MEAN CONCENTRATION STIMULATION POINT AND APPLICATION INTERVAL OF NEMARIOC-AL PHYTONEMATICIDE IN THE MANAGEMENT OF *MELOIDOGYNE JAVANICA* ON SWEET POTATO CULTIVAR 'BOPHELO'

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

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DECLARATION

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

Candidate: Elias Mphashi Sebothoma	Signature	Date
Supervisor: Professor P.W. Mashela	Signature	Date

DEDICATION

To my beloved parents, Mr N.P. Sebothoma and Mrs E.T. Sebothoma, and my siblings.

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I thank my Helper, Protector the Almighty God for showering His choicest blessings to me, for providing me with strength to complete this research and for protecting me throughout the studies, without His Grace I could not have made it. I would like to extend my sincere gratitude to my supervisor, Professor P.W. Mashela for introducing me to scientific writing, his tireless guidance, positive criticism, assistance, being patient and encouraging me throughout my studies. Special thanks to research assistant Mr P.E. Tseke for his assistance and technical support, without his contribution this study would not have been successful. I would also like to express my gratitude to Mrs P. Kgopa for her support and motivation. I appreciate with thanks, Agricultural Research Council Vegetable and Ornamental Plants Institute (ARC-VOPI) for providing me with sweet potato cuttings used in this study. I also extend my sincere thanks to the Green Biotechnologies Research Centre of Excellence (GBRCE) and Limpopo Agro-Food Technology Station (LATS) for providing technical assistance with processing and analysing samples. I am deeply grateful to Mr M.K. Ralefatane, Mr L.T. Letsoalo, Mr E.M. Letsoalo, Mrs S.M. Seabela, Ms M.A. Mawasha and Ms S.R. Mawasha for helping me during establishment and harvesting of my experiments. I am honoured to have been a member of the research team at the GBRCE. I gratefully acknowledge my beloved parents, Mr N.P. Sebothoma and Mrs E.T. Sebothoma for granting me the opportunity to further my studies, for their patience and moral support during the entire period of my study. I also thank my brothers, Benny, Stanley, and William Sebothoma and my little sister Violet, for their assistance and support; may God bless them. Thanks

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ABSTRACT

Phytonematicides have allelochemicals as active ingredients and could be highly phytotoxic on crops being protected against nematode damage. In order to avoid phytotoxicity, the application concentration, technically referred to as mean concentration stimulation point (MCSP), along with the application interval, have to be empirically established. The Curve-fitting Allelochemical Response Data (CARD) computer-based model was adopted at the Green Biotechnologies Research Centre of Excellence (GBRCE) for developing the MCSP. The MCSP is computed from the CARD-generated biological indices and was technically defined as a phytonematicide concentration that could manage the nematode population densities without causing phytotoxicity to the test crop and it is plant-specific. The MCSP and application interval had been empirically established for different crops, but they had not been established for sweet potatoes. Therefore, the objective of the study was to determine the MCSP for Nemarioc-AL phytonematicide on *Meloidogyne javanica*-infected sweet potato cv. 'Bophelo' and its application interval. Sweet potato cuttings were planted in 25-cm-diameter plastic bags containing steam-pasteurised loam soil and Hygromix at 3:1 (v/v) ratio. Each plant was inoculated with 5 000 eggs and second-stage juveniles (J2) of *M. javanica*, with seven treatments, namely, 0, 2, 4, 8, 16, 32 and 64% Nemarioc-AL phytonematicide, arranged in a randomised complete block design, with five replicates. At 56 days after the initiation of treatment, the MCSP values for plant variables and plant physiology variables were 1.92 and 3.08% Nemarioc-AL phytonematicide, respectively. The overall sensitivity values for plant variables and plant physiology variables were 0 and 1 unit, respectively, showing that the sweet potato cv. 'Bophelo'

was highly sensitive to the product. Nematode variables with increasing concentrations of Nemarioc-AL phytonematicide exhibited positive and quadratic relations. The life cycle of *M. javanica* and the derived MCSP were used to empirically establish the application interval. Briefly, the location and most materials and methods were as outlined above except that ‘weeks-per-month-of-30 days’, with the MCSP being applied on 0, 7.5, 15, 22.5 and 30 days (0, 1, 2, 3 and 4 weeks) serving as treatments, replicated eight times. At 56 days after the treatments, plant variables and increasing application interval exhibited positive quadratic relations with the average of 2.55 ‘week-of-30-day-month’ translating to 19 days (2.55/4 × 30), with nematode variables exhibiting negative quadratic relationships. In conclusion, when the MCSP of Nemarioc-AL phytonematicide on sweet potato cv. 'Bophelo' at 1.92% was applied every 19 days, it would not be phytotoxic, but it would be able to suppress nematode population densities of *M. javanica*. The MCSP for essential nutrient elements could be reduced to that of plant growth variables, since the products are not intended for use as fertilisers.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Sweet potato (*Ipomoea batatas* L.) is one of the widely cultivated crops among the smallholder farmers and is one of the most important crops in South Africa (Motsa *et al*., 2015). According to Motsa *et al.* (2015), sweet potatoes contain high concentrations of carbohydrates when compared to other starchy crops. Sweet potatoes contain vitamin C, vitamin B, vitamin E and almost all macro- and micro-nutrients (Motsa *et al.,* 2015). Orange-fleshed sweet potato cultivars were introduced as alternative to industrial food fortification supplementation during the process of biofortification (Vasconcelos *et al.*, 2017). Biofortified sweet potato cultivars contain high β-carotene content, which is a precursor to vitamin A (Laurie, 2010).

Makhwedzhana (2018) discovered that sweet potato cultivar 'Bophelo' was highly susceptible to root-knot (*Meloidogyne* species) nematodes, especially *M. incognita* race 2. *Meloidogyne* species could induce yield and quality losses in sweet potato, thereby affecting the marketability of the tubers (Mohandas and Siji, 2012). Conventionally, methyl bromide, Nemacur and Aldicarb were used to manage nematode population densities, with double sweet potato marketable yield and 40% reduced cracked tubers (Mohandas and Siji, 2012). However, most of these products had since been withdrawn from the agrochemical markets (Mashela *et al*., 2008). Therefore cucurbitacin-containing phytonematicides were researched and developed as the alternative

nematode management strategies. However, the active ingredients of these products as allelochemicals, could be highly phytotoxic to crops being protected against nematode damage (Mashela *et al*., 2017).

1.1.1 Description of the research problem

Allelochemicals are naturally allelopathic and therefore, phytonematicides could be highly phytotoxic to plants (Mashela *et al*., 2017). Phytotoxicity of Nemafric-BL, Nemarioc-AL phytonematicides, neem (*Azadirachta indica* A. Juss.), wild garlic (*Tubalghia violacea* Harv.) and oil from clove (*Eugenia caryophyllata* L.) had been observed in various crops (Mashela *et al*., 2017). Due to zero tolerance on phytotoxicity by various registration authorities of agricultural inputs, literature is replete with efficacy trials on phytonematicides (Mashela *et al*., 2017). In South Africa, Act No. 36 of 1947 (as amended) provides clear guidelines for the registration of agricultural inputs (Mashela *et al*., 2017), with much emphasis being placed on avoiding the phytotoxicity.

Biological indices, D_m, R_h, D_0, D_{50} and D_{100}, generated by the Curve-fitting Allelochemical Response Data (CARD) (Liu *et al*., 2003) have been used to develop the mean concentration stimulation point [MCSP = $D_m + (R_h/2)$] (Mashela *et al*., 2016). The MCSP is a concentration of a phytonematicide that could stimulate plant growth, without suppressing nematode population densities (Mashela *et al*., 2016). The MCSP is empirically determined and it is crop-specific (Mashela *et al*., 2017).

1.1.2 Impact of the research problem

In granular formulation, Nemarioc-AG phytonematicide inhibit germination and emergence of both monocotyledonous and dicotyledonous seeds from 50 to 100% (Mafeo *et al*., 2011). In liquid formulation, the cucurbitacin-containing phytonematicides were highly phytotoxic to geranium (*Pelargonium sidoides* DC.) plants (Sithole, 2016), tomato plants (Pelinganga, 2013; Tseke *et al*., 2013), potato plants (Thopola *et al*., 2018), citrus seedlings (Mathabatha *et al*., 2016) and butternut squash (Lebea, 2017) with the phytotoxicity being density-dependent. Worldwide, the registration authorities of agricultural inputs view phytotoxicity with zero tolerance (OECD, 2017).

1.1.3 Possible causes of the research problem

Granular and liquid formulations of cucurbitacin-containing phytonematicides have inherent characteristics of density-dependent growth (DDG) patterns, with the efficacy of any given concentration being dependent upon the DDG phase in which such a concentration was located (Mashela *et al*., 2017). Generally, when entities are plotted against increasing concentrations of phytonematicides, positive or negative quadratic relationships, depending on the phase in which concentrations succeeding zero occur, would be observed (Mamphiswana *et al*., 2010; Pelinganga, 2013; Sithole, 2016). The DDG patterns demonstrate that phytotoxicity is concentration-dependent (Mashela *et al*., 2017).

1.1.4 Proposed solutions

The dosage model concept addresses phytotoxicity using the CARD computer-based model, which is a four-step model, namely, (a) MCSP, (b) application interval, (c) application frequency and (d) dosage (Mashela *et al*., 2017). The CARD model is indispensable in determining whether the allelochemical-containing product used is phytotoxic or non-phytotoxic, with the focus phase for phytonematicides being the stimulation phase. The midpoint of the stimulation phase – the MCSP, constitutes that concentration of the phytonematicide which could stimulate plant growth and concomitantly suppress nematode population densities (Mashela *et al*., 2017). The development of MCSP requires empirically-based data and it is crop-specific. Using the concept of 'weeks-per-month-of-30 days', with 30-days being the life cycle of *Meloidogyne* species, the application interval that could disrupt the life cycle of *Meloidogyne* species was computed (Mashela *et al*., 2017). Similarly, the application interval of the citrus nematode (*Tylenchulus semipenetrans* Cobb 1913), was established using 'weeks-per-month-of-42 days' (Mathabatha, 2018: unpublished), where 42 days is the life cycle of *T. semipenetrans*. This concept was developed to enhance the capability of a phytonematicide to break the life cycles of nematodes since second-stage juveniles (J2) hatch consequentially. The application intervals are empirically determined, with the MCSP being applied at 0, 7.5, 15, 22.5 and 30 days for *Meloidogyne* species and at 0, 10.5, 21, 31.5 and 42 days for *T. semipenetrans* (Mashela *et al*., 2017).

