INFLUENCE OF PRIMING POTATO (*SOLANUM TUBEROSUM*) SEEDS IN SOLUTIONS OF THREE PHYTONEMATICIDES ON POTATO GROWTH AND NEMATODES

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DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE MASTER OF AGRICULTURAL MANAGEMENT (PLANT PRODUCTION), 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, Tshegofatso Eva Thopola, declare that the dissertation hereby submitted to the University of Limpopo, for the degree Master of Agricultural Management (Plant Production) 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 Lord and Savior, my mother, Professor Kefilwe Magdeline Thopola, my two beautiful kids, Deo Bokang Anza Thopola, Zamar Rendani Thopola and to my two brothers Mohau Brain Thopola and Paballo Emmanuel Thopola.

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ABSTRACT

Although potato seed priming in water is not allowed for quality-related reasons in tubers of the produced crop, it was viewed as necessary to use the technique as a carrier of active ingredients of phytonematicides, with the hope that should the technique work, then other solutions could be used for priming of potato tuber seeds. The objectives of this study were to investigate the feasibility of using potato seed tubers as carriers of cucurbitacin A, cucurbitacin B and momordin from triterpenoid-containing phytonematicides to improve management of nematode population densities in potato plants under greenhouse, microplot and field conditions, respectively. In single treatments ($A_1B_0M_0$, $A_0B_1M_0$ and $A_0M_0B_1$), potato seed tubers were without any phytotoxicity in 3% solutions, in any two permutations ($A_1B_1M_0$, $A_1B_0M_1$ and $A_0M_1B_1$) at 1.5% each and at three permutations ($A_1B_1M_1$) at 1% each, for 7 h and then dried under shade for 2 h prior to planting. Twenty-cm-diameter plastic pots were filled with 2 700 ml growing medium under greenhouse conditions and placed on benches at 0.3 m × 0.2 m spacing. Under microplot 30-cm-diameter plastic pots were used and pots were then inserted into 20-cm-deep holes at 0. 5 m × 0.5 m spacing and under field conditions potato seed tubers were set at 30-cm-depth with a 0.6 m × 0.6 m spacing. A 2 × 2 × 2 factorial experiment was laid out in a randomised complete block design, with the eight treatments replicated 7 times. Nemarioc-AL (A), Nemafric-BL (B) and Mormodica (M) phytonematicides served as first, second and third factors, respectively. At 56 days after applying treatments, the $A_1B_1M_1$ interactions were not significant on all plant variables under greenhouse and field conditions however under microplot the interaction was significant ($P \leq 0.05$) on fresh tuber mass, fresh root mass and dry root mass, contributing 28, 26 and 26% in Total treatment variation (TTV) of the respective variables. In contrast, the $A_1B_1M_1$

interactions were highly significant ($P \leq 0.01$) on chlorophyll content, contributing 43 and 40% in TTV. Generally, relative to untreated control, the second and first order interactions, along with individual treatments, significantly increased fresh tuber mass by 31% relative to the untreated control, except for Nemarioc-AL × Mormodica and Nemafric-BL × Mormodica interactions which were not different to the untreated. The $A_0B_1M_1$ interaction was highly significant on plant height, stem diameter, chlorophyll content, dry shoot mass, dry root mass and fresh tuber mass, contributing 45, 36, 37, 35, 60 and 35% in TTV of the respective variables under greenhouse conditions similar to the microplot experiment, the interaction relative to the untreated control, also did not have any effect on plant variables. However, under field conditions the first order interaction, $A_0B_1M_1$, was highly significant on dry root mass, contributing 60% in TTV on the variable. Relative to untreated control, the interaction reduced dry root mass by 14%, which was not different to the effect of Mormodica phytonematicide at 13%, but was significantly different to that of Nemafric-BL phytonematicide. $A_1B_0M_1$ interaction had significant effects on fresh tuber mass, contributing 33% in TTV on the variable. Relative to the untreated control, the interaction increased fresh tuber mass (yield) by 32%, which was not different to that of Nemarioc-AL phytonematicide at 40%, but significantly different to that of Mormodica phytonematicide at 16%. Nemafric-BL and Mormodica phytonematicides under greenhouse conditions, each reduced dry shoot mass by 18 and 22%, respectively, whereas their interaction effects on the variable did not differ significantly from the untreated control. Similarly, under microplot conditions Mormodica phytonematicide alone significantly reduced plant height by 12%, although this was not different from the effects of Nemafric-BL phytonematicide. Also, the effects of Nemafric-BL phytonematicide on plant height was not different to that of the untreated control. However, Mormodica phytonematicide increased FSM,

DSM and FTM by 31, 33 and 19%, respectively. Mormodica phytonematicide effect on the variables was significantly different to the untreated control. The phytonematicide also reduced FRM and DRM by 17% and the effect on the two variable which significantly differed from the untreated control. The second order interaction were not significant on nematode variable under greenhouse and field conditions, but the interaction was significant only on total nematodes in roots, reproductive potential (RP) and final population (Pf) of *Meloidogyne* species on roots of potato plants and in the soil under microplot conditions, contributing 11, 13 and 10% in TTV on the variables, respectively. Relative to untreated control Nemarioc-AL × Nemafric-BL × Mormodica phytonematicide interaction reduced total nematode, RP and PF by 18, 64 and 18%, respectively, whereas their effects on the variables differed significantly from untreated control. Generally, Nemafric-BL × Mormodica interaction consistently in all three experiment reduced all nematodes variables. Nemarioc-AL × Nemafric-BL × Mormodica interactions were only significant on Na, contributing 7% in TTV o the variable. Relative to untreated control the interaction reduced Na by 33% and effects on the variable was significantly different to those of untreated control also Nemarioc-AL, Nemafric-BL and Mormodica alone. However, the second order interactions were not significant in greenhouse and field conditions. The $A_0B_1M_1$ first order interaction although the interactive effects, contributed highly in TTV on Na and Zn in potato tuber tissues, relative to untreated control, the effects were rather negligent at 2 and 4%, respectively. In all different conditions of the study validating that potato seed tubers could be used as carriers of active ingredients of phytonematicides when used through the priming technology. The Nemafric-BL and Mormodica phytonematicide interactions consistently reduced population densities of the *Meloidogyne* species and increased yield under microplot and field experiments.

CHAPTER 1
RESEARCH PROBLEM

Background

International agreements to withdraw the highly effective methyl bromide and related fumigant nematicides due to their ozone-depleting properties and subsequent effects on global warming, have had deleterious concerns in management of plant nematodes (Mashela *et al*., 2011). Following the withdrawal of methyl bromide in 2005, there was an unheralded need for alternative products for managing nematodes in crop husbandry (Caboni and Ntalli, 2014). Use of organic amendments as an alternative to methyl bromide received much attention after the adoption of the Montreal protocol in the early 1990s (Bello, 1998). Unfortunately for such products to be successful in management of plant nematode, extremely large quantities of the material were required (Mankau, 1968; Mankau and Minter, 1962; McSorley and Gallaher, 1995; Muller and Gooch, 1982; Rodriguéz-Kabana, 1986; Stirling, 1991), which translated to unavailability and transport cost challenges (Mashela *et al*., 2017). In addition to inconsistent results in nematode suppression (McSorley, 2003), most organic amendments reduced soil Ph (Stirling, 1991). Research on phytonematicides as currently described, was initiated, *inter alia,* to ameliorate the drawbacks of conventional organic amendments in managing plant nematodes (Mashela, 2002), which ranged from reducing excessively large quantities, reduction in soil Ph, negative period, phytotoxicity and most importantly, the issue of inconsistent results. In addition to resolving the listed issues using computer-based algorithm, the Curve-fitting Allelochemical Response Dose (CARD) model (Liu *et al*., 2003), application technologies, namely, the ground leaching technology (GLT) and botinemagation

technology, were designed, researched and developed (Mashela, 2002; Mashela *et al*., 2017).

The GLT system involves placing small quantities of granulated phytonematicides around the seedling at transplanting, with the active ingredient leached into the rhizosphere through irrigation water (Mashela, 2002). In contrast, in botinemagation technology, the phytonematicides in liquid formulations are applied through irrigation water using any irrigation system, but preferably drip irrigation system (Mashela *et al*., 2017). The GLT system is, especially in large commercial farming systems, labour intensive, whereas the botinemagation systems requires initial high investment (Mashela *et al*., 2017). Consequently, the need for research and development of user-friendly application technologies for phytonematicides exists.

1.1.1 Description of the research problem

In plant production, yield reduction due to nematodes is a function of the initial nematode population density at transplanting or sowing (Seinhorst, 1965). In other words, an appropriate system should be able to deliver the active ingredients in the rhizosphere in small quantities at all times from transplanting until the next application time. The existing application technologies for phytonematicides place the active ingredients in the rhizosphere during irrigation, with limited exposure to rhizosphere as the soil gradually dries up, with the subsequent placement of active ingredients being dependent on irrigation intervals. The phytonematicides used in the GLT system in granular (G) formulation include Nemarioc-AG and Nemafric-BG phytonematicides (Mashela *et al*., 2017), whereas in the botinemation the products are Nemarioc-AL and Nemafric-BL phytonematicides, in liquid (L) formulation. In both systems, the

efficacy of the irrigation system and the irrigation intervals are important. In both the GLT and the botinemation systems, temporary blockage of drip holes could have serious consequences on management of nematode population densities as outlined by Seinhorst (1965). Additionally, as long as the plant grows, an ideal technology should have the capability to timeously release sufficient quantities of the active ingredients into the rhizosphere to supress nematode population densities, but without inducing phytotoxicity.

Worldwide, the need to have alternatives that are user-friendly in management of plant nematodes is increasing. The withdrawal of synthetic fumigant nematicides from the agro-chemical markets due to their environment-unfriendliness (Mashela *et al*., 2015), had serious effects on crops that do not have resistance to plant nematodes. Potato (*Solanum tuberosum* L.) is such a crop since it does not have any genotypes with resistance to root-knot (*Meloidogyne* species) nematodes (Pofu *et al*., 2012), with yield reduction due to nematodes being as high as 50% to complete crop failure (Lamberti, 1979; Pofu *et al*., 2012).

1.1.2 Impact of the research problem

The lack of user-friendly application technologies could limit the widespread adoption of the developed phytonematicides, particularly in potato production since most the crop is grown under large fields, where the use of the GLT system would not be ideal due to its high cost. Also, the botinemagation would not be ideal since most farmers use sprinkler irrigation systems, which would render the entire field to be exposed to cucurbitacins, which would eventually pollute the water systems.

1.1.3 Possible causes of the research problem

The lack of suitable placement technology of phytonematicides could be ascribed to the fact that the technology is recent and in the recent past, the crop was reliant on methyl bromide for nematode management, with most farmers believing that nematodes were not a challenge in potato production (Mashela *et al*., 2017). Unfortunately, the conditions that favour successful potato production are also favourable for multiplication and survival of plant nematodes (Jones, 1970). For example, hatch and movement to roots occur most rapidly in sandy soils and this contributes to crops grown on such soils, like potato, being those likely to suffer the heaviest damage by plant-parasitic nematodes (Trudgill and Ritz 1999).

1.1.4 Possible solutions of the research problem

The potato seed tubers, upon sowing, as plants grow, generate roots which eventually generate numerous other tubers that would serve as the subsequent fresh tuber mass (yield). In most cases, the potato seed tuber remains where it was originally set, with the new fresh tubers being raised up, thus, the need for ridging. Since the potato seed tuber remains the major source of roots, any chemical compounds being leached out of the former, would be dripping onto the developing roots, where second-stage juveniles (J2) would be penetrating the root system.

1.2 Problem statement

The investigation intended to explore the use of the potato seed tubers as carriers of active ingredients, namely, cucurbitacin A ($C_{27}H_{40}O_9$), cucurbitacin B ($C_{32}H_{48}O_8$) and momordin ($C_{41}H_{64}O_{13}$) from Nemarioc-AL, Nemafric-BL and Mormodica phytonematicides, respectively. Since chemicals compounds move from high

concentration to low concentration, during priming, cucurbitacins and mormodin would move from solutions into the potato seed tubers, whereas after setting they would move from the tubers into the surrounding zones, which would later include the rhizosphere.

1.3 Rationale of the study

The priming technology was chosen as an appropriate application technology for active ingredients cucurbitacins and momordin from the three phytonematicides due to the extended survival of the potato seed tubers. Due to their allelopathic nature (Rice, 1984), the resultant plants could suffer from allelopathy, with reduced marketable yield. However, since the marketable yield constitutes tubers, which are modified stems, such potato tubers could be more highly tolerant than potato roots when subjected to increasing concentrations of cucurbitacin-containing phytonematicides (Pelinganga *et al*., 2012). In the current study, the Mean Concentration Stimulation Point (MCSP), which were previously obtained in tomato (*Solanum lycorpersicum* L.), of approximately 3% (Pelinganga, 2012), would be used since potato plants are also in the same family as tomato plants. The 3% was chosen despite previous observations which suggested that the MCSP values were plant-specific (Mashela *et al*., 2017). Among other reasons, the value was chosen to allow permutations for second order interactions to be at 1% each main factor. However, should the permutations consistently suppress nematode population densities in potato husbandry under various conditions, the existence of phytotoxicity, if any, would be resolved using the Curve-fitting Allelochemical Response Dose (CARD) algorithm (Liu *et al*., 2003), as described previously (Mashela *et al*., 2017), and permutations assessed again. Finally, comparison of the magnitude for nematode suppression

using the priming technology and the conventional application methods, namely, GLT and botinemagation, would provide some clues as to whether the technology was worth investing resources and time in it.

1.4 Purpose of the study

1.4.1 Aim

Development of user-friendly and cost effective technology for placing cucurbitacins and momordin in the rhizosphere of potatoes using potato seed tubers.

1.4.2 Objectives

1. To investigate whether using potato seed tubers as carriers of cucurbitacin A, cucurbitacin B and momordin from triterpenoid-containing phytonematicides would not be phytotoxicity but improve management of nematode population densities in potato plants under greenhouse conditions.
2. To determine whether using potato seed tubers as carriers of cucurbitacin A, cucurbitacin B and momordin from triterpenoid-containing phytonematicides would avoid phytotoxicity and improve management of nematode population densities in potato plants under micro-plot conditions.
3. To establish whether using potato seed tubers as carriers of cucurbitacin A, cucurbitacin B and momordin from triterpenoid-containing phytonematicides would avoid phytotoxicity and improve management of nematode population densities in potato plants under field conditions.

1.5 Null hypotheses

1. Using potato seed tubers as carriers of cucurbitacin A, cucurbitacin B and momordin from triterpenoid-containing phytonematicides would avoid phytotoxicity and improve management of nematode population densities in potato plants under greenhouse conditions.
2. Using potato seed tubers as carriers of cucurbitacin A, cucurbitacin B and momordin from triterpenoid-containing phytonematicides would avoid phytotoxicity and improve management of nematode population densities in potato plants under microplot conditions.
3. Using potato seed tubers as carriers of cucurbitacin A, cucurbitacin B and momordin from triterpenoid-containing phytonematicides would avoid phytotoxicity and improve management of nematode population densities in potato plants under field conditions.

1.6 Reliability, validity and objectivity

In the current study, reliability of data was based on statistical analysis of data at the probability level of 5%, whereas validity was achieved through repeating the experiments in time (Leedy and Ormrod, 2005). Additionally, the factorial set of treatments would be another way of increasing the range of validity (Little and Hills, 1981). The objectivity was achieved by discussing the findings on the basis of empirical evidence as shown by statistical analyses, with findings compared and contrasted with findings in other studies.

1.7 Bias

In this study, bias was minimised by ensuring that the experimental error in each experiment was reduced through increased replications and randomisation of the treatments (Leedy and Ormrod, 2005). Additionally, the discussion of data was limited to treatments that were significant at the probability level of 5%.

1.8 Scientific significance of the study

The study intends to establish whether priming potato seeds with Nemarioc-AL, Nemafric-BL and Mormodica phytonematicides could be used as an alternative technique in the management of *Meloidogyne* species under various conditions, with the magnitudes being compared with those under the GLT and botinemagation. The information would be useful in potato husbandry since there are no nematode resistant cultivars.

1.9 Structure of dissertation

Following the description and detailed outlines of the research problem (Chapter 1), work done and not yet done on the research problem were reviewed (Chapter 2). Then, each of the three subsequent research chapters (Chapter 3; Chapter 4; Chapter 5) addressed each of the three objectives. In the final chapter (Chapter 6), a summary of findings in all research chapters would be provided and then integrated to provide the significance of the findings and recommendations with respect to future research, culminating in conclusions that tie the entire study to provide a take-home message. Literature citation and referencing followed the Harvard style of using author-alphabets. In the next chapter, literature on the work done on the research problem was provided.

CHAPTER 2

LITERATURE REVIEW

2.1 Work done on the problem statement

2.1.1 Application of phytonematicides

Phytonematicides are researched globally as an alternative to synthetic chemical nematicides. Phytonematicides are produced in different range of forms, which include aqueous plant extracts (Chedekal, 2013; Rossner and Zebitz, 1987), methanol plant extracts (Usman, 2013), ethanol plant extracts (Khan *et al*., 2008), oil cakes (Muller and Gooch, 1982), essential oils (Myer *et al*., 2008), fermented crude plant extracts (Mashela, 2002; Pelinganga and Mashela, 2012; Pelinganga et al., 2013), powders (Ahmad *et al*., 2013) and granules (Mashela *et al*., 2011). Different plant species are researched and found to have nematicidal effects.

