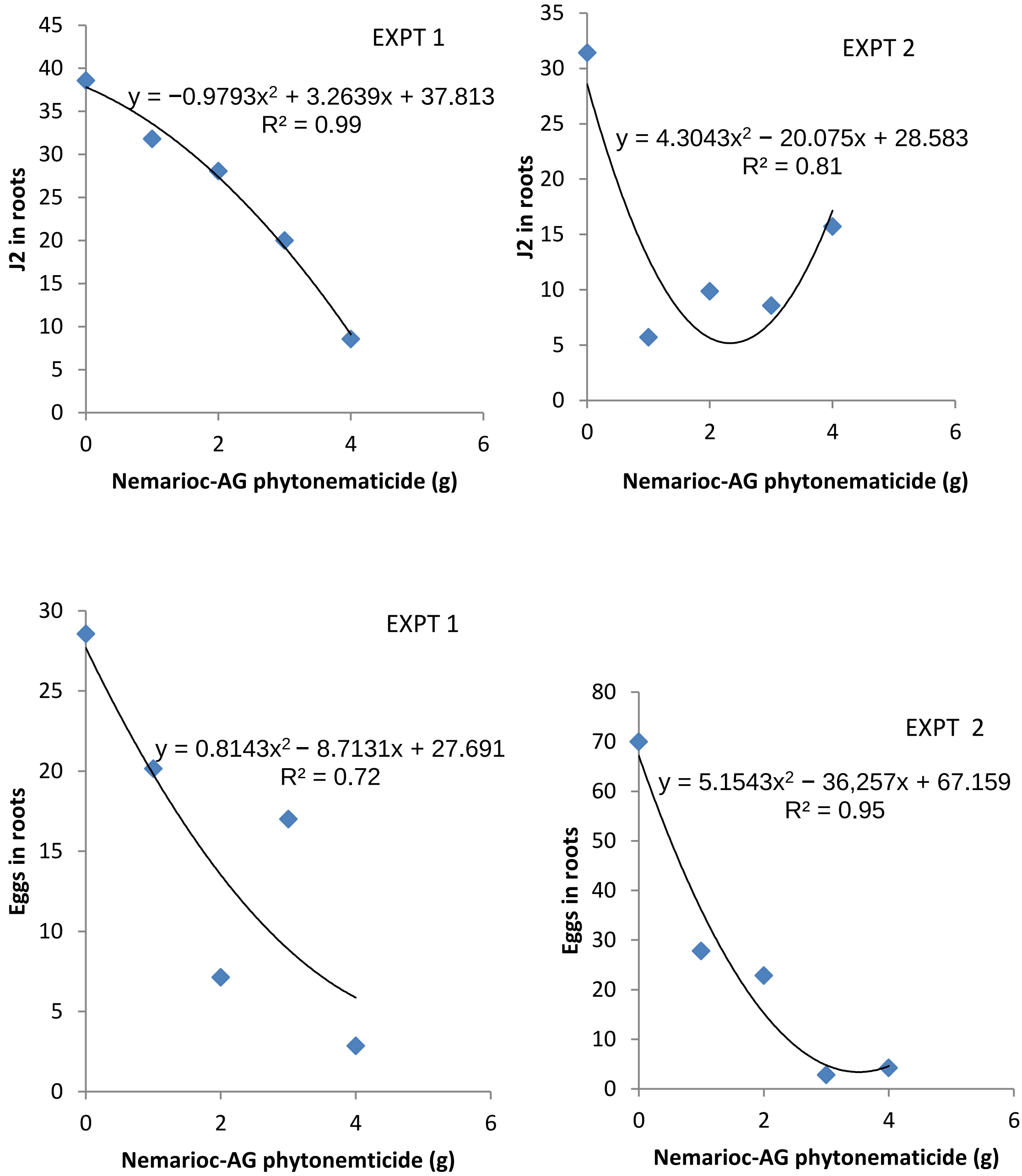


Figure 3.6 Response of J2 in roots and eggs in roots of potato to increasing concentrations of Nemarioc-AG phytonematicide at 56 days after inoculation.

