HOST-STATUS AND HOST-SENSITIVITY OF SWEET POTATO CULTIVAR 'BLESBOK' TO *MELOIDOGYNE JAVANICA* AND RELATED MANAGEMENT STRATEGIES OF *MELOIDOGYNE INCOGNITA*

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DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE MASTER OF AGRICULTURAL MANAGEMENT IN PLANT PRODUCTION, IN THE FACULTY OF SCIENCE AND AGRICULTURE, AT THE UNIVERSITY OF LIMPOPO, SOUTH AFRICA.

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2020

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DECLARATION

I, Ndemedzo Vincent Makhado declare that the dissertation hereby submitted to the University of Limpopo, for the degree Master of Agricultural Management in 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.

Candidate: Ndemedzo Vincent Makhado _____ Signature _____ Date

DEDICATION

I dedicate this dissertation to my grandmother, Mrs Sarah Mukondeleli, great-grandmother Mrs Sophia Mukondeleli and my family.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank the Almighty God for giving me the opportunity, strength and encouragement, especially during all the challenging moments, thereby enabling me to complete this project. I could never have done this without the faith I have in Him. Words alone are not enough to express my heartfelt gratitude towards my supervisory team, Dr K.M. Pofu and Prof P.W. Mashela, for introducing me to the field of research, scientific writing and providing me with much needed professional guidance on various aspects of this research, along with financial assistance for all the consumables and analytical work. It was indeed a privilege and nobility to have been trained by these legends, and I want to say "*Gratias tibi*" for your effort in demonstrating that hard work pays and close guidance, all which enabled me to reach this milestone. My sincere and pensive thanks to my beloved parents, Mr N.P. Makhado and Mrs N.F. Makhado for their unconditional love, unwavering support and continuous encouragement throughout my years at tertiary institutions and throughout the entire process of research and writing this dissertation. This accomplishment would not have been possible without your love. You have always envisaged my success and sacrificed yourselves to raise me to be a better man. Dad and Mom, I hope by this small token of success, I will make you proud. I would like to express my appreciation to my loving grannies Mrs Sarah and Sophia Mukondeleli, and younger sister, Fhumulani Vidah Makhado, for your endless sacrifices, support and prayers. Special thanks to the whole team of the Green Biotechnologies Research Centre of Excellence for the indispensable technical assistance. I will forever be indebted to the Agricultural Research Council through Dr Pofu and the National Research Foundation for financial support during my Master's degree programme.

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ABSTRACT

Root-knot (*Meloidogyne* species) nematodes are host to most plant species, with the success of most crops being dependent upon proper nematode management tactics. Sweet potato (*Ipomoea batatas* L.) is highly susceptible to root-knot nematodes, with physical damage being visible on roots. The withdrawal of highly effective fumigant synthetic nematicides from the agrochemical markets resulted in a need to investigate alternative strategies for managing high nematode population densities, with the use of nematode resistance being the most preferred strategy. The objectives of this study were (1) to establish whether sweet potato cv. 'Blesbok' would be resistant to *M. javanica* under greenhouse conditions, (2) to investigate whether cucurbitacin-containing phytonematicides would be comparable to Velum synthetic nematicide in suppressing *Meloidogyne* species. For Objective 1, treatments comprised 0, 5, 25, 125, 625, 3125 and 15625 eggs and second-stage juveniles (J2), had six replications and validated in time. Uniform sweet potato cuttings were transplanted in 20-cm-diameter plastic pots, filled with steam pasteurised (300°C for 1 hour) loam soil. At 56 days after inoculation, plant growth, plant nutrient and nematode variables were assessed using analysis of variance and subjected to lines of the best fit. Treatments had significant ($P \leq 0.05$) effects on eggs and highly significant ($P \leq 0.01$) effects on J2, final nematode population densities (Pf) and the reproductive factor (RF), contributing 39, 45, 42 and 92% in total treatment variation (TTV) of the respective variables. Treatments did not have significant effects on plant variables. Calcium, K, Mg and Fe versus *M. javanica* levels each exhibited negative quadratic relations, with the models being explained by associations from 59 to 96%. In contrast, Zn versus *M.*

javanica levels exhibited positive quadratic relation, with the model being explained by 80 and 98% association and optimised at 125 *M. javanica* units. For Objective 2, four treatments, namely, untreated control, Nemarioc-AL phytonematicide, Nemafric-BL phytonematicide and Velum had 10 replications and also validated in time. The plantlets with well-developed root system were transplanted under field conditions. Data for Object 2 did not comply with the requirements for ANOVA and were therefore subjected to Principal Component Analysis (PCA). Nemafric-BL phytonematicide treatment in both experiments reduced eggs, J2 in roots and J2 in soil and RP of *Meloidogyne* species, with the results being comparable to those of Velum synthetic nematicide. Nemarioc-AL phytonematicide reduced J2 in roots and in soil of *Meloidogyne* species, without affecting eggs in roots and RP. Nemafric-BL phytonematicide and Velum each increased plant growth variables in Experiment 1 and Experiment 2, whereas Nemarioc-AL phytonematicide did not have significant effects on plant growth variables. Velum chemical nematicide stimulated the accumulation of most essential nutrient elements in leaf tissues of the test cultivar, followed by Nemafric-BL phytonematicide, whereas Nemarioc-AL phytonematicide had no significant effects on the accumulation of essential nutrient elements. The study had two major outcomes, namely, (1) that the efficacy of Nemafric-BL phytonematicide was comparable to that of Velum chemical nematicide in suppression of population densities of *Meloidogyne* species in cv. 'Blesbok' under field conditions and (2) that cv. 'Blesbok' was tolerant to *M. javanica* and therefore, it was not necessary to investigate the mechanisms of nematode resistance.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Sweet potato (*Ipomoea batatas* L.) is one of the high yielding crops with high nutritional benefits than most other staple starchy foods (DAFF, 2011; Nungo *et al*., 2007), with added benefits accrued from biofortification (Bouis *et al*., 2011). Sweet potato is a widely cultivated crop among the smallholder farmers and is one of the most important crops for sustainable food security. Currently in South Africa, Limpopo (Burgersfort, Hoedspruit, Marble hall and Levumbu), Mpumalanga (Nelspruit), Kwazulu-Natal and Western Cape provinces are the major production areas of sweet potato (DAFF, 2011). Most sweet potatoes produced in South Africa are destined to domestic markets (DAFF, 2015). However, the crop has a wide range of pests, with the root-knot (*Meloidogyne* species) nematodes being one of the most important economic pests. Globally, the genus *Meloidogyne* is widely distributed and has over 98 species, with *M. arenarea, M. javanica, M. incognita, M. entorolobii* and *M. hapla* being among the major species (Elling, 2013; Onkendi and Moleleki, 2013). Worse, within certain *Meloidogyne* species there are biological races, which had been identified using differential host plants and molecular approaches. Notwithstanding, most farmers, especially in developing countries, are not aware of this notorious soil-borne pest due to its microscopic sizes. Yield of sweet potato was reduced from 10 to 48% in the Philippines (Gapasin, 1981) and by approximately 6% in South Africa (Kleynhans, 1991).

1.1.1 Description of the research problem

Following the withdrawal of synthetic fumigant nematicides from the agrochemical markets, particularly methyl bromide in 2005 (Mashela *et al*., 2015), alternative commercial synthetic chemical nematicides had been costly and inaccessible to most average farmers in South Africa. In contrast, nematode population densities had been increasing and more aggressive due to shorter life cycles associated with climate change (Okulewicz, 2017). Generally, in South Africa, *M. javanica, M. incognita* races 2 and 4 and *M. enterolobii* occur in various crop- producing regions as thermophilic *Meloidogyne* species (Kleynhans *et al.,* 1996; Rashidifard *et al*., 2018). Thermophilic *Meloidogyne* species have short life cycle, with that of *M. enterolobii*, one of the most aggressive *Meloidogyne* species, being approximately 15 days (Jaiswal and Singh, 2010).

At least 2 500 smallholder farmers in South Africa are reliant on sweet potato for income (Laurie *et al.,* 2017), whereas at least 2 000 ha land in South Africa are under sweet potato production (Domola, 2003; Laurie, 2004). All sweet potato-producing regions in South Africa have high population densities of *Meloidogyne* species (Pofu *et al*., 2017). *Meloidogyne* species had been shown to be capable of reducing plant growth in sensitive crops by as high as 50% to complete crop failure (MacQuin, 2014; Pofu *et al*., 2012).

Due to the withdrawal of chemical fumigant nematicides from the agrochemical markets (Mashela *et al.,* 2011), various alternatives, including nematode resistance, are being investigated and introduced for managing nematode population densities (Mashela *et al*., 2017). However, in order to manage *Meloidogyne* species

successfully through the use of nematode resistant genotypes, it is important that the resistant materials be identified. In South Africa, not until recently (Pofu *et al*., 2017), the host-status of commercially used sweet potato cultivars to nematodes was not known, although much breeding work for quality and yield was being done under the Sweet Potato Biofortification Programme of the Agricultural Research Council (ARC) (Laurie *et al*., 2015). After the empirical-demonstration that cultivars such as 'Bosbok', 'Mvuvhelo', 'Ribbok' and 'W-119' were non-host to local *Meloidogyne* species (Pofu *et al*., 2017), the ARC sweet potato breeders became interested in including nematode resistance in the breeding programme.

1.1.2 Impact of research problem

Damage due to nematodes on sweet potatoes are conspicuous, with symptoms including severe, very deep longitudinal cracks and huge visible galls (Bridge, 1978), which affect the marketability of the produce, thereby resulting in reduced profits. In addition to economic losses from the markets, damaged roots store poorly (Bridge, 1978). Globally, yield losses due to *Meloidogyne* species in sweet potato had been estimated at 6, 15, 24 and 6% for South Africa, South America, West Africa and Southeast Asia, respectively (Kleynhans, 1991). The increase in global temperature due to climate change is expected to affect nematode population densities by reducing their life cycles and altering host plant physiology (Ibrahim *et al.,* 2019). In certain crops such as tomato plants, it had been shown that nematode resistance is broken as temperatures increase (Dropkin, 1969; Eddaoudi *et al*., 1997; Melakeberham, 1998; Silva *et al*., 2019; Williamson, 1998). Consequently, it is important to continue assessing various plant genotypes for nematode resistance and where these are not available, attempt should be made to introgress genes from resistant cultivars into high

yielding nematode-susceptible sweet potato cultivars through plant breeding technologies (Mashela *et al*., 2016).

1.1.3 Possible causes of the research problem

Existing trends suggest that nematode population densities, along with crop losses due to damage by plant-parasitic nematodes, are increasing. Yield losses due to nematode damaged increased to US$157 billion (Elling, 2013; Onkendi *et al*., 2014) and relative to US$126 billion (Chitwood, 2003) before the withdrawal of highly effective fumigant nematicides. During the cited timeframe, the relative increase in yield losses was 37% (Mashela *et al*., 2016). Generally, prior to the withdrawal of fumigant nematicides, which were easily accessible and cheap, most farmers did not appreciate other alternative strategies in the management of plant-parasitic nematodes. Ever since the withdrawal of fumigant nematicides, alternative synthetic nematicides, due to high demands, are expensive and therefore, highly inaccessible to the smallholder farmers and for large commercial agronomic crops such as sweet potato plants (Pofu *et al.,* 2017). Additionally, the emergence of certain *Meloidogyne* species, such as *M. enterolobii*, which is believed to be one of the most aggressive *Meloidogyne* species (Coyne *et al*., 2018; Onkendi and Moleleki, 2013), could also be viewed as one of the causes of the existing challenges, that is, lack of empirically-based alternatives to synthetic fumigant nematicides. Although there had been attempts to breed for pathogens such as bacterial and fungal diseases in sweet potato (Liu, 2017; Sseruwu, 2012), nematodes were not included at the ARC Sweet Potato Biofortification Programme, until it was shown that there were local sweet potato cultivars with the potential of having resistance to *Meloidogyne* species (Pofu *et al.,* 2017).

1.1.4. Possible solutions of research problem

Identification of nematode-resistant genotypes involves screening of multiple genotypes in close liaison with plant breeders in sweet potato production. Once non-host status is empirically-established, cultivars with non-host status should be subjected to nematode-resistance trials. In the event the test cultivar is not resistant to the test nematodes, should it be of high economic potential in terms of other breeding attributes, alternative management strategies, such as the use of synthetic chemical nematicides and cucurbitacin-containing phytonematicides, should be investigated.

1.1.5. General focus of the study

The study focused on the host-status and host-sensitivity of sweet potato cv. 'Blesbok' to *M. javanica,* which was previously shown to be a host to *M. incognita* races 2 and 4, but non-host to *M. javanica* (Pofu *et al*., 2017). Additionally, the cucurbitacin-containing phytonematicides were investigated as an alternative management strategy against *M. incognita* race 2.

1.2 Problem statement

Cream-fleshed sweet potato cv. 'Blesbok' has high yield, good storage life and high fibre content (Leighton, 2008). Root tuber shape of sweet potato cv. 'Blesbok' is long and curved. In a host suitability study of 12 sweet potato cultivars in South Africa, it was shown that cv. 'Blesbok' was a non-host to *M. javanica,* but a host to *M. incognita* race 2 and *M. incognita* race 4 (Pofu e*t al*., 2017). Following the observation, it was then incumbent that the cultivar be subjected to nematode resistance trial, namely, host-status and host-sensitivity, to *M. javanica*. Also, should it be resistant, a further

study would be necessary to establish whether the resistance was pre- or post-infectional nematode resistance (Kaplan and Davis, 1987). Only post-infectional nematode resistance can be introgressed in plant breeding (Kaplan and Davis, 1987; Thurau *et al*., 2010). Additionally, since it was shown that the cultivar was a host to *M. incognita* races 2 and 4, the nematode-cultivar dynamics would be investigated under cucurbitacin-containing phytonematicide as an alternative management strategy.

1.3 Rationale of the study

Sweet potato is highly susceptible to *Meloidogyne* species, with serious damage being caused on root tubers (Suzuki *et al*., 2012). Due to banishment of the use of harmful nematicides that aided in the increase of global warming, detrimental to human health and non-target organisms, new alternatives had to be developed to mitigate the reduction in the yields due to root-knot nematodes (Phan *et al*., 2018). However, few chemical nematicide such as Velum are still available in the market. Velum is a new nematicide with fungicidal activity and it is widely used to control wide range of nematodes (Bayer Crop Science, 2015). Generally, nematode resistant offers environment-friendly solutions and is compatible with other interventions such as biological agents, phytonematicides and crop rotations (Starr *et al.*, 2002). The first step towards using nematode resistance in plant breeding is to assess the host suitability of many cultivars with economic attributes or which have already shown some resistance to other pathogens such as bacteria and fungi. During this first step, due to differences in root sizes of the different cultivars, the reproductive potential (RP = Pf/g fresh root) would be used to assess the host suitability of the cultivars, where Pf is eggs in roots + second-stage juveniles (J2) in roots (Pofu *et al*., 2017). Once cultivars with non-host status had been identified, a single cultivar should be subjected

to a series of inoculation level, with the assessment being done using the reproductive factor (Pf/Pi), where Pf is eggs in roots + J2 in roots + J2 in soil, and Pi is the inoculation level, which in most cases comprises eggs + J2. The final assessment would be done using the concepts of susceptible or tolerant or resistant host (Seinhorst, 1965). Since in the previous study (Pofu *et al*., 2017), sweet potato cv. 'Blesbok' was shown to be non-host to *M. javanica*, there was no need to conduct screening and the cultivar was then subjected to host-status and host-sensitivity trial. As stated earlier, should the cultivar be a resistant host to *M. javanica*, it would then be necessary to establish whether resistance was pre-infectional or post-infectional, to provide information on whether the nematode resistant genes could be introgressed or not. However, for developing alternative nematode management strategies using cucurbitacin-containing phytonematicides, the first step would be to develop the mean concentration stimulation point (MCSP) (amount to be applied) using from the Curve-fitting Allelochemical Response Data (CARD) algorithm model (Liu *et al*., 2003; Mashela *et al*., 2017), then followed by application interval which focuses mainly on breaking the life cycle of test nematode (weeks-per-month-of-30 days) (Mashela *et al*., 2017). In the previous studies, MCSP on sweet potato production was developed at 3% for Nemafric-BL and Nemarioc-AL phytonematicide with the application interval of 19 days under greenhouse conditions (Sebothoma, 2019; Selomo, 2019), and there was a need to test them under field conditions.

