HOST-STATUS AND HOST-SENSITIVITY OF HYBRID SORGHUM-SUDAN GRASS TO TROPICAL *MELOIDOGYNE* SPECIES AND RACES AND INFECTION OF NEMATODE-SUSCEPTIBLE SWEET POTATO FROM RESIDUAL SOIL NEMATODES

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[redacted]

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

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DECLARATION

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

Candidate: Vision Tabi Selapa	Signature	Date
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DEDICATION

I dedicate this mini-dissertation to my loving family.

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ABSTRACT

Worldwide, root-knot (*Meloidogyne* species) nematodes are considered to be the most important and damaging genus in crop husbandry. The existence of a wide host range, over 2000 plants, and several biological races, makes the management of this nematode genus difficult with nematode-resistant crops. Hybrid Sorghum-Sudan grass (*Sorghum bicolor* × *Sorghum Sundanese*) has been classified as being resistant to certain *Meloidogyne* species and races, with a wide range of uses in crop rotation intended to manage nematode population densities. However, due to the ability of nematodes to enter chemiobiosis when gradually exposed to chemicals, this hybrid might not be effective in managing nematode population densities for the subsequent highly susceptible sweet potato (*Ipomoea batatas* L.) cultivars. The objective of the study was to determine whether hybrid Sorghum-Sudan grass would suppress *M*. *javanica* (Trial 1), *M*. *incognita* race 2 (Trial 2) and *M*. *incognita* race 4 (Trial 3) population densities, allowing a nematode susceptible sweet potato cv. ′Beauregard′ as successor crop to be cultivated without suffering nematode damage. The hybrid Sorghum-Sudan grass study was conducted under greenhouse conditions, with seven inoculation levels, namely, 0; 5; 25; 125; 625; 3 125 and 15 625 eggs and second-stage juveniles (J2) of each nematode species or race, arranged in randomised complete block design, with six replications and validated in time. Plant growth, foliar nutrient elements and nematodes were collected at 56 days after inoculation and prepared for analysis using standard methods. The reproductive factor (RF) at all levels was zero, whereas nematode inoculation at all levels did not have any effect on plant growth of the hybrid Sorghum-Sudan grass. However, the nematode levels affected the accumulation of nutrient elements and the quality of forage. After cultivating the susceptible sweet potato cultivar in pots

previously with hybrid Sorghum-Sudan grass at increasing levels of *M. javanica* alone, that is in Trial 1, similar results were observed with respect to RF and lack of nematode damage to plant growth. Consequently, the hybrid was suitable for use in crop rotation with sweet potato for the purpose of managing nematode population densities of thermophilic *Meloidogyne* species and/or races.

CHAPTER 1

RESEARCH PROBLEM

1.1 Background

The root-knot (*Meloidogyne* species) nematodes, mainly *M. javanica* and *M. incognita* in subtropical regions of South Africa have a wide distribution on a wide range of host plants, which are over 2000 plants (Kleynhans *et al*., 1996; Onkendi *et al*., 2014). Since, the withdrawal of fumigant chemical nematicides from the agrochemical markets, it is has been difficult to manage plant nematode due to the existence of biological races and numerous host plants (Mashela *et al*., 2011). Biological races are morphologically identical, with separation achieved through differential host plants and molecular markers (Pofu, 2012). In South Africa, *M. javanica* and *M. incognita* race 2 occur as separate or mixed populations in various agro-ecological systems, whereas *M. incognita* race 4 occurs predominantly in the former tobacco-producing regions (Kleynhans *et al*., 1996).

1.1.1 Description of the research problem

Nematodes, primarily the root knot nematodes, cause yield losses in agricultural crops with incidents from as high as from 50% to total crop failure, especially in crops without nematode resistance, such as watermelon (*Citrullus lanatus*) and potato (*Solanum tuberosum*) (Mashela *et al*., 2017a). The root-knot (*Meloidogyne* species) nematodes are the most common in tropical and sub-tropical regions of South Africa (Pofu *et al*., 2010; Troung *et al*., 2015). Plant resistance is the most preferred strategy for managing plant nematodes due to its compatibility with many other nematode management strategies (Pofu, 2012). Crops like hybrid Sorghum-

Sudan grass which are known to be resistant, exude chemicals that suppress plant nematodes in the rhizosphere (Chiuta, 2021). However, most nematode species have the ability to enter cryptobiosis, which exists in different forms, with that induced by chemicals referred to as chemiobiosis (Mashela *et al*., 2015). In most cases, plants that induce cryptobiosis have self-protection mechanism against nematode damage, without protecting the successor crops when cultivated in the same location (Chiuta, 2021). Currently, it is not documented whether the tropical *Meloidogyne* species would enter chemiobiosis after exposure to the hybrid Sorghum-Sudan grass and thereby fail to protect potato when cultivated as a successor crop.

1.1.2 Impact of the research problem

Nematodes are the most documented soil pests that have high impact on the productivity of crops. Prior to the withdrawal of fumigant chemical nematicides, worldwide surveys suggested that on average, yield loss in major crops was about 12.3% (Sasser and Freckman, 1987), which could be much higher after the international withdrawal of the products in 2005. Moreover, plant-parasitic nematode such as *Meloidogyne* species have a wide host range, making it difficult to estimate crop losses due to this genus, although economic losses are estimated at over several billion U.S. dollars (Elling, 2013; Sasser and Freckman, 1987). According to Chitwood (2002), nematode yield losses increased after the withdrawal of methyl bromide technology, with the pre-withdrawal losses being estimated at over US$125 billion, whereas three and eight years later the estimated yield losses stood at US$157 and US$173 billion, respectively (Abad *et al*., 2008; Elling, 2013; Mashela *et al*., 2017a). According to Lima *et al*. (2018), *Meloidogyne* species alone lead to an

average yield crop loss from 10% to complete crops failure, with monetary losses estimated at US $78 billion on a worldwide scale.

1.1.3 Possible causes of the research problem

Prior to the withdrawal of fumigant nematicides, other nematode management strategies were ignored, with the belief that actually nematodes were not economic pests on certain crops (Mashela e*t al*., 2017a,b). However, after the withdrawal, surveys demonstrated that nematode damage were responsible for high crop losses (Abad *et al*., 2008; Elling, 2013), resulting in a shift to investigating alternative nematode management strategies (Mashela *et al*., 2017a,b).

1.1.4 Proposed solution of research problem

The inclusion of nematode resistance technology in crop rotation systems is one of the recommended strategies intended for managing nematode population densities, due to its compatibility with many other strategies (Khanzada *et al*., 2012). Two mechanisms of nematode resistance, pre-infectional nematode resistance and post-infectional nematode resistance, had been reported (Chiuta, 2021). However, plants with pre-infectional nematode resistance are believed not to be efficient in suppressing nematode population densities, whereas those with post-infectional nematode resistance are believed to be highly effective in nematode reduction (Chiuta, 2021). Sorghum has pre-infectional nematode resistance, whereas Sudan grass has post-infectional nematode resistance (Chiuta, 2021), with hybrid Sorghum-Sudan grass (*Sorghum bicolor x Sorghum Sundanese*) possessing both pre- and post-infectional nematode resistance mechanisms, which could accord it to be highly effective in managing nematode population densities (Mashela *et al*., 2017a).

Consequently, the use of hybrid Sorghum-Sudan grass could be suitable for reducing the nematode population densities in the soil for the subsequent production of highly nematode susceptible cultivars as successor crops in context of crop rotations intended to manage nematode population densities (Ana *et al.*, 2010).

1.1.5 General focus of the study

The current study intended to establish the host-status and host-sensitivity in hybrid Sorghum-Sudan grass to selected thermophilic *Meloidogyne* species and biological races that occur in sweet potato-producing regions of South Africa, with the subsequent production of sweet potato as a successor crop.

1.2 Problem statement

Generally, the use of nematode resistance for managing nematode population densities should follow the identification of existing *Meloidogyne* species and biological races (Timper, 2014), with the initial plant being resistant to all existing species and biological races. Hybrid Sorghum-Sudan grass is widely used in crop rotations intended to manage population densities of *Meloidogyne* species. In the USA, empirical evidence suggested that a number of *Sorghum* species were non-host to *M. incognita* races 1, *M. incognita* race 3 and *M. javanica* (McSorley and Gallaher, 1991; McSorley *et al*., 1994), with limited information on hybrid Sorghum-Sudan grass.

1.3 Rationale of the study

Hybrid Sorghum-Sudan grass has been classified to be resistant to *Meloidogyne* species and it is widely used as a nematode resistant hybrid in crop rotations

intended to manage population densities of nematodes (Ramatsitsi, 2017). According to McSorley *et al*. (1994), hybrid Sorghum-Sudan grass might be a useful crop to use in rotation to manage nematode population densities in soils infested with *Meloidogyne* species, thereby improving yield of subsequent crops. The hybrid Sorghum-Sudan grass was initially classified as having pre-infection nematode resistance (Ramatsitsi, 2017), which implies that second-stage juveniles (J2) are not allowed to enter the root systems. However, a recent study (Chiuta, 2021) suggested that the hybrid has both pre- and post-infectional nematode resistance mechanisms. Additionally, due to the ability of nematodes to enter cryptobiosis in pre-infectional nematode resistance mechanism, it is imperative that any argument that would involve recommendations of technologies to farmers be based on empirical evidence (Mashela and Pofu, 2016).

1.4 Purpose of the study

1.4.1 Aim

The establishment of host-status and host-sensitivity in hybrid Sorghum-Sudan grass to selected *Meloidogyne* species and biological races.

1.4.2 Objectives

I. Determine whether hybrid Sorghum-Sudan grass would suppress *M. javanica, M. incognita* race 2 and *M. incognita* race 4 each without suffering plant growth damage under greenhouse conditions.

II. Establish whether the nematode-susceptible successor sweet potato crop would have damaging nematode population densities when cultivated in pots where hybrid Sorghum-Sudan grass was previously inoculated with increasing levels of

M. *javanica*.

1.4.3 Null hypotheses

I. Hybrid Sorghum-Sudan grass would not suppress *M. javanica, M. incognita* race 2 and *M. incognita* race 4 each without suffering plant growth damage under greenhouse conditions.

II. The nematode-susceptible successor sweet potato crop would not have damaging nematode population densities when cultivated in pots where hybrid Sorghum-Sudan grass was previously inoculated with increasing levels of *M. javanica*.

1.5 Reliability, validity and objectivity

Reliability was ensured by using appropriate statistical levels of significance ($P \leq 0.05$). Validity was ensured by conducting the same experiment at the same location during different times. Objectivity was achieved by discussing the findings on the basis of empirical evidence as shown by statistical analyses, with findings compared and contrasted with findings in other studies (Little and Hills, 1981).

1.6 Bias

Bias is described as any influence, conditions or set of conditions that singly or altogether distort the data (Leedy and Ormrod, 2005). In this study, bias was avoided by using replications and randomisation ensuring that experimental error in each experiment are minimised.

1.7 Scientific significance of the study

Findings would provide information on the efficacy of hybrid Sorghum-Sudan grass on suppression of the South African *Meloidogyne* species or races population densities in context of crop rotations intended to manage nematode numbers.

1.8 Structure of the mini-dissertation

Following the Research Problem (Chapter 1), the work done and not yet done on the research problem was reviewed (Chapter 2). Then, each of the two subsequent chapters addressed each of the two objectives in sequence (Chapter 3-4). Finally, in (Chapter 5), findings in all chapters were summarised and integrated to provide the significance of the findings and recommendations with respect to future research and culminated in conclusions which tied the entire study together. Literature citation and referencing followed the Harvard style as prescribed by Senate-approved policy framework of the University of Limpopo. The analysed data were included as appendices for easy reference.

CHAPTER 2
LITERATURE REVIEW

2.1 Work done on the problem statement

Nematodes are multicellular animals in the group ecdysozoa, which are animals that regularly mould during their developmental stages (Singh and Phulera, 2015). Generally, nematodes are referred to as tube-in-tube animal, which is non-segmented, with bilateral symmetry, without the true respiratory and circulatory systems (Chitwood, 2002). Nematodes are categorised into free-living and parasitic nematodes, classified as zoo and plant nematodes, which cost billions of USA dollar losses in agriculture. According to studies after the 2005 withdrawal of fumigant nematicides from the agrochemical markets, plant nematodes had since caused huge losses in crops, amounting to over US$250 billion of dollars (Abad *et al*., 2008; Jones *et al*., 2017; Nicol *et al*., 2011; Pereira da Silva *et al*., 2018). Nematode damage is either physical through probing by the stylets or chemicals released from the ventral glands during movement to sedentary sites and dorsal glands during sedentary stages. In most cases, it is difficult to notice the damage through above-ground symptoms since the symptoms are similar to those associated with water and mineral nutrient deficiencies that lead to chlorosis, stunted top-growth, failure to respond to fertilisers and limited recovery from wilting (Singh and Phulera, 2015). Underground symptoms might include root galls, stunted root growth, necrotic root lesions, deformed roots, cracks on modified stems like tubers and root rotting (Singh and Phulera, 2015).

Globally, *Meloidogyne* species have the largest number of species, with the widest host range, estimated at above 2000 (Ganaie and Khan, 2016; Hunt and Handoo, 2009). Five *Meloidogyne* species, namely, *M. javanica, M. arenaria, M. incognita, M. hapla*, and *M. enterolobii*, each with biological races, which are morphologically similar within a species, but can be identified using differential host plants and molecular approaches (Mashela *et al.,* 2017b). The existence of biological races complicates the development of efficient nematode management strategies, particularly the use of nematode resistance technologies (Ganaie and Khan, 2016).

Generally, host-status in plant nematodes is assessed using either reproductive potential (RP = Pf/g fresh roots) among many cultivars inoculated at one level or reproductive factor (RF = Pf/Pi), where Pf is the final nematode population density and Pi is the initial nematode population density (Pofu *et al*., 2010; Seinhorst, 1965). The RP is used as indicator for host-status during screening a large number of cultivars, whereas RF is used as indicator for establishing host-status when assessing the degree of nematode resistance in a single cultivar exposed to different levels of inoculation (Mashela and Pofu, 2016). During the use of RP, host sensitivity is not established, whereas during the use of RF host sensitivity is established. Mostly, when using both host-status and host-sensitivity for establishing the degree of nematode resistance, the plant classified as being either resistant, tolerant or susceptible to the test nematode (Seinhorst, 1967).

Susceptible hosts are plants that allow M*eloidogyne* species to form the giant cells, with feeding occurring to allow nematode growth, development and reproduction (Hussey and Grundler, 1998). Tolerant hosts are plants that allow the penetration of

J2, subsequent development of J2 to adults, feeding and then reproduction, whereas the plant does not endure nematode damage (Seinhorst, 1967; Trudgill, 1992). In contrast, resistant hosts do not allow J2 to penetrate the roots of the host plant with pre-infectional nematode resistance, but if penetration is allowed in plants with post-infectional nematode resistance, either movement or feeding fails, resulting in J2 not developing to maturity (Seinhorst, 1967; Taylor and Sasser, 1978).

