Influence of Cucurbitacin-containing Phytonematicides on Growth, Yield and Foliar Nutrient Elements in Watermelon Production

by

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DECLARATION

I declare that the influence of cucurbitacin-containing phytonematicides on growth, yield and foliar nutrient elements in watermelon production herby submitted to the University of Limpopo, for the degree of Agricultural Management (Plant Production) has not previously been submitted by me for a degree at this or any other University; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

Ramadimetja N. N. Date

DEDICATION

To my kids, Mennute and Jane and my husband Lucas

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ABSTRACT

Nemafric-BL and Nemarioc-AL phytonematicides, which are being researched and developed to serve as alternatives to methyl bromide, have not been tested against plant growth and accumulation of essential nutrient elements in watermelon (*Citrullus lanatus*) cultivars. The objectives of this study were two-fold, to determine the (1) overall growth responses and accumulation of selected essential nutrient elements in watermelon cultivars ‘Congo’ and ‘Charleston Gray’ and suppression of *Meloidogyne javanica* in response to increasing concentrations of Nemafric-BL and Nemarioc-AL phytonematicides, and (2) efficacy of Velum, Nemafric-BL and Nemarioc-AL phytonematicides on growth of watermelon, accumulation of selected essential nutrient elements in leaf tissues of cv. ‘Congo’ and the suppression of population densities of *Meloidogyne* species. Objective 1 and Objective 2 were achieved under greenhouse and field conditions, respectively. At 56 and 90 days after initiating the greenhouse and field treatments, respectively, the data were collected and subjected to statistical analyses. Nemafric-BL phytonematicide significantly affected growth of watermelon cultivars ‘Charleston Gray’ and ‘Congo’, whereas Nemarioc-AL phytonematicide did not have any significant effects on the plant variables of both cultivars. Generally, plant growth variables and increasing concentrations of Nemafric-BL phytonematicide had density-dependent growth (DDG) patterns, which were quantified through either positive or quadratic equations (Chapter 3). In contrast, both phytonematicides had significant effects on selected nutrient elements in leaf tissues of both watermelon cultivars. The affected nutrient

elements, P, Mn and Na versus increasing concentrations of phytonematicides exhibited the DDG patterns, which were also quantified through either positive or quadratic equations (Chapter 3). The phytonematicides were consistent in suppressing nematode numbers in both watermelon cultivars. Comparison of synthetic nematicide Velum and the two phytonematicides under field conditions suggested that, relative to untreated control, the three products each stimulated growth of watermelon cv. 'Congo'. The efficacy of Nemafric-BL and Nemarioc-AL phytonematicides on suppression of population densities of *Meloidogyne* species was comparable to that of Velum. In conclusion, Nemafric-BL and Nemarioc-BL phytonematicides were highly effective in managing population densities of *Meloidogyne* species in watermelon cultivars and also affected the partitioning of selected nutrient elements in tissues. The study provided essential information that could assist in decision-making in nematode management in watermelon production, particularly in fertiliser application.

CHAPTER 1
RESEARCH PROBLEM

1.1 Background

1.1.1 Description of the research problem

The fumigant nematicides were withdrawn from the agrochemical markets due to their environment-unfriendliness (Mashela *et al*., 2015). However, prior to the cut-off date in 2005, systemic chemical nematicides were not being used in managing root-knot (*Meloidogyne* species) in watermelon (*Citrullus lanatus*) production due to their unacceptable high chemical residues in fruits (Galt, 2009; Thies *et al*., 2009). All commercially available *C. lanatus* cultivars are highly susceptible to infection by *Meloidogyne* species, with yield reduction being from as high as 50% to complete crop failure (Thies and Levi, 2007). *Citrullus* species are grouped within the Cucurbitaceae family, where few plant species have resistance to *Meloidogyne* species (Pofu, 2012). Unfortunately, no nematode-resistant genotypes exist in *Citrullus* species (Thies and Levi, 2007), and therefore, successful production of watermelon cultivars dictates that nematode population densities be suppressed through alternative management strategies.

Watermelon production in South Africa is concentrated in Northern Cape (84%), Western Cape (8%), Eastern Cape (7%) and Limpopo Province (0.34%) (Pofu, 2012), constituting 1.4% of the world watermelon produce, with China being the largest international watermelon producer. Worldwide, there are more than 60 *Meloidogyne* species, with hosts spanning over 3000 plant species (Pofu, 2012). Among 60 *Meloidogyne* species, *M. incognita* is internationally the most aggressive root-knot nematode, followed by *M. javanica*. In South Africa, *M. incognita* is the

most aggressive, followed by *M. javanica* (Kleynhans *et al*., 1996), with the two nematode species occurring as mixed populations. In any cultigen, where nematode resistance genotypes are not known like in *Citrullus* species, population densities of *Meloidogyne* species have to be managed if production has to be profitable.

At the Green Technologies Research Centre, University of Limpopo, a team of researchers observed that fruits of wild cucumber (*Cucumis myriocarpus)* and wild watermelon *(Cucumis africanus)* had potent nematicidal properties (Mashela *et al*., 2015). The two *Cucumis* species are indigenous to Botlokwa in Limpopo Province, South Africa (Kristkova *et al*., 2003). The potential uses of ground fruits from the two *Cucumis* species to serve as alternative products in managing plant-parasitic nematodes had been tested under a wide range of conditions in both granular and liquid formulations (Mashela *et al*., 2015). Nemarioc-AL (L = liquid formulation) or Nemarioc-AG (G = granular formulation) phytonematicide is produced from ground fruit of *C. myriocarpus*, whereas Nemafric-BL or Nemafric-BG phytonematicide is produced from ground fruit of *C. africanus* (Mashela *et al*., 2015). However, the two products could be highly phytotoxic to crops being protected against nematodes.

1.1.2 Impact of the research problem

Most crops are highly sensitive to the phytotoxic effects in Nemafric-BL and Nemarioc-AL phytonematicides (Mafeo *et al*., 2011a, Mashela and Dube, 2014; Pelinganga and Mashela, 2012; Tseke, 2013). Internationally, most registration agencies have zero tolerance to agricultural products with phytotoxicity. Phytotoxicity from phytonematicides could reduce plant growth from as high as 35% to complete

crop failure as shown in most products that could not be used after successful *in vitro* efficacy trials (Mashela *et al*., 2015).

1.1.3 Possible causes of the research problem

In Nemafric-BL and Nemarioc-AL phytonematicides the active ingredients had been identified as cucurbitacin B and cucurbitacin A in *C. africanus* and *C. myriocarpus* fruits (Chen *et al*., 2005) and, therefore, the trade names Nemafric-BL and Nemarioc-AL phytonematicides, respectively. The active ingredients in phytonematicides are technically the allelochemicals, which are toxic to plants from unrelated plant species (Rice, 1984).

1.1.4 Proposed solutions

The concept of Mean Concentration Stimulation Point (MCSP) was developed in an attempt to manage phytotoxicity in crops being protected against nematodes using phytonematicides (Mashela *et al*., 2015). The MCSP is crop-specific and could eventually be used to establish the application interval and the dosage model for a specific nematode and crop (Mashela *et al*., 2015). Therefore, the starting point in the use of any phytonematicide in the management of plant-parasitic nematodes is to establish the sensitivity of the crop to be protected to the target phytonematicide.

1.1.5 General focus of the current study

The primary focus of the current study would be to assess the overall sensitivity of widely used commercial *C. lanatus* cultivars in Limpopo Province to Nemarioc-AL and Nemafric-BL phytonematicides. The secondary focus would be to assess the responses of the accumulation of selected nutrient elements in leaves of *C. lanatus*

cultivars in response to the application of the two phytonematicides, along with the assessment of the potential chemical residues in watermelon cultivars under when nematodes are managed in the crop under field conditions.

1.2 Problem statement

Cucumis species, which are being developed as phytonematicides for watermelon production, produce large quantities of cucurbitacins, which are the bitterest chemical compounds known to humankind. The research intends to investigate whether cucurbitacin-containing phytonematicides applied on watermelon cultivars would not affect plant growth and accumulation of selected essential nutrient elements.

1.3 Rationale for the study

The non-phytotoxic concentrations of the two products on watermelon cultivars would allow for the eventual determination of the application interval and thereby the dosage model, which would be essential in environmental impact assessment (Mashela *et al.*, 2015). Phytotoxicity of the cucurbitacin-containing phytonematicides would not be ideal for the products to be registered for watermelon production.

1.4 Purpose of the study

1.4.1 Aim

The aim of the study was to develop an alternative management strategy for population densities of *Meloidogyne* species in watermelon production using phytonematicides.

1.4.2 Objectives

1. To determine the overall sensitivity and accumulation of selected essential nutrient elements in watermelon cultivars ‘Congo’ and ‘Charleston Gray’ in response to increasing concentrations of Nemafric-BL and Nemarioc-AL phytonematicides under greenhouse conditions.
2. To compare the efficacy of Velum, Nemafric-BL phytonematicide and Nemarioc-AL phytonematicide on growth of watermelon, accumulation of selected essential nutrient elements in leaf tissues and the suppression of population densities of *Meloidogyne* species under field conditions.

1.5 Reliability, validity and objectivity

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

1.6 Bias

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

1.8 Structure of the dissertation

Following the description and detailed outlining of the research problem (Chapter 1), the work done and not yet done on the research problem was reviewed (Chapter 2). Then, each of the two subsequent chapters (Chapters 3 and 4) addressed each of the objectives in sequential order. In the final chapter (Chapter 5), the findings of the study would be summarised and integrated to provide the significance of the findings and recommendations with respect to future research, culminating in a conclusion that would tie up the entire study. The citations and references used the Harvard-author style as prescribed by the Senate of the University of Limpopo.

CHAPTER 2
LITERATURE REVIEW

2.1 Work done on the research problem

2.1.1 Cucurbitaceae technologies

A number of technologies had been developed from the Cucurbitaceae family in respect to serving as alternatives used in nematode management and had since been referred to as the Cucurbitaceae technologies (Mashela *et al*., 2015). The wild cucumber (*Cucumis myriocarpus)* and wild watermelon *(C. africanus)* are indigenous to Limpopo Province, South Africa (Kristkova *et al*., 2003).

Ground leaching technology: In this technology, the fruits from the *Cucumis* species, namely, *C. africanus* and *C. myriocarpus* are ground and applied around the stem of the seedling at transplanting (Mashela, 2002). The active ingredients, the cucurbitacins, are leached from the powdered material into the rhizosphere. Fruits from *C. myriocarpus* contain cucurbitacin A, which is slightly polar and therefore, soluble in water (Van Wyk *et al*., 2002). However, this chemical compound is unstable, and soon breaks down to cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) (Van Wyk *et al*., 2002). In contrast, fruit from *C. africanus* contain cucurbitacin B ($C_{32}H_{48}O_8$), which is nonpolar and stable (Chen *et al.,* 2005). In *C. africanus* the cucurbitacins are equally distributed in the whole plant (Chen *et al*., 2005), whereas in *C. myriocarpus* the materials are primarily localised in roots and fruits (Van Wyk *et al.,* 1997, 2002). In granular formulation, phytonematicides from fruits of *C. africanus* and *C. myriocarpus* are referred to as Nemafric-BG and Nemarioc-AG phytonematicides, respectively (Mashela *et al*., 2015), with first and last letter in the prefix representing the active ingredient and formulation, respectively.

Botinemagation technology: In this technology, the active ingredients in the ground materials are extracted through fermentation using effective microbes (Ncube, 2008) prior to application using irrigation water (Mashela *et al*., 2015). The products are therefore referred to as Nemafric-AL and Nemarioc-AL phytonematicides, where L represents liquid formulation.

Intergeneric grafting technology: Generally, grafting is done in plants within the same genus, but different plant species (species-species) and this is being referred to as interspecfic grafting. This form of grafting is characterised by high compatibility between the rootstocks and scions (Pofu, 2012). In contrast, grafting plants within the same family (genus-genus) is referred to as intergeneric grafting, which is characterised by excessively high levels of incompatibilities (Pofu, 2012). Grafting *C. africanus* on *C. myriocarpus* would constitute interspecific grafting, whereas grafting *Citrullus* species on any of the two *Cucumis* species would constitute intergeneric grafting. Pofu *et al*. (2012) demonstrated that when *C. lanatus* cultivars 'Charleston Gray' and 'Congo' were intergenerically grafted onto *Cucumis* seedling rootstocks, the latter retained their resistance to *Meloidogyne* species, with the scions flowering earlier with higher fruit yield. However, nematode resistance was lost when large quantities of honeydew were produced under attack by the greenhouse whiteflies (Pofu and Mashela, 2013).

2.1.2 Efficacy of phytonematicides from fruits of *Cucumis* species

In granular formulations the products reduced population densities of *Meloidogyne* species from 80 to 100% (Mafeo, 2012; Mashela, 2002; Mashela *et al*., 2011). Similar high efficacies were observed in liquid formulations (Mashela *et al*., 2015; Pelinganga and Mashela, 2012; Pelinganga *et al*., 2012; Seshweni, 2016; Sithole *et al*., 2016). The efficacy of the two products on nematode suppression and crop yield were not different to those of synthetic nematicides such as aldicarb and fenamiphos (Mashela *et al*., 2008) and Velum (Seshweni, 2016).

2.1.3 Challenges in cucurbitacin-containing phytonematicides

The active ingredients in phytonematicides from fruits of *Cucumis* species are cucurbitacins, which are highly oxidised tetracyclic triterpenoids in plants within the Cucurbitaceae Family (Chen *et al*., 2005; Eslin, 2006). Allelochemicals as active ingredients in phytonematicides are naturally phytotoxic to other plant species during interference (Khosa, 2013; Ntalli and Caboni, 2012; Okwute, 2012; Wuyts *et al*., 2006). In banana (*Musa acuminata*) trial, application of 200-400 g powdered neem seed kernels per mat at 6-month application interval induced phytotoxicity – characterised by complete wilting prior to fruiting (Musabuyinna *et al*., 2000). Additionally, in survivor plants the inflorescence failed to emerge (Musabuyinna *et al*., 2000), resulting in a condition called chocking, where the inflorescence could not emerge through the whorl of the pseudostem. Wild garlic (*Tulbaghia violacea*) bulbs contain sacrid volatile oils and sulpho-oxides – both being derivatives of allicin (Vijayalakshmi *et al*., 1996). Crude extracts of garlic bulb at 50% concentration reduced population densities of plant-parasitic nematodes, but was highly phytotoxic to tomato seedlings (Egunjobi and Afolami, 1976; Sukul *et al*., 1974). However, at

20% concentration there were no noticeable effects on tomato plant growth, while the product suppressed population densities of *M. incognita* (Agbenin *et al*., 2005). Oil from clove (*Eugenia caryophyllata*), when drenched using 0.1%, 0.2% and 0.3% concentrations at 0, 2, 5 and 7 days prior to transplanting cucumber (*Cucumis sativus*), muskmelon (*C. melo*), pepper and tomato seedlings, all concentrations were highly phytotoxic to all crops, while reducing nematode populations (Meyer *et al*., 2008).

