INTEGRATED SYSTEM FOR THE MANAGEMENT OF *MELOIDOGYNE JAVANICA* IN POTATO PRODUCTION

by

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DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo, for the degree of Masters of Agricultural Management (Plant Production) has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

_____ _____

Seshweni MD (Ms) Date

DEDICATION

To the family I have always wanted to be my family

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ABSTRACT

Cultivated potato (*Solanum tuberosum* L.) cultigens do not have resistant genotypes to root-knot (*Meloidogyne* species) nematodes. Currently, efforts are underway to introgress nematode resistance in potato breeding programmes, whereas other environment-friendly nematode management strategies are being assessed in various cultigens. Nemafric-BL and Nemarioc-AL phytonematicides have being researched and developed for managing the root-knot nematode whereas Biocult Mycorrhizae are intended to enhance crop productivity through improved absorption of P, which is inherently low in most South African soils. The objectives of the study, therefore, were: (1) to determine the interactive effects of Nemacur (N), Biocult Mycorrhizae (B) and Nemarioc-AL or Nemafric-BL phytonematicide (P) on population densities of *M. javanica* and growth of potato plants, (2) to investigate the effects of Nemacur (N), Velum (V), Biocult Mycorhizae (B) and Nemarioc-AL or Nemafric-BL phytonematicide (P) on population densities of *M. javanica* and growth of potato plants. For the microplot experiment, potato cv. 'Mondial G3' seeds were sown in 25-cm-diameter plastic pots with 5 000 ml steam-pasteurised river sand and Hygromix-T at 3:1 (v/v) growing mixture in autumn (March-May) 2015. Pots were buried 80% deep into the soil in with 0.5 m inter-row and 0.5 m intra-row spacing. Potato cv. 'Mondial G3' seeds were dipped in a mixture of Mancozeb with a wettener for disease management prior to sowing. Appropriate treatments were applied soon after emergence of leaves. Each plant was inoculated by dispensing a mixture of 5 000 eggs and *M. javanica* J2. Eight treatments, control ($N_0B_0P_0$), Nemacur ($N_1B_0P_0$), Biocult ($N_0B_1P_0$), phytonematicide ($N_0B_0P_1$), Nemacur × Biocult ($N_1B_1P_0$), Nemacur × phytonematicide ($N_1B_0P_1$), Biocult × phytonematicide ($N_0B_1P_1$) and Nemacur × Biocult × phytonematicide ($N_1B_1P_1$), were arranged in a randomised complete block

design (RCBD) with 8 replications (n= 64). Under field conditions the study was conducted in summer (October 2015 - January 2016), with 30-cm furrows dug and potato seeds placed in the soil with 30 cm inter-row and 40 cm intra-row spacing. The four treatments, namely, (1) untreated control, (2) Nemacur or Velum (3) Biocult Mycorrhizae and (4) Nemarioc-AL or Nemafric-BL phytonematicide, were arranged in RCBD, replicated three times for the Velum experiment and five times for the Nemacur experiment. At 56 days after inoculation, the second order interaction ($N_1B_1P_1$) was highly significant ($P \leq 0.01$) for eggs in root and total nematodes, contributing 13 and 12% to total treatment variation (TTV) of the two variables, respectively, in the Nemarioc-AL phytonematicide study. Relative to untreated control, the second order interaction ($N_1B_1P_1$) reduced eggs in root and total nematodes by 42 and 36%, respectively. In both Nemarioc-AL and Nemafric-BL phytonematicide experiments, the combination of phytonematicide and Biocult Mycorrhizae reduced gall rating. Nemacur, Biocult and Nemarioc-AL phytonematicide, the treatment effects were highly significant on eggs, J2 in root and total nematodes, contributing 53, 68 and 57% to TTV of the three variables, respectively. Nemacur, Biocult and Nemafric-BL phytonematicide treatments each was not significant ($P \leq 0.05$) for nematodes variables. Both treatments for Nemacur, Biocult and Nemarioc-AL or Nemafric-BL phytonematicides were significant for gall rating, contributing 92 and 70% to TTV of the variable, respectively. In Nemarioc-AL phytonematicide, relative to the untreated control, gall rating was reduced by 48 to 56%, whereas in Nemafric-BL phytonematicide the variable was reduced by 33 to 56%. In the Velum study, Biocult and Nemarioc-AL or Nemafric-BL phytonematicide, the treatment effects in both experiments were highly significant ($P \leq 0.01$) on eggs in root, contributing 88% to TTV of the variable. Both treatments from Nemarioc-AL

and Nemafric-BL phytonematicides had no significant effects on all plant variables measured. In microplot, the second order interaction (Nemacur × Biocult × Nemarioc-AL phytonematicide) was highly significant for nematode eggs in root and total nematode. In a three-way matrix, the $N_1B_1P_1$ interaction had the highest effects on eggs, followed by Biocult alone, then Nemacur alone and then the phytonematicide. The same trend was observed in the three-way matrix for total nematodes. However, in two-way matrix for eggs, Biocult outperformed Nemacur, as was the phytonematicide on J2. In another microplot study, the second order interaction (Nemacur × Biocult × Nemafric-BL phytonematicide) was significant for J2 in soil and roots, with the three-way matrix showing, that Biocult alone had higher effects than the $N_1B_1P_1$ interaction on J2 in root. A three-way matrix also showed that Nemacur was outperformed by the phytonematicide alone, Biocult alone and the interactions on J2 in soil. In conclusion, Nemarioc-AL and Nemafric-BL phytonematicides could each be used with Biocult Mycorrhizae in the management of population densities of *M. javanica* in potato production since the impact from Nemacur which is a synthetic nematicide does not have that much difference from that of phytonematicides interacted with Biocult Mycorrhizae.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

The withdrawal of synthetic nematicides from the agro-chemical markets (Mashela *et al*., 2015), had serious consequences on cash crops, including potato (*Solanum tuberosum* L.), which relied heavily on the products (Dinh *et al.,* 2015). Although environment-friendly nematode management strategies such as the use of botanicals (Bello, 1998; Mashela, 2002; Mashela and Mpati, 2002; Mashela and Mphosi, 2002; Mashela and Nthangeni, 2002; Mashela *et al.*, 2008; Rajendran and Sarita, 2005; Sukul *et al*., 2001), organic soil amendments (Nagesh and Reddy, 1997; Singh *et al*., 2001; Vedhera *et al*., 1998) and nematodes resistance (Pofu *et al*., 2011a) had been developed for various crops. However, not much work on alternatives to synthetic nematicides had been done in potato production in South Africa. In the late 1980s global losses in potato due to nematode damage stood at approximately US$6 billion annually (Sasser and Freckman, 1987), whereas in South Africa the losses were at 17% (Keetch, 1989). In the UK alone potato losses due to the potato cyst (*Globodera rostochiensis* Wollenweber*, G. pallida* Stone) nematodes account for approximately US$70 million per annum or 9% of the UK production (DEFRA, 2010). In a recent South African Plant-Parasitic Nematode Survey (SAPPNS) database (Marais *et al*., 2015), it was shown that root-knot (*Meloidogyne* species) nematodes, including the cyst (*Heterodera* species) nematodes occur in most major potato-producing districts of South Africa.

Reasons for slow pace in adopting alternative nematode management strategies in potato production are not clear. However, there are untested beliefs in South Africa

that the potato cyst nematode had been eliminated through quarantine (EPPO, 2008). Development of nematode management strategies, using tested alternative strategies in crops (Pofu *et al*., 2011b) is essential in the future development of appropriate management strategies of *Meloidogyne* species in potato production. The strategies could include the management of *Meloidogyne species* using nematode resistance (Pofu *et al*., 2011b), phytonematicides (Mashela *et al*., 2013) and various other alternative products such as Biocult Mycorrhizae which are available on agrochemical markets in South Africa. The widely tested Nemarioc-AL and Nemafric-BL phytonematicides are being researched and developed for management of nematodes in South Africa (Mashela *et al*., 2015).

1.2 Problem statement

The effect of nematodes on potato is well-known and data concerning plant-parasitic nematodes that are associated with potato are well-documented. However, due to the widespread uses of synthetic nematicides, research on the use of alternative environment-friendly products on potato production had not been investigated. Adoption of alternatives to synthetic nematicides in potato production in South Africa had been slow. Locally-available products and strategies could be tested on managing population densities of *M. javanica* in potato production.

1.3 Rationale of the study

Information suggested that nematodes in all potato-producing regions of South Africa are challenge, with limited research on the use of alternative management strategies been a priority. The successful suppression of population densities of *M. javanica* on potato would enhance the potential use of available products combined or separately

in management of *Meloidogyne* species. The potato industry is important in South Africa in the context of the Presidential Outcomes in the South African National Development Plan. The product Mycorrhizae Biocult contains *Glomus* species, which are Vesicular-Abuscular Mycorrhiza that lives symbiotically by absorbing phosphorus for plants in exchange for carbohydrates. Also, the product contains *Trichoderma* species, which has disease-suppressing properties.

1.4 Purpose of the study

1.4.1 Aim

The aim was to develop a sustainable alternative management system for managing population densities of *Meloidogyne* species in potato production as alternative to synthetic chemical nematicides.

1.4.2 Objectives

1. To determine the interactive effects of Nemacur (N), Biocult Mycorrhizae (B) and Nemarioc-AL or Nemafric-BL phytonematicide (P) on population densities of *M. javanica* and growth of potato plants.
2. To investigate the effects of Nemacur (N) or Velum (V), Biocult Mycorhizae (B) and Nemarioc-AL or Nemafric-BL phytonematicide (P) on population densities of *M. javanica* and growth of potato plants.

1.4.3 Hypotheses

1. The interactive effect of Nemacur, Biocult Mycorrhizae and Nemarioc-AL or Nemafric-BL phytonematicide would have an effect on population densities of *M. javanica* and growth of potato plants.

2. The effects of Nemacur or Velum, Biocult Mycorrhizae and Nemarioc-AL or Nemafric-BL phytonematicide would have an effect on population densities of *M. javanica* and growth of potato plants.

1.5 Reliability, validity and objectivity

In this study, reliability of data were based on statistical analysis of data at the probability level of 5%, validity was achieved through repeating the experiments in time, while objectivity will be achieved by ensuring that the findings are discussed on the basis of empirical evidence, in order to eliminate all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

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

1.7 Structure of dissertation

Following the description and detailed outlining of the research problem (Chapter 1), the work done and not yet done on the research problem was reviewed (Chapter 2). Then, each of the two subsequent chapters (Chapter 3, 4) addressed each of the two objectives, sequentially. In the final chapter (Chapter 5), findings in all chapters were summarised and integrated to provide the significance of the findings and recommendations with respect to future research, culminating in a conclusion which tied together the entire study. In the citations and references the Havard style, approved by the Senate of the University of Limpopo, was used.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Cultivated potato (*Solanum tuberosum* L.) plants do not have resistant genotypes to root-knot (*Meloidogyne* species) nematodes (Jones, 2006). Efforts are underway to introgress nematode resistance in potato breeding programmes in South Africa (Dinh *et al*., 2015). However, additional work was being done in South Africa to develop non-chemical and environment-friendly nematode management strategies. Most of the phytonematicide strategies were being researched on annual crops (Mashela *et al*., 2015), whereas research on perennial crops focused primarily on nematode resistant rootstocks (Duncan, 2005, 2009). In low-input agricultural farming systems, farmers use organic amendments to supress plant-parasitic nematodes and to provide essential nutrient elements (Stirling, 2014). However, the use of conventional organic amendments to suppress plant-parasitic nematodes had many drawbacks (Mankau, 1968; Mankau and Minteer, 1962; McSorley and Gallaher, 1995a, b; Muller and Gooch, 1982; Stirling, 2014). The drawbacks included large quantities of organic material, high transport costs to transport the organic material to the fields, longer periods to decompose, reduction of soil pH and inconsistent results in nematode suppression (Mashela, 2002). Phytonematicides were researched and developed to ameliorate the drawbacks of conventional organic amendments Indigenous under the auspices of the Cucurbitaceae Technologies (Mashela *et al*., 2015), since the focus was exclusively on using the materials from *Cucumis* species indigenous to Limpopo Province, South Africa.

2.1.1 Status of nematodes on potato in South Africa

According to the South African Plant-Parasitic Nematode Survey (SAPPNS) and the National Collection of Nematodes (NCN) database, approximately 453 plant-parasitic nematodes were reported previously in South Africa with 21% associated with potato crops. The survey by Marias *et al*. (2015), clarified the previous contradictions which suggested that information on the distribution of plant-parasitic nematodes in South Africa was scanty (Decraemer, 1995; Kleynhans *et al*., 1996).

Among the sixteen potato-producing regions, Mpumalanga and Gauteng Provinces had 50 and 40 different nematode species associated with potatoes, respectively (Marias *et al*., 2015). Contrary to what is in the previous records (Decraemer, 1995); Kleynhans *et al*., 1996), evidence exist that the potato cyst nematode (*Globodera rostochiensis* Wollenweber) and the cyst nematodes (*Heterodera* species) are prevalent in certain cooler potato-producing regions of South Africa (Knoetze, 2014). Previous claims suggested the two nematode genera had been eliminated in South Africa through quarantine efforts.