The J2 penetrate the root system at the elongation zone through the apoplastic pathway and move towards the embryonic zone to get into the vascular bundle and then move upwards to the elongation zone, where a feeding site is formed in one cell through mitosis without cytokinesis (Mashela *et al*., 2017b). During this movement, nematode resistant plants release chemicals collectively called plant genes, whereas nematodes release chemicals called gene products, which counter the gene products. At the feeding site, a single cell is converted into a multicellular structure called the giant cell, with the root galls being formed in response to physical force originating from the developing female bodies (Hussey and Grundler, 1998; Mashela *et al*., 2017b).

Hybrid Sorghum-Sudan exudes two specific chemicals, durrihn ($C_{14}H_{17}NO_7$) and sorgoloene ($C_{22}H_{29}O_4$), each with nematicidal properties (Chitwood, 2002; Wang *et al*., 2002). Sorgoleone is mainly from sorghum, whereas durrihn is primarily released by Sudan grass (Czarnota *et al*., 2003; Dayan *et al*., 2010). The two chemicals are released by different parts of the root system, with dhurinn mostly produced in the epidermal cells of roots in hybrid Sorghum-Sudan grass and then degrades into hydrogen cyanide (HCN), known for its toxicity to nematodes (Curto *et al*., 2012). In

contrast, sorgoleone is produced by living root hairs, without being converted to HCN (Weston *et al*., 2013). Sorgoleone binds with soil particles and organic matter and can remain in the soil for up to 7 weeks after release and incorporation, from where it is further released into soil solutions (Weston *et al*., 2013). In contrast, dhurrin is released by seedlings a few days after germination, with the release decreasing rapidly as the plant grows (Curto *et al*., 2012).

Worldwide, crop rotation is believed to be ineffective in areas where *Meloidogyne* species are involved due to the existence of a wide host range, including most of the economic crops (Agenbag, 2016; Fourie *et al*., 2015; Grabau and Noling, 2019; Onkendi *et al*., 2014). Additionally, producers have a limited number of resistant crops from which to choose crops that make economic sense for inclusion in crop rotation systems (Agenbag 2016; Fuller *et al*., 2008). Hybrid Sorghum-Sudan grass, due to its nematode-suppressive attributes had been frequently used as a cover crop and rotational crop (McSorley and Gallaher, 1991; McSorley *et al*., 1994). Hybrid Sorghum-Sudan grass, in addition to suppressing nematodes, has the ability to increase soil organic matter when used in crop rotation systems (Timper, 2014).

After the withdrawal of fumigant nematicides from the agrochemical markets due to their environment-unfriendliness, research focus shifted much to alternative management strategies such as crop rotations (Kratochvil *et al*., 2004; McSorley, 2011; Ramatsitsi, 2017; Seshweni, 2016). Another major emerging strategy had been the use of phytonematicides, especially the cucurbitacin-containing phytonematicides (Mashela, 2002; Mashela *et al*., 2017a).

2.2 Work not yet done on the problem statement

Limpopo Province, with subtropical climate, is one of the six major sweet potato-producing regions in South Africa. The province is renowned for its thermophilic *Meloidogyne* species, with biological races (Kleynhans *et al*., 1996). Although nematode resistance had been established in hybrid Sorghum-Sudan grass, that resistance had not been established on *Meloidogyne* biological races that exist in Limpopo Province. Thus, it would not be wise to recommend the use of the hybrid for managing thermophilic *Meloidogyne* biological races for the successful production of sweet potatoes in context of crop rotation systems without proper empirically-based evidence.

2.3 Addressing the identified gaps

Hybrid Sorghum-Sudan grass was first inoculated with increasing levels of *Meloidogyne* species and biological races. After harvest, nematode-susceptible sweet potato cultivar 'Beauregard' was then grown in pots with increasing levels of *M. javanica* to establish whether the cross-over residual nematodes would allow the cultivation of the cultivar without nematode damage. Generally, the damage induced by nematodes is proportional to Pi at planting (Seinhorst, 1965).

CHAPTER 3

INFLUENCE OF SORGHUM-SUDAN GRASS ON NEMATODE POPULATION DENSITIES AND ACCUMULATION OF NUTRIENT ELEMENTS IN LEAF TISSUES

3.1 Introduction

Hybrid Sorghum-Sudan grass (*Sorghum bicolor* × *Sorghum Sundanese*) was bred for use as a nematode-resistant crop for managing population densities of root-knot (*Meloidogyne* species) nematodes. The hybrid did not support the development of *M. incognita* and *M. javanica* second-stage juveniles (J2) to reproducing adults (Makuba, 2015). The hybrid contains pre-infectional nematode resistance mechanism, which prevents J2 from penetrating the root system due to chemical substances which are exuded in the rhizosphere (Ramatsitsi, 2017). According to Wang *et al*. (2002), the hybrid Sorghum-Sudan releases chemical compounds known as dhurrin and sorgolene, which have nematicidal properties (Clark, 2007). Nematode infection also leads to drastic changes in accumulation of nutrient elements in leaf tissues of various crops (Mashela, 1992; Melakeberhan *et al*.,1987; Santana-Gomes *et al*., 2013), which could affect the quality of the produce. Also, nematode species and/or biological races express different behaviours in resistant crops (Brida *et al*., 2017; Curto *et al*., 2012; Karajeh *et al*., 2011; Weston *et al*., 2013), but such behaviours have not been investigated in hybrid Sorghum-Sudan grass and thermophilic *Meloidogyne* species in South Africa. The objective of this study was, therefore, to determine whether hybrid Sorghum-Sudan grass would suppress *M*. *javanica*, *M*. *incognita* race 2 and *M*. *incognita* race 4 population densities, with nematode infection affecting accumulation of certain essential nutrient elements in leaf tissues of the hybrid.

3.2 Materials and methods

3.2.1 Description of the study site

Three parallel trials (Trial 1: *M*. *javanica*, Trial 2: *M*. *incognita* race 2 and Trial 3: *M*. *incognita* race 4) were conducted at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, Limpopo Province, South Africa (23° 53′ 10″S, 29° 44′ 15″ E) under greenhouse conditions, during February-April 2018 and validated in 2019. The greenhouse size was 20 m × 100 m, with the roof covered with a green net to allow 65% photosynthetically active radiation to pass through. Ambient day/night temperatures averaged 28/11°C, with maximum daily temperatures controlled using thermostatically activated fans on the northern side wall. Wet walls on the southern side wall ensured that relative humidity was retained between 60 and 70%.

3.2.2 Treatments and research design

The treatments, namely, 0, 5, 25, 125, 625, 3 125 and 15 625 eggs and J2 for each nematode species/races, were arranged in randomised complete block design, with six replications. Blocking was done for wind streams caused by heat-extracting fans and shading by the greenhouse walls in the morning and in the late afternoon. When required for inoculation, eggs and J2 were extracted from roots of tomato cv. 'Floradade' by shaking in 1% NaClO solution and rinsing with tapwater in a 25-µm opening sieve.

3.2.3 Procedures

In each trial, 20-cm-diameter plastic pots were filled with steam-pasteurised (300^0C for 1 h) loam soil (65% sand, 30% silt and 5% clay), irrigated to field capacity and

placed on the greenhouse benches at 0.2 m × 0.2 m spacing. Two hybrid seeds were sown per pot and seedlings thinned to one at two leaf-stage. Seedlings were inoculated at different inoculation level by dispensing approximate numbers of *Meloidogyne* eggs and J2 using a 20-ml-plastic syringe by placing into 5-cm-deep holes around the cardinal points of stems and filling up the holes with the growing medium. Seedlings were irrigated using 200 ml chlorine-free tapwater every other day and fertilised once at five leaf-stage using 5 g NPK 2:3:2 (26) + 5% Zn + 5% S + 5% Ca fertiliser mixture, with 1 g N:P:K 2:1:2 (43) per plant to provide a total of 0.175 mg N, 0.16 mg K and 0.16 mg P, 0.45 mg Mg, 0.378 mg Fe, 0.0375 mg Cu, 0.175 mg Zn, 0.5 mg B, 1.5 mg Mn and 0.035 mg Mo per ml tapwater added to provide essential macro- and micro-nutrient elements except Ca (Figure 3.1).

Figure 3.1. Hybrid Sorghum-Sudan grass inoculated with *Meloidogyne incognita* race 2.

3.2.4 Data collection

Plant variables: At 56 days after inoculation, plant height was measured from the soil level to the tip of the flag leaf using a measuring tape. Leaf chlorophyll content was

measured using a SPAD-502 chlorophyll meter (Konica Minolta, Beijing, China) of three healthy mature leaves and recorded as the average of three readings. Stem diameter was recorded with a Digital Vernier Calibre® (mm) at the crown. Leaf number was counted and shoot weight determined after oven-drying at 60°C for 72 h. Root system was removed from pots, immersed in water to remove excess soil particles, all roots for treatment zero were oven dried at 52°C for 72 h and weighed to facilitate extrapolation of dry root mass for inoculated roots. Root galls were assessed using the North Carolina Differential Scale of 1-5, where 1 = no galls, 2 = 1-10 galls, 3 = 11-31 galls, 4 = 31-100 galls and 5 = greater than 100 galls per root system (Taylor and Sasser, 1978).

Nutrient element variables: About 0.4 g ground healthy matured leaves of hybrid Sorghum-Sudan grass were digested in 75 ml vessel with 5 ml of 70% nitric acid (HNO_3) and 3 ml of 30% hydrogen peroxide (H_2O_2) using microwave digester (Perlain Elmer, Titan MPS). The vessels were then inserted into the microwave digester to whirl for 46 minutes under temperature ranging up to 260°C. Subsequently the vessels we placed in the laminar flow hood and allowed to cool down for 5 minutes. Samples from the vessels were transferred into 50 ml centrifuging tubes and stored in the refrigerator before analysing them. Calcium, Fe, K, Mg and Zn elements were analysed from leaf samples using the Inductively Coupled Plasma Optical Emission Spectrometry (Shimandzu, ICPE-9000).

Nematode variables: Nematodes were extracted from total root system per plant by maceration and blending for 60 s in 1% NaCIO solution (Hussey and Barker, 1973). The material was passed through top-down nested 75-µm and 25-µm mesh sieves,

remaining content of the 25-µm were bottled into 100 ml plastic containers. Nematodes were separated from the debris of the aliquot through the sugar-floatation and centrifuging method (Marais *et al*., 2017). A sugar stock solution was prepared by dissolving 624 g sugar/L tapwater, 45 ml of the stock solution was added into the centrifuge tubes and stirred once prior to centrifuging for 3 minutes at 1800 rpm. The aliquot was then decanted onto 25-µm opening sieve with sugar rinsed off the nematodes through running tapwater, eggs and J2 were collected into 100 ml plastic container for storage and then counting. From the 100 ml aliquot eggs and J2 were counted from 5 ml aliquot sample using a stereomicroscope. Nematode numbers from roots were converted to nematodes per total root system per plant and expressed as reproductive factor (RF = Pf/Pi), which is a proportion of the final nematode population density (Pf) to the initial nematode population density (Pi).

3.2.5 Data analysis

Prior to analysis of variance (ANOVA), all treatments were expressed as exponents of 5 (5^0, 5^1, 5^2, 5^3, 5^4, 5^5 and 5^6) and transformed through $\log_5(x)$ to generate a geometric series of 0, 1, 2, 3, 4, 5 and 6 in order to ensure that the x-axis data were expressed with equidistance in-between (Causton, 1977; Mashela *et al*., 2020). Data were then subjected to ANOVA through Statistix 10.0 software. The mean sum of squares was partitioned to provide the contribution of sources of variation in the total treatment variation (TTV) of the variables (Gomez and Gomez, 1984). Treatment means were separated using Fisher's Least Significant Difference test at 5% level of probability and when necessary, data were further subjected to lines of the best fit.

3.3 Results

3.3.1 Nematodes and plant variables

***Meloidogyne incognita* race 2**: In hybrid Sorghum-Sudan grass, treatment effects had highly significant ($P \leq 0.01$) effects on RF, contributing 80 and 46% in TTV of the variable in Experiment 1 and Experiment 2, respectively (Table 3.1). In both experiments, mean RF values of *M. incognita* race 2 on Sorghum-Sudan grass at all inoculation level were zero (Table 3.2). Incidentally, at all inoculation levels, infection by *M. incognita* race 2 did not have significant effects on all growth variables of the test plant (Table 3.3).

***Meloidogyne incognita* race 4**: Treatment effects had highly significant effects on RF, contributing 84 and 72% in TTV of the variable in Experiment 1 and Experiment 2, respectively (Table 3.4), with the mean RF values being zero (Table 3.5). In both experiments the inoculation effects had no significant effects on growth variables of Sorghum-Sudan grass (Table 3.6).

Meloidogyne javanica: In Experiment 1, RF value of *M. javanica* on Sorghum-Sudan grass was highly significant, with treatments in the experiment contributing 58% in TTV of the variable, whereas in Experiment 2 it was not significant respectively (Table 3.7). In this experiment the RF values were zero (Table 3.8). In both experiments, *M. javanica* infection at all levels of inoculation did not affect plant growth variables of the test hybrid Sorghum-Sudan grass (Table 3.9).

Table 3.1. Partitioning mean sum of squares of dry root mass (DRM), number of eggs per plant, number of second-stage juveniles (J2), total and reproductive factor (RF) of hybrid Sorghum-Sudan grass at 56 days after inoculation with *Meloidogyne incognita* race 2.

Source		DRM		Eggs		J2		Total (Pf)		RF	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Experiment 1											
Replication	5	43.929	34	0.254	11	0.205	8	0.323	9	8.331	10
Treatment	6	53.812	41^{ns}	1.605	73***	2.251	85***	2.884	82***	68.285	80***
Error	30	32.438	25	0.353	16	0.208	9	0.331	9	8.126	10
Total	41	130.178	100	2.212	100	2.664	100	3.538	100	84.742	100
Experiment 2											
Replication	5	22.288	37	0.257	10	0.101	5	0.188	5	261.71	27
Treatment	6	17.999	30^{ns}	1.977	80***	1.905	90***	3.180	90***	454.406	46***
Error	30	20.088	33	0.257	10	0.101	5	0.188	5	261.710	27
Total	41	60.375	100	2.491	100	2.108	100	3.535	100	977.826	100

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

Table 3.2. Responses for eggs, second-stage juveniles (J2), total nematodes and reproductive factor (RF) of *Meloidogyne incognita* race 2 on hybrid Sorghum-Sudan grass at 56 days after inoculation (n = 41).