Sensitivities of seedlings to clove oil from *E. caryophyllata* varied with plant species, with tomato seedlings being the most sensitive among all the test plants (Meyer *et al*., 2008). Generally, at transplanting, seedlings from various crops were all affected by oil at 0.2% and 0.3% concentrations. The product contains eugenol ($C_{10}H_{12}O_2$) – a member of the phenylpropanoids class of chemical compounds, as an active ingredient, which is naturally herbicidal at low concentrations (Bainard *et al*., 2006; Boyd and Brennan, 2006; Boyd *et al*., 2006; Waliwitiya *et al*., 2005; Walter *et al.*, 2001; Tworkoski, 2002). Incidentally, oil cakes from different plant species have high levels of phytotoxicity to various crops at various concentrations (Haseeb *et al*., 1980; Mian and Rodriguez-Kabana, 1982a, b; Muller and Gooch, 1982; Parmar, 1996). Ahmad *et al*. (2013) demonstrated that ground leaves of adulsa (*Justicia adhatoda*) at 3% (w/w) concentration were highly phytotoxic to tomato seedlings. Similar phytotoxic effects were observed from high concentrations of *L. camara* root extracts on various plant species (Shaukat *et al*., 2003).

Nemarioc-AG phytonematicide was shown to be highly phytotoxic to eight monocotyledonous and ten dicotyledonous crops, with most crops failing to emerge

when 5 g crude extracts were applied as pre-emergent drenches (Mafeo and Mashela, 2009a, b, 2010). Similarly, both Nemafric-BL and Nemarioc-AL phytonematicides were highly phytotoxic to tomato seedlings when applied at above 10% concentration after transplanting (Pelinganga and Mashela, 2012; Pelinganga *et al*., 2013a, b). Except in rare cases such as pyrethrins that account for 80% global uses of botanical pesticides, in purified form most active ingredients of phytonematicides, including azadirachtin-containing products, are not effective on nematode suppression, while they are highly phytotoxic to crops (Wuyts *et al*., 2006; Okwute, 2012). Subsequently, most active ingredients in phytonematicides are applied as crude extracts.

2.1.4 Managing phytotoxicity in cucurbitacin-containing phytonematicides

Due to phytotoxicity and its zero tolerance in most legislative frameworks on products used in agriculture, literature is replete with efficacy trials which do not go beyond *in vitro* status. Using the concept of density-dependent growth (DDG) patterns, there are basically three concentration ranges, namely, stimulation, neutral and inhibition concentration ranges (Liu *et al*., 2003). Using the latter, Mashela *et al*. (2015) developed the concept of Mean Concentration Stimulation Point (MCSP), which is the middle point of the stimulation phase within the DDG patterns (Liu *et al*., 2003; Mashela *et al*., 2015).

The MCSP is the concentration of the phytonematicides that is non-phytotoxic concentration to the plant that is being protected against nematode damage, while it consistently suppresses nematode numbers (Mashela *et al*., 2015). Actually, the MCSP is the concentration that should be applied at each application time and it is

used to estimate the application interval and then the dosage (Mashela *et al*., 2015). The latter is important in assessing the environmental impact of the phytonematicides.

2.1.5 Quality protocols in manufacturing phytonematicides

Using the concentration of cucurbitacin A and B, Shadung (2016) developed the quality protocols of Nemafric-BL and Nemarioc-AL phytonematicides from the production of fruits in the field, through drying of fruit pieces to storage of inventories and final products. As part of the protocols, Shadung *et al*. (2015) demonstrated that the optimum quantities of cucurbitacins in fruits occur in fully mature fruits, with the suitable drying temperature being at 52ºC, whereas lower temperatures result in growth of fungal diseases during drying and higher temperatures result in reduced cucurbitacin levels. During storage of inventories, regardless of whether the containers were airtight sealed or not, the concentration of the cucurbitacins and storage period exhibited quadratic relations (Shadung and Mashela, 2016), with the cucurbitacins still within the stimulation range of the DDG patterns at six months after storage.

2.1.6 Propagation of *Cucumis* species

Mafeo (2005) demonstrated that seeds of *C. myriocarpus* contained some dormancy, which was later confirmed by Maila (2015) in seeds of both *C. myriocarpus* and *C. africanus*. Generally, seeds of *C. africanus* are the most difficult to germinate (Pofu, 2012). Maila *et al*. (2016) demonstrated that dormancy in seeds of both *Cucumis* species was induced by chemicals and seed testa, a phenomenon referred to as chemical and physical dormancies, respectively. In seeds of *C.*

myriocarpus, leaching promoted germination, whereas in *C. africanus* both leaching and scarification improved germination.

2.2 Work not yet done on the research problem

The management of *Meloidogyne* species using phytonematicides as alternatives to methyl bromide in watermelon production had not been documented. Also, the influence of Nemafric-BL and Nemarioc-AL phytonematicides on growth responses and accumulation of nutrient elements in watermelon cultivars had not been documented. Additionally, the efficacy of the two phytonematicides and available synthetic chemical nematicides had not been compared on watermelon production.

2.3 Addressing the identified gaps

Nemafric-BL and Nemarioc-AL phytonematicides consistently suppressed nematode population densities of *Meloidogyne* species in various crops. The two phytonematicides stimulated growth of plants at low concentrations, but inhibited growth at high concentrations, within the context of DDG patterns, resulting in the concept of MCSP, where plant growth variables were used to generate the biological indices (Liu *et al.*, 2003). The identified gaps in the current review were lack of information on the influence of the two phytonematicides at various concentrations on essential nutrient elements in leaf tissues of crops.

CHAPTER 3
INFLUENCE OF CUCURBITACIN-CONTAINING PHYTONEMATICIDES ON GROWTH OF WATERMELON CULTIVARS

3.1 Introduction

The density-dependent growth (DDG) patterns, stimulation, neutral and inhibition phases (Liu *et al*., 2003) are the main characteristics of increasing allelochemicals concentration in plant growth. Mean Concentration Stimulation Point (MCSP) is the phytonematicide concentration that stimulates growth of the protected plant species while suppressing nematode numbers (Mashela *et al*., 2015). The MCSP of each phytonematicide is plant-specific and therefore should be empirically-developed for each plant species. Sithole (2016) demonstrated the MCSP in African geranium for Nemarioc-AL/Nemafric-BL was 2.63%, whereas Mathabatha *et al*. (2016) observed that for Nemarioc-AL/Nemafric-BL phytonematicide on *Citrus volkameriana* the value was 6.83%. The objective of this study was to determine the responses of growth and accumulation of essential nutrient elements in watermelon cultivars to Nemafric-BL and Nemarioc-AL phytonematicides under greenhouse conditions.

3.2 Materials and methods

3.2.1 Description of the study site

Watermelon cultivars ‘Congo’ and ‘Charleston Gray’ greenhouse experiments were conducted in summer (November-December) 2015 at the Green Biotechnologies Research Centre (GTRC), University of Limpopo, Limpopo Province, South Africa (S23°53'10" E29°44'15"). The greenhouse was 50 m long and 24 m wide, with a wet wall on one end and heat extraction fans on the opposite wall. Thus, conditions were not homogeneous inside the facility.

3.2.2 Preparation of seedlings

Seeds of watermelon cultivars ‘Charleston Gray’ and ‘Congo’ were separately sown in 200-hole seedling trays containing Hygromix-T growing mixture (Hygrotech Pty. Limited, Pretoria, South Africa) and allowed to germinate, emerge and grow for four weeks. Seedlings in trays were exposed to the environmental factors outside the greenhouse on a steel hardening-off table by withholding irrigation, thereby enhancing suberisation and greening of etiolated seedling stems. The exposure was monitored closely during the first day, with trays moved to the shade when 50% seedlings were tipping over and then irrigated. After short recovery of the seedlings on the same day, the seedling trays were returned to the hardening-off bench. Seedlings were transplanted at 7 days after hardening-off.

3.2.3 Preparation of materials

Fruits for preparing Nemarioc-AL and Nemafric-BL phytonematicides were harvested from *Cucumis myriocarpus* and *C. africanus*, respectively, on cultivated plots, washed, cut into pieces and dried at 52ºC for 72 h (Mashela *et al*., 2015). Also, the two phytonematicides were prepared as described previously (Mashela *et al.*, 2015).

Loam soil (25% clay, 7% silt and 68% sand) was steam-pasteurised for 3 h at 300ºC and cooled overnight. The 18-cm-diameter plastic pots were filled with pasteurised loam and Hygromix-T at 4:1 (v/v) ratio. The growing medium was irrigated to full capacity and allowed for drainage overnight. Hardened-off five-week-old seedlings were transplanted and returned to the greenhouse, with two ‘Charleston Gray’ trials for each phytonematicide and two ‘Congo’ trials for each phytonematicide running concurrently. Three days after transplanting, each seedling in each trial was

inoculated with 5000 eggs and second-stage juveniles (J2) of *M. javanica* by placing in a 5-cm deep furrow around the stem using a 20-ml plastic syringe. The inoculum was covered by the growing medium.

3.2.4 Treatments and research design

The treatment levels, comprising 0.0, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 and 25.6% Nemafric-BL or Nemarioc-AL phytonematicide, were arranged in a randomised complete block design, with five replications (n = 45). Each treatment was applied weekly. The first treatments were applied seven days after planting, the total of eight application frequencies during the growing season. On application days, irrigation was replaced by treatments.

3.2.5 Cultural practices

Starting from transplanting, seedlings were irrigated with 300 ml chlorine-free tapwater every other day. Fertilisation comprised 2 g 2:3:2 (22) N:P:K and 2 g superphosphate (10.6%), each applied at 5-cm away from the trunk at transplanting. The first top dressing was done at four weeks after transplanting using 2 g Limestone Ammonium Nitrates (LAN) and 2 g N:P:K 2:3:4 (30), each applied separately in a furrow around the stem and covered with soil. A weekly spraying programme was designed for late blight, early blight, anthracnose, downy mildew and powdery mildew, which comprised alternating mancozeb (Dithane M45), copper oxychloride and Bravo as per label instruction.

3.2.6 Data collection

At harvest, 56 days after initiating the treatments, plant length was measured from the crown to the tip of the longest runner, shoots cut at the soil level and stem diameters measured 5 cm above the severed ends using a Digital Vernier Caliber. Shoots were oven-dried at 70°C for 72 h and weighed. Ten mature and healthy leaves were collected per plant, rinsed in distilled water, with excess water removed by pressing between tissue papers and leaves dried at 70ºC for 72 h. Leaves were ground in a Wiley Mill, prepared for quantification of nutrient elements using ICP (Shadung 2016). Root systems were removed from pots, immersed in water to remove soil particles, blotted dry and weighed to facilitate the calculation of nematode density per total roots per plant. Root galls, when necessary, were assessed using the North Carolina Differential Scale of 1 = no galls, 2 = 1 - 10 galls, 3 = 11 - 100 galls and 4 = > 100 galls (Taylor and Sasser, 1978). All collected roots were separately weighed. Nematodes were extracted from 5 g roots per plant by maceration and blending for 30 seconds in 1% NaOCl (Hussey and Barker, 1973) and passed through top-down nested 150-µm, 45-µm and 25-µm mesh sieves. Contents of the 25-µm mesh sieve were poured into 100-ml plastic containers for counting under a stereomicroscope.

Soil per pot was thoroughly mixed and a representative sample of 250-ml soil was collected. Nematodes were extracted from soil samples using the modified sugar-floatation and centrifugation method (Coolen and D'Herde, 1972). Briefly, the soil sample was washed through a 45-µm-aperture sieve into a bucket, which was then filled with water and mixed in a swill. After the swill had stopped, the aliquot was poured through a 25-µm sieve, with the contents being washed into 100-ml plastic

centrifuge tubes. A teaspoon of kaolin was then added in each tube and contents centrifuged at 1 750 RPM for five minutes. The kaolin solution was then decanted with nematodes having settled at the bottom of the tubes with soil particles. A 469 g sugar/L tapwater was poured into the centrifuge tubes and stirred once prior to centrifuging for one minute at 1 750 RPM. The aliquot was then decanted onto 25-µm sieve; sugar was rinsed off the nematodes, which were then collected from the 25-µm sieve into 100-ml plastic containers for counting under a stereomicroscope. During counting, which was completed in less than 10 days, samples were stored at 5^{o}C. Nematode numbers from roots were converted to nematodes per total root system per plant, whereas soil nematode numbers were converted to 2 700 L soil per pot. Reproductive factors, described as final population/initial population numbers, were computed.

3.2.7 Data analysis

Prior to analysis of variance (ANOVA), nematode data were transformed through $\log_{10}$ (x + 1) to homogenise the variances (Gomez and Gomez, 1984). Data were subjected to ANOVA through the SAS software (SAS Institute, 2008) to determine the effects of increasing concentrations of phytonematicides to plant variables and essential nutrient elements (Appendix 3.1-3.41). Mean separation for significant ($P \leq 0.05$) treatments was achieved through using Waller-Duncan Multiple Range test. Due to the importance of the neutral phase in the DDG pattern trials (Mashela *et al*., 2015); in the event treatment means were not significant ($P \leq 0.05$), lines of the best fit were determined for the variables. Unless otherwise stated, only treatments that were significant at the probability level of 5% were discussed.

3.3 Results

3.3.1 Plant variables

Nemafric-BL phytonematicide treatment effects were significant on dry shoot mass, stem diameter and vine length, but were not significant on root gall and fresh root mass (Table 3.1). In contrast, Nemarioc-AL phytonematicide did not have any significant effects on the same plant variables. At low concentration Nemafric-BL phytonematicide slightly increased dry shoot mass, stem diameter and vine length whereas at high concentrations the product reduced the variables, but treatments had no effects on fresh root mass and gall rating (Table 3.2). Both Nemafric-BL and Nemarioc-AL phytonematicide did not have significant effects on plant growth variables of cv. 'Charleston Gray' (Table 3.3). Treatments had no significant effects on all plant variables of cv. 'Congo' (Table 3.4-3.6).

The vine length versus increasing concentrations of Nemafric-BL phytonematicide exhibited positive quadratic relation, with the association explaining 86% of the observed model (Figure 3.1). Using the $x = – b_1/2b_2$ relation, vine length was optimised at 2.76% Nemafric-BL phytonematicide. Similarly, both dry shoot mass and stem diameter versus increasing concentrations of Nemafric-BL phytonematicide each exhibited positive quadratic relations, with the associations explaining 91% (Figure 3.2) and 97% (Figure 3.3) of the observed models. Dry shoot mass and stem diameter were optimised at 2.41% and 2.48% Nemafric-BL phytonematicide, respectively.

Table 3.1 Partitioning of sources of variation in selected growth variables of watermelon cv. 'Charleston Gray' in response to Nemafric-BL and Nemarioc-AL phytonematicides (n = 45).