1.4 Purpose of the study

1.4.1 Aim

Assessment of the degree of nematode resistance, mechanism of resistance and related nematode management strategies in sweet potato cv. 'Blesbok' to *Meloidogyne* species.

1.4.2 Objectives

1. To establish whether sweet potato cv. 'Blesbok' would be resistant to *M. javanica* under greenhouse conditions.
2. To determine whether resistance in cv. ‘Blesbok’ to *M. javanica* would constitute post-infectional nematode resistance mechanism.
3. To investigate whether cucurbitacin-containing phytonematicides would be comparable to Velum chemical nematicide in managing *Meloidogyne* species.

1.4.3 Null hypotheses

1. Sweet potato cv. 'Blesbok' would be resistant to *M. javanica* under greenhouse conditions.
2. Resistance in cv. 'Blesbok' to *M. javanica* would not constitute post-infectional nematode resistance mechanism.
3. Cucurbitacin-containing phytonematicides would not be comparable to Velum chemical nematicide in management of *Meloidogyne* species.

1.5 Reliability, validity and objectivity

The reliability of data was based on statistical analysis of data on the probability level of 5%, experiments were repeated in time to attain validity and the findings were discussed on the basis of empirical evidence to attain objectivity in order to obliterate all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

In this study bias was lessen through reducing experimental error by increasing the number of replications. Each treatment was also randomised inside the selected experimental design (Leedy and Ormrod, 2005).

1.7 Scientific significance of the study

In the main, should the findings suggest that the formulated Null hypotheses be rejected, the test cultivar would then be viewed as being resistant to the test *Meloidogyne* species, with further investigations showing whether the cultivars had pre- or post-infectional nematode resistance mechanism. Should the cultivar have post-infectional nematode resistance, this would be significant since only this type of nematode resistance could be introgressed during plant breeding. However, should the Null hypothesis for nematode resistance be accepted, mechanisms of nematode resistance would not be investigated. Further, in the final Null hypothesis, the findings would be important in that they would clarify the question as to whether the test phytonematicides were comparable or not to one of the available synthetic chemical nematicide, Velum, which is inaccessible to most farmers, especially the small-scale farmers, due to its high cost. Sweet potato cultivar 'Blesbok' is the major commercial cultivar grown in South Africa.

1.8 Structure of the dissertation

Following the description and detailed outlining of 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, Chapter 4) addressed each of the two objectives, sequentially. In the last chapter (Chapter 5), findings in all chapters were summarized and integrated to provide the significance of the findings and recommendations with the respect to the future research and conclusion. Literature citations and referencing followed the Harvard style using the author-alphabet. In the next chapter, a detailed literature review on the research problem, with specific focus on the work done and not done, would be done.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Root-knot (*Meloidogyne* species) nematodes are widely spread in the tropical and temperate regions around the globe (Pofu *et al*., 2010; Troung *et al.,* 2015). This nematode genus causes serious challenges in many staple food crops. Root-knot nematodes are the most damaging nematode species, with the success of most crops in tropical and subtropical regions being dependent upon proper nematode management tactics (Fourie *et al*., 2015; Sikora and Fernandez, 2005). The *Meloidogyne* species is very prevalent in conditions that are suitable for the growth of the sweet potato crop which are tropical and subtropical regions. Troung *et al.* (2015) postulated that climate change speed up reproduction of nematode by increasing temperature and increase into high populations posing severe damage to sweet potato crop. Management of root-knot nematodes was never regarded as a challenge until the banishment of effective synthetic chemical nematicides from the agro-chemical markets (Mashela *et al.,* 2015). According to Mafeo and Mashela (2009a); Mashela (2002) the prominence on crop improvement strategies has gradually been moving from chemical to non-chemical approaches for sustainable agriculture. Following the banishment of synthetic chemical nematicides there is a need to develop nematode management strategies using tested alternative strategies in crops (Pofu *et al.,* 2017) which would be essential in the future development of appropriate management strategies of *Meloidogyne* species in sweet potato production.

2.2 Work done on problem statement

2.2.1 Conventional concepts

Nematode-plant relations could be established using three different empirically-based trials, namely, host-suitability, host-status and host-sensitivity and mechanism of nematode resistance (Mashela *et al.,* 2015; Seinhorst, 1967). Each is unique and serves a specific purpose. In host suitability, commonly referred to as screening and is conducted to see if the cultivars do allow nematode to reproduce or not. Many cultivars are screened at a go using one level of nematode infection and reproductive potential (RP) is used as an assessment tool. The RP is generated through dividing final population (Pf) by fresh root mass (g) (Pofu *et al*., 2017). The RP helps to compare the nematodes since the cultivars cannot be compared due to their genetically differences. This information is essential since it provides knowledge about the relationship between nematode species and cultivar and it can only be acquired through screening process. However, screening does not provide information on the degree of nematode resistance which emanates from host-status and host-sensitivity concepts (Mashela *et al*., 2015; Seinhorst, 1967).

Seinhorst (1965) conceptualised nematode-plant relations for the description and determination of susceptible, tolerant and resistant hosts. Actually, the Seinhorst (1965) model should be used after establishing the host suitability, using individually only those cultivars that were non-host during screening. Nematode resistance is determined by subjecting cultivars to known nematode species for host-status and host-sensitivity tests. In nematode resistance trials, a series of nematode levels are used which include measure of nematode reproductive factor (RF) and plant damage due to nematode infection (Pofu *et al*., 2017). Reproductive factor (RF) is used as an assessment tool in nematode resistance trials, which emanates from the proportion of

final nematode population density (Pf) to initial nematode population density (Pi) to measure nematodes in a given host (Seinhorst, 1967; Windham and Williams, 1988).

In order to interpret RF values without confounding, it requires a good understanding of the equilibrium (E) point on reproduction of nematodes. Seinhorst (1967) stated that at E, final population is equal to initial population (Pf = Pi) can be below or above unity, whereas beyond E point, RF values would always be below one due to competition for infection sites and food resources. In contrary at E point, nematode population are at the lowest competition and if the plant is susceptible, RF values is consistently greater than one (Pofu, 2012). Generally, when RF values are below one, it implies that the nematode failed to reproduce on the given host, whereas values beyond one indicate that the nematode was able to reproduce on the given host. According to Seinhorst (1967) when the RF is greater than one and nematode reduces plant growth, the plant is said to be susceptible, when RF is greater than one and nematode does not reduce plant growth, the plant is said to be tolerant and when RF is less than one and nematode infection does not reduce plant growth, the plant is said to be resistant.

Mechanism of nematode resistance is another important concept in nematode-plant relations. Plants which are resistant to root-knot nematodes may exhibit resistance against plant-parasitic nematodes which can be either pre- or post-infectional nematode resistance (Pofu and Mashela, 2011). Understanding the mechanism of nematode resistance improve the uses of the plant species in introgression since only post-inflectional nematode resistance can be introgressed. The body of nematode is protected with sensory organs which are used to sense chemicals in lesser quantities which guide the direction nematode should move. Chemo-attractants and chemo-

repellents attract and repel nematode, respectively (Wuyts *et al.,* 2006; Zhao *et al.,* 2000). Mechanisms of nematode resistance in nematode transgenic plants suggested that at a molecular level, plants used three different strategies to prevent nematode infection (Mashela *et al*. 2016).

2.2.2 *Meloidogyne* species in sweet potato

Sweet potato root-knot nematode host-status is dependent on sweet potato genotypes and *Meloidogyne* populations (Cervantes-Flores and Yencho, 2002). Mashela *et al*. (2016) indicated that nematode-plant relations are important in successful co-existence. However, the genetic complexity of sweet potato complicates the plant-pathogen interactions (Cervantes-Flores and Yencho, 2002). The degree of nematode resistance is described in using two concepts, namely, host-status and host-sensitivity (Seinhorst, 1967). According to Seinhorst (1967) the degree of nematode resistance is indicated in one of the three ways, namely, susceptible, tolerant and resistant, which had been widely used in nematode-plant relations (Mashela *et al.*, 2017).

Susceptible host is defined as plant that allows nematode penetration and reproduction, and suffer the damage in terms of growth and yield (Trudgill, 1992). Growing sweet potato cultivars which are susceptible to *Meloidogyne* species could result in low yield, since nematodes reproduction is very high during infection stages due to the availability of penetration sites (Karuri *et al*., 2017). Susceptible sweet potato cultivars affected by nematodes suffer severe yield losses of about 10.2%; decrease the quality of storage roots by triggering the formation of cracks and reduced marketability of storage roots (Lawrence *et al.,* 1986; Nicol *et al*., 2011; Overstreet *et al*., 2009; Suzuki *et al*., 2012). According to Barker *et al.* (1994), economic losses due

to nematodes in sweet potato could rise if there are no effective management strategies. Nematode infection could also increase the damage from secondary pathogens such as fungi and bacteria. The cracks caused by nematode allow certain secondary organisms to enter the roots and cause rotting during storage. Also, high temperature can lead to increased fungal pathogens which could further reduce sweet potato yields due to combination of fungi and nematodes (Pritchard, 2011). According to Kistner *et al*. (1993), marketable yield decreased by 11.4% as a result of damage caused by *M. javanica* and *M. incognita* in South Africa. Nkosi (2018) tested nematode resistance on sweet potato cv. ′Mafutha′ against *M. javanica, M. incognita* races 2 and 4, and showed that the cultivar was a susceptible host to all three tested nematode species. Host-status and host-sensitivity of sweet potato cv. ′Bophelo′ was assessed against *M incognita* race 2 and the cultivar was shown to be a susceptible host (Makhwedzhana, 2018). Cervantes-Flores and Yencho (2002) found that cultivars ′Beauregard′, ′Nancy Hall′ and ′L86-33′ were highly susceptible to *Meloidogyne* species. Karuri *et al*. (2017) conducted a survey of root-knot nematode resistance in sweet potato cultivars from Kenyan fields and showed that 11% test sweet potato cultivars were good host to *Meloidogyne* species.

Tolerant host is defined as the ability of plant to allow nematode penetration and reproduction without incurring growth reduction and yield loss (Roberts, 1992; Seinhorst, 1967; Trudgill, 1985). Crozzoli *et al.* (1994) reported tolerance among three selections of sweet potato as these supported nematode penetration and reproduction. Cultivars which are tolerant to nematodes cannot be used in crop rotation systems intended to manage nematode population densities since such cultivars could result in build-up of nematodes for successor crops (Pofu *et al.,* 2012).

Resistance to nematode infection is exhibited as a decrease or inhibition of nematode penetration and reproduction (Corbett *et al*., 2011; Trudgill, 1992) or prevention of feeding site establishment (Corbett *et al*., 2011; Williamson and Kurmar, 2006). Reduction in the severity of root galling and necrosis are the characteristic of resistance in sweet potato genotypes (Cervantes-Flores *et al.,* 2002; Piedra-Buena *et al*., 2013). According to Cervantes-Flores and Yencho (2002) and Okada *et al*. (2017), resistance is the most economically and sustainable method of managing nematode population densities. Sweet potato genotypes have limited resistant dominant genes, with strong resistance effects and massive genes with weak resistance effects (Okada *et al.,* 2017). Maseko (2018) tested host-status and host-sensitivity of sweet potato cv. 'Bophelo' 'Mvuvhelo' and 'Bosbok' against *M. javanica* where, the reproductive factor was less than one and nematode infection did not reduce plant growth, which indicated that all cultivars were resistant to *M. javanica*. Also, Makhwedzhana (2018) established nematode resistance against *M. incognita* in sweet potato cultivars 'Bophelo', 'Mvuvhelo' and 'Bosbok', and found that cultivars 'Mvuvhelo' and 'Bosbok' were resistant to *M. incognita*. Ten varieties of sweet potatoes in Nigeria were evaluated for resistance to root-knot nematodes, with nine varieties falling within the susceptibility group (Atungwu *et al*., 2013). A survey of root-knot nematode resistance in sweet potato cultivars in Kenya suggested that 68% test sweet potato cultivars were highly resistant to *Meloidogyne* species (Karuri *et al*., 2017).

2.2.3 Mechanism of nematode resistance

Nematode resistance mechanisms comprise pre-infectional nematode resistance and post-infectional nematode resistance (Kaplan and Davis, 1987). Only post-infectional

nematode resistance genes can be introgressed into nematode susceptible cultivars (Haussmann *et al.,* 2004; Thurau *et al*., 2010). Consequently, after establishing that a cultivar is resistant to a given nematode species, there is need to establish the mechanism of nematode resistance in order provide plant breeders with information as to whether the test cultivar was the candidate of introgression or not.

Pre-infectional nematode resistance: Gapasin (1986) observed that three sweet potato cultivars, namely, 'Jasper', 'Jewel' and 'W-86', had pre-infectional nematode resistance to *M. incognita* and *M. javanica*. Generally, in pre-infectional nematode resistance, chemicals are released into the rhizosphere so that J2 are prevented from penetrating the root systems (Ferraz and Brown, 2002). The root exudates could either be attractive or repellent to J2 (Huang, 1985). In different plant species, root exudates such as dhurrin, sorgoleone, glucose and Alpha-terthienyl had been shown to contain some nematicidal effects on *Meloidogyne* species (Chitwood, 2003; Czarnota *et al*., 2003; Gommers and Bakker, 1988; Ntalli and Caboni, 2012). Mashela and Pofu (2016) observed that J2 of *Meloidogyne* species failed to penetrate roots of sweet stem sorghum cv. ′Ndendane-X1‵ and concluded that the cultivar had pre-infectional nematode resistance to *M. javanica* and *M. incognita* race 2. Penetration of *M. incognita* into roots of Sunn hemp was prevented by chemicals which were released to the rhizosphere (McSorley and Gallaher, 1991; Roberts, 1992). Selected rapeseed cultivars ′Dwarf Essex′, ′Elena′, ′Indore′, ′Jupiter′, ′Cascade′, ′Bridger′ and ′Humus′ were shown to prevent penetration of *Meloidogyne* species into their root system (Bernard and Montgomery-Dee, 1993).

Post-infectional mechanism of resistance: In this classification, the J2 are allowed to penetrate the root systems, but whilst on their way to the infection sites or after attempting to establish the infection site, the plant activate chemicals referred to as plant genes to attack J2, while the J2 release gene product (Mashela *et al.,* 2016). Mashela *et al.* (2016) reviewed three identified approaches through which plants at a molecular level can resist nematode attack, namely, anti-gene product, anti-plant gene and RNA-interference.

Anti-gene products: During the formation of nematode feeding site, the secretion of gene products is crucial to allow nematode development subsequent stages (Curtis, 2008; Siddique *et al*., 2014). During the migration phases from penetration to the feeding site, which constitutes the most damaging phase, the plant produce peroxidase, chitinase, lipoxygenase, extension and proteinase inhibitors, which are called plant genes (Gardener *et al.,* 2015; Gheysen and Fenoll, 2002; Hewezi and Baum, 2015). During this period, J2 activate chemicals from the ventral gland referred to as gene products (Mashela *et al*., 2016), which downregulate or upregulate the plant genes, thus conferring the relations be either favourable or unfavourable (Gardener *et al*., 2015; Gheysen and Fenoll, 2002; Hawezi and Baum, 2015). Generally, when the gene products-plant genes relations are unfavourable, nematodes are failing to feed, develop and then to reproduce (Mashela *et al*., 2016), whereas favourable gene-gene interactions result in opposite effects. Plant genes with capabilities to silence the gene products are collectively called the resistance (R) genes (Seinhorts, 1967; Trudgill, 1992). The R genes had since been transferred as transgenes into commercial cultigens that conventionally had no resistant genotypes to plant-parasitic nematodes

to develop nematode resistance transgenic plants (Jones *et al*., 1998; Mashela *et al.,* 2016).