Treatment	Eggs	J2	Total (Pf)	RF	Eggs	J2	Total (Pf)	RF
	Experiment 1				Experiment 2			
0	0	0	0	0	0	0	0	0
5	10	3	13	2.6	0	0	0	0
25	0	0	0	0	0	0	0	0
125	0	0	0	0	0	0	0	0
625	0	0	0	0	0	0	0	0
3125	13	7	20	0.01	0	0	0	0
15625	97	97	193	0.01	523	77	600	0.04
$LSD_{0.05}$	67	53	106	0.224	790	57	828	0.04

Table 3.3. Partitioning mean sum of squares for plant height (PH), chlorophyll content (CC), stem diameter (STD), number of leaves (NOL), fresh root mass (FRM) and dry shoot mass (DSM) in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source		PH (mm)		CC		STD (mm)		NOL (NOL/plant)		FRM (g/plant)		DSM (g/plant)	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
						Experiment 1							
Replication	5	756.01	48	45.80	16	19.76	26	0.001	20	43.93	34	0.02	24
Treatment	6	566.05	36^{ns}	167.29	57^{ns}	28.30	38^{ns}	0.003	40^{ns}	53.81	41^{ns}	0.05	48^{ns}
Error	30	255.62	16	78.92	27	27.01	36	0.003	40	32.44	25	0.03	28
Total	41	1577.67	100	292.01	100	75.07	100	0.007	100	130.18	100	0.10	100
						Experiment 2							
Replication	5	50.59	14	18.75	34	0.01	66	0.0007	46	22.29	37	0.001	66
Treatment	6	82.05	23^{ns}	15.44	27^{ns}	0.00	15^{ns}	0.0003	16^{ns}	18.00	30^{ns}	0.0001	16^{ns}
Error	30	218.41	62	21.77	39	0.00	19	0.0006	38	20.09	33	0.0002	18
Total	41	351.05	100	55.96	100	0.02	100	0.0015	100	60.38	100	0.0009	100

** Highly significant P ≤ 0.01. *significant P ≤ 0.05. ^{ns} not significant.

Table 3.4. Partitioning mean sum of squares of number of dry root mass (DRM), number of eggs per plant, number of second-stage juveniles (J2), total and reproductive factor (RF) of *Meloidogyne incognita* race 4 on hybrid Sorghum-Sudan grass at 56 days after inoculation.

Source	DRM			Eggs		J2		Total (Pf)		RF	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
					Experiment 1						
Replication	5	50.062	48	0.411	6	0.209	4	1.750	3	0.087	4
Treatment	6	25.757	24^{ns}	6.764	91**	4.523	92**	47.601	84**	1.908	93**
Error	30	29.568	28	0.250	4	0.196	4	7.046	13	0.064	3
Total	41	105.387	100	7.425	100	4.928	100	56.398	100	2.059	100
					Experiment 2						
Replication	5	11.117	22	0.480	13	0.253	11	0.498	13	265.782	23
Treatment	6	16.514	33^{ns}	2.594	71**	1.704	73**	2.876	72**	588.661	50^{ns}
Error	30	22.486	45	0.568	16	0.368	16	0.620	16	327.431	28
Total	41	50.118	100	3.643	100	2.325	100	3.993	100	1181.874	100

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

Table 3.5. Responses for eggs, second-stage juveniles (J2), total (Pf) and reproductive factor (RF) *Meloidogyne incognita* race 4 on hybrid Sorghum-Sudan grass after 56 days of inoculation (n = 41).

Treatment	Eggs	J2	Total (Pf)	RF	Eggs	J2	Total (Pf)	RF
	Experiment 1				Experiment 2			
0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0
125	7	7	14	0.11	0	0	0	0
625	60	30	90	0.14	0	0	0	0
3125	110	50	160	0.05	380	63	443	0.14
15625	697	283	980	0.06	277	82	358	0.02
$LSD_{0.05}$	517.35	305.90	823.25	0.36	655.51	108.35	757.45	0.21

Table 3.6. Partitioning mean sum of squares for plant height (PH), chlorophyll content (CC), stem diameter (STD), number of leaves (NOL), fresh root mass (FRM) and dry shoot mass (DSM) in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source		PH (mm)		CC		STD (mm)		NOL (NOL/plant)		FRM (g/plant)		DSM (g/plant)	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
						Experiment 1							
Replication	5	326.67	53	46.54	44	6.44	35	1.24	51	50.06	48	0.0009	40
Treatment	6	85.14	14 ^{ns}	25.71	24 ^{ns}	6.12	34 ^{ns}	0.49	20 ^{ns}	25.76	24 ^{ns}	0.0006	20 ^{ns}
Error	30	200.35	33	33.42	32	5.59	31	0.68	28	29.57	28	0.0010	40
Total	41	612.16	100	105.67	100	18.15	100	2.41	100	105.39	100	0.0030	100
						Experiment 2							
Replication	5	56.18	13	10.92	15	0.0010	70	0.44	44	11.12	22	0.016	44
Treatment	6	213.87	48 ^{ns}	32.83	44 ^{ns}	0.0001	10 ^{ns}	0.21	22 ^{ns}	16.51	33 ^{ns}	0.009	23 ^{ns}
Error	30	171.13	39	30.91	41	0.0003	20	0.34	34	22.49	45	0.012	33
Total	41	441.17	100	74.66	100	0.0140	100	0.99	100	50.12	100	0.037	100

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

Table 3.7. Partitioning mean sum of squares of number of eggs per plant, number of second-stage juveniles (J2), total (Pf), and reproductive factor (RF) of hybrid Sorghum-Sudan grass after 56 days of inoculation with *Meloidogyne javanica.*

Source		Eggs		J2		Total (Pf)		RF	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
				Experiment 1					
Replication	5	0.294	15	0.125	8	0.247	9	6.079	18
Treatment	6	1.329	68**	1.251	77**	2.027	76**	20.127	58**
Error	30	0.324	17	0.251	15	0.395	15	8.471	24
Total	41	1.947	100	1.627	100	2.669	100	34.677	100
				Experiment 2					
Replication	5	0.076	33	0.042	33	0.087	33	0.285	33
Treatment	6	0.077	34ns	0.042	34ns	0.087	34ns	0.285	34 ns
Error	30	0.076	33	0.042	33	0.087	33	0.285	33
Total	41	0.229	100	0.126	100	0.260	100	0.856	100

**Highly significant P ≤ 0.01, *significant P ≤ 0.05, ns not significant.

Table 3.8. Responses for eggs, second-stage juveniles (J2), total (Pf) and reproductive factor (RF) of *Meloidogyne javanica* on hybrid Sorghum-Sudan grass at 56 days after inoculation (n = 41).

Treatment	Eggs	J2	Total (Pf)	RF
		Experiment 1		
0	0	0	0	0
5	0	0	0	0
25	0	0	0	0
125	0	7	7	0.06
625	0	0	0	0
3125	63	20	83	0.03
15625	47	23	70	0.01
$LSD_{0.05}$	85	28	105	0.09

Table 3.9. Partitioning mean sum of squares for plant height (PH), chlorophyll content (CC), stem diameter (STD), number of leaves (NOL), fresh root mass (FRM) and dry shoot mass (DSM) in hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation.

Source		PH (mm)		CC		STD (mm)		NOL (NOL/plant)		FRM (g/plant)		DSM (g/plant)	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
						Experiment 1							
Replication	5	245.29	43	15.21	27	0.01	70	0.0016	60	57.30	31	0.012	42
Treatment	6	179.57	31 ^{ns}	17.96	32 ^{ns}	0.00	13 ^{ns}	0.0007	28 ^{ns}	96.58	51 ^{ns}	0.011	38 ^{ns}
Error	30	150.49	26	23.73	42	0.00	17	0.0003	12	33.86	18	0.007	20
Total	41	575.35	100	56.90	100	0.02	100	0.0027	100	187.74	100	0.028	100
						Experiment 2							
Replication	5	901.07	69	68.22	44	0.016	62	0.003	49	123.92	66	0.06	67
Treatment	6	132.42	10 ^{ns}	45.10	29 ^{ns}	0.004	18 ^{ns}	0.002	31 ^{ns}	18.92	10 ^{ns}	0.01	13 ^{ns}
Error	30	273.09	21	41.50	27	0.005	20	0.001	20	43.92	24	0.02	20
Total	41	1306.58	100	154.83	100	0.030	100	0.006	100	186.76	100	0.09	100

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

3.3.2 Nutrient elements in leaf tissues

***Meloidogyne incognita* race 2**: In hybrid Sorghum-Sudan grass leaf tissues, treatment effects were significant ($P \leq 0.05$) effects on Ca, Fe, K, Mg and Zn in TTV of the variable in Experiment 1 and Experiment 2, respectively (Table 3.10). In both experiments, mean for all values of *M. incognita* race 2 on Sorghum-Sudan grass leaf tissues at all level were not significantly different from one another (Table 3.13).

***Meloidogyne incognita* race 4**: Treatment effects had no significant effects on all nutrient elements tested in Experiment 1 and Experiment 2, respectively (Table 3.11), with the mean in both experiments had no significant effects on nutrient elements of Sorghum-Sudan grass leaf tissues (Table 3.14).

Meloidogyne javanica: In Experiment 1 and Experiment 2, nutrient element variables of *M. javanica* on Sorghum-Sudan grass leaf tissues were not significant, respectively (Table 3.12). In both experiments, *M. javanica* infection at all levels of inoculation did not affect nutrient elements of the test hybrid Sorghum-Sudan grass leaf tissues (Table 3.15).

Table 3.10. Partitioning mean sum of squares for calcium (Ca), iron (Fe), potassium (K), magnesium (Mg) and zinc (Zn) in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source		Ca		Fe		K		Mg		Zn	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
					Experiment 1						
Replication	5	299.40	40	7639.83	37	37.29	51	23.72	40	3987.17	30
Treatment	6	90.51	12*	6111.7	30*	7.05	9*	9.07	16*	4098.67	31*
Error	30	360.20	48	6941.98	33	29.28	40	26.11	44	5079.29	39
Total	41	750.11	100	20693.51	100	73.62	100	58.93	100	13165.13	100
					Experiment 2						
Replication	5	888.33	75	11145.4	55	7.63	8	14.01	16	14282.4	43
Treatment	6	58.70	5*	3926.6	20*	55.61	57*	39.66	46*	9741.0	29*
Error	30	229.59	20	5083.6	25	33.46	35	33.33	38	9226.3	28
Total	41	1176.62	100	20155.6	100	96.69	100	87.00	100	33249.7	100

** Highly significant P ≤ 0.01, *significant P ≤ 0.05.

Table 3.11. Partitioning mean sum of squares for calcium (Ca), iron (Fe), potassium (K), magnesium (Mg) and zinc (Zn) in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	Ca			Fe		K		Mg		Zn	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
					Experiment 1						
Replication	5	1.476	21	2464.1	9	3.240	15	0.263	22	6611.5	20
Treatment	6	2.028	28*	6935.2	27*	5.459	26*	0.253	22*	4197.9	12*
Error	30	3.696	51	16785.7	64	12.433	59	0.662	56	24031.7	70
Total	41	7.201	100	26185.0	100	21.132	100	1.178	100	34841.1	100
					Experiment 2						
Replication	5	1.611	26	15657.4	46	8.520	34	2.940	38	9881.0	24
Treatment	6	1.860	30*	7821.4	23*	7.599	31*	2.371	30*	14594.8	36*
Error	30	2.698	44	10305.6	31	8.627	35	2.481	32	15998.5	40
Total	41	6.169	100	33784.4	100	24.746	100	7.792	100	40474.3	100

** Highly significant P ≤ 0.01, *significant P ≤ 0.05.

Table 3.12. Partitioning mean sum of squares for calcium (Ca), iron (Fe), potassium (K), magnesium (Mg) and zinc (Zn) in hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation.

Source		Ca		Fe		K		Mg		Zn	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
					Experiment 1						
Replication	5	99.731	38	4609.9	23	5.807	31	22.126	52	6599.43	46
Treatment	6	77.001	30*	10636.7	52*	3.638	20*	11.736	27*	4840.57	34*
Error	30	82.250	32	5036.7	25	9.161	49	8.912	21	2774.50	20
Total	41	258.982	100	20283.3	100	18.606	100	42.774	100	14214.50	100
					Experiment 2						
Replication	5	25.794	11	12619.7	46	10.984	30	44.128	49	6317.52	54
Treatment	6	76.309	32*	8285.7	31*	10.313	29*	29.147	32*	2362.8	20*
Error	30	139.379	57	6124.3	23	14.604	41	16.762	19	3100.24	26
Total	41	241.482	100	27029.7	100	35.901	100	90.037	100	11780.56	100

** Highly significant P ≤ 0.01, *significant P ≤ 0.05.

Table 3.13. Responses for calcium (Ca), iron (Fe), potassium (K), magnesium (Mg) and zinc (Zn) in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Treatment	Ca	Fe	K	Mg	Zn	Ca	Fe	K	Mg	Zn
	Experiment 1					Experiment 2				
0	36.41	205.17	11.79	10.81	139.58	11.11	185.06	10.08	8.99	193.13
5	31.28	176.00	11.81	10.09	158.44	11.08	142.67	6.13	5.85	132
25	35.61	198.67	14.19	12.64	161.02	15.56	139.29	7.16	6.67	136.67
125	25.09	130.00	12.56	8.68	166.90	15.22	163.19	3.97	3.52	243.96
625	31.97	129.77	11.51	10.42	207.08	13.19	157.56	2.85	2.50	178.5
3125	35.49	157.63	12.67	11.52	178.42	17.95	190.81	11.32	9.46	207.96
15625	32.54	136.98	14.03	10.99	210.63	8.99	115.94	8.70	6.40	157
$LSD_{0.05}$	22.38	28.24	2.38	3.02	40.03	7.87	34.99	2.73	2.81	43.26

Table 3.14. Responses for calcium (Ca), iron (Fe), potassium (K), magnesium (Mg) and zinc (Zn) in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Treatment	Ca	Fe	K	Mg	Zn	Ca	Fe	K	Mg	Zn
	Experiment 1					Experiment 2				
0	2.16	32.96	3.58	2.97	123.21	1.87	50.24	3.34	2.00	80.17
5	2.60	85.95	4.80	2.52	124.34	2.81	108.49	6.46	4.05	99.08
25	2.78	138.45	6.42	2.62	192.54	2.06	42.35	4.16	2.56	70.33
125	2.90	87.19	4.66	3.00	147.85	3.35	137.62	6.20	2.78	201.79
625	2.45	78.84	4.93	2.88	163.00	3.22	124.65	5.74	2.81	179.48
3125	2.18	96.44	4.29	2.63	168.52	2.83	94.90	4.69	2.47	130.08
15625	3.87	124.24	5.90	2.55	178.77	2.89	78.08	4.98	2.79	118.90
$LSD_{0.05}$	1.27	32.76	1.16	0.06	18.79	0.94	19.70	1.46	0.86	19.14

Table 3.15. Responses for calcium (Ca), iron (Fe), potassium (K), magnesium (Mg) and zinc (Zn) in hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation.