1.1.5 General focus of the study

The phytotoxicity challenges of Nemarioc-AL phytonematicide on sweet potato cultivar 'Bophelo' could be solved by empirically-deriving MCSP and application intervals. In such studies, cuttings of cv. 'Bophelo' would be inoculated with *M. javanica* in order to establish whether nematodes were also suppressed during the establishment of non-phytotoxicity variables.

1.2 Problem statement

The active ingredient of Nemarioc-AL phytonematicide is cucurbitacin A ($C_{32}H_{46}O_9$), which is an allelochemical. Due to its polarity, the compound is slightly soluble in water and it is also highly unstable and hydrolysis to cucumin and leptodermin (Chen *et al*., 2005). Due to its dependence on DDG phases, the product could either stimulate plant growth, neutral or inhibit plant growth, with the latter being undesirable since it would be viewed as being phytotoxic. Due to plant- and nematode-specificities of phytonematicides, empirically-designed trials are necessary to generate MCSP and the application interval sweet potato cultivar 'Bophelo' and suppression of population densities of *M. javanica*.

1.3 Rationale of the study

Phytotoxicities of phytonematicides should be managed at all costs in order to enhance the registration of the products and for their sustainable use in agricultural systems (OECD, 2017). Due to reliance on data from poorly designed experiments, most agricultural products are registered and the deregistered due to undesirable effects

such as phytotoxicity (OECD, 2017). In cucurbitacin-containing phytonematicides, the test plant is subjected to increasing concentrations of the product in pots under greenhouse conditions to generate MCSP, which is then used under microplot conditions to establish the application interval. Upon the establishment of the two variables, it becomes feasible to determine the application frequency when the life cycle of the crop is known and then the dosage model. The latter is required to indicate how much of the active ingredient had been disposed into the soil environment after the application period.

1.4 Purpose of the study

1.4.1 Aim

Development of dosage model for application of cucurbitacin-containing phytonematicide on sweet potatoes for managing *Meloidogyne* species.

1.4.2 Objectives

1. To determine whether increasing concentration of Nemarioc-AL phytonematicide would generate MCSP on sweet potato cv. 'Bophelo' for managing population densities of *M. javanica*.
2. To establish whether the MCSP of Nemarioc-AL phytonematicide on sweet potato cv. 'Bophelo' could be used to derive the application interval of the product for managing population densities of *M. javanica*.

1.5 Hypotheses

1. Increasing concentration of Nemarioc-AL phytonematicide would generate MCSP on sweet potato cv. 'Bophelo' for managing population densities of *M. javanica*.
2. The MCSP of Nemarioc-AL phytonematicide on sweet potato cv. 'Bophelo' could be used to derive the application interval of the product for managing population densities of *M. javanica*.

1.6 Reliability, validity and objectivity

Reliability of data was based on statistical analysis of data at the probability level of 5%, validity was achieved through repeating the experiments at the same location during different times, whereas objectivity was achieved by ensuring that the findings are discussed on the basis of empirical evidence, in order to eliminate all forms of subjectivity (Leedy and Ormrod, 2005).

1.7 Bias

Bias was minimised by ensuring that the experimental error in each experiment was reduced through replications. Also, treatments were assigned randomly within the selected research designs (Leedy and Ormrod, 2005).

1.8 Scientific significance of the study

The CARD computer-based model would help to compute the MCSP which would be used to determine the application interval in the management of *M. javanica* on sweet potato. The findings of this study would come up with non-phytotoxic concentration that

could be used in suppression of nematodes in sweet potato, facilitating the registration of the product in accordance with OECD (2017). This would also provide farmers with an environment-friendly product for management of nematodes.

1.9 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 problem statement was reviewed (Chapter 2). Then, each of the subsequent chapters (Chapter 3 and 4) addressed each of the objectives in sequence. In the final chapter (Chapter 5), findings in all chapters were summarised and integrated to provide the significance and future recommendations with respect to future research, culminating in a conclusion which tied the entire study together. Citations in the text and reference listing adopted the Harvard referencing style that uses the author-alphabet as approved by the Senate of the University of Limpopo.

CHAPTER 2

LITERATURE REVIEW

2.1 Work done on the problem statement

2.1.1 Phytotoxicity of phytonematicides

Mashela *et al.* (2017) stated that the active ingredients of the phytonematicides are allelochemicals, therefore, phytonematicides could be highly phytotoxic to the protected crops if the right concentration is not used. Mashela *et al.* (2017) further argued that phytotoxicity in phytonematicides could limit the potential registration and widespread adoption of these materials for managing *Meloidogyne* species. According to Mafeo and Mashela (2009), in granular formulation, Nemarioc-AG phytonematicide at 150 g was highly phytotoxic and also inhibited germination of maize (*Zea mays* L.), finger millet (*Eleusine coracana* L.), sorghum (*Sorghum bicolor* L.) and onion (*Allium cepa* L.). Mafeo and Mashela (2010) demonstrated that drench-application of Nemarioc-AG phytonematicide at sowing was highly phytotoxic to eight monocotyledonous and ten dicotyledonous plants. Nemarioc-AG phytonematicide also suppressed seedling growth and inhibited germination of onion, leek (*Allium ampeloprasum* L.) and chive (*Allium schoenoprasum* L.) by as high as 50 to 100% when applied at the rate of 2 g/plant (Mafeo *et al.*, 2011).

In liquid formulation, Nemarioc-AL and Nemafric-BL phytonematicides at 10% concentration were reported to be highly phytotoxic to tomato (*Solanum lycopersicum* L.) seedlings after transplanting (Pelinganga and Mashela, 2012). Sithole (2016)

reported that Nemarioc-AL phytonematicide was highly phytotoxic to geranium (*Pelargonium sidoides* DC.) reducing plant growth by 5 to 10% at a concentration of 10%.

2.1.2 Mean concentration stimulation point

Mashela *et al*. (2017) adapted the Curve-fitting Allelochemical Response Data (CARD) model (Liu *et al*., 2003) to come up with the mean concentration stimulation point (MCSP). The CARD computer-based model generates biological indices which are used to compute MCSP. The biological indices include: (a) threshold stimulation (D_m) which represent the allelochemical concentration where stimulation phase begin, (b) saturation point (R_h) which is 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), (f) the sensitivity index (k) which provides the level of sensitivity of an organism to the test product and (g) the coefficient of determination (R^2) provides the degree of the strength of the CARD model (Liu *et al*., 2003).

The MCSP was originally derived using the two biological indices, D_m and R_h, through the relation: $MCSP = D_m + (R_h/2)$, MCSP which is a concentration of a phytonematicide that would stimulate plant growth, while inhibiting nematode population densities (Mashela *et al*., 2017). Pelinganga (2013) used increasing concentrations, 0, 2, 4, 8, 16, 32 and 64% Nemarioc-AL phytonematicide in managing population densities of *M.*

incognita race 2 on tomato (*Solanum lycopersicum* L.) cultivar 'Floradade' under greenhouse conditions, with the MCSP established at 2.99% for Nemarioc-AL phytonematicide. Tseke *et al*. (2013) established the MCSP at 4.4% Nemarioc-AL phytonematicide on tomato cultivar 'Floradade' under greenhouse conditions. Sithole (2016) established the 6.18% MCSP for Nemarioc-AL phytonematicide on geranium plants (*Pelargonium sidoides* DC.) under micro-plot conditions. Mashitoa (2016) reported the 18.10% MCSP for Nemarioc-AL phytonematicide on beetroot cv. 'Detroit Dark Red' (*Beta vulgaris* L.). Lebea (2017) showed that the MCSP of Nemarioc-AL phytonematicide on butternut squash (*Cucurbita pepo* L.) cv. 'Caserta' at 11.85% under micro-plot conditions. Mathabatha *et al*. (2016) established the MCSP of Nemarioc-AL phytonematicide at 8.6% on citrus rootstock under greenhouse conditions.

The CARD computer-based model also provides the information on the sensitivity of the crop to the product being used to protect it against pests, through the use of sensitivity index (k). According to Liu *et al*. (2003), sensitivity indices close to zero meant that the plant organ is highly sensitive to the allelochemical used, and those far away from zero meant that the plant organ was tolerant to the material. Most crops exposed to Nemarioc-AL phytonematicide were shown to be highly sensitive to the product. Pelinganga (2013) reported k values ranging from 1 to 2 units for tomato plant variables exposed to increasing concentrations of Nemarioc-AL phytonematicide on weekly basis over 56 days. Previously, the exposure of tomato plants to increasing concentration of Nemarioc-AL phytonematicide resulted in k values of 2, 1, 0 and 2 units for dry root mass, dry shoot mass, plant height and stem diameter for tomato plant, respectively

(Tseke *et al*., 2013). Sithole (2016) reported k values of 0 and 3 for dry root mass and plant height, respectively, when geranium plants were exposed to increasing concentration of Nemarioc-AL phytonematicide. Mashitoa (2016) reported that the beetroot plants were highly sensitive to increasing concentration of Nemarioc-AL phytonematicide, with the overall sensitivity of 0 units. According to Lebea (2017), butternut squash was found to be highly sensitive to increasing concentration of Nemarioc-AL phytonematicide under micro-plot conditions, with overall sensitivity of 0 units. Mathabatha *et al*. (2017) reported k values of 1, 0, 1 and 0 units for dry shoot mass, dry root mass, plant height and stem diameter, respectively, when citrus rootstock were exposed to increasing concentration of Nemarioc-AL phytonematicide.

