# INTERACTIVE EFFECTS OF CUCURBITACIN-CONTAINING PHYTONEMATICIDES AND BIOMUTI ON GROWTH OF CITRUS ROOTSTOCK SEEDLINGS AND ACCUMULATION OF NUTRIENT ELEMENTS IN LEAF TISSUES

BY

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# DECLARATION

I, Tlou Mokoele declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree Master of Science in Agriculture (Horticulture) has not been submitted previously by me or anybody for a degree at this or any other University. Also, this is my work in design and in execution, while related materials contained herein had been duly acknowledged.

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| Candidate: Tlou Mokoele | Date |
| Supervisor: Professor P.W. Mashela | Date |

# DEDICATION

To my beloved grandparents, Mamoliki Ruth and the late Phuti Johannes Mokoele

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ABSTRACT

Cucurbitacin-containing phytonematicides and a variety of unidentified soil microbes in suppressive soils (Biomuti) had been consistent in suppression of population densities of root-knot (*Meloidogyne spp*.) nematodes on various crops. However, information on suppressive effects of cucurbitacin-containing phytonematicides and Biomuti on citrus growth and suppression of the citrus nematode (*Tylenchulus semipenetrans*) had not been documented. The objective of this study therefore, was to determine the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides and Biomuti on growth and nutrient elements in leaf tissues of *Poncirus trifoliata* rootstock seedlings under greenhouse and field conditions. Uniform six-month-old citrus rootstock seedlings [Du Roi Nursery (Portion 21, Junction Farm, Letsitele)] were transplanted in 4 L plastic bags filled with growing mixture comprising steam-pasteurised (300°C for 1 h) loam and compost (cattle manure, chicken manure, sawdust, grass, woodchips and effective microorganisms) at 4:1 (v/v) ratio and placed on greenhouse benches. A 2 × 2 × 2 factorial experiment with the first, second and third factors being Nemarioc-AL phytonematicide (A) and Nemafric-BL phytonematicide (B) and Biomuti (M), were arranged in randomized complete block design, with 10 blocks*.* The treatment combinations were $A_0B_0M_0$, $A_1B_0M_0$, $A_0B_1M_0$, $A_0B_0M_1$, $A_1B_1M_0$, $A_1B_0M_1$, $A_0B_1M_1$ and $A_1B_1M_1$, with 1 and 0 signifying with and without the indicated factor. Treatments were applied at 3% dilution for each product as substitute to irrigation at a 17-day application interval. Under greenhouse conditions, seedlings were irrigated every other day with 300 ml chlorine-free tap water. Under field conditions, the study was executed using similar procedures to those in the greenhouse trial, except that the citrus seedlings were transplanted directly into the soil of a prepared field and seedlings were irrigated using drip irrigation for 2 h every

other day. At 64 days after transplanting, plant growth variables were measured and foliar nutrient elements were quantified using the Inductively Coupled Plasma Optical Emission Spectrometry (ICPE-9000). Data were subjected to analysis of variance using SAS software. Significant second and first order interactions were further expressed using the three-way and two-way tables, respectively. At 64 days after the treatments, under greenhouse conditions Nemarioc-AL × Nemafric-BL × Biomuti interaction was not significant ($P \leq 0.05$) on plant variables of seedling rootstocks in both experiments. In contrast, the Nemarioc-AL × Biomuti interaction was highly significant ($P \leq 0.01$) on stem diameter, contributing 52% in TTV of the variable in Experiment 1 (Table 3.1), whereas in Experiment 2 the interaction was highly significant on dry shoot mass, contributing 33% in TTV of the variable (Table 3.2). Relative to untreated control, the two-way matrix showed that the Nemarioc-AL × Biomuti interaction, Nemarioc-AL phytonematicide and Biomuti each increased stem diameter by 1%, 12% and 5%, respectively (Table 3.3). Relative to untreated control, the two-way matrix table showed that Nemarioc-AL × Biomuti interaction increased dry shoot mass by 10%, whereas Nemarioc-AL phytonematicide and Biomuti each increased dry shoot mass by 23% and 17%, respectively (Table 3.4). Nemarioc-AL × Nemafric-BL × Biomuti interaction was not significant ($P \leq 0.05$) for all plant growth variables in both experiments. However, Nemarioc-AL × Nemafric-BL interaction was significant for leaf number and stem diameter contributing 45% and 29% in TTV of the respective variables in Experiment 2 (Table 4.1). Relative to untreated control, two-way matrix table showed that the Nemarioc-AL × Nemafric-BL interaction and Nemafric-BL phytonematicides each increased stem diameter by 8% and 11% respectively, whereas Nemarioc-AL phytonematicides reduced stem diameter by 2% (Table 4.2). Also using two-way matrix table showed that Nemarioc-AL and Nemafric-

BL phytonematicides each increased leaf number by 1% and 7% respectively, whereas the Nemarioc-AL × Nemafric-BL interaction increased leaf number by 6% (Table 4.2). Nemafric-BL × Biomuti interaction was significant for stem diameter contributing 29% in TTV of the respective variable in Experiment 2 (Table 4.1). Using two-way matrix table showed that Nemafric-BL × Biomuti interaction and Nemafric-BL phytonematicide each increased stem diameter by 7%, whereas Biomuti alone reduced stem diameter by 6% (Table 4.3). Under greenhouse conditions, the second order Nemarioc-AL × Nemafric-BL × Biomuti interaction was highly significant for foliar Mg, contributing 5% in TTV of the variable in Experiment 1 (Table 3.4). Relative to untreated control, the three-way matrix table showed that the three factors, Nemafric-BL phytonematicide and Biomuti each reduced Mg by 33%, 35% and 53%, respectively, whereas Nemarioc-AL phytonematicide increased Mg by 12% (Table 3.5). Nemarioc-AL × Biomuti interaction was highly significant for foliar Mg, contributing 9% in TTV of the variable in Experiment 1 (Table 3.4). Relative to untreated control, the two-way matrix table showed that the Nemarioc-AL × Biomuti interaction and Nemafric-BL phytonematicide reduced Mg by 42% and 12%, respectively, whereas Nemarioc-AL phytonematicide alone increased Mg by 14% (Table 3.6). Nemarioc-AL × Biomuti interaction was highly significant for foliar Ca and Mg, contributing 59 and 4% in TTV of the respective variables in Experiment 1 (Table 3.4). Also using two-way matrix table showed that Nemarioc-AL phytonematicide and Biomuti separately reduced Ca by 12% and 22% respectively, whereas the Nemarioc-AL × Biomuti interaction increased Ca by 1% (Table 3.7). Relative to untreated control, the Nemarioc-AL × Biomuti interaction, Nemarioc-AL phytonematicide and Biomuti reduced foliar Mg by 26%, 21% and 33%, respectively (Table 3.7). Nemafric-BL × Biomuti interaction was highly significant for foliar Mg and P, contributing 50 and 21%

in Experiment 1, whereas in Experiment 2 the interaction was significant for foliar Ca and Mg, contributing 41% and 38% in TTV of the respective variables (Table 3.4). Relative to untreated control, the two-way matrix table showed that Nemafric-BL phytonematicide and Biomuti individually reduced Mg by 60% and 51%, respectively, whereas the Nemafric-BL × Biomuti interaction reduced Mg by 38% (Table 3.8). Also, in the two-way matrix table the Nemafric-BL × Biomuti interaction and Nemafric-BL phytonematicide each reduced Mg by 13% and 2%, respectively, whereas Biomuti alone increased P by 17% (Table 3.8). Relative to untreated control, Nemafric-BL phytonematicide and Biomuti reduced Ca by 29% and 18%, respectively, whereas Nemafric-BL × Biomuti interaction reduced Ca by 14% (Table 3.9). Using two-way matrix table showed that Nemafric-BL phytonematicide and Biomuti separately reduced Mg by 21%, whereas the Nemafric-BL × Biomuti interaction reduced Mg by 16% (Table 3.9). Interaction of Nemarioc-AL × Nemafric-BL × Biomuti had no significant effect on K, Na and Zn in both experiments. Under field conditions, the second order Nemarioc-AL × Nemafric-BL × Biomuti interaction was not significant for all the nutrient elements in Experiment 1. Nemarioc-AL × Biomuti was significant for Ca, K and highly significant for Mg and P, contributing 31, 8, 23 and 19% in TTV of the respective variables in Experiment 1 (Table 4.4). Relative to untreated control, two-way matrix table showed that Nemarioc-AL phytonematicide and Biomuti each increased Ca by 15% and 26% repectiviely, whereas the Nemarioc-AL × Biomuti increased Ca by 17% (Table 4.5). Interaction of Nemarioc-AL × Biomuti, Nemarioc-AL phytonematicide and Biomuti each reduced Mg by 48%, 70% and 37% (Table 4.5). Also using two-way matrix table showed that Nemarioc-AL phytonematicide and Biomuti each increased P by 4% and 5% respectively, whereas the Nemarioc-AL × Biomuti interaction increased P by 50% (Table 4.5). Realative to untreated control, Biomuti and Nemarioc-AL phytonematicide each reduced K by 10% and 5% respectively, whereas the Nemarioc-AL × Nemafric-BL interaction reduced K by 38% (Table 4.7). Nemafric-BL × Biomuti interaction was highly significant for Mg and Zn, contributing 11% and 29% in TTV of the respective variables in Experiment 1 (Table 4.4). Relative to untreated control, two-way matrix table showed that Nemarioc-AL phytonematicide and Biomuti separately increased Mg by 1% and 19% respectiviely, whereas the Nemafric-BL × Biomuti interaction reduced Mg by 43% (Table 4.6). Nemafric-BL × Biomuti interaction, Nemafric-BL phytonematicide and Biomuti each reduced Zn by 35%, 31% and 64% (Table 4.6). Using three-way matrix table showed that the Nemarioc-AL × Nemafric-BL × Biomuti, Nemarioc-AL × Nemafric-BL, Nemarioc-AL × Biomuti and Nemafric-BL × Biomuti interactions each increased Ca by 44%, 18%,10% and 24% (Table 4.8). Further the matrix showed that Nemarioc-AL, Nemafric-BL phytonematicides and Biomuti each increased Ca by 25%, 31% and 23% (Table 4.8). Under both greenhouse and field conditions, although second and first order interactions were not consistent of various variables, results demonstrated that the three products interacted significantly for various products. In conclusion, the study suggested that these innovative products could be used in combination with Biomuti to stimulate plant growth but had antagonistic effects on accumulation of nutrient elements in *P. trifoliata* rootstock seedlings, suggesting that the products should be applied separately.

