INTERACTIVE EFFECTS OF *MELOIDOGYNE* SPECIES AND SUGARCANE APHID (*MELANAPHIS SACCHARI)* ON NEMATODE RESISTANCE IN SWEET STEM SORGHUM AND EFFECTS OF TERPENOID-CONTAINING PHYTONEMATICIDES ON BOTH PESTS

by

KOENA GIDEON MALEKA

THESIS

Submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

AGRICULTURE (PLANT PRODUCTION)

in the

FACULTY OF SCIENCE AND AGRICULTURE

(School of Agricultural and Environmental Sciences)

at the

UNIVERSITY OF LIMPOPO, SOUTH AFRICA

SUPERVISOR: PROF P.W. MASHELA

CO-SUPERVISOR: DR K.M. POFU

2020

TABLE OF CONTENTS Page

DECLARATION

I, Maleka Koena Gideon, declare that the thesis hereby submitted to the University of Limpopo, for the degree Doctor of Philosophy in Agriculture (Plant Production) has not been submitted previously by me or anybody 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 had been duly acknowledged.

Maleka KG

Date

DEDICATION

To my lovely wife, Refiloe Raisibe Maleka and my beloved sons (Thapelo and Thato Maleka) and daughter (Magau Maleka). My beloved parents, Paulina Phuti and the late Johannes Matseba Maleka, may his soul rest in peace.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my gratitude to my supervisors, Professor P.W. Mashela and Dr K.M. Pofu. Their patience had considerably added more value to research and writing of this thesis. It is indeed through their persistence and kindness that I completed my thesis. To Professor P.W. Mashela, words cannot express my sincere gratitude for the academic and financial support. I am humbled by the amount of support and priceless scientific knowledge he imparted to me. Dr K.M. Pofu showed me the generosity of continuously providing much needed research consumables and editorial comments. I must acknowledge members of the Green Biotechnologies Research Centre of Excellence, for all the technical support. I also recognise that this research would not have been possible without the financial assistance of National Research Foundation of South Africa. This thesis has been, without doubt, the single largest test of my own commitment, patience and perseverance. Though you may not see your name here, please know that your various contributions have not gone unnoticed or unappreciated. My deepest gratitude goes to my family for their indefatigable love and unconditional support throughout my life and my studies. Finally, I give all glory to the Almighty God, the source of all wisdom.

LIST OF TABLES

LIST OF FIGURES Page

LIST OF APPENDICES Page

ABSTRACT

Worldwide, both root-knot (*Meloidogyne* species) and sugarcane aphid (*Melanaphis sacchari* Zehntner), are economic pests on sugarcane and sorghum crops. In most cases, each of the two pests is managed using host plant resistance due to the economic benefits derived from this management strategy. The highly nematode resistant sweet stem sorghum (*Sorghum bicolor* L.) cv. 'Ndendane-X1' used in ethanol production, is highly sensitive to sugarcane aphid, with some indication that the latter could interfere with nematode resistance in the sorghum cultivar. This study had four objectives which collectively intended to investigate the interactive effects of infection by three *Meloidogyne* species and infestation by aphid under different conditions on resistance to nematode in a nematode-resistant sorghum cultivar. The research objectives were achieved through four trials. In each trial a 2 × 2 factorial experiment, each with and without nematode and aphid as first and second factors, respectively, were conducted. Treatments were arranged in a randomised complete block design, with six replications, and each experiment validated in time. At 150 days, after emergence, the nematode × aphid interaction significantly reduced sucrose by 17, 74 and 42% in *Meloidogyne enterolobii, Meloidogyne incognita* and *Meloidogyne javanica* trials, respectively. Aphid infestation of sorghum significantly increased the reproductive potentials of the three respective *Meloidogyne* species by 196, 320 and 152%, but significantly, reduced plant growth variables from 20-44 and 48-51% in two respective trials. The mineral nutrients S and Zn were reduced in leaf tissues of the test cultivar in Trial 1, whereas Ca and Zn were respectively reduced by 24 and 51% in Trial 2 and by 52 and 51% in Trial 3. Since the reproductive potential values for *Meloidogynqe* species on the test sorghum cultivar were greater than unity and nematode infection reduced the plant variables, cv. 'Ndendane-X1' lost resistance to

the test *Meloidogyne* species. In achieving Objective 2, procedures were similar to those in Objective 1 except that the study was conducted under field conditions under mixed nematode populations of *M. enterolobii, M. incognita* and *M. javanica*. Sorghum seedlings were raised at 0.3 m × 0.3 m inter and intra row spacings. Soon after emergence, plants were thinned to one per station, randomly selected for nematode and nematode-aphid treatments. Mixed populations of *Meloidogyne* species (*M. enterolobii, M. incognita* and *M. javanica*) at approximately 1:1:1 (v/v) ratio were applied at 300 eggs + J2 per plants after thinning at the five plants which were used as nematode alone treatments. The latter were also infested with 20 sugarcane aphids to constitute a nematode + aphid treatments. Buffer zone plants separating the treatments were monitored for aphids and stock borer, which were sprayed when necessary. At 150 days after infestation, relative to nematode alone, nematode-aphid significantly reduced degrees Brix from 13% to 61%, but significantly increased the reproductive potential of mixed *Meloidogyne* species and root galls by 279 and 199%, respectively. Also, the combined effect significantly reduced plant growth variables from 35 to 55% and the mineral nutrient elements in leaf tissues of the cultivar from 33 to 73%. At 150 days after the treatment, the second and first order interaction (Nemarioc-AL × Nemafric-BL × Mordica and Nemafric-BL × Mordica) had significantly increased sucrose content from 48 to 66%, increased plant growth variables from 49 to 163%, increased accumulation of certain nutrient elements from 164 to 206%. The terpenoid-containing phytonematicides could have potential future application in the husbandry of ethanol-producing sweet stem sorghum cultivars in relation to increasing sucrose above the 16% minimum for premium delivery fees and increased plant growth. Under field conditions, in pest-free condition (Objective 3), drenched terpenoid-containing phytonematicides significantly increased sucrose content at

middle and bottom part of SSS cv. 'Ndendane-X1' by 66 and 48%. However, these products did not significantly increase plant variables, except tiller number, which was increased by 163 under first order interaction from Nemafric-BL and Mordica phytonematicides. Similarly, nutrient elements variables had generally not been increased by the interaction of these products, except Ca and K, which were increased by 206 and 164%. In achieving Objective 4, a 2 × 2 × 2, with the first, second and third factor being Nemarioc-AL (with and without), Nemafric-BL (with and without) and Mordica (with and without) phytonematicides, respectively. on sorghum cultivar infected with a mixture of *Meloidogyne* species and infested with aphids, under microplot conditions, untreated control sucrose content remained below the standard of 16 degrees Brix, whereas the second order interaction increased the variable far above the standard, along with various plant growth variables also increased. However, both nematode and aphid population densities were significantly reduced by the interactions. Findings in this thesis constituted the first report where aphid infestation broke resistance to *Meloidogyne* species in sweet stem sorghum cv. 'Ndendane-X1'. Therefore, the successful use of nematode resistance in the cultivar in areas with high nematode population densities would depend upon the effective management of the sugarcane aphid population densities. Also, the three terpenoid-containing phytonematicides would when combined or used alone have the potential future in the husbandry of sweet stem sorghum cultivars intended for ethanol production and suppression of nematode population densities.

LIST OF PUBLICATIONS EMANATED FROM THE THESIS

a. Maleka, K.G., P.W. Mashela and K.M. Pofu. 2020. Influence of sugarcane aphid (*Melanaphis sacchari*) infestation on resistance of sweet stem sorghum (*Sorghum bicolor*) to *Meloidogyne* species under field conditions.
a. Maleka, K.G., P.W. Mashela and K.M. Pofu. 2020. Interference of sugarcaneaphid with sugar content in sweet stem sorghum and its resistance to *Meloidogyne* species under microplot conditions. Research on Crops 21(3): 621-626.

CHAPTER 1
GENERAL INTRODUCTION TO RESEARCH PROBLEM

1.1 Introduction

Plant defence systems such as host resistance are dependent upon physiological activities that produce either primary or secondary metabolites, which manifest through the availability of energy and organic carbon (Narayani and Srivastava, 2017). Primary metabolites such as carbohydrates, vitamins, proteins (or amino acids) and/or mineral elements, have their accumulation being reliant on solar energy captured through photosynthesis (Bisen *et al*., 2012). Roots as sources for most primary metabolites are dependent upon leaves for photosynthates, whereas leaves are dependent upon roots for water and minerals, all of which constitute mutual interdependencies (Wardlaw, 1990). Such interdependencies are important in pest-plant interactions, particularly when pests simultaneously infest leaves and roots, with aphids and nematodes being examples. Aphids (Hemiptera: *Aphidoidae*), mites (Arthropoda: *Tetranychidae*), mealybugs (Hemiptera: *Pseudococcida*e), greenhouse whiteflies (Scientific name: *Trialeurodes vaporariorum* Westwood) and scale insects (Hemiptera: *Coccoidea*), are phloem-feeders, with mouth parts transformed into stylets with lumens, which are inserted into phloem vessels to suck sucrose, thus, the name, sucking insects. In plants, sucrose as a product of photosynthesis is translocated from photosynthesising organs to sinks (Salisbury and Ross, 1992). During feeding, due to high turgor pressure inside the phloem vessels (Salisbury and Ross, 1992), sucrose is lost as honeydew, which is eventually covered by sooty mould on plant surfaces (Singh *et al*., 2004). The mould further reduces the photosynthetic area and capacity of the affected plant surfaces. Sucrose losses could affect plant defence systems, which are dependent upon primary metabolites (Mashela *et al*.,

2016). Subsequently, factors such as climate, pests, poor cultural practices, soil type and water quality, could affect plant defence systems.

Climate change predictions suggested that most plant pests would experience significantly reduced ontogenies due to high temperatures by the year 2030 (Both *et al*., 2009). Therefore, most plant pests, including nematodes, would have high population densities and probably with increased aggressiveness due to inter- and intra-specific competition for space and food (Walther *et al*., 2002). In crop husbandry, plants with resistance to pests would be much preferred as management strategy since most of the previously preferred pesticides had been withdrawn from the agrochemical markets due to their being environment-unfriendly (Mashela *et al*., 2015). However, increasing temperatures would not only affect the ontogenies of plant pests, but would also affect plant resistance as shown in resistance to the root-knot (*Meloidogyne* species) nematodes in the late 1960s. During that time, Dropkin (1969) observed that when 3-day-old tomato seedlings with Mi-1 resistance were exposed to temperature above 33°C for four days after inoculation with *Meloidogyne* species and retained at 27°C for 1 month, nematode resistance was completely lost. That constituted the first record which showed that highly nematode resistance in plants could be compromised by environmental factors, with numerous confirmations (Mashela *et al*., 1992). Various predictions suggest that with global warming, temperatures, by 2030, would be as high as 38°C inland South Africa.

Twenty-three years later, Mashela *et al*. (1992) demonstrated that exposure of nematode resistant citrus seedlings to cyclic salinity broke resistance to the citrus

nematode (*Tylenchulus semipenetrans* Cobb 1913). Incidentally, global-warming with high incidents of flooding and high evapotranspiration, would be characterised by repeated incidents of cyclic salinity. Actually, citrus-producing regions with cyclic salinity such as south-eastern Florida, USA, have high population densities of *T. semipenetrans* (Duncan *et al*., 1995). After demonstrating that wild watermelon (*Cucumis africanus* L.F.) and wild cucumber (*Cucumis myriocarpus* Naude.) were highly resistant to thermophilic *Meloidogyne* species such as *M. incognita* and *M. javanica* (Pofu *et al.*, 2010). Later, Liu *et al*. (2015) confirmed that *C. africanus* was higly resistance to *Meloidogyne* species, whereas *C. myriocarpus* was moderately resistant to the test nematode. Interestingly, Pofu *et al.* (2011) observed that *T. vaporariorum* broke resistance to *M. javanica* in *C. africanus* under greenhouse conditions. In an overview on plant genes involved in nematode resistance in transgenic plants (Mashela *et al*., 2017a), emphasis was on illustrating that all chemical compounds involved in plant genes that confer nematode resistance in plants have their origin from photosynthates. Consequently, any factor that could disturb the abundance and/or availability of the photosynthates, might result in the loss of resistance to nematodes.

Highly nematode-resistant sorghum cv. 'Ndendane-X1' genotype, was observed to be highly susceptible to sugarcane aphids (Mashela and Pofu, 2016). Sweet stem sorghum (*Sorghum bicolor* L.) belongs to Poaceae family and has dual uses for food (grain) and biofuel (stalk) production as a substitute for fossil energy (Mashela and Pofu, 2016). The plant attained international attention since future use over fossil-fuel energy was deemed to be one of the major contributors to the formation of greenhouse layer (Kamrun, 2011; Reddy e*t al*., 1995). Sweet sorghum has high sugar content (Lee

and Bressan, 2006), with degrees Brix (°Bx) ranging from 14 to 23% (Vinutha *et al*., 2014). The recommended minimum °Bx for biofuel production in sorghum cultivars is 16% and above (Tran *et al*., 2017).

1.2 Problem statement

A highly nematode-resistant sweet stem sorghum (SSS) cv. 'Ndendani-X1' was identified in South Africa as being suitable for the production of ethanol for biofuel due to its high sucrose content (Mashela and Pofu, 2016). However, preliminary observations under field conditions suggested that the cultivar was highly susceptible to damage by sugarcane aphid (*Melanaphis sacchari* Zehntner), which is also widely spread in sugarcane fields in South Africa. Since cv. 'Ndendani-X1' was highly resistant to various *Meloidogyne* species (Mabuka, 2013), the observation that it was sensitive to aphids was in agreement with the principle that the plant genes that confer nematode resistance in pest-plant relations do not necessarily confer insect resistance or *vice versa* (Mashela *et al*., 2016). Due to energy-demanding nature of nematode resistance, whether pre-infectional or post-infectional nematode resistance (Kaplan and Davis, 1987), the efficacy of resistance is exclusively dependent upon the photosynthates. Observations that resistance can be lost under conditions such as high temperature (Dropkin, 1969), cyclic salinity (Mashela *et al*., 1992) and certain sucking insects (Pofu *et al*., 2011), all could be characterised by having the ability to deplete carbohydrates. Although *Meloidogyne* species and *T. semipenetrans* are consistently suppressed by cucurbitacin-containing phytonematicides (Mashela *et al*., 2017b), the efficacy of these products on insect pests had been inconsistent. Aphid-nematode interaction on nematode resistant SSS cv. 'Ndendane-X1' had not been investigated, which would also generate knowledge on the compatibility of nematode

resistance with other management strategies when a plant is challenged by competing pests.

1.3 Rationale

Sweet stem sorghum cv. 'Ndendane-X1', with high economic potential in context of climate change, has been identified as having distinctive attributes required for the potential production of biofuel, with °Bx being as high as 20% (Mashela and Pofu, 2016). The cultivar was shown to have pre-infectional resistance to *Meloidogyne* species, but with high susceptibility to sugarcane aphids. Worldwide, both aphids and *Meloidogyne* species are of great economic importance and separately cause huge crop losses (McDonald and Nicol, 2005). Thermophilic *Meloidogyne* species such as *M*. *incognita* race 2, *M. incognita* race 4, *M. javanica* and *M. entorolobii* are the most common and predominant in tropical and subtropical regions (Collett, 2020; Onkendi *et al*., 2014), where the production of SSS is common (Singh *et al*., 2004). Prior to the withdrawal of methyl bromide from the agrochemical markets in 2005, crop yield losses due to nematodes were at US$126 billion (Chitwood, 2003), whereas three and eight years after the withdrawal the losses were recorded at US$157 (Abad *et al*., 2008) and US$173 billion (Elling, 2013), respectively, accounting for relative increases in yield loss of 25 and 37%, respectively (Mashela *et al.*, 2016). Similarly, sugarcane aphid is endemic in sorghum- and sugarcane-producing regions (Singh *et al*., 2004). Like the SSS, the sugarcane aphid is indigenous to Africa, but the pest is currently also widely distributed in Asia, North America, South America and Australia, actually wherever sorghum is cultivated (Singh *et al*., 2004). Sugarcane aphid infestation is characterised by large quantities of honeydew, with resultant global grain yield losses

ranging from 20 to 40% (Chapin *et al*., 2000), whereas aphid-virus transmitted diseases account for 34-67% losses (Riedell *et al*., 2003).

Following the withdrawal of synthetic chemical pesticides from the agrochemical markets, the use of resistant cultivars has received much attention as an alternative management strategy of pests, particularly the plant nematodes (Mashela *et al*., 2015). The use of nematode resistance is preferred since it is compatible with many other nematode management strategies such as the use of biocontrol agents like *Steinernema* species and *Trichoderma* species (Madaure *et al*., 2018), along with phytonematicides such as the cucurbitacin-containing phytonematicides *(*Mashela *et al.,* 2017b*).* Cucurbitacin-containing phytonematicides have been produced from fruits of *C. africanus and C. myriocarpus* as Nemarioc-AL and Nemafric-BL phytonematicides, respectively (Mashela *et al*., 2017b). The active ingredients of the respective phytonematicides are cucurbitacin A ($C_{32}H_{46}O_8$) and cucurbitacin B ($C_{32}H_{46}O_8$) (Mashela *et al*., 2015), which are triterpenoids (Van Wyk and Wink, 2004). The two phytonematicides have been widely tested against thermophilic *Meloidogyne* species and *T. semipenetrans*, with consistent results on nematode suppression, whereas their counterpart, Mordica phytonematicide, produced from bitter gourd (*Momordica balsamina* L.) that contains mormodin ($C_{42}H_{66}O_{13}$) active ingredient (Thakur *et al*., 2009). All three plant species are in the family Cucurbitaceae. However, Mordica phytonematicide had not been widely tested against suppression of plant nematodes, except under *in vitro* conditions (Thakur *et al*., 2009). The cucurbitacin-containing phytonematicides were shown to be highly compatible with nematode resistance in SSS for the purpose of managing population densities of *Meloidogyne*

species (Mabuka, 2013). However, the interactive effects of the three products have not been tested as phytonematicides or as phytoinsecticides.

Mechanisms of nematode resistance include pre-infectional and post-infectional nematode resistance (Kaplan and Davis, 1987). In pre-infectional nematode resistance, the pre-formed chemical compounds with origin from metabolites are released into the rhizosphere primarily as secondary metabolites, thereby repelling the second-stage juveniles (J2) from the rhizosphere. Penetration of roots further releases chemicals which promote J2 hatch and in the absence of the activity, developing eggs still in J1 enter the dauer stage (Cassada and Russell, 1975), whereas other J2 fail to hatch, resulting in chemiobiosis, which is one form of cryptobiosis (Gallaher *et al*., 1991). In contrast, in post-infectional nematode resistance, J2 penetrate the root systems (Kaplan and Davis, 1987), with various plant genes triggered, resulting in J2 being trapped inside the root system (Mashela *et al*., 2017a). Incidentally, the plant genes in this form of resistance are also products of chemical compounds with their origin from photosynthates, which are activate when a foreign entity is detected. Consequently, exposure of nematode resistant plants to sucking insect pests in areas with nematode population densities, could predispose plants to damage by nematodes due to loss of nematode resistance. Such a hypothesis has not been tested on SSS cv. 'Ndendane-X1', which has high pre-infectional nematode resistant capabilities to *Meloidogyne* species.

1.4 Aim

To investigate aphid-nematode interaction in sweet stem sorghum and to develope management protocols of aphids using cucurbitacin-containing products.

1.4.1 Objectives

1. To investigate whether the sugarcane aphid would interact with *Meloidogyne* species to reduce sucrose content and then nematode resistance in SSS cv. 'Ndendani-X1'.
2. To investigate whether the combined effects of mixed population of *Meloidogyne* species and sugarcane aphid would break pre-infectional nematode resistance in SSS cv. 'Ndendani-X1' under field conditions.
3. To investigate whether the interactive effects of Nemarioc-AL, Nemafric-BL and Mordica phytonematicides would stimulate sugar content and plant growth variables of cv. 'Ndendane-X1' under pest-free conditions.
4. To determine whether the interactive effects of Nemarioc-AL, Nemafric-BL and Mordica phytonematicides would stimulate sucrose content and plant growth variables of SSS cv. ‘Ndendane-X1’ but inhibited *Meloidogyne* species through direct contact and sugarcane aphid through induced systemic plant substances under microplot conditions.

1.4.2 Null hypotheses

1. Sugarcane aphid would not interact with *Meloidogyne* species to reduce sucrose content and then nematode resistance in SSS cv. 'Ndendani-X1'.
2. Combined effects of mixed population of *Meloidogyne* species and sugarcane aphid would not break pre-infectional nematode resistance in SSS cv. 'Ndendani-X1' under field conditions.

3. The interactive effects of Nemarioc-AL, Nemafric-BL and Mordica phytonematicides would not stimulate sugar content and plant growth variables of cv. 'Ndendane-X1' under pest-free conditions.
4. The interactive effects of Nemarioc-AL, Nemafric-BL and Mordica phytonematicides would not stimulate sucrose content and plant growth variables of SSS cv. ‘Ndendane-X1’ nor inhibited *Meloidogyne* species through direct contact and sugarcane aphid through induced systemic plant substances under microplot conditions.

1.5 Reliability, validity and objectivity

Reliability was ensured by using statistical levels of significance ($P \leq 0.05$). Validity was achieved through repeating the experiments in time, whereas the objectivity was ensured that findings were discussed on the basis of empirical evidence, as shown in the statistical analyses, in order to eliminate all forms of subjectivity.

1.6 Bias

Bias was reduced to the smallest possible level by ensuring that the experimental error in each experiment was minimised through (a) using a high number of replications in each experiment, and (b) by assigning treatments to experimental plots at random within the selected research designs.

1.7 Significance of the study

Aphids and nematodes are serious pests of sucrose-producing crops. Due to the withdrawal of environment-unfriendly pesticides from the agrochemical markets, host resistance to pests is preferred as being the most environment-friendly and user-

friendly pest management strategy. However, plant genes responsible for insect suppression are not responsible for nematode suppression, *vice versa*. The significance of this study therefore, would be that, should aphid infestation break resistance to *Meloidogyne* species, aphid population densities should then be managed where nematode resistance is being used to manage nematode population densities.

1.8 Format of the thesis

Chapter 1 provided a detailed outline of the research problem, whereas Chapter 2 contains literature review on the research problem. Each research chapter comprised a single objective as outlined under subsection 1.41. In the Introduction of each research chapter, the null hypothesis was spelt out soon after the objective. Also, each research chapter ended with the synthesis regarding new findings and conclusion, along with a statement on whether the null hypothesis was accepted or rejected on the basis of the overall findings. Additionally, to improve the flow of the content in the thesis, a sentence was included in the subsection to link up the current and the next chapter by paraphrasing what would be done in the subsequent chapter. The final chapter of the thesis provided a summary of the findings, the significance of the findings, recommendations with respect to closing the perceived gaps and then conclusions. Literature citation and referencing styles followed the Harvard format of the author-alphabet.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Sweet stem sorghum cv. 'Ndendane-X1' is highly resistant to thermophilic root-knot (*Meloidogyne* species) nematodes (Mabuka, 2013; Mashela and Pofu, 2016). The cultivar, like other sorghum cultivars, has pre-infectional nematode resistance (Kaplan and Keen, 1980), where sorgolene ($C_{42}H_{66}O_{13}$) chemical compound is released into the rhizosphere (Czarnota *et al*., 2003; Dayan *et al*., 2010). Since this mechanism of resistance is reliant on carbohydrates, any factor that could temper with the sucrose source, might directly affect the resistance to nematodes (Mashela and Nthangeni, 2002). The objective of this study was to review literature on the aphid-nematode interaction on nematode resistance and productivity of nematode-resistant sweet stem sorghum cultivars and the sustainable management strategies against both pests, in order to have a view on the work done and not yet done on the research problem.

2.2 Work done in the research problem

2.2.1 Plant nematodes

Plant nematodes live in the soil or in plant tissue, and typically do not move great distances. Anthropogenic movement of the nematodes over great distances might occur through nematode-contaminated materials such as planting material, soil, machinery, organic fertilizers and flooded water. The majority of plant nematodes affect crops through feeding on belowground plant parts (modified stems and roots), whereas fewer nematodes feed on aboveground parts (stems, leaves, seeds). Thus, plant nematodes can have direct impact on economically important crop produce, with others having the ability to serve as virus vectors which cause complex diseases in

plants (Lamberti and Roco, 1987). One genus, *Bursaphelenchus xylophilus* (Steiner and Bührer) feeds on barks of pine forest trees; where it causes wide economic losses through tree wilting (Fukuda *et al*., 1997). Plant nematodes damage plants through physical and chemical means (Mashela *et al*., 2016) and by facilitating infestation by secondary pathogens such as fungi, bacteria and viruses (Powel, 1971). Nematodes can cause significant damage to most cultivated crops, but due to their microscopic size, they remain invisible to the unaided eye (Ngangbam and Devi, 2012). Plant nematodes in the phylum Nematoda are classified into two orders: Dorylaimida and Tylenchida. All the five genera in the order Dorylaimida (*Longidorus, Paralongidorus, Paratrichodorus Trichodorus* and *Xiphinema*) are migratory ectoparasites, with the ability to transmit phytopathogenic nepo- and tobra-viruses (Lamberti and Roco, 1987). In contrast, most economically important genera of migratory endoparasites (*Anguina, Aorolaimus, Ditylenchus, Hirschmanniella, Hoplolaimus Pratylenchus and Radopholus)* belong to the order Tylenchida, where their movement and feeding inside plant organs could induce cell death and tissue necrosis. Other plant nematodes in the order Tylenchida might feed from the outside of the affected plant organ and include *Aphelenchoides, Criconemella, Helicotylenchus, Hemicriconemoides, Hemicycliophora, Scutellonema Tylenchorhynchus, Tylenchulus, Rhadinaphelenchus* and *Rotylenchulus* (Hunt *et al*., 2005).

The most important nematode families of the order Tylenchida, either as models of plant-pathogen interaction or as crop pests, constitute sedentary endo-parasitic nematodes in the *Heteroderidae* and *Nacobbidae* families. The family *Heteroderidae* includes the most widespread cyst nematodes in the genera *Globodera* and *Heterodera* and the root-knot (*Meloidogyne* species) nematodes, where the second-

stage juveniles (J2), hatched from eggs, are the ′infective ` stage. The J2 move through the soil in order to locate new host roots and penetrate the roots at the elongation zone and migrate to the tip of the root using stylets and chemicals from the ventral gland. Once at the tip of the root, J2 enter the xylem and move upward to the elongation zone, where the feeding sites are established using stylets and chemicals from the dorsal glands (Mashela *et al*., 2016). The nematode and the root cells release chemicals which either enhance compatibility or incompatibility. In the event of compatibility, J2 mould and grow to young adults, which in case of cyst nematodes form the feeding cells through symplasia, whereas *Meloidogyne* species form the feeding cells called giant cells through mitosis without cytogenesis. In *Meloidogyne* species, as the young females feed on the established giant cells, they assume obesity and physically push out the root cells to form root galls, with eggs being laid in the gelatineous matrix which occurs as a visible brownish and later black structure outside the rear part of the female body on the root galls. *Meloidogyne* species differ from most other sedentary endoparasites by having extensive host ranges, with over 3 000 plant species serving as hosts (Rizvi and Rizvi, 1992).