2.1.3 Application interval of phytonematicides

The concept of application interval focused mainly on breaking the life cycle of the test nematodes (Mashela *et al*., 2017). Pelinganga (2013) developed the concept of 'weeks-per-month-of-30 days' to enhance the capability of a phytonematicides to break the life cycle of *Meloidogyne* species, which under optimum conditions is approximately 30 days (Mashela *et al*., 2017). Pelinganga (2013) empirically used the following concentrations, 3% Nemarioc-AL and 3% Nemafric-BL, with the application intervals, 0, 1, 2, 3 and 4 'weeks-per-month-of-30 days' and discovered the integrated mean time intervals of 2.614 and 2.139 'weeks-per-month-of-30 days', which translated to 20 and 16 days application time intervals, respectively.

Mathabatha (2018: unpublished) observed the following application intervals, 2.19 and 2.51 ‘weeks-per-month-of-30 days’ for Nemarioc-AL phytonematicide for *Swingle citrumelo* citrus rootstock under greenhouse conditions when managing *T. semipenetrans*, which translated to 22 and 26 days, respectively. Mathabatha (2018: unpublished) had the mean application interval of 2.28 and 2.30 ‘weeks-per-month-of-30 days’ for both Nemarioc-AL and Nemafric-BL phytonematicides, respectively, on Carrizo citrange citrus rootstock under greenhouse conditions, which translated to a 24-day application period for both products. According to Mathabatha (2018: unpublished), the application intervals for the two respective products on rough lemon rootstock under greenhouse conditions were also optimised at 2.28 and 2.30 weeks, which translated to 24 days.

2.2 Work not done on the problem statement

The development of non-phytotoxic concentration and application interval of Nemarioc-AL phytonematicide in management of *M. javanica* on sweet potato cultivar 'Bophelo' had not been documented. The CARD model is a reliable tool for generating the biological indices (D_m and R_h) used in the computation of the MCSP and subsequently the application interval, application frequency and then the dosage model.

CHAPTER 3

MEAN CONCENTRATION STIMULATION POINT OF NEMARIOC-AL PHYTONEMATICIDE ON SWEET POTATO CULTIVAR 'BOPHELO'

3.1 Introduction

Owing to the international withdrawal of most synthetic chemical nematicides from the agrochemical markets due to their environment-unfriendliness, with methyl bromide being the last to be withdrawn in 2005 (Mashela *et al*., 2008). Cucurbitacin-containing phytonematicides were researched and developed as an alternative to methyl bromide for managing root-knot (*Meloidogyne* species) nematodes (Mashela *et al*., 2017). Nemarioc-AL phytonematicide comprises fermented crude extracts from dried fruit of wild cucumber (*Cucumis myriocarpus* Naude.), with the active ingredient cucurbitacin A ($C_{32}H_{46}O_9$), which is slightly soluble in water due to its partial polarity, but it rapidly oxidises to cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Chen *et al*., 2005).

Nemarioc-AL phytonematicide consistently suppressed population densities of *Meloidogyne* species under different environmental conditions and cropping systems (Mashela *et al*., 2017). However, the product could be highly phytotoxic to crops that it should be protecting against nematode damage (Mashela *et al*., 2016). Phytotoxicity challenges could be resolved using the Curve-fitting Allelochemical Response Data (CARD) computer-based model (Liu *et al*., 2003), as adapted for cucurbitacin-containing phytonematicides (Mashela *et al*., 2017). The CARD-generated model generates biological indices, which were used to formulate the mean concentration stimulation

point [MCSP = D_m + (R_h/2)], which is the concentration to be applied without inducing phytotoxicity (Mashela *et al*., 2017). The MCSP is plant-specific and it had not been documented for Nemarioc-AL phytonematicide on sweet potato. The objective of the study was to determine whether increasing concentration of Nemarioc-AL phytonematicide would generate MCSP on sweet potato cv. 'Bophelo' for managing population densities of *M. javanica*.

3.2 Materials and methods

3.2.1 Description of the study site

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 greenhouse temperature was set at 25°C and controlled using thermostatically-activated fans on one side of the greenhouse wall, with the wet wall being on the opposite side. During the extraction of warm air, the windblown currents created heterogeneous conditions inside the greenhouse. Consequently, the created heterogeneous conditions were taken into account when designing the experimental layout in the greenhouse under description. The study was conducted in summer-autumn (January-March: Experiment 1) 2017 and validated in autumn (March-May) 2018 (Experiment 2).

3.2.2 Treatments and research design

The treatments comprised 0, 2, 4, 8, 16, 32 and 64% Nemarioc-AL phytonematicide arranged in a randomised complete block design, with five replications, due to the created heterogeneous conditions mentioned above, blocking was done (Figure 3.1).

Figure 3.1 Greenhouse layout of the experiment.

3.2.3 Procedures

Nemarioc-AL phytonematicide was prepared using the locally-adapted method of Kyan *et al*. (1999). Briefly, 20 L–hermetically sealed plastic containers were filled with 16 L chlorine-free water. Approximately, 80 g dried and ground *C. myriocarpus* fruit, mixed with 300 ml effective microorganisms (EM), 624 g brown sugar and 300 ml molasses. Allowance for released CO_2 to escape from the container was provided through an airtight 5 mm diameter tube with one end glued to a hole on the lid of the 20 L container, dangling into a 1-L bottle half-filled with chlorine-free tapwater. The system was placed

in a room with ambient temperature of 37.5 ± 2°C for 14 days to allow for the fermentation-induced pH to drop to below 3.7 (Kyan *et al*., 1999).

Sweet potato cuttings were obtained from the Agricultural Research Council-Vegetable and Ornamental Plants (ARC-VOP) and locally multiplied on plots for experimental use at the GBRCE. Uniform 30-cm long sweet potato cuttings were stimulated to root by dipping their lower ends in Seradix rooting hormone with one-third of the lower end of cutting then placed in water for 5 days. Cuttings with well-developed root system were transplanted into 25-cm-diameter plastic bags filled with 5.9 L of growing mixture made from steam-pasteurised (300°C for 1 h) loam soil and Hygromix (Hygrotech, Pretoria North) at 3:1 (v\v) ratio. Containers with plants were placed on greenhouse benches at 0.30 m × 0.30 m spacing.

Meloidogyne javanica inoculum was prepared by extracting eggs and second-stage juveniles (J2) from roots of greenhouse-raised nematode-susceptible tomato (*Solanum lycopersicum* L.) cv. 'Floradade' in 1% NaOCl solution (Hussey and Parker, 1973). At transplanting each plant was fertilised with 5 g NPK 2:3:2 (22) + 0.5% S + 5% Zn + 5% Ca fertiliser and 2 g NPK 2:3:2 (43) Multifeed (Nulandies, Johannesburg) to provide 0.47 N, 0.43 P, 0.43 K, 121 Mg, 1 Fe, 0.10 Cu, 0.47 Zn, 1.34 B, 4.02 Mn and 0.09 mg Mo per ml tapwater, without Ca (Mashela *et al*., 2017). Seven days after transplanting, each plant was inoculated with 5 000 eggs and J2 *M. javanica* by dispensing using a 20-ml-plastic syringe, with inocula placed in 5-cm-deep holes on the cardinal sides of the vine. Holes with inoculum were filled using the growing mixture. Each plant was

irrigated with 500 ml chlorine-free water every other day. Once a week, irrigation was substituted for with the same quantity of the appropriate phytonematicide treatment. Pest management comprised regular monitoring and application of control measures when necessary. Scouting for insect pests was carried out on daily basis. Cyperin 200 EC (Cypermethrin and Spidermetrien), Mulan 20 SP (Acetamiprid), aphicide plus (Imidacloprid), Malasol (Mercaptothion) were used to control green aphids (*Myzus persicae* Sulz), greenhouse whiteflies (*Trialeurodes vaporarorium* Westwood) and red spider mites (*Tetranychus urticae* Koch).

3.2.4 Data collection

Plant variables: At 56 days after initiating the treatments, vine length was measured from the crown to the terminal end of the flag leaf. Stem diameter was measured at 3 cm above the cut ends using a digital vernier caliper (DC – 515). Chlorophyll content was measured using chlorophyll meter (Minolta spad – 502). Root system was removed from the soil, immersed in water to remove soil particles, blotted dry and weighed to facilitate the extraction of nematodes per total root system. Root galls were assessed using the North Carolina Differential Rating Scale at 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls and 5 ≥ 100 galls per root system (Taylor and Sasser, 1978). Shoots were oven-dried at 72°C for 72 h and weighed. Mature dried leaves were finely ground in a Wiley mill to pass through a 1 mm opening sieve.

Essential nutrient element variables: Approximately, 0.40 g dried leaf material was digested in 40 ml 5% nitric acid (HNO_3) solution, followed by placing the container on a vortex to allow for complete wetting of the mixture. The material was magnetically stirred, thereafter incubated in a 95°C water-bath for 60 minutes. The samples were allowed to cool down at room temperature, filtered and then decanted into 50 ml tubes which were covered with a foil. Nutrient elements, Fe, K, Na and Zn in leaf tissues were analysed using the Inductively Coupled Plasma Optical Emission Spectrometry (ICPE-9000).

Nematode variables: Nematodes were extracted from 5 g roots per plant through maceration and blending for 60 seconds in 1% NaOCl solution (Hussey and Barker, 1973), whereas J2 from soil samples were extracted from a 250 ml soil subsample/pot using the modified sugar-floatation and centrifugation method (Jenkins, 1964). Final nematode population (Pf) was determined by adding total nematodes in soil to total nematodes in the root.