# CHAPTER 1
# RESEARCH PROBLEM

## 1.1 Background

Citriculture is one of the world's largest agricultural industries in the world, with South Africa ranking 12th in terms of production (Pretorius and Le Roux, 2017). Most citrus trees are raised on grafted rootstocks due to numerous abiotic and biotic factors that have negative impact on plant growth and fruit quality (Cohn, 1972). The citrus nematode (*Tylenchulus semipenetrans* Cobb 1913) is considered one of the major biotic challenges in citrus production because it causes significant economic losses in all citrus producing regions of the world (Duncan, 2005). Fruit losses induced by *T. semipenetrans* in severely-infested Florida citrus orchards ranged from 10 to 30% (Verdejo-Lucas and McKenry, 2004). However, yield losses in arid and high salinity-affected western states of the USA could be as high as 50% (Duncan, 2005). Cohn (1972) estimated annual global citrus yield losses due to the citrus nematode from 9 to 12%.

### 1.1.1 Description of the research problem

Worldwide, *T. semipenetrans* is one of the most damaging pests of citrus (Duncan and Cohn, 1990) and it causes serious damage in citrus trees (Milne, 1982). Slow decline of citrus is a disease associated with infection by *T. semipenetrans* in citrus (Duncan, 2005; Verdejo-Lucas and McKenry, 2004), characterised by leaf chlorosis, leaf abscission, die-back of young twigs, smaller leaves or fruit and imbalances of essential nutrient elements (Mashela, 1992). Management of *T. semipenetrans* depended on the use of grafted nematode-resistant rootstocks, since the withdrawal of synthetic nematicides (Duncan, 2009). Due to host differentiation, three biotypes of this nematode are recognized, namely, citrus, mediterranean and poncirus biotypes

(Verdejo-Lucas, 1992). Certain races are able to invade nematode-resistant rootstocks, which have been a primary means of pest management for the citrus nematode (Inserra *et al*., 1980; O'Bannon and Esser, 1985).

Duncan and Cohn (1990) postulated that nematode population densities that suppress tree growth and fruit yield were influenced by factors that included aggressiveness of the nematode population, soil characteristics, susceptibility of the rootstock, presence of other pathogens and orchard management practices. Studies have since shown that *T. semipenetrans* interacted with salinity in a manner which resulted in cyclic nematode population densities (Mashela *et al*., 1992a). *Tylenchulus semipenetrans* infection interferes with salt-tolerance in salt-tolerant citrus rootstocks (Mashela and Nthangeni, 2002a), whereas salinity interfered with nematode-resistance (Mashela *et al*., 1992b). Thus, even if nematode-resistant rootstocks were considered the most effective method to manage plant-parasitic nematodes, amendment of new virulent isolates of *T. semipenetrans* that are capable of overcoming resistance in resistant genotypes might contravene the perceived economic importance of these rootstocks (Inserra *et al*., 1980).

The phytonematicides constitute allelochemicals, which are secondary metabolites produced during respiration, with limited physiological roles (Shrivasta and Jha, 2016). Such allelochemicals had been defined as non-nutritional chemicals produced by one plant and affects the growth, germination, health and behaviour of another plant (Day *et al*., 2003). Interactions of allelochemicals with plants and microbes involve stimulatory as well as inhibitory effects (Molisch, 1937). Mashela *et al*. (2013) reported that wild watermelon (*Cucumis africanus)* and wild cucumber (*Cucumis myriocarpus)* fruit extracts possess nematicidal properties. The fruit crude extracts from the two *Cucumis* species had been used to produce Nemarioc-AL and Nemafric-BL phytonematicides through the fermented crude extract technology (Mashela *et al.,* 2015).

Nemarioc-AL and Nemafric-BL phytonematicides contain active ingredients which are allelochemical compounds that could be phytotoxic. Mashela *et al*. (2015) used the stimulation concentration point to develop the concept of Mean Concentration Stimulation Point [MCSP = $D_m + (R_h/2)$]. The MCSP values stimulate plant growth, but suppress nematode population densities (Mashela *et al*., 2015). The use of plant extracts that contain nematicidal properties could be effective, cheaper, healthier and safer control measures than the synthetic chemical nematicides (El-Zawahry *et al*., 2014).

The use of phytonematicides as an alternative strategy in suppression of nematode numbers is gaining international recognition due to their environment-friendliness (Ballesteros *et al.,* 1992). Mashela *et al*. (2015) argued that phytonematicides were introduced to lessen conventional organic amendments problems in suppression of plant-parasitic nematodes such as (a) inconsistent results in nematode suppression, (b) large quantities (10–500 t/ha) which were required to achieve nematode suppression, (c) unavailability of the materials; (d) high transport costs to haul the materials from the production site to that of use; (e) negative period, with the subsequent time-lag to allow for microbial decomposition in order to avoid negative periods and (f) decrease in soil pH, which inherently imbalances the availability of essential nutrient elements in the soil. Nemarioc-AL/AG and Nemafric-BL/BG phytonematicides had been consistent in suppressing nematode numbers in various crops, with occasional incidents of stimulated plant growth (Mashela *et al.,* 2015).

1.1.2 Impact of the research problem

*Tylenchulus semipenetrans* is one of the major parasitic pests in the plant rhizosphere that causes serious damage to citrus trees. The existence of this nematode could cause long-term production losses in all citrus-producing regions (Duncan and Cohn, 1990). Verdejo-Lucas and McKenry (2004) postulated that the estimated damage induced by *T. semipenetrans* ranged from 10 to 30%. However, the intensity of damage depends on the susceptibility of the rootstock, degree of aggressiveness of the nematode, soil characteristics, the level of infection, the presence of other pathogens and orchard management practices (Duncan and Cohn, 1990).

The host range of *T. semipenetrans* includes all Citrus species and most hybrids of citrus with other members of the Rutaceae family, such as trifoliata orange [*Poncirus trifoliata* (L.) Raf]. Non-rutaceous plants, such as grape (*Vitis vinifera* L.), olive (*Olea europea* L.) and persimmon (*Diospyros* spp.), are also hosts of the citrus nematode (Verdejo-Lucas *et al.,* 2000). *Poncirus trifoliata* rootstock was the rootstock of choice for most South African citrus farmers (Rabe and Von Broembsen, 1991). However, *P. trifoliata* was declared not to be resistant to the local biotype and it also accumulated salt ions (Rabe and Von Broembsen, 1991). Areas with salinity problems might possibly increase severity of citrus decline (Duncan *et al*., 1995).

*Tylenchulus semipenetrans* numbers were increased by Nemarioc-AG phytonematicide which was phytotoxic to rough lemon (*Citrus jambhiri)* (Maile *et al*., 2013). In phytonematicides, nematode numbers could either be stimulated, neutral or inhibited through the density-dependent growth patterns, with apparent inconsistent results in nematode suppression (Mashela *et al*., 2017). Also, phytotoxicity from phytonematicides had been documented from crude extracts of various plants on a wide range of crops (Akhtar and Malik, 2000; Musabyimana *et al.,* 2000; Susan *et al.,* 2008). Mafeo (2012) postulated that the phytotoxicity of phytonematicides was mostly depended upon the developmental stage of plants, with germination being highly sensitive to cucurbitacin-containing phytonematicides.

#### 1.1.3 Possible causes of the research problem

The suspension of synthetic chemical nematicides due to their various negative impacts on the environment, particularly breakdown of ozone layer and high levels of chemical residues in produce led to increased research and development of different approaches to replace synthetic chemical nematicides (Mashela *et al*., 2015). The invasion and reproduction of *T. semipenetrans* are influenced by soil type and rootstocks (Iqbal *et al.,* 2007). However, there are limited rootstocks which are resistant or tolerant to diseases or pests in any other area, lacking wide range rootstock selection (Verdejo-Lucas and Mckenry, 2004). Options for managing *T. semipenetrans* such as use of organic amendments and nematode-resistant rootstocks have had serious limitations as shown above.

#### 1.1.4 Proposed solutions

Development of nematode management approaches, using tested alternative tactics used in other crops (Pofu and Mashela, 2011), is indispensable in the future development of appropriate management strategies of *T. semipenetrans* in citrus production. Duncan (2015) stated that there is urgent need for discovery and registration of new nematicides and nematode-resistant rootstocks for use in citrus. The approaches such as use of crude extracts from plants developed locally (Mashela, *et al*., 2013) and various potential replacements to methyl bromide developed (Bello *et al*., 1998). The widely tested Nemarioc-AL and Nemafric-BL phytonematicides are being researched and developed for management of nematodes in South Africa (Mashela *et al*., 2015), along with or without Biomuti which is a product being tested by the ARC. The components of Biomuti have not yet been established. The product has the ability to aid recovery of depleted soil as well as fortification and also consist of unconfirmed nematode inhibiting effects. However, these three products will increase the uptake of nutrients, improve growth of citrus seedlings and increase soil biological activity in addressing issues in an environment-friendly way.

### 1.1.5 General focus of the study

The study intends to improve citrus seedling growth, increase nutrient uptake and manage *T. semipenetrans* population densities by establishing the appropriate interactive concentration of Nemarioc-AL, Nemafric-BL phytonematicides and Biomuti. The latter is the product of the Agricultural Research Council, with unknown constituents.

## 1.2 Problem statement

The withdrawal of synthetic nematicides due to their environment-unfriendly led to limited options for managing the *T. semipenetrans*. Information on the interactive effects of phytonematicides and Biomuti on growth of *P. trifoliata* citrus seedling, nutrient elements and successful suppression of *T. semipenetrans* is scant. The researcher intends to establish the appropriate interactive dosage of Nemarioc-AL and Nemafric-BL phytonematicides and Biomuti, whether the collaborative use of these three sustainable products would be beneficial on growth, nutrient uptake and also serve as successful suppressants of *T. semipenetrans* population densities on *P. trifoliata* citrus rootstock seedlings.