2.1.2 Nematode management in potato production

Due to the patchiness of information on nematode-potato relations in South Africa (Decraemer, 1995; Kleynhans *et al*., 1996), the management of nematodes in potato production had never been viewed as a priority. However, the withdrawal of most synthetic nematicides from agrochemical markets, with the cut-off date for methyl bromide in 2005 (Mashela *et al*., 2015), necessitated the review of the views on nematode-potato relations in South Africa. Currently, efforts are underway through

the Agricultural Research Council and Universities supported by Potato South Africa, to develop suitable and sustainable management strategies for potato production.

2.2 Existing alternatives

2.2.1 Phytonematicides

Two phytonematicides, Nemarioc-AL and Nemafric-BL, derived from crude extracts of wild cucumber (*Cucumis myriocarpus* Naude.) and wild watermelon (*Cucumis africanus* L.f.) fruits, respectively, are being researched and developed in South Africa (Mashela *et al*., 2015) Detailed agronomics of the two *Cucumis* species included development of sexual and asexual propagation protocols (Mafeo and Mashela, 2009a), *in vitro* mass propagation along with irrigation and fertilisation protocols (Nkgapele *et al*., 2011a, b; Mafeo, 2005). Cucurbitacins are used in plant defense against nematodes, fungi and insects (Chen *et al*., 2005). Orally-administered, decoctions of crude extracts of *C. africanus* and *C. myriocarpus* fruits control intestinal roundworms in humans (*Ascaris lumbricoides* L.), dogs (*Toxocara canis* Wener, *Toxascaris leonine* Wener), chickens (*Ascaridia galli* Schrank) and other domesticated animals (Mashela *et al*., 2011). Motivated by these observations, Mashela (2002) opted to use crude extracts from *C. myriocarpus* fruit to suppress population levels of *Meloidogyne* species in pot trials, with results showing at least 90% suppression of the nematodes. Two different products, namely Nemarioc-AL and Nemafric-BL phytonematicides, had since been developed as liquid (L) and as granular (G) formulations (Mashela *et al*., 2015).

Ground leaching technology (GLT): This technology involves the application of ground materials from selected plant organs in small quantities. The latter has both

suppressive effect on nematodes and “fertiliser effect” on growth of plants (Mashela, 2002; Mashela and Nthangeni, 2002; Mashela *et al*., 2010). The GLT system was developed to ameliorate the drawbacks of conventional organic amendments, namely, inconsistent nematode suppression results, large quantities of materials required to suppress nematodes, with the resultant high transport costs and availability, negative period and changes in soil pH (Mashela, 2002).

In GLT, mature fruits of *C. africanus* and *C. myriocarpus* were cut into small pieces, dried at 52 °C for 72 h and ground in a Wiley mill to pass through a 1-mm-pore sieve (Mashela, 2002). The material is applied soon after transplanting without first being subjected to microbial degradation (Mashela, 2002; Mashela and Mphosi, 2002). The GLT system is naturally labour-intensive and could, therefore, be costly for large-scale commercial farmers. Development of bio-nematicides from fermented crude extracts of *C. africanus* and *C. myriocarpus* fruits would enhance the application of GLT through irrigation water in commercial farming agriculture. Mashela and Pofu (2012) demonstrated that the material promoted nodulation of *Bradyrhizobium japonicum* Kirchner in cowpea (*Vigna unguiculata* L.) production. Also, the independence of the GLT system from microbial activities was demonstrated through elimination of *Bacillus* species in predictive models when using crude extracts of castor (*Ricinus communis* L.) bean (Mashela and Nthangeni, 2002; Mofokeng *et al*., 2004) and fever tea (*Lippia javanica* L.) leaves (Mashela *et al*., 2010).

Botinemagation: Botinemagation is defined as the use of phytonematicides through irrigation systems (Mashela *et al*., 2011). Only cucurbitacin A in *C. myriocarpus* fruit is soluble in water due to its partial polarity (Chen *et al*., 2005). However, it was

uncertain whether crude extracts of *C. africanus* fruit could also serve as fermented crude extracts in suppression of nematodes since cucurbitacin B is insoluble in water. Thus, fermented crude extracts of *C. africanus* and *C. myriocarpus* fruits were tested separately and reduced nematode population densities by 89% (range 80 to 100%) and 69% (range 52 to 79%), respectively (Pelinganga *et al*., 2011). At low dilutions both materials had fertiliser effect on tomato plants, while at high dilutions each was phytotoxic. Results of the study (Pelinganga *et al*., 2011) demonstrated that the two materials could serve as potent bio-nematicides at low concentrations. Pelinganga and Mashela (2012), after establishing the stimulatory concentrations, devised the concept of a "30-day week-month" to determine the application intervals 16 and 17 days for *C. myriocarpus* and *C. africanus* fruits, respectively. The optimum application interval of Nemarioc-AL 3% was at 16 days. At this interval, the material would be able to disrupt the life cycle of *M. incognita* race 2 in tomato production, without reducing growth of tomato plants (Pelinganga *et al*., 2013a). In both Nemafric-BL and Nemarioc-AL phytonematicides Mean Concentration Stimulation Point (MCSP) values were established as being equivalent to 3% concentration (Pelinganga *et al*., 2013b). This means, for every 3 L stock solution of Nemafric-BL or Nemarioc-AL phytonematicides, 100 L chlorine-free water is required for application through drip irrigation, termed Botinemagation (Mashela *et al*., 2015).

Post-emergent application: Crude extracts of *C. myriocarpus* fruit suppressed plant-parasitic nematodes in greenhouse and microplot trials by over 90% (Mashela, 2002; Mofokeng *et al*., 2004; Shakwane *et al*., 2004), and in field trials by over 80% (Mashela, 2007). Additionally, relative to untreated controls, the crude extracts increased soil electrical conductivity from 95 to 160%, but had no significant effect on

soil pH. Also, the material improved fruit yield and growth of tomato and dry shoot mass of citrus seedlings in various trials (Mashela, 2007; 2002; Mashela *et al*., 2008; Mphosi, 2004). Regardless of the organic amendment source, when used as post-emergent bio-nematicide, the material had fertiliser effect and had no effect on soil pH, with the exception of *L. javanica* leaves, which reduced soil pH (Mashela *et al*., 2010). In contrast, relative to untreated control, crude extracts of *C. myriocarpus* fruit alone drastically reduced all plant variables except plant height, which suggested that the product may be phytotoxic to citrus plants.

Pre-emergent application: Crop yield losses are, incidentally proportional to initial population densities (Pi) of nematodes (Seinhorst, 1967). Ideally, the use of a material in GLT system should be as a pre-emergent bio-nematicide in order to keep the Pi at the lowest level possible. However, a preliminary study suggested that the crude extracts of *C. myriocarpus* fruits used in the GLT system, may not be suitable for use as pre-emergent nematicides to reduce Pi levels in the Solanaceae family due to allelopathic effects on germinating seedlings (Mafeo and Mashela, 2009a). In vitro, seed germination assays suggested that at 5 g crude extracts of *C. myriocarpus* fruit were highly phytotoxic to tomato, watermelon and butternut squash (Mafeo and Mashela, 2009a), along with maize (*Zea mays* L.), finger millet (*Eleusine coracana* L), sorghum (*Sorghum bicolor* L.) and onion (*Allium cepa* L.) (Mafeo and Mashela, 2009b). In greenhouse trials, the material completely inhibited seedling emergence of all dicotyledonous crops tested (Mafeo and Mashela, 2010). Crude extracts of *C. myriocarpus* fruit stimulated growth of various organs in maize, millet and sorghum, whereas at high dosages the material inhibited growth of the three test crops. Estimated mean dosage response for stimulation for maize, millet and

sorghum being was found to be 1.13, 0.86 and 1.12 g, respectively. At the dosage of 2 g/plant where crude extracts of ground *C. myriocarpus* fruits suppressed *M. incognita* race 2, when applied as a pre-plant bio-nematicide the material had either 50% or 100% inhibition of growth in chive (*Allium schoenoprasum* L.), leek (*Allium porrum* L.) and onion during the 18-day testing period (Mafeo *et al*., 2011).

Drawbacks of phytonematicides: The drawback of the GLT system was its high labour costs since products were manually applied, which rendered the system less appealing to large commercial tomato producers (Mashela *et al*., 2011). Additionally, phytonematicides could be highly phytotoxic, with various statutory bodies having zero tolerance on agricultural inputs which have phytotoxic attributes (EPPO, 2010). However, all agricultural inputs, when wrongly used, can be stressful to crops.

Managing drawbacks of phytonematicides: An alternative technology, referred to as botinemagation (Mashela *et al*., 2011), was developed for use in large-scale tomato farming systems, where crude extracts from fermented plant organs were used through drip irrigation systems. Using dried fruits of *C. myriocarpus* and *C. africanus* fruits, fermented crude extracts as liquid formulations consistently reduced population densities of *Meloidogyne* species in tomato production (Pelinganga *et al*., 2013a, b). Due to phytotoxicity and its zero tolerance in most legislative frameworks on products used in agriculture, literature is replete with efficacy trials which do not go beyond *in vitro* status. Using the concept of DDG patterns, there are basically three concentration phases, namely, stimulation, neutral and inhibition phases. Using the latter, the concept of mean concentration stimulation point [$MCSP = D_m + (R_h/2)$] was developed in an attempt to answer the questions of how much concentration of

Nemarioc-AL or Nemafric-BL phytomaticide to apply, which was followed by that of the application interval. The two questions were empirically answered (Mashela *et al.*, 2015), with avoidance of phytotoxicity and the efficacy of the products on nematode suppression being enhanced (Pelinganga *et al*., 2013a).

2.2.2 Biological control

Mycorrhizal fungi: Mycorrhizal fungi work by excreting powerful chemicals into the soil which improve plant growth through increased nutrient uptake in exchange for photosynthetic carbon from their host (Smith *et al*., 2010). Also, they can alleviate plant stress caused by abiotic as well as biotic factors, including plant-parasitic nematodes (Gianinazzi *et al*., 2010; Singh *et al.,* 2011; Vos *et al*., 2012a). Mycorrhizal fungi can suppress plant-parasitic nematodes, as has been previously reviewed (Hol and Cook, 2005; Pinochet *et al.,* 1996). Under *in vitro*, greenhouse and field conditions, various workers demonstrated how mycorrhizal fungi had protective effects against plant-parasitic nematodes on cultigens such as banana (*Musa acuminata* L.), coffee (*Coffea arabica* L.) and tomato (Alban *et al*., 2013; Calvet *et al*., 2001; Koffi *et al*., 2013; Vos *et al*., 2012b).

Trichoderma is a species-rich genus of fungi belonging to the Ascomycota phylum. *Trichoderma* species are remarkable for their rapid growth, capability of using diverse substrates and their tolerance to noxious chemicals (Kubicek *et al*., 2003). Some *Trichoderma* species are of economic importance because of their production of enzymes and antibiotics and are used as biocontrol agents (Gams and Bissett, 1998; Kubicek *et al*., 2003; Sivasithamparam and Ghissalberti, 1998). *Trichoderma* is not only a biological control agent, but also a plant growth enhancer, which is

supported by reports on growth promotion of several species of plants treated with *Trichoderma* species (Hoyos-Carvajal *et al*., 2009). Nzanza (2011) investigated seedling growth and development of tomato as influenced by *T. harziam* and arbuscular mycorrhizal fungi and demonstrated that the combination of the two products improved growth and development of tomato seedlings.

2.2.3 Crop rotation systems

Crop rotation with non-host or poor host plants is one of the most important cultural methods to reduce soil population densities of the nematodes and thus allow the following host crop to grow and yield satisfactory (Nusbaum and Ferris, 1973). However, crop rotations have economic costs for the grower. Therefore, the inclusion in a rotation of resistant crop cultivars can be an option, as it does not require significant changes in farming operations or in market supply (Ornat and Sorribas, 2008). Antagonistic crops to nematodes are those that are considered to produce toxic substances, usually, while the crops are growing or after incorporation into the soil. In practical nematode management strategies, the use of this approach relies on pre-plant cover crops, intercropping or green manures. Marigold (*Tagetes patula* L.), neem (*Azadirachta indica* A. Juss), sunn hemp (*Crotalaria juncea* L.), castor bean (*Ricinus communis* L.), partridge pea (*Chanaecrista fasciculate* Michx.), asparagus (*Asparagus officinalis* L.), rapeseed (*Brassica napus* L.) and sesame (*Sesamum indicum* L.) have been extensively studied and used as antagonistic crops for nematode control.