The ground leaching technology (GLT) involved the use of Nemarioc-AG or Nemafric-BG phytonematicide in granular (G) formulation at approximately 2 g phytonematicide/plant, which translated to 8 kg for 4 000 tomato plants per ha (Mashela, 2002; Mashela and Nthangeni, 2002). Nemarioc-AG phytonematicide reduced population density of *Meloidogyne* species by 90%, 90% and 80% under greenhouse (Mashela, 2002), microplot (Mofokeng *et al*., 2004; Shakwane *et al*., 2005) and field conditions (Mashela, 2007), respectively. The material was applied around the crop seedling at transplanting or post-emergence after which it was soon covered by soil. Most other plant species which were tested were not suitable for use in the GLT system because a large quantiy of the dried granuale will be requirment. For example, *Brassica* species, which are widely used in biofumigation for nematode management (Bello, 1998), require microbial degradation first to release the active

ingredient with nematicidal properties, and were therefore not suitable in the GLT system (Mashela *et al*., 2011).

Botinemagation technology involved the application of Nemarioc-AL or Nemafric-BL phytonematicide through irrigation system (Mashela *et al*., 2015). Nemarioc-AL phytonematicide reduced *M. incognita* population density in roots and soil under greenhouse conditions by 97-99% and 47-90%, respectively, under microplot conditions by 61% and 52%, respectively, and under field conditions by 79-85% and 79-85%, respectively (Pelinganga *et al*., 2012; Pelinganga *et al*., 2013a; Pelinganga, 2013b). Nemafric-BL phytonematicide, when applied through botinemagation, reduced *M. incognita* population density in roots and soil under greenhouse conditions by 85-97% and 45-96%, respectively, under microplot conditions by 72% and 77%, respectively, and under field conditions 79-85 and 79-85%, respectively (Pelinganga *et al*., 2012; Pelinganga *et al*., 2013a; Pelinganga, 2013b).

2.1.2 Economic importance of potato

Potato (*Solanum tuberosum* L.) originated in the tropical areas of the high altitude of the Andes. Currently, the crop is being grown throughout the world, but is of particular importance in the temperate climates. World production is estimated at 308 million ton fresh tubers from 19 million ha (FAOSTAT, 2001). Potato is a cholesterol-free as well as high in fibre, Vitamin C and essential minerals (McGill *et al*., 2013). It is also one of the most important vegetable crop in South Africa and the world's recognised stable food consumed by many people (Hijmans, 2001).

Currently, the potato industry contributes to approximately 43% of major vegetable production, 15% horticultural produce and 4% total agricultural products (Schulze, 2016). Limpopo Province is the main potato production region in South Africa, producing approximately 21% of the total gross (Agriorbit, 2017). Potato is one of the crops that are crucial in terms of food security, taking into account the rapid human population growth and increased hunger rates (IPC, 2011). Due to the progressive increase in the global human population, estimated to reach 9 billion in 2050, it is of vital importance that agricultural and horticultural crops be produced and utilised optimally.

2.1.3 Economically important nematode in potato production

Management of plant-parasitic nematodes in cropping systems is indispensable if crop enterprises are to be profitable and improve food security on a global scale. Due to various setbacks on nematode resistance, organic amendments and/or other biological agents were tested on a grand scale for the suppression of population nematode densities (Mashela *et al*., 2015). Notably, higher plants, biocontrol agents and fungi have since provided a broad spectrum of active compounds for use in nematode management (Chitwood, 2002; Okwute 2012; Chedekal, 2013). Several plant-parasitic nematodes have been reported to be noxious to potato crops worldwide, notably the cyst nematodes (*Globodera* species) (Jatala and Bridge, 1990; Jensen *el al*., 1979). In addition to the cyst nematodes, some nematode species cause damage in almost all major potato growing regions (Greco, 1993). Root-knot *(Meloidogyne* species) nematodes can cause an estimated annual loss of US$157 billion globally (Abad *et al*., 2008). Detailed estimates of crop losses due to nematodes

suggested that *Meloidogyne* species were a major contributor towards overall yield losses in the USA (Koenning *et al*., 1999).

Current estimated annual crop losses due to nematode damage in South Africa stand at 14% (Swart, 2010). However, in most cases, the impact of *Meloidogyne* species is grossly underestimated since there are no genotypes with resistance to *Meloidogyne* species (Jones *et al*., 2011; Onkendi *et al*., 2014; Pofu and Mashela, 2018).

2.1.4 Density-dependent growth patterns in phytonematicides

The concept of density-dependent growth (DDG) patterns is not unique to phytonematicides. Biological entities respond to various abiotic and/or biotic factors through a myriad of complex processes and mechanisms, with the DDG patterns being dominant. For example, when various plant nematodes infect plants at population densities below the tolerance limit, plant growth was invariably stimulated (Wallace, 1973), whereas at high population densities, growth was inevitably reduced (Seinhorst, 1967). In soil allelochemical residue (SAR) trials, it was shown that the SAR effects from one phytonematicide stimulated growth of the successor crop, whereas such effects consistently reduced population density of *Meloidogyne* species (Mashela and Dube, 2014). Mashela and Dube (2014) further stated that, for phytonematicdes to be successful, their inhibition concentration range to nematodes should overlap the stimulation range to the test crop being protected against nematodes.

Cucurbitacins, which are the active ingredients of the test phytonematicides, were shown to have the potential to induce bioactivities of healthy animal cells (Lee *et al*.,

2010). For example, at high concentrations, cytotoxicity occurred, whereas at low concentrations division of healthy cells was stimulated, thereby rendering the materials cancerous (Lee *et al*., 2010). However, in crops, the stimulated effects on cells was viewed as a “fertiliser effect” (Mashela, 2002), and desirable. Generally, the efficacy of phytonematicides is dependent upon the concentration of allelochemicals in the organ used for processing the intended products. The accumulation of secondary metabolites in organs varies from season to season (Mudau *et al*., 2008), with high inconsistent results in nematode suppression and high phytotoxicities during certain seasons (Mashela *et al*., 2015).

The two major application technologies of phytonematicides, were researched and developed for ameliorating the disadvantages of using organic amendments in managing nematode population densities (Chapter 1). They required expensive irrigation systems, large quantieties of the amendments leading to high transportation costs. However, certain crops which are grown under large commercial farming systems, for example in areas with high rainfall such as in eastern part of Free State Province, irrigation systems are not required. Consequently, for potato production in particular, the priming technology as previously introduced (Chapter 1), would be most appropriate.

2.2 Work not done on problem statement

Priming technology on potato seed tuber had not been investigated as to whether it would suppress nematode population density without inducing phytotoxicity. Such a study would be conducted under greenhouse, microplot and field conditions, due to the existence of diverse environmental and soil conditions.

CHAPTER 3

POTATO SEED TUBERS AS CARRIERS OF ACTIVE INGREDIENTS OF PHYTONEMATICIDES UNDER GREENHOUSE CONDITIONS

3.1 Introduction

The existing triterpenoid-containing phytonematicides, Nemarioc-AL, Nemafric-BL and Mormodica phytonematicides, have been researched and developed from fruits of wild cucumber (*Cucumis myriocarpus* Naude.), wild watermelon (*Cucumis africanus* L.F.) and bitter gourd (*Mormodica balsamina* L.) (Chen *et al*., 2005). The active ingredients in the products are triterpenoids cucurbitacin A ($C_{27}H_{40}O_9$), cucurbitacin B ($C_{32}H_{48}O_8$) and momordin ($C_{41}H_{64}O_{13}$), respectively. In phytonematicide form, the products had been consistent in suppressing population densities of root-knot (*Meloidogyne* species) nematodes in various crops (Jeffrey, 1978; Mashela *et al*., 2017).

Each of the phytonematicides could be applied using the ground leaching technology (GLT) system (Mashela, 2002) or botinemagation system (Mashela *et al*., 2017). Generally, the GLT system is labour-intensive and is therefore not suitable for large commercial farming scales (Mashela *et al*., 2017). However, the GLT system had been successfully used in smallholder farming systems (Mafeo, 2005). In this system, products in granular formulation are applied at transplanting in a circular furrow around the stem and then covered, with the active ingredients being released into the rhizosphere through leaching. In contrast, in botinemagation, phytonematicides in liquid formulations are applied through irrigation water, where the application could be repeated during the production of certain crops, as dictated by the application concentration and the application intervals (Mashela *et al*., 2017). Since this system

requires infrastructure for fermentation and irrigation, it could be costly for adoption in smallholder farming systems. Consequently, there is a need for researching and developing application systems that would be less costly, but user-friendly.

Currently, there are limited products for use in the management of plant-parasitic nematodes in potato (*Solanum tuberosum* L.) production. Potato seed tubers used at planting are not part of the harvestable produce, but these are central in the productivity of the produce from the potato plant. At harvest, most of the potato seed tubers are still in good form, but can be easily distinguished from the new tubers. The potato seed tubers can imbibe water and therefore, could serve as carriers of active ingredients of the triterpenoid-containing phytonematicides, and then release them into the rhizosphere of the growing potato plants. The objective of this study was to determine the potential of using potato seed tubers as carriers of cucurbitacin A, cucurbitacin B and momordin from triterpenoid-containing phytonematicides without phytotoxicity and improve management of nematode population densities in potato plants under greenhouse conditions.

3.2 Materials and methods

3.2.1 Location of the study

The study was conducted at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, South Africa (23°53’10” S, 29°44’15” E). The greenhouse was 30 m × 16 m, with a wet wall on the southern side and fans on the northern side. These thermostat-controlled fans extracted hot air from the greenhouse to the exterior, unfortunately, creating heterogeneous conditions within the greenhouse. The latter necessitated experimental designs that would account for the

created variability. Day/night ambient temperatures averaged 25/18°C during summer (November-January), with relative humidity ranging from 70 to 75%. The experiment was initiated in summer 2017 (Experiment 1) and validated in 2018 (Experiment 2).

3.2.2 Treatments and experimental design

A 2 × 2 × 2 factorial experiment was laid out in a randomised complete block design, with the eight treatments replicated 7 times. Nemarioc-AL (A), Nemafric-BL (B) and Mormodica (M) phytonematicides served as first, second and third factors, respectively. The eight treatments included untreated control ($A_0B_0M_0$), $A_0B_0M_1$, $A_0B_1M_0$, $A_0B_1M_1$, $A_1B_0M_0$, $A_1B_0M_1$, $A_1B_1M_0$ and $A_1B_1M_1$.

3.2.3 Procedures

Twenty-cm-diameter plastic pots were filled with 2 700 ml growing medium that comprised pasteurised (300 °C for 1 h) loam soil (35% clay, 15% silt and 50% sand), river sand and Hygromix-T (Hygrotech, Pretoria North, South Africa) per pot in 2:1:1 (v/v) ratio. Pots were placed on greenhouse benches at 0.3 m × 0.2 m spacing. The three respective phytonematicides were prepared using dried fruits from their respective cultivated crops as described previously (Mashela *et al*., 2017). Briefly, mature fruits were separately collected, washed in tapwater, chopped into small pieces and dried in air-forced ovens at 52º C for 72 h. The materials were ground in a Wiley mill through a 1-mm-mesh sieve. The ground materials were separately stored at room temperature in hermitically sealed plastic bags for future use.

Approximately 80 g ground material of *C. myriocarpus* fruit powder and 40 g *C. africanus* and *M. balsamina* were separately fermented in 20 L-plastic containers with

16 L chlorine-free tapwater as previously described (Mashela *et al*., 2017). Briefly, the containers were hermetically-sealed, with allowance for released CO_2 to escape provided through an airtight 5 mm diameter tube with one end glued to a hole on the lid of the container, and with the outlet end dangling into a litre bottle half-filled with tapwater. Approximately 300 ml molasses, 100 g brown sugar and 300 ml effective microorganisms (EM) were added into the container (Pelinganga *et al*., 2012). After a 14-day incubation period, when Ph was at least ±3.7 (Kyan *et al*., 1999), each phytonematicide was ready for use. In single treatments ($A_1B_0M_0$, $A_0B_1M_0$ and $A_0M_0B_1$), potato seed tubers were submerged in 3% solutions, in any two permutations ($A_1B_1M_0$, $A_1B_0M_1$ and $A_0M_1B_1$) at 1.5% each and at three permutations ($A_1B_1M_1$) at 1% each. Treatmnts were then left for 7 h and then dried under a shaded room temperature at 27º C for 2 h.

Figure 3.1 Priming potato seed tubers in permutations of Nemarioc-AL × Nemafric-BL × Mormodica phytonematicide solutions.

Nematode inoculation was prepared by extracting eggs and second-stage juveniles (J2) of greenhouse-grown nematode-susceptible tomato cv. 'Floradade' in 1% NaOCl solution (Hussey and Barker, 1973). At 60% emergence, each pot was inoculated by dispensing a mixture of 5 000 *M. javanica* eggs + J2 using a 20-ml plastic syringe by placing into 5-cm-deep holes on the cardinal points of stems or around the centre of the pot and thereafter, covered with the growing medium.

3.2.4 Cultural practices

Plants were irrigated using 350 ml tapwater every other day. Plants were fertilised at 100% emergence with 2.5 g 2:3:2 (22) NPK + 0.5% Ca + 0.5% Fe + 0.5% Zn fertiliser mixture to provide a total of 155 mg N, 105 mg P and 130 mg K per ml water and after 30 days top-dressed with 1 g 2:1:2 (43) Multifeed (Nulandies, Johannesburg) which provided a total of 0.175 mg N, 0.16 mg K, 0.16 mg P, 0.45 mg, 0.378 mg Fe, 0.0375 mg Cu, 0.175 mg Zn, 0.5 mg B, 1.5 mg Mn and 0.035 mg Mo per ml water, without Ca. Population densities of leaf miner (*Tuta absaluta* Meyrick 1917*)* and the greenhouse whiteflies (*Trialeurodes vaporariorum* Westwood) were managed using Steward and Cypermethrin as per label instruction.

3.2.5 Data collection

At 56 days after setting the primed potato seed tubers, plant height was measured from the crown to the tip of the flag leaf and chlorophyll content on three matured leaves per plant was measured using a chlorophyll meter (Minolta Spad-502). The stem diameter was measured at 5 cm from the distal end of the severed stem using a Vernier caliper. Tubers were separated from shoots and roots, with roots shaken in water to remove soil particles, pattered dry using the laboratory towel and then

weighed. Eggs and juveniles were extracted from the total root system of each plant through the maceration and blending method for 30 s in 1% NaOCl solution (Hussey and Barker, 1973). The aliquots were passed through 75 and 25-µm nested sieves, with nematodes being collected from the 25-µm mesh sieve. Soil per pot was thoroughly mixed and a 250 g soil sample was collected for nematode assay in the soil. In both root and soil samples, the extracts were mixed with kaolin and further centrifuged using the modified sugar-floatation method to separate nematodes from debris and soil particles, respectively (Jenkins, 1964). Fresh shoot and additional roots from five plants with known fresh mass were oven-dried at 52 °C for 72 h for dry mass determination.

Mature potato leaf tubers were chopped into small pieces and dried in air-forced ovens at 52 ºC for 72 h (Mashela *et al*., 2017), with the material ground using A43 Monlinex coffee grinder. Approximately 0.4 g powdered materials in vessels were digested in 75 ml 70% nitric acid (HNO_3) and 30 ml hydrogen peroxide (H_2O_2) using microwave digester (Perlain Elmer, Titan MPS). The vessels were then inserted into the microwave digester, sealed and whirled for 46 minutes at 260 °C. Thereafter, the vessels were placed in the laminar flow hood and allowed to cool down for 5 minutes, with materials transferred into 50 ml centrifuge tubes and stored in the cold room before analysing the nutrient elements using Inductively Coupled Plasma Optical Emission Spectrometry (Shimadzu, ICPE-9000).

3.2.6 Data analysis

Nematode data were transformed using $\log_{10}(x + 1)$ prior to analysis. Nematode and plant data were subjected to factorial analysis of variance using Statistix 10.1 software. The degrees of freedom and their associated mean sum of squares were partitioned to provide the total treatment variation (TTV) for separate sources (Steyn *et al*., 2003). Significant second and first order interactions were further expressed using either three-way or two-way matrix tables, respectively, thereby allowing for the determination of the direction and magnitude of the effects of the permutations relative to the untreated control. Unless otherwise stated, treatment effects were discussed at the probability level of 5%.

3.3 Results

Nematode and plant data were assessed for seasonal interactions and since the interactions were not significant ($P \leq 0.05$), data were pooled ($n = 112$) and subjected to factorial analysis of variance through Statistix 10.1 software.