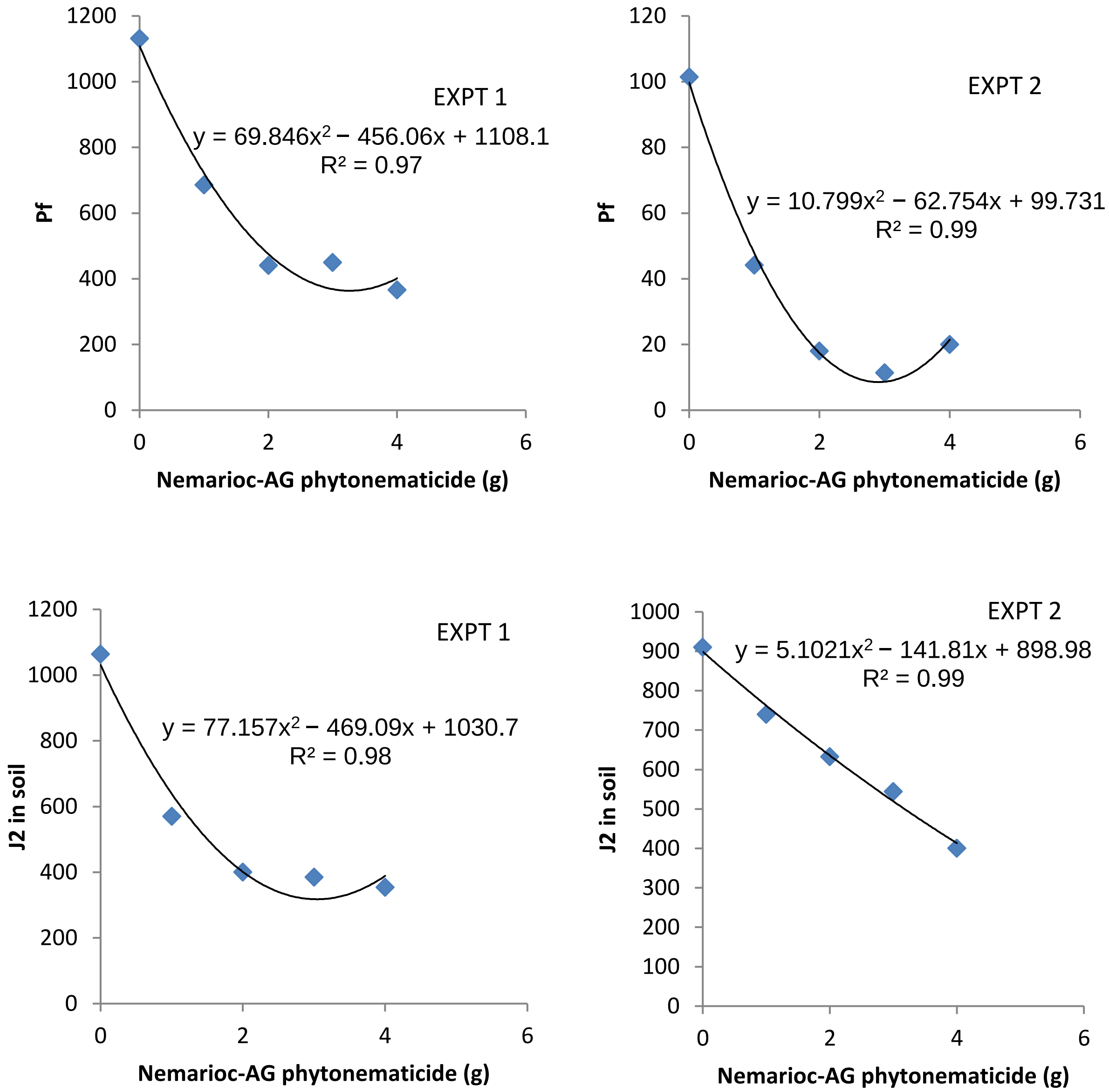


Figure 3.7 Responses of Pf and J2 in soil of potato to increasing concentrations of Nemarioc-AG phytonematicide at 56 days after inoculation.

3.3.2 Post-emergent application of Nemarioc-AG phytonematicide

Plant responses to post-emergent application of Nemarioc-AG phytonematicide: In Experiment 1, chlorophyll content and plant height exhibited quadratic relations over increasing concentrations of Nemarioc-AG phytonematicide (Figure 3.8). The MCSP was established at 1.57 g (Table 3.2). Biological indices where negative quadratic relations were exhibited were excluded in the calculation of MCSP since the former did not have the stimulation phase. The model explained the relationship by 98 and 78% in chlorophyll content and plant height, respectively. Chlorophyll content and plant height had sensitivity (k value) of k = 1, respectively, with the overall sensitivity (∑k) being equal to 2. In Experiment 2, all plant variables were excluded in the MCSP, because they did not have stimulation phase.