Sunn hemp is often cultivated as a cover crop for direct seeding, intercrops or soil amendment and is considered an antagonistic crop for most plant-parasitic

nematodes, especially root-knot nematodes (Wang *et al*., 2002). Population densities of *M. incognita* were affected by 26 previous cover crops of *Crotalaria junceain* North Florida (Wang *et al*., 2002). Viaene and Abawi (1998) recommended the use of some *Crotalaria* species from Senegal as pre-crops for providing green manure, whereas at the same time they decreased the level of root-knot nematodes and increased the level of beneficial mycorrhizal fungi. Marigolds (*Tagetes* species) have been shown to suppress plant parasitic nematodes, such as root-lesion (*Pratylenchus* species) and root-knot nematodes. Most antagonistic plants cultivated as pre-plant cover crops may be followed by soil incorporation of the biomass with a subsequent reduction of plant parasitic nematode numbers and the enhancement of nematode antagonists. However, it should be noted that grower acceptance of new strategies using antagonistic plants is based on economic and logistical considerations, as well as efficacy. Too often the large amounts of biomass required restricted the use of the approach to cheap sources of local species/waste products.

2.2.4 Quarantine

The nematode species under quarantine in South Africa for potato include *Globodera* species and *Heterodera* species (Marias *et al*., 2015). Despite previous suggestions that these nematode species have been successfully eliminated in South Africa, Marias *et al*. (2015) demonstrated that the two nematode species are still widely distributed in the Western Cape and Eastern Cape Provinces, with limited distribution in Gauteng and Mpumalanga Provinces. Although, it has been documented that the unlisted provinces were not quarantined for the two nematode species, one should indicate that data recorded by Marias *et al*. (2015) only provided a picture of the areas were samples were collected for the surveys.

2.3 Work not yet done on the research problem

The challenges currently facing the potato producing farmers mainly involve limited management strategies for use as alternatives to the synthetic chemical nematicides. The potato industry had been highly reliant of synthetic chemical nematicides to the extent that alternatives such as nematode resistance were ignored for a long period at international scale. Internationally, with the cut-off date of the withdrawal of methyl bromide in 2005, much research work had been focusing on managing nematode population densities using environment-friendly sustainable products. The proposed research intended to investigate alternative products and technologies that had been developed in other crop industries for use in potato production for managing population densities of *Meloidogyne* species.

CHAPTER 3
INTEGRATED MANAGEMENT STRATEGIES OF NEMATODES ON POTATO PLANTS: MICROPLOT STUDIES

3.1 Introduction

Additive and/or synergistic interactions between phytonematicides and other sustainable products with nematicidal properties may offer a powerful and reliable strategy for sustainable management of nematode population densities (Mashela *et al*., 2015). The intensity and the direction of interactions are established using statistical tools and matrix tables (SSC, 2000). Using two- or three-matrix tables Nemarioc-AG (G = granular formulation), phytonematicide, Aldicarb and Nemacur had the highest impact on nematode numbers when combined than when each was alone (Mashela *et al*., 2008). Nzanza (2011) showed that *Trichoderma* and AMF synergistically improved most of growth variables in tomato seedlings.

Nemacur which had been widely used in nematode management is being withdrawn from the agrochemical markets (Kline and Company, 2005). Environment-friendly products with nematicidal properties are being tested for use in nematode management (Archana and Prasad, 2014). Biocult Mycorrhizae, which contains *Glomus* species and *Trichoderma* species, along with Nemarioc-AL phytonematicide, are examples of such products (Mafeo and Mashela, 2009a, b; Nzanza, 2011). Nemarioc-AL (L = liquid formulation) phytonematicide is a fermented product developed from dried fruit of wild cucumber (*Cucumis myriocarpus* Naude.) indigenous to Limpopo Province (Mashela *et al*., 2015). The active ingredient in fruit of *C. myriocarpus* is cucurbitacin A (Chen *et al*., 2005), which oxidises rapidly to cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Chen *et al*., 2005). Nemafric-BL phytonematicide is fermented from dried fruit of wild watermelon (*Cucumis africanus*

L.f.), also indigenous to Limpopo Province (Mashela *et al*., 2011). The active ingredient in fruit of *C. africanus* is cucurbitacin B ($C_{38}H_{40}O_8$) (Chen *et al*., 2005). The interactive effects among phytonematicides, from *Cucumis* species, Biocult Mycorrhizae and Nemacur have not been documented. The objective of this study was to determine the interactive effects of Nemacur (N), Biocult Mycorrhizae (B) and phytonematicides (P) from *Cucumis* species on population densities of *M. javanica* and growth of potato plants.

3.2 Materials and methods

3.2.1 Location of the study

The study was conducted under microplot conditions at the Green Technologies Research Centre (GTRC), University of Limpopo, Limpopo Province, South Africa (23°53'10"S, 29°44'15"E). The location has hot and dry summers, with daily maximum temperature from 28 to 38°C. The average annual rainfall is less than 500 mm which occurs mainly in summer. The two parallel experiments of Nemarioc-AL phytonematicide with Nemacur and Biocult Mycorrhizae, along with that of Nemafric-BL phytonematicide with the other two products were initiated during autumn (March-May) in 2015.

3.2.2 Experimental design

A 2 × 2 × 2 factorial experiment was laid out in a randomised complete block design, with eight replications (Figure 3.1). The first, second and third factors comprised Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, respectively. The eight treatments included (a) untreated control ($N_0B_0P_0$), (b) Nemacur alone ($N_1B_0P_0$), (c) Biocult Mycorrhizae alone ($N_0B_1P_0$), (d) Nemarioc-AL phytonematicide

alone ($N_0B_0P_1$), (e) $N_1B_1P_0$, (f) $N_1B_0P_1$, (g) $N_0B_1P_1$ and (h) $N_1B_1P_1$). A similar but parallel study was conducted using Nemafric-BL phytonematicide. Unless otherwise stated, all procedures for Nemafric-BL phytonematicide with the two products were similar to those of Nemarioc-AL phytonematicide with the two products.

3.2.3 Sampling

Phytonematicides were prepared by collecting fruits from *C. myriocarpus* and *C. africanus* from separate cultivated fields at GRTC after fruits had matured (Mafeo and Mashela, 2009a, b). Fruits were cut into pieces to increase the drying surface and dried at 52ºC in air-forced ovens (Mashela, 2002). Dried fruits were ground in a Wiley mill to pass through a 1-mm sieve and fruits from each *Cucumis* species were fermented to produce Nemarioc-AL phytonematicide or Nemafric-BL phytonematicide as described by (Mashela *et al.,* 2015).

Twenty-five-cm-diameter plastic pots were filled with steam-pasteurised (300 °C for 1 h) Hutton sandy loam (65% sand, 30% clay, 5% silt), containing 1.6% organic C, with electrical conductivity (EC) of 0.148 $dS.m^{-1}$ and river sand were mixed with a Hygromix-T (Hygrotech, Pretoria North, South Africa) in a 2:1:1 (v/v) ratio. Pots were buried 80% deep into the soil in microplots with 0.5 m x 0.5 m spacing (Figure 3.1). Potato cv. ‘Mondial G3’ seeds were dipped in a mixture of Mancozeb with a wettener for disease management prior to planting. *Meloidogyne javanica* inoculum was prepared by extracting eggs and second-stage juveniles (J2) from roots of greenhouse-grown nematode-susceptible kenaf (*Hibiscus cannabinus* L.) in 1% NaOCl (Hussey and Barker, 1973). Appropriate treatments were applied soon after emergence of leaves. Each plant was inoculated by dispensing a mixture of 5 000 *M.*

javanica eggs and J2 using a 20-ml plastic syringe by placing into 5-cm-deep holes on cardinal points of the stems per replication and covered with the growing medium.

Figure 3.1 Establishment of potatoes on microplots.

3.2.4 Data collection

At 56 days after inoculation with nematodes, plant length was measured from the soil level to the tip of the flag leaf. Shoots were cut at the soil level, chlorophyll content was measured using a chlorophyll meter (MINOLTA, SPAD-502) and stem diameter measured 5-cm from the cut end of the stem using a digital vernier caliper. Fresh shoots were oven-dried for 72 h at 52°C for dry matter determination. Root systems were removed from pots, immersed in water to remove soil particles, blotted dry and fresh mass measured to facilitate the calculation of nematode density per total root system per plant. Fully-developed root galls were assessed using the North Carolina Differential Scale at 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-

100 galls and 5 ≥ 100 galls per root system (Taylor and Sasser, 1978). Nematodes were extracted from total root system per plant by maceration and blending for 30s in 1% NaOCl solution (Hussey and Barker, 1973). The aliquot was passed through top-down nested 63 and 25-µm screen opening sieves. Contents of the 25-µm screen sieve were poured into 100 ml plastic containers for counting under a stereomicroscope.

Soil per pot was thoroughly mixed and a 250 ml soil sample was collected. Nematodes were extracted from soil samples using the modified sugar-floatation and centrifugation method (Jenkins, 1964). Briefly, the soil sample was poured into a 4 L bucket and stirred, once the swill had stopped, the aliquot was poured through 63 and 25-µm screen sieves, with the contents being washed into 50 ml plastic centrifuge tubes. Approximately 3 g kaolin was then added in each tube and contents centrifuged at 1 750 RPM for five minutes. Kaolin solution was then decanted with nematodes having settled at the bottom of the tubes with soil particles. A 480 g sugar/l tap water was poured into the centrifuge tubes and stirred once prior to centrifuging for 60 s at 1 750 RPM. The aliquot was then decanted onto 25-µm sieve with sugar being rinsed off the nematodes, which were then collected from the sieve into 100-ml plastic containers for counting under a stereomicroscope. Nematode numbers from roots were converted to nematodes per total root system per plant, whereas soil nematode numbers were converted to 5 litre soil per pot to estimate the final nematode population density (Pf).

3.2.5 Data analysis

Data for plant and nematode variables were subjected to a factorial analysis of variance through the SAS software (SAS Institute, 2008). Prior to ANOVA, nematode population density data were transformed through $\log_{10}(x + 1)$ to normalise the variances (Gomez and Gomez, 1984). Significant second and first order interactions were further expressed using the three-way and two-way matrix, respectively, in order to allow for the determination of the magnitude and direction of the main factors relative to untreated controls (Steyn *et al*., 2003).

3.3 Results

3.3.1 Nematode variables

Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide: Effects of the second order ($N_1B_1P_1$) interaction were highly significant on eggs in root and total nematodes, contributing 13 and 12% in total treatment variation (TTV) of the two variables, respectively (Tables 3.1). The first order ($N_1B_0P_1$) and $N_1B_1P_0$ interactions had significant effects on J2 in root, contributing 15 and 17% in TTV of the two variables, respectively (Table 3.1). The second order ($N_1B_1P_1$), followed by the first order ($N_0B_1P_1$) interactions had the highest impact on nematode eggs in root (Table 3.2), whereas the effects of Nemacur alone was equivalent to that of Nemarioc-AL phytonematicide. Similar trends were observed on total nematodes (Table 3.3). On J2 in root, combining Nemacur and Nemarioc-AL phytonematicide had more or less similar effects than when the products were used separately (Table 3.4). Biocult Mycorrhizae alone had higher impact than when combined with Nemacur or Nemacur used alone (Table 3.5).

Table 3.1 Responses of eggs in root, J2 in root and total nematodes of potato to Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonemacide interactions under microplot conditions (n = 60).

Source	DF	Eggs in root		J2 in root		Total nematode	
		MS	%	MS	%	MS	%
Replication	7	0.13786	1	0.0394	1	0.0914	1
Nemacur (N)	1	1.54862	14***	0.2972	9***	1.1789	13***
Biocult (B)	1	3.34390	30***	1.1142	32***	2.7198	30***
Nemarioc-AL (P)	1	2.53257	23***	0.4013	12*	1.9399	21***
N × B	1	0.95072	8***	0.5654	17**	0.9796	11***
N × P	1	1.00004	9***	0.5150	15**	1.0250	11***
B × P	1	0.12628	1ns	0.0057	1ns	0.0268	0ns
N × B × P	1	1.41196	13***	0.3223	9ns	1.0409	12***
Error	48	0.08244	1	0.1309	4	0.0639	1
Total	60	11.1421	100	3.3915	100	9.0661	100

ns Not significant, * slightly significant at P ≤ 0.10, ** Significant at P ≤ 0.05 and

*** Highly significant at P ≤ 0.01.

Table 3.2 Three-way matrix for eggs in root as affected by second order interaction of Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide at 56 days after treatment application (n = 60).

		Phytonematicide (P)			
Nemacur (N)	Biocult (B)	P_0	Impact (%)	P_1	Impact (%)
N_0	B_0	3.6022	−	2.7196	−25
N_0	B_1	2.6649	−26	2.2122	−38
N_1	B_0	2.4662	−31	2.7119	−24
N_1	B_1	2.6443	−27	2.0939	−42

Impact (%) = [(treatment/control) – 1] x 100.

Table 3.3 Three-way matrix for total nematodes as affected by second order interaction of Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide at 56 days after treatment application (n = 60).

		Phytonematicide (P)			
Nemacur (N)	Biocult (B)	P_0	Impact (%)	P_1	Impact (%)
N_0	B_0	3.6562	−	2.8165	−22
N_0	B_1	2.7560	−24	2.3558	−35
N_1	B_0	2.5982	−28	2.8050	−23
N_1	B_1	2.7331	−25	2.3304	−36

Impact (%) = [(treatment/control) – 1] x 100.