3.2.5 Data analysis

Plant variables and essential nutrient elements were subjected to CARD computer-based model to generate the biological indices (Liu *et al.,* 2003), for the calculation of MCSP value for the phytonematicide using:

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

where D_m is the threshold stimulation point and R_h is the saturation point (Mashela *et al.,* 2016). Biological indices where variables displayed negative quadratic relations were

excluded in the calculation of the MCSP since the former did not have the stimulation phases. The CARD model was also used to get the sensitivity values (k-values), which were further used to calculate the overall sensitivity (∑k) values (Table 3.1). Prior to subjecting the data to the CARD model, the geometric series treatment values were log-transformed using $\log_2 2^x$ to generate 0, 1, 2, 3, 4, 5 and 6% to promote equidistance between adjacent x-axis values (Mashela *et al*., 2017). Nematode variables were subjected to analysis of variance (ANOVA) through the Statistix 10.0 software, with significant variables further subjected to lines of the best fit (Appendices 3.1-3.4) (Gomez and Gomez, 1984).

3.3 Results

Seasonal interactions were not significant on all variables and therefore, the data for the two seasons (Experiment 1 and Experiment 2) were pooled (n = 70) and re-analysed (Gomez and Gomez, 1984).

3.3.1 Plant growth variables

The CARD-generated biological indices (Table 3.1) and associated concentration response curves (Figure 3.2) for dry shoot mass, dry root mass, vine length and gall rating were explained by 96, 93, 98 and 99% coefficients of determination, respectively. Biological indices where negative quadratic relations were exhibited were excluded in the calculation of the MCSP since the former did not have the stimulation phases. The MCSP value of Nemarioc-AL phytonematicide on plant growth variables was established at 1.92% (Table 3.1). The sensitivity (k) values for the respective plant

variables were all 0 unit, resulting in the overall sensitivity (∑k) value of 0 unit (Table 3.1). In the current study, plant variables exhibited positive quadratic relations in context of density-dependent growth (DDG) patterns when plotted against increasing concentrations of Nemarioc-AL phytonematicide (Figure 3.2). Nemarioc-AL phytonematicide stimulated plant growth at lower concentrations in most plant variables, while at higher concentrations inhibition occurred (Figure 3.2).

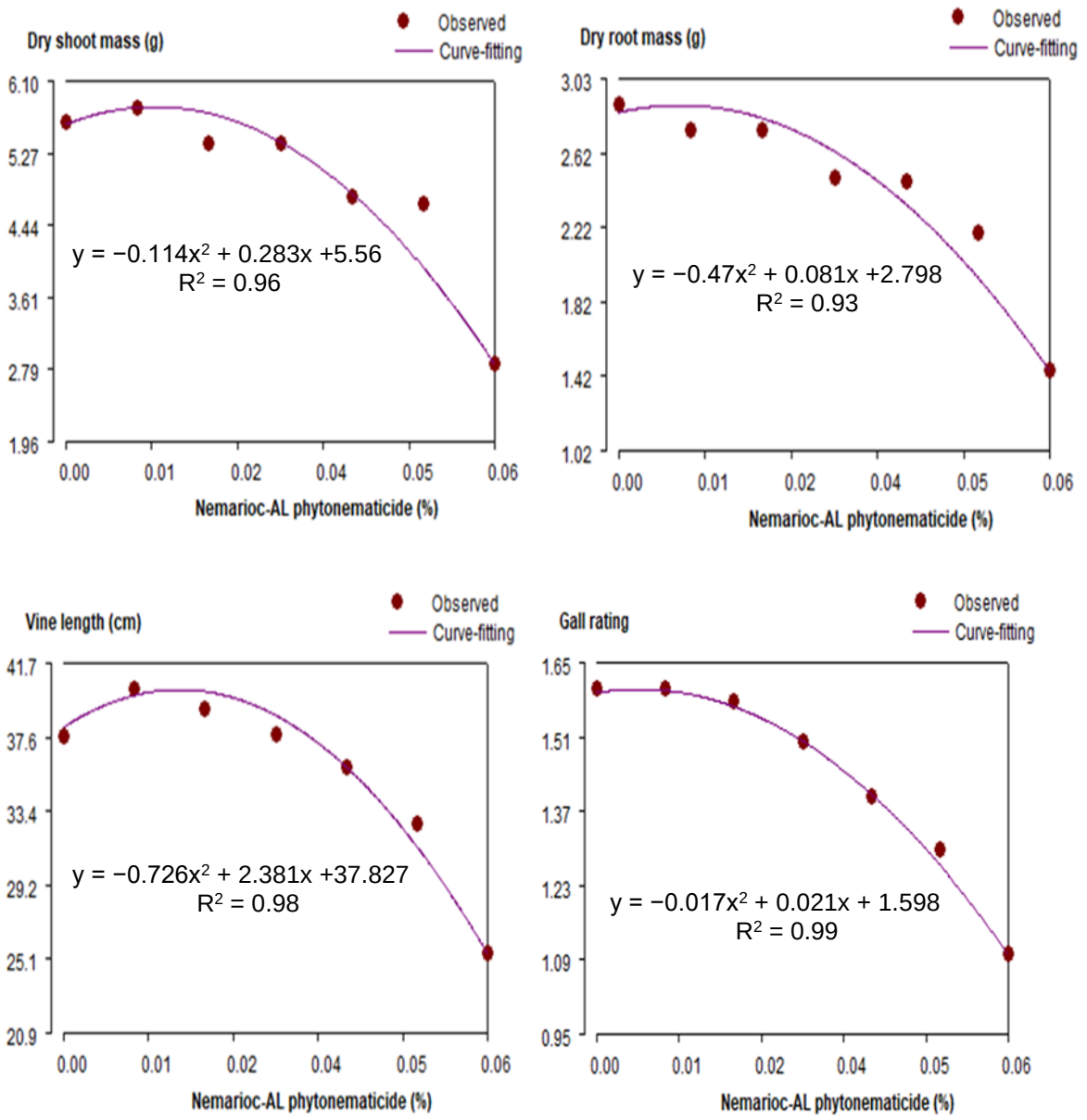

Figure 3.2 Responses of sweet potato cv. 'Bophelo' dry shoot mass, dry root mass, vine length and gall rating to increasing concentrations of Nemarioc-AL phytonematicide.

Table 3.1 Biological indices for dry shoot mass (DSM), dry root mass (DRM), vine length (VNL) and gall rating (GLR) of sweet potato to increasing concentrations of Nemarioc-AL phytonematicide at 56 days after initiation of treatments.

Biological index	DSM	DRM	VNL	GLR	Mean
Threshold stimulation (D_m)	1.822	1.514	1.780	0.619	1.434
Saturation (R_h)	0.560	0.113	2.506	0.700	0.970
0% inhibition (D_0)	3.645	3.028	3.56	1.237	2.868
50% inhibition (D_{50})	6.246	6.608	6.976	7.486	6.829
100% inhibition (D_{100})	7.8	8.6	8.9	10.3	8.9
R^2	0.96	0.93	0.98	0.99	
k-value	0	0	0	0	
Overall sensitivity			$\sum k = 0$		

MCSP = $D_m + (R_h/2) = 1.434 + (0.970/2) = 1.434 + 0.485 = 1.92\%$.

3.3.2 Essential nutrient elements

All essential nutrient elements in leaf tissues of cv. ′Bophelo′ measured, when plotted against increasing concentrations of Nemarioc-AL phytonematicide exhibited positive quadratic relationships, with the models for Fe, K, Na and Zn explained by 97, 55, 78 and 87%, respectively (Figure 3.3). The sensitivity values for Fe, K, Na and Zn were 0, 1, 0 and 0 unit, respectively, with the ∑k of 1 unit (Table 3.2). The MCSP for the essential nutrient elements in leaf tissues of cv. ′Bophelo′ was 3.08% (Table 3.2).

3.3.3 Nematode variables

Nematode variables and increasing concentrations of Nemarioc-AL exhibited quadratic relationships. The J2 and eggs in roots and final population of nematodes exhibited negative quadratic relationships, with the models explained by 81, 75 and 83%, respectively, whereas J2 in soil exhibited positive quadratic relationship with the model explained by 89% (Figure 3.4).

