NON-PHYTOTOXIC CONCENTRATION AND APPLICATION INTERVAL OF NEMARIOC-AL PHYTONEMATICIDE IN MANAGEMENT OF *MELOIDOGYNE JAVANICA* ON POTATO CULTIVAR 'MONDIAL G3'

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

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DECLARATION

I, Selaelo Patrisia Kobe, 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 execution, and related materials contained herein had been duly acknowledged

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DEDICATION

To my mother, Tlou Selina Kobe, my father, Mokibelo Samuel Kobe, with love.

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ABSTRACT

Potato (*Solanum tuberosum* L.) is highly susceptible to root-knot (*Meloidogyne* species) nematodes, with no known nematode resistant genotypes. In Limpopo Province, two cucurbitacin-containing phytonematicides had been researched and developed. The active ingredients of the cucurbitacin-containing phytonematicides are cucurbitacins, which are allelochemicals that could induce phytotoxicity on crops being protected against nematode damage. The objectives of this study were to determine: (1) mean concentration stimulation point (MCSP) of Nemarioc-AL phytonematicide on potato cultivar 'Mondial G3' for managing *M. javanica* and (2) application interval of Nemarioc-AL phytonematicide on potato cultivar 'Mondial G3'. Sprouted tubers were planted in 10 cm deep/pot with each pot filled with steam-pasteurised soil and Hygromix at 3:1 (v/v) ratio in the field under microplot conditions. After 100% emergence (2 weeks), each plant was inoculated with 5 000 *M. javanica* eggs and second-stage juveniles (J2). Seven treatments, namely, 0, 2, 4, 8, 16, 32 and 64% Nemarioc-AL phytonematicide were arranged in a randomised complete block design, with 11 replications. In Objective 2, four treatments, namely, 1, 2, 3 and 4 weeks were arranged in randomised complete block design, with 15 replications. Plant variables and nutrient elements were subjected to the Curve-fitting Allelochemical Response Data (CARD) model to generate biological indices used to compute MCSP using the relation MCSP = $D_m + R_h/2$ and the overall sensitivity value ($\sum k$). The MCSP for plant variables and nutrient elements, were empirically derived as 4.31% and 1.33%, with the $\sum k$ of 18 and 4 units, respectively. Nematode variables and increasing concentrations of Nemarioc-AL phytonematicide exhibited negative quadratic relations where eggs, J2 in soil and roots and total population (Pf) were optimised at

14.43, 28.23, 23.30 and 13.55%. To conduct Objective 2 which is application interval, empirically derived MCSP value of 4.31% from Objective 1 was used. Application interval was optimised using the concept of 1, 2, 3, and 4 weeks in weeks-per-month-of-30-days. The application interval of 4.31% was established at 2.43 weeks which translated to 18 days [(2.43 weeks/4 weeks) × 30 days]. All nematode variables in Objective 2 were not significantly different at all intervals. In, conclusion Nemarioc-AL phytonematicide can be used at 4.31% concentration to control nematodes population densities without being phytotoxic to crops at 18 days application interval.

CHAPTER 1
RESEARCH PROBLEM

1.1. Background

1.1.1 Description of the research problem

Potato (*Solanum tuberosum* L.) has no known genotypes that are resistant to root-knot (*Meloidogyne* species) nematodes (Pofu *et al.,* 2012; Thies and Levi, 2007; Tseke and Mashela, 2018). The successful production of this crop is limited by plant-parasitic nematodes, in particular *Meloidogyne* species (Onkendi and Moleleki, 2013). Due to lack of genotypes with nematode resistance in potato cultivars, yield reduction can be from as high as 50% to complete crop failure (Pofu *et al.,* 2012). *Meloidogyne* species pose a significant threat to crop production in Africa due to huge losses resulting from their damage (Karuri *et al.,* 2017). Internationally, *M. incognita* is ranked as the most aggressive nematode followed by *M. javanica* (Kleynhans *et al.,* 1996). However, in South Africa, *M. javanica* had been regarded as the most aggressive than *M. incognita* (Kleynhans *et al.,* 1996). Synthetic nematicides were withdrawn from the agrochemical markets because they posed environmental hazards (Mashela *et al.,* 2017). The use of botanicals had been receiving enormous attention since the global withdrawal of synthetic chemical nematicides. Following the withdrawal of methyl bromide in 2005, alternatives were researched and developed, with particular emphasis on sustainable products such as phytonematicides (Mashela *et al.,* 2017).

In Limpopo Province, two cucurbitacin-containing phytonematicides were widely investigated researched and developed (Mashela *et al.,* 2017). Fermented crude extracts of dried fruit from wild cucumber (*Cucumis myriocarpus* Naude.) are generally used to develop Nemarioc-AL phytonematicide, with active ingredient cucurbitacin A ($C_{32}H_{46}O_9$), which is soluble in water and highly unstable degradable to cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) chemical compounds (Chen *et al.,* 2005, Mashela *et al.,* 2017). This phytonematicide can be highly toxic, even to the target crops (Pelinganga and Mashela, 2012). Phytotoxicity from cucurbitacin-containing phytonematicide has been reported in a wide range of crops (Mafeo, 2012; Pelinganga and Mashela, 2012). The Curve-fitting Allelochemical Response Data (CARD) computer-based model, developed in Australia (Liu *et al.,* 2003), has been adapted to develop the Mean Concentration Stimulation Point (MCSP), which is the optimal to suppress nematode population density but without phytotoxicity (Mashela *et al.,* 2017).

1.1.2 Impact of the research problem

The nematicides introduced to manage nematode population densities had drawbacks where most of the effective cucurbitacin-containing phytonematicide had phytotoxicity to crops (Mashela *et al.,* 2015). Stringent regulations governing agriculture pesticides resulted in their de-registration (OECD, 2017). As a result, most products intended to manage nematode population densities could not go beyond *in vitro* trial stages due to phytotoxicity to the test plant (EPPO, 2010).

1.1.3 Possible causes of the research problem

The active ingredients in phytonematicides are allelochemicals, which are known to be used by plants in allelopathy (Mashela *et al.,* 2015). Allelochemicals have adverse effects on different plant species (Pelinganga and Mashela, 2012), but there could also be auto-allelopathy that occurs within the same plant species (Mashela *et al.,* 2015). Adoption of phytonematicides had been limited by increases in the incidents of phytotoxicity and perceived inconsistent results in nematode suppression of soil amended with phytonematicides (Mashela *et al.,* 2015).

1.1.4 Proposed solutions

The CARD model could be helpful in establishing the amount of the phytonematicide to be applied at any one time, with the MCSP, generated through the use of the CARD model providing that envisaged solution. The determination of MCSP allows the optimal concentration of nematicide to be determined. However, once the MCSP had been empirically-established, the outstanding issue would be when should the derived quantity be applied, which then calls for the empirical determination of the application interval.

1.1.5 General focus of the study

The primary focus of the study would be the establishment of the MCSP of Nemarioc-AL phytonematicide on potato and the overall sensitivity of potato to this product (Mashela *et al.,* 2017). Once established, the MCSP would be used to establish the application interval using the life cycle of the target nematode as a yard stick (Pelinganga *et al*., 2013).

1.2 Problem statement

Indigenous *Cucumis* fruits were used to develop phytonematicides for the management of nematodes. However, the introduced phytonematicides could not be adopted for use in the management of crops due to incidence of phytotoxicity on targeted crops (Sithole, 2016). Crude extracts of Nemarioc-AG phytonematicide were previously shown to be highly phytotoxic to eight monocotyledonous and ten dicotyledonous crops when applied as pre-emergent drenches (Mafeo and Mashela, 2010). The successful use of Nemarioc-AL phytonematicide on potato dictates that its MCSP and the application interval be empirically established.

1.3 Rationale of the study

Lack of alternative strategies to manage *Meloidogyne* species effectively contribute greatly to incidents of food crisis across the globe (Onkendi *et al.,* 2014). The major drawback of phytonematicides in nematode management was their high incidences of phytotoxicity to target (Pelinganga *et al.,* 2013). However, it was shown that phytotoxicity was concentration-specific and crop-specific, with the CARD model used to overcome the challenge (Mashela *et al.,* 2015). The empirical development of the MCSP and the application interval is central to the successful use of Nemarioc-AL phytonematicide for the management of plant-parasitic nematodes.

1.4. Purpose of the study

1.4.1 Aim

To determine non-phytotoxic concentration and the application interval of cucurbitacin-containing phytonematicide in the management of *Meloidogyne* species in potato production.

1.4.2 Objectives

1. To assess whether a MCSP of Nemarioc-AL phytonematicide on potato cv. 'Mondial G3' would be established for managing population densities of *M. javanica*.
2. To investigate whether the MCSP of Nemarioc-AL phytonematicide on potato cultivar 'Mondial G3' could be suitable for the establishment of the application interval using the life cycle of *M. javanica.*

1.4.3 Hypotheses

1. MCSP of Nemarioc-AL phytonematicide on potato cv. 'Mondial G3' would be established for managing population densities of *M. javanica*.
2. MCSP of Nemarioc-AL phytonematicide on potato cultivar 'Mondial G3' could be suitable for the establishment of the application interval using the life cycle of *M. javanica*.

1.5 Reliability, validity and objectivity

In this study, the reliability of data was based on statistical analysis of data at the probability level of 5%. Validity was achieved through repeating the experiments over two seasons. 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 ensuring that in each experiment, experimental error is reduced through adequate replications. Treatments were assigned randomly within the selected research designs (Leedy and Ormrod, 2005).

1.7 Scientific significance of the study

The use of plant material for the management of nematodes is environmentally friendly compared to synthetic nematicides ((Mashela *et al.,* 2017). Since the withdrawal of synthetic nematicides, crop yield has decreased globally and that affect crop production and development of alternative management strategies is required. The study intends to develop MCSP and application interval of Nemarioc-AL phytonematicide upon which the application would induce non-phytotoxicity. The outcomes of this study would improve the use of Nemarioc-AL phytonematicide for management of *M. javanica* by potato farmers.

1.8 Structure of the 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 outlined as literature review (Chapter 2). Then the subsequent chapters (Chapter 3, Chapter 4) addressed each Objective. In the final chapter (Chapter 5), the significance of findings was summarised and integrated to provide their significance with recommendations for future research. In the study, citations in text and references followed the Harvard style of author-alphabet as approved by the Senate of the University of Limpopo.

CHAPTER 2
LITERATURE REVIEW

2.1 Work done on problem statement

In South Africa, 453 plant-parasitic nematodes had been reported on crops, with 21% associated with potatoes (Marais *et al*., 2015). Synthetic nematicides such as methyl bromide were widely used in the management of plant-parasitic nematodes in potato production. However, due to their environment-unfriendliness, the products had since been withdrawn from the agrochemical markets (Mashela *et al.,* 2017). Various alternatives are currently in use, with phytonematicides from fruits of wild cucumber (*Cucumis myrioca*rpus Naude.) and wild watermelon (*Cucumis africanus* L.), namely, Nemarioc-AL and Nemafric-BL phytonematicides, respectively (Mashela *et al.*, 2017), being widely used in South Africa. The two *Cucumis* species have the centres of biodiversity in Limpopo Province, South Africa (Mashela *et al.,* 2015). The letters A and B in the listed phytonematicides denote the active ingredients, cucurbitacin A and B, respectively, whereas L and G denote liquid and granular formulations, respectively (Chen *et al.,* 2005; Mashela *et al.,* 2015; Tseke, 2013). However, since the active ingredients are allelochemicals, the products could result in allelopathy to target crops.

The amount to be applied, technically referred to as mean concentration stimulation point (MCSP) and the application interval for any of the two phytonematicides, are crop-specific (Mashela *et al*., 2017) and should be established empirically to avoid phytotoxicity (Pelinganga *et al.,* 2013). Most registration authorities have zero tolerance on phytotoxicity of agricultural inputs and therefore, it had been imperative that MCSP values and the associated application intervals be established for each crop and phytonematicide.

2.1.1 Phytotoxicity of plant extracts

Many tested botanicals were effective in controlling nematodes but were implicated to be phytotoxic to test crops (Oka *et al.,* 2012). Phytonematicides are phytotoxic to other plants due to the active ingredients they contain which are the allelochemicals (Wuyts *et al.,* 2006). In granular formulation, Nemarioc-AG phytonematicide was associated with inhibition of seed germination, increased seedling mortalities and suppression of seedling growth in both monocotyledonous and dicotyledonous plants (Mafeo *et al.,* 2011b). Later, Anese *et al.* (2015) confirmed that allelochemicals from plant extracts could inhibit seed germination and reduce growth of seedlings in different crops. Phytotoxicity of different plant extracts could be attributed to different chemical composition and concentrations of the active ingredients (Taye *et al.,* 2012).

Plant extracts from the stem bark of drymis (*Drymis brasielensis* G. Forst.) reduced growth of lettuce (*Lactuca sativa* L.) seedlings due to phytotoxic effects (Anese *et al.,* 2015). Ahmad *et al.* (2010) demonstrated that at high concentrations, plants that had toxic chemicals might produce phytotoxic symptoms, probably due to auto-allelopathy. Zasada *et al*. (2002) reported that phytotoxicity was commonly exhibited at higher concentrations that exerted high inhibition on *M. javanica* by extracts of neem (*Azadirachta indica* A. Juss.), nerium (*Nerium oleander* L.) and ivy (*Hedera helix* L.). At concentrations above 10% aqueous extracts of neem basil, nitta (*Parkia timoriana* DC.), pepper (*Capsicum* *spp.*) and ginger (*Zingiber officinale* Roscoe), were highly phytotoxic on tomato (*Solanum lycorpesicum* L.) (Agbenin *et al.,* 2005; Bawa *et al.,* 2014). The effects of allelochemicals on plants and nematodes suppression are concentration specific, but higher concentrations are required to induce phytotoxicity than nematicidal effects (Mashela *et al.,* 2015). Ahmad *et al.* (2010) demonstrated that before the extracts of tick berry (*Lantana camara* L.*)* could be applied, the optimum concentration should be determined to avoid phytotoxicity of the extracts towards the targeted crop. Malatji (2017) demonstrated that Nemalan, a fermented crude extract from *L. camara* under microplot and greenhouse conditions, had the MCSP of 5.76 and 5.31% on tomato plants, respectively. Population densities of *M. incognita* were reduced by leaf extracts of *L. camara*, Mexican marigold (*Tagetes erecta* L.) and pyrethrum (*Tanacetum cinerariifolium* Trevir.) flower extracts at 3 and 5%, respectively (Taye *et al.,* 2012).