Table 3.4 Two-way matrix for J2 in root as affected by first order interaction of Nemacur and Nemarioc-AL phytonematicide at 56 days after treatment application (n = 60).

Nemacur (N)	Phytonematicide (P)			
	P_0	Impact (%)	P_1	Impact (%)
N_0	2.2493	−	1.9011	−15
N_1	1.9239	−14	1.9456	−14

Impact (%) = [(treatment/control) – 1] x 100.

Table 3.5 Two-way matrix for J2 in root as affected by first order interaction of Nemacur and Biocult Mycorrhizae at 56 days after treatment application (n = 60).

Nemacur (N)	Biocult Mycorrhizae (B)			
	B_0	Impact (%)	B_1	Impact (%)
N_0	2.3081	−	1.8424	−20
N_1	1.9739	−14	1.8956	−17

Impact (%) = [(treatment/control) – 1] x 100.

Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide: The second order ($N_1B_1P_1$) interaction was significant for J2 in root and J2 in soil, contributing 19 and 7%, respectively in total treatment variation (TTV) (Table 3.6). The second order ($N_1B_1P_1$), followed by ($N_1B_0P_1$), ($N_1B_1P_0$) and ($N_0B_1P_1$) interaction had the highest

impact on J2 in soil (Table 3.7), where the effects of Nemacur alone was equivalent to that of ($N_0B_1P_1$) on J2 in root (Table 3.8).

3.3.2 Plant variables

Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide: The second order interaction ($N_1B_1P_1$) was not significant ($P \leq 0.05$) on plant variables. In contrast, the $N_0B_1P_1$ interaction was highly significant for gall rating and significant for chlorophyll content, contributing 18 and 41% in TTV, respectively (Table 3.9). The $N_1B_1P_0$ interaction had similar effects and contributed significantly by 18% in TTV to gall rating (Table 3.9). The first order ($N_1B_1P_0$) interaction had the highest impact on gall rating (Table 3.11), followed by the first order ($N_0B_1P_1$) interaction (Table 3.10). The effects of Biocult Mycorrhizae alone were higher than that of Nemacur alone (Table 3.11). The first order ($N_0B_1P_1$) interaction had the lowest impact on chlorophyll content (Table 3.12). Biocult Mycorrhizae alone had the effects higher than those of Nemarioc-AL phytonematicide alone (Table 3.12).

Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide: The first order $N_1B_1P_0$ interaction was highly significant on stem diameter contributing 38% in TTV of the variable (Table 3.13). The $N_0B_1P_1$ interaction was significant for gall rating and contributed 13% in TTV. The first order ($N_0B_1P_1$) interaction had an impact on gall rating with Nemafric-BL phytonematicide alone having the highest impact on gall rating (Table 3.14). The first order ($N_1B_1P_0$) interaction had the lowest impact on stem diameter. Nemacur alone had the highest impact on stem diameter followed by Biocult Mycorrhizae alone (Table 3.15).

Table 3.6 Responses of eggs in root, J2 in root, eggs and J2 in root and J2 in soil of potato to Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonemacides interactions under microplot conditions (n = 62).

		Eggs in root		J2 in root		Eggs and J2 in root		J2 in soil	
Source	DF	MS	%	MS	%	MS	%	MS	%
Replication	7	0.07441	1	0.35957	8	0.09340	2	0.00435	0
Nemacur (N)	1	0.93456	16^{**}	0.28864	7^{ns}	0.78527	15^{**}	2.05773	7^{**}
Biocult (B)	1	1.9221	33^{***}	0.42097	10^{ns}	1.52429	29^{***}	7.67751	24^{***}
Phytonematicide (P)	1	0.10558	12^{ns}	0.59494	14^{*}	0.18823	4^{ns}	7.67751	22^{***}
N × B	1	0.00443	0^{ns}	0.10526	2^{ns}	0.00046	0^{ns}	2.04649	7^{**}
N × P	1	2.14931	36^{***}	1.33109	31^{***}	1.94093	37^{***}	2.04649	7^{**}
B × P	1	0.19011	3^{ns}	0.15699	4^{ns}	0.08065	2^{ns}	7.65577	24^{***}
N × B × P	1	0.30824	5^{ns}	0.79335	19^{**}	0.39625	8^{ns}	2.05773	7^{**}
Error	48	0.21659	4^{ns}	0.20745	5^{ns}	0.20325	3^{ns}	0.48183	2^{ns}
Total	62	5.90533	100	4.25826	100	5.21273	100	31.70541	100

^{ns} Not significant, * slightly significant at P ≤ 0.10, ** Significant at P ≤ 0.05, *** Highly significant at P ≤ 0.01.

Table 3.7 Three-way matrix for J2 in soil as affected by second order interaction of Nemacur (N), Biocult Mycorrhizae (B) and Nemafric-BL phytonematicide (P) at 56 days after treatment application (n = 62).

		Phytonematicide			
Nemacur	Biocult	P_0	Impact (%)	P_1	Impact (%)
N_0	B_0	2.1220	−	1.39E−16	−100
N_0	B_1	−2.78E−17	−100	−8.33E−17	−100
N_1	B_0	0.6750	−68	−5.83E−16	−100
N_1	B_1	2.78E−17	−100	−1.98E−03	−100

Impact (%) = [(treatment/control) – 1] x 100.

Table 3.8 Three-way matrix for J2 in root as affected by second order interaction of Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide at 56 days after treatment application (n = 62).

		Phytonematicide (P)			
Nemacur (N)	Biocult (B)	P_0	Impact (%)	P_1	Impact (%)
N_0	B_0	2.7099	−	2.0989	−22
N_0	B_1	2.3393	−13	1.9780	−27
N_1	B_0	1.9760	−27	2.3976	−11
N_1	B_1	2.2191	−18	1.9907	−26

Impact (%) = [(treatment/control) – 1] x 100.

Table 3.9 Responses of gall rating and chlorophyll content of potato to Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide interactions under microplot conditions (n = 60).

		Gall rating		Chlorophyll content	
Source	DF	MS	%	MS	%
Replication	7	0.033394	3	5.5850	5
Nemacur (N)	1	0.03412	3^{***}	0.0487	0^{ns}
Biocult (B)	1	0.24726	25^{***}	25.6403	22^{ns}
Phytonematicide (P)	1	0.15408	16^{***}	7.7952	7^{ns}
N × B	1	0.17334	18^{***}	1.5249	1^{ns}
N × P	1	0.02741	3^{ns}	14.5533	13^{ns}
B × P	1	0.17431	18^{***}	47.1609	41**
N × B × P	1	0.1108	11^{ns}	0.5509	0^{ns}
Error	48	0.02410	2	12.0908	11
Total	60	0.97936	100	114.95	100

^{ns} Not significant, ** Significant at P ≤ 0.05 *** Highly significant at P ≤ 0.01.

Table 3.10 Two-way matrix for gall rating as affected by first order interaction of Biocult Mycorrhizae and Nemarioc-AL phytonematicide at 56 days after treatment application (n = 60).

Biocult (B)	Phytonematicide (P)			
	P_0	Impact (%)	P_1	Impact (%)
B_0	0.4770	−	0.2683	−44
B_1	02414	−49	0.2478	−48

Impact (%) = [(treatment/control) – 1] x 100.

Table 3.11 Two-way matrix for gall rating as affected by first order interaction of Nemacur and Biocult Mycorrhizae at 56 days after treatment application (n = 60).

Nemacur (N)	Biocult Mycorrhizae (B)			
	B_0	Impact (%)	B_1	Impact (%)
N_0	0.4201	−	0.2147	−52
N_1	0.2953	−34	0.2744	−39

Impact (%) = [(treatment/control) – 1] x 100.

Table 3.12 Two-way matrix for chlorophyll content as affected by first order interaction of Biocult Mycorrhizae and Nemarioc-AL phytonematicide at 56 days after treatment application (n = 60).

Biocult (B)	Phytonematicide (P)			
	P_0	Impact (%)	P_1	Impact (%)
B_0	44.779	−	46.729	−2
B_1	44.705	−6	47.194	−1

Impact (%) = [(treatment/control) – 1] x 100.

Table 3.13 Responses of gall rating and stem diameter of potato to Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide interactions under microplot conditions (n = 62).

Source	DF	Gall rating		Stem diameter	
		MS	%	MS	%
Replication	7	0.04791	10	0.73621	12
Nemacur (N)	1	0.10170	20**	0.56150	9ns
Biocult (B)	1	0.02473	5ns	0.83521	13ns
Phytonematicide (P)	1	0.12238	24**	0.19796	3ns
N × B	1	0.01791	4ns	2.37900	38***
N × P	1	0.05724	11ns	0.34760	6ns
B × P	1	0.06345	13*	0.82149	13ns
N × B × P	1	0.04558	9ns	0.08787	1ns
Error	48	0.02198	4	0.33735	5
Total	62	0.5288	100	06.30418	100

ns Not significant, * slightly significant at P ≤ 0.10, ** Significant at P ≤ 0.05 *** Highly significant at P ≤ 0.01.

Table 3.14 Two-way matrix for gall rating as affected by first order interaction of Biocult Mycorrhizae and Nemafric-BL phytonematicide at 56 days after treatment application (n = 62).

	Phytonematicide (P)			
Biocult (B)	P_0	Impact (%)	P_1	Impact (%)
B_0	0.3777	−	0.2258	−40
B_1	0.2744	−27	0.2497	−33

Impact (%) = [(treatment/control) – 1] x 100.

Table 3.15 Two-way matrix for stem diameter as affected by first order interaction of Nemacur and Biocult Mycorrhizae at 56 days after inoculation (n = 62).

	Biocult (B)			
Nemacur (N)	B_0	Impact (%)	B_1	Impact (%)
N_0	3.7675	−	4.2300	12
N_1	4.3463	15	4.0297	6

Impact (%) = [(treatment/control) – 1] x 100.

3.4 Discussion

3.4.1 Nematode variables

In the Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide trial, the highly significant effects of the second order interaction Nemacur × Biocult Mycorrhizae × Nemarioc-AL phytonematicide, along with the first order interaction of

Nemacur with any other main factor on suppression of *M. javanica* stages confirmed observations by others (Kagai *et al*., 2012) when combining Nemacur with other organic materials on suppression of root-knot nematodes. In their study, Kagai *et al*. (2012) observed that combining leaves of *Lantana camara* with Nemacur reduced root-knot nematodes by 80-83%, whereas *L. camara* alone achieved 73% nematode reduction. However, for the practical application purposes, inclusion of Nemacur in botanicals is no longer feasible since the product had been withdrawn from the agrochemical markets due to its widespread toxicity to non-target organisms.

The significant pairwise treatments involving Biocult Mycorrhizae and Nemarioc-AL phytonematicide were, however, important for the South African potato industry since the two products meet the requirements of locality. In order to enhance successful adoption of materials in managing nematode population densities using botanicals, their availability in local areas is important in terms of affordability, especially for resource poor smallholder farmers (Mashela *et al*., 2015). The fact that the first order interaction, Biocult Mycorrhizae × Nemarioc-AL phytonematicide, was not significant on any of the nematode variables implies that it would serve no purpose in combining the two locally-produced products for managing population densities of *M. javanica* in potato production.

In other interactive experiments of dried *Cucumis myriocarpus* fruit, fever tea (Lippia *javanica*) leaf and castor bean (*Ricinus communis*) fruit in tomato (*Solanum lycopersicon* L.) production, the second order interaction was highly significant on suppression of *M. incognita* (Mashela *et al*., 2007). The pairwise comparisons of the three organic products demonstrated that the highest magnitude on suppression was

achieved when the three products were operating together, which was explained in terms of the various active ingredients in the materials (Mashela *et al*., 2007). In the second order interaction ($N_1B_1P_1$) of the current study, Biocult Mycorrhizae alone had higher effects on suppression of *M. javanica* than Nemarioc-AL phytonematicide did, whereas each performed much better than the combined effects of $N_1B_1P_1$. Although the effects of Biocult Mycorrhizae alone on suppression of nematodes were higher than those of Nemacur, the magnitude for the latter was higher than that of Nemarioc-AL phytonematicide.

In granular formulation (G), Nemarioc-AG phytonematicide was as efficient as Nemacur and Aldicarb synthetic nematicides in nematode suppression (Mashela *et al*., 2008). Ononuju *et al*. (2014) investigated the interaction of nematophagous fungi and furadan synthetic nematicide on suppression of *M. incognita* in okra, and observed that the interaction drastically reduced nematode population densities in the soil.

In the Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide trial, the second order ($N_1B_1P_1$) had the highest impact on J2 in soil than the first order interactions $N_1B_0P_1$, $N_1B_1P_0$ and $N_0B_1P_1$, whereas the effects of Nemacur alone were equivalent to those of $N_0B_1P_1$ on J2 in root. In this trial, therefore, results suggested that it would be better to combine Biocult Mycorrhizae and Nemafric-BL phytonematicide in nematode management in potato production. In most of the Nemarioc-AL and Nemafric-BL phytonematicides, the two phytonematicides had been consistent in nematode suppression, with differences in magnitudes explained on the basis of their active ingredients (Mashela *et al*., 2015). In Nemarioc-AL

phytonematicide the active ingredient, cucurbitacin A ($C_{32}H_{46}O_8$), is soluble in water, whereas in Nemafric-BL phytonematicide, the active ingredient, cucurbitacin B ($C_{32}H_{48}O_8$), is insoluble in water (Mashela *et al*., 2015).

