PRE- AND POST-EMERGENT APPLICATION EFFECTS OF NEMAFRIC-BG PHYTONEMATICIDE ON GROWTH OF POTATO CULTIVAR 'MONDIAL G3' AND SUPPRESSION OF *MELOIDOGYNE JAVANICA*

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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 AGRICULTURE AND ENVIRONMENTAL SCIENCES, FACULTY OF SCIENCE AND AGRICULTURE, UNIVERSITY OF LIMPOPO, SOUTH AFRICA

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DECLARATION

I, Tiego Isaac Huma, declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of 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 execution, and related materials contained herein had been duly acknowledged.

		06/09/19
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Co-Supervisor: Professor P.W. Mashela	Signature	Date

DEDICATION

To my exquisite mother, Mrs Huma Maruwane Stephinan, the mother I have always wanted to be my mother.

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ABSTRACT

Available potato (*Solanum tuberosum* L.) cultivars do not have any genotype that is resistant to the root-knot (*Meloidogyne* species) nematodes. Due to the susceptibility of potato cultivars to *Meloidogyne* species, alternative management strategies had to be researched and developed after the withdrawal of methyl bromide from the agro-chemical markets, amongst which were the cucurbitacin-containing phytonematicides. However, of the available application methods of phytonematicides, the ground leaching technology (GLT) and botinemagation technology were not suitable for use in most high-rainfall potato-producing regions, where production is under rain-fed conditions. The objective of the study, therefore, was to determine whether pre- and post-emergent application of Nemafric-BG phytonematicide would have effects on growth of potato and suppression of *M. javanica* population densities. Parallel pot trials of pre- and post-emergent application of Nemafric-BL phytonematicide were conducted under greenhouse conditions in autumn (February-April: Experiment 1) 2017 and validated (Experiment 2) in 2018. Each plant was inoculated with 3000 *M. javanica* eggs and second-stage juveniles (J2). Five treatments, namely, 0, 2, 4, 8 and 16 g concentration of Nemafric-BG phytonematicide, arranged in randomised complete block design, were either applied mixed with seed tubers for pre-emergent or spread on the soil surface after emergence for post-emergent trials. In all cases, plant growth variables were assessed using the Curve-fitting Allelochemical Response Data (CARD) model, whereas nutrient elements (Fe, K, Na and Zn) and nematode variables were assessed using analysis of variance, with data subjected to lines of the best fit. In pre-emergent application trial, plant height (R^2 = 0.98) and fresh root mass (R^2 = 0.99) exhibited quadratic relations, characterised by density dependent growth patterns with increasing concentrations of Nemafric-BG

phytonematicide in Experiment 1, similar trends were also observed on plant height (R^2 = 0.99) and root mass (R^2 = 0.99) in Experiment 2. In contrast, in post-emergent application trial, plant height (R^2 = 0.97), fresh root mass (R^2 = 0.99) and dry shoot (R^2 = 0.98) exhibited quadratic relations in Experiment 1, which ascribed to DDG patterns, similar trends were also observed in Experiment 2 on plant height (R^2 = 0.99), fresh root mass (R^2 = 0.96) and dry shoot (R^2 = 0.99) of potato cv. 'Mondial G3'. In pre-emergent application trials, Mean Concentration Stimulation Point (MCSP) = 24.18 and 7.82 g, respectively, in Experiment 1 and Experiment 2, with ∑k being equivalent to 20 and 6 units for potato to the product, respectively, in Experiment 1 and Experiment 2. In contrast, post-emergent application trials, MCSP = 9.87 and 12.10 g, respectively, in Experiment 1 and Experiment 2, whereas the ∑k value for potato to the product was 11 and 6 units, respectively in Experiment 1 and Experiment 2. Increasing concentrations of the phytonematicide significantly ($P \leq 0.05$) affected the selected nutrient elements. In pre-emergent application trials, K (R^2 = 0.96) Na (R^2 = 0.90) and Zn (R^2 = 0.83) each with increasing Nemafric-BG phytonematicide concentrations exhibited positive quadratic fashion, while Fe (R^2 = 0.87) exhibited negative quadratic relations in Experiment 1. In Experiment 2, K (R^2 = 0.99), Na (R^2 = 0.90) and Zn (R^2 = 0.97) contents each in leaf tissues against the increasing concentrations of the phytonematicide exhibited negative quadratic relations, while Fe (R^2 = 0.88) exhibited positive quadratic relations. In post-emergent trials, Fe (R^2 = 0.91, Na (R^2 = 0.90) and Zn (R^2 = 0.99) contents in leaf tissues against increasing Nemafric-BG phytonematicide concentration exhibited negative quadratic relations, whereas K (R^2 = 0.86) exhibited positive quadratic relation in Experiment 1. In Experiment 2, Fe (R^2 = 0.93), K (R^2 = 0.92), Na (R^2 = 0.79) and Zn (R^2

= 0.89) contents in leaf tissues of potato exhibited positive quadratic, respectively. In pre-emergent trial for Experiment 1, eggs in roots (R^2 = 0.78), J2 in roots (R^2 = 0.85), J2 in soil (R^2 = 0.97) and Pf (R^2 = 0.78) of *M. javanica* against increasing pre-emergent application concentrations of Nemafric-BG phytonematicide exhibited negative quadratic relations, characterised by DDG patterns. Similar trends were observed on eggs in roots (R^2 = 0.82), J2 in roots (R^2 = 0.99), J2 in soil (R^2 = 0.84) and Pf (R^2 = 0.85) in Experiment 2. In contrast, in post-emergent application trial, eggs in roots (R^2 = 0.87), J2 in roots (R^2 = 0.99), J2 in soil (R^2 = 0.91) and Pf (R^2 = 0.99) of *M. javanica* against increasing post-emergent application concentrations of Nemafric-BG phytonematicide also exhibited negative quadratic relations in Experiment 1, which ascribed to DDG patterns. Similar trends were also observed on eggs in roots (R^2 = 0.72), J2 in roots (R^2 = 0.68), J2 in soil (R^2 = 0.85) and Pf (R^2 = 0.83) in Experiment 2. Results from the study demonstrated that Nemafric-BG phytonematicide stimulated plant growth at lower concentration and the product does not have any detrimental effects in accumulation of nutrient elements in leaf tissues. Therefore, it is concluded, that the product could be applied at the recommended rates of 7.82 and 9.87 g/plant in pre and post-emergent application, respectively, for the management of root-knot nematodes, provided the active ingredient does not accumulate in potato tubers or have any detrimental effects in accumulation of nutrient elements in tubers and temper with nutritional value of potatoes.

CHAPTER 1
RESEARCH PROBLEM

1.1Background

1.1.1 Description of the research problem

Potato (*Solanum tuberosum* L.) cultivars do not have known genotypes that are resistant to root-knot (*Meloidogyne* species) nematodes (Berthou *et al*., 2003; García *et al*., 2014; Jones, 2006). Root-knot nematodes are the most damaging nematode species, with the success of most crops in tropical and subtropical regions being dependent upon proper nematode management tactics (Fourie *et al*., 2015; Sikora and Fernandez, 2005). Internationally, *M. incognita* is the most widespread and aggressive tropical root-knot nematode, with numerous hosts and biological races (Sasser, 1979). However, in South Africa, *M. javanica* is more aggressive than *M. incognita* (Kleynhans *et al*., 1996).

Following the global withdrawal of synthetic nematicides from agrochemical markets due to their environment-unfriendliness, alternatives for managing nematode numbers were widely researched and developed (Mashela *et al*., 2015). In Limpopo Province, South Africa, a cucurbitacin-B containing phytonematicide, Nemafric-BG phytonematicide was developed (Mashela *et al*., 2015). However, due to the presence of allelochemicals in Nemafric-BG phytonematicide, the use of this phytonematicide in managing nematode population densities is hindered by its high level of phytotoxicity (Mashela *et al*., 2015). Therefore, there is a need for detailed research in optimisation of its concentration on various crops.

1.1.2 Impact of the research problem

Phytotoxicity due to phytonematicides has been reported in tomato, sweet potato (*Ipomoea batatas* L.), nightshade (*Atropa belladonna* L.), cowpea (*Vigna unguiculata* (L.) Walp.), wild geranium (*Pelargonium sidoides* DC.), potato (*Solanum tuberosum* L.) and other crops protected against nematodes with the use of cucurbitacin-containing phytonematicides (Mashela, 2014; Mashela and Dube, 2014; Mashela *et al*., 2015; Sithole *et al*., 2016; Tseke, 2013). Generally, phytotoxicity could reduce plant growth from as high as 50% to complete crop failure (Lamberti, 1979; Mashela *et al*., 2011; Pofu *et al*., 2010). In most cases, the test phytonematicides had not gone beyond *in vitro* efficacy trials due to phytotoxicity challenges (EPPO, 2010).

1.1.3 Possible causes of the research problem

Global yield loss due to nematode damage prior to withdrawal of synthetic nematicides had been estimated at US$126 billion per annum (Chitwood, 2003), with percentage yield losses ranging from 6 to 20% (Sithole, 2016). Cucurbitacin-containing phytonematicides have been successfully used in suppression of *Meloidogyne* species in vegetable cultivation (Mashela *et al*., 2015). However, the active ingredients in cucurbitacin-containing phytonematicides are allelochemicals, with the potential challenge related to phytotoxicity in many crop species (Pelinganga and Mashela, 2012), with auto-allelopathy occurring within the same species (Mafeo and Mashela, 2010; Mafeo and Mphosi, 2012). Allelopathy from certain phytonematicides could reduce plant growth of different plant species by as high as 50% to complete crop failure (Mashela *et al*., 2015). Internationally, the use of agricultural inputs to protect crops against pests has zero tolerance towards phytotoxicity (EPPO, 2010).

1.1.4 Proposed solution

High incidence of phytotoxicity in nematode suppression with phytonematicides had been restricting the approval of phytonematicides in numerous countries. The effectiveness of phytonematicides depend on allelochemicals as active ingredients, which are phytotoxic in nature (Mashela *et al*., 2017). Plants respond to increasing concentrations of allelochemicals through density-dependent growth patterns, which have three phases, viz., stimulation, neutral and inhibition, with each phase having a range of concentrations (Salisbury and Ross, 1992). The stimulation phase of these materials is used to generate non-phytotoxic concentrations of phytonematicides on various commercial crop cultivars (Mafeo *et al*., 2011b; Pelinganga and Mashela, 2012). Curve-fitting allelochemical response dosage (CARD) model was adopted at the Green Biotechnologies Research Centre of Excellence (GBRCE) for development of phytonematicides. This computer-generated concept of mean concentration stimulation point (MCSP) is used to generate a non-phytotoxic concentration on various phytonematicides (Mashela *et al*., 2015; Sithole *et al*., 2016). This computer-based model has since been used to ameliorate the incidence of phytotoxicity on various crops and inconsistent results on nematode suppression.

1.1.5 General focus of the study

This study will provide both small- and large-scale farmers with a suitable pre- and post-emergent application concentration of Nemafric-BG phytonematicide, which will effectively suppress nematode without inducing phytotoxicity to plants. This research will lead to improved potato production in South Africa, with the use of phytonematicides for management of root-knot nematodes instead of using synthetic nematicides, which are environmentally unfriendly. The aim of this research study is to develop pre- and post-emergence application concentration of Nemafric-BG phytonematicide, which will not be phytotoxic to potato but suppress *Meloidogyne javanica* and stimulate potato growth, and this will result in the eventual registration of this product on Potato, for management of *Meloidogyne species* in accordance with Act No. 36 of 1947 as amended in 2012.

1.2 Problem statement

Allelochemicals are produced as secondary metabolites with no physiological roles in plants, they are compartmentalised to avoid toxicity to cells. Most allelochemicals affect biological systems through density-dependent growth (DDG) patterns (Liu *et al*., 2003), which have three phases, stimulation, neutral and inhibition phases. The stimulation phase of these materials is used to generate non-phytotoxic concentrations of phytonematicides on numerous commercial crop cultivars (Pelinganga and Mashela, 2012). The computer-generated concept of mean concentration stimulation point (MCSP) is used to generate a non-phytotoxic concentration of Nemafric-BG phytonematicide (Mashela *et al*., 2015; Sithole *et al*., 2016). Active ingredient of Nemafric-BG phytonematicide, cucurbitacin-B ($C_{32}H_{46}O_8$), is allelochemical compound (Chen *et al*., 2005), with the potential of inducing phytotoxicity on nematode-susceptible potato.

1.3 Rationale of the study

Potato plants are amongst the top commercial crops produced in South Africa (Pieterse and Theron, 2003), without genotypes resistant to root-knot nematodes (Berthou *et al*., 2003; García *et al*., 2014; Jones, 2006; Shabeg *et al*., 2016). Following the global withdrawal of methyl bromide from agro-chemical companies in 2005 (Collangea *et al*., 2011; Mashela *et al*., 2015), it would not be feasible to successfully produce potatoes without using alternative strategies to manage nematodes. However, due to the allelopathic nature of phytonematicides (Sithole *et al*., 2016), it would be prudent to develop pre- and post-emergent application concentration for Nemafric-BG phytonematicides on potato. Development of pre- and post-emergent application concentrations and overall sensitivity values for Nemafric-BG phytonematicide would allow for the eventual registration of this product on potato, for management of *Meloidogyne* species in accordance with Act No. 36 of 1947 as amended in 2012.

1.4 Purpose of the study

1.4.1 Aim

Development of pre- and post-emergent non-phytototoxic concentration of cucurbitacin-containing phytonematicide that would suppress *Meloidogyne* species.

1.4.2 Objective

To determine whether pre- and post-emergent applications of Nemafric-BG phytonematicide would have effects on growth of potato and suppression of *M. javanica* population densities.

1.4.3 Hypothesis

Pre- and post-emergent applications of Nemafric-BG phytonematicide would have effects on growth of potato and suppression of *M. javanica* population densities.

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

1.7 Scientific significance of the study

The study would provide both small- and large-scale potato farmers with a suitable application concentration for Nemafric-BG phytonematicide, which would effectively suppress nematode without inducing phytotoxicity to potato plants. The study would also provide information to improve potato production in South Africa, with the use of phytonematicides for managing population densities of root-knot nematodes instead of using synthetic nematicides, which were shown to be environment-unfriendly. The study intended to develop pre- and post-emergent application concentration of Nemafric-BG phytonematicide, which would not be phytotoxic to potato plants, but would suppress *M. javanica* population densities. Results would assist in the eventual registration process of the product for use on potato, for managing *Meloidogyne* species as promulgated in Act No. 36 of 1947 as amended in 2012.

1.8 Structure of the mini-dissertation

Following the description and detailed outlining of the research problem (Chapter 1), the work done on the research problem was outlined as Literature Review (Chapter 2). Then, each of the two components of the objective of the study constituted the subsequent chapter (Chapter 3), with the final chapter (Chapter 4), summarising the findings and their significance, potential future recommendations, along with the conclusions.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Potato (*Solanum tuberosum* L.) does not have resistant genotypes to root-knot (*Meloidogyne* species) nematodes (Jones, 2006) and this has resulted in development of management strategies for root-knot nematodes. The emphasis on crop improvement strategies has progressively been shifting from chemical to non-chemical approaches for sustainable agriculture (Mafeo and Mashela, 2009a, b; Mashela, 2002; Mashela *et al*., 2017). Following the withdrawal of highly effective synthetic nematicides from agro-chemical markets, the use of phytonematicides had been in the forefront as a management approach of choice in reducing nematodes population densities below injury level, because they are not toxic to human health and environment (Mashela *et al*., 2015). Small quantities (0.20 to 0.71 t/ha) of granular wild watermelon (*Cucumis africanus* L.) (Mabuka, 2013) and wild cucumber (*Cucumis myriocarpus* Naude.) (Mashela, 2002) fruits were successfully used to suppress the southern root-knot nematode (*Meloidogyne* species), with evidence of fertiliser effect on various crops.