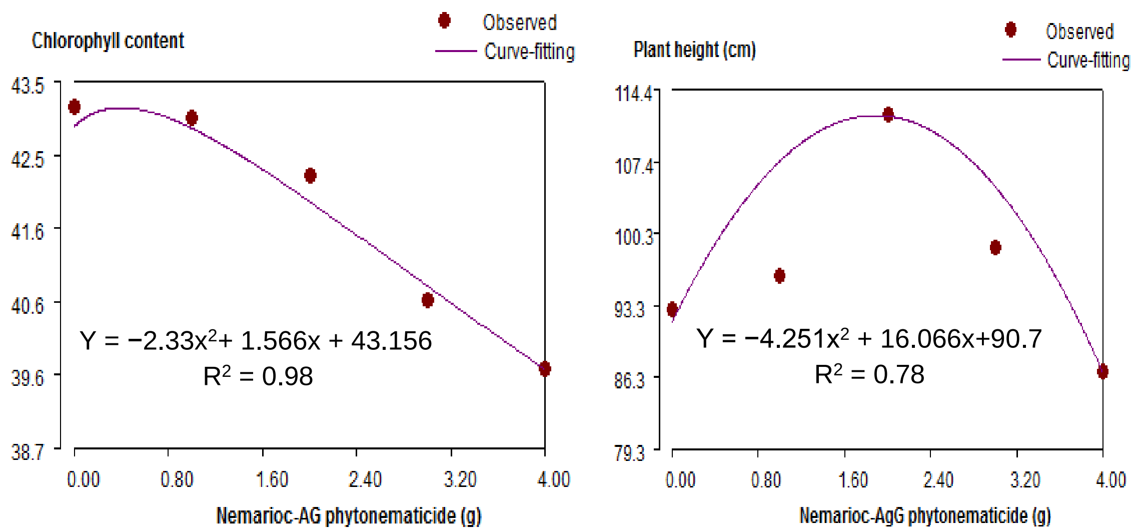


Figure 3.8 Responses of chlorophyll content and plant height of potato to increasing concentrations of Nemarioc-AG phytonematicide in Experiment 1 at 56 days after inoculation.

Table 3.2 Biological indices for chlorophyll content and plant height of potato to increasing concentrations of Nemarioc-AG phytonematicide at 56 days after inoculation.

Biological index	Chlorophyll content	Plant height	Mean
Threshold stimulation (D_m)	0.263	1.891	1.077
Saturation point (R_h)	0.399	1.518	0.9585
0% inhibition (D_0)	0.958	3.78	2.369
50% inhibition (D_{50})	28.875	5.566	17.2205
50% inhibition (D_{100})	103.8	6.5664	55.1832
R^2	0.98	0.78	
k value	1	1	
Overall sensitivity ∑k = 2			
MCSP = D_m + (R_h/2) = 1.077 + (0.9585/2) = 1.077 + 0.479 = 1.57 g			

Nutrient element responses to post-emergent application of Nemarioc-AG phytonematicide: Osmoticum ions, In Experiment 1 and Experiment 2, K over increasing concentration of Nemarioc-AG phytonematicide on potato exhibited negative quadratic relation in Experiment 1 and positive quadratic relation in Experiment 2, with models being explained by 97 and 89%, respectively (Figure 3.9). In both experiments, Na over increasing concentration of Nemarioc-AG phytonematicide exhibited positive and negative quadratic relations, with models being explained by 91 and 95%, respectively (Figure 3.9).

Micronutrient elements: In Experiment 1, Fe over increasing concentration of Nemarioc-AG phytonematicide on potato exhibited negative quadratic relations, with models being explained by 96% (Figure 3.10), In Experiment 2, Fe over increasing concentration of Nemarioc-AG phytonematicide was excluded, because the $R^2 \leq 0.25$. In both Experiment 1 and Experiment 2, Zn over increasing concentration of Nemarioc-AG phytonematicide exhibited positive quadratic relations, with models being explained by 93 and 93% (Figure 3.10).

