INTERACTION OF VESICULAR ARBUSCULAR MYCORRHIZA, NEMATODE AND PHYTONEMATICIDES ON GROWTH AND NUTRITIONAL CONTENT OF CLEOME GYNANDRA

BY

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DECLARATION

I declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Agriculture (Agronomy) has not previously been submitted previously by me for a degree at this or any other university; that it is my work in design and in execution, and that all materials contained herein has been duly acknowledged.

Rabothata Masia Rodney (M.R) Date

DEDICATION

To the family I have always wanted to be my family.

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ABSTRACT

Cleome gynandra is increasingly becoming an important strategy for achieving food and nutrition security among rural households in many developing countries. Root-knot (*Meloidogyne* species) nematodes, with limited nematode management strategies, limit the successful production of this vegetable crop. Nemafric-BL and Nemarioc-AL phytonematicides are separately being developed in South Africa for sustainable crop production systems. However, the two products have not been simultaneously tested for managing the notorious *Meloidogyne* species and absorption of phosphorus, with a combination of Vesicular arbuscular mycorrhiza (VAM). The objective of this study therefore was to determine the interactive effects of VAM and each of the two phytonematicides on nutrient content, growth of *C. gynandra.* A 2 × 2 × 2 factorial experiment, with the first, second and third factors being VAM (V), nematode (N) and Nemafric-BL phytonematicide (P). The eight treatments included (1) untreated control ($V_0N_0P_0$), (2) nematodes alone ($V_0N_1P_0$), (3) VAM alone ($V_1N_0P_0$) (4) Nemarioc-AL phytonematicide alone ($V_0N_0P_1$), (5) $V_1N_1P_0$, (6) $V_0N_1P_1$, (7) $V_1N_0P_1$ and (8) $V_1N_1P_1$, were laid out in a randomised complete block design, with ten replications. The same layout experiment was done for the Nemarioc-AL phytonematicide trial which had a similar layout. Seedlings were irrigated with 250 ml chloride-free tapwater every other day for 56 days. Multifeed and NPK (2:3:2(22) fertilisers were applied at transplanting.

The second order interaction ($V_1N_1P_1$), was highly significant ($P \leq 0.01$) for plant height contributing 54% in TTV (Total Treatment Variation) of the variable. Among the main factors (N, P and V), only nematode had highly significant effects on stem diameter. All interactions of VAM, nematode and Nemarioc-AL phytonematicide and main factors each had no significant effect on Cleome. The second order ($V_1N_1P_1$)

and the first order interaction ($V_1N_1P_1$) did not have significant effects on the three nutrient elements except for the first order interaction ($V_1N_0P_1$) which was significant on foliar Zn contributing 42% in TTV of the variable. Also nematode had highly significant effect on foliar K and significant effect on foliar Zn contributing 49 and 31% in TTV of the respective variables. Using the two-way table, VAM and Nemafric-BL phytonematicide each increased foliar Zn by 27% and 29%, respectively. The second and first order interactions of VAM, N and Nemarioc-AL phytonematicide and the main factors did not have significant effect on foliar K, Fe and Zn. The second order interaction of VAM, nematode and Nemafric-BL phytonematicid**e** had significant effects on gall rating, contributing 2% in TTV of the variable. VAM, nematode and Nemarioc-AL phytonematicide showed that the second and first order interaction except for $V_1N_0P_1$ interaction on gall rating, were not significant for nematode variables. The $V_1N_0P_1$ interaction contributed 20% in TTV of gall rating. Using a two-way table, VAM and phytonematicide each increased root galls by 7% and 74%, respectively. Combined, VAM and phytonematicide reduced root galls by 64%. The innovative products interacted together and that Nemafric-BL and Nemarioc-AL phytonematicides and VAM alone could be used in managing nematodes.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

Indigenous vegetables such as Cleome (*Cleome gynandra)* are rich in vitamins, minerals, proteins and anti-oxidants (van den Heever, 1995); while on the other hand they are drought tolerant. Inland South Africa predictions suggested that temperatures would increase by 2% in 2030, whereas rainfall would decline by 10% (IPCC, 2007). Generally, the scarcity of water is due to the greater frequency and severity of droughts in most semi-arid areas, and excessive heat conditions, all of which could limit plant growth and yields (Parry *et al.,* 2007). Smallholder farmers are mostly affected by water deficits (Altieri and Koohafkan, 2008). Reduced yields have since become an economic problem since the presidential outcomes for agriculture, namely, food security, job creation and wealth generation would be directly affected.

Cleome species are reported to be tolerant to harsh environmental conditions and can survive in low rainfall areas (van Rooyen, 2001). Thus, *Cleome* species as an alternative vegetable could reduce the reliance on regular irrigation. However, yield of *C. gynandra* could be affected by the root-knot (*Meloidogyne* species*)* nematodes, which infect a wide range of plants, thereby causing yield losses (Anwar and McKenry, 2010; Cetintas and Yarba, 2010). According to Ntidi *et al*. (2015), *Meloidogyne* species in South Africa infected indigenous vegetables in different regions. After these underutilised crops are infected by nematodes, yield and quality are reduced (Wang *et al.,* 2008), leading to decreased yields and economic losses (Ntidi *et al.,* 2015). The management of nematodes in indigenous crops is essential

because of their high-value as a source of essential nutrients and functional products (Gowen, 1997).

Most synthetic nematicides have been withdrawn from the agrochemical markets due to them being environment unfriendly. The synthetic nematicides that were used in managing plant-parasitic nematodes had environmental problems in many crop production systems, which led to their withdrawal from the agrochemical markets (Mashela *et al.,* 2008). Therefore, there has since been need for developing other alternative management strategies that could substitute synthetic nematicides such as methyl bromide. Nemafric-BL and Nemarioc-AL phytonematicides have been researched and developed as alternatives to methyl bromide to manage nematode populations on tomato plants in Limpopo Province, South Africa (Pelinganga and Mashela, 2012; Pelinganga *et al.,* 2012). Nemafric-BL and Nemarioc-AL phytonematicides consistently suppressed population densities of *Meloidogyne* species under diverse environments in tomato production.

Phosphorus is naturally the most limiting plant nutrient in South African soils (Brady and Weil, 2000). The introduction of vesicular arbuscular mycorrhiza (VAM) in cropping systems could enhance the sustainable utilisation of scarce resources such as phosphorous (Ramos-Zapata *et al*., 2009; Xian-Can *et al*., 2010). Most small scale farmers are classified as resource-poor and cannot afford continuous acquisition of resources such as superphosphate fertilisers and synthetic nematicides to ensure adoption of best agricultural practices. Therefore, underutilised vegetable crops such as *Cleome* species and the reduced reliance on fertilisers could offer an opportunity for increased food security in marginal communities. The interactive effects of VAM and phytonematicides on *C. gynandra*

should be investigated in order to provide cleome-producing smallholder farmers with empirically-based information regarding the envisaged problem statement.

1.2 Problem statement

Due to increasing unfavourable conditions for exotic crops, climate-smart agriculture has since dictated the increased focus on adapted underutilised plant species. However, production information for most of the plant species with potential for improving food security, job creation and wealth generation is not empirically-supported. Information on growth and accumulation of essential nutrient elements in *C. gynandra* under VAM, nematode infestation and phytonematicide interactions has not been documented. The study was intended to investigate the growth and accumulation of selected essential nutrient elements in a *Cleome* species under various permutations of VAM, nematode and phytonematicide in order to enhance the development of best agricultural production practices for this underutilised indigenous crop.

1.3 Rationale of the study

Research on growth responses and accumulation of essential nutrient elements of indigenous underutilised crops such as *C. gynandra* could promote their production and therefore food security. Due to the labour-intensive nature of pre-harvest production and post-harvest handling practices, indigenous vegetables are well-positioned to promote job creation and wealth generation in the contest of the presidential outcomes outlined by the National Development Plan framework for the agricultural sector. Investigation of the interactive effects of VAM, nematode and phytonematicide would enhance decision-making among resource-poor farmers in

marginal communities on *C. gynandra* could serve as an alternative crop in their production systems.

1.4 Purpose of the study

1.4.1 Aim

The aim of the study was to develop production systems that would contribute to the potential database of *C. gynandra* as an alternative crop under diverse interactive systems.

1.4.2 Objectives

1. To determine whether the growth and nutrient content of *C. gynandra* would increase under interaction systems of VAM, nematode and Nemafric-BL phytonematicide.
2. To investigate whether the growth and nutrient content of *C. gynandra* would increase under interaction systems of VAM, nematode and Nemarioc-AL phytonematicide.