3.3.1 Effects on plant variables

The $A_1B_1M_1$ interactions were not significant on all plant variables. However, the $A_0B_1M_1$ interaction was highly significant ($P \leq 0.01$) on plant height, stem diameter, chlorophyll content, dry shoot mass, dry root mass and fresh tuber mass, contributing 45, 36, 37, 35, 60 and 35% in TTV of the respective variables (Appendix 3.1). Generally, relative to the untreated control, the $A_0B_1M_1$ interaction did not reduce or increase plant variables. However, the two-way interaction continue to suggest that both Nemarioc-AL and Mormodica phytonematicides each reduced the plant variables.

Nemafric-BL and Mormodica phytonematicides each reduced dry shoot mass by 18 and 22%, respectively, whereas their interaction effects on the variable did not differ significantly from the untreated control (Table 3.1). Mormodica phytonematicide alone significantly reduced plant height by 12%, although this was not significantly different from the effects of Nemafric-BL phytonematicide. Also, the effects of Nemafric-BL phytonematicide on plant height was not significantly different to that of the untreated control.

Table 3.1 Two-way matrix for dry shoot mass and plant height as affected by first order interaction of Nemafric-BL and Mormodica phytonematicides on potato plants at 56 days after priming application.

	Mormodica (M)							
	Dry shoot mass				Plant height			
Nemafric-BL (B)	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]
B_0	6.6[a]	–	5.1[b]	–22	70.2[a]	–	61.9[b]	–12
B_1	5.4[b]	–18	6.5[a]	–2	66.6[ab]	–5	70.7[a]	1

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

Nemafric-BL and Mormodica phytonematicides each significantly reduced fresh root mass by 30%, whereas the effects of the interaction was not significantly different from the untreated control (Table 3.2). Similar effects were observed on dry root mass.

Table 3.2 Two-way matrix for fresh root mass and dry root mass as affected by first order interaction of Nemafric-BL and Mormodica phytonematicides on potato plants at 56 days after priming application.

	Mormodica (M)							
	Fresh root mass				Dry root mass			
Nemafric-BL (B)	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]
B_0	17.6^{a}	–	12.2^{b}	–30	1.4^{ab}	–	1.0^{c}	–30
B_1	13.3^{b}	–30	18.8^{a}	7	1.1^{bc}	–24	1.5^{a}	6

[x]Column means with the same letter were not different ($P \leq 0.05$) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Relative to untreated control, Mormodica phytonematicide reduced chlorophyll content by 8%, which was not significantly different to that of Nemafric-BL phytonematicide alone (Table 3.3). Although the interaction increased chlorophyll content, the effects were not different to those of untreated control and Nemafric-BL phytonematicide alone. Both Nemafric-BL and Mormodica phytonematicides each significantly reduced the stem diameter, whereas the interaction effect on the variable were not different to those of untreated control.

Table 3.3 Two-way matrix for chlorophyll content and stem diameter as affected by second order interaction of Nemafric-BL and Mormodica phytonematicides on potato plants at 56 days after priming application.

	Mormodica (M)							
	Chlorophyll content				Stem diameter			
Nemafric-BL (B)	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]
B_0	25.8[a]	–	23.6[b]	–8	5.7[a]	–	5.1[b]	–11
B_1	24.8[ab]	–4	26.5[a]	3	5.0[b]	–12	5.6[ab]	–1

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

The interaction of Nemarioc-AL and Nemafric-BL phytonematicides significantly increased fresh tuber mass, but the effects were not significantly different to those of untreated control or Nemafric-BL phytonematicide alone (Table 3.4). Although Nemarioc-AL phytonematicide alone reduced fresh tuber mass by 20%, the effects were not different to those of untreated control and Nemafric-BL phytonematicide alone. Nemarioc-AL and Nemafric-BL phytonematicides reduced stem diameter by 14 and 11%, respectively, but the effects were not significantly different to those of the interaction. The interaction of Nemafric-BL and Mormodica phytonematicides increased fresh tuber mass, but the effects were not different to those of untreated control or Nemafric-BL phytonematicide alone (Table 3.5). Mormodica phytonematicide alone reduced fresh tuber mass by 27%, but the effects on the variable were not different to those of Nemafric-BL phytonematicide alone.

Table 3.4 Two-way matrix for fresh tuber mass and stem diameter as affected by second order interaction of Nemarioc-AL and Nemafric-BL phytonematicides on potato plants at 56 days after priming application.

	Nemafric-B L (B)							
	Fresh tuber mass				Stem diameter			
Nemarioc-AL (A)	B_0[x]	R.I. (%)[y]	B_1	R.I. (%)[y]	B_0	R.I. (%)[y]	B_1	R.I. (%)[y]
A_0	101.6[ab]	–	87.5[ab]	–14	5.8[a]	–	5.1[b]	–11
A_1	80.8[b]	–20	109.4[a]	8	4.9[b]	–14	5.5[ab]	–5

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Table 3.5 Two-way matrix for fresh tuber mass as affected by first order interaction of Nemafric-BL and Mormodica phytonematicides on potato plants at 56 days after treatment applications.

	Mormodica (M)			
Nemafric-BL (B)	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]
B_0	105. 7[a]	–	76.7[b]	–27
B_1	87.8[ab]	–16	109.1[a]	3

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Mormodica phytonematicide alone increased chlorophyll content, but the effects were not significantly different to those of untreated control and Nemarioc-AL

phytonematicide alone, but were significantly different to those of the interaction of Nemarioc-AL and Mormodica phytonematicides (Table 3.6).

Table 3.6 Two-way matrix for chlorophyll content as affected by first order interaction of Nemarioc-AL and Mormodica phytonematicides on potato plants at 56 days after priming application.

	Mormodica (M)			
Nemarioc-AL (A)	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]
A_0	24.6[ab]	–	26.0[a]	6
A_1	26.0[ab]	–5	24.2[b]	–2

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

3.3.2 Selected nutrient elements

The $A_1B_1M_1$ first order interaction was significant on Na and Zn in potato tubers, contributing 63 and 54% in TTV of the respective variables. However, all other interactions and single treatments did not have significant effects on nutrient elements. Relative to untreated control, the first order interaction for Nemafric-BL and Mormodica phytonematicides reduced Na and Zn by 2 and 4%, respectively, in potato tubers (Table 3.7).

Table 3.7 Two-way matrix for Na and Zn as affected by first order interaction of Nemafric-BL and Mormodica phytonematicides at 56 days after priming application.

Nemafric-BL (A)	Mormodica (M)			
	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]
	Na			
A_0	2.41[ab]	–	2.67[a]	11
A_1	2.58[ab]	7	2.35[b]	–2
	Zn			
A_0	1.43[ab]	–	1.55[a]	9
A_1	1.49[ab]	4	1.38[b]	–4

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

3.3.3 Effects on nematode variables

None of the interactions affected eggs in roots, whereas Mormodica and Nemarioc-AL phytonematicides had significant effects on eggs, contributing 48 and 20% in TTV on the respective variables (Appendix 3.2). The interaction of Mormodica and Nemafric-BL phytonematicides, Mormodica, Nemafric-BL and Nemarioc-AL phytonematicides had highly significant effects on J2 in roots, contributing 22, 20, 16 and 29% in TTV on the respective variables (Appendix 3.2). In contrast, the three first order interactions had significant effects on PF, contributing 17, 49 and 11% in TTV on the respective variables. All interactions did not have significant effects on RP, whereas the main factors from below upward had significant effects on RP, contributing 31, 22 and 28% in TTV on the respective variables. All first order interactions from below upward had highly significant effects on PF, contributing 19, 45, 13 and 12% in TTV on the respective variables.

Relative to untreated control, Nemarioc-AL and Mormodica phytonematicides significantly reduced eggs in roots by 25 and 30%, respectively (Table 3.8), where the two factors did not have different effects on the variable. The interactive effects of Nemafric-BL and Mormodica phytonematicides, reduced J2 in roots by 34, 35 and 37%, respectively (Table 3.9). Relative to untreated control, Nemafric-BL phytonematicide reduced total nematodes by 37%, which was not significantly different to effects of Mormodica phytonematicide on the variable. Relative to untreated control, Nemarioc-AL phytonematicide, Nemafric-BL phytonematicide and their interaction reduced total nematodes by 29, 31 and 28%, respectively, but the effects were not significantly different (Table 3.10). Similarly, Nemarioc-AL phytonematicide, Mormodica phytonematicide and the interaction reduced total nematode by 47, 30 and 8%, respectively, but only the effects of Nemarioc-AL and Mormodica phytonematicides were different to those of the untreated control, whereas those of the interaction were not different. Relative to untreated control, all treatments each reduced J2 from 29 to 33% (Table 3.11) and RP from 22 to 35% (Table 3.12).

Table 3.8 Relative impact of Nemarioc-AL and Mormodica phytonematicides on eggs in the roots of potato plants at 56 days after priming application.

Treatment	Eggs in roots	R.I. (%)[y]
Control	0.81[a]	–
Nemarioc-AL	0.61[b]	–25
Mormodica	0.56[b]	–30

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Table 3.9 Two-way matrix for nematodes on second-stage juveniles and total nematodes in the roots as affected by first order interaction of Nemafric-BL (B) and Mormodica phytonematicides on potato plants at 56 days after priming application.

		Mormodica (M)						
		J2 in roots			Total nematodes			
Nemafric-BL (B)	N_0	R.I. (%)[y]	M_1	R.I. (%)[y]	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]
B_0	2.00[a]	–	1.25[b]	–37	2.73[a]	–	2.29[ab]	–16
B_1	1.29[b]	–35	1.31[b]	–34	1.71[b]	–37	2.42[a]	–12

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Table 3.10 Two-way matrix for nematodes on Total nematodes in the roots as affected by first order interactions of Nemarioc-AL × Nemafric-BL and Nemarioc-AL × Mormodica phytonematicides on potato plants at 56 days after priming application.

Nemarioc-AL (A)	Nemafric-BL (B)				Mormodica (M)			
	B_0	R.I. (%)[y]	B_1	R.I. (%)[y]	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]
A_0	2.93[a]	–	2.01[b]	–31	2.90[a]	–	2.04[bc]	30
A_1	2.09[b]	–29	2.11[b]	–28	1.54[c]	–47	2.66[ab]	8

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Table 3.11 Relative impact of Nemarioc-AL, Nemafric-BL and Mormodica on second-stage juveniles (J2) in roots on potato plants at 56 days after priming application.

Treatment	J2	R.I. (%)[y]
Control	0.83[a]	–
Nemarioc-AL	0.55[b]	–33
Control	0.81[a]	–
Nemafric-BL	0.57[b]	–29
Control	0.82[a]	–
Mormodica	0.57[b]	–31

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100

Table 3.12 Relative impact of Nemarioc-AL, Nemafric-BL and Mormodica phytonematicides on reproductive potential (RP) of *Meloidogyne* species in the roots of potato plants at 56 days after priming application.

Treatment	RP	R.I. (%)[y]
Control	1.03[a]	–
Nemarioc-AL	0.77[b]	–25
Control	1.01[a]	–
Nemafric-BL	0.79[b]	–22
Control	1.03[a]	–
Mormodica	0.77[b]	–35

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Relative to untreated control, Nemafric-BL and Mormodica phytonematicides reduced PF by 20 and 12%, respectively, which were not significantly different to the effect of the interaction, and was also not different to the untreated control (Table 3.13). Nemarioc-AL and Mormodica phytonematicides significantly reduced PF by 26% and 17%, respectively, with interaction effects not being different from those of Mormodica phytonematicide and the interaction (Table 3.14). Nemarioc-AL phytonematicide, Nemafric-BL phytonematicide and the interaction reduced PF by 18, 17 and 16%, respectively, with the effects not being different from each other (Table 3.15). Similarly, Nemarioc-AL and Nemafric-BL phytonematicides reduced Pf by 10 and 8%, respectively (Table 3.16).

Table 3.13 Two-way matrix for final population (PF) density in the roots previously treated with Nemafric-BL and Mormodica phytonematicides on potato plants at 56 days after priming application.

	Mormodica (M)			
Nemafric-BL (B)	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]
B_0	3.06[a]	–	2.70[b]	–12
B_1	2.46[b]	–20	2.83[ab]	–7

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Table 3.14 Two-way matrix for final population (PF) in the roots as affected by first order interaction of Nemarioc-AL and Mormodica phytonematicides on potato plants at 56 days after priming application.

	Mormodica (M)			
Nemarioc-AL (A)	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]
A_0	3.17[a]	–	2.62[bc]	–17
A_1	2.34[c]	–26	2.90[ab]	–9

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Table 3.15 Two-way matrix for nematode final population (PF) in the roots as affected by first order interaction of Nemarioc-AL and Nemafric-BL phytonematicides on potato plants at 56 days after priming application.

	Nemafric-BL (B)			
Nemarioc-AL (A)	B_0	R.I. (%)[y]	B_1	R.I. (%)[y]
A_0	3.16[a]	–	2.64[b]	–17
A_1	2.59[b]	–18	2.65[b]	–16

[x]Columns means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Table 3.16 Relative impact of Nemarioc-AL and Nemafric-BL phytonematicides on final population (PF) on potato plants at 56 days after priming application.

Treatment	PF	R.I. (%)[y]
Control	2.90[a]	–
Nemarioc-AL	2.62[b]	–10
Control	2.88[a]	–
Nemafric-BL	2.64[b]	–8

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

3.4 Discussion

3.4.1 Effects on plant variables

The interactive effects had significant effects on plant growth variables, with the interactions at times slightly reducing plant growth variables. The issue of phytotoxicity of cucurbitacin-containing phytonematicides, as shown by the reductive effects, had been resolved using the Curve-fitting Allelochemical Response Dose (CARD) algorithm model (Liu *et al*., 2003; Mashela *et al*., 2017). Consequently, there is no Mean Concentration Stimulation Point (MCSP) for priming of potato seed tubers as used in other crops using botinemagation (Mashela *et al*., 2017).

The use of the CARD model on priming of potato tubers would also provide information on the sensitivity (k) and overall sensitivity (Σk) of potatoes to the combined products, namely, Nemarioc-AL and Mormodica phytonematicides at 1:1 (v/v) ratio. At the appropriate MCSP, the products would not be phytotoxic to potato seed tubers, but would have nematicidal effects on plant nematodes (Mashela *et al*., 2017). After establishing the MCSP for priming potato seed tubers, quality studies on the produce should follow since certain growers had since claimed that priming of potato seed tubers results in poor quality produce, without specifying whether this was eating or storage quality.

3.4.2 Effects on nematode variables

Primed potato seeds for various products had significant effects on different components of PF, namely, eggs, J2 and PF itself, with results showing that the products reduced nematode population densities. Results in the current study, agree with those in other studies (Mashela and Mpati, 2002; Rabothata, 2017) where the

interactive effects of the test phytonematicides had limited effects on population densities of nematodes. Generally, alone the phytonematicides had relatively high effects on nematode numbers when compared to the interactive effects of the products. Ideally, the MCSP using the CARD model should be generated for each product, perhaps starting with the most investigated products, namely, Nemarioc-AL and Nemafric-BL phytonematicides (Mashela *et al*., 2017).

3.4.3 Selected nutrient elements

Although the interactive effects of Nemafric-BL and Mormodica phytonematicides, contributed highly in TTV of Na and Zn in potato tuber tissues, relative to untreated control, the effects were rather negligible. In the current study, the effects of the treatments on leaf tissues was not determined, whereas in other studies the cucurbitacin-containing phytonematicides had significantly high effects on nutrient elements as shown by the magnitude relative to untreated control (Maake, 2018; Mokoele, 2019; Rabothata, 2017).

3.5 Conclusion

Post-priming of potato seed tubers using the three triterpenoid-containing phytonematicides, effects were observed from emergence through harvest on plant and nematode variables. Essentially, results of the current study suggested that seed potato priming with phytonematicides could be a potential method for the application of phytonematicides for the management of nematode population densities in potato production. However, there was high variability, suggesting that it would be product to assess the MCSP for each product on potato tubers through the CARD model to

establish the sensitivity biological indices of each product as done in the ground leaching technology and botinemagation systems.

CHAPTER 4
POTATO SEED TUBERS AS CARRIERS OF ACTIVE INGREDIENTS OF PHYTONEMATICIDES UNDER MICROPLOT CONDITIONS

4.1 Introduction

Potato seed (S*olanum tuberosum* L.) tubers successfully served as carriers of active ingredients of terpenoid-containing phytonematicides for suppression of nematode population densities under greenhouse conditions (Chapter 3). However, in the study, the concentration of Nemarioc-AL, Nemafric-BL and Mormodica phytonematicides separately or collectively had tendencies towards phytotoxicity. Generally, under greenhouse conditions, in addition to restricted root growth in pots, temperature in pots and ambient temperature could be such that they interact with cucurbitacins to affect the efficacy of the products. Various studies have demonstrated that the efficacy of cucurbitacins is dependent on temperature, with temperature above 25° C promoting the efficacy of the products, whereas that below 25° C inhibits efficacy of the products and encourages the degeneration of the products (Mashela *et al*., 2017).