The life cycle of plant nematodes consists of the egg and four juvenile stages, with the four stages separated by a mould. Following embryogenesis, the first mould occurs within the egg, giving rise to (J2), each with a stylet and therefore feeds on root hairs after hatching. Depending on the availability of the suitable temperature and moisture, J2 emerges out from an egg and moves freely in soil searching for new roots of the same plant or some other adjacent plants. A second mould occurs giving rise to J3. The third mould follows quickly and juveniles develop to J4. In case of *Meloidogyne* species, sex differentiation occurs after the third mould. Females acquire a V-shaped

genital primordium, while in males the tails are I-shaped. The J3 and J4 retain the old cuticles as a result of superimposed moulding. The pointed tail of J2 is still visible and hence these are also called the spike-tailed stages. During moulding from J2 to J3, the stylet is discarded, so that the J3 and J4 are without stylets and therefore are non-feeding stages (Askary, 2008). At the last mould, the adult female becomes sac-like, the stylet reappears and the reproductive system becomes fully developed with the vulval opening marking its appearance. Adult males remain vermiform, coiled inside the J4 cuticle, emerge out and migrate out of root into the soil, do not feed and are therefore short-lived. The time required for a complete life cycle varies depending on the environmental factors, the host and *Meloidogyne* species. Usually, thermophilic *Meloidogyne* species have short life cycles, for instance, for *Meloidogyne entorolobii* the life cycle is approximately 15 days (Ashokkumar *et al*., 2019; Collett, 2020; Costa *et al*., 2020), *M. incognita* (Chitwood) 37 days (Ibrahim and El-Saedy, 1987) and *M. javainca* Chitwood) 27 days (Bird, 1959). Yield loss due to nematode damage in crops without nematode resistance could amount to as high as 50% to total crop failure (Chitwood, 2003; Trudgill, 1992). The total annual yield loss related to plant nematodes was in the late 1980s projected at 12.3% (Sasser and Freckman, 1987). Worldwide, annual crop losses due to plant nematodes had been estimated at US$123 billion (Chitwood, 2003), which was prior to the withdrawal of fumigant nematicides in 2005. Three and eight years after the withdrawal, the crop losses were estimated at US$157 billions (Abad *et al*., 2008) and US$173 (Elling, 2013) billions, respectively. In addition to direct costs, root-knot nematodes cause indirect costs because of the quarantine status of some *Meloidogyne* species. For example, *M. chitwoodi* and *M. enterolobii* are increasingly regulated because they are aggressive nematodes for potato plants and other economically important cash crops (Watkins *et al*., 2012).

2.2.2 Loss of nematode resistance in plants

The initial report on interference of nematode resistance was related to the breakdown of gene *Mi* in tomato seedlings post-exposure to temperature above 28°C (Dropkin, 1969). The gene *Mi*, which confers resistance to several species of root-knot nematode, is present in many modern tomato cultivars (Milligan *et al*., 1998). The gene *Mi* is a member of the leucine zipper, nucleotide binding, leucine-rich repeat Family of plant genes (Williamson *et al*., 1994). However, the breaking of nematode resistance by temperature was not unique to gene *Mi.* Nematode resistance was also broken in alfalfa (*Medicago sativa* L.) plant (Griffin and Waite, 1971), common bean (*Phaseolus vulgaris* L.) and grape (*Vitis vinifera* L.) rootstocks when exposed to 32, 26 and above 27°C, respectively (Ferris *et al*., 1993). Generally, under high temperature, the breakdown of carbohydrates is high, which could translate to depletion of plant genes (Mashela *et al*., 2016), resulting in the elimination of nematode resistance. Increased temperature due to climate change could increase challenges in the breakdown of nematode resistance. At the same time, such increases could shorten the ontogenies of most plant nematodes (Chakraborty and Newton, 2011). Growth stages and development of plants are sensitive to temperature and could be drastically affected by nematode damage post nematode resistance losses (Chakraborty and Datta, 2003). New nematode races, with increased fecundity could also evolve rapidly after loss of nematode resistance (Chakraborty *et al*., 2013), leading to reduced crop productivity, high food insecurity and/or limited availability and accessibility. Various races have been identified using differential host plants and molecular approaches among *Meloidogyne* species (Pofu, 2012). By definition, nematode races are morphological identical.

Nematode damage in certain plants had been associated with abiotic factors such as salinity. Densities of the citrus nematode (*Tylenchulus semipenetrans* Cobb 1913) and its associated slow decline symptoms were observed to be dominant in citrus-producing areas with high salinity (Duncan *et al*., 1995). Climate change had been shown to cause recurrent floods, followed by high evapotranspiration, which are associated with increased salinity challenges (Wheeler, 2011). In addition to breaking resistance to *T. semipenetrans* in resistant citrus rootstocks exposed to cyclic salinity (Mashela, 1992), *T. semipenetrans* was shown to break salt tolerance in highly salt tolerant citrus rootstocks (Mashela and Nthangeni, 2002). In short, salinity breaks nematode resistance, whereas nematode infection breaks salt tolerance. Generally, there are no citrus rootstocks that contain both nematode resistance and salt tolerance (Mashela and Nthangeni, 2002).

Most biological races in plant nematodes were ascribed to loss of nematode resistance in plants, although such reports were not supported by empirical studies. Nematode resistance in highly nematode resistant wild watermelon (*Cucumis africanus* L.F.) was lost when the plants were heavily infested by the greenhouse whiteflies (*Trialeurodes vaporariorum* Westwood) under greenhouse conditions, whereas in the control greenhouse, nematode resistance was not lost (Pofu *et al*., 2011). In plants with lost nematode resistance, shoots were covered with sooty mould, which suggested that the whiteflies were sucking honeydew from underneath the leaves. The latter was linked to loss of carbohydrate sources required to synthesis of gene products, which are responsible for conferring resistance.

2.2.3 Cultural practices and loss of nematode resistance

Generally, a balance between the root and the shoot, as conceptualised as root/shoot ratio, plays an indispensable role in nematode resistance. Using a split-root experiment, Mashela and Nthangeni (2002) demonstrated that root pruning, that is, reduced root/shoot ratio, resulted in effects similar to those of high infection with population densities of *T. semipenetrans*, where females after maturity and death, have root-pruning effects (O'Bannon and Esser, 1985). Generally, the symptoms of slow decline of citrus, induced by *T. semipenetrans*, were described as having similar symptoms as those of nutrient deficiencies (Mashela, 1992). In the early 1980s, mechanical weed control was the most common in citriculture. As in citrus orchards with high nematode population densities, after mechanical weed control, trees expressed K deficiency symptoms, with such trees failing to respond to fertiliser application during attempts to correct K deficiency (Fouche *et al*., 1977). In his classical work in salinity-nematode-citrus interactions, Mashela (1992) demonstrated the link between nematode infection and nutrient deficiencies, resulting in recommendations that were intended to scale down the practice of mechanical weed control in citrus orchards. Earlier, Duncan and McSorley (1987) had shown that the root systems of two parallel adjacent citrus trees were spreading outside of the canopy, with much damage being incurred during weed control using weeding discs. Consequently, Mashela (1992) in view of the overall observations in relation to root-pruning and accumulation of Na and Cl ions in leaf tissues of citrus trees, suggested that it would be prudent if weeds between the rows were mowed instead of using discs.

In plant nematodes, the chemical compounds that are able to trigger nematode resistance have been code-named plant genes (Gheysen and Fenoll, 2002), whereas

their counterparts in nematodes, which either downgrade or upgrade the reactions of plant genes, are gene products (Seah *et al*., 1998). The plant genes are primarily produced during biosynthesis of secondary metabolites (Favery *et al*., 1998; Fenoll *et al*., 1997 and Gheysen, 1998; Kaloshian *et al*., 1996; Vercauteren *et al*., 2002; Veremis and Roberts, 1996). Any factor that would interfere with either photosynthesis or respiration, resulted in Mashela *et al*. (2016) formulating the hypothesis which suggested that such factors would directly affect nematode resistance. All secondary metabolites have three things in common, namely, carbon, hydrogen and oxygen, with the carbon numbers being the determining factor of the structural size of the chemical compound. Triterpenoids, for example, have over 30-C skeletons, resulting in large molecules such as those of the cucurbitacins (Van Wyk and Wink, 2004).

Factors that affect the amount of photosynthetic products could be those that reduce water absorption and then increasing the closure of stomata and the stomatal conductance (Kozlowski and Pallardy, 1997; Lou, 2002 and Yashiroda, 1960), which could include root pruning through soil-borne pests and diseases. Alternatively, sucking pests such as aphids (*Aphididae*), mealybugs (*Pseudococcidae*), scales (*Coccoidea*) and greenhouse whiteflies, could tap directly into the phloem vessels, which translocate sucrose from the shoots to other organs, including roots. Such sucking insects are wasteful since most result in honeydew, which contains large quantities of sucrose, being released as waste. Further, the contamination of honeydew with sooty mould, reduces the available photosynthetic area, all of which could contribute to reduced photosynthates for biosynthesis of secondary metabolites, which could translate to reduced plant genes and therefore, loss of nematode resistance.

2.2.4 Need for alternatives in managing nematode population densities

After the withdrawal of methyl bromide from the agrochemical markets due to its contribution to the thinning of ozone layer by its bromide as a halogen, along with its biocidal nature on soil microbes, various alternatives were investigated for managing nematode population densities (McSorley, 2011). Bello (1998) reviewed a wide range of alternatives to fumigant nematicides, with organic amendments being in the forefront. However, due to a wide range of disadvantages that were inherently associated with organic amendments (Mashela, 2002), various other nematode management alternatives, including phytonematicides (Mashela *et al*., 2017b) and nematode resistance (Mashela *et al*., 2015), were investigated. The phytonematicides were investigated and developed with the main purpose being that of ameliorating the previously identified drawbacks of conventional organic amendments (Mashela, 2002). In contrast, nematode resistance was investigated for its user-friendliness and its compatibility with many other nematode management strategies (Roberts, 1992; Silva *et al*., 2013; Sone, 2010; Starr *et al*., 2002; Zhang *et al*., 2016). Although in the current review both strategies were reviewed, the review was limited to the test crop, as well as phytonematicides, which were used in the current study.

2.2.5 The sweet stem sorghum

Fossil fuel, due to the greenhouse gases that it releases into the atmosphere, is greatly contributing to global warming. Currently, the demand for fossil fuel withdrawal is no longer exclusively from the environmentalists, but from all humanity, due to its contribution towards global warming. Sweet stem sorghum is energy-rich carbohydrate source and due to its being classified as a non-food, it qualifies for the production of biofuel for mitigation against climate change (Rooney *et al*., 2007). Use

of biofuel could drastically reduce the gaseous emissions that are responsible for the formation of greenhouse layers (Rolando *et al*., 2015), which promote the advance of climate change. Plants are deemed suitable for biofuel production when their degrees Brix (°Bx) range from 14 to 19% (Almodares and Sharif, 2007). Sweet stem sorghum (SSS) cv. 'Ndendane-X1', was shown to contain °Bx as high as 20% under optimal conditions (Mashela and Pofu, 2016), with another study reporting that its °Bx on various parts of a single plant ranged from 14 to 20% (Mabuka, 2013). Consequently, the cultivar has high potential for biofuel production.

Another important attribute observed in this cultivars was its high resistance to certain thermophilic *Meloidogyne* species and biological races (Mashela and Pofu, 2016), especially *M. incognita* race 2, *M. incognita* race 4 and *M. javanica*. In South Africa, *M. incognita* race 2 and *M. javanica* occur as pure or mixed populations (Clark, 2007; Khan and Khan, 1991), making it difficult to introduce nematode resistance crops, without detailed identification trials. In contrast, *M. incognita* race 4 occurs in cotton-producing regions (Kleynhans *et al*., 1996), where it is a major nematode species of cotton. A recent study (Pretorius, 2018) suggested that *M. enterolobii* was actually widely distributed in South Africa, but did not occur as pure cultures. Also, due to its short lifespan, in most cases it is viewed as the most aggressive. Severity of damage induced by plant nematodes can be attributed to mechanical damages to the roots, subsequent infection by opportunists (Spaull and Cadet, 1990) and soil type (Trudgill, 1992). Root deformation leads to limited water and nutrient uptake, with severe dysfunction affecting photosynthesis.

Although SSS cv. 'Ndendane-X1' was identified as being resistant to most thermophilic *Meloidogyne* species (Mabuka, 2013; Mashela and Pofu, 2016), it was observed as being highly susceptible to sugarcane aphid (*Melanaphis sacchari* Zehntner), which occurs mostly in the genera *Saccharum* and *Sorghum* (Singh *et al*., 2004). *Melanaphis sacchari* is widely distributed in sugarcane and sorghum-producing countries (CIE, 1981; Eastop, 1955, Mead, 1978). Such countries are predominantly in most continents of the world, with the pest reported in Africa (Angola, Botswana, Egypt, Ethiopia, Kenya, Nigeria, South Africa, Sudan, Tanzania, Uganda and Zimbabwe), Asia (Bhutan, China, India, Indonesia, Japan, Pakistan, Philippines, Taiwan and Thailand), North America (Haiti, Jamaica, Mexico and United States of America), South America (Argentina, Brazil, Colombia, Ecuador, Peru, Trinidad, Tobago and Venezuela) and Australia (Singh *et al*., 2004). The range of economic damage due to aphid infestation on sorghum had been reported as being from minor to severe in Botswana and Zimbabwe (Flattery, 1982; Page *et al*., 1985). In South Africa, where sugarcane and sorghum production had been intensive, aphid damage had been recorded as being from as high as 60% to complete crop failure (Van Rensburg, 1973). In India, early sowing of sorghum resulted in 13-15% aphid-yield losses, whereas late sowing resulted in 30-35% yield losses (Balikai, 2007). Heavy aphid infestation occurs during all plant growth stages, with serious economic damage might occur during the final growth stages (Teetes *et al*., 1995; Van Rensburg and Van Hamburg 1975), especially when plants are moisture-stressed (Raetano and Nakano, 1994). Such damage is due to its reproductive capacity and the increased release of phloem sap, technically referred to as honey-dew. As alluded to earlier, the honeydew supports the growth of sooty mould, which is a collective term for different Ascomycete fungi, that include genera such as *Alternaria and Cladosporium* , both with high reproductive

capacities and great dispersion capabilities (Agrios, 2005; Villanueva *et al*., 2014). Technically, sooty mould does not have registered fungicides and therefore, aphids should be managed to ameliorate the challenge.

2.2.6 Interactive effects of nematodes and sugarcane aphid

In general, the aphid genus *Melanaphis* has over 20 identified species, with the host range restricted to four plant genera, namely, *Sorghum*, *Oryza*, *Panicum* and *Pennisetum*, all in the family Graminaeae (Commonwealth Institute of Entomology, 1981; Singh *et al*., 2004) and in the genus *Saccharum* of the family Poaceae. Sugarcane is (*Saccharum officinarum* L.**)** is widely cultivated, providing around 70% of the global sugar (Singh *et al*., 2004). Sugarcane yields the highest number of calories per unit area of cultivation for any of the sucrose-producing plants. Although host plants are typically infested soon after emergence, significant infestations might occur during late growth stages, especially when it is dry due to concentrated sucrose in the phloem vessels (Van Rensburg, 1973). Due to increasing temperatures at a global scale, ontogenies for most pests, including those of aphids and nematodes, have been increasingly shortened (Mashela and Pofu, 2016). The two pests are regularly managed, because among other economic losses, they are both vectors of different plant viruses. The sugarcane aphid transmits three virus diseases, namely, millet red leaf virus (*Luteovirus* species), sugarcane yellow leaf virus (*Polerovirus* species), Mosaic of abaca (*Potyvirus* species) (Singh *et al*., 2004). In contrast, a total of over 70 viruses are vectored within four nematode genera, namely, *Xiphinema*, *Longidorus*, *Paralongidorus* and *Paratrichodorus* (Lamberti and Roca, 1987). However, the genus *Meloidogyne* is not a virus vector.

Meloidogyne species interacted with (*Schizaphis rufula* Walker), on Marram grass (*Ammophila arenaria* L.) with the aphid having reduced preference for nematode-infected plants (Vandegehuchte *et al*., 2010). In contrast, when *Meloidogyne* species interacted with green peach aphid (*Myzus persicae* Sulzer) on tobacco (*Nicotiana tabacum* L.), the aphid growth rate and fecundity were reduced, without any reciprocal effects on nematode performance (Kaplan *et al*., 2011). The interactive effects of *M. incognita* or soybean cyst (*Heterodera glycine* Ichinohe*)* with leaf chewing insects on soybean (*Glycine max* L.), increased nematode fecundity as measured through the reproductive potential (Alston *et al*., 1993). The interaction of *M. incognita* and a stalk-borer (*Ostrinia nubilalis* Hübner) on maize reduced nematode root penetration (Tiwari *et al*., 2009). In all cases, the resistance level of the plants to pests was not declared, suggesting that interference with nematode resistance in the test plants was a peripheral issue.

2.2.7 Potential use of botanicals in management of aphids

Synthetic chemical insecticides are commonly used to manage aphid population densities in various farming systems (Nzanza and Mashela, 2012). However, the efficacy of these insecticides is compromised due to the reproduction form in aphids, namely, parthenogenesis, which enhances the development of aphid resistance to the products (James, 2003; Yang *et al*., 2010). Additionally, most insecticides are inaccessible due to high costs and are also environment-unfriendly (Mashela *et al*., 2015). Therefore, development of botanicals has received much attention to serve as alternative to the synthetic chemical products (Nzanza and Mashela, 2012; Pelinganga and Mashela, 2012). To date, the use of phytonematicides from neem (*Azadirachta indica* A. Juss) leaf and wild garlic (*Allium sativum* L.) (Nzanza and Mashela, 2012),

along with oils of cumin (*Cuminum cyminum* L.), anise (*Pimpinella anisium* L.), oregano (*Origanum syriacum* L.) and eucalyptus (*Eucalyptus camaldulensis* Dehn.) (Basedow *et al*., 2002), have been the most preferred due to their effectiveness in reducing aphid population densities without adverse environmental effects. For instance, in various crops, the use of such products had been associated with stronger and superior quality seedlings (Giannuzzi *et al*., 2001), higher crop uniformity (Waterer and Coltman, 1988), better mineral nutrient uptake (Bethlenfalvay *et al*., 1988; Chandanie *et al*., 2009; Marschner and Dell, 1994), improved tolerance to soil-borne diseases (Pozo and Azcón-Aguilar, 2007) and reduction of general plant stress, thereby increasing yields (Chandanie *et al*., 2009; Lovato *et al*., 1996). However, most of the mechanisms involved in the listed ameliorative effects are undocumented.

Due to the increasing need to develop mitigation and adaptive strategies against climate change, it had been imperative that synthetic chemical pesticides be substituted by sustainable and degradable plant-based products. Use of phytonematicides would meet some of the demands articulated in favour of reducing CO_2 emissions from fossil oil (Mashela *et al*., 2017b). The advantages of using phytonematicides include their being environment-friendly, high degree of biodegradability and high potential for local production from indigenous plants, thereby increasing the local economies (Mashela, 2002). Sweet stem sorghum cv. 'Ndendane-X1', with its high Brix content, could also be a suitable alternative for ethanol production which could be an alternative to fossil oil. The cucurbitacin-containing phytonematicides, Nemarioc-AL and Nemafric-BL phytonematicides, have been successful in management of population densities of plant nematodes (Mashela *et al*., 2017b).

2.3 Work not yet done in the research problem

The interactive effects of either *M. sacchari* or thermophilic *Meloidogyne* species on resistant sorghum cultivars, had not been documented. Should the *M. sacchari* and thermophilic *Meloidogyne* species interact to break nematode resistance in SSS cv. 'Ndandane-X1', it would imply the need to manage aphid population densities in order to retain high Brix content and nematode resistance in the cultivar, along with in sugarcane cultivars. Also, should all the cucurbitacin-containing phytonematicides suppress aphid population densities, this would suggest the existence of broad spectrum bioactivities in the active ingredients of the three products. In the following chapter the interactive effects of aphids and three thermophilic *Meloidogyne* species on nematode resistance in sweet stem sorghum (SSS) cv. 'Ndendani-X1' would be investigated.

CHAPTER 3
MELANAPHIS SACCHARI AFFECTS SUCROSE CONTENT IN SWEET STEM SORGHUM AND ITS RESISTANCE TO *MELOIDOGYNE* SPECIES

3.1 Introduction

Sweet stem sorghum (*Sorghum bicolor* L.) cv. 'Ndendane-X1' is highly resistant to root-knot (*Meloidogyne* species) nematodes (Mashela and Pofu, 2016), but highly susceptible to sugarcane aphids (*Melanaphis sacchari* Zehntner) (Mashela and Pofu, 2016). Nematode resistance is an active process, where products of photosynthesis origin are essential in biosynthesis of active ingredients (Ahn and Lee, 2003; Mashela *et al*., 2016). In plant nematodes there are two forms of nematode resistance mechanisms, namely, pre- and post-infectional nematode resistance (Kaplan and Davis, 1987). Roots of *Sorghum* species, which include sweet stem sorghum (SSS), exude sorgolene ($C_{42}H_{66}O_{13}$) as an active ingredient against nematodes (Dayan *et al*., 2010). Sorgolene repels second-stage juveniles (J2) from penetrating the root system and, thereby, confers nematode resistance in certain *Sorghum* cultivars. Since aphids feed on sucrose in the phloem sap (Hewer *et al*., 2010), it is probable that aphid-infection in nematode resistant sorghum cultivars could result in the loss of nematode resistance. Previously, the focus in nematode-aphid interactions was mainly on the effects of nematodes on aphid performance (Bezemer *et al*., 2005; Hol *et al*., 2010; Kaplan *et al*., 2011; Wurst and Van der Putten, 2007), except for *Heterodera schachtii* Schmidt interacted with *Brevicoryne brassicae* L. in *Arabidopsis thaliana* L, with nematode infection being reduced (Kutyniok and Muller, 2012). In such interactions, aphid performance was primarily measured using aphid growth rate and/or fecundity.

The interaction of *M. incognita* with another phloem-feeding insect, the greenhouse whiteflies (*Trialeurodes vaporariorum* Westwood), broke nematode resistance in a highly nematode resistant wild watermelon (*Cucumis africanus* L.F.) (Pofu *et al.*, 2013). However, no information existed on whether *Meloidogyne* species could interact with aphids on nematode resistance in sweet stem plants such as Sorghum and *Saccharum* species. The objective of this study was to determine the effect of sugarcane aphid on sucrose content and nematode resistance present in a sweet stem sorghum cv.'Ndendani-X1'. The null hypothesis suggested that the sugarcane aphid would not have effect on sucrose content and nematode resistance present in a sweet stem sorghum cv.'Ndendani-X1'.

3.2 Materials and methods

3.2.1 Location of the study

Three separate trials, each for *M. enterolobii* (Trial 1), *M. incognita* (Trial 2) and *M. javanica* (Trial 3), were initiated under microplot conditions at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, South Africa (23°53'10''S, 29°44'15''E). The location had mean annual rainfall of less than 450 mm, with the annual distribution skewed to summer, whereas minimum/maximum temperatures average 18/38°C. Experiments in each trial were separately initiated in summer (October-January) 2018 and validated in summer 2019. The site consisted of Hutton form with loam soil, comprising 65% sand, 30% clay, 5% silt, 1.6% organic C, EC 0.148 dS/m and pH (H_2O) of 6.5.

3.2.2 Treatments, experimental design and procedures

In each trial, 2 × 2 factorial experiment, with the first factor being nematode (with and

without) and second factor the aphid (with and without), was conducted. The treatments, untreated control (A_0N_0), nematode alone (A_0N_1), aphid alone (A_1N_0) and nematode + aphid (A_1N_1), were arranged in a randomised complete block design (RCBD), with six replications (Figure 3.1).

Figure 3.1 Sweet stem sorghum cv. 'Ndendane-X1' established under micro-plot conditions at GBRCE for the study.

Blocking was done against shading from windbreak trees in the morning or in the afternoon. Thirty-cm-diameter plastic pots were filled with 10 000 ml steam-pasteurised (300°C for 1 h) Hutton soil derived from the site and mixed with river sand at 3:1 (v/v) ratio. Pots were inserted into 20-cm-deep holes at 0.3 m × 0.3 m spacing. Three SSS cv. 'Ndendane-X1' seeds were sown per pot and at two true-leaf-stage, seedlings were thinned to one per pot. In each trial, eggs + second-stage juveniles (J2) for an appropriate nematode species or race were extracted from roots of

greenhouse-grown nematode-susceptible tomato cv. 'Floradade' in 1% NaOCl solution (Hussey and Barker, 1973). Each seedling was inoculated by dispensing 5 000 eggs + J2 using 20 ml plastic syringe into 5-cm-deep holes around the stems of appropriate treatments. Twenty sugarcane aphids, reared on aphid-susceptible SSS raised on a field 2 km from the trial site, were picked up into vials with leaf pieces from the ventral sides of leaves using a soft brush at three weeks after inoculation with nematodes and placed in whorls of appropriate treatments.

Seven days after inoculation with nematodes, each seedling was fertilised with 5 g 2:1:2 (43) Multifeed fertiliser (Nulandies, Johannesburg) to provide a total of 0.70 mg N, 0.64 mg K and 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 per ml water. Each seedling was also fertilised with 2 g NPK 2:3:2 (22) + 0.5% S + 5% Zn + 5% Ca, primarily to supply Ca. Each seedling was originally irrigated with 2 litres chlorine-free tapwater every third day using drip irrigation system that had an output of one litre per hour during vegetative growth. At initiation of the reproductive stage, each plant was irrigated with 4 litres water per week. Plants were sprayed once for stalk borer (*Papaipema nebris* Guenée) using at 5 ml Bulldock Beta 125 SC/10 litre chlorine-free water a week before infestation with aphids. The A_0N_0 and A_0N_1 treatments were monitored for aphid infestation, which were managed using malasol when necessary, during which time the A_1N_0 and A_1N_1 were temporarily covered with transparent plastic bags to protect them from the spray drifts.