1.4.3 Hypotheses

1. Growth and nutrient content of *C. gynandra* would increase under interaction systems of VAM, nematode and Nemafric-BL phytonematicide.
2. Growth and nutrient content of *C. gynandra* would increase under interaction systems of VAM, nematode and Nemarioc-AL phytonematicide.

1.5 Reliability, validity and objectivity

Reliability of data was based on statistical analysis of data at the probability level of 5%, validity was achieved through the use of factorial experiment, whereas the objectivity was achieved through ensuring that the findings were discussed on the basis of empirical evidence, in order to eliminate all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

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

1.7 Structure of the mini-dissertation

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

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Cultivation of indigenous vegetables, referred to as underutilised crops in South Africa, is increasingly becoming important under climate-smart agriculture. However, information on best agricultural practices for this group of alternative crops is scanty. The ensuring review intended to assess what has already been done and not done on indigenous alternative vegetable with reference to cleome (*Cleome gynandra*).

2.2 Work done on cleome

2.2.1 Origin and distribution of *Cleome gynandra*

Cleome gynandra belongs to the *Capparaceae* family and is indigenous to South Africa (van Rensburg *et al.,* 2007). However, the plant has since been distributed to the tropics and subtropics of Africa. In South Africa, the plant occurs on agricultural land and near human settlements, mostly as weeds. *Cleome gynandra* is less common under highly humid climatic areas, but it tolerates some drought. The plant extends from Limpopo, North-West, Gauteng, Mpumalanga, KwaZulu-Natal, Free State and Northern Cape to Namibia (van den Heever and Venter, 2006; van Rooyen, 2001). Among poor rural communities, young leaves are collected cooked, and eaten like spinach (van den Heever and Venter, 2006).

2.2.2 Environmental requirements

Cleome species grows best during summer and is sensitive to cold since it does not grow well when temperatures drop below 15°C. *Cleome* species prefer well-drained medium-textured soils and do not grow well in poorly drained or heavy clay soils

(Mnzava *et al.,* 2004). The plant requires full exposure to sunlight and performs poorly when shaded. *Cleome* species grow best when adequately supplied with water. The plant tolerates a degree of water stress, but prolonged water stress promotes flowering and senescence (Cook and Fairweather, 2005). Application of fertilisers containing appreciable amounts of nitrogen could delay flowering and increased number and size of leaves (van Rensburg *et al.,* 2007). The plant species is harvested by uprooting and ratoon harvesting (Mnzava *et al.,* 2004; van den Heever and Coertze, 1996).

2.2.3 Nutritional status of cleomes

Deficiencies of iron, zinc, calcium and vitamins are widespread, with over 300 million people affected every year, and a much greater number being at risk of malnutrition deficiencies (Habwe *et al*., 2009). Malnutrition deficiencies increase the vulnerability to infectious diseases, causing numerous human deaths (Davis, 1996). Micronutrient deficiencies affect mainly pregnant women and children and contribute significantly to the global disease burden of children by limiting proper cognitive development, impairing physical development and increasing susceptibility to infectious diseases (Asare-Marfo *et al.*, 2013). Most countries in Sub-Saharan Africa are still struggling to address problems of under-nutrition and micronutrient deficiencies (Lopriore and Muehlhoff, 2003). Indigenous leafy vegetables are increasingly being recognised as possible contributors of both micronutrients and bioactive compounds to human diets of populations in Africa (Smith and Eyzaguirre, 2007).

The tender leaves or young shoots and often the flowers, are boiled and consumed as a potherb, tasty relish, stew or side dish (van den Heever and Venter, 2006).

Fresh leaves are used as ingredients in other mashed foods, whereas dried leaves are ground and incorporated in weaning foods. The leaves are rather bitter due to phenolic compounds, and for this reason are cooked with other leafy vegetables such as cowpea (*Vigna unguiculata*), amaranth (*Amaranthus* species) and blackjack (*Solanum nigrum*) (Mishra *et al.,* 2011). African indigenous leafy vegetables such as *Cleome* species, are an important source of essential nutrient like vitamin A, potassium, zinc and iron (Aphane *et al.,* 2002). Such leafy vegetables had been recognised as possible major contributors of bio-active phytochemicals to the human body in marginal communities. *Cleome gynandra* has been described as one of the most important alternative vegetable crop and its status had since risen significantly to be ranked among the recognised vegetables with high nutritional, medicinal and economic potential (Onim and Mwaniki, 2008). The health and economic benefits of this vegetable have been explored extensively in the past (Ojiewo *et al.,* 2010).

The value and important role indigenous vegetables could play in the survival of populations at risk and help reduce malnutrition. Thus these indigenous vegetables are significant contributors to food security and nutrition for smallholder farmers (Onim and Mwaniki, 2008). *Cleome gynandra* is rich in micronutrients such as iron, zinc, vitamins and contains non-nutrient substances called phytochemicals, which help protect people against non-communicable diseases (Yang and Keding, 2009). High in vitamins and micronutrients, cleome contributes to a healthy diet for many rural Africans with limited food budgets (Oniang'o *et al*., 2006). It is known to have high levels of beta-carotene and vitamin C (Chweya and Mnzava, 1997). In most cases, vitamin C is lost during cooking; however, cleome retains the vitamin better

than most other vegetables. It also contains significant amounts of calcium, magnesium and iron (Mhlonto *et al.,* 2007).

2.2.4 Medicinal uses of cleomes

Cleome gynandra has been reported to relieve constipation and to facilitate child birth (van den Heever and Venter, 2007). The vegetable is commonly used as a herbal remedy for a number of ailments including rheumatic and inflammatory conditions (Narendhirakannan *et al.,* 2005). Leaves are used in many countries for ear aches, epileptic fits, stomach ache and constipation (Mishra *et al.,* 2011).

2.2.5 Cleome-insect interactions

Cleome species are mainly attacked by several insects such as pentatomids (*Acrosternum gramineum*), locusts (*Schistocera gregaria*) and hurricane bugs (*Bagrada* species). *Cleome* species have insecticidal and insect repellent properties *(Nyalala and Grout, 2007).* Spraying aqueous plant extracts from *Cleome* species can considerably reduce aphid and thrip populations. Cabbage aphid (*Brevicoryne brassicae*) is a serious pest causing stunted growth and wrinkling of the leaves and growing tips (Cole and Jackman, 1980). The hurricane bug may similarly affect cleome plant-the attacks are more prevalent during dry periods, but can be effectively controlled with insecticides (Chweya and Mnzava, 1997). Intercropping *Cleome* species with cabbage could also reduce diamondback moth and thrip attacks (Schippers, 2002). *Cleome* species possess strong ability to produce strong odour and there has been fewer reports of arthropod pests on them compared to the exotic ones (Nchore *et al*., 2010).

2.2.6 Cleome-mite interaction

Cleome species are commonly called spider plants because of the visible spider threads on the plants. The threads are caused by a mite referred to red spider mite (*Tetranychus urticae*). The mite can be managed using commercial miticides, while crude extracts from leaves of *Cleome* species also have miticidal properties (Nyalala and Grout, 2007).

2.2.7 Cleome-pathogen interaction

The main fungal diseases associated with *Cleome* species are powdery mildew (*Sphaerotheca fuliginea*, *Oidiopsis taurica*) and leaf spot (*Cercospora uramensis*) (Rubaihayo, 1997). Cleome species have strong antifungal activity against soil-borne pathogens due to its glucosinolate components (Lazzeri and Manici, 2001).

2.2.8 Cleome-nematode interaction

Root-knot (*Meloidogyne* species) nematodes attack a wide range of vegetable crops causing yield and quality losses. *Cleome* species are also referred to as weed and however, susceptible to plant-parasitic nematodes may maintain nematode populations between and during crop growing seasons (Ntidi, 2008). Such weeds are an important reservoir for nematodes during periods when crop hosts. *Cleome* species and *Amaranthus* species have been reported to be susceptible to nematodes (Nchore *et al*. 2010). Therefore, crops like these are susceptible to the root-knot nematodes and could be cultivated in areas where the nematodes are widely distributed. This would ensure sustainable food, income and nutrition security among rural and urban households.

2.2.9 Cleome-VAM Symbiosis

Vesicular-arbuscular mycorrhizas are the mutualistic associations between soil fungi and plant roots (Sayeeda *et al.,* 2013). *Cleome* species can receive mineral nutrients obtained from the soil by the fungus while, in exchange, the fungus obtain photosynthetically derived carbon compounds from the plant. The primary function of mycorrhiza is the acquisition of mineral nutrients from the soil. Increased plant uptake of nutrients would result in improved growth of the *Cleome* species (Wallander, 2000). Micronutrients such as zinc and copper have limited diffusion in solution in many soils. VAM facilitates heavy metal uptake by forming chelates that solubilises metals and increase their bioavailability in soil (Vivas *et al.,* 2003).