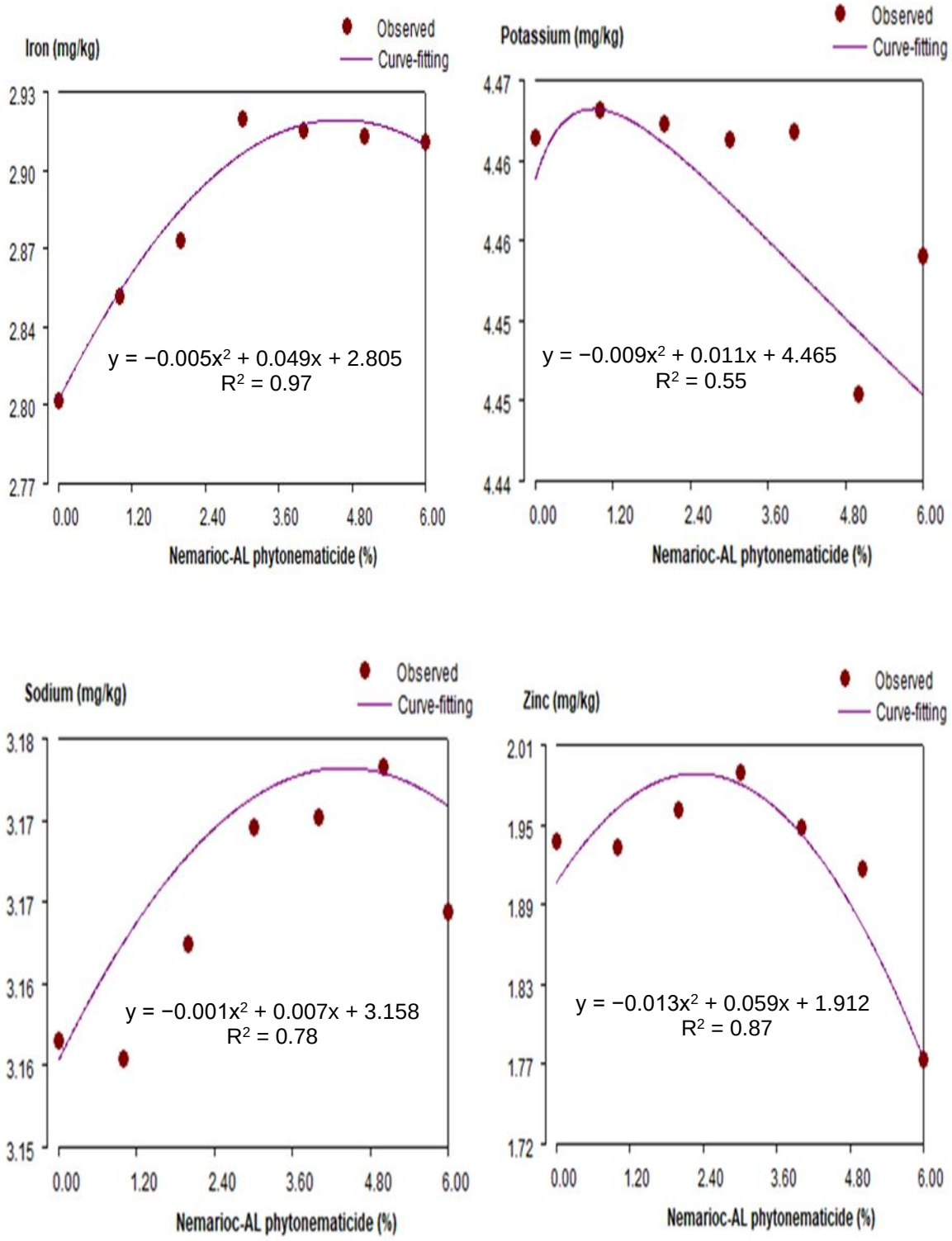

Figure 3.3 Responses of sweet potato iron, potassium, sodium and zinc to increasing concentrations of Nemarioc-AL phytonematicide.

Table 3.2 Biological indices for iron, potassium, sodium and zinc of sweet potato to increasing concentrations of Nemarioc-AL phytonematicide at 56 days after initiation of treatments.

Biological index	Fe	K	Na	Zn	Mean
Threshold stimulation (D_m)	4.608	0.901	4.403	2.291	3.051
Saturation (R_h)	0.113	0.004	0.016	0.068	0.050
0% inhibition (D_0)	9.216	2.613	8.807	4.582	6.305
50% inhibition (D_{50})	21.478	0	48.69	11.181	20.337
100% inhibition (D_{100})	28	0	66.9	14.7	27.4
R^2	0.97	0.55	0.78	0.87	
k-value	0	1	0	0	
Overall sensitivity			$\sum k = 1$		

MCSP = $D_m + (R_h/2) = 3.051 + (0.050/2) = 3.051 + 0.025 = 3.08\%$.

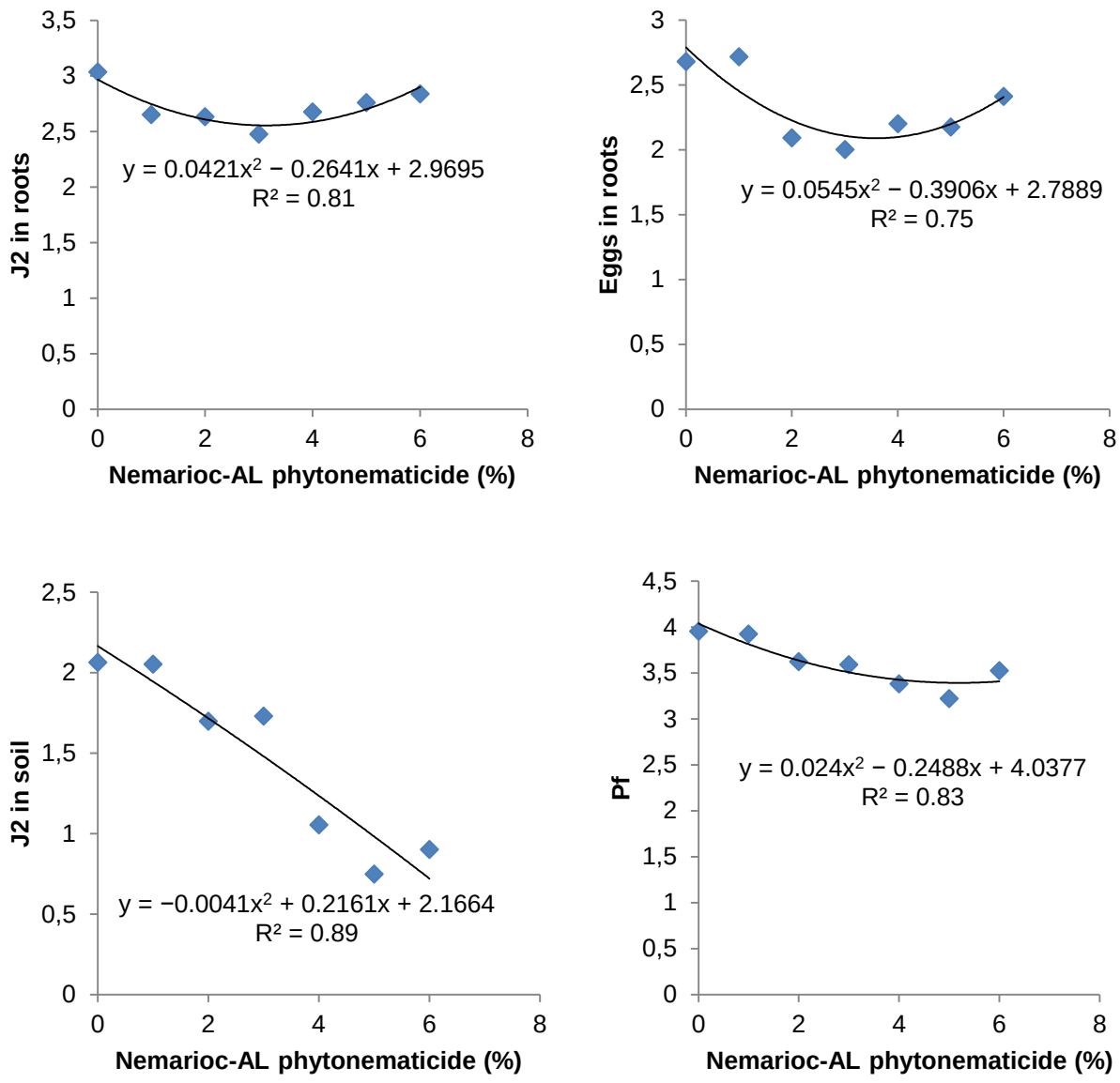

Figure 3.4 Responses of second-stage juveniles in roots, eggs in roots, J2 in soil and final population of *Meloidogyne javanica* to increasing concentrations of Nemarioc-AL phytonematicide.

3.4 Discussion

3.4.1 Plant growth variables

The derived MCSP of 1.92% Nemarioc-AL phytonematicide on sweet potato cv. 'Bophelo' was less than the one of 2.99% derived for tomato plants cv. 'Floradade' (Pelinganga, 2013) and 2.97% for citrus (*Swingle citrumelo*) seedling rootstock (Mathabatha *et al*., 2017). The value was also lower than those in other studies with tomato (Tseke *et al*., 2013), geranium (*Pelargonium sidoides* DC.) (Sithole, 2016) and citrus rootstock (*Volkameriana citrus* Pasq.) (Mathabatha *et al*., 2016), which were 4.40, 6.18 and 8.6% Nemarioc-AL phytonematicide, respectively. The observed differences in MCSP values in the current study and the previous studies might be due to the fact that MCSP is crop-specific (Mashela *et al*., 2017).

According to Liu *et al*. (2003), sensitivity indices close to zero suggest that the crop was highly sensitive to the test product, whereas those further from zero meant that the plant was tolerant. In this study, ∑k was 0 unit, suggesting that the sweet potato cv. 'Bophelo' was highly sensitive to Nemarioc-AL phytonematicide. Similarly, Mashitoa (2016) observed ∑k of 0 unit for beetroot cv. 'Detroit Dark Red' (*Beta vulgaris* L.) exposed to Nemarioc-AL phytonematicide under greenhouse conditions. The ∑k of 3 units was observed for geranium (Sithole, 2016), thereby suggesting that the crop was tolerant to the product. Also, the ∑k value of sweet potato in the current study was much lower than those of the product on tomato plants (Pelinganga, 2013; Tseke *et al*., 2013).

Generally, the DDG patterns are characterised by the stimulation, neutral and inhibition concentration ranges, with the stimulation being used in phytonematicides, whereas the inhibition concentrations are suitable for use in the development of herbicides (Liu *et al.,* 2003). Mashela *et al*. (2017) described four possible unique scenarios when plants were exposed to increasing concentrations of Nemarioc-AL phytonematicide, namely, (a) positive linear if stimulation concentrations were involved, (b) neutral, (c) negative linear if inhibition concentrations are involved and (d) quadratic relations when the stimulation, neutral and inhibition concentrations are involved. The measured plant variables in the current study clearly defined the stimulation and inhibition phases in the context of DDG, whereby the plant variables and increasing concentrations of Nemarioc-AL phytonematicide exhibited a negative quadratic relationship.

3.4.2 Essential nutrient elements

The computed MCSP for the nutrient elements was 3.08% Nemarioc-AL phytonematicide, which was comparable to the MCSP values of 2.99 and 2.97% Nemarioc-AL phytonematicide on tomato plants cv. 'Floradade' (Pelinganga, 2013) and citrus seedling rootstock (Mathabatha *et al*., 2017). In the current study, all assessed essential nutrient elements in leaf tissues confirmed the existence of all three phases of DDG pattern. The measured nutrient elements and increasing concentration of Nemarioc-AL phytonematicide exhibited positive quadratic relationships. The current study agreed with the observation, whereby K and Fe in leaf tissues of green bean (*Phaseolus vulgaris* L.) under greenhouse conditions exhibited a positive quadratic

relationships with increasing concentrations of Nemarioc-AL phytonematicide, with models explained by 97 and 98%, respectively (Mashela and Pofu, 2017).