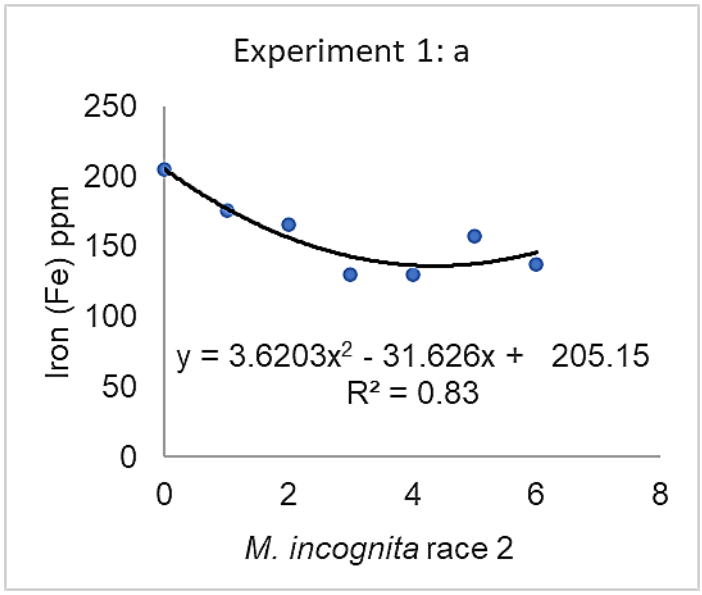
Treatment	Ca	Fe	K	Mg	Zn	Ca	Fe	K	Mg	Zn
	Experiment 1					Experiment 2				
0	27.53	157.65	10.49	9.94	138.67	23.96	175.33	8.74	14.48	131.27
5	23.17	113.88	9.50	10.83	192.40	30.42	150.39	11.20	13.58	140.60
25	27.15	59.73	11.37	9.94	150.83	21.62	191.27	10.37	11.96	159.54
125	22.56	88.42	9.70	7.07	222.71	25.85	98.58	11.57	9.79	159.58
625	18.12	45.94	9.00	7.57	169.21	27.58	128.42	12.59	10.24	182.58
3125	19.85	37.55	9.88	8.40	192.29	26.90	106.83	12.43	10.81	152.31
15625	25.56	72.78	9.57	9.73	168.75	31.98	181.79	11.00	15.43	124.04
$LSD_{0.05}$	10.69	23.68	1.57	1.52	12.11	6.92	22.27	2.51	2.83	15.65

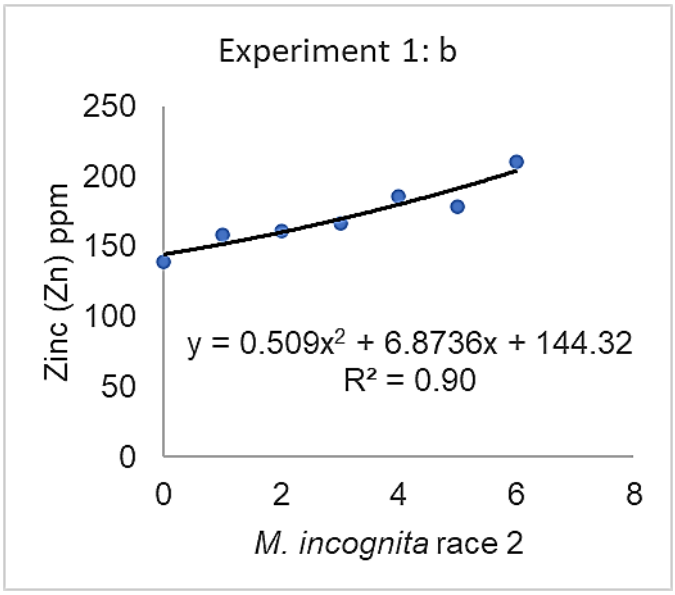
***Meloidogyne incognita* race 2**: Iron and Ca in leaf tissues of hybrid Sorghum-Sudan grass against increasing inoculation level of *M. incognita* race 2 in Experiment 1 (E1) (Figure 3.2), along with K and Mg in Experiment 2 (E2) (Figure 3.3), exhibited a negative quadratic relation, with the associations explained by 83 and 71% for Fe and Ca, respectively (E1) and 81% and 90% for K and Mg, respectively (E2). Meanwhile, Zn and Ca versus increasing levels of *M. incognita* exhibited a positive quadratic relation, with associations explained by 90% (E1) and 81% (E2), respectively. All these nutrients elements are optimized at Fe (4) and Zn (7); and K (3) and Ca (1) (Table 3.16). Finally, K and Mg for E1 and Fe and Zn for E2 versus nematode levels had no significant relationships since R^2 was less than 0.25.

***Meloidogyne incognita* race 4**: in leaf tissues of the test hybrid against increasing inoculation level of *M. incognita* race 4 in E1 (Figure 3.4), exhibited a negative quadratic relation. Meanwhile, Fe and Zn versus increasing levels of *M. incognita* exhibited positive quadratic relationships, with associations explained by 78 and 92% (Figure 3.4); and Fe (95 %), K (83%), Zn (93%) and Ca (84%) (Figure 3.5) (E2). All these nutrient elements were optimised at Ca (0.04 nematodes), Fe (6 nematodes) and Zn (15 nematodes) for E1 and Fe (3 nematodes), K (3 nematodes), Zn (4 nematodes) and Ca (4 nematodes) for E2 (Table 3.17). Finally, K for (E1) and Mg for E1 and Fe and Zn for E2 had no significant relationships since R^2 was less than 0.25.

Meloidogyne javanica: Iron, Mg and Ca in leaf tissues of hybrid Sorghum-Sudan grass against increasing inoculation level of *M. javanica* in both E1 and E2, exhibited a negative quadratic relation, with the associations explained by 96, 81 and 73%, respectively, for E1 (Figure 3.6) and 73, 81 and 38%, respectively, in E2 (Figure 3.7).

Meanwhile, Zn and K versus increasing levels of *M. javanica* exhibited positive quadratic relations, with associations explained by 94% for E1 and 77% in E2 while K was 77% for both E1 and E2. All these nutrient elements were optimised at Fe (4 nematodes), Mg (3 nematodes), Zn (4 nematodes) and Ca (3 nematodes) in E1 (Table 3.16) and Fe (3 nematodes), K (4 nematodes), Mg (3), Zn (3 nematodes) and Ca (2 nematodes) (Table 3.18). Finally, K versus nematodes in E1 had no significant relations since R^2 was less than 0.25.





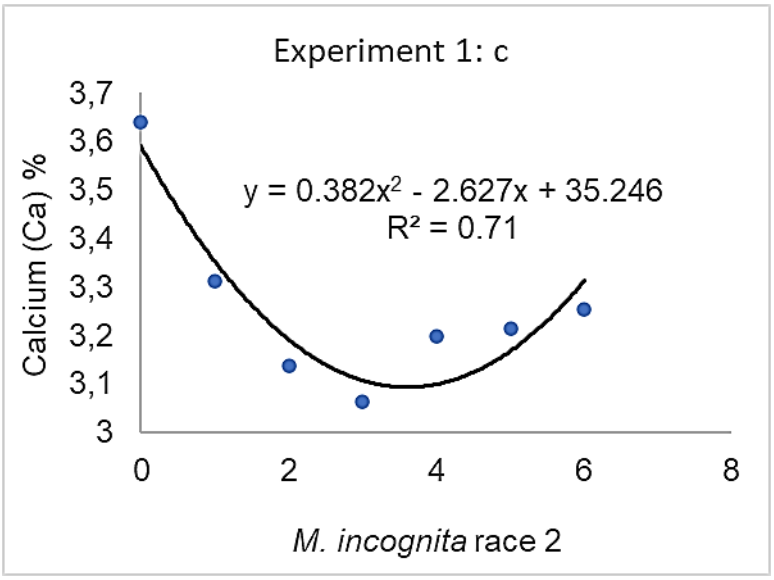
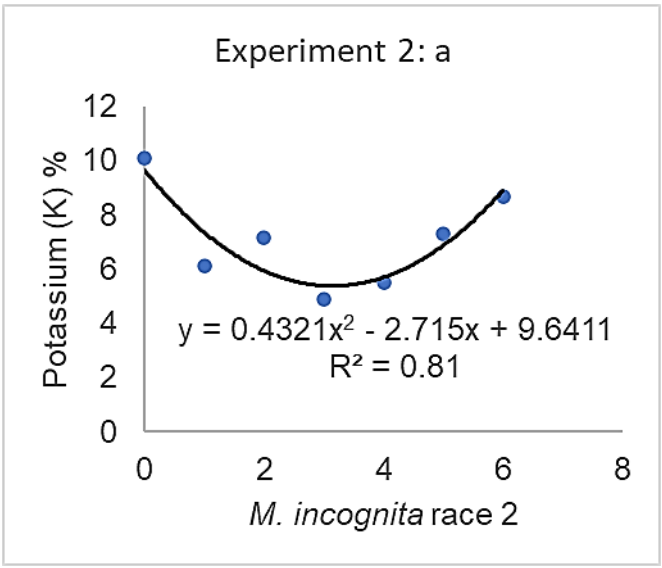
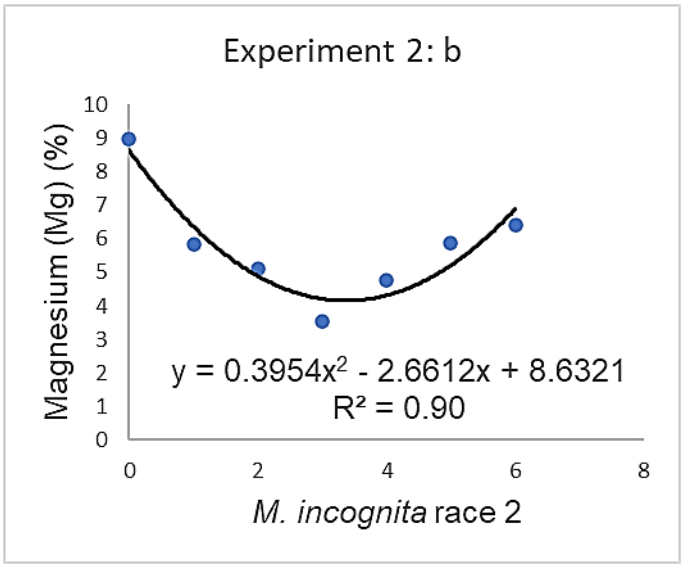


Figure 3.2. The relationship between *Meloidogyne incognita* race 2 (Experiment 1) and nutrient elements (Fe, Zn and Ca) in leaf tissues of hybrid Sorghum-Sudan grass.





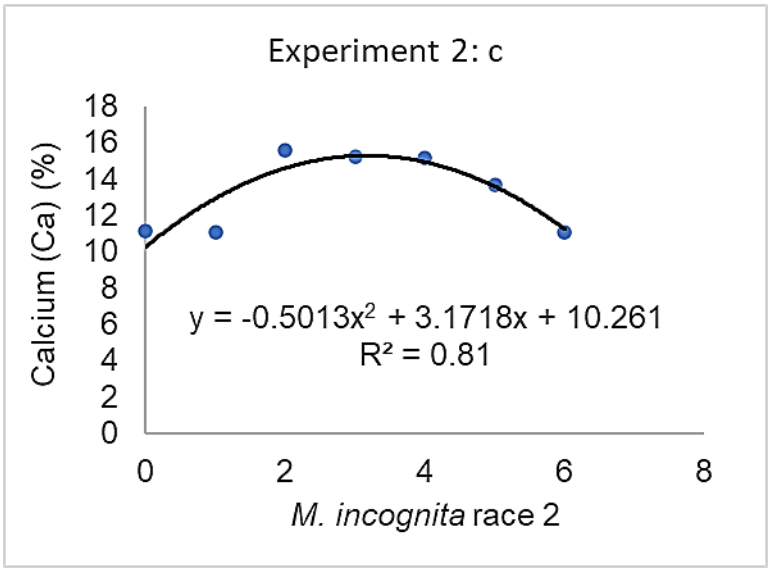
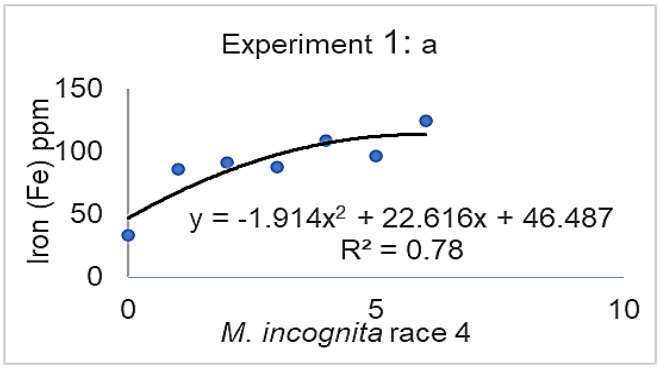


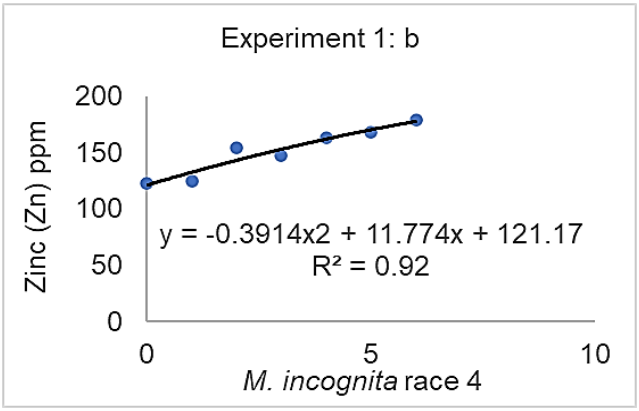
Figure 3.3. The relationship between *Meloidogyne incognita* race 2 (Experiment 2) and nutrient elements (Fe, Mg and Ca) in leaf tissues of hybrid Sorghum-Sudan grass.

Table 3.16. Optimisation models of nutrient elements in leaf tissues of hybrid Sorghum-Sudan grass of affected by different levels of *Meloidogyne incognita* race 2.

	Model	R^2	x^Y
Element	Experiment 1		
Fe	$y = 3.6203x^2 – 31.626x + 205.15$	0.83	4
Zn	$y = 0.509x^2 + 6.8736x + 144.32$	0.90	7
Ca	$y = 0.382x^2 – 2.627x + 35.246$	0.71	4
	Experiment 2		
K	$y = 0.4321x^2 – 2.715x + 9.6411$	0.81	3
Mg	$y = 0.3954x^2 - 2.6612x + 8.6321$	0.90	3
Ca	y = -0.5013x2 + 3.1718x + 10.261	0.81	1

[Y]Calculated optimum treatment level (*Meloidogyne* species) $x = -b_1/2b_2$, where b_1 = coefficient of x and b_2 = coefficient of x^2 on the quadratic equation, then x was the optimum inoculum level.