Anti-gene strategy: In this strategy, plant genes and gene products can have either upregulation or downregulation effects (Seah *et al.,* 1998). The unregulated plant genes improve compatibility of plant-nematode interaction (Hewezi and Baum, 2015; Mentelin *et al*., 2015). Host plant genes that enhance the nematode feeding and secretions to allow compatibility between nematodes and plants, are silenced. Phytotoxic chemical compounds that destroy nematode feeding structures, namely, syncytium and giant cells are upregulated (Mashela *et al*., 2016). Furthermore, specific plant genes are produced to shelter the nematode, but such chemicals can be suppressed thereby leaving nematode body exposed (Hewezi and Baum, 2015). Failure to develop and maintain the feeding structures inhibit nematode development and such anti-plant gene approaches had been successfully used (Mashela *et al*., 2016).

RNA-interference strategy: The RNA interference (RNAi) genes have precise selective for the target organisms with slightly off-target effects (McDowell and Woffenden, 2003). The RNAi disrupts the nematode gene products through a host-induced gene silencing approach (Hewezi and Baum, 2015; Mentelin *et al*., 2015; Williamson and Hussey, 1996). The released cathepsin L-like cysteine proteinases in nematode resistant transgenic plants were shown to be an attractive group of candidate genes for RNAi-induced downregulation due to their level of specificity to the target gene products, resulting in immediate silencing of host-induced nematode gene products (Mashela *et al*., 2016).

2.2.4 Comparing test phytonematicides with synthetic chemical nematicides

In South Africa, Nemarioc-AL and Nemafric-BL phytonematicides, with active ingredients cucurbitacin A ($C_{32}H_{46}O_9$) and cucurbitacin B ($C_{32}H_{46}O_8$), are produced from fruits of wild cucumber (*Cucumis myriocarpus* Naude*.)* and wild watermelon (*Cucumis africanus* L*.)*, are being researched and developed for managing nematode population densities for different nematode species on a wide range of crops (Mashela *et al.,* 2015). The two phytonematicides had been developed as liquid (L) and as granular (G) formulations, namely, Nemarioc-AL and Nemarioc-AG phytonematicides, respectively. Technically called the nemarioc-group phytonematicides and the Nemafric-BL and Nemafric-BG phytonematicides, being the nemafric-group phytonematicides. The success of the phytonematicides is dependent upon the allelochemicals as active ingredients, which are naturally phytotoxic to plants (Mashela *et al*., 2015). Conventionally, the liquid and granular phytonematicides are applied through botinemagation and the ground leaching technology (GLT). In the GLT, ground materials are applied in small quantities around the stem, with active ingredients released into the rhizosphere through irrigation water (Mashela, 2002; Mashela *et al*., 2011). In botinemagation, the ground fruits are first fermented (Mashela *et al.,* 2015, 2017), with product in liquid formulation applied through irrigation system at low concentration (Mashela *et al*., 2011, 2017). The first report on comparing cucurbitacin-containing phytonematicides and commercial nematicides was in 2008, where the efficacy of Nemarioc-AG phytonematicide on suppression of population densities of *Meloidogyne* species was comparable to those of aldicarb and fenamiphos on tomato plants (Mashela *et al*., 2008). Velum contains an active ingredient Floupyram, with both nematicidal and fungal properties (Bayer Crop Science, 2015),

and the nematicidal efficacy was comparable to those of the cucurbitacin-containing phytonematicides on potato plants under field conditions (Seshweni, 2017).

Challenges in use of phytonematicides: The active ingredients of phytonematicides are allelochemicals and therefore, the correct concentration of phytonematicides should be used to avoid the phytotoxicity of the products to the protected plants (Mashela *et al*., 2017). The potential registration (certification) and widespread adoption of the phytonematicides for managing *Meloidogyne* species could be limited by their inherent phytotoxicity. Mafeo (2012) indicated that inhibition of seed germination in response to the allelochemicals released by Nemarioc-AG phytonematicide was consistent with reports in literature.

At 5 g Nemarioc-AG phytonematicide, inhibited germination of tomato (*Solanum lycopesicon* L.), butternut squash (*Cucurbita moschata* L.), maize (*Zea mays* L.), finger millet (*Eleusine coracana* L.), sorghum (*Sorghum bicolor* L.), leek (*Allium ampeloprasum* L.) and onion (*Allium cepa* L.) (Mafeo and Mashela 2009b, 2010). The listed crops constituted both monocotyledonous and dicotyledonous crops. Similar phytotoxicity results were observed in sweet stem sorghum (Mabuka, 2015), medicinal plants (*Pelargonium sidoides)* (Sithole, 2016) and in citrus seedlings (Maile, 2013).

Mitigation strategies for phytotoxicity: Due to zero tolerance on phytotoxicity from agricultural inputs, literature is replete with *in vitro* nematode- phytonematicide trials where the product is highly effective in killing nematodes, but could not be registered due to its high phytotoxicity levels (Mashela *et al.,* 2017). In South Africa, Act no 36 of 1947 and related amendments or regulations, prescribe requirements for the

registration of agricultural inputs (Mashela *et al.,* 2017) with emphasis on circumvention of phytotoxicity.

In cucurbitacin-containing phytonematicides, the innovators (Mashela *et al*., 2017) refined the Curve-fitting Allelochemical Response Dose (CARD) algorithm computer model (Liu *et al.,* 2003), for developing the concept Mean Concentration Stimulation Point (MCSP). The MCSP is the non-phytotoxic concentration that would consistently suppress nematode population densities, at the same time having the potential to stimulate plant growth. According to the conceptual model of MCSP (Mashela *et al*., 2017), the latter could be computed using two biological indices D_m (threshold stimulation) and R_h (saturation point) through the relations:

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

The relationship, cordially referred to as Mashela's first law of phytonematicides, is the opposite of the biological index D_{50} (Mashela *et al.,* 2017), which is the concentration of a phytonematicide that would inhibit plant growth by 50%. This MCSP had been intended to reduce nematode population densities, without inducing phytotoxicity. The MCSP values for Nemarioc-AL and Nemafric-BL phytonematicides on sweet potato cv. 'Bophelo' were 1.92 and 3.08%, respectively (Sebothoma, 2019; Selomo, 2019). Generally, for most plant species, the MCSP values are from 1 to 3%, with most nematode species that are grouped as r strategists being susceptible to MCSP value of 2% (Mashela *et al*., 2017). R strategist nematodes are small in size, with short life span and high reproduction rate (Katz and Trudgill, 1999).

The CARD model also gives two other biological indices, which are critical in the development of phytonematicides, namely, the sensitivity index (k) and the overall sensitivity (Σk). The Σk is inversely proportional to the sensitivity of the crop to the test phytonematicide. For example, Σk value of zero depicts susceptibility, where further from zero depicts tolerance (Liu *et al*., 2003).

Application interval of phytonematicides: Once the MCSP value is empirically-established to avoid phytotoxicity, another question arises: When should MCSP be applied in order to avoid phytotoxicity, but achieving nematode suppression? Pelinganga and Mashela (2012) pioneered the concept of a "weeks per-month-of-nematode ontogeny", or Mashela's second law of phytonematicides. The law states that: "Application of liquid phytonematicides should be repeated twice within the life cycle (ontogeny) of the test nematode". This law allows for managing what was previously referred to "inconsistent results" of phytonematicides (McSorley, 2003), thus, rendering the products not suitable for use as commercial products. The application interval of Nemarioc-AL phytonematicide on sweet potato cv. 'Bophelo' was optimized at 2.55 'weeks-per-month-of-days' (2.55/4 × 30), translating to 19 days (Sebothoma, 2019). The application interval of citrus nematode (*Tylenchulus semipenetrans*), was established using 'weeks-per-month of 42 days' (Mathabatha, 2020, unpublished), where 42 days constituted the life cycle of *T. semipenetrans*.

2.3 Work not done on problem statement

The host-status and host-sensitivity of sweet potato cv. 'Blesbok' to *M. javanica* had not been documented. Similarly, the efficacy of Nemarioc-AL and Nemafric-BL

phytonematicides in management of *Meloidogyne* species on sweet potato cv. 'Blesbok' would be compared with that of Velum under field conditions.

2.4 Addressing the identified gaps

In order to address the identified gaps, this research study focused on establishment of nematode resistance to *M. javanica* in sweet potato cv. 'Blesbok' and its mechanism of resistance. The efficacy of cucurbitacin-containing phytonematicides would be comparable to that of Velum chemical nematicide in management of *Meloidogyne* species population densities in the cultivar.

2.5 Summary of identified gaps

The establishment of host-status and host-sensitivity and its mechanisms of resistance to *M. javanica* in sweet potato cv. 'Blesbok' and related management strategies were identified as the existing gaps. The outcome in this study would provide evidence on nematode resistance and whether the mechanism of resistance in the test cultivar was pre- or post-infectional nematode resistance. The efficacy of cucurbitacin-containing phytonematicides in management of *Meloidogyne* species in sweet potato production would be compared with that of Velum, which had been unavailable to smallholder farmers due to its high cost. Cultivar 'Blesbok' contribute 70-80% of the total production to the South African sweet potato industry.

CHAPTER 3

HOST-STATUS AND HOST-SENSITIVITY OF SWEET POTATO CULTIVAR 'BLESBOK' TO *MELOIDODYNE JAVANICA*

3.1 Introduction

Most sweet potato (*Ipomoea batatas* L.) producing regions in South Africa are heavily infested with tropical root-knot (*Meloidogyne* species) nematodes (Pofu *et al*., 2017). In South Africa, yield losses due to *Meloidogyne* species in sweet potato was estimated at 6% during the availability of methyl bromide (Kleynhans, 1991). Currently, with the withdrawal of methyl bromide from the agrochemical markets in 2005, the situation had been worsening (Mashela *et al*., 2017).

In sweet potato production, *M. javanica* is the most disastrous pest (Gomes *et al*., 2015). Also, it is well-established that contrary to the international records (Taylor and Sasser, 1978), the South African population densities of *M. javanica* are more aggressive than those of *M. incognita* (Kleynhans *et al.,* 1996). Globally, nematode resistant cultivars are widely used in management of population densities of *Meloidogyne* species (Khanzada *et al.,* 2012; Moens *et al*., 2009). Recent evidence in South Africa (Makhwedzhana, 2018; Maseko, 2018; Pofu *et al*., 2017), suggest that nematode resistant germplasm is available in sweet potato cultivars.

The advent of biofortification has resulted in increased use of orange-fleshed sweet potato cultivars (Laurie *et al*., 2015). Such cultivars with nematode resistance are scarce including the local cv. 'Mvuvhelo' and the exotic cv. 'W-119' (Makhwedzhana, 2018; Pofu *et al*., 2017). Another local cultivar with nematode resistance which is

commercially available is 'Bosbok' (Makhwedzhana, 2018; Maseko, 2018). Also, the purple-red skinned and white flesh local sweet potato cv. 'Blesbok' was tested not resistant to races of *M. incognita* (Pofu *et al*., 2017), but without information of nematode resistance to *M. javanica*. The objective of this study was to determine the host-status and host-sensitivity of sweet potato cv. 'Blesbok' to *M. javanica* population densities.

3.2 Materials and Methods

3.2.1 Description of study location

The study was conducted at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, in Limpopo Province of South Africa (23° 53′ 10″S, 29° 44′ 15″ E) under greenhouse conditions. The size of greenhouse was 20 m × 100 m with the roof covered with a green net to allow 65% photosynthetically active radiation to pass through. Ambient day/night temperatures averaged 28/21 °C respectively, with maximum temperatures controlled using thermostatically activated fans on the northern side wall and wet walls on the southern side wall to ensure that relative humidity is retained between 60 and 70%. The trial for *M. javanica* was conducted during (March to May) 2018 and validated in 2019.

3.2.2 Treatments and research design

Treatments, namely, 0, 5, 25, 125, 625, 3125 and 15625 eggs + second-stage juveniles (J2) *M. javanica* were laid out in a randomised complete block design, with six replications. The trial was blocked because conditions inside the greenhouse were heterogeneous due to the size of greenhouse and wind-blowing generated currents during the heat extraction.

Legend 3.1 Experimental set-up sweet potato cultivar inoculated with *Meloidogyne javanica* under greenhouse conditions.

3.2.3 Procedures and cultural practices

Twenty cm diameter pots were filled with steam pasteurised (300°C for 60 minutes) sandy clay loam soil (30% clay, 5% silt, 65% sand). Pots were arranged on greenhouse benches lifted with bricks at 0.30 m × 0.25 m spacing and irrigated to full capacity using 200 ml tap water. Rooted 'Blesbok' plantlets, obtained from the Agricultural Research Council-Vegetable and Ornamental Plants (ARC-VOP), were transplanted at one cutting per pot. Inocula of *M. javanica* cultured nematode-susceptible tomato plant cv. 'Floradade' was prepared by extracting eggs and second-stage juveniles (J2) in 1% NaOCl solution (Hussey and Barker, 1973). Seven days after transplanting, inoculation for the respective levels was achieved using a 20ml plastic syringe by placing into 5 cm deep holes on the cardinal points of the crown. Six cv. 'Beauregard' plants were used as a nematode-susceptible standard (Cervantes-Flores *et al*., 2002), inoculated with 15 625 eggs + J2, to aid in assessing the viability

of the inocula. Irrigation was done using 200 ml tapwater at every other day. Plants were fertilised once at 4 weeks after transplanting using 2 g NPK 2:1:2 (43) to provide 0.70 mg N, 0.64 mg K, 0.64 mg P, 1.8 mg, 1.5 mg Fe, 0.15 mg Cu, 0.7 mg Zn, 2 mg B, 6 mg Mn and 0.14 mg Mo/ml tapwater and 2 g NPK 2:3:2 (26) + 0.5% Zn + 5% S + 5% Ca to provide all essential nutrients elements. Insect pests and diseases scouted and monitored on daily basis. Comite EC was sprayed to control the observed mites at application rate of 10 ml per 1000 ml of water. Cypermethrin was applied for aphids and whitefly observed at application rate of 1 ml per L chlorine free tapwater. Funginex was sprayed to control powdery mildew disease at an application rate of 2.5 ml per 2000 ml of water.

3.2.4 Data collection

Plant variables: At 56 days after inoculation, vine length was measured from the crown to the tip of the leaf and cut at the soil level, with vines being oven dried at 52 °C for 72 hours to constant weight. Stem diameter was measured using digital Vernier caliper at the crown. Root system was removed from pots, immersed in water to remove excess soil particles, all roots for treatment zero were oven dried at 52 °C for 72 hours and weighed to facilitate extrapolation of dry root mass. Root galls were assessed using the North Carolina Differential Scale of 0-5 where 1 = no galls, 2 = 1-10 galls, 3 = 11-31 galls, 4 = 31-100 galls and 5 = being galls that are greater than 100 per root system (Taylor and Sasser, 1978). Soil per pot was thoroughly mixed and 250 ml soil sample was collected.

Nutrient elements variables: About 0.4 g ground healthy matured leaves of sweet potato cv. 'Blesbok' plant were digested in 75 ml vessel with 5 ml of 70% nitric acid

(HNO_3) and 3 ml of 30% hydrogen peroxide (H_2O_2) using microwave digester (Perlain Elmer, Titan MPS). The vessels were then inserted into the microwave digester to whirl for 46 minutes under temperature ranging up to 260 °C. Subsequently the vessels we placed in the laminar flow hood and allowed to cool down for 5 minutes. Samples from the vessels were transferred into 50 ml centrifuging tubes and stored in the refrigerator before analysing them. Ca, K, Mg, Fe and Zn elements were analysed from sweet potato leaf samples using the Inductively Coupled Plasma Optical Emission Spectrometry (Shimadzu, ICPE-9000).