Nematicidal effects of garlic were tested, and been found to be phytotoxic (Agbenin *et al.,* 2005). Toxicity of many plant extracts on different plant species was extensively reported (Okeniyi *et al.,* 2013). Extracts from leaves, roots and seeds of sweet basil inhibited germination, shoot and root elongation of agriculturally important crops exhibiting phytotoxicity to maize, wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.), lentil (*Lens esculenta* L.), barley (*Hordeum vulgare* L.), okra (*Abelmoschus esculentus* L.), gram (*Cicer arietinum* L.) and mustard (*Brassica campestris* L.) (Verma *et al.,* 2012). Generally, when cucumber seedlings were treated with leaf powder of myrtle (*Myrtus. communis* L.*)* to control nematode population densities of *M. javanica* under *in vitro* and *ex vitro* conditions, the product reduced the number of root galls (Oka *et al.,* 2012). Extracts of *D.*

brasiliensis from different plant parts of shown to be phytotoxic on two tested crops which are radish and wheat (*Triticum aestivum* L.) (Anese *et al.,* 2015). In contrast extracts of garlic and castor been were effective for controlling *M. incognita* on tomato and showed no phytotoxicity when compared with findings in the previous studies (El-Nagdi and Youssef, 2013).

2.1.2 Efficacy of plant extracts/ botanicals

In nematode management different botanicals have been tested and studied extensively (El-Nagdi and Youssef, 2013; Khan *et al.,* 2008). Pelinganga and Mashela (2012) found that diluted fermented crude extracts computed from biological indices of the Curve-fitting Allelochemical Response Data (CARD) was 2.64% for tomato plants. However, in other studies it was shown that as low as 2% phytonematicide could reduce final nematodes population density of *M. incognita* (Mashela *et al.*, 2017). Dosages of *C. africanus* were found to be phytotoxic to tomato plants but CARD model demonstrated that the material stimulated plant growth at concentrations below 10% (Pelinganga *et al.,* 2012).

Extracts from neem (*Azadirachta indica*), ashwaganda (*Withania somnifera* L.), marigold (*Tagetes erecta* L.) and eucalyptus (*Eucalyptus atriodora* Hook) reduced nematode population densities on papaya seedlings (*Carica papaya* L.) (Khan *et al.,* 2008). At 5% concentration, the extracts of African basil (*Ocimum gratissimum* L.) were effective in reducing population densities of root-knot nematodes on eggplant (*Solanum melongena* L.) (Claudius-cole *et al.,* 2010). Lower concentrations of pawpaw siam weed (*Chromolaena odorata* L.), lemon grass (*Cymbopogon citratus* DC.), mango (*Mangifera* *indica* L.), basil (*Ocimum basilicum* L.), bitter leaf (*Vernonia amygdalina* Delile), neem (*Azarachta indica* A. Juss.), moringa (*Moringa oleifera L.)* and black pepper (*Piper nigrum* L.) had nematicidal effects on root-knot nematodes (Aghale *et al.,* 2016). In addition to nematode suppression, yield of tomato seedlings was effectively increased when leaf extracts of *L. camara* were used at 3 and 5% (Taye *et al.,* 2012).

In vitro trials demonstrated that more than 90% nematode numbers could be suppressed by more than 90% by phytonematicides (Okwute, 2012). However, a large number of botanicals that exhibited nematicidal properties did not make it beyond *in vitro* trials because of high levels of phytotoxicity (Mashela *et al*., 2017). Asif *et al.* (2017) demonstrated that plant extracts did not only reduce nematode population densities, but could also improve plant growth as shown in the early 2000s (Mashela, 2002). Taye *et al.* (2012) reported that the increase in yield of tomato seedlings and the reductions in nematode numbers showed that the tested plant extracts had nematicidal potential.

2.1.3 Curve-fitting Allelochemical Response Data (CARD) model

The MCSP is referred to as the concentration of the phytonematicide which stimulates plant growth but suppresses population densities of the test nematode (Mashela *et al.,* 2017; Pelinganga *et al.,* 2013). The CARD model was developed in Australia with seven biological indices (Liu *et al.,* 2003), namely, threshold stimulation (D_m) – allelochemical concentration starting point of stimulation phase, saturation point (R_h) – point at which stimulation phase ends and neutral phase starts, inhibition at 0% (D_0) – point at which neutral phase ends, inhibition at 50% (D_{50}) – concentration at half distance of the inhibition, inhibition at 100% (D_{100}) – end point of inhibition phase, sensitivity value (k) provides the level of sensitivity of an organism to the test product and overall sensitivity value ($\sum k$) – the summation of sensitivity values (Mashela *et al.,* 2017). The two biological indices, namely, Dm and Rh, were used to develop the concept of MCSP where the relation MCSP = $D_m + (R_h/2)$ (Mashela *et al.,* 2017). Mashela *et al.* (2015) demonstrated that following the density-dependent patterns, plant responses can either be stimulated, neutral or inhibited to a degree of response dependent on the concentration of the phytonematicides. The MCSP values were empirically derived for Nemarioc-AL and Nemafric-BL phytonematicides on tomato seedlings as 2.99 and 2.64%, respectively (Pelinganga, 2013), for *Citrus volkameriana* seedling rootstocks as 8.6 and 6.3% (Mathabatha *et al.,* 2016), for squash or zucchini (*Cucumis pepo*) as 2.83 and 11.85%, respectively (Lebea, 2017) and for wild geranium (*Pelargonium sidoides* DC.) as 6.2 and 2.9%, respectively (Sithole, 2016). Mashela *et al*. (2017) argued that since nematodes were reduced at lower values than the MCSP values, when the values were higher than three, they could still be reduced to 3% since MCSP was not intended for use as "fertiliser".

2.1.4 Application interval of phytonematicides

The responses of plants to phytonematicides and its specificity was shown to be associated with the application interval (Mashela *et al.,* 2015). Generally, in most phytonematicides, the products are applied at weekly or biweekly interval, without empirical basis (Pelinganga, 2013). For instance, Nemalan, produced from the fermentation of *L. camara* was applied at 7 days (weekly) (Nzanza *et al.,* 2013).

In cucurbitacin-containing phytonematicides, the application interval had been based on the life-cycle of the specific nematode. The life-cycle of *Meloidogyne* species in tropical and subtropical areas is approximately 30 days (Mashela *et al.,* 2015). Active ingredients could be applied through botinemagation, which was described as the use of botanicals in managing nematode population densities through irrigation systems (Mashela *et al.,* 2017). The MCSP is computed using the CARD model, whereas the application interval could not use the CARD model since the x-axis did not represent increasing concentrations of phytonematicides (Liu *et al.,* 2003), but represented increase in time (Mashela *et al.,* 2017). Application interval for *Meloidogyne* species was developed over a “week-of-30-day-month” (Pelinganga *et al.,* 2013). Application interval is determined over a period of 30 days to break the lifecycle of the nematode. Application interval is calculated using mean of optimised plant growth variables/4 weeks × 30 days.

Eventually, the application interval was used to compute the application frequency, which was shown to be the proportion of the crop life cycle (days) to the application interval (days), which was a unit-less variable and a constant for a given plant species (Mashela *et al.,* 2015). The MCSP (%) and application frequency (AF) are required for computation of the dosage model [D = MCSP (%) × AF].

2.2 Work not done on problem statement

Nemarioc-AL phytonematicide, as shown in various studies above, had been successful in suppressing nematode population densities using the empirically based concepts of

MCSP and application interval, which are plant- and nematode-specific. However, the MCSP and application interval of Nemarioc-AL phytonematicide and *M. javanica* on potato cultivar 'Mondial G3' have not been widely documented.

2.3 Addressing identified gaps

The identified gap was that the MCSP and application interval of Nemarioc-AL phytonematicide and *M. javanica* on potato cultivar 'Mondial G3' had not been documented. According to cited literature, the MCSP for Nemarioc-AL phytonematicide should first be empirically determined on potato cv. ′Mondial′, with the derived value being used to determine the application interval using the life cycle of the nematode within the concept of “week-of-30-day-month”.

CHAPTER 3
MEAN CONCENTRATION STIMULATION POINT OF NEMARIOC-AL PHYTONEMATICIDE ON POTATO CULTIVAR 'MONDIAL G3'

3.1 Introduction

Worldwide, since the withdrawal of fumigant synthetic chemical nematicide in 2005 from the agrochemical markets, botanicals had been in the forefront for testing as alternatives for use in the management of root-knot (*Meloidogyne* species) nematodes (Mashela *et al.,* 2017). In South Africa, fruits of wild cucumber (*Cucumis myriocarpus* Naude.) were extensively tested as dried and fermented crude extracts and was technically referred to as Nemarioc-AL phytonematicide in liquid formulation (Pelinganga and Mashela, 2012) and Nemarioc-AG phytonematicide in granular formulation (Mashela, 2002). Generally, the liquid and granular formulations were formulated for smallholder and large commercial farming systems (Mashela *et al*., 2017).

Nemarioc-AL phytonematicide had been highly effective in the management of *Meloidogyne* species on different crops under diverse conditions (Mashela *et al*., 2017). However, the product tends to induce phytotoxicity on certain plants and requires the development of non-phytotoxic concentrations (Mashela *et al.,* 2017). The latter, technically referred to as Mean Concentration Stimulation Point (MCSP), is generated using biological indices from the Curve-fitting Allelochemical Response Data (CARD) computer-based model (Mashela *et al*., 2015), which was developed in Australia (Liu *et al*., 2003). The MCSP is that concentration of the phytonematicide that would suppress population densities of nematodes without inducing phytotoxicity when applied at empirically-based time intervals (Mashela *et al.,* 2017). Generally, the MCSP is crop-specific within the stimulation phase of the cucurbitacin-containing phytonematicides and should therefore be established for separate plant species (Mashela *et al*., 2015). Potato (*Solanum tuberosum* L.) plants, within the same genus as tomato (*Solanum lycopersicum* L.), are highly susceptible to *Meloidogyne* species (Onkendi and Moleleki, 2013; Tseke and Mashela, 2018). Currently, there is no potato genotype that is resistant to *Meloidogyne* species (Marais *et al.,* 2015). Due to various successful incidents in the management of *Meloidogyne* species in tomato production using Nemarioc-AL phytonematicide, it was assumed that the product could also be useful in the management of this genus in potato production. However, due to the crop-specificity of the product, it was imperative that the MCSP of Nemarioc-AL phytonematicide on potato cv. 'Mondial G3' be established prior to its use in potato production.

3.2 Materials and methods

3.2.1 Description of study site

The study was conducted under microplot 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 study was initiated during autumn (February-April) in 2017 (Experiment 1) and repeated in 2018 (Experiment 2).

3.2.2 Treatments and research design

Treatments comprised 0, 2, 4, 8, 16, 32 and 64% Nemarioc-AL phytonematicide, arranged in a randomised complete block design, where blocking was against the

Casuariana cunninghamiana windbreak trees that shaded the trial before 10h00 am, with 11 replications.

3.2.3 Procedures

Matured fruit of *C. myriocarpus* were collected locally and cut into small pieces and dried in air-forced oven at 52°C for 72 h (Mashela *et al.,* 2017). The material was ground in a Wiley mill through a 1-mm sieve and then finely powdered using A43 Monlinex coffee powder. Ground material was stored at a room temperature in hermitically sealed plastic bags for future use. Approximately, 80 g ground material of *C. myriocarpus* powdered meal was fermented in 20 L hermetically-sealed plastic container with 16 L chlorine-free water. Approximately 300 ml molasses, 100 g sugar and 300 ml effective micro-organisms (EM) were added into the container (Pelinganga *et al.,* 2012; Tseke, 2013). The released CO_2 from the container was allowed to escape through an airtight system comprising a tube with one end glued to a hole on the lid of 20 L container whereas the outlet end dangled into a 2 L bottle half- filled with tapwater.

Microplot experiment was established by inserting 20-cm-diameter plastic pots into 15-cm-deep holes at 1.0 m × 1.0 m spacing (Figure 3.1). Each pot was filled with steam pasteurised soil and Hygromix at 3:1 (v/v) ratio with one sprouted tuber inserted to 10 cm deep/pot. *Meloidogyne javanica* inoculum was prepared by extracting eggs and second-stage juveniles (J2) from roots of greenhouse-grown nematode-susceptible tomato cv. 'Floradade' in 1% NaOCl solution (Hussey and Baker, 1973). After 100% emergence (2 weeks), each pot was infested with 5 000 *M. javanica* eggs and J2 using a 20 ml syringe by placing into 3 cm-deep holes on the cardinal points of the plants. Nemarioc-AL phytonematicide was applied weekly at appropriate concentrations as a substitute for irrigation. Plants were fertilised at 100% emergence with 5 g NPK 2:3:2 (22) + 0.5% S + 5% Zn + 5% Ca/plant and 1 g NPK 2:1:2 (43)/L water for providing a mixture of macro- and micro-nutrient elements, without Ca. The plants were sprayed every week for disease management by altering Steward, Bravo, Funginex and Dithane M45, whereas insect pests were scouted and monitored on daily basis.

Figure 3.1 Trial layout of Nemarioc-AL phytonematicide on potato cv. 'Mondial G3' under microplot conditions.

3.2.4 Data collection

At 56 days after initiating the treatments, plant height was measured from the crown to the tip of the flag leaf and the stem diameter was measured at 3 cm above the severed ends using a digital Vernier calliper. Chlorophyll content on matured leaves per plant was measured using chlorophyll meter. Tubers were harvested and weighed, shoots were separated from the tubers and oven dried at 52°C for 72 h and dry shoot and tuber mass was weighed. Leaf samples were analysed for Fe, K, Na and Zn (ICPE-9000). Approximately, 0.4 g ground leaf tissues of potato were digested in a vessel with two reagents namely 5.0 ml HNO_3 (nitric acid) and 3.0 ml H_2O_2 (hydrogen peroxide) in a microwave digester. The mixture was stirred with clean PTFE and allowed to settle for 10 minutes before closing the vessel.

Roots were removed from soil and immersed in water to remove soil particles and weighed to facilitate calculation of nematode densities per total roots per plant. Nematodes were extracted from the entire root system using maceration and blending method for 60 seconds in 1% NaOCl solution (Hussey and Barker, 1973). The aliquot was passed through 75- and 25-µm nested sieves and nematodes were collected from the 25-µm mesh sieve. Soil/pot was mixed thoroughly, and a 250 ml soil sample was collected with J2 extracted from soil samples using the sugar floatation and centrifugation method (Jenkins, 1964). Eggs and J2 from root samples and soil samples were counted from a 5-ml aliquot under the stereomicroscope (Leica Zoom 2000-Model no: Z45V240Vac) at 5.0 magnification. Nematode numbers from root samples were converted to nematodes per total root system per plant, whereas J2 from soil sample were converted to the volume of the growing mixture 2700 mg/pot to allow for the determination of the final nematode population density (Pf).