		Nemafric-BL phytonematicide									
		Dry shoot mass (g/plant)		Fresh root mass (g/plant)		Stem diameter (mm/plant)		Vine length (cm/plant)		Root gall (total root system/plant)	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Rep	4	1.719	72	1.748	61	0.35880	46	845.740	67	0.001790	27
Treat	8	0.498	21***	0.710	24^{ns}	0.28771	37**	302.644	24**	0.002459	36^{ns}
Error	32	0.176	7	0.426	15	0.13697	17	122.672	9	0.002512	37
Total	44	2.385	100	2.884	100	0.78348	100	1271.056	100	0.006761	100
		Nemarioc-AL phytonematicide									
Rep	4	0.3564	30	4.6339	52	0.02979	7	1113.99	53	0.06359	87
Treat	8	0.4072	34^{ns}	2.0325	23^{ns}	0.11975	29^{ns}	509.45	24^{ns}	0.00385	5^{ns}
Error	32	0.4263	36	2.2433	25	0.25876	64	496.96	23	0.00536	8
Total	44	1.1900	100	8.9097	100	0.4083	100	2120.4	100	0.0728	100

DF = Degrees of freedom; ***Highly significant at P ≤ 0.01; **Significant at P ≤ 0.05; ^{ns}Not significant at P ≤ 0.05.

Table 3.2 Influence of Nemafric-BL phytonematicide to selected growth variables of watermelon cv. 'Charleston Gray' under greenhouse conditions (n = 45).

Treatment (%)	Dry shoot mass (g/plant)		Fresh root mass (g/plant)		Stem diameter (mm/plant)		Vine length (cm/plant)		Root gall (total root system/plant)	
	Mean[y]	Impact (%)[z]	Mean	Impact (%)	Mean	Impact (%)	Mean	Impact (%)	Mean	Impact (%)
0.0	2.7[ab]	–	4.2	–	3.4	–	90.3[abc]	–	0.7	–
0.2	2.6[ab]	–4	4.1	–2	3.6	6	90.5[abc]	0	0.7	0
0.4	2.8[a]	4	4.3	2	3.3	–3	92.8[ab]	3	0.7	0
0.8	2.6[ab]	–4	4.5	8	3.5	2	97.1[a]	7	0.7	0
1.6	2.7[ab]	0	4.1	–2	3.6	6	89.7[abc]	–0.6	0.7	0
3.2	2.8[a]	4	4.1	–2	3.7	8	92.9[ab]	3	0.7	0
6.4	2.0 [c]	–26	3.7	–12	3.0	–12	74.7[d]	–17	0.7	0
12.8	2.2[bc]	–19	3.5	–16	3.3	–3	77.5[cd]	–14	0.7	0
25.6	2.2[bc]	–19	3.4	–18	1.1	–65	80.4[bcd]	–11	0.7	0

[y]Column means followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan multiple range test.

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

Table 3.3 Partitioning of sources of variation in selected nutrient elements in leaf tissues of watermelon cv. 'Charleston Gray' in response to Nemafric-BL and Nemarioc-AL phytonematicides (n = 45).

Treatment (%)	Dry shoot mass (g/plant)		Fresh root mass (g/plant)		Stem diameter (mm/plant)		Vine length (cm/plant)		Root gall (total root system/plant)	
	Variable[y]	Impact (%)[z]	Variable	Impact (%)	Variable	Impact (%)	Variable	Impact (%)	Variable	Impact (%)
0.0	1.2	–	2.9	–	2.7	–	59.5	–	0.6	–
0.2	1.1	–8	2.3	–21	2.7	0	72.1	21	0.6	0
0.4	1.0	–17	1.7	–41	3.0	11	82.1	38	0.6	0
0.8	1.1	–8	2.0	–31	2.8	4	82.4	38	0.6	0
1.6	1.7	42	3.3	14	3.0	11	72.7	22	0.7	2
3.2	1.6	33	3.6	23	3.0	11	62.6	5	0.7	2
6.4	1.4	17	2.6	–10	2.9	7	73.4	23	0.6	0
12.8	1.4	17	2.4	–17	3.0	11	58.8	–1	0.6	0
25.6	0.9	–25	1.9	–34	3.1	15	55.3	–7	0.6	0
$LSD_{0.05}$	1.0		2.1		0.7		29.3		0.2	

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

Table 3.4 Partitioning of sources of variation in selected growth variables of watermelon cv. 'Congo' in response to Nemafric-BL and Nemarioc-AL phytonematicides (n = 45).

Source	DF	Dry shoot mass (g/plant)		Fresh root mass (g/plant)		Stem diameter (mm/plant)		Vine length (cm/plant)		Root gall (total root system/plant)	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Nemafric-BL phytonematicide											
Rep	4	2.39300	66	2.393	64	0.45028	50	844.385	51	0.003804	28
Treat	8	0.58256	16^{ns}	0.444	12^{ns}	0.24099	27^{ns}	516.403	31^{ns}	0.004893	36^{ns}
Error	32	0.67138	18	0.931	24	0.20056	23	309.428	18	0.004908	36
Total	44	3.64694	100	3.767	100	0.89183	100	1670.216	100	0.013605	100
Nemarioc-AL phytonematicide											
Rep	4	0.14022	15	1.748	61	0.14159	17	1014.12	54	0.001956	26
Treat	8	0.47689	48^{ns}	0.710	25^{ns}	0.25027	31^{ns}	367.74	20^{ns}	0.002048	27^{ns}
Error	32	0.36710	37	0.426	14	0.42669	52	494.91	26	0.003615	47
Total	44	0.98421	100	2.885	100	0.81855	100	1876.77	100	0.007619	100

DF = degree of freedom, MSS = Mean sum of squares, TTV = Total treatment variation, ^{ns}Not significant at P ≤ 0.05.

Table 3.5 Influence of Nemafric-BL phytonematicides to selected growth variables of watermelon cv. 'Congo' under greenhouse conditions (n = 45).

Treatment (%)	Dry shoot mass (g/plant)		Fresh root mass (g/plant)		Stem diameter (mm/plant)		Vine length (cm/plant)		Root gall (total root system/plant)	
	Variable[y]	Impact (%)[z]	Variable	Impact (%)	Variable	Impact (%)	Variable	Impact (%)	Variable	Impact (%)
0.0	2.6	–	4.1	–	3.3	–	70.0	–	0.6	–
0.2	2.6	0	4.2	2	3.4	3	83.3	19	0.7	1
0.4	2.9	12	3.7	–10	3.6	9	84.8	21	0.6	0
0.8	2.3	–12	3.9	–4	3.7	12	92.0	31	0.6	0
1.6	2.7	4	4.0	–2	3.9	18	80.8	15	0.7	5
3.2	3.1	19	4.1	0	3.8	15	81.0	16	0.6	0
6.4	2.5	–4	3.7	–9	3.6	9	70.3	0	0.6	0
12.8	2.5	–4	4.3	5	3.9	18	84.0	20	0.7	1
25.6	1.9	–27	4.4	6	3.4	3	58.5	–16	0.7	1
$LSD_{0.05}$	1.5	–	1.0	–	0.9	–	24.6	–	0.3	–

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

Table 3.6 Influence of Nemarioc-AL phytonematicide to selected growth variables of watermelon cv. ‘Congo’ under greenhouse conditions (n = 45).

Treatment (%)	Dry shoot mass (g/plant)		Fresh root mass (g/plant)		Stem diameter (mm/plant)		Vine length (cm/plant)		Root gall (total root system)	
	Variable[y]	Impact (%)[z]	Variable	Impact (%)	Variable	Impact (%)	Variable	Impact (%)	Variable	Impact (%)
0.0	0.9	–	4.2	–	2.8	–	45.7	–	1.6	–
0.2	1.4	55	4.1	–2	3.4	23	65.0	42	0.6	0
0.4	1.0	11	4.3	2	3.2	15	67.0	46	0.6	0
0.8	1.1	22	4.5	7	2.8	1	58.5	28	0.7	0
1.6	1.4	55	4.1	–2	2.9	3	63.5	39	0.6	0
3.2	0.8	–10	4.1	–2	3.2	15	48.0	5	0.9	0
6.4	1.8	91	3.7	–11	3.0	7	68.9	51	0.6	0
12.8	1.5	66	3.5	–16	2.9	3	53.4	17	1.6	0
25.6	1.2	34	3.4	–18	3.0	7	51.9	13	0.6	0
$LD_{0.05}$	1.1		1.9		0.9		24.2		1.4	

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

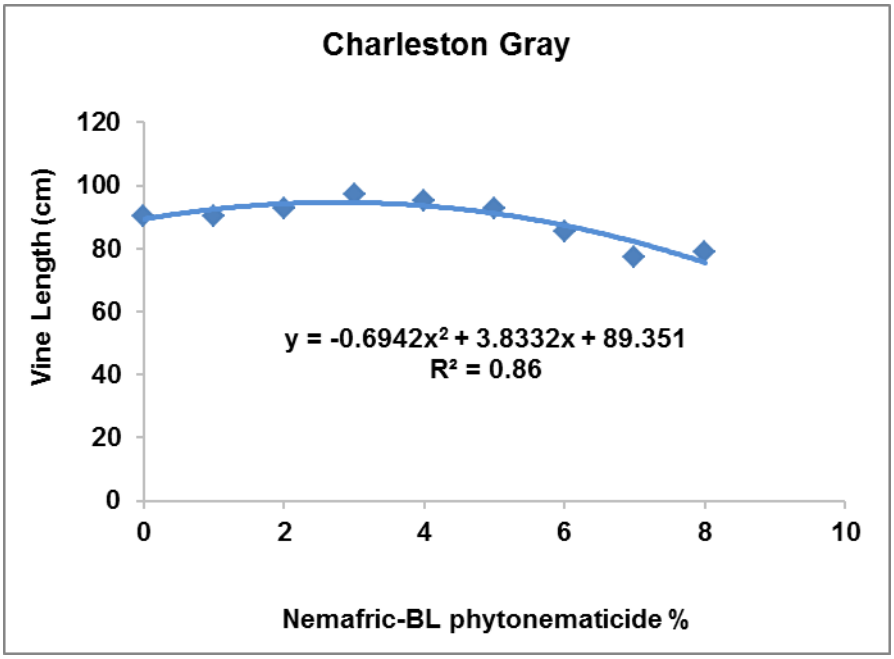

Figure 3.1 Vine length of 'Charleston Gray' and Nemafric-BL phytonematicide.

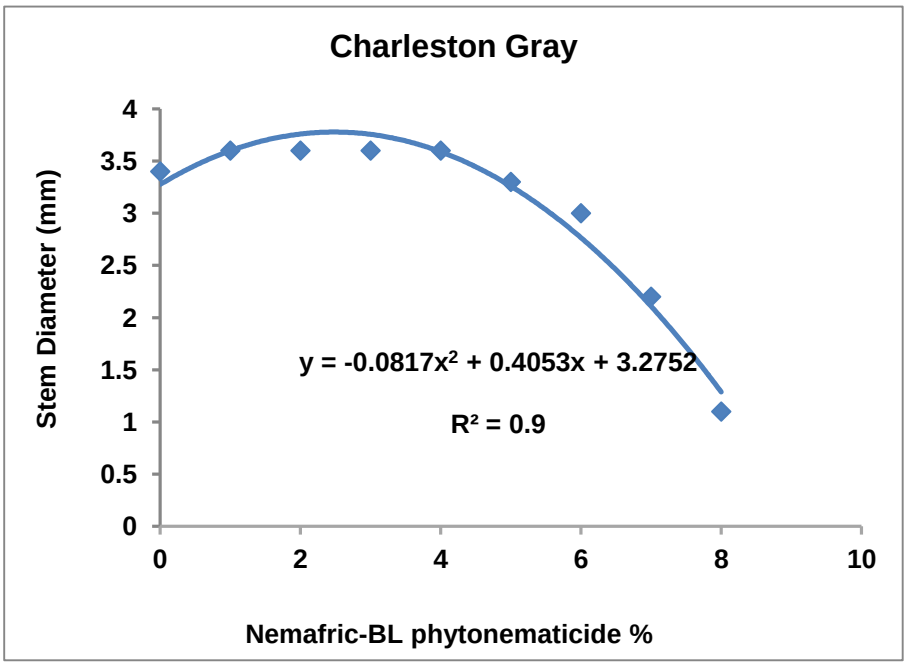

Figure 3.2 Stem diameter of 'Charleston Gray' and Nemafric-BL phytonematicide.

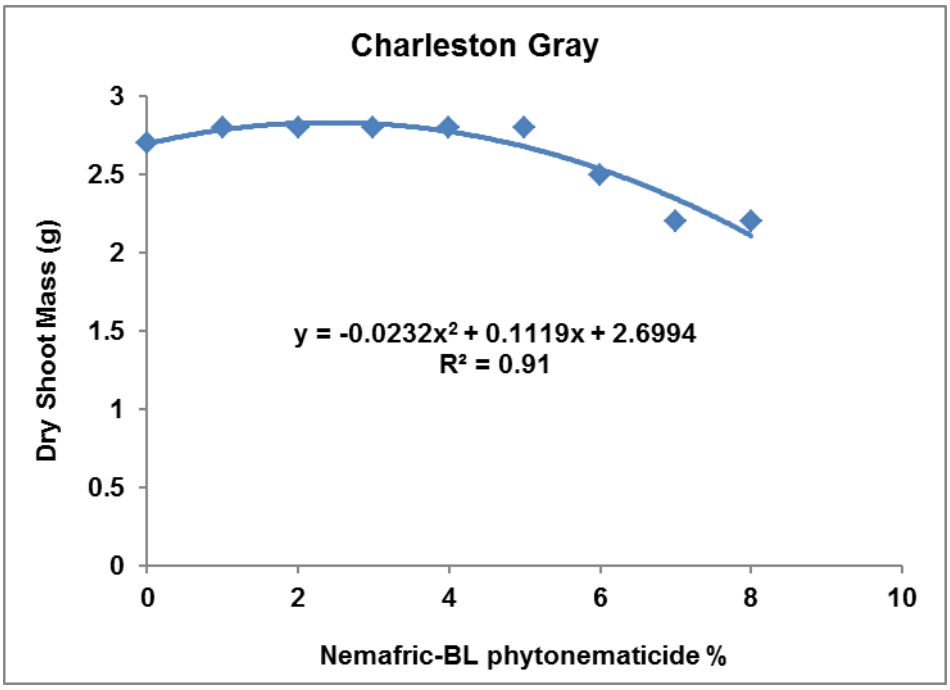

Figure 3.3 Dry shoot mass of 'Charleston Gray' and Nemafric-BL phytonematicide.