Nematode variables: Nematodes were extracted from total root system per plant by maceration and blending for 60 s in 1% NaOCl solution (Hussey and Barker, 1973). The material was passed through top-down nested 75 µm and 25 µm mesh sieves, remaining content of the 25 µm were bottled into 100 ml plastic containers. Nematode was separated from the debris of the aliquot through the sugar-floatation and centrifuging method (Jenkins, 1964). The nematodes in the soil were also extracted using modified sugar-floatation and centrifugation method (Jenkins, 1964). In short, 5 litre soil solution was prepared using 250 ml soil sample, mixed with water in a swill. After the swill, had stopped, the aliquot was poured through nested 75 µm and 25 µm mesh sieves, remaining content of the 25 µm were bottled into 100 ml plastic centrifuge tubes. A spatula (2½ g) of kaolin was added in each tube after the samples were centrifuged at 1 800 rpm for 4 minutes after the solution was decanted with remaining nematodes and soil particles settled at the base of the tubes. A sugar stock solution was prepared by dissolving 624 g sugar/L tape water, 45 ml of the stock solution was added into the centrifuge tubes and stirred once prior to centrifuging for 3 minutes at 1 800 rpm. The aliquot was then decanted onto 25 µm sieve with sugar

rinsed off the nematodes through running tapwater, the remaining water was collected into 100 ml plastic container for further analysis. From the 100 ml aliquot eggs and J2 were counted from 5 ml aliquot sample with the use of stereomicroscope. Nematode numbers from roots were converted to nematodes per total root system per plant and soil nematode numbers were converted to total nematodes per pot soil. Reproductive factors (RF) were computed using proportion of final nematode numbers (Pf) to initial nematode numbers (Pi).

3.2.5 Data analysis

Final estimated nematode population density (Pf) was generated by converting nematodes counts from both soil and roots samples to total root system and total pot soil. The reproductive factor (RF = Pf/Pi) was generated by dividing final population density (Pf) by initial population density (Pi). Collected data was subjected to analysis to variance using SAS software (SAS, 2008), were data was significant, means were separated using Waller-Duncan multiple range test at the probability level of 5%. Prior to the analysis of variance nematode data were transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984), but untransformed means were reported. The degrees of freedom and their associated mean sum of squares were partitioned to provide the total treatment variation (TTV) of different variables. Nutrient elements were assessed through analysis of variance using SAS software (SAS, 2008) and data were further subjected to lines of the best fits.

3.3 Results

Nematode responses to inoculation of *Meloidogyne javanica*: Treatments had significant effect ($P \leq 0.05$) on eggs and highly significant effect ($P \leq 0.01$) on J2, Pf

and RF, contributing 39, 45, 42 and 92%, respectively, in total treatment variation (TTV) of the respective variables in Experiment 1. Whereas in Experiment 2 treatments had significant effect on J2 and highly significant effect on eggs, Pf and RF, contributing 68, 73, 73, 72 and 97%, respectively, in TTV of the respective variables. At inoculation less than 125 eggs + J2, RF values were greater than one, whereas at inoculation greater than 625 eggs + J2 the RF values were less than one in both experiments. Mean RF gradually declined as inoculum levels increased of the J2 of *M. javanica* were able to penetrate roots of sweet potato cv. 'Blesbok' and developed to mature females that produced eggs (Table 3.1 to 3.2).

3.1 Partitioning mean sum of squares of eggs, second-stage juveniles (J2) in roots, J2 in soil, final population (Pf) and reproductive factor (RF) of *Meloidogyne javanica* to sweet potato cv. 'Blesbok' under greenhouse conditions.

Source		Eggs		J2(root)		J2(soil)		Pf		RP	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
					Experiment 1						
Replication	5	1.59582	46	1.13513	46	0	0	1.71953	47	0.05355	5
Treatment	6	1.37697	39**	1.12524	45***	0	0	1.54540	42***	1.08367	92***
Error	30	0.51377	15	0.22831	9	0	0	0.40620	11	0.04477	3
Total	41	3.48656	100	2.48868	100	0	0	3.67113	100	1.18199	100
					Experiment 2						
Replication	5	0.19134	14	0.06295	10	0.08490	5	0.14349	12	0.01504	2
Treatment	6	1.03723	73***	0.44934	68**	1.15537	73***	0.90778	72***	1.03876	97***
Error	30	0.18847	13	0.15166	22	0.33994	22	0.20139	16	0.01222	1
Total	41	1.41704	100	0.66395	100	1.58021	100	1.25266	100	1.06602	100

Significant P ≤ 0.05, * highly significant P ≤ 0.01.

Table 3.2. Response of initial nematode numbers (Pi), eggs in root, second-stage juveniles (J2) in roots, J2 in soil, final nematode population density (Pf) and reproductive factor (RF) of cultivar 'Blesbok' inoculated with *Meloidogyne javanica* under greenhouse conditions.

Pi	Eggs	J2 (roots)	Pf	RF	Eggs	J2 (roots)	J2 (soil)	Pf	RF
		Experiment 1					Experiment 2		
5	1.64^{ab} (47)	1.32^{cd} (20)	1.81^{bc} (67)	1.14^{a} (13.40)	1.53^{b} (37)	1.37^{b} (23)	0^{b}	1.76^{b} (60)	1.09^{a} (12.00)
25	1.13^{b} (43)	1.03^{d} (27)	1.28^{c} (70)	0.43^{b} (2.78)	1.75^{b} (40)	1.42^{b} (27)	0^{b}	1.80^{b} (67)	0.55^{b} (2.68)
125	2.15^{a} (363)	2.03^{ab} (133)	2.51^{ab} (496)	0.63^{bc} (3.97)	2.03^{b} (110)	1.74^{ab} (60)	0.49^{ab} (10)	2.23^{b} (180)	0.31^{c} (1.44)
625	2.47^{a} (350)	2.18^{a} (200)	2.67^{a} (550)	0.23^{cd} (0.88)	1.66^{b} (130)	1.39^{b} (43)	0^{b}	1.84^{b} (173)	0.10^{d} (0.28)
3 125	2.20^{a} (383)	1.64^{abc} (87)	2.28^{ab} (470)	0.06^{d} (0.15)	2.02^{b} (110)	1.62^{ab} (47)	0.44^{ab} (7)	(2.18)^{b} (163)	0.02^{d} (0.05)
15 625	1.88^{ab} (153)	1.52^{bcd} (57)	1.99^{abc} (210)	0.00^{d} (0.01)	2.63^{a} (550)	2.08^{a} (160)	(1.10)^{a} 30	(2.78)^{a} (740)	0.02^{d} (0.05)
				Cultivar 'Beauregard'					
^{Z}15 625	57 853	6 983	64 836	4.15	27 428	4 108	380	31 844	2.04

Plant responses to inoculation of *Meloidogyne javanica*: In both Experiment 1 and Experiment 2, treatment had no damage effect on the tested cultivar on all plant variables measured, namely, vine length, dry shoot mass, dry root mass, number of tubers, dry tuber mass and chlorophyll content of both Experiment 1 and Experiment 2 (Table 3.3 to 3.5).

3.3 Partitioning mean sum of squares of vine length (VNL), stem diameter (STD), dry root mass (DRM) and dry shoot mass (DSM) of sweet potato cv 'Blesbok' inoculated with *Meloidogyne javanica* under greenhouse conditions.

Source		VNL		STD		DRM		DSM	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
					Experiment 1				
Replication	5	3562.56	50	0.82	9	1.96	43	15.39	31
Treatment	6	1073.87	14^{ns}	5.69	62^{ns}	1.23	27^{ns}	10.49	21^{ns}
Error	30	2571.57	36	2.69	29	1.34	30	23.49	48
Total	41	7208.00	100	9.20	100	4.53	100	49.37	100
					Experiment 2				
Replication	5	2039.74	50	2.27	45	8.39	26	2.60	46
Treatment	6	387.75	10^{ns}	0.89	18^{ns}	4.77	15^{ns}	1.73	30^{ns}
Error	30	1675.13	40	1.90	37	19.11	59	1.35	24
Total	41	4102.62	100	5.06	100	32.27	100	5.68	100

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

3.4 Partitioning mean sum of squares of number of tubers (NOT), dry tuber mass (DRT) and chlorophyll content (CHC) of sweet potato cv 'Blesbok' inoculated with *Meloidogyne javanica* under greenhouse conditions.

Source		NOT		DRT		CHC	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
				Experiment 1			
Replication	5	0.14	52	12.51	46	96.92	28
Treatment	6	0.04	15^{ns}	3.22	12^{ns}	130.94	38^{ns}
Error	30	0.09	33	11.59	42	118.42	34
Total	41	0.27	100	27.32	100	346.28	100
				Experiment 2			
Replication	5	0.21	6	0.29	6	36.05	24
Treatment	6	2.32	65^{ns}	3.07	65^{ns}	64.69	42^{ns}
Error	30	1.03	29	1.38	29	51.25	34
Total	41	3.56	100	4.74	100	151.99	100

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

Table 3.5 Response of initial nematode numbers (Pi), vine length (VNL), stem diameter (STD), dry root mass (DRM), dry shoot mass (DSM) number of tubers (NOT), dry tuber mass (DRT) and chlorophyll content (CHC) of sweet potato cv. 'Blesbok' to *Meloidogyne javanica* under greenhouse conditions.

Pi	VNL (cm)	STD (mm)	DRM (g)	DSM (g)	NOT	DRT (g)	CHC	VNL (cm)	STD (mm)	DRM (g)	DSM (g)	NOT	DRT (g)	CHC
	Experiment 1							Experiment 2						
0	91.83	5.03	2.28	4.55	0.33	3.17	33.70	110.00	3.39	2.02	7.97	0.83	0.91	33.87
5	61.50	3.19	1.38	6.59	0.16	2.00	27.43	100.33	3.52	1.21	9.55	1.83	2.07	33.00
25	68.50	3.56	1.56	4.21	0.20	2.28	26.22	108.83	3.10	1.30	10.02	0.00	0.00	31.62
125	81.33	4.50	2.44	7.47	0.24	1.72	37.80	122.17	3.79	1.33	8.80	0.17	0.23	36.30
625	90.67	5.85	2.39	6.86	0.40	2.14	37.13	99.33	3.43	2.53	9.48	0.33	0.21	37.48
3 125	97.67	5.27	1.50	5.37	0.26	2.20	36.67	101.17	2.96	1.46	10.39	0.33	0.21	27.40
15 625	74.00	5.26	1.76	4.44	0.25	0.72	33.35	108.14	2.84	2.30	8.29	0.83	0.62	33.68
LSD	62.77	35.18	60.98	85.92	114.28	167.63	32.79	38.14	41.19	61.41	47.44	164.08	193.80	21.47

Nutrient elements responses to inoculation of *Meloidogyne javanica*: Nutrient elements and increasing of *M. javanica* treatment showed quadratic relationships. Ca, K, Mg, and Fe each in leaf tissues of sweet potato cv. 'Blesbok' against the increasing of *M. javanica* treatment exhibited negative quadratic relations in Experiment 1 and 2 (Fig 3.1 to 3.2). The quadratic relationships of four respective nutrient elements with increasing *M. javanica* population densities were explained by 83, 96, 59 and 71% in Experiment 1, whereas in Experiment 2 the models were explained by 96, 77, 95 and 91%. Using the relations $X= -b_1/2b_2$ (Gomez and Gomez 1984), Ca, K, Mg and Fe in the leaf tissues were optimised at 3, 3, 3, and 2 level of *M. javanica* nematode (Table 3.6) in Experiment 1, whereas in Experiment 2 were optimised at 1, 1, 1 and 3 (Table 3.6), respectively. In contrast, Zn content in leaf tissues of sweet potato cv. 'Blesbok' with increasing of *M. javanica* treatment exhibited positive quadratic relation, with model explained by 80 and 98% in Experiment 1 and Experiment 2 (Fig 3.3). Zinc was minimised at 4 and 3 level of *M. javanica* nematode in Experiment 1 and Experiment 2, respectively.

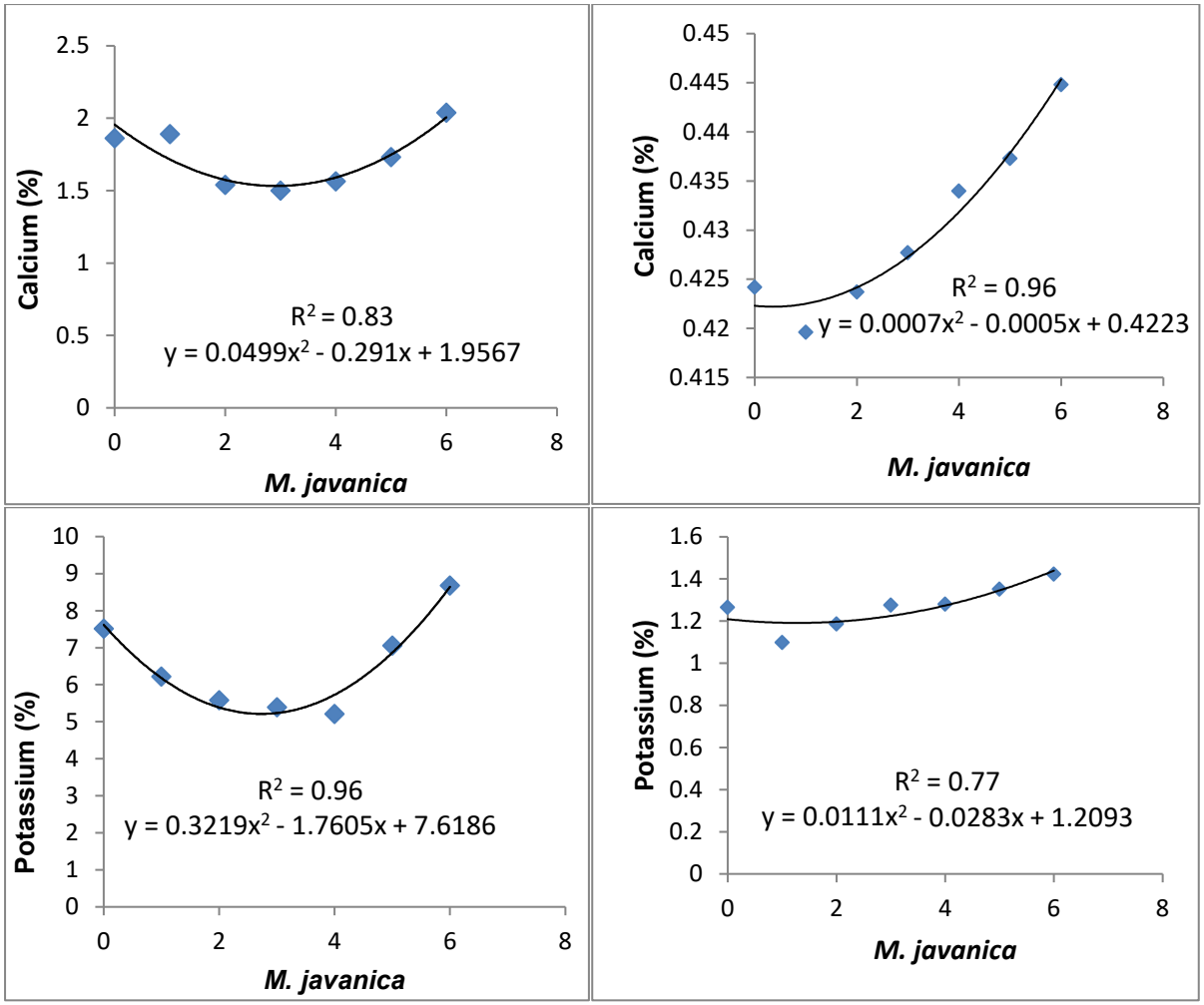

Fig 3.1 Response of calcium and potassium in leaf tissues of sweet potato cv. 'Blesbok' against increasing level of *M. javanica* at 56 days after initiation of treatments for Experiment 1 and 2.