2.3 Work not done on *Cleome* species

In South Africa, Nemarioc-AL and Nemafric-BL phytonematicides are being researched and developed for use as alternative to synthetic chemical nematicides (Mashela *et al*., 2015). The interaction among VAM, *Meloidogyne* species and the two phytonematicides remains as one of the gap where some work had not been done on *Cleome* species.

CHAPTER 3
RESPONSES OF *CLEOME GYNANDRA* TO NEMATODES AND SUSTAINABLE PRODUCTS

3.1 Introduction

Vesicular arbuscular mycorrhiza (VAM) in plant systems is responsible for improving the absorption of phosphorus (Abbott and Robson, 1982). The root-knot (*Meloidogyne* species) nematodes, mainly *M. incognita* races 2 and 4 and *M. javanica,* widely distributed in South Africa (Kleynhans *et al.,* 1996), could prevent absorption of nutrient elements. More than 60 *Meloidogyne* species are internationally parasitic to over 2 000 plant species (Koenning *et al.,* 1999). Prior to the withdrawal of methyl bromide from the agrochemical markets in 2005, yield losses per annum due to nematode damage were estimated globally at US$126 billion (Chitwood, 2003). Three and eight years after the withdrawal, crop losses had increased to US$157 billion (Abad *et al.,* 2008) and US$173 billion (Elling, 2013), respectively. The latter translates to relative increase in yield losses of 25 and 37%, respectively. However, the described losses did not include those among the underutilised alternative crops such as indigenous leafy vegetables like *Cleome gynandra*.

Consequently, the use of sustainable products such as VAM and phytonematicides for absorption of selected nutrient elements had hardly been assessed. The widely used phytonematicides in South Africa in resource-poor farming systems are Nemafric-BL and Nemarioc-AL phytonematicides, from fruits of Cucumis species indigenous to Limpopo Province (Mashela *et al*., 2015), (Mashela 2016). The objective of this study, therefore, was to determine interactive effects of VAM,

nematode and phytonematicides on growth and accumulation of selected nutrient elements in *C. gynandra*.

3.2 Materials and methods

3.2.1 Location of the study

A greenhouse experiment was initiated during (January-March 2016) at the Green Technologies Research Centre (GTRC), University of Limpopo, South Africa (23°53′10″S, 29°44′15″E). The ambient, day/night temperatures averaged 28/21°C, with maximum temperatures controlled using thermostatically-activated fans. The green house was 30 m × 16 m, with a wet wall on the southern side and fans at the northern side. These fans cooled the greenhouse by sucking the exterior hot air, thereby creating homogeneous conditions in the greenhouse. The two parallel experiments of VAM (V), nematode (N) and Nemafric-BL phytonematicide (P) or V, N and Nemarioc-AL phytonematicide (P) were conducted alongside each other.

3.2.2 Treatments and experimental design

Twenty-cm-diameter plastic pots were filled with steam-pasteurised river sand, loam soil (20% clay, 40% silt and 2% sand) and Hygromix-T at 2:1:1 (v/v) ratio. The pots were placed on greenhouse benches at 0.3 m and 0.2 m spacing. Seeds of *C. gynandra* were collected from pods of the previously field raised plants and sown in seedling trays containing Hygromix-T. Uniform six-week old seedlings were hardened-off by withholding irrigation on seedlings placed outside the greenhouse (Pelinganga, 2013). Each seedling was transplanted into a 20-cm-diameter plastic pot containing the growing mixture described above.

The experiment was arranged in a 2 × 2 × 2 factorial experiment, with the first, second and third factors being VAM, nematode and any of the two phytonematicides. Treatments were laid out in a randomised complete block design, with ten replications. The eight treatments included (1) control ($V_0N_0P_0$), (2) nematode alone ($V_0N_1P_0$), (3) VAM alone ($V_1N_0P_0$) (4) Nemarioc-AL phytonematicide alone ($V_0N_0P_1$), (5) $V_1N_1P_0$, (6) $V_0N_1P_1$, (7) $V_1N_0P_1$ and (8) $V_1N_1P_1$. The same layout experiment was used in the Nemarioc-AL phytonematicide trial.

3.2.3 Procedures

Nematode inoculation was prepared by extracting eggs and second-stage juveniles (J2) of *M. javanica* from roots of nematode-susceptible tomatoes cv. 'Floradade' collected at the GTRC in 1% NaOCl solution (Hussey and Barker, 1973). *Cucumis africanus* and *C. myriocarpus* fruits were collected from locally cultivated plants after fruit maturity and cut into pieces and dried in air-forced ovens at 52°C for 72 h (Mafeo and Mashela, 2009). Approximately 40 g ground materials of *C. africanus* fruit and 40 g *C. myriocarpus* fruit were each fermented in 20 L air-tight sealed plastic containers, respectively, with 16 L chlorine-free tapwater. An equivalent of 300 g molasses, 100 g brown sugar and 300 g effective microorganisms (EM) were fermented in 20 L container for 14 days at room temperature (Mashela *et al.,* 2015). The EM culture in South Africa comprises strains of yeast, lactic acid bacteria, photosynthetic bacteria, actinomycete bacteria and minor strains of fungi (Mashela *et al.,* 2015). Allowance for the released CO_2 to escape from the container was provided through an air-tight 5-mm-diameter tube with one end glued to a hole on the lid of the 20 L container, with the outlet end being dangled into a 1 L bottle which was half-filled with chlorine-free tapwater.

A 30-ml-plastic syringe was used to place 5000 *M. javanica* eggs and J2 into 3-cm-deep holes on cardinal points of stem. After transplanting the seedlings, 3 g VAM was applied in a farrow around each seedling. Seven days after inoculation with nematodes, 3% Nemarioc-AL and Nemafric-BL phytonematicides in each experiment was applied at 250 ml solution. In the first order and second order combinations treatments were halved and applied at one-third each, respectively. Treatments were applied once.

3.2.4 Cultural practices

Seedlings were irrigated with 250 ml chloride-free tapwater every other day for 56 days. Approximately 5 g Multifeed (Nulandies, Johannesburg) was applied at transplanting to provide 1.21 Mg, 0.43 K, 0.47 N, 0.43 P, 1 Fe, 4.02 Mg, 0.47 Zn, 0.10 Cu, 1.34 B and 0.09 mg Mo per ml (NPK Analysis 2:1:2 (43). The seedlings were also fertilised with 2.5 g N:P:K 2:3:2(22) to provide a total of 155 mg N, 105 mg P and 130 mg K. In addition to that, 5 g of 2:3:2(26)+ 0.5% ZN + 5% S + 5% Ca fertiliser mixture per plant was applied which provided 155 mg of N, 105 mg P, and 130 mg K per ml of water. The major pest, red spider mites were monitored and scouted for and when 10 pests per plant where observed, they were sprayed with a miticide namely the Ludwig's Organic, which was applied at 50 ml/10 L chlorine free water. The spray was combined with a wetting agent referred to as G49.

Legend 3.1 Establishment of *Cleome gynandra* treated with vesicular arbuscular mycorrhiza, *Meloidogyne javanica* and Nemafric-BL phytonematicide.

Legend 3.2 Establishment of *Cleome gynandra* treated with vesicular arbuscular mycorrhiza, *Meloidogyne javanica* and Nemarioc-AL phytonematicide.

3.2.5 Data collection

At 56 days after initiating the treatments, plant height was measured from the crown to the terminal end of the flag leaf and chlorophyll content was measured from three mature and healthy leaves using digital chlorophyll meter. Stems were cut at the crown and stem diameter measured at 3 cm above the severed ends using a digital vernier caliper. Shoots were weighed after cutting and then oven-dried at 52°C for 72 h and weighed for dry mass.

Root systems were removed from pots and immersed in tapwater to remove soil particles, blotted dry and fresh mass measured to facilitate the calculation of nematode density per total root system per plant. Fully-developed root galls were assessed using the North Carolina Differential Scale at 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls and 5 ≥ 100 galls per root system (Taylor and Sasser, 1978).