3.2.5 Data analysis

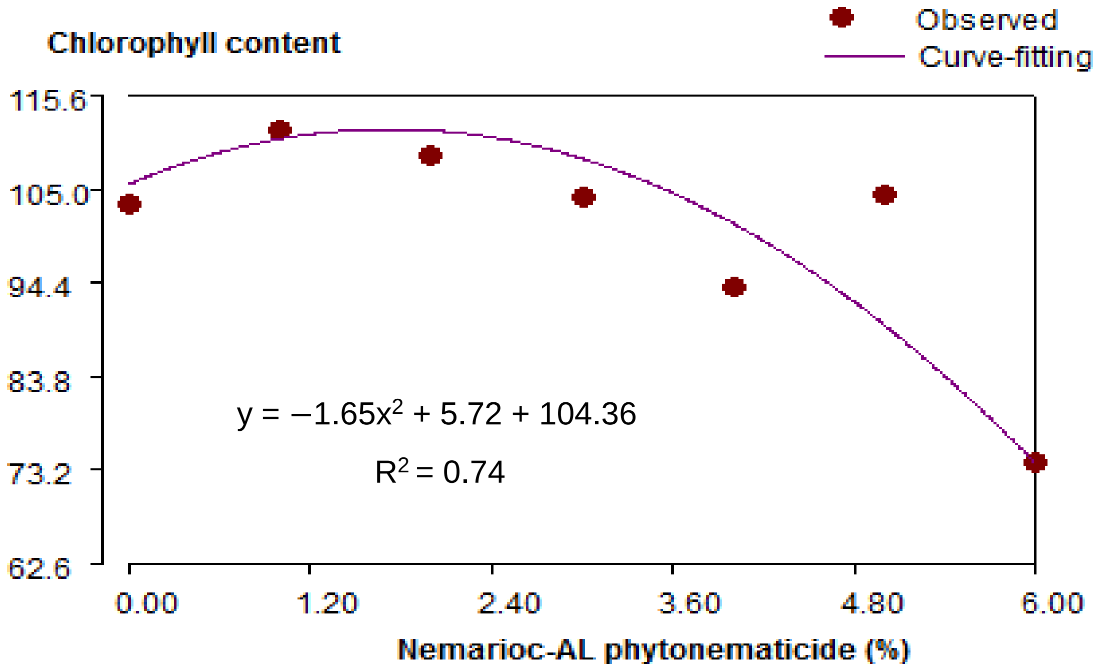
Data were analysed using Statistix 10.0 software to generate the plant growth, nematodes and nutrient element means. Plant growth and nutrient element means were subjected to the CARD model to generate the density-dependent growth (DDG) curves and the biological indices, where D_m and R_h, were used for calculating MCSP values (Liu *et al.,* 2003; Mashela *et al.,* 2017). Discrete nematode and nutrient elements data were transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984). Prior to CARD model the concentration variables were log-transformed using $\log_2 2^x$ to generate 0, 1, 2, 3, 4, 5 and 6% phytonematicide which provided equal distances (x-axis), thereby avoiding the original challenges of points (y-axis) being overcrowded close to x = 0 (Causton, 1997). Means of nematode variables were subjected to the lines of the best fit.

3.3 Results

The seasonal interaction was not significant for all plant variables and therefore, the data for the two seasons were pooled (n = 154) and re-analysed as described.

3.3.1 Plant growth variables.

Chlorophyll content, stem diameter, dry root mass and dry tuber mass versus increasing concentrations of Nemarioc-AL phytonematicide exhibited positive quadratic relation with the relations explained by 74, 84, 58 and 91% associations, respectively (Figure 3.2; Figure 3.3). The generated biological indices from the CARD model were used for computation of MCSP. Using the relation MCSP = $D_m + R_h$, the MCSP value was 4.31% with the overall sensitivity ($\sum k$) of 18 units (Table 3.1). Plant height and dry shoot mass versus increasing concentration of Nemarioc-AL phytonematicide exhibited negative quadratic relations which could not be used for calculating MCSP (Figure 3.3).



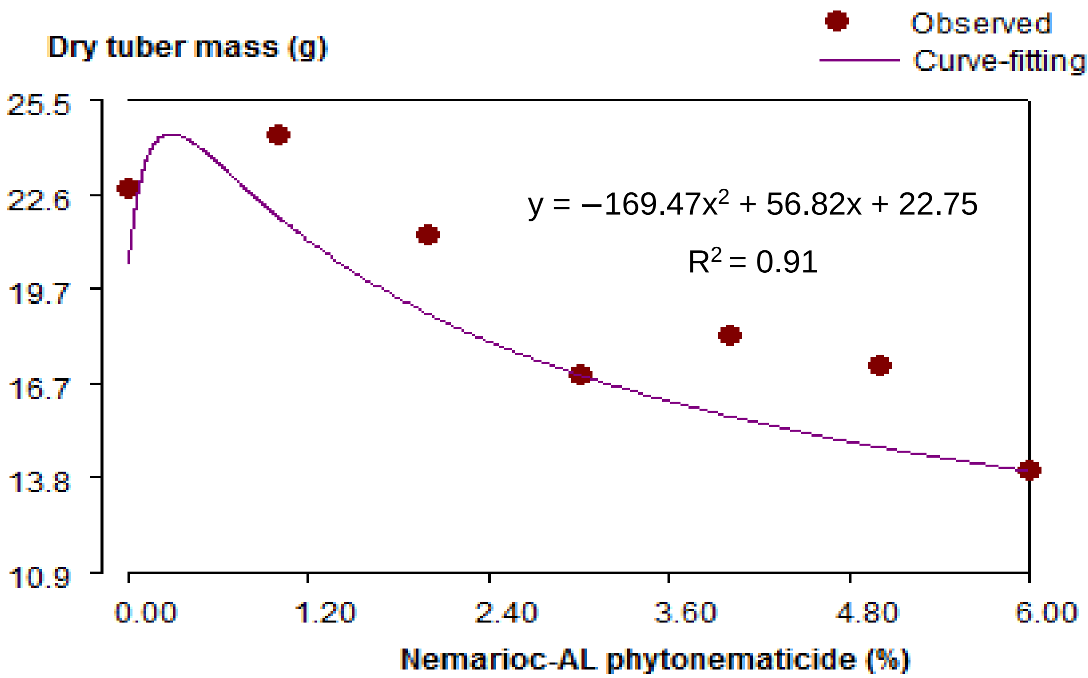
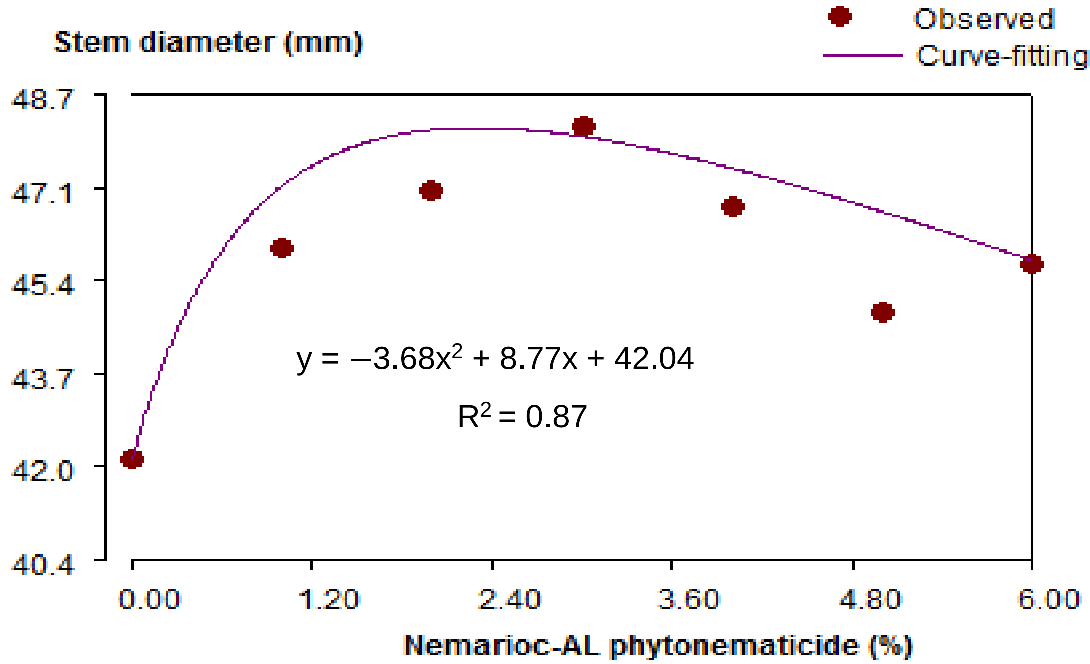


Figure 3.2 Response of chlorophyll and dry tuber mass of potato cv. 'Mondial G3' to concentrations of Nemarioc-AL phytonematicide at 56 days after the treatments.



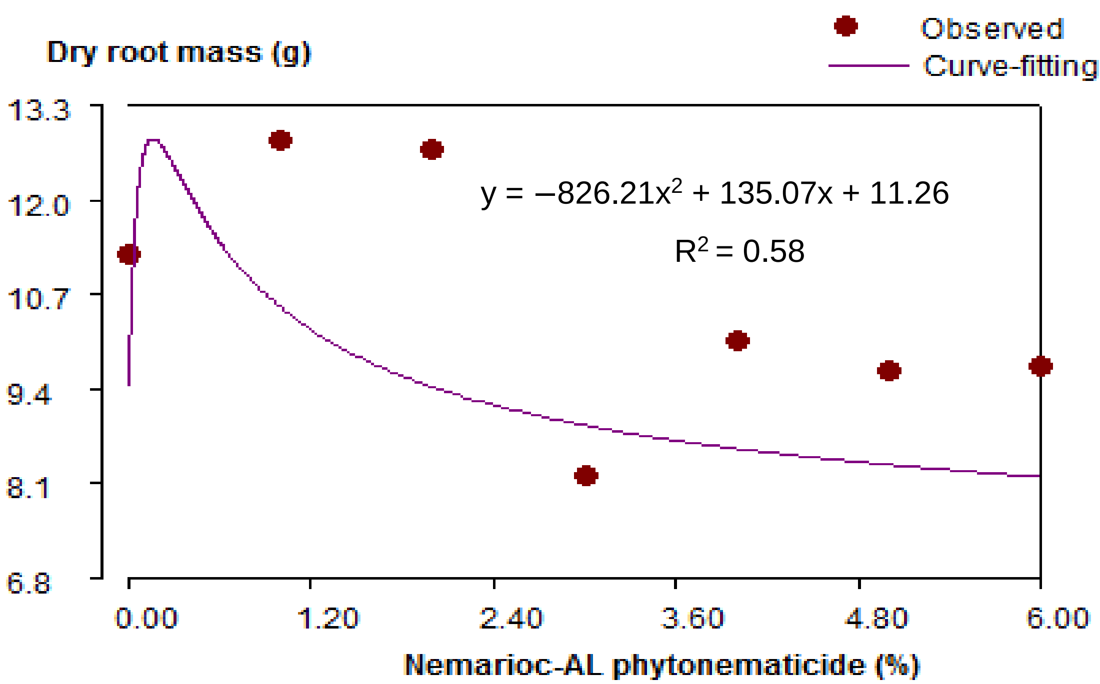


Figure 3.3 Response of stem diameter and dry root mass of potato cv. 'Mondial G3' to concentrations of Nemarioc-AL phytonematicide at 56 days after the treatments.

Table 3.1 Biological indices for chlorophyll (CHL), stem diameter (STD), dry tuber mass (DTM) and dry root mass (DRM) on potato cv. 'Mondial G3' to increasing concentrations of Nemarioc-AL phytonematicide at 56 days after treatments.

Biological indices	CHL	STD	DTM	DRM	Mean
Threshold stimulation (D_m)	1.73	2.29	2.82	0.16	1.75
Saturation point (R_h)	4.95	5.23	4.76	5.52	5.11
0% inhibition (D_0)	3.46	9.85	1.41	1.96	4.17
50% inhibition (D_{50})	7.61	46.64	14.38	–	22.87
100% inhibition (D_{100})	9.9	117.8	–	–	63.85
R^2	0.74	0.87	0.91	0.58	
k value	0	1	5	12	
Overall sensitivity ($\sum k$) = 18					
MCSP = D_m + R_h/2 = 1.75 + 5.11/2 = 4.31.					

3.3.2 Nematode variables

Eggs in roots, J2 in roots and soil and final nematode population versus increasing concentration of Nemarioc-AL phytonematicide exhibited negative quadratic relation with the models explained by 81, 87, 91 and 95%, respectively (Figure 3.4, Figure 3.5, Figure 3.6 and Figure 3.7). Using $x = -b_1/2b_2$ the nematodes variables were optimised at 14.43, 28.23, 23.30 and 13.55% in eggs, J2 in roots, J2 in sol and final nematode population, respectively (Table 3.2).

Table 3.2 Optimisation models of *Meloidogyne javanica* in root and soil to increasing concentrations of Nemarioc-AL phytonematicide at 56 days after treatments.

Variable	Model	R^2	x (%)^z
Eggs	$y = 0.0091x^2 - 0.1314x + 2.5128$	0.81	14.43
J2 in roots	$y = 0.0034x^2 - 0.096x + 2.5407$	0.87	28.23
J2 in soil	$y = 0.0033x^2 - 0.0769x + 3.376$	0.92	23.30
Final nematode population	$y = 0.0074x^2 - 0.1003x + 3.5472$	0.95	13.55

^{z}Calculated optimum response concentration $x = -b_1/2b_2$.