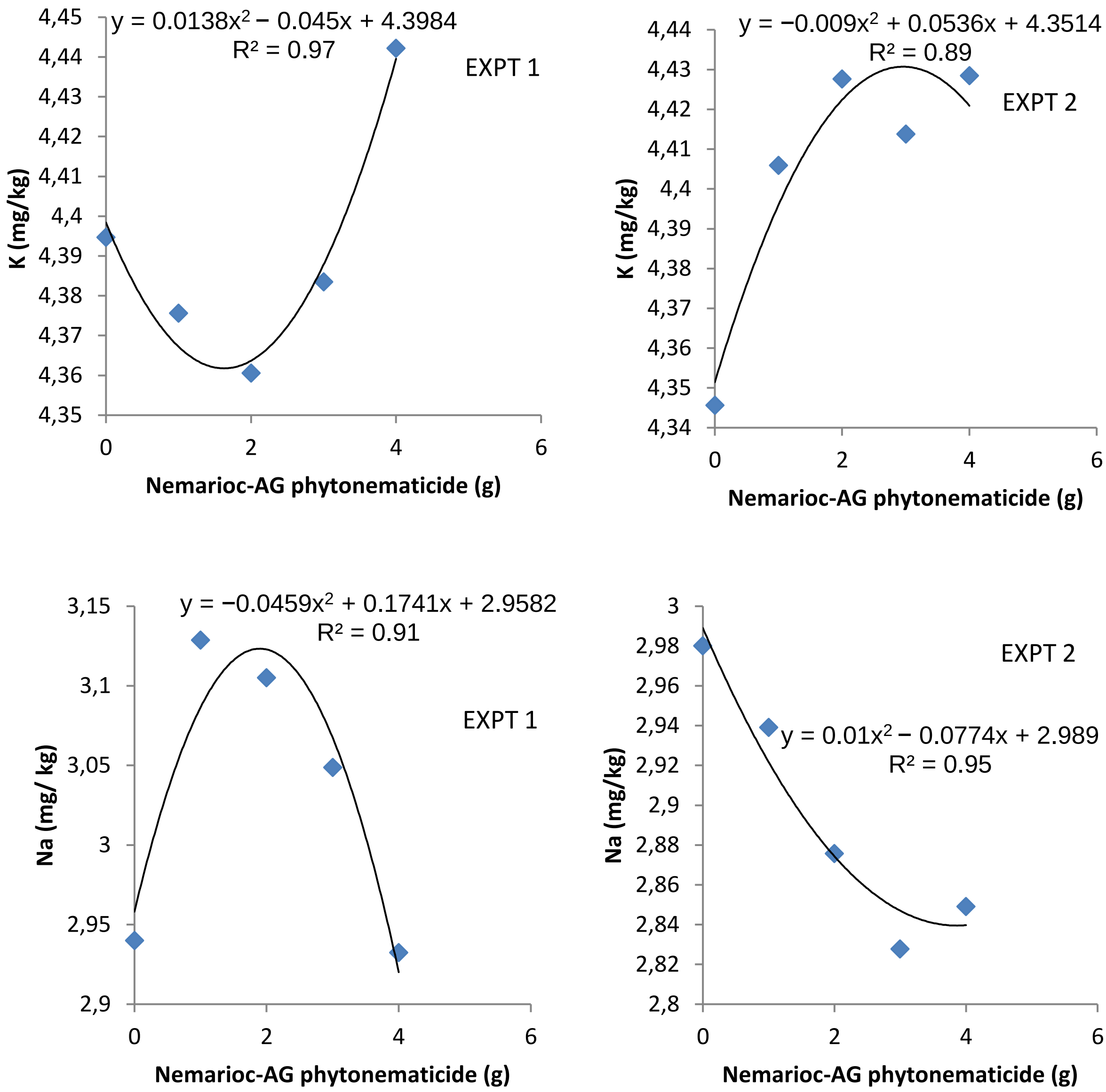
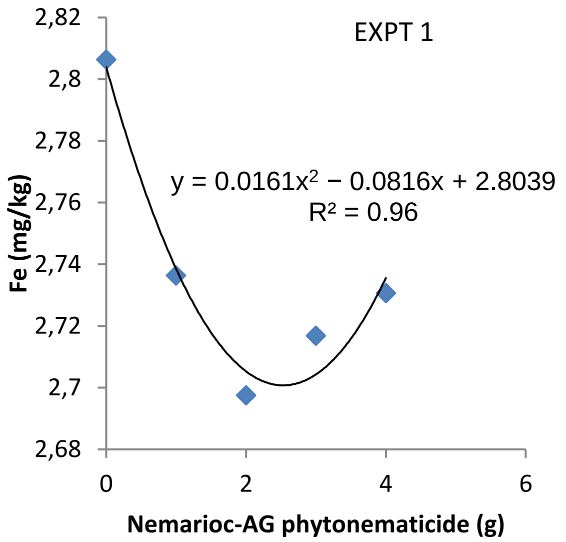
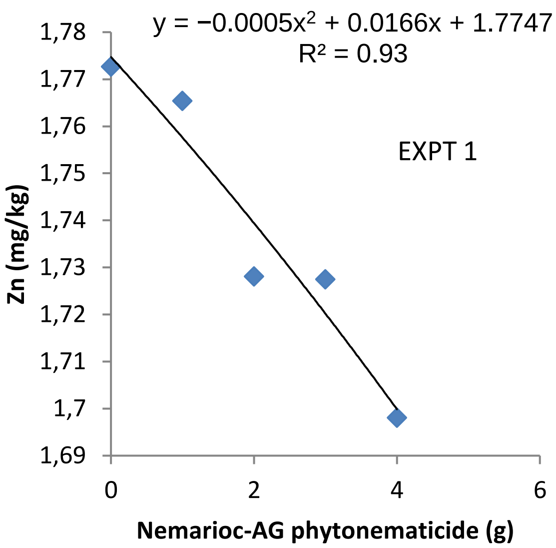


Figure 3.9 Responses of K and Na in leaf tissues of potato to increasing concentrations of Nemarioc-AG phytonematicide at 56 days after inoculation.