Nematodes were extracted from 5 g roots/plant through the maceration and blending method in 1% NaOCl (Hussey and Barker, 1973). The materials were passed through top-down nested 75-µm and 25-µm opening sieves. Contents of the 25-µm opening sieve were poured into 100-ml-plastic containers, bought to 100-ml mark and tightly closed for counting under a stereomicroscope. Shoots and the remaining roots were dried at 52°C for 72 h. Dry roots were adjusted for 5 g fresh roots from which nematode eggs and J2 were extracted. Soil per pot was thoroughly mixed and a 250-ml soil sample collected, with nematodes extracted using the modified sugar-floatation and centrifugation method (Jenkins, 1964). Soil per pot was thoroughly mixed and a 250 ml soil sample was collected. Nematode J2 were extracted from

soil samples using the modified sugar-floatation and centrifugation method (Jenkins, 1964). The soil sample was poured into a 4 L bucket and stirred, once the swill had stopped, water was poured through 63-µm and 25-µm screen sieves, with the contents being washed into 50 ml plastic centrifuge tubes with 3 g kaolin. The contents in each tube were centrifuged at 1 750 RPM for five minutes. Kaolin solution was then decanted with nematodes having settled at the bottom of the tubes with soil particles.

A 480 g sugar/L tapwater was poured into the centrifuge tubes and stirred once prior to centrifuging for 60 s at 1 750 RPM. The aliquot was decanted onto 25-µm screen sieve with sugar being rinsed off the nematodes, which were then collected from the screen sieve into 100 ml plastic containers and brought to 100 ml mark for counting under a stereomicroscope. Nematode eggs and J2 from roots were converted to nematodes per total root system per plant, whereas soil nematode numbers were converted to 5 L soil per pot to estimate the final nematode population densities (Pf).

Mature leaves were ground into powder using a mortar and pestle. Only 0.1 g material of each sample put into a separate beaker and treated with 45 ml distilled water mixed with 83 ml HNO_3. The mixture was stirred and incubated in 90°C water bath for 60 minutes. The samples were allowed to cool at room temperature and filtered through filter paper into a 100 ml volumetric flask and made up to mark with de-ionized water, the sample covered with a foil. Samples were then subjected to Atomic Absorption Spectrometry (ASS) to quantify potassium, zinc and ion elements at Limpopo Agrofood Technology Station (LATS).

3.2.6 Data analysis

Data were subjected to analysis of variance (ANOVA) through the 2008 SAS software (SAS Institute, 2008). Significant second and first order interactions were further expressed using the three and two-way tables, respectively, in order to allow for the determination of the magnitude and direction of the effects of the main factors relative to the control (Steyn *et al*., 2003).

3.3 Results

3.3.1 Plant growth variables

VAM, nematode and Nemafric-BL phytonematicide: The second order interaction ($V_1N_1P_1$), was highly significant ($P \leq 0.01$) for plant height, contributing 54% in TTV of the variable (Table 3.1). Among the main factors, only nematode had significant effects on stem diameter. A three-way table matrix suggested that the three factors together, VAM alone and nematode alone reduced plant height of cleome (Table 3.2). However, the matrix further indicated that $V_1N_1P_0$ had no impact on plant height, with the highest negative impact being on the VAM factor alone. The interaction $V_1N_1P_1$ also reduced plant height, while $V_1N_0P_1$ and $V_0N_1P_1$ each increased plant height (Table 3.2). Nematode alone had highly significant effects on stem diameter, contributing 37% in TTV of the variable (Table 3.1). Using two sample t-test, infection by *Meloidogyne* species reduced stem diameter by 37%.

VAM, nematode and Nemarioc-AL phytonematicide: All interactions and the main factors each had no significant effects on cleome. The in (Table 3.3) results showed that dry root mass, dry shoot mass, plant height, stem diameter and chlorophyll

content in *C. gynandra* were not affected by the main factors VAM, nematode and Nemarioc-AL.

Table 3.1 Responses of dry root mass, dry shoot mass, plant height, stem diameter and chlorophyll content in *Cleome gynandra* to vesicular arbuscular mycorrhiza (VAM), nematodes and Nemafric-BL phytonematicide (n = 80).

Source		Dry root mass		Dry shoot mass		Plant height		Stem diameter		Chlorophyll content	
	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Replication	9	0.46	16	28.22	9	0.01	8	0.97	3	238.9	24
VAM (V)	1	0.98	34 ^{ns}	71.80	23 ^{ns}	0.02	15 ^{ns}	3.81	13 ^{ns}	140.7	14 ^{ns}
Nematode (N)	1	0.13	4 ^{ns}	9.99	3 ^{ns}	0.00	0 ^{ns}	11.26	37***	143.3	15 ^{ns}
Phytonematicide (P)	1	0.23	8 ^{ns}	0.85	0 ^{ns}	0.00	0 ^{ns}	0.01	0 ^{ns}	2.66	0 ^{ns}
V × N	1	0.002	0 ^{ns}	13.33	5 ^{ns}	0.00	0 ^{ns}	0.19	0 ^{ns}	3.44	0 ^{ns}
V × P	1	0.008	0 ^{ns}	17.13	6 ^{ns}	0.00	0 ^{ns}	4.43	15 ^{ns}	292.2	30 ^{ns}
N × P	1	0.21	7 ^{ns}	69.18	23 ^{ns}	0.02	15 ^{ns}	2.66	9 ^{ns}	25.16	3 ^{ns}
V × N × P	1	0.75	26 ^{ns}	74.48	24 ^{ns}	0.07	54 **	5.43	18 ^{ns}	0.27	0 ^{ns}
Error	63	0.15	5	22.12	7	0.01	8	1.52	5	132.6	14
Total	79	2.92	100	307.1	100	0.13	100	30.28	100	979.23	100

TTV = Total treatment variation

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

Table 3.2 Three-way matrix for plant height in of *Cleome gynandra* affected by second order interaction of vesicular arbuscular mycorrhiza (VAM), nematodes and Nemafric-BL phytonematicide at 56 days after treatment application (n = 80).

		Phytonematicide (P)			
VAM	Nematode	P_0	RI (%)	P_1	RI (%)
V_0	N_0	2.0397	−	2.0253	−1
V_0	N_1	2.0160	−1	2.0519	1
V_1	N_0	1.9374	−5	2.0572	1
V_1	N_1	2.0316	0	1.9559	−4

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

Table 3.3 Responses of dry root mass, dry shoot mass, plant height, stem diameter and chlorophyll content in *Cleome gynandra* to vesicular arbuscular mycorrhiza (VAM), nematodes and Nemarioc-AL phytonematicide (n = 80).

Source		Dry root mass		Dry shoot mass		Plant height		Stem diameter		Chlorophyll content	
	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Replication	4	43.26	11	205.57	11	0.03	2	5243.7	11	151.29	28
VAM (V)	1	52.06	13 ^{ns}	283.12	16 ^{ns}	0.02	1 ^{ns}	5128.9	11 ^{ns}	49.51	9 ^{ns}
Nematode (N)	1	46.14	12 ^{ns}	181.50	10 ^{ns}	0.01	0 ^{ns}	4900.6	10 ^{ns}	8.88	2 ^{ns}
Phytonematicide (P)	1	49.64	13 ^{ns}	152.62	8 ^{ns}	1.93	93 ^{ns}	5577.1	12 ^{ns}	13.99	3 ^{ns}
V × N	1	43.21	11 ^{ns}	101.02	6 ^{ns}	0.02	1 ^{ns}	5329.4	11 ^{ns}	39.84	7 ^{ns}
V × P	1	40.01	10 ^{ns}	316.41	17 ^{ns}	0.00	0	5134.4	11 ^{ns}	192.38	35 ^{ns}
N × P	1	29.23	8 ^{ns}	330.89	18 ^{ns}	0.02	1 ^{ns}	5401.5	12 ^{ns}	2.76	1 ^{ns}
V × N × P	1	42.10	11 ^{ns}	50.72	3 ^{ns}	0.02	0 ^{ns}	5285.4	11 ^{ns}	6.35	1 ^{ns}
Error	28	42.46	11	191.61	11	0.03	2	5295.4	11	79.08	14
Total	39	388.11	100	1813.46	100	2.08	100	47296	100	544.08	100

TTV = Total treatment variation

^{ns}Not significant P ≤ 0.05.

3.3.2 Essential nutrient elements

VAM, nematode and Nemafric-BL phytonematicide: The second order ($V_1N_1P_1$) and the first order interactions did not have effects on the three nutrient elements except for the first order interaction V1N0P1, which had significant effects on foliar Zn, contributing 42% in TTV of the variable (Table 3.4). Also, nematode had highly significant effect on foliar Fe and significant effect on foliar Zn, contributing 49 and 31%, respectively, in TTV of the respective variables. Using the two-way table, VAM and Nemafric-BL phytonematicide each increased foliar Zn by 27% and 29%, respectively (Table 3.5). The $V_1N_0P_1$ interactions increased foliar Zn by 33%.