3.4.3 Nematode variables

Nematode variables and increasing concentrations of Nemarioc-AL phytonematicide exhibited positive and negative quadratic relationships, clearly corroborating the context of DDG patterns for the respective assessed variables. Tseke and Mashela (2018) observed that *M. incognita* in roots and soil with increasing concentrations of Nemarioc-AL phytonematicide exhibited positive and negative quadratic relationships, with the models explained by 96 and 93%, respectively.

3.5 Conclusion

The MCSP on sweet potato cv. 'Bophelo' when using plant growth variables was established at 1.92% Nemarioc-AL phytonematicide. In contrast, when using plant physiology variables the MCSP was established at 3.08% Nemarioc-AL phytonematicide. Sweet potato cv. 'Bophelo' was highly sensitive to the product and the MCSP for plant growth variables and plant physiology variables could be reduced to 2% Nemarioc-AL phytonematicide, since the products are not intended for use as fertilisers. Nemarioc-AL phytonematicide could successfully be used in management of population densities of *Meloidogyne* species after the determination of application interval.

CHAPTER 4

APPLICATION INTERVAL OF NEMARIOC-AL PHYTONEMATICIDE IN SWEET POTATO CULTIVAR 'BOPHELO'

4.1 Introduction

The mean concentration stimulation point (MCSP) was established as the first step toward managing phytotoxicity in the use of Nemarioc-AL phytonematicide in the management of population densities of root-knot (*Meloidogyne* species) nematodes on sweet potato (Chapter 3). The MCSP, described as the concentration of phytonematicide which could be applied on plant without inducing phytotoxic, but being successful in suppressing the nematode population densities (Mashela *et al*., 2017), was established at 1.92% for Nemarioc-AL phytonematicide on sweet potato cv. 'Bophelo'. Subsequently, the MCSP is used in the establishment of the application interval.

In addition to the MCSP, the life cycle of the test nematode is used in the empirical determination of the application interval (Mashela *et al*., 2017). Mashela *et al*. (2017) developed the concept 'weeks-per-month-of-30 days' for *Meloidogyne* species, which was equivalent to 0, 7.5, 15, 22.5 and 30 days or 0, 1, 2, 3 and 4 weeks derived from the concept 'weeks-per-month-of-30 days' (Mashela *et al*., 2017). The objective of this study was to determine the application interval in weeks for Nemarioc-AL phytonematicide on sweet potato cv. 'Bophelo' for managing population densities of *M. javanica*.

4.2 Materials and methods

4.2.1 Description of the study site

The study site and conditions were as described previously (Chapter 3). The study was conducted in spring-summer (November-January: Experiment 1) 2017/2018 and repeated in summer-autumn (February-April: Experiment 2) 2018.

4.2.2 Treatments and research design

Treatments, namely, 0, 7.5, 15, 22.5 and 30 days of application interval were arranged in a randomised complete block design (RCBD), with eight replications for experiment 1 and seven replications for experiment 2 (Figure 4.1).

Figure 4.1 Greenhouse layout of the experiment.

4.2.3 Procedures

Nemarioc-AL phytonematicide and sweet potato cuttings were prepared using the locally developed procedure as described previously (Chapter 3). Sweet potato cuttings were collected and prepared as described previously (Chapter 3). Cuttings with well-developed root systems were transplanted into 20-cm-diameter plastic bags with approximately 2.7 L steam-pasteurised (300°C for 1 h) loam soil and Hygromix (Hygrotech, Pretoria North) at 3:1 (v\v) ratio. Containers with plants were placed on the greenhouse benches at 0.25 m intra-spacing and 0.25 m inter-spacing. At seven days after transplanting, each plant was inoculated with 5000 eggs and second-stage juveniles (J2) *M. javanica*. Treatments were initiated at seven days after inoculation, with 1.92% Nemarioc-AL phytonematicide concentration applied during the computed time-frames in days. Cultural practices such as fertilisation and pest management were as described previously (Chapter 3), whereas each plant was irrigated with 250 ml chlorine-free tapwater every other day.

4.2.4 Data collection

At 56 days after inoculation, plant, nutrient and nematode variables were measured and recorded as previously described (Chapter 3).

4.2.5 Data analysis

The treatments 0. 7.5, 15, 22.5 and 30 days were expressed as 0, 1, 2, 3, and 4 weeks. Plant variables were subjected to analysis of variance (ANOVA) through the Statistix 10.0 software. Significant plant variables were further subjected to lines of the best fit (Appendices 4.1-4.4) (Gomez and Gomez, 1984).

$$Y = b_2x^2 + b_1x + a$$

where Y = plant growth responses, x = application interval with $x = -b_1/2b_2$ value for optimum application time interval. Nematode variables were also subjected to ANOVA through the Statistix 10.0 software, with significant variables further subjected to lines of the best fit (Appendices 4.5-4.8) (Gomez and Gomez, 1984). Essential nutrient elements were subjected to analysis of variance ANOVA through the Statistix 10.0 software.

4.3 Results

Seasonal interactions were not significant for all variables and therefore, the data for the two experiments were pooled (n = 75) and re-analysed (Gomez and Gomez, 1984).

4.3.1 Plant growth variables

Vine length, stem diameter, dry root mass and gall rating exhibited density-dependent growth (DDG) patterns when plotted against increasing application interval of 1.92% Nemarioc-AL phytonematicide (Figure 4.2). The relationships between plant variables and application intervals in weeks exhibited positive quadratic equations, where the models were explained by 98, 89, 96 and 93% coefficient of determination, respectively

(Figure 4.2). Using $x = -b_1/2b_2$ relation, vine length, stem diameter, dry root mass and gall rating were optimised at different application intervals in weeks, with the mean application interval of 2.55 weeks (Table 4.1), which translated to 19 days (2.55/4 × 30).

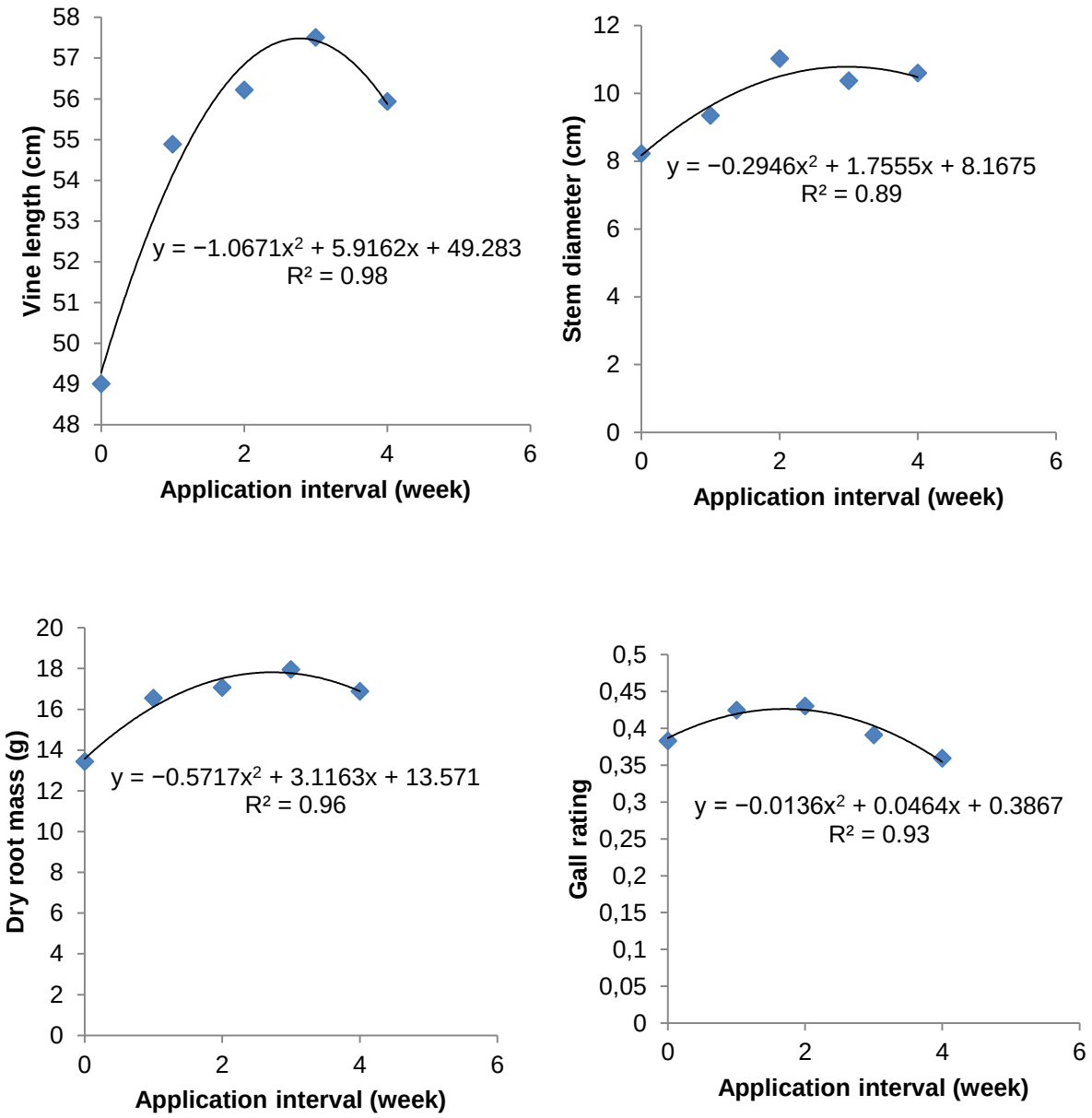

Figure 4.2 Responses of vine length, stem diameter, dry root mass and gall rating to increasing application intervals of Nemarioc-AL phytonematicide on sweet potato cultivar 'Bophelo'.

Table 4.1 Optimisation of application interval for Nemarioc-AL phytonematicide on vine length (VNL), stem diameter (STD), dry root mass (DRM) and gall rating (GLR) for sweet potato cultivar 'Bophelo'.