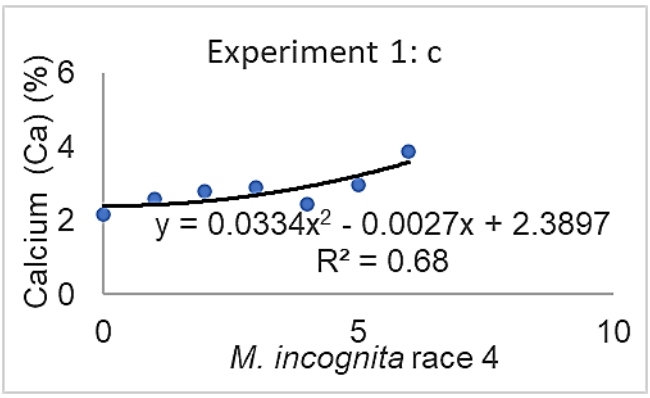
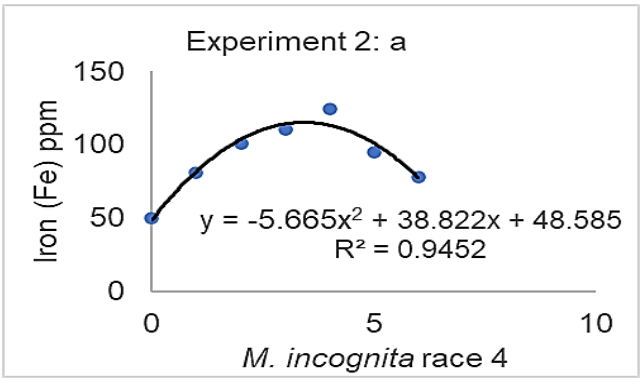
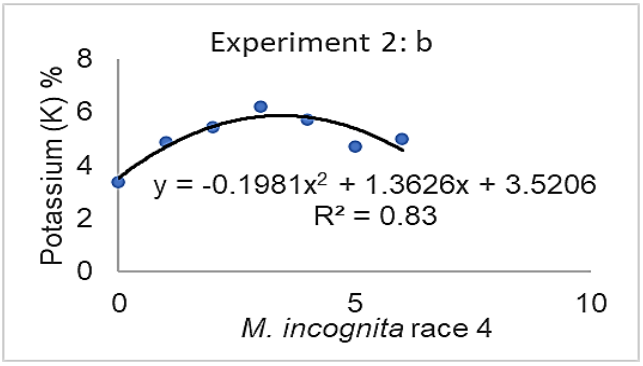
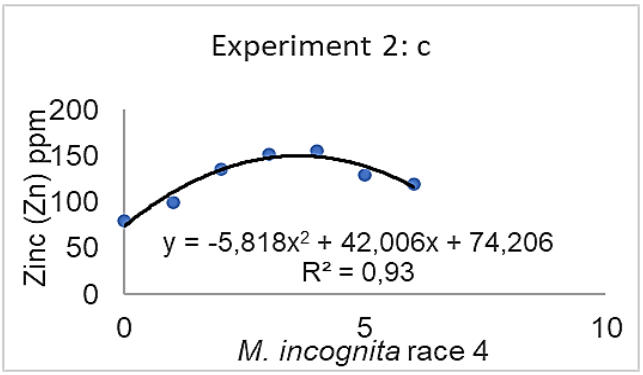


Figure 3.4. The relationship between *Meloidogyne incognita* race 4 (Experiment 1) and nutrient elements (Fe, Zn and Ca) in leaf tissues of hybrid Sorghum-Sudan grass.







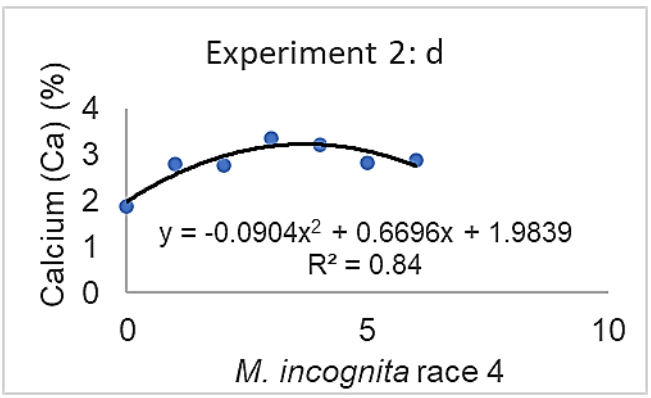


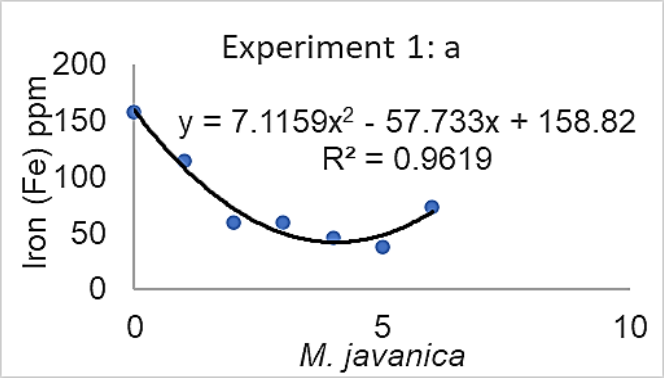
Figure 3.5. The relationship between *Meloidogyne incognita* race 4 (Experiment 2) and nutrient elements (Fe, K, Zn and Ca) in leaf tissues of hybrid Sorghum-Sudan grass.

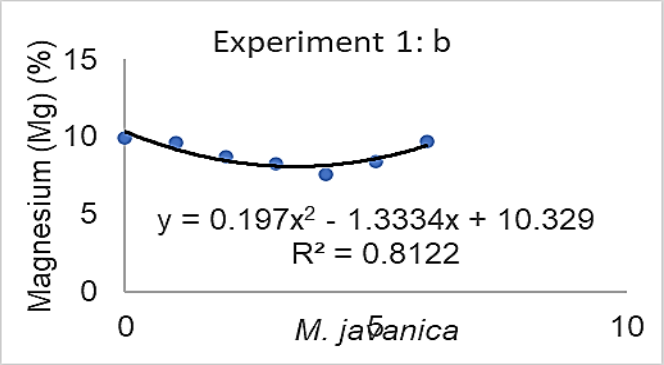
Table 3.17. Optimisation models of nutrient elements in leaf tissues of hybrid Sorghum-Sudan grass of affected by different levels of *Meloidogyne incognita* race 4.

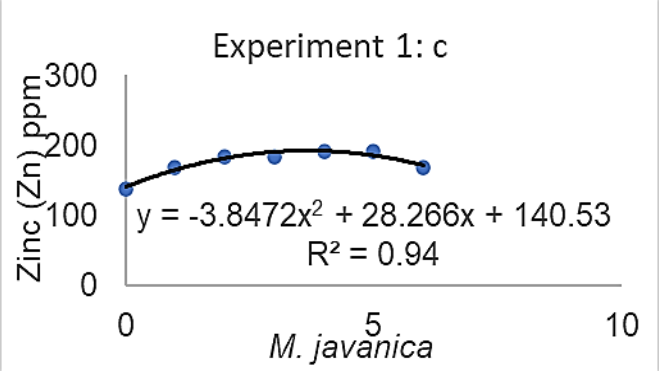
	Model	R^2	x^Y (%)^Z
Element	Experiment 1		
Fe	$y = –1.9144x^2 + 22.616x + 46.487$	0.78	6 (15 625)
Zn	$y = –0.3914x^2 + 11.774x + 121.17$	0.92	15 (39063)
Ca	$y = 0{,}0334x^2 - 0{,}0027x + 2{,}3897$	0.68	0.04 (0)
	Experiment 2		
Fe	$y = –5.6645x^2 + 38.822x + 48.585$	0.95	3 (125)
K	$y = –0.1981x^2 + 1.3626x + 3.5206$	0.83	3 (125)
Zn	$y = –5.818x^2 + 42.006x + 74.206$	0.93	6 (15 625)
Ca	$y = -0.0904x^2 + 0.6696x + 1.9839$	0.84	4 (625)

[Y]Calculated optimum treatment level (*Meloidogyne* species) x = −b1/2b2, where b1 = coefficient of x and b2 = coefficient of x2 on the quadratic equation, then x was the optimum inoculum level.

[Z]Outside brackets are the log (x+1) (transformed) data and inside are the untransformed data.







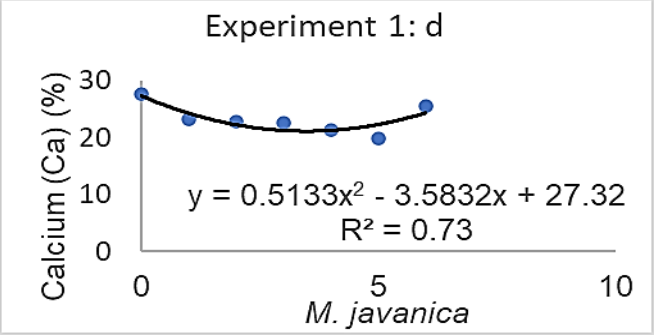
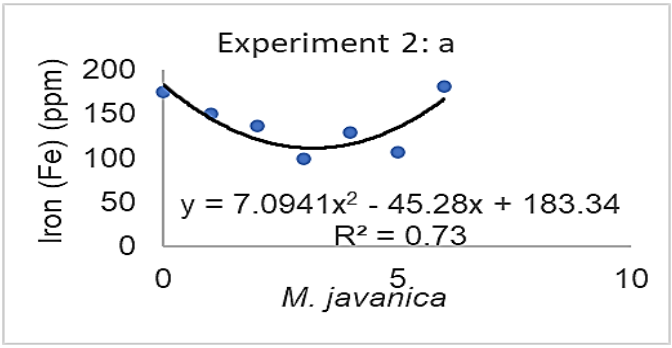
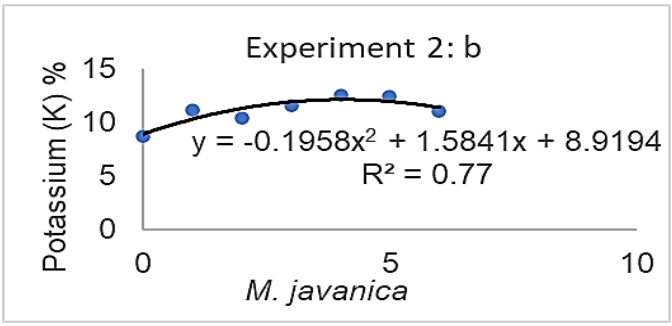
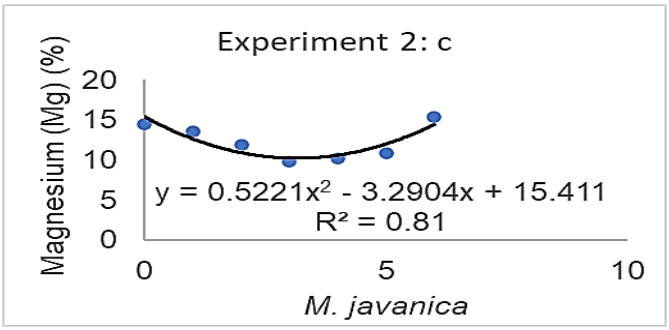
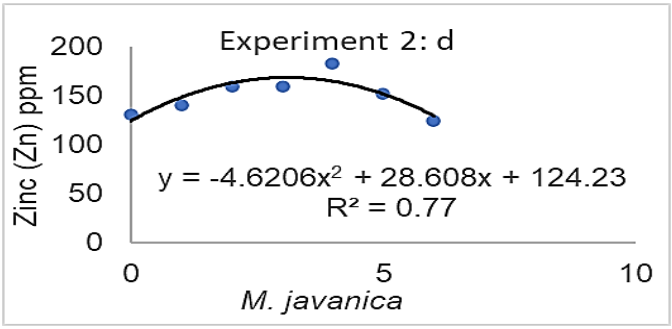


Figure 3.6. The relationship between *Meloidogyne javanica* (Experiment 1) and nutrient elements (Fe, Mg, Zn and Ca) in leaf tissues of hybrid Sorghum-Sudan grass.









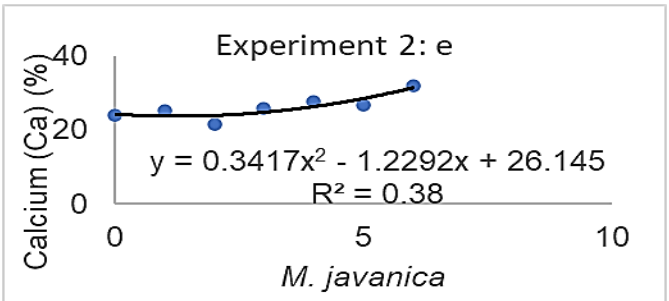


Figure 3.7. The relationship between *Meloidogyne javanica* (Experiment 2) and nutrient elements (Fe, K, Mg, Zn and Ca) in leaf tissues of hybrid Sorghum-Sudan grass.

Table 3.18. Optimisation models of nutrient elements in leaf tissues of hybrid Sorghum-Sudan grass of affected by different levels of *Meloidogyne javanica.*

	Model	R^2	x^Y (%)^Z
Element	Experiment 1		
Fe	$y = 7.1159x^2 – 57.733x + 158.82$	0.96	4 (625)
Mg	$y = 0.197x^2 - 1.3334x + 10.329$	0.81	3 (125)
Zn	$y = –3.8472x^2 + 28.266x + 140.53$	0.94	4 (625)
Ca	$y = 0.5133x^2 - 3.5832x + 27.32$	0.73	3 (125)
	Experiment 2		
Fe	$y = 7.08x^2 – 45.209x + 183.36$	0.73	3 (125)
K	$y = –0.1958x^2 + 1.5841x + 8.9194$	0.77	4 (625)
Mg	$y = 0,5221x^2 – 3.2904x + 15.411$	0.81	3 (125)
Zn	$y = –4.6206x^2 + 28.608x + 124.23$	0.77	3 (125)
Ca	$y = 0.3417x^2 – 1.2292x + 26.145$	0.38	2 (25)

[Y]Calculated optimum treatment level (*Meloidogyne* species) $x = -b_1/2b_2$, where b_1 = coefficient of x and b_2 = coefficient of x^2 on the quadratic equation, then x was the optimum inoculum level.

[Z]Outside the brackets are the log(x+1) (transformed) data and inside are the untransformed data.