## 1.3 Rationale of the study

The study intends to successfully improve citrus rootstock seedlings growth, increase nutrient uptake and manage *T. semipenetrans* population densities by using locally-tested products due to the recent withdrawal of synthetic nematicides from the agrochemical markets, however most of the management approaches had been focusing on environment-friendly nematode management tactics (Ujvary, 2002). The successful interaction of Nemarioc-AL, Nemafric-BL and Biomuti would enhance the potential status of these three sustainable products mixed or applied separately in citrus production to mitigate the drawbacks of conventional organic amendments (Mashela, 2002). Following the withdrawal of synthetic nematicides, options for managing *T. semipenetrans* population is scant. The study intends to determine the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides and Biomuti on growth and nutrient elements in leaf tissues of *Poncirus trifoliata* rootstock seedlings.

## 1.4 Purpose of the study

### 1.4.1 Aim

The aim of the study constituted the establishment of appropriate combinations of cucurbitacin-containing phytonematicides and Biomuti for management of *T. semipenetrans* on citrus seedlings.

### 1.4.2 Objectives

1. To determine the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides and Biomuti on growth and nutrient elements in leaf tissues of *P. trifoliata* under greenhouse conditions.
2. To investigate the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides and Biomuti on growth and nutrient elements in leaf tissues of *P. trifoliata* under field conditions.

## 1.5 Reliability, validity and objectivity

In this study, reliability of data was based on statistical analysis of data at the probability level of 5%, whereas validity was achieved through using factorial experiments (Leedy and Ormrod, 2005). Objectivity was ensured by discussing results on the basis of empirical evidence (Leedy and Ormrod, 2005).

## 1.6 Bias

Bias was described as any influence, conditions or set of conditions that singly or altogether distort the data (Leedy and Ormrod, 2005). In this study, bias was minimised by ensuring that the experimental error in each experiment was reduced through increased blocks and randomisation of the treatments.

## 1.7 Scientific significance of the study

The two phytonematicides and Biomuti have different active ingredients. The findings of the study would show whether the products should be combined or used singularly in management of nematodes in citrus production.

## 1.8 Structure of mini-dissertation

Following detailed outlining of the research problem (Chapter 1), the work done and not yet done on the research problem would be reviewed (Chapter 2). Then, each of the two subsequent chapters (Chapters 3 and 4) would address each of the two objectives in sequence. In the final chapter (Chapter 5), findings in all chapters would be summarised and integrated to provide the significance of the findings and recommendations with respect to future research, culminating in conclusions which would tie the entire study together. The mini-dissertation adopted the Harvard referencing style and citation in text, as approved by the Senate of the University of Limpopo.

# CHAPTER 2
# LITERATURE REVIEW

## 2.1 Work done on the problem statement

Non-nutritional chemicals produced by one organism that affects the growth, germination, health and behaviour of other organisms are known as allelopathy (Day *et al*., 2003). Molisch (1937) postulated that they constitute of chemical interaction among all plants and microbes involving stimulatory as well as inhibitory effect. An allelopathic quality reduces the vigour of native plant species and limits their productivity and interferes with harvesting (Sharma *et al*., 2005). Interaction of cucurbitacin-containing phytonematicides with other sustainable products that have nematicidal properties may offer a highly effective and reliable strategy for sustainable management of nematode population densities (Mashela *et al*., 2015). Sikora and Fernandez (2005) postulated that nematicides might consistently play a major role in the reduction of nematode populations.

### 2.1.1 Under greenhouse conditions

Nemarioc-AL and Nemafric-BL phytonematicides are being researched and developed as alternatives to environment-friendly synthetic nematicides in South Africa for use in production of various crops (Mashela and Dube, 2014). Efficacy trials using any of the two products had been conducted against root knot nematodes (*Meloidogyne* spp.) and the *Tylenchulus semipenetrans* under diverse conditions (Mashela *et al.,* 2015). Chokoe (2017) examined non-phytotoxic concentration of Nemarioc-AL and Nemafric-BL phytonematicides separately on green bean, reported reduction of nematode variables as high as 100% by the two phytonematicides under greenhouse and microplot conditions. Nemarioc-AL and Nemafric-BL phytonematicides reduced root galls by 28 to 72 and 43 to 67%, respectively (Mashitoa, 2017).

Mashela and Pofu (2012) asserted that cowpea treated with *C. myriocapus* increased fresh and dry shoot which demonstrated the “fertilizer effect”. However, issues of phytotoxicity in phytonematicides limit the adoption and use of these products as alternative products for managing nematode numbers (Mashela *et al.,* 2015). Mathabatha *et al*. (2016) established the MCSP values on *Citrus volkameriana* for Nemarioc-AL and Nemafric-BL phytonematicides as 8.6 and 6.3%, respectively. However, other studies established MCSP values for beetroot (*Beta vulgaris*) were 18.1 and 6.4% (Mashitoa, 2017), for green bean (*Phaseolus vulgaris*) were 2.11 and 0.27% (Chokoe, 2017) for Nemarioc-AL and Nemafric-BL phytonematicides under greenhouse conditions. Lower MCSP values were observed specifically for Nemafric-BL phytonematicide as compared to Nemarioc-AL phytonematicide in most trials.

The interaction between nematodes and Nemarioc-AG phytonematicide had highly significant effects on dry root mass, dry shoot mass, plant height and stem diameter (Maile, 2013). The interaction had no effect on all plant variables, except that the interaction had negligence effect and reduced plant height and stem diameter by 3 and 2%. Nematodes had been reported to stimulate growth at population densities below the damage threshold (Mashela *et al*., 2010; Wallace, 1973), whereas nematode population densities above the damage threshold, result reduction of plant growth (Maile, 2013). Rabothata (2017) tested the interaction of vesicular arbuscular mycorrhiza (VAM), nematode and Nemafric-BL phytonematicide for their effects on growth and nutritional content of *Cleome gynandra* and observed that the second order interaction for VAM, nematode and Nemafric-BL phytonematicide was highly significant for plant height, contributing 54% in TTV of the variable. However, the interaction reduced plant height by 4%.

Sithole (2016) suggested that plant height responded rapidly to higher concentrations of Nemafric-BL phytonematicide than other plant organs in *Pelargonium sidoides*. However, plant height in other studies was shown to suggest phytotoxicity of cucurbitacin-containing phytonematicides on *C. gynandra* (Rabothata, 2017), tomato (Maake, 2017; Nyamandi, 2017). Treatment combination between nematode and Nemarioc-AL phytonematicide reduced final nematode population densities of *Meloidogyne* species and nodule numbers by 79 and 20%, respectively (Mashela and Pofu, 2012). Nematode and *Bradyrhizobium* reduced fresh shoot mass, dry shoot mass and nodule mass of cowpea (*Vigna unguiculata*) by 57, 59 and 64%, respectively (Mashela and Pofu, 2012).

The interaction between VAM and Nemafric-BL phytonematicide increased Zn by 33% in foliar tissues of cleomes, but reduced root galls by 64% (Rabothatha, 2017). In contrast the interaction between VAM, nematode and Nemafric-BL phytonematicides had no effects on the nutrient elements but had significant effects on gall rating contributing 2% in TTV of the variable. *Trichoderma* species are widely used organisms for biological disease control and for plant improvement (Harman *et al.,* 2004). A number of *Trichoderma* strains colonize plant roots of dicots and monocots (Harman and Shoresh, 2007). Nzanza (2011) studied the interaction of *Tichoderma harzianum* and VAM and observed that *T. harzianum* and VAM synergistically improved most of the growth variables in tomato (*Solanum lycopersicum* L.) seedlings. Results suggested that the two products when combined improved shoot length, root length, dry shoot mass and dry root mass on tomato plants (Nzanza, 2011).

*Lantana camara* has many negative impacts encompassing the potential to disrupt succession cycle, displacing native biota resulting in decreased biodiversity (Day *et al*., 2003; Ghisalberti, 2000). Extracts from the plant can be employed to combat antimicrobial, fungicidal, insecticidal and nematicidal problems (Shrivastava and Jha, 2016). Udo *et al.* (2014) observed that two control agents *L. camara* and *Paecilomyces lilacinus* were highly effective in nematode suppression as shown by their reduction of root galls and egg mass, with improvement of plant growth when used at 0.80 g $mL^{-1}$ leaf extract in combination with double application of the bionematicide. Rape seed (*Brassica napus* L.) and *L. camara* leaf extracts exhibited 92 and 93% inhibition of J2 hatch and 62 and 73% J2 mortality, respectively (Feyisa *et al*., 2016).

Decaying leaves used in combination with *P. aeruginosa* distinctly suppressed nematode population densities of *M. javanica* and subsequent J2 development in Mungbean (Ali *et al.,* 2001). *Pseudomonas aeruginosa* used in combination with *L. camara* extract resulted in significant reduction of root galls and shoot mass (Ali *et al*., 2001). Ali *et al*. (2001) observed that growth-promoting bacteria such as *P. aeruginosa* with decaying *L. camara* leaves provided superior suppression of *Meloidogyne javanica* and enhanced shoot growth. *Lantana camara* when combined with nemacur reduced root-knot nematode numbers by between 79.8 and 82.6% (Kagai *et al.,* 2012). *Lantana camara* with nemacur increased cumulative flower yield, plant height and also reduced root galling by 79.3% (Kagai *et al.,* 2012).

In agriculture numerous plant extracts are already used for crop and post-harvest control against pests (Colin and Pussemier, 1992). Neem (*Azadirachta indica*) extract is also one of the most important plant extract which is regarded as cost-effective and environmentally friendly (Sarawaneeyaruk *et al.,* 2015). In general, most pests are vulnerable to the physiological effects of *A. indica* (Mordue-Luntz and Nisbet, 2000). Treatment combination between leaf extracts of marigold (*Tagetes erecta*) and *A. indica* displayed 100% inhibition of egg hatch and larva mortality (Feyisa *et al*., 2016). *Azadirachta indica* and Confidor® (a.i. Imidacloprid) when combined reduced final nematode numbers and improved yield of tomato plants (Gurjar *et al.,* 2016).

Maile (2013) who studied the response of *T. semipenetrans* to crude extracts of indigenous *Cucumis* fruits with and without effective microorganisms in citrus production observed that the interaction between effective microorganisms (EM), nematodes and *C. myriocarpus* improved plant growth of rough lemon, contributing 4 and 5% in TTV of dry root mass and shoot mass, respectively. Relative to untreated control, EM when combined with Nemafric-BG phytonematicide increased dry root mass and rot/shoot ratio by 18 and 25%, respectively (Maile, 2013).