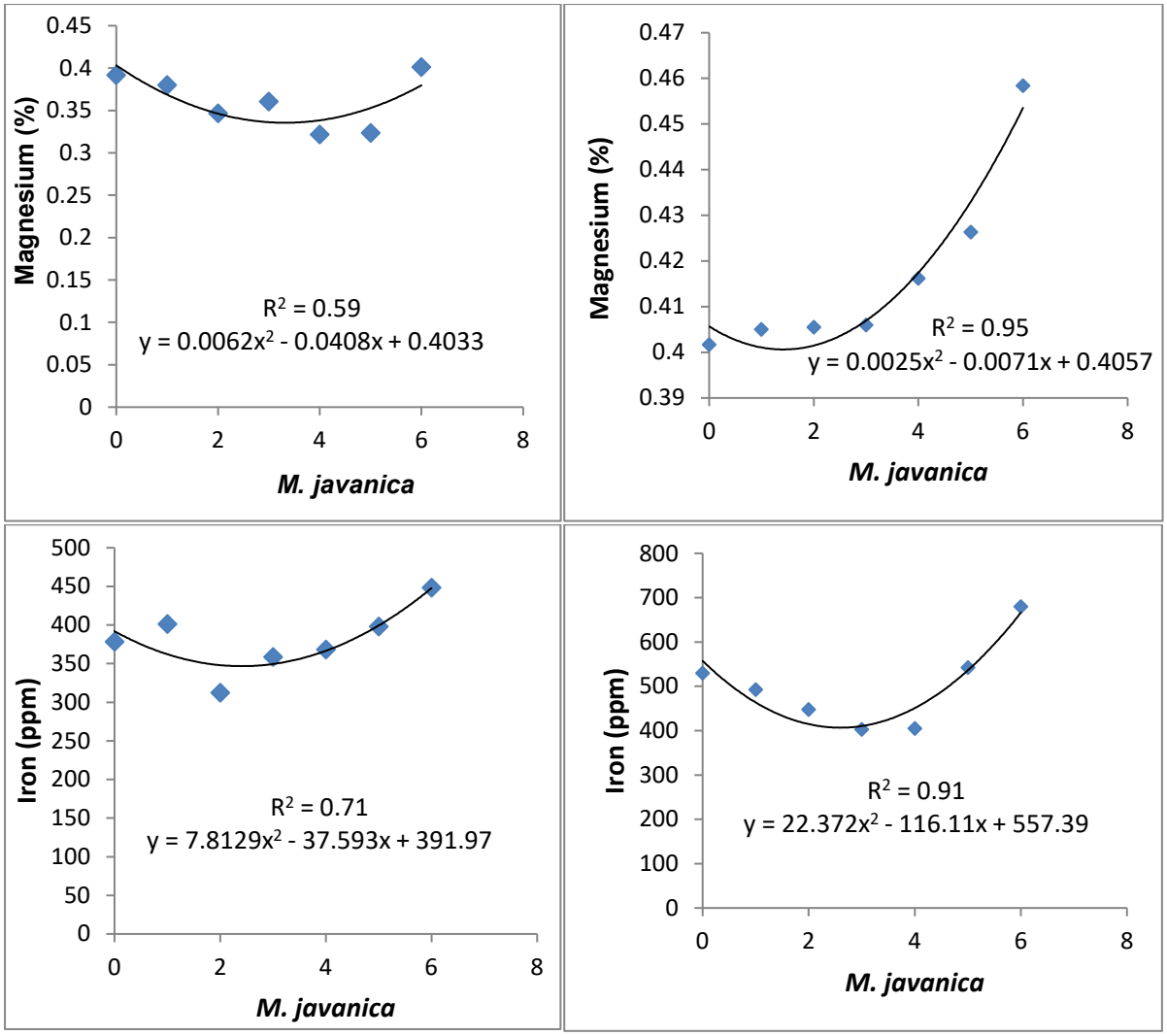

Fig 3.2 Response of magnesium and iron in leaf tissues of sweet potato cv. 'Blesbok' against increasing level of *M. javanica* at 56 days after initiation of treatments for Experiment 1 and 2.

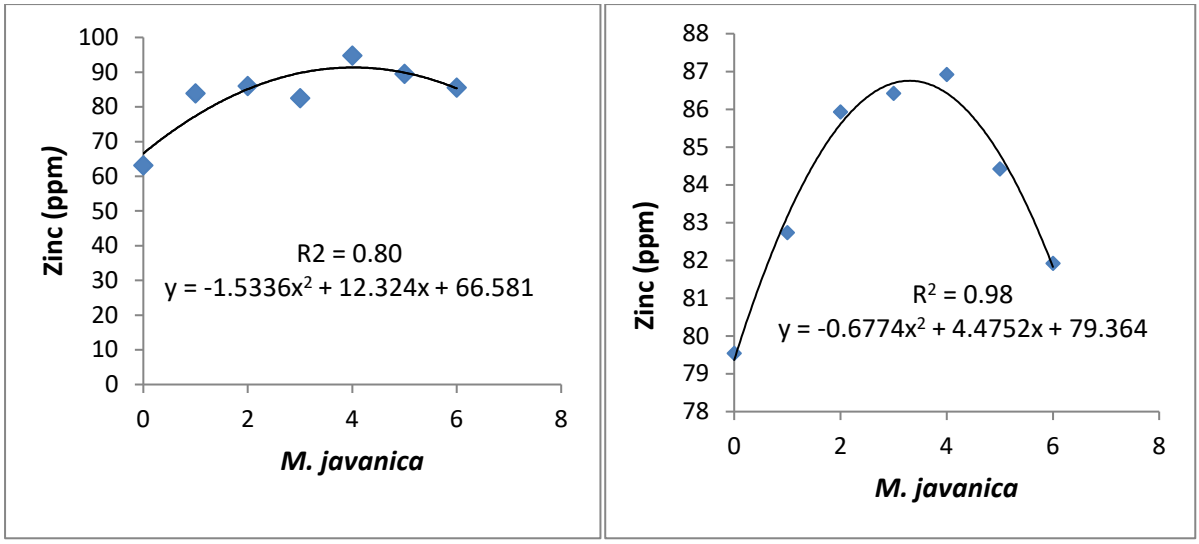

Fig 3.3 Response of zinc in leaf tissues of sweet potato cv. 'Blesbok' against increasing level of *M. javanica* at 56 days after initiation of treatments for Experiment 1 and Experiment 2.

Table 3.6 Optimisation models of selected nutrient elements in leaf tissues of sweet potato cv. 'Blesbok' as affected by *M. javanica*.

Element	Model	R^2	X	Model	R^2	x
	Experiment 1			Experiment 2		
Ca	$Y = 0.0499x^2 – 0.2910x + 1.9567$	0.83	3	$Y = 0.0007x^2 – 0.0005x + 0.4223$	0.96	1
K	$Y = 0.3219x^2 – 1.7605x + 7.6186$	0.96	3	$Y = 0.0111x^2 – 0.0283x + 1.2093$	0.77	1
Mg	$Y = 0.0062x^2 – 0.0408x + 0.4033$	0.59	3	$Y = 0.0025x^2 – 0.0071x + 0.4057$	0.95	1
Fe	$Y = 7.8129x^2 – 37.593x + 391.97$	0.71	2	$Y = 22.372x^2 – 116.11x + 557.39$	0.98	3
Average			3			2

3.4 Discussion

In the current study, at inoculation level of ≤ 125 eggs + J2 and ≥ 625 eggs + J2 the RF values were greater than one and less than one, respectively. The RF for the cultivar 'Beauregard' denoted that the inocula were viable. The RF values below unity denote that the test nematode failed to feed and reproduce on the test plants, whereas values above unity denoted that the nematodes established feeding sites and reproduced on the test plants (Windham and Williams, 1988). Observations in the current study agreed with those of Atungwu *et al.* (2013), where cv. 'Blesbok' had RF values of the test nematodes, namely, *M. javanica*, *M. incognita* races 2 and 4, and *M. arenaria*, being greater than one. The RF values of greater than one in this study indicated that *M. javanica* is able to feed and reproduce on this cultivar at lower numbers, but were not able at high numbers.

According to Seinhorst (1965) nematode resistance is interpreted using two concepts: host-status and host-sensitivity. The RF is used to describe host-status, which is a measure of the reproduction potential of a nematode in a host plant (Windham and Williams, 1988). The RF serves as an indicator of whether a plant is host or non-host to the test nematode (Seinhorst, 1967).

During screening, Pofu *et al.* (2017) indicated that sweet potato cv. 'Blesbok' was non-host to *M. javanica*, but was a host to *M. incognita* races 2 and 4. In screening tests for non-host one inoculation level with eggs and second-stage juveniles (J2) is used. The disadvantage of screening is that the nematode inoculation level could have been above the Seinhorst's equilibrium (E) point, where reproductive potential is inevitably always below one due to competition for infection sites and other resources (Seinhost,

1965). Mashela *et al.* (2015) indicated that screening is an ideal tool for use to provide preliminary results on host-status, but it does not provide information on host-sensitivity, which is essential for making inferences about the degree of nematode resistance. Hence the degree of nematode resistance has to be established for those cultivars shown to be non-host during screening.

Host-sensitivity refers to host response to nematodes (Seinhorst, 1967), which is described by three concepts; resistance host, tolerant host or susceptible host. Generally, when the RF is greater than one and nematode infection reduced plant growth variables, the plant is a susceptible host to the test nematode. Similarly, when RF is greater than one, but nematode infection did not reduce any plant growth variable, the plant is tolerant host. In contrast, when RF is less than one and nematode infection did not reduce plant growth variables, the plant could be viewed as a resistant host (Seinhorst, 1967). In this study root galls were not observed, although this is not a requirement in host-status studies. In *Cucumis* species (Pofu, 2012; Ramatsitsi, 2017) and in *Zea mays* (ARC, 2013) *Meloidogyne* species were observed to develop and reproduce without inducing root galls. Therefore, the absence of root galls does not mean that reproduction and juvenile hatch did not occur (Fourie *et al.,* 2015). Karuri *et al.* (2017) did not observe any root galls on susceptible cultivars of sweet potato cv. 'EM7', with the highest number of eggs. According to Sikora and Fernandez (2005), at some substantial levels, nematode infections have no effect on plant growth.

According to Melakeberham *et al.* (1985), the nutrient element status changes when plants are infected by nematode. However, in most nematode resistance studies, nutrient elements are hardly assessed, which could explain the paucity of such data.

In the current study, results showed that at low inoculation levels, nematode infection stimulated accumulation of nutrient elements in sweet potato cv. 'Blesbok', whereas at high nematode levels, accumulation was inhibited, which is a rare occurrence suggesting limited similar studies in nematode-plant relations. This study results confirmed Fatemy and Evans (1986) hypothesis that the nematode infection reduce the uptake of the nutrients of the cation which are taken actively. Fatemy and Evans (1986) observed similar results were infected potato plants had lower concentration of K and Mg in their dry matter compared to control. Also, the negative quadratic relationship in K confirmed observation by Malebekerham *et al.* (1985) when *Phaseolus vulgaris* exposed to increasing nematodes densities, where the treatment exhibited the quadratic relationship. Nematode can disrupt accumulation of nutrients elements for its own growth which would reduce the supply of nutrients available to the leaves (Oteifa and Elgindi, 1962). Positive quadratic relationship was observed when increasing the level of nematode population densities increases the accumulation of Zn. The optimum level of those negative quadratic relationships was also provided at which the selected essentials nutrients elements would be at optimum. Ca, K, Mg and Fe were optimised at 3, 3, 3 and 2 in Experiment 1 whereas in Experiment 2 were optimised at 1, 1, 1 and 3 levels of *M. javanica,* respectively. In contrast, Zn were minimised at 4 and 3 levels *M. javanica* in Experiment 1 and Experiment 2, respectively. In order to maximise those nutrient elements which exhibited negative quadratic relationships, the test nematode population densities should be managed at 2 nematodes.

3.5 Conclusion

Sweet potato cv. 'Blesbok' was tolerant to *M. javanica* since RF values were above unity at low inoculation levels, but below unity at high inoculation level, whereas all plant variables were not affected by nematode infection. Observed infection of the test cultivar by *M. javanica* changed the accumulation of essential nutrient elements following the DDG patterns. In conclusion, cv. 'Blesbok' is not suitable for use in crop rotation systems intended to suppress population densities of *M. javanica* since it would result in the build-up of nematode numbers for the successor crops. The successful production of cv. 'Blesbok' in areas with high population densities of *M. javanica* would therefore rely on the existence of alternative management strategies of nematodes, which is the focus for the next research chapter.

CHAPTER 4

EFFICACY OF PHYTONEMATICIDES AND VELUM ON SUPPRESSION OF *MELOIDOGYNE* SPECIES IN SWEET POTATO PRODUCTION

4.1 Introduction

Previously, it was shown that sweet potato (*Ipomoea batatas*) cv. 'Blesbok' was tolerant to *Meloidogyne javanica*, with indication that different nematode levels affected the partitioning of various nutrient elements (Chapter 3). In nematode-susceptible crops, the nemarioc-group (Nemarioc-AL and Nemarioc-AG) and nemafric-group (Nemafric-BL and Nemafric-BG) phytonematicides, had been successfully used as alternatives to systematic fumigant nematicides in nematode management (Mashela *et al.,* 2015). In tomato production, the efficacy of Nemarioc-AG phytonematicide in nematode suppression was comparable to aldicarb and fenamiphos synthetic chemical nematicides under field conditions (Mashela *et al.,* 2008), which have since been withdrawn from the agrochemical markets. In potato production, the efficacies of Nemarioc-AL and Nemafric-BL phytonematicides were comparable with that of Velum, suggesting the high potential use of the products under field conditions. Therefore, the objective of this study was to investigate whether the efficacy of Nemarioc-AL and Nemafric-BL phytonematicides would be comparable to that of Velum in sweet potato production in the management of population densities of *Meloidogyne* species under field conditions.

4.2 Materials and Methods

4.2.1 Description of study location

The study was conducted at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, in Limpopo Province of South Africa (23°53′10″ S, 29°44′15″ E) under field conditions. The trial was conducted during autumn (February-April) 2018 and validated in 2019, soon after summer with maximum temperature ranging from 28 °C to 38 °C and less than 500 mm rainfall. The field had Hutton soil (65% sand, 30% clay, 5% silt) containing 1.6% organic C, with EC= 0.148 dS m^{-1} and pH = 7.77. The two trials were 10 m away from each other and 5 m away from *Casuarina cunninghamiana* windbreak trees. The size of the plot was 10 m × 5 m.

4.2.2 Treatments and research design

Treatments, namely, Nemarioc-AL phytonematicide, Nemafric-BL phytonematicide, Velum and untreated control, were laid out in a randomised complete block design, with 10 replications. Treatments in each trial were blocked due to heterogeneous conditions caused by the windbreak trees.

Legend 4.1 Experimental set-up of sweet potato cultivar 'Blesbok' under field conditions.

4.2.3 Procedures and cultural practices

The sweet potato plantlets, originally from the ARC-VOP, were multiplied under greenhouse conditions. The size of greenhouse was 100 m × 20 m with a roof covered by a green net to allow approximately 84% incident radiation to pass through. Thermostatically activated fans on one end and the wet wall on the other end to retain ambient temperature at 26 °C and ensuring that relative humidity was retained from 70 to 80%. The conditions inside the greenhouse were heterogeneous due to the size of greenhouse and the wind-blown generated. During summer (November to January) the greenhouse minimum/maximum temperature average 28/21 °C, whereas winter (May to July) the minimum/maximum average 18/5 °C. Uniform 25 cm sweet potato plantlets were stimulated to root by dipping their lower ends in Seradix rooting hormone with one-third of the lower end of cutting placed in water for 7 days. Plantlets with well-developed root system were transplanted directly in the field with 0.3 m × 0.6 m spacing and each plot had 40 plants. Each experimental site was irrigated using drip irrigation at two days before planting and sampled for initial nematode population density (Pi). Soil samples consisted of 3 composited cores (2.5 cm × 30 cm) per plot, which were collected in a systematic pattern. Total soil cores were mixed and second-stage juveniles (J2) extracted from 250 ml soil subsample using the sugar floatation and centrifugation method (Jenkins, 1964), with the average Pi being 170 J2 *Meloidogyne* species per 250 ml soil subsample.