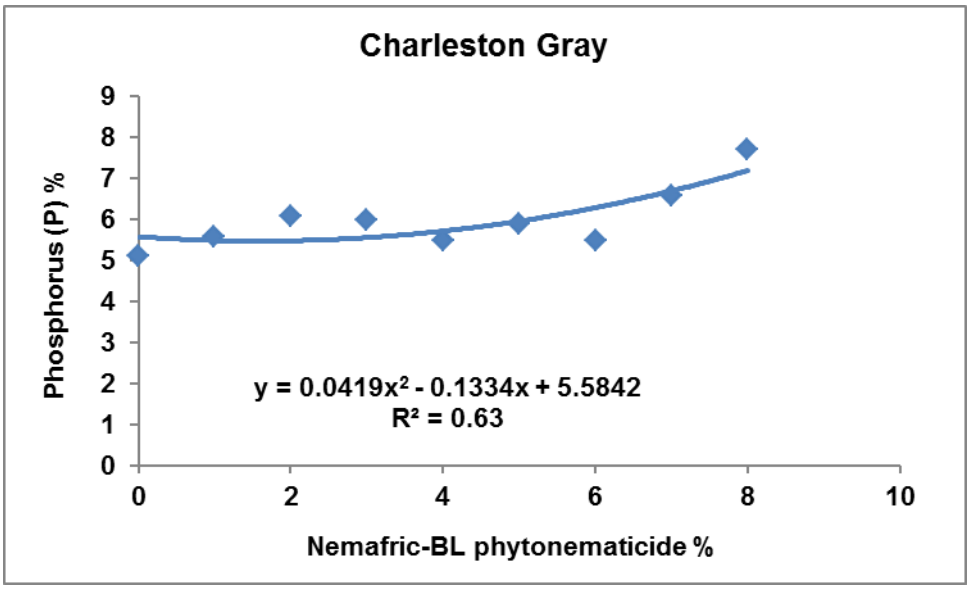

Figure 3.4 Phosphorus in leaf tissues of 'Charleston Gray' and Nemafric-BL phytonematicide

3.3.2 Essential nutrient element variables

In cv. ‘Charleston Gray’, Nemafric-BL phytonematicide had highly significant effects on P in leaf tissues, whereas Nemarioc-AL phytonematicide had similar effects on Mn and Na in leaf tissues (Table 3.7). Generally, Nemafric-BL phytonematicide increased P in leaves of cv. ‘Charleston Gray’, whereas Nemarioc-AL phytonematicide reduced Mn and Na in leaves of the cultivar (Table 3.8).

In cv. ‘Congo’, Nemafric-BL and Nemarioc-AL phytonematicides each had highly significant effects on P, Mn and Na in leaf tissues (Table 3.7). The responses of P and Na to increasing concentrations of Nemafric-BL phytonematicide were not consistent, whereas those of Mn increased and gradual decline with increasing concentration of the product (Table 3.7). The responses of P to increasing concentrations of Nemarioc-AL phytonematicide increased and gradually declined, whereas those of Mn and Na in leaf tissues of cv. ‘Congo’ were consistently reduced with increasing concentrations of the product (Table 3.8).

Table 3.7 Partitioning of sources of variation in selected nutrient elements in leaf tissues of watermelon cv. ‘Charleston Gray’ in response to Nemafric-BL and Nemarioc-AL phytonematicides (n = 45).

		Nemafric-BL phytonematicide		Nemarioc-AL phytonematicide			
		P (ppm)		Mn (ppm)		Na (ppm)	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	4	0.64789	10	0.25299	33	8.1731	13
Treat	8	3.89017	61**	0.39208	52**	40.5339	68**
Error	32	1.80089	29	0.11491	15	11.0727	19
Total	44	6.33895	100	0.75998	100	59.7797	100

**Highly significant at P ≤ 0.05.

Table 3.8 Influence of Nemafric-BL and Nemarioc-AL phytonematicides to selected nutrient elements in leaf tissues of watermelon cv. 'Charleston Gray' (n = 45).

Treatment (%)	Nemafric-BL phytonematicide		Nemarioc-AL phytonematicide			
	P (ppm)		Mn (ppm)		Na (ppm)	
	Variable	Impact (%)	Variable[y]	Impact (%)[z]	Variable	Impact (%)[x]
0.0	5.1[b]	–	8.0[c]	–	7.7[a]	–
0.2	4.8[b]	–6	0.0[c]	–100	5.9[abc]	–23
0.4	6.1[ab]	20	0.5[ab]	–94	6.5[ab]	–16
0.8	6.0[b]	17	0.6[ab]	–93	1.8[cd]	–77
1.6	5.5[b]	8	0.8[a]	–90	2.6[bcd]	–66
3.2	5.9[b]	16	0.4[abc]	–95	1.2[d]	–84
6.4	5.5[b]	8	0.4[abc]	–95	1.8[cd]	–77
12.8	4.9[b]	–4	0.6[ab]	–93	0.0[d]	–100
25.6	7.7[a]	51	0.3[bc]	–96	6.5[ab]	–16
$LSD_{0.05}$	1.7288	-	0.4367	-	4.2868	-

[y]Column means followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan multiple range test.

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

Table 3.9 Partitioning of sources of variation in selected nutrient elements in leaf tissues of watermelon cv. 'Congo' under greenhouse conditions in response to Nemafric-BL and Nemarioc-AL phytonematicides (n = 45).

Nemafric-BL phytonematicide							
		Mn (ppm)		Na (ppm)		P (ppm)	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Rep	4	0.12557	10	1.9143	4	3.5162	17
Treat	8	0.90170	71**	33.4906	67**	14.5007	73**
Error	32	0.24814	19	14.2598	29	1.9384	10
Total	44	1.27541	100	49.6647	100	19.9553	100
Nemarioc-AL phytonematicide							
Rep	4	0.03267	8	2.4635	4	0.90473	8
Treat	8	0.27013	71**	57.8247	92**	8.89755	79**
Error	32	0.07854	21	2.2995	4	1.49819	13
Total	44	0.38134	100	62.5877	100	11.30047	100

**Highly significant at P ≤ 0.05.

Table 3.10 Influence of Nemafric-BL phytonematicides to selected essential nutrient elements in leaf tissues of watermelon cv. 'Congo' (n = 45).

Treatment	Mn (ppm)		Na (ppm)		P (ppm)	
(%)	Mean[y]	Impact (%)[z]	Mean	Impact (%)	Mean	Impact (%)
0.0	0.0338[d]	–	8.492[ab]	–	5.4320[cd]	–
0.2	0.2984[cd]	78	9.726[ab]	14	4.1200[d]	–24
0.4	0.3470[cd]	92	5.942[bc]	–30	7.3340[b]	35
0.8	0.6250[bcd]	1.7	11.400[a]	34	6.0640[bc]	11
1.6	1.0960[ab]	3.1	10.034[ab]	18	4.8300[cd]	–11
3.2	1.4040[a]	4.0	7.050[abc]	–17	6.3420[bc]	16
6.4	0.3826[cd]	1.0	7.844[abc]	–7	5.0080[cd]	–7
12.8	0.5890[bcd]	1.6	3.334[c]	–60	5.3320[cd]	–2
25.6	0.7706[abc]	2.1	10.928[a]	28	9.8280[a]	80

[y]Column means followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

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

Table 3.11 Influence of Nemarioc-AL phytonematicide to selected nutrient elements in leaf tissues watermelon cv. 'Congo' (n = 45).

Treatment	Mn (ppm)		Na (ppm)		P (ppm)	
(%)	Mean[y]	Impact (%)[z]	Mean	Impact (%)	Mean	Impact (%)
0.0	0.0240[cd]	–	6.0460[bc]	–	4.5400[c]	–
0.2	0.0000[d]	–100	6.4240[b]	6	5.1960[bc]	14
0.4	0.1792[bcd]	647	4.3514[c]	–28	8.1100[a]	78
0.8	0.3248[abcd]	1253	1.2920[d]	–78	6.1650[b]	36
1.6	0.6760[a]	2717	0.0254[d]	–99	4.7140[bc]	3
3.2	0.3958[ab]	1549	0.0000[d]	–100	6.1340[b]	35
6.4	0.3804[abc]	1485	0.0000[d]	–100	6.2280[b]	37
12.8	0.4166[ab]	1636	0.0000[d]	–100	3.8540[c]	–15
25.6	0.6000[a]	2400	8.5620[a]	41	4.1820[c]	–8

[y]Column means followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

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

Table 3.12 Influence of Nemafric-BL phytonematicide to selected essential nutrient elements in watermelon cultivar 'Charleston Gray' (n = 45).

Treatment (%)	Ca (ppm)	P (ppm)	Mg (ppm)	K (ppm)	Mn (ppm)	Na (ppm)	Fe (ppm)	Zn (ppm)
0.0	22.154	5.142[b]	25.580	57.120	0.577	9.266	19.500	7.858
0.2	28.040	4.876[b]	25.180	60.160	0.542	8.582	19.920	3.826
0.4	30.040	6.146[ab]	24.520	63.140	0.220	7.610	19.480	2.236
0.8	26.704	6.014[b]	24.320	48.700	0.346	6.128	18.880	5.790
1.6	31.000	5.506[b]	25.580	48.600	0.982	4.034	16.120	4.386
3.2	34.000	5.960[b]	27.120	53.640	0.716	5.822	18.580	4.448
6.4	24.906	5.556[b]	25.620	50.160	0.523	7.862	22.954	10.028
12.8	32.280	4.932[b]	24.860	46.840	0.324	5.276	19.300	6.302
25.6	30.500	7.754[a]	26.220	49.260	0.641	9.808	21.500	4.346

Column means followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan multiple range test.

Table 3.13 Influence of Nemarioc-AL phytonematicide to selected essential nutrient elements in watermelon cultivar 'Charleston Gray' (n = 45).

Concentration (%)	Ca (ppm)	P (ppm)	Mg (ppm)	K (ppm)	Mn (ppm)	Na (ppm)	Fe (ppm)	Zn (ppm)
0.0	46.788	4.272	26.080	99.52	0.008^{c}	7.752^{a}	22.040	0.000
0.2	42.300	5.650	27.140	71.62	0.000^{c}	5.978^{abc}	22.680	0.000
0.4	31.366	7.040	24.960	53.04	0.560^{ab}	6.538^{ab}	23.040	0.212
0.8	32.146	4.784	25.260	103.30	0.603^{ab}	1.888^{cd}	22.700	0.000
1.6	35.660	4.630	24.900	77.06	0.838^{a}	2.600^{bcd}	20.508	4.418
3.2	29.326	6.974	24.760	105.60	0.407^{abc}	1.246^{d}	22.120	2.654
6.4	22.870	4.896	25.400	84.78	0.423^{abc}	1.888^{cd}	23.160	3.332
12.8	23.354	5.728	24.160	91.62	0.632^{ab}	0.033^{d}	22.640	2.792
25.6	29.292	5.536	25.540	95.22	0.329^{bc}	6.546^{ab}	23.620	0.000

Column means followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

Table 3.14 Influence of Nemafric-BL phytonematicide to selected nutrient elements in leaf tissues of watermelon cultivar ‘Congo’ (n = 45).

Treatment (%)	Ca (ppm)	P (ppm)	Mg (ppm)	K (ppm)	Mn (ppm)	Na (ppm)	Fe (ppm)	Zn (ppm)
0.0	29.998	5.432	23.480	61.360	0.033	8.492	19.540	7.889
0.2	27.080	4.120	23.760	49.480	0.298	9.726	18.158	9.294
0.4	30.400	7.334	25.420	48.200	0.347	5.942	19.120	3.016
0.8	21.820	6.064	27.040	42.420	0.625	11.400	16.060	5.932
1.6	19.572	4.830	23.840	52.480	1.096	10.034	21.340	8.440
3.2	28.070	6.342	24.460	54.640	1.404	7.050	23.120	6.280
6.4	34.480	5.008	25.000	52.620	0.382	7.844	21.106	7.266
12.8	25.422	5.332	25.540	51.540	0.589	3.334	17.732	2.604
25.6	27.860	9.828	25.500	48.420	0.770	10.928	18.600	1.406

Column means followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

Table 3.15 Influence of Nemarioc-AL phytonematicide to nutrient elements manganese, sodium and phosphorus of watermelon cultivar ‘Congo’ (n = 45).

Treatment (%)	C (ppm)	P (ppm)	Mg (ppm)	K (ppm)	Mn (ppm)	Na (ppm)	Fe (ppm)	Zn (ppm)
0.0	37.260	4.540^{c}	24.960	88.660	0.024^{cd}	6.046^{bc}	22.440	0.496
0.2	25.874	5.196b^{c}	25.880	82.860	0.000^{d}	6.424^{b}	24.080	0.746
0.4	21.760	8.110^{a}	25.880	68.580	0.179^{bcd}	4.351^{c}	22.640	2.660
0.8	22.020	6.156^{b}	26.500	79.960	0.324^{abcd}	1.292^{d}	22.920	2.510
1.6	46.500	4.714b^{c}	26.380	87.800	0.676^{a}	0.025^{d}	22.500	2.796
3.2	28.180	6.134^{b}	25.520	82.160	0.395^{ab}	0.000^{d}	22.460	2.278
6.4	32.366	6.228^{b}	24.540	88.160	0.380^{abc}	0.000^{d}	23.320	0.000
12.8	31.636	3.854^{c}	26.060	84.440	0.416^{ab}	0.000^{d}	22.800	5.738
25.6	38.520	4.182^{c}	25.900	82.520	0.600^{a}	8.562^{a}	22.760	1.136
$LSD_{0.05}$	28.765	–	2.443	23.770	–	–	1.969	4.512

Column means followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

In leaf tissues of cv. ‘Charleston Gray’, P versus increasing concentration of Nemafric-BL phytonematicide exhibited positive quadratic relation, with the association explaining 63% of the observed model (Figure 3.4). Phosphorus in leaf tissues of cv. ‘Charleston Gray’ was optimised at 1.59% Nemafric-BL phytonematicide. In contrast, both Mn and Na versus increasing concentrations of Nemarioc-AL phytonematicide each exhibited negative quadratic relations, with the associations explaining 86% (Figure 3.5) and 81% (Figure 3.6) of the observed models. The minimum inhibition concentrations of Mn and Na in leaf tissues of ‘Charleston Gray’ were achieved at 5.44% and 4.60% Nemarioc-AL phytonematicide, respectively.

In leaf tissues of cv. ‘Congo’, P versus increasing concentration of Nemafric-BL phytonematicide exhibited positive quadratic relation, with the association explaining 75% of the observed model (Figure 3.7). Phosphorus in leaf tissues of cv. ‘Congo’ was optimised at 2.30% Nemafric-BL phytonematicide. Similarly, both Mn and Na versus increasing concentrations of Nemafric-BL phytonematicide each exhibited positive quadratic relations, with the associations explaining 55% (Figure 3.8) and 60% (Figure 3.9) of the observed models. Manganese and Na in leaf tissues of ‘Congo’ were optimised at 14.29% and 2.56% Nemafric-BL phytonematicide, respectively.