Table 3.4 Responses of potassium, iron and zinc in leaves of *Cleome gynandra* to vesicular arbuscular mycorrhiza (VAM), nematodes and Nemafric-BL phytonematicide (n = 80).

Source	DF	K		Fe		Zn	
		MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Replication	9	185.492	19	0.09	6	0.02	3
VAM (V)	1	113.31	11^{ns}	0.13	9^{ns}	0.004	1^{ns}
Nematode (N)	1	33.78	3^{ns}	0.73	49^{***}	0.27	31^{**}
Phytonematicide(P)	1	16.19	2^{ns}	0.16	11^{ns}	0.021	2^{ns}
V × N	1	6.49	1^{ns}	0.11	7^{ns}	0.001	1^{ns}
V × P	1	38.62	4^{ns}	0.08	5^{ns}	0.36	42^{**}
N × P	1	323.16	32^{ns}	0.04	3^{ns}	0.00	0^{ns}
V × N × P	1	169.39	17^{ns}	0.06	5^{ns}	0.10	12^{ns}
Error	63	111.21	11	0.07	5	0.07	8
Total	79	997.67	100	1.49	100	0.87	100

TTV = Total treatment variation.

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

Table 3.5 Two-way matrix for zinc in leaves of *Cleome gynandra* to vesicular arbuscular mycorrhiza and Nemafric-BL phytonematicide (n = 80).

VAM	Phytonematicide (P)			
	P_0	Impact %	P_1	Impact %
V_0	0.5275	–	0.6820	29
V_1	0.6720	27	0.6995	33

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

VAM, nematode and Nemarioc-AL phytonematicide: In this study, the second and first order interactions and the main factors did not have significant effect on foliar K, Fe and Zn (Table 3.6).

Table 3.6 Responses of potassium, iron and zinc in leaves of *Cleome gynandra* to vesicular arbuscular mycorrhiza (VAM), nematodes and Nemarioc-BL phytonematicide (n = 80).

Source	DF	K		Fe		Zn	
		MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Replication	9	273.62	13	0.09	16	0.06	14
VAM (V)	1	94.96	4^{ns}	0.24	40^{ns}	0.04	9^{ns}
Nematode (N)	1	309.06	14^{ns}	0.00	0^{ns}	0.11	24^{ns}
Phytonematicide(P)	1	23.29	1^{ns}	0.02	4^{ns}	0.00	0^{ns}
V × N	1	9.09	1^{ns}	0.00	0^{ns}	0.00	0^{ns}
V × P	1	688.20	32^{ns}	0.00	0^{ns}	0.08	17^{ns}
N × P	1	215.96	10^{ns}	0.04	7^{ns}	0.03	7^{ns}
V × N × P	1	347.95	16^{ns}	0.11	18^{ns}	0.09	21^{ns}
Error	63	194.73	9	0.09	15	0.04	8
Total	79	2156.8	100	0.59	100	0.45	100

^{ns} Not significant P ≤ 0.05.

3.3.3 Nematode effect

VAM, nematode and Nemafric-BL phytonematicide: The second order interaction had significant effects on gall rating, contributing 2% in TTV of the variable. Nematode, phytonematicides, V × N and N × P also had significant effect on gall rating (Table 3.7). The three-way table suggested that the phytonematicide as the main factor was highly significant on J2 in roots, contributing 51% in TTV of the variable.

VAM, nematode and Nemarioc-AL phytonematicide: The second and first order interaction except for the $V_1N_0P_1$ interaction on gall rating, were not significant for nematode variables (Table 3.8). The $V_1N_0P_1$ interaction contributed 20% in TTV of gall rating. Using a two-way table, VAM and phytonematicide each increased root galls by 7% and 74% respectively (Table 3.9). Combined, VAM and phytonematicide increased root galls by 64%. Nemarioc-AL phytonematicide had highly significant effects on J2 in roots, but had significant effect on total nematode, contributing 51% and 51% in TTV of the respective variables. Relative to the untreated control, Nemarioc-Al phytonematicide reduced J2 and total nematodes by 23 and 39% respectively.

Table 3.7 Responses of Gall rating, J2 in roots, eggs and J2 and total nematodes in soil in of *Cleome gynandra* to vesicular arbuscular mycorrhiza(VAM) , nematodes and Nemafric-BL phytonematicide interactions (n = 80).

		Gall rating		J2 in roots		Total nematodes	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Replication	9	0.01156	1	0.01057	10	0.13918	11
VAM (V)	1	0.02116	1 ^{ns}	0.01597	15 ^{ns}	0.15454	12^{ns}
Nematode (N)	1	0.5993	29***	0.00757	7 ^{ns}	0.10768	9 ^{ns}
Phytonematicide (P)	1	0.70805	1***	0.03751	36**	0.47441	38**
V × N	1	0.04527	2**	0.00039	1 ^{ns}	0.00106	0 ^{ns}
V × P	1	0.02116	1 ^{ns}	0.01597	15 ^{ns}	0.15454	12 ^{ns}
N × P	1	0.5993	29***	0.00757	7 ^{ns}	0.10768	9 ^{ns}
V ×N × P	1	0.04527	2**	0.00039	1 ^{ns}	0.00106	0 ^{ns}
Error	63	0.01154	1	0.00882	9	0.10831	9
Total	79	2.06261	100	0.10476	100	1.24846	100

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

Table 3.8 Three-way matrix for gall in *Cleome gynandra* affected by second order interaction of vesicular arbuscular mycorrhiza (VAM), nematodes and Nemafric-BL phytonematicide at 56 days after treatment application (n = 80).

		Phytonematicide (P)			
VAM	Nematode	P_0	RI (%)	P_1	RI (%)
V_0	N_0	0.2207	−	0.6414	191
V_0	N_1	0.2812	27	0.3613	64
V_1	N_0	0.0151	−93	0.1406	−36
V_1	N_1	0.1556	−29	0.0310	−86

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

Table 3.9 Responses of Gall rating, J2 in roots, eggs and J2 and total nematodes in soil in of *Cleome gynandra* to vesicular arbuscular mycorrhiza(VAM) nematodes and Nemarioc-AL phytonematicide interactions (n = 80).

		Gall rating		J2 in roots		Total nematodes	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Replication	9	0.03533	5	0.0157	7	0.16535	7
VAM (V)	1	0.01456	20 **	0.01729	7 ^{ns}	0.15499	6 ^{ns}
Nematode (N)	1	0.03533	8 ^{ns}	0.00671	3 ^{ns}	0.1073	5 ^{ns}
Phytonematicide (P)	1	0.01456	20 **	0.11893	51***	1.24311	51***
V × N	1	0.03533	8 ^{ns}	0.01729	7 ^{ns}	0.15499	6 ^{ns}
V × P	1	0.01456	20 **	0.01729	7 ^{ns}	0.15499	6 ^{ns}
N × P	1	0.01456	8 ^{ns}	0.00671	3 ^{ns}	0.1073	5 ^{ns}
V × N × P	1	0.00775	8 ^{ns}	0.01729	7 ^{ns}	0.15499	6 ^{ns}
Error	63	0.18128	3	0.01849	8	0.18792	8 ^{ns}
Total	79	0.35326	100	0.2357	100	2.43094	100

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

Table 3.10 Two-way matrix for gall ratting in *Cleome gynandra* to vesicular arbuscular mycorrhiza and Nemafric-BL phytonematicide (n = 80).

	Phytonematicide (P)			
VAM	P_0	RI (%)	P_1	RI (%)
V_0	0.6841	-	0.0211	−97
V_1	0.6900	0.9	0.0301	−96

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

3.4 Discussion

3.4.1 Plant growth variables

VAM, nematode and Nemafric-BL phytonematicide: The second order interaction, VAM × nematode × Nemafric-BL phytonematicide, on plant height confirmed the observation of Goswami *et al*. (2006), where they observed that the root length and weight of tomato plant significantly increased where the tomato plants were treated with *Paecilomyces lilacinus* and *Trichoderma viride* in combination with mustard cake and furadan. Similar effects of phytonematicide on plant growth were observed when the same product and Nemarioc-AL phytonematicide were used on tomato plant (Mashela *et al*., 2008) observed that the use of ground *C. myriocarpus* increased (72-94%) fruit yield. However, a three-way table matrix was further used to analyse the data. The data demonstrated the interaction reduced plant height, which suggested that the product may be phytotoxic to *Cleome* species. The phytonematicide alone reduced the plant height showing that the sustainable product could also not be used alone in *Cleome* species.