In low input agricultural farming systems, farmers use organic amendments to manage root-knot nematodes and to provide essential nutrient elements (Stirling, 2014). Yet, the use of conventional organic amendments to suppress root-knot nematodes had many drawbacks (Mashela *et al*., 2017; Stirling, 2014), which includes large quantities of organic material, high transport costs to transport the organic material to the fields, longer periods to decompose, reduction of soil pH and inconsistent results in nematode suppression (Mashela, 2002). Ground leaching technology (GLT) system was introduced as one of the interventions to manage nematodes in crop production, in order to ameliorate drawbacks associated with application of conventional organic amendments. Briefly, the technology involves using small quantities (0.20-0.72 t/ha) of ground fruits of *C. myriocarpus* and *C. africanus* plants to suppress plant-parasitic nematodes (Mafeo and Mashela, 2009a, b; Mashela 2002; Mashela *et al*., 2015). However, the successful use of phytonematicides in many countries is restricted by their ability to induce phytotoxicity to crops protected against nematodes damage (Mashela *et al*., 2015). The review on the research problem in this study focused on three aspects: (i) what had already been done on the research problem, (ii) what had not yet been done on the research problem and (iii) addressing the identical gaps.

2.2 Work done on the problem statement

2.2.1 Pre-emergent application of phytonematicides

In this intervention, the phytonematicides were applied at planting and had been mainly Nemarioc-AG phytonematicide (Mafeo, 2012). Materials used in this intervention, were either mechanically mixed throughout the soil profile, applied in aqueous solutions or mixed with seeds or with fertilisers in granular form. Most non-fumigant nematicides are applied at planting or even at post-planting due to their limited phytotoxicities. Phytonematicides that inhibit germination would obviously not be suitable for use at planting. Inhibition of seed germination in response to allelochemicals released by phytonematicides produced from *C. africanus* and *C. myriocarpus*, have been consistently reported in literature (Mafeo, 2012; Mashela *et al*., 2017).

Yield loss in crop production, is proportional to the initial population densities (Pi) of nematodes (Seinhorst, 1967). Preferably, the use of phytonematicides in GLT systems should be as a pre-emergent phytonematicide in order to keep initial nematode population density at the lowest level possible. In vitro, seed germination tests suggested that at 5 g crude extracts of *C. myriocarpus* fruit (Nemarioc-AG phytonematicide) were highly phytotoxic to tomato, watermelon and butternut squash (Mafeo and Mashela, 2009a), maize, finger millet, sorghum and onion (Mafeo and Mashela, 2009b). Under greenhouse conditions, the material completely inhibited seedling emergence of all dicotyledonous crops tested (Mafeo and Mashela, 2010).

In the study conducted by Mafeo and Mashela (2009b), increasing concentrations of Nemarioc-AG phytonematicide explained 91%, 97% and 91% of the total treatment variation in inhibition of seed germination in tomato, watermelon and butternut squash, respectively. Results in that study suggested that Nemarioc-AG phytonematicide had allelopathic effect on seed germination of test plants and hence, the material was not suitable for use as a pre-emergent phytonematicide. However, validation studies were initiated using the Curve-fitting Allelochemical Response Dosage (CARD) computer model (Liu *et al*., 2003) to determine concentrations where Nemarioc-AG phytonematicide stimulated, had no effect and inhibited germination of various crops in order to establish the pre-emergent application concentration for Nemarioc-AG phytonematicide that will not be phytotoxic to the test plants, while suppressing nematode population densities (Mafeo and Mashela, 2010; Mafeo *et al*., 2010).

2.2.2 Post-emergent application of phytonematicides

Mashela (2002) introduced the ground leaching technology (GLT) system as one of the post-emergent mediations to manage root-knot (*Meloidogyne* species) nematodes in crop production, with the view of overcoming drawbacks related to application of conventional organic amendments. Briefly, the technology involves using small quantities (0.20-0.72 t/ha) of powdered organs from selected plants to suppress plant-parasitic nematodes. In this intervention, the materials were applied after emergence of the crops protected against nematodes and had been mainly Nemarioc-AG/AL and Nemafric-BG/BL phytonematicides (Mashela *et al.*, 2017).

In the GLT system ground fruits of *C. myriocarpus* Naude. and *C. africanus* L. consistently suppressed nematode (*Meloidogyne* species) population densities in greenhouse trials by over 90% (Mashela, 2002), in micro-plot trials by over 90% (Mofokeng *et al*., 2004; Shakwane *et al*., 2004) and in field trials by over 80% (Mashela *et al*., 2011), the product improved tomato fruit yield and plant growth (Mashela, 2007; 2002; Mashela *et al*., 2008; Mphosi, 2004). Pelinganga *et al*. (2011) observed similar results when using fruits of *C. myriocarpus* and *C. africanus* in the fermented crude extract technology. The two *Cucumis* products reduced nematode population densities by over 69% and by over 89%, respectively, through botinemagation (Pelinganga *et al*., 2011). Sithole (2016), also observed similar results when using Nemarioc-AL and Nemafric-BG phytonematicides, the product reduced nematode numbers by over 90%. However, the products inhibited plant growth. The use of granular *C. africanus* fruit (Nemafric-BG phytonematicide) for suppression of plant-parasitic nematodes is successful on *Meloidogyne species* in crop production under diverse conditions (Mashela *et al*., 2011). Nemafric-BG phytonematicide reduced nematodes in roots by 80%, but increases juveniles in soil by 178%, however plant growth was inhibited when used as post-emergent phytonematicide on citrus plants under greenhouse conditions, whereas Nemafric-BL phytonematicide did not have any effect on plant growth and nematode suppression on citrus plants under greenhouse condition (Maile, 2013). Regardless of the organic amendment source, when used as post-emergent phytonematicide, the material had fertiliser effect and had no side effect on soil pH, with the exception of Fever tea (*Lippia javanica*) leaves, which reduced soil pH (Mashela *et al*., 2010). In contrast, relative to untreated control, Nemarioc-AG and Nemafric-BG phytonematicides drastically reduced numerous plant variables, which suggested that the products might be phytotoxic to crops protected against nematode damage (Mabuka, 2013; Mashela *et al*., 2015).

Researchers at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo adopted the use of computer-based Curve-fitting Allelochemical Response Data (CARD) model (Liu *et al*., 2003) to develop the mean concentration stimulation point (MCSP) in order to overcome phytotoxicity in phytonematicide. The CARD model quantifies the three DDG patterns using biological indices (Liu *et al*., 2003). The biological indices include: (a) threshold stimulation (D_m) the allelochemical concentration where stimulation phase begin, (b) saturation point (R_h) the concentration at which stimulation ends or where the neutral phase start, (c) 0% inhibition (D_0) the concentration at which neutral phase ends, (d) 50% inhibition (D_{50}) the concentration at half the distance of the inhibition phase, (e) 100% inhibition (D_{100}) the concentration that terminates the inhibition phase. In the development of MCSP, two biological indices, D_m and R_h, are used through the relation: MCSP = $D_m + (R_h/2)$, which is the concentration at which a given phytonematicide would not be phytotoxic to the crop being protected against nematode damage, while suppressing nematode population densities (Mashela *et al*., 2015). Along with the biological indices, the CARD model provide the sensitivity index (k), which provides information of the sensitivity of the crop to the product being used to protect it against nematodes. Generally, the lower the k value, the higher is the sensitivity of the plant to the material (Liu *et al*., 2003). Pelinganga *et al*. (2012) reported k values which ranged from 0 to 1 for tomato plant variables when exposed to Nemafric-AL phytonematicide. Tseke *et al*. (2013) observed k values of 2, 1, 0 and 2 for dry root mass, dry shoot mass, plant height and stem diameter for tomato plant, respectively, when using Nemarioc-AL phytonematicide on tomato plants.

2.3 Work not yet done on the problem statement

2.3.1 Pre-emergent application of Nemafric-BG phytonematicide

The MCSP is phytonematicide specific, plant species-specific and application time-specific. Therefore, the MCSP value of Nemafric-BG phytonematicide on potato cv. 'Mondial G3' had to be empirically developed if the product had to be successfully used in nematode suppression in potato production as a pre-emergent phytonematicide.

2.3.2 Post-emergent application of Nemafric-BG phytonematicide

The degree of phytotoxicity of Nemafric-BG phytonematicide on potato cv. 'Mondial G3' remains undocumented. Due to the economic attributes and lack of nematode resistant genotypes of potato, MCSP values for Nemafric-BG phytonematicide would be established for post-emergent application. In order to successfully investigate whether Nemafric-BG phytonematicide would be useful as post-emergent phytonematicide in management of root-knot nematode in potato production, a series of experiments would be conducted to determine the appropriate MCSP values for post-emergent application on this crop.

2.4 Addressing the identified gaps

Generally, biological systems respond to extrinsic or intrinsic factors in accordance to the density-dependent growth pattern, which is characterised by specific concentration for stimulation, inhibition and saturation of growth (Mashela *et al*., 2015). In order to successfully investigate whether Nemafric-BG phytonematicide could be used as pre- and post-emergent phytonematicide, a series of experiments need to be conducted, to determine the appropriate concentrations of Nemafric-BG phytonematicide for various crops in relation to density dependent growth pattern responses. In order to determine the application concentrations, one has to establish the position where the normal concentration used in GLT for suppression of *M. javanica* stood in relation to DDG responses (Salisbury and Ross, 1992), which required the use of computer based- CARD model (Liu *et al*., 2003).

CHAPTER 3

EFFECTS OF PRE- AND POST-EMERGENT APPLICATION OF NEMAFRIC-BG PHYTONEMATICIDE ON GROWTH OF POTATO CULTIVAR 'MONDIAL G3' AND NEMATODE SUPPRESSION

3.1 Introduction

Potato (*Solanum tuberosum* L.) does not have known genotypes that are resistant to root- knot (*Meloidogyne* species) nematodes (Berthou *et al*., 2003; Jones, 2006; García *et al*., 2014). Root-knot nematodes are a serious problem in potato production, infestations may result in unusable tubers, in addition to causing the plant to wilt, grow stunted or yellow (García *et al*., 2014). In South Africa, potato production losses due to plant parasitic nematode species in 1989 were estimated to be 16.7%, accounting for $7 Million annually (Keetch, 1989; Onkendi *et al*., 2014). In the recent past, synthetic nematicides such as aldicarb and nemacur were used to manage *Meloidogyne* species in potato production. However, due to their toxicity and adverse effects on the environment, a large number of synthetic nematicides have been withdrawn from the agrochemical market (Collangea *et al*., 2011; Mashela *et al*., 2015). This had since propelled the use of botanicals such as cucurbitacin-containing phytonematicides to the forefront as important nematode management strategy.

Due to the presence of allelochemicals in phytonematicides, the successful use of cucurbitacin-containing phytonematicides in managing nematode population densities could be hindered by their high level of phytotoxicity (Mashela *et al*., 2015). A computer based Curve-fitting Allelochemical Response Data (CARD) model (Liu *et al*., 2003) has been adopted to develop non-phytotoxic concentration on various phytonematicides (Mashela *et al*., 2015). The objective of this study was to determine whether pre- and post-emergent applications of Nemafric-BG phytonematicide would have effects on growth of potato and suppression of *M. javanica* population densities.

3.2 Materials and methods

3.2.1 Description of the study area

The study was conducted under greenhouse conditions at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, South Africa (23°53'24.6"S, 29°44'33.4"E). The available greenhouse was 100 m × 20 m in size, with thermostatically activated fans on one end and the wet wall on the other end, for moderating inside temperatures. In summer, the greenhouse maximum/minimum temperatures averaged 28/21°C, whereas in winter the minimum/maximum temperatures averaged 5/18°C. The parallel trials (Trial 1: Pre-emergent, Trial 2: Post-emergent) were conducted in autumn (March-May: Experiment 1) 2017 and repeated in 2018 (March-May: Experiment 2).

3.2.2 Treatments and research design

Treatments, comprising 0, 2, 4, 8 and 16 g Nemafric-BG phytonematicide, were arranged in randomised complete block design, with seven replicates.

3.2.3 Procedures

Nemafric-BG phytonematicide was prepared using the locally developed method (Mashela *et al*., 2015). Briefly, the method comprised of cutting fruits of *C. africanus* into pieces, drying them at 52°C and ground into granules. Experiments were established by placing 20-cm-diameter plastic pots on greenhouse benches at 0.3 m × 0.3 m spacing, each filled with 2 700 ml steam-pasteurised (300°C for 1 h) sandy loam soil and Hygromix (Hygrotech, Pretoria North) at 3:1 (v/v) ratio.

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 trial, similar treatments were applied on the surface of the growing medium after 100% emergence of the sprouted tubers and slightly covered with the growing medium.

Meloidogyne javanica inocula were prepared by extracting eggs and second-stage juveniles (J2) from roots of greenhouse-grown nematode-susceptible tomato cultivar 'Floradade' in 1% NaOCl solution (Hussey and Barker, 1973). Two weeks after 100% emergence, each seedling was inoculated with 3 000 *M. javanica* eggs and J2 using a 20-ml plastic syringe by placing inocula into 3-cm-deep holes on the cardinal points of plants. After inoculation, plants were fertilised with 2.5 g N:P:K 2:3:2 (22) per plant, which provided a total of 155 mg N, 105 mg P and 130 mg K per ml of water. Multifeed 2:1:2 (43) was applied per plant to provide a total of 0.175 mg N, 0.16 mg K and 0.16 mg P, 0.45 mg 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 tap water (Mashela, 2002). Each plant was irrigated with 250 ml chlorine-free tap water every other day. Weekly sprays for disease management comprised alternating Mycoguard, Bravo, Funginex and Dithane M45, whereas insect pests were scouted and monitored on daily basis and controlled when populations go beyond 10 insects per plant.

Figure 3.1 Effects of Nemafric-BG phytonematicide on growth of potato cv. 'Mondial G3' and suppression of *Meloidogyne javanica* under greenhouse conditions.

3.2.4 Data collection

At 56 days after initiation of treatments, plant height was measured from the crown to the tip of the plant, with chlorophyll content on three matured leaves per plant measured using chlorophyll meter (Minolta Spad-502). Shoots were separated from the roots and oven dried at 70°C for 72 hours and weighed. For nutrient content analysis in leaf tissues, a microwave digester (PerkinElmer, Tatan MPS) was used for the preparation and approximately 0.4 g ground leaf materials of potato cv. Mondial G3 plant were digested in 75 ml vessel with 5 ml of 70% nitric acid (HNO_3) and 3 ml of 30% hydrogen peroxide (H_2O_2) (Campbell and Plank, 1998). The mixture was vortexed and samples allowed to cool for at least 10 minutes prior to closing the vessel, which were then inserted into the microwave digester 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. 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. Potato leaf samples were then analysed for K, Fe, Zn and Na using inductively coupled plasma spectrometry (Shimadzu, ICPE-9000).

Root systems were removed from shoots, immersed in water to remove soil particles, blotted dry and weighed to facilitate the calculation of nematode population densities per total roots per plant. Root galls were assessed using the North Carolina Differential Rating Scale of 1 = 0 galls, 2 = 1-10 galls, 3 = 11-31 galls, 4 = 32-100 galls, 5 = >100 galls (Taylor and Sasser, 1978). Eggs and J2 were extracted from total root system per plant using maceration and blending method for 60 s in 1% NaOCl solutions (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. Soil per pot was thoroughly mixed and a 250 ml soil sample was collected, with nematodes extracted using the sugar-floatation and centrifugation method (Jenkins, 1964). Eggs and J2 from root samples and J2 from soil samples were counted from a 5 ml aliquot of each sample with the use of a stereomicroscope (Carl Zeiss Microscopy GmbH, Jana). The J2 from soil were converted to 2 700 ml soil per pot and used to determine the final nematode population densities (Pf). The latter was used to compute the reproductive factor (RP = Pf/Pi), which is a proportion of Pf and the initial nematode population densities (Pi).