Each plant was irrigated using 500 ml chlorine-free tap water every other day. Every 19 days, irrigation was substituted with that same quantity of the appropriate phytonematicides and Velum treatments. Treatments were applied using 500 ml beaker and chlorine-free water was applied as control. Plants were fertilised at 5 days and top-dressed at 30 days after transplanting using 5 g NPK 2:1:2 (43) to provide

0.70 mg N, 0.64 mg K, 0.64 mg P, 1.8 mg, 1.5 mg Fe, 0.15 mg Cu, 0.7 mg Zn, 2 mg B, 6 mg Mn and 0.14 mg Mo/ml tap water and 2 g NPK 2:3:2 (26) + 0.5% Zn + 5% S + 5% Ca, respectively, to provide all essential nutrients elements. Pests and diseases were monitored on daily basis, but none was observed. Weeds were removed once using hand held hoes soon after the second fertiliser application.

Nemarioc-AL and Nemafric-BL phytonematicides were produced from wild cucumber (*Cucumis myriocarpus* Naude) and wild watermelon (*Cucumis africanus* L.), respectively, as previously explained (Mashela *et al*., 2017). Briefly, mature fruits were collected from cultivated fields and cut into pieces, dried at 52 °C for 72 h before grinding in Wiley mill. Approximately 80 g dried *C. myriocarpus* powdered fruit were mixed with 300 ml effective microorganisms (EM), 100 g brown sugar and 300 ml molasses in a 20 L hermetically sealed plastic container, filled with 16 L chlorine-free tap water. During fermentation, the released CO_2 was allowed to pass through from the container using an airtight 5-mm-diameter tube with one end glued to a hole on the lid of the 20 L container, dangling into a 1 L bottle half-filled with chlorine free tap water. The mixture was placed in a room with ambient temperature of 37.5 ± 2 °C for 14 days to allow fermentation-induced pH to drop to below 3.7 (Kyan, 1999). The fermentation procedure for Nemafric-BL phytonematicide was similar to that prescribed above for Nemarioc-AL phytonematicide except that 40 g dried *C. africanus* fruit material was used.

4.2.4 Data collection

At 56 days after initiating treatments, vine length was measured from the crown to the tip of the flag leaf using measuring tape. Stem diameter was measured at 3 cm above the soil surface using Vernier calliper, with stems being severed from roots at the soil level. Shoots and sampled roots were oven dried at 52 °C for 72 h, whereas roots were removed using hand-held forks and immersed in a 20 L half-filled with tap water to remove soil particles. Nematode eggs and J2 were extracted from 10 g roots per plant by using maceration and blending method for 60 seconds in 1% NaOCl (Hussey and Barker, 1973) and passed through nested 75 µm and 25 µm sieves. Approximately 500 ml soil sample/plant was collected around roots, with nematode juveniles extracted from 250 ml soil subsample (Jenkins, 1964). In either root or soil samples, nematodes were counted from 5 ml aliquot using a stereomicroscope.

4.2.5 Data analysis

Treatment effects on nematode, plant growth and essential nutrient elements were subjected to analysis of variance (ANOVA) using SAS software (SAS, 2008). However, since other nematode and plant variable data did not meet the normality requirements of ANOVA, the data were further subjected to principle component analysis (PCA). PCA analysis was done in XLSTAT (Addinsoft, 2007). Regarding the results for the F1 and F2, the results were based on the score for the eigenvalues.

4.3 Results

In Experiment 1, both principal component (PC) 1 and PC2 accounted for 98.73% variability on nematode data, whereas PC1 alone accounted for 60.09% in variability of nematode data, whereas PC2 accounted for 38.64% in nematode data (Figure 4.1). PC1 was positively correlated with all measured nematode data, namely, J2 in soil, J2

in roots, eggs in roots, ring nematode (*Criconema* spp.), spiral (*Helicotylenchus* spp.) and reproductive potential, with corresponding control and Nemarioc-AL phytonematicide being on the positive side, whereas Velum chemical nematicide and Nemafric-BL phytonematicide on the negative side. PC2 was positively correlated with J2 in soil, J2 in roots and *Criconema* but negatively correlated with eggs, spiral and reproductive potential corresponding with Nemafric-BL phytonematicide and control treatments were on the positive side of PC2, whereas Velum chemical nematicide and Nemarioc-AL phytonematicide were on the negative side (Figure 4.1).

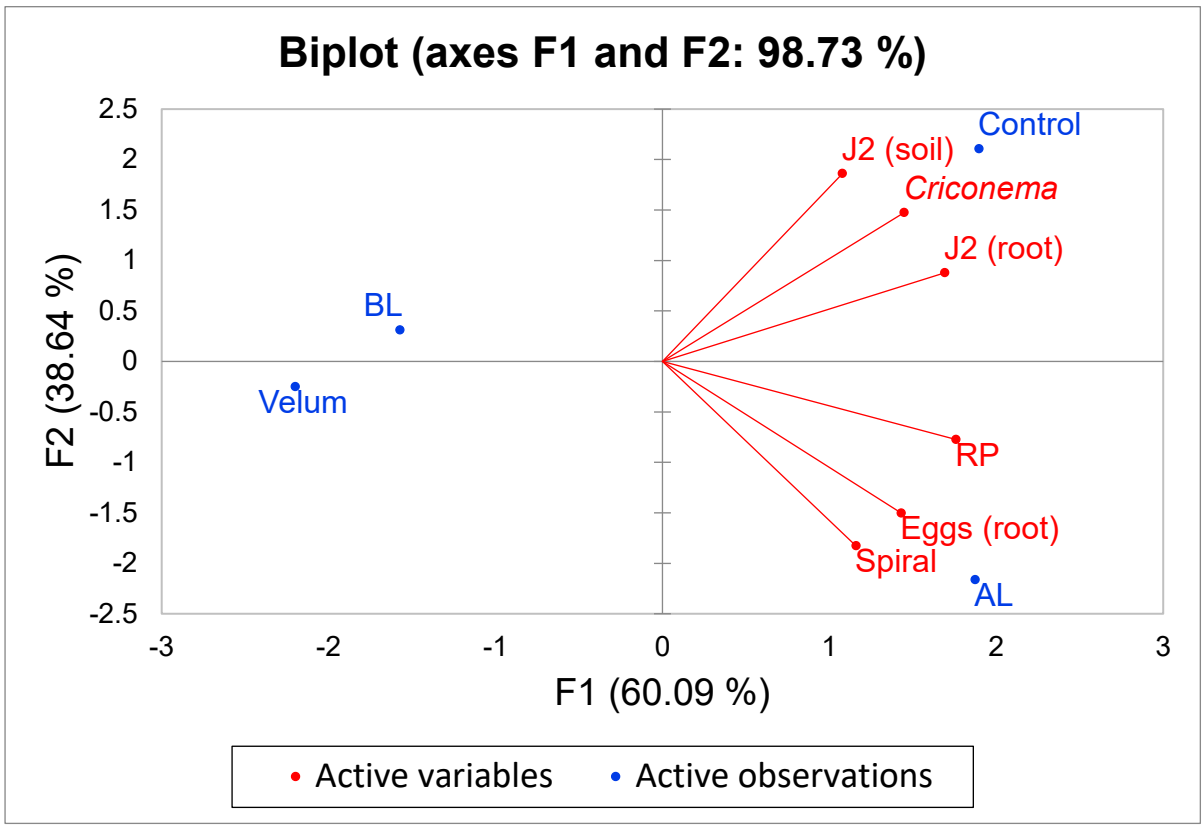

Figure 4.1. Principal components analysis of eggs in root, second-stage juveniles (J2) in soil, J2 in root, reproductive potential (RP), spiral and *Criconema*, where AL = Nemarioc-AL and BL = Nemafric-BL phytonematicides in Experiment 1.

In Experiment 2, PC1 and PC2 contributed 100% on nematode data, whereas PC1 was responsible for 78.28% variability in nematode data (Figure 4.2). PC1 was

positively correlated with C*riconema* and negatively correlated spiral nematode corresponding Nemarioc-AL-, Nemafric-BL phytonematicides and Velum chemical nematicide on the positive side but control on the negative side. PC2 was responsible for 21.72% variability in nematode data. PC2 was positively correlated with *Criconema* and spiral nematodes with control, Nemarioc-AL phytonematicide and Velum chemical nematicide on the positive side, whereas Nemafric-BL phytonematicide was on the negative side (Figure 4.2).

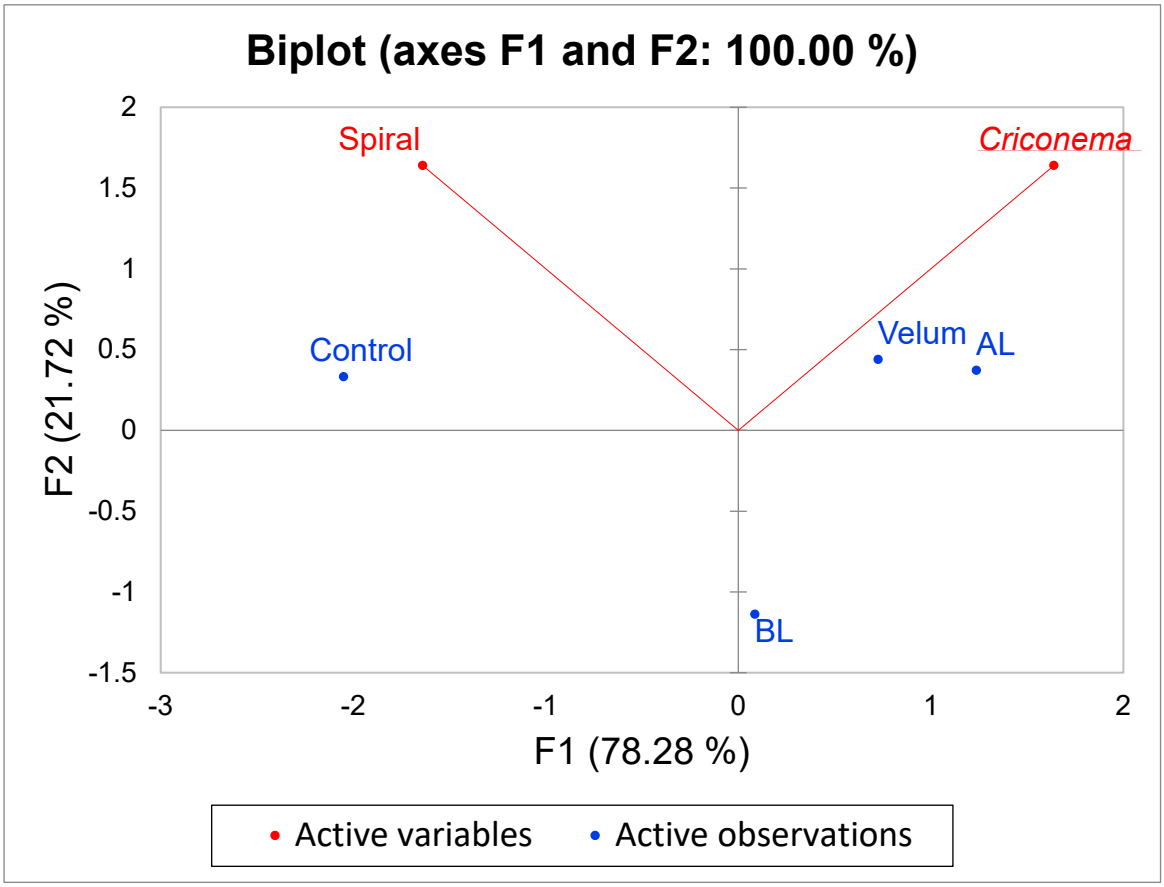

Figure 4.2. Principle component analysis of spiral (*Helicotylenchus* spp) and ring (*Criconema)* in Experiment 2.

In Experiment 1, PC1 and PC2 accounted for 95.75% variability of plant growth data, whereas PC1 alone accounted for 82.42% variability in plant growth data, whereas PC2 accounted for 13.34% in plant variable data (Figure 4.3). PC1 was positively correlated with dry shoot mass, dry root mass, fresh root mass, plant height, chlorophyll content and stem diameter with corresponding Nemafric-BL

phytonematicide and Velum chemical treatments on the positive side, whereas Nemarioc-AL phytonematicide and the control on the negative side. PC2 was positively correlated with stem diameter, chlorophyll content and plant height, but negatively correlated to dry shoot mass, fresh root mass and dry root mass with Nemarioc-AL and Nemafric-BL phytonematicides treatments on the positive side, whereas Velum chemical nematicide and control where on the negative side (Figure 4.3).

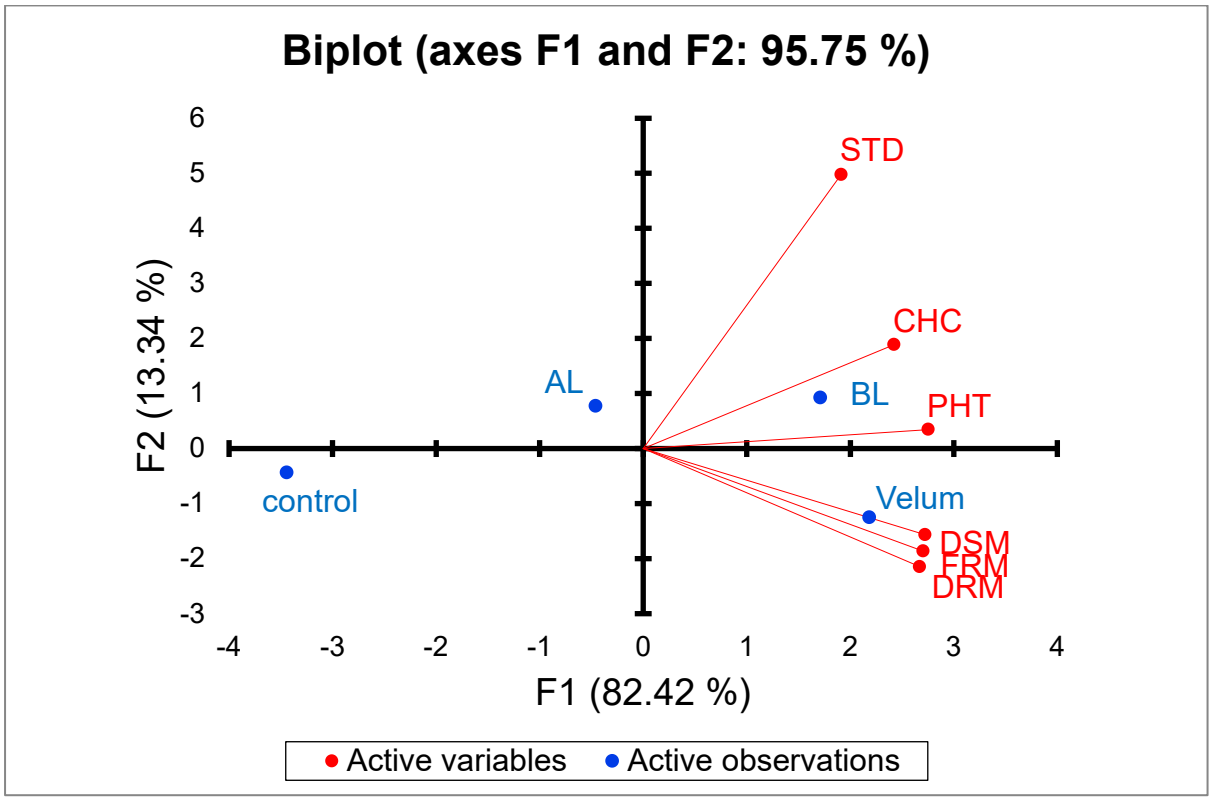

Figure 4.3 Principal component analysis of stem diameter, chlorophyll content, plant height, dry shoot mass, fresh root mass and dry root mass in Experiment 1.

In Experiment 2, PC1 and PC2 accounted for 85.53% variability on the plant growth data, whereas PC1 alone was accounted for 49.58% variability in the plant growth data, whereas PC2 was accounted for 35.95% variability in plant growth data (Figure 4.4). PC1 was positively correlated with DSM, DRM, CHC, PHT and FRM, but negatively correlated with STD, with corresponding Nemafric-BL phytonematicide,

Velum synthetic and control on the positive side, whereas Nemarioc-AL phytonematicide on the negative side. PC2 was positively correlated with STD, DRM, PHT, and CHC, but negatively correlated with DSM and FRM, corresponding with Nemafrioc-BL and Nemarioc-AL phytonematicides on the positive side of PC2 whereas Velum chemical nematicide and control were on the negative side (Figure 4.4).