2.1.2 Under field conditions

Duke (2010) reported that allelopathic effects can be and have been measured in controlled environment settings such as laboratory and greenhouse, but replicating the effects in the field has been and continues to be difficult. Similarly, Lebea (2017) indicated that the MCSP and ∑k values appeared to be location specific since they were not similar in various locations. Under field conditions, Nemarioc-AL and Nemafric-BL phytonematicides had no significant effect on all plant variables in beetroot (*Beta vulgaris* L.) and butternut squash (*Cucurbita pepo* L.) (Mashitoa, 2017; Lebea, 2017). Similarly, Maake (2017) observed that the interaction between Nemarioc-AL and Nemafric-BL phytonematicides had no significant effect on all plant growth variables of tomato plants, except for dry shoot mass, where the combined effects reduced the variable by 8% under microplot conditions. The interaction of Nemarioc-AL and Nemafric-BL phytonematicides increased Na and S by 12 and 41% under field conditions (Maake, 2017). Also, the interaction of Nemarioc-AL and Nemafric-BL phytonematicides increased K by 8%, but reduced Mg, Mn and S by 14, 82 and 1%, respectively (Maake, 2017).

In Nemarioc-AL phytonematicide reduced gall rating by 48 to 56%, whereas relative to untreated control, Nemafric-BL phytonematicide reduced gall rating from 33 to 56% in *P. sidoides* plants (Seshweni, 2016). Neem extracts also enhanced the performance of other organic amendments when used in combination with other products under field conditions (Oka *et al*., 2007). The mixture of neem leaf and wild garlic plant extracts (GarNeem-FPE) at the ZZ2 was more effective in reducing population densities of whiteflies (*Bemisia tabaci*, Homoptera: Aleyrodidae) and aphids (*Myzus persicae,* Homoptera: Aphidae) than either plant extract applied in isolation (Nzanza and Mashela, 2012). Nzanza and Mashela (2012) demonstrated that as seasons continued the number of aphids and whiteflies increased from 3.30 to 8.17 (148%) and from 6.70 to 16.97 (153%), in winter and summer, respectively. However, it was suggested that the fermented plant extracts had repellent effects as opposed to other mechanisms such as anti-feedant activities (Nzanza and Mashela, 2012).

In the study were various oil-seed cakes such as *A. indica*, castor (*Ricinus communis*), groundnut (*Arachis hypogaea*), linseed (*Linum usitatissimum*), sunflower (*Helianthus annuus*) and soybean (*Glycine max*) in combination with *P. fluorescens* resulted in less multiplication rate of nematodes (Rizvi *et al.,* 2012). The interactive effects of different oil seed cakes with *P. fluorescens* lead to significant improvement of plant-growth parameters such as plant weight, percent pollen fertility, pod numbers, rootnodulation and chlorophyll content of chickpea, with the most effective combination of *P. fluorescens* which was observed with neem cake (Rizvi *et al.,* 2012).

The combination of treatments significantly improved the plant growth parameters of tobacco (*Nicotiana tabacum* L.*)* (Raveendra *et al.*, 2011). Relative to untreated control, the combination of neem cake and Furadan® 3GR and neem cake and marigold each significantly increased plant height, dry shoot mass, number of leaves/plant, root length and dry root mass in *N. tabacum* (Raveendra *et al.*, 2011). Similarly, the combination of neem cake and Thimet® 100G (a.i. phorate) significantly increased the plant variables mentioned above except number of leaves (Raveendra *et al.*, 2011). Madaure and Mashela (2017) demonstrated that the second order interaction among Nemafric-BL phytonematicide, *T*. *harzianum* and *Steinernema feltiae* improved plant growth, whereas the nematode variables were not significantly affected by the three products under field condition which were infested with *Meloidogyne* species. The second order interaction in Nemarioc-AL phytonematicide, Nemacur and Biocult had significant effect for eggs in roots and total nematodes, reducing the variables by 42 and 36%, respectively (Seshweni, 2016).

## 2.2 Work not yet done on the problem statement

The two Nemarioc-AL and Nemafric-BL phytonematicides and Biomuti, produced in South Africa, had been dubbed sustainable products that could be used in climate-smart agriculture for sustainable nematode management, since the materials contain different active ingredients. Information on the interactive effects of the three products on growth, accumulation of essential nutrient elements and suppression of *T. semipenetrans* in commonly used citrus rootstocks seedling in South Africa had not been documented. These products contain different active ingredients and have the potential to serve as bio-nematicide and bio-stimulants, since they are environment-friendly and they might be adopted in low-input agricultural systems. The objective of the study was to determine the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides and Biomuti on growth and nutrient elements in leaf tissues of *Poncirus trifoliata* rootstocks seedlings under greenhouse and field conditions.

# CHAPTER 3
# INTERACTIVE EFFECTS OF NEMARIOC-AL AND NEMAFRIC-BL PHYTONEMATICIDE AND BIOMUTI ON CITRUS SEEDLINGS UNDER GREENHOUSE CONDITIONS

## 3.1 Introduction

Worldwide, agricultural production had been challenged by increasing population densities of nematodes (Nicol *et al.,* 2011). Due to the recent withdrawal of fumigant synthetic nematicides from the agrochemical markets, most of the management approaches had been focusing on environment-friendly nematode management tactics (Ujvary, 2002). The crop losses induced by citrus *T. semipenetrans* in severely-infested Florida citrus orchards ranged from 10 to 30% (Verdejo-Lucas and McKenry, 2004). In arid and high salinity-affected western states of the USA, yield losses due to *T. semipenetrans* infection could be as high as 50% (Duncan, 2005). Cohn (1972) estimated annual global citrus yield losses due to *T. semipenetrans* infection to be in the range of 9 to 12%. For many years after World War II, citriculture had been much reliant on synthetic chemical pesticides for the management of population densities of *T. semipenetrans* (Gamliel *et al.,* 2000). The suspension of synthetic nematicides from the agrochemical markets led to increased research and development of different approaches as alternatives to synthetic nematicides (Mashela *et al*., 2015).

Uses of plant extracts that contain nematicidal chemicals had been viewed as being safer than the use of synthetic chemical nematicides, which had been environment-unfriendly (El-Zawahry *et al*., 2014). In some cases, the use of environment-friendly products with nematicidal properties could be viewed as potential replacement for the environment-unfriendly synthetic chemical nematicides (Mashela *et al*., 2015). The two Nemarioc-AL and Nemafric-BL phytonematicides and Biomuti, produced in South Africa, had been dubbed sustainable products that could be used in climate-smart agriculture for sustainable nematode management. However, the interactive effects of the three products on growth and accumulation of essential nutrient elements in commonly used citrus rootstock seedlings in South Africa had not been documented. The objective of the study was to determine the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides and Biomuti on growth and nutrient elements in leaf tissues of *Poncirus trifoliata* (L.) rootstock seedlings under greenhouse conditions.

## 3.2 Materials and methods

### 3.2.1 Description of the study area

A greenhouse trial was conducted at the Green Biotechnologies Research Centre (GBRC), University of Limpopo, South Africa (23°53'10"S, 29°44'15"E) during autumn (January-March) 2016 and repeated in (July-September) spring 2017. The roof of the greenhouse was covered with a green net to allow for 85% incident radiation to pass through. Ambient temperature was retained at 25°C using thermostat-controlled fans on the northern side of the greenhouse, with the wet wall installed at the southern side to ensure that relative humidity was retained between 70 and 80%. The length and width of the greenhouse were 100 m and 20 m, respectively, with hot air being sucked out by fans, which created heterogenous conditions within the greenhouse.

### 3.2.2 Treatments and research design

In the 2 × 2 × 2 factorial experiment, the first order, second and third factors comprised Nemarioc-AL (A) phytonematicide, Nemafric-BL (B) phytonematicide and Biomuti (M), respectively. The eight treatment combinations, namely, $A_0B_0M_0$, $A_1B_0M_0$, $A_0B_1M_0$, $A_0B_0M_1$, $A_1B_1M_0$, $A_1B_0M_1$, $A_0B_1M_1$ and $A_1B_1M_1$, were arranged in a randomised complete block design, with 10 blocks. Phytonematicides and Biomuti were each applied at 3%. In first and second order interactions, treatments were reduced by half and one-third, respectively.

### 3.2.3 Procedures

The greenhouse trials were established by transplanting six-month-old uniform citrus rootstock seedlings into 5 L black polyethylene bags filled with the growing mixture comprising pasteurised (300°C for 1 h) loam soil and compost (cattle manure, chicken manure, sawdust, grass, woodchips and Effective microorganisms) from ZZ2 at 4:1: (v/v) ratio. The bags were placed on the greenhouse benches at 0.25 m inter-row and 0.25 m intra-row spacing (Legend 3.1).

Two days after transplanting, each seedling was fertilised using 5 g 2:3:2 (26) NPK + 0.5% Zn + 5% S + 5% Ca which provided 74.3 g N, 111.4 g P, 74.3 g K, 5 g Zn and 50 g Ca per seedling. Also, 1 g 2:1:2 (43) Multifeed (Nulandies, Johannesburg) fertiliser to provide a total of 0.175 mg N, 0.16 mg K and 0.16 mg P, 0.45 mg Mg, 0.378 mg Fe, 0.0375 mg Cu, 0.175 mg Zn, 0.5 mg B, 1.5 mg Mn and 0.035 mg Mo per ml water. Kirchhoffs Ludwig's organic insecticide spray plus (a.i. canola oil, garlic extract, Pyrethrins and Piperonyl butoxide) was used to control mites and later hand picking was done of giant swallowtail butterfly since they didn't appear in more than 10 rootstock seedlings. Phytonematicides were prepared by collecting mature fruits from wild cucumber (*Cucumis myriocarpus* Naudin.) and wild watermelon (*Cucumis africanus* L.F.) from cultivated fields at GBRCE and prepared prior to drying as described previously (Mafeo and Mashela, 2009).