3.2.5 Data analysis

Plant variables were subjected to Curve-fitting Allelochemical Response Data (CARD) model to generate appropriate biological indices (Liu *et al*., 2003), which allowed for the calculation of Mean Concentration Stimulation Point (MCSP), sensitivity values and overall values ($\sum k$) (Mashela *et al*., 2017). whereas nutrient elements (Fe, K, Na and Zn) and nematode variables were assessed using analysis of variance, with data subjected to lines of the best fit. Unless otherwise stated, treatments discussed were significant at the probability level of 5%.

3.3 Results

3.3.1 Pre-emergent application effects

Curve-fitting Allelochemical Response Data

Plant height and fresh root mass had density-dependent growth (DDG) patterns with increasing concentrations of Nemafric-BG phytonematicide in both experiments (Figure 3.2). The respective biological indices for the two plant variables as produced by the CARD model for each of the two curves in both Experiment 1 and 2, were summarised (Table 3.1). In Experiment 1 and 2, plant height and fresh root mass over increasing concentrations of Nemafric-BG phytonematicide exhibited quadratic relations (Figure 3.3). In Experiment 1, the model explained the relationships by 98 and 99% in plant height and fresh root mass, respectively (Table 3.1), whereas in Experiment 2 the model explained the relationships by 99% in both respective variables (Table 3.1). In both experiments, increasing concentrations of Nemafric-BG phytonematicide did not have significant effects on stem diameter, dry shoot mass, chlorophyll content and gall rating. In Experiment 1, using the relation MCSP = D_m + (R_h/2), MCSP for pre-emergent application was equal to 24.18 g (Table 3.1), whereas in Experiment 2 MCSP was equal to 7.82 g (Table 3.1).

Sensitivity

In Experiment 1, plant height and fresh root mass had sensitivity (k) values of 5 and 15, respectively, with the overall sensitivity (Σk) of potato cv. 'Mondial G3' being equivalent to 20 (Table 3.1). Whereas in Experiment 2, plant height and fresh root mass had k values of 4 and 2, respectively, with the Σk being equivalent to 6 (Table 3.1).

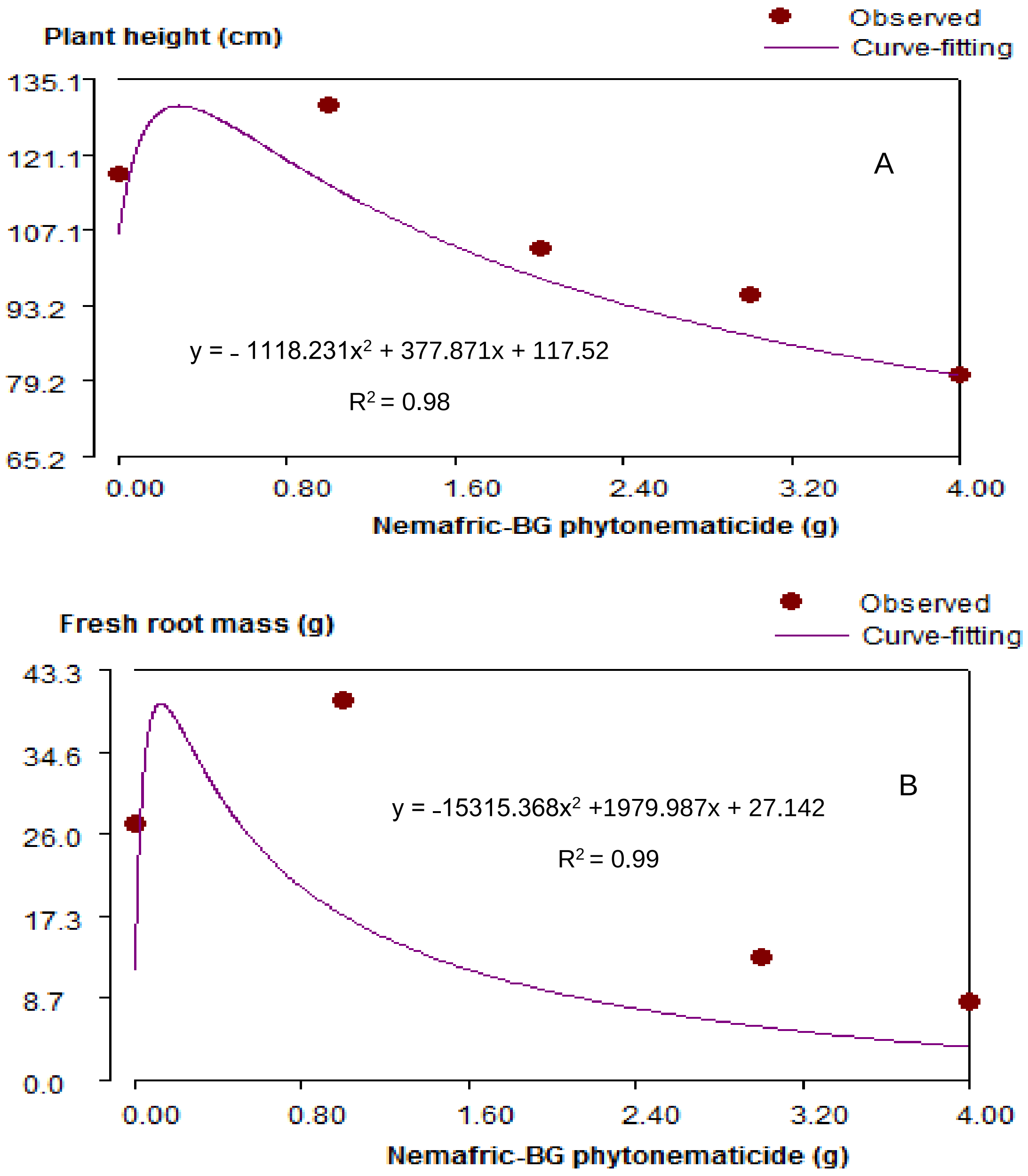

Figure 3.2 Responses of plant height (A) and fresh root mass (B) of potato cv. 'Mondial G3' to increasing pre-emergent application concentrations of Nemafric-BG phytonematicide in Experiment 1 at 56 days after initiation of treatments.

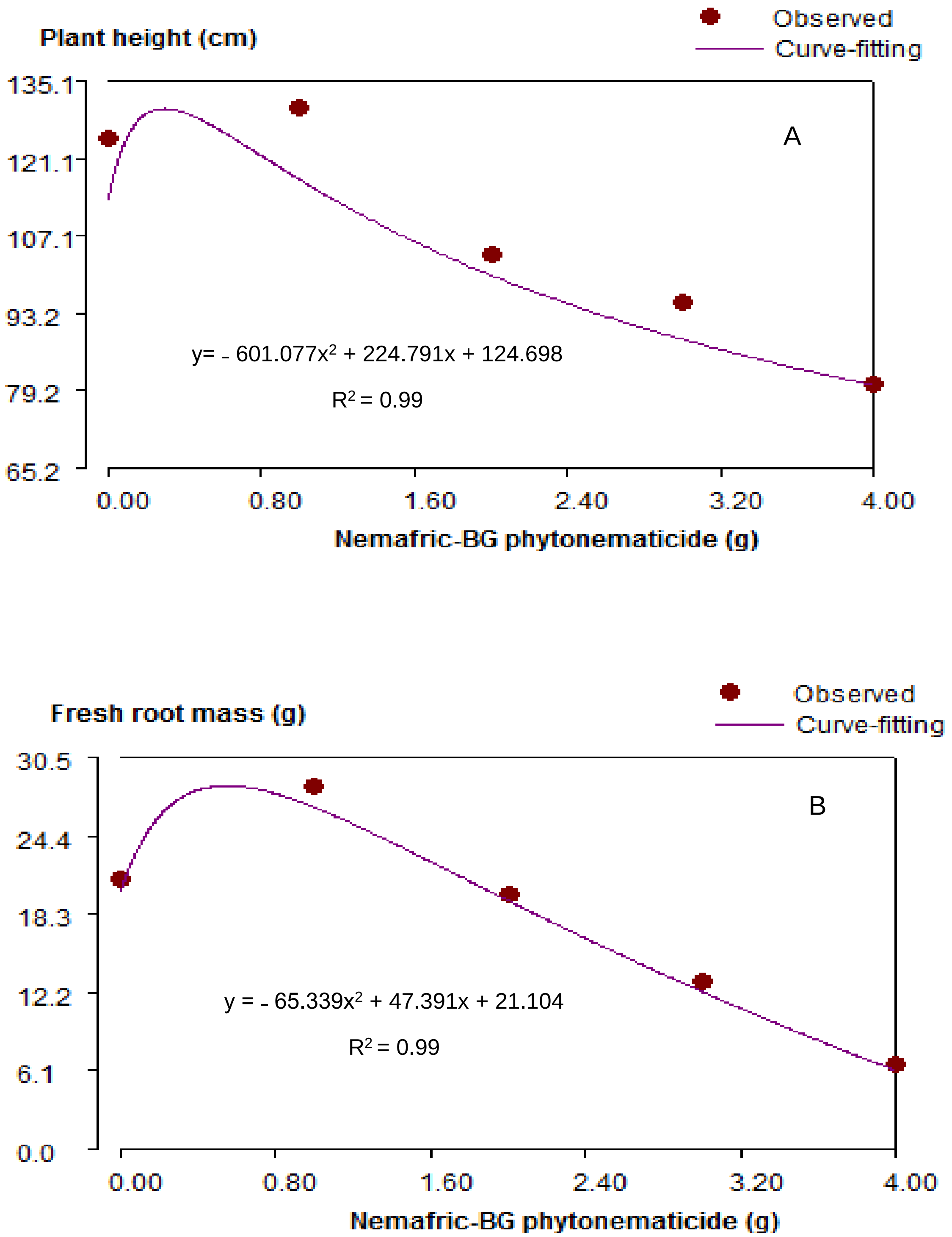

Figure 3.3 Responses of plant height (A) and fresh root mass (B) of potato cv. 'Mondial G3' to increasing pre-emergent application concentrations of Nemafric-BG phytonematicide in Experiment 2 at 56 days after initiation of treatments.

Table 3.1 Biological indices for plant height (PHT) and fresh root mass (FRM) of potato cultivar 'Mondial G3' exposed to increasing pre-emergent application concentrations of Nemafric-BG phytonematicide.

Experiment 1			
Biological indices	PHT (cm)	FRM (g)	Mean
Threshold stimulation(D_m)	0.285	0.124	0.2045
Saturation point (R_h)	31.922	63.994	47.958
0% inhibition(D_0)	1.452	1.577	1.5145
50% inhibition (D_{50})	8.286	2.915	5.6005
100% inhibition (D_{100})	148.7	7.1	77.9
R^2	0.98	0.99	
Sensitivity (k)	5	15	
Overall sensitivity (∑k) = 20			
MCSP = D_m + (R_h/2) = 0.2045 + (47.958/2) = 24.18 g			
Experiment 2			
Threshold stimulation(D_m)	0.292	0.548	0.4185
Saturation point (R_h)	21.017	8.593	14.805
0% inhibition(D_0)	1.171	1.902	1.5365
50% inhibition (D_{50})	6.794	3.343	5.0685
100% inhibition (D_{100})	64.4	5.2	34.8
R^2	0.99	0.99	
Sensitivity (k)	4	2	
Overall sensitivity (∑k) = 6			
MCSP = D_m + (R_h/2) = 0.4185 + (14.805/2) = 7.82 g			

Nutrient element variables

In Experiment 1, Iron (Fe) content in leaf tissues of potato cv. 'Mondial G3' with increasing concentrations of Nemafric-BG phytonematicide exhibited negative quadratic relation, with the model explained by 87% association (Figure 3.4). Using $X = -b1/2b2$ relation (Gomez and Gomez, 1984), Fe in leaf tissues was optimised at 9.08 g concentration of Nemafric-BG phytonematicide (Table 3.2). In contrast, K, Na and Zn each with increasing Nemafric-BG phytonematicide concentrations exhibited positive quadratic relations, with the models explained by 96 90 and 83% associations, respectively (Figure 3.4). K, Na and Zn were optimised at 7.84, 7.89 and 2.28 g concentration of Nemafric-BG phytonematicide, respectively in Experiment 1 (Table 3.5). Whereas in Experiment 2, K, Na and Zn contents each in leaf tissues of potato cv. 'Mondial G3' against the increasing concentrations of Nemafric-BG phytonematicide exhibited negative quadratic relations (Figure 3.5). The quadratic models for the three respective nutrient elements with increasing Nemafric-BG phytonematicide concentrations were explained by 99, 90 and 97% associations. Using the relation $X = -b1/2b2$, K, Na and Zn in leaf tissues were optimised at 6.34, 9.11 and 7.13 g concentrations of Nemafric-BG phytonematicide (Table 3.2). In contrast, Fe with increasing concentrations of Nemafric-BG phytonematicide exhibited positive quadratic relation, with the model explained by 88% association. Fe was optimised at 0.03 g concentration of Nemafric-BG phytonematicide.

Table 3.2 Optimisation model of selected nutrient elements in leaf tissues of potato cv. 'Mondial G3' as affected by pre-emergent application concentrations of Nemafric-BG phytonematicide.

Element	Model	R^2	x (g)
	Experiment 1		
Fe	$Y = 11.914x^2 – 216.39x + 1222$	0.87	9.08
K	$Y = - 16.897x^2 + 264.81x +20519$	0.96	7.84
Na	$Y = - 2.7216x^2 + 42.954x + 1113.7$	0.90	7.89
Zn	$Y = - 1.52x^2 + 6.9221x + 88.26$	0.83	2.28
	Experiment 2		
Fe	$Y = –7.4185x^2 + 128.33x + 559.77$	0.88	0.03
K	$Y = 19.754x^2 – 250.52x + 21252$	0.99	6.34
Na	$Y = 3.7463x^2 – 68.284x + 1328.4$	0.90	9.11
Zn	$Y = 0.1428x^2 – 2.0315x + 93.053$	0.97	7.13

x = −b1/2b2.