The observation that Biocult Mycorrhizae could reduce population densities of *M. javanica* supported those of others (Sharma *et al*., 1995; Suresh *et al*., 1985). Sharma *et al*. (1995) studied the effect of Vesicular Abuscular Mycorrhiza (VAM) fungus (*Glomus fasciculatum*) on the survival and development of *M. incognita* in tomato cv. “Pusa Ruby” and noted that VAM fungi reduced both reproduction and development of nematodes. Suresh *et al*. (1985) observed that root extracts from mycorrhizal plants colonised by *G. fasciculatum* resulted in 50% mortality of *M. incognita* J2 in tomato roots in four days, whereas penetration of colonised roots by nematode J2 was not prevented.

In Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide trial, the reduction of the population density of *M. javanica* on potato seedlings by the second order interaction ($N_1B_1P_1$) confirmed similar observations when synthetic nematicides, phytonematicides and nematophus fungus had second order interactions on nematode population densities (Brodie and Good, 1973; Chitwood, 2002; Mateille *et al*., 1995). Combining Furadan and *Glomu*s species in the first season of Dolichos bean reduced the number of galls by 93 and 73% after six and nine weeks, respectively (Ahmed *et al*., 2009). Also, Shaukat *et al*. (2010) observed that plant extracts and Fertinemakil plus were effective in reducing population densities of *Hemidesmus indicus, Rotylenchulus reniformis* and *M. incognita*.

Verma and Khan (2004) in a greenhouse study to manage root-knot nematode, *M. incognita* in tulsi (*Ocimum canum* [*O. americanum*]) found that treatments green chopped leaves of neem, datura, eucalyptus, tulsi, parthenium, madar, sadabahar, subabul, mint and clerodendrum, significantly reduced the nematode fecundity and improved plant growth at varying levels. In the same study, a significant reduction in root-knot galls per root system, egg masses per root system and final nematode populationby 72, 74, 74 and 77%, respectively (Verma and Khan, 2004).

3.4.2 Plant variables

In Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide trial, the first order interactions $N_0B_1P_1$ and $N_1B_1P_0$ have had highly significant effects on gall rating, stem diameter and chlorophyll content in potato plants. The interactions decreased galls, chlorophyll content and increased stem diameter on the potato plant. The results confirmed those of Nargis *et al*. (2005), where leaf and stem extracts of marigold had the best effects on most plant growth characters, as well as suppressing the galling incidence. Most phytonematicides have the ability to stimulate and inhibit plant growth under the auspices of density-dependent (DDG) growth patterns as shown with Nemarioc-AL and Nemafric-BL phytonematicides (Mashela *et al*., 2015). The DDG patterns, with three phases, namely, stimulation, neutral and inhibition phases, are concentration- and plant-specific (Mashela *et al*., 2016).

The observation where Biocult Mycorrhizae improved growth of potato in the current study confirmed those of other effective microorganisms. For instance, Hafeez *et al*. (2000) reported that treatments of tomato plants with *Paecilomyces lilacinus* and

Trichoderma harzianum amended with organic substrate resulted in the minimum number of galls per plant. Spiegel and Chet (1998) also reported that *T. harzianum* improved growth and higher yield of *M. javanica* infected plants and decreased the root galling index and the number of eggs per g of root. Goswami *et al*. (2006) observed that root length and weight of tomato plants were significantly increased when plants were treated with *P. lilacinus* and *T. viride* in combination with mustard cake and Furadan, with overall promotion of shoot growth. Nagesh *et al*. (2001) noted that supplementing neem oil-cakes with inorganic fertilisers as nitrogen, phosphorus and potassium, had an additive effect on the mycelial growth and sporulation of *P. lilacinus*, increasing the antagonistic potential of the fungus against population densities of *M. incognita*. In the current study, the $N_0B_1P_1$ interaction reduced chlorophyll content of potato plants, which confirmed the observations where *Bacillus* species and *R. communis* fruit meal interacted to reduce certain variables in tomato plants (Mashela and Nthangeni, 2002).

In Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide trial, the interaction results between Nemacur and Biocult Mycorrhizae on stem diameter confirmed observations by Archana and Prasad (2014), where the use of botanicals increased stem diameter of pigeon pea (*Cajanus cajan*). Also, Reddy *et al*. (1995) noted that neem cake in combination with VA mycorrhiza increased shoot length, shoot mass and root mass in pigeon peas. Sharon *et al.* (2001) demonstrated that reduction of *M. javanica* by *T. harzianum* on pigeon peas resulted in increase of shoot mass. Seed soaking with aqueous extracts of neem and karanj seed kernel at 20% proved to be most effective among various plant products tested in improving plant growth of cowpea and minimising infection of plant-parsitic nematodes (Ram

and Baheti, 2003). The effects of neem cake and *Cymbopogon flexuosus* (leaves), *Cymbopogon winterianus* (leaves), *Spilanthes acmella* [*Blainvillea acmella*] (shoots) and *Costus speciosus* (shoots and rhizomes) dried powder on cowpea plants reduced *M. incognita* population densities and improved plant growth and productivity (Pandey, 2002).

3.5 Conclusion

The results of this study demonstrated that Biocult Mycorrhizae and Nemarioc-AL phytonematicide should not be combined to derive maximum efficacy in the suppression of population densities of *Meloidogyne* species in potato prooduction. In contrast, Biocult Mycorrhizae and Nemafric-BL phytonematicide have to be combined in order to achieve maximum suppression of nematode population densities. The influence of the interactions on growth and productivity of potato cv. ‘Modial G3’ exhibited a tendency towards the DDG patterns, as shown by stimulation on some variables and inhibition on others.

CHAPTER 4
INTEGRATED MANAGEMENT STRATEGIES OF NEMATODES ON POTATO PLANTS: FIELD STUDIES

4.1 Introduction

Nemarioc-AL and Nemafric-BL phytonematicides are potent phytonematicides (Mashela *et al*., 2015), which consistently reduced population densities in various crops (Mafeo and Mashela, 2009a; Pelinganga *et al*., 2011). Recently (Chapter 3), the two products were shown to be effective in suppressing root-knot (*Meloidogyne javanica* Treub.) nematodes under microplot conditions. Similarly, Biocult Mycorrhizae suppressed nematode numbers (Chapter 3). The synthetic nematicide Velum contains an active ingredient, Floupyram, has both nematicidal and fungal properties (Bayer Crop Science, 2015). However, the effects of this synthetic nematicide with other environment-friendly products on suppression of nematode population densities and growth of potato plants had not been documented. The objective of this study was to determine the effects of Nemacur (N), Velum (V), Biocult Mycorrhizae (B), Nemarioc-AL phytonematicide (P) and Nemafric-BL phytonematicide (P) on population densities of the root-knot nematodes and growth of potato seedlings.

4.2 Materials and methods

4.2.1 Description of plant growing conditions

The study was conducted under field conditions at the Green Technologies Research Centre (GTRC), University of Limpopo, South Africa (23°53'10"S, 29°44'15"E). Soil at the location was predominantly Hutton sandy loam (65% sand, 30% clay, 5% silt), with organic C = 1.6%, EC = 0.148 dS/m and pH (H_2O) = 6.5. The experiments were both conducted in summer (October 2015-January 2016). The

location has hot dry summers, with daily maximum temperature from 28 to 38 °C. The average annual rainfall at the site was 500 mm.

4.2.2 Treatments, research design and procedures

The preparation of the phytonematicide was done as described previously (Chapter 3). Seeds of potato cv. 'Mondial G3' were dipped in a mixture of mancozeb and a wettener for disease management prior planting. Thirty centimetre furrows were dug and the seeds of potato placed in the soil with 30 cm inter x 40 cm intra row spacing. Randomised complete block design (RCBD) experiments, with four treatments, namely, (1) untreated control ($N_0B_0P_0$), (2) Nemacur or Velum (3) Biocult Mycorrhizae and (4) Nemarioc-AL or Nemafric-BL phytonematicide were replicated five times for the Nemacur experiment and three times for the Velum experiment. Preparation of inoculum with nematodes was as explained previously (Chapter 3).

4.2.3 Data collection

Fifty-six days after inoculation with nematodes in both experiments, shoots were cut at the soil level, oven-dried for 72 h at 52°C for recording of dry matter. Root systems were removed from soil, immersed in water to remove soil particles, blotted dry and fresh mass measured to facilitate the calculation of nematode density per total root system per plant. Extraction of nematodes and gall rating was done as described previously (Chapter 3). Nematode numbers from roots were used to calculate the reproductive potential (RP) in the roots.

4.2.4 Data analysis

Prior to analysis of variance (ANOVA), nematode and gall rating were separately transformed through $\log_{10}$ (x + 1) to normalise the variances (Gomez and Gomez, 1984). Data were subjected to ANOVA through the SAS software (SAS Institute, 2008) to determine the effects of Nemarioc-AL, Nemafric-BL phytonematicide, Nemacur, Velum and Biocult Mycorrhizae on eggs and juveniles on root, reproductive potential, dry shoot mass, dry root mass, tuber mass and galling were recorded. Mean separation for significant ($P \leq 0.05$) treatments was achieved through the Fisher's least significant difference test. Unless otherwise stated, only treatments that were significant at the probability level of 5% were discussed.

4.3 Results

4.3.1 Comparison of Nemacur with phytonematicides

Nematode variables Nemarioc-AL phytonematicide: The treatment effects were highly significant ($P \leq 0.01$) on population densities of eggs, juveniles in root and total nematode, contributing 53, 68 and 57% in total treatment variation of the nematodes in roots, respectively (Table 4.1). Relative to untreated control, Nemarioc-AL phytonematicide reduced eggs, juveniles in roots and total nematodes by 80, 85 and 81%, respectively (Table 4.2).

Table 4.1 Sources of variation as affecting eggs in root (Eggs), J2 in root (J2), total nematodes in root (Total) and reproductive potential (RP) at 56 days after Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide application (n = 20).

		Eggs		J2		Total		RP	
Source	DF	MS	%	MS	%	MS	%	MS	%
Rep	4	3.9735	45	1.6721	27	3.7793	40	9406.9	33
Trt	3	4.6122	53***	4.2877	68***	5.3684	57***	11048.4	39ns
Error	12	0.1529	2	0.3370	5	0.2238	2	7876.9	28
Total	19	8.7386	100	6.2967	100	9.3715	1	28332.2	100

ns Not significant, *** Highly significant at P ≤ 0.01.

Table 4.2 Responses of eggs in root, J2 in root and total nematodes in root to Nemarioc-AL phytonematicide, Nemacur and Biocult Mycorrhizae on potato cv. 'Mondial G3' in the field conditions (n = 12).

	Eggs in root		J2 in root		Total in root	
Treatment	Variable	(%)	Variable	(%)	Variable	(%)
Control	3.12[a]±0.22	−	2.95[a]±0.55	−	3.39[a]±0.25	−
Nemacur	2.35[a]±0.00	−24	1.34[b]±0.49	−54	2.40[b]±0.49	−29
Biocult	0.71[b]±0.45	−77	0.42[b]±0.28	−85	0.73[c]±0.39	−78
Nemarioc-AL	0.61[b]±0.00	−80	0.42[b]±0.42	−85	0.64[c]±0.42	−81

Impact (%) = [(treatment/control) – 1] x 100.

Nematode variables Nemafric-BL phytonematicide: Treatment effects were not significant (P ≤ 0.05) on population densities *of M. javanica* on potato (Appendix 4.1).

Plant variables Nemarioc-AL phytonematicide: Treatment effects were highly significant (P ≤ 0.01) on gall rating of the potato roots, contributing 92% to total treatment variation (Table 4.3). All other plant variables measured were not significant (P ≤ 0.05). Relative to untreated control, Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide reduced gall rating by 56, 48 and 56%, respectively (Table 4.4).

Table 4.3 Sources of variation for tuber mass (TBM), dry shoot mass (DSM), dry root mass (DRM) and gall rating (GLR) at 56 days after Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematcide application (n= 20).

		TBM		DSM		DRM		GLR	
Source	DF	MS	%	MS	%	MS	%	MS	%
REP	4	7143.4	14	91.6465	57	0.5784	51	0.0048	4
Trt	3	18971.1	37^{ns}	12.3806	8^{ns}	0.3181	28^{ns}	0.1063	92***
Error	12	24920.2	49	57.7345	36	0.2322	21	0.0044	4
Total	19	51034.7	100	161.7616	100	1.1287	100	0.1156	100

^{ns} Not significant, *** Highly significant at P ≤ 0.01.

Table 4.4 Responses of gall rating in root to Nemarioc-AL phytonematicide, Nemacur and Biocult Mycorrhizae on potato cv. 'Mondial G3' in the field conditions (n = 12).