Fruits from the two *Cucumis* species were cut separately into pieces and each dried at 52°C for 72 h in air-forced ovens (Mafeo and Mashela, 2009). Thereafter, dried fruits were separately ground in a Wiley mill to pass through a 1-mm opening sieve. Approximately 80 g *C. myriocarpus* powdered fruit and 40 g *C. africanus* powdered fruit were each placed in separate 20 L air-tight sealed plastic containers (Mashela *et al*., 2016). In each air-tight container, 300 ml molasses, 100 g brown sugar and 300 ml effective microorganisms (EM) and 16 L chlorine-free tapwater were added. Allowance of gasses to escape from each container was provided through a 5-mm-diameter tube, with one end glued to a hole on the lid of the 20 L container, with the outlet-end dangling into a 2 L bottle that was half-filled with chlorine-free tapwater (Mashela *et al*., 2016). The products were ready to be used after a 14-day fermentation period, when pH was at 3.7 or less.

Legend 3.1 Citrus seedlings treated with Nemarioc-AL and Nemafric-BL phytonematicides with and without Biomuti under greenhouse conditions.

### 3.2.4 Data collection

At 63 days after initiating treatments, plant height was measured from the soil level to the terminal end of the flag leaf. Chlorophyll content was measured using a chlorophyll meter (MINOLTA, SPAD-502) and stem diameter measured 5-cm from the cut end of the stem using a digital vernier caliper. Leaf number was counted per plant and shoots were cut at the soil level. Fresh shoots were oven-dried for 72 h at 52°C for dry matter determination.

Mature oven dried leaves were separated from stems and ground using a mortar and pestle. Approximately 0.10 g ground dried leaves were digested in 40 ml of 5% nitric acid ($HNO_3$), followed by placing the container on a vortex meter to allow for complete wetting. The mixture was magnetically stirred, thereafter incubated in a 95°C water bath for an hour, cooled down at room temperature overnight. Samples were then filtered and poured into 50 ml centrifuge tubes which are covered with a foil. Samples were then submitted to Limpopo Agro-Food Technology Station (LATS) and calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P) and zinc (Zn) quantified using the Inductively Coupled Plasma Optical Emission Spectrometry (ICPE-9000).

### 3.2.5 Data analysis

Data were subjected to factorial analysis of variance (ANOVA) through the SAS software (SAS Institute, 2008). Prior to ANOVA, leaf number data were transformed through $\log_{10}(x + 1)$ to normalise the variances (Gomez and Gomez, 1984). Degrees of freedom and their associated mean sum of squares were partitioned to provide the total treatment variation (TTV) for different sources of variation. Significant second and first order interactions were further expressed using the three-way and two-way matrix tables, respectively, in order to allow for the determination of the magnitude and direction of the main factors relative to untreated controls (Steyn *et al*., 2003). Unless otherwise stated, results were discussed at the probability level of 5%.

## 3.3 Results

### 3.3.1 Interactive effects on plant growth variables

Nemarioc-AL × Nemafric-BL × Biomuti interaction was not significant ($P \leq 0.05$) on plant variables of citrus rootstock seedlings in both experiments. In contrast, the Nemarioc-AL × Biomuti interaction was highly significant ($P \leq 0.01$) on stem diameter, contributing 52% in TTV of the variable in Experiment 1 (Table 3.1), whereas in Experiment 2 the interaction was highly significant on dry shoot mass, contributing 33% in TTV of the variable (Table 3.2). Relative to untreated control, the two-way matrix showed that the Nemarioc-AL × Biomuti interaction, Nemarioc-AL phytonematicide and Biomuti each increased stem diameter by 1, 12 and 5%, respectively (Table 3.3). The Nemarioc-AL × Biomuti interaction and Biomuti each had a negligent effect on stem diameter although the direction of the effect suggested that the material increased the variable (Table 3.3). Relative to untreated control, the two-way matrix table showed that Nemarioc-AL × Biomuti interaction increased dry shoot mass by 10%, whereas Nemarioc-AL phytonematicide and Biomuti each increased dry shoot mass by 23 and 17%, respectively (Table 3.4).

### 3.3.2 Interactive effects on essential nutrient elements

The second order Nemarioc-AL × Nemafric-BL × Biomuti interaction was highly significant for foliar Mg, contributing 5% in TTV of the variable in Experiment 1 (Table 3.4). Relative to untreated control, the three-way matrix table showed that the three factors, Nemafric-BL phytonematicide and Biomuti each reduced Mg by 33, 35 and

53%, respectively, whereas Nemarioc-AL phytonematicide increased Mg by 12% (Table 3.5). Nemarioc-AL × Biomuti interaction was highly significant for foliar Mg, contributing 9% in TTV of the variable in Experiment 1 (Table 3.4). Relative to untreated control, the two-way matrix table showed that the Nemarioc-AL × Biomuti interaction and Nemafric-BL phytonematicide reduced Mg by 42 and 12%, respectively, whereas Nemarioc-AL phytonematicide alone increased Mg by 14% (Table 3.6). Nemarioc-AL × Biomuti interaction was highly significant for foliar Ca and Mg, contributing 59 and 4% in TTV of the respective variables in Experiment 1 (Table 3.4). Also using two-way matrix table showed that Nemarioc-AL phytonematicide and Biomuti separately reduced Ca by 12 and 22% respectively, whereas the Nemarioc-AL × Biomuti interaction increased Ca by 1% (Table 3.7). Relative to untreated control, the Nemarioc-AL × Biomuti interaction, Nemarioc-AL phytonematicide and Biomuti reduced foliar Mg by 26, 21 and 33%, respectively (Table 3.7).

Nemafric-BL × Biomuti interaction was highly significant for foliar Mg and P, contributing 50 and 21% in Experiment 1, whereas in Experiment 2 the interaction was significant for foliar Ca and Mg, contributing 41 and 38% in TTV of the respective variables (Table 3.4). Relative to untreated control, the two-way matrix table showed that Nemafric-BL phytonematicide and Biomuti individually reduced Mg by 60 and 51%, respectively, whereas the Nemafric-BL × Biomuti interaction reduced Mg by 38% (Table 3.8). Also, in the two-way matrix table the Nemafric-BL × Biomuti interaction and Nemafric-BL phytonematicide each reduced Mg by 13 and 2%, respectively, whereas Biomuti alone increased P by 17% (Table 3.8). Relative to untreated control, Nemafric-BL phytonematicide and Biomuti reduced Ca by 29 and 18%, respectively, whereas Nemafric-BL × Biomuti interaction reduced Ca by 14% (Table 3.9). Using two-way matrix table showed that Nemafric-BL phytonematicide and Biomuti separately reduced Mg by 21%, whereas the Nemafric-BL × Biomuti interaction reduced Mg by 16% (Table 3.9). Interaction of Nemarioc-AL × Nemafric-BL × Biomuti had no significant effect on K, Na and Zn in both experiments.

Table 3.1 Partitioning mean sum of squares for cucurbitacin-containing phytonematicides (A, B) and Biomuti (M) on of dry shoot mass (DSM), plant height (PH), leaf number(LN), chlorophyll content (CC) and stem diameter (SD) on citrus seedlings under greenhouse conditions.

| | | DSM | | PH | | $LN^{Z}$ | | CC | | SD | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | DF | MSS | TTV (%) | MSS | TTV (%) | MSS | TTV (%) | MSS | TTV (%) | MSS | TTV (%) |
| Experiment 1 | | | | | | | | | | | |
| Block | 9 | 2.962 | 18 | 357.758 | 84 | 0.032 | 15 | 107.323 | 22 | 1.796 | 21 |
| A | 1 | 2.861 | $17^{ns}$ | 0.210 | $0^{ns}$ | 0.056 | $27^{ns}$ | 106.953 | $22^{**}$ | 1.056 | $12^{ns}$ |
| B | 1 | 1.396 | $9^{ns}$ | 5.253 | $1^{ns}$ | 0.002 | $1^{ns}$ | 83.436 | $17^{ns}$ | 0.219 | $3^{ns}$ |
| M | 1 | 3.515 | $21^{ns}$ | 36.585 | $9^{ns}$ | 0.019 | $9^{ns}$ | 0.703 | $0^{ns}$ | 0.453 | $5^{ns}$ |
| A × B | 1 | 0.988 | $6^{ns}$ | 9.870 | $2^{ns}$ | 0.002 | $1^{ns}$ | 9.453 | $2^{ns}$ | 0.047 | $1^{ns}$ |
| A × M | 1 | 2.839 | $17^{ns}$ | 0.105 | $0^{ns}$ | 0.035 | $17^{ns}$ | 57.970 | $11^{ns}$ | 4.508 | $52^{***}$ |
| B × M | 1 | 0.352 | $2^{ns}$ | 0.528 | $0^{ns}$ | 0.001 | $0^{ns}$ | 10.440 | $2^{ns}$ | 0.019 | $0^{ns}$ |
| A × B × M | 1 | 0.476 | $3^{ns}$ | 0.703 | $0^{ns}$ | 0.040 | $19^{ns}$ | 95.266 | $19^{ns}$ | 0.084 | $1^{ns}$ |
| Error | 63 | 1.083 | 7 | 17.556 | 5 | 0.023 | 11 | 25.964 | 5 | 0.436 | 5 |
| Total | 79 | 16.362 | 100 | 428.568 | 100 | 0.209 | 100 | 497.508 | 100 | 8.718 | 100 |
| Experiment 2 | | | | | | | | | | | |
| Block | 9 | 17.175 | 36 | 1337.66 | 62 | 0.024 | 13 | 30.353 | 8 | 1.867 | 43 |
| A | 1 | 4.622 | $10^{ns}$ | 49.93 | $2^{ns}$ | 0.001 | $1^{ns}$ | 43.808 | $11^{ns}$ | 0.806 | $18^{ns}$ |
| B | 1 | 0.621 | $1^{ns}$ | 380.19 | $18^{***}$ | 0.007 | $4^{ns}$ | 144.184 | $36^{ns}$ | 0.007 | $0^{ns}$ |
| M | 1 | 0.234 | $0^{ns}$ | 58.14 | $3^{ns}$ | 0.055 | $30^{**}$ | 0.685 | $0^{ns}$ | 0.086 | $2^{ns}$ |
| A × B | 1 | 4.985 | $10^{ns}$ | 162.45 | $7^{ns}$ | 0.020 | $11^{ns}$ | 24.865 | $6^{ns}$ | 0.444 | $10^{ns}$ |
| A × M | 1 | 15.940 | $33^{***}$ | 6.16 | $0^{ns}$ | 0.049 | $27^{ns}$ | 117.613 | $29^{ns}$ | 0.554 | $13^{ns}$ |
| B × M | 1 | 0.597 | $1^{ns}$ | 45.90 | $2^{ns}$ | 0.012 | $7^{ns}$ | 0.200 | $0^{ns}$ | 0.000 | $0^{ns}$ |
| A × B × M | 1 | 2.142 | $4^{ns}$ | 74.88 | $3^{ns}$ | 0.002 | $1^{ns}$ | 2.592 | $1^{ns}$ | 0.049 | $1^{ns}$ |
| Error | 63 | 2.300 | 5 | 57.79 | 3 | 0.014 | 8 | 40.177 | 10 | 0.547 | 13 |
| Total | 79 | 48.616 | 100 | 2173.1 | 100 | 0.184 | 100 | 404.477 | 100 | 4.362 | 100 |

$^{ns}$Not significant at $P \leq 0.05$, **Significant at $P \leq 0.05$, ***Highly significant at $P \leq 0.01$.