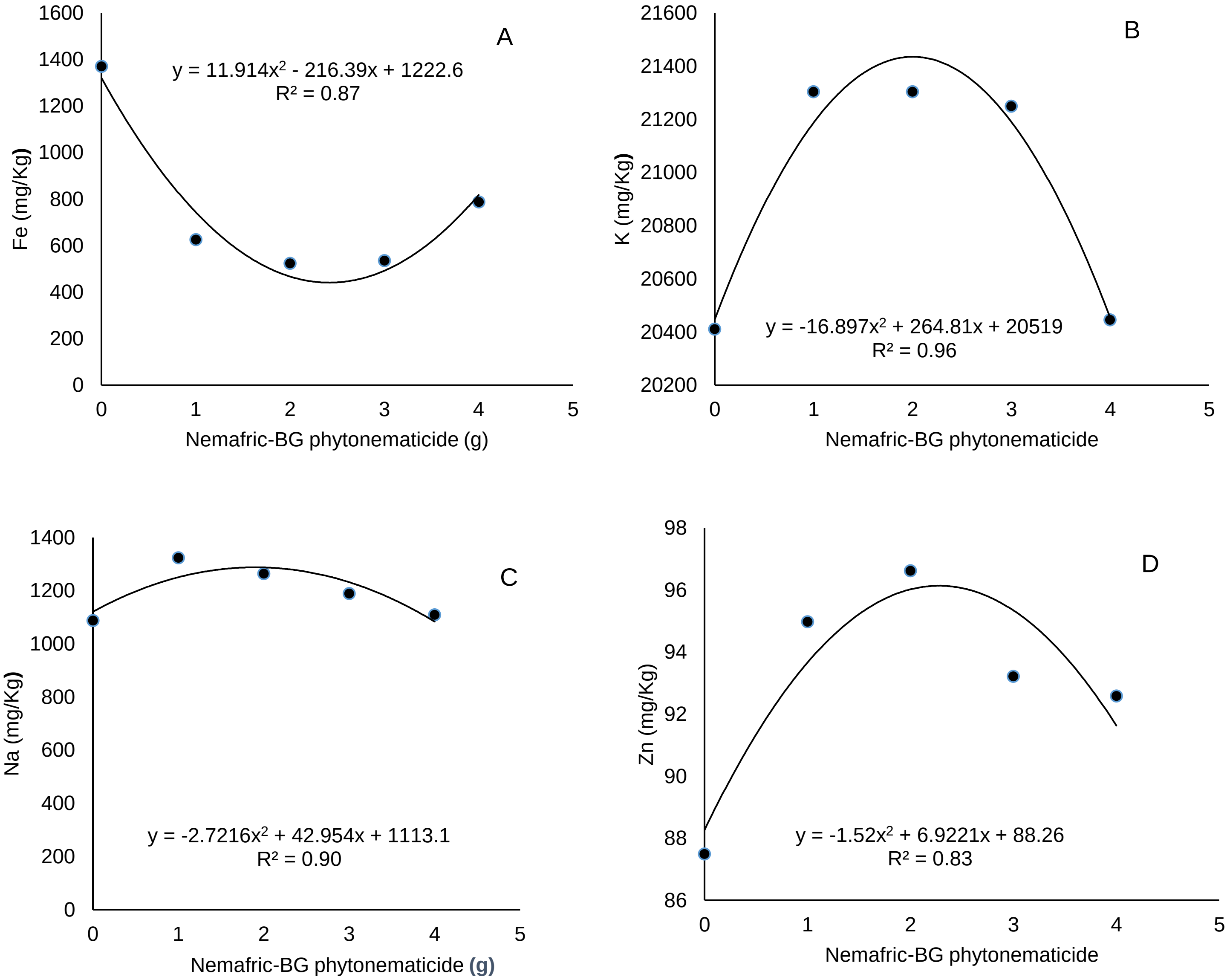

Figure 3.4 Response of Fe(A), K (B), Na (C) and Zn (D) in leaf tissues of potato cv. 'Mondial G3' to increasing pre-emergent application concentrations of Nemafric-BG phytonematicide in Experiment 1 at 56 days after initiation of treatments

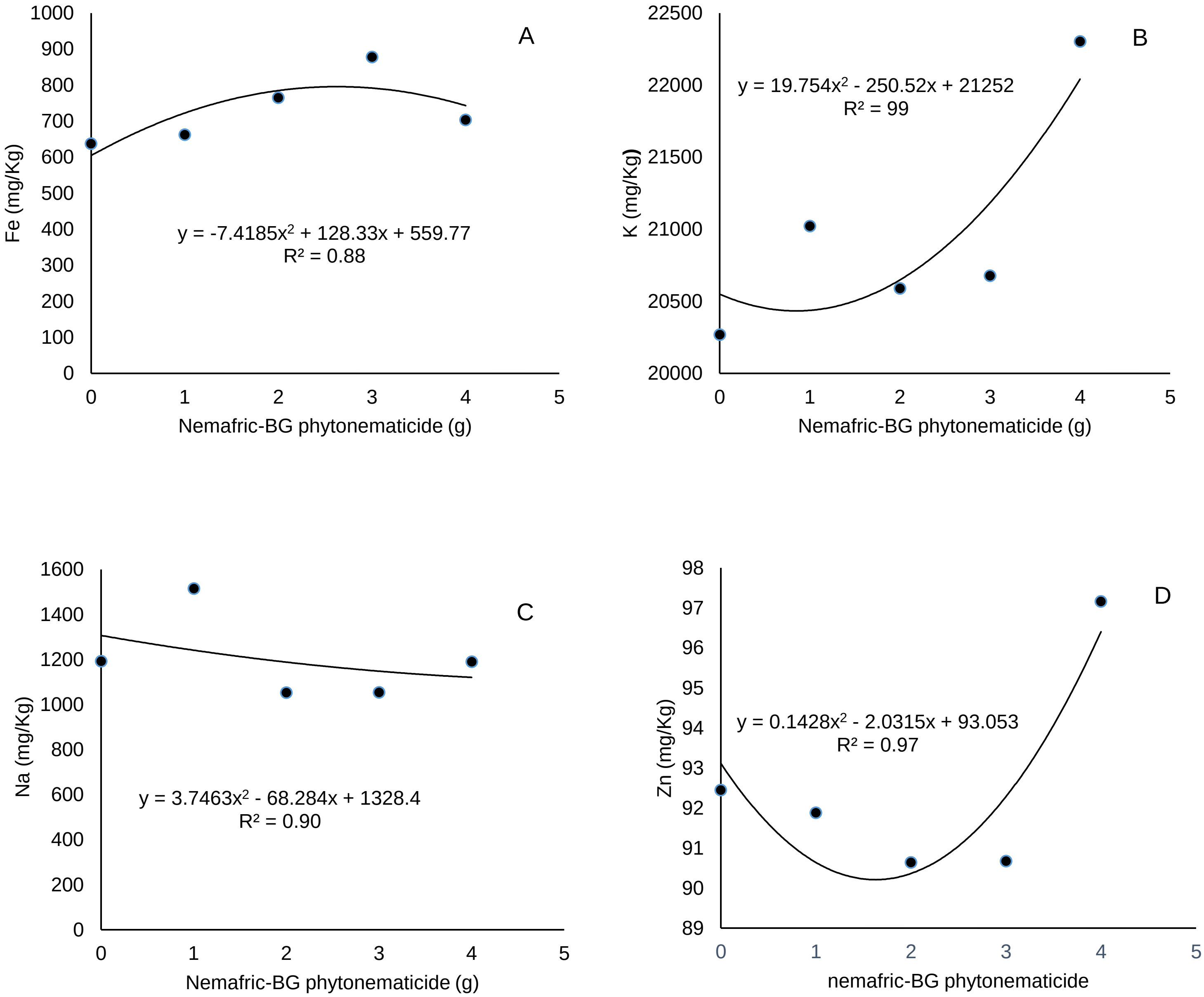

Figure 3.5 Response of Fe (A), K (B), Na (C) and Zn (D) in leaf tissues of potato cv. 'Mondial G3' to increasing pre-emergent application concentrations of Nemafric-BG phytonematicide in Experiment 2 at 56 days after initiation of treatments.

Nematode variables

In Experiment 1 and Experiment 2, eggs in roots, J2 in roots, J2 in soil and Pf of *M. javanica* against increasing pre-emergent application concentrations of Nemafric-BG phytonematicide exhibited negative quadratic relations (Figure 3.6-3.8). The quadratic models for the four respective nematode variables with increasing concentrations of Nemafric-BG phytonematicide were explained by 78, 85, 97 and 78% associations in Experiment 1 (Figure 3.6-3.7). Whereas in Experiment 2, the model explained the relations by 82, 99, 84 and 85%, respectively (Figure 3.8).

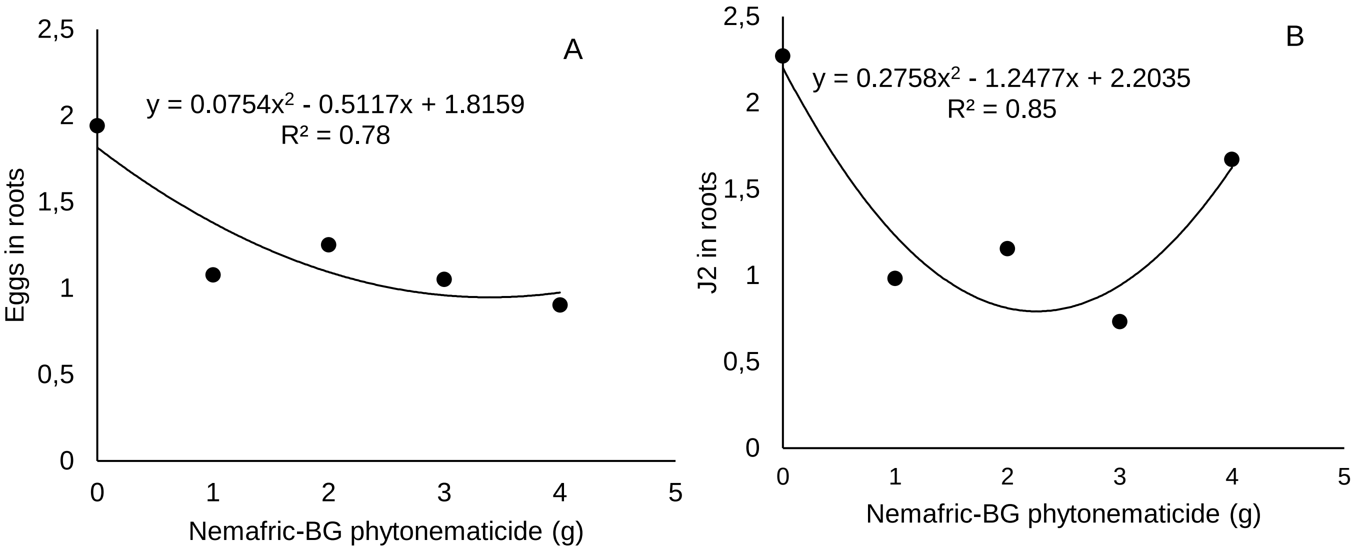

Figure 3.6 Response of *Meloidogyne javanica* eggs in roots (A) and second stage juveniles (J2) in roots (B) to increasing pre-emergent application concentrations of Nemafric-BG phytonematicide in Experiment 1 at 56 days after initiation of treatments.

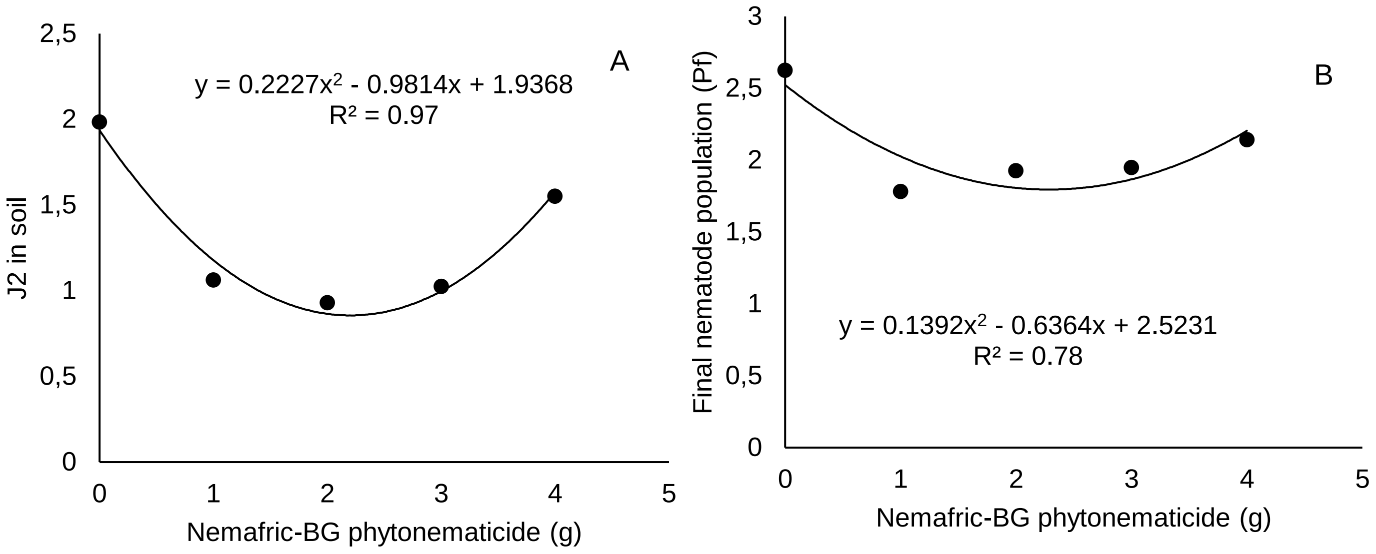

Figure 3.7 Response of *Meloidogyne javanica* second stage-juveniles (J2) in soil (A) and final nematode population (Pf) (B) to increasing pre-emergent application concentrations of Nemafric-BG phytonematicide in Experiment 1 at 56 days after initiation of treatments.

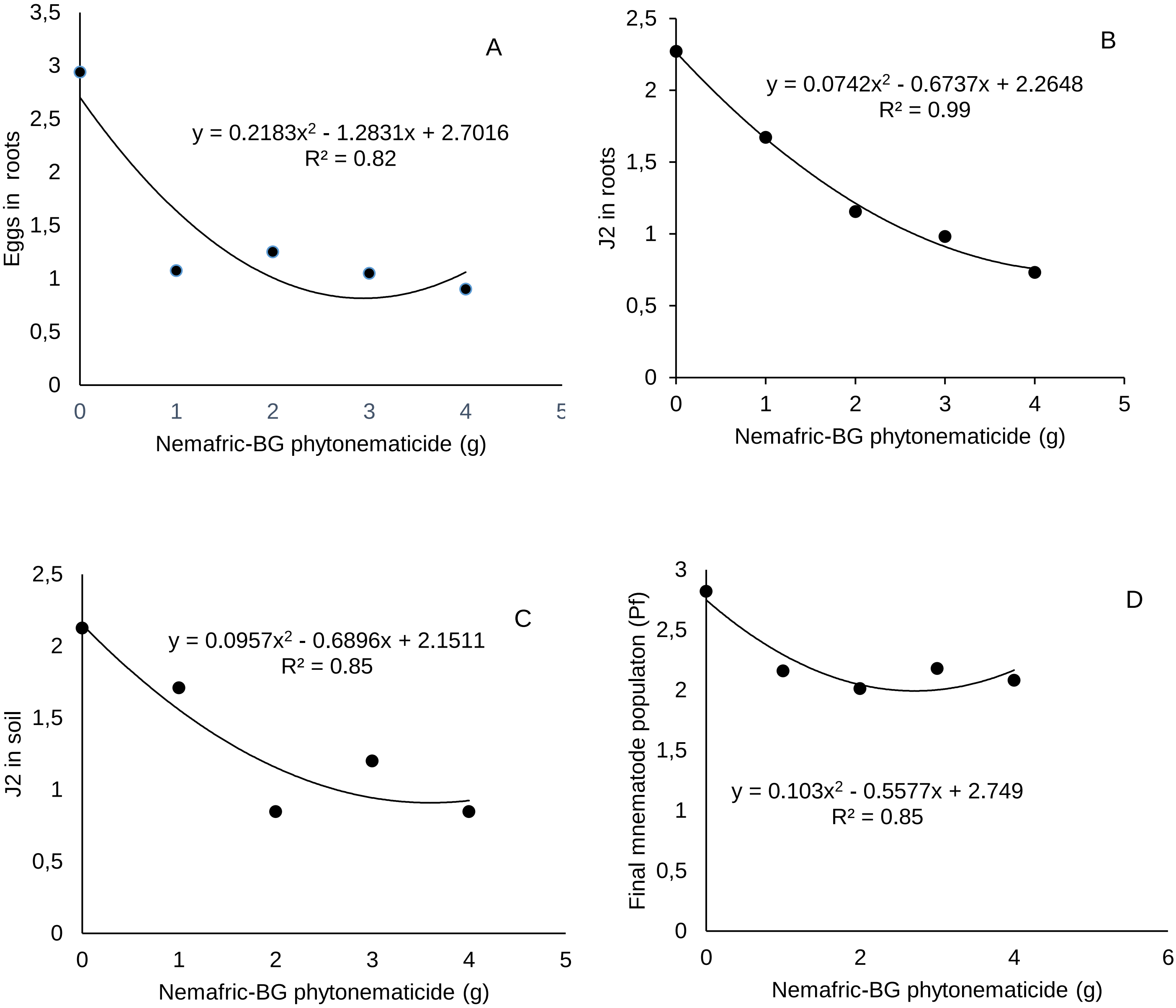

Figure 3.8 Response of *Meloidogyne javanica* eggs in roots (A), second stage-juveniles (J2) in roots (B), second-stage juveniles (J2) in soil (C) and final nematode population (Pf) (D) to increasing pre-emergent application concentrations of Nemafric-BG phytonematicide in Experiment 2 at 56 days after initiation of treatments.