Generally, crude extracts of Nemafric-BL phytonematicide haVE been shown to be phytotoxic to various crops (Pelinganga and Mashela, 2013). This could be attributed to nematicidal properties of phytonematicides which haVE been reported to be phytotoxic when not used properly (Mashela *et al.*, 2008; Pelinganga *et al.,* 2011).

The VAM alone had improved plant growth of *Cleome* species, suggesting that the product can be used alone in cleome production. The combination of VAM and phytonematicide had the least effect on plant growth compared to when the products where used individually. The VAM was observed to be beneficial to growth of the tomato plants (Nzanza, 2011).

Nematode alone reduced stem diameter, which confirmed the observation of Mashela *et al*. (2011), where *Meloidogyne* species reduced stem diameters in tomato plants. There are various abiotic and biotic factors that reduce root/shoot ratio, resulting in increased accumulation of non-structural carbohydrates, with the result that stem diameter declines (Mashela and Nthangeni, 2002). The reduction of stem diameter is a physical mechanism used in reducing the concentrations of non-structural carbohydrates in order to avoid damaging osmotic potentials in root cells, which was observed under nematode infection and root pruning (Mashela and Nthangeni, 2002).

VAM, nematode and Nemarioc-AL phytonematicide: Findings in VAM, nematodes and Nemarioc-AL phytonematicide were different from those of VAM, nematodes and Nemafric-BL phytonematicide since the interaction had no significant effects on any plant variable. Nemarioc-AL phytonematicide contains large quantities

of cucurbitacin A (Mashela *et al*., 2015). Cucurbitacin A, in fruit and roots of *C. myriocarpus* is the only cucurbitacin that is water-soluble and breaks down to two components, cucumin and leptodermin, which are tetracyclic triterpenoids (Chen *et al*., 2005). Curcubitacin are found to be poisonous especially in fruits of plants.

Studies had since shown that increasing concentrations of phytonematicides, which are allelochemicals, affect plant growth through the density-dependent growth (DDG) patterns (Mashela *et al*., 2015). Generally, the effects of bioactive compounds on plant growth could be stimulatory, neutral or inhibitory (Liu *et al*., 2003; Mashela *et al*., 2015). The stimulatory and inhibitory effects occur when increasing concentrations had significant effects on plant variables (Mashela *et al.,* 2015), with positive curvilinear quadratic relations, which allow the generation of optimum effects using the $x = -b_1/2b_2$ relation (Mashela *et al*., 2015). However, neutral effects occur when phytonematicides treatments did not have any significant effects on plant growth variables. The absence of significant effects on plant variables in the VAM, nematode and Nemarioc-AL phytonematicide could be viewed in terms of saturation as depicted in DDG patterns (Liu *et al*., 2003), which had since been confirmed in various studies (Dube and Mashela, 2016; Mashela *et al*., 2015, Mathabatha *et al*., 2015; Sithole *et al*., 2016).

3.4.2 Essential nutrient elements

VAM, nematode and Nemafric-BL phytonematicide: The interaction results between VAM and phytonematicides on foliar Zn agreed with observations by Wellings *et al.* (1991), where VAM improved Zn uptake and growth of pigeon pea

(*Cajanus cajan*) in vertisol. Nematode effects in this study on foliar K and Zn confirmed observations by Streeter *et al*. (2001), where the presence of nematodes led to Zn deficiency in *Medicago truncatula*. Also, a study conducted by Mashela *et al.* (2016) showed that the sensitivity of the pseudo-stems to nematode infection was confirmed, with 23–45% reduced potassium in pseudo-stem tissues, but had no effect on other mineral malnutrition elements in African ginger. The results also showed that VAM and Nemafric-BL phytonematicide increased foliar Zn. Thus, the results confirm observations by Kucey and Janzen (1987), where VAM increased plant dry matter production under all sets of growth conditions resulting in increased P, Zn, Cu and Fe in field beans (*Vicia faba*) and of P and Zn in wheat *(Triticum aestivum)*. Thus, VAM and Nemafric-BL phytonematicide could be both combined or used individually in increasing Zn production of *Cleome* species. This could be essential since Zn is important for nerve function and male fertility and plays a big role for normal sexual development especially for the development of testes and ovaries, it is also essential for reproduction, healthy functioning of the heart and normal growth (Ayoola and Adeyeye, 2010).

VAM, nematode and Nemarioc-AL phytonematicide: The second and first order interactions and the main factors did not have significant effect on foliar K, Fe and Zn. Currently, it is not clear whether responses of K, Fe and Zn in plants subjected to various concentrations of phytonematicide could be viewed as was plant growth above. However, since plant was not affected by the treatments, and the accumulation of the three elements was also not affected, there could be an association.

3.4.3 Nematode effects

VAM, nematode and Nemafric-BL phytonematicide: The results of gall rating in the second order interaction between VAM, nematode and Nemafric-BL phytonematicide agrees with the observations of Hafeez *et al*. (2000), where soil amended with *Paecilomyces lilacinus* and *T. harzianum* had reduced root galls per plant. Spiegel and Chet (1998) also reported that *T. harzianum* reduced *M. javanica* root galling index and the number of eggs per g of root.

The results in VAM, nematodes and Nemafric-BL phytonematicide, where the reduction of the population density of *M. javanica* on (Table 3.1) was not significant *in* second order interaction ($V_1N_1P_1$) treatment, similar observations when synthetic nematicides, phytonematicides and nematophagouus fungus had second order interactions on nematode population densities (Chitwood, 2003). Reduction of nematode numbers by ($V_0N_1P_1$) treatment in cleome roots was consistent with observation in other studies, where the materials reduced *Meloidogyne* species in roots of tomato plants (Mashela and Mpati, 2002).

VAM, nematode and Nemarioc-AL phytonematicide: The results of the first order interaction between VAM and Nemarioc-AL phytonematicide agrees with that of Mashela *et al*. (2007) where other interactive experiments of dried *Cucumis myriocarpus* fruit, fever tea (Lippia *javanica*) leaf and castor bean (*Ricinus communis*) fruit in tomato (*Solanum lycopersicon*) production, the second order interaction was highly significant on suppression of *M. incognita*. Galls on the plant roots interfere with nutrients and water absorption leading to discoloration of the

leaves displaying symptoms that resemble those of nutrient deficiencies (Onyango *et al.,* 2013).

3.5 Conclusion

The two products Nemarioc-AL and Nemafric-BL when combined with VAM and nematode, had different effects on plant growth and nematode numbers. VAM, nematode and Nemafric-BL phytonematicide interactions contributed towards improved improving the growth of *Cleome* species. Also, the Nemafric-BL phytonematicide contributed in the reduction of nematodes number (Table 3.7). VAM increased the nutrient uptake and availability of selected nutrient elements in *Cleome* species. In most cases, the efficacy of nematode suppression of the two sustainable products is productive, whereas the efficacy of the combination of the two products with VAM brought inconsistent results. Results of the study showed that the innovative products should be used alone in managing nematodes in cleome production.

CHAPTER 4
SUMMARY, SIGNIFICANCE OF FINDINGS, FUTURE RECOMMENDATIONS AND CONCLUSIONS

4.1 Summary

The interaction of vesicular arbuscular mycorrhiza (VAM), Nemafric-BL phytonematicide and nematodes or VAM, Nemarioc-AL phytonematicide and nematodes contributed towards the suppression of high nematode numbers of *Meloidogyne javanica*, but not on improvement of plant variables (Table 3.7 and 3.9), such as dry shoot weight and dry root mass and chlorophyll content. However, they could only improve plant height. Results of the study showed that the innovative products interacted together and that Nemafric-BL and Nemarioc-AL phytonematicides and VAM alone could be used in managing nematodes. Thus, the factors contributed towards the suppression of high nematode numbers of *M. javanica*. The permutations of VAM, nematode and phytonematicide invariably contributed towards the reduction of most variables of *C. gynandra*. Results of the study, therefore, suggested that the two innovative products should be used separately in managing nematodes in cleome production. After analysis, the responses of K, Fe and Zn in leaves of *C. gynandra* to VAM, nematode and Nemafric-BL phytonematicide could have been differentiated by planting conditions such as climate, soil fertilisation, genetic and environmental factors. However, the results showed that *C. gynandra* is a rich source of minerals and the findings have indicated that this underutilised vegetable could make significant contribution to the daily recommended dietary allowances for the nutrients.

4.2 Significance of findings

The findings of the research indicated that VAM, Nemafric-BL phytonematicide or Nemarioc-AL phytonematicide should each be used separately in the management of nematode densities in cleome production. The reasons why the two products (VAM and phytonematicides) were not compatible, along with reasons for poor inoculation of roots with VAM have generated new areas for future research.