3.2.3 Data collection

Plant variables: At 150 days after inoculation with nematodes, plant height measured from the soil surface to the tip of the head using measuring tape. Stem diameter was measured at 5 cm above the severed ends using a digital Vernier calliper. Tiller number, along with internode number in both primary and tiller stalk, were recorded. The panicle was cut from mother plant shoots and tiller, to measure with fresh biomass measured. The remaining roots were dried in air-forced ovens (AD 200 AGRI-DRYER, White River, South Africa) at 52°C for 72 h, for determination of dry biomass and recorded

Sugar content was measured and expressed in Degrees Brix. Stems were marked into three classes, namely, top (internode just below the peduncle), bottom (internode just above the severed-end of the stem) and middle (middle distance between the top and bottom internodes). The middle part of each class was cut using a knife that was soaked in distilled water and wiped using laboratory towel prior to re-use, with the juice from the top-end of the cut portion squeezed using a pair of pliers onto the eye of a hand-held digital refractometer for determining the degrees Brix (^{o}Bx).

Nutrient element variables: Healthy and mature leaves were sampled per plant and wiped with disposable laboratory towel moistened with distilled water, dried at 70°C for 72 h and ground in a Wiley mill to pass through a 0.75 mm sieve. Approximately 0.4 g leaves tissue samples were digested in 75 ml vessel with 5 ml 70% nitric acid (HNO_3) and 30% hydrogen peroxide (H_2O_2) at 3 ml using microwave digester (Perlain Elmer, Titan MPS). The vessels were placed in the microwave digester to whirl for 46 minutes at approximately 260°C. and then placed in the laminar flow hood to cool down for 5 minutes. Samples from the vessels were transferred into 50 ml centrifuge

tubes, sealed and stored in the cold room at 5ºC prior to analysis. Aliquots were quantified for Zn, Fe, K, P, Mg, Mn, Ca, Cu, S and Na nutrient elements using the Inductively Coupled Plasma Optical Emission Spectrometry (Shimandzu, ICPE-9000).

Nematode variables: Roots were emptied from pots and separately immersed in tapwater to remove soil particles, blotted dry, chopped into small pieces and mixed. A sample of approximately 10 g fresh roots was measured, with eggs + J2 extracted using the maceration and blending method in 1% NaOCl (Hussey and Barker, 1973). The material was passed through top-down nested 150- and 25-µm pore sieves, with contents of the 25-µm opening sieve subjected to sugar and centrifugation method, with kaolin added in each centrifuge (Marais *et al*., 2017). Contents were passed through top-down nested 150- and 25-µm pore sieves, with the contents of the latter poured into 100-ml-plastic containers, brought to 100-ml mark, tightly closed and counted from 5 ml aliquot under a 60 x magnification stereomicroscope. Nematode eggs and J2 from roots were converted to nematodes per 10 g roots or expressed as reproductive potential (RP = eggs + J2/ g roots).

3.2.4 Data analysis

The Shapiro-Wilk test was performed on the standardised residuals of data to test for deviations from normality (Shapiro and Wilk, 1965) and also tested for homogeneity of treatment combination variances using the Levene test (Levene, 1960). The standardised residuals were acceptably normal with homogeneous treatment variances. Plant and nematode RP variables were subjected to analysis of variance (ANOVA) using Statistix 10 software. In cases where treatment interaction effects were significant, the results were further expressed using matrix tables to allow for the

determination of the magnitude and direction of interactive effects relative to untreated control (Steyn *et al*., 2003). Significant treatment means were separated using appropriate tests at the probability level of 5%. Unless otherwise stated, treatment means were discussed at the probability level of 5%.

3.3 Results

In each trial, seasonal effects for variable were not significant and therefore, data were pooled (n = 48) and reanalysed. In all three *Meloidogyne* species, the interactive effects of nematode and sugarcane aphid population density had significant effects on degree Brix (°Bx) in parts of sweet stem sorghum (Appendix 3.1). Relative to nematode alone, the interactive effects of sugarcane aphid with *M. enterolobii*, *M. incognita* and *M. javanica* significantly reduced °Bx by 17, 74 and 42%, respectively (Table 3.1).

Table 3.1 Interactive effects of *Meloidogyne* species with aphids on degree Brix in middle sweet stem sorghum at 150 days after inoculation with nematodes on microplots (n = 48).

	Aphid			
	A_0[x]	R.I. (%)[y]	A_1	R.I. (%)
Meloidogyne enterolobii				
N_0	18.42[a] ± 1.2	–	18.33[a] ± 2.2	–1
N_1	18.25[a] ± 0.6	–1	15.71[b] ± 1.3	–17
Meloidogyne incognita				
N_0	18.73[a] ± 0.3	–	18.21[a] ± 0.4	–1
N_1	12.00[b] ± 0.7	–34	4.71[c] ± 1.7	–74
Meloidogyne javanica				
N_0	17.96[a] ± 1.3	–	14.83[b] ± 0.7	–20
N_1	10.12[d] ± 0.4	–42	12.96[c] ± 0.3	–42

[x]Column means ± standard error followed by the same letter were not different (P ≤ 0. 05) according to Fisher's Least Significant Different test.

[y]R.I = Relative impact (%) = [(interaction value/control value) − 1] × 100.

A_0 = without aphid, A_1 = with aphid, N_0 = without nematode, N_1 = with nematode.

In three *Meloidogyne* species, the interactive effects of nematode and aphid population density had significant effects on reproductive potential of *Meloidogyne* species (Appendix 3.2). Relative to nematode alone, the interactive effects of *M. enterolobii*, *M. incognita* and *M. javanica* with sugarcane aphid increased reproductive potential by 196, 320 and 152%, respectively (Table 3.2).

Table 3.2 Reproductive potential of *Meloidogyne enterolobii*, *M. incognita* and *M. javanica* as affected by nematode and aphid interaction on sweet stem sorghum at 150 days after inoculation with nematodes on microplots (n = 48).

Interaction factor		*M. enterolobii*	*M. incognita*	*M. javanica*
Nematode (N)	Aphid (A)	RP[x]	RP	RP
N_1	A_0	$11.78^{b} \pm 0.4$	$7.21^{b} \pm 1.2$	$9.97^{b} \pm 1.3$
N_1	A_1	$34.95^{a} \pm 0.3$	$30.28^{a} \pm 0.4$	$25.09^{a} \pm 0.5$
Relative impact (%)[y]		196	320	152

[x]Column means ± standard error followed by the same letter were not different (P ≤ 0. 05) according to Student t-test.

[y]R.I = Relative impact (%) = [(interaction value/control value) − 1] × 100.

A_0 = without aphid, A_1 = with aphid, N_0 = without nematode, N_1 = with nematode.

The interactive effects of *Meloidogyne* species and sugarcane aphid population density had significant effects on plant variables (Appendix 3.3). Relative to untreated control, the interactive effects of *M. enterolobii* with aphid significantly reduced dry root mass, plant height and stem diameter by 44, 33 and 20%, respectively. In contrast, the interactive effects of *M. incognita* with sugarcane aphid had significant effects on dry root mass and peduncle length (Appendix 3.4), Relative to untreated control, the interactive effects of *M. incognita* with sugarcane aphid reduced the variables by 48 and 48%, respectively (Table 3.3).

Table 3.3 Interactive effects of *Meloidogyne* species with aphid on selected plant growth variables of sweet stem sorghum at 150 days after inoculation with nematodes on microplots (n = 48).

	Aphid			
	A_0[x]	R.I. (%)[y]	A_1	R.I. (%)
Meloidogyne enterolobii				
	Dry root mass (g/plant)			
N_0	656.45[a] ± 0.3	–	492.85[b] ± 1.2	–25
N_1	418.05[b] ± 1.4	–36	370.08[b] ± 0.2	–44
	Plant height (cm)			
N_0	32.50[a] ± 2.2	–	30.08[a] ± 1.2	–7
N_1	31.75[a] ± 0.5	–2	21.62[b] ± 0.5	–33
	Stem diameter (mm)			
N_0	22.71[a] ± 0.6	–	20.28[b] ± 1.3	–10
N_1	21.49[ab] ± 0.7	–5	18.03[ab] ± 2.2	–20
Meloidogyne incognita				
	Dry root mass (g)			
N_0	631.21[a] ± 0.6	–	307.31[b] ± 1.3	–51
N_1	367.18[b] ± 0.7	–42	325.26[b] ± 0.8	–48
	Head/panicle length (cm)			
N_0	63.04[a] ± 0.2	–	34.76[b] ± 0.7	–45
N_1	26.12[b] ± 1.7	–59	32.97[b] ±1.5	–48

[x]Column means ± standard error followed by the same letter were not different ($P \leq 0.05$) according to Fisher's Least Significant Different test.

[y]R.I = Relative impact (%) = [(interaction value/control value) − 1] × 100.

A_0 = without aphid, A_1 = with aphid, N_0 = without nematode, N_1 = with nematode.

Although other nutrient elements (Cu, K, P, Mg, Mn, and Na) in leaf tissues of SSS cv. 'Ndendane-X1' were not significantly affected by the interactions, the interaction had significant effects on S, Zn, Ca and Fe (Appendix 3.5). Relative to untreated control, *M. enterolobii* and sugarcane aphid population density interacted to significantly reduce S and Zn by 40 and 47%, respectively. *Meloidogyne incognita* interacted with sugarcane aphid to reduce Ca and Zn by 24 and 51%, respectively, whereas *M. javanica* and aphid reduced Fe and Zn by 52% (Table 3.4).

Table 3.4 Interactive effects of *Meloidogyne* species with aphids on selected nutrient elements in sweet stem sorghum leaf tissues at 150 days after inoculation with nematodes on microplots (n = 48).

	Aphid			
	A_0[x]	R.I. (%)[y]	A_1	R.I. (%)
Meloidogyne enterolobii				
		S (mg/kg)		
N_0	501.46[a] ± 3.2	–	455.10[a] ± 1.2	–9
N_1	465.10[a] ± 0.7	–7	300.31[b] ± 0.8	–40
		Zn (mg/kg)		
N_0	78.135[a] ± 0.4	–	42.271[c] ± 2.2	–46
N_1	61.219[b] ± 0.6	–21	41.677[c] ± 0.2	–47
Meloidogyne incognita				
		Ca (mg/kg)		
N_0	995.84[a] ± 1.2	–	567.50[ab] ± 1.5	–43
N_1	786.76[ab] ±1.3	–20	754.83[b] ± 0.5	–24
		Zn (mg/kg)		
N_0	185.98[a] ± 0.5	–	80.01[c] ± 0.8	–56
N_1	107.93[b] ± 1.5	–41	91.03[c] ± 0.2	–51
Meloidogyne javanica				
		Fe (mg/kg)		
N_0	216.56[a] ± 0.9	–	141.49[b] ± 1.9	–34
N_1	215.59[a] ± 1.3	–0.4	104.75[c] ± 0.5	–52

[x]Column means ± standard error followed by the same letter were not different (P ≤ 0. 05) according to Fisher's Least Significant Different test.
[y]R.I = Relative impact (%) = [(interaction value/control value) − 1] × 100.
A_0 = without aphid, A_1 = with aphid, N_0 = without nematode, N_1 = with nematode

3.4 Discussion

Two mechanisms of nematode resistance exist, namely, pre-infectional and post-infectional nematode resistance (Kaplan and Davis, 1987). In both mechanisms, chemical compounds with the origin from photosynthesis are involved. Such chemicals are first translocated through the phloem vessels to sinks as sucrose (Salisbury and Ross, 1992.), which is a reducing sugar with the ability to change osmotic potential and therefore, turgor pressure in plant cells. In pre-infectional nematode resistance, which occurs in SSS, the mechanism involves the release of copious concentration of sorgolene ($C_{42}H_{66}O_{13}$) into the rhizosphere (McSorley *et al*., 1994), which has nematicidal properties with attributes for the repellent of J2 from the rhizosphere (Hewezi and Baum, 2015). Sorgolene, as a secondary metabolite, originates from photosynthesis and is technically the tetraterpene, which is derived from the precursors with 40-C atoms (Van Wyk and Wink, 2004). The tetraterpenes as terpenoids are chemically reactive, with their functional groups have capabilities to form covalent bonds with free amino groups of proteins as well as the SH groups of proteins (Van Wyk and Wink, 2004). There is evidence that sorgolene, although it is released as a repellent chemical product, it actuals kills J2 in the soil (Huang *et al*. 1986), thereby preventing J2 from penetrating the root system. In the current study, the reduction of sucrose by the sugarcane aphid as shown by the reduced degrees Brix and honeydew on SSS leaf surfaces, along with sooty mould which reduced the photosynthesis area of leaves, could have had a bearing on the sorgolene quantity available to prevent J2 from penetrating the root system. Most unfortunately, in pre-infectional nematode resistance, once the exuded chemicals are by-passed, there is not another means of resistance to J2 inside the root system (Mashela *et al*., 2016).

Wondafrash *et al*. (2013) demonstrated that there were mediated interactions, where the induced systemic effects from belowground pests could affect the aboveground pests, *vice versa*. Naturally, phloem feeders like aphids, insert their stylets directly into the phloem sap for feeding, with large content of the sap being released onto the plant surfaces, which was later covered by sooty mould (Singh *et al*., 2004). Constituents of sucrose are required for the biosynthesis of both primary and secondary metabolites. Most importantly, the loss of sucrose as observed in the current study would eventually have direct effects on the concentration of exudates such as sorgolene, thereby affecting its nematicidal properties to J2. The reduction in sorgolene in the current study was characterised through measurements of °Bx in various parts of SSS, which was significantly reduced in aphid-infested plants. In another nematode-aphid interactive study (Kutyniok and Muller, 2012), it was shown that aphids changed the glucosinolate content in the phloem sap. Glucosinolates constitute a natural class of organic compounds that contain sulphur and nitrogen and occur as derivatives of glucose and an amino acid (Agerbirk and Olsen, 2012). The *M. incognita* × aphid interaction in the current study reduced S in leaf tissues of SSS, which could translate into affecting the concentration of the glucosinolate in the phloem sap. Glucosinolates are enzymatically hydrolysed to produce sulphate ions, D-glucose and characteristic degradation products such as isothiocyanates, which have strong nematicidal properties (Pastorczyk and Bednarek, 2016).

Meloidogyne species and sugarcane aphid interacted to significantly increase the reproductive potential (fecundity) values of the test *Meloidogyne* species on SSS cv. 'Ndendane-X1', at the same time, the interaction significantly reduced plant growth variables. In nematode resistant plants, the reproductive potential values above unity,

with nematode infection reducing plant growth variables, both denote the loss of nematode resistance for the test nematode (Dropkin, 1959; Pofu *et al*., 2013). In the current study, the cultivar lost its resistance to all three thermophilic *Meloidogyne* species. Following the first observation of loss in nematode resistance in *C. africanus* to *M. incognita*, which was heavily infested with the greenhouse whiteflies under greenhouse conditions (Pofu *et al*., 2013), the observation in the current study constitutes the second report where a nematode interacted with a sucking insect to break nematode resistance in plants. The significance of this observation is that both *Meloidogyne* species (Dana, 2003) and the sugarcane aphid (Singh *et al*., 2004) are worldwide key pests of sugarcane and SSS, where nematode resistance (Dana, 2003) and aphid resistance (Singh *et al*., 2004) are preferred strategies for managing population densities of each pest. Findings in the current study could also be applicable in other plants with resistance to *Meloidogyne* species, where sucking insects are key pests. In crop production, sucking insects could include aphids, mealybugs, scales and whiteflies.

In other nematode-aphid interactions on various crops, the main focus was on aphid performance as measured through growth rate and fecundity, along with the related mechanisms involved (Bezemer *et al*., 2005; Hol *et al*., 2010; Kaplan *et al*., 2011; Wurst and Van der Putten, 2007), but hardly on nematode performance. The suggested mechanisms in reduced aphid fecundity included changes in nutritional quality of phloem sap (Wurst and Van der Putten, 2007), where reduced amino acids (Bezemer *et al*., 2005; Hol *et al*., 2010; Kaplan *et al*., 2011) or changes in glucosinolate content (Kutyniok and Muller, 2012) were implicated. In some cases, the nematode-aphid interactions resulted in lower aphid growth rates (Kaplan *et al*., 2011) or there

were no significant effects on aphid performance (Kabouw *et al*., 2011; Kutyniok and Muller, 2012), without any effects on nematode population densities (Kaplan *et al*., 2011), except in Kutyniok and Muller (2012), where nematode infection was reduced. In the cited nematode-aphid interactions, it was hardly explicit whether the test plants were resistant to the test nematodes.

Wondafrash *et al*. (2013) observed that aphids had no significant effects on nematode performance. In nematode-chewing insect interaction, the interaction had no effects on caterpillar development (Carter-Wientjes *et al*., 2004; Kaplan *et al*., 2008; Olson *et al*., 2008), but increased caterpillar performance in terms of foraging (Kaplan *et al*., 2008). Although in most nematode-insect interactive studies the focus was primarily on how such interactions affected the performance of the insect, leaf chewers in such interactions either increased nematode performance in terms of reproductive potential (Alston *et al*., 1993), reduced nematode root penetration due to systemic induced defence (Tiwari *et al*., 2009) or had no effects on nematode performance (Fu *et al*., 2001). In the study by Kaplan *et al*. (2011), although the nematode-aphid interaction reduced aphid growth rate and fecundity, the reciprocal effects on nematodes were not studied. In the listed interactions the test nematodes included migratory and sedentary nematodes, with a wide range of aphid genera and species as summarised in a review by Wondafrash *et al*. (2013). Overall, *Pratylenchus* species as migrating endoparasitic nematodes generally reduced aphid fecundity, whereas *Heterodera, Globodera* and *Meloidogyne* species as sedentary endoparasitic nematodes reduced aphid performance by decreasing the attractiveness of the plants by inducing systemic defense systems (Wondafrash *et al*., 2013).

The reduced Ca and Fe in some of the nematode-aphid interactions in the current study could also explain some of the observed results, primarily those associated with reduced nematode resistance. Generally, nematode resistance could be induced by both chemicals at a molecular level and physical structures in plants (Mashela *et al*., 2016; Wondafrash *et al*., 2013). During root penetration of J2 from soil solutions at the elongation zone, the latter is preferred due to limited lignin, which confers much physical resistance to J2. Similarly, Ca constitutes a high percentage of the middle lamella between adjacent cell walls (Salisbury and Ross, 1992.), which also confers some strength to adjacent cells, and therefore resistance to J2 (Mashela *et al*., 2016). The reduction of Ca could adversely affect the thickness of the cell walls, thereby reducing physical resistance to J2 penetration. The Fe ion, in addition to serving important roles as a catalyst in biosynthesis of primary molecules (Salisbury and Ross, 1992.), it is an integral catalyst in the biosynthesis of various molecules that are indispensable in plant systemic induced responses that mediate interactions between belowground and aboveground pests (McMahon *et al*., 2002).

3.5 Conclusion

The effect of sugarcane aphid on SSS cv. 'Ndendane-X1' compromised the preinfection nematode resistance, which is dependent upon sorgolene for its efficacy. Sorgolene is a secondary metabolite, with a large number of carbon, hydrogen and oxygen atoms. The breakdown in nematode resistance could be attributed to the fact that sorgolene as a secondary metabolite has its origin from the photosynthates and aphids feed directly from the phloem sap, which contains sucrose. Apparently, the successful use of nematode resistance in SSS cv. 'Ndendane-X1' in areas with high population densities of *Meloidogyne* species would depend upon the effective

management of aphid population densities. The null hypothesis which suggested that the sugarcane aphid would not have effect on sucrose content and nematode resistance present in a sweet stem sorghum cv.'Ndendani-X1' was therefore rejected. In the next research chapter, the combined effects of the three thermophilic *Meloidogyne* species and sugarcane aphid on nematode resistance in SSS cv. 'Ndendani-X1' were investigated under field conditions.

CHAPTER 4

MELANAPHIS SACCHARI BREAKS ROOT KNOT RESISTANCE PRESENT IN SWEET STEM SORGHUM CV 'NDENDANE-X1' UNDER MIXED INOCULATION OF *MELOIDOGYNE* SPECIES

4.1 Introduction

The semi-arid regions, where sweet stem sorghum (*Sorghum bicolor* L.) cv. 'Ndendane-X1', with high resistance to root-knot (*Meloidogyne* species) nematodes, is being cultivated for ethanol production, have high population densities of thermophilic *Meloidogyne* species (Mashela and Pofu, 2016). Such regions are also renowned for the high population density of sugarcane aphid (*Melanaphis sacchari* Zehntner), which is a key pest in sorghum and sugarcane (*Saccharum officinarum* L.*)* production (Singh *et al*., 2014). The highly nematode resistant sweet stem sorghum (SSS) cv. 'Ndendane-X1', is highly susceptible to sugarcane aphid (Mashela and Pofu, 2016). Generally, nematode resistance is an active process, where products of photosynthetic origin are essential in producing plant genes (Mashela *et al*., 2016). Roots of nematode resistant SSS cultivars exude sorgolene ($C_{42}H_{66}O_{13}$), which is an active ingredient with capabilities to repel second-stage juveniles (J2) from the rhizosphere (Huang *et al*., 1986). Sorgolene is one of the seven classes of the terpenoids with 42-C atoms (Van Wyk and Wink, 2004), derived from the precursors with 40-C atoms, with functional groups that have antimicrobial activities (Van Wyk and Wink, 2004), which include nematicidal activities (Czarnota *et al*., 2003; Dayan *et al*., 2010). Nematode resistance in *Sorghum* species had been shown to be highly variable from season to season in crop rotation systems intended to manage nematode population densities (McSorley, 211), without any information on the

explanation of the probable causes. Generally, sorghum cultivars which were shown to be highly resistant to *Meloidogyne* species under greenhouse conditions, were observed to have no nematode resistance under field conditions (McSorley, 2011). In McSorley (2011) study in northern Florida, USA, with tropical climate, aphid population densities were neither mentioned nor controlled. Because aphids feed from sucrose in the phloem sap (Singh *et al*., 2004), high population densities of sugarcane aphid could interfere with sorgolene (Chapter 3), which emanates from the photosynthesis as a secondary metabolite (Dayan *et al*., 2010). Sorgolene, as alluded to earlier (Chapter 3), requires an excessively large number of carbon, hydrogen and oxygen atoms, which have the origin from photosynthesis. Previously, Pofu *et al*. (2013) demonstrated that the greenhouse whiteflies (*Trialeurodes vaporariorum* Westwood) could break nematode resistance to *Meloidogyne* species in wild watermelon (*Cucumis africanus* L.F.), which has post-infectional nematode resistance (Kaplan and Davis, 1987). In plants with such resistance mechanism, J2 penetrate the root system, where plant genes are either downregulated or upregulated by the gene products in the nematode to resist or allow mutual existence (Mashela *et al*., 2016). Such plant genes can be triggered by an aboveground feeder, with the induced chemicals systematically moved from foliage to roots, *vice versa*, to have adverse effects, on root pests (Wondafrash *et al*., 2013).

In another form of nematode resistance, namely, pre-infectional nematode resistance, J2 are not allowed to penetrate the root systems (Kaplan and Davis, 1987). Currently, there is no information on how the interaction of *Meloidogyne* species and sugarcane aphid would affect nematode resistance in plants with pre-infectional nematode resistance, where chemical compounds with nematicidal properties are released into

the rhizosphere to induce bioactive behaviours on J2 that prevent root penetration. The objective of this study was to determine if sugarcane aphid breaks root-knot nematode resistance present in sweet stem sorghum cv. 'Ndendani-X1'under mixed inoculation of *Meloidogyne* species. The null hypothesis suggested that sugarcane aphid would not breaks root-knot nematode resistance present in sweet stem sorghum cv. 'Ndendani-X1'under mixed inoculation of *Meloidogyne* species

4.2 Materials and methods

4.2.1 Location of the study

A trial was conducted in a field with mixed *Meloidogyne* species, at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, South Africa (23°53'10''S, 29°44'15''E). The location had mean annual rainfall of less than 500 mm, with the annual distribution skewed towards summer (November-January), whereas the minimum/maximum temperature averaged 18/38°C. The experiment was initiated in late spring (August-October) 2017 and validated in 2018. The site comprised Hutton form with loam soil (65% sand, 30% clay, 5% silt) that contained 1.6% organic C, EC 0.148 dS/m and pH (H_2O) of 6.5.

4.2.2 Treatments, experimental design and procedures

Plants were in a 0.3 m × 0.3 m spacing, with 10 plants in a 3 m long row of nematode alone and a 3 m long row of nematode + aphid constituting a replication. The two treatments were separated by three border rows of SSS on all cardinal sides to allow for spraying of aphid and stalk borer (*Papaipema nebris*) on border plants. Treatments, within the border rows, were arranged in a randomised complete block design, with six replications. Blocking was done against shading from the windbreak trees in the

morning or in the afternoon. Six seeds were sown per station and seedlings thinned to one per sowing station at two true-leaf-stage. The site was sampled for initial nematode population density (Pi) of *Meloidogyne* species at thinning, with J2 extracted from a 250 ml soil subsample using sugar floatation method (Jenkins, 1964).

Figure 4. 1 Sweet stem sorghum cv. 'Ndendane-X1' in mixed populations of *Meloidogyne* species under field trial.

Three days after thinning, each seedling was fertilised with 5 g 2:1:2 (43) Multifeed fertiliser (Nulandies, Johannesburg) to provide a total of 0.70 mg N, 0.64 mg K and 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 per ml water (Mashela, 2002). Each seedling was also fertilised with 2 g NPK 2:3:2 (22) + 0.5% S + 5% Zn + 5% Ca, primarily to supply Ca. During vegetative growth, from sowing to the initiation of the reproductive stage, seedlings were irrigated every third day with 2 litres chlorine-free tapwater, and thereafter with 4 litres water per week. Two weeks after thinning, 20 adult aphids, reared on aphid-susceptible SSS cultivated

at the University Experimental Farm (13 km away), were picked up from the abaxial surface of older leaves using a soft brush into sealed 10ml vials with leaf pieces, placed in a cooler box and transported to the experimental site. Aphids were released by placing each vial upside down in the whorl of an appropriate treatment. Border rows were sprayed once prior to aphid infestation for stalk borer (*Papaipema nebris*) using 5 ml Bulldock Beta 125 SC/10 litre chlorine-free water. Infestation of border rows by sugarcane aphid was monitored daily, with entities sprayed with 5 ml Malasol/10 litre water, whenever more than 20 aphids were observed within the border row plants. During such sprays, temporary barriers were erected to ensure that treatments were not contaminated by spray drifts.

4.2.3 Data collection

Plant variables: At 150 days after inoculation with nematodes, plant height measured from the soil surface to the top of the panicle using measuring tape. Stem diameter was measured at 5 cm above the severed ends using a digital Vernier calliper. tiller number, along with internode number in both primary and tiller stalk, were recorded. The panicle was cut from mother plant shoots and tillers, with fresh mass measured. Peduncle length was measured from the base of the tassel to the apex of the top internode.

Degrees Brix: Stems were marked into three classes, namely, top (internode just below the peduncle), bottom (internode just above the severed-end of the stem) and middle (middle distance between the top and bottom internodes). The middle part of each class was cut using a knife that was soaked in distilled water and wiped using laboratory towel prior to re-use, with the juice from the top-end of the cut portion

squeezed using a pair of pliers onto the eye of a hand-held digital refractometer for determining the degrees Brix. Shoots of primary stalk and tiller plants, along with the remaining roots were dried in air-forced ovens (AD 200 AGRI-DRYER, White River, South Africa) at 52°C for 72 h, for determination of dry shoot and dry root mass.