Variable	Quadratic relation	R^2	x^z
VNL	$y = -1.0671x^2 + 5.9162x + 49.283$	0.98	2.772
STD	$y = -0.2946x^2 + 1.7555x + 8.1675$	0.89	2.979
DRM	$y = -0.5717x^2 + 3.1163x + 13.571$	0.96	2.725
GLR	$y = -0.0136x^2 + 0.0464x + 0.3867$	0.93	1.706
Mean integrated application interval (weeks)			2.546

$^z x = -b_1/2b_2$.

4.3.2 Essential nutrient elements

Increasing application interval of Nemarioc-AL phytonematicide did not significantly affect the accumulation of all measured essential nutrient elements in the leaves of sweet potato plant (Appendices 4.9-4.12).

4.3.3 Nematode variables

Second-stage juveniles and eggs in roots, J2 in soil and final population of *M. javanica* over increasing application interval of Nemarioc-AL phytonematicide exhibited negative quadratic relationships, with the models explained by 76, 99, 85 and 85% coefficient of determination, respectively (Figure 4.3).

Table 4.2 Partitioning mean sum of squares for iron, potassium, sodium and zinc at application interval of 1.92% Nemarioc-AL phytonematicide on sweet potato cultivar 'Bophelo' leaves at 56 days after initiation of treatments.

		Fe		K		Na		Zn	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	14	0.02415	42	0.01555	47	0.01111	66	0.01845	68
Treatment	4	0.01248	22^{ns}	0.01213	37^{ns}	0.00200	12^{ns}	0.00224	8^{ns}
Error	56	0.02056	36	0.00530	16	0.00362	22	0.00656	24
Total	74	0.05719	100	0.03298	100	0.01673	100	0.02725	100

^{ns}Not significant at P ≤ 0.05.

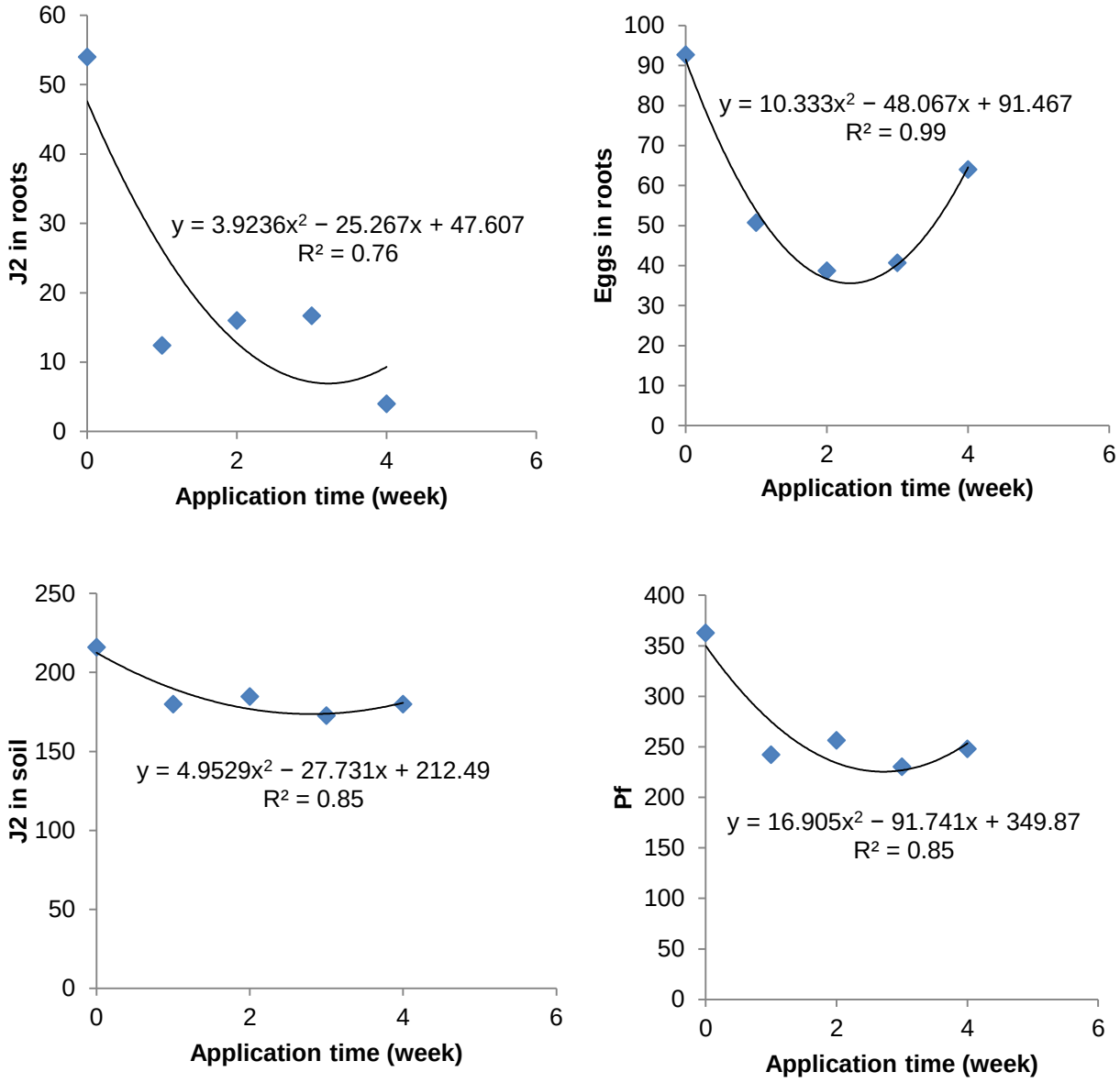

Figure 4.3 Responses of second-stage juveniles in roots, eggs in roots, second-stage juveniles in soil and final population of *Meloidogyne javanica* to increasing application intervals of Nemarioc-AL phytonematicide on sweet potato cultivar 'Bophelo'.

4.4 Discussion

4.4.1 Plant growth variables

In the current study, plant variables when plotted against increasing application intervals of 1.92% Nemarioc-AL phytonematicide displayed density-dependent growth (DDG) patterns. Application intervals of Nemarioc-AL phytonematicide at both lower and higher time intervals showed that there were stimulation and inhibition on the growth of sweet potato, respectively. Pelinganga (2013) reported that Nemarioc-AL phytonematicide when applied at 3% on tomato plant had attributes of inducing DDG patterns as the application interval increases, with the relationship explained for dry root mass, dry fruit mass, dry shoot mass, plant height and stem diameter by 92, 65, 64, 88 and 65%, respectively.

In the current study, various plant organs exhibited strong positive quadratic relations and were optimised at different application time intervals, with the computed integrated mean application interval converted to 19 days, which was more or less similar to that of 20 and 16 days for Nemafric-BL and Nemarioc-AL phytonematicides, respectively (Pelinganga, 2013). Mathabatha (2018: unpublished) demonstrated that when 2.97% Nemarioc-AL phytonematicide was applied over increasing application intervals to breakdown the life cycle of *Tylenchulus semipenetrans* and the integrated mean application interval was found to be 24 days for all citrus rootstocks.

4.4.2 Essential nutrient elements

In the current study, increasing application interval of Nemarioc-AL phytonematicide did not have an effect on essential nutrient elements. Numerous studies were done on the effect of phytonematicides on the accumulation of the essential nutrient elements, however there is no report on the effect of the increasing application interval of Nemarioc-AL phytonematicide on essential nutrient elements. Mashela and Pofu (2017) reported that increasing concentrations of Nemarioc-AL and Nemafric-BL phytonematicides also affected Ca, K, Na, Fe and Zn accumulation on the leaf tissues of green bean (*Phaseolus vulgaris* L.) under greenhouse conditions. In various interactive studies (Maake, 2018; Nyamandi, 2017; Pelinganga, 2013; Rabothata, 2017; Shadung, 2016), the two cucurbitacin-containing phytonematicides were shown to have significant effects on different essential nutrient elements.

4.4.3 Nematode variables

In the current study, increasing application intervals of Nemarioc-AL phytonematicide and nematode variables exhibited negative quadratic relationships. The observations were in agreement with report on tomato plants when exposed to increasing application interval of Nemarioc-AL phytonematicide, whereby the treatments and *M. incognita* race 2 exhibited negative quadratic relations, with models explained by 90 and 96%, respectively (Pelinganga *et al.*, 2013). The nematode population in the current study, decreases as the application intervals of Nemarioc-AL phytonematicide increases.

4.5 Conclusion

The application interval of Nemarioc-AL phytonematicide on sweet potato cv. 'Bophelo' for the management of population densities of *Meloidogyne* species was established at 2.55 weeks, which translated to 19 day application interval. The nutrient contents in leaves of sweet potato were not affected by the increasing application intervals of Nemarioc-AL phytonematicide. The obtained application interval for Nemarioc-AL phytonematicide on sweet potato could be able to disrupt the life cycle of the test nematode, without inducing toxicity to the test crop.