3.3.2 Post-emergent application effects

Curve-fitting allelochemical response data

Plant height, fresh root mass and dry shoot mass of potato cv. 'Mondial G3' had density-dependent growth (DDG) patterns with increasing concentrations of Nemafric-BG phytonematicide in both experiments (Figure 3.9-3.10). The respective biological indices for the two plant variables as produced by the CARD model for each of the two curves in both Experiment 1 and 2, were summarised (Table 3.3). In Experiment 1, treatments exhibited quadratic relations on plant height, fresh root mass and dry shoot mass (Figure 3.9), with the model explaining the relationship by 97, 99 and 98%, respectively (Table 3.3), whereas in Experiment 2, increasing concentrations of Nemafric-BG phytonematicide exhibited strong positive quadratic relations on plant height, fresh root mass and dry shoot mass (Figure 3.10), with the model explaining the relationship by 99,96 and 99%, respectively (Table 3.3). In both experiments, increasing concentrations of Nemafric-BG phytonematicide did not have significant effects on stem diameter, chlorophyll content and gall rating. In Experiment 1, using the relation MCSP = D_m + (R_h/2) relation, MCSP for post-emergent application was derived at 9.87 g, whereas in Experiment 2, MCSP was equal to 12.10 g (Table 3.3).

Sensitivity

In Experiment 1, when exposing potato plants to increasing concentrations of Nemafric-BG phytonematicide, plant height, fresh root mass and dry shoot mass had sensitivity values (k) values of 2, 8 and 1, respectively, with overall sensitivity (Σ_k) of potato cv. 'Mondial G3' being equivalent to 11 units (Table 3.3). In Experiment 2, plant height, fresh root mass and dry shoot mass had k values of 5, 0 and 1, respectively, with the Σk being equivalent to 6 units (Table 3.3).

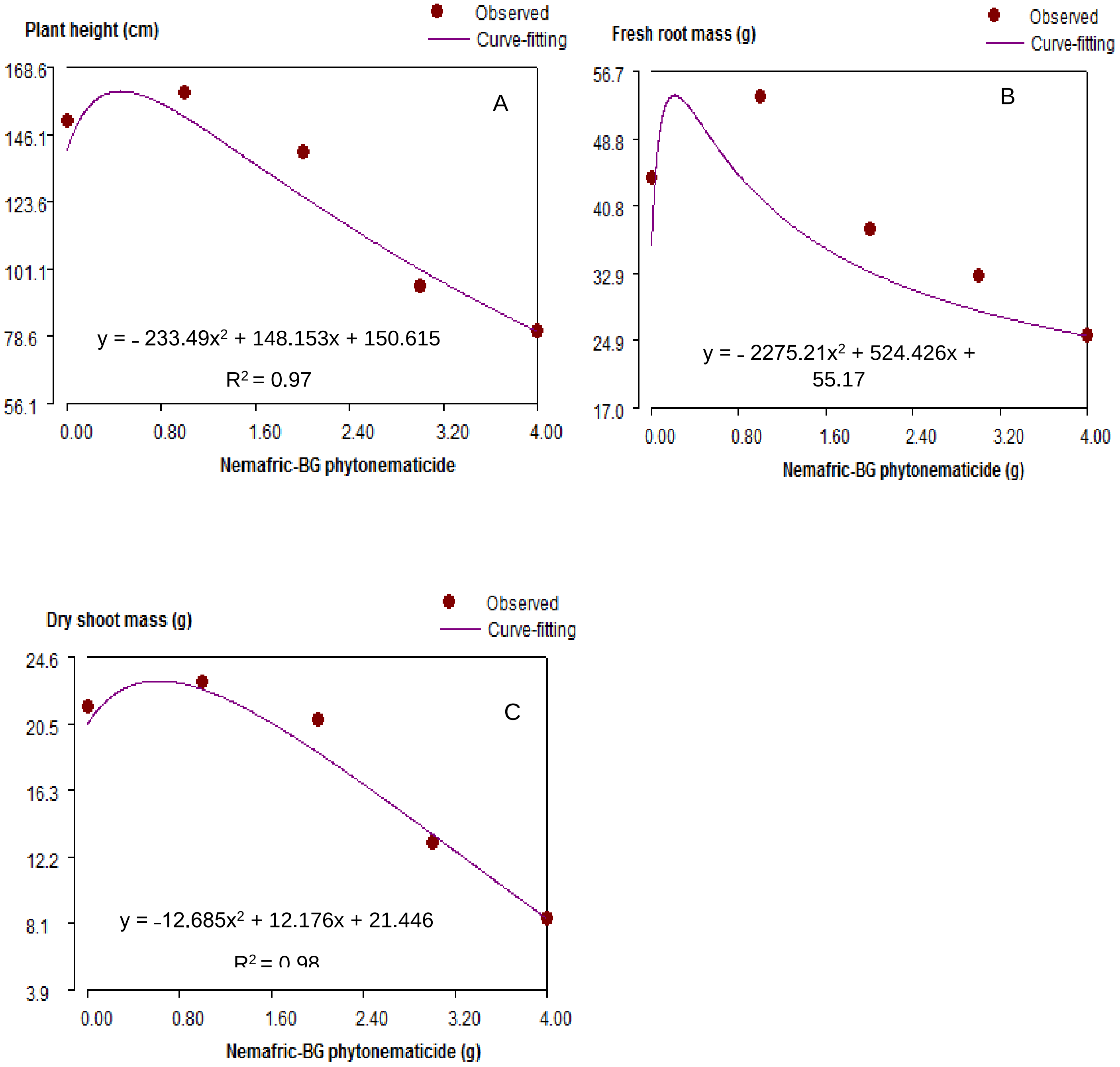

Figure 3.9 Responses of plant height (A), fresh root mass (B) and dry shoot mass (C) of potato cv 'Mondial G3' to increasing post-emergent application concentrations of Nemafric-BG phytonematicide in Experiment 1 at 56 days after initiation of treatments.

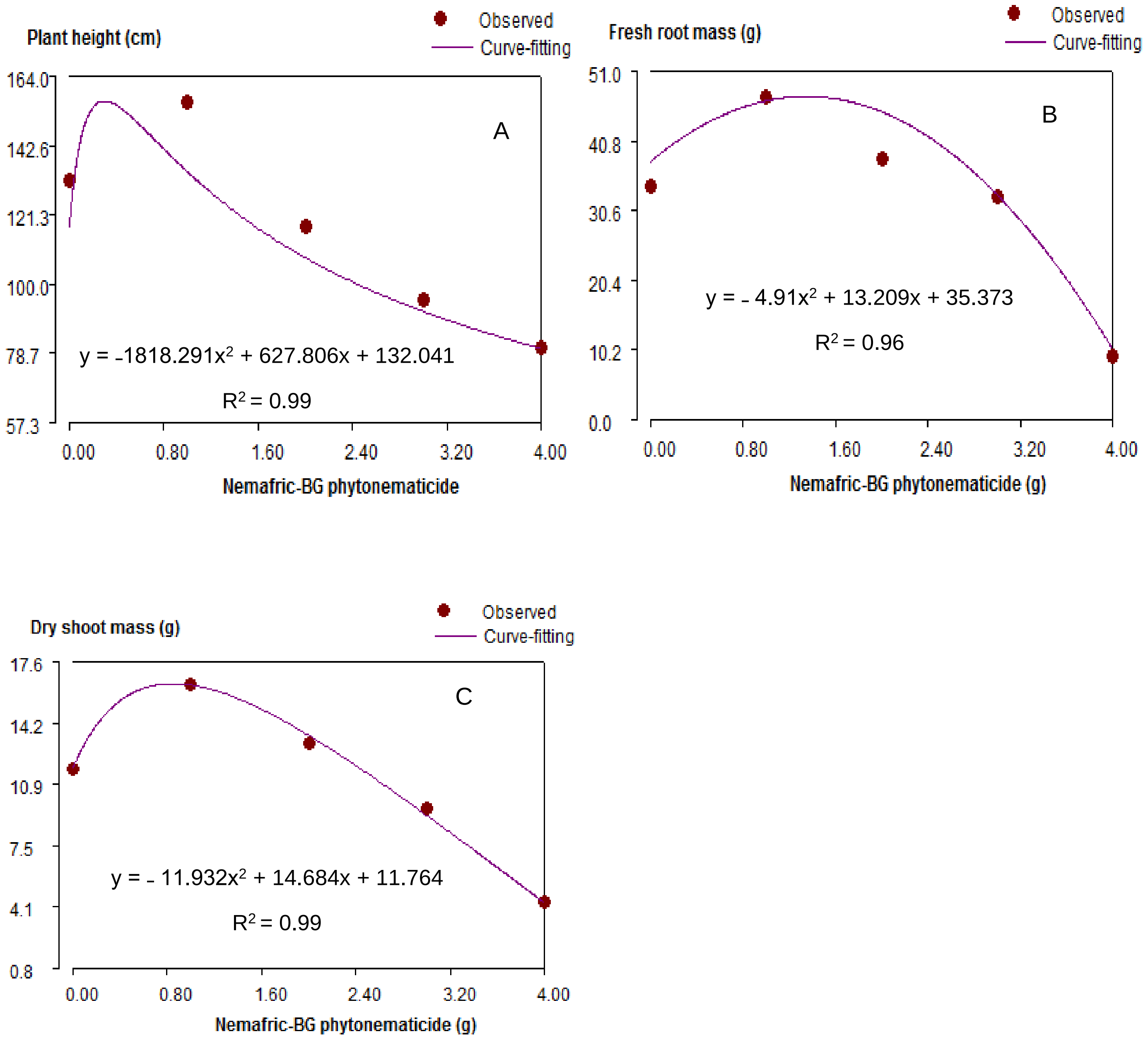

Figure 3.10 Responses of plant height (A), fresh root mass (B) and dry shoot mass (C) of potato cv 'Mondial G3' to increasing post-emergent application concentrations of Nemafric-BG phytonematicide in Experiment 2 at 56 days after initiation of treatments.

Table 3.3 Biological indices for plant height (PHT), fresh root mass (FRM) and dry shoot mass (DSM) of potato cultivar 'Mondial G3' exposed to increasing post-emergent application concentrations of Nemafric-BG phytonematicide

Experiment 1				
Biological indices	PHT (cm)	FRM (g)	DSM (g)	Mean
Threshold stimulation(D_m)	0.453	0.209	0.616	0.426
Saturation point (R_h)	23.501	30.219	2.922	18.881
0% inhibition(D_0)	1.426	1.56	1.611	1.532
50% inhibition (D_{50})	4.115	5.188	3.559	4.287
100% inhibition (D_{100})	8.6	42.7	5.5	18.933
R^2	0.97	0.99	0.98	
Sensitivity (k)	2	8	1	
Overall sensitivity (∑k) = 11				
MCSP = $D_m + (R_h/2) = 0.426 + (18.881/2) = 9.87$ g				
Experiment 2				
Threshold stimulation(D_m)	0.296	1.345	0.85	0.830
Saturation point (R_h)	54.191	8.884	4.518	22.531
0% inhibition(D_0)	1.576	2.69	2.423	2.230
50% inhibition (D_{50})	5.148	3.671	3.706	4.175
100% inhibition (D_{100})	23.6	4.3	5.0	10.967
R^2	0.99	0.96	0.99	
Sensitivity (k)	5	0	1	
Overall sensitivity (∑k) = 6				
MCSP = $D_m + (R_h/2) = 0.830 + (22.531/2) = 12.10$ g				

Nutrient element variables

In Experiment 1, Fe, Na and Zn contents each in leaf tissues of potato cv. 'Mondial G3' against increasing Nemafric-BG phytonematicide concentrations exhibited negative quadratic relations (Figure 3.11). The quadratic models for the three respective nutrient elements with increasing concentrations of Nemafric-BG phytonematicide, were explained by 91, 90 and 99% associations. Using $X = -b_1/2b_2$ relations (Gomez and Gomez, 1984), Fe, Na and Zn in leaf tissues of potato cv. 'Mondial G3' were optimised at 9.87, 10.69 and 10.51 g concentrations of Nemafric-BG phytonematicide, respectively (Table 3.4). In contrast, K content in leaf tissues of potato cv. 'Mondial G3' with increasing concentrations of Nemafric-BG phytonematicide exhibited positive quadratic fashion, with the model explained by 86% associations (Figure 3.11). K in leaf tissues was optimised at 9.47 g concentration of Nemafric-BG phytonematicide (Table 3.4), whereas in Experiment 2, Fe, K, Na and Zn contents in leaf tissues of potato cv. 'Mondial G3' exhibited positive quadratic, respectively (Figure 3.12). Models for the four respective nutrient elements were explained by 93, 92, 79 and 89 % associations. Using the relation, $X = -b_1/2b_2$, Fe, K, Na and Zn were optimised at 1.83, 20.26, 2.01 and 2.12 g concentrations of Nemafric-BG phytonematicide, respectively (Table 3.4).

Table 3.4 Optimisation model of selected nutrient elements in leaf tissues of potato cv. 'Mondial G3' as affected by post-emergent application concentrations of Nemafric-BG phytonematicide.

	Model	R^2	x (g)
Element	Experiment 1		
Fe	$Y = 1.7385x^2 – 34.33x + 492.76$	0.91	9.87
K	$Y = − 80.544x^2 + 1525.3x + 22339$	0.86	9.47
Na	$Y = 2.2547x^2 – 48.211x + 1450.3$	0.90	10.69
Zn	$Y = 0.4109x^2 – 8.6339x + 114.29$	0.99	10.51
	Experiment 2		
Fe	$Y = –32.753x^2 +120.12x + 560.48$	0.93	1.83
K	$Y = –8.492x^2 + 344.13x + 24131$	0.92	20.26
Na	$Y = –57.214x^2 + 230.2x + 1278$	0.79	2.01
Zn	$Y = −4.6364x^2 + 20.305x + 76.374$	0.89	2.12

$x = −b_1/2b_2$.