Nematode variables: Roots of the three sampled plants per plot were dug using a fork, immersed in tapwater to remove soil particles, blotted dry, chopped into small pieces and mixed. Eggs + J2 were extracted from a sample of approximately 10 g fresh roots using the maceration and blending method in 1% NaOCl solution as explained previously (Chapter 3). Also, one litre soil sample was collected from the three sowing stations of the sampled plants, mixed and 250 ml subsample used for the extraction of final J2 population density (Pf-J2) using the centrifugation method (Jenkins, 1964), In either case, the material was passed through top-down nested 150- and 25-µm pore sieves, with contents of the 25-µm opening sieve further subjected to sugar and centrifugation method, with kaolin added in each centrifuge tube (Marais *et al*., 2017). Contents were passed through top-down nested 150- and 25-µm pore sieves, with contents of the latter poured into 100-ml-plastic containers, brought to 100-ml mark, tightly closed and stored at 13°C prior to counting from 5 ml aliquot under a 60 × magnification stereomicroscope. Nematode eggs and J2 from roots were expressed as reproductive potential (RP = eggs + J2/g roots), whereas J2 from soil were expressed as (RP = Pf-J2/Pi-J2).

Nutrient elements: Three healthy and mature leaves from the three plants each were wiped with disposable laboratory towel moistened with distilled water, dried at 70°C for 72 h and ground in a Wiley mill to pass through a 0.75 mm sieve. Approximately

0.4 g leaf tissue samples were subjected to microwave digestion method by immersing materials in a mixture of nitric acid (5 ml HNO_3) and hydrogen peroxide (3 ml H_2O_2) in a Perkin Elmer titan MPS for 45 minutes. Aliquots were then incubated in a warm water bath for an hour at 95°C, left to cool down at room temperature, filtered and the container covered with a foil. Samples were then submitted to Limpopo Agro-food Station (LATS) where they were subjected to Atomic Absorption Spectrometry (AAS) to quantify for Zn, Fe, K, P, Mg, Mn, Ca, Cu, S and Na nutrient elements through SW-864 EPA method 3050-B using Inductively Coupled Plasma Optical Emission Spectrometry (ICPE-9000).

4.2.4 Data analysis

Data for each variable were assessed for seasonal differences using the Levene-test (Levene, 1960) and since seasonal effects were not significant at the probability of 5%, data were pooled (n = 48) and subjected to Student t-test analysis using Statistix 10.1 software. Unless otherwise stated, treatment means were discussed at the probability level of 5%.

4.3 Results

Treatments had highly significant effects on top degrees Brix and middle degree Brix, but significant ($P \leq 0.05$) on bottom degree Brix of SSS cv. 'Ndendane-X1' (Appendix 4.1). Relative to nematode alone, nematode + aphid reduced degree Brix in top, middle and bottom parts of SSS cv. 'Ndendane-X1' by 61, 33 and 13%, respectively (Table 4.1). Treatment effects on reproductive potential of *Meloidogyne* species on SSS cv. 'Ndendane-X1' and on the related root galls were highly ($P \leq 0.01$) significant (Appendix 4.2). Relative to nematode alone, nematode + aphid increased the

reproductive potential of *Meloidogyne* species and root galls by 279 and 199%, respectively (Table 4.2), along with J2 in soil samples.

Table 4.1 Combined effects of *Meloidogyne* species and aphids relative to those of *Meloidogyne* species alone on degree Brix of sweet stem sorghum from three stem parts at 150 days after seedling emergence under field conditions (n = 48).

Treatment	Top Brix (%)	Middle Brix (%)	Bottom Brix (%)
Nematode	$12.00^{a} \pm 0.9$	$15.35^{a} \pm 0.6$	$11.91^{a} \pm 0.8$
Nematode + aphid	$4.71^{b} \pm 1.7$	$10.32^{b} \pm 0.7$	$10.38^{b} \pm 0.8$
Relative impact (%)[y]	-61^{***x}	-33^{***}	-13^{**}

[y]Relative impact (%) = [(interaction value/control value) − 1] × 100.

[x*** and **]Significant at P ≤ 0.01 and P ≤ 0.05, respectively, according to Student t-test.

Table 4.2 Combined effects of *Meloidogyne* species and aphids relative to those of *Meloidogyne* species alone on nematode reproductive potential and root galls on sweet stem sorghum at 150 days after seedling emergence under field conditions (n = 48).

Treatment	Reproductive potential	Root galls
Nematode	$0.68^{b} \pm 0.2$	$1.17^{b} \pm 0.2$
Nematode + aphid	$1.88^{a} \pm 0.1$	$3.50^{a} \pm 0.3$
Relative impact (%)[y]	279^{***}	199^{***}

[y]Relative impact (%) = [(interaction value/control value) − 1] × 100.

[x***] Significant at P ≤ 0.01 according to Student t-test.

The treatment effects were significant on plant height, internode number, peduncle length and stem diameter (Appendix 4.3). Relative to nematode alone, nematode + aphid reduced plant height, internode number, peduncle length and stem diameter by 47%, 41%, 55% and 35%, respectively (Table 4.3). Treatment effects had highly significant effects on Ca, but significantly affected K and Zn in leaf tissues of SSS cv. 'Ndendane-X1' (Appendix 4.4). Relative to nematode alone, nematode + aphid reduced Ca, K and Zn in leaf tissues of the test cultivar by 73%, 43% and 33%, respectively (Table 4.4).

Table 4.3 Combined effects of *Meloidogyne* species and aphids relative to those of *Meloidogyne* species on plant growth variables of sweet stem sorghum at 150 days after seedling emergence under field conditions (n = 48).

Treatment	Plant height (cm)	Internode number	Peduncle length (cm)	Stem diameter (mm)
Nematode	101.0[a] ± 2.7	13.7[a] ± 0.6	29.1[a] ± 4.5	21.3[a] ± 2.5
Nematode +Aphid	53.9[b] ± 1.9	8.1[b] ± 0.5	13.1[b] ± 0.5	13.8[b] ± 0.4
Relative impact (%)	–47[**]	–41[**]	–55[***x]	–35[**]

[y]Relative impact (%) = [(interaction value/control value) − 1] × 100.

[x]*** and **Significant at P ≤ 0.01 and P ≤ 0.05, respectively, according to Student t-test.

Table 4.4 Combined effects of *Meloidogyne* species and aphids relative to those of *Meloidogyne* species selected nutrient elements in sweet stem sorghum leaf tissues at 150 days after seedling emergence under field conditions (n = 48).

Treatment	Ca (mg/kg)	K (mg/kg	Zn (mg/kg)
Nematode	$876.6^{b} \pm 0.7$	$3397.0^{a} \pm 1.7$	$57.4^{a} \pm 2.0$
Nematode + Aphid	$430.3^{c} \pm 0.8$	$1930.1^{b} \pm 4.2$	$38.6^{b} \pm 2.8$
Relative impact (%)	-73^{***x}	-43^{**}	-33^{**}

[y]Relative impact (%) = [(interaction value/control value) − 1] × 100.

[x]*** and ** Significant at P ≤ 0.01 and P ≤ 0.05, respectively, according to Student t-test.

4.4 Discussion

The phloem-sap feeders like aphids have evolved with their mouth parts being converted into needle-like structures with lumen, biologically referred to as stylets, which are inserted into the phloem for sap-sucking. Unfortunately, during the process of feeding, large content of sucrose is released onto the plant surface, which is later covered by sooty mould (Narayana, 1975). Constituents of sucrose are required for the biosynthesis of secondary metabolites, which are the primary products used in plant defense systems. The loss of sucrose as observed in our study confirmed observations that sugarcane aphid significantly reduced total sugars and polyphenols (Balikai, 2007), along with crude fibre and carbohydrate content (Van den Berg *et al*., 2003). This reduction of sugars would eventually have detrimental effects chemical compounds that confer nematode resistance such as sorgolene ($C_{42}H_{66}O_{13}$), which are released into the rhizosphere to suppress nematode population densities. Under both treatments, degrees Brix content was below the 16-°Bx permissible for ethanol production in SSS cultivars (Liang *et al*., 2010). Practically, both pests should be

managed if SSS cultivars have to meet the minimum requirement for premium when the cultivars are produced for ethanol production, either using non-plant based strategies such as insecticides or using plant-based strategies such as nematode resistance.

The increase in reproductive potential in roots and increased root galls of *Meloidogyne* species when combined with aphids under field conditions, along with the reduction of various plant growth variables, our proposed hypotheses, which states that the reduction of photosynthates, would interfere with plant resistance, since the latter is dependent on the products with origin from the former. In the current study under field conditions, the highly nematode resistant SSS cv. 'Ndendane-X1' (Mashela and Pofu, 2016), obviously lost its resistance to *Meloidogyne* species, as shown by high reproductive potential and root galls. On a number of occasions, when using sorghum cultivars with high nematode resistance in northern Florida, USA, Gallaher *et al.* (1991) observed that certain sorghum cultivars with known resistance to *Meloidogyne* species readily lost nematode resistance under field conditions. However, at times, the same sorghum cultivar retained its nematode resistance during certain seasons. Incidentally, outbreaks of sugarcane aphid in northern Florida occurred for the first time on sorghum in 1977 (Zapata *et al*., 2016). Generally, aphids infest sorghum soon after emergence, with population density gradually increasing to reach a peak during the grain filling stages, when most of the damage occurs (Van Rensburg, 1973). Generally, the sugarcane aphid feeds on both the ventral and dorsal surfaces of older sorghum leaves, with honeydew dripping onto leaves below, which are eventually, covered by sooty mould (Narayana, 1975), which further reduces the photosynthetic

capacity of affected plants and therefore support the proposed hypotheses, where nematode-aphid interaction breaks nematode resistance.

After the withdrawal of most biocidal fumigant nematicides from the agrochemical markets, both *Meloidogyne* species and sugarcane aphid population densities are globally being managed using nematode resistance (Dana, 2003; Singh *et al*., 2004). Although plant genes such as Mi genes in plants with post-infectional nematode resistance mechanism, were observed to have the capability to suppress aphids (Rossi *et al*., 1998; Vos *et al*., 1998), in plants with pre-infectional mechanisms of nematode resistance like sorghum and sugarcane, such attributes have not been reported. For instance, although the test cultivar in the current study is highly resistant to *Meloidogyne* species, it is highly susceptible to sugarcane aphid infestation (Mashela and Pofu, 2016). In other nematode-aphid interaction studies, interactions either had no significant effects on nematode population densities (Kaplan *et al*., 2011; Wondafrash *et al*., 2013) or reduced nematode infection (Kutyniok and Muller, 2012).

The reduction of Ca, K and Zn in the current study under nematode + aphid treatment confirmed observations where aphids significantly reduced total minerals (Balikai, 2007). Calcium contributes to the biosynthesis of the middle lamella, which confer strength to cell walls (Salisbury and Ross, 1992.), which could be providing a multidimensional aspect on the observed reduction in nematode resistance since during penetration J2 had to move through the cell walls (Mashela *et al*., 2016). The reduced Zn could explain the reduced panicle length in the current study, which is generally associated with a rosette of leaves in plants with deficient Zn (Salisbury and Ross, 1992.). Although K is related to grain filling (Salisbury and Ross, 1992), in the

current study, its effects as measured through panicle mass was not significant under field conditions.

4.5 Synthesis and conclusion

In various sorghum varieties and sugarcane hybrids were nematode resistance was relied upon for some time, nematode resistance was lost, for unexplained reasons. In some cases, without the use of differential plant tests or molecular approaches, it was concluded that nematode races were induced by unexplained factors. In the current study, where findings supported those in microplots (Chapter 3), it could be said with certainty that nematode-aphid interaction was responsible for loss of nematode resistance in sorghum cv. 'Ndendane-X1'. The reduction in photosynthates as measured through the decrease in degree Brix, was characterised by the increased reproductive potential values of *Meloidogyne* species and reduction of plant growth variables, which supported the view of broken nematode resistance under nematode + aphid population densities. The null hypothesis which suggested that the combined effects of mixed population of *Meloidogyne* species and sugarcane aphid would not break pre-infectional nematode resistance in SSS cv. 'Ndendani-X1' under field conditions was therefore, rejected. In conclusion, the successful use of nematode resistance in SSS cv. 'Ndendane-X1' in areas with high population densities of *Meloidogyne* species would depend upon the effective management of aphid population densities. In the ensuing research chapter, the interactive effects of Nemarioc-AL, Nemafric-BL and Mordica phytonematicides, which are terpenoid-containing products, with the history of suppressing nematode population densities, were investigated as to whether they would stimulate sugar content and plant growth variables in sorghum cv. 'Ndendane-X1' under pest-free conditions.

CHAPTER 5
TERPENOID-CONTAINING PHYTONEMATICIDES INCREASE SUGAR CONTENT AND GROWTH IN SWEET STEM SORGHUM

5.1 Introduction

Sweet stem sorghum (SSS) cv. 'Ndendane-X1' was selected for ethanol production due to its high degrees Brix (°Bx), which was greater than 19°Bx at the time of selection (Mashela and Pofu, 2016). SSS cultivars for ethanol production should have at least 15°Bx (Vasilakoglou *et al*., 2011), with growers receiving premium for cultivars with at least 16°Bx. Due to various unknown reasons, sugar content is unstable, with the cv. 'Ndendane-X1' at times having °Bx less than the 15°Bx minimum required for ethanol production. Mabuka (2013) previously observed that cultivation of cv. 'Ndendane-X1' on sowing stations where nematode population densities were previously managed using terpenoid-containing phytonematicides had significantly higher °Bx than from those sown on untreated control plots.

The terpenoids (or terpenes) comprise seven classes of chemical compounds, most with antimicrobial activities (Van Wyk and Wink, 2004). The cucurbitacin-containing terpenoids, technically the triterpenes are derived from the precursors with 30-C atoms, whereas the momordin-containing terpenoids are the tetraterpene, from precursors with 40-C atoms. The cucurbitacin-group have two phytonematicides, Nemarioc-AL and Nemafric-BL phytonematicides, containing the active ingredients cucurbitacin A ($C_{32}H_{46}O_9$) and cucurbitacin B ($C_{32}H_{46}O_8$), respectively. In contrast, the momordin-group has Mordica phytonematicide, containing momordin ($C_{42}H_{66}O_{13}$) active ingredient. Generally, the terpenoids have two primary functional groups,

namely, (i) the aldehyde function that form covalent bonds with free amino groups of proteins and (ii) the terminal or exocyclic methylene groups, which couple to SH groups of proteins (Van Wyk and Wink, 2004). Additionally, the peroxides (inner oxides) of terpenoids are chemically reactive and can bind to proteins.

The terpenoids were also shown to have the potential to improve soil health when exuded from roots of *Cucumis africanus* L.F. (Mashela and Dube, 2014), with limited information on how the chemical compounds achieve this important attribute. Following the observation that the residual effects of Nemarioc-AL phytonematicide increased sugar content in cv. 'Ndendane-X1' (Mashela and Dube, 2014), the question was raised on whether the three commercially used terpenoid-containing phytonematicides would not improve the sugar content of the cultivar, along with its plant growth variables much better when combined than when operating alone. The objective of this study was to determine if application of terpenoid-containing phytonematicides increase sugar content and growth in sweet stem sorghum cv. 'Ndendane-X1'. The null hypothesis suggested that the application of terpenoid-containing phytonematicides do not increase sugar content and growth in sweet stem sorghum cv. 'Ndendane-X1'.

5.2 Materials and methods

5.2.1 Location of the study

The experiment was conducted under micro-plot conditions at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, South Africa (23°53'10"S, 29°44'15"E). The location had mean annual rainfall of less than 500 mm, which occur mostly during summer (November-January), whereas

minimum/maximum temperature averaged 28/38°C. The experiment was established in early summer 2018 and validated in 2019.

5.2.2 Treatments, experimental design and procedures

A 2 × 2 × 2 factorial experiments, with the first, second and third factor comprising Nemarioc-AL (with and without), Nemafric-BL (with and without) and Mordica (with and without) phytonematicides, respectively. The eight treatments, comprising $A_0B_0M_0$, $A_1B_0M_0$, $A_0B_1M_0$, $A_0B_0M_1$, $A_1B_1M_0$, $A_1B_0M_1$, $A_0B_1M_1$ and $A_1B_1M_1$, were arranged in a randomised complete block design, with three replications. Blocking was done for shading from windbreak trees.

Figure 5.1 Sweet stem sorghum cv. 'Ndendane-X1' at 150 days after planting.

Thirty-cm-diameter plastic pots, each filled with pasteurised (300°C for 1 h) loam soil (65% sand, 30% clay, 5% silt, 1.6% organic C, EC = 0.148 dS/m, pH (H_2O) = 6.5), which was derived from the experimental site. Six SSS seeds of cv.'Ndendane-X1' were sown per pot and thinned to one seedling per pot at two true-leaf stage. The terpenoid phytonematicides were separately prepared from fruits of wild cucumber (*Cucumis myriocarpus* Naude.), wild watermelon (*Cucumis africanus* L.F.) and bitter gourd (*Momordica balsamina* L.) as Nemarioc-AL, Nemafric-BL and Mordica phytonematicides, respectively, through effective microorganism fermentation (Mashela *et al*., 2017b). Weekly treatments were applied through botinemagation as substitute for 1 litre water/plant irrigation every third day. The main factors ($A_1B_0M_0$, $A_0B_1M_0$ and $A_0B_0M_1$) were each combined at 3%, whereas the first order interactions ($A_1B_1M_0$, $A_1B_0M_1$, $A_0B_1M_1$) were each at 1.5% and the second order interaction ($A_1B_1M_1$) were each at 1%. Plants were irrigated using a drip irrigation system for 3 h every other third day until the end of vegetative reproduction, where irrigation interval increased to one week for 3 h. Each seedling was fertilised with 5 g 2:1:2 (43) Multifeed fertiliser (Nulandies, Johannesburg) which provide a total of 0.70 mg N, 0.64 mg K and 0.64 mg P, 1.8 mg Mg, 1.5 mg Fe, 0.15 mg Cu, 0.7 mg Zn, 2 mg B, 6 mg Mn and 0.14 mg Mo per ml water (Mashela, 2002). Plants were provided with 5 g NPK 2:3:2 (22) + 0.5% S + 5% Zn + 5% Ca fertiliser to supply the crops with calcium (Ca) element every two weeks after transplanting.

5.2.3 Data collection

Plant variables: At 150 days after inoculation with nematodes, shoots were cut at the soil surface and plant height measured from the cut end to the top of the panicle of both primary and tiller stalks using measuring tape. Stem diameter was measured at

5 cm above the severed ends using a digital Vernier calliper. tiller number, along with internode number in both primary and tiller stalk, were recorded. The panicle was cut from mother plant shoots and tillers, with fresh mass measured. Peduncle length was measured from the base of the tassel to the apex of the top internode. Stems were marked into three classes, namely, top (internode just below the peduncle), bottom (internode just above the severed-end of the stem) and middle (middle distance between the top and bottom internodes). The middle part of each class was cut using a knife that was soaked in distilled water and wiped using laboratory towel prior to re-use, with the juice from the top-end of the cut portion squeezed using a pair of pliers onto the eye of a hand-held digital refractometer for determining the degrees Brix (°Bx). Shoots of primary stalk and tiller plants, along with the remaining roots were dried in forced air-ovens (AD 200 AGRI-DRYER, White River, South Africa) at 52°C for 72 h, for determination of dry mass.

Nutrient elements: Two healthy and mature leaves from the three plants each were wiped with disposable laboratory towel moistened with distilled water, dried at 70°C for 72 h and ground in a Wiley mill to pass through a 0.75 mm sieve. Approximately 0.4 g leaf tissue samples were subjected to microwave digestion method by immersing materials in a mixture of nitric acid (5 ml HNO_3) and hydrogen peroxide (3 ml H_2O_2) in a PerkinElmer titan MPS for 45 minutes. Aliquots prior quantification of Zn, Fe, K, P, Mg, Mn, Ca, Cu, S and Na nutrient elements through SW-864 EPA method 3050B using Inductively Coupled Plasma Optical Emission Spectrometry (ICPE-9000).

5.2.4 Data analysis

The Shapiro-Wilk test was performed on the standardised residuals of data to test for deviations from normality (Shapiro and Wilk, 1965) and tested for homogeneity of treatment combination variances (Levene, 1960). The standardised residuals were acceptably normal with homogeneous treatment variances. Nematode reproduction potential and plant variables were subjected to analysis of variance (ANOVA) using Statistix 10.1 software. In cases where treatment interaction effects were significant, the results were further expressed using matrix tables to allow for the determination of the magnitude and direction of interactive effects relative to untreated control (Steyn *et al*., 2003). Significant treatment means were separated using appropriate tests at the probability level of 5%. Unless otherwise stated, treatment means were discussed at the probability level of 5%.

5.3 Results

Degrees Brix: The second order interaction from Nemarioc-AL, Nemafric-BL and Mordica phytonematicides had significant effects on MP middle Brix and MP bottom Brix, contributing 41 and 37% in TTV of the respective variables (Appendix 5.1). On the contrary, first order interaction from Nemafric-BL × Mordica, Nemarioc-AL × Mordica and Nemarioc-AL × Nemafric-BL phytonematicides did not have significant effects on all plant variables. Nemafric-BL and Nemarioc-AL phytonematicides main factors had no significant effects on the variables, whereas Mordica phytonematicide main factor had significant effects on MP bottom Brix, contributing 40% in TTV of the variable. Relative to untreated control, alone or combined, the treatments had a tendency to increase mother plant middle and bottom Brix with the second order interactions increasing the variables at each location by 66 and 48% (Table 5.1). Other

combinations did not have clear trends, although there were most cases where the products increased the degrees Brix.

Table 5.1 Interactive effects of Nemarioc-AL, Nemafric-BL and Mordica phytonematicides on degree Brix in sweet stem sorghum at 150 days after application of treatments (n = 48).

		Mordica			
Nemarioc-AL	Nemafric-BL	M_0[x]	R.I. (%)[y]	M_1	R.I. (%)
		Plant middle °Bx			
AL_0	BL_0	11.42[c] ± 1.8	–	18.17[ab] ± 0.2	59
AL_0	BL_1	15.92 [b] ± 0.9	39	17.42[ab] ± 1.5	52
AL_1	BL_0	17.25[ab] ± 0.9	51	17.33[ab] ± 1.7	51
AL_1	BL_1	16.50[b] ± 0.85	44	19.00[a] ± 1.9	66
		Plant bottom °Bx			
AL_0	BL_0	12.93[c] ± 1.6	–	18.43[ab] ± 0.1	43
AL_0	BL_1	17.60[ab] ± 1.0	36	17.43[ab] ± 0.7	35
AL_1	BL_0	17.10[ab] ± 1.0	32	17.37[b] ± 0.9	34
AL_1	BL_1	16.50[b] ± 0.3	27	19.16[a] ± 0.7	48

[x]Column means ± standard error followed by the same letter were not different (P ≤ 0. 05) according to Fisher's Least Significant Difference test.

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

M_0 = without Mordica, M_1 = with Mordica, AL_0 = without Nemarioc-AL, AL_1 = with Nemarioc-AL, BL_0 = without Nemafric-BL, BL_1 = with Nemafric-BL.

Plant variables: The second order interaction (Nemarioc-AL × Nemafric-BL × Mordica phytonematicides) did not have significant effects on plant variables. Similarly, Nemafric-BL × Mordica, Nemarioc-AL × Mordica and Nemarioc-AL × Nemafric-BL phytonematicides interactions did not have significant effects on plant variables. However, Nemafric-BL × Mordica phytonematicides had significant effects on tiller number, contributing 13% in total treatment variation (TTV) of the variable (Appendix 5.3). Mordica phytonematicide as a main factor had no significant effects on all plant variables, except for tiller number, contributing 21% in TTV of the variable. However, Nemafric-BL phytonematicide as a main factor had significant effects on mother plant internode number and tiller number, contributing 29 and 52% in TTV of the respective variables. In contrast, Nemarioc-AL phytonematicide as a main factor had significant effects on mother plant height, mother plant panicle mass, mother plant peduncle length and mother plant internode number, contributing 37, 33, 49 and 39% in TTV of the respective variables. Relative to untreated control, Nemafric-BL × Mordica phytonematicides interaction increased tiller number by 163% (Table 5.2). Relative to untreated control, Nemafric-BL phytonematicide as a main factor increased mother plant internode number by 24%, whereas Nemarioc-AL phytonematicide increased mother plant height, mother plant panicle mass, mother plant peduncle length and mother plant internode number by 37%, 30%, 37% and 39%, respectively.

Table 5.2 Interactive effects of Nemafric-BL and Mordica phytonematicides on tiller number of sweet stem sorghum at 150 days after application of treatments.

	Mordica			
Nemafric –BL	M_0[x]	R.I. (%)[y]	M_1	R.I. (%)
BL_0	1.17[b] ± 0.6	–	1.33[b] ± 1.1	14
BL_1	1.75[b] ± 0.1	49	3.08[a] ± 0.8	163

[x]Column means ± standard error followed by the same letter were not different (P ≤ 0. 05) according to Fisher's Least Significant Difference test.

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

M_0 = without Mordica, M_1 = with Mordica, BL_0 = without Nemafric-BL, BL_1 = with Nemafric-BL.

Nutrient elements: Generally, the second order interaction Nemarioc-AL × Nemafric-BL × Mordica did not have significant effects on essential nutrient elements, except for Ca and K, contributing 26 and 20% in TTV on the respective variables (Appendix 5.4). In contrast, the first order interaction from Nemafric-BL × Mordica phytonematicides had significant effects on Ca, contributing 29% in TTV on the variable. The Nemarioc-AL × Mordica and Nemarioc-AL × Nemafric-BL interactions did not have significant effects on all essential nutrient elements. The main factors Mordica and Nemafric-BL phytonematicides had significant effects on Ca, contributing 14 and 22% in TTV on the variable, respectively. Nemarioc-AL phytonematicide had significant effects on Ca and K, contributing 24 and 40% in TTV on the respective variables Relative to untreated control, the combined effects of the products increased Ca and K in leaf tissues of SSS, with the second order interactions increasing Ca and K by 206 and 164%, respectively (Table 5.3). However, the second order interactions were not

significantly to most first order interactions of Mordica phytonematicide with the cucurbitacin-containing phytonematicides.

Table 5.3 Interactive effects of Nemarioc-AL, Nemafric-BL and Mordica phytonematicides on calcium and potassium in sweet stem sorghum leaf tissues at 150 days after application of the treatments (n = 48).