In contrast, microplot conditions could also subject the plants to root-bounding, with uncontrolled ambient temperature, which could have a modulating effect on the temperature of the growing medium in pots. Consequently, microplot conditions could offer conditions which are closely mimicking uncontrolled environmental conditions. Therefore, the results of such study where potato seed tubers are used as carriers of cucurbitacins would provide some information on how the product would affect the suppression of nematodes and the potential phytotoxicity of the products. The objective of this study was to establish whether using potato seed tubers as carriers of cucurbitacin A ($C_{32}H_{46}O_9$), cucurbitacin B ($C_{32}H_{46}O_8$) and momordin ($C_{42}H_{66}O_{13}$)

from triterpenoid-containing phytonematicides would improve management of nematode population densities in potato plants under microplot conditions.

4.2 Materials and methods

4.2.1 Location of the study

The study was conducted under microplot conditions at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, South Africa (23°53'10" S, 29°44'15" E). Soil at the location is predominantly Hutton sandy loam comprising 65% sand, 30% clay, 5% silt, with organic C being at less than 1.6%, electrical conductivity at 0.148 Ds/m and Ph (H_2O) at 6.5. The microplot experiment was conducted in summer (October-December) 2017 and validated in autumn (March-June) 2018. The location has hot dry summers, with daily maximum temperature from 28 to 38° C and daily minimum temperatures from 10 to 18° C. The average annual rainfall at the site was less than 500 mm, with high distribution percentage rainfall in summer.

4.2.2 Treatments and experimental design

A 2 × 2 × 2 factorial experiment was laid out in a randomised complete block design, with eight treatments, replicated 7 times. Nemarioc-AL (A), Nemafric-BL (B) and Mormodica (M) phytonematicides served as first, second and third factors, respectively. The eight treatments included untreated control ($A_0B_0M_0$), Nemarioc-AL phytonematicide alone ($A_1B_0M_0$), Nemafric-BL phytonematicide alone ($A_0B_1M_0$), Mormodica phytonematicide alone ($A_0B_0M_1$), $A_1B_1M_0$, $A_1B_0M_1$, $A_0B_1M_1$ and $A_1B_1M_1$). Seed tubers of cv. 'Mondial G3' were primed and dried as described previously (Chapter 3).

4.2.3 Procedures

Thirty-cm-diameter plastic pots were filled with growing medium that comprised pasteurised (300 °C for 1 h) loam soil (35% clay, 15% silt and 50% sand, Ph(H_2O) 6.4, EC = 1.24 S/m^3), river sand and Hygromix-T (Hygrotech, Pretoria North, South Africa) in 2:1:1 (v/v) ratio. Primed potato seed tubers were each set in pots that were then inserted into 20-cm-deep holes at 0. 5 m × 0.5 m spacing (Figure 4.1). The preparation of phytonematicides and nematode inoculum [eggs + second-stage juveniles (J2)] were as described previously (Chapter 3).

4.2.4 Cultural practices

Plants were irrigated using drip irrigation that discharged 1 000 ml/h. Soon after seed tuber setting, pots were irrigated for 2 h to increase moisture to field capacity, which was followed by 1 h irrigation every other day. At 100% emergence, each plants were fertilised with 5 g 2:3:2 (22) NPK + 0.5% Ca + 0.5% Fe + 0.5% Zn fertiliser mixture to provide a total of 155 mg N, 105 mg P and 130 mg K per ml water. At 30 days after the first application, each plant was top-dressed with 3 g 2:1:2 (43) Multifeed (Nulandies, Johannesburg) to provide a total of 0.175 mg N, 0.16 mg K, 0.16 mg P, 0.45 mg Mg, 0.378 mg Fe, 0.0375 mg Cu, 0.175 mg Zn, 0.5 mg B, 1.5 mg Mn and 0.035 mg Mo per ml water, without Ca. Pests observed were those observed in chapter 3 and were controlled as described previously (Chapter 3).

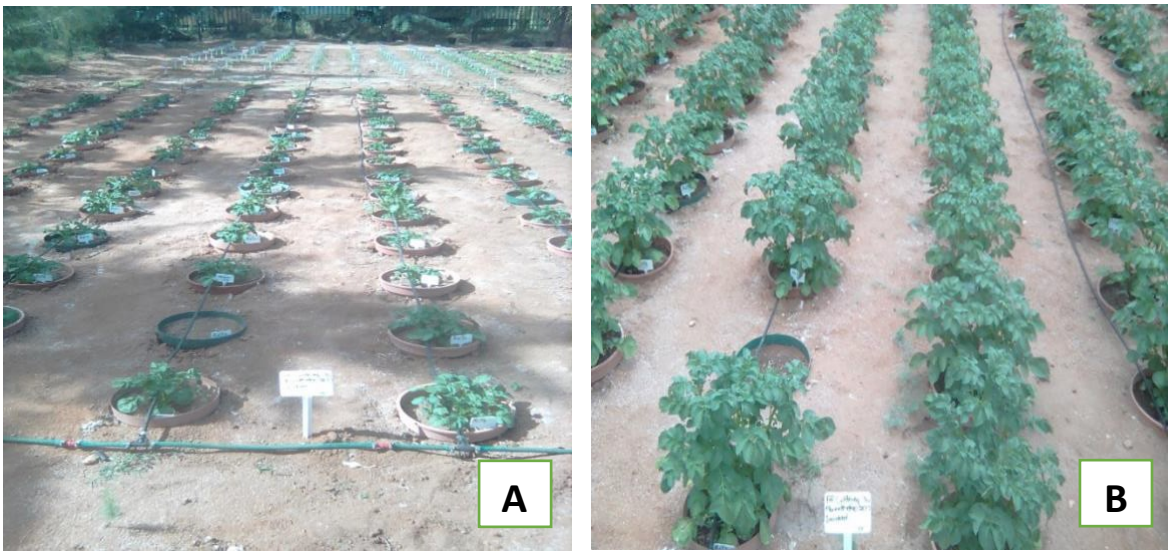

Figure 4.1 Establishment of potato experiment at 100% emergence (A) and at 4 weeks after 100% emergence (B).

At 100% emergence, each plant was inoculated using 5 000 eggs + J2 as described previously (Chapter 3). Plants were monitored for insect pests which were sprayed when more than 10 entities were observed in the entire plot. A disease spraying programme was designed as described previously (Chapter 3).

4.2.5 Data collection

Plant and nematode variables were collected as described previously (Chapter 3).

4.2.6 Data analysis

Nematode and plant data were assessed for seasonal interactions and since the interactions were not significant ($P \leq 0.05$), data were pooled ($n = 112$) and subjected to factorial analysis of variance through Statistix 10.1 software. The degrees of freedom and their associated mean sum of squares were partitioned to provide the total treatment variation (TTV) for separate sources (Steyn *et al*., 2003). Significant

second and first order interactions were further expressed using three-way and two-way matrix tables, respectively, thereby allowing for the determination of the direction and magnitude of permutations relative to the untreated control. Unless otherwise stated, treatment effects were discussed at the probability level of 5%.

4.3 Results

4.3.1 Interactive effects on plant growth variables

The second order interaction (Nemarioc-AL × Nemafric-BL × Mormodica) was significant on fresh tuber mass, fresh root mass and dry root mass, contributing 28, 26 and 26% in TTV of the respective variables (Appendix 4.2). In contrast, Nemarioc-AL × Nemafric-BL and Nemafric-BL × Mormodica interactions were highly significant ($P \leq 0.01$) on chlorophyll content, contributing 43 and 40% in TTV of the variables, respectively (Appendix 4.1). Mormodica phytonematicide had highly significant effects on fresh shoot mass, dry shoot mass, fresh tuber mass, fresh root mass and dry root mass, contributing 44, 37, 47, 48 and 48% in TTV of the respective variables (Appendix 4.1-4.2). Generally, relative to untreated control, the second and first order interactions, along with individual treatments, significantly increased fresh tuber mass, except for Nemarioc-AL × Mormodica and Nemafric-BL × Mormodica interactions which were not different to the untreated (Table 4.1).

Table 4.1 Three-way matrix for fresh tuber mass as affected by second order interaction of Nemarioc-AL, Nemafric-BL and Mormodica phytonematicides on potato plants at 56 days after treatment.

		Mormodica (M)			
Nemarioc-AL (A)	Nemafric-BL (B)	M_0[x]	R.I. (%)[y]	M_1	R.I. (%)[y]
A_0	B_0	733.8[d]	–	1091.1[a]	49
A_1	B_0	905.5[bc]	23	883.9[bcd]	20
A_0	B_1	872.4b[cd]	18	970.9[ab]	32
A_1	B_1	784.0[cd]	7	965.1[ab]	31

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Relative to untreated control the interaction reduced fresh tuber mass (FTM) and dry root mass (DRM) by 33% (Table 4.2; Table 4.3). The interaction effects on the variable did not significantly differ to the untreated control. Relative to untreated control Nemarioc-AL × Nemafric-BL phytonematicide interaction reduced chlorophyll content by 1%, whereas their interaction effects on the variable did not differ significantly from the untreated control (Table 4.4).

Table 4.2 Three-way matrix for fresh root mass (FRM) as affected by second order interaction of Nemarioc-AL, Nemafric-BL and Mormodica phytonematicides on potato plants at 56 days after treatment.

		Mormodica (M)			
Nemarioc-AL (A)	Nemafric-BL (B)	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]
A_0	B_0	32.473[a]	–	23.046[bc]	–29
A_1	B_0	23.786[bc]	–26	23.733[bc]	–26
A_0	B_1	27.680[abc]	–14	25.023[bc]	–22
A_1	B_1	29.687[ab]	–8	21.563[c]	–33

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Table 4.3 Three-way matrix for dry root mass (DRM) as affected by second order interaction of Nemarioc-AL, Nemafric-BL and Mormodica phytonematicides on potato plants at 56 days after treatment.

		Mormodica (M)			
Nemarioc-AL (A)	Nemafric-BL (B)	M_0	R.I. (%)[y]		R.I. (%)[y]
A_0	B_0	6.938[a]	–	4.923[bc]	–28
A_1	B_0	5.082[bc]	–26	5.070[bc]	–26
A_0	B_1	5.914[abc]	–14	5.347[bc]	–22
A_1	B_1	6.342[ab]	–8	4.607[c]	–33

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Table 4.4 Two-way matrix for chlorophyll content as affected by first order interaction of Nemarioc-AL and Nemafric-BL phytonematicides on potato plants at 56 days after treatment.

	Nemafric-BL (B)			
Nemarioc-AL (A)	B_0	R.I. (%)[y]	B_1	R.I. (%)[y]
A_0	38.289[a]	–	35.636[c]	7
A_1	35.829[bc]	–6	37.729[ab]	1
B_0	38.004[a]	–	36.193[ab]	3
B_1	35.361[b]	–7	37.925[a]	–0

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Table 4.5 Two-way matrix for chlorophyll content as affected by first order interaction of Nemafric-BL and Mormodica phytonematicides on potato plants at 56 days after treatment.

	Nemafric-BL (B) Mormodica (M)			
Nemafric-BL (A)	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]
A_0	38.004[a]	–	36.193[ab]	3
A_1	35.361[b]	–7	37.925[a]	–0

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Similarly, Nemafric-BL × Mormodica phytonematicide interactions effects on chlorophyll did not differ significantly with those from untreated control, Nemarioc-AL and Nemafric-BL phytonematicides alone (Table 4.5). Mormodica phytonematicide increased FSM, DSM and FTM by 31, 33 and 19%, respectively. Mormodica phytonematicide effect on the variables was significantly different to the untreated control (Table 4.6; Table 4.7). However, relative to untreated control, the phytonematicide reduced FRM and DRM by 17% each and the effects on the two variables significantly differed from the untreated control.

Table 4.6 Relative impact of Mormodica phytonematicide on fresh shoot mass (FSM) and dry shoot mass (DSM) of potato plants at 56 days after treatment.

	FSM		DSM	
Treatment	Variable	R.I. (%)[y]	Variable	R.I. (%)[y]
Control	351.53[b]	-	44.361[b]	-

Phytonematicide	459.36[a]	31	58.859[a]	33

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Table 4.7 Relative impact of Mormodica on fresh tuber (FTM), fresh root mass (FRM) and dry root mass (DRM) of potato plants at 56 days after treatment.

Treatment	FTM		FRM		DRM	
	Variable	R.I. (%)[y]	Variable	R.I. (%)[y]	Variable	R.I. (%)[y]
Control	823.92[b]	-	28.406[a]	-	6.069[a]	-
Phytonematicide	977.73[a]	19	23.341[b]	–17	4.987[b]	–17

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

4.3.2 Interactive effects on nematodes

Nemarioc-AL × Nemafric-BL × Mormodica phytonematicide interaction was significant only on total nematodes in roots, RP and PF of *Meloidogyne* species on roots of potato plants and in the soil. The combination of the three products contributed 11, 13 and 10% in TTV on the respective variables (Appendix 4.3). Nemafric-BL × Mormodica phytonematicide interactions were also significant on total nematodes, RP, PF and eggs in the roots, contributing 32, 32, 9 and 39% in TTV on the variables (Appendix 4.3), respectively. Mormodica phytonematicide alone was highly significant on final population contributing 64% in TTV on the variable (Appendix 4.3).

Relative to untreated control Nemarioc-AL × Nemafric-BL × Mormodica phytonematicide interaction reduced total nematode, RP and PF by 18, 64 and 18%, respectively, whereas their effects on the variables differed significantly from untreated control (Table 4.10; Table 4.11; Table 4.12). Relative to untreated control Nemafric-

BL × Mormodica phytonematicide reduced eggs, total nematodes, RP and PF by 2, 14, 35 and 13% respectively, which significantly differed with the untreated control (Table 4.8; Table 4.9). However, the effect of Nemafric-BL × Mormodica phytonematicide on eggs was not different to that of the untreated control (Table 4.8). Relative to untreated control Mormodica phytonematicide reduced PF by 10% and the effects on the variable was significantly different to that of untreated control (Table 4.13).

Table 4.8 Two-way matrix for eggs and total nematodes in roots (eggs &J2) as affected by first order interaction of Nemafric-BL and Mormodica phytonematicides on population density of nematodes 56 days after treatment.

	Mormodica (M)							
	Eggs				Total nematodes in roots			
Nemafric-BL (A)	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]
B_0	1.765[a]	–	1.268[b]	–28	3.641[a]	–	2.961[b]	–18
B_1	1.590[ab]	–10	1.723[a]	–2	3.183[b]	–13	3.141[b]	–14

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Table 4.9 Two-way matrix for reproductive potential (RP) and final population (PF) as affected by first order interaction of Nemafric-BL and Mormodica phytonematicides on population density of nematodes 56 days after treatment.

	Mormodica (M)							
	RP				PF			
Nemafric-BL (A)	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]
B_0	1.462[a]	-	1.065[b]	–27	4.262[a]	-	3.711[c]	–13
B_1	1.069[b]	–26	0.941[b]	–35	3.972[b]	–6	3.688[c]	–13

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Table 4.10 Three-way matrix for total nematodes in roots as affected by second order interaction of Nemarioc-AL, Nemafric-BL and Mormodica phytonematicides on potato plants at 56 days after treatment.

		Mormodica (M)			
Nemarioc-AL (A)	Nemafric-BL (B)	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]
A_0	B_0	3.737[a]	–	2.847[d]	–23
A_1	B_0	3.544[ab]	–5	3.076[cd]	–17
A_0	B_1	3.126[cd]	–16	3.237[bc]	–13
A_1	B_1	3.240[bc]	–13	3.046[cd]	–18

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Table 4.11 Three-way matrix for reproductive potential (RP) as affected by second order interaction of Nemarioc-AL, Nemafric-BL and Mormodica phytonematicides on potato plants at 56 days after treatment.

		Mormodica (M)			
Nemarioc-AL (A)	Nemafric-BL (B)	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]
A_0	B_0	1.547a	–	0.814d	–47
A_1	B_0	1.378ab	–11	1.068cd	–31
A_0	B_1	1.029cd	–33	1.139bc	–26
A_1	B_1	1.110bcd	–28	0.990d	–64

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Table 4.12 Three-way matrix for final population (PF) as affected by second order interaction of Nemarioc-AL, Nemafric-BL and Mormodica phytonematicides on potato plants at 56 days after treatment.

		Mormodica (M)			
Nemarioc-AL (A)	Nemafric-BL (B)	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]
A_0	B_0	4.436[a]	-	3.588[d]	–19
A_1	B_0	4.087[b]	–8	3.787[cd]	–15
A_0	B_1	3.983[bc]	–10	3.785[cd]	–15
A_1	B_1	3.960[bc]	–11	3.636[d]	–18

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Table 4.13 Relative impact of final population (PF) as affected by Mormodica on potato plants at 56 days after treatment.

Treatment	Variable	R.I. (%)[y]
Control	4.117[a]	-
Mormodica (M)	3.699[b]	-10

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

4.3.3 Interactive effects on selected nutrients

Nemarioc-AL × Nemafric-BL × Mormodica interactions were only significant on Na, contributing 7% in TTV of the variable. Relative to untreated control the interaction

reduced Na by 33% and effects on the variable was significantly different to those of untreated control also Nemarioc-AL, Nemafric-BL and Mormodica alone (Table 4.14).

Table 4.14 Three-way matrix for sodium (Na) as affected by first order interaction of Nemarioc-AL, Nemafric-BL and Mormodica.