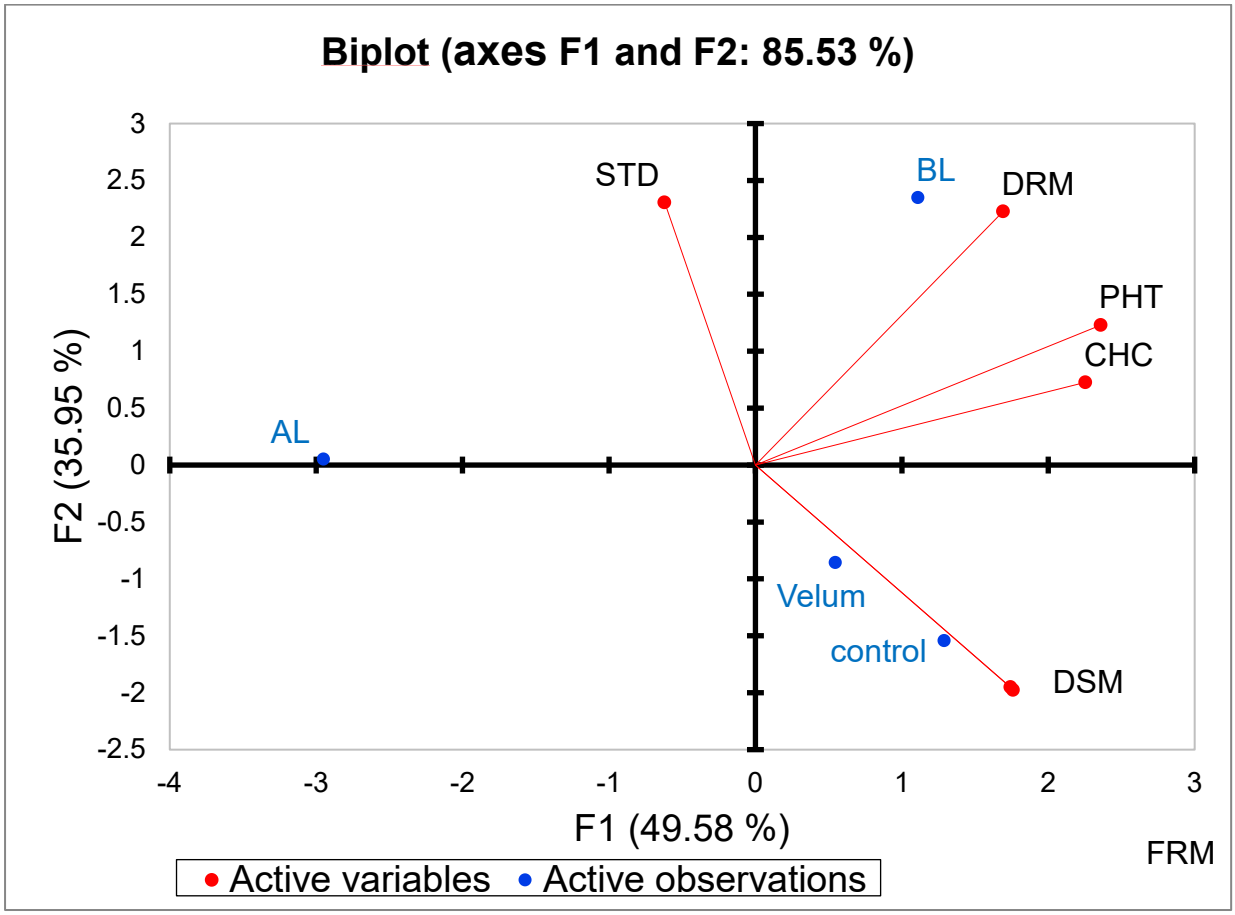

Figure 4.4 Principal components analysis of stem diameter, chlorophyll content, plant height, dry shoot mass, fresh root mass and dry root mass in Experiment 2.

In Experiment 1, PC1 and PC2 accounted for 83.51% variability on the essential nutrient elements data, whereas PC1 alone was accounted 62.73% variability in essential nutrient elements data, whereas PC2 accounted 20.71% variability on essential nutrient elements data (Figure 4.5). PC1 was positively correlated with calcium, magnesium, potassium and iron but negatively correlated with zinc,

corresponding with Velum chemical nematicide and control on the positive side. PC2 was positively correlated with calcium, potassium and zinc but negatively correlated with magnesium and iron corresponding with Nemafric-BL phytonematicide treatment being on the positive side, whereas control, Nemarioc-AL phytonematicide and Velum chemical nematicide treatments were on the negative side (Figure 4.5).

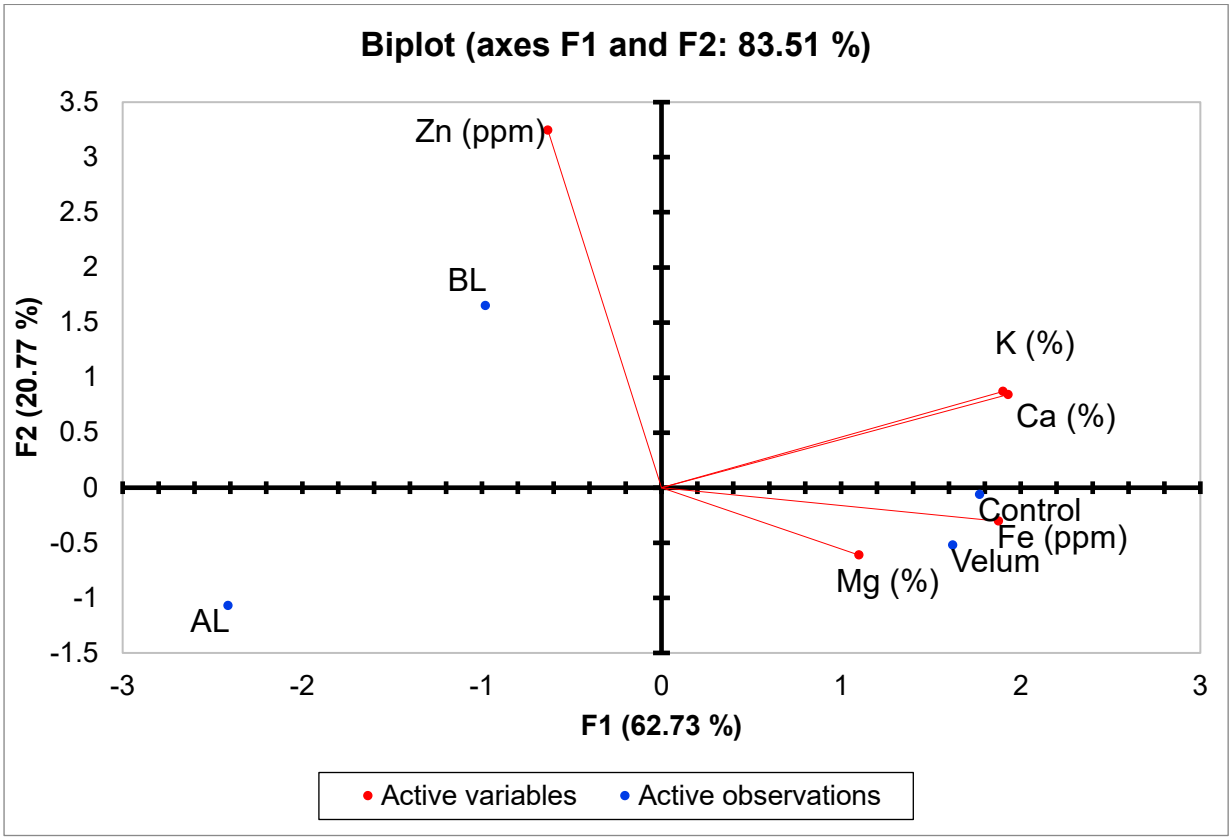

Figure 4.5 Principal components analysis for magnesium, iron, calcium, potassium and zinc in Experiment 1.

In Experiment 2, PC1 and PC2 accounted for 99.95% variability on the essential nutrient elements data, whereas PC1 alone was accounted for 95.04% variability in the essential nutrient elements data, whereas PC2 accounted for 4.91% variability in essential nutrient elements data (Figure 4.6). PC1 was positively correlated with all measured essential nutrient elements, namely, calcium, potassium, magnesium, iron and zinc with Velum chemical nematicide, control and Nemafric-BL phytonematicide, whereas Nemarioc-AL phytonematicide was on the negative side. It was positively correlated with potassium and zinc with Nemafric-BL phytonematicide corresponding

on the positive side, whereas calcium, magnesium and iron were negatively correlated with control, Velum synthetic nematicide, Nemarioc-AL phytonematicide and control corresponding on the negative side of PC2 (Figure 4.6).

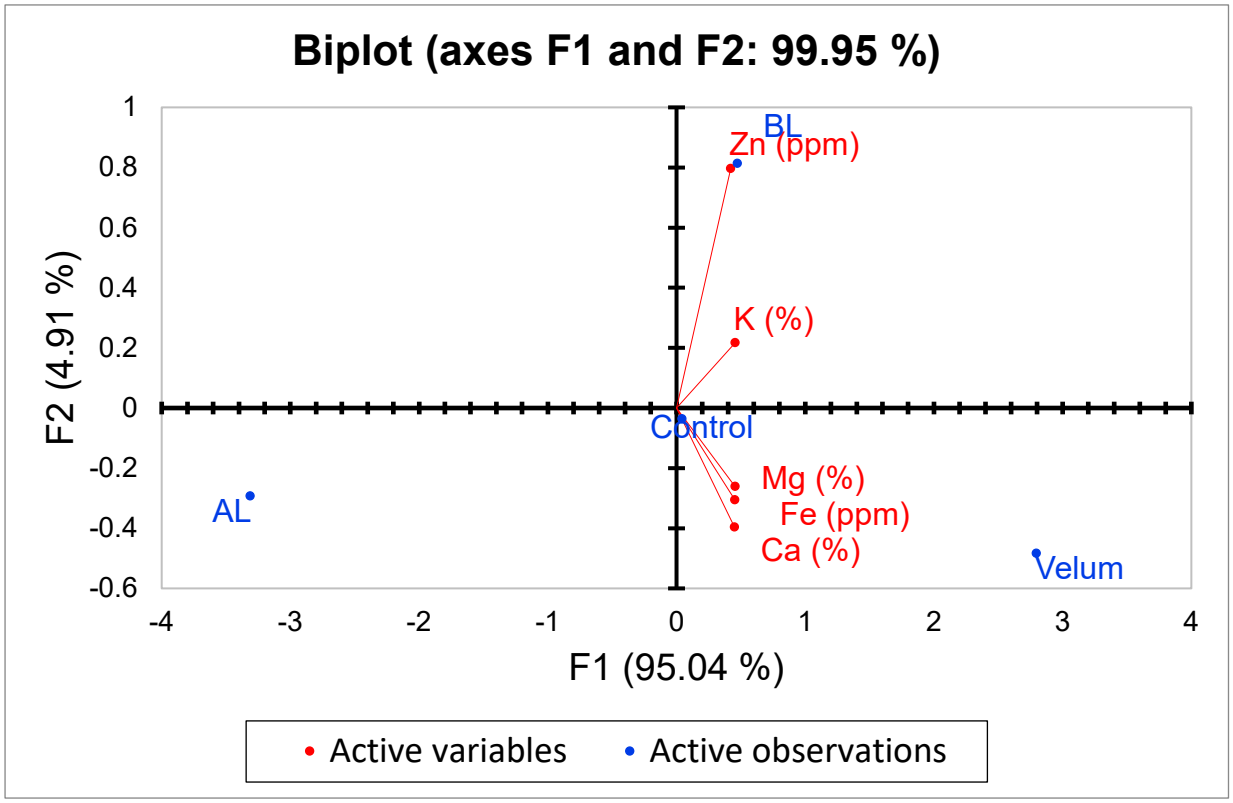

Figure 4.6 Principal components analysis of magnesium, iron, calcium, potassium and zinc in Experiment 2.

4.4 Discussion

Nematode variables: The efficacy of phytonematicides on suppressing nematode population densities were labelled 'inconsistent' since the materials sometimes stimulate (Kimpinski *et al.,* 2003), had no effect (McSorley and Gallaher, 1995) or inhibited (Mashela *et al.,* 2015) nematodes population densities. According to Mashela *et al*. (2015), increasing concentrations of phytonematicides, which are allelochemicals, disrupt nematode population densities through the DDG patterns. However, the effects of allelochemicals on plant phytotoxicity and nematodes are plant-specific (Mashela *et al*., 2015). Phytonematicides had been consistent in suppression of nematode population densities.

All measured nematode data, namely, J2 in soil, J2 in roots and eggs in roots of *Meloidogyne* species, ring nematode (*Criconema* spp.), spiral (*Helicotylenchus* spp.) and the related RP values, corresponded to Velum and Nemafric-BL phytonematicide on the negative side. In this study, Nemafric-BL phytonematicide treatments had effects similar to those of Velum on eggs in roots, J2 in roots, J2 in soil and RP. The results of this study were in agreement with that of Dana (2004) using PCA, where aldicarb chemical nematicide and organic amendments such as mixture of extra filter cake and furfural; trash and filter cake; Thune and filter cake and filter cake suppressed *Meloidogyne* species. Dana (2004) noted that aldicarb and organic amendments had strong negative relationship to *Meloidogyne* species which was well evidenced by PCA. Rodriquez-Kabana *et al*. (1993) emphasized that a furfural contains nematicidal properties.

In the current study, Nemarioc-AL phytonematicide reduced juveniles of plant-parasitic nematodes (*Meloidogyne* species). Similar results were observed by Mwamba (2016), where extracts from *Asteraceae* plants were shown by PCA analysis that PC1 gave 56.6% and 28.5% for PC2 accounting for about 85.1% variability of organic compounds which managed *Meloidogyne* species. The *Asteraceae* plants consist monoterpenes and sesquiterpenes as active ingredients which have insecticidal, nematicidal and repellent properties to most pests (Vasudevan *et al*., 1997). Plants that contain sesquiterpenoids are phytotoxic to nematodes (Chitwood, 2003). Nemarioc-AL phytonematicide consists of cucurbitacin A ($C_{32}H_{46}O_9$) that affect J2 hatch, J2 mobility and J2 mortality (Dube, 2016) and also have the capabilities of destroying the cuticles of J2 in *Meloidogyne* species (Mashela *et al*., 2020).

However, eggs, RP of *Meloidogyne* species and the spiral nematodes were not affected by Nemafric-AL phytonematicide. Generally, it appears that the cucurbitacin-containing phytonematicides would hardly affect the K strategists such as the *Helicotylenchus* species (Mashela *et al.*, 2020). Similar findings were noted by Dana (2004) using aldicarb and organic amendments, where *Helicotylenchus* species had strong negative correlated with *Meloidogyne* species on sugarcane production. This observation indicate that the product had increased the *Helicotylenchus* species which is known to promote plant growth while displacing plant parasitic nematodes since it feeds on the root elongation (penetration) zone, which made *Meloidogyne* species think that it is feeding site for another nematode. This was depicted on the untreated control where there were no *Meloidogyne* species found due to the *Helicotylenchus* species presence. K-strategies nematodes are bigger in size, have long life cycles (Stirling, 2014) and have low reproductive rates. The K-strategies nematodes cannot

be affected by phytonematicides at low concentration since they have structures and chemicals which accord them much more tolerance to adversarial environmental conditions (Wang *et al*., 2009).

In this study, eggs were not affected by Nemarioc-AL phytonematicide. This findings are not in agreement with other observations where Nemarioc-AL phytonematicide reduced all nematode stages including eggs with 100% (Mashela *et al*., 2015), and reduced eggs with 91-100% (Lebea, 2017). The increase of eggs when using Nemarioc-AL phytonematicide confirmed the observation by Dube (2016) when using pure cucurbitacin A and the results revealed that at low concentration the product inhibited juvenile hatch, whereas at high level the activity was stimulated. The *Meloidogyne* species lay their eggs in gelatinous matrix (egg masses) that are usually found outside of galled roots and are protected by chitin. When first-stage juvenile (J1) which occur inside the eggs is gradually exposed to adverse conditions like those of nematicide and/or botanicals, J1 enters survival stage known as dauer stage, where it stops metabolic activities (Mashela, 2007). After, when the conditions are favourable the juvenile exit the survival stage and assume normal activities.