3.4 Discussion

For all three test nematodes on hybrid Sorghum-Sudan grass, nematode and plant results could be explained using the host-status and host-sensitivity concepts (Seinhorst, 1965). The fact that the RF values were zero at all levels of inoculation, with the test nematodes not affecting growth of the test hybrid, the hybrid was confirmed as being resistant to all test thermophilic *Meloidogyne* species and biological races. Hybrid Sorghum-Sudan grass releases a chemical compound called dhurrin and sorgolene as root exudate from different parts of the root system (Weston *et al*., 2013). Observations in the current study agree with those where the hybrid was shown to be highly resistant to *M. javanica* in the trial where 25 nematode-resistant plant species were used as cover crops under field conditions (Valenzuela and Smith, 2002). In all plant species, RF had zero values.

Nutrients availability in the plant tissues is affected by all the amount of the nutrients in the soil, moreover, the health of the root. When the root system is not damaged by the nematodes in this case the available nutrients in the leaf tissues if not affected. In the current study the TTV and the response table suggest that all *Meloidogyne* species had no effect to the hybrid Sorghum-Sudan grass leaf nutrient accumulation. However, various studies shows that one to three of the nutrient element studies have traits of affecting the nutrient element. For example, Chiuta (2021) found that at UL location Na content was significantly high in potato-(Velum)-potato and differed from the other three cropping sequences which were not significantly different from each other.

The assessed nutrient elements versus increasing nematode levels demonstrated responses that ranged from negative to positive quadratic relations, with limited associations which were not significant. The quadratic relationships could be better explained using the density-dependent growth (DDG) pattern concepts. Mashela *et al*. (2015) explained the responses of DDG patterns as comprising three phases, namely, the stimulation, neutral and inhibition phases. In positive quadratic relations, nematodes at lower levels stimulated the accumulation of nutrient elements or plant growth variables, whereas at higher levels the accumulation or plant growth was inhibited. The observation of nutrient element responses implies that although RF values were at zero values and plant growth was not affected by nematode levels, at the physiological level the hybrid incurred some form of effect as shown by the quadratic models. Positive quadratic relations suggested that there was improvement in accumulation of the affected nutrient elements, whereas negative quadratic relations suggested inhibition of the accumulation of the affected nutrient element and therefore, deterioration in quality. Current results agreed with those in Ramputla (2019) on chilli pepper, where increasing nematode levels did not have effects on plant variables, but improved the accumulation of Ca, Fe, K, Mg and Zn. Generally, chilli pepper cultivars are moderately resistant to *Meloidogyne* species (Gisbert *et al*., 2013), but suffer at the physiological level when exposed to increasing level of nematodes. In banana plants, increasing levels of *M. incognita* were shown to reduce the accumulation of N, P, K and Mg, although Ca was increased in the leaf tissues (Devarajan *et al*., 2003; Santana-Gomes *et al*., 2013). The effects of nematodes on the accumulation of nutrient elements in plants were first observed in citrus induced by the citrus nematode (*Tylenchulus semipenetrans* Cobb 1913) in Florida, USA, which drastically changed certain cultural practices in

citriculture (Mashela, 1992). The current physiological observation of nutrient element accumulation responding to increasing nematode level in nematode resistant hybrid Sorghum-Sudan suggested that the phenomenon in plant-nematode relations could be widespread. Most nutrient elements are readily mobilised from organ to organ, depending on the prevailing conditions that affect the physiology of the plants (Santana-Gomes *et al*., 2013; Taiz and Zeiger, 2010).

Zinc in leaf tissues of the hybrid versus increasing nematode numbers in various trials during the current study exhibited positive quadratic relations, which suggested that at low nematode densities, the presence of nematode would be beneficial in the accumulation of Zn in leaf tissues of the hybrid, thereby increasing the quality of the forage, whereas at high inoculation densities the opposite might be true. According to Barker and Pilbeam (2007), plants deficient in Zn contain low levels of superoxide dismutase and encourages inhibition of metabolic processes which also leads to loss of membrane integrity. The accumulation of Zn in plant tissues improves the amino acids in the root exudates, which might weaken the attraction of nematodes towards roots (Cakmak and Marschner, 1988; Santana-Gomes *et al*., 2013).

3.5 Conclusion

Hybrid Sorghum-Sudan grass was resistant to *M*. *javanica*, *M*. *incognita* race 2 and *M*. *incognita* race 4. However, as observed in the accumulation of various nutrient elements, the nematode levels affected the accumulation of nutrient elements and therefore the quality of forage. Apparently, when the test hybrid is used as forage, supplementation of certain nutrient elements would be necessary, depending on the infestation level of nematodes. Due to the ability of nematodes to enter various

survival stages, it could be that the hybrid leaves behind a high level of nematode residues in the soil, which had been referred to as cross-over (Chiuta, 2021). Consequently, it was necessary to investigate whether it would be safe to cultivate nematode susceptible cultivars after the test hybrid in context of crop rotations intended for managing nematode population densities.

CHAPTER 4

INFECTION OF NEMATODE-SUSCEPTIBLE SWEET POTATO CULTIVAR FROM RESIDUAL SOIL NEMATODES AFTER HYBRID SORGHUM-SUDAN GRASS

4.1 Introduction

Crop rotation using nematode resistant hybrid Sorghum-Sudan grass for managing nematode population densities of root-knot (*Meloidogyne* species) nematodes became increasingly important after the withdrawal of fumigant chemical nematicides in 2005. Cultivars ‘Trudan 8’, ‘Sordan 79’ and ‘SS-222’ were shown to be poor hosts to various M*eloidogyne* species and biological races (Clark, 2007). Hybrids of Sorghum-Sudan grass release both dhurhin and sorgolene into the rhizosphere, which accord the hybrids the pre-infectional nematode resistance mechanism (Bertin *et al.,* 2003; Chiuta, 2021; Dayan *et al*., 2010; Hennion *et al*., 2019; Weston *et al*., 2013; Weston and Mathesius, 2014). In a wide range of experiments, Chiuta (2021) demonstrated that due to the ability of most nematodes to enter cryptobiosis, plants with pre-infectional nematode resistance mechanism, which does not allow nematodes to penetrate the root systems, leave a large number of residual nematodes for the next crop and, therefore, such plants are not suitable for use in crop rotations intended to manage nematode population densities. However, hybrid Sorghum-Sudan grass is believed to have both pre-infectional and post-infectional nematode resistance mechanisms (Chiuta, 2021). Internationally, *M. incognita* is the major root-knot nematode species that causes widespread damage in crops (Taylor and Sasser, 1978), whereas in South Africa *M. javanica* was viewed as the major aggressive *Meloidogyne* species (Kleynhans *et al*., 1996), until the discovery of *M. enterolobii* (Collett, 2021). The objective of this study was therefore to establish

whether the nematode-susceptible successor sweet potato cv. 'Beauregard' would be damaged by the residual nematode population densities following the cultivation in pots where hybrid Sorghum-Sudan grass was previously inoculated with increasing population densities of *M. javanica*.

4.2 Materials and methods

4.2.1 Description of the study site

The experiments were conducted at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, in Limpopo Province, South Africa (23°53'10"S, 29°44'15"E). Minimum and maximum ambient temperatures ranged from 19 to 20°C and from 25 to 27°C, respectively, with the maxima controlled using thermostatically activated fans. Relative humidity was kept at 60-70% through the wet wall. The study was conducted mid-February to mid-May then late-June to late-September 2020. After harvesting hybrid Sorghum-Sudan grass on the degree of nematode resistance to *Meloidogyne* species, soil infested with *M. javanica* (Chapter 3) was returned to their respective 20-cm diameter pots and kept moist through irrigation once weekly for a month.

4.2.2 Treatments and research design

The growing mixture was not re-infested, but the treatments 0, 5, 25, 125, 625, 3 125 and 15 625 eggs + second-stage juveniles (J2) were retained and arranged in randomised complete block design, with six replications. Blocking was done since conditions inside the greenhouse were heterogeneous due to wind currents which were generated by fans during extraction of heat.

4.2.3 Procedures

Approximately 20-cm long cuttings of highly susceptible nematode cultivar 'Beauregard', were inserted into 20-cm-diameter plastic pots, containing the growing mixture with residual *M. javanica* nematodes derived from previously inoculated pots with hybrid Sorghum-Sudan grass (Chapter 3). The pots were placed on the greenhouse benches at 0.2 m × 0.2 m spacing. Sweet potato cuttings were irrigated using 200 ml tapwater every other day and fertilised a month after setting using 5 g NPK 2:3:2 (26) + 5% Zn + 5% S + 5% Ca fertiliser mixture, with 1 g N:P:K 2:1:2 (43) per plant to provide a total of 0.175 mg N, 0.16 mg K and 0.16 mg P, 0.45 mg Mg, 0.378 mg Fe, 0.0375 mg Cu, 0.175 mg Zn, 0.5 mg B, 1.5 mg Mn and 0.035 mg Mo per ml tapwater added to provide essential macro- and micro-nutrients except for Ca.

4.2.4 Data collection

At 56 days after setting, length of the leading vine was measured from the soil level to the tip of the vine using a measuring tape. Leaf chlorophyll content was measured on three mature and healthy leaves using a SPAD-502 chlorophyll meter (Konica Minolta, Beijing, China, recorded and averaged prior to analysis of variance (ANOVA). Leaf number was counted per plant. Dry shoot weight was recorded after shoots were oven-dried at 60°C for 72 h. Stem diameter was measured using a Digital Vernier Calibre® (mm) at 5-cm above the severed end. Root system was removed from pots, immersed in water to remove excess soil particles, with all roots measured for fresh mass and those from 0 nematode treatment oven-dried at 60°C for 72 h and weighed to facilitate extrapolation of dry root mass. Root galls were assessed using the North Carolina Differential Scale of 0-5 where 1 = no galls, 2 = 1-

10 galls, 3 = 11-31 galls, 4 = 31-100 galls and 5 = being galls that are greater than 100 per root system (Taylor and Sasser, 1978).

Nematodes were extracted from the total root system per plant using maceration and blending for 60 sin 1% NaCIO solution (Hussey and Barker, 1973). The material was passed through top-down nested 75-µm and 25-µm mesh sieves, with the remaining content from the 25-µm sieve being stored in 100 ml plastic containers. Nematodes were separated from the debris of the aliquot through the sugar-floatation and centrifugation method (Marais *et al*., 2017). A sugar stock solution was prepared by dissolving 624 g sugar/L tapwater, 45 ml of the stock solution was added into centrifuge tubes and stirred once prior to centrifuging for 3 minutes at 1 800 rpm. The aliquot was then decanted onto 25-µm sieve with sugar rinsed off the nematodes through running tapwater, the remaining water was collected into 100 ml plastic container for further analysis. From the 100 ml aliquot eggs and J2 were counted from 5 ml aliquot sample using 60 magnification × stereomicroscope. Nematode numbers from roots were converted to nematodes per total root system per plant as the final nematode population density (Pf) and expressed as the reproductive factor (RF = Pf/Pi), which is a proportion of Pf to initial nematode population density (Pi).

4.2.5 Data analysis

Treatment data (x-axis) were expressed as exponents of base 5 as explained previously (Chapter 5) to ensure that the intervals had the same distances. Variable data were subjected to ANOVA using Statistix 10.0 software. Mean separation was achieved using Fisher's least significant difference test at the probability level of 5%.

Prior to the analysis of variance nematode data were transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984).

4.3 Results

Hybrid Sorghum-Sudan grass: The treatment effects on eggs, J2 in roots and Pf were highly significant (P≤ 0.01), contributing 92, 87 and 92% in total treatment variation (TTV) of the respective variables, without significant effects on J2 in the soil and RF (Table 4.1). At all levels of inoculation, the RF values were zero including that on the standard tomato cv. ‘Floradade’ (Table 4.2). Treatments had a significant effect on dry shoot mass and dry root mass, contributing 61 and 65% in TTV of the respective variables, but there were no significant effects on plant height, stem diameter and chlorophyll content (Table 4.3). Generally, there was an increase in dry shoot mass and dry root mass with increase in inoculum nematode levels (Table 4.3).

Sweet potato experiment:The treatment effects on eggs, J2 in roots and Pf were highly significant (P≤ 0.01), contributing 71, 67 and 70% in total treatment variation (TTV) of the respective variables, without significant effects on RF (Table 4.4). At all levels of inoculation, the RF values were zero including that on the susceptible standard cv. 'Beauregard' (Table 4.5). At all the inoculation level, infection by *M. javanica* infection did not have significant effects on hybrid Sorghum-Sudan grass growth variable (Table 4.6 and Table 4.7).

Table 4.1. Analysis of variance for eggs, second-stage juveniles (J2) in roots and soil, final nematode population density (Pf) and the reproductive factor (RF) of mixed *Meloidogyne* species on hybrid Sorghum-Sudan grass at 56 days after inoculation.

Source		Eggs		J2 in roots		J2 in soil		Pf		RF	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	5	0.33	4	0.21	7	0.34	23	0.40	4	1.35	32
Treatment	6	8.23	92**	2.85	87**	0.67	46ns	9.16	92**	1.35	32ns
Error	30	0.4	5	0.20	6	0.46	31	0.39	4	1.58	36
Total	41	8.98	100	3.26	100	1.47	100	9.95	100	4.28	100

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

Table 4.2. Responses of eggs, second-stage juvenile (J2) in roots and soil, final nematode population density (Pf) and reproductive factor (RF) of mixed *Meloidogyne* species on hybrid Sorghum-Sudan grass at 56 days after inoculation.

Treatment	Eggs	J2 in roots	J2 in soil	Pf	[y]RF
0	0	0	0	0	0
5	5	2	0	7	1.33
25	2	2	0	4	0.13
125	8	2	0	10	0.08
625	7	7	18	32	0.05
3125	215	22	36	273	0.09
15625	2487	83	54	2624	0.17
		Tomato cv. 'Floradade'			
[z]F3125	955	62	36	1053	0.34

[y]RF=Pf/Pi and [z] cv. 'Floradade' as a susceptible standard.

Table 4.3. Partitioning mean sum of squares of plant height (PH), stem diameter (STD), chlorophyll content (CC), dry shoot mass (DSM) and dry root mass (DRM) of mixed *Meloidogyne* species on hybrid Sorghum-Sudan grass at 56 days after inoculation.

Source		VL (cm)		STD (mm)		CC		DSM (g/plant)		DRM (g/plant)	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	5	37078.6	32	5.439	53	42.631	38	22.177	19	10.992	9
Treatment	6	39257.7	34^{ns}	2.592	25^{ns}	12.106	11^{ns}	72.172	61*	78.043	65*
Error	30	38904.2	34	2.212	22	57.699	51	24.184	20	30.864	26
Total	41	115240.5	100	10.242	100	112.437	100	188.528	100	119.898	100

^{ns} Not significant at P ≤ 0.05.

Table 4.4. Analysis of variance for eggs, second-stage juveniles (J2) in roots, final nematode population density (Pf) and the reproductive factor (RF) of mixed *Meloidogyne* species on sweet potato at 56 days after inoculation.