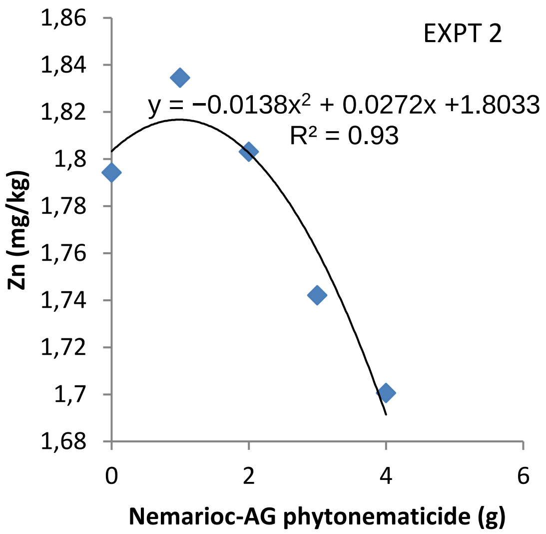


Figure 3.10 Responses of Fe and Zn, in leaf tissues of potato to increasing concentrations of Nemarioc-AG phytonematicide at 56 days after inoculation.

Nematode responses to post-emergent application of Nemarioc-AG phytonematicide: In Experiment 1, J2 in roots and soil, Pf and eggs in roots over increasing concentration of Nemarioc-AG phytonematicide on potato exhibited negative quadratic relations, respectively, with models being explained by 92, 98, 96 and 98% respectively (Figure 3.11 and Figure 3.12). In Experiment 2, all nematode variables were not significant at $R^2 \leq 0.25$.

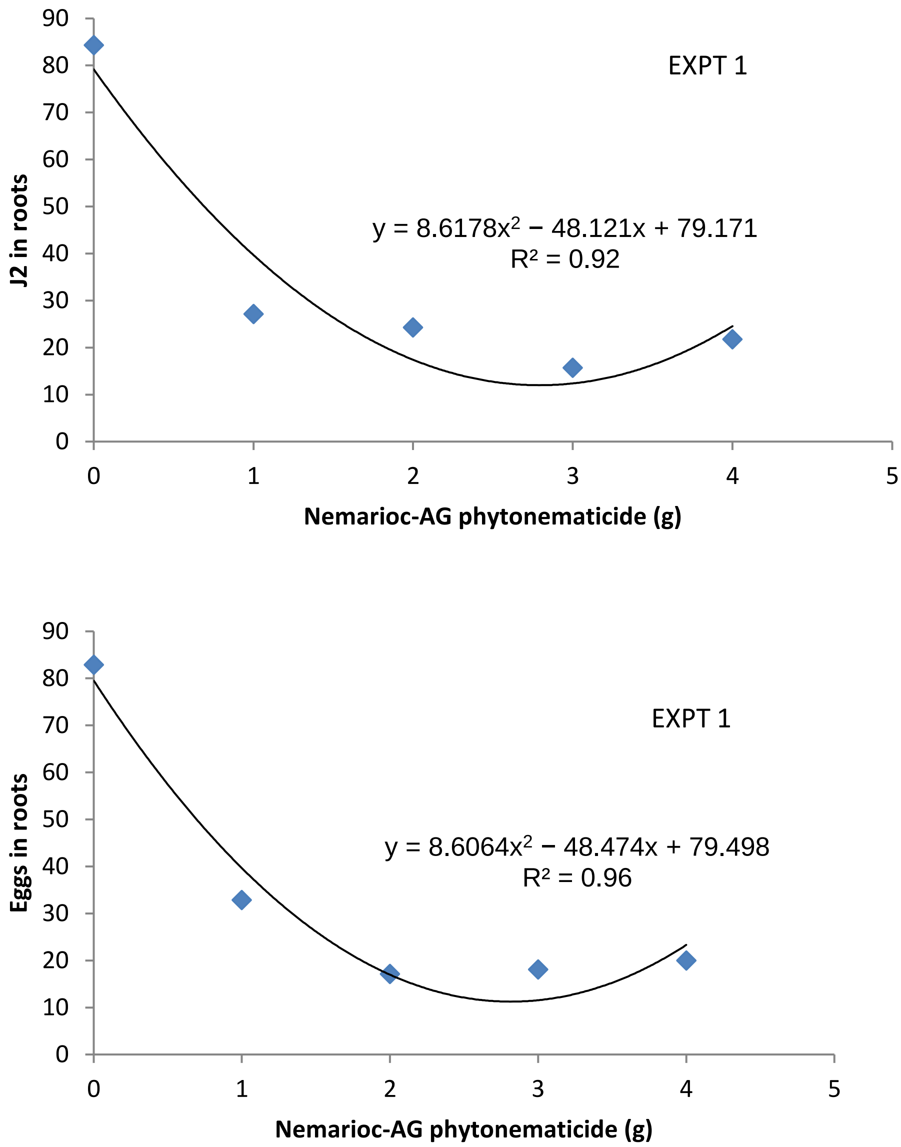
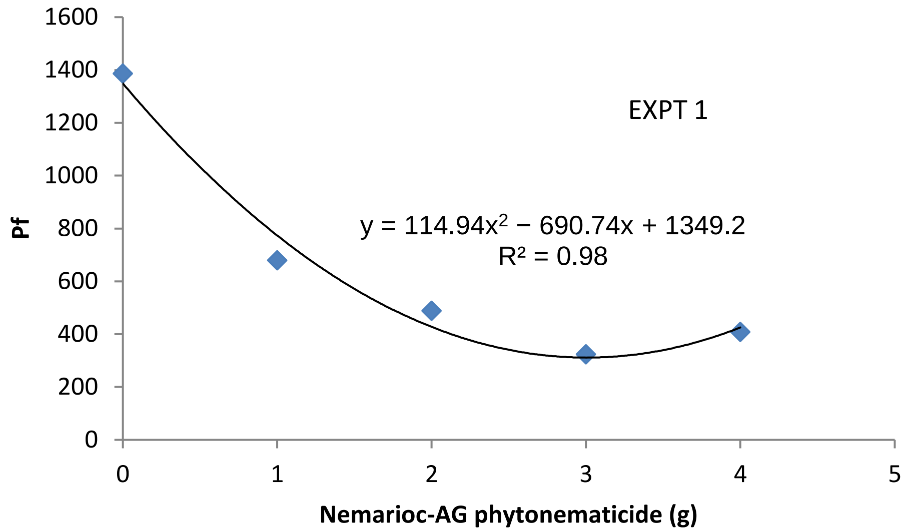