		Mormodica (M)			
Nemarioc-AL (A)	Nemafric-BL (B)	M_0	R.I. (%)[y]	M_1	R.I. (%)[y]
A_0	B_0	6.938[a]	–	4.923[bc]	–28
A_1	B_0	5.082[bc]	–26	5.070[bc]	–26
A_0	B_1	5.914[abc]	–14	5.347[bc]	–22
A_1	B_1	6.342[ab]	–8	4.607[c]	–33

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

4.4 Discussion

4.4.1 Plant growth variables

Generally, allelopathic inhibitors interfere with key physiological processes in receptor plants, resulting in reduction of plant growth and development (Ashrafi *et al*., 2008; Inderjit and Duke, 2005). According to Pelinganga and Mashela (2012), the established MCSP would stimulate plant growth and consistently suppressed nematode population densities. Phytotoxicity, in addition to being density-dependent, also depends on the stage of growth of a plant (Einhellig, 1985; Nawaz *at el*., 2013).

Priming is not only a simple, low-cost and easily performable technology, but can be used on-farm, if refined and empirically-developed to avoid phytotoxicity. Mashela *et al*. (2010) noted that phytonematicides had a 'fertiliser effect', which had since been shown to be the stimulation effects which were not related to the nutrient elements. An interesting phenomenon in the current study was the stimulation of the fresh potato tuber mass (yield) by the products, whereas the same products suppressed root growth. Although the disparity could not be explained here, it could be that the roots were more sensitive to the products than the tubers, which constitute modified stems.

Different organs have different sensitivities to the products (Mashela *et al*., 2017). The products, in other crops, could either stimulate, have no effect or inhibit growth of different organs (Mafeo, 2010; Pelinganga, 2013) as observed in the current study. Such observations confirmed the existence of the stimulation phase in density-dependent growth patterns in response to increasing concentrations of allelochemicals as shown in the CARD model (Lui *et al*., 2003; Mashela *et al*., 2017).

Chlorophyll is the core component of pigment-protein complexes embedded in the photosynthetic membranes and play a major role in the photosynthesis (Campbell, 1990). Any changes in chlorophyll content are expected to affect photosynthesis either positively or negatively. Reduced chlorophyll content in allelochemical-treated plants had been frequently reported in phytonematicides (Manuel *et al*., 2011; Mafeo, 2005; Seshweni, 2016). Others (Einhellig and Rasmussen, 1979; Patterson, 1981) also observed that phenolic acids such as p-coumaric acid and vanillic acid reduced chlorophyll content in leaves of soybean plants, with the resultant decrease in biomass. Similar results were also observed in other plants such as *Parthenium hysterphorus* (Kanchan and Chandra, 1980) and *Cucumis sativus* (Pramanik *et al*., 2001). In contrast, Mashela *et al*. (2013) observed that residual chemical effects increased chlorophyll content on tomato plants growing on soil where Nemafric-BL phytonematicide at 3 and 6% were previously applied in the preceding crop. The findings showed that the interactions hardly reduced chlorophyll content relative to the untreated control, whereas individual materials reduced the variable.

4.4.2 Interactive effects on nematode population densities

The cucurbitacin-containing phytonematicides, when applied either using the ground leaching or botinemagation technology, have been consistent in suppression of nematode population densities at low concentrations (Mashela *et al*., 2017). In the current study, the priming technology was able to successfully suppress nematode population densities under microplot conditions, although the magnitudes were lower than those observed in other two technologies. The highest magnitude in the current study under priming was 64%, whereas in the other technologies the reduction effects

could be as high as 92-100% (Seshweni, 2016; Tseke *et al*., 2013). The reduction of population densities of *Meloidogyne* species using the priming technology with respect to the second order interaction (Nemarioc-AL × Nemafric-BL × Mormodica) in the current study confirmed observations in the previous study (Chapter 3).

The contribution of Mormodica phytonematicide in second and first order interactions in the current study confirmed those in the previous study (Chapter 3). Although in the current and previous studies the magnitudes were not as impressive as in the other application technologies, it had been noteworthy that momordin from Mormodica phytonematicide could be playing a role that had not been observed previously (Mashela *et al*., 2017), with its role in combination with cucurbitacin-containing phytonematicides being worth investigating in the ground leaching and botinemagation technologies.

4.4.3 Nutrient elements in leaf tissues of potato tubers

In the current study, as observed in the previous study (Chapter 3), the second order interaction (Nemarioc-AL × Nemafric-BL × Mormodica) decreased Na in leaf tissues of potato tubers, which also agreed with observations in another study (Maake, 2018), where Nemarioc-AL and Nemafric-BL phytonematicides reduced Na in leaf tissues by 30 and 39%. Nyamandi (2017) observed that Nemarioc-AL × Biomuti interaction did not have significant effects on nutrient elements under microplot conditions, which confirmed observations in the current study. In other interactive studies, Nemarioc-AL × Biomuti interaction increased Ca and reduced Mg in leaf tissues of citrus rootstock seedlings (Mokoele, 2019), which could be suggesting that the interactions could be specific. Since treatments did not affect Fe, K and Zn nutrient elements in leaf tissues

of potato plants, the observation could be viewed as being favourable since phytonematicides are not intended to serve as fertilisers, but products for managing nematode population densities.

4.5 Conclusion

The interaction of the three products contributed towards improved potato yield under microplot conditions, although there was some evidence of phytotoxicity on roots and chlorophyll content. Results in the current study suggested that the three products could be used in priming potato seed tubers for the management of plant nematodes under microplot conditions. In the next chapter, the efficacy of the products when applied through priming would be investigated under field conditions.

CHAPTER 5
POTATO SEED TUBERS AS CARRIERS OF ACTIVE INGREDIENTS OF PHYTONEMATICIDES UNDER FIELD CONDITIONS

5.1 Introduction

Priming potato seed tubers in solutions of Nemarioc-AL, Nemafric-BL and Mormodica phytonematicides interacted to affect potato plants and final nematode population density under greenhouse (Chapter 3) and microplot (Chapter 4) conditions. Generally, Nemarioc-AL and Nemafric-BL phytonematicides hardly interacted with each other when used in ground leaching technology or botinemagation technology (Rabothata, 2017), with the use of Mormodica phytonematicide improving the interaction of the two products under both greenhouse and microplot conditions. Currently, it is not clear how this product modulates the interactive effects of the other two products when used in the priming technology under both conditions.

Generally, under both greenhouse and microplot conditions, root growth is limited due to the use of containers (Sebati, 2018), which could also exacerbate the influence of the products on inhibition of root growth as observed previously (Chapter 3; Chapter 4). Under field conditions, where the imposed restrictions by the plastic containers do not exist, leached active ingredients from the products could move away from the root system and thereby enhancing root growth, which could be at the expense of promoting nematode population densities, and thereby, the reduction in tuber yield. The objective of this study was to establish whether using potato seed tubers as carriers of cucurbitacin A ($C_{32}H_{46}O_9$), cucurbitacin B ($C_{32}H_{46}O_8$) and momordin ($C_{42}H_{66}O_{13}$) from triterpenoid-containing phytonematicides would avoid improve management of nematode population densities in potato plants under field conditions.

5.2 Materials and methods

5.2.1 Location of the study

A field study was conducted at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, South Africa (23°53'10" S, 29°44'15" E). Soil at the location was predominantly Hutton sandy loam, comprising 65% sand, 30% clay, 5% silt, with organic C being at less than1.6%, EC at 0.148 Ds/m and Ph(H_2O) at 6.5. The field experiment was conducted in autumn (February-April) 2017 and validated in 2018. The location has hot dry summers, with daily maximum temperature ranging from 28 to 38° C and daily minimum temperatures from 10 to 18° C. The average annual rainfall at the site was less than 500 mm, with the distribution being skewed to summer (November-January).

5.2.2 Treatments and experimental design

A 2 × 2 × 2 factorial experiment was laid out in a randomised complete block design, with eight treatments, replicated 7 times. Nemarioc-AL (A), Nemafric-BL (B) and Mormodica (M) phytonematicides served as first, second and third factors, respectively. The eight treatments comprised untreated control ($A_0B_0M_0$), Nemarioc-AL phytonematicide ($A_1B_0M_0$), Nemafric-BL phytonematicide ($A_0B_1M_0$), Mormodica phytonematicide ($A_0B_0M_1$), $A_1B_1M_0$, $A_1B_0M_1$, $A_0B_1M_1$ and $A_1B_1M_1$.

5.2.3 Procedures

Primed potato seed tubers (Chapter 3) were set at 30-cm-depth with a 0.6 m × 0.6 m spacing in a field with a history of high population density of *Meloidogyne* species. The

preparation of phytonematicides and nematode inoculum [eggs + second-stage juveniles (J2)] were as described previously (Chapter 3).

Figure 5.1 Harvesting of potatoes under field conditions.

5.2.4 Cultural practices

Plants were irrigated every other day using drip irrigation that discharged 1 000 ml/h. At 100% emergence, each plant was fertilised with 5 g 2:3:2 (22) NPK + 0.5% Ca + 0.5% Fe + 0.5% Zn fertiliser mixture to provide a total of 155 mg N, 105 mg P and 130 mg K per ml water. At 30 days after the first application, each plant was top-dressed with 3 g 2:1:2 (43) Multifeed (Nulandies, Johannesburg) to provide a total of 0.175 mg N, 0.16 mg K, 0.16 mg P, 0.45 mg Mg, 0.378 mg Fe, 0.0375 mg Cu, 0.175 mg Zn, 0.5

mg B, 1.5 mg Mn and 0.035 mg Mo per ml water, without Ca. Insect pests and diseases were managed as described previously (Chapter 3).

5.2.5 Data collection

Plant growth and nutrient element data, along with nematode data, were collected as described previously (Chapter 3).

5.2.6 Data analysis

Nematode data were transformed using $\log_{10}(x + 1)$ prior to analysis to homogenise the variances (Little and Hills, 1978). Nematode and plant data were subjected to factorial analysis of variance through Statistix 10.1 software. The degrees of freedom and their associated mean sum of squares were partitioned to provide the total treatment variation (TTV) for separate sources of variance (Steyn *et al*., 2003). Significant second and first order interactions were further expressed using three-way and two-way matrix tables, respectively, thereby allowing for the determination of the direction and magnitude of combinations relative to untreated control. Unless otherwise stated, treatment effects were discussed at the probability level of 5%.

5.3 Results

5.3.1 Interactive effects on plant and nutrient element variables

The second order interaction ($A_1B_1M_1$) did not have significant effects on any plant variable. The first order interaction, Nemafric-BL × Mormodica, was highly significant ($P \leq 0.01$) on dry root mass, contributing 60% in TTV on the variable (Appendix 5.1). Relative to untreated control, the interaction reduced dry root mass by 14%, which was not different to the effect of Mormodica phytonematicide at 13%, but was

significantly different to that of Nemafric-BL phytonematicide (Table 5.2) Nemarioc-AL × Mormodica interaction had significant effects on fresh tuber mass, contributing 33% in TTV on the variable (Appendix 5.1). Relative to the untreated control, the interaction increased fresh tuber mass (yield) by 32% (Table 5.1), which was not different to that of Nemarioc-AL phytonematicide at 40%, but significantly different to that of Mormodica phytonematicide at 16%, which was not different to the untreated control (Table 5.2).

Table 5.1 Two-way matrix for fresh tuber mass in as affected by first order interaction of Nemarioc-AL × Mormodica phytonematicide on potato plants at 56 days after treatment.

	Mormodica (M)			
Nemarioc-AL (A)	M_0[x]	R.I. (%)	M_1	R.I. (%)
A_0	273.42[b]	–	318.43[b]	16
A_1	379.69[a]	40	361.28[a]	32

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.
[y]Relative impact (%) = [(treatment/control) – 1] × 100.

Table 5.2 Two-way matrix for dry root mass as affected by first order interaction of Nemarioc and Mormodica phytonematicide on potato plants at 56 days after treatment.

Nemafric-BL (B)	Mormodica (M)			
	M_0[x]	R.I.(%)[y]	M_1	R.I. (%)
B_0	27.77[a]	–	24.23[bc]	–13
B_1	27.21[ab]	–2	23.96[c]	–14

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

The first order interactions, Nemarioc-AL × Mormodica and Nemarioc-AL × Nemafric-BL, had significant effect on chlorophyll content, each contributing 15% in TTV on the variable (Appendix 5.1). In both cases, the significant effects were from Nemarioc-AL phytonematicides, whereas those of the interaction, Mormodica and Nemafric-BL phytonematicide were not different to that of untreated control (Table 5.4). Treatments did not have significant effects on K, Na, Fe and Zn in leaf tissues of potato plants (data not shown).

Table 5.3 Two-way matrix for chlorophyll content as affected by second order interaction of Nemarioc-AL × Mormodica and Nemarioc-AL × Nemafric-BL phytonematicides on potato plants at 56 days after treatment.

	Mormodica (M)			
Nemarioc-AL (A)	M_0[x]	R.I. (%)	M_1	R.I. (%)
A_0	39.21[a]	–	38.86[ab]	–1
A_1	38.54[b]	–2	38.92[ab]	–1
	Nemafric-BL (B)			
	B_0	R.I.(%)	B_1	R.I. (%)
A_0	29.22[a]	–	38.85[ab]	–1
A_1	38.56[b]	–2	38.90[ab]	–1

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[y]Relative impact (%) = [(treatment/control) – 1] × 100.

5.3.2 Interactive effects on nematode variables

The second order interaction ($A_1B_1M_1$), had no significant effects on all nematode variables under field conditions. However, the first order interaction, Nemafric-BL × Mormodica, had highly significant effects on nematode eggs in roots, contributing 45% in TTV on the variable, with Mormodica phytonematicide significantly contributing 26% in TTV on the variable (Appendix 5.2). Relative to untreated control, Nemafric-BL × Mormodica interaction had no effect on eggs of *Meloidogyne* species in roots, whereas Nemafric-BL and Mormodica phytonematicides significantly reduced eggs by 27 and 30%, respectively (Table 5.4).

Table 5.4 Three-way matrix for eggs in roots as affected by first order interaction Nemafric-BL and Mormodica phytonematicides on potato plants at 56 days after treatment.

	Mormodica (M)			
Nemafric-BL (B)	M_0 [x]	R.I. (%)	M_1	R.I. (%)
B_0	1.230[ab]	-	0.867[b]	–30
B_1	0.907[b]	–27	1.522[a]	24

[x]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test

[y]Relative impact (%) = [(treatment/control) – 1] × 100

5.4 Discussion

5.4.1 Interactive effects on plant and nutrient element variables

Under field conditions, the second order interaction ($A_1B_1M_1$) did not have significant effects on any plant variable, which contradicted observations under greenhouse (Chapter 3) and microplot (Chapter 4) conditions. Due to the diversity of conditions under field conditions, it is probable that Mormodica phytonematicide, which had been viewed as the modulator (Chapter 3, Chapter 4), could not play such a role under field conditions due to numerous factors, particularly the microorganisms, which were not controlled under the current location. Notwithstanding, the effects of the first order interaction, Nemafric-BL × Mormodica, on dry root mass, with the reduction on dry root mass, confirmed those observed under controlled environments (Chapter 3; Chapter 4). This observation, as inferred previously (Chapter 3; Chapter 4), demonstrated that the active ingredients in the primed seed tuber, had bioactivities in the roots, where *Meloidogyne* species infest the plant (Dongmei *et al*., 2019). The latter agrees with the observation, discussed later, where the phytonematicides, applied through priming, reduced nematode population densities.

The reduced dry root mass was indicative of the existence of phytotoxicity when the triterpenoid-containing phytonematicides were applied using the priming technology. In ground leaching technology and botinemagation technology, phytotoxicity was managed using the concept Mean Concentration Stimulation Point (MCSP), derived from biological indices of the Curve-fitting Allelochemical Response Dose (CARD) algorithm model (Liu *et al*., 2003; Mashela *et al*., 2017). The MCSP, technically referred to as the First law of phytonematicides (Mashela *et al*., 2017), is the concentration that should be used to manage nematode population densities without inducing phytotoxicity (Mashela *et al*., 2017). In the current study, the MCSP values used for the three products were those derived for tomato (*Solanum lycopersicum* L.) plants in the previous study (Pelinganga, 2012). The observed phytotoxicity suggested that the MCSP values for the three products should be empirically-developed for each product prior to using their respective permutations in factorial studies, thereby confirming the suggestion that MCSP values were plant-specific (Mashela *et al*., 2017).

Despite the reduction of roots, the products consistently increased fresh tuber mass, regardless of the condition under which the study was carried out. This observation is important since the produce in potato constitutes the fresh tubers, which is a modified stem. The biological index, sensitivity index, in the CARD model, had previously shown that various organs in different plants have various sensitivity indices (Lie *et al*., 2003; Mafeo *et al*., 2012; Mashela *et al*., 2017), with roots being the most sensitive.