4.3 Future recommendations

Vesicular arbuscular mycorrhiza (VAM), Nemafric-BL phytonematicide, Nemarioc-AL phytonematicide can be used alone to reduce the nematodes levels in cleome production. VAM alone can be used in order to improve the availability of micronutrient elements in *Cleome* species. In addition to potential research areas listed above, there could still be much more work to be done on the health benefits of cleome when using the phytonematicides as innovative products for managing nematodes. For instance, the issue of cucurbitacin A and B chemical residues in leaves of cleome would have to be empirically-addressed.

4.4 Conclusions

Nemarioc-AL and Nemafric-BL phytonematicides should not be used in combination with the other control agents like VAM to manage population densities of *M. javanica* in cleome production The results those of a wide range of studies where combining the two phytonematicides with other products did not confer additional benefits.

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APPENDICES

APPENDIX 3.1 Analysis of variance table for plant height of *Cleome gynandra* on Vesicular arbuscular mycorrhiza, *Meloidogyne javanica* and Nemafric-BL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	0.14	0.01		
VAM (V)	1	0.02	0.02	1.80	0.1841
Nematode (N)	1	0.00	0.00	0.00	0.9700
Phytonematicide (P)	1	0.00	0.00	0.34	0.5611
B × N	1	0.00	0.00	0.01	0.9295
B × P	1	0.00	0.00	0.04	0.8415
N × P	1	0.02	0.02	1.67	0.2012
B × N × P	1	0.07	0.07	4.80	0.0322
Error	63	0.98	0.01		
Total	79				

APPENDIX 3.2 Analysis of variance for stem diameter *of Cleome gynandra* on Vesicular arbuscular mycorrhiza, *Meloidogyne javanica* and Nemafric-BL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	8.74	0.97		
VAM (V)	1	3.81	3.81	2.50	0.11
Nematode (N)	1	11.26	11.26	7.40	0.00
Phytonematicide (P)	1	0.01	0.01	0.01	0.93
B × N	1	0.19	0.19	0.13	0.71
B × P	1	4.43	4.43	2.91	0.09
N × P	1	2.66	2.66	1.75	0.19
B × N × P	1	5.43	5.43	3.57	0.06
Error	63	95.95	1.5		
Total	79				

APPENDIX 3.3 Analysis of variance for chlorophyll of *Cleome gynandra* on Vesicular arbuscular mycorrhiza, *Meloidogyne javanica* and Nemafric-BL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	2150.34	238.92		
VAM (V)	1	140.74	140.74	1.06	0.30
Nematode (N)	1	143.35	143.35	1.08	0.30
Phytonematicide (P)	1	2.66	2.65	0.02	0.88
B × N	1	3.44	3.44	0.03	0.87
B × P	1	292.29	292.28	2.20	0.14
N × P	1	25.17	25.16	0.19	0.66
B × N × P	1	0.28	0.27	0.00	0.96
Error	63	8359.39	132.68		
Total	79				

APPENDIX 3.4 Analysis of variance for dry shoot of *Cleome gynandra* on Vesicular arbuscular mycorrhiza, *Meloidogyne javanica* and Nemafric-BL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	253.06	28.1180		
VAM (V)	1	71.81	71.8096	3.25	0.0764
Nematode (N)	1	9.99	9.9909	0.45	0.5040
Phytonematicide (P)	1	0.85	0.8548	0.04	0.8448
B × N	1	13.34	13.3369	0.60	0.4404
B × P	1	17.13	17.1335	0.77	0.3822
N × P	1	69.19	69.1855	3.13	0.0818
B × N × P	1	74.48	74.4826	3.37	0.0713
Error	63	1393.85	22.1246		
Total	79				

APPENDIX 3.5 Analysis of variance for dry root of *Cleome gynandra* on Vesicular arbuscular mycorrhiza, *Meloidogyne javanica* and Nemafric-BL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	4.17	0.46		
VAM (V)	1	0.09	0.09	0.65	0.4234
Nematode (N)	1	0.13	0.13	0.87	0.3559
Phytonematicide (P)	1	0.23	0.23	1.54	0.2191
B × N	1	0.00	0.00	0.02	0.8940
B × P	1	0.00	0.00	0.05	0.8180
N × P	1	0.21	0.21	1.40	0.2409
B × N × P	1	0.52	0.52	3.44	0.0684
Error	63	9.56	0.15		
Total	79				

APPENDIX 3.6 Analysis of variance for plant height of *Cleome gynandra* on Vesicular arbuscular mycorrhiza, *Meloidogyne javanica* and Nemarioc-AL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	0.34	0.03		
VAM (V)	1	0.02	0.02	0.67	0.41
Nematode (N)	1	0.01	0.01	0.40	0.52
Phytonematicide (P)	1	1.92	1.92	0.00	0.99
B × N	1	0.02	0.02	0.83	0.36
B × P	1	7.59	7.59	0.24	0.62
N × P	1	0.02	0.02	0.90	0.34
B × N × P	1	0.02	0.02	0.70	0.40
Error	63	2.00	0.03		
Total	79	2.46			

APPENDIX 3.7 Analysis of variance for stem diameter *of Cleome gynandra* on Vesicular arbuscular mycorrhiza, *Meloidogyne javanica* and Nemarioc-AL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	47194	5243.78		
VAM (V)	1	5129	5128.96	0.97	0.32
Nematode (N)	1	4901	4900.64	0.93	0.33
Phytonematicide (P)	1	5577	5577.13	1.05	0.30
B × N	1	5329	5329.46	1.01	0.31
B × P	1	5134	5134.41	0.97	0.32
N × P	1	5402	5401.53	1.02	0.31
B × N × P	1	5285	5285.48	1.00	0.32
Error	63	333612	5295.42		
Total	79	417563			

APPENDIX 3.8 Analysis of variance for chlorophyll of *Cleome gynandra*, vesicular arbuscular mycorrhiza *Meloidogyne javanica* and Nemarioc-AL at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	1361.68	151.29		
VAM (V)	1	49.52	49.51	0.63	0.43
Nematode (N)	1	8.88	8.88	0.11	0.73
Phytonematicide (P)	1	13.99	13.99	0.18	0.67
B × N	1	39.85	39.84	0.50	0.48
B × P	1	192.39	192.38	2.43	0.12
N × P	1	2.76	2.76	0.03	0.85
B × N × P	1	6.35	6.35	0.08	0.77
Error	63	4982.11	79.08		
Total	79	6657.54			

APPENDIX 3.9 Analysis of variance for dry shoot of *Cleome gynandra* on vesicular arbuscular mycorrhiza, *Meloidogyne javanica* and Nemarioc-AL at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	1850.2	205.57		
VAM (V)	1	283.1	283.12	1.48	0.22
Nematode (N)	1	181.5	181.50	0.95	0.33
Phytonematicide (P)	1	152.6	152.62	0.80	0.37
B × N	1	101.0	101.02	0.53	0.47
B × P	1	316.4	316.41	1.65	0.20
N × P	1	330.9	330.89	1.73	0.19
B × N × P	1	50.7	50.72	0.26	0.60
Error	63	12071.4	191.61		
Total	79	15337.9			

APPENDIX 3.10 Analysis of variance for dry root of *Cleome gynandra* on vesicular arbuscular mycorrhiza, *Meloidogyne javanica* and Nemarioc-AL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	389.36	43.26		
VAM (V)	1	52.07	52.06	1.23	0.27
Nematode (N)	1	46.15	46.14	1.09	0.30
Phytonematicide (P)	1	49.64	49.64	1.17	0.28
B × N	1	43.22	43.21	1.02	0.31
M × P	1	40.02	40.01	0.94	0.33
N × P	1	29.23	29.23	0.69	0.40
B × N × P	1	42.11	42.10	0.99	0.32
Error	63	2675.08	42.46		
Total	79	3366.87			

APPENDIX 3.11 Analysis of variance for gall rating of *Cleome gynandra* on vesicular arbuscular mycorrhiza, *Meloidogyne javanica* and Nemafric-BL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	0.10401	0.01156		
VAM (V)	1	0.02116	0.02116	1.83	0.1805
Nematode (N)	1	0.59930	0.59930	51.95	0.0000
Phytonematicide (P)	1	0.70805	0.70805	61.38	0.0000
B × N	1	0.04527	0.04527	3.92	0.0520
M × P	1	0.02116	0.02116	1.83	0.1805
N × P	1	0.59930	0.59930	51.95	0.0000
B × N × P	1	0.04527	0.04527	3.92	0.0520
Error	63	0.72680	0.01154		
Total	79	2.87033			