			Mordica		
Nemarioc -AL	Nemafric -BL	M_0[x]	R.I. (%)[y]	M_1	R.I. (%)
			Calcium (mg/kg)		
AL_0	BL_0	25000[c] ± 1.1	–	47100[b] ± 1.2	88
AL_0	BL_1	52100[b] ± 0.4	108	47100[b] ± 0.5	88
AL_1	BL_0	49300[ab] ± 0.4	97	47190[b] ± 1.5	89
AL_1	BL_1	51000[ab] ± 0.8	103	76500[a] ± 0.7	206
			Potassium (mg/kg)		
AL_0	BL_0	10420[d] ± 2.0	–	16140[cd] ± 0.4	55
AL_0	BL_1	22520[abc] ± 0.9	116	19550[abc] ± 1.3	88
AL_1	BL_0	24900[ab] ± 0.2	139	20490[bc] ± 0.2	96
AL_1	BL_1	22080[abc] ± 0.4	111	27500[a] ± 0.1	164

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

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

M_0 = without Mordica, M_1 = with Mordica, AL_0 = without Nemarioc-AL, AL_1 = with Nemarioc-AL, BL_0 = without Nemafric-BL, BL_1 = with Nemafric-BL.

5.4 Discussion

Degrees *Brix*: The second order interaction from Nemarioc-AL, Nemafric-BL and Mordica phytonematicides had significant effects on °Bx in SSS cv. 'Ndendane-X1'. Although the exact mechanism is not yet clear, this was an important observation since when compared with untreated control the increment was substantial, from 48 to 66%. Although in the current study the main factors alone did not have significant effects on °Bx, Mashela and Dube (2014) observed in another study that the residual effects of Nemarioc-AL phytonematicide increased the °Bx in the test cultivar. In various other studies (Mashela *et al*., 2017b), it was observed that the cucurbitacin-containing phytonematicides increase chlorophyll content in leaf tissues of different plants, which could possibly explain the increased °Bx in the current study. Photosynthates are translocated from shoots to other organs as sucrose (Salisbury and Ross, 1992.), with the excess needed for sustenance being stored as starch, which is easy to hydrolyse to ethanol during the fermentation process (Nzanza and Mashela, 2012). Although the mechanism involved in improvement of °Bx is not yet clear, it would be important that additional studies be conducted to investigate whether there were no terpenoid chemical residues in sucrose of SSS cultivars and how, if any, the residues would affect the quality of ethanol.

Plant growth variables: The significant effects of second order interactions on selected plant growth variables agreed with observations of the two cucurbitacin-containing phytonematicides with other products (Madaure *et al*., 2018; Mashela *et al*., 2015, 2017; Rabothata, 2017). The second order interaction of Nemarioc-AL phytonematicide, Nemafric-BL phytonematicide and *Steinernema feltiae* Filipjev (Madaure *et al*., 2018) or *Trichoderma harzianum* Rifai (Madaure *et al.*, 2018) was either significant or neutral on various plant growth variables, with limited information

on the mechanisms involved. In the current study, the major focus was on investigating whether the developed methodology using the total Mean Concentration Stimulation Point of 3% for the three phytonematicides would hold for assessing the potential phytotoxicity without resorting to the Curve-fitting Allelochemical Response Dose (CARD) computer based model (Mashela *et al*., 2017b). In the CARD model, which uses the principles of the algorithm (Liu *et al*., 2003), three concentration-based phases, namely, stimulation, neutral and inhibition phases, had been identified. Basically, most of the outcomes of botanical-based treatments occur within the three phases as depicted by the stimulation (+), neutral (0) and inhibition (−) responses, respectively. In most cases, the neutral phase is implicated when the treatment did not have a significant effect on the test variable, as observed on a number of occasions in the current and other studies (Mashela *et al*., 2017b).

Generally, when the effect of a phytonematicide has stimulation and inhibition effects, relative to the untreated control, the effects would be depicted by positive and negative effects, respectively. All plant growth variables in the current study depicted either relative positive or neutral effects as separated by appropriate post-hoc tests. The developed procedure was user-friendly since it did not require the development of the MCSP as required when using the CARD model (Mashela *et al*., 2017b). According to the first law of phytonematicides (*i.e.* MCSP), the concentration that would not induce phytotoxicity, but stimulate or be neutral on plant growth, occurs from 1 to 3% phytonematicide (Mashela *et al*., 2015, 2017). The latter allowed permutations which were used when one, two or three products were used. Allow this technique provides some information in relation to the suitability of any of the three products, alone or combined, the technique does not substitute the empirical development of the MCSP,

and its added advantage of providing sensitivity and overall sensitivity values about the test variable and test entity, respectively.

In the current study, all SSS plant variables were stimulated by the phytonematicides alone or when combined, confirming one previous study (Mabuka, 2013), which showed that cucurbitacin-containing phytonematicides had stimulation effects on cv. ′Ndendane-X1′. In several other studies it was shown that the stimulation effects were due to increased cell division in organs (Chen *et al*., 2005), which in animals is viewed as being cancerous (Lee *et al*., 2010) because they could not adjust their morphometric when exposed to the products (Mashela *et al*., 2020). The observed effects, when viewed together using a holistic approach, could suggest that the terpenoids, due to the large number of carbon atoms, when used as drenched phytonematicides, could be improving organic C in the soil, which improves the overall performance of SSS. The improvement of organic C, has been linked to the improvement of soil health (Mashela and Dube, 2014), which translated into improved plant growth and accumulation of various nutrient elements in leaf tissues of various plants (Mashela and Pofu, 2017). In a recent study (Mashela *et al*., 2020), it was demonstrated that using wild watermelon (*Cucumis africanus* L.F.) in crop rotation systems intended to manage nematode population densities of *Meloidogyne* species, significantly improved organic C, when compared with other crops. Roots of *C. africanus* contain large quantities of cucurbitacin B ($C_{32}H_{46}O_8$), which could explain the improvement in organic C from degradation of roots, which, therefore, increased carbon sequestration in the soil. Apparently, the three terpenoid-containing phytonematicides improve soil health through carbon sequestration, the concept which implies the retaining of organic C in the soil as opposed to releasing it into the

atmosphere where it forms greenhouse layer (Sandra *et al*., 2012), responsible for global warming.

Another observation worth mentioning in the current study, was the influence of the second order interactions and certain first order interactions on accumulation of K and Ca in leaf tissues of SSS. The effects consistently increased the two nutrient elements, with the increment ranging from 14 to 206%. Increased K could be associated with increased panicle mass of both the mother plant and tillers as observed in the current study since K is essential in seed filling and development (Salisbury and Ross, 1992). Also, high K content is essential in plants during conversion of sucrose to starch, where conversion enzymes use K as a catalyst (Salisbury and Ross, 1992.).

5.5 Synthesis and conclusion

Observations in the current study suggested that the three terpenoid-containing phytonematicides, collectively or singularly, increased sucrose in stems and plant growth components, along with improving soil health in areas without sugarcane aphids and *Meloidogyne* species. In conclusion, the three products, collectively and singularly, have practical future application in the production of SSS destined for ethanol production. The null hypothesis which suggested that the interactive effects of Nemarioc-AL, Nemafric-BL and Mordica phytonematicides would not stimulate sugar content and plant growth variables of cv. 'Ndendane-X1' under pest-free conditions was therefore, rejected. In the ensuing research chapter, the combined effects of the three terpenoid-containing phytonematicides were further studied on stimulation of sugar content and growth variables of cv. 'Ndendane-X1' was studied from sorghum plants infested with sugarcane aphid and infected with *Meloidogyne* species.

CHAPTER 6
INTERECTIVE EFFECTS OF TERPENOID-CONTAINING PHYTONEMATICIDES TO SWEET STEM SORGHUM INOCULATED WITH *MELOIDOGYNE INCOGNITA* AND *MELANAPHIS SACCHARI*

6.1 Introduction

Nematode resistance in sweet stem sorghum (SSS) cv. 'Ndendane-X1' was previously (Chapter 3) compromised by the interactive effects of the root-knot (*Meloidogyne* species) nematodes and sugarcane aphid (*Melanaphis sacchari* Zehntner). Therefore, successful production of SSS cv. 'Ndendane-X1' would depend upon the effective management of nematode and sugarcane population densities. Generally, the management of nematode population densities using phytonematicides is preferred since the products are both user-friendly and environment-friendly. The cucurbitacin-containing products, namely, Nemarioc-AL and Nemafric-BL phytonematicides, derived from fruits of wild cucumber (*Cucumis myriocarpus* Naude.) and wild watermelon (*Cucumis africanus* L.F.), with active ingredients cucurbitacin A ($C_{32}H_{46}O_9$) and cucurbitacin B ($C_{32}H_{46}O_8$), had limited significant interactions under different environment on productivity of crops and/or suppression of nematodes (Mashela *et al*., 2015; Pelinganga and Mashela, 2012; Pelinganga *et al*., 2012). Under the hierarchy of terpenoids, the active ingredients of the two products are chemically referred to as triterpenoids. Another terpenoid-containing phytonematicide, derived from fruit of bitter gourd (*Momordica balsamina* L.), Mordica phytonematicide, has momordin ($C_{42}H_{66}O_{13}$) as an active ingredient, chemically referred to as tetraterpenoid. Generally, the terpenoids have similar functional groups, with the main targets in entities being lipids and proteins (Van Wyk and Wink, 2004).

Belowground and aboveground pests were previously shown to have capabilities to interact with each other through various mechanisms (Bezemer *et al*., 2005; Hol *et al*., 2010; Kaplan *et al*., 2011; Kutyniok and Muller, 2012; Wurst and Van der Putten, 2007). In most cases, the interactive effects involved the production of induced systemic effects from root to leaf tissues, *vice versa* (Wondafrash *et al*., 2013). The highly efficient fumigant nematicides, which were biocidal to microorganisms in the soil (Van Gundy *et al*., 1964), resulted in soil degradation, which eventually resulted in poor soil health and reduced crop yield. However, since sugarcane aphid breaks nematode resistance, where plant resistance is used to manage nematode densities, it would be imperative that aphid be managed using environment-friendly soft insecticides, with the combination of the latter with any of the terpenoid-containing phytonematicides being ideal. The interactive effects of the three terpenoid-containing drenched phytonematicides in environments infested with *Meloidogyne* species and sugarcane aphids on the productivity of SSS and the performance of nematodes and aphids had not been documented. The objective of the study was to determine the interactive effects of Nemarioc-AL, Nemafric-BL and Mordica phytonematicides to sucrose content and plant growth variables of SSS cv. 'Ndendane-X1', but inhibited *Meloidogyne* species and sugarcane aphid under microplot conditions. The null hypothesis suggested that the interactive effects of Nemarioc-AL, Nemafric-BL and Mordica phytonematicides would not stimulate sucrose content and plant growth variables of SSS cv. 'Ndendane-X1' nor inhibit *Meloidogyne* species and sugarcane aphid under microplot conditions.

6.2 Materials and methods

6.2.1 Location of the study

An experiment was conducted under micro-plot conditions at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, South Africa (23°53'10"S, 29°44'15"E) in November-March 2018 and validated in 2019. The location had mean annual rainfall of less than 500 mm, which occurred mostly in summer (November-January), whereas minimum/maximum temperatures averaged 28/38°C.

6.2.2 Treatments, experimental design and procedures

A 2 × 2 × 2 factorial experiments, with the first, second and third factor comprising Nemarioc-AL, Nemafric-BL and Mordica phytonematicides, respectively. The eight treatments, comprising $A_0B_0M_0$, $A_1B_0M_0$, $A_0B_1M_0$, $A_0B_0M_1$, $A_1B_1M_0$, $A_1B_0M_1$, $A_0B_1M_1$ and $A_1B_1M_1$, were arranged in a randomised complete block design, with three replications. Blocking was done for shading from windbreak trees. Thirty-cm-diameter plastic pots, each filled with pasteurised (300°C for 1 h) loam soil, derived from the site of the experiments. Six SSS seeds cv.'Ndendane-X1' were sown per pot and thinned to one seedling per pot at two true-leaf stage. Mature fruits from *C. africanus, C. myriocarpus* and *M. balsamina* were separately collected from cultivated fields, cut into pieces and dried at 52ºC for 72 h in air-forced ovens (Mafeo and Mashela, 2009). Each plant material was fermented using effective microorganisms (EM) as described by (Mashela *et al*., 2017b). Eggs and second-stage juveniles (J2) of *M. incognita* race 2, when required, were extracted from roots of greenhouse-grown nematode-susceptible tomato cv. 'Floradade' in 1% NaOCl solution (Hussey and Barker, 1973) and rinsed of NaOCl in 25-µm opening sieves. Sugarcane aphids were reared on SSS at 1.5 km away from the experimental sites. After thinning, each seedling was inoculated by dispensing 5 000 eggs + J2 using 20 ml plastic syringe into 5-cm-deep

holes around the stem and each infested with 20 sugarcane aphids in whorls using a soft brush, with both entities being non-treatments.

Figure 6.1 Sweet stem sorghum cv. 'Ndendane-X1' established for the study.

The phytonematicide treatments in each trial were applied weekly, with the main factors ($A_1B_0M_0$, $A_0B_1M_0$, $A_0B_0M_1$) being at 3%, whereas the main factors in the first order interactions ($A_1B_1M_0$, $A_1B_0M_1$, $A_0B_1M_1$) were at 1.5% and the second order interaction ($A_1B_1M_1$) at 1% each. Plants were irrigated using a drip irrigation system for 1 h every other day until the first signs of reproduction and then irrigation interval was increased to one week at 3 h operational period. Each seedling was fertilised with 5 g 2:1:2 (43) Multifeed fertiliser (Nulandies, Johannesburg) which provide a total of 0.70 mg N, 0.64 mg K and 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 per ml water (Mashela, 2002). Plants were provided

with 5 g NPK 2:3:2 (22) + 0.5% S + 5% Zn + 5% Ca fertiliser to supply the crops with calcium (Ca) element every two weeks after transplanting.

6.2.3 Data collection and analysis

At 150 days after the treatments, degrees Brix, plant growth and nutrient element variables, along with the reproductive potential of *Meloidogyne* species, were collected as described previously (Chapter 3). Two plants per plot was randomly selected for sampling of sugarcane aphid, making a total number of 16 plants for sampling. Collection of sugarcane aphids was done on two leafs per sampled plant, making a total of 32 leaf for sampling. Counting was conducted every other week for 21 weeks, starting when plants were at two true-leaf-stage, up to termination of the experiment at 150 days (Church and Strickland, 1954). Each leaf sample was examined separately on the ventral side t and the aphid population on each leaf counted. The recorded values were transformed through $\log_{10}(x + 1)$ prior to subjection to analysis of variance to normalise the variances and both untransformed and transformed data were recorded. The mean number of population density of aphid/ leaf were calculated.

The Shapiro-Wilk test was performed on the standardized residuals to test for deviations from normality (Shapiro and Wilk, 1965) and data were also tested for homogeneity of the treatment variances (Levene, 1960). The standardised residuals were acceptably normal with homogeneous treatment variances and therefore, data were subjected to analysis of variance using the Statistix 10.1 software. Seasonal effects were not significant and data were pooled (n = 48) and subjected to analysis of variance. Unless otherwise stated, treatments were discussed at the probability level of 5%.

6.3 Results

Degrees Brix: The interactive effects of Nemarioc-AL, Nemafric-BL and Mordica phytonematicides had significant effects on mother plant middle sucrose content in degrees Brix (°Bx) of SSS with *Meloidogyne* species and sugarcane aphid on microplot, contributing 26% in TTV on the variable (Appendix 6.1). Relative to untreated control, the second order interaction significantly increased sucrose content in SSS middle stem by 34%, but which was not significantly different to Mordica phytonematicide (Table 6.1).

Table 6.1 Second order interaction of terpenoid-containing phytonematicides on mother plant middle sucrose content [degrees Brix (°Bx)] in sweet stem sorghum with *Meloidogyne incognita* and *Melanaphis sacchari* for 150 days under microplot conditions (n = 48).

		Mordica			
Nemarioc-AL	Nemafric-BL	M_0[x]	R.I. (%)[y]	M_1	R.I. (%)
AL_0	BL_0	$15.83^{b} \pm 0.1$	−	$18.58^{ab} \pm 0.2$	17
AL_0	BL_1	$16.02^{b} \pm 0.4$	1	$17.07^{b} \pm 0.7$	8
AL_1	BL_0	$17.52^{b} \pm 0.5$	11	$16.48^{b} \pm 1.2$	4
AL_1	BL_1	$17.02^{b} \pm 0.2$	7	$21.27^{a} \pm 0.3$	34

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

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

M_0 = without Mordica, M_1 = with Mordica, AL_0 = without Nemarioc-AL, AL_1 = with Nemarioc-AL, BL_0 = without Nemafric-BL, BL_1 = with Nemafric-BL.

The interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides had significant effects on mother plant middle sucrose content and mother plant top

sucrose content, contributing 25 and 49% in TTV of the respective variables (Appendix 6.1). Relative to untreated control, the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides increased sucrose in mother plant middle and top parts by 27 and 15%, respectively. In contrast, the interactive effects of Nemarioc-AL with Mordica phytonematicides and Nemafric-BL with Mordica phytonematicides had significant effects on mother plant bottom sucrose content, contributing 31 and 21% in TTV on the respective variables. Relative to untreated control, the interactive effects of Mordica and Nemarioc-AL phytonematicides increased sucrose in mother plant bottom parts by 87%, whereas Mordica and Nemafric-BL phytonematicides increased the variable by 60% (Table 6.2).

Table 6.2 First order interaction of terpenoid-containing phytonematicides on sucrose content [degrees Brix (°Bx)] of sweet stem sorghum with *Meloidogyne incognita* and *Melanaphis sacchari* for 150 days under microplot conditions (n = 48).

	Nemafric-BL			
	BL_0[x]	R.I. (%)[y]	BL_1	R.I. (%)
Nemarioc-AL	Mother plant middle sucrose content (°Bx)			
AL_0	$14.57^{c} \pm 0.3$	−	$16.33^{b} \pm 0.7$	12
AL_1	$15.59^{bc} \pm 0.5$	7	$18.54^{a} \pm 2.2$	27
	Mother plant top sucrose content (°Bx)			
AL_0	$14.18^{b} \pm 1.2$	−	$14.88^{b} \pm 0.5$	5
AL_1	$15.83^{b} \pm 0.3$	12	$16.29^{a} \pm 0.4$	15
	Mordica			
	M_0	R.I. (%)	M_1	R.I. (%)
Nemarioc-AL	Mother plant bottom sucrose content (°Bx)			
AL_0	$7.067^{b} \pm 0.3$	−	$7.925^{b} \pm 0.7$	12
AL_1	$8.123^{b} \pm 0.1$	14	$14.789^{a} \pm 0.2$	87
Nemafric-BL	Mother plant bottom sucrose content (°Bx)			
BL_0	$7.517^{b} \pm 0.5$	−	$9.237^{b} \pm 1.2$	23
BL_1	$8.534^{b} \pm 0.2$	13	$12.007^{a} \pm 0.2$	60

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

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

M_0 = without Mordica, M_1 = with Mordica, AL_0 = without Nemarioc-AL, AL_1 = with Nemarioc-AL, BL_0 = without Nemafric-BL, BL_1 = with Nemafric-BL.

Plant variables: The interactive effects of Nemarioc-AL, Nemafric-BL and Mordica phytonematicides with *Meloidogyne* and sugarcane aphid on microplots had significant effects on mother plant panicle mass, mother plant stem diameter, tiller 1 stem diameter and mother plant internode number, contributing 11, 6, 17 and 31% in TTV on the respective variables (Appendix 6.2). The interactive effects of Nemarioc-AL, Nemafric-BL and Mordica phytonematicides with *Meloidogyne* and sugarcane aphid had significant effects on mother plant panicle mass, contributing 11% in TTV of the respective variables (Appendix 6.3). The first order interaction Nemarioc-AL × Mordica had significant effects on mother plant height and S1 plant height, contributing 19 and 16% in TTV on the respective variables. The Nemarioc-AL × Nemafric-BL phytonematicides interaction had significant effects on tiller 1 plant height and tiller 1 panicle mass, contributing 14 and 29% in TTV on the respective variables (Appendix 6.3). Similarly, the Nemafric-BL × Mordica phytonematicides interaction had significant effects on mother plant stem diameter and S1 stem diameter, contributing 7 and 22% in TTV on the respective variables. The Nemarioc-AL × Mordica phytonematicides and Nemarioc-AL × Nemafric-BL phytonematicides each had significant effects on tiller 1 stem diameter, contributing 26 and 13% in TTV of the respective variables.

Generally, the main factors alone or combined, increased mother plant panicle mass in SSS cv. 'Ndendane-X1', with the largest relative mass increase of 370% being under the second order interaction (Table 6.3). Although there was variability in mother plant panicle mass of SSS, in all cases the variable was significantly higher than that of untreated control. Relative to untreated control, second order interaction of Nemarioc-AL, Nemafric-BL and Mordica phytonematicides increased mother plant stem diameter by 61% (Table 6.4). Tiller 1 stem diameter increased by 78% (Table 6.5),

whereas mother plant internode number increased by 91% (Table 6.6) all under second order interaction. Relative to untreated control, first order interaction for Nemarioc-AL and Mordica phytonematicides increased mother plant peduncle length by 97% (Table 6.7). Relative to untreated control, first order interaction for Nemarioc-AL and Mordica phytonematicides increased mother plant height and tiller 1 plant height by 123 and 584%, respectively (Table 6.8). Alone, the main factors did not have significant effects on the variables. Similarly, first order interaction for Nemarioc-AL and Nemafric-BL phytonematicides significantly increased tiller 1 plant height and tiller 1 panicle mass by 674% and 404% (Table 6.9), with main factors significantly increasing the individual variables. Also, each main factor increased the variable by 59 and 65%, respectively.

Table 6.3 Second order interaction of terpenoid-containing phytonematicides on mother plant panicle mass (g) in sweet stem sorghum with *Meloidogyne incognita* and *Melanaphis sacchari* for 150 days under microplot conditions (n = 48).

		Mordica			
Nemarioc-AL	Nemafric-BL	M_0[x]	R.I. (%)[y]	M_1	R.I. (%)
AL_0	BL_0	$13.73^{d} \pm 1.5$	−	$31.33^{c} \pm 0.7$	280
AL_0	BL_1	$51.33^{bc} \pm 0.5$	148	$67.91^{b} \pm 0.8$	242
AL_1	BL_0	$52.30^{bc} \pm 0.9$	172	$47.88^{bc} \pm 0.3$	245
AL_1	BL_1	$48.67^{bc} \pm 2.5$	96	$120.33^{a} \pm 0.5$	370

[x]Column means ± standard error followed by the same letter were not different ($P \leq 0.05$) according to Fisher's Least Significant Difference test.
[y]R.I. = [y]Relative impact (%) = [(treatment/control) − 1] × 100.
M_0 = without Mordica, M_1 = with Mordica, AL_0 = without Nemarioc-AL, AL_1 = with Nemarioc-AL, BL_0 = without Nemafric-BL, BL_1 = with Nemafric-BL.

Table 6.4 Second order interaction of terpenoid-containing phytonematicides on mother plant stem diameter (mm) in sweet stem sorghum with *Meloidogyne incognita* and *Melanaphis sacchari* for 150 days under microplot conditions (n = 48).

Nemarioc-AL	Nemafric-BL	Mordica			
		M_0[x]	R.I. (%)[y]	M_1	R.I. (%)
AL_0	BL_0	1.1110[b] ± 0.2	−	1.1350[b] ± 0.8	2
AL_0	BL_1	1.1450[b] ± 1.5	3	1.3150[b] ± 0.4	18
AL_1	BL_0	1.2312[b] ± 0.2	10	1.2150[b] ± 0.1	9
AL_1	BL_1	1.3150[b] ± 1.7	18	1.7930[a] ± 0.7	61

[x]Column means ± standard error followed by the same letter were not different (P ≤ 0. 05) according to Fisher's Least Significant Difference test.

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

M_0 = without Mordica, M_1 = with Mordica, AL_0 = without Nemarioc-AL, AL_1 = with Nemarioc-AL, BL_0 = without Nemafric-BL, BL_1 = with Nemafric-BL.

Table 6.5 Second order interaction of terpenoid-containing phytonematicides on tiller 1 stem diameter (mm) in sweet stem sorghum with *Meloidogyne incognita* and *Melanaphis sacchari* for 150 days under microplot conditions (n = 48).

		Mordica			
Nemarioc-AL	Nemafric-BL	M_0[x]	R.I. (%)[y]	M_1	R.I. (%)
AL_0	BL_0	1.0483[b] ± 2.2	−	1.2242[b] ± 1.7	16
AL_0	BL_1	1.3450[b] ± 2.0	28	1.2965[b] ± 0.3	17
AL_1	BL_0	1.2317[b] ± 1.4	17	1.3807[b] ± 2.2	32
AL_1	BL_1	1.2040[b] ± 2.5	15	1.8649[a] ± 2.2	78

[x]Column means ± standard error followed by the same letter were not different (P ≤ 0. 05) according to Fisher's Least Significant Difference test.

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

M_0 = without Mordica, M_1 = with Mordica, AL_0 = without Nemarioc-AL, AL_1 = with Nemarioc-AL, BL_0 = without Nemafric-BL, BL_1 = with Nemafric-BL.

Table 6.6 Second order interaction of terpenoid-containing phytonematicides on mother plant internode number in sweet stem sorghum with *Meloidogyne incognita* and *Melanaphis sacchari* for 150 days under microplot conditions (n = 48).

		Mordica			
Nemarioc-AL	Nemafric-BL	M_0[x]	R.I. (%)[y]	M_1	R.I. (%)
AL_0	BL_0	$6.08^{b} \pm 2.8$	−	$6.71^{b} \pm 0.5$	10
AL_0	BL_1	$7.54^{b} \pm 0.5$	24	$7.96^{b} \pm 1.6$	31
AL_1	BL_0	$8.46^{ab} \pm 0.4$	39	$7.63^{b} \pm 0.6$	25
AL_1	BL_1	$8.92^{ab} \pm 0.3$	47	$11.59^{a} \pm 0.5$	91

[x]Column means ± standard error followed by the same letter were not different (P ≤ 0. 05) according to Fisher's Least Significant Difference test.

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

M_0 = without Mordica, M_1 = with Mordica, AL_0 = without Nemarioc-AL, AL_1 = with Nemarioc-AL, BL_0 = without Nemafric-BL, BL_1 = with Nemafric-BL.

Table 6.7 Second order interaction of terpenoid-containing phytonematicides on mother plant peduncle length in sweet stem sorghum with *Meloidogyne incognita* and *Melanaphis sacchari* for 150 days under microplot conditions (n = 48).

		Mordica			
Nemarioc-AL	Nemafric-BL	M_0[x]	R.I. (%)[y]	M_1	R.I. (%)
AL_0	BL_0	14.400[c] ± 1.7	−	16.200[c] ± 3.9	16
AL_0	BL_1	16.133[c] ± 2.8	12	17.017[c] ± 2.2	18
AL_1	BL_0	15.600[c] ± 1.4	8	19.500 [c] ± 2.9	76
AL_1	BL_1	14.500[c] ± 2.3	1	28.500[a] ± 2.8	97

[x]Column means ± standard error followed by the same letter were not different (P ≤ 0. 05) according to Fisher's Least Significant Difference test.