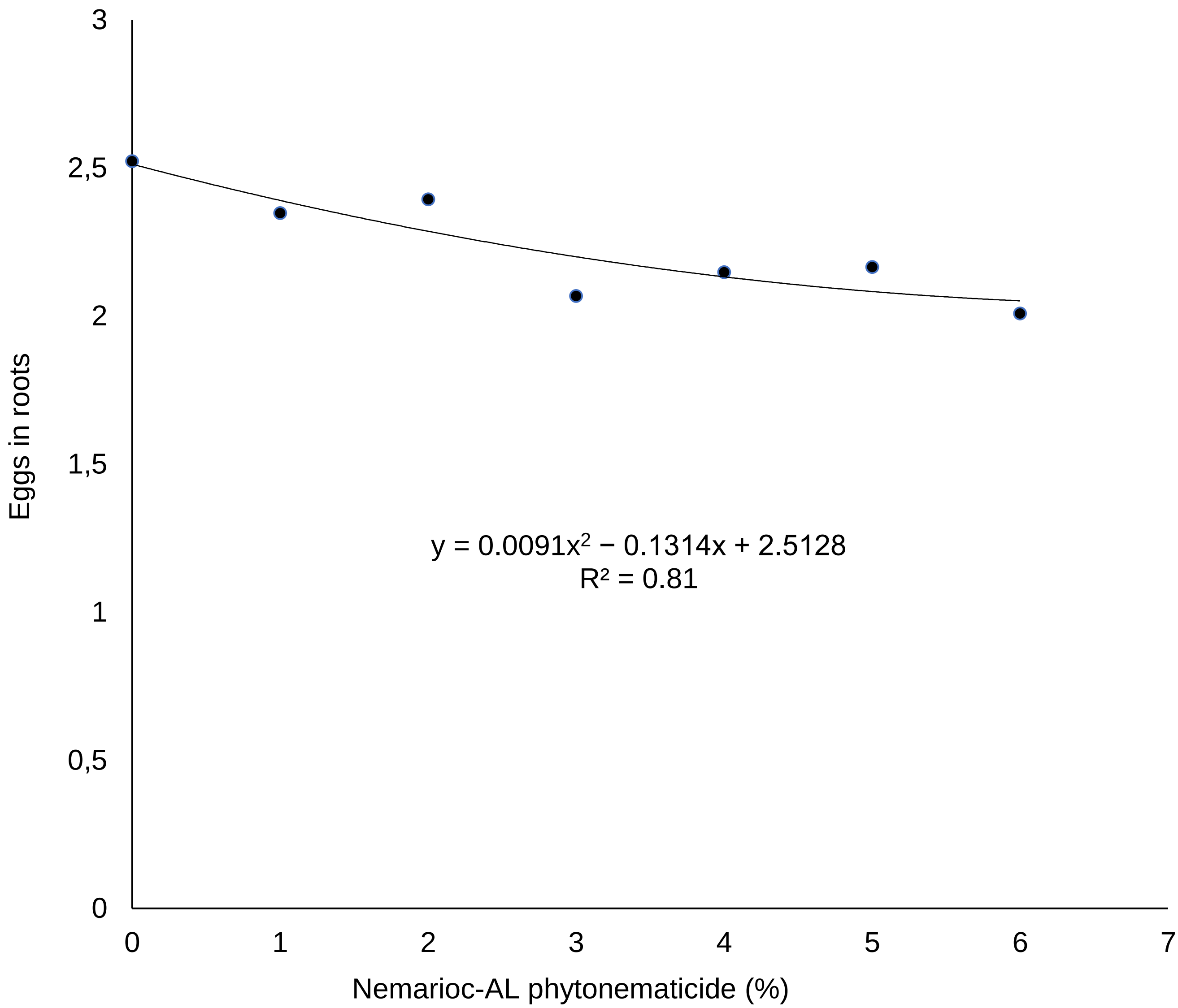


Figure 3.4 Influence of Nemarioc-AL phytonematicide on final nematode population of *Meloidogyne javanica* at 56 days after treatment.

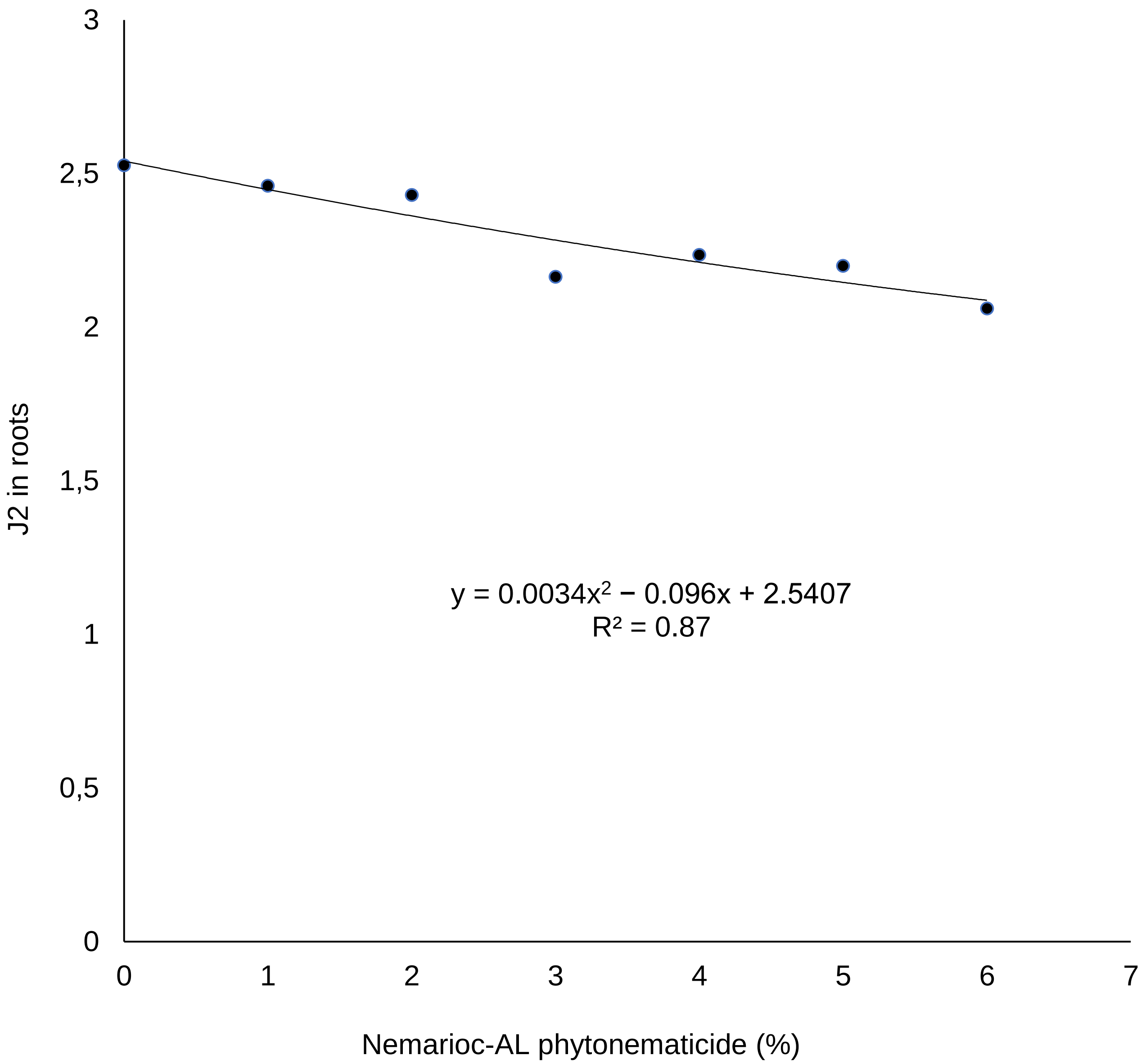


Figure 3.5 Influence of Nemarioc-AL phytonematicide on J2 in roots of *Meloidogyne javanica* at 56 days after treatment.

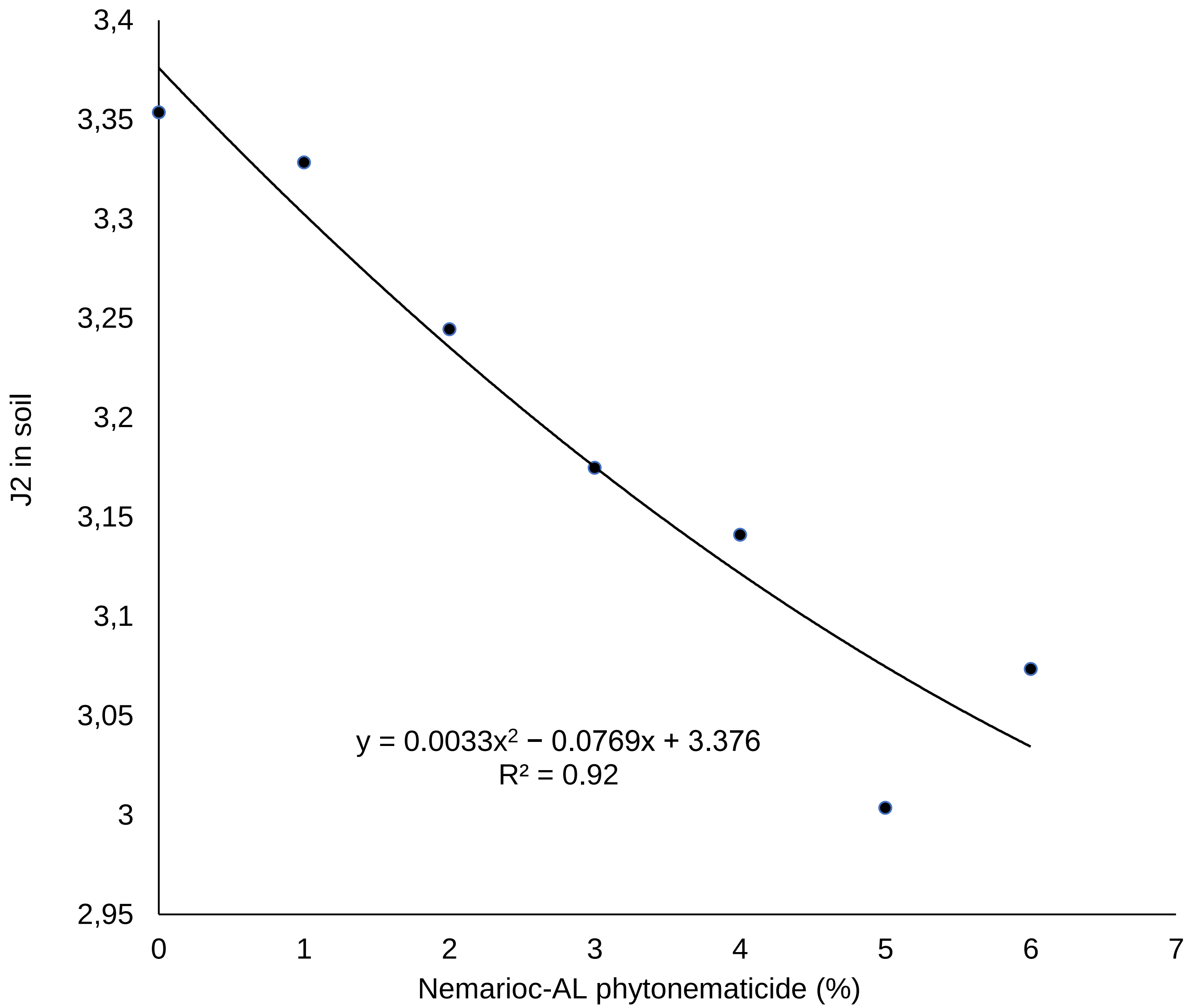


Figure 3.6 Influence of Nemarioc-AL phytonematicide on J2 in soil of *Meloidogyne javanica* at 56 days after treatments.

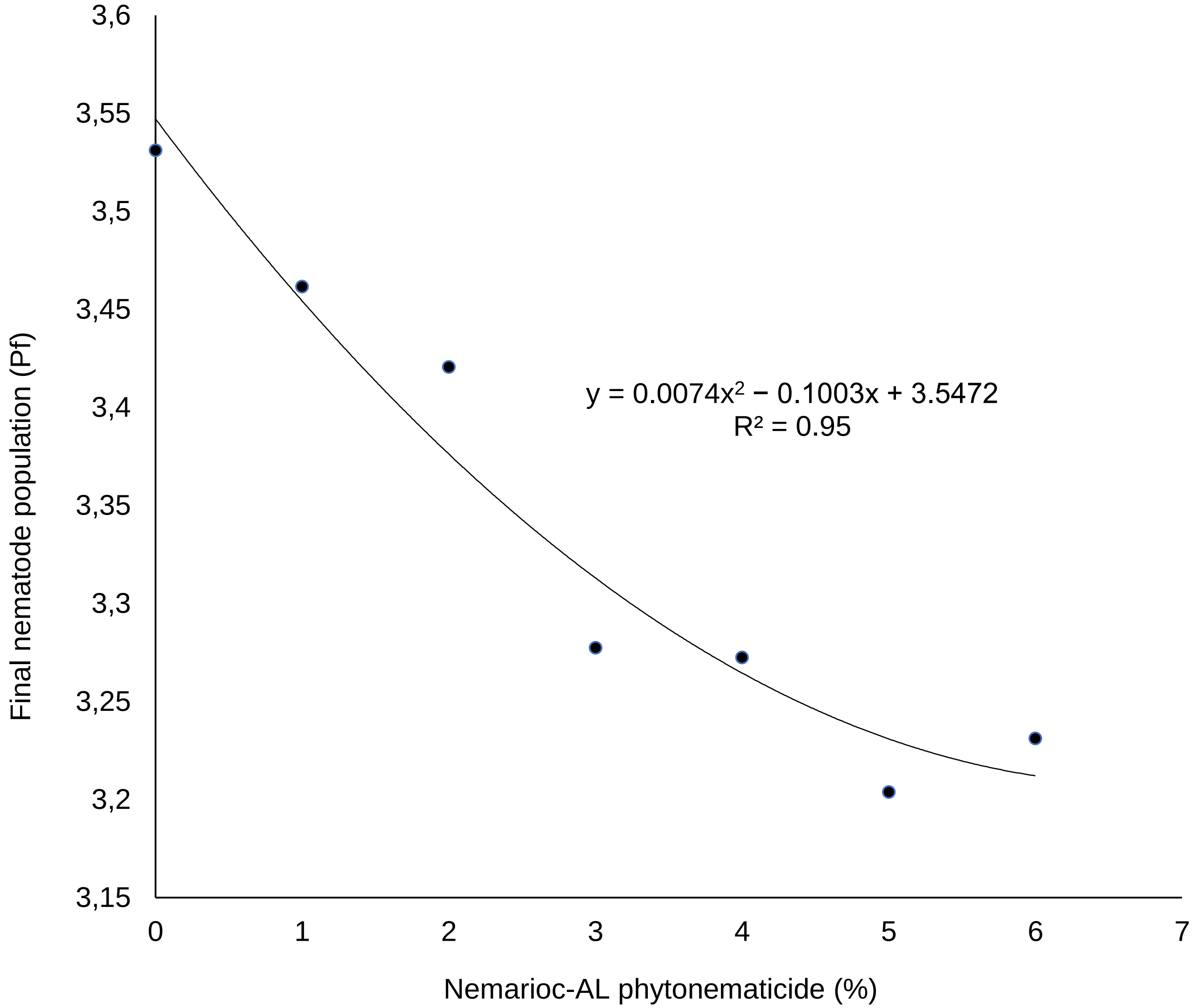


Figure 3.7 Influence of Nemarioc-AL phytonematicide on final nematode population of *Meloidogyne javanica* at 56 days after treatments.