$^{Z}$ Transformation using $\log_{10}(x+1)$.

Table 3.2 Two-way matrix for stem diameter as affected by Nemarioc-AL (A) × Biomuti (M) interaction under greenhouse conditions in Experiment 1.

| | Biomuti (M) | | | |
|---|---|---|---|---|
| Nemarioc-AL (A) | $M_0$ | R.I. (%) | $M_1$ | R.I. (%) |
| $A_0$ | 5.644 | – | 5.9525 | 5 |
| $A_1$ | 6.349 | 12 | 5.7075 | 1 |

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

Table 3.3 Two-way matrix for dry shoot mass as affected by Nemarioc-AL (A) × Biomuti (M) interaction under greenhouse conditions in Experiment 2.

| | Biomuti (M) | | | |
|---|---|---|---|---|
| Nemarioc-AL (A) | $M_0$ | R.I. (%) | $M_1$ | R.I. (%) |
| $A_0$ | 5.934 | – | 6.935 | 23 |
| $A_1$ | 7.307 | 17 | 6.522 | 10 |

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

Table 3.4 Partitioning sum of squares for cucurbitacin-containing phytonematicides (A, B) and Biomuti (M) on calcium (Ca), potassium(K), magnesium (Mg), Sodium (Na), zinc (Zn) and phosphorus (P) in leaf tissue of citrus seedlings under greenhouse conditions in Experiment 1.

| | | Ca | | K | | Mg | | Na | | Zn | | P | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | DF | MSS | %$^{e}$ | MSS | %$^{e}$ | MSS | %$^{e}$ | MSS | %$^{e}$ | MSS | %$^{e}$ | MSS | %$^{e}$ |
| Experiment 1 | | | | | | | | | | | | | |
| Block | 9 | 462.42 | 4 | 1525.41 | 7 | 11.13 | 1 | 169.07 | 10 | 0.04 | 11 | 1.29 | 1 |
| A | 1 | 532.05 | $5^{ns}$ | 8933.13 | $41^{**}$ | 15.92 | $1^{ns}$ | 463.20 | $27^{ns}$ | 0.01 | $3^{ns}$ | 52.10 | $33^{***}$ |
| B | 1 | 443.35 | $4^{ns}$ | 3789.92 | $18^{ns}$ | 282.45 | $21^{***}$ | 521.32 | $30^{ns}$ | 0.15 | $42^{ns}$ | 43.60 | $28^{***}$ |
| M | 1 | 380.67 | $3^{ns}$ | 867.71 | $4^{ns}$ | 107.73 | $8^{***}$ | 5.23 | $0^{ns}$ | 0.00 | $0^{ns}$ | 1.50 | $1^{ns}$ |
| A × B | 1 | 120.96 | $1^{ns}$ | 1245.28 | $6^{ns}$ | 117.80 | $9^{***}$ | 363.72 | $22^{ns}$ | 0.00 | $0^{ns}$ | 3.56 | $2^{ns}$ |
| A × M | 1 | 6395.14 | $59^{***}$ | 2051.83 | $9^{ns}$ | 60.48 | $4^{***}$ | 4.02 | $0^{ns}$ | 0.03 | $8^{ns}$ | 14.69 | $9^{ns}$ |
| B × M | 1 | 291.20 | $3^{ns}$ | 1492.56 | $7^{ns}$ | 684.61 | $50^{***}$ | 0.94 | $0^{ns}$ | 0.08 | $22^{ns}$ | 32.90 | $21^{***}$ |
| A × B × M | 1 | 1675.64 | $15^{ns}$ | 126.48 | $1^{ns}$ | 79.55 | $5^{***}$ | 1.98 | $0^{ns}$ | 0.02 | $6^{ns}$ | 3.10 | $2^{ns}$ |
| Error | 63 | 620.74 | 6 | 1501.45 | 7 | 11.62 | 1 | 197.28 | 11 | 0.03 | 8 | 4.77 | 3 |
| Total | 79 | 10922 | 100 | | 100 | 1371.29 | 100 | 1726.76 | 100 | 0.36 | 100 | 157.51 | 100 |
| Experiment 2 | | | | | | | | | | | | | |
| Block | 9 | 1585.70 | 6 | 2857.72 | 11 | 10.18 | 5 | 11.44 | 17 | 0.91 | 12 | 4885.58 | $11^{ns}$ |
| A | 1 | 444.40 | $2^{ns}$ | 455.25 | $2^{ns}$ | 15.30 | $8^{ns}$ | 14.67 | $22^{ns}$ | 0.88 | $12^{ns}$ | 4903.72 | $11^{ns}$ |
| B | 1 | 5550.40 | $23^{ns}$ | 1328.78 | $5^{ns}$ | 29.15 | $15^{ns}$ | 0.46 | $1^{ns}$ | 0.59 | $8^{ns}$ | 4766.73 | $11^{ns}$ |
| M | 1 | 107.10 | $0^{ns}$ | 9613.24 | $39^{**}$ | 29.15 | $15^{ns}$ | 8.90 | $13^{ns}$ | 1.56 | $20^{ns}$ | 4335.29 | $10^{ns}$ |
| A × B | 1 | 2.20 | $0^{ns}$ | 1589.90 | $6^{ns}$ | 10.09 | $5^{ns}$ | 3.36 | $5^{ns}$ | 0.59 | $8^{ns}$ | 4443.40 | $10^{ns}$ |
| A × M | 1 | 855.70 | $3^{ns}$ | 121.92 | $0^{ns}$ | 13.89 | $7^{ns}$ | 0.01 | $0^{ns}$ | 0.60 | $8^{ns}$ | 4890.74 | $11^{ns}$ |
| B × M | 1 | 10146.60 | $41^{**}$ | 1380.79 | $6^{ns}$ | 69.55 | $38^{**}$ | 6.90 | $10^{ns}$ | 1.21 | $16^{ns}$ | 4843.08 | $11^{ns}$ |
| A × B × M | 1 | 3676.90 | $15^{ns}$ | 5220.33 | $22^{ns}$ | 1.83 | $2^{ns}$ | 11.29 | $17^{ns}$ | 0.39 | $5^{ns}$ | 4883.91 | $11^{ns}$ |
| Error | 63 | 2199.6 | 9 | 2307 | 9 | 9.56 | 5 | 10.37 | 15 | 0.81 | 11 | 4844.47 | 11 |
| Total | 79 | 24568.6 | 100 | 24875.12 | 100 | 188.7 | 100 | 674 | 100 | 7.54 | 100 | 4279692 | 100 |

$^{ns}$ Not Significant at $P \leq 0.05$, ** Significant at $P \leq 0.05$ and *** Highly Significant at $P \leq 0.01$.
$^{e}$ Total treatment variation.

Table 3.5 Three-way matrix for magnesium (Mg) as affected by Nemarioc-AL (A) × Nemafric-BL (B) × Biomuti (M) interaction in leaf tissues of citrus seedlings under greenhouse conditions in Experiment 1.

| | | Nemafric-BL (B) | | | |
|---|---|---|---|---|---|
| Nemarioc-AL (A) | Biomuti (M) | $B_0$ | R.I. (%) | $B_1$ | R.I. (%) |
| $A_0$ | $M_0$ | 15.029[a] | – | 9.842[b] | –35 |
| $A_0$ | $M_1$ | 7.113[b] | –53 | 9.638[b] | –36 |
| $A_1$ | $M_0$ | 16.819[a] | 12 | 2.789[c] | –81 |
| $A_1$ | $M_1$ | 8.392[b] | –44 | 10.052[b] | –33 |

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

Table 3.6 Two-way matrix for magnesium (Mg) as affected by Nemarioc-AL (A) × Nemafric-BL (B) interaction under greenhouse conditions in Experiment 1.

| | Nemafric-BL (B) | | | |
|---|---|---|---|---|
| Nemarioc-AL (A) | $B_0$ | R.I. (%) | $B_1$ | R.I. (%) |
| $A_0$ | 11.071[ab] | – | 9.740[b] | –12 |
| $A_1$ | 12.605[a] | 14 | 6.421[c] | –42 |

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

Table 3.7 Two-way matrix for calcium (Ca) and magnesium (Mg) as affected by Nemarioc-AL (A) × Biomuti (M) interaction under greenhouse conditions in Experiment 1.

| | Biomuti | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ca | | | | Mg | | | |
| Nemarioc-AL (A) | $M_0$ | R.I. (%) | $M_1$ | R.I. (%) | $M_0$ | R.I. (%) | $M_1$ | R.I. (%) |
| $A_0$ | 102.23$^{a}$ | – | 79.99$^{b}$ | –22 | 12.435$^{a}$ | – | 8.375$^{b}$ | –33 |
| $A_1$ | 89.51$^{ab}$ | –12 | 103.03$^{a}$ | 1 | 9.804$^{b}$ | –21 | 9.222$^{b}$ | –26 |

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

Table 3.8 Two-way matrix for magnesium (Mg) and phosphorus (P) as affected by Nemafric-BL (B) × Biomuti (M) interaction under greenhouse conditions in Experiment 1.