Source		Eggs		J2 in roots		Pf		RF	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	5	0.31	8	0.24	10	0.32	8	0.01	21
Treatment	6	2.62	71**	1.63	67**	2.88	70**	0.02	41ns
Error	30	0.79	21	0.56	23	0.94	22	0.02	38
Total	41	3.71	100	2.43	100	4.14	100	0.04	100

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

Table 4.5. Responses of final eggs, final population second-stage juvenile (J2) in roots, final nematode population density (Pf) and the reproductive factor (RF) of mixed *Meloidogyne* species on sweet potato at 56 days after inoculation.

Treatment	Eggs	J2 in roots	Pf	[y]RF
0	0	0	0	0
5	0	0	0	0
25	0	0	0	0
125	3	3	7	0
625	87	10	97	0
3125	70	87	157	0
15625	327	90	417	0
		Cultivar 'Beauregard'		
[Z]B3125	390	90	433	0

[y]RF=Pf/Pi and [z] cv. ‘Beauregard’ as a susceptible standard.

Table 4.6. Partitioning mean sum of squares of vine length (VL), stem diameter (STD), chlorophyll content (CC), dry shoot mass (DSM) and dry root mass (DRM) of mixed *Meloidogyne* species on sweet potato at 56 days after inoculation.

Source		VL (cm)		STD (mm)		CC		DSM (g/plant)		DRM (g/plant)	
	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	5	137.052	55	10.766	65	203.501	35	2.902	57	24.354	44
Treatment	6	61.753	25^{ns}	2.794	17^{ns}	198.609	34^{ns}	1.563	30^{ns}	19.676	36^{ns}
Error	30	48.557	20	2.997	18	179.093	31	0.674	13	10.683	20
Total	41	247.362	100	16.557	100	581.203	100	5.139	100	54.714	100

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

Table 4.7. Responses of plant vine length (VL), stem diameter (STD), chlorophyll content (CC), dry shoot mass (DSM) and dry root mass (DRM) mixed *Meloidogyne* species on hybrid Sorghum-Sudan grass at 56 days after inoculation.

Pi	VL (cm)	STD (mm)	CC	DSM (g/plant)	DRM (g/plant)
0	17.08	3.81	35.98	0.58	4.41
5	18.33	3.32	32.15	0.74	3.04
25	17.50	3.93	34.10	1.42	3.99
125	20.00	4.48	43.32	1.03	5.17
625	23.58	4.63	43.03	1.59	7.23
3125	15.73	3.56	39.27	0.35	2.25
15625	13.50	2.64	27.73	0.27	2.08
$LSD_{0.05}$	11.98	3.90	18.95	1.89	2.79

4.4 Discussion

Based on the results in the current study, hybrid Sorghum-Sudan grass did not leave substantial residual nematodes as a source of infection on the subsequent sweet potato cultivar. Chiuta (2021) observed similar results on potato as a successor crop when the nematode resistant plant wild watermelon (*Cucumis africanus* L.) was used for managing nematode population densities. In the study, Chiuta (2021) observed that for plants with pre-infectional nematode resistance such as *Sorghum bicolor* cv. 'Ndendani-X1', a subsequent potato crop was heavily infected with *M. enterolobii* under field conditions. Chiuta (2021) concluded that nematode-resistant plants with pre-infectional nematode resistance were having auto-protection mechanism which were not suitable for protecting the successor crops in crop rotation sequences. Usually, nematode resistance mechanisms occur in two forms, namely, pre-infectional and post-infectional nematode resistance mechanisms (Kaplan and Davis, 1987).

In pre-infectional nematode resistance mechanism, J2 are either repelled by chemicals exuded in the rhizosphere and therefore nematodes are not allowed to penetrate the root systems (Kaplan and Davis, 1987). Nematodes have developed various survival strategies towards detrimental environmental factors, with such survival strategies collectively referred to as cryptobiosis, which occur in various forms (Mashela, 2007). Chemiobiosis is one form of cryptobiosis which is induced by the gradual exposure of nematode eggs and or juveniles to chemicals, such as those from root exudates. In contrast to pre-infectional nematode resistance mechanism, in post-infectional nematode mechanism, J2 penetrate the root system, where gene products released by J2, activate plant genes released by plants to induce chemical

substances which kill J2 or isolate J2 in such a way that they could not develop further to maturity and then reproduce (Mashela *et al*., 2017b). Generally, hybrid Sorghum-Sudan grass has pre-infectional nematode resistance, with claims that the hybrid also has post-infectional nematode resistance mechanism (Chiuta, 2021). Most importantly, the hybrid releases two types of chemicals, namely, dhurinn and sorgolene, released from different parts of the root system (Wang *et al*., 2002).

In the current study it was demonstrated that the hybrid Sorghum-Sudan grass was able to substantially reduce the cross-over nematode residues for either sweet potato or tomato cultivars which are highly susceptible to the test nematodes as shown by the RF values of zero. Most Sudan grass hybrids were shown in various studies to be poor hosts of *Meloidogyne* species (Djian-Caporalino *et al*., 2019). In a Brazilian study, Lima De Brida *et al*. (2017) observed that Sudan grass hybrids 'BRS-610, 'BRS-800', and '307.343' were resistant to *M. incognita* and *M. javanica*. Additionally, hybrid Sorghum-Sudan grass during on-farm surveys showed that the hybrid was a poor host for *Meloidogyne* species (Kratochvil *et al*., 2004). In another study (MacGuidwin and Layne, 1995), both Sudan grass and Sorghum-Sudan grass hybrid cultivars failed to suppress nematodes in Wisconsin, USA, where potato was used as a successor crop.

Generally, above the damage threshold level nematode infection reduces plant growth variables, whereas below the damage threshold nematode infection levels may or may not stimulate plant growth. Makhwedzhana (2018) observed that plant growth in certain nematode-resistant sweet potato cultivars was stimulated by nematode infection, whereas those in nematode-susceptible sweet potato cultivars

were significantly reduced. In the current study, residual nematodes after hybrid Sorghum-Sudan grass increased dry shoot mass and dry root mass. Other plant variables such as plant height, stem diameter and chlorophyll content, were not affected by the residual nematodes. The observation could further be explained using the concept of density-dependent growth (DDG) patterns (Makhwedzhana, 2018; Mashela *et al*., 2015; Pofu *et al*., 2016). The stem diameter observation in the current study was not consistent with observation on infection by *Meloidogyne* species, which consistently reduces stem diameter in various plant species (Mofokeng, 2005). However, it should be reiterated that the nematode cross-over residues from hybrid Sorghum-Sudan grass was low to the extent that it hardly induced effects on sweet potato as a successor crop.

4.5 Conclusion

Hybrid Sorghum-Sudan grass successfully reduced the cross-over residue nematodes for the successful production of sweet potato cv. 'Beauregard', which is highly susceptible to *Meloidogyne* species. Consequently, the hybrid could be used in crop rotation systems where nematode susceptible crops such as root and tuber crops are to be used as successor crops.

CHAPTER 5

SUMMARY OF FINDINGS, SIGNIFICANCE, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary of findings

Hybrid Sorghum-Sudan grass did not allow the development of *Meloidogyne javanica, M. incognita* race 2 and *M. incognita* race 4 as shown by the reproductive factor (RF) values which were less than one, namely, zero, without suffering any plant growth damage at all levels of inoculation. The hybrid Sorghum-Sudan grass was classified as being a resistant host to the test *Meloidogyne* species. Infection of nematode-susceptible sweet potato from cross-over residual soil nematodes was not successful due to root exudates that did not allow the penetration and development of the nematodes in the successor susceptible sweet potato.

5.2 Significance of findings

Hybrid Sorghum-Sudan grass could be used in crop rotations systems intended to manage population densities of thermophilic *Meloidogyne* species and races. The observation on cv. 'Beauregard' subjected to *M. javanica*, suggested that nematode susceptible crops could be used as successor crops after the use of hybrid Sorghum-Sudan grass.

5.3 Recommendations

The nematode resistance mechanism in hybrid Sorghum-Sudan grass against *M. enterolobii* is currently not clear. Consequently, future studies should assess the mechanism involved in nematode resistance of hybrid Sorghum-Sudan grass to

Meloidogyne species and also host-status and host-sensitivity to *M. enterolobii*, which is an emerging nematode risk in South Africa. Also, it is important that the effect of using this hybrid on soil health be investigated.

5.4 Conclusions

Hybrid Sorghum-Sudan grass was shown to be resistant to *M. incognita* race 2, *M. incognita* race 4 and *M*. *javanica*. The hybrid hardly accumulated cross-over nematode residues as shown in the successor sweet potato cv. 'Beauregard', which was subjected to previously inoculated increasing nematode population densities of *M. javanica* on hybrid Sorghum-Sudan grass. Consequently, hybrid Sorghum-Sudan grass might possibly be viewed as being suitable for use in crop rotation systems intended to manage population densities of *M. javanica* in tropical areas of Limpopo Province, South Africa.

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APPENDICES

Appendix 3.1 Analysis of variances for plant height of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	1633.35	326.67		
Trt	6	510.6	85.143	0.42	0.8564
Error	30	6010.34	200.345		
Total	41	8154.54			

Appendix 3.2 Analysis of variances for chlorophyll content of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	232.69	46.538		
Trt	6	154.28	25.713	0.77	0.5999
Error	30	1002.64	33.4214		
Total	41	1389.61			

Appendix 3.3 Analysis of variances for stem diameter of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	32.205	6.4411		
Trt	6	36.702	6.11695	1.09	0.3883
Error	30	167.643	5.5881		
Total	41	236.55			

Appendix 3.4 Analysis of variances for number of leaves of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	6.1905	1.2381		
Trt	6	2.9524	0.49206	0.72	0.6359
Error	30	20.4762	0.68254		
Total	41	29.619			

Appendix 3.5 Analysis of variances for fresh root mass of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	111.44	22.2881		
Trt	6	107.994	17.999	0.9	0.5105
Error	30	602.643	20.0881		
Total	41	822.078			

Appendix 3.6 Analysis of variances for dry shoot mass of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	0.0465	0.0093		
Trt	6	0.0359	0.0059	0.6	0.726
Error	30	0.2976	0.0099		
Total	41	0.38	0.025		

Appendix 3.7 Analysis of variances for plant height of hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	4505.3	901.066		
Trt	6	794.5	132.423	0.48	0.8143
Error	30	8192.7	273.091		
Total	41	13492.6			

Appendix 3.8 Analysis of variances for chlorophyll content of hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	341.11	68.2226		
Trt	6	270.62	45.1035	1.09	0.3927
Error	30	1245.05	41.5015		
Total	41	1856.78			

Appendix 3.9 Analysis of variances for stem diameter of hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	48.732	9.74641		
Trt	6	14.241	2.37343	0.82	0.5615
Error	30	86.571	2.88571		
Total	41	149.544			

Appendix 3.10 Analysis of variances for number of leaves of hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	10.119	2.02381		
Trt	6	7.6667	1.27778	1.59	0.1833
Error	30	24.0476	0.80159		
Total	41	41.8333			

Appendix 3.11 Analysis of variances for fresh root mass of hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	619.6	123.92		
Trt	6	113.52	18.919	0.43	0.8525
Error	30	1317.56	43.919		
Total	41	2050.67			

Appendix 3.12 Analysis of variances for dry shoot mass of hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	55.931	11.1861		
Trt	6	11.8	1.9667	0.61	0.7224
Error	30	97.143	3.2381		
Total	41	164.873			

Appendix 3.13 Analysis of variances for plant height of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	3780	756.006		
Trt	6	3396.3	566.048	2.21	0.0691
Error	30	7668.4	255.615		
Total	41	14844.8			

Appendix 3.14 Analysis of variances for chlorophyll content of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	229.02	45.804		
Trt	6	1003.74	167.29	2.12	0.080
Error	30	2367.47	78.916		
Total	41	3600.23			

Appendix 3.15 Analysis of variances for stem diameter of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	98.8	19.7594		
Trt	6	169.78	28.2969	1.05	0.415
Error	30	810.33	27.0111		
Total	41	1078.91			

Appendix 3.16 Analysis of variances for number of leaves of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	0.007	0.001		
Trt	6	0.016	0.003	1.01	0.439
Error	30	0.079	0.003		
Total	41	0.101	0.007		

Appendix 3.17 Analysis of variances for fresh root mass of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	111.44	22.29		
Trt	6	107.99	18.00	0.9	0.5105
Error	30	602.64	20.09		
Total	41	822.08			

Appendix 3.18 Analysis of variances for dry shoot mass of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	0.123	0.025		
Trt	6	0.295	0.049	1.7	0.154
Error	30	0.866	0.029		
Total	41	1.285	0.103		

Appendix 3.19 Analysis of variances for plant height of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	280.9	56.181		
Trt	6	1283.21	213.868	1.25	0.3097
Error	30	5133.74	171.125		
Total	41	6697.85			

Appendix 3.20 Analysis of variances for stem diameter of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	18.6425	3.7285		
Trt	6	2.9196	0.4866	0.39	0.8796
Error	30	37.4398	1.24799		
Total	41	59.0018			

Appendix 3.21 Analysis of variances for chlorophyll content of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	54.61	10.9217		
Trt	6	196.97	32.8282	1.06	0.4067
Error	30	927.35	30.9116		
Total	41	1178.93			

Appendix 3.22 Analysis of variances for number of leaves of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	2.1905	0.4381		
Trt	6	1.2857	0.21429	0.63	0.7021
Error	30	10.1429	0.3381		
Total	41	13.619			

Appendix 3.23 Analysis of variances for fresh root mass of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	219.64	43.9289		
Trt	6	322.87	53.8119	1.66	0.1657
Error	30	973.13	32.4376		
Total	41	1515.64			

Appendix 3.24 Analysis of variances for dry shoot mass of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	0.0465	0.0093		
Trt	6	0.0359	0.0059	0.6	0.726
Error	30	0.2976	0.0099		
Total	41	0.38			

Appendix 3.25 Analysis of variances for plant height of hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	1226.43	245.286		
Trt	6	1077.44	179.573	1.19	0.3367
Error	30	4514.79	150.493		
Total	41	6818.65			

Appendix 3.26 Analysis of variances for chlorophyll content of hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	76.063	15.2126		
Trt	6	107.747	17.9578	0.76	0.6093
Error	30	712.028	23.7343		
Total	41	895.838			

Appendix 3.27: Analysis of variances for stem diameter of hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	286.5	57.2999		
Trt	6	579.48	96.5794	2.85	0.0256
Error	30	1015.83	33.8611		
Total	41	1881.81			

Appendix 3.28 Analysis of variances for fresh root mass of hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	619.6	123.92		
Trt	6	113.52	18.919	0.43	0.853
Error	30	1317.56	43.919		
Total	41	2050.67			