3.3.3 Nutrient elements variables

Iron, K and Zn in leaf tissues of potato cultivar 'Mondial G3' versus increasing concentrations of Nemarioc-AL phytonematicide exhibited positive quadratic relations with the models explained by 95, 68 and 80%, respectively (Figure 3.8 and Figure 3.9). The generated biological indices from the CARD model were used for computation of MCSP (Table 3.3). Sodium exhibited negative quadratic relation with model explained by 86% and cannot be used for calculation of MCSP. Using the relations MCSP = $D_m + R_h/2$ the MCSP value was 1.33% with the overall sensitivity ($\sum k$) of 4 units (Table 3.3).

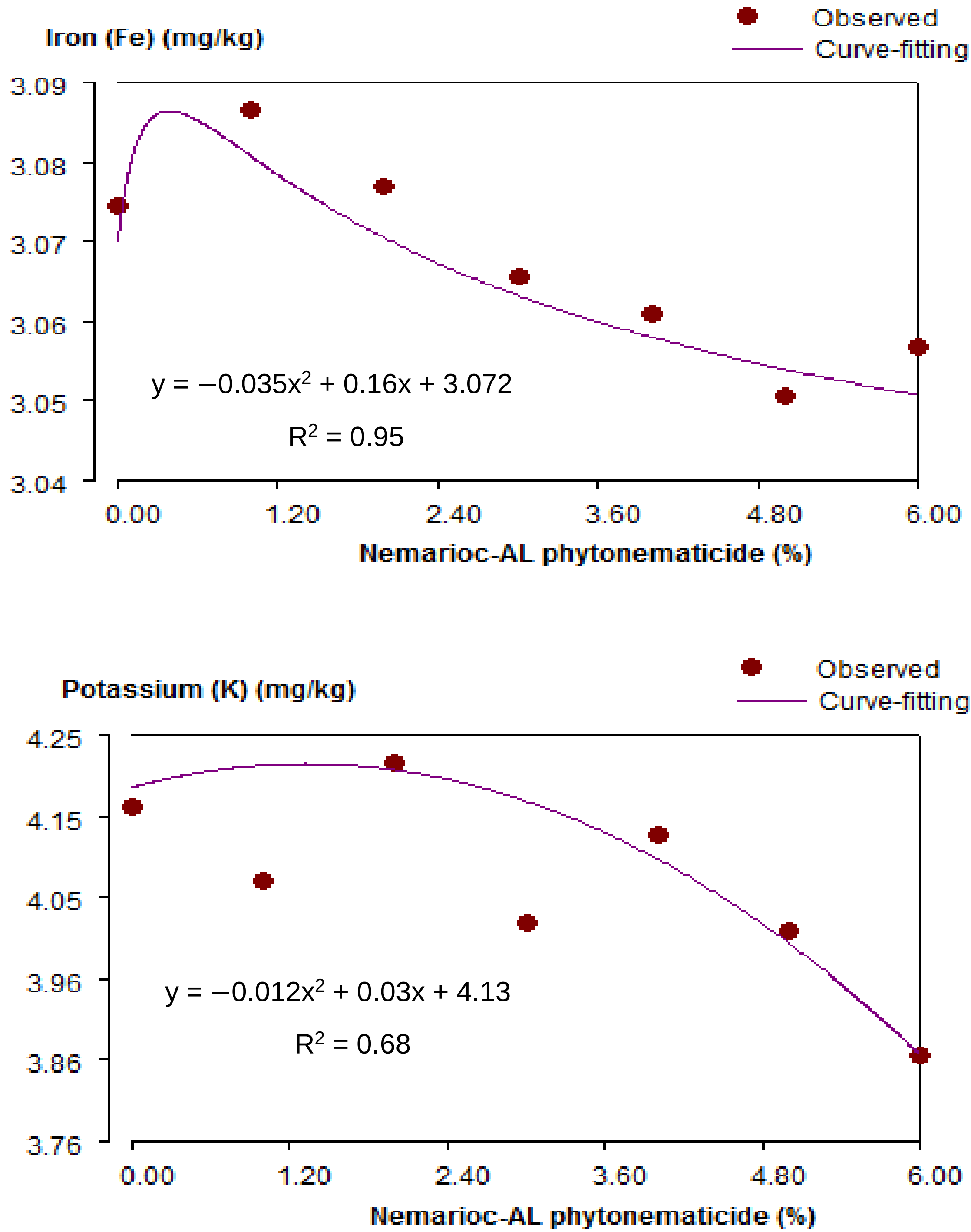


Figure 3.8 Responses of Fe and K in leaf tissues of potato cultivar 'Mondial G3' to increasing concentrations of Nemarioc-AL phytonematicide.

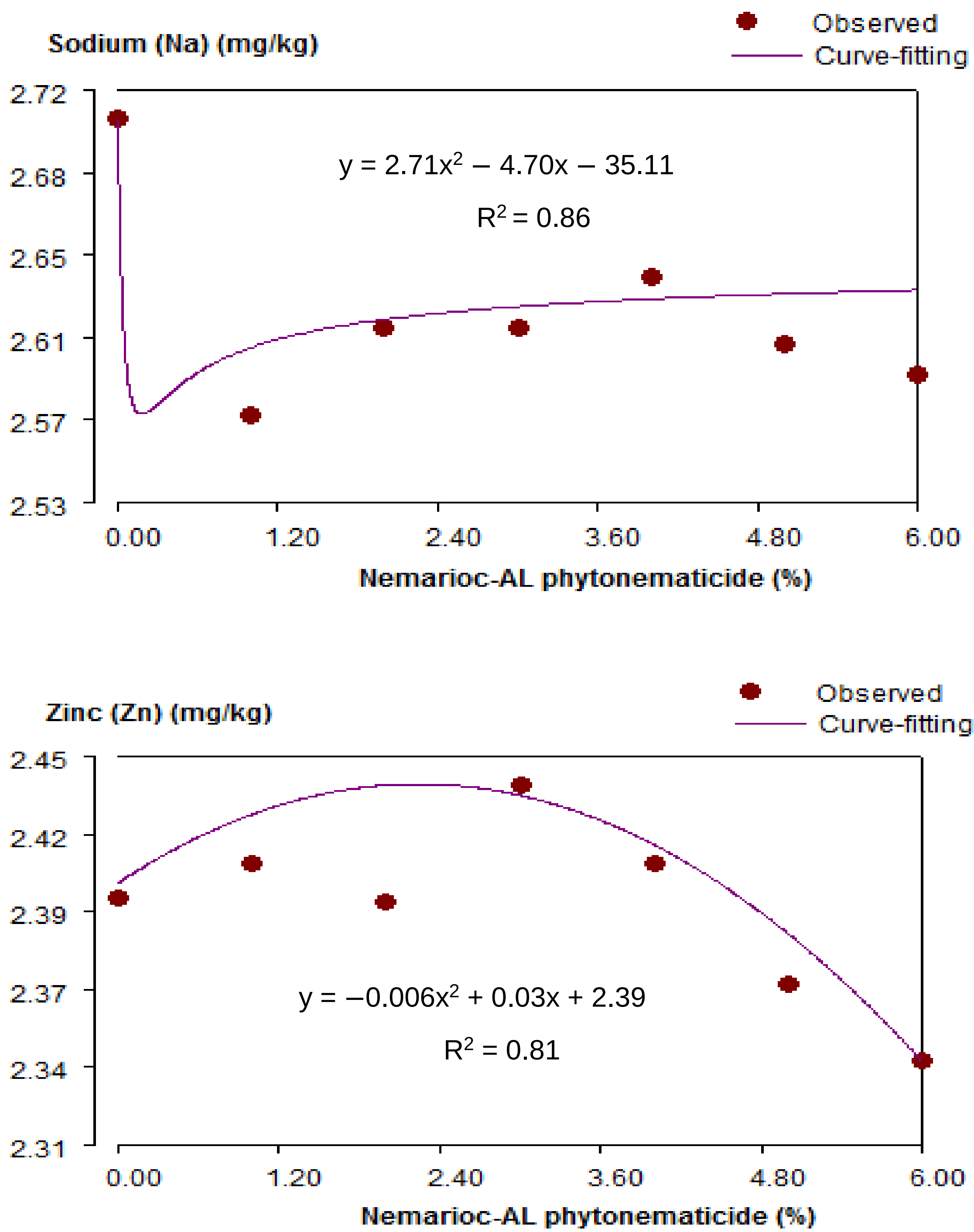


Figure 3.9 Responses of Na and Zn in leaf tissues of potato cultivar 'Mondial G3' to increasing concentrations of Nemarioc-AL phytonematicide.

Table 3.3 Biological indices for iron (Fe), potassium (K) and zinc (Zn) on potato cv. 'Mondial G3' to increasing concentrations of Nemarioc-AL phytonematicide at 56 days after treatments.

Biological index	Fe	K	Zn	Mean
Threshold stimulation (D_m)	0.39	1.32	2.24	1.32
Saturation point (R_h)	0.02	0.02	0.03	0.02
0% inhibition (D_0)	2.04	2.65	4.48	3.05
50% inhibition (D_{50})	–	14.71	16.94	15.82
100% inhibition (D_{100})	–	20.20	22.9	21.55
R^2	0.95	0.68	0.81	
k value	4	0	0	
Overall sensitivity ($\sum k$) = 4				
MCSP = $D_m + (R_h/2) = 1.32 + (0.02/2) = 1.33\%$.				

3.4 Discussion

3.4.1 Plant variables

Chlorophyll content, stem diameter, dry root mass and dry tuber mass exhibited positive quadratic relations when plotted against increasing concentrations of Nemarioc-AL phytonematicide. High co-efficient of determination (R^2) for the CARD model on plant variables suggested strong density-dependent growth (DDG) patterns of potato and increasing concentrations of Nemarioc-AL phytonematicide. Similar results were reported where plant variables of tomato cv. 'Floradade' exhibited positive quadratic equations showing three phases of DDG pattern (Tseke and Mashela, 2018). These findings were supported by Pelinganga, (2013) where four plant variables, namely, dry shoot mass, dry root mass, plant height and stem diameter exhibited positive quadratic relations of tomato and increasing concentrations of crude extracts of *C. africanus* and *C. myriocarpus*. Furthermore, supported by Mafeo (2012), who reported high co-efficient of determination in variables of chive and increasing concentrations of crude extracts of *C. myriocarpus* ranging from 94-99%.

As concentrations of Nemarioc-AL phytonematicide increased, the existence of DDG patterns showing three phases confirming those of Mafeo (2012). Crude extracts of neem leaf at low concentrations stimulated growth of maize and tomato seedlings, whereas at high concentrations plant growth was reduced significantly (Agbenin *et al.,* 2005) which supported the inhibition phase observed at higher concentrations of Nemarioc-AL phytonematicide in this study.

The MCSP value for Nemarioc-AL phytonematicide on plant variables of potato cv. 'Mondial G3' was empirically derived as 4.31% being lower than that for *P. sidoides***,** which was at 6.18% (Sithole, 2016). The MCSP for tomato was at 2.63% (Pelinganga and Mashela, 2012), which was lower to MCSP derived in this study but the MCSP of 2.63% reduced nematode population densities which can be adopted as a concentration that can manage *Meloidogyne* species since these phytonematicides are not used as a fertiliser effect. In contrast increasing concentrations of Nemarioc-AL phytonematicide on beetroot (*Beta vulgaris* L.) resulted in MCSP which was much higher at 18.1% (Mashitoa, 2017). Mafeo (2012) explained that the relationships generated by CARD model is dependent of k value which is sensitivity and the lower the k value the more the sensitive is the plant and ‘the higher the k value the less sensitive is the plant to the material. Since the overall sensitivity index of potato on Nemarioc-AL phytonematicide was high at $\sum k = 18$, it shows that the plant was less sensitive to the test product.

Meyer *et al*. (2008) demonstrated that phytotoxic effects of plant extracts differ between crops where phytotoxic level depends on sensitivity of the crop to increasing concentrations of crude extracts. Overall sensitivity values corresponded with the results in three crops where overall sensitivity values for Nemarioc-AG phytonematicide were at 24 and 22 on chive (*Allium schoenoprasum*) and onion, respectively (Mafeo *et al.,* 2011a). Clove (*Syzygium aromaticum* L.) oil showed different effects on various plant type where musk melon (*Cucumis melo* L.) and pepper seedlings was least affected but showed significant effects on shoots heights and fresh mass compared to controls (Meyer *et al.,* 2008). Stimulated plant variables at low concentrations confirmed observations made in previous studies where Nemarioc-AL phytonematicide was used. Lower concentrations of Nemarioc-AL phytonematicide stimulated growth of potato cv. 'Mondial G3' supported by (Mafeo, 2012; Pelinganga 2013) which confirmed that small quantities of crude extracts from *Cucumis* species invariably stimulated growth of tomato while increasing concentrations reduced growth. Emergence of monocotyledonous and dicotyledonous plants was inhibited by increasing concentrations of Nemarioc-AG phytonematicide (Mafeo, 2012). Amongst the test crops from three different families (*Allieceae, Gramineae* and *Solanaceae*), onion was highly sensitive to Nemarioc-AG phytonematicide, whereas tomato and millet were the least sensitive (Mafeo, 2012).

3.4.2 Nematode variables

Nematodes in roots and soil with increasing concentrations of Nemarioc-AL phytonematicide exhibited negative quadratic relations because these variables were not significant. Based on three phases of DDG pattern nematodes can either be stimulated, neutral or inhibited (Tseke and Mashela, 2018). The results of this study contradicted that of Dube (2016), where increasing concentration of Nemarioc-AL phytonematicide at lower concentrations, stimulation of eggs and J2 in roots were observed whereas in this study all nematode variables were inhibited from lower to higher concentrations. Nemarioc-AL phytonematicide over increasing concentrations consistently reduced *M. javanica* on *P. sidoides* in roots and in soil (Sithole, 2016). Generally, efficacy of plant extracts is dependent to concentration and duration of exposure of the nematode to the extract (Agbenin *et al.,* 2005).