Figure 3. 11 Responses of J2 in roots and eggs in roots of potato to increasing concentration of Nemarioc-AG phytonematicide at 56 days after inoculation.



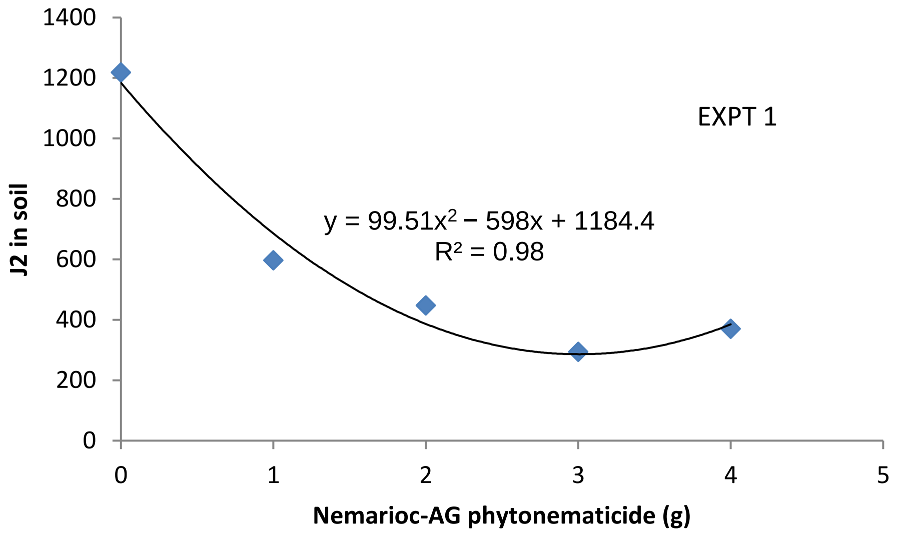


Figure 3.12 Responses of J2 in soil and Pf of potato to increasing concentration of Nemarioc-AG phytonematicide at 56 days after inoculation.

3.4 Discussion

3.4.1 Pre-emergent application of Nemarioc-AG phytonematicide

Plant responses to pre-emergent application of Nemarioc-AG phytonematicide: In the current study, chlorophyll content, dry shoot mass and gall rating exhibited density-dependent growth (DDG) pattern over increasing concentrations of Nemarioc-AG phytonematicide. The model was explained with R^2 values ranging from 63 to 98%, with strong DDG patterns as observed in seedlings from Alliaeceae, Graminae and Solanaceae families exposed to increasing concentration of Nemarioc-AG phytonematicide (Mafeo, 2012). Generally, at low concentration the material stimulated growth of various organs, whereas at high concentration the material inhibited growth (Mashela *et al*., 2016).

The value of MCSP was generated at 1.95 g Nemarioc-AG phytonematicide for potato cv. 'Mondial G3'. The generated MCSP for Nemarioc-AG phytonematicide on potato was higher than those of maize (*Zea mays*), millet (*Pennisetum glaucum*) and sorghum (*Sorghum bicolor*), which were at 0.86, 1.13 and 1.12 g, respectively (Mafeo *et al*., 2011b). The derived MCSP for Nemarioc-AG phytonematicide on potato was also higher than those of eggplant (*Solanum melongena* L.), pepper (*Capsicum annuum* L.) and tomato (*Solanum lycorpesicum*L.), which were at 0.74, 1.11 and 0.53 g, respectively (Mafeo, 2012). The overall sensitivity value of potato to the product was equal to zero ($\sum k = 0$), which suggested that potato plants were highly sensitive to this product (Liu *et al*., 2003). The latter confirmed who showed that the sensitivity of organisms increased as $\sum k$ approached zero. Crops, such as beetroot (*Beta vulgaris* L.), also had overall sensitivities of zero to increasing concentration of Nemarioc-AG phytonematicide. Mafeo *et al*. (2011b) observed overall sensitivity values of 24, 49 and 22 units, respectively, on chive (*Allium schoenoprasum*), leek (*Allium ampeloprasum*) and onion (*Allium cepa*) seedlings exposed to increasing concentration of Nemarioc-AG phytonematicide during germination. Other plants of Solanaceae family such as eggplant (*Solanum melongena*), pepper (*Capsicum annuum)* and tomato (*Solanum. lycorpesicum*) had overall sensitivity of 9, 32 and 51, respectively (Mafeo, 2012). High MCSP values are undesirable since they would eventually result in phytotoxicity.