In the current study, as observed previously (Chapter 3; Chapter 4), the first order interactions, Nemarioc-AL × Mormodica and Nemarioc-AL × Nemafric-BL, reduced chlorophyll content. However, in the current study, as opposed to the previous observations under controlled conditions, the reductive effect was attributed to Nemarioc-AL phytonematicide, whereas the other two factors in the interaction did not have significant effects on the variable. In context of density-dependent growth patterns, it was previously shown that the two cucurbitacin-containing phytonematicides either have stimulatory (Mafeo and Mashela, 2012), neutral (Lebea, 2017) or inhibitory (Pelinganga *et al*. 2012) effects on chlorophyll content. Obviously, the inhibitory effects, which represent phytotoxicity, are not desirable for this application technique.

The observation that the treatments did not have significant effects on K, Na, Fe and Zn was contrary to effects observed in the previous priming studies (Chapter 3; Chapter 4) and in ground leaching technology (Mashela *et al*., 2017) and botinemagation technology (Pelinganga *et al*., 2012), where the treatments either stimulated or inhibited the accumulation of nutrient elements. However, in the other application technologies, nutrient elements were assessed when the phytonematicides were applied at a geometric series where the MCSP was being derived and not during the validation of the MCSP. In most cases, during the validation of the MCSP, the effects of the products on nutrient elements in leaf tissues were not significantly different from those of untreated controls (Maake, 2016; Pelinganga, 2013).

5.4.2 Interactive effects on nematode variables

Under field conditions, the second order interaction ($A_1B_1M_1$) did not have significant effects on all nematode variables, which contradicted observations under controlled conditions. This observation could, as indicated earlier, be attributed to the existence of micro-organisms, which, probably interfered with the second order interaction. Nemafric-BL × Mormodica interaction and Mormodica phytonematicide, in agreement with findings in the previous studies (Chapter 3; Chapter 4), significantly reduced nematode eggs in roots, but not J2 as in the previous studies. The potential mechanism involved in the reduction of eggs was not consistant in the current study, and it is not clear why Nemarioc-AL phytonematicide did not suppress nematode numbers as observed in the previous studies (Chapter 3; Chapter4).

The magnitudes with which the product reduced nematode numbers in the current and previous studies under priming technology were not as big as those under the ground leaching and the botinemagation technologies (Mashela *et al*., 2017). However, the most important issue here was that priming allows the active ingredients to be in the rhizosphere during emergence. In plant nematodes, the initial nematode population density in the rhizosphere of the emerging plantlet is the most important in relation to yield reduction (Seinhorst, 1967). Consequently, since the technology has the capability of ensuring that the initial nematode population density could be kept in check, it is important that the technology be viewed seriously.

5.5 Conclusion

Phytotoxicity of roots, increased potato yield and suppression of nematode eggs in roots were consistent with observations under controlled conditions. Treatment effects

in the current study suggested strong effects of effective micro-organisms on some of the components of the permutations, especially on Mormodica phytonematicide. Results in the current study suggest the priming of potato seed tubers could be suitable as an application technology of phytonematicides.

CHAPTER 6

SUMMARY OF FINDINGS, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

6.1 Summary of findings

Observations in the current study sugest that priming of potato seed tubers in phytonematicide solutions prior to setting under greenhouse, microplot and field conditions consistently suggested that the technology could be suitable for application of phytonematicides in the management of root-knot (*Meloidogyne* species) nematodes in cultivation of potato (*Solanum batatas* L.). Improved fresh tuber yield under the technology was desirable since it translates to increased profits. However, the consistent reduction in root growth associated with the use of this technology greenhouse, microplot and field conditions could suggest phytotoxicity of cucurbitacins released into the rhizospehere, which was undesirable. The consistent reduction in Na in leaf tissues of potato under greenhouse and microplot conditions, although not observed under field conditions, could be essential if the element was also reduced in the tubers. The reduction in nematode population densities using the priming technology under various conditions, although under field conditions the reduction was restricted to nematode eggs, was important since the technology is primarily designed for managing nematodes.

6.2 Significance of findings

In the current study, it was shown under various conditions that potato seed tubers could be used as carriers of active ingredients in the application of phytonematicides for managing nematode population densities when used through the priming

technology. The priming technology adds to the existing two application technologies of phytonematicides, namely, the ground leaching technology and botinemagation, which are labour-intensive and infrastructure-demanding, respectively. Importantly, the priming technology consistently reduced nematode population densities on potato plants, which was important since the crop does not have genotypes with resistance to *Meloidogyne* species. The effects also showed that Mormodica phytonematicide served an important role of modulating the efficacy of both Nemarioc-AL and Nemafric-BL phytonematicides in nematode suppression and improvement of potato yield tubers.

6.3 Recommendations

Instead of using Mean Concentration Stimulation Point (MCSP) of the three phytonematicides on tomato plants which induced phytotoxicity on roots of potato, it would be imperative that the MCSP for the three products on potato plants be established and further interactive studies be conducted to provide definitive solution to the problem statement which was being investigated. Additionally, the irrigation interval for each MCSP value should be established in using the priming technology for the cultivation of potatoes. Also, the chemical residues in produce should be established since bioactivities of fresh tubers in response to the treatments was observed through stimulated tuber growth. Obviously, the storability of primed tubers and subsequent fresh tubers, along with their storage and eating qualities should be empirically-established.

6.4 Conclusions

Although the priming technology had the limitation of phytotoxicity on roots, all the other positive attributes suggested that the need to fine-tune the technology using the Curve-fitting Allelohemical Response Dose (CARD) models as previously achieved in the GLT and botinemation phytonematicide application technologies. The current study demonstrated for the first time that Nemarioc-AL, Nemafric-BL and Mormodica phytonematicides, alone or combined, could be applied using priming technology for the management of nematode population densities under different conditions in the production of potatoes

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APPENDICES

Appendix 3.1 Partitioning mean sum of squares for Nemarioc-AL(A), Nemafric-BL (B) and Mormodica (M) phytonematicides on plant height (PHT),) stem diameter (STD), chlorophyll content (CHL), dry shoot mass (DSM), dry root mass (DRM) and fresh tuber mass (FTM) of primed potato seed tubers inoculated with *Meloidogyne* Species.

Source	DF	PLH		STD		CHL		DSM		DRM		FTM	
		MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]
Block	13	301.0	13[ns]	1.554	5[ns]	14.07	5[ns]	30.13	23[ns]	1.388	16[ns]	8996.6	18[ns]
Nem-AL (A)	1	98.8	4[ns]	1.311	4[ns]	2.20	1[ns]	7.06	5[ns]	0.000	0[ns]	8.4	0[ns]
Nem-BL (B)	1	190.3	8[ns]	0.078	0[ns]	26.13	9[ns]	0.12	0[ns]	0.229	3[ns]	1474.7	3[ns]
Mormodica (M)	1	121.3	5[ns]	0.003	0[ns]	0.94	0[ns]	0.94	1[ns]	0.000	0[ns]	417.2	1[ns]
A × B	1	24.5	1[ns]	10.345	34[***]	7.87	3[ns]	32.34	25[ns]	0.655	7[ns]	12720.8	25[***]
A × M	1	24.5	1[ns]	2.684	9[ns]	71.20	25[**]	1.99	2[ns]	0.305	4[ns]	2713.7	5[ns]
B × M	1	1062.7	45[**]	10.825	36[***]	107.44	37[***]	45.37	35[**]	5.224	60[***]	17652.3	35[***]
A × B × M	1	355.0	15[ns]	2.223	7[ns]	45.90	16[ns]	3.39	3[ns]	0.477	5[ns]	4382.8	9[ns]
Error	91	167.7	7[ns]	1.408	5[ns]	11.74	4[ns]	9.84	7[ns]	0.441	5[ns]	1787.9	4[ns]
Total	111	2345.8	100	30.431	100	287.49	100	131.18	100	8.719	100	50154.4	100

[y]TTV = (MSS/TOTAL) × 100.

[***]Highly significant at P ≤ 0.01, [**]Significant at P ≤ 0.05, [ns]Not significant at P ≥ 0.05.

Appendix 3.2 Partitioning mean sum of squares for Nemarioc-AL (A), Nemafric-BL (B) and Mormodica (M) phytonematicides on eggs, juveniles (J2) total nematodes, reproductive potential (RP) and final population (PF) affected by primed potato seed tubers.

Source	DF	Eggs in roots		J2 in roots		Total nematodes		RP		PF	
		MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]
Block	13	0.496	4^{ns}	0.599	3^{ns}	0.227	0^{ns}	0.304	5^{ns}	0.136	1^{ns}
Nem-AL (A)	1	2.503	20^{**}	5.222	29^{***}	3.886	7^{ns}	1.827	28^{**}	2.188	12^{**}
Nem-BL (B)	1	0.499	4^{ns}	2.806	16^{**}	5.600	10^{ns}	1.425	22^{**}	1.510	8^{**}
Mormodica (M)	1	6.051	48^{***}	3.655	20^{***}	0.463	1^{ns}	1.999	31^{**}	0.001	0^{ns}
A × B	1	0.284	2^{ns}	0.687	4^{ns}	6.153	11^{**}	0.146	2^{ns}	2.416	13^{***}
A × M	1	0.095	1^{ns}	0.436	3^{ns}	27.394	49^{***}	0.133	2^{ns}	8.657	45^{***}
B × M	1	0.950	8^{ns}	3.919	22^{***}	9.307	17^{**}	0.191	3^{ns}	3.759	19^{***}
A × B × M	1	1.266	10^{ns}	0.120	1^{ns}	1.794	3^{ns}	0.221	3^{ns}	0.002	0^{ns}
Error	91	0.383	3^{ns}	0.417	2^{ns}	1.522	3^{ns}	0.193	3^{ns}	0.325	2^{ns}
Total	111	12.527	100	17.861	100	56.346	100	6.439	100	18.994	100

[y]TTV = (MSS/TOTAL) × 100.

***Highly significant at P ≤ 0.01, **Significant at P ≤ 0.05, [ns]Not significant at P ≤ 0.05.

Appendix 3.3 Partitioning mean sum of squares for Nemarioc-AL (A), Nemafric-BL (B) and Mormodica (M) phytonematicides on iron (Fe), potassium (K), sodium (Na) and zinc (Zn) as affected by primed potato seed tubers.

Source	DF	Fe		K		Na		Zn	
		MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]
Block	13	0.041	8[ns]	0.022	8[ns]	0.281	11[ns]	0.081	11[ns]
Nem-AL (A)	1	0.086	17[ns]	0.008	3[ns]	0.067	3[ns]	0.002	0[ns]
Nem-BL (B)	1	0.124	24[ns]	0.003	1[ns]	0.167	6[ns]	0.093	14[ns]
Mormodica (M)	1	0.071	14[ns]	0.010	4[ns]	0.012	0[ns]	0.001	0[ns]
A × B	1	0.074	14[ns]	0.042	16[ns]	0.000	0[ns]	0.010	1[ns]
A × M	1	0.026	5[ns]	0.088	34[ns]	0.124	5[ns]	0.049	7[ns]
B × M	1	0.000	0[ns]	0.058	23[ns]	1.662	63[**]	0.390	54[**]
A × B × M	1	0.054	11[ns]	0.000	0[ns]	0.029	1[ns]	0.019	3[ns]
Error	91	0.038	3[ns]	0.027	11[ns]	0.303	11[ns]	0.074	10[ns]
Total	111	0.514	100	0.258	100	2.645	100	0.719	100

[y]TTV = (MSS/TOTAL) × 100.

[***]Highly significant at P ≤ 0.01, [**]Significant at P ≤ 0.05, [ns]Not significant at P ≥ 0.05.

Appendix 3.4 Analysis of variance for plant height (PLH) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicides, under greenhouse conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	3913.6	301.05		
Nemarioc-AL	1	98.8	98.81	0.59	0.4447
Nemafric-BL	1	190.3	190.32	1.13	0.2896
Mormodica	1	121.4	121.39	0.72	0.3971
A×B	1	24.5	24.52	0.15	0.7031
A×M	1	24.7	24.70	0.15	0.7020
B×M	1	1062.7	1062.72	6.34	0.0136
A×B×M	1	355.0	3555.00	2.12	0.1491
Error	91	15261.1	167.70		
Total	111	21052.1	2345.8		

Appendix 3.5 Analysis of variance for stem diameter (STD) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under greenhouse conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	20.205	1.5542		
Nemarioc-AL	1	1.312	1.3116	0.93	0.3371
Nemafric-BL	1	0.078	0.0782	0.06	0.8142
Mormodica	1	0.003	0.0030	0.00	0.9633
A×B	1	10.346	10.3457	7.35	0.0080
A×M	1	2.685	2.6846	1.91	0.1708
B×M	1	10.825	10.8253	7.69	0.0067
A×B×M	1	2.223	2.2233	1.58	0.2122
Error	91	128.153	1.4083		
Total	111	175.830	30.431		

Appendix 3.6 Analysis of variance for chlorophyll (CHL) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under greenhouse conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	182.96	14.07		
Nemarioc-AL	1	2.20	2.20	0.19	0.6661
Nemafric-BL	1	26.13	26.13	2.23	0.1392
Mormodica	1	0.95	0.94	0.08	0.7771
A×B	1	7.88	7.87	0.67	0.4150
A×M	1	71.20	71.20	6.06	0.0157
B×M	1	107.45	107.44	9.15	0.0032
A×B×M	1	45.90	45.90	3.91	0.0511
Error	91	1068.74	11.74		
Total	111	1513.40	287.49		

Appendix 3.7 Analysis of variance for dry shoot mass (DSM) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under greenhouse conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	391.72	30.13		
Nemarioc-AL	1	7.07	7.06	0.72	0.3991
Nemafric-BL	1	0.13	0.12	0.01	0.9098
Mormodica	1	0.95	0.94	0.10	0.7573
A×B	1	32.35	32.34	3.29	0.0731
A×M	1	1.99	1.99	0.20	0.6540
B×M	1	45.38	45.37	4.61	0.0344
A×B×M	1	3.39	3.39	0.34	0.5586
Error	91	895.56	9.84		
Total	111	1378.52	131.18		

Appendix 3.8 Analysis of variance for dry root mass (DRM) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under greenhouse conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	18.0453	1.388		
Nemarioc-AL	1	0.0010	0.000	0.00	0.9627
Nemafric-BL	1	0.2295	0.229	0.52	0.4726
Mormodica	1	0.0005	0.000	0.00	0.9740
A×B	1	0.6558	0.655	1.49	0.2259
A×M	1	0.3056	0.305	0.69	0.4074
B×M	1	5.2246	5.224	11.84	0.0009
A×B×M	1	0.4771	0.477	1.08	0.3011
Error	91	40.1391	0.441		
Total	111	65.0783	8.719		

Appendix 3.9 Analysis of variance for fresh tuber mass (FTM) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under greenhouse conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	116956	8996.6		
Nemarioc-AL	1	8	8.4	0.00	0.9455
Nemafric-BL	1	1475	1474.7	0.82	0.3662
Mormodica	1	417	417.2	0.23	0.6302
A×B	1	12721	12720.8	7.12	0.0090
A×M	1	2714	2713.7	1.52	0.2211
B×M	1	17652	17652.3	9.87	0.0023
A×B×M	1	4383	4382.8	2.45	0.1209
Error	91	162696	1787.9		
Total	111	319021	50154.4		

Appendix 3.10 Analysis of variance for nematode eggs of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under greenhouse conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	6.4489	0.496		
Nemarioc-AL	1	2.5035	2.503	6.52	0.0123
Nemafric-BL	1	0.5000	0.499	1.30	0.2567
Mormodica	1	6.0516	6.051	15.77	0.0001
A×B	1	0.2843	0.284	0.74	0.3916
A×M	1	0.0951	0.095	0.25	0.6198
B×M	1	0.9504	0.950	2.48	0.1190
A×B×M	1	1.2661	1.266	3.30	0.0726
Error	91	34.9185	0.383		
Total	111	53.0184	12.527		

Appendix 3.11 Analysis of variance for J2 in roots of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under greenhouse conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	7.7876	0.599		
Nemarioc-AL	1	2.2225	5.222	12.50	0.0006
Nemafric-BL	1	2.8066	2.806	6.72	0.0111
Mormodica	1	3.6553	3.655	8.75	0.0039
A×B	1	0.6872	0.687	1.65	0.2029
A×M	1	0.4360	0.436	1.04	0.3097
B×M	1	3.9198	3.919	9.38	0.0029
A×B×M	1	0.1203	0.120	0.29	0.5928
Error	91	38.0141	0.417		
Total	111	62.6495	17.861		

Appendix 3.12 Analysis of variance for total nematodes of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under greenhouse conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	32.6761.	0.227		
Nemarioc-AL	1	3.8863	3.886	1.666	0.0644
Nemafric-BL	1	5.6251	5.600	1.898	0.0567
Mormodica	1	0.4632	0.463	0.995	0.7888
A×B	1	6.1531	6.153	2.897	0.0543
A×M	1	27.3943	27.394	12.878	0.0019
B×M	1	9.3071	9.307	8.785	0.0437
A×B×M	1	1.7943	1.794	0.098	0.6957
Error	91	52.6632	1.522		
Total	111	139.9627	56.346		