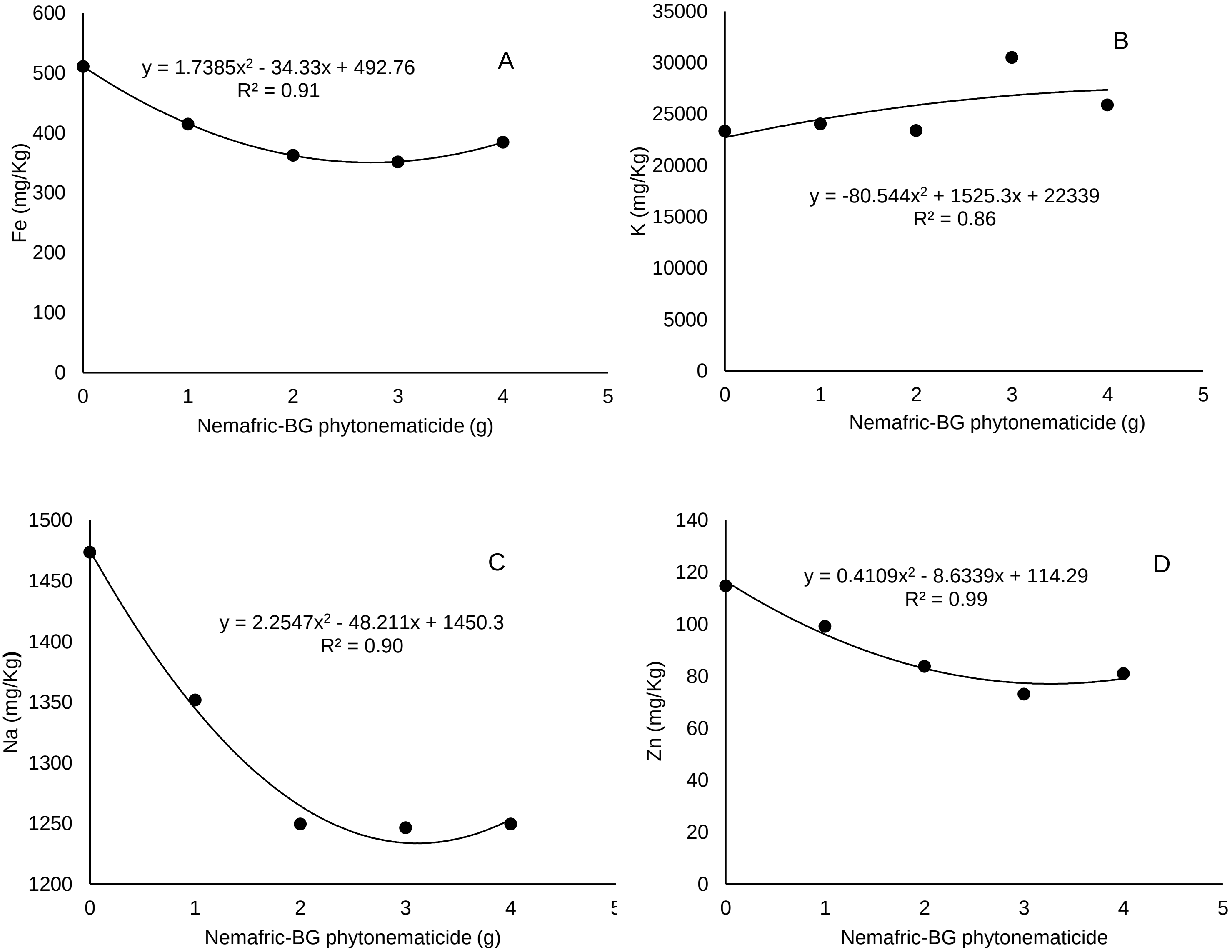

Figure 3.11 Response of Fe (A), K (B), Na (C) and Zn (D) in leaf tissues of potato cv. 'Mondial G3' to increasing post-emergent application concentrations of Nemafric-BG phytonematicide in Experiment 1 at 56 days after initiation of treatments.

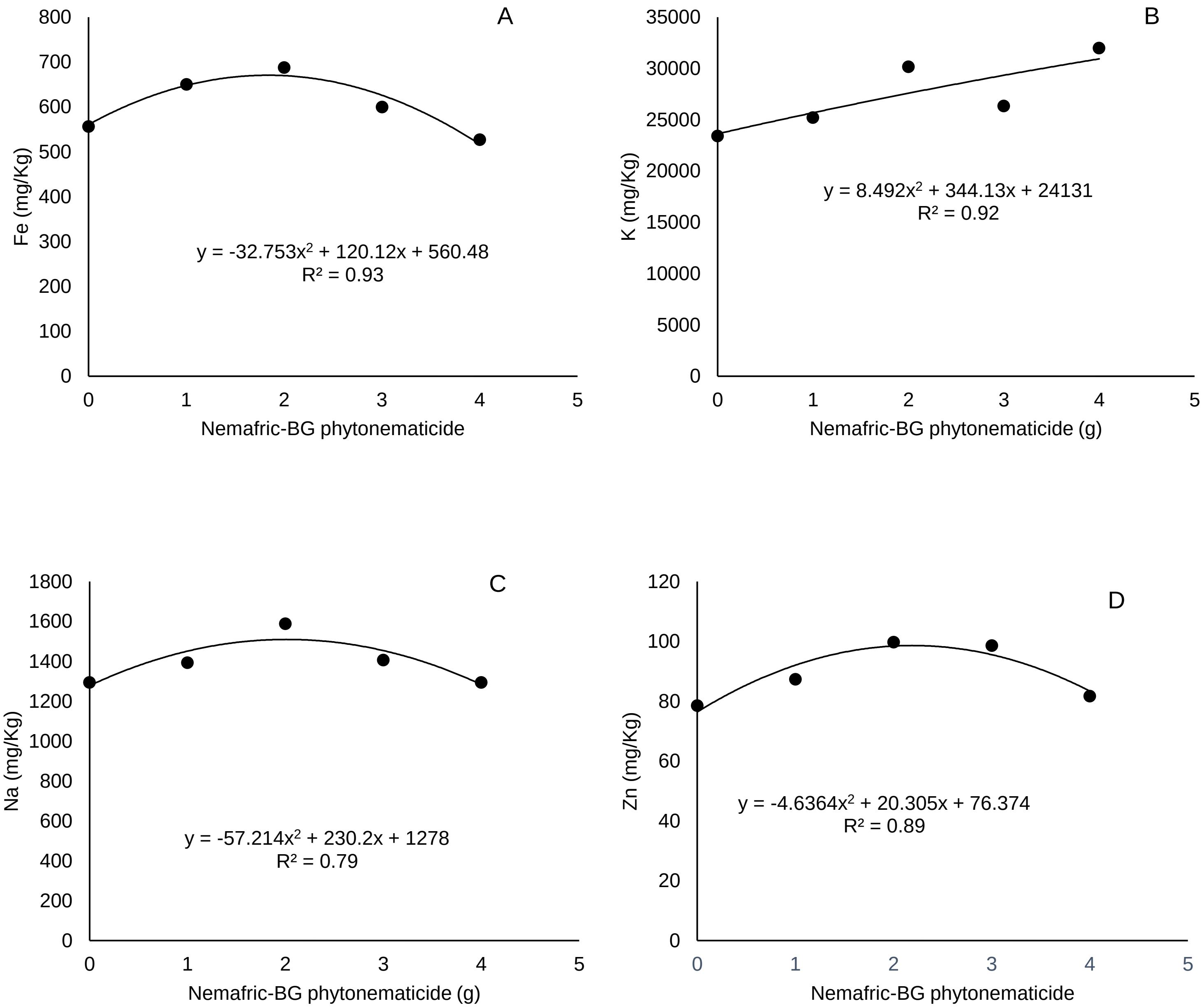

Figure 3.12 Response of Fe (A), K (B), Na (C) and Zn (D) in leaf tissues of potato cv. 'Mondial G3' to increasing post-emergent application concentrations of Nemafric-BG phytonematicide in Experiment 2 at 56 days after initiation of treatments.

Nematode variables: In both Experiment 1 and 2, eggs in roots, J2 in roots, J2 in soil and Pf of *M. javanica* against increasing post-emergent application concentrations of Nemafric-BG phytonematicide exhibited negative quadratic relations (Figure 3.13 - 3.15). The quadratic models for the four respective nematode variables with increasing concentrations of Nemafric-BG phytonematicide were explained by 87, 99, 91 and 99% associations, respectively in Experiment 1 (Figure 3.13 - 3.14). Whereas in Experiment 2, associations for the four respective variables were explained by 72, 68, 85 and 83% (Figure 3.15).

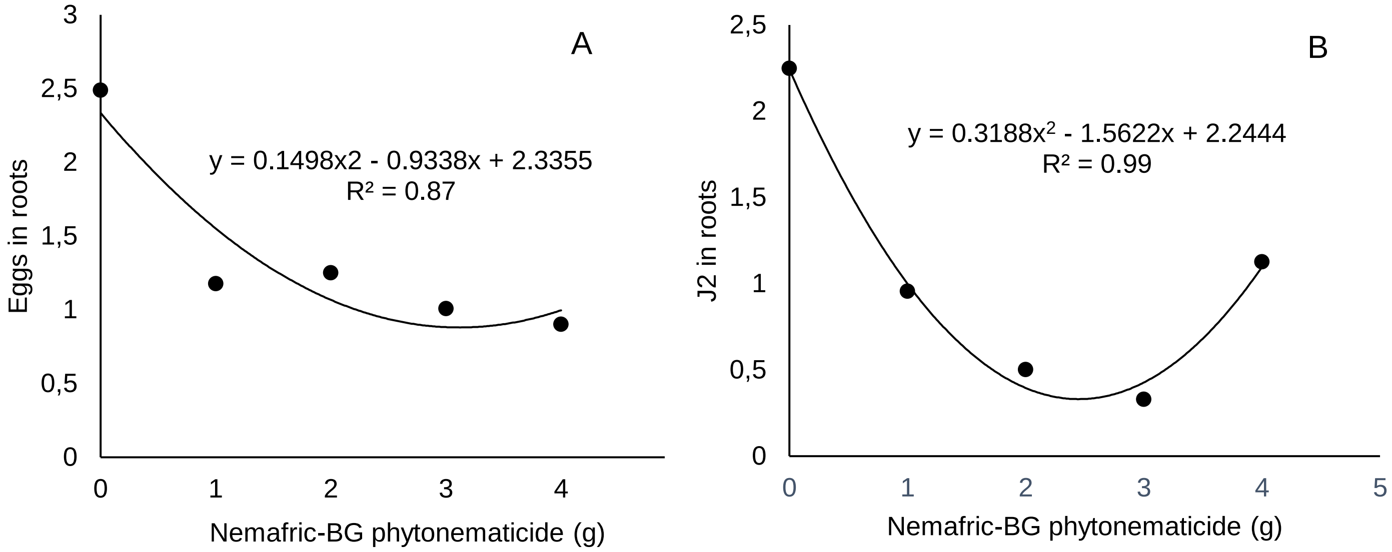

Figure 3.13 Response of *Meloidogyne javanica* eggs in roots (A) and second-stage juveniles (J2) in roots (B) to increasing post-emergent application concentrations of Nemafric-BG phytonematicide in Experiment 1 at 56 days after initiation of treatments.

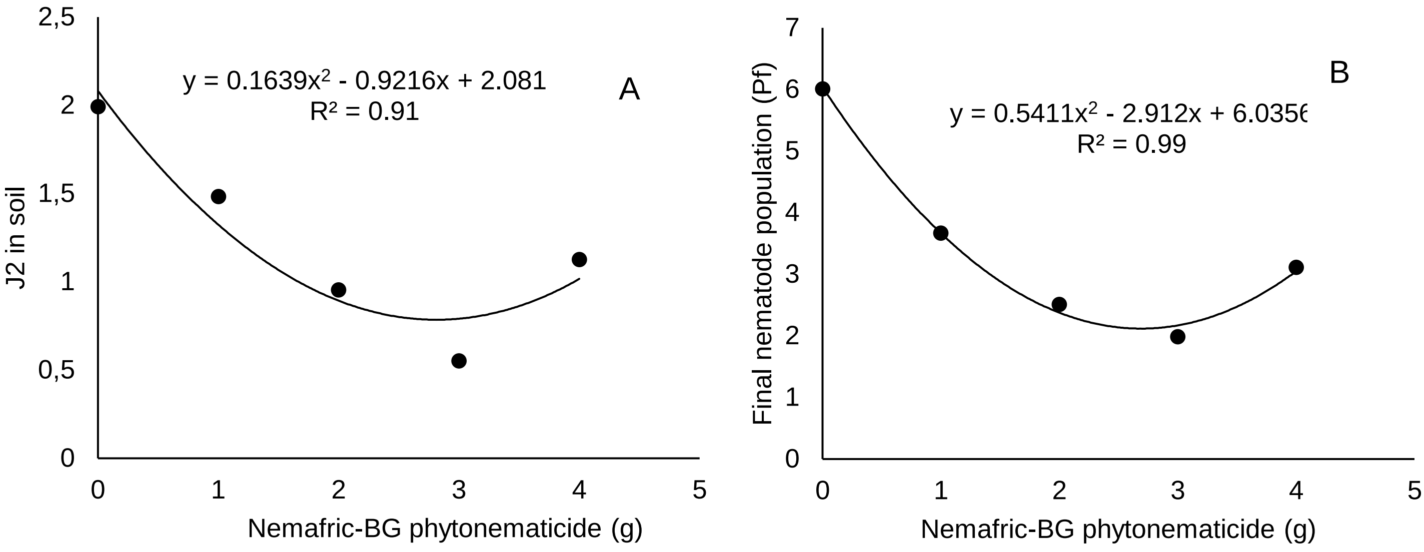

Figure 3.14 Response of *Meloidogyne javanica* second-stage juveniles (J2) in soil (A) and final nematode population (Pf) (B) to increasing post-emergent application concentrations of Nemafric-BG phytonematicide in Experiment 1 at 56 days after initiation of treatments.

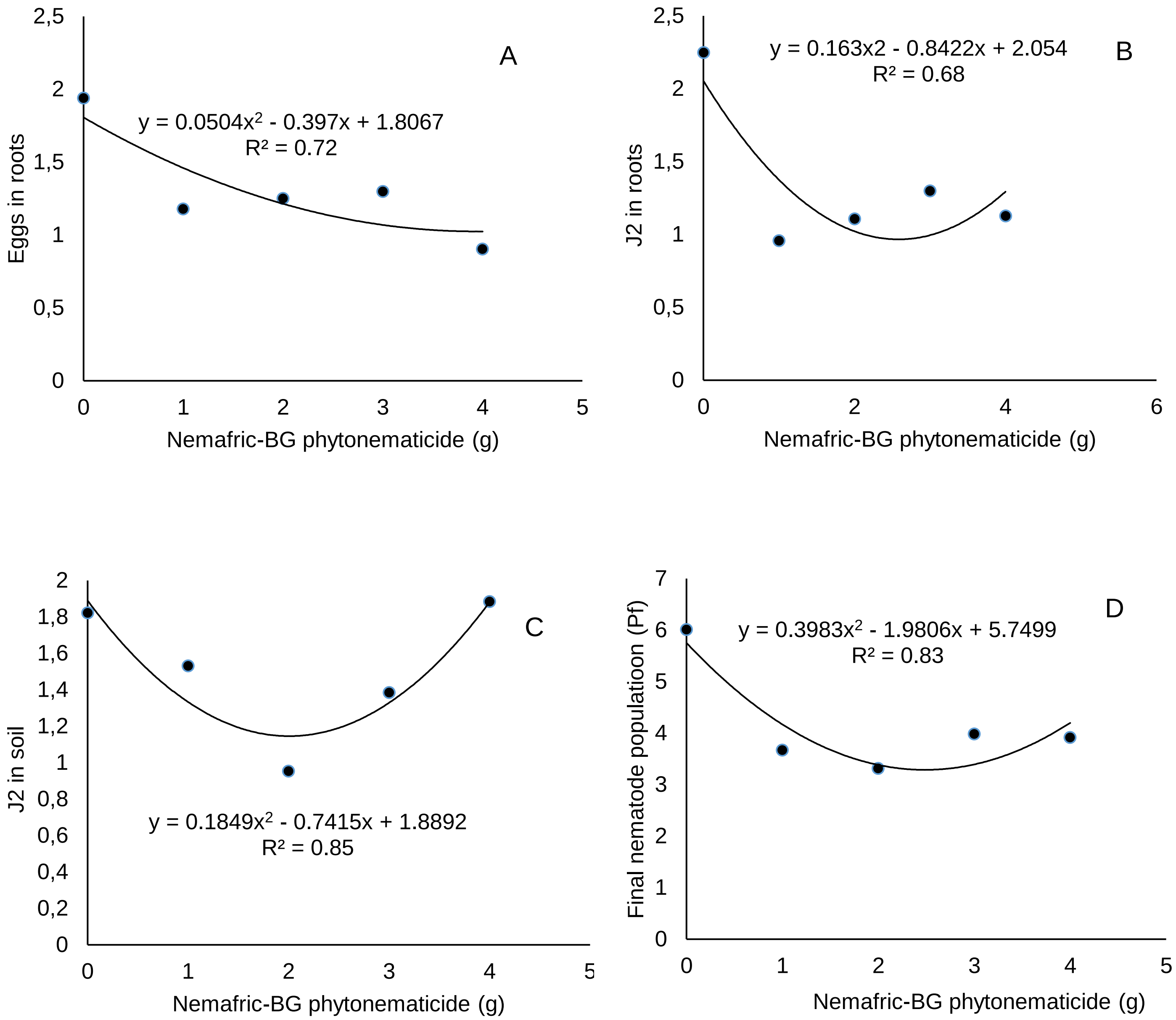

Figure 3.15 Response of *Meloidogyne javanica* eggs in roots (A), second-stage juveniles (J2) in roots (B), second-stage juveniles (J2) in soil (C) and final nematode population (Pf) (D) to increasing post-emergent application concentrations of Nemafric-BG phytonematicide in Experiment 2 at 56 days after initiation of treatments.