Nutrient element responses to pre-emergent application of Nemarioc-AG phytonematicide: The observations showed that an increased in concentration of Nemarioc-AG phytonematicide reduced accumulation of nutrient elements in leaf tissues. The results confirmed observations by Khosa (2005), when exposing tomato plant to increasing concentrations of Nemarioc-AG phytonematicide. Generally, various plant organs have different sensitivities to Nemarioc-AG phytonematicide (Mafeo, 2012), with findings in this study suggesting that nutrient elements in leaf tissues in potato could also be responding differently to various levels of Nemarioc-AG phytonematicide. The reduction of accumulation of nutrients elements is known to have an impact on the tested crop (Mashela and Nthangeni, 2002). Decrease in Fe content in leaves of potato observed in the current study, might be due to Fe existing as Fe^{3+} in the soil bound with Fe hydroxides, suggesting that plants needed to mobilize Fe in the soil by first making it soluble before it could be absorbed and transported (Jeong and Guerinot, 2009).

Nematode responses to pre-emergent application of Nemarioc-AG phytonematicide: In this study, J2 in roots, eggs in roots, Pf and J2 in soil over increasing concentration of Nemarioc-AG phytonematicide on potato exhibited positive and negative quadratic relations. Mashela *et al*. (2017) explained that a positive quadratic relationship between variables and increasing concentrations of a particular phytonematicide, suggested that the concentrations used were within the stimulation range and a negative quadratic relationship suggests that concentrations of allelochemicals were already in inhibition range. The positive relationship observed in this study confirms observation under *in vitro* trials, at low concentration of cucurbitacin-containing phytonematicides (Dube, 2016). The negative quadratic relation observed over increasing concentration of Nemarioc-AG phytonematicide agreed with the inhibition effects of cucurbitacin-containing phytonematicides on *Meloidogyne* species (Mashela *et al*. 2016).

3.4.2 Post-emergent application of Nemarioc-AG phytonematicide

Plant growth responses to post-emergent application of Nemarioc-AG phytonematicide: In the current study, chlorophyll and plant height exhibited DDG pattern over increasing concentrations of Nemarioc-AG phytonematicide. Using the curve-fitting allelochemical response dosage (CARD) model, the variables and the concentrations of phytonematicides, as shown above (section 3.4.1), are characterised by quadratic relationships. The model explained the relationship by 98 and 78%, which agreed with the strong DDG patterns observed when tomato plants were exposed to increasing concentration of crude extracts of *Tulbaghia violacea* (Malungane, 2014). In the current study, the MCSP was generated at 1.57 g Nemarioc-AG phytonematicide, with the overall sensitivity value of the product on potato being 2, which suggested that potato plants were tolerant (Liu *et al*., 2003). Rice (1984) indicated that the degree of sensitivity in plants to allelochemicals was plant- specific, with seedlings being highly tolerant than other stages in the life of a given plant species.

Nutrient element responses to post-emergent application of Nemarioc-AG phytonematicide: In this study, osmoticum ions (K and Na) over increasing concentration of Nemarioc-AG phytonematicide exhibited negative and quadratic relations. Positive quadratic relationship in K and negative quadratic relationship in Na confirmed observations by Mashela and Pofu (2017) when exposing green beans to increasing concentrations of other cucurbitacin-containing phytonematicide, Nemarioc-AL phytonematicide, where the treatment exhibited the quadratic relationship. The stimulation range for accumulation of the selected nutrient elements in leaf tissues of green beans was within approximately 3% concentration of Nemarioc-AL phytonematicide (Mashela and Pofu, 2017). The value of 3% is equivalent to an empirically-established concentration for nematode management in tomato plants (Mashela *et al*., 2016). Micronutrient elements (Fe and Zn) over increasing concentration of Nemarioc-AG phytonematicide on potato exhibited negative and positive quadratic relations. The observations were also in agreement with other observations (Mashela and Pofu, 2017).