In leaf tissues of cv. ‘Congo’, P versus increasing concentration of Nemarioc-AL phytonematicide exhibited positive quadratic relation, with the association explaining 81% of the observed model (Figure 3.10). Phosphorus in leaf tissues of cv. ‘Congo’ was optimised at 3.50% Nemarioc-AL phytonematicide. Similarly, both Mn versus

increasing concentrations of Nemarioc-AL phytonematicide exhibited positive quadratic relations, with the associations explaining 90% of the observed model (Figure 3.11). Manganese in leaf tissues of ‘Congo’ was optimised at 8.25%. In contrast, Na and increasing concentration of Nemarioc-AL phytonematicide exhibited negative quadratic relation, with the association explaining 88% of the observed model (Figure 3.12). The minimum inhibition concentration for Na was attained at 4.85% Nemarioc-AL phytonematicide.

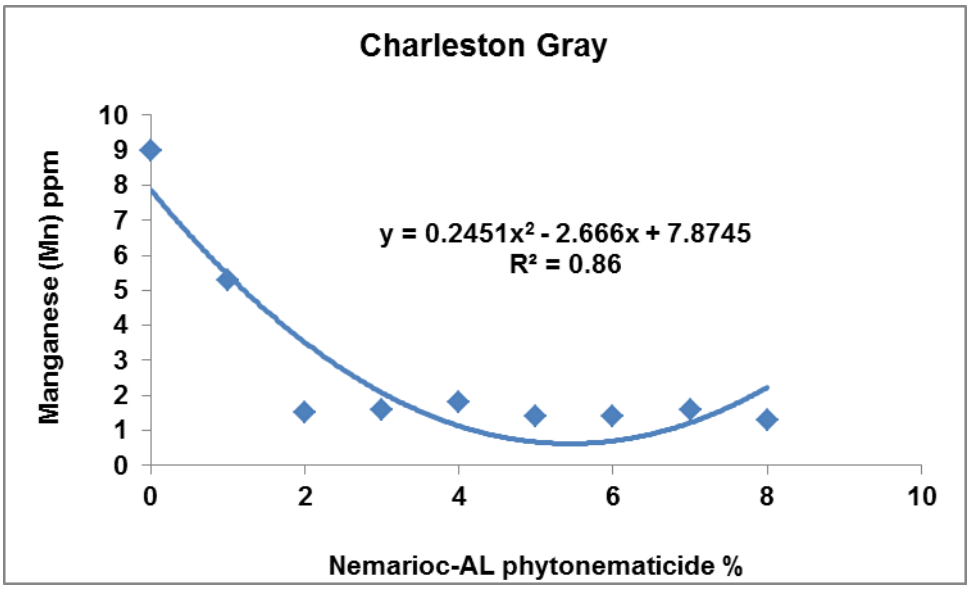

Figure 3.5 Manganese in leaf tissues of 'Charleston Gray' and Nemarioc-AL phytonematicide.

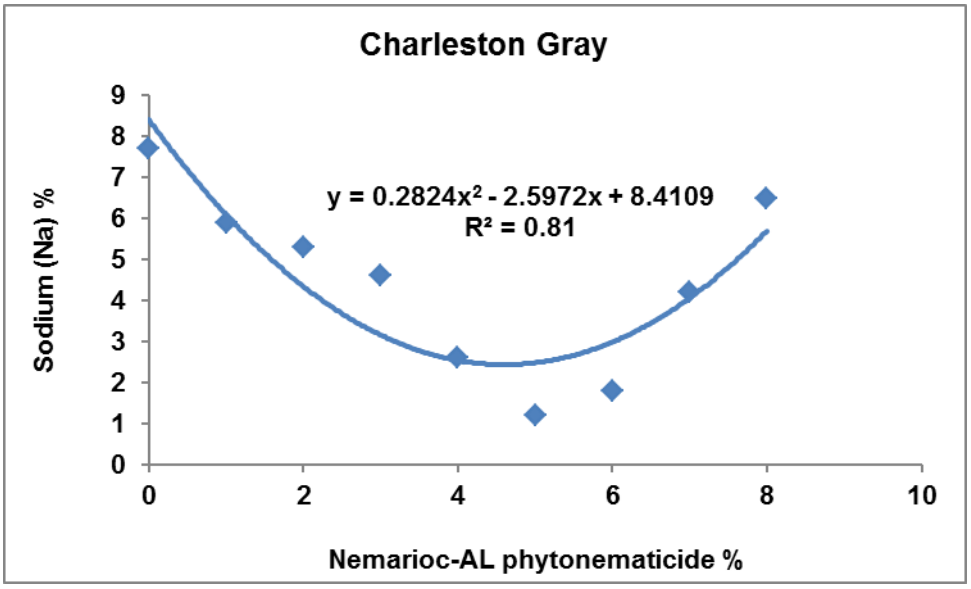

Figure 3.6 Sodium in leaf tissues of 'Charleston Gray' and Nemarioc-AL phytonematicide.

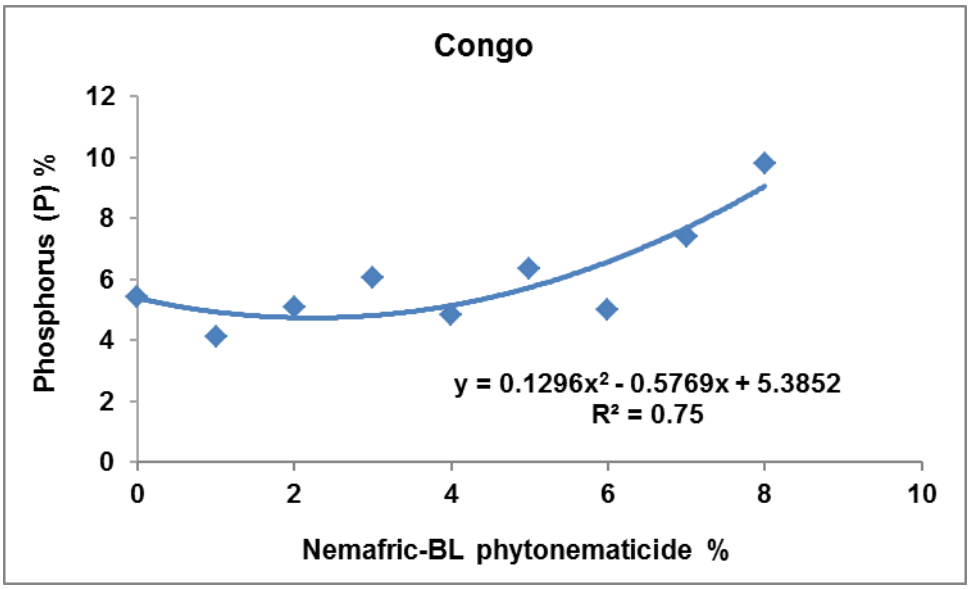

Figure 3.7 Phosphorus in leaf tissues of 'Congo' and Nemafric-BL phytonematicide.

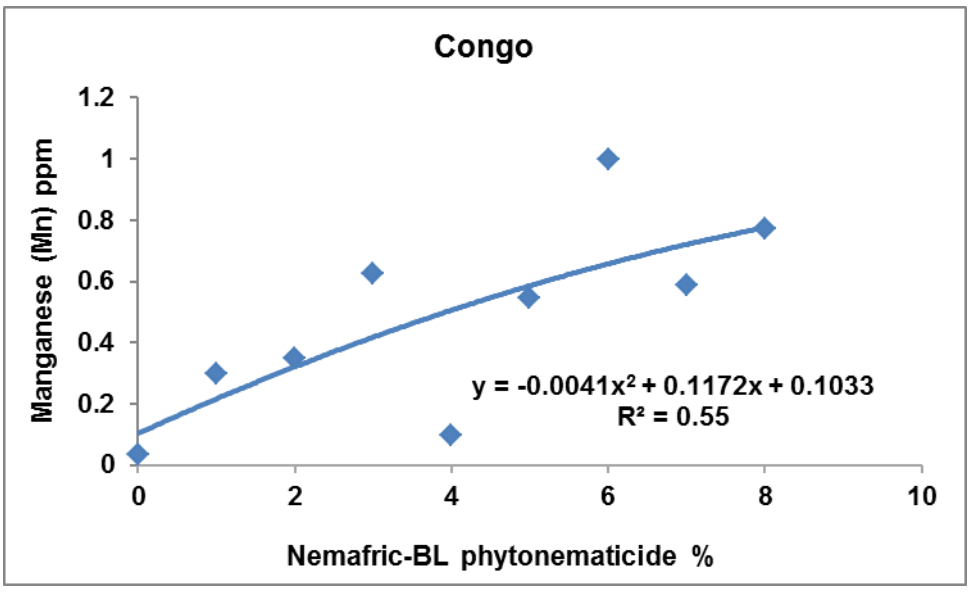

Figure 3.8 Manganese in leaf tissues of 'Congo' and Nemafric-BL phytonematicide.

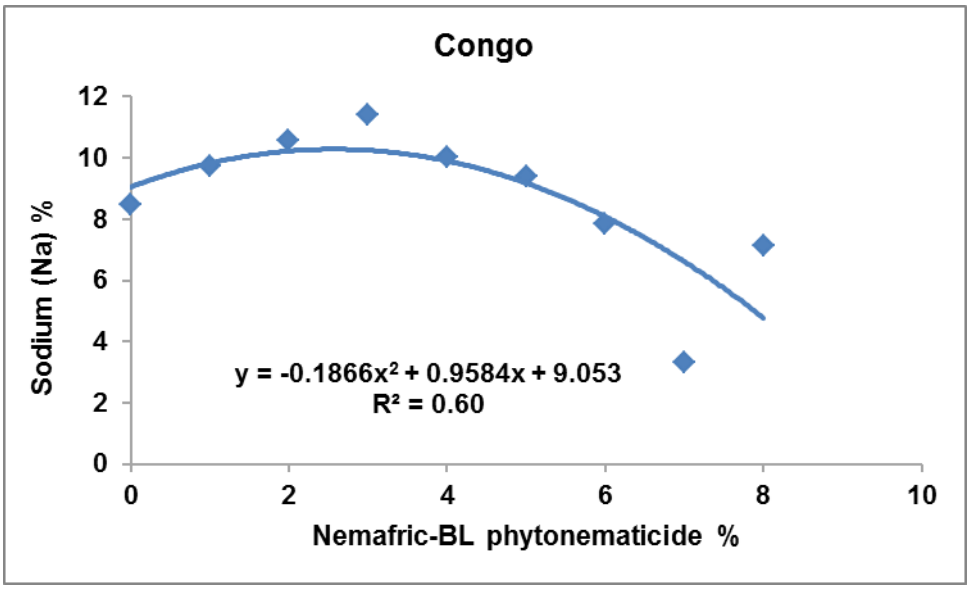

Figure 3.9 Sodium in leaf tissues of 'Congo' and Nemafric-BL phytonematicide.

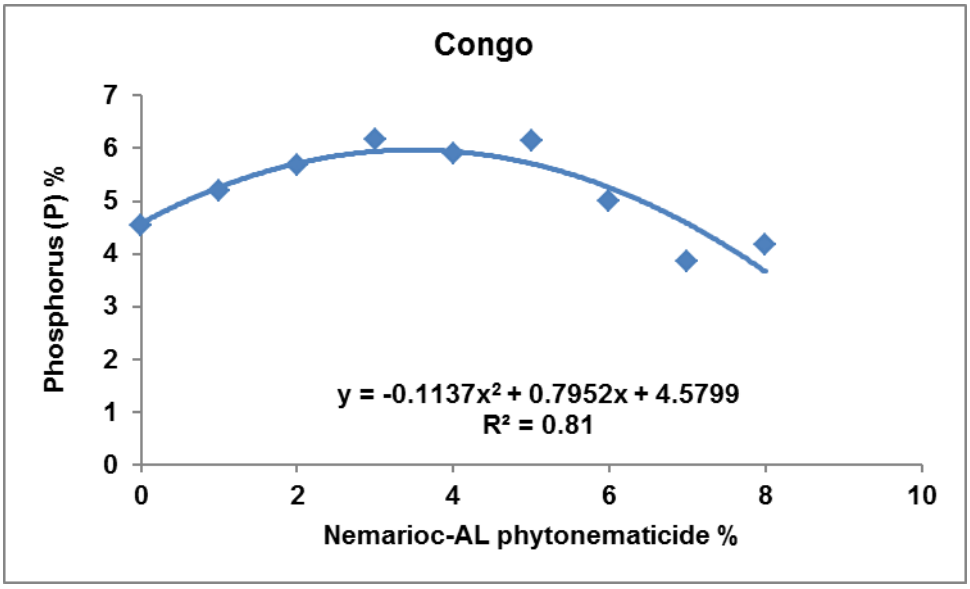

Figure 3.10 Phosphorus in leaf tissues of 'Congo' and Nemarioc-AL phytonematicide

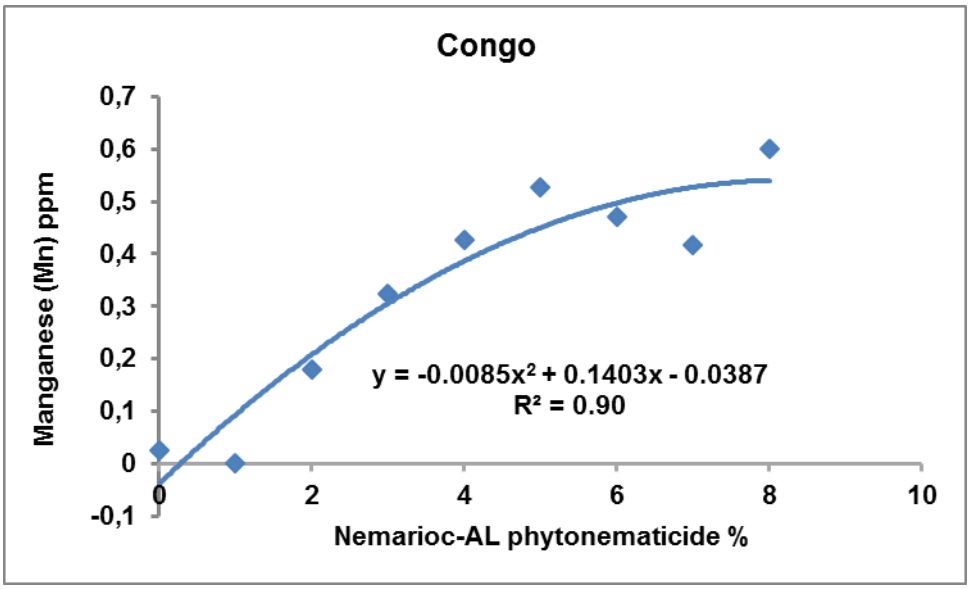

Figure 3.11 Manganese in leaf tissues of 'Congo' and Nemarioc-AL phytonematicide.