APPENDIX 3.12 Analysis of variance for juveniles in roots of *Cleome gynandra* on Vesicular arbuscular mycorrhiza, *Meloidogyne javanica* and Nemafric-BL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	0.09509	0.01057		
VAM (V)	1	0.01597	0.01597	1.81	0.1832
Nematodes (N)	1	0.00757	0.00757	0.86	0.3578
Phytonematicides (P)	1	0.03751	0.03751	4.25	0.0433
B × N	1	0.00039	0.00039	0.04	0.8346
M× P	1	0.01597	0.01597	1.81	0.1832
N × P	1	0.00757	0.00757	0.86	0.3578
B × N × P	1	0.00039	0.00039	0.04	0.8346
Error	63	0.55558	0.00882		
Total	79	0.73604			

APPENDIX 3.13 Analysis of variance for total nematodes in soil of *Cleome gynandra* on Vesicular arbuscular mycorrhiza, *Meloidogyne javanica* and Nemafric-BL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	1.25264	0.13918		
VAM (V)	1	0.15454	0.15454	1.43	0.2368
Nematodes (n)	1	0.10768	0.10768	0.99	0.3225
Phytonematicides (P)	1	0.47441	0.47441	4.38	0.0404
B × N	1	0.00106	0.00106	0.01	0.9217
M× P	1	0.15454	0.15454	1.43	0.2368
N × P	1	0.10768	0.10768	0.99	0.3225
B × N × P	1	0.00106	0.00106	0.01	0.9217
Error	63	6.82348	0.10831		
Total	79	9.07707			

APPENDIX 3.14 Analysis of variance for gall rating of *Cleome gynandra* on vesicular arbuscular mycorrhiza. *Meloidogyne javanica* and Nemafric-BL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	0.08374	0.00930		
VAM (V)	1	0.03533	0.03533	4.56	0.0366
Nematodes (N)	1	0.01456	0.01456	1.88	0.1753
Phytonematicides (P)	1	0.03533	0.03533	4.56	0.0366
B × N	1	0.01456	0.01456	1.88	0.1753
B × P	1	0.03533	0.03533	4.56	0.0366
N × P	1	0.01456	0.01456	1.88	0.1753
B × N × P	1	0.01456	0.01456	1.88	0.1753
Error	63	0.48806	0.00775		
Total	79	0.73603			

APPENDIX 3.15 Analysis of variance for juveniles in roots of *Cleome gynandra* on vesicular arbuscular mycorrhiza, *Meloidogyne javanica* and Nemarioc-AL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	0.14133	0.01570		
VAM (V)	1	0.01729	0.01729	0.93	0.3373
Nematodes (N)	1	0.00671	0.00671	0.36	0.5492
Phytonematicides (P)	1	0.11893	0.11893	6.43	0.0137
B × N	1	0.01729	0.01729	0.93	0.3373
B × P	1	0.01729	0.01729	0.93	0.3373
N × P	1	0.00671	0.00671	0.36	0.5492
B × N × P	1	0.01729	0.01729	0.93	0.3373
Error	63	1.16502	0.01849		
Total	79	1.50785			

APPENDIX 3.16 Analysis of variance for total nematodes in soil of *Cleome gynandra* on vesicular arbuscular mycorrhiza. *Meloidogyne javanica* and Nemarioc-AL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	1.4881	0.16535		
VAM (V)	1	0.1550	0.15499	0.82	0.3672
Nematodes (N)	1	0.1073	0.10730	0.57	0.4527
Phytonematicides (P)	1	1.2431	1.24311	6.62	0.0125
B × N	1	0.1550	0.15499	0.82	0.3672
B × P	1	0.1550	0.15499	0.82	0.3672
N × P	1	0.1073	0.10730	0.57	0.4527
B × N × P	1	0.1550	0.15499	0.82	0.3672
Error	63	11.8388	0.18792		
Total	79	15.4046			

APPENDIX 3.17 Analysis of variance for potassium in leaves of *Cleome gynandra* on vesicular arbuscular mycorrhiza, *Meloidogyne javanica* and Nemafric-BL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	1669.43	185.492		
VAM (V)	1	113.31	113.31	1.02	0.3167
Nematodes (N)	1	33.79	33.78	0.30	0.5835
Phytonematicides (P)	1	16.19	16.19	0.15	0.7041
B × N	1	6.49	6.49	0.06	0.8099
B × P	1	38.63	38.62	0.35	0.5577
N × P	1	323.17	323.16	2.91	0.0932
B × N × P	1	169.39	169.39	1.52	0.2217
Error	63	7006.55	111.21		
Total	79	9376.94			

APPENDIX 3.18 Analysis of variance for iron in leaves of *Cleome gynandra* on vesicular arbuscular mycorrhiza, Meloidogyne *javanica* and Nemafric-BL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	0.84464	0.09		
VAM (V)	1	0.13122	0.13	1.87	0.1764
Nematodes (N)	1	0.73728	0.73	10.50	0.0019
Phytonematicides (P)	1	0.16562	0.16	2.36	0.1295
B × N	1	0.10513	0.11	1.50	0.2256
B × P	1	0.08065	0.08	1.15	0.2878
N × P	1	0.04140	0.04	0.59	0.4453
B × N × P	1	0.06728	0.06	0.96	0.3313
Error	63	4.42157	0.07		
Total	79	6.59479			

APPENDIX 3.19 Analysis of variance for zinc in leaves of *Cleome gynandra* on vesicular arbuscular mycorrhiza, Meloidogyne *javanica* and Nemafric-BL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	0.26828	0.02		
VAM (V)	1	0.00496	0.004	0.07	0.7970
Nematodes (N)	1	0.27495	0.27	3.70	0.0590
Phytonematicides (P)	1	0.02145	0.02	0.29	0.5931
B × N	1	0.00190	0.00	0.03	0.8735
B × P	1	0.36856	0.36	4.96	0.0296
N × P	1	0.00003	0.00	0.00	0.9837
B × N × P	1	0.10011	0.10	1.35	0.2503
Error	63	4.68389	0.074		
Total	79	5.72414			

APPENDIX 3.20 Analysis of variance for potassium in leaves of *Cleome gynandra* on vesicular arbuscular mycorrhiza, *Meloidogyne javanica* and Nemarioc-AL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	2462.6	273.62		
VAM (V)	1	95.0	94.96	0.49	0.4875
Nematodes (N)	1	309.1	309.06	1.59	0.2124
Phytonematicides (P)	1	23.3	23.29	0.12	0.7306
B × N	1	9.1	9.09	0.05	0.8297
B × P	1	688.2	688.20	3.53	0.0647
N × P	1	216.0	215.96	1.11	0.2963
B × N × P	1	347.9	347.95	1.79	0.1861
Error	63	12267.9	194.73		
Total	79	16419.0			

APPENDIX 3.21 Analysis of variance for iron in leaves of *Cleome gynandra* on vesicular arbuscular mycorrhiza, *Meloidogyne javanica* and Nemafric-BL phytonematicide at 56 days after inoculation (n = 80).

Source	DF	SS	MS	F	P
Rep	9	0.84800	0.09		
VAM (V)	1	0.23871	0.24	2.65	0.1088
Nematodes (N)	1	0.00066	0.00	0.01	0.9320
Phytonematicides (P)	1	0.02145	0.02	0.24	0.6275
B × N	1	0.00105	0.00	0.01	0.9144
B × P	1	0.00190	0.00	0.02	0.8850
N × P	1	0.03828	0.04	0.42	0.5172
B × N × P	1	0.10440	0.11	1.16	0.2861
Error	63	5.68363	0.09		
Total	79	6.93809			

APPENDIX 3.22 Analysis of variance for zinc in leaves of *Cleome gynandra* on vesicular arbuscular mycorrhiza, Meloidogyne *javanica* and Nemarioc-AL phytonematicide at 56 days after inoculation (n = 80

Source	DF	SS	MS	F	P
Rep	9	0.55948	0.06216		
VAM (V)	1	0.04186	0.04186	1.13	0.2913
Nematodes (N)	1	0.10585	0.10585	2.86	0.0955
Phytonematicides (P)	1	0.00003	0.00003	0.00	0.9769
B × N	1	0.00010	0.00010	0.00	0.9584
B × P	1	0.07750	0.07750	2.10	0.1525
N × P	1	0.03160	0.03160	0.86	0.3586
B × N × P	1	0.09591	0.09591	2.60	0.1122
Error	63	2.32825	0.03696		
Total	79	3.24059			