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

M_0 = without Mordica, M_1 = with Mordica, AL_0 = without Nemarioc-AL, AL_1 = with Nemarioc-AL, BL_0 = without Nemafric-BL, BL_1 = with Nemafric-BL..

Table 6.8 First order interactions of terpenoid-containing phytonematicides on mother plant height and tiller 1 plant height in sweet stem sorghum with *Meloidogyne incognita* and *Melanaphis sacchari* for 150 days under microplot conditions (n = 48).

	Mordica			
	M_0[x]	R.I. (%)[y]	M_1	R.I. (%)
Nemarioc-AL	Mother plant height (cm)			
AL_0	89.42[b] ± 2.4	−	93.42[b] ± 0.3	4
AL_1	94.58[b] ± 1.2	6	199.09[a] ± 0.8	123
	Tiller 1 plant height (cm)			
AL_0	15.48[b] ± 0.6	−	16.32[b] ± 0.4	5
AL_1	35.25[b] ± 0.8	129	105.42[a] ± 0.3	584

[x]Column means ± standard error followed by the same letter were not different (P ≤ 0. 05) according to Fisher's Least Significant Difference test.

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

M_0 = without Mordica, M_1 = with Mordica, AL_0 = without Nemarioc-AL, AL_1 = with Nemarioc-AL.

Table 6.9 First order interactions of terpenoid-containing phytonematicides on Tiller 1 panicle mass (g) and tiller 1 plant height in sweet stem sorghum with *Meloidogyne incognita* and *Melanaphis sacchari* for 150 days under microplot conditions (n = 48).

	Nemafric-BL			
	BL_0[x]	R.I. (%)[y]	BL_1	R.I. (%)
Nemarioc-AL	Tiller 1 panicle mass (g)			
AL_0	12.70[b] ± 2.2	−	21.03[b] ± 2.1	65
AL_1	20.23[b] ± 1.3	59	64.08[a] ± 0.6	404
	Tiller 1 plant height (cm)			
AL_0	15.42[b] ± 2.3	−	17.42[b] ± 0.7	138
AL_1	36.75[b] ± 0.3	13	103.92[a] ± 0.3	674

[x]Column means ± standard error followed by the same letter were not different (P ≤ 0. 05) according to Fisher's Least Significant Difference test.

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

AL_0 = without Nemarioc-AL, AL_1 = with Nemarioc-AL, BL_0 = without Nemafric-BL, BL_1 = with Nemafric-BL.

Essential nutrient element variables: The second order interaction for the terpenoid-containing phytonematicides had significant effects on Mg, contributing 12% in TTV on the variable (Appendix 6.4). The first order interaction Nemarioc-AL × Mordica and Nemarioc-AL and Nemafric-BL phytonematicides had significant effects on Ca, contributing 13 and 23%, respectively, in TTV on the variable. Mordica phytonematicide had significant effects on Ca and Mg, contributing 26 and 46% in TTV on the respective variables, whereas Nemafric-BL phytonematicide also had significant effects on Ca and Mg, contributing 27 and 27% in TTV on the variable.

Relative to untreated control, the second order interaction resulted in the highest accumulation of Mg in leaf tissues of SSS, followed by most other first order interactions and then the main factors (Table 6.9). The first order interaction of Mordica and Nemarioc-AL phytonematicides interaction had the highest effect on Ca in SSS leaf tissues, followed by that of Mordica and Nemarioc-AL phytonematicides, which accumulated significantly higher Ca than the untreated control (Table 6.11). Similarly, the Nemafric-BL × Nemarioc-AL interaction had the highest effect on Ca in leaf tissues of SSS, followed by both Nemafric-BL and Nemarioc-AL phytonematicides, which accumulated more Ca than that in untreated control.

Table 6.10 Second order interaction of terpenoid-containing phytonematicides on magnesium (mg/kg) in leaf tissues of sweet stem sorghum with *Meloidogyne incognita* and *Melanaphis sacchari* for 150 days under microplot conditions (n = 48).

		Mordica			
Nemarioc-AL	Nemafric-BL	M_0[x]	R.I. (%)[y]	M_1	R.I. (%)
AL_0	BL_0	$2423.3^{d} \pm 0.5$	−	$5506.7^{bc} \pm 2.2$	127
AL_0	BL_1	$6360.0^{b} \pm 0.6$	162	$6396.7^{b} \pm 0.2$	163
AL_1	BL_0	$3576.7^{cd} \pm 1.2$	47	$6270.0^{b} \pm 0.9$	158
AL_1	BL_1	$4125^{bcd} \pm 0.2$	70	$9450.0^{a} \pm 0.7$	289

[x]Column means ± standard error followed by the same letter were not different (P ≤ 0. 05) according to Fisher's Least Significant Difference test.

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

M_0 = without Mordica, M_1 = with Mordica, AL_0 = without Nemarioc-AL, AL_1 = with Nemarioc-AL, BL_0 = without Nemafric-BL, BL_1 = with Nemafric-BL.

Table 6.11 Second order interaction of terpenoid-containing phytonematicides on calcium in leaf tissues of sweet stem sorghum with *Meloidogyne incognita* and *Melanaphis sacchari* for 150 days under microplot conditions (n = 48).

	Mordica			
	M_0[x]	R.I. (%)[y]	M_1	R.I. (%)
Nemarioc-AL	Ca (mg/kg)			
AL_0	3398.3[c] ± 0.8	−	4773.3[b] ± 0.5	40
AL_1	4244.2[bc] ± 0.5	25	6717.5[a] ± 0.2	98
	Nemafric-BL			
	BL_0[x]	R.I. (%)[y]	BL_1	R.I. (%)
Nemarioc-AL	Ca (mg/kg)			
AL_0	3156.7[c] ± 0.1	−	4601.7[b] ± 0.4	46
AL_1	4415.8[b] ± 1.2	40	6959.2[a] ± 0.2	120

[x]Column means ± standard error followed by the same letter were not different (P ≤ 0. 05) according to Fisher's Least Significant Difference test.

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

M_0 = without Mordica, M_1 = with Mordica, AL_0 = without Nemarioc-AL, AL_1 = with Nemarioc-AL, BL_0 = without Nemafric-BL, BL_1 = with Nemafric-BL.

Aphid population density: The second order interaction of the three phytonematicides had significant effects on aphid population density, contributing 35% in TTV of the variable (Appendix 6.5). The first order interactions Nemafric-BL × Mordica, Nemarioc-AL × Mordica and Nemarioc-AL × Nemafric -BL had significant effects on aphid population density, contributing 20, 16 and 22%, respectively, in TTV of the variable (Appendix 6.5). Relative to untreated control, the Nemarioc-AL × Nemafric-BL ×

Mordica phytonematicides interaction reduced population density of aphids by 92%, whereas the Nemafric-BL × Mordica, Nemarioc-AL × Mordica and Nemarioc-AL × Nemafric-BL interaction reduced the variable by 75, 79 and 80%, respectively (Table 6.12).

Table 6.12 Second order interaction of terpenoid-containing phytonematicides on sugarcane aphid population density in sweet stem sorghum with *Meloidogyne incognita* and *Melanaphis sacchari* for 150 days under microplot conditions (n = 48).

		Mordica			
Nemarioc-AL	Nemafric-BL	M_0[x]	R.I. (%)[y]	M_1	R.I. (%)
AL_0	BL_0	205(2.315[a] ± 2.9)	−	69(1.8439[c] ± 0.3)	−66
AL_0	BL_1	61(1.792[d] ± 0.3)	−70	43(1.6432[f] ± 0.7)	−79
AL_1	BL_0	77(1.8921[b] ± 0.4)	−62	51(1.7145[e] ± 1.2)	−75
AL_1	BL_1	42(1.6330[f] ± 0.2)	−80	16(1.2292[g] ± 0.2)	−92

[x]Untransformed data outside brackets, whereas within brackets data were transformed using $\log_{10}(x + 1)$ to homogenise the variance.

[y]Column means ± standard error followed by the same letter were not different (P ≤ 0. 05) according to Fisher's Least Significant Difference test.

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

M_0 = without Mordica, M_1 = with Mordica, AL_0 = without Nemarioc-AL, AL_1 = with Nemarioc-AL, BL_0 = without Nemafric-BL, BL_1 = with Nemafric-BL.

Nematode variables: The second order interaction of the phytonematicides had significant effects on reproductive potential, contributing 26% in TTV on the variable

(Appendix 6.6). Relative to untreated control, Nemarioc-AL × Nemafric-BL × Mordica reduced reproductive potential by 81%, whereas Nemarioc-AL × Mordica, Nemafric-BL × Mordica and Nemarioc-AL× Nemafric-BL phytonematicides reduced the variable by 64, 66 and 64%, respectively (Table 6.13).

Table 6.13 Second order interaction of terpenoid-containing phytonematicides on reproductive potential (eggs + juveniles/g root) of *Meloidogyne incognita* on sweet stem sorghum with *Meloidogyne incognita* and *Melanaphis sacchari* for 150 days under microplot conditions (n = 48).

		Mordica			
Nemarioc-AL	Nemafric-BL	M_0[x]	R.I. (%)[y]	M_1	R.I. (%)
AL_0	BL_0	31.13[a] ± 3.3	−	17.91[b] ± 0.2	−42
AL_0	BL_1	19.67[b] ± 0.4	−37	10.73[c] ± 0.7	−66
AL_1	BL_0	19.77[b] ± 0.3	−36	11.33[c] ± 1.2	−64
AL_1	BL_1	10.10[c] ± 0.3	−68	5.83[d] ± 0.2	−81

[x]Column means ± standard error followed by the same letter were not different (P ≤ 0. 05) according to Fisher's Least Significant Difference test.

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

M_0 = without Mordica, M_1 = with Mordica, AL_0 = without Nemarioc-AL, AL_1 = with Nemarioc-AL, BL_0 = without Nemafric-BL, BL_1 = with Nemafric-BL.

6.4 Discussion

Degrees Brix: Due to increased °Bx in various parts of the stem of SSS, especially in the middle part, it is apparent that some chemical modifications, which is one attribute

related to momordin (Minami *et al*., 1998), could be in place during the interactive effects of the three main factors.

Plant growth variables: The second order interaction had significant effects on four plant variables, with the first order interactions occurring in each of the cucurbitacin-containing phytonematicides with Mordica phytonematicide exclusively, confirming observations in reproductive potential of *Meloidogyne* species. The ability of momordin to undergo various modifications when interacting with other chemicals (Minami *et al*., 1998), could probably explain how Mordica phytonematicide and Nemarioc-AL or Nemafric-BL phytonematicide separately interacted to promote plant growth variables in SSS. Generally, all observed interactions increased the test variables, which could imply that the concentration was within the stimulation phase as explained earlier in the current chapter.

In the current study, the two cucurbitacin-containing phytonematicides had significant interactions on certain plant variables, with comparatively much higher magnitudes than when the products each interacted with Mordica phytonematicide. For instance, Nemarioc-AL and Nemafric-BL phytonematicides together significantly increased tiller 1 plant height and tiller 1 panicle mass by 674 and 404%, respectively. Even in the presence of aphids and nematodes, treatments increased °Bx in SSS cv. 'Ndendane-X1', with the largest increase of 34% being under the second order interaction, with effects of Mordica phytonematicide alone on °Bx not being different to that under second order interaction.

Nutrient element variables: The second order interaction for the three terpenoid-containing phytonematicides increased accumulation of Mg in leaf tissues of SSS, whereas the related first order interactions increased accumulation of Mg and Ca in leaf tissues (Maake, 2018). Currently, the mechanism involved in increased accumulation of certain nutrient elements and the reduction of Na (Chapter 3), is not clear. In the current study, increased accumulation of Mg was important since it serves as a central element in the structure of the chlorophyll (Taiz and Zeiger, 2006), and its accumulation in leaf tissues could explain increased chlorophyll content in other studies where plants were subjected to the test phytonematicides (Mashela *et al*., 2013). Similarly, Ca is important in the lignification of organs, which could also serve as a physical barrier for J2 penetration into roots (Gheysen and Fenoll, 2002).

Sugarcane aphid population density: The second order interaction of the three drenched phytonematicides reduced aphid population density by 69% under field conditions. Also, all the three first order interactions significantly reduced aphid population densities. This constituted the first report where the interaction of the three products reduced aphid population densities, whereas in previous studies where aphids interacted with nematodes (Chapter 3 and 4), the focus was primarily either on aphid performance (Wondafrash *et al*., 2013) or nematode performance (Pofu *et al*., 2011), without attempts to use any product that could manage both nematodes and aphids. In most studies (Maake, 2018), Nemarioc-AL and Nemafric-BL phytonematicides hardly interacted to suppress nematode population densities, whereas each interacted with Mordica phytonematicide to have substantial effects on reduction (63-70%) of the reproductive potential of nematodes. Cucurbitacin A ($C_{32}H_{46}O_9$) and cucurbitacin B ($C_{32}H_{46}O_8$) are soluble and insoluble in water,

respectively (Chen *et al*., 2005). Cucurbitacin A is also unstable and disintegrates rapidly into cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$), which could provide some clue as to why Nemarioc-AL and Nemafric-BL phytonematicides hardly interacted with each other to supress nematode population densities as observed in numerous other studies (Mashela *et al*., 2011). The observed successful suppression of nematodes through Nemarioc-AL and Nemafric-BL phytonematicides although it confirmed many other studies as cited by Mashela *et al*. (2017), is important in SSS since the plant species possess pre-infectional nematode resistance mechanism, which counter the effects of the reduced sorgolene concentration in the rhizosphere. The reduction of aphid population densities suggested that the products changed the quality of the phloem sap, which was previously shown to be important in improving the fecundity of aphids (Douglas, 1993). Thus, the phytonematicides supplement the cultivar in nematode suppression, ensuring that there was limited residual nematode population density for the successor crop, as observed in most crops with pre-infectional nematode resistance. Mashela and Pofu (2016) suggested that the nematode resistance in SSS cv. 'Ndendane-X1' was not useful in crop rotation systems since most J2 never entered the root systems and succumb to plant genes, but along with the unhatched J2, remain in the soil.

The role played by momordin ($C_{42}H_{66}O_{13}$) in the interactions as observed in the current study is not clear. Momordin is a potent inhibitor of protein synthesis that inactivate ribosomes in eukaryotes (Husain *et al*., 1994; Minami and Funatsu, 1993; Ortigao and Better, 1992). Previous studies (Dube, 2016; Shadung, 2016) demonstrated that there was hardly any cucurbitacin residues in produce where crops were treated with Nemarioc-AL and Nemafric-BL phytonematicides for managing nematode population

densities. However, such tests have not been conducted for momordin chemical residues in phloem sap.

Nematode reproductive potential: The second order interaction suppressed the reproductive potential of mixed nematode population densities. The mechanism involved in nematode suppression are beginning to emerge. Recently, Mashela *et al.* (2020) showed that during short exposure of K nematode strategists (Phillips and Trudgill, 1983.) such as *S. feltiae* to increasing cucurbitacin-containing concentration of phytonematicides, such nematodes adjust various body parts in order to avoid damage by hydrostatic pressure on internal organs within the pseudocoelom, which translates to turgor pressure in plant cells (Mashela and Nthangeni, 2002). The adjustment of morphometric gives the K nematode strategists some tolerance level to cucurbitacin-containing phytonematicides. However, the nematode strategists like *Meloidogyne* species were not tolerant to the test phytonematicides.

Generally, the top layer of nematode cuticles comprises lipids, with clear indication that the terpenoids are highly lipophilic (Van Wyk and Wink, 2004). Most importantly, approximately 80% of the nematode cuticle consists of proteins (Wang *et al*., 2009), with recent observations showing that total proteins and increasing terpenoid-containing phytonematicide significantly exhibited negative quadratic relations in *Meloidogyne* species (Mashela *et al*., 2020), which agreed with the roles played by the aldehyde and exocyclic methylene groups in interference with either amino acids or proteins in living entities (Van Wyk and Wink, 2004).

6.5 Synthesis and conclusion

Most of the second order interactions of the three terpenoid-containing phytonematicides or any of the first order interactions, had relatively higher stimulation effects on the plant variables, higher inhibition effects on nematode variable and sugarcane aphid population density. The combined effects of the three terpenoid-containing phytonematicides on sucrose content, was such that the treated SSS cv. 'Ndendane-X1' would be classified as premium intake at the mill since the °Bx was above 16%. The reason for this stimulation could not be exclusively associated with the reduction of nematodes and sugarcane aphids since such effects were observed under pest-free conditions (Chapter 5). However, in the current study (Chapter 6), where untreated control plants were exposed to nematodes and aphids, which were high, variables were reduced, and thereby explaining the higher magnitudes where the plants were exposed to the phytonematicides in soil-drenched forms. Apparently, when nematode resistance alone is used to manage nematode population densities, it would be imperative to manage sugarcane aphid using environment-friendly soft insecticides. Although in some instances sugarcane aphid had been managed using plant resistance (Wondafrash *et al.*, 2013), it is not known how such resistance would respond when plants were exposed to *Meloidogyne* species with and without the test phytonematicides.

In conclusion, the combined effects of the three terpenoid-containing phytonematicides restored nematode resistance in the test SSS cultivar, in addition to inducing substances which suppressed the test aphid population densities. Although findings in the current study had practical future applications, in all cases, the mechanisms involved were not yet clear. The null hypothesis which suggested that the interactive effects of Nemarioc-AL, Nemafric-BL and Mordica phytonematicides

would not stimulate sucrose content and plant growth variables of SSS cv. 'Ndendane-X1' nor inhibited *Meloidogyne* species through direct contact and sugarcane aphid through induced systemic plant substances under microplot conditions, was therefore, rejected. In the ensuing chapter, the summary of the findings, the significance of the findings, the future recommendations and the overall conclusions of the study, were provided.

CHAPTER 7

SUMMARY OF FINDINGS, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS, CONCLUSIONS

7.1 Summary of findings

A highly nematode-resistant sweet stem sorghum (SSS) cv. ‘'Ndendane-X1', which is suitable for the production of ethanol is highly susceptible to damage by population density of *M. sacchari*. Nematode resistance, whether pre-infectional or post-infectional nematode resistance, dependent upon photosynthates and that resistance can be lost under different environmental conditions, such sucking insects. Although *Meloidogyne* species and *T. semipenetrans* are consistently suppressed by terpenoid--containing phytonematicides, the efficacy of the products on insect pests had been inconsistent. This study was conducted to investigate aphid-nematode interaction in SSS and the development of management protocols of aphids using terpenoid-containing phytonematicides. Under microplot conditions, the interactive effects of nematode and sugarcane aphid population density in all three *Meloidogyne* species (*M. enterolobii*, *M. incognita* and *M. javanica*) reduced sucrose content and other plant variables, while stimulating reproductive potential. Nutrient elements (S, Zn, Ca and Fe) in leaf tissues of the cultivar were significantly reduced as affected by the interactions of nematode and sugarcane aphid population density in all three *Meloidogyne* species. However, other nutrient elements (Cu, K, P, Mg, Mn, and Na) were not significantly affected by the interactions. Similar results were obtained under field condition, where combined effects of mixed population of *Meloidogyne* species and sugarcane aphid interaction had significantly reduced degrees Brix on the entire stem of the cultivar. In this instance, *the* treatment significantly increased reproductive potential of *Meloidogyne* species and related root galls on the cultivar. Subsequently,

in Chapter 5, it was observed that, both plant and nematode variables were increased and reduced, respectively, when plants were exposed to interaction of the three terpenoid-containing phytonematicides in regions without sugarcane aphids and *Meloidogyne* species. In Chapter 6, the second order interactions of the three terpenoid-containing phytonematicides or any of the first order interactions, stimulation plant variables, while reducing nematode variable and sugarcane aphid population density. Results of the study showed that the nematode-aphid interaction was responsible for loss of nematode resistance in sorghum cv. 'Ndendane-X1'. The reduction in degree Brix in both Chapter 3 and 4, was clearly accompanied by the increased in reproductive potential values, respectively. However, second order interactions of the three terpenoid-containing phytonematicides appeared to have improve plant growth and accumulation of nutrient elements, while reducing nematode variable and sugarcane aphid population density. Consequently, sugarcane aphid broke down nematode resistance to all three *Meloidogyne* species and Mixed population of *Meloidogyne* species in SSS sugarcane cv. 'Ndendane-X1' Therefore, aphid population densities must be managed using terpenoid-containing phytonematicides, if SSS cv. 'Ndendani-X1' is to be sustainably produced.

7.2 Significance of findings

Findings in the study provided the first evidence that the sugarcane aphid on sorghum breaks resistance to the root-knot nematode. Consequently, in order to retain nematode resistance in sorghum, it is important that population densities of aphids be reduced either through the use of aphid-resistant cultivars or insecticides. The resistance to the nematode is dependent upon sorgolene, whereas the aphid depletes the photosynthates that serve as source for sorgolene in sorghum roots. The two

cucurbitacin-containing phytonematicides, Nemarioc-AL and Nemafric-BL phytonematicides, hardly interacted with other in improvement of plant variables and sugar content in sorghum. Consequently, there was no need to combine the two products in nematode management. In contrast, mormodin from oleanolic acid, a triterpenoid interacted with each of the cucurbitacin- containing phytonematicides to improve the productivity of sorghum in relation to plant variables and degrees Brix. Thus, mormodin could be combined with any of the two phytonematicides to improve their efficacy in improving the productivity of sorghum.

7.3 Recommendations

In future it would be necessary to further investigate the modulation role that wasbeing played by momordin in each of the cucurbitacin-containing phytonematicide. Additionally, since in the absence of nematodes and aphids, the products stimulated plant growth variables and sucrose, the products could be used in sorghum production for the observed improvements and then establishing the mechanisms involved.

7.4 Conclusions

Reproductive potential value was higher than one, whereas plant growth was reduced under microplot and field conditions. In plant parasitic nematode, when the reproductive potential is greater than one and the plant suffers yield loss, the plant is said to be susceptible host. Therefore, aphids broke nematode resistance to *M. incognita* race 2, 4 and *M. javanica* on the test SSS cultivar. In conclusion, sugarcane aphids must be controlled in order to retain nematode resistance in SSS cv. 'Ndendane-X1'. Also, as shown by reduced reproductive potential and aphid population density, Nemafric-BL, Nemarioc-AL and Mordica phytonematicides

appeared to be effective management strategies against both pests. Furthermore, these products increased plant variables and nutrient elements of the test crop.

REFERENCES

Abad, P., Gouzy, J. and M.J. Aury. 2008. Genome sequence of the metazoan *Meloidogyne incognita*. *Nature Biotechnology* 26:909–915.

Agerbirk, N. and C.E. Olsen. 2012. Glucosinolate structures in evolution. *Phytochemistry*77 16–45.

Agrios, G.N. 2005. Plant Pathology. Elsevier Academic Press. Burlington, Mass.

Ahn, J. H. and J.S. Lee. 2003. Sugar acts as a regulatory signal on the wound-inducible expression of SbHRGP3: GUS in transgenic plants. *Plant Cell Report* 22:286–293.

Almodares, A. and M.E. Sharif. 2007. Effects of irrigation on biomass of sugar beet and sweet sorghum cultivars. *Journal of Environmental Biology* 28:213–218.

Alston, D.G., Schmitt, D.P., Bradley, J.R. and H.D. Coble. 1993. Multiple pest interactions in soybean: Effects on *Heterodera* egg populations and crop yield. *Journal of Nematology* 25:42–49.

Ashokkumar, N., Poornima, K. and P. Kalaiarasan. 2019. Embryogenesis, penetration and post penetration development of *Meloidogyne enterolobii* in guava (*Psidium guajava* L.). *Annals of Plant Protection Sciences* 27:140–145.

Askary, T.H. 2008. Studies on root-knot nematode infesting pigeon pea and its integrated management. PhD Thesis, Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, India.

Balikai, R.A. 2007. Eco-biology and management of aphid, *Melanaphis sacchari* (Zehntner) in rabi sorghum. *Agricultural Reviews* 1:28.

Basedow, T., Ossiewatsch, H.R., Bernal-Vega, J.A., Kollmann, S., El-Shafie, H.A. and C.M. Nicol. 2002. Control of aphids and whiteflies, with different neem

preparations in laboratory, greenhouse and field effect and limitations. *Z. Pflanz. Pflanz* 109:612–623.

Bello, A. 1998. Biofumigation and Integrated Crop Management. In: Bello, J.A., Gonzalez, M.A. and R. Rodriguez-Kabana (Eds.). Alternatives to Methyl Bromide for the Southern European Countries. Valencia, Spain.

Bethlenfalvay, G.J., Brown, M.S., Ames, R.N. and R.S. Thomas. 1988. Effects of drought on host and endophyte development in mycorrhizal soybeans in relation to water use and phosphate uptake. *Plant physiology* 72:565–571.

Bezemer, T.M. and N.M. Van Dam. 2005. Linking aboveground and belowground interactions via induced plant defenses. *Trends in Ecology and Evolution* 20:617–624.

Bird, A.F. 1959. Development of the root-knot nematodes *Meloidogyne javanica* (Treub) and *Meloidogyne hapla* Chitwood in the tomato. *Nematologica* 4:32–42.

Bisen, P.S., Debnath, M. and B.B. Prasad. 2012. Microbes: Concepts and Applications. Wiley, Blackwell.

Both, C., Van Asch, M., Bijlsma, R.G., Burg Van Den, A.B. and M.E. Visser. 2009. Climate change and unequal phenological changes across four trophic levels: constraints or adaptations. *Journal of Animal Ecology* 78:73–83.

Carter-Wientjes, C.H., Russin, J.S., Boethel, D.J., Griffin, J.L. and E.C. McGawley. 2004 Feeding and maturation by soybean looper (*Lepidoptera: Noctuidae*) larvae on soybean affected by weed, fungus, and nematode pests. *Journal of Economic Entomology* 97:14–20.

Cassada, R.C. and R.L. Russell. 1975. The dauerlarva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. *Developmental Biology* 46:326–342.

Chakraborty, S. and S. Datta. 2003. How will plant pathogens adapt to host plant resistance at elevated CO2 under a changing climate? *The New Phytologist* 159:733–742.

Chakraborty, S. and C. Newton. 2011. Climate change, plant diseases and food security: An overview. *Plant Pathology* 60:2–14.

Chakraborty, U., Chakraborty, B.N., Chakraborty, A.P. and P.L. Dey. 2013. Water stress amelioration and plant growth promotion in wheat plants by osmotic stress tolerantbacteria. *World Journal of Microbiology and Biotechnology* 29:789–803.

Chandanie, W.A., Kubota, M. and M. Hyakumachi. 2009. Interaction between the *arbuscular mycorrhizal* fungus *Glomus mosseae* and plant growth-promoting fungi and their significance for enhancing plant growth and suppressing damping-off of cucumber (*Cucumis sativus* L.). *Applied Soil Ecology* 41:336–341.

Chapin, F.S., Eugster, III. W. and J.P. McFadden. 2000. Summer differences among arctic eco in regional climate. *Journal of Climate* 13:2002–2010.