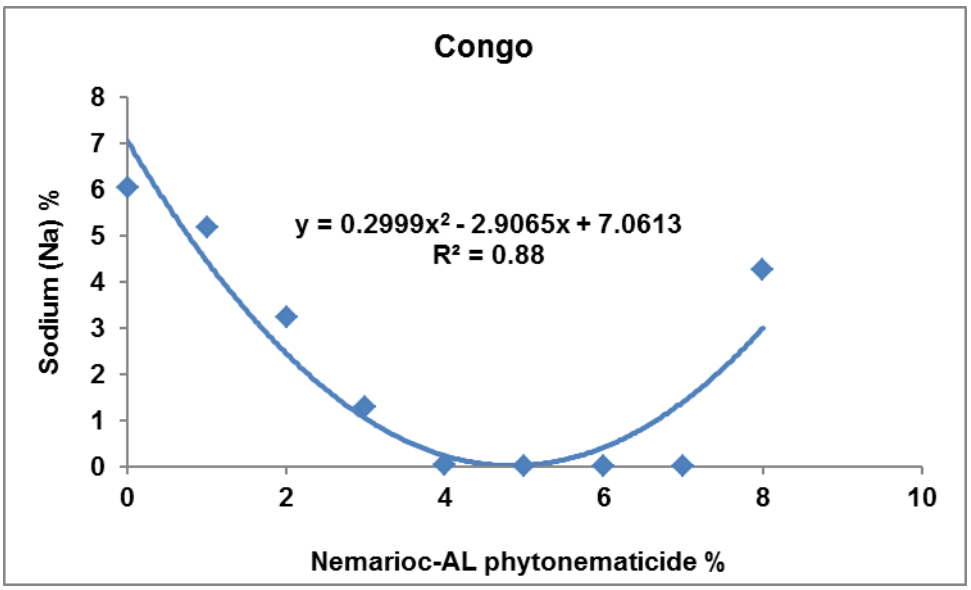

Figure 3.12 Sodium in leaf tissues of 'Congo' and Nemarioc-AL phytonematicide.

3.3.3 Nematode variables

Nemarioc-AL and Nemafric-BL phytonematicides had significant effects on *M. javanica* variables under greenhouse conditions, contributing 74-87% and 73-85% in TTV of the nematode variables for ‘Charleston Gray’, respectively (Table 3.16) and 64-81% and 77-82% in TTV of the nematode variables for ‘Congo’, respectively (Table 3.17). Relative to the untreated control, increasing concentration of phytonematicides did not have significant differences on nematode population densities, but these were significantly different to those under untreated control (Data not shown).

Table 3.16 Partitioning sources of variation in various stages of *Meloidogyne javanica* on roots and related soil of watermelon cv. 'Charleston Gray' under greenhouse conditions in response to Nemafric-BL and Nemarioc-AL phytonematicides (n = 45).

Nemafric-BL											
		J2 in soil		J2 in roots		Eggs in root		Total eggs and J2		Total nematodes	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Rep	4	1.849	4	1.605	4	5.077	13	0.280	12	2.722	9
Treat	8	36.404	87***	27.724	74***	29.946	74***	1.704	76***	25.123	78***
Error	32	3.658	9	8.280	22	5.433	13	0.271	12	4.308	13
Total	44	41.912	100	37.609	100	40.456	100	2.2555	100	32.154	100
Nemarioc-AL											
Rep	4	2.427	9	2.916	9	1.918	6	2.612	9	2.705	8
Treat	8	23.294	79***	25.534	83***	20.611	73***	23.430	85***	27.485	85***
Error	32	3.627	12	2.367	8	5.866	21	1.726	6	2.278	7
Total	44	29.348	100	30.817	100	28.395	100	27.768	100	32.461	100

***Highly significant at P ≤ 0.01.

Table 3.17 Partitioning sources of variation in various stages of *Meloidogyne javanica* on roots and related soil of watermelon cv. 'Congo' under greenhouse conditions in response to Nemafric-BL and Nemarioc-AL phytonematicides (n = 45).

Nemafric-BL											
		J2 in soil		J2 in roots		Eggs in root		Total eggs and J2		Total nematodes	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Rep	4	0	0	7.577	10	4636	5	7.358	10	7.358	10
Treat	8	0	0	47.594	65***	77636	81***	48.285	64***	48.285	64***
Error	32	0	0	18.683	25	1391	14	19.095	26	19.095	26
Total	44	0	0	73.853	100	96183	100	74.738	100	74.738	100
Nemarioc-AL											
Rep	4	0.733	7	1.3750	4	1.429	7	1.435	4	1.399	4
Treat	8	8.061	77***	27.891	77***	16.279	82***	28.596	78***	29.291	78***
										1	
Error	32	1.709	16	6.783	19	2.280	11	6.768	18	6.859	18
Total	44	10.504	100	36.049	100	19.988	100	36.828	100	37.549	100

***Highly significant at P ≤ 0.01.

3.4 Discussion

3.4.1 Plant variables

Non-significant effects of the two phytonematicides on a number of growth and nutrient element variables on watermelon cultivars ‘Charleston Gray’ and ‘Congo’ under greenhouse conditions confirmed observations in monocotyledonous and dicotyledonous plants (Mafeo *et al*., 2011a, b), tomato plants (Pelinganga *et al*., 2013b), citrus seedling rootstocks (Mathabatha *et al*., 2016), African ginger (Mashela *et al*., 2016) and pelargonium (Sithole *et al*., 2016). The effects of increasing concentrations of phytonematicides are characterised by three phases, namely, stimulation, neutral and inhibition phases (Liu *et al.,* 2003; Mashela *et al*, 2015). Generally, when the responses are under stimulation, inhibition or all three phases, the treatment effects would be reviewed as being significant ($P \leq 0.05$), whereas when the responses are under the neutral phase the treatment effects would not be significant ($P \geq 0.05$) as shown when assessing the effects of various concentrations of the two phytonematicides on various stages of *Meloidogyne* species (Dube, 2016).

Dry shoot mass, stem diameter and vine length of cv. ‘Charleston Gray’ versus increasing concentrations of Nemafric-BL phytonematicide exhibited quadratic relationship which characterised the DDG patterns (Mashela *et al*., 2015; Salisbury and Ross, 1992). The DDG patterns are the main feature that characterise the responses of organisms to increasing concentrations of allelochemicals (Liu *et al*., 2003). Allelochemicals are the active ingredients for the two phytonematicides (Mashela *et al*., 2015).

Different variable responses between Nemafric-BL and Nemarioc-AL phytonematicide could be due to the chemistry of their active ingredients. Nemafric-BL phytonematicide contain cucurbitacin B ($C_{32}H_{46}O_8$) which is not soluble in water and it is highly stable. In contrast, Nemarioc-AL phytonematicide contains cucurbitacin A ($C_{32}H_{46}O_9$), which is partially soluble in water and breaks down to cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$) constituents (Chen *et al*., 2005). Due to the observed DDG patterns under Nemafric-BL phytonematicide, the three variables could be subjected to the Curve-fitting Allelochemical Response Data (CARD) to generate the biological indices (Liu *et al*., 2003). The minimum (D_m) and maximum (R_h) of the stimulation range could be used to estimate the Mean Concentration Stimulation Point [$MCSP = D_m + R_h/2$] (Mashela *et al*., 2015).

3.4.2 Essential nutrient elements

The stimulation effect on tomato plant growth, first observed in cucurbitacin-containing phytonematicides, was referred to as the fertiliser effect (Mashela, 2002). A similar effect was later observed in tomato plants treated with cucurbitacin-free phytonematicides (Khosa, 2013). In both cases, detailed analyses of essential nutrient elements in leaf tissues did not support the fertiliser effect concept since the treatment resulted in negligent changes in essential nutrient elements. Consequently, the current study could be viewed as the first report to quantify changes in essential nutrient elements with increasing concentration of phytonematicides.

Depending on the cultivar or the phytonematicides, the P, Na and Mn in leaf tissues were either increased or decreased by increasing concentrations of phytonematicide.

Phosphorus is used in protein and nucleoprotein synthesis and metabolic transfer processes such as ADP and ATP in photosynthesis and respiration (Salisbury and Ross, 1992). Also, P is one of the major components of phospholipids. In leaf tissues of 'Congo' versus increasing levels of Nemarioc-AL phytonematicide, P in leaf tissues increased following the DDG patterns (Liu *et al*., 2003; Mashela *et al*., 2015; Salisbury and Ross, 1992), which are characterised by the three phases that were adequately described earlier and the quadratic equations. In leaf tissues of 'Congo', P was optimised at 3.5% Nemarioc-AL phytonematicide, which was too close to the 3% Nemarioc-AL phytonematicide used as a standard in tomato plants (Mashela *et al*., 2015). Consequently, the saturation for P in leaf tissues could be easily achieved when using Nemarioc-AL phytonematicide, which suggests the need to establish the MCSP of the product on watermelon. The latter agreed with the view that MCSP was plant- and phytonematicide-specific (Mashela *et al*., 2015), but it is not currently clear how the P responses affect the physiological activities of the plant.

The major role of Mn is in chlorophyll synthesis and it also acts as a coenzyme in various physiochemical processes in plants (Salisbury and Ross, 1992). The response of Mn was cultivar-specific, with the element in leaf tissues increasing and decreasing in 'Congo' and 'Charleston Gray', respectively. As in P, the responses of Mn to increasing concentration of phytonematicide had DDG patterns, with negative and positive quadratic relations, respectively. In 'Congo', Mn was optimised at high levels (8.25%) for Nemarioc-AL phytonematicide; there was no likelihood of the element being affected when the concentration of the product was at 3%. As in P, it is not yet clear how the Mn responses affect the physiological activities in the two watermelon cultivars. The responses of Na to increasing concentrations of

phytonematicides were also cultivar-specific and phytonematicide-specific as observed in Mn. Also, Na in tissues of ‘Congo’ and ‘Charleston Gray’ increased and decreased with increasing concentration of phytonematicide. Sodium is an essential nutrient element in C4 plants, but watermelon caries out C3-type photosynthesis (Miyake and Yokota, 2000). In C3 plants, Na in leaf tissues can reduce the lifespan of leaves through phytotoxicity (Salisbury and Ross, 1992). However, it is currently not known how the responses of Na would affect the physiological activities of the two watermelon cultivars.

3.4.3 Nematode variables

The reduction of *Meloidogyne* species under both Nemafric-BL and Nemarioc-AL phytonematicides was consistent with observations made in other crops where nematodes were managed using the two phytonematicides in granular and liquid formulation (Dube, 2016; Mafeo, 2012; Mashela, 2017; Maile, 2015; Pelinganga, 2013; Tseke, 2013).

3.5 Conclusion

The DDG patterns observed in plant growth variables of the two watermelon cultivars agreed with previous observations in various crops. However, the DDG patterns observed in accumulation of essential nutrient elements were the first in this study and since it is a physiological process, it could serve as an appropriate standard for stabling the MCSP as opposed to the reliance on plant growth variables. However, the challenge is that there was no consistent in the responses of the essential nutrient elements to increasing concentrations of phytonematicides.

CHAPTER 4
COMPARING EFFICACY OF PHYTONEMATICIDES AND VELUM ON GROWTH OF WATERMELON ‘CONGO’ AND NEMATODE SUPPRESSION

4.1 Introduction

Methyl bromide cut-off date for use under field conditions was in 2005, where it was internationally withdrawn from the agrochemical markets (Mashela *et al*., 2015). Since then, a large number of products had been researched and developed as alternatives to methyl bromide. In South Africa, nematologists had been active in research and development of such products. The most successful products had been the products from plants, with Nemafric-BL and Nemarioc-AL phytonematicides being among those products that are in the forefront of alternatives to methyl bromide (Mashela *et al*., 2015). Another product, which was previously introduced to the agrochemical markets as a fungicide/insecticide – Velum, was shown to possess nematicidal properties. Seshweni (2017) tested the product against suppression of root-knot (*Meloidogyne* species) nematodes on potato (*Solanum tuberosum*) plants, but just like the two cucurbitacin-containing phytonematicides, it had not been tested on the protection of watermelon (*Citrullus lanatus*) cultivars against nematode damage. Further, the efficacy of the two products on growth of watermelon cultivars and nematode suppression had not been documented. The objective of this study, therefore, was to compare the efficacy of Velum, Nemafric-BL phytonematicide and Nemarioc-AL phytonematicide on growth of watermelon, accumulation of selected essential nutrient elements in leaves and the suppression of population densities of *Meloidogyne* species under field conditions.

4.2 Materials and methods

4.2.1 Description of study site

The study was conducted under field conditions from mid-summer (Nov 2015) to mid-autumn (February 2016) at the Green Biotechnologies Research Centre (GTRC), University of Limpopo, South Africa (S23°53'10" E29°44'15"). The location has semi-arid climate, with high incidence of summer rainfall. The study was repeated from mid-spring (August 2016) to mid-summer (November 2016).

4.2.2 Land preparation

The land was manually prepared using hand forks. A drip irrigation system was laid out to allow for one litre water per hole of drip irrigation and the 0.90 m × 1.2 m inter- and intra-row spacing. Soil samples were collected for soil texture, nutrient analysis and initial population densities of nematodes (Pi). Nematode Pi was assessed using methods explained previously (Chapter 3). Meanwhile, watermelon cv. 'Congo' seeds were sown in 200-hole seedling trays containing Hygromix-T growing mixture (Hygrotech, Pretoria North). Hardening-off of the seedlings was achieved as explained previously (Chapter 3). Fourteen days after transplanting, each seedling was inoculated with 5000 eggs and second-stage juveniles (J2) to augment Pi which was found to be low at the site.

4.2.3 Treatments, research design and procedures

At seven days after transplanting, the four treatments, namely, untreated control, Velum, Nemafric-BL phytonematicide and Nemarioc-AL phytonematicide, were arranged in a randomised complete block design, with 12 replications. Treatments were initiated at seven days after transplanting and applied weekly for 56 days. Each

phytonematicide was applied weekly at 2% per seedling using 500 ml chlorine-free tapwater, with Velum applied at 0.08 ml/15 L chlorine-free tapwater.

4.2.4 Cultural practices

Prior to transplanting, each planting station was irrigated for 2 weeks by a total of 600 mm water and at, 25 mm after transplanting on weekly basis until harvest. Fertilisation comprised 5 g 2:3:2 (22) N:P:K fertiliser mixture and 5 g superphosphate (10.6%), each applied at 5-cm away from the trunk at transplanting. The first top dressing was done at 4 weeks after transplanting using 5 g LAN and 5 g N:P:K 2:3:4 (30), applied separately in a furrow around the stem and covered with soil. The second top-dressing was achieved at six weeks after transplanting by applying 5 g LAN and 5 g potassium nitrate.