Treatment	Variable	Impact (%)
Control	0.69^{a}±0.57	−
Nemacur	0.30^{b}±0.00	−56
Biocult Mycorrhizae	0.36^{b}±0.33	−48
Nemarioc-AL	0.30^{b}±0.67	−56

Impact (%) = [(treatment/control) – 1] x 100.

Plant variables Nemafric-BL phytonematicide: Treatment effects were significant ($P \leq 0.05$) on gall rating of the potato roots, contributing 92% to total treatment variation (Table 4.5). All other plant variables measured were not significant ($P \leq 0.05$). Relative to untreated control, treatments Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide decreased gall rating by 56, 33 and 42%, respectively (Table 4.6).

Table 4.5 Sources of variation for tuber mass (TBM), dry shoot mass (DSM), dry root mass (DRM) and gall rating (GLR) at 56 days after Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide application (n =20).

		TBM		DSM		DRM		GLR	
Source	DF	MS	%	MS	%	MS	%	MS	%
Rep	4	2608.4	11	14194.7	77	0.51047	32	0.02361	20
Trt	3	10742.1	46^{ns}	2901.1	16^{ns}	0.50477	32^{ns}	0.08297	70**
Error	12	10132.4	43	14.07.1	8	0.58204	36	0.01242	10
Total	19	23482.9	100	18502.9	100	1.59728	100	0.119	100

^{ns} Not significant, ** Significant at P ≤ 0.05.

Table 4.6 Responses of gall rating on root to Nemafric-BL phytonematicide, Nemacur and Biocult Mycorrhizae on potato cv. 'Mondial G3' in the field conditions (n = 12).

Treatment	Variable	Impact (%)
Control	0.69^{a}±0.57	−
Nemacur	0.30^{b}±0.00	−56
Biocult Mycorrhizae	0.46^{b}±0.57	−33
Nemafric-BL	0.40^{b}±0.67	−42

Impact (%) = [(treatment/control) – 1] x 100.

4.3.2 Comparison of Velum with phytonematicides

Nematode variables Nemarioc-AL phytonematicide: The treatment effects were highly significant (P ≤ 0.01) on population densities of eggs in root, contributing 88% in total treatment variation of the eggs in root (Table 4.7). Relative to untreated

control, the treatments reduced population densities of nematodes with Biocult Mycorrhizae having the lowest reduction of 48% and both Velum and Nemarioc-AL having reduced eggs in root by 100%, respectively (Table 4.8).

Table 4.7 Sources of variation as affecting eggs in root, J2 in root, total nematodes in root and reproductive potential (RP) at 56 days after Velum, Biocult Mycorrhizae and Nemarioc-AL phytonematicide application (n = 12).

		Eggs in root		J2 in root		Total in root		RP	
Source	DF	MS	%	MS	%	MS	%	MS	%
Rep	2	0.3140	6	0.5083	29	1.4451	38	1.6241	18
Trt	3	4.7280	88***	0.0988	6ns	0.8332	48ns	5.6061	61ns
Error	6	0.3144	6	1.1717	66	0.5739	15	1.8860	21
Total	11	5.3563	100	1.7788	100	3.8522	100	9.1163	100

ns Not significant, *** Highly significant at P ≤ 0.01.

Table 4.8 Responses of eggs in root to Nemarioc-AL phytonematicide, Velum and Biocult Mycorrhizae on potato cv. 'Mondial G3' in the field conditions (n = 20).

Treatment	Variable	(%)
Control	2.02^{a}±1.15	−
Velum	0.00^{b}±0.01	−100
Biocult	1.05^{c}±0.14	−48
Nemrioc-AL	0.00^{b}±0.06	−100

Impact (%) = [(treatment/control) – 1] x 100.

Nematode variables Nemafric-BL phytonematicide: The treatment effects were highly significant (P ≤ 0.01) on eggs in root and reproductive potential, contributing 88% in total treatment variation respectively and significant (P ≤ 0.05) for total nematodes in root, contributing 77% (Table 4.9). Relative to untreated control, Velum had the highest reduction on eggs in root reducing by 100%, Nemafric-BL phytonematicide had the highest on total nematodes reducing by 84% and Biocult Mycorrhizae had the highest reduction of 71% on reproductive potential (Table 4.10).

Table 4.9 Sources of variation as affecting eggs in root, J2 in root, total nematodes in root (Total) and reproductive potential (RP) at 56 days after Velum, Biocult Mycorrhizae and Nemafric-BL phytonematicide application.

		Eggs in root		J2 in root		Total		RP	
Source	DF	MS	%	MS	%	MS	%	MS	%
Rep	2	0.0991	2	0.2785	9	0.4239	8	0.438	2
Treatment	3	4.7223	88***	1.7281	55ns	4.0705	77**	19.478	88***
Error	6	0.5580	10	1.1199	36	0.7784	15	2.2146	10
Total	11	5.3794	100	3.1265	100	5.2728	100	22.1306	100

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

Table 4.10 Responses of eggs in root, total in root and reproductive potential (RP) to Nemafric-BL phytonematicide, Velum and Biocult Mycorrhizae on potato cv. 'Mondial G3' in the field conditions (n = 20).

	Eggs in root		Total in root		RP	
Treatment	Variable	(%)	Variable	(%)	Variable	(%)
Control	2.30^{a}±43.15	−	2.55^{a}±4.24	−	0.56^{a}±0.21	−
Velum	0.00^{d}±0.01	−100	1.47^{ab}±1.6	−42	0.21^{b}±0.11	−62
Biocult	1.33^{bc}±23.14	−42	1.12^{b}±0.28	−56	0.96^{b}±0.44	−71
Nemafric	0.74^{c}±29.06	−67	0.38^{b}±0.14	−84	0.83^{b}±0.59	−48

Impact (%) = [(treatment/control) – 1] x 100.

Plant variables Nemarioc-AL and Nemafric-BL Phytonematicide: Both treatment effects from Nemarioc-AL and Nemafric-BL phytonematicide had no significance (P ≤ 0.05) on all plant variables measured (Appendix 4.20 and 4.21).

4.4 Discussion

4.4.1 Comparison of Nemacur with phytonematicides

The highly effective effects of Nemarioc-AL phytonematicide on eggs in root, juveniles in root and total nematode numbers confirmed the previously observed consistent results of this product on nematode population densities (Mashela *et al*., 2015). The relatively high impacts of this product on various population densities of nematodes also confirmed observations of this product on nematodes, whereas the magnitudes of reduction were at least 80% (Dube and Mashela, 2016; Mashela *et al*., 2015; Pelinganga, 2013). In all nematode stages, confirming previous observations (Chapter 3), Biocult Mycorrhizae and Nemarioc-AL phytonematicide have had similar effects on eggs in root, J2 in root and total nematodes in root, which were comparable to those of Nemacur. Similarities on gall rating among the three products also confirmed observations that the efficacy of Nemarioc-AG (G = granular formulation) phytonematicide was not different to that of Nemacur and Aldicarb (Mashela *et al*., 2008).

In the current study, the Nemafric-BL phytonematicide treatment was not effective on population densities *of M. javanica* on potato, which confirmed the saturation effects of phytonematicides on nematodes (Dube, 2016). Also, the saturation observations in the current study supported those of McSorley (2011), who dubbed the efficacy of botanicals on nematode resistance to be ‘inconsistent’ since the materials sometimes stimulated (Belair and Tremblay, 1995; Kimpinski *et al*., 2003), had no effect (Jafee *et al*., 1994; McSorley and Gallaher, 1995a; Thoden *et al*., 2011) or inhibited (Mashela *et al*., 2011) nematode population densities. Generally, it had since been shown that increasing concentrations of phytonematicides, which are

allelochemicals, affect nematode population densities through the density-dependent growth (DDG) population densities (Mashela *et al*., 2015). The DDG concept was adapted from the Curve-fitting Allelochemical Response Dosage (CARD) computer-based model (Liu *et al*., 2003). The DDG patterns have three phases, stimulation, neutral and inhibition phases (Liu *et al*., 2003). According to this model, at the stimulation concentration, nematode populations are stimulated as observed in Europe (Belair and Tremblay, 1995; Kimpinski *et al*., 2003), in the saturation phase botanicals would be viewed as having no effect on nematode numbers as observed in Florida, USA (Jafee *et al*., 1994; McSorley and Gallaher, 1995a; Thoden *et al*., 2011), whereas in the inhibition phase the materials would be viewed as having suppressive effects on nematode numbers (Mashela *et al*., 2011). The concept of the DDG patterns had since resolved the issue of “inconsistent results in nematode suppression by organic amendments” (Mashela *et al*., 2015).

The absence of significant effects on plant variables could in the Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide and in Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide could be viewed in terms of saturation as explained earlier in this study. However, in other cases stimulation was observed (Mashela *et al*., 2015), whereas in others saturation was observed (Mashela *et al*., 2015).

4.3.2 Comparison of Velum with phytonematicides

As observed in the study under Nemacur and phytonematicides above, in this study, Velum and Nemarioc-AL phytonematicide had highly significant effects on eggs in root. However, in this study, the efficacy of Biocult Mycorrhizae on nematode egg

suppression was much less than that of Velum and Nemarioc-AL phytonematicide – both of which reduced eggs by 100%. Similarly, the treatments did not have influence on J2 in root, total in root and reproductive potential, all of which could be explained as above. Similar effects were observed in the Velum- Biocult Mycorrhizae-Nemafric-BL phytonematicide trial, except that Velum reduced eggs by 100%, whereas the effects of Biocult Mycorrhizae and Nemafric-BL phytonematicide. Similarly, the treatments did not have any effect on plant variables, which could be explained as above.

4.5 Conclusion

In this study, the tested products had influence on nematode and plant variables as depicted by the DDG patterns. The latter had since put to rest the concept of “inconsistent results” when dealing with phytonematicides. In most cases, the efficacy of Velum on nematode suppression was much better than that of the two sustainable products, whereas the efficacy of the latter was comparable to that of Nemacur, which had been withdrawn from the agrochemical markets.

CHAPTER 5
SUMMARY, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary

The suggestion that nematodes were not a serious soil-borne pest in potato production was shown to be a fallacy in the recent data-based study in South Africa (Marais *et al*., 2015). The several trials conducted The matrix developed for the second order interactions of synthetic nematicides (Nemacur or Velum), Biocult Mycorrhizae and phytonematicide (Nemarioc-AL, Nemafric-BL) on nematode population densities, demonstrated that Bicocult Mycorrhizae and any of the two phytonematicides could be strong candidates for use in sustainable management of the root-knot (*Meloidogyne* species) in potato production (Chapters 3 and 4). The two products, namely, Biocult Mycorrhizae and the listed phytonematicides are being developed and researched in South Africa as alternatives to synthetic chemical nematicides which are internationally being withdrawn from the agrochemical markets (Mashela *et al*., 2015).

5.2 Significance of findings

The current research demonstrated that biocontrol agents such as Biocult Mychorhizzae, Nemarioc-AL and Nemafric-BL phytonematicides have the potential for use as alternatives to synthetic chemical nematicides in potato production. The advantage with the three products was that they are locally developed and could be affordable to poor-resource smallholder farmers who are venturing into potato production.

5.3 Recommendations

Nemarioc-AL and Nemafric-BL phytonematicides could be applied in potato production to manage population densities of root-knot nematodes. However, further studies are required to establish the residue levels of cucurbitacins in potato tubers and the soil residual effects for the predecessor crops. Also, to reduce the soil residual effects, it is important that appropriate crop rotation systems be developed for inclusion in potato production for the management of root-knot nematodes.

5.4 Conclusions

Plant-parasitic nematodes, particularly the root-knot nematodes, are currently a challenge in potato production in South Africa. Nemarioc-AL and Nemafric-BL phytonematicides could be used in combination with the other control agents to manage population densities of *Meloidogyne* species, provided that there are no chemical cucurbitacin residuals in tubers, which could be detrimental to consumer health.