Nematode responses to post-emergent application of Nemarioc-AG phytonematicide: In this study J2 in roots and soil, Pf and eggs in roots exhibited negative quadratic relations, this confirms with the existence of DDG patterns (Liu and An, 2005). Similar observations were made, when tomato (*Solanum lycorpesicum* L.) plants were exposed to increasing concentration of Nemarioc-AG phytonematicide, with model explained by 93% (Mashela, 2017). In the GLT system, increasing concentration of Nemarioc-AG phytonematicide inhibited *Meloidogyne* species population densities in field trials by over 80% (Mashela *et al*., 2007). Similarly, Nemarioc-AG phytonematicide consistently suppressed *M. incognita* race 2 on tomato (*Solanum lycorpesicum* L.) to increasing concentration of Nemarioc-AG phytonematicide (Mashela, 2002). Negative quadratic relations observed on J2 in soil to increasing concentration of Nemarioc-AG phytonematicide confirmed observation by Tseke and Mashela (2018) when determining efficacy of Nemarioc-AL phytonematicide on *Meloidogyne* species in tomato plant production. These negative quadratic relations suggested that the concentrations of allelochemicals were already in inhibition range. In contrast, Maile (2013) observed that increasing concentration of Nemarioc-AG phytonematicide increased population densities of the citrus nematode (*Tylenchulus semipenetrans* Cobb) on citrus seedlings. This explains that the growth of nematode population densities is cyclic (Pofu, 2008).

3.5 Conclusion

Pre-emergent: In conclusion, Nemarioc-AG phytonematicide when applied in granular formulation as pre-emergent treatment, stimulated chlorophyll content, dry shoot mass and gall ratings. With exception of gall rating, the stimulation of plant growth variables was a positive attribute. MCSP of 1.95 g of Nemarioc-AG phytonematicide was obtained which would stimulate growth and also not be detrimental to potato plants. Accumulation of essential nutrient elements in potato leaf tissues and increasing concentration of phytonematicide concentrations exhibited density-dependent growth patterns. Nemarioc-AG phytonematicide was suitable for use as pre-emergent treatment in management of nematodes on potato.

Therefore, it would be necessary to empirically develop its application interval on the test crop.

Post-emergent: In conclusion, Nemarioc-AG phytonematicide when applied in granular formulation as post-emergent treatment, stimulated plant height and chlorophyll content. MCSP of 1.57 g of Nemarioc-AG phytonematicide was obtained which would stimulate growth and also not be detrimental to potato plants. The product inhibited population densities of *M. incognita*, therefore, this product is suitable for use as post-emergent in management of nematodes on the test crop.

CHAPTER 4
SUMMARY OF FINDINGS, SIGNIFICANCE, RECOMMENDATIONS AND CONCLUSIONS

4.1 Summary of findings

Nemarioc-AG phytonematicide has potential of inducing phytotoxicity to the crops, and also reduce nematode population densities. The study was therefore conducted, to determine whether Nemarioc-AG phytonematicide could serve as pre- and post-emergent phytonematicide without inducing phytotoxicity while suppressing population densities of *M. incognita*. MCSP of 1.95 g of Nemarioc-AG phytonematicide at pre-emergent application was obtained with overall sensitivity being equal to zero ($\sum k = 0$), which showed that the tested crop was highly sensitive to the concentration used. Therefore, all nutrients elements to increasing concentration of Nemarioc-AG phytonematicide exhibited negative quadratic relations, with model ranging from 63 to 98%. The treatment reduced population densities of *M*. *incognita*. Furthermore, when Nemarioc-AG phytonematicide was applied as post-emergent the treatment, MCSP of 1.57 g of Nemarioc-AG phytonematicide was obtained with overall sensitivity being equal to two ($\sum k = 2$), which also shows that the tested crop was tolerant to the concentration used. Positive and negative quadratic relations were exhibited; models were ranging from 89 to 97%. The product reduced population densities of *M. incognita*.

4.2 Significance

Meloidogyne incognita causes more damage to crops, with most nematicides withdrawn from the agrochemical markets due to their environment-unfriendliness. Planting potato with Nemarioc-AG phytonematicide would help potato producers. Since Nemarioc-AG phytonematicide is locally developed it could be affordable to smallholder farmers who are interested in potato production. The environment would also not be affected since product is environment-friendly. The MCSP was obtained, together with overall sensitivity. The MCSP obtained would help in stimulating growth of the test crop and also reduce the population density of *M. incognita*.

4.3 Recommendations

Nemarioc-AG phytonematicide could be used as pre- and post-emergent treatment in management of *M. incognita* on potato. The product can be applied at 1.95 g as pre-emergent and 1.57 g as post-emergent, which would stimulate plant growth and at the same time suppress nematode population densities. Therefore, it would be necessary to empirically develop application interval on the test crop for both pre- and post-emergence application, using the MCSP obtained in this study.

4.4 Conclusions

Increasing concentration of Nemarioc-AG phytonematicide had stimulated certain plant variables and inhibited population densities of *M. incognita* in pre- and post-emergent application; therefore, this product is suitable for use as pre-and post-emergent in management of nematodes on the test crop.

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