In all nematode variables optimum values were high where J2 in roots was higher with the minimum nematode population achieved at 28.23% which disagreed with Tseke and Mashela (2017), where J2 in eggs and roots were optimised at very low concentration, 0.22% and Nemarioc-AL phytonematicide suggested stimulation. However, the nematicidal properties of crude extracts of *C. myriocarpus* have been reported against *Meloidogyne* species on different plant species (Mafeo and Mashela, 2010; Mashela *et al.,* 2011; Mashela *et al.,* 2015; Mashela *et al.,* 2017; Sithole *et al.,* 2016). Nemafric-BL phytonematicide was shown to reduce nematode numbers on tomato plant at low concentrations compared to high concentrations (Tseke and Mashela, 2018) which agree with the results of the current study. In all nematode variables, the optimum concentration of Nemarioc-AL phytonematicide reduced nematode on the test plant compared to higher concentrations. Similarly, Nemarioc-AL phytonematicide reduced nematode population on tomato plant (Pelinganga *et al.,* 2013).

Mashela *et al*. (2011) demonstrated that at low concentrations crude extracts of *C. myriocarpus* suppressed nematode numbers and improved growth of tomato. According to Pelinganga *et al.* (2012), nematode population densities were reduced in roots and soil when fermented crude extracts of *C. myriocarpus* were applied at 0, 10, 20, 30, 40, 50 and 60%. *Meloidogyne incognita* was consistently reduced in roots and soil by ground *C. myriocarpus* and castor bean (*Ricinus communis* L.) (Mashela *et al.,* 2010). Agbenin *et al.* (2005), observed that at 20% crude extracts of garlic (*Allium sativum* L.) bulb successfully reduced nematode population without having effects on tomato plant growth which differ with the results of this study where concentration that reduced population densities of nematodes without reducing plant growth was very low at 4.31% and these differences are explained by concentrations being crop-specific.

3.4.3 Nutrient elements

Quadratic relations were positive against the increasing concentrations of Nemarioc-AL phytonematicide exhibiting three phases of DDG patterns namely stimulation, neutral and inhibition phase. Mashela and Pofu (2017) observed that Na, Fe and K against increasing concentrations of Nemarioc-AL phytonematicide exhibited positive quadratic relations whereas Na and Zn exhibited negative quadratic relations which agree with the observations in this study except for Zn which exhibited positive quadratic relations in this study. Results observed in this study shows that Fe, K and Zn start off with stimulation through neutral to the inhibition phase except for Na which start by reducing over increasing concentrations of Nemarioc-AL phytonematicide. Na in leaf tissues of potato exhibited negative quadratic relation where stimulation phase was not observed which disagree with the results reported by Mashela and Pofu (2017) where Nemafric-BL phytonematicide on green bean exhibited positive quadratic relation showing three phases of DDG pattern starting off with stimulation phase. The CARD model demonstrated that the MCSP of Nemarioc-AL phytonematicide to the essential nutrients was empirically derived as 1.33% which is comparable to the lowest concentrations that have been derived and recommended in previous studies in the range of 2-3 % (Sithole, 2016; Tseke, 2013).

3.5 Conclusion

Nemarioc-AL phytonematicide exhibited phytotoxicity over increasing concentrations on potato cv 'Mondial G3' and CARD demonstrated that the non-phytotoxic concentration is 4.31%. The achieved MCSP for management of *M. javanica* population densities on potato cv. 'Mondial G3' which was at 4.31% should be adopted and used since nematode population densities were reduced at 4.31% while stimulating growth of potato plant. It is important that MCSP of Nemarioc-AL phytonematicide be established on different cultivars of potato and under different environmental conditions.

CHAPTER 4
APPLICATION INTERVAL OF NEMARIOC-AL PHYTONEMATICIDE ON POTATO CULTIVAR 'MONDIAL G3'

4.1 Introduction

Many botanicals have been tested in granular and liquid formulations for use in nematode management (Ahmad *et al.,* 2010; Mashela *et al.,* 2015; Moosavi, 2012). Most plant extracts that had nematicidal properties and reduced nematode population densities successfully, were phytotoxic to the protected crops (Anese *et al.,* 2015; Mashela *et al.,* 2017; Pelinganga *et al.,* 2012; Zasada *et al.,* 2002). Phytotoxicity had been resolved using the Curve-fitting Allelochemical Response Data model (CARD), from which two biological indices were used to compute the mean concentration stimulation point (MCSP), which was established as 4.31% for Nemarioc-AL phytonematicide on potato cv. 'Mondial G3'. Upon the establishment of the MCSP, this should be used, along with the life cycle of the nematode to derive the application interval (Mashela *et al*., 2017). The objective of the study was to investigate the application interval of Nemarioc-AL phytonematicide on potato cv. 'Mondial G3' for managing *Meloidogyne javanica* under microplot conditions.

4.2 Materials and methods

4.2.1 Description of study site

The study was conducted under microplot conditions at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, South Africa (23°53'10''S, 29°44'15''E). The study was initiated in spring (August-September) 2017 (Experiment 1) and validated in spring 2018 (Experiment 2).

4.2.2 Treatments and research design

Treatments, namely, application time interval at 1-, 2-, 3- and 4-weeks-of-30-day month (Mashela *et al.,* 2017), arranged in a randomised complete block design, with eight replications for Experiment 1 and seven replications for Experiment 2.

4.2.3 Procedures

The experiments were established under microplot conditions (Figure 4.1) as described previously (Chapter 3). Also, fertilisation, nematode inoculation, irrigation, disease management and preparation of Nemarioc-AL phytonematicide, were as described previously (Chapter 3). The phytonematicide was applied at 4.31% Nemarioc-AL phytonematicide at 7.5, 15, 22.5 and 30 days (Mashela *et al*., 2017).

Figure 4.1 Trial layout of Nemarioc-AL phytonematicide on potato cv. 'Mondial G3' under microplot conditions.

4.2.4 Data collection

At 56 days after the treatments, plant, minerals and nematode variables were collected as described previously (Chapter 3).

4.2.5 Data analysis

Nematode variables were subjected to analysis of variance (ANOVA) through Statistix 10.0. Plant variables data were analysed using Statistix 10.0 to generate means. The degrees of freedom and associated mean sum of squares for nematode variables were partitioned to provide the total treatment variation (TTV) for different sources of variation. Discrete nematode and nutrient elements data were transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984). Plant variables means were subjected to lines of the best fit using plant growth response to increasing time application intervals and modelled by regression curve estimations resulting in quadratic equation $Y = b_2x^2 + b_1x + a$ relation, where Y = plant growth response, x = application time interval and $x = -b_1/2b_2$ for optimum application time interval (Pelinganga, 2013). Means of nutrient elements were subjected to the lines of the best fit.

4.3 Results

The seasonal interaction was not significant for all plant variables and therefore, the data for the two seasons were pooled (n = 60) and re-analysed as described.

4.3.1 Plant variables

Plant height, stem diameter and dry shoot mass versus application interval exhibited positive quadratic relations, with the models being explained by 74% (Figure 4.2), 96% (Figure 4.3) and 89% (Figure 4.4), respectively. The three plant variables were optimised at different application intervals with the mean of 2.43 weeks/month, which translated to 18 days (2.43/4 × 30 days) (Table 4.1).

Table 4.1 Quadratic relationship, coefficient of determination and computed optimum application time to 4.31% of Nemarioc-AL phytonematicide on plant height, stem diameter and dry shoot mass of potato 'Mondial G3' at 56 days after treatments.

Variable	Formula	R^2	x^z
Plant height (cm)	$y = -2.108x^2 + 8.346x + 34.05$	0.74	1.97
Stem diameter (mm)	$y = -0.225x^2 + 0.934x + 3.68$	0.96	2.12
Dry shoot mass (g)	$y = -0.694x^2 + 4.464x + 5.71$	0.90	3.21
Mean integrated application time interval 2.43			

^{z}Calculated optimum response concentration, $x = -b_1/2b_2$.

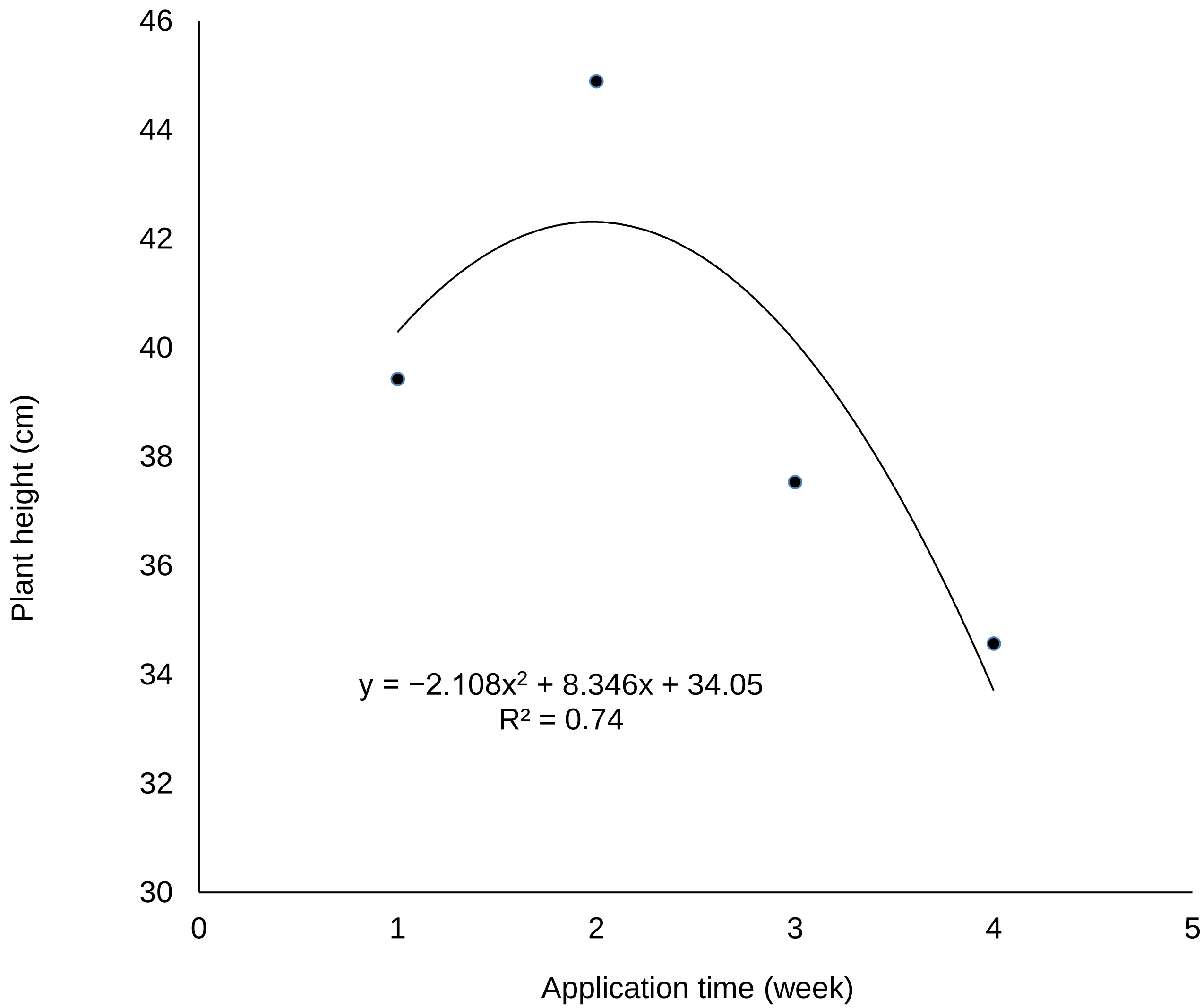


Figure 4.2 Influence of application time of 4.31% Nemarioc-AL phytonematicide on plant height of potato cv. 'Mondial G3' at 56 days after treatment.

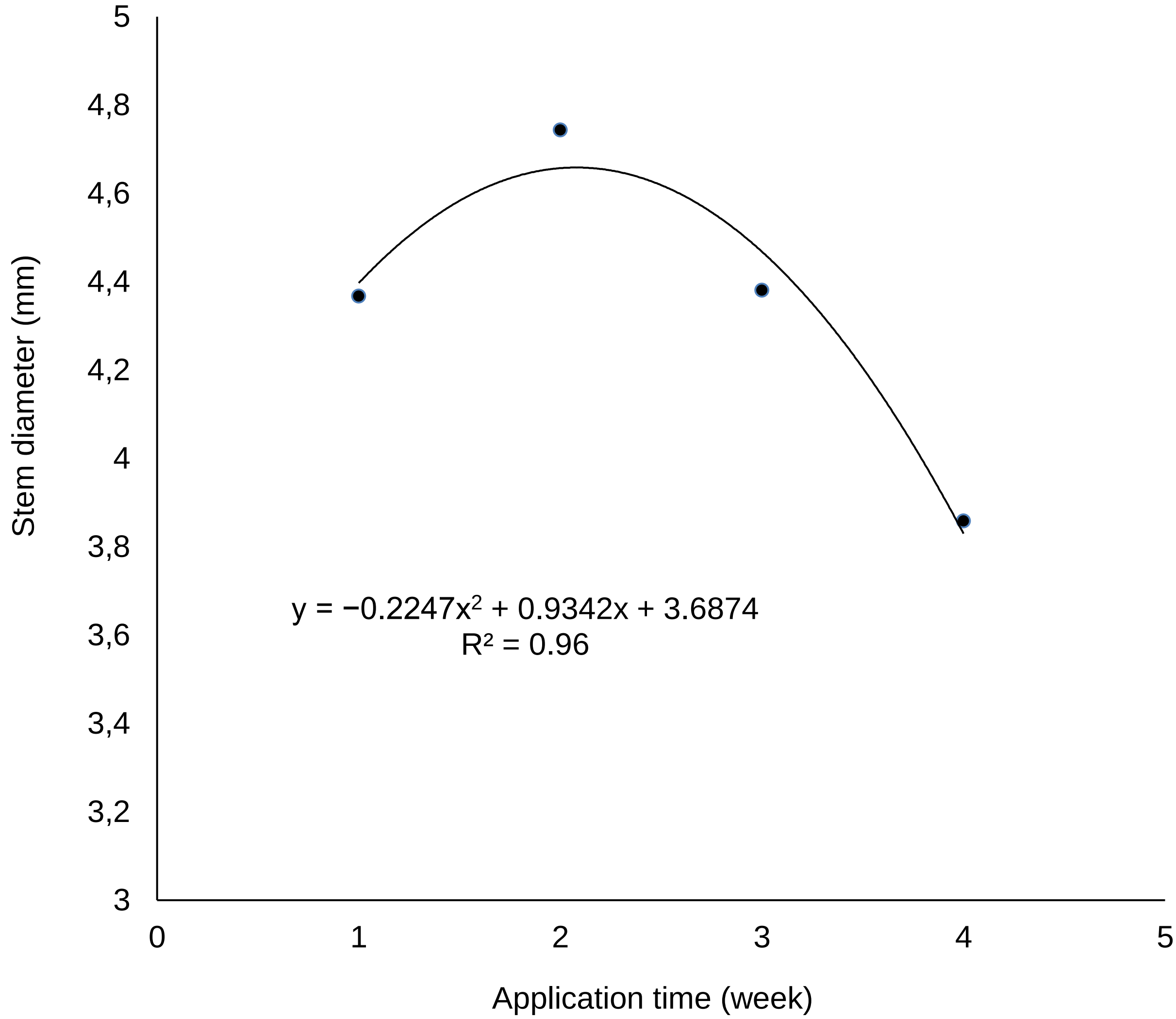


Figure 4.3 Influence of application time of 4.31% Nemarioc-AL phytonematicide on plant height of potato cv. 'Mondial G3' at 56 days after treatment.