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APPENDICES

Appendix 3.1 Analysis of variance for eggs in root of potato on Nemacur, Biocult Mycorrhizae and Nemarioc-AL under microplot conditions at 56 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Rep	7	0.96503	0.13786		
Nemacur (N)	1	1.54862	1.54862	18.78	0.0001
Biocult Mycorrhizae (B)	1	3.34390	3.34390	40.56	0.0000
Phytonematicide (P)	1	2.53257	2.53257	30.72	0.0000
N × B	1	0.95072	0.95072	11.53	0.0014
N × P	1	1.00004	1.00004	12.13	0.0011
B × P	1	0.12628	0.12628	1.53	0.2221
N × B × P	1	1.41969	1.41969	17.22	0.0001
Error	46	3.79234	0.08244		
Total	60				

Appendix 3.2 Analysis of variance for J2 in root of potato on Nemacur, Biocult Mycorrhizae and Nemarioc-AL under microplot conditions at 56 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Rep	7	0.27574	0.03939		
Nemacur (N)	1	0.29719	0.29719	2.27	0.1387
Biocult Mycorrhizae(B)	1	1.11424	1.11424	8.51	0.0054
Phytonematicide (P)	1	0.40132	0.40132	3.07	0.0866
N × B	1	0.56542	0.56542	4.32	0.0433
N × P	1	0.51504	0.51504	3.94	0.0533
B× P	1	0.00571	0.00571	0.04	0.8354
N × B × P	1	0.32232	0.32232	2.46	0.1234
Error	46	6.02014	0.13087		
Total	60				

Appendix 3.3 Analysis of variance for eggs and J2 in root of potato on Nemacur, Biocult Mycorrhizae and Nemarioc-AL under microplot conditions at 56 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Rep	7	0.64032	0.09147		
Nemacur (N)	1	1.17659	1.17659	18.37	0.0001
Biocult Mycorrhizae (B)	1	272287	2.72287	42.52	0.0000
Phytonematicide (P)	1	1.94083	1.94083	30.31	0.0000
N × B	1	0.98189	0.98189	15.33	0.0003
N × P	1	1.02716	1.02636	16.03	0.0002
B × P	1	0.02716	0.02716	0.42	0.5182
N × B × P	1	1.03927	1.03927	16.23	0.0002
Error	46	2.94589	0.06404		
Total	60				

Appendix 3.4 Analysis of variance for total nematodes of potato on Nemacur, Biocult Mycorrhizae and Nemarioc-AL under microplot conditions at 56 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Rep	7	0.63944	0.09135		
Nemacur (N)	1	1.17890	1.17890	18.44	0.0001
Biocult Mycorrhizae (B)	1	2.71978	2.71978	42.54	0.0000
Phytonematicide (P)	1	1.93985	1.93985	30.34	0.0000
N × B	1	0.97961	0.97961	15.32	0.0003
N × P	1	1.02497	1.02497	16.03	0.0002
B × P	1	0.02683	0.02683	0.42	0.5203
N × B × P	1	1.04090	1.04090	16.28	0.0002
Error	46	2.94076	0.06393		
Total	60				

Appendix 3.5 Analysis of variance for eggs in root of potato on Nemacur, Biocult Mycorrhizae and Nemafric-BL under microplot conditions at 56 days after inoculation (n = 62).

Source	DF	SS	MS	F	P
Rep	7	0.5208	0.07441		
Nemacur (N)	1	0.9346	0.93456	4.31	0.0432
Biocult Mycorrhizae (B)	1	1.9222	1.92221	8.87	0.0045
Phytonematicide (P)	1	0.1056	0.10558	0.49	0.4884
N × B	1	0.0044	0.00443	0.02	0.8869
N × P		2.1493	2.14931	9.92	0.0028
B × P	1	0.1901	0.19011	0.88	0.3535
N × B × P	1	0.3082	0.30824	1.42	0.2388
Error	48	10.3965	0.21659		
Total	62				

Appendix 3.6 Analysis of variance for J2 in root of potato on Nemacur, Biocult Mycorrhizae and Nemafric-BL under microplot conditions at 56 days after inoculation (n = 62).

Source	DF	SS	MS	F	P
Rep	7	2.51700	0.35957		
Nemacur (N)	1	0.28864	0.28864	1.39	0.2440
Biocult Mycorrhizae (B)	1	0.42097	0.42097	2.03	0.1608
Phytonematicide (P)	1	0.59494	0.59494	2.87	0.0968
N × B	1	0.10526	0.10526	0.51	0.4797
N × P	1	1.33109	1.33109	6.42	0.0146
B × P	1	0.15699	0.15699	0.76	0.3887
N × B × P	1	0.79335	0.79335	3.82	0.0564
Error	48	9.95779	0.20745		
Total	62				

Appendix 3.7 Analysis of variance for eggs and J2 in root of potato on Nemacur, Biocult Mycorrhizae and Nemafric-BL under microplot conditions at 56 days after inoculation (n = 62).

Source	DF	SS	MS	F	P
Rep	7	0.65377	0.09340		
Nemacur (N)	1	0.78527	0.78527	3.86	0.0551
Biocult Mycorrhizae (B)	1	1.52429	1.52429	7.50	0.0086
Phytonematicide (P)	1	0.18823	0.18823	0.93	0.3407
N × B	1	0.00046	0.00046	0.00	0.9624
N × P	1	1.94093	1.94093	9.55	0.0033
B × P	1	0.08065	0.08065	0.40	0.5317
N × B × P	1	0.39625	0.39625	1.95	0.1691
Error	48	975619	0.20325		
Total	62				

Appendix 3.8 Analysis of variance for J2 in soil of potato on Nemacur, Biocult Mycorrhizae and Nemafric-BL under microplot conditions at 56 days after inoculation (n = 62).

Source	DF	SS	MS	F	P
Rep	7	0.0304	0.00435		
Nemacur (N)	1	2.0577	2.05773	4.27	0.0442
Biocult Mycorrhizae (B)	1	7.6775	7.67751	15.93	0.0002
Phytonematicide (P)	1	7.6775	7.67751	15.93	0.0002
N × B	1	2.0465	2.04649	4.25	0.0448
N × P	1	2.0465	2.04649	4.25	0.0448
B × P	1	7.6558	7.65577	15.89	0.0002
N × B × P	1	2.0577	2.05773	4.27	0.0442
Error	48	23.1280	0.48183		
Total	62				

Appendix 3.9 Analysis of variance for total nematodes of potato on Nemacur, Biocult Mycorrhizae and Nemafric-BL under microplot conditions at 56 days after inoculation (n = 62).

Source	DF	SS	MS	F	P
Rep	7	0.64793	0.09256		
Nemacur (N)	1	0.51546	0.51546	2.61	0.1129
Biocult Mycorrhizae (B)	1	1.48112	1.48112	7.49	0.0087
Phytonematicide (P)	1	0.17322	0.17322	0.88	0.3539
N × B	1	0.02102	0.02102	0.11	0.7458
N × P	1	1.50431	1.50431	7.61	0.0082
B × P	1	0.07191	0.07191	0.36	0.5493
N × B × P	1	0.21277	0.21277	1.08	0.3047
Error	48	9.48990	0.19771		
Total	62				

Appendix 3.10 Analysis of variance for fresh shoot mass of potato on Nemacur, Biocult Mycorrhizae and Nemarioc-AL under microplot conditions at 56 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Rep	7	10330.8	1475.83		
Nemacur (N)	1	377.4	377.43	0.18	0.6699
Biocult Mycorrhizae (B)	1	20.8	20.81	0.01	0.9202
Phytonematicide (P)	1	31.0	30.96	0.02	0.92027
N × B	1	301.1	301.15	0.15	0.7033
N × P	1	51.6	51.57	0.03	0.8747
B × P	1	1390.4	1390.40	0.68	0.4145
N × B × P	1	310.9	310.89	0.15	0.6988
Error	46	94328.8	2050.63		
Total	60				

Appendix 3.11 Analysis of variance for fresh root mass of potato on Nemacur, Biocult Mycorrhizae and Nemarioc-AL under microplot conditions at 56 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Rep	7	1555.81	222.258		
Nemacur (N)	1	663.50	663.505	8.49	0.0055
Biocult Mycorrhizae (B)	1	0.12	0.118	0.00	0.9691
Phytonematicide (P)	1	212.68	212.682	2.72	0.1059
N × B	1	80.92	80.917	1.04	0.3143
N × P	1	0.04	0.036	0.00	0.9830
B × P	1	60.20	60.202	0.77	0.3847
N × B × P	1	120.18	120.178	1.54	0.2213
Error	46	3595.94	78.173		
Total	60				

Appendix 3.12 Analysis of variance for tuber mass of potato on Nemacur, Biocult Mycorrhizae and Nemarioc-AL under microplot conditions at 56 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Rep	7	81641	11663.0		
Nemacur (N)	1	289	289.2	0.02	0.8993
Biocult Mycorrhizae (B)	1	1670	1670.2	0.09	0.7611
Phytonematicide (P)	1	40	40.0	0.00	0.9625
N × B	1	6844	6844.2	0.038	0.5389
N × P	1	4355	4354.6	0.24	0.6238
B × P	1	11653	11652.8	0.65	0.4233
N × B × P	1	23733	23733.2	1.33	0.2549
Error	46	821395	17856.4		
Total	60				

Appendix 3.13 Analysis of variance for stem diameter of potato on Nemacur, Biocult Mycorrhizae and Nemarioc-AL under microplot conditions at 56 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Rep	7	350.83	50.118		
Nemacur (N)	1	42.95	42.952	0.77	0.3846
Biocult Mycorrhizae (B)	1	112.54	112.541	2.02	0.1621
Phytonematicide (P)	1	50.84	50841	0.91	0.3445
N × B	1	30.84	30.841	0.55	0.4607
N × P	1	70.90	70.904	1.27	0.2652
B × P	1	55.67	55.667	1.00	0.3229
N × B × P	1	60.51	60.514	1.09	0.3029
Error	46	2564.01	55.739		
Total	60				

Appendix 3.14 Analysis of variance for gall rating of potato on Nemacur, Biocult Mycorrhizae and Nemarioc-AL under microplot conditions at 56 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Rep	7	0.23757	0.03394		
Nemacur (N)	1	0.03412	0.03412	1.42	0.2402
Biocult Mycorrhizae (B)	1	0.24726	0.24726	10.26	0.0025
Phytonematicide (P)	1	0.15408	0.15408	6.39	0.0149
N × B	1	0.17334	0.17334	7.19	0.0101
N × P	1	0.02741	0.02741	1.14	0.2917
B × P	1	0.17431	0.17431	7.23	0.0099
N × B × P	1	0.01108	0.01108	0.46	0.5011
Error	46	1.10855	0.02410		
Total	60				

Appendix 3.15 Analysis of variance for chlorophyll content of potato on Nemacur, Biocult Mycorrhizae and Nemarioc-AL under microplot conditions at 56 days after inoculation (n = 60).

Source	DF	SS	MS	F	P
Rep	7	39.095	5.5850		
Nemacur (N)	1	0.049	0.0487	0.00	0.9497
Biocult Mycorrhizae (B)	1	25.640	25.6403	2.12	0.1521
Phytonematicide (P)	1	7.795	7.7952	0.64	0.4261
N × B	1	1.55	1.5249	0.13	0.7241
N × P	1	14.553	14.5533	1.20	0.2783
B × P	1	47.161	47.1609	3.90	0.0543
N × B × P	1	0551	0.5509	0.05	0.8319
Error	46	556.178	120908		
Total	60				

Appendix 3.16 Analysis of variance for fresh shoot mass of potato on Nemacur, Biocult Mycorrhizae and Nemafric-BL under microplot conditions at 56 days after inoculation (n = 62).

Source	DF	SS	MS	F	P
Rep	7	2679.1	3.82.72		
Nemacur (N)	1	1062.2	1062.24	2.07	0.1569
Biocult Mycorrhizae (B)	1	10.5	10.52	0.02	0.8868
Phytonematicide (P)	1	642.2	642.15	1.25	0.2691
N × B	1	204.7	204.68	0.40	0.5309
N × P	1	383.3	383.31	0.75	0.390
B × P	1	3572.5	3572.5	6.95	0.0112
N × B × P	1	240	24.03	0.05	08297
Error	48	24656.9	51368		
Total	62				

Appendix 3.17 Analysis of variance for fresh root mass of potato on Nemacur, Biocult Mycorrhizae and Nemafric-BL under microplot conditions at 56 days after inoculation (n = 62).

Source	DF	SS	MS	F	P
Rep	7	2291.49	327.356		
Nemacur (N)	1	232.09	232.094	1.47	0.2308
Biocult Mycorrhizae (B)	1	36.00	35.998	0.23	0.6348
Phytonematicide (P)	1	168.54	168.545	0.07	0.3061
N × B	1	134.48	134.480	0.85	0.3601
N × P	1	97.23	97.232	0.62	0.4359
B × P	1	82.24	82.240	0.52	0.4735
N × B × P	1	40.95	40.951	0.26	0.6125
Error	48	7561.42	157.530		
Total	62				

Appendix 3.18 Analysis of variance for tuber mass of potato on Nemacur, Biocult Mycorrhizae and Nemafric-BL under microplot conditions at 56 days after inoculation (n = 62).

Source	DF	SS	MS	F	P
Rep	7	32352	4621.7		
Nemacur (N)	1	10722	10722.3	2.11	0.1530
Biocult Mycorrhizae (B)	1	1615	1615.4	0.32	0.5756
Phytonematicide (P)	1	837	837.0	0.16	0.6868
N × B	1	3406	3405.6	0.67	0.4172
N × P	1	7706	7706.0	1.52	0.2243
B × P	1	54709	54709.4	10.76	0.0019
N × B × P	1	433	433.2	0.09	0.7716
Error	48	244078	5085.0		
Total	62				

Appendix 3.19 Analysis of variance for stem diameter of potato on Nemacur, Biocult Mycorrhizae and Nemafric-BL under microplot conditions at 56 days after inoculation (n = 62).