3.4 Discussion

3.4.1 Pre-emergent application effects

Curve-fitting allelochemical response data

Plant height and fresh root mass had density-dependent growth (DDG) patterns with increasing concentrations of Nemafric-BG phytonematicide in both experiments. The DDG patterns characterise most biological responses when exposed to increasing concentrations of allelochemicals (Liu *et al*., 2003). When using other cucurbitacin-containing phytonematicides, similar results were observed when exposing maize, millets and sorghum plants to increasing pre-emergent application concentrations of Nemarioc-AG phytonematicide (Mafeo *et al*., 2011b). The 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*., 2016) had similar DDG patterns when exposed to increasing concentrations of Nemarioc-AL phytonematicide.

The plant height and dry shoot mass over increasing concentrations of Nemafric-BG phytonematicide exhibited positive quadratic relations. 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%, 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%, respectively (Tseke *et al*., 2013). Pelinganga (2013), also observed similar trend on plant height of tomato plants when exposed to increasing concentrations of fermented crude extracts of *C. myriocarpus*, with the relation explained by 97%. Similarly, Mafeo *et al*. (2011a) when exposing chive, leek and onion seedlings to different levels of Nemafric-AG phytonematicide, seedling height, radicle length, coleoptile length and coleoptile diameter exhibited quadratic relations. In the early stages of the ground leaching technology (GLT) system, Mashela (2002) observed stimulated growth on tomato plants at lower concentrations, which was referred to as a “fertiliser effect”, however, it was not supported by empirical evidence of accumulated essential nutrient elements. Later, it was confirmed that at small quantities of cucurbitacin-containing phytonematicides, the materials invariably stimulated growth of potato plants (Mafeo, 2012; Pelinganga, 2013), which confirmed the existence of the stimulation phase in density-dependent growth patterns in response to low concentrations of allelochemicals (Lui *et al*., 2003).

The fact that increasing concentrations of Nemafric-BG phytonematicide had no effect on stem diameter, dry shoot mass, chlorophyll content and gall rating in the current study, could be suggesting that the organs were exposed to saturation concentration ranges by harvest time (Mashela *et al.*, 2015). When using other cucurbitacin-containing phytonematicide, similar observations were made on geranium plants (Sithole *et al*., 2016), tomato plants (Pelinganga *et al*., 2013; Tseke *et al*., 2013) when exposed to Nemarioc-AL phytonematicide and various commercial plants (Mafeo, 2012), when exposed to increasing concentrations of Nemarioc-AG phytonematicide. In the early stages of the ground leaching technology (GLT) system, Mashela (2002) referred to the observed stimulated growth in tomato plants as a “fertiliser effect”, which was, however, not supported by empirical evidence of accumulated essential nutrient elements. The stimulated growth of selected plant variables in this study confirmed observations in various studies where cucurbitacin containing phytonematicides were used (Mashela *et al*., 2015; Pelinganga and Mashela, 2012;

Pelinganga *et al*., 2011, 2012; Tseke *et al*., 2013). In most cases, when concentrations were within the stimulation range, growth is always stimulated, with the neutral range having no effect, whereas within the inhibition range, growth is reduced (Mashela *et al*., 2015). In the neutral range, plant growth levels off, with rapid growth of untreated control plants so that the treatment effects could not result in significant effects in short-term trials (Mashela *et al*., 2015). The three phases, in perspective of the DDG patterns had since been used to mitigate the issue of 'inconsistent results' of phytonematicides in root-knot nematodes management (Mashela *et al*., 2015; McSorley, 2011).

In the current study when using the two biological indices (D_m and R_h), the MCSP for pre-emergent application of Nemafric-BG phytonematicide was 24.18 g in Experiment 1, whereas MCSP was 7.82 g in Experiment 2. The observed MSCP were quite high when compared with those derived for maize, millet and sorghum, which were at 1.13, 0.86 and 1.12 g, respectively, for another ground cucurbitacin-containing phytonematicide, Nemarioc-AG phytonematicide when used as pre-emergent phytonematicide (Mafeo *et al*., 2011b). The practical importance of MCSP is that it is the concentration of the phytonematicide which stimulates plant growth, while at the same time suppressing nematodes population densities (Pelinganga and Mashela, 2012; Pelinganga *et al*., 2012; Tseke *et al*, 2013).

In this study, the overall sensitivity index (Σk) of potato cv. 'Mondial G3' to increasing concentrations of Nemafric-BG phytonematicide was at 20 and 6 units, respectively in Experiment 1 and 2, therefore it is suggested that potato plants are less sensitive to the product when used as a pre-emergent phytonematicide (Mashela *et al*., 2015). When using other cucurbitacin-containing phytonematicides, Mafeo (2012) showed that 18 crops had different sensitivities (k) values to Nemarioc-AG phytonematicide, with vibrant stimulatory and inhibitory concentrations. At low k values, the product is highly phytototoxic 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 *Pelargonium sidoides* plants when treated with Nemarioc-AL phytonematicide (Sithole *et al*., 2016). Generally, the degree of sensitivity in plants to cucurbitacins is plant-stage-specific, with seedlings being highly tolerant than other stages in the life of a given plant species (Mashela *et al*., 2015).

Nutrient element variables

The evaluated nutrient elements and increasing Nemafric-BG phytonematicide concentrations exhibited quadratic relations, which are the main features of density dependent growth (DDG) patterns (Liu *et al*., 2003; Mashela *et al*., 2017; Salisbury and Ross, 2005). DDG patterns, are characterised by three phases, viz., stimulation, neutral and inhibition phases (Liu *et al*., 2003), which provided much perception into how phytonematicides affect plant growth, nematode suppression (Dube, 2016; 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; Mokoele, 2018; Shadung, 2016). Similar results were observed on bioactivities of phytonematicides (Dube, 2016).

In the current study, in Experiment 1, response of Fe in leaf tissues against increasing concentrations of Nemafric-BG phytonematicide, along with those of K, Na and Zn in

Experiment 2, started from inhibition through the neutral to the stimulation phases. In contrast, K and Na in Experiment 1, along with Fe in Experiment 2, started from stimulation through the neutral to the inhibition phases. The stimulation concentration range for accumulation of selected nutrient elements in leaf tissues of potato cv. 'Mondial G3' was within the approximately 1.9 g concentration of this phytonematicide, which is the empirically established concentration for management of nematodes in potato husbandry, in this study. The stimulated accumulation for selected nutrient elements could be useful in the interpretation of the fertiliser effects of phytonematicides, which were hardly obvious when the material was applied at one concentration (Mashela, 2002).

The observed quadratic models also provided optimum concentrations at which the selected nutrient elements would be at the optimum contents. Generally, the optimum phytonematicide concentrations coincide with the neutral phase (Liu *et al*., 2003), the leaf tissues could be said to be saturated with the selected nutrient elements. In Experiment 1, the optima for Fe (9.08 g), K (7.84 g) Na (7.89 g) and Zn (2.28), and in Experiment 2 the optimum for Fe (0.03 g), K (6.34 g), Na (9.11 g) and Zn (7.13 g), were all further 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. The optimum concentrations 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. It could be improbable that accumulated concentrations in soils where nematodes are controlled using Nemafric-

BG phytonematicide could ever reach the optimum phytonematicide concentration. The increase in Na content in Experiment 1, could be undesirable to plants, because it is typically viewed as a non-essential nutrient element or waste ions that C3 plants, such as potato do not need. Mashela and Pofu (2017) observed similar trend in accumulation of Na in leaf tissues of green beans when using Nemafric-BL phytonematicide under greenhouse conditions. Shadung (2016) also observed accumulation of Na in leaf tissues of tomato plant when using Nemafric-BL and Nemarioc-AL phytonematicides, where the two products increased Na in leaf tissues by 54 and 38%, respectively. Research has shown that Na is not an essential element for plants but it can be utilised in small quantities, similar to micronutrients, to help in metabolism and synthesis of chlorophyll. In some crops, it can be utilised as a partial replacement for K and aids in the opening and closing of stomata, which helps regulate internal water balance (Zalesny *et al*., 2007). In C3 plants this element participates as an osmotically active ion (Salisburg and Ross, 2005). Generally, increasing Na content in leaves of certain plants such as citrus can be highly phytotoxic in leaves, with physiological phytotoxicity content being as low as 0.10 % Na in leaf tissues (Robinson, 1981). The increase in Na content is ideal in C4 crops, where it plays an essential role in activities of phosphoenolpyruvate carboxylase (Shomer-llan and Waisel, 1973).

Nematode variables

In the current study, eggs in roots, J2 in roots, J2 in soil and Pf of *M. javanica* against increasing pre-emergent application concentrations of Nemafric-BG phytonematicide exhibited negative quadratic relations, in both Experiment 1 and 2. Nemafric-BG phytonematicide had the attributes of other allelochemicals in terms of inducing density-dependent growth patterns in nematode variables as concentrations increased. Although this feature characterises most biological responses when exposed to intrinsic and or extrinsic increasing concentrations of allelochemicals (Salisbury and Ross, 1992). It is confirmed that at small quantities of crude extracts from *Cucumis* species, the materials invariably reduced nematode population densities (Mafeo, 2012; Pelinganga, 2013), which confirmed the existence of the inhibition phase in density-dependent growth patterns in response to lower concentrations of allelochemicals (Lui *et al*., 2003). In the current study, in both Experiment 1 and 2, response of eggs in roots, J2 in roots, J2 in soil and Pf of *M. javanica* against increasing concentrations of Nemafric-BG phytonematicide, started from inhibition through the neutral to the stimulation phases. The inhibition concentration range for nematode variables was within 24.18 and 7.82 g concentration of this phytonematicide, respectively in Experiment 1 and 2, which is the empirically established concentration for management of nematodes in potato husbandry, in this study when the product is used as a pre-emergent phytonematicide. Similarly, Nemafric-BG phytonematicide reduced J2 in soil at 2 g and in roots at 10 g by 24% and 85% on sweet sorghum var. ndendane-X1 under field conditions (Mabuka, 2013). Sithole *et al.* (2016), when using other cucurbitacin-containing phytonematicides, Nemarioc-AL phytonematicide on geranium plants for management of *M. javanica* the product reduced J2 in soil, at 6% concentration of the phytonematicide by 100% and j2 in roots by as high as 81%, whereas Nemafric-BL phytonematicide reduced J2 in roots by as high as 70 and 96%, respectively in Experiment 1 and 2 (Sithole, 2016). Increasing concentrations of Nemarioc-AL phytonematicide reduced final population of *M. javanica* on green beans at 0.8 and 1.6% concentrations of the product by 40 and 71%, respectively. In contrast Nemafric-BL phytonematicide reduced final population of nematodes on green beans at 0.8% concentration of the phytonematicide by 72% (Chokoe, 2017). Sithole (2016) observed that Nemafric-BL phytonematicide reduced Pf at 2% concentration of the phytonematicide by 95%.

3.4.2 Post-emergent application effects

Curve-fitting allelochemical response data

In this study, plant height, fresh root mass and dry shoot mass of potato cv. 'Mondial G3' had density-dependent growth (DDG) patterns with increasing post-emergent application concentrations of Nemafric-BG phytonematicide in both experiments. The DDG patterns characterise most biological responses when exposed to increasing concentrations of allelochemicals (Liu *et al*., 2003). Similar results were observed when sweet stem sorghum var. ndendane-X1 was exposed to increasing post-emergent application concentrations of Nemafric-BG phytonematicide under field conditions (Mabuka, 2013). In contrast, when using other cucurbitacin containing phytonematicides, similar results were observed when tomato plants (Pelinganga *et al*., 2012; Tseke *et al*., 2013), geranium plants (Sithole *et al*., 2016), Volkameriana citrus rootstock (Mathabatha *et al*., 2016) were exposed to Nemafric-BL and Nemarioc-AL phytonematicides and other plant species (Inderjit *et al*., 1999) to increasing concentrations of allelochemicals. Similarly, growth of maize, millets, sorghum plants (Mafeo *et al*., 2011b) and certain plant species from Alliaceae family, such as onion (*Allium cepa* L.), leek (*Allium fistosum* L.) and chive (*Allium schoenoprasum* L.), also had similar DDG patterns when exposed to increasing concentrations of Nemarioc-AG phytonematicide (Mafeo *et al*., 2011a). In Experiment 1, treatments exhibited quadratic relations on plant height, fresh root mass and dry shoot mass, with the model explaining the relationship by 97, 99 and 98%, respectively, whereas in Experiment 2, increasing concentrations of Nemafric-BG phytonematicide exhibited strong positive quadratic relations on plant height, fresh root mass and dry shoot mass, with the model explaining the relationship by 99,96 and 99%, respectively. High coefficients of determination (R^2) for the CARD models in plant variables suggested strong density-dependent relationships between growth of potato cv. 'Mondial G3' and increasing concentrations of Nemafric-BG phytonematicide when used as a post-emergent phytonematicide (Liu *et al*., 2003). When using other cucurbitacin-containing phytonematicides, similar observations were made by Pelinganga (2013), where dry root mass, dry shoot mass, plant height and stem diameter of tomato plants had high R^2 for the CARD models, when exposed to increasing concentrations of Nemafric-BL and Nemarioc-AL phytonematicides. The stimulated growth of selected plant variables in this study confirmed observations in various studies where this phytonematicide was used (Mabuka, 2013; Maile, 2013; Mashela *et al*., 2015).

In the current study Nemafric-BG phytonematicide when used as a post-emergent phytonematicide, the product had stimulatory effects on plant height, fresh root mass and dry shoot mass at 2 g concentration. Whereas, when exposing rough lemon seedlings to 2 g post-emergent application of Nemafric-BG phytonematicide, the product reduced plant height and stem diameter by 2 and 3%, respectively (Maile, 2013). When sweet stem sorghum var. ndendane-X1 was exposed to increasing concentrations of Nemafric-BG phytonematicide under field conditions, plant height was stimulated at 2, 4, 8 and 10 g by 4, 3, 8 and 0.09%, respectively and reduced at 6 g by 1 %, whereas dry shoot mass was stimulated by 0.09, 6, 7, 19 and 6% at 2, 4, 6, 8 and 10 g concentration of the product, respectively (Mabuka, 2013). These contradictions were in agreement with the hypothesis proposed by Mashela *et al*. (2015); Pelinganga and Mashela (2012) and Rice (1984) that allelopathy was concentration-specific, organ-specific and plant-specific.