Fruit flies were controlled weekly using Lebaycid insecticide as per label instruction. Daily scouting was done in the experimental plots to check for availability of pests, weeds and rotten fruit, with rotten fruit removed weekly and stored in tied black plastic bags until disposal. A weekly spraying programme was designed for late blight, early blight, anthracnose, downy mildew and powdery mildew, which comprised alternating mancozeb (Dithane M45), copper oxychloride and Bravo as per label instruction. Weeds were manually controlled using hand-hoes when the transplants were still young and thereafter pulled out by hand when necessary.

4.2.5 Data collection

At 90 days after transplanting, stem diameter, fruit number, fruit mass and vine length were measured. Fruit were harvested and fruit mass recorded. Total soluble

solids (TTS) (% Brix) was estimated using a hand-held refractometer (Bellingham and Stanley, UK). Vines were dried at 70ºC for 72 h and selected essential nutrient determined as explained previously (Chapter 3).

4.2.6 Data analysis

Data was subjected to analysis of variance (ANOVA) through SAS software (Appendix 4.1-4.13). In variables where the treatment effects were significant ($P \leq 0.05$), the mean sum of squares (MSS) were partitioned to generate the total treatment variation to the affected variable (Gomez and Gomez, 1984). Treatment means for significant treatment effects were separated using Fisher's Least Significant Difference test at the probability level of 5%.

4.3 Results

Seasonal interactions were not significant, and therefore, data for the two seasons were pooled and re-subjected to analysis of variance (n = 96). The treatment effects were not significant on growth plant variables of cv. 'Congo'. Treatment effects were significant on fruit number, fruit mass and vine length, but not on dry shoot mass and TTS (% Brix) (Table 4.1). Relative untreated control, Velum, Nemafric-BL and Nemarioc-AL nematicides increased plant growth of cv. 'Congo', with fruit number increased by 264, 254 and 133%, respectively, whereas, fruit mass was increased by 85, 68 and 43%, respectively, and vine length by 32, 48 and 29%, respectively. In fruit number and fruit mass, the effects of Velum and the two cucurbitacin-containing phytonematicides were not different from one another, but were all significantly different to those of untreated control. Nemafric-BL phytonematicide resulted in the

highest vine length when compared with untreated control, which was, however, not different to that of Velum and Nemarioc-AL phytonematicide.

The treatments significantly affected P in leaf tissues of cv. ‘Congo’, but had no significant effects on Ca, Mg, K, Mn, Na, Fe and Zn in leaf tissues of the cultivar. Relative to the untreated control, Velum, Nemafric-BL and Nemarioc-AL products increased P in leaf tissues of cv. ‘Congo’ by 7, 13 and 34%, respectively (Table 4.2). Treatment effects on J2 in soil, J2 in root, eggs in root, eggs and J2 and total nematode were highly significant, contributing 78, 60, 73, 23 and 69% in TTV of the respective variables (Table 4.3). Effects of the three products were not significantly different from one another, but were different from the untreated control (data not shown).

Table 4.1 Influence of Velum and two phytonematicide on growth of watermelon cultivar ‘Congo’ under field conditions (n = 96)

Treatment	Dry shoot mass (g/plant)	Fruit number[z] /plant	Fruit mass (g/plant)	Total soluble solids	Vine length (cm/plant)
Control	19.23	0.483[b]	1272.0[b]	19.1	42.9[b]
Velum	20.20	1.625[a]	2350.4[a]	20.3	56.5[ab]
Nemafric-BL	21.03	1.708[a]	2131.4[a]	19.9	63.7[a]
Nemarioc-AL	19.75	1.125[a]	1824.7[a]	20.0	55.4[ab]
$LSD_{0.05}$	6.11	–	–	3.12	–

[z]Column means followed by the same letter were not different (P ≤ 0.05) according to Fisher’s Least Significant Difference test.

Table 4.2 Influence of Velum, Nemafric-BL and Nemarioc-AL phytonematicides to accumulation of nutrient elements in watermelon cultivar ‘Congo’ (n = 96).

Treatment	Ca (ppm)	P (ppm)	Mg (ppm)	K (ppm)	Mn (ppm)	Na (ppm)	Fe (ppm)	Zn (ppm)
Control	32.128	5.176[c]	26.079	37.562	0.448	8.407	16.643	7.530
Velum	34.117	5.513[bc]	25.317	39.013	0.672	8.032	18.767	8.203
Nemafric-BL	30.628	5.865[b]	26.050	40.125	0.412	9.713	17.314	6.801
Nemarioc-AL	30.242	6.927[a]	25.208	44.654	0.384	8.325	18.397	7.165
$LSD_{0.05}$	9.546	–	1.882	5.922	0.449	1.494	3.320	4.090

Column means followed by the same letter were not different (P ≤ 0.05) according to Fisher’s Least Significant Difference test.

Table 4.3 Partitioning of sources of variation in various nematode stages in roots of watermelon cultivar ‘Congo’ and related soil in response to Velum and Nemafric-BL and Nemarioc-AL phytonematicides (n = 96).

		J2 in soil		J2 in root		Eggs in root		Eggs and J2 in root		Total nematodes	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Rep	23	7.923	21	9.397	37	2.092	23	0.129	22	18.325	30
Treat	3	29.692	78***	15.128	60***	6.771	73***	0.417	23**	41.981	69***
Error	69	0.312	1	0.081	3	0.384	4	0.232	4	0.738	1
Total	95	37.927	100	25.332	100	9.247	100	0.569	100	61.044	100

4.4 Discussion

The effects of Velum on growth of watermelon were comparable with those of Nemafric-BL and Nemarioc-AL phytonematicides, which confirmed similar observations made in potato-treated with Velum and the two phytonematicides (Seshweni, 2017). Additionally, observations on Velum and the two phytonematicides agreed with those of Nemarioc-AL phytonematicide, which had comparable effects with aldicarb and fenamiphos on tomato production in suppression of population densities of *M. incognita* (Mashela *et al*., 2008).

Generally, cucurbitacin-containing phytonematicides, when applied at the Mean Concentration Stimulation Point (MCSP) (Mashela *et al*., 2015), result in stimulation of plant growth (Mashela, 2002). All the three products tested in the current study stimulated growth of watermelon cv. ‘Congo’, confirming observations when using the three products in potato production (Seshweni, 2017). Although the treatment effects on sugar content were not significant, the variable was not reduced, which confirmed the improvement of cucurbitacin-containing phytonematicides on TSS in sweet stem sorghum (Mashela and Pofu, 2016). Currently, it is not clear how cucurbitacins improve TSS in watermelon fruits. Due to their big molecular structures and probably their non-polarity, the cucurbitacins do not have the ability to pass through plasmalemma in the symplastic pathways and therefore, could hardly have residues in aboveground parts (Dube, 2016; Shadung, 2016). Because systemic nematicides such as aldicarb and fenamiphos had fatal residues challenges in watermelon fruits (Pofu, 2012), it is important that the cucurbitacin residues in fruits of this crop be empirically-tested.

The influence of Nemafric-BL and Nemarioc-AL phytonematicides on P in leaf tissues of watermelon confirmed observations under greenhouse conditions (Chapter 3), whereas in both 'Charleston Gray' and 'Congo' cultivars, P in leaf tissues of watermelon and increasing concentration of either phytonematicide exhibited positive quadratic relations (Chapter 3). Similar effects on P versus increasing concentrations in cucurbitacin-containing phytonematicides were observed in cowpea and green bean trials (Mashela: Pers. Comm.). Apparently, among all the tested essential nutrient elements tested against increasing concentrations of cucurbitacin-containing phytonematicides, P was the most sensitive to these products. Phosphorus, as indicated earlier (Chapter 3), is used in protein and nucleoprotein synthesis and metabolic transfer processes such as ADP and ATP in photosynthesis and respiration.

Comparative effects of Velum to Nemafric-BL and Nemarioc-AL phytonematicides on various stages of *Meloidogyne* species confirmed those of the three products on nematodes in potato production (Seshweni, 2017). Additionally, the observations confirmed those of Nemarioc-AL phytonematicide with aldicarb and fenamiphos on *Meloidogyne* species in tomato production (Mashela *et al.*, 2008). Active saponins from alfalfa (*Medicago sativa*) were shown to reduce population densities of *M. incognita* significantly more than fenamiphos (D'Addabbo *et al*., 2010).

4.5 Conclusion

The efficacy of Nemafric-BL and Nemarioc-AL phytonematicides was comparable to that of Velum in suppression of *Meloidogyne* species under field conditions. In

addition, the three products had stimulative effects on growth of watermelon, which is a common phenomenon in cucurbitacin-containing phytonematicides.

CHAPTER 5

SUMMARY OF FINDINGS, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary of findings

Nemafric-BL phytonematicide significantly affected growth of watermelon cultivars 'Charleston Gray' and 'Congo', whereas Nemarioc-AL phytonematicide did not have any significant effects on the plant variables of both cultivars. Generally, plant growth variables and increasing concentrations of Nemafric-BL phytonematicide had density-dependent growth (DDG) patterns, which were quantified through either positive or quadratic equations (Chapter 3). In contrast, both phytonematicides had significant effects on selected nutrient elements in leaf tissues of both watermelon cultivars. The affected nutrient elements, P, Mn and Na versus increasing concentrations of phytonematicides exhibited the DDG patterns, which were also quantified through either positive or quadratic equations (Chapter 3). The phytonematicides were consistent in suppressing nematode numbers in both watermelon cultivars. Comparison of synthetic nematicide Velum and the two phytonematicides under field conditions suggested that, relative to untreated control, the three products each stimulated growth of watermelon cv. 'Congo'. The efficacy of Nemafric-BL and Nemarioc-AL phytonematicides on suppression of population densities of *Meloidogyne* species was comparable to that of Velum.

5.2 Significance of findings

Watermelon cultivars are highly sensitive to damage by *Meloidogyne* species and currently there is no genotype resistance to nematodes. The findings demonstrated that the two phytonematicides consistently reduced population densities of

Meloidogyne species. Also, the findings showed for the first time that Nemafric-BL and Nemarioc-AL phytonematicides could either increase or decrease certain essential nutrient elements in DDG patterns. The generated positive quadratic relations provide an opportunity to establish the optimum concentrations, which could assist in decision-making in relation to fertilisation of the crop. Under both greenhouse and field conditions, P was affected by the phytonematicide treatments, which could imply that this essential nutrient element was the most sensitive to cucurbitacin-containing phytonematicides. The study also confirmed that the efficacy of the two phytonematicides on suppression of *Meloidogyne* species was comparable to that of the commercially available synthetic nematicide Velum.

5.3 Recommendations

Further studies would be necessary to investigate the sensitivity of P to increasing concentration of cucurbitacin-containing phytonematicides in relation to the actual physiological activities. This could also be extended to other affected nutrient elements. Additionally, the potential chemical residues of the cucurbitacins in watermelon fruits should form part of a future investigation, since the chemical compounds at low concentrations are cancerous (Lee *et al*., 2010).

5.4 Conclusions

Nemafric-BL and Nemarioc-AL phytonematicides were highly effective in managing population densities of *Meloidogyne* species in watermelon cultivars. These cucurbitacin-containing phytonematicides also affected the partitioning of selected nutrient elements in in leaf tissues of watermelon. The study provided essential

information that could assist in decision-making in nematode management in watermelon production, particularly in management of fertiliser application.

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APPENDICES

Appendix 3.1 Analysis of variance for dry shoot mass in watermelon cultivar ‘Charleston Gray’ treated with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	6.8778	1.71944		
Treatment	8	3.9191	0.48989	2.78	0.0186
Error	32	5.6342	17.607		
Total	44	16.4311			

Appendix 3.2 Analysis of variance for fresh root mass in watermelon cultivar ‘Charleston Gray’ with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	6.9933	1.79833		
Treatment	8	5.6818	0.71022	1.67	0.1448
Error	32	13.6227	0.42571		
Total	44	26.2978			

Appendix 3.3 Analysis of variance for stem diameter in watermelon cultivar ‘Charleston Gray’ with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	1.43520	0.35880		
Treatment	8	2.30164	2.8771	2.10	0.0653
Error	32	4.38300	0.13697		
Total	44	8.11984			

Appendix 3.4 Analysis of variance for vine length in watermelon cultivar ‘Charleston Gray’ with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	3382.96	845.740		
Treatment	8	2421.16	302.644	2.47	0.0331
Error	32	3925.52	122.672		
Total	44	9729.63			

Appendix 3.5 Analysis of variance for root galls in watermelon cultivar ‘Charleston Gray’ with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	0.00716	0.001790		
Treatment	8	0.01967	0.002459	0.98	0.4698
Error	32	0.08038	0.002512		
Total	44	0.10722	0.006761		

Appendix 3.6 Analysis of variance for dry shoot mass in watermelon cultivar ‘Charleston Gray’ with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	1.4258	0.35644		
Treatment	8	3.2578	0.40722	0.96	0.4871
Error	32	13.6422	0.42632		
Total	44	18.3258			

Appendix 3.7 Analysis of variance for Fresh root mass in watermelon cultivar ‘Charleston Gray’ with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	18.536	4.63389		
Treatment	8	16.260	2.03250	0.91	0.5236
Error	32	71.784	2.2432		
Total	44	106.580			

Appendix 3.8 Analysis of variance for stem diameter in watermelon cultivar ‘Charleston Gray’ with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	0.2979	0.02979		
Treatment	8	0.11975	0.11975	0.46	0.8729
Error	32	0.25876	0.25876		
Total	44	0.4083			

Appendix 3.9 Analysis of variance for vine length in watermelon cultivar ‘Charleston Gray’ with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	4456.0	11113.99		
Treatment	8	4075.6	509.45	1.03	0.4378
Error	32	1590.7	496.96		
Total	44	24434.3			

Appendix 3.10 Analysis of variance for root galls in watermelon cultivar ‘Charleston Gray’ with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	0.25436	0.06359		
Treatment	8	0.03080	0.00385	0.72	0.6742
Error	32	0.00536	0.0536		
Total	44	0.45680			

Appendix 3.11 Analysis of variance for dry shoot mass in watermelon cultivar ‘Congo’ with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	9.5720	2.39300		
Treatment	8	4.6604	0.58256	0.87	0.5531
Error	32	21.4840	0.67138		
Total	44	35.7164			

Appendix 3.12 Analysis of variance for fresh root mass in watermelon cultivar ‘Congo’ with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	7.0236	1.75589		
Treatment	8	3.5498	0.44372	0.48	0.8634
Error	32	29.7824	0.93070		
Total	44	40.3558			

Appendix 3.13 Analysis of variance for stem diameter in watermelon cultivar ‘Congo’ with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	1.8011	0.45028		
Treatment	8	1.9279	0.24099	1.20	0.3290
Error	32	6.4159	0.20050		
Total	44	10.1449			

Appendix 3.14 Analysis of variance for vine length in watermelon cultivar ‘Congo’ with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	3377.5	844.385		
Treatment	8	4131.2	516.403	1.6	0.1447
Error	32	9901.7	309.428		
Total	44	17410.5			