Plant growth variables: In this study, Nemafric-BL phytonematicide and Velum chemical nematicide increase plant growth variables. The results of this study confirmed observation by Montes-Molina *et al.* (2008) where bean plants were grown in the soil which was treated with Mataraton and lambda cyalothrin. Mataraton and lambda cyalothrin treatments were found in the upper quadrant with most measured plant growth variables and they had positive correlation with PC1, but, nodule weight and number of nodule were positively correlated with PC2.

The plants which were treated with Velum chemical nematicide and Nemafric-BL phytonematicide developed better and their chlorophyll content, plant height, dry shoot mass, fresh root mass and dry root mass were increased than those that were treated with Nemarioc-AL and untreated control. The results were in agreement with the hypothesis stated by Farooq *et al*. (2013) that allelochemicals promote plant growth at low concentration. Sithole (2016) indicated that Nemafric-BL phytonematicide at low concentration plant height response very fast compared to other plant growth variables. The increase in root mass by Nemafric-BL phytonematicide and Velum chemical nematicide agreed with the observation of Ertani *et al*. (2018) where seaweeds (*Laminaria* and *Ascophyllum nodosum* spp.*)* extracts enhanced the root system development and plant nutrition which was well evidenced by the PCA analysis, presented gradient where the untreated plants displayed negative correlation. In other studies, seaweed extracts increased plant growth, germination rate, chlorophyll synthesis, fruit quality and post-harvest shelf life (Calvo *et al*., 2014; Goni *et al*., 2016). Seaweed extracts are also known to have functional groups which are corresponding to lipids and phenolics (Ertani *et al*., 2018).

Nemarioc-AL phytonematicide had weak negative correlation with all plant growth variables. This simply means that the material did not have any effect on the growth of the plant. These results were in agreement with the hypothesis stated by Pelinganga *et al*. (2011) and Rice (1984) that the allelopathy was concentration specific, organ specific and plant specific. Rice (1984) stated that the degree of sensitivity in plants to allelochemicals is plant specific. Mashela *et al* (2015) noted that if plant variables are

not affected by phytonematicides it believed that the organs at harvest were still at saturation point.

Essential nutrient elements: The result of this study indicated similar observations where PC1 and PC2 contributed 45.56% and 11.06% accounting for 56.62% variability of total variables (Bessa *et al*., 2016). The PC1 and PC2 in this study elucidated the relationship between different variables. In the current study, Velum chemical nematicide enhanced most of the essential nutrient element variables, followed by Nemafric-BL phytonematicide which had strong positive correlation with Zn. Calcium, Mg and Fe had very strong positive with Velum chemical nematicide. Similar observations were confirmed on maize plants (Ertani *et al.,* 2018) when using seaweed extracts where PC1 explained 42.21% of variability and were highly correlated with N, P, K and S, whereas PC2 explained 20.88% and was negatively correlated with B, Cu, Fe, Mn and Zn of the total variability of essential nutrient elements. According to Nzanza (2006), Mg is the most important element of the chlorophyll molecule and an enzyme activator of energy transfer reaction. The increase of calcium is very vital since its influence on plant quality is easily visible. Calcium is involved in the cell elongation, division, and activation of many important enzymes (Foth and Ellis, 1988). Iron regulates respiration, photosynthesis, and reduces NO_3^- and S which are essential to the plant growth (Mamatha, 2007).

Nemafric-BL phytonematicide enhanced Zn accumulation which was shown by strong positive correlation. Zinc is the most important metal component and activator of most enzymes which are involved in metabolic activities and biochemical pathways (Grotz and Guerinot, 2002; Kabata-Pendias and Pendias, 2001), and it also affects the

formation of chlorophyll and auxins (Mamatha, 2007). However, high quantities of metals such as Zn may induce oxidative stresses which result in changes on the capacity of certain antioxidant enzymes such as catalase, peroxidase, superoxide dismutase and glutathione-ascorbate cycle enzymes (Remans *et al*., 2012).

Nemafric-BL phytonematicide had no significant effect on the accumulation of most essential nutrient elements which was shown by weak correlation. Comprehensive continuous nutrient analysis in different plant organs could not associated with the observed stimulated growth with essential nutrient elements (Mashela and Nthangeni, 2002). Rabothata (2017) observed similar results where Nemafric-BL phytonematicide did not have effects on the accumulation of essential nutrients elements in leaf tissues of *Cleome gynandra* which agrees with the results of this study were Nemafric-BL phytonematicide did not have significant effects on calcium, potassium, magnesium and iron in the leaf tissues of sweet potato plants.

In the current study, Nemarioc-AL phytonematicide had no significant effects on essential nutrient elements, which confirmed other observations (Sebothoma, 2019) where the application interval of Nemarioc-AL phytonematicide had no effect on essential nutrient elements in leaf tissues of sweet potato cv. 'Bophelo'. Mashela *et al*. (2015) and Liu *et al*. (2003) noted that the mode of action which include stimulation, neutral or inhibition depend on the level of concentration and sensitivity of the target plant organs.

4.5 Conclusion

In conclusion, the efficacy of Nemafric-BL phytonematicide was comparable to that of Velum chemical nematicide in suppression of *Meloidogyne* species in sweet potato

production and can be used as an alternative to the chemical nematicide since it is available in local areas and affordable to smallholder farmers. However, the Nemarioc-AL phytonematicide stimulated *Helicotylenchus* species is believed to displace *Meloidogyne* species in the rhizosphere. Therefore, Nemafric-BL phytonematicide could serve as potent bionematicide at low concentration in sweet potato production.

CHAPTER 5

SUMMARY OF FINDINGS, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary of findings

The study focused on the investigating the host-status and host-sensitivity of sweet potato cv. 'Blesbok' and mechanism of nematode resistance to *Meloidogyne javanica* and related management strategies to *Meloidogyne* species on the cultivar. The findings of this study demonstrated that sweet potato cv. 'Blesbok' was tolerant host to *M. javanica* since it allowed reproduction at lower inoculation level, but had no significant effects on plant growth variables. Consequently, mechanisms of nematode resistance were not further investigated. Also, increasing nematode population densities resulted in inducing the imbalances of nutrient elements in the test sweet potato cultivar. Findings in the study also indicated that the efficacy of Nemafric-BL phytonematicide was comparable to that of Velum in suppression of *Meloidogyne* species in sweet potato production under field conditions.

5.2 Significance of findings

Sweet potato cv. 'Blesbok' should not be used in managing population densities of *Meloidogyne* species, especially *M. javanica*, since it would result in the build-up of nematode population densities for subsequent crops. Due to its effects on nutrient elements, it is increasingly imperative that appropriate fertilisation strategies be developed in areas where sweet potatoes are subjected to high nematode population densities. The comparability in the efficacy of the phytonematicides and Velum in

nematode suppression, augers well for the registration of the former products for use in nematode management.

5.3 Recommendations

The mechanism through which *M. javanica* stimulated and then inhibited the accumulation of nutrient elements following the DDG patterns in sweet potato cultivars, would enhance the understanding of the related interactions. Observations in the study suggested that large nematodes such as *Helicotylenchus* species were not sensitive to phytonematicides at the concentration used for smaller nematodes like *Meloidogyne* species and therefore, studies should be conducted for establishing appropriate concentrations for the former. Also, further experiments should be conducted to quantify yield reduction due to nematodes.

5.4 Conclusions

Sweet potato cv. 'Blesbok' was tolerant to infection by *M. javanica,* although the accumulation of nutrient elements was highly susceptible to nematode infection. The cv. 'Blesbok' should never be included in crop rotation programmes for suppressing *Meloidogyne* species since it would result in nematode build-up for the successor crops. Nemafric-BL phytonematicide was comparable to Velum in suppression of *Meloidogyne* species. Therefore, Nemafric-BL phytonematicide could be used as an alternative to Velum in management of nematode population densities in areas where *M. javanica* were shown to be high.

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APPENDICES

Appendix 1. Analysis of variances for the eggs of *Meloidogyne javanica* on sweet potato cv. 'Blesbok' under greenhouse conditions at 56 days after inoculation for Experiment 1.

Source	DF	SS	MSS	F	P
Replication	5	7.9791	1.59582		
Treatment	5	6.8849	1.37697	2.68	0.0451
Error	25	12.8443	0.51377		
Total	35	27.7083			

Appendix 2. Analysis of variances for the eggs of *Meloidogyne javanica* on sweet potato cv. 'Blesbok' under greenhouse conditions at 56 days after inoculation for Experiment 2.

Source	DF	SS	MSS	F	P
Replication	5	0.9567	0.19134		
Treatment	5	5.1862	1.03723	5.50	0.0015
Error	25	4.7118	0.18847		
Total	35	10.8546			

Appendix 3. Analysis of variances for the second stage juveniles (J2) in roots of *Meloidogyne javanica* on sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation for Experiment 1.

Source	DF	SS	MSS	F	P
Replication	5	5.6757	1.13513		
Treatment	5	5.6262	1.12524	4.93	0.0028
Error	25	5.7078	0.22831		
Total	35	17.0097			

Appendix 4. Analysis of variances for the second stage juveniles (J2) in roots of *Meloidogyne javanica* on sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation for Experiment 2.

Source	DF	SS	MSS	F	P
Replication	5	0.31477	0.06295		
Treatment	5	2.24671	0.44934	2.96	0.0310
Error	25	3.79140	0.15166		
Total	35	6.35288			

Appendix 5. Analysis of variances for the second stage juveniles (J2) in soil of *Meloidogyne javanica* on sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation for Experiment 2.

Source	DF	SS	MSS	F	P
Replication	5	0.4245	0.08490		
Treatments	5	5.7768	1.15537	3.40	0.0137
Error	25	8.4986	0.33994		
Total	35	14.6999			

Appendix 6. Analysis of variances for the final population (Pf) of *Meloidogyne javanica* on sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation for Experiment 1.

Source	DF	SS	MSS	F	P
Replication	5	8.5976	1.71953		
Treatments	5	7.7270	1.54540	3.80	0.0106
Error	25	10.1549	0.40620		
Total	35	26.4795			

Appendix 7. Analysis of variances for the final population (Pf) of *Meloidogyne javanica* on sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation for Experiment 2.

Source	DF	SS	MSS	F	P
Replication	5	0.7174	0.14349		
Treatments	5	4.5389	0.90778	4.51	0.0046
Error	25	5.0349	0.20139		
Total	35	10.2912			

Appendix 8. Analysis of variances for the reproductive factor (RF) *Meloidogyne javanica* on sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation for Experiment 1.

Source	DF	SS	MSS	F	P
Replication	5	0.26774	0.05355		
Treatments	5	5.41833	1.08367	24.20	0.0000
Error	25	1.11926	0.04477		
Total	35	6.80533			

Appendix 9. Analysis of variances for the reproductive factor (RF) *Meloidogyne javanica* on sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation for Experiment 2.

Source	DF	SS	MSS	F	P
Replication	5	0.07520	0.01504		
Treatments	5	5.19378	1.03876	84.97	0.0000
Error	25	0.30561	0.01222		
Total	35	5.57459			

Appendix 10. Analysis of variances for the vine length (VNL) of sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation with *Meloidogyne javanica* for Experiment 1.

Source	DF	SS	MSS	F	P
Replication	5	17813	3562.56		
Treatments	5	6443	1073.87	0.42	0.8614
Error	25	77147	2571.57		
Total	35	101403			

Appendix 11. Analysis of variances for the vine length (VNL) of sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation with *Meloidogyne javanica* for Experiment 2.

Source	DF	SS	MSS	F	P
Replication	5	10198.7	2039.74		
Treatments	5	2326.5	387.75	0.23	0.9630
Error	25	50253.8	1675.13		
Total	35	62779.0			

Appendix 12. Analysis of variances for the stem diameter (STD) of sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation with *Meloidogyne javanica* for Experiment 1.

Source	DF	SS	MSS	F	P
Replication	5	4.081	0.81623		
Treatments	5	34.119	5.68645	2.11	0.0814
Error	25	80.824	2.69412		
Total	35	119.023			

Appendix 13. Analysis of variances for the stem diameter (STD) of sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation with *Meloidogyne javanica* for Experiment 2.

Source	DF	SS	MSS	F	P
Replication	5	11.3597	2.27193		
Treatments	5	5.3493	0.89155	0.47	0.8259
Error	25	57.0534	1.90178		
Total	35	73.7623			

Appendix 14. Analysis of variances for the chlorophyll content (CHC) of sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation with *Meloidogyne javanica* for Experiment 1.

Source	DF	SS	MSS	F	P
Replication	5	484.61	96.922		
Treatments	5	785.65	130.941	1.11	0.3822
Error	25	3552.49	118.416		
Total	35	4822.75			

Appendix 15. Analysis of variances for the chlorophyll content (CHC) of sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation with *Meloidogyne javanica* for Experiment 2.

Source	DF	SS	MSS	F	P
Replication	5	180.24	36.0473		
Treatments	5	388.16	64.6930	1.26	0.3040
Error	25	1537.36	51.2454		
Total	35	2105.76			

Appendix 16. Analysis of variances for the dry shoot mass (DSM) of sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation with *Meloidogyne javanica* for Experiment 1.

Source	DF	SS	MSS	F	P
Replication	5	76.971	15.3941		
Treatments	5	62.937	10.4896	0.45	0.8415
Error	25	704.615	23.4872		
Total	35	844.523			

Appendix 17. Analysis of variances for the dry shoot mass (DSM) of sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation with *Meloidogyne javanica* for Experiment 2.

Source	DF	SS	MSS	F	P
Replication	5	41.942	8.3883		
Treatments	5	28.601	4.7669	0.25	0.9557
Error	25	573.290	19.1097		
Total	35	643.833			

Appendix 18. Analysis of variances for the dry root mass (DRM) of sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation with *Meloidogyne javanica* for Experiment 1.

Source	DF	SS	MSS	F	P
Replication	5	9.7814	1.95629		
Treatments	5	7.4062	1.23437	0.92	0.4956
Error	25	40.3281	1.34427		
Total	35	57.5157			

Appendix 19. Analysis of variances for the dry root mass (DRM) of sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation with *Meloidogyne javanica.*

Source	DF	SS	MSS	F	P
Replication	5	12.9980	2.59961		
Treatments	5	10.3570	1.72617	1.52	0.2053
Error	25	34.0474	1.13491		
Total	35	57.4024			

Appendix 20. Analysis of variances for the number of tubers (NOT) of sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation with *Meloidogyne javanica* for Experiment 1.

Source	DF	SS	MSS	F	P
Replication	5	0.68641	0.13728		
Treatments	5	0.23350	0.03892	0.44	0.8473
Error	25	2.66359	0.8879		
Total	35	3.58349			

Appendix 21. Analysis of variances for the number of tubers (NOT) of sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation with *Meloidogyne javanica* for Experiment 2.

Source	DF	SS	MSS	F	P
Replication	5	1.0476	0.20952		
Treatments	5	13.9048	2.31746	2.25	0.0658
Error	25	30.9524	1.03175		
Total	35	45.9048			

Appendix 22. Analysis of variances for the dry tuber mass (DRT) of sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation with *Meloidogyne javanica* for Experiment 1.

Source	DF	SS	MSS	F	P
Replication	5	62.561	12.5122		
Treatments	5	19.335	3.2225	0.28	0.9429
Error	25	347.806	11.5935		
Total	35	429.702			

Appendix 23. Analysis of variances for the dry tuber mass (DRT) of sweet potato cv 'Blesbok' under greenhouse conditions at 56 days after inoculation with *Meloidogyne javanica* for Experiment 2.

Source	DF	SS	MSS	F	P
Replication	5	1.4332	0.28664		
Treatments	5	18.4133	3.06888	2.22	0.0681
Error	25	41.4053	1.38018		
Total	35	61.2518			