In both experiments, increasing concentrations of Nemafric-BG phytonematicide did not have significant effects on stem diameter, chlorophyll content and gall rating. Lack of significant effects on certain plant variables to increasing levels of Nemafric-BG phytonematicide confirmed reports of Kohli *et al*. (2001), who observed that at 2% crude extracts of yellow nuts edge (*Cyperus esculentus* L.), had no effect on germination of lettuce (*Lactuca sativa* L.), whereas at 5% the extracts inhibited germination. Similarly, Ghafarbi *et al*. (2012) witnessed that exposing eight selected plant species to seed extracts from wheat (*Triticum aestivum* L.) had no effect on plant variables. The fact that increasing concentrations of Nemafric-BG phytonematicide had no effect on stem diameter, chlorophyll content and gall rating in this study, suggested that the organs were, by harvest time at saturation concentration (Mashela *et al.*, 2015). Similar observations were made on chlorophyll content, dry shoot mass, dry tuber mass and dry root mass of geranium plants (Sithole *et al*., 2016), number of flowers and fruit mass of tomato plants (Pelinganga *et al*., 2013) and various commercial plants (Mafeo, 2012). Mabuka (2013) observed that Nemafric-BG phytonematicide did not have effect on chlorophyll content, dry shoot mass and plant height of sweet stem sorghum var. ndendane-X1 when used as post emergent phytonematicide under field conditions. These contradictions were in agreement with the hypothesis proposed by Mashela *et al*. (2015); Pelinganga and Mashela (2012) and Rice (1984) that allelopathy was concentration-specific, organ-specific and plant-specific.

In Experiment 1, using the relation MCSP = D_m + (R_h/2) relation, MCSP for post-emergent application was derived at 9.87 g, whereas in Experiment 2, MCSP was equal to 12.10 g. Which appeared to have been different to those derived for potato plants at 24.18 g (in Experiment 1) and 7.82 g (in Experiment 2) when Nemafric-BG phytonematicide is used as pre-emergent phytonematicide (Table 3.1). In the current study, the MCSP value for post-emergent phytonematicides appeared to have been high when compared with those derived for maize, millet and sorghum, which were at 1.13, 0.86 and 1.12 g, respectively for Nemarioc-AG phytonematicide as pre-emergent phytonematicide (Mafeo *et al*., 2011b). Because the overall sensitivity index (Σk) of potato cv. 'Mondial G3' to Nemafric-BG phytonematicide when used as a post-emergent phytonematicide, was at 11 and 6 units in Experiment 1 and 2, respectively. Consequently, the plants would be moderately sensitive to the product (Mafeo, 2012; Mashela *et al*., 2015; Sithole *et al*., 2016; Tseke *et al*., 2013). Plant sensitivity is indirectly proportional to k values, with zero suggesting the highest sensitivity to allelochemicals used, while high k values suggested decreased sensitivities (Liu *et al*., 2003). Other researchers have demonstrated that tomato (Pelinganga and Mashela, 2012; Tseke *et al*., 2013) and geranium (Sithole, 2016) plants exhibited moderate sensitivity to Nemafric-BL phytonematicide when used as post-emergent phytonematicide. However, in this study plant height and dry shoot mass in Experiment 1 and fresh shoot mass and dry shoot mass in Experiment 2 were highly sensitive to Nemafric-BG phytonematicide, when used as post-emergent phytonematicide. These observations support the fact that k values are affected by various factors, such as age of the test plant and/or organ of the test plant (Pelinganga, 2013).

Nutrient element variables

The response of the assessed nutrient elements to increasing Nemafric-BG phytonematicide concentrations exhibited quadratic relations, which showed density dependent growth (DDG) patterns (Liu *et al*., 2003; Salisbury and Ross, 2005; Mashela *et al*., 2017), except K in Experiment 1, which exhibited positive linear relationship. DDG pattern are characterised by three phases, namely., stimulation, neutral and inhibition phases (Liu *et al*., 2003), which provided much understanding into how phytonematicides affect plant growth, nematode suppression (Dube, 2016; Mashela *et al*., 2016; Shadung, 2016) and nutrient elements accumulation (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 (Shadung, 2016; Mashela *et al*., 2017; Mashela and Pofu, 2017; Mokoele, 2018). Similar results were observed on bioactivities of phytonematicides (Dube, 2016). In this study, in Experiment 1, response of Fe, Na and Zn in leaf tissues against increasing concentrations of Nemafric-BG phytonematicide, along with Fe in Experiment 2, started from inhibition through the neutral to the stimulation phases. In contrast, K in Experiment 1, along with Zn in Experiment 2, started from stimulation through the neutral to the inhibition phases, while K showed stimulation across all levels of the phytonematicide. The stimulation concentration range for accumulation of selected nutrient elements in leaf tissues of potato cv. 'Mondial G3' was within the approximately 9.87 and 12.10 g concentration of this phytonematicide in Experiment 1 and 2, which is the empirically established concentration for management of nematodes in potato husbandry, in this study. The stimulated accumulation for selected nutrient elements could be useful in the interpretation of the fertiliser effects of phytonematicides, which were hardly obvious when the product was applied at one concentration (Mashela, 2002). The observed quadratic models also provided optimum concentrations at which the selected nutrient elements would be at the optimum contents. Generally, the optimum phytonematicide concentrations coincide with the neutral phase (Liu *et al*., 2003), the leaf tissues could be said to be saturated with the selected nutrient elements.

In Experiment 1, the optimum for Fe (9.87 g), K (9.47 g), Na (10.69 g) and Zn (10.51 g), and in Experiment 2 the optimum for Fe (1.83 g), K (20.26 g), Na (2.01 g) and Zn (2.12 g), were all higher than the concentrations used on potato cv. 'Mondial G3' in nematode suppression, when using the phytonematicide as pre- and post-emergent phytonematicide in this study. Mashela and Pofu (2017) observed similar results when using Nemafric-BL and Nemarioc-AL phytonematicides as post-emergent phytonematicides on green beans under greenhouse conditions. The optimum concentrations 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. It could be improbable that accumulated concentrations in soils where nematodes are controlled using Nemafric-BG phytonematicide could ever reach the optimum phytonematicide concentration. Observed positive linear relationship on K against increasing concentrations of Nemafric-BG phytonematicide, shows a high accumulation in K element in leaf tissues of potato cv. 'Mondial G3' as phytonematicide concentrations increases. Pelinganga (2013) also observed accumulation of K in leaf tissues of tomato plant when using Nemafric-BL phytonematicide, where the product increased K in leaf tissues by 81% at 2% concentration of the phytonematicide. All these observations are in agreement with the fact that, Potassium exists in plant as an ion, and does not form stable complexes. Potassium is characterised by high mobility in plants at all levels (cells, tissues, long-distance transport), which is easily transported from roots to shoots. Potassium transfers easily with growth centre and is preferentially distributed in younger tissues (Mikkelsen, 2007).

Nematode variables

In both Experiment 1 and 2, eggs in roots, J2 in roots, J2 in soil and Pf of *M. javanica* against increasing post-emergent application concentrations of Nemafric-BG phytonematicide exhibited negative quadratic relations. The inhibited growth pattern of nematode variables in this study confirmed observations in various studies where this phytonematicide was used (Mashela *et al*., 2015). In most cases, when concentrations were within the inhibition range, nematode numbers are invariably reduced, with the neutral range having no effect whereas within the stimulation range, nematode populations are stimulated (Mashela *et al*., 2015). In the neutral range, nematode population densities levels off, with nematode populations of untreated control plants "catching up" so that the treatment effects would not result in significant effects in short-term experiments (Mashela *et al*., 2015). The three phases, in context of density-dependent growth (DDG) patterns had since laid to rest what had been perceived as inconsistent results of organic amendments in management of plant-parasitic nematodes (Mashela *et al*., 2015). In the current study, in both Experiment 1 and 2, response of eggs in roots, J2 in roots, J2 in soil and Pf of *M. javanica* against increasing post-emergent application concentrations of Nemafric-BG phytonematicide, started from inhibition through the neutral to the stimulation phases. Mabuka (2013) observed similar trends on Pf in roots and soil, when sweet stem sorghum var. ndendane-X1 was exposed to increasing post-emergent application concentrations of Nemafric-BG phytonematicide for management of *Meloidogyne* species under field conditions, where Pf in roots and soil were reduced by 77-85% and 24-65%, respectively. In contrast, when 2 g Nemafric-BG phytonematicide was used as a post-emergent phytonematicide on rough lemon seedlings for management of *Tylenchulus semipenetrans* under greenhouse conditions*,* the product reduced J2 in roots by 80% and increased J2 in soil and Pf by 178 and 70%, respectively (Maile, 2013). These inconsistent results in nematode management support the argument that when nematodes are stimulated, at the exposure to a certain concentration of allelochemical, it is suggested that the concentration used was at the stimulation range (Mashela *et al*., 2015).

3.5 Conclusions

The non-phytotoxic concentration of Nemafric-BG phytonematicide on potato cultivar 'Mondial G3' was 24.18 and 7.82 g, respectively in Experiment 1 and 2, when used as a pre-emergent phytonematicide and when used as post-emergent phytonematicide it was 9.87 and 12.10 g, respectively in Experiment 1 and 2. However, since *M. javanica* population densities were reduced at low MCSP values, it would be ideal to adopt the lowest concentration, such as 7.82 g and 9.87 for pre- and post-emergent application, respectively for Nemafric-BG phytonematicide in the management of nematodes on potato. In this way, the initial non-phytotoxic concentration (D0), which would occur at 24.18 and 12.10 for pre- and post-emergent application, respectively, would not be attained due to accumulation in soil solution. The fact that the optimum concentrations for selected nutrient elements are comparatively higher as compared with the concentrations used in nematode suppression, concludes that the product could not have detrimental effects on nutrient elements when used as either pre- or post-emergent phytonematicide. However, it is important that residues of this phytonematicide on potato tubers should be established, since at low concentrations, cucurbitacins could be cancerous to human beings

CHAPTER 4

SUMMARY OF FINDINGS, SIGNIFICANCE, RECOMMENDATIONS AND CONCLUSIONS

4.1 Summary of findings

Nemafric-BG phytonematicide produced in South Africa, is one of the sustainable products that could be used in climate-smart agriculture for sustainable nematode management. However, lack of information on how this product affect the accumulation of essential nutrient element in crops and phytotoxicity are major challenges in the implementation of phytonematicides as alternative to aldicarb and nemacur for managing nematode population densities in potato production. The study was initiated to determine non-phytotoxic concentration and overall sensitivity of Nemafric-BG phytonematicide using the Curve-fitting Allelochemical Response Dosage (CARD) computer-based model, which provided seven biological indices (Liu *et al*., 2003; Mashela *et al.,* 2017). The two biological indices (D_m and R_h) were used to compute the Mean Concentration Stimulation Point (MCSP) for potato (*Solanum tuberosum* L.) cv. 'Mondial G3' when exposed to increasing concentrations of Nemafric-BG phytonematicides in the management of *Meloidogyne javanica* Treub., whereas the k values were used to determine the overall sensitivity of potato cv. 'Mondial G3' to the phytonematicide. Under greenhouse conditions, Nemafric-BG phytonematicide had some effects on plant growth, nematodes and accumulation of nutrient elements in potato plants.

In pre-emergent application experiments, Nemafric-BG phytonematicide had significant effects on plant height and fresh shoot mass of potato cv. 'Mondial G3', whereas the product had no effect on other plant variables. In post-emergent application experiments, the product had significant effects on plant height, fresh root mass and dry shoot mass, whereas other plant variables were not significantly affected by this phytonematicide. In both pre-and post-emergent experiments, plant variables and increasing concentrations of Nemafric-BG phytonematicide had density-dependent growth (DDG) patterns, which had three phases, stimulation, saturation/neutral and inhibition. The CARD model was used to compute the biological indices for stimulation, saturation and inhibition phases, with Mean Concentration Stimulation Point [MCSP = $D_m + (R_h/2)$] for Nemafric-BG phytonematicide being 24.18 and 7.82 g when the product is used as a pre-emergent phytonematicide, respectively, in Experiment 1 and 2. and when the product was used as a post-emergent phytonematicide, MCSP was derived at 9.87 and 12.10 g, respectively, in Experiment 1 and 2. In pre-emergent application trials, potato cv. 'Mondial G3' was moderately sensitive to Nemafric-BG phytonematicide, with overall sensitivity of 20 and 6 units, respectively, in Experiment 1 and 2, whereas in post-emergent application trials potato cv. 'Mondial G3' had overall sensitivity of 11 and 6 units, respectively, in Experiment 1 and 2 (Liu *et al*., 2003; Mashela *et al*., 2017).

When the product was used as a pre-emergent phytonematicide on potato, in Experiment 1, the relationship between Fe, K and Na contents each in leaf tissues of potato cv. 'Mondial G3' and increasing Nemafric-BG phytonematicide concentrations was explained by 87, 96 and 90%, respectively. Whereas in Experiment 2 the relationship against increasing phytonematicide concentrations and Fe, K, Na and Zn, was explained by 88, 99, 90 and 97% respectively. In contrast, in post-emergent application trials, Fe, K, Na and Zn against increasing phytonematicide concentration relationship were explained by 91, 86, 90 and 90%, respectively, whereas in Experiment 2, Fe, K and Zn against increasing phytonematicide concentrations were associated by 86, 92 and 93%, respectively.

In the current study, eggs in roots, J2 in roots, J2 in soil and Pf of *M. javanica* against increasing pre-emergent application concentrations of Nemafric-BG phytonematicide exhibited negative quadratic relations, in both pre- and post-emergent trials. In all trials response of nematode variables to increasing concentrations of the phytonematicide, started from inhibition through the neutral to the stimulation phases. The results demonstrated that nematodes are reduced at lower concentrations and stimulated at higher concentration.

4.2 Significance of the findings

In transplanted seedlings, cucurbitacin-containing phytonematicides could be easily used in ground leaching technology (GLT), which is post-emergent. In the current study, Nemafric-BG phytonematicide could be used as either pre- or post-emergent phytonematicide. The significance of using phytonematicide in granular formulation is that it should be applied once with the efficacy that lasted for the entire growing season for annual crops like potatoes. In the current cultivar, the MCSP values for pre- and post-emergent applications were 7.82 g and 9.87 g, respectively.

4.3 Recommendations

Nemafric-BG phytonematicide could be applied at concentration of 7.82 g and 9.87 g/ plant, when using the product as a pre- and post-emergent phytonematicide, respectively, which should be validated under various conditions. Since the tuber is the edible part of the plant, residues of this phytonematicide on potato tubers should be established, since at low concentrations, cucurbitacin-B could be cancerous to human beings (Lee *et al*., 2010). Effects of essential nutrient elements in potato tubers should also be established, in order to find out if phytonematicides have any detrimental effect in accumulation of nutrient elements in tubers. Duration of allelochemical residues in the soil should also be established, in order to investigate the effects of soil allelochemical residue (SAR) of Nemafric-BG phytonematicide on soil health, growth of following crops and population densities of *Meloidogyne* species (Mashela and Dube, 2014).

4.4 Conclusions

Nemafric-BG phytonematicide could, upon validation be suitable for use through Ground Leaching Technology system on potato to manage nematodes. This product could be used as pre-emergent phytonematicide and post-emergent phytonematicide, since results from the study demonstrated that potato plants are less sensitive to the product. It is concluded, that the product could be applied at the recommended 7.82 and 9.87 g/ plant in pre and post-emergent application, respectively, for management of root-knot nematodes, provided the active ingredient does not accumulate in potato tubers or have any detrimental effects in accumulation of nutrient elements in tubers and temper with nutritional value of potatoes.

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