Source	DF	SS	MS	F	P
Rep	7	5.1535	0.73621		
Nemacur (N)	1	0.5615	0.56150	1.66	0.2032
BiocultMycorrhizae (B)	1	0.0835	0.08352	0.25	0.6211
Phytonematicide (P)	1	0.1980	0.19796	0.59	0.4474
N × B	1	2.3790	2.37900	7.05	0.0107
N × P	1	0.3476	0.34760	1.03	0.3152
B × P	1	0.8215	0.82149	2.44	0.1252
N × B × P	1	0.0879	0.08787	0.26	0.6121
Error	48	16.1930	0.33735		
Total	62				

Appendix 3.20 Analysis of variance for gall rating of potato on Nemacur, Biocult Mycorrhizae and Nemafric-BL under microplot conditions at 56 days after inoculation (n = 62).

Source	DF	SS	MS	F	P
Rep	7	0.33539	0.04791		
Nemacur (N)	1	0.10170	0.10170	4.63	0.0365
BiocultMycorrhizae (B)	1	0.02473	0.02473	1.13	0.2941
Phytonematicide (P)	1	0.12238	0.12238	5.57	0.0224
N × B	1	0.01791	0.01791	0.81	0.3712
N × P	1	0.05724	0.05724	2.60	0.1131
B × P	1	0.6345	0.06345	2.89	0.0958
N × B × P	1	0.04558	0.04558	2.07	0.1564
Error	48	1.05502	0.02198		
Total	62				

Appendix 3.21 Analysis of variance for chlorophyll content of potato on Nemacur, Biocult Mycorrhizae and Nemafric-BL under microplot conditions at 56 days after inoculation (n = 62).

Source	DF	SS	MS	F	P
Rep	7	111.35	15.9067		
Nemacur(N)	1	65.06	65.0574	2.93	0.0936
Biocult Mycorrhizae (B)	1	32.94	32.9411	1.48	0.2295
Phytonematicide (P)	1	32.66	32.6576	1.47	0.2315
N × B	1	59.02	59.0164	2.65	0.1098
N × P	1	41.22	41.2167	1.85	0.1797
B × P	1	10.26	10.2572	0.46	0.5003
N × B × P	1	9.89	9.8933	0.44	0.5079
Error	48	1067.32	22.2358		
Total	62				

Appendix 4.1 Sources of variation as affecting eggs in root, J2 in root, total nematodes in roots and reproductive potential (RP) at 56 days after Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide application.

		Eggs in roots		J2 in roots		Total in roots		RP	
Source	DF	MS	%	MS	%	MS	%	MS	%
Rep	4	1.157	32	0.384	12	1.752	39	35.558	17
Trt	3	0.668	18^{ns}	1.681	51^{ns}	1.161	26^{ns}	106.833	50^{ns}
Error	12	1.797	50	1.245	38	1.629	36	69.434	33
Total	19	3.6211	100	3.309	100	4.542	100	2.11.825	100

*** highly significant at P ≤ 0.01, ** significant at P ≤ 0.05, ^{ns} not significant.

Appendix 4.2 Analysis of variance for eggs in root of potato to Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	4	1.2558	0.31396		
TRT	3	14.1840	4.72798	15.04	0.0002
Error	12	3.7724	0.31437		
Total	19	19.2122			

Appendix 4.3 Analysis of variance for J2 in root of potato to Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	4	2.0332	0.50830		
TRT	3	0.2965	0.09884	0.08	0.9673
Error	12	14.0600	1.17167		
Total	19	16.3897			

Appendix 4.4 Analysis of variance for eggs and J2 in root of potato to Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	4	5.7804	1.44511		
TRT	3	5.4996	1.83319	3.19	0.0625
Error	12	6.8862	0.57385		
Total	19	18.1663			

Appendix 4.5. Analysis of variance for reproductive potential of potato to Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	4	6.4965	1.62413		
TRT	3	16.8184	5.60612	2.97	0.0744
Error	12	22.6323	1.88602		
Total	19	45.9471			

Appendix 4.6 Analysis of variance for fresh shoot mass of potato to Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	4	161597	40399.2		
TRT	3	22110	7369.9	0.64	0.6033
Error	12	138041	11503.4		
Total	19	321747			

Appendix 4.7 Analysis of variance for fresh root mass of potato to Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	4	1307.02	326.756		
TRT	3	1999.31	666.435	6.05	0.0094
Error	12	1320.80	110.066		
Total	19	4627.13			

Appendix 4.8 Analysis of variance for tuber mass of potato to Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	4	16534	4133.5		
TRT	3	52014	17338.2	3.41	0.0532
Error	12	61057	5088.1		
Total	19	129606			

Appendix 4.9 Analysis of variance for dry shoot mass of potato to Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	4	1615.97	403.992		
TRT	3	221.10	73.699	0.64	0.6033
Error	12	1380.41	115.034		
Total	19	3217.47			

Appendix 4.10 Analysis of variance for dry root mass of potato to Nemacur, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	4	52.281	13.0702		
TRT	3	79.972	26.6574	6.05	0.0094
Error	12	52.832	4.4027		
Total	19	185.085			

Appendix 4.11 Analysis of variance for eggs in root of potato to Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	4	0.3963	0.09907		
TRT	3	14.1669	4.72231	8.46	0.0027
Error	12	6.6964	0.55803		
Total	19	21.2596			

Appendix 4.12 Analysis of variance for J2 in root of potato to Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	4	1.1140	0.27850		
TRT	3	5.1843	1.72809	1.54	0.2542
Error	12	13.4389	1.11991		
Total	19	19.7372			

Appendix 4.13 Analysis of variance for eggs and J2 in root of potato to Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	4	1.6959	0.42398		
TRT	3	12.2115	4.07050	5.23	0.0154
Error	12	9.3403	0.77836		
Total	19	23.2477			

Appendix 4.14 Analysis of variance for reproductive potential of potato to Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	4	1.7521	0.4380		
TRT	3	58.4341	19.4780	8.80	0.0023
Error	12	26.5751	2.2146		
Total	19	86.7612			

Appendix 4.15 Analysis of variance for fresh shoot mass of potato to Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	4	106821	26705.3		
TRT	3	38921	12973.7	0.58	0.6391
Error	12	268318	22359.9		
Total	19	414060			

Appendix 4.16 Analysis of variance for fresh root mass of potato to Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	4	98638	24659.5		
TRT	3	81524	27174.8	1.01	0.4225
Error	12	323141	26928.4		
Total	19	503303			

Appendix 4.17 Analysis of variance for tuber mass of potato to Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	4	40804.6	10201.2		
TRT	3	10153	3384.3	1.00	0.4282
Error	12	40809.4	3400.8		
Total	19	91767.1			

Appendix 4.18 Analysis of variance for dry shoot mass of potato to Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	4	17091.4	4272.85		
TRT	3	6227.4	2075.78	0.58	0.6391
Error	12	42930.9	3577.58		
Total	19	66249.7			

Appendix 4.19 Analysis of variance for dry root mass of potato to Nemacur, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	4	98638	24659.5		
TRT	3	81524	27174.8	1.01	0.4225
Error	12	323141	26928.4		
Total	19	503303			

Appendix 4.20 Sources of variation for dry shoot mass (DSM), dry root mass (DRM) and tuber mass (TBM) at 56 days after Velum, Biocult Mycorrhizae and Nemarioc-AL phytonematicide application.

		DSM		DRM		TBM	
Source	DF	MS	%	MS	%	MS	%
Rep	2	403.992	68	13.0702	30	4133.5	16
Treatment	3	73.699	12^{ns}	26.6574	60^{ns}	17338.2	65^{ns}
Error	6	115.034	19	4.4027	10	5088.1	19
Total	11	592.725	100	44.1303	100	26559.8	100

^{ns} Not significant, *** Highly significant at P ≤ 0.01.

Appendix 4.21 Sources of variation for dry shoot mass (DSM), dry root mass (DRM) and tuber mass (TBM) at 56 days after Velum, Biocult Mycorrhizae and Nemafric-BL phytonematicide application.

		DSM		DRM		TBM	
Source	DF	MS	%	MS	%	MS	%
Rep	2	4272.85	43	986.38	31	10201.2	60
Treatment	3	2075.78	21^{ns}	1086.99	35^{ns}	3384.3	20^{ns}
Error	6	3577.58	36	1077.14	34	3400.8	20
Total	11	9926.21	100	3150.51	100	16986.3	100

^{ns} Not significant, *** Highly significant at P ≤ 0.01.

Appendix 4.22 Analysis of variance for eggs in root of potato to Velum, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	7.9470	3.97350		
TRT	3	13.8367	4.61223	30.17	0.0005
Error	6	0.9172	0.15286		
Total	11	22.7008			

Appendix 4.23 Analysis of variance for J2 in root of potato to Velum, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	3.3441	1.67205		
TRT	3	12.8630	4.28767	12.72	0.0052
Error	6	2.0217	0.33695		
Total	11	18.2288			

Appendix 4.24 Analysis of variance for eggs and J2 in root of potato to Velum, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	7.5586	3.77930		
TRT	3	16.1053	5.36842	23.99	0.0010
Error	6	1.3426	0.22377		
Total	11	25.0065			

Appendix 4.25 Analysis of variance for reproductive potential of potato to Velum, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	18813.7	9406.9		
TRT	3	33145.3	11048.4	1.40	0.3305
Error	6	47260.2	78767		
Total	11	99219.3			

Appendix 4.26 Analysis of variance for fresh shoot mass of potato to Velum, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	18329.3	9164.65		
TRT	3	3714.2	1238.06	0.21	0.8830
Error	6	34640.7	5773.45		
Total	11	56684.2			

Appendix 4.27 Analysis of variance for fresh root mass of potato to Velum, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	321.327	160.663		
TRT	3	265.069	88.356	1.37	0.0072
Error	6	387.073	64.512		
Total	11	973.469			

Appendix 4.28 Analysis of variance for tuber mass of potato to Velum, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	14287	7143.4		
TRT	3	56913	18971.1	0.76	0.5556
Error	6	149521	24920.2		
Total	11	220721			

Appendix 4.29 Analysis of variance for dry shoot mass of potato to Velum, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	183.293	91.6465		
TRT	3	37.142	12.3806	0.21	0.8830
Error	6	346.407	57.7345		
Total	11	566.842			

Appendix 4.30 Analysis of variance for dry root mass of potato to Velum, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	1.15678	0.57839		
TRT	3	0.95425	0.31808	1.37	0.3390
Error	6	139346	0.23224		
Total	11	3.50449			

Appendix 4.31 Analysis of variance for gall rating of potato to Velum, Biocult Mycorrhizae and Nemarioc-AL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	0.00958	0.00479		
TRT	3	0.31903	0.10634	23.94	0.0010
Error	6	0.02665	0.00444		
Total	11	0.35526			

Appendix 4.32 Analysis of variance for eggs in root of potato to Velum, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	2.3146	1.15729		
TRT	3	2.0015	0.66718	0.37	0.7770
Error	6	10.7798	1.79663		
Total	11	15.0959			

Appendix 4.33 Analysis of variance for J2 in root of potato to Velum, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	0.7673	0.38367		
TRT	3	5.0425	1.68085	1.35	0.3442
Error	6	7.4724	1.24541		
Total	11	13.2823			

Appendix 4.34 Analysis of variance for eggs and J2 in root of potato to Velum, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	3.5042	1.75211		
TRT	3	3.4836	1.16121	0.71	0.5791
Error	6	9.7740	1.62900		
Total	11	16.7618			

Appendix 4.35 Analysis of variance for reproductive potential of potato to Velum, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	71.117	35.558		
TRT	3	320.499	106.833	1.54	0.2985
Error	6	416.605	69.434		
Total	11	808.221			

Appendix 4.36 Analysis of variance for fresh shoot mass of potato to Velum, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	16798.4	8399.22		
TRT	3	5149.9	1716.65	2.06	0.2068
Error	6	4995.7	832.62		
Total	11	26944.1			

Appendix 4.37 Analysis of variance for fresh root mass of potato to Velum, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	102.095	51.0475		
TRT	3	151.430	50.4767	0.87	0.5079
Error	6	349.225	58.2042		
Total	11	602.750			

Appendix 4.38 Analysis of variance for tuber mass of potato to Velum, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	5216.7	2608.4		
TRT	3	32226.4	10742.1	1.06	0.4329
Error	6	60794.1	10132.4		
Total	11	98237.3			

Appendix 4.39 Analysis of variance for dry shoot mass of potato to Velum, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	28389.3	14194.7		
TRT	3	8703.4	2901.1	2.06	0.2068
Error	6	8442.8	1407.1		
Total	11	45535.5			

Appendix 4.40 Analysis of variance for dry root mass of potato to Velum, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	1.02095	0.51047		
TRT	3	1.51430	0.50477	0.87	0.5079
Error	6	3.49225	0.58204		
Total	11	6.02750			

Appendix 4.41 Analysis of variance for gall rating of potato to Velum, Biocult Mycorrhizae and Nemafric-BL phytonematicide, under field conditions at 56 days after initiation of treatments and inoculation.

Source	DF	SS	MS	F	P
Rep	2	0.00958	0.00479		
TRT	3	0.31903	0.10634	23.94	0.0010
Error	6	0.02665	0.00444		
Total	11	0.35526			