CHAPTER 5

SUMMARY OF FINDINGS, SIGNIFICANCE, FUTURE RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary of findings

Since the active ingredients in phytonematicides developed as alternative to synthetic chemicals are naturally phytotoxic to plants (Mashela *et al*., 2017), empirical data on mean concentration stimulation point (MCSP) and application interval is of paramount importance in the development of phytonematicides. The Curve-fitting Allelochemical Response Data (CARD) computer-based model has proven to be a valuable tool in this respect. The CARD-model generates biological indices critical in the computation of MCSP, a non-phytotoxic phytonematicide concentration. Biological indices where variables displayed negative quadratic relations were excluded in the calculation of the MCSP since the former did not have the stimulation phases. The study was set to determine the MCSP and application interval of Nemarioc-AL phytonematicide on sweet potato cv. 'Bophelo'. The CARD computer-based model generated seven biological indices, of which the first two were used to establish the MCSP when sweet potato cv. 'Bophelo' were exposed to increasing concentrations of Nemarioc-AL phytonematicide. The MCSP values of Nemarioc-AL phytonematicide for plant variables and plant physiology variables were established at 1.92 and 3.08%, respectively. In the current study, the overall sensitivities for plant variables and plant physiology variables were 0 and 1 unit, respectively. The obtained overall sensitivities from CARD computer-based model clearly indicate that sweet potato cv. 'Bophelo' was highly sensitive to the

product. In Experiment 1, all essential nutrient elements in leaf tissues when plotted against increasing concentrations of Nemarioc-AL phytonematicide exhibited positive quadratic relationships, whereas for Experiment 2, treatment effects were not significant for essential nutrient elements. In Experiment 1, J2 and eggs in roots and final population of nematodes exhibited negative quadratic relationships, with the models explained by 81, 75 and 83%, respectively, and whereas, J2 in soil exhibited positive quadratic relationship with the model explained by 89%. In Experiment 2, all nematode variables over increasing application interval of Nemarioc-AL phytonematicide exhibited negative quadratic relationships, with the models explained by 76, 99, 85 and 85% coefficient of determination, respectively. The MCSP established was used to determine the integrated application interval of the product, which was optimized at 2.55 ‘weeks-per-month-of-30 days’, translating to 19 days (2.55/4 × 30).

5.2 Significance

Using the biological indices, CARD computer-based model helped to compute the MCSP which was used to determine the application interval in the management of *M. javanica* on sweet potato cv. 'Bophelo'. The findings of this study came up with non-phytotoxic concentration that can be used in the suppression of nematodes in sweet potato and also facilitating the registration of the product in accordance with OECD (2017). This would also provide farmers with an environment-friendly and affordable product for management of nematodes. The observed quadratic relations on plant variables in this study allowed for the determination of the application interval at 1.92% Nemarioc-AL phytonematicide.

5.3 Future recommendations

Nemarioc-AL phytonematicide could be applied at 1.92% for sweet potato production under greenhouse conditions. The MCSP and application interval should be validated under field and microplot conditions for sweet potato cv. 'Bophelo'. According to Lee *et al*. (2010), at low concentrations the cucurbitacins could be carcinogenic, therefore, the empirical study should be conducted to analyse the cucurbitacin A chemical residues on sweet potato tubers, this is very critical since the tubers come in direct contact with the product. It would also be imperative to assess the environmental impact of Nemarioc-AL phytonematicide in terms of cucurbitacin A in the soil, more especially on microorganisms such as earthworms and other beneficial organisms.

5.4 Conclusions

The MCSP of Nemarioc-AL phytonematicide on sweet potato cv. 'Bophelo' when using plant growth variables was established at 1.92% from Dm and Rh of variables that had positive quadratic relations. In contrast, when using plant physiology variables the MCSP was established at 3.08% from Dm and Rh of variables that had positive quadratic relations. The MCSP for plant growth variables and plant physiology variables could be reduced to 2%, since the products are not intended for use as fertilisers. The obtained application interval of 19 days for Nemarioc-AL phytonematicide on sweet potato could be able to disrupt the life cycle of the test nematode, without inducing toxicity to the test crop.

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APPENDICES

Appendix 3.1 Analysis of variance for second-stage juviniles (J2) in roots inoculated with *Meloidogyne javanica* under increasing concentrations of Nemarioc-AL phytonematicide on sweet potato cultivar 'Bophelo' at 56 days after initiation of treatments (n = 70).

Source	DF	SS	MS	F	P
Replication	9	1.2439	0.13821		
Treatment	6	3.5790	0.59651	1.72	0.1345
Error	54	18.7480	0.34718		
Total	69	23.5709	1.0819		

Appendix 3.2 Analysis of variance for eggs in roots inoculated with *Meloidogyne javanica* under increasing concentrations of Nemarioc-AL phytonematicide on sweet potato cultivar 'Bophelo' at 56 days after initiation of treatments (n = 70).

Source	DF	SS	MS	F	P
Replication	9	3.7786	0.41985		
Treatment	6	10.1620	1.69366	3.26	0.0083
Error	54	28.0708	0.51983		
Total	69	42.0114	2.63334		

Appendix 3.3 Analysis of variance for second-stage juveniles (J2) in soil inoculated with *Meloidogyne javanica* under increasing concentrations of Nemarioc-AL phytonematicide on sweet potato cultivar 'Bophelo' at 56 days after initiation of treatments (n = 70).

Source	DF	SS	MS	F	P
Replication	9	0.8061	0.08956		
Treatment	6	14.6065	2.43442	7.53	0.0000
Error	54	17.4620	0.32337		
Total	69	32.8746	2.84735		

Appendix 3.4 Analysis of variance for final population (Pf) of *Meloidogyne javanica* under increasing concentrations of Nemarioc-AL phytonematicide on sweet potato cultivar 'Bophelo' at 56 days after initiation of treatments (n = 70).

Source	DF	SS	MS	F	P
Replication	9	0.48121	0.05347		
Treatment	6	4.28371	0.71395	8.25	0.0000
Error	54	4.67085	0.08650		
Total	69	9.43578	0.85392		

Appendix 4.1 Analysis of variance for vine length of sweet potato cultivar 'Bophelo' at 56 days after initiation of treatments (n = 75).

Source	DF	SS	MS	F	P
Replication	14	3116.93	222.638		
Treatment	4	1249.19	312.297	4.47	0.0033
Error	56	3915.62	69.922		
Total	74	8281.74	604.857		

Appendix 4.2 Analysis of variance for stem diameter of sweet potato cultivar 'Bophelo' at 56 days after initiation of treatments (n = 75).

Source	DF	SS	MS	F	P
Replication	14	220.804	15.7717		
Treatment	4	80.729	20.1822	3.61	0.0109
Error	56	312.964	5.5886		
Total	74	614.497	41.5425		

Appendix 4.3 Analysis of variance for dry root mass of sweet potato cultivar 'Bophelo' at 56 days after initiation of treatments (n = 75).

Source	DF	SS	MS	F	P
Replication	14	422.19	30.1567		
Treatment	4	178.58	44.6442	3.99	0.0064
Error	56	626.71	11.1913		
Total	74	1227.48	85.9922		

Appendix 4.4 Analysis of variance for gall rating of sweet potato cultivar 'Bophelo' at 56 days after initiation of treatments (n = 75).

Source	DF	SS	MS	F	P
Replication	14	0.04300	0.00307		
Treatment	4	0.10915	0.02729	3.60	0.0111
Error	56	0.42419	0.00757		
Total	74	0.57634	0.03793		

Appendix 4.5 Analysis of variance for second-stage juveniles (J2) in roots inoculated with *Meloidogyne javanica* at application interval of 1.92% Nemarioc-AL phytonematicide on sweet potato cultivar 'Bophelo' at 56 days after initiation of treatments. (n = 75).

Source	DF	SS	MS	F	P
Replication	14	14288.0	1020.57		
Treatment	4	22701.3	5675.33	5.67	0.0007
Error	56	56098.7	1001.76		
Total	74	93088.0	7697.66		

Appendix 4.6 Analysis of variance for eggs in roots inoculated with *Meloidogyne javanica* at application interval of 1.92% Nemarioc-AL phytonematicide on sweet potato cultivar 'Bophelo' at 56 days after initiation of treatments. (n = 75).

Source	DF	SS	MS	F	P
Replication	14	23427	1673.33		
Treatment	4	29453	7363.33	3.13	0.0214
Error	56	131587	2349.76		
Total	74	184467	11386.42		

Appendix 4.7 Analysis of variance for second-stage juveniles (J2) in roots inoculated with *Meloidogyne javanica* at application interval of 1.92% Nemarioc-AL phytonematicide on sweet potato cultivar 'Bophelo' at 56 days after initiation of treatments. (n = 75).

Source	DF	SS	MS	F	P
Replication	14	584133	41723.8		
Treatment	4	19596	4898.9	0.09	0.9867
Error	56	3218331	57470.2		
Total	74	3822060	104092.9		

Appendix 4.8 Analysis of variance for final population (Pf) of *Meloidogyne javanica* at application interval of 1.92% Nemarioc-AL phytonematicide on sweet potato cultivar 'Bophelo' at 56 days after initiation of treatments. (n = 75).

Source	DF	SS	MS	F	P
Replication	14	630155	45011.1		
Treatment	4	174137	43534.3	0.66	0.6238
Error	56	3705549	66170.5		
Total	74	4509841	154715.9		

Appendix 4.9 Analysis of variance for iron at application interval of 1.92% Nemarioc-AL phytonematicide on sweet potato cultivar 'Bophelo' at 56 days after initiation of treatments (n = 75).

Source	DF	SS	MS	F	P
Replication	14	0.34313	0.02415		
Treatment	4	0.04991	0.01248	0.61	0.6593
Error	56	1.15138	0.02056		
Total	74	1.54442	0.05719		

Appendix 4.10 Analysis of variance for potassium at application interval of 1.92% Nemarioc-AL phytonematicide on sweet potato cultivar 'Bophelo' at 56 days after initiation of treatments (n = 75).

Source	DF	SS	MS	F	P
Replication	14	0.21768	0.01555		
Treatment	4	0.04850	0.01213	2.29	0.0712
Error	56	0.29681	0.00530		
Total	74	0.56299	0.03298		

Appendix 4.11 Analysis of variance for sodium at application interval of 1.92% Nemarioc-AL phytonematicide on sweet potato cultivar 'Bophelo' at 56 days after initiation of treatments (n = 75).

Source	DF	SS	MS	F	P
Replication	14	0.15559	0.01111		
Treatment	4	0.00798	0.00200	0.55	0.6987
Error	56	0.20266	0.00362		
Total	74	0.36623	0.01673		

Appendix 4.12 Analysis of variance for zinc at application interval of 1.92% Nemarioc-AL phytonematicide on sweet potato cultivar 'Bophelo' at 56 days after initiation of treatments (n = 75).

Source	DF	SS	MS	F	P
Replication	14	0.25830	0.01845		
Treatment	4	0.00895	0.00224	0.34	0.8490
Error	56	0.36738	0.00656		
Total	74	0.63463	0.02725		