Appendix 3.13 Analysis of variance for reproductive potential (RP) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under greenhouse conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	3.8987	0.304		
Nemarioc-AL	1	1.8274	1.827	2.23	0.0567
Nemafric-BL	1	1.4252	1.425	2.88	0.0554
Mormodica	1	1.9993	1.999	3.65	0.8274
A×B	1	0.1464	0.146	0.78	0.0912
A×M	1	0.1332	0.133	0.43	0.6610
B×M	1	0.1912	0.191	0.98	0.6286
A×B×M	1	0.2212	0.221	0.22	0.8794
Error	91	5.1933	0.193		
Total	111	15.0359	6.439		

Appendix 3.14 Analysis of variance for final population (PF) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under greenhouse conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	6.8874	0.136		
Nemarioc-AL	1	2.1885	2.188	1.06	0.0511
Nemafric-BL	1	1.5104	1.510	0.57	0.0444
Mormodica	1	0.0012	0.001	0.39	0.1279
A×B	1	2.4162	2.416	1.80	0.0298
A×M	1	8.6571	8.657	6.28	0.0310
B×M	1	3.7593	3.759	2.04	0.0116
A×B×M	1	0.0022	0.002	0.04	0.8794
Error	91	8.5673	0.325		
Total	111	33.9896	18.994		

Appendix 3.15 Analysis of variance for iron (Fe) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under greenhouse conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	7.6516	0.041		
Nemarioc-AL	1	0.0864	0.086	0.41	0.2933
Nemafric-BL	1	0.1241	0.124	0.85	0.2888
Mormodica	1	0.0711	0.071	0.37	0.3042
A×B	1	0.0744	0.074	0.56	0.2987
A×M	1	0.0263	0.026	0.18	0.3169
B×M	1	0.0001	0.000	0.09	0.3477
A×B×M	1	0.0542	0.054	0.29	0.3082
Error	91	10.9899	0.038		
Total	111	19.5659	0.514		

Appendix 3.16 Analysis of variance for potassium (K) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under greenhouse conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	7.4444	0.022		
Nemarioc-AL	1	0.0084	0.008	0.16	0.2190
Nemafric-BL	1	0.0031	0.003	0.10	0.3746
Mormodica	1	0.0103	0.010	0.21	0.4513
A×B	1	0.0424	0.042	1.09	0.8523
A×M	1	0.0882	0.088	2.11	0.9021
B×M	1	0.0580	0.058	1.22	0.8421
A×B×M	1	0.0002	0.000	0.03	0.2180
Error	91	13.8979	0.027		
Total	111	21.5529	0.258		

Appendix 3.17 Analysis of variance for sodium (Na) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under greenhouse conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	8.6667	0.281		
Nemarioc-AL	1	0.0673	0.067	0.66	0.4190
Nemafric-BL	1	0.1675	0.167	2.18	0.6746
Mormodica	1	0.0122	0.012	0.88	0.3513
A×B	1	0.0002	0.000	0.09	0.8523
A×M	1	0.1243	0.124	2.76	0.8021
B×M	1	1.6623	1.662	6.55	0.0121
A×B×M	1	0.0291	0.029	0.22	0.6180
Error	91	14.2334	0.303		
Total	111	24.7955	2.645		

Appendix 3.18 Analysis of variance for zinc (Zn) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under greenhouse conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	11.6544	0.081		
Nemarioc-AL	1	0.0023	0.002	0.19	0.6651
Nemafric-BL	1	0.0931	0.093	0.93	0.3954
Mormodica	1	0.0013	0.001	0.05	0.8274
A×B	1	0.0103	0.010	0.43	0.7912
A×M	1	0.0492	0.049	0.67	0.6610
B×M	1	0.3903	0.390	4.94	0.0286
A×B×M	1	0.0194	0.019	0.70	0.8794
Error	91	53.0677	0.074		
Total	111	65.2686	0.719		

Appendix 4.1 Partitioning mean sum of squares for Nemarioc-AL (A), Nemafric-BL (B) and Mormodica (M) phytonematicides on plant height (PHT),) fresh shoot mass (FSM), dry shoot mass (DSM) and chlorophyll content (CHL) of primed potato seed tubers inoculated with *Meloidogyne* Species.

Source	DF	PHT		FSM		DSM		CHL	
		MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]
Block	13	1042.54	46[ns]	132072	18[ns]	2763.39	17[ns]	27.276	8[ns]
Nem-AL (A)	1	4.13	0[ns]	1113	0[ns]	15.44	0[ns]	0.947	0[ns]
Nem-BL (B)	1	59.02	3[ns]	7779	1[ns]	6.28	0[ns]	3.975	1[ns]
Mormodica (M)	1	69.30	4[ns]	325601	44**	5886.13	37**	5.806	2[ns]
A × B	1	45.39	2[ns]	51695	7[ns]	2215.21	14[ns]	145.145	43***
A × M	1	0.43	0[ns]	57332	8[ns]	206.39	1[ns]	4.204	1[ns]
B × M	1	306.57	14[ns]	92183	13[ns]	2037.82	13[ns]	133.984	40***
A × B × M	1	520.73	23[ns]	15341	3[ns]	1953.57	12[ns]	0.586	0[ns]
Error	91	173.03	8[ns]	49946	6[ns]	1015.27	6[ns]	14.818	5[ns]
Total	111	2221.14	100	733062	100	16099.50	100	336.741	100

[y]TTV = (MSS/TOTAL) × 100.

[***]Highly significant at P ≤ 0.01, [**]Significant at P ≤ 0.05, [ns]Not significant at P ≥ 0.05.

Appendix 4.2 Partitioning mean sum of squares for Nemarioc-AL (A), Nemafric-BL (B) and Mormodica (M) phytonematicides on stem diameter (STD), fresh tuber mass (FTM), fresh root mass and dry root mass (DRM) of primed potato seed tubers inoculated with *Meloidogyne* Species.

Source	DF	STD		FTM		FRM		DRM	
		MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]
Block	13	10.825	34[ns]	121174	9[ns]	39.699	3[ns]	1.815	3[ns]
Nem-AL (A)	1	1.144	4[ns]	29491	2[ns]	156.374	10[ns]	7.140	10[ns]
Nem-BL (B)	1	0.732	2[ns]	835	0[ns]	1.467	0[ns]	0.068	0[ns]
Mormodica (M)	1	6.072	20[ns]	662355	47**	718.420	48**	32.788	48**
A × B	1	4.989	16[ns]	6027	0[ns]	75.014	5[ns]	3.423	5[ns]
A × M	1	0.546	2[ns]	153654	11[ns]	26.715	2[ns]	1.222	5[ns]
B × M	1	2.957	9[ns]	5544	0[ns]	2.964	0[ns]	0.133	0[ns]
A × B × M	1	0.319	1[ns]	372765	28**	385.469	26**	17.601	26**
Error	91	3.659	12[ns]	47360	3[ns]	96.409	6[ns]	4.401	6[ns]
Total	111	31.243	100	1399205	100	1502.531	100	68.591	100

[y]TTV = (MSS/TOTAL) × 100.

[***]Highly significant at P ≤ 0.01, [**]Significant at P ≤ 0.05, [ns]Not significant at P ≥ 0.05.

Appendix 4.3 Partitioning mean sum of squares for Nemarioc-AL, Nemafric-BL and Mormodica (M) phytonematicide on Iron (Fe), potassium (K), sodium (Na) and zinc (Zn) as affected by primed potato seed tubers.

Source	DF	Fe		K		Na		Zn	
		MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]
Block	13	0.015	4^{nsy}	0.005	3^{ns}	0.217	2^{ns}	0.094	5^{ns}
Nem-AL (A)	1	0.000	20^{ns}	0.012	29^{ns}	0.803	24^{ns}	0.066	28^{ns}
Nem-BL (B)	1	0.036	4^{ns}	0.000	16^{ns}	0.003	10^{ns}	0.045	22^{ns}
Mormodica (M)	1	0.024	17^{ns}	0.007	20^{ns}	0.010	29^{ns}	0.033	31^{ns}
A × B	1	0.002	2^{ns}	0.004	4^{ns}	0.004	4^{ns}	0.212	2^{ns}
A × M	1	0.001	1^{ns}	0.000	3^{ns}	0.001	5^{ns}	0.043	2^{ns}
B × M	1	0.022	8^{ns}	0.000	22^{ns}	0.248	16^{ns}	0.109	3^{ns}
A × B × M	1	0.022	10^{ns}	0.014	1^{ns}	1.128	7^{**}	0.024	3^{ns}
Error	91	0.018	3^{ns}	0.004	2^{ns}	0.228	2^{ns}	0.148	3^{ns}
Total	111	0.144	100	0.050	100	2.64672	100	0.77772	100

[y]TTV = (MSS/TOTAL) × 100.

[***]Highly significant at P ≤ 0.01, [**]Significant at P ≤ 0.05, [ns]Not significant at P ≥ 0.05.

Appendix 4.4 Partitioning mean sum of squares for Nemarioc-AL (A), Nemafric-BL (B) and Mormodica (M) phytonematicide on eggs, juveniles (J2) total nematodes in roots (eggs & J2), reproductive potential (RP) and final population (PF) affected by primed potato seed tubers.

Source	DF	Eggs in roots		J2 in roots		Total nematodes		RP		PF	
		MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]
Block	13	1.490	15[ns]	0.619	18[ns]	0.581	7[ns]	0.469	8[ns]	0.140	2
Nem-AL (A)	1	1.953	20[ns]	0.140	0[ns]	0.003	0[ns]	0.000	0[ns]	0.182	2
Nem-BL (B)	1	0.612	6[ns]	2.090	1[ns]	0.539	6[ns]	0.506	9[ns]	0.499	7
Mormodica (M)	1	0.826	8[ns]	2.358	44[ns]	3.638	41[ns]	1.938	33[ns]	4.885	64***
A × B	1	0.090	1[ns]	1.511	7[ns]	0.022	0[ns]	0.040	1[ns]	0.000	0
A × M	1	0.080	1[ns]	0.694	8[ns]	0.024	0[ns]	0.065	1[ns]	0.313	4
B × M	1	3.905	39**	0.940	13[ns]	2.847	32***	1.867	32***	0.683	9**
A × B × M	1	0.250	2[ns]	2.124	3[ns]	0.925	11**	0.745	13**	0.796	10**
Error	91	0.738	7[ns]	0.669	6[ns]	0.214	3[ns]	0.161	3	0.149	2
Total	111	9.944	100	11.145	100	8.793	100	5.800	100	7.645	100

[y]TTV = (MSS/TOTAL) × 100.

[***]Highly significant at P ≤ 0.01, [**]Significant at P ≤ 0.05, [ns]Not significant at P ≥ 0.05.

Appendix 4.5 Analysis of variance for plant height (PLH) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under microplot conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	13553.1	1042.54		
Nemarioc-AL	1	4.1	4.13	0.02	0.8776
Nemafric-BL	1	59.0	59.02	0.34	0.5607
Mormodica	1	69.3	69.30	0.40	0.5284
A×B	1	45.4	45.39	0.26	0.6098
A×M	1	0.4	0.43	0.00	0.9606
B×M	1	306.6	306.57	1.77	0.1865
A×B×M	1	520.7	520.73	3.01	0.0862
Error	91	15745.8	173.03		
Total	111	30304.5	2221.14		

Appendix 4.6 Analysis of variance for fresh shoot mass (FSM) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under microplot conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	1716930	132072		
Nemarioc-AL	1	1113	1113	0.02	0.8817
Nemafric-BL	1	7779	7779	0.16	0.6940
Mormodica	1	325601	325601	6.52	0.0123
A×B	1	51695	51695	1.04	0.3117
A×M	1	57332	57332	1.15	0.2868
B×M	1	92183	92183	1.85	0.1776
A×B×M	1	15341	15341	0.31	0.5808
Error	91	4545095	49946		
Total	111	6813069	733062		

Appendix 4.7 Analysis of variance for dry shoot mass (DSM) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under microplot conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	35924	2763.39		
Nemarioc-AL	1	15	15.44	0.02	0.9021
Nemafric-BL	1	6	6.28	0.01	0.9375
Mormodica	1	5886	5886.13	5.80	0.0181
A×B	1	2215	2215.21	2.80	0.1431
A×M	1	206	206.39	0.20	0.6532
B×M	1	2038	2037.82	2.01	0.1600
A×B×M	1	1954	1953.57	1.92	0.1688
Error	91	92390	1015.27		
Total	111	140635	16099.50		

Appendix 4.8 Analysis of variance for chlorophyll (CHL) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under microplot conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	354.59	27.276		
Nemarioc-AL	1	0.95	0.947	0.06	0.8010
Nemafric-BL	1	3.98	3.975	0.27	0.6058
Mormodica	1	5.81	5.806	0.39	0.5329
A×B	1	145.15	145.145	9.80	0.0024
A×M	1	4.20	4.204	0.28	0.5956
B×M	1	133.98	133.984	9.04	0.0034
A×B×M	1	0.59	0.586	0.04	0.8428
Error	91	1348.42	14.818		
Total	111	1997.65	336.741		

Appendix 4.9 Analysis of variance for stem diameter (STD) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under microplot conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MSS	F	P
Rep	13	35924	10.825		
Nemarioc-AL	1	15	1.144	0.02	0.9021
Nemafric-BL	1	6	0.732	0.01	0.9375
Mormodica	1	5886	6.072	5.80	0.0181
A×B	1	2214	4.989	2.18	0.1431
A×M	1	206	0.546	0.20	0.6532
B×M	1	2038	2.957	2.01	0.1600
A×B×M	1	1954	0.319	1.92	0.1688
Error	91	92390	3.659		
Total	111	140635	31.243		

Appendix 4.10 Analysis of variance for fresh tuber mass (FTM) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under microplot conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	1575259	121174		
Nemarioc-AL	1	29491	29491	0.62	0.4321
Nemafric-BL	1	835	835	0.02	0.8947
Mormodica	1	662355	662355	13.99	0.0003
A×B	1	6027	6027	0.13	0.7221
A×M	1	153654	153654	3.24	0.0750
B×M	1	5544	5544	0.12	0.7330
A×B×M	1	372765	372765	7.87	0.0061
Error	91	4309734	47360		
Total	111	7115664	1399205		

Appendix 411 Analysis of variance for fresh root mass (FRM) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under microplot conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	516.1	39.699		
Nemarioc-AL	1	156.4	156.374	1.62	0.2061
Nemafric-BL	1	1.5	1.467	0.02	0.9021
Mormodica	1	718.4	718.420	7.45	0.0076
A×B	1	75.0	75.14	0.78	0.3801
A×M	1	26.7	26.715	0.28	0.5999
B×M	1	3.0	2.964	0.03	0.8612
A×B×M	1	385.5	385.469	4.00	0.0485
Error	91	8773.2	96.409		
Total	111	10655.7			

Appendix 4.12 Analysis of variance for dry root mass (DRM) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under microplot conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	23.595	1.815		
Nemarioc-AL	1	7.141	7.140	1.62	0.2060
Nemafric-BL	1	0.068	0.068	0.02	0.9013
Mormodica	1	32.789	32.788	7.45	0.0076
A×B	1	3.423	3.423	0.78	0.3802
A×M	1	1.222	1.222	0.28	0.5995
B×M	1	0.133	0.133	0.03	0.8624
A×B×M	1	17.601	17.601	4.00	0.0435
Error	91	400.515	4.401		
Total	111	486.488	68.591		

Appendix 4.13 Analysis of variance for nematode eggs of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under microplot conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	19.3728	1.490		
Nemarioc-AL	1	1.9533	1.953	2.65	0.1073
Nemafric-BL	1	0.6123	0.612	0.83	0.3649
Mormodica	1	0.8268	0.826	1.12	0.2927
A×B	1	0.0907	0.090	0.12	0.7267
A×M	1	0.0809	0.080	0.11	0.7413
B×M	1	3.9052	3.905	5.29	0.0237
A×B×M	1	0.2508	0.250	0.34	0.5615
Error	91	67.1842	0.738		
Total	111	94.2770	9.944		

Appendix 4.14 Analysis of variance for J2 in the roots of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under microplot conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	8.0520	0.619		
Nemarioc-AL	1	0.1407	0.140	0.21	0.6477
Nemafric-BL	1	2.0900	2.090	3.12	0.0807
Mormodica	1	2.3589	2.358	3.52	0.0638
A×B	1	1.5117	1.511	2.26	0.1365
A×M	1	0.6945	0.694	1.04	0.3112
B×M	1	0.9401	0.940	1.40	0.2392
A×B×M	1	2.1240	2.124	3.17	0.0783
Error	91	60.9465	0.669		
Total	111	78.8585	11.145		

Appendix 4.15 Analysis of variance for total nematodes of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under microplot conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	7.5613	0.581		
Nemarioc-AL	1	0.0031	0.003	0.01	0.9052
Nemafric-BL	1	0.5396	0.539	2.51	0.116
Mormodica	1	3.6386	3.638	16.92	0.0001
A×B	1	0.0222	0.022	0.10	0.7484
A×M	1	0.0240	0.024	0.11	0.7389
B×M	1	2.8475	2.847	13.24	0.0005
A×B×M	1	0.9255	0.925	4.30	0.0408
Error	91	19.5644	0.214		
Total	111	35.1263	8.793		