| | Biomuti | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mg | | | | P | | | |
| Nemafric-BL (B) | $M_0$ | R.I. (%) | $M_1$ | R.I. (%) | $M_0$ | R.I. (%) | $M_1$ | R.I. (%) |
| $B_0$ | 15.924[a] | – | 7.752[bc] | –51 | 9.125[b] | – | 10.680[a] | 17 |
| $B_1$ | 6.315[c] | –60 | 9.845[b] | –38 | 8.930[b] | –2 | 7.921[b] | –13 |

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

Table 3.9 Two-way matrix for calcium (Ca) and magnesium (Mg) as affected by Nemafric-BL (B) × Biomuti (M) interaction under greenhouse conditions in Experiment 2.

| | Biomuti | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ca | | | | Mg | | | |
| Nemafric-BL | $M_0$ | R.I. (%) | $M_1$ | R.I. (%) | $M_0$ | R.I. (%) | $M_1$ | R.I. (%) |
| $B_0$ | 135.88[a] | – | 111.04[ab] | –18 | 14.694[a] | – | 11.622[b] | –21 |
| $B_1$ | 96.70[b] | –29 | 116.91[ab] | –14 | 11.622[b] | –21 | 12.280[b] | –16 |

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

## 3.4 Discussion

### 3.4.1 Interactive effects on plant growth

In the current study the results depicted a number of significant and non-significant interactions. The Nemarioc-AL × Nemafric-BL × Biomuti interaction was not significant ($P \leq 0.05$) on plant variables of rootstock seedlings in both experiments. Generally, cucurbitacin-containing phytonematicides from fruit of wild cucumber and wild watermelon contain active ingredients that consist of cucurbitacin A and B, respectively (Mashela *et al*., 2015). Cucurbitacin A is considered being partially polar and slightly soluble in water and breaks down to two components which are tetracyclic triterpenoids namely, cucumin and leptodermin (Chen *et al*., 2005), whereas cucurbitacin B is considered non-polar and insoluble in water (Jeffrey, 1978). In the endodermis of roots the non-polar molecules, for instance, glucose cannot be transported through the bipolar membranes of the symplastic pathways into the vesicular bundle (Campbell, 1990). Allelochemicals serve as active ingredients in crude plant extracts (Rice, 1984; Inderjit and Malik, 2002).

Increasing concentrations of allelochemicals affect plants and microorganisms through the density-dependent growth (DDG) patterns (Liu *et al.,* 2003). The DDG patterns, with three phases, namely, stimulation, neutral and inhibition phases, the occurrence of these phases is considered phytonematicide- and plant-specific (Mashela *et al*., 2016). The interaction of cucurbitacin-containing phytonematicides and Biomuti showed that by the time of harvest they were actually operating at stimulation phase which is shown by increase in stem diameter. In the current study, the other variables were not affected by the interactions between Nemarioc-AL, Nemafric-BL phytonematicides and Biomuti thus, the study suggested that the organs at harvest time were still on the saturation phase (Mashela *et al*., 2015). The organs had different responses or sensitivities to the products.

Nemarioc-AL × Biomuti interaction was highly significant on stem diameter, but the interaction had a negligent contribution in TTV of the stem diameter in Experiment 1. However, the variable was increased by the interaction, which contradicted previous other observations where Nemarioc-AL × Biomuti interaction did not affect plant growth variables of tomato plants (Nyamandi, 2017). Seshweni (2016) observed that in Nemacur, Biocult and Nemarioc-AL phytonematicides trial, the first order interactions had highly significant effects on gall rating, stem diameter and chlorophyll content in potato plants. However the interaction increased stem diameter and chlorophyll of potato plants (Seshweni, 2016). However, Maile (2013) reported the first observations where biological activities in citrus-nematode relations interacted to reduce stem diameter. The increase of stem diameter could be due to alllelochemicals that have a stimulation effect as shown in crops such as tomato (Mashela, 2002; Mashela *et al*., 2007; Pelinganga *et al.*, 2012).

The Nemarioc-AL × Biomuti interaction increased dry shoot mass which contradicted observations where Nemarioc-AL × Biomuti interaction reduced dry shoot mass and number of galls of tomato plants under field conditions. Under microplot trial, the interaction did not have an effect on plant growth variables (Nyamandi, 2017). The interaction of concentration × application interval of crude extract of *C. myriocarpus* had significant effects but the interaction had no effect on dry shoot and fruit number, whereas under *C. africanus* the interaction had no significant effects on dry fruit, dry shoot, fruit number, plant height and stem diameter. Also the current study contradicted individual studies on green bean (*Phaseolus vulgaris* L.) (Chokoe, 2017) where Nemarioc-AL phytonematicide significantly reduced dry shoot mass, whereas on beetroot (*Beta vulgaris* L.) (Mashitoa, 2017) and watermelon cv. 'Charleston Gray' (Nhlane, 2017) Nemarioc-AL phytonematicide had no significant effect on dry shoot mass. The increase in dry shoot mass could be explained by the enhancement of source strength through $CO_2$ enrichment throughout the prolonged period (Marcelis, 1996).

3.4.2 Interactive effects on nutrient elements

The second order Nemarioc-AL × Nemafric-BL × Biomuti interaction was highly significant for foliar Mg of citrus rootstock seedlings in Experiment 1, which could not be associated with any such interaction for foliar Mg in plants. However, this second order interaction drastically reduced foliar Mg, thereby confirming observations where Mg was reduced by the Biomuti × Mycoroot × Nemafric-BL interaction (Nyamandi, 2017). Nhlane (2017) also observed that increasing concentrations of Nemarioc-AL phytonematicide consistently reduced Mg and Na in leaf tissues of watermelon cv. 'Congo'. Magnesium is an essential nutrient element and plays significant role in photosynthesis, enzyme catalyst and nucleic acid anabolism (Tanoi and Kobayashi, 2015). Alterations in photosynthetic carbon metabolism and restriction of $CO_2$ fixation due to Mg deficiency could possibly lead to production of reactive oxygen species (Cakmak and Kirkby, 2008). In the absence of Mg, the plant hormone ethylene responsible for fruit ripening, leaf senescence and stress signalling, in inherently increased (Hermans *et al*., 2004).

In the current study, Nemarioc-AL × Biomuti interaction slightly increased Ca and considerably reduced Mg in leaf tissues of citrus rootstock seedlings in Experiment 1. The current study contradicted observation where Nemarioc-AL × Biomuti interaction did not have significant effect on nutrient elements under microplot and field conditions (Nyamandi, 2017). Low accumulation of Ca in leaf tissues of rootstock seedlings could lower the plant resistance to a number of pathogens (Delian *et al*., 2016). It enhances resistance against pathogens (Usten *et al*, 2006), and also play key role in growth and development of fruits (Kadir, 2004). Antagonistic relationship of nutrient elements such as Ca, Mg and K result in unavailability of the depressed nutrient element (Kasinath *et al.,* 2014).

Nemafric-BL × Biomuti interaction was highly significant for foliar Mg and P in leaf tissues of rootstock seedlings in Experiment 1. Phosphorus and magnesium were reduced by Nemafric-BL × Biomuti interaction and this contradicted other observations where Nyamandi (2017), observed that Nemafric-BL × Biomuti interaction did not have significant effect on nutrient elements, except decreasing P in leaf tissues of tomato plants under microplot and field conditions. Magnesium is also known as the forgotten element (Cakmak and Yazieci, 2010), which serve as the cofactor and activator of enzymes (Tang and Luan, 2017). Phosphorus is the most important nutrient element which is unavailable in soil of most regions (Dunlop, 1999). Nhlane (2017) observed that P was the most sensitive nutrient element to increasing concentrations of phytonematicides. Generally, low accumulation of P in plant tissues reduces the development of new tissues, which would eventually lead to stunted plant growth (McCauley *et al*., 2009).

Interaction of Nemafric-BL × Biomuti was highly significant in Experiment 2, with the interaction reducing Ca and Mg in leaf tissues of citrus rootstock seedlings. The current study contradicted observations by Nyamandi (2017) who observed that Nemafric-BL × Biomuti interaction did not have significant effect on nutrient elements, except decreasing P in leaf tissues of tomato plants. Calcium, magnesium, and potassium compete among each other and the accumulation of any one of them could lessen the uptake rate of the other two (Malvi, 2011). Sufficient supply of the required Ca might enhance root development and leaf growth (Del-Amor and Marcelis*,* 2003).

Interaction of Nemarioc-AL × Nemafric-BL × Biomuti had no significant effect on K, Na and Zn in both experiments, which was in agreement with observations by Nhlane (2017) who observed that Nemarioc-AL and Nemafric-BL phytonematicides and Velum had no significant effects on Ca, Mg, K, Mn, Na, Fe and Zn in leaf tissues of the cultivar cv. 'Congo'. Also, *C. myriocarpus* fruit species had no significant effect on accumulation of nutrient elements in tomato plants (Mashela, 2002). Potassium is a key nutrient in the plants that plays a role in osmoregulation which maintains high cell turgor pressure which affects cell elongation for growth (Malvi, 2011). Bergmann (1992) reported that high concentrations of K might induce Ca and Mg deficiencies resulting in damage to plants. Zinc plays a major role in promoting growth hormones and starch formation (Malvi, 2011). Deficiency of this element reduces growth, chlorophyll synthesis and also plants lose their tolerance to stress (Kawachi *et al*., 2009). The availability of Zn to plants is generally inadequate due to its immobile nature and the adsorptive properties of soil (Sharma *et al.,* 2012). Nutrient availability for uptake by plants in most cases is affected by the extent of the availability of elements in the soil (Mashela *et al*., 2013).

## 3.5 Conclusion

Interactions of phytonematicides and Biomuti had different effects on plant growth and accumulation of nutrient elements in citrus rootstock seedlings. All the interactions had no significant effect on chlorophyll, leaf number and plant height except the interaction between Nemarioc-AL phytonematicide and Biomuti which had an effect on numerous plant growth variables as compared to other combinations. Nutrient elements were generally reduced by all second order Nemarioc-AL × Nemafric-BL × Biomuti interaction and first order Nemarioc-AL × Nemafric-BL, Nemafric-BL × Biomuti, Nemarioc-AL × Biomuti interactions except the interaction between Nemarioc-AL phytonematicide and Biomuti which increased Ca in leaf tissues of citrus rootstock seedlings. However, the phytonematicides when interacted together with Biomuti improved plant growth in *P. trifoliata* rootstock seedlings, but had antagonistic effects on most nutrient elements, suggesting that the products should be applied separately.