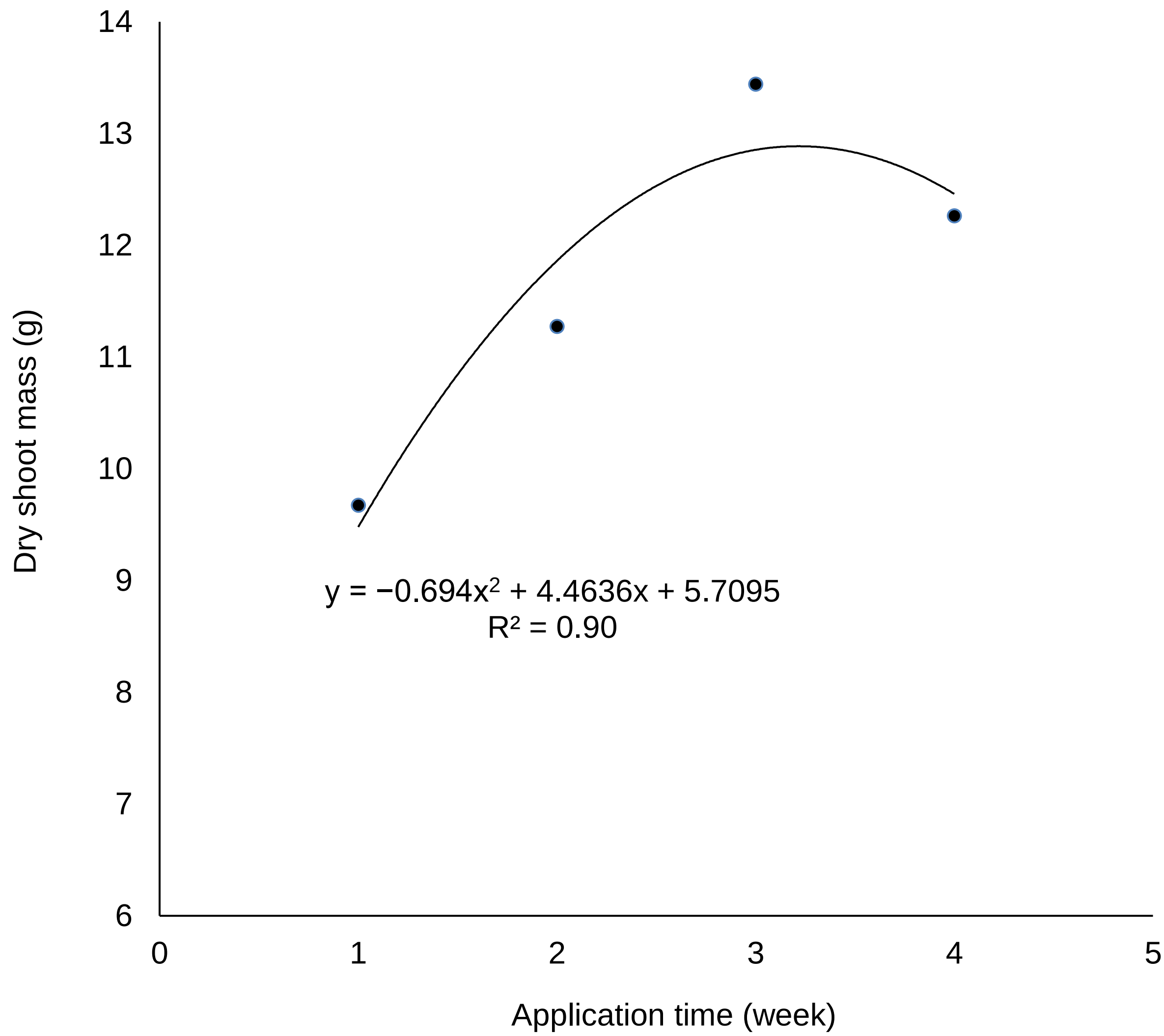


Figure 4.4 Influence of application time of 4.31% Nemarioc-AL phytonematicide on dry shoot mass of potato cv. 'Mondial G3' at 56 days after treatment.

4.3.2 Nematode variables

Nemarioc-AL phytonematicide at 4.31% applied at 1, 2, 3 and 4 weeks interval did not have significant effect on all eggs, J2 in roots and soil and total nematode population (Table 4.2) (Appendix 4.1-4.4).

Table 4.2 Partitioning mean sum of squares for *Meloidogyne javanica* eggs, second stage juveniles (J2) and final population (Pf) at application interval of 4.31% Nemarioc-AL phytonematicide on potato cultivar 'Mondial G3' 56 days after treatments.

		$Eggs_{roots}$		$J2_{roots}$		$J2_{soil}$		Pf	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	14	0.46	37	0.15	16	1.44	60	0.56	56
Treatment	3	0.29	23^{ns}	0.28	31^{ns}	0.26	11^{ns}	0.17	17^{ns}
Error	42	0.49	40	0.48	53	0.71	29	0.27	27
Total	59	1.24	100	0.91	100	2.41	100	1.0	100

^{ns}Not significant at $P \leq 0.05$.

4.3.3 Nutrient elements

Iron, Na and K in each leaf tissues of potato cultivar 'Mondial G3' against application time interval of Nemarioc-AL phytonematicide exhibited positive quadratic relations with the models explained by 83, 92, and 99% respectively (Figure 4.5; Figure 4.6; Figure 4.7) except for Zn which exhibited negative quadratic relation (Figure 4.8).

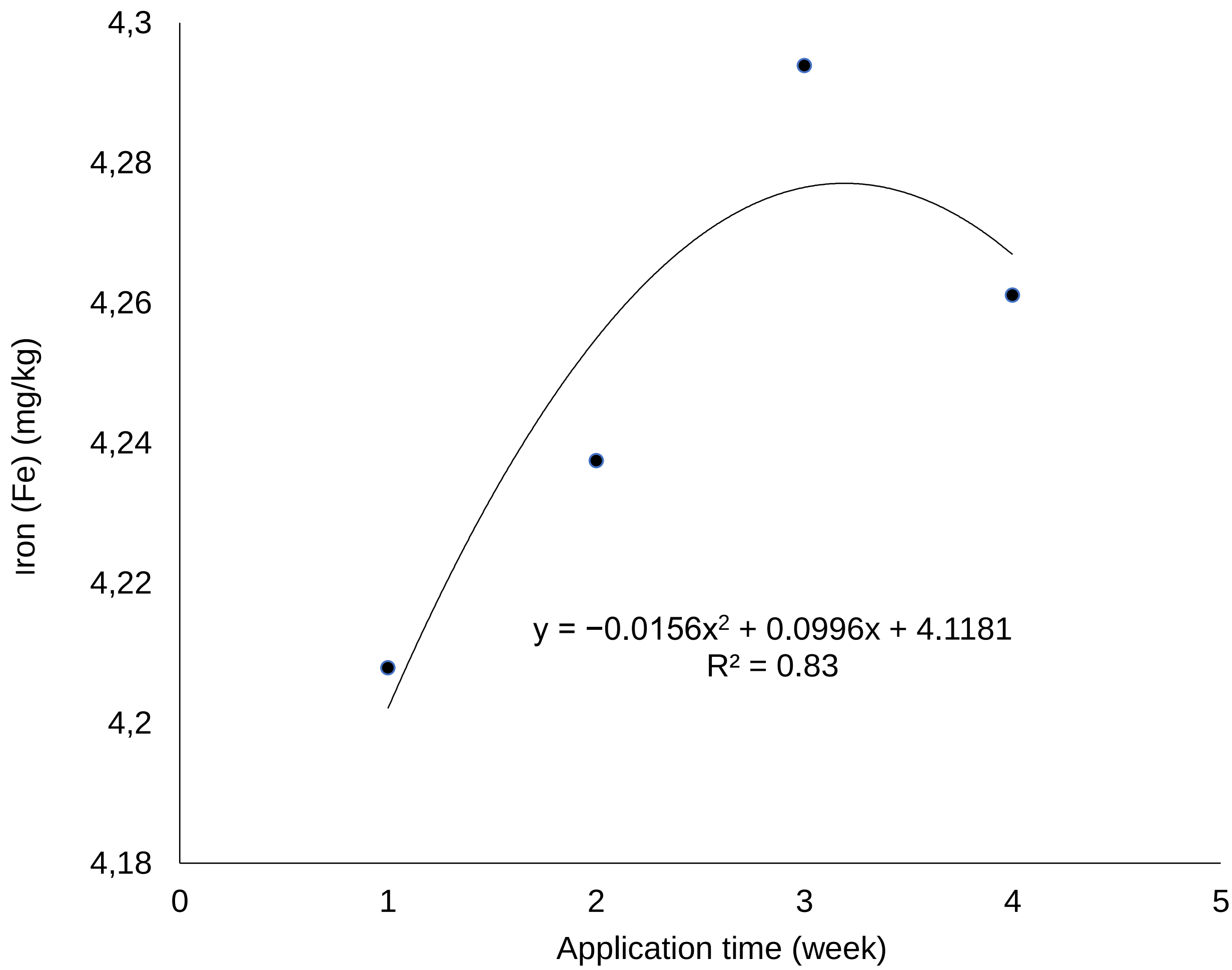


Figure 4.5 Responses of iron (Fe) in leaf tissues of potato cv. 'Mondial G3' to increasing application intervals of Nemarioc-AL phytonematicide at 56 days after treatment.

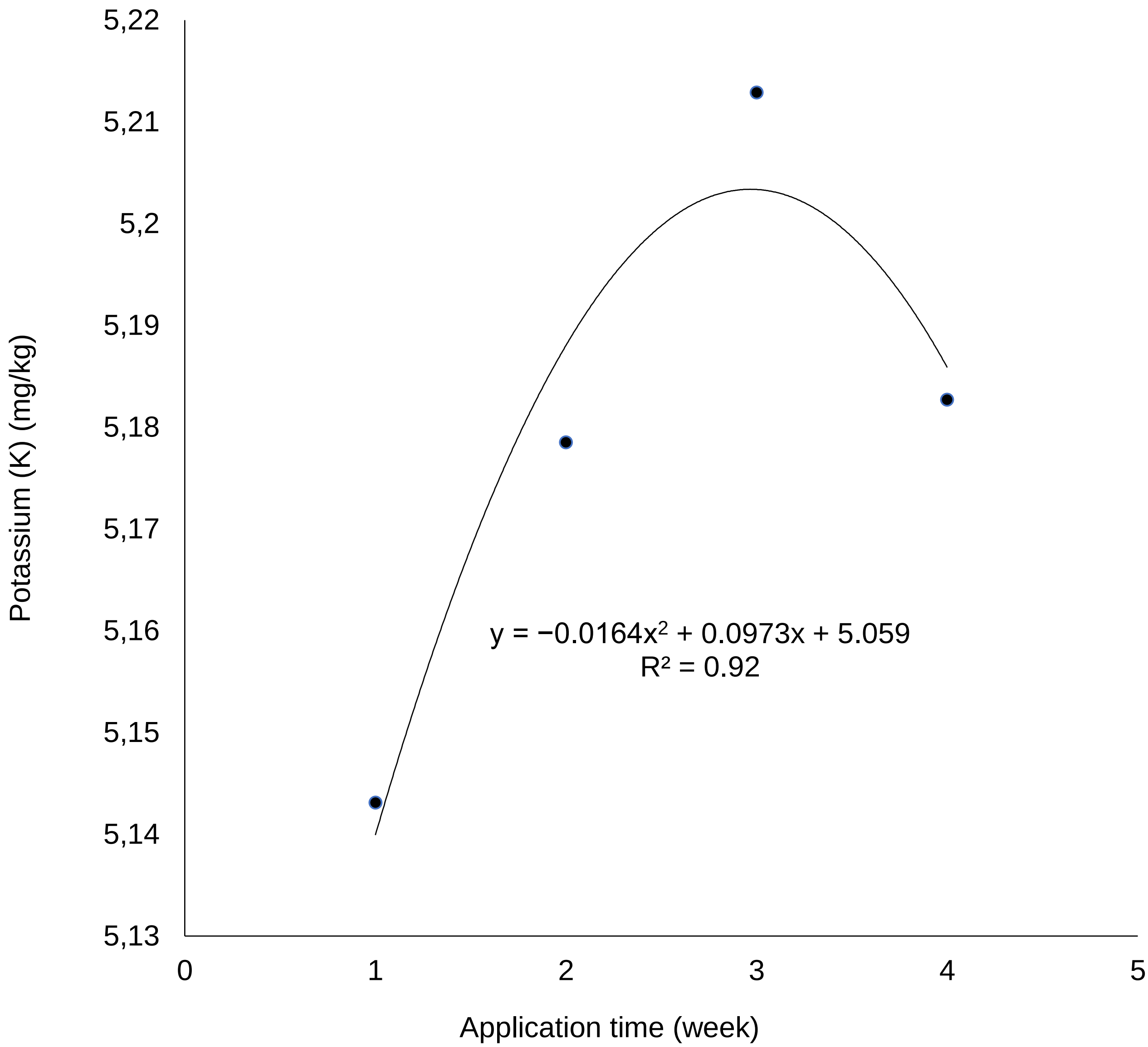


Figure 4.6 Response of potassium (K) in leaf tissues of potato cv. 'Mondial G3' to increasing application intervals of Nemarioc-AL phytonematicide at 56 days after treatment.