Appendix 4.16 Analysis of variance for reproductive potential (RP) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under microplot conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	6.4489	0.469		
Nemarioc-AL	1	2.5035	0.000	0.01	0.9878
Nemafric-BL	1	0.5063	0.506	0.65	0.5667
Mormodica	1	0.9381	0.938	0.91	0.3343
A×B	1	0.0404	0.040	0.06	0.8433
A×M	1	0.0652	0.065	0.10	0.7987
B×M	1	2.8672	2.867	13.87	0.0026
A×B×M	1	0.7454	0.745	3.01	0.0411
Error	91	21.5787	0.161		
Total	111	34.7305	5.800		

Appendix 4.17 Analysis of variance for final population (PF) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under microplot conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	1.8296	0.140		
Nemarioc-AL	1	0.1827	0.182	1.23	0.2712
Nemafric-BL	1	0.4995	0.499	3.35	0.0705
Mormodica	1	4.8857	4.885	32.76	0.0000
A×B	1	0.0008	0.000	0.01	0.9403
A×M	1	0.3134	0.313	2.10	0.1506
B×M	1	0.6833	0.683	4.58	0.0350
A×B×M	1	0.7969	0.796	5.34	0.0231
Error	91	13.5702	0.149		
Total	111	22.7622	7.645		

Appendix 4.18 Analysis of variance for iron (Fe) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under microplot conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	0.20405	0.015		
Nemarioc-AL	1	0.00023	0.000	0.01	0.9097
Nemafric-BL	1	0.03655	0.036	2.02	0.1582
Mormodica	1	0.02446	0.024	1.36	0.2474
A×B	1	0.00273	0.002	0.15	0.6984
A×M	1	0.00158	0.001	0.09	0.7681
B×M	1	0.02266	0.022	1.26	0.2655
A×B×M	1	0.02206	0.022	1.22	0.2719
Error	91	1.64266	0.018		
Total	111	1.95699	0.144		

Appendix 4.19 Analysis of variance for potassium (K) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under microplot conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	0.07601	0.005		
Nemarioc-AL	1	0.01299	0.012	3.19	0.0776
Nemafric-BL	1	0.00002	0.000	0.00	0.9476
Mormodica	1	0.00754	0.007	1.85	0.1772
A×B	1	0.00448	0.004	1.10	0.2975
A×M	1	0.00068	0.000	0.17	0.6849
B×M	1	0.00035	0.000	0.09	0.7693
A×B×M	1	0.01499	0.014	3.68	0.0583
Error	91	0.37105	0.004		
Total	111	0.48810	0.050		

Appendix 4.20 Analysis of variance for sodium (Na) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under microplot conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	2.8275	0.217		
Nemarioc-AL	1	0.8031	0.803	3.51	0.0642
Nemafric-BL	1	0.0032	0.003	0.01	0.9062
Mormodica	1	0.0109	0.010	0.05	0.8275
A×B	1	0.0042	0.004	0.02	0.8922
A×M	1	0.0014	0.001	0.01	0.9372
B×M	1	0.2487	0.248	1.09	0.2999
A×B×M	1	1.1287	1.128	4.93	0.0288
Error	91	20.8265	0.228		
Total	111	25.8544	2.64672		

Appendix 4.21 Analysis of variance for zinc (Zn) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under microplot conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	1.2303	0.094		
Nemarioc-AL	1	0.0666	0.066	0.45	0.5042
Nemafric-BL	1	0.0451	0.045	0.30	0.5825
Mormodica	1	0.0331	0.033	0.22	0.6376
A×B	1	0.2129	0.212	1.44	0.2335
A×M	1	0.0434	0.043	0.29	0.5897
B×M	1	0.1092	0.109	0.74	0.3926
A×B×M	1	0.0248	0.024	0.17	0.6832
Error	91	13.4701	0.148		
Total	111	15.2355	0.77772		

Appendix 5.1 Partitioning mean sum of squares for Nemarioc-AL (A), Nemafric-BL (B) and Mormodica (M) phytonematicides on dry shoot mass (DSM), dry root mass (DRM) and fresh tuber mass (FTM), plant height, (PHT) and chlorophyll content (CHL) of primed potato seed tubers inoculated with *Meloidogyne* Species.

		DSM		DRM		FTM		PHT		CHL	
Source	DF	MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]	MS	TTV (%)[y]	MSS	TTV (%)[y]
Block	13	15.746	12	3.180	13	23215.7	10	142.997	66	12.628	54
Nem-AL (A)	1	23.850	18^{ns}	2.697	11^{ns}	71448.5	32**	21.95	10^{ns}	2.220	10 ^{ns}
Nem-BL (B)	1	1.308	1^{ns}	0.217	1^{ns}	2132.4	1^{ns}	8.032	4^{ns}	0.007	0 ^{ns}
Mormodica (M)	1	2.114	2^{ns}	0.030	0^{ns}	1230.1	0^{ns}	1.208	0^{ns}	0.007	0 ^{ns}
A × B	1	29.971	22*	0.499	2^{ns}	13991.7	6^{ns}	12.984	6^{ns}	3.593	15*
A × M	1	0.685	1^{ns}	0.239	0^{ns}	73330.5	33**	0.307	0^{ns}	3.614	15*
B × M	1	15.726	12^{ns}	14.732	60**	21.9	0^{ns}	2.477	1^{ns}	0.432	2 ^{ns}
A × B × M	1	34.348	26*	1.593	7^{ns}	30538.2	14^{ns}	3.430	2^{ns}	0.078	0 ^{ns}
Error	91	7.984	6	1.525	6	8787.1	4	23.196	11	0.878	4
Total	111	131.732	100	24.712	100	224696.1	100	216.581	100	23.457	100

[y]TTV = (MSS/TOTAL) × 100.

[***]Highly significant at P ≤ 0.01, [**]Significant at P ≤ 0.05, [ns]Not significant at P ≥ 0.05.

Appendix 5.2 Partitioning mean sum of squares for Nemarioc-AL, Nemafric-BL and Mormodica (M) phytonematicide on eggs, juveniles (J2) total nematodes in roots (eggs & J2) and reproductive potential (RP) affected by primed potato seed tubers.

		Eggs		J2		Total N		RP	
Source	DF	MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]
Block	13	1.77106	12	0.51715	8	0.60979	10	0.38541	10^{ns}
Nem-AL (A)	1	1.08300	7^{ns}	0.52530	8^{ns}	0.66872	11^{ns}	0.64783	18^{ns}
Nem-BL (B)	1	0.76563	5^{ns}	1.68543	26^{ns}	1.13685	19^{ns}	1.05468	29^{ns}
Mormodica (M)	1	0.44625	3^{ns}	0.92185	14^{ns}	0.52779	9^{ns}	0.06266	2^{ns}
A × B	1	1.78446	12^{ns}	0.02729	2^{ns}	0.02077	3^{ns}	0.08558	3^{ns}
A × M	1	0.06497	1^{ns}	0.12173	2^{ns}	0.06992	1^{ns}	0.03399	1^{ns}
B × M	1	6.70379	45^{***}	1.50499	23^{ns}	1.73858	30^{ns}	0.81469	23^{ns}
A × B × M	1	1.06927	7^{ns}	0.62795	10^{ns}	0.57467	10^{ns}	0.18254	5^{ns}
Error	91	1.16785	8	0.46192	7	0.48815	7	0.31249	8
Total	111	14.85628	100	6.39361	100	5.88535	100	3.57987	100

[y]TTV = (MSS/TOTAL) × 100.

[***]Highly significant at P ≤ 0.01, [**]Significant at P ≤ 0.05, [ns]Not significant at P ≤ 0.05.

Appendix 5.3 Partitioning mean sum of squares for Nemarioc-AL, Nemafric-BL and Mormodica (M) phytonematicide on Iron (Fe), potassium (K), sodium (Na) and zinc (Zn) as affected by primed potato seed tubers.

Source	DF	Fe		K		Na		Zn	
		MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]	MSS	TTV (%)[y]
Block	13	0.017	19[ns]	0.032	3[ns]	0.033	2[ns]	0.019	5[ns]
Nem-AL (A)	1	0.000	1[ns]	0.028	29[ns]	0.079	24[ns]	0.049	28[ns]
Nem-BL (B)	1	0.000	1[ns]	0.015	16[ns]	0.012	10[ns]	0.006	22[ns]
Mormodica (M)	1	0.032	40[ns]	0.042	20[ns]	0.002	29[ns]	0.003	31[ns]
A × B	1	0.014	15[ns]	0.000	4[ns]	0.025	4[ns]	0.001	2[ns]
A × M	1	0.001	1[ns]	0.005	3[ns]	0.003	5[ns]	0.019	2[ns]
B × M	1	0.004	5[ns]	0.018	22[ns]	0.038	16[ns]	0.101	3[ns]
A × B × M	1	0.004	4[ns]	0.007	1[ns]	0.008	7[ns]	0.051	3[ns]
Error	91	0.012	14[ns]	0.029	2[ns]	0.020	2[ns]	0.024	3[ns]
Total	111	0.088	100	0.153	100	0.225	100	0.278	100

[y]TTV = (MSS/TOTAL) × 100.

[***]Highly significant at P ≤ 0.01, [**]Significant at P ≤ 0.05, [ns]Not significant at P ≤ 0.05.

Appendix 5.4 Analysis of variance for dry shoot mass (DSM) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	204.698	15.746		
Nemarioc-AL	1	23.850	23.850	2.99	0.0873
Nemafric-BL	1	1.309	1.308	0.16	0.6865
Mormodica	1	2.114	2.114	0.26	0.6081
A×B	1	29.971	29.971	3.75	0.0558
A×M	1	0.685	0.685	0.09	0.7703
B×M	1	15.726	15.726	1.97	0.1639
A×B×M	1	34.349	34.348	4.30	0.0409
Error	91	726.540	7.984		
Total	111	1039.242	131.732		

Appendix 5.5 Analysis of variance for dry root mass (DRM) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	41.342	3.180		
Nemarioc-AL	1	2.697	2.697	1.77	0.1870
Nemafric-BL	1	0.218	0.217	0.14	0.7064
Mormodica	1	0.030	0.030	0.02	0.8884
A×B	1	0.500	0.499	0.33	0.5686
A×M	1	0.240	0.239	0.16	0.6928
B×M	1	14.732	14.732	9.66	0.0025
A×B×M	1	1.594	1.593	0.04	0.3095
Error	91	138.850	1.525		
Total	111	200.201	24.712		

Appendix 5.6 Analysis of variance for fresh tuber mass (FTM) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	301804	23215.7		
Nemarioc-AL	1	71449	71448.5	8.13	0.0054
Nemafric-BL	1	2132	2132.4	0.24	0.6235
Mormodica	1	1230	1230.1	0.14	0.7092
A×B	1	13992	13991.7	1.59	0.2102
A×M	1	73331	73330.5	8.35	0.0048
B×M	1	22	21.9	0.00	0.9603
A×B×M	1	30538	30538.2	3.48	0.0655
Error	91	799626	8787.1		
Total	111	1222675	224696.1		

Appendix 5.7 Analysis of variance for plant height (PHT) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	1858.96	142.997		
Nemarioc-AL	1	21.95	21.95	0.95	0.3333
Nemafric-BL	1	8.03	8.032	0.35	0.5577
Mormodica	1	1.21	1.208	0.05	0.8200
A×B	1	12.98	12.984	0.56	0.4563
A×M	1	0.31	0.307	0.01	0.9087
B×M	1	2.48	2.477	0.11	0.7446
A×B×M	1	3.43	3.430	0.15	0.7015
Error	91	2110.86	23.196		
Total	111	4020.21	216.581		

Appendix 5.8 Analysis of variance for chlorophyll (CHL) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	164.164	12.628		
Nemarioc-AL	1	2.220	2.220	2.53	0.1153
Nemafric-BL	1	0.007	0.007	0.01	0.9283
Mormodica	1	0.007	0.007	0.01	0.9283
A×B	1	3.594	3.593	4.09	0.0460
A×M	1	3.615	3.614	4.12	0.0454
B×M	1	0.432	0.432	0.49	0.4848
A×B×M	1	0.079	0.078	0.09	0.7650
Error	91	79.903	0.878		
Total	111	254.021	23.457		

Appendix 5.9 Analysis of variance for nematode eggs of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	5.56621	1.77106		
Nemarioc-AL	1	1.08300	1.08300	5.88	0.077
Nemafric-BL	1	0.76563	0.76563	2.16	1.098
Mormodica	1	0.44625	0.44625	1.10	2.171
A×B	1	1.78446	1.78446	6.55	0.061
A×M	1	0.06497	0.06497	0.23	3.269
B×M	1	6.70379	6.70379	15.43	0.001
A×B×M	1	1.06927	1.06927	4.21	0.071
Error	91	8.18166	1.16785		
Total	111	25.66524	14.85628		

Appendix 5.10 Analysis of variance for J2 in roots of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	7.8259	0.51715		
Nemarioc-AL	1	0.5253	0.52530	0.82	0.238
Nemafric-BL	1	1.6854	1.68543	3.97	0.079
Mormodica	1	0.9219	0.92185	1.33	0.066
A×B	1	0.0273	0.02729	0.08	0.887
A×M	1	0.1217	0.12173	0.49	0.975
B×M	1	1.5050	1.50499	3.53	0.065
A×B×M	1	0.6286	0.62795	0.96	0.173
Error	91	11.6298	0.46192		
Total	111	24.8709	6.39361		

Appendix 5.11 Analysis of variance for total nematodes of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	13.6752	0.60979		
Nemarioc-AL	1	0.6687	0.66872	1.99	0.893
Nemafric-BL	1	1.1369	1.13685	4.21	0.096
Mormodica	1	0.5278	0.52779	1.42	0.863
A×B	1	0.0208	0.02077	0.27	0.996
A×M	1	0.0699	0.06992	0.96	0.785
B×M	1	1.7386	1.73858	4.83	0.086
A×B×M	1	0.5747	0.57467	1.59	0.898
Error	91	22.9879	0.48815		
Total	111	41.4005	5.88535		

Appendix 5.12 Analysis of variance for nematode reproductive potential (RP) to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	3.887633	0.38541		
Nemarioc-AL	1	0.647831	0.64783	0.97	0.865
Nemafric-BL	1	1.054684	1.05468	2.09	0.090
Mormodica	1	0.062661	0.06266	0.06	0.061
A×B	1	0.085582	0.08558	1.12	0.293
A×M	1	0.033995	0.03399	0.01	0.057
B×M	1	0.814693	0.81469	1.05	0.233
A×B×M	1	0.182544	0.18254	0.09	0.174
Error	91	6.667443	0.31249		
Total	111	13.437066	3.57987		

Appendix 5.13 Analysis of variance for iron (Fe) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	0.22906	0.017		
Nemarioc-AL	1	0.00047	0.000	0.04	0.8485
Nemafric-BL	1	0.00076	0.000	0.06	0.8077
Mormodica	1	0.03272	0.032	2.55	0.1136
A×B	1	0.01410	0.014	1.10	0.2970
A×M	1	0.00120	0.001	0.09	0.7600
B×M	1	0.00489	0.004	0.38	0.5384
A×B×M	1	0.00407	0.004	0.32	0.5745
Error	91	1.16639	0.012		
Total	111	1.45367	0.088		

Appendix 5.14 Analysis of variance for potassium (K) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	0.42795	0.032		
Nemarioc-AL	1	0.02874	0.028	0.97	0.3274
Nemafric-BL	1	0.01587	0.015	0.54	0.4662
Mormodica	1	0.04290	0.042	1.45	0.2321
A×B	1	0.00005	0.000	0.00	0.9684
A×M	1	0.00574	0.005	0.19	0.6609
B×M	1	0.01809	0.018	0.61	0.4367
A×B×M	1	0.00786	0.007	0.26	0.6080
Error	91	2.69769	0.029		
Total	111	3.24489	0.153		

Appendix 5.15 Analysis of variance for sodium (Na) of potato to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	0.44094	0.033		
Nemarioc-AL	1	0.07978	0.079	3.99	0.0589
Nemafric-BL	1	0.01263	0.012	0.63	0.4291
Mormodica	1	0.002	0.002	0.00	0.9908
A×B	1	0.02586	0.025	1.29	0.2587
A×M	1	0.003	0.003	0.18	0.6731
B×M	1	0.038	0.038	1.91	0.1707
A×B×M	1	0.008	0.008	0.44	0.5105
Error	91	1.82191	0.020		
Total	111	2.43164	0.225		

Appendix 5.16 Analysis of variance for zinc (Zn) to Nemarioc-AL, Nemafric-BL and Mormodica phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	13	0.25093	0.019		
Nemarioc-AL	1	0.04944	0.049	1.98	0.1626
Nemafric-BL	1	0.00693	0.006	0.28	0.5993
Mormodica	1	0.00315	0.003	0.13	0.7233
A×B	1	0.00109	0.001	0.04	0.8350
A×M	1	0.01967	0.019	0.79	0.3769
B×M	1	0.10186	0.101	4.08	0.0572
A×B×M	1	0.05199	0.051	2.08	0.1523
Error	91	2.27007	0.024		
Total	111	2.75513	0.278		