Chen, J.C., Chiu, M.H., Nie, R.L., Cordell, G.A. and S.X. Qiu. 2005. Cucurbutacins and cucurbitane glycosides: Structures and biological activities. *Natural Product Reports* 22:386–399.

Chitwood, D.J. 2003. Research on plant-parasitic nematode biology conducted by the United States department of agriculture-agricultural research service. *Pest Management Science* 59:748–753.

Church, B.M. and A.H. Strickland. 1954. Sampling cabbage aphid populations on brussels sprouts. *Plant Phathology* 1111:3059–1365.

Commonwealth Institute of Entomology. 1981. Sugarcane Aphid (*Melanaphis sacchari*: zehntner). Distribution Maps of Pests. Farnham Royal, UK.

Clark, A. 2007. Managing Cover Crops Profitably. National SARE Outreach Handbook Series Book 9. National Agriculture Laboratory: Beltsville, Maryland.

Collett, R.L. 2020. A comparative study of the development and reproduction of *Meloidogyne enterolobii* and other thermophilic South African *Meloidogyne* species. Master Dissertation, North-West University, South Africa.

Costa, M.G.S., Garcia, M.J.D.M., Perdoná M.J. and S.R.S. Wilcken. 2020. Resistance of macadamia walnut against *Meloidogyne enterolobii* and *Meloidogyne javanica.Phytoparasitica* 48:397–405.

Czarnota, M.A., Rimando, A.M. and L.A. Weston. 2003. Evaluation of root exudates of seven sorghum accessions*. Journal of Chemical Ecology* 29:2073–2083.

Dana, P. 2003. Effect of soil factors on parasitic nematodes in sugarcane in KwaZulu-Natal, South Africa. PhD Thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Dayan, F.E., Rimando, A.M., Pan, Z., Baerson, S.R., GImsing, A.L. and S.O. Duke. 2010. Sorgoleone. *Phytochemistry* 71:1032–1039.

Douglas, A.E. 1993.The nutritional quality of phloem sap utilized by natural aphid popu lations. *Ecological Entomology* 18:31–38.

Dropkin, V.H. 1959. Varietal response of soybeans to *Meloidogyne* - a bioassay system for separating races of root-knot nematodes. *Phytopathology* 49:18–23.

Dropkin, V.H. 1969. The necrotic reaction of tomatoes and other hosts resistant to *Meloidogyne incognita. Journal of Phytopathol*ogy 59:1632–1637.

Dube, Z.P. 2016. Nemarioc-AL and Nemafric-BL phytonematicides; bioactivities in *Meloidogyne incognita*, tomato crop, soil type and organic matter. PhD Thesis, University of Limpopo. Sovenga, South Africa.

Duncan, L.W. and R. McSorley. 1987. Modelling Nematode Populations. in: Veech, J.A., and D.W. Dickson (Eds.). Vistas on Nematology. Society of Nematologists: Hyattsville, Maryland.

Duncan, L.W., Mashela, P.W., Ferguson, J., Graham, J., Abou-setta, M. and M. El-morshedy. 1995. Estimating crop loss in orchards with patches of mature citrus trees infected by *Tylenchulus semipenetrans. Nematropica* 25:43–51.

Eastop, V.F. 1955. Notes on East African aphids. VI. Cereal and grassroot feeding species. *East African Agricultural Journal* 20: 209–212.

Elling, A.A. 2013. Major emerging problems with minor *Meloidogyne* species. *Phytopathology* 103:1092–1102.

Favery, B., Lecomte, P., Gil, N., Bechtold, N., D. Bouchez *et al*., 1998. RPE, a plant gene involved in early development steps of nematode feeding cells. The *EMBO Journal* 17:6799–6811.

Fenoll, C., Aristizábal, F.A., Sanz-Alférez. S. and F.F. de Campo. 1997. Regulation of Gene Expression in Feeding Sites. In: Fenoll, C, Grundler, F.M.W. and S.A. Ohl (Eds.). Cellular and molecular aspects of plant-nematode interactions, Dordrecht.

Ferris, V.R., Ferris, J.M. and J. Faghihi. 1993. Variation in DNA in plant parasitic nematodes. *Fundamental and Applied Nematology* 16:177–184.

Flattery, K.E. 1982. An assessment of pest damage of grain sorghum in Botswana. *Experimental Agriculture* 18:319–328.

Fouche, P.S., Bester, D.H. and G.H. Veldman. 1977. The influences of potassium applications and nematocides on the potassium nutrition of valencia orange trees. *Journal of the American Society for Horticultural Science* 102:546–547.

Fu, S., Kisselle, K.W., Coleman, D.C., Hendrix, P.F. and D.A. Crossley. 2001. Short-term impacts of aboveground herbivory (grasshopper) on the abundance and 14C activity of soil nematodes in conventional tillage and no-till agroecosytems. *Soil Biology and Biochemistry* 33:1253–1258.

Fukuda, K., Ichihara, Y. and K. Suzuki. 1997. Incidence of the induced resistance of pine wilt disease. *Transaction of the Japan Society* 108:355–356.

Gallaher, R.N., McSorley, R. and D.W. Dickson. 1991. Nematode densities associated with corn and sorghum cropping systems in Florida. *Journal of Nematology* 23:668–672.

Gheysen, G. and C. Fenoll. 2002. Gene expression in nematode feeding sites. *Annual Review of Phytopathology* 40:191–219.

Gheysen, G. 1998 Chemical Signals in the Plant-Nematode Interaction: a Complex System. In: Romeo, J.T., Downum, K.R. and R. Verpoorte. (Eds.). Phytochemical signals and plant-microbe interactions, Springer US.

Giannuzzi, S., Schuepp, H., Barea, J.M. and K. Haselwandter. 2001. Mycorrhizal Technology in Agriculture: From Genes to Bioproducts. Bikhausser, Bassel, Switzerland.

Griffin, G.D. and W.W. Waite. 1971. Attraction of *Ditylenchulus dipsaci* and *Meloidogyne*

hapla by resistant and susceptible alfalfa seedlings. *Journal of Nematology* 3:215.

Hewezi, T. and T.J. Baum. 2015. Gene Silencing in Nematode Feeding Sites. In: Escobar, C. and C. Fenoll. (Eds.). Plant nematode interactions: a view on compatible interrelationships. Elsevier, New York.

Hewer, A., Will, T. and A.J.E. Van Bel. 2010. Plant cues for aphid navigation in vascular tissues. *Journal of Experimental Botany* 213:4030–4042.

Hol, W.H.G., De Boer, W., Termorshuizen, A.J., Meyer, K.M., Schneider, J.H.M., Van Dam, N.M., *et al*. 2010. Reduction of rare soil microbes modifies plant–herbivore interactions. *Ecology Letters* 13:292–301.

Huang, S.P., Dellavecchia, P.T. and P.E. Ferreira. 1986. Varietal response and estimates of heritability of resistance to *Meloidogyne javanica* incarrots. *Journal of Nematology* 18:496–501.

Husain, J.; Tickle, I. J. and S.P. Wood. 1994. "Crystal structure of momordin, a type I ribosome inactivating protein from the seeds of *Momordica charantia*". *FEBS Letters* 342:154–158.

Hunt, E.R., Cavigelli, M., Daughtry, C.S.T., McMurtrey, J.E. and C.L. Walthall. 2005. Evaluation of digital photography from model aircraft for remote sensing of crop biomass and nitrogen status. *Precision Agriculture* 6:359–378.

Hussey, R.S. and K.R. Barker. 1973. Comparison of methods for collecting inocula of *Meloidogyne* spp. including technique. *Plant Disease* 57:1025–1028.

Ibrahim, I.K.A. and M.A. El-Saedy. 1987. Development of *Meloidogyne incognita* and *M. javanica* in soybean roots. *Nematologica Mediterranea* 15:47–50.

James, D.G. 2003. Field evaluation of herbivore induced plant volatiles as attractants for beneficial insects: Methyl salicylate and the green lacewing, *Chrysopa nigricornis*. *Journal of Chemical Ecology* 29:1601–1609.

Jenkins, W.R. 1964. A rapid centrifugal-floatation technique for separating nematodes from soil. *Plant Disease Reporter* 48:692.

Kabouw, P., Kos, M., Kleine, S., Vockenhuber, E.A., Van Loon, J.J.A., Van Der Putten, W.H., *et al*. 2011. Effects of soil organisms on aboveground multitrophic interactions are consistent between plant genotypes mediating the interaction. *Entomology of Oilseed Brassica Crop* 139:197–206.

Kaloshian, I., Williamson, V.M., Miya, O., Lawn, D. and B.B. Westerdahl. 1996. Resistance breaking nematodes identified in California tomatoes. *California Agriculture* 50:18–19.

Kamrun, N. 2011. Cultivation of *Jatropha curcas* L. in Bangladesh. In: Müller, V. (Ed.). A sustainable solution to the energy, environmental and socioeconomic crisis. Saarbrücken, Germany.

Kaplan, D.T. and N.T. Keen. 1980. Mechanisms conferring plant incompatibility to nematodes. *Review of Nematology* 3:123–134.

Kaplan, D.T. and E.L. Davis. 1987. Mechanisms of Plant Incompatibility with Nematodes. In: Veech, J.A. and D.W. Dickson (Eds.). Vistas on nematology. Society of Nematologists, Hyattsville, Maryland.

Kaplan, I., Halitschke, R., Kessler, A., Rehill, B.J., Sardanelli, S. and R.F. Denno. 2008.Physiological integration of roots and shoots in plant defense strategies links above- and belowground herbivory. *Ecology Letters*. 11:841–851.

Kaplan, I., Sardanelli, S., Rehill, B.J. and R.F. Denno. 2011. Toward a mechanistic understanding of competition in vascular-feeding herbivores: an empirical test of the sink competition hypothesis. *Oecologia* 166:627–636.

Khan, A.A. and M.W. Khan. 1991. Reaction of cauliflower cultivars to *Meloidogyne javanica* and races of *Meloidogyne incognita. Nematropica* 21:161–166.

Kleynhans, K.P.N., Van den berg, E., Swart, A., Marias, M. and N.H Buckley. 1996. Plant Nematodes in South Africa. Plant Protection Research Institute, Pretoria.

Kozlowski, T.T. and S.G. Pallardy. 1997. Physiology of Woody Plants. Academic Press, San Diego.

Kutyniok, M. and C. Muller. 2012. Crosstalk between above- and belowground herbivores is mediated by minute metabolic responses of the host *Arabidopsis thaliana*. *Journal of experimental Botany* 63:6199–6210.

Lamberti, F. and F. Roca. 1987. Present Status of Nematodes as Vectors of Plant Viruses In: Veech, J.A. and D.W. Dickson. (Eds.). Vistas on Nematology. Hyattsville, Maryland.

Lee, T.S.G. and E.A. Bressan. 2006. The potential of ethanol production from sugarcane in Brazil. *Sugar Technology* 8:195–198.

Lee, D.H., Iwanski, G.B. and N.H. Thoennissen. 2010. Cucurbitacin: Ancient compound shedding new light on cancer treatment. *Scientific World Journal* 10:413–418.

Levene, H. 1960. In Contributions to Probability and Statistics: Essays in Honor of Hotelling, H. and Olkin, I. (Eds.). Stanford University Press.

Liang, Y. Sarkany, N. Cui, Y. Yesuf, J. Trushenski, J. and J.W. Blackburn. 2010. Use of sweet sorghum juice for lipid production by *Schizochytrium limacinum* SR21. *Bioresource Technology* 101:3623-3627.

Liu, D. L., An, M., Johnson, I.R. and J.V. Lovett. 2003. Mathematical modelling of allelopathy. III. A model for curve-fitting allelochemical dose responses. *Non-linearity in Biology, Toxicology and Medicine* 1:37–50.

Liu, B., Ren, J., Zhang, Y., An, J., Chen, M., Chen, H., Xu, C. and H. Ren. 2015. A new grafted rootstock against root-knot nematode for cucumber, melon and watermelon. *Agronomic Sustainable Development* 35:251–259.

Lou, C.H. 2002. Signal transport and integral behaviour in maintaining water economy in higher plant. *Chinese Bulletine of Botany* 17:475–477.

Lovato, P.E., Gininazzi-Pearoon, V., Trouvelot, A. and S. Gininazzi. 1996. The state of art of mycorrhizas and micro propagation. *Advances in Horticultural Sciences* 10:46–52.

Maake, M.V. 2018. Interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides on growth and foliar nutrient elements of tomato cultivar 'htx 14' plants. Master Dissertation, University of Limpopo. Sovenga, South Africa.

Mabuka, K. 2013. Integrated management strategies for *Meloidogyne* species in *Solanum lycopersicum* production systems. Master Dissertation, University of Limpopo. Sovenga, South Africa.

Madaure, T.T., Mashela, P.W. and D. De Waele. 2018. Interactive effects of Nemarioc-AL phytonematicide, *Steinernema feltiae* and *Trichoderma harzianum* on the reproduction of *Meloidogyne incognita* race 2 under greenhouse conditions. *Soil and Plant Science* 69:3.

Mafeo, T.P. and P.W. Mashela. 2009. Responses of monocotyledonous crops to crude extracts of *Cucumis myriocarpus* fruit as a pre-emergent bionematicide. *African Crop Science Conference Proceedings* 9:631–634.

Marais, M., Swart, A., Fourie, H., Berry, S.D., Knoetze, R. and A.P. Malan. 2017. Techniques and Procedures. In: Fourie, H., Spaull, V.W., Jones, R.K., Daneel, M.S. and D. De Waele. (Eds.). Nematology in South Africa: a view from the 21st century. Springer International Publishing: Switzerland.

Marschner, H. and B. Dell. 1994. Nutrient uptake in mycorrhizal symbiosis. *Plant Soil* 159:89–102.

Mashela, P.W. 1992. Interactions of *Tylenchulus semipenetrans*, citrus rootstocks and salinity. Thesis, University of Florida, Gainesville, Florida, USA.

Mashela, P.W., Duncan, L.W., Graham, J.H. and R. McSorley. 1992. Leaching soluble salts increases population densities of *Tylenchulus semipenetrans*. *Journal of Nematology* 24:103–108.

Mashela, P.W. 2002. Ground wild cucumber fruits suppress numbers of *Meloidogyne incognita* on tomato in microplots. *Nematropica* 32:13–19.

Mashela, P.W. and M.E. Nthangeni. 2002. Osmolyte allocation in response to *Tylenchulus semipenetrans* infection, stem girdling and root pruning in Citrus. *Journal of Nematology* 34:273–277.

Mashela, P.W., De Waele, D. and K.M. Pofu. 2011. Use of indigenous *Cucumis* technologies as alternative to synthetic nematicides in management of root-knot nematodes in low-input agricultural farming systems: *A Review. Scientific Research Essay* 6:6762–6768.

Mashela, P.W., Pofu, K.M. and Z.P. Dube. 2013. Post-application residual effect of 3 and 6% nemafric-BL phytonematicide on growth and brix of sweet sorghum and population density of *Meloidogyne* species. *African Crop Science Conference Proceedings* 11:339–342.

Mashela, P.W and Z.P. Dube. 2014. Soil allelochemical residue effects of Nemafric-BL and Nemarioc-AL phytonematicides on soil health, growth of sweet sorghum and *Meloidogyne* species. *Acta Agriculturae Scandinavica, Section B - Soil and Plant Science* 64:1.

Mashela, P.W., Dube, Z.P. and K.M. Pofu. 2015. Managing Phytotoxicity and Inconsistent Nematode Suppression in Soil Amended with Phyto-nematicides. In: Meghvansi, M.K. and A. Varma. (Eds.). Organic amendments and soil suppressiveness in plant disease management. Springer International. Publishing, Heidelberg, Switzerland.

Mashela, P.W., Ndhlala A.R, Pofu, K.M. and Z.P. Dube. 2016. Phytochemicals of Nematode-Resistant Transgenic Plant. In: Sumita, Jha. (Ed.). Transgenesis and secondary metabolism. Springer International, Switzerland.

Mashela, P.W., Ndhlala, A.R., Pofu, K.M. and Z.P. Dube. 2017a. Phytochemicals of Nematode-Resistant Transgenic Plants. In: S. Jha (Ed.). Transgenesis and Secondary Metabolism. Springer International publishing, Switzerland.

Mashela, P.W., De Waele, D., Dube, Z., Khosa, M.C., Pofu, K.M., Tefu, G., *et al*., 2017b. Alternative Nematode Management Strategies. In: Nematology in South Africa:View from the 21st Century. Fourie, H., Spaull, V.W., Jones, R.K., Daneel, M.S., De Waele, D. (Eds.). Springer International Publishing: Heidelberg,Switzerland.

Mashela, P.W. and K.M. Pofu. 2017. Influence of cucurbitacin-containing phytonematicides on selected nutrient elements in leaf tissues of green bean under greenhouse conditions. *Acta Agriculturae Scandinavica, Section B – Soil and Plant Science* 67:1–5.

Mashela, P.W., Shokoohi, E. and K.M. Pofu. 2020. Morphological adjustment in free-living *Steinernema feltiae* infective juveniles to increasing concentration of Nemafric-BL phytonematicide. *Plos One* 15:1.

McDonald, A.H. and J.M. Nicol. 2005. Nematode Parasites of Cereals. In: Luc, M., Sikora, R.A. and J.Bridge. (Eds.). Plant-parasitic nematodes in subtropical and tropical agriculture. CAB International, Wallingford.

McMahon, M.J., Kofranek, A.M. and V.E. Rubatzky. 2002. Hartmann's Plant Science: Growth, Development and Utilisation of Cultivated Plants. Prentice Hall, Upper Saddle River, New Jersey.

McSorley, R. 2011. Overview of organic amendments for management of plant-parasitic nematodes, with case studies from Florida. *Journal of Nematology* 43:69–81.

McSorley, R., Dickson, D.W. and J.A. De Brito. 1994. Host-status of selected tropical rotation crops to four populations of root-knot nematodes. *Nematropica* 24:45–53.

Mead, F.W. 1978. Sugarcane aphid, *Melanaphis sacchari* (Zehntner) - Florida – new continental United States record. *Cooperative Plant Pest Report* 3:475.

Milligan, S., Bodeau, J., Yaghoobi, J., Kaloshian, I., Zabel, P. and V.M. Williamson. 1998.The root-knot nematode resistance gene Mi from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. *Plant Cell* 10:1307–1319.

Minami, Y. and G. Funatsu. 1993. "The complete amino acid sequence of momordin-a, a ribosome-inactivating protein from the seeds of bitter gourd (*Momordica charantia*)". *Bioscience, Biotechnology, and Biochemistry* 57:1141–1144.

Minami, M., Rafiqul, I. and F. Gunki. 1998. Chemical Modifications of Momordin and Luffin-a, Ribosome-Inactivating Proteins from the Seeds of *Momordica charantia* and *Luffa cylindrical*. *Bioscience, Biotechnology, and Biochemistry* 62:959–964.

Narayani, M. and S. Srivastava. 2017. Elicitation: a stimulation of stress in in vitro plant cell/tissue cultures for enhancement of secondary metabolite production. *Phytochemistry Reviews* 16:1227–1252.

Narayana, D. 1975. Screening for aphids and sooty molds in sorghum. *Sorghum Newsletter* 18:21–22.

Ngangbam, A.K. and N.B. Devi. 2012. An approach to the parasitism genes of the root-knot nematode. *Journal of Plant Pathology* 1:81–87.

Nzanza, B. and P.W. Mashela. 2012. Control of whiteflies and aphids in tomato (*Solanum lycopersicum* L.) by fermented plant extracts of neem leaf and wild garlic. *African Journal of Biotechnology* 11:16077–16082.

O'bannon, J.H. and R.P. Esser. 1985. Citrus Declines Caused by Nematodes in Florida. II Physiological Races. Nematology Circular Number 116. Florida Department of Agriculture Consumer Services. Division Plant Industries, Gainesville, Florida, USA.

Olson, D.M., Davis, R.F., Wackers, F.L., Rains, G.C. and T. Potter. 2008. Plant-herbivore-carnivore interactions in cotton, *Gossypium hirsutum*: Linking belowground and aboveground. *Journal of Chemical Ecology* 34:1341–1348.

Onkendi, M.E. Mariette, Marais., Kariuki, George. Muhia. and L. Moleleki. 2014.The threat of root-knot nematodes (*Meloidogyne* spp.) in Africa: A review. *Plant Pathology* 63:727–737.

Ortigao, M. and M. Better. 1992. "Momordin II, a ribosome inactivating protein from Momordica balsamina, is homologous to other plant proteins". *Nucleic Acids Research* 20: 4662.

Page, S.L., Mguni, C.M. and S.Z. Sithole. 1985. Pests and Diseases of Crops in Communal Areas of Zimbabwe. Overseas Development Administration Technical Report, St. Albans, England.

Pastorczyk, M. and P. Bednarek. 2016. The Function of Glucosinolates and Related Metabolites in Plant Innate Immunity. In: S. Kopriva (Ed.). Advances in botanical research London, UK.

Pelinganga, O.M. and P.W. Mashela. 2012. Mean dosage stimulation range of allelochemicals from crude extracts of *Cucumis africanus* fruit for improving growth of tomato plant and suppressing *Meloidogyne incognita* numbers. *Journal of Agricultural Science* 12:8–12.

Pelinganga, O.M., Mashela, P.W., Nzanza, B. and M.S. Mphosi. 2012. Baseline information on using fermented crude extracts from *Cucumis africanus* fruit for suppression of *Meloidogyne incognita* and improving growth of tomato plant. *African Journal Biotechnology* 11:1407–1413.

Phillips, M.S. and D.L. Trudgill. 1983. Variations in the ability of *Globodera pallida* to produce females on potato clones bred from *Solanum vernei* or *Solanum tuberosum andigena*. *Nematologica* 29:217–226.

Pofu, K.M., Mashela, P.W. and N.M. Mokgalong. 2010. Host-status and host sensitivity of *Cucumis africanus* and *Cucumis myriocarpus* to *Meloidogyne incognita* race 2 under greenhouse conditions. *African Journal of Agricultural Research* 5:1504–1508.

Pofu, K.M., Mashela, P.W., Mokgalong, N.H. and T.P. Mafeo. 2011. Flowering and productivity of watermelon cultivars in inter-generic grafting on nematode resistant *cucumis* seedling rootstock in *Meloidogyne* infested field. *Conference Proceeding* 10:421–424.

Pofu, K.M., Mashela, P.W. and T.P Mafeo. 2013. Interaction of greenhouse whitefly (*Trialeurodes vaporariorum*) and root-knot nematode (*Meloidogyne javanica*) on nematode-resistance in wild watermelon. *International Society for Horticultural Science* 1:431–438.

Powel, N.T. 1971. Interactions between nematodes and fungi in disease complexes. *Annual Review of Phytopathology* 9:253–274.

Pozo, M.J. and C. Azcón-Aguilar. 2007. Unraveling mycorrhiza-induced resistance. *Plant Biology* 10:393–398.

Pretorius, M. 2018. The abundance, identity and population dynamics of Meloidogyne species. associated with maize in South Africa. Master Dissertation, University of North west, South Africa.

Rabothata, M.R. 2017. Interaction of *Vesicular Arbuscular mycorrhiza*, nematode and phytonematicides on growth and nutritional content of *Cleome gynandra*. Master Mini Dissertation, University of Limpopo. Sovenga, South Africa.

Raetano, C.G. and O. Nakano. 1994 Influence of climatic conditions on the occurrence of sugarcane aphid, *Aphis sacchari* (Zehntner) (Hemiptera: *Aphididae*) on sugarcane. *Cientifica Jaboticabal* 22:303–306.

Reddy, P.P., Rao, M.S., Mohandas, S. and M. Nagesh. 1995. Integrated management of citrus nematode, *Tylenchulus semipenetrans* Cobb using VA mycorrhiza, *Glomus fasciculatum* (Thaxt.) Gerd, Trappe and oil cakes. *Journal of Pest Management in Horticultural Ecosystems* 1:37–41.

Riedell, W.E., Kieckhefer, R.W. and L.S. Hesler. 2003. Root and shoot responses to oat aphids and dwarf virus in spring wheat. *Crop Science* 43:1380–1386.

Rizvi, S.J.H. and V. Rizvi. 1992. Allelopathy: Basic and Applied Aspects. Chapman and Hall, London.

Roberts, P.A. 1992. Current status of the availability, development and use of host plant resistance to root-knot nematodes. *Journal of Nematology* 24:213–227.

Rolando, J.L., Ramírez, D.A., Yactayo, W., Monneveux, P.and R. Quiroz. 2015. Leaf greenness as a drought tolerance related trait in potato (*Solanum tuberosum* L.) *Environmental and Experimental Botany* 110:27–35.

Rossi, M., Goggin, F.L., Milligan, S.B., Kaloshian, I., Ullman, D.E. and V.M. Williamso.1998. The nematode resistance gene Mi of tomato confers resistance against the potato aphid. *Proceding of the National Academy of Sciences* 95:9750–9754.

Rooney, W., Blumenthal, L., Jurg, B.B. and J.E. Mullet. 2007. Designing sorghum as a dedicated bioenergy feedstock. *Biofuel, Bioproducts and Biorefining* 1:157–2007.

Salisbury, F.B. and C.W. Ross. 1992. Plant Physiology. Wadsworth, Belmont Californi.

Sandra, C., Theodor F., Amir, K., Michele, P. and S.A. João de Moraes. 2012. Soil Organic Carbon Accumulation and Greenhouse Gas Emission Reductions from Conservation Agriculture. Integrated Crop Management. *A Literature Review*:16

Sasser, J.N. and D.W. Freckman. 1987. A World Perspective on Nematology. In Veech, J.A. and D.W. Dickson. (Eds.). Vistas on nematology. Hyattsville, Maryland, USA.

Seah, S., Sivasithamparam, K., Karakousis, A. and E. Lagudah. 1998. Cloning and char cterisation of a family of de disease resistance gene analogs from wheat and barley. *Theoretical and Applied Genetics* 97:937–945.

Shadung, K.G. 2016. Quality protocols for Nemarioc-AL and Nemafric-BL phytonematicides and potential chemical residues in tomato fruits. PhD Thesis, University of Limpopo. Sovenga, South Africa.

Shapiro, S.S. and M.B. Wilk. 1965. An analysis of varience test for normality. *Biometrics* 52:591.

Silva, R.V., Oliveira, R.D.L., Ferreira, P.S., Ferreira, A.O. And F.A. Rodrigues. 2013. Defense responses to *Meloidogyne exigua* in resistant coffee cultivar and non-host plant. *Tropical Plant Pathology* 38:114–121.

Singh, B.U., Padmaja, P.G. and N. Seetharama. 2004 Biology and management of the sugarcane aphid, (*Melanaphis sacchari* (Zehntner) (*Homoptera: Aphididae*), in sorghum: *A review Crop Protection* 23:739–755.

Sone, N.N.A. 2010. Resistance of Carrot Cultivars to *Meloidogyne chitwoodi.* Nematology, Department of Biology. University of Ghent, Belgium.