Appendix 3.29 Analysis of variances for number of leaves of hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	6.119	1.22381		
Trt	6	3.4762	0.57937	2.35	0.0554
Error	30	7.381	0.24603		
Total	41	16.9762			

Appendix 3.30 Analysis of variances for dry shoot mass of hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	0.053	0.011		
Trt	6	0.012	0.002	0.77	0.599
Error	30	0.078	0.003		
Total	41	0.143			

Appendix 3.31 Analysis of variances for plant height of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	252.96	50.593		
Trt	6	492.28	82.047	0.38	0.8886
Error	30	6552.18	218.406		
Total	41	7297.42			

Appendix 3.32 Analysis of variances for chlorophyll content of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	93.742	18.7484		
Trt	6	92.63	15.4383	0.71	0.6449
Error	30	653.245	21.7748		
Total	41	6818.65			

Appendix 3.33 Analysis of variances for stem diameter of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	0.06194	0.01239		
Trt	6	0.01694	0.00282	0.81	0.5701
Error	30	0.10448	0.00348		
Total	41	0.18336			

Appendix 3.34: Analysis of variances for number of leaves of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	2.6905	0.5381		
Trt	6	1.1429	0.19048	0.43	0.8497
Error	30	13.1429	0.4381		
Total	41	16.9762			

Appendix 3.35 Analysis of variances for fresh root mass of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	111.44	22.29		
Trt	6	107.99	18.00	0.9	0.5105
Error	30	602.64	20.09		
Total	41	822.08			

Appendix 3.36 Analysis of variances for dry shoot mass of hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	0.003	0.00061		
Trt	6	0.001	0.00014	0.85	0.544
Error	30	0.005	0.00017		
Total	41	0.009			

Appendix 3.37 Analysis of variances for calcium in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	14.97	2.99397		
Trt	6	5.431	0.90512	0.25	0.010
Error	30	108.059	3.60197		
Total	41	128.46			

Appendix 3.38 Analysis of variances for iron in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	38199	7639.83		
Trt	6	36670	6111.7	0.88	0.03
Error	30	208259	6941.98		
Total	41	283129			

Appendix 3.39 Analysis of variances for potassium in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	186.45	37.2902		
Trt	6	42.28	7.0468	0.24	0.04
Error	30	878.48	29.2825		
Total	41	1107.21			

Appendix 3.40 Analysis of variances for magnesium in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	1.18757	0.23751		
Trt	6	0.54402	0.09067	0.35	0.03
Error	30	7.83343	0.26111		
Total	41	9.56502			

Appendix 3.41 Analysis of variances for zinc in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	19936	3987.17		
Trt	6	24592	4098.67	0.81	0.026
Error	30	152379	5079.29		
Total	41	196907			

Appendix 3.42 Analysis of variances for calcium in hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	1.2897	0.25794		
Trt	6	4.5786	0.76309	0.55	0.03
Error	30	41.8137	1.39379		
Total	41	47.682			

Appendix 3.43 Analysis of variances for iron in hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation

Source	DF	SS	MS	F	P
Reps	5	23049	4609.9		
Trt	6	63820	10636.7	2.11	0.02
Error	30	151102	5036.7		
Total	41	237972			

Appendix 3.44 Analysis of variances for potassium in hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation

Source	DF	SS	MS	F	P
Reps	5	29.034	5.80684		
Trt	6	21.829	3.63814	0.4	0.029
Error	30	274.839	9.16131		
Total	41	325.702			

Appendix 3.45 Analysis of variances for magnesium in hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation

Source	DF	SS	MS	F	P
Reps	5	1.10631	0.22126		
Trt	6	0.70415	0.11736	1.32	0.028
Error	30	2.67356	0.08912		
Total	41	4.48401			

Appendix 3.46 Analysis of variances for zinc in hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation

Source	DF	SS	MS	F	P
Reps	5	32997	6599.43		
Trt	6	29043	4840.57	1.74	0.014
Error	30	83235	2774.5		
Total	41	145276			

Appendix 3.47 Analysis of variances for calcium in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	0.0738	0.01476		
Trt	6	0.1217	0.02028	0.55	0.026
Error	30	1.10889	0.03696		
Total	41	1.30439			

Appendix 3.48 Analysis of variances for iron in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	12320	2464.1		
Trt	6	41611	6935.2	0.41	0.018
Error	30	503571	16785.7		
Total	41	557503			

Appendix 3.49 Analysis of variances for potassium in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	16.199	3.2397		
Trt	6	32.756	5.4593	0.44	0.02
Error	30	372.981	12.4327		
Total	41	421.935			

Appendix 3.50 Analysis of variances for magnesium in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	0.01317	2.63E-03		
Trt	6	0.01518	2.53E-03	0.38	0.038
Error	30	0.19865	6.62E-03		
Total	41	0.22699			

Appendix 3.51 Analysis of variances for zinc in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	33058	6611.5		
Trt	6	25187	4197.9	0.17	0.01
Error	30	720951	24031.7		
Total	41	779196			

Appendix 3.52 Analysis of variances for calcium in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	44.419	8.88384		
Trt	6	3.522	0.58706	0.26	0.019
Error	30	68.877	2.2959		
Total	41	116.819			

Appendix 3.53 Analysis of variances for iron in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	54142	10828.4		
Trt	6	25030	4171.7	0.8	0.015
Error	30	155866	5195.5		
Total	41	235038			

Appendix 3.54 Analysis of variances for potassium in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	30.3	6.0592		
Trt	6	347.87	57.9783	1.78	0.013
Error	30	976.46	32.5486		
Total	41	1354.62			

Appendix 3.55 Analysis of variances for magnesium in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	0.7176	0.14352		
Trt	6	2.359	0.39316	1.18	0.037
Error	30	10.0366	0.33455		
Total	41	13.1132			

Appendix 3.56 Analysis of variances for zinc in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 2 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	62889	12577.9		
Trt	6	65010	10834.9	1.08	0.032
Error	30	301344	10044.8		
Total	41	429242			

Appendix 3.57 Analysis of variances for calcium in hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	4.9865	0.99731		
Trt	6	4.62	0.77001	0.94	0.043
Error	30	24.6751	0.8225		
Total	41	34.2817			

Appendix 3.58 Analysis of variances for iron in hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation

Source	DF	SS	MS	F	P
Reps	5	63099	12619.7		
Trt	6	49714	8285.7	1.35	0.024
Error	30	183728	6124.3		
Total	41	296541			

Appendix 3.59 Analysis of variances for potassium in hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation

Source	DF	SS	MS	F	P
Reps	5	54.919	10.9838		
Trt	6	61.881	10.3134	0.71	0.036
Error	30	438.118	14.6039		
Total	41	554.918			

Appendix 3.60 Analysis of variances for magnesium in hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation

Source	DF	SS	MS	F	P
Reps	5	2.20638	0.44128		
Trt	6	1.74884	0.29147	1.74	0.014
Error	30	5.02847	0.16762		
Total	41	8.98369			

Appendix 3.61 Analysis of variances for zinc in hybrid Sorghum-Sudan grass to *Meloidogyne javanica* levels under greenhouse conditions after 56 days of inoculation

Source	DF	SS	MS	F	P
Reps	5	31588	6317.52		
Trt	6	14177	2362.8	0.76	0.013
Error	30	93007	3100.24		
Total	41	138772			

Appendix 3.62 Analysis of variances for calcium in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	0.08056	0.01611		
Trt	6	0.11159	0.0186	0.69	0.027
Error	30	0.8093	0.02698		
Total	41	1.00145			

Appendix 3.63 Analysis of variances for iron in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	78287	15657.4		
Trt	6	46928	7821.4	0.76	0.026
Error	30	309167	10305.6		
Total	41	434382			

Appendix 3.64 Analysis of variances for potassium in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	42.599	8.51981		
Trt	6	45.594	7.59905	0.88	0.0208
Error	30	258.799	8.62664		
Total	41	346.993			

Appendix 3.65 Analysis of variances for magnesium in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	0.14701	0.0294		
Trt	6	0.14224	0.02371	0.96	0.034
Error	30	0.74427	0.02481		
Total	41	1.03351			

Appendix 3.66 Analysis of variances for zinc in hybrid Sorghum-Sudan grass to *Meloidogyne incognita* race 4 levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	49405	9881		
Trt	6	87569	14594.8	0.91	0.024
Error	30	479955	15998.5		
Total	41	616929			

Appendix 4.1 Analysis of variances for eggs on hybrid Sorghum-Sudan grass to mixed *Meloidogyne* species levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	1.6267	0.32534		
Trt	6	49.4117	8.23528	19.59	0.001
Error	30	12.6125	0.42042		
Total	41	63.6509	8.98104		

Appendix 4.2 Analysis of variances for J2 in roots hybrid Sorghum-Sudan grass to mixed *Meloidogyne* species levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	1.0586	0.21173		
Trt	6	17.0892	2.8482	14.04	0.001
Error	30	6.0864	0.20288		
Total	41	24.2343	3.26281		

Appendix 4.3 Analysis of variances for J2 in soil hybrid Sorghum-Sudan grass to mixed *Meloidogyne* species levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	1.7199	0.34397		
Trt	6	4.0296	0.67161	1.47	0.2216
Error	30	13.6959	0.45653		
Total	41	19.4454	1.47211		

Appendix 4.4 Analysis of variances for final population (Pf) hybrid Sorghum-Sudan grass to mixed *Meloidogyne* species levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	1.9999	0.39998		
Trt	6	54.9458	9.15764	23.49	0.003
Error	30	11.6965	0.38988		
Total	41	68.6422	9.9475		

Appendix 4.5 Analysis of variances for reproductive factor (RF) hybrid Sorghum-Sudan grass to mixed *Meloidogyne* species levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
Reps	5	6.7518	1.35036		
Trt	6	8.1018	1.3503	0.85	0.5403
Error	30	47.5159	1.58386		
Total	41	62.3695	4.28452		

Appendix 4.6 Analysis of variances for plant height on hybrid Sorghum-Sudan grass to mixed *Meloidogyne* species levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
REP	5	185393	37078.6		
TRT	6	235546	39257.7	1.01	0.438
Error	30	1167125	38904.2		
Total	41	1588064			

Appendix 4.7 Analysis of variances for stem diameter on hybrid Sorghum-Sudan grass to mixed *Meloidogyne* species levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
REP	5	27.193	5.43864		
TRT	6	15.553	2.5922	1.17	0.3472
Error	30	66.347	2.21155		
Total	41	109.093			

Appendix 4.8 Analysis of variances for chlorophyll content on hybrid Sorghum-Sudan grass to mixed *Meloidogyne* species levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
REP	5	213.16	42.6313		
TRT	6	72.64	12.1064	0.21	0.9709
Error	30	1730.97	57.6989		
Total	41	2016.76			

Appendix 4.9 Analysis of variances for fresh root mass of hybrid Sorghum-Sudan grass to mixed *Meloidogyne* species levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
REP	5	54.96	10.9915		
TRT	6	468.26	78.0432	2.53	0.0422
Error	30	925.91	30.8637		
Total	41	1449.13			

Appendix 4.10 Analysis of variances for dry shoot mass of hybrid Sorghum-Sudan grass to mixed *Meloidogyne* species levels under greenhouse conditions after 56 days of inoculation.

Source	DF	SS	MS	F	P
REP	5	110.86	22.1727		
TRT	6	433.03	72.1716	2.98	0.0209
Error	30	725.52	24.1839		
Total	41	1269.41			

Appendix 4.11 Analysis of variances for eggs on sweet potato plant under greenhouse conditions after overwintering of previously inoculated hybrid Sorghum-Sudan grass.

Source	DF	SS	MS	F	P
REP	5	1.5283	0.30566		
TRT	6	15.6937	2.61562	3.3	0.0129
Error	30	23.7774	0.79258		
Total	41	40.9994	3.71386		

Appendix 4.12 Analysis of variances for J2 in roots on sweet potato plant under greenhouse conditions after overwintering of previously inoculated hybrid Sorghum-Sudan grass.

Source	DF	SS	MS	F	P
REP	5	1.1923	0.23846		
TRT	6	9.7989	1.63315	2.9	0.0237
Error	30	16.8894	0.56298		
Total	41	27.8806	2.43459		

Appendix 4.13 Analysis of variances for final population on sweet potato plant under greenhouse conditions after overwintering of previously inoculated hybrid Sorghum-Sudan grass.

Source	DF	SS	MS	F	P
REP	5	1.5923	0.31845		
TRT	6	17.2995	2.88325	3.07	0.0184
Error	30	28.1848	0.93949		
Total	41	47.0766	4.14119		

Appendix 4.14 Analysis of variances for reproductive factor on sweet potato plant under greenhouse conditions after overwintering of previously inoculated hybrid Sorghum-Sudan grass.

Source	DF	SS	MS	F	P
REP	5	0.0482	0.00964		
TRT	6	0.11042	0.0184	1.09	0.3891
Error	30	0.50504	0.01683		
Total	41	0.66366	0.04487		

Appendix 4.15 Analysis of variances for stem diameter on sweet potato plant under greenhouse conditions after overwintering of previously inoculated hybrid Sorghum-Sudan grass.

Source	DF	SS	MS	F	P
REP	5	53.828	10.7657		
TRT	6	16.766	2.7944	0.93	0.4863
Error	30	89.91	2.997		
Total	41	160.504			

Appendix 4.16 Analysis of variances for of vine length (VL) on sweet potato plant under greenhouse conditions after overwintering of previously inoculated hybrid Sorghum-Sudan grass

Source	DF	SS	MS	F	P
REP	5	685.26	137.052		
TRT	6	370.52	61.753	1.27	0.2998
Error	30	1456.72	48.557		
Total	41	2512.5			

Appendix 4.17 Analysis of variances for fresh root mass of sweet potato plant under greenhouse conditions after overwintering of previously inoculated hybrid Sorghum-Sudan grass.

Source	DF	SS	MS	F	P
REP	5	121.772	24.3544		
TRT	6	118.056	19.6759	1.84	0.1244
Error	30	320.495	10.6832		
Total	41	560.323			

Appendix 4.18 Analysis of variances for chlorophyll content (CC) on sweet potato plant under greenhouse conditions after overwintering of previously inoculated hybrid Sorghum-Sudan grass.

Source	DF	SS	MS	F	P
REP	5	1017.51	203.501		
TRT	6	1191.65	198.609	1.11	0.3804
Error	30	5372.8	179.093		
Total	41	7581.96			

Appendix 4.19 Analysis of variances for dry shoot mass of sweet potato plant under greenhouse conditions after overwintering of previously inoculated hybrid Sorghum-Sudan grass.

Source	DF	SS	MS	F	P
REP	5	14.5116	2.90232		
TRT	6	9.3754	1.56256	2.32	0.0587
Error	30	20.216	0.67387		
Total	41	44.103			