Appendix 3.15 Analysis of variance for root galls in watermelon cultivar ‘Congo’ with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	0.01234	0.003804		
Treatment	8	0.03914	0.004893	1.00	0.4572
Error	32	0.15704	0.004908		
Total	44	0.20852	0.013605		

Appendix 3.16 Analysis of variance for dry shoot mass in watermelon cultivar ‘Congo’ with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	0.5609	0.14022		
Treatment	8	3.8151	0.47689	1.30	0.271
Error	32	11.7471	0.36710		
Total	44	16.1231			

Appendix 3.17 Analysis of variance for fresh root mass in watermelon cultivar ‘Congo’ with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	6.9933	1.74833		
Treatment	8	5.6818	0.71022	1.67	0.1448
Error	32	13.6227	0.42571		
Total	44	26.2978			

Appendix 3.18 Analysis of variance for stem diameter in watermelon cultivar ‘Congo’ with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	0.5663	0.14159		
Treatment	8	2.0021	0.25027	0.59	0.7814
Error	32	13.6541	0.42669		
Total	44	16.2225			

Appendix 3.19 Analysis of variance for vine length in watermelon cultivar ‘Congo’ with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	4056.5	1014.12		
Treatment	8	2941.9	367.74	0.74	0.6535
Error	32	15837.2	494.91		
Total	44	228.35			

Appendix 3.20 Analysis of variance for root galls in watermelon cultivar ‘Congo’ with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	0.00782	0.003804		
Treatment	8	0.01638	0.004893	0.57	0.7970
Error	32	0.11566	0.004908		
Total	44	0.13987			

Appendix 3.21 Analysis of variance for Ca in leaf tissues of watermelon cultivar ‘Charleston Gray’ with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	1881.26	470.316		
Treatment	8	563.51	70.438	0.39	0.9203
Error	32	5844.91	182.654		
Total	44				

Appendix 3.22 Analysis of variance for P in leaf tissues of watermelon cultivar ‘Charleston Gray’ with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	2.5916	0.64789		
Treatment	8	31.1214	3.89017	2.16	0.0585
Error	32	57.6285	1.80089		
Total	44	91.3415			

Appendix 3.23 Analysis of variance for Mg in leaf tissues of watermelon cultivar ‘Charleston Gray’ with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	76.778	19.1944		
Treatment	8	30.035	3.7544	0.75	0.6469
Error	32	159.958	4.9987		
Total	44	266.771			

Appendix 3.24 Analysis of variance for K in leaf tissues of watermelon cultivar ‘Charleston Gray’ with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	336.23	84.057		
Treatment	8	1346.38	168.297	0.84	0.5755
Error	32	6416.81	200.525		
Total	44	8099.42			

Appendix 3.25 Analysis of variance for Mn in leaf tissues of watermelon cultivar 'Charleston Gray' with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	0.4223	0.10558		
Treatment	8	2.1246	0.26558	0.55	0.8119
Error	32	15.5285	0.48527		
Total	44	18.0755			

Appendix 3.26 Analysis of variance for Na in leaf tissues of watermelon cultivar 'Charleston Gray' with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	80.281	20.0703		
Treatment	8	151.704	18.9630	0.95	0.4894
Error	32	637.408	19.9190		
Total	44	869.394			

Appendix 3.27 Analysis of variance for Fe in leaf tissues of watermelon cultivar 'Charleston Gray' with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	51.42	12.8549		
Treatment	8	143.71	17.9639	0.54	0.8150
Error	32	1058.11	33.0660		
Total	44	1253.24			

Appendix 3.28 Analysis of variance for Zn in leaf tissues of watermelon cultivar 'Charleston Gray' with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	31.12	7.7792		
Treatment	8	219.59	27.4485	0.70	0.6853
Error	32	1246.87	38.9646		
Total	44	1497.57			

Appendix 3.29 Analysis of variance for Ca in leaf tissues of watermelon cultivar 'Charleston Gray' with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	1282.9	320.720		
Treatment	8	2541.8	317.730	0.43	0.8952
Error	32	23732.5	741.642		
Total	44	27557.3			

Appendix 3.30 Analysis of variance for P in leaf tissues of watermelon cultivar 'Charleston Gray' with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	3.619	0.90473		
Treatment	8	71.180	8.89755	5.94	0.001
Error	32	47.942	1.49819		
Total	44	122.741			

Appendix 3.31 Analysis of variance for Mg in leaf tissues of watermelon cultivar 'Charleston Gray' with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	18.331	4.58278		
Treatment	8	29.511	3.68889	1.01	0.4507
Error	32	117.309	3.66590		
Total	44	165.151			

Appendix 3.32 Analysis of variance for K in leaf tissues of watermelon cultivar 'Charleston Gray' with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	1205.9	301.487		
Treatment	8	5335.7	666.965	2.02	0.0752
Error	32	10544.2	329.506		
Total	44	17085.9			

Appendix 3.33 Analysis of variance for Mn in leaf tissues of watermelon cultivar 'Charleston Gray' with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	1.01197	0.25299		
Treatment	8	3.13662	0.39208	3.41	0.006
Error	32	3.67697	0.11491		
Total	44	7.82556			

Appendix 3.34 Analysis of variance for Na in leaf tissues of watermelon cultivar ‘Charleston Gray’ with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	32.693	8.1731		
Treatment	8	324.271	40.5339	3.66	0.0039
Error	32	354.325	11.0727		
Total	44	711.289			

Appendix 3.35 Analysis of variance for Fe in leaf tissues of watermelon cultivar ‘Charleston Gray’ with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	30.347	7.58681		
Treatment	8	31.988	3.99854	0.65	0.7264
Error	32	195.486	6.10894		
Total	44	257.822			

Appendix 3.36 Analysis of variance for Zn in leaf tissues of watermelon cultivar ‘Charleston Gray’ with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	29.753	7.4383		
Treatment	8	127.657	15.9571	1.24	0.3074
Error	32	410.997	12.8437		
Total	44	568.407			

Appendix 3.37 Analysis of variance for Ca in leaf tissues of watermelon cultivar 'Congo' with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	401.68	100.419		
Treatment	8	812.83	101.603	0.53	0.8236
Error	32	6113.64	191.051		
Total	44	7328.14			

Appendix 3.38 Analysis of variance for P in leaf tissues of watermelon cultivar 'Congo' with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	14.065	3.5162		
Treatment	8	116.005	14.5007	7.48	0.001
Error	32	62.028	1.9384		
Total	44	192.098			

Appendix 3.39 Analysis of variance for Mg in leaf tissues of watermelon cultivar 'Congo' with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	27.375	6.84367		
Treatment	8	51.312	6.41400	1.42	0.2269
Error	32	144.741	4.52317		
Total	44	223.428			

Appendix 3.40 Analysis of variance for K in leaf tissues of watermelon cultivar ‘Congo’ with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	994.87	248.717		
Treatment	8	1077.95	134.744	0.79	0.6165
Error	32	5470.03	170.938		
Total	44	7542.85			

Appendix 3.41 Analysis of variance for Mn in leaf tissues of watermelon cultivar ‘Congo’ with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	0.5023	0.1255		
Treatment	8	7.2136	0.90170	3.63	0.0041
Error	32	7.9404	0.24814		
Total	44	15.6563			

Appendix 3.42 Analysis of variance for Na in leaf tissues of watermelon cultivar ‘Congo’ with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	7.657	1.9143		
Treatment	8	267.925	33.4906	2.35	0.0412
Error	32	456.314	14.2578		
Total	44	731.896			

Appendix 3.43 Analysis of variance for Fe in leaf tissues of watermelon cultivar 'Congo' with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	626.55	156.638		
Treatment	8	183.64	22.955	0.72	0.6763
Error	32	1026.71	32.085		
Total	44	1836.90			

Appendix 3.44 Analysis of variance for Zn in leaf tissues of watermelon cultivar 'Congo' with nine concentrations of Nemafric-BL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	141.56	35.3892		
Treatment	8	316.06	39.5080	0.87	0.5527
Error	32	1456.03	45.5011		
Total	44	1913.66			

Appendix 3.45 Analysis of variance for Ca in leaf tissues of watermelon cultivar 'Congo' with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	167.1	167.1		
Treatment	8	2678.0	334.748	0.67	0.7126
Error	32	15954.4	498.574		
Total	44	18799.5			

Appendix 3.46 Analysis of variance for P in leaf tissues of watermelon cultivar ‘Congo’ with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	3.619	0.90473		
Treatment	8	71.180	8.89755	5.94	0.0001
Error	32	47.942	1.49819		
Total	44	122.741			

Appendix 3.47 Analysis of variance for K in leaf tissues of watermelon cultivar ‘Congo’ with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	24.936	6.23411		
Treatment	8	16.255	2.03189	0.56	0.7985
Error	32	115.152	2.03189		
Total	44	156.343	3.59849		

Appendix 3.48 Analysis of variance for Mn in leaf tissues of watermelon cultivar ‘Congo’ with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	0.03267	0.03267		
Treatment	8	2.16105	0.27013	3.44	0.005
Error	32	2.51318	0.7854		
Total	44	4.80492			

Appendix 3.49 Analysis of variance for Na in leaf tissues of watermelon cultivar 'Congo' with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	9.854	2.4635		
Treatment	8	462.598	57.8247	25.15	0.001
Error	32	73.582	2.2995		
Total	44	546.034			

Appendix 3.50 Analysis of variance for Fe in leaf tissues of watermelon cultivar 'Congo' with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	21.214	5.30356		
Treatment	8	11.140	1.39250	0.60	0.7742
Error	32	74.818	2.33806		
Total	44	107.172			

Appendix 3.51 Analysis of variance for Zn in leaf tissues of watermelon cultivar 'Congo' with nine concentrations of Nemarioc-AL phytonematicide.

Source	DF	SS	MSS	F	P
Replication	4	40.008	10.0021		
Treatment	8	119.729	14.9662	1.22	0.3193
Error	32	392.639	12.2700		
Total	44	552.377			

Appendix 4.1 Analysis of variance for dry shoot mass of watermelon cultivar ‘Congo’ to Velum and Nemarioc-AL and Nemafric-BL phytonematicides.

Source	DF	SS	MSS	F	P
Replication	23	1811.03	78.741		
Treatment	3	42.16	14.053	0.12	0.9451
Error	69	7764.35	112.527		
Total	95	9617.55			

Appendix 4.2 Analysis of variance for fruit number of watermelon cultivar ‘Congo’ to Velum and Nemarioc-AL and Nemafric-BL phytonematicides.

Source	DF	SS	MSS	F	P
Replication	23	43.740	1.90172		
Treatment	3	4.948	1.64931	0.92	0.4344
Error	69	123.302	1.64931		
Total	95	171.990			

Appendix 4.3 Analysis of variance for fruit mass of watermelon cultivar ‘Congo’ to Velum and Nemarioc-AL and Nemafric-BL phytonematicides.

Source	DF	SS	MSS	F	P
Replication	23	1.168	5077231		
Treatment	3	1.575	5251666	1.90	0.1384
Error	69	1.912	2770691		
Total	95	3.237			

Appendix 4.4 Analysis of variance for fruit number of watermelon cultivar ‘Charleston Gray’ to Velum and Nemarioc-AL and Nemafric-BL phytonematicides.

Source	DF	SS	MSS	F	P
Replication	23	0.78250	0.03402		
Treatment	3	0.07141	0.02380	0.50	0.1384
Error	69	3.28773	0.04765		
Total	95	4.14164			

Appendix 4.5 Analysis of variance for fruit mass of watermelon cultivar ‘Charleston Gray’ to Velum and Nemarioc-AL and Nemafric-BL phytonematicides.

Source	DF	SS	MSS	F	P
Replication	23	2.171	9438781		
Treatment	3	1.165	3883885	1.09	0.3610
Error	69	2.468	3577376		
Total	95	4.756			

Appendix 4.6 Analysis of variance for Ca in leaf tissues of watermelon cultivar 'Congo' to Velum and Nemarioc-AL and Nemafric-BL phytonematicides.

Source	DF	SS	MSS	F	P
Replication	23	8331.7	362.247		
Treatment	3	222.6	74.191	0.27	0.8468
Error	69	18959.1	274.769		
Total	95	27513.3			

Appendix 4.7 Analysis of variance for P in leaf tissues of watermelon cultivar 'Congo' to Velum and Nemarioc-AL and Nemafric-BL phytonematicides.

Source	DF	SS	MSS	F	P
Replication	23	20.495	0.8911		
Treatment	3	41.458	13.8193	10.00	0.001
Error	69	95.399	1.3826		
Total	95	157.353			

Appendix 4.8 Analysis of variance for Mg in leaf tissues of watermelon cultivar 'Congo' to Velum and Nemarioc-AL and Nemafric-BL phytonematicides.

Source	DF	SS	MSS	F	P
Replication	23	291.74	12.6843		
Treatment	3	15.59	5.1970	0.49	0.6929
Error	69	737.35	10.6863		
Total	95	1044.68			

Appendix 4.9 Analysis of variance for K in leaf tissues of watermelon cultivar ‘Congo’ to Velum and Nemarioc-AL and Nemafric-BL phytonematicides.

Source	DF	SS	MSS	F	P
Replication	23	2312.6	100.547		
Treatment	3	675.2	225.080	2.13	0.1046
Error	69	7298.1	105.769		
Total	95	10285.9			

Appendix 4.10 Analysis of variance for Mn in leaf tissues of watermelon cultivar ‘Congo’ to Velum and Nemarioc-AL and Nemafric-BL phytonematicides.

Source	DF	SS	MSS	F	P
Replication	23	21.7078	0.94382		
Treatment	3	1.2421	0.41402	0.68	0.5672
Error	69	42.0061	0.60878		
Total	95	64.9560			

Appendix 4.11 Analysis of variance for Na in leaf tissues of watermelon cultivar ‘Congo’ to Velum and Nemarioc-AL and Nemafric-BL phytonematicides.

Source	DF	SS	MSS	F	P
Replication	23	244.798	10.6434		
Treatment	3	40.132	13.3773	1.99	0.1241
Error	69	464.674	6.7344		
Total	95	749.604			

Appendix 4.12 Analysis of variance for Fe in leaf tissues of watermelon cultivar 'Congo' to Velum and Nemarioc-AL and Nemafric-BL phytonematicides.

Source	DF	SS	MSS	F	P
Replication	23	486.86	21.1676		
Treatment	3	68.77	22.9217	0.69	0.5614
Error	69	2293.36	33.2371		
Total	95	2848.98			

Appendix 4.13 Analysis of variance for Zn in leaf tissues of watermelon cultivar 'Congo' to Velum and Nemarioc-AL and Nemafric-BL phytonematicides.

Source	DF	SS	MSS	F	P
Replication	23	814.90	35.4304		
Treatment	3	25.76	8.5870	0.17	0.9162
Error	69	3481.86	50.4618		
Total	95	4322.52			