Spaull, V.W. and P. Cadet. 1990. Nematode Parasites of Sugarcane. In: Luc, M., Sikora, R.A. and J. Bridge. (Eds.). Plant-Parasitic Nematodes in Subtropical and Tropical Agriculture. CAB International, Wallingford.

Steyn, N.P., Nel, J.H. and A. Casey. 2003. Secondary data analyses of dietary surveys undertaken in South Africa to determine usual food consumption of the population. *Public Health Nutrition* 6:631–644.

Starr, J.L., Cook, R. and J. Bridge. 2002. Plant Resistance to Plant-Parasitic Nematodes. Biddles Guildford: United Kingdom.

Taiz, L. and E. Zeiger. 2006. Plant physiology. Sinauer Associates Inc., Massachusetts, USA.

Teetes, G.L., Manthe, C.S., Peterson, G.C., Leuschner. K. and B.B. Pendleton. 1995. Sorghum resistant to the sugarcane aphid, *Melanaphis sacchari* (Homoptera: Aphididae), in Botswana and Zimbabwe. *International Journal of Tropical Insect Science*16:63–71.

Thakur, G.S., Bag, M. and B.S. Sarodiya. 2009. *Momordica. Balsamina:* A medicinal plant for healthcare management. *Current Pharm Biotechnology* 10:667–82.

Tiwari, S., Youngman, R.R., Lewis, E.E. and J.D. Eisenback. 2009. European corn borer (*Lepidoptera: Crambidae*) stalk tunneling on root-knot nematode (*Tylenchida: Heteroderidae*) fitness on corn. *Journal of Economic Entomology* 102:602–609.

Tran, T.M., Hampton, C.S., Brossard, T.W., Harmata, M., Robertson, J.D. and S.S. Jurisson. 2017. In vivo transport of three radioactive fluorinated deoxysucrose analogs by the maize sucrose transporter. *Plant Physiology Biochemistry* 115: 1–11.

Trudgill, D.L. 1992. Resistance to and tolerance of plant-parasitic nematodes in plants. *Annual Review of Phytopathology* 29:167–192.

Van Dam, N.M., Tytgat, T.O.G. and J.A. Kirkegaard. 2009. Root and shoot glucosinolates: A comparison of their diversity, function and interactions in natural and managed ecosystems. *Phytochemistry Reviews* 8:171–186.

Vandegehuchte, M. L., De La Pena, E. and D. Bonte. 2010. Interactions between root and shoot herbivores of *Ammophila arenaria* in the laboratory do not translate into correlated abundances in the field. *Oikos* 119:1011–1019.

Van Gundy, S.D.; Martin, J.P. and P.H. Tsao. 1964. Some soil factors influencing reproduction of the citrus nematode and growth reduction of sweet orange seedlings. *Phytopathology* 54:294–299.

Van Rensburg, N.J. 1973. Notes on the occurrence and biology of the sorghum aphid in South Africa. *Journal Entomological Society of Southern African* 36:293–298.

Van Rensburg, N. J. and H. Van Hamburg. 1975 Grain Sorghum Pests: An Integrated Control Approach. Stellenbosch. Entomological Society of South Africa, Pretoria.

Van Wyk, B.E. and M. Wink. 2004. Medicinal Plants of the World. Timber Press: Portland, Orego.

Vasilakoglou, I., Dhima, K., Karagiannidis, N. and T. Gatsis. 2011. Sweet sorghum productivity for biofuels under increased soil salinity and reduced irrigation. *Field Crop Research* 120:38–46.

Vercauteren, I., De Almeida, E.J., De Groodt, R. and G. Gheysen. 2002. An *Arabidopsis thaliana* pectin acetylesterase gene is up-regulated in nematode feeding sites induced by root-knot and cyst nematodes. *Molecular. Plant–Microbe Interaction* 15:404–407.

Veremis, J.C. and P.A. Roberts. 1996. Identification of resistance to *Meloidogyne javanica* in the *Lycopersicon peruvianum* complex. *Theoretical and Applied Genetics* 93:894–901.

Villanueva, R.T., Brewer, M., Way, M.O., Biles, S., Sekula, D., Bynum, E. *et al*. 2014. Relationship between NDVI and sugarcane aphid injury to sorghum plants in 26 grain sorghum fields. *Journal of Economic Entomology* 108.

Vinutha, K.S., Rayaprolu, L., Yadagiri, K., Umakanth, A.V., Patil, J.V. and P.S. Rao. 2014. Sweet sorghum research and development in India: status and prospects. *Sugar Technology* 16:133–143.

Vos, P., Simons, G., Jesse, T., Wijbrandi, J., Heinen, L., Hogers, R. *et al*. 1998. The tomato Mi-1 gene confers resistance to both root-knot nematodes and potato aphids. *Nature Biotechnology* 16:1365–1369.

Walther, G.R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. *et al.* 2002. Ecological responses to recent climate change. *Nature* 416:389–395.

Wang, B., Liu, X., Wu, W., Liu, X. and S, Li. 2009. Purification, characterization, and gene cloning of an alkaline serine protease from a highly virulent strain of the nematode-endoparasitic fungus *Hirsutella rhossiliensis*. *Microbiological Research* 164:665–673.

Waterer, D.R. and R.R. Coltman. 1988. Phosphorus concentration and application interval influence growth and mycorrhizal infection of tomato and onion transplants. *Journal of the American Society for Horticultural Science* 113:704–798.

Watkins, P.R., Huesing, J.E., Margam, V., Murdock, L.L., and T.J.V. Higgins. 2012. Insects, Nematodes, and other Pests. In: Altman, A., Hasegawa, P.M. (Eds.). Plant Biotechnology and Agriculture — Prospects for the 21st Century, Elsevier.

Wardlaw, I.F. 1990. The control of carbon partitioning in plants. *New Phytologist* 116:341–381.

Wheeler, D. 2011. Quantifying Vulnerability to Climate Change: Implications for Adaptation Assistance. Center for Global Development, Washington.

Williamson, V.M., Lambert, K.N. and I. Kaloshian. 1994. Molecular Biology of Nematode Resistance in Tomato. In: Lamberti, F., Giorgi, C.D. and D.M. Bird. (Eds.). Advances in Molecular Plant Nematology. Plenum Press, New York.

Wondafrash, M., Van Dam N.M. and T.O.G. Tytgat. 2013. Plant systemic induced responses mediate interactions between root parasitic nematodes and aboveground herbivorous insects. *Frontiers in Plant Science* 4:87.

Wurst, S., and W.H. Van der Putten. 2007. Root herbivore identity matters in plant-mediated interactions between root and shoot herbivores. *Basic and Applied Ecology* 8:491–499.

Yang, N.W., Li, A.L., Wan, F.H., Liu, W.X. and D. Johnson. 2010. Effects of plant essential oils on immature and adult sweet potato whitefly, *Bemisia tabaci* biotype. *Belchim Crop Protection* 29:1200–1207.

Zapata, S.D, Villanueva, R., Sekula, D., Esparza-Diaz, G., Duke, K. and M. Mutaleb. 2016. The Economic Impact of the Sugarcane Aphid on Sorghum Production. 2016 SAEA (Southern Agricultural Economics Association) Annual Meeting, San Antonio, Texas, USA.

Zhang, H., Li, C., Davis, E.L., Wang, J., Griffin, J.D., Kofsky, J. and S. Bao-HUA. 2016 Genome-wide association study of resistance to soybean cyst nematode (*Heterodera glycines*) in wild soybean (*Glycinesoja*). *Frontiers in Plant Science* 7:1214.

Appendix 3.1 Partitioning mean sum of squares for degree Brix of middle sweet stem sorghum in *Meloidogyne* species and aphid interaction in field trial.

		M. enterolobii		*M. incognita*		*M. javanica*	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	11	3.399	1	4.749	1	2.627	1
Nematode (N)	1	1180.08	77**	440.488	94**	257.924	72**
Aphid (A)	1	154.08	10**	0.035	3^{ns}	12.344	4*
N × A	1	165.02	11 **	11.054	2*	74.455	22**
Error	33	11.34	1	2.342	0	2.273	1
Total	47	1513.92	100	478.888	100	351.825	100

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

Appendix 3.2 Partitioning mean sum of squares for reproductive potential (RP) of sweet stem sorghum in *Meloidogyne* species and aphid interaction in field trial.

		M. enterolobii		*M. incognita*		*M. javanica*	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	11	408	4	88	2	0.05421	1
Nematode (N)	1	6296	61**	3632	70**	9.37311	80**
Aphid (A)	1	1555	15^{ns}	689	13**	0.47812	8**
N × A	1	1669	16**	683	13**	0.47812	10**
Error	33	422	4	105	2	0.05711	1
Total	47	10349	100	5196	100	10.44067	100

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

Appendix 3.3 Partitioning mean sum of squares for root mass (RM), mother plant (MP) plant height (MP-PH) and MP stem diameter (MP-SD) of sweet stem sorghum in *Meloidogyne enterolobii* and aphid interaction on microplot trial.

		RM (g)		MP-PH (cm)		MP-SD (mm)	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	11	34795	6	37.5	2	18.19	6
Nematode (N)	1	40110	6^{ns}	799.3	45**	6.345	2 ^{ns}
Aphid (A)	1	134281	21*	455.0	25**	208.4	66**
N × A	1	391317	62**	404.3	24**	72.38	23**
Error	177	30069	5	77.9	4	12.32	4
Total	191	630572	100	1774	100	317.6	100

*Significant at P ≤ 0. 05, **Significant at P ≤ 0.01, ^{ns}Not significant at P ≤ 0.05.

Appendix 3.4 Partitioning mean sum of squares for dry root mass (DRM) and mother plant (MP) peduncle length (MP-PL) of sweet sorghum in *Meloidogyne incognita* and aphid interaction on microplot trial.

		DRM (g)		MP-PL (cm)	
Source	DF	MSS	TTV (%)	MSS	TTV (%)
Replication	11	14010	2	2003.6	5
Nematode (N)	1	181671	22**	17779.6	41*
Aphids (A)	1	401458	47**	5453.7	12ns
N × A	1	238549	28**	14640.7	34*
Error	177	11560	1	3541.5	8
Total	191	847248	100	43419.1	100

*Significant at P ≤ 0.05, nsNot significant at P ≤ 0.05.

Appendix 3.5 Partitioning mean sum of aquares for sulphur (S) and zinc (Zn) of sweet stem sorghum in *Meloidogyne enterolobii* and aphid interaction under microplot trial.

		S (mg/kg)		Zn (mg/kg)	
Source	DF	MSS	TTV (%)	MSS	TTV (%)
Replication	11	6793	2	82.82	1
Nematode (N)	1	109610	36**	799.31	7*
Aphid (A)	1	133748	44**	9209.56	82**
N × A	1	42082	14 *	919.84	8*
Error	33	8624	2	202.66	2
Total	47	300857	100	11214	100

Significant at P ≤ 0. 05, **Significant at P ≤ 0.01, nsNot significant at P ≤ 0.05.

Appendix 3.6 Partitioning mean sum of squares for calcium (Ca), manganese (Mn), potassium (K) and zinc (Zn) of sweet stem sorghum in *Meloidogyne incognita* and aphid interaction under microplot trial.

		Ca (mg/kg)		Mn (mg/kg)		K (mg/kg)		Zn (mg/kg)	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Rep	11	10403	8	138.46	1	35056	10	4612.3	5
Nematode (N)	1	1419	0 ^{ns}	3930.87	50**	54504	15**	13479.6	15*
Aphid (A)	1	63554	48*	3240.24	42**	57706	2^{ns}	45287.1	50**
N × A	1	47143	36*	371.99	5 ^{ns}	2.2070	62^{ns}	23802.0	26**
Error	33	10019	8	124.73	1	42342	12	3129.9	3
Total	47	1312643	100	7806.29	100	35837484	100	90310.9	100

*Significant at P ≤ 0. 05, **Significant at P ≤ 0.01, ^{ns}Not significant at P ≤ 0.05.

Appendix 3.7 Petitioning mean sum of squares for iron (Fe), magnesium (Mg) zinc (Zn) and calcium (Ca) of sweet stem sorghum in *Meloidogyne javanica* and aphid interaction under microplot trial.

		Fe (mg/kg)		Mg (mg/kg)		Zn (mg/kg)		Ca (mg/kg)	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Rep	11	1120	1	12929	11	861	10	21541	2
Nematode (N)	1	4266	4*	19767	17^{ns}	2796	32**	39127	19**
Aphid (A)	1	1036	91**	74883	63**	2923	33**	14244	70**
N × A	1	3839	3*	16863	1^{ns}	1817	21*	13242	7^{ns}
Error	33	879	1	88465	7	458	5	36938	2
Total	47	1137	100	118112	100	8858	100	20066	100

Appendix 4.1 Partitioning mean sum of squares for mother plant (MP) top juice Brix (MP-TB), MP middle juice Brix (MP-MB), MP bottom juice Brix (MP-BB) and number of root galls (NRG) of sweet stem sorghum in mixture of *Meloidogyne* species and aphid interaction in field trial.

		MP-TB (%)		MP-MB (%)		MP-BB (%)	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	11	3.399	1	4.749	1	2.627	1
Nematode (N)	1	1180.08	77**	440.488	94**	257.924	72**
Aphid (A)	1	154.08	10**	0.035	3ns	12.344	4*
N × A	1	165.02	11 **	11.054	2*	74.455	22**
Error	33	11.34	1	2.342	0	2.273	1
Total	47	1513.92	100	478.888	100	351.825	100

Appendix 4.2 Partitioning mean sum of squares for reproductive potential (RP) and number of root gall in sweet stem sorghum in mixture of *Meloidogyne* species and aphid interaction in field trial.

		NRG		RP	
Source	Df	MSS	TTV (%)	MSS	TTV (%)
Replication	11	0.2027	2	0.05421	1
Nematode (N)	1	28.5208	47**	9.37311	80**
Aphid (A)	1	6.0208	10**	0.47812	8**
N × A	1	22.6875	39**	0.47812	10**
Error	33	0.334	2	0.05711	1
Total	47	57.7658	100	10.44067	100

*Significant at P ≤ 0.05, **Significant at P ≤ 0.01, nsNot significant at P ≤ 0.05.

Appendix 4.3 Partitioning mean sum of squares for mother plant (MP) plant height (MP-PH), MP peduncle length (MP-PL), MP internode number (MP-IN) and MP stem diameter (MP-SD) of sweet stem sorghum in mixture of *Meloidogyne* species and aphid interaction in field trial.

		MP-PH (cm)		MP-PL (cm)		MP-IN		MP-SD (mm)	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	11	1192	1	26.16	0	2.74	0	10.96	1
Nematode (N)	1	41890	21**	305.02	3**	4.68	1 ns	0.09	1 ns
Aphid (A)	1	121807	60**	7475.02	90**	999.18	91**	5066.60	91**
N × A	1	34561	17**	221.02	6**	13.02	7**	27.14	6**
Error	33	1016	1	18.82	1	1.76	1	7.02	1
Total	47	200466	100	8046.04	100	1021.41	100	5111.81	100

**Significant at P ≤ 0.01, nsNot significant at P ≤ 0.05.

Appendix 4.4 Partitioning mean sum of squares for calcium (Ca), potassium (K) and zinc (Zn) of sweet stem sorghum in mixture of *Meloidogyne* species and aphid interaction under field trial.

		Ca (mg/kg)		K (mg/kg)		Zn (mg/kg)	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	11	20	1	1775981	1	248.27	2
Nematode (N)	1	103453	22**	26079549	22**	4243.63	27**
Aphid (A)	1	6628378	65**	79424082	67**	9343.01	54**
N × A	1	282225	10 **	105117	7*	1956.17	6*
Error	33	311	1	2732309	2	101.57	1
Total	47	7014387	100	118543407	100	15893	100

Significant at P ≤ 0. 05, **Significant at P ≤ 0.01, nsNot significant at P ≤ 0.05.

Appendix 5.1 Partitioning mean sum of squares for mother plant top Brix (MP-TB), MP-middle Brix (MP-MB) and MP bottom Brix (MP-BB) of sweet stem sorghum in three percent of Nemarioc-AL (AL), Nemafric-BL (BL) and Mordica (M) phytonematicides in micro plot trial.

		MP-TB (%)		MP-MB (%)		MP-BB (%)	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	5	12.97	7	3.88	1	4.52	5
AL	1	15.76	25 ^{ns}	0.02	2 ^{ns}	0.30	1 ^{ns}
BL	1	0.13	10 ^{ns}	0.08	3 ^{ns}	0.19	2 ^{ns}
M	1	7.92	12 ^{ns}	15.24	33 ^{ns}	20.02	40 *
AL × BL	1	0.05	3 ^{ns}	0.19	2 ^{ns}	0.14	2 ^{ns}
AL × M	1	3.26	5 ^{ns}	0.74	2 ^{ns}	0.44	1 ^{ns}
BL × M	1	1.88	3 ^{ns}	2.50	5 ^{ns}	2.00	4 ^{ns}
AL × BL × M	1	14.63	23 ^{ns}	18.81	41 *	18.50	37 *
Error	35	7.11	11	4.30	9	4.36	9
Total	47	63.70	100	45.77	100	50.47	100

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

Appendix 5.2 Partitioning mean sum of squares for mother plant (MP) plant height (MP-PH), MP panicle mass (MP-PM) and MP peduncle length (MP-PL) of sweet stem sorghum in three percent of Nemarioc-AL (AL), Nemafric-BL (BL) and Mordica (M) phytonematicides in a microplot trial.

		MP-PH (cm)		MP-PM (g)		MP-PL (cm)	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	5	6595	6	9431	8	93.97	3
AL	1	41713	37*	36244	33*	1630.62	49**
BL	1	37241	33ns	17929	16ns	588.55	18ns
M	1	5874	5ns	15491	14ns	235.19	7ns
AL × BL	1	475	0ns	6054	5ns	19.33	0ns
AL × M	1	31.7	0ns	2446	2ns	6.31	0ns
BL × M	1	3317	3ns	6345	6ns	253.18	8ns
AL × BL × M	1	7475	7ns	9579	9ns	291.15	9ns
Error	35	11342	10	7441	8	226.29	7
Total	47	114064	100	110961	100	3344.59	100

*Significant at P ≤ 0.05, **Significant at P ≤ 0.01, nsNot significant at P ≤ 0.05.

Appendix 5.3 Partitioning mean sum of squares for MP internodes number (MP-IN) and Tiller no. of sweet stem sorghum in three percent of Nemarioc-AL (AL), Nemafric-BL (BL) and Mordica (M) phytonematicides in a microplot trial.

		MP-IN		Tiller no.	
Source	DF	MSS	TTV (%)	MSS	TTV (%)
Replication	5	24.60	5	0.23	1
AL	1	205.47	39*	1.33	4ns
BL	1	151.76	29*	16.33	52**
M	1	24.78	5ns	6.75	21**
AL × BL	1	8.78	2ns	0.33	1ns
AL × M	1	1.90	0ns	0.75	2ns
BL × M	1	32.47	6ns	4.08	13*
AL × BL × M	1	41.19	8ns	0.75	2ns
Error	35	37.74	7	0.78	2
Total	47	528.68	100	31.33	100

*Significant at P ≤ 0.05, **Significant at P ≤ 0.01, nsNot significant at P ≤ 0.05.

Appendix 5.4 Partitioning mean sum of squares for calcium (C), Copper (Cu) and potassium (K) of sweet stem sorghum in three percent of Nemarioc-AL (AL), Nemafric-BL (BL) and Mordica (M) phytonematicides in micro plot trial.

		Ca (mg/kg)		Cu (mg/kg)		K (mg/kg).	
Source	DF	MSS	TTV	MSS	TTV	MSS	TTV
Replication	5	4.6191	5	72.6	61	84.795	6
AL	1	20.7244	24**	1.19	1ns	521.328	40**
BL	1	25.3752	29**	2.38	2 ns	291.802	22**
M	1	12.2412	14**	1.19	1 ns	10.763	1 ns
AL × BL	1	0.1141	0ns	1.19	1 ns	95.511	7 ns
AL × M	1	0.2945	0ns	2.38	2 ns	2.223	0 ns
BL × M	1	0.0027	0ns	34.51	29*	1.030	0 ns
AL × BL× M	1	22.4680	26**	0. 3852	0ns	258.397	20**
Error	35	1.5216	2	7.14	6	44.480	3
Total	47	87.3608	100	119	100	1310.329	100

*Significant at P ≤ 0.05, **Significant at P ≤ 0.01, nsNot significant at P ≤ 0.05.

Appendix 6.1 Partitioning mean sum of squares for mother plant (MP) top sucrose (MP-TS), MP middle sucrose (MP-MS) and MP bottom sucrose (MP-BS) content of sweet stem sorghum in three percent of Nemarioc AL (AL), Nemafric-BL (BL) and Mordica (M) phytonematicides in micro plot trial.

		MP-TS (%)		MP-MS (%)		MP-BS (%)	
Source	DF	MSS	TTV	MSS	TTV	MSS	TTV
Replication	5	9.0927	11	5.9677	4	1.3324	1
AL	1	0.5002	1^{ns}	9.1002	6^{ns}	26.3589	24^{*}
BL	1	0.8269	1^{ns}	6.5269	5^{ns}	8.3084	7^{ns}
M	1	11.3102	14^{ns}	24.5102	17^{*}	0.2094	0^{ns}
AL × BL	1	40.8852	49^{**}	35.8802	25^{**}	10.2953	9^{ns}
AL × M	1	12.3019	15^{ns}	0.2552	0^{ns}	35.0721	31^{**}
BL × M	1	0.2552	0^{ns}	17.8852	13^{ns}	23.7586	21^{*}
AL × BL× M	1	1.7252	2^{ns}	36.5752	26^{**}	0.9549	1^{ns}
Error	35	6.0240	7	5.5869	4	5.4860	5
Total	47	82.9215	100	142.2877	100	111.776	100

*Significant at P ≤ 0.05, **Significant at P ≤ 0.01, nsNot significant at P ≤ 0.05.

Appendix 6.2 Partitioning mean sum of squares for mother plant (MP) panicle mass (MP-PM), mother plant stem diameter (MP-SD), tiller 1 stem diameter (S1-SD) and mother plant internode number (MP-IN) of sweet stem sorghum in three percent of Nemarioc AL (AL), Nemafric-BL (BL) and Mordica (M) phytonematicides in micro plot trial.

		MP-PM		MP-SD (mm)		S1-SD (mm)		MP-IN	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Rep	5	185.4	0	3.03	1	6.99	1	0.25	0
AL	1	8249.3	20**	57.94	21**	57.18	7^{ns}	56.33	24**
BL	1	15336	37**	90.69	33**	77.39	9*	48.00	20**
M	1	7715.5	19**	59.89	22**	20.81	3^{ns}	48.00	20**
AL × BL	1	21.6	0^{ns}	12.87	5^{ns}	107.37	13**	3.00	1^{ns}
AL × M	1	819.7	2^{ns}	11.70	4^{ns}	213.06	26**	1.33	1^{ns}
BL × M	1	4224.8	10*	19.68	7*	178.52	22**	5.33	2^{ns}
AL × BL× M	1	4459.8	11*	17.49	6*	139.162	17**	75.00	31**
Error	35	626.3	2	4.08	1	17.240	2	2.250	1
Total	47	41638.4	100	277.4222	100	817.754	100	239.4999	100

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

Appendix 6.3 Partitioning mean sum of squares for mother plant (MP) plant height (MP-PH), tiller 1 plant height (S1-PH), Mother plant peduncle length (MP-PL) and tiller 1 panicle mass (S1-PM) of sweet stem sorghum in three percent of Nemarioc-AL (AL), Nemafric-BL (BL) and Mordica (M) phytonematicides in a microplot trial.

		MP-PH (cm)		S1-PH (cm)		MP-PL		S1-PM	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	5	20150	11	7640	5	3.35	1	2945.61	11
AL	1	30305	16^{ns}	8034	5^{ns}	235.48	25**	7673.49	27*
BL	1	23669	12^{ns}	47062	31**	77.22	9**	3785.37	14^{ns}
M	1	35322	19^{ns}	8992	6^{ns}	213.73	24**	1.41	0^{ns}
AL × BL	1	153	0^{ns}	20460	14**	61.46	7**	8169.30	29**
AL × M	1	36857	19*	23452	16**	180.62	20**	1834.22	7^{ns}
BL × M	1	27165	14^{ns}	21973	15**	25.35	3*	477.04	2^{ns}
AL × BL × M	1	7353	4^{ns}	9947	7^{ns}	101.83	11**	1837.94	7^{ns}
Error	35	9346	5	3318	2	6.32	1	1307.81	5
Total	47	20150	100	150882	100	905.39	100	26724.38	100

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

Appendix 6.4 Partitioning mean sum of squares for calcium (C) and magnesium (Mg) of sweet stem sorghum in three percent of Nemarioc-AL phytonematicide, Nemafric-BL phytonematicide and Mordica phytonematicide in micro plot trial.

		Ca (mg/kg)		Mg (mg/kg)	
Source	DF	MSS	TTV	MSS	TTV
Replication	5	1.48	1	2.48	1
AL	1	3.61	2^{ns}	5.61	3 ^{ns}
BL	1	47.72	27**	54.89	27**
M	1	44.42	26**	93.04	46**
AL × BL	1	39.24	23**	0.90	0 ^{ns}
AL × M	1	23.35	13**	17.99	9 ^{ns}
BL × M	1	5.88	3^{ns}	0.12	0 ^{ns}
AL × BL× M	1	6.09	4^{ns}	24.18	12*
Error	35	2.07	1	5.24	3
Total	47	173.89	100	204.49	100

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

Appendix .6.5 Partitioning mean sum of squares for aphid population density of sweet stem sorghum in three percent of Nemarioc-AL (AL), Nemafric-BL (BL) and Mordica (M) phytonematicides in a microplot trial.

Source	DF	MSS	TTV (%)
Replication	5	1.48	1
AL	1	0.02	1
BL	1	4.68	1
M	1	13.02	2*
AL × BL	1	540.02	22**
AL × M	1	295.02	16**
BL × M	1	379.68	20**
AL × BL × M	1	667.52	35**
Error	35	1.32	2
Total	47	1902.79	100

*Significant at P ≤ 0.05, **Significant at P ≤ 0.01, nsNot significant at P ≤ 0.05.

Appendix 6.6 Partitioning mean sum of squares for reproductive potential of sweet stem sorghum in three percent of Nemarioc-AL (AL), Nemafric-BL (BL) and Mordica phytonematicides in micro plot trial.

Source	DF	MSS	TTV
Replication	5	0.01979	2
AL	1	0.00027	0^{ns}
BL	1	0.02670	3^{ns}
M	1	0.13180	14^{ns}
AL × BL	1	0.15486	16^{ns}
AL × M	1	0.20838	22^{*}
BL × M	1	0.10983	12^{ns}
AL × BL × M	1	0.24965	26^{**}
Error	35	0.05064	5
Total	47	0.95192	100

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