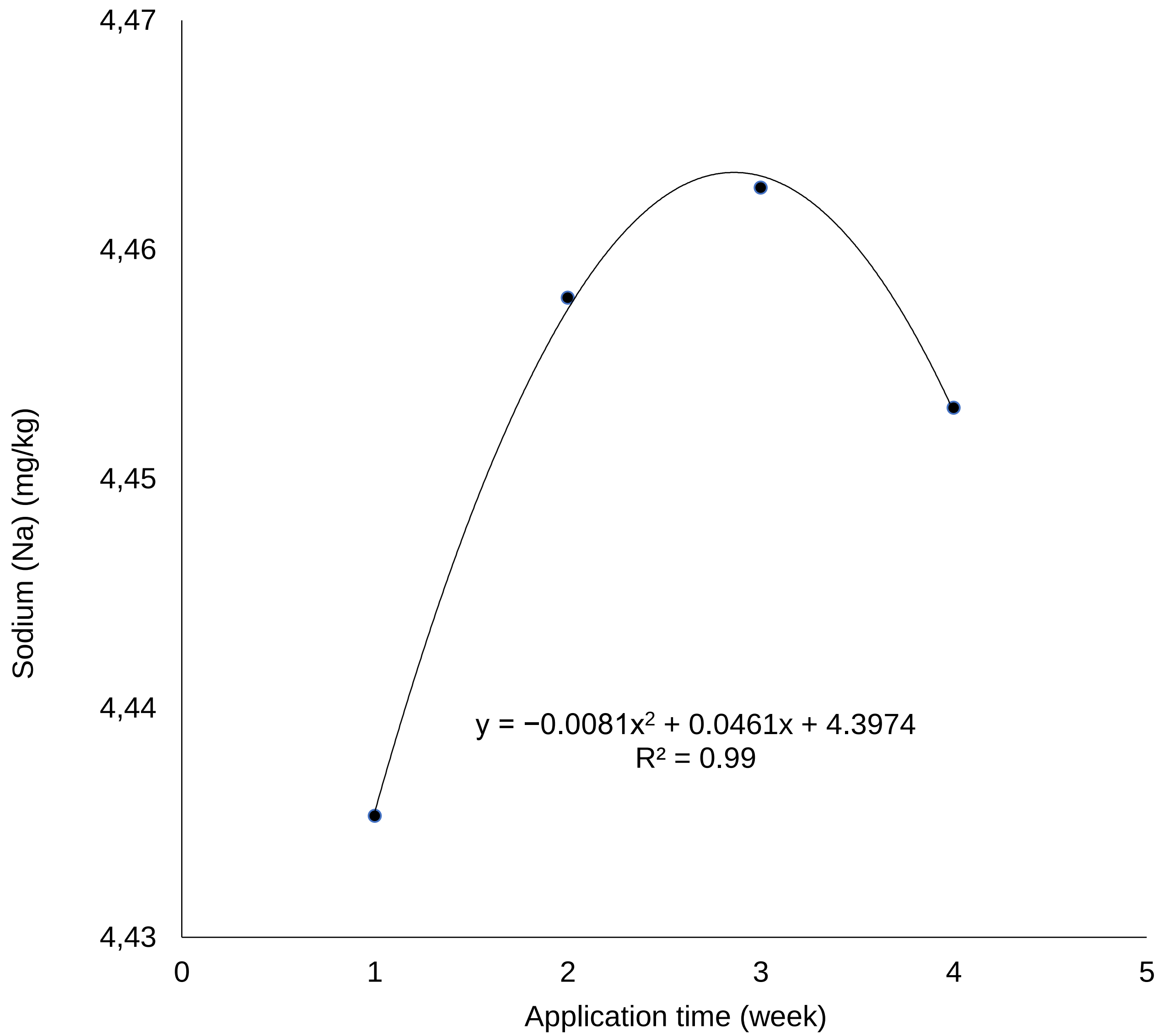


Figure 4.7 Response of sodium (Na) in leaf tissues of potato cv. 'Mondial G3' to increasing application intervals of Nemarioc-AL phytonematicide at 56 days after treatment.

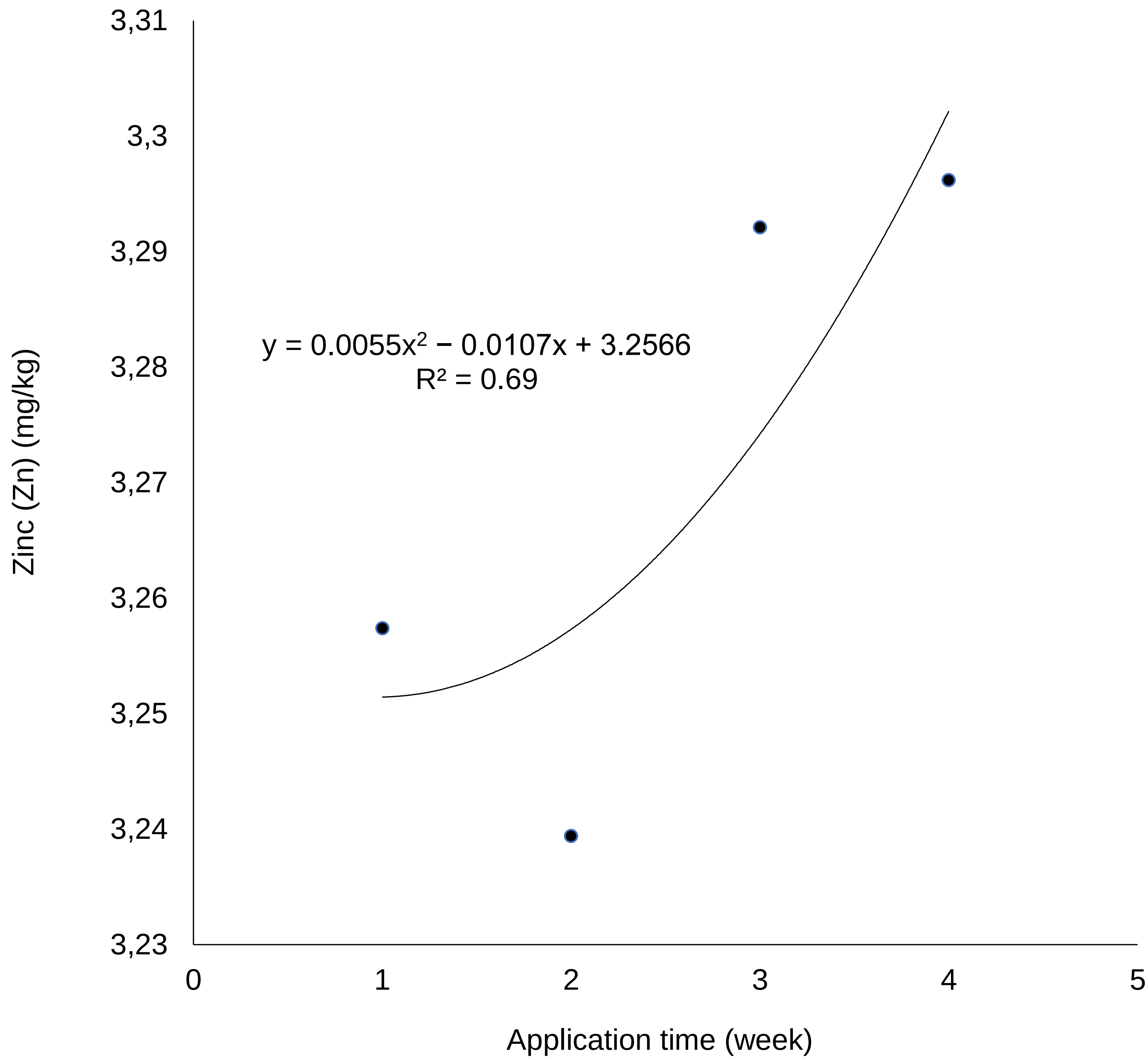


Figure 4.8 Response of zinc (Zn) in leaf tissues of potato cv. 'Mondial G3' to increasing application intervals of Nemarioc-AL phytonematicide at 56 days after treatment.

4.4 Discussion

4.4.1 Plant variables

The three significant plant variables were optimised at different application interval with the integrated mean of 2.43. The application interval of Nemarioc-AL phytonematicide when applied at MCSP of 4.31% on potato cv. ′Mondial G3′ was optimised to provide an application interval of 18 days. Results of this study agreed with those of Pelinganga (2013), who observed that when Nemarioc-AL phytonematicide was applied at MCSP of 3% on tomato-infested with 1500 eggs and J2 of *M. incognita* had the application interval of 18 days. However, at MCSP of 3%, Nemafric-BL phytonematicide on tomato had the application interval of 18 days (Pelinganga, 2013). The current observation was remarkable since both potato and tomato belong to the *Solanaceae* Family. In the current study, when Nemarioc-AL phytonematicide could be applied at the MCSP of 4.31% and the application interval of 18 days, the product would not be phytotoxic to potato cv. 'Mondial G3'. Application time interval for Nemarioc-AL phytonematicide on potato would mitigate phytotoxicity based on plant growth while nematode numbers are reduced.

4.4.2 Nematode variables

The efficacy of plant extracts is dependent on the concentrations and time of exposure to nematodes (Agbenin *et al.,* 2005; Akhter and Khan, 2018). There was no significant difference on eggs, second stage juveniles (J2) in roots and soil and total nematode population (Pf). When determining application interval using phytonematicide plant growth is primary while nematode suppression is secondary (Pelinganga *et al.,* 2013). Pelinganga (2013) explained that application interval is influenced by the level of phytotoxicity of the material to the test crop. The application time interval at 18 days of Nemarioc-AL phytonematicide applied at 4.31% would improve growth of the plant while nematode population densities are suppressed. As a result, 4.31% Nemarioc-AL phytonematicide would be acceptable for controlling *M. javanica* on potato cv. 'Mondial G3'.

4.4.3 Nutrient elements

The optimised nutrient elements in leaf tissues of potato showed density-dependent growth (DDG) patterns characterised by three phases, namely, stimulation, neutral and inhibition phase. The observed nutrient elements started off from stimulation phase through inhibition phase except Zn which started by decreasing. Similar results were observed in another study (Mashela and Pofu, 2017), where Zn and increasing concentrations of Nemarioc-AL phytonematicide exhibited negative quadratic relation which also occurred when the phytonematicide was applied at 4.31% at different time intervals. The influence of MCSP of Nemarioc-AL phytonematicide applied at different on essential nutrient elements was not documented. However, Na exhibited positive quadratic relation over increasing application intervals which disagreed with observations in another study (Mashela and Pofu, 2017) where Na over increasing concentration exhibited negative quadratic relation in green bean (*Phaseolus vulgaris* L.) leaf tissues.

4.5 Conclusion

The optimum application time interval of Nemarioc-AL phytonematicide was at 18-day interval. At 18 days interval Nemarioc-AL phytonematicide applied at 4.31% would not be phytotoxic to potato plants while nematode population densities are suppressed.

CHAPTER 5
SUMMARY OF FINDINGS, SIGNIFICANCE, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary of findings

Many plant extracts have been used successfully to manage nematode population densities but phytotoxicity remained a paramount challenge because it inhibits growth of the protected crop. The Curve-fitting Allelochemical Response Data (CARD) model was used in this study to determine a non-phytotoxic concentration referred to as Mean Concentration Stimulation Point (MCSP) of Nemarioc-AL phytonematicide for managing *Meloidogyne javanica* on potato. MCSP was computed by using two biological indices (D_m and R_h). In the current study the MCSP = $D_m + R_h/2$ for plant variables and nutrient elements were empirically derived at 4.31 and 1.33%. with sensitivity values $\sum k = 18$ and $\sum k = 4$, respectively. Nematode variables exhibited negative quadratic relations where eggs, J2 in soil and roots and total population (Pf) were optimised at 14.43, 28.23, 23.30 and 13.55%. The MCSP value of 4.31% was used to establish the application interval of Nemarioc-AL phytonematicide on the test potato cultivar as 18 days.

5.2 Significance

The outcomes of the study would improve the use of Nemarioc-AL phytonematicide for management of nematode population densities on potato plants. The derived MCSP at 4.31%, when used in conjunction with the empirically-established application interval of 18 days, would not induce phytotoxicity on potato plants.

5.3 Future recommendations

The MCSP of 4.31% Nemarioc-AL phytonematicide at the application interval of 18 days on potato should be validated under field conditions in different potato-producing regions. Also, since tubers would come into contact with cucurbitacin A, it would be necessary that cucurbitacin chemical residues be determined in accredited laboratories.

5.4 Conclusions

Nemarioc-AL phytonematicide when tested under various conditions and cultivars at appropriate empirically established application time intervals could be useful for use by smallholder farmers through irrigation who cannot afford expensive method of controlling methods. For potato cv. 'Mondial G3' Nemarioc-AL phytonematicide could be used at the MCSP of 4.31% when applied at the application interval of 18 days.

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APPENDICES

Appendix 4.1 Analysis of variance of eggs in roots of potato treated with 4.31% of Nemarioc-AL phytonematicide applied at 1, 2, 3 and 4 weeks in a 30-day month period.

Source	DF	SS	MS	F	P
Replication	14	6.46	0.46		
Treatment	3	0.87	0.29	0.59	0.63
Error	42	20.82	0.49		
Total	59	28.15			

Appendix 4.2 Analysis of variance of second stage juveniles (J2) in roots of potato treated with 4.31% of Nemarioc-AL phytonematicide applied at 1, 2, 3 and 4 weeks in a 30-day month period.

Source	DF	SS	MS	F	P
Replication	14	2.10	0.15		
Treatment	3	0.86	0.28	0.60	0.62
Error	42	20.15	0.48		
Total	59	23.12			

Appendix 4.3 Analysis of variance of second stage juveniles (J2) in soil of potato treated with 4.31% of Nemarioc-AL phytonematicide applied at 1, 2, 3 and 4 weeks in a 30-day month period.

Source	DF	SS	MS	F	P
Replication	14	20.12	1.44		
Treatment	3	0.78	0.26	0.36	0.78
Error	42	29.72	0.71		
Total	59	50.60			

Appendix 4.4 Analysis of variance of total nematode population (Pf) of potato treated with 4.31% of Nemarioc-AL phytonematicide applied at 1, 2, 3 and 4 weeks in a 30-day month period.

Source	DF	SS	MS	F	P
Replication	14	7.81	0.56		
Treatment	3	0.52	0.17	0.63	0.59
Error	42	11.44	0.27		
Total	59	19.77			