# CHAPTER 4
# INTERACTIVE EFFECTS OF NEMARIOC-AL AND NEMAFRIC-BL PHYTONEMATICIDE AND BIOMUTI ON CITRUS SEEDLINGS UNDER FIELD CONDITIONS

## 4.1 Introduction

Plant extracts are gaining much focus in research and development as environment-friendly strategies for management of nematode population densities (Maji *et al.,* 2005). Integration of phytonematicides with Biomuti, a recently developed product by the Agricultural Research Council (ARC) with unknown active ingredients, could be easily adopted in low-input agricultural systems. However, the product had been shown to reduce number of root galls on tomato plants (Nyamandi, 2017). Cucurbitacin-containing phytonematicides at rates applied where shown to stimulate (Mashela, 2002; Pelinganga *et al*., 2012), inhibit (Mafeo *et al.,* 2011; Pelinganga and Mashela, 2012) and no significant effects (Lebea, 2017; Mashela and Pofu, 2017) on plant growth.

Under greenhouse conditions, cucurbitacin-containing phytonematicides and Biomuti combined were shown to be effective in improvement of plant growth (Chapter 3). However the three products generally had an antagonistic effect on accumulation of nutrient elements (Chapter 3). The use of cucurbitacin-containing phytonematicides and Biomuti on growth and accumulation of nutrient elements under field conditions had not been documented. The objective of the study was to determine the interactive effects of Nemarioc-AL and Nemafric-BL phytonematicides and Biomuti on growth and nutrient elements in leaf tissues of *Poncirus trifoliata* (L.) rootstock seedlings under field conditions.

## 4.2 Materials and methods

### 4.2.1 Description of the study area

The study was conducted under field conditions during autumn (January-March) 2016 and repeated in spring 2017 at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, South Africa (23°53'10"S, 29°44'15"E). Soil at the location is predominantly Hutton sandy loam (65% sand, 30% clay, 5% silt), with organic C = 1.6%, EC = 0.148 dS/m and pH ($H_2O$) = 6.5. The location has hot dry summers, with daily maximum temperature from 28 to 38°C. The location has semi-arid climate, with average incidence of annual rainfall approximately 500 mm.

### 4.2.2 Treatments and research design

A 2 × 2 × 2 factorial experiment with eight treatments combinations were laid out in a randomised complete block design with 10 blocks (n = 80) as previously described (Chapter 3).

### 4.2.3 Procedures

The field trials were established by planting citrus rootstock seedlings directly in the field with 35 cm separating the plants inter and intra row space (Legend 4.1). Fertilisation and disease management were as explained previously (Chapter 3). When necessary, plants were irrigated with 300 ml through drip irrigation for 2 h every other day. Phytonematicides were prepared as described previously (Chapter 3).

### 4.2.4 Data collection

At 63 days after initiation of treatments, plant variables and nutrient elements were prepared, collected and recorded as previously described (Chapter 3).

4.2.5 Data analysis

Data were subjected to analysis of variance (ANOVA) procedure using SAS software (SAS Institute, 2008) as previously described (Chapter 3).

Legend 4.1 Citrus seedlings treated with Nemarioc-AL and Nemafric-BL phytonematicides with and without Biomuti under field conditions.

4.3 Results

4.3.1 Interactive effects on plant growth

Nemarioc-AL × Nemafric-BL × Biomuti interaction was not significant for all plant growth variables in both experiments (Table 4.1). However, Nemarioc-AL × Nemafric-

BL interaction was significant for leaf number and stem diameter, contributing 45 and 29% in total treatment variation (TTV) of the respective variables in Experiment 2 (Table 4.1). Relative to untreated control, Nemarioc-AL × Nemafric-BL interaction and Nemafric-BL phytonematicide increased stem diameter by 8 and 11%, respectively, whereas Nemarioc-AL phytonematicide reduced stem diameter by 2% (Table 4.2). Also, Nemarioc-AL and Nemafric-BL phytonematicides each increased leaf number by 1 and 7%, respectively, whereas Nemarioc-AL × Nemafric-BL interaction increased leaf number by 6% (Table 4.2). Nemafric-BL × Biomuti interaction was significant for stem diameter, contributing 29% in TTV of the variable in Experiment 2 (Table 4.1). Using two-way matrix table showed that Nemafric-BL × Biomuti interaction and Nemafric-BL phytonematicide increased stem diameter by 7% each, whereas Biomuti reduced stem diameter by 6% (Table 4.3).

### 4.3.2 Interactive effects on nutrient elements

Nemarioc-AL × Nemafric-BL × Biomuti interaction was not significant for all the nutrient elements in Experiment 1. Nemarioc-AL × Biomuti interaction was significant for Ca and K and highly significant for Mg and P, contributing 31, 8, 23 and 19% in TTV of the respective variables in Experiment 1 (Table 4.4). Relative to untreated control, Nemarioc-AL phytonematicide and Biomuti each increased Ca by 15 and 26%, respectively, whereas Nemarioc-AL × Biomuti interaction increased Ca by 17% (Table 4.5). Nemarioc-AL × Biomuti interaction, Nemarioc-AL phytonematicide and Biomuti reduced Mg by 48, 70 and 37%, respectively (Table 4.5). Also, Nemarioc-AL phytonematicide and Biomuti increased P by 4 and 5%, respectively, whereas the Nemarioc-AL × Biomuti interaction increased P by 50% (Table 4.5). Nemarioc-AL × Biomuti interaction was significant for K and highly significant for Mg, contributing 8%

and 23% in TTV of the respective variables in Experiment 1 (Table 4.4). Relative to untreated control, Biomuti and Nemarioc-AL phytonematicide reduced K by 10 and 5%, respectively, whereas the Nemarioc-AL × Nemafric-BL interaction reduced K by 38% (Table 4.6). Relative to untreated control, Biomuti and Nemarioc-AL phytonematicide reduced Mg by 37 and 70%, respectively, whereas the Nemarioc-AL × Nemafric-BL interaction reduced Mg by 48% (Table 4.6).

Nemafric-BL × Biomuti interaction was highly significant for Mg and Zn, contributing 11 and 29% in TTV of the respective variables in Experiment 1 (Table 4.4). Relative to untreated control, Nemarioc-AL phytonematicide and Biomuti increased Mg by 1 and 19%, respectively, whereas Nemafric-BL × Biomuti interaction reduced Mg by 43% (Table 4.7). Nemafric-BL × Biomuti interaction, Nemafric-BL phytonematicide and Biomuti reduced Zn by 35, 31 and 64%, respectively (Table 4.7). Nemarioc-AL × Nemafric-BL × Biomuti, Nemarioc-AL × Nemafric-BL, Nemarioc-AL × Biomuti and Nemafric-BL × Biomuti interactions each increased Ca by 44, 18, 10 and 24%, respectively (Table 4.8). Nemarioc-AL phytonematicide, Nemafric-BL phytonematicide and Biomuti each increased Ca by 25, 31 and 23%, respectively (Table 4.8).

Table 4.1 Partitioning mean sum of squares for cucurbitacin-containing phytonematicides (A, B) and Biomuti (M) on dry shoot mass (DSM), plant height (PH), leaf number (LN), chlorophyll (CC) and stem diameter (SD) of citrus seedlings under field conditions in Experiment 1.

| | | DSM | | PH | | $LN^{Z}$ | | CC | | SD | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | DF | MSS | TTV (%) | MSS | TTV (%) | MSS | TTV (%) | MSS | TTV (%) | MSS | TTV (%) |
| Experiment 1 | | | | | | | | | | | |
| Block | 9 | 6.378 | $15^{ns}$ | 276.256 | $14^{ns}$ | 0.037 | $10^{ns}$ | 134.727 | $15^{ns}$ | 1.390 | $12^{ns}$ |
| A | 1 | 0.343 | $1^{ns}$ | 79.401 | $4^{ns}$ | 0.008 | $2^{ns}$ | 233.586 | $26^{ns}$ | 0.347 | $3^{ns}$ |
| B | 1 | 25.696 | $60^{**}$ | 785.631 | $39^{**}$ | 0.093 | $24^{ns}$ | 12.403 | $1^{ns}$ | 6.492 | $57^{**}$ |
| M | 1 | 0.007 | $0^{ns}$ | 533.028 | $27^{ns}$ | 0.014 | $4^{ns}$ | 80.601 | $9^{ns}$ | 0.553 | $5^{ns}$ |
| A × B | 1 | 0.023 | $0^{ns}$ | 20.100 | $1^{ns}$ | 0.001 | $0^{ns}$ | 92.235 | $10^{ns}$ | 0.020 | $0^{ns}$ |
| A × M | 1 | 2.949 | $7^{ns}$ | 68.635 | $3^{ns}$ | 0.074 | $19^{ns}$ | 21.321 | $2^{ns}$ | 0.005 | $0^{ns}$ |
| B × M | 1 | 0.157 | $0^{ns}$ | 40.186 | $2^{ns}$ | 0.036 | $9^{ns}$ | 25.878 | $3^{ns}$ | 0.675 | $6^{ns}$ |
| A × B × M | 1 | 2.823 | $7^{ns}$ | 1.275 | $0^{ns}$ | 0.095 | $25^{ns}$ | 190.653 | $22^{ns}$ | 0.573 | $6^{ns}$ |
| Error | 63 | 4.229 | 10 | 200.756 | 10 | 0.028 | 7 | 90.674 | 10 | 1.244 | 11 |
| Total | 79 | 42.505 | 100 | 2005.268 | 100 | 0.387 | 100 | 882.078 | 100 | 11.299 | 100 |
| Experiment 2 | | | | | | | | | | | |
| Block | 9 | 3.023 | $11^{ns}$ | 209.217 | $11^{ns}$ | 0.023 | $9^{ns}$ | 173.138 | $11^{ns}$ | 1.134 | $6^{ns}$ |
| A | 1 | 2.898 | $11^{ns}$ | 126.756 | $7^{ns}$ | 0.037 | $15^{ns}$ | 16.200 | $1^{ns}$ | 1.439 | $7^{ns}$ |
| B | 1 | 0.046 | $0^{ns}$ | 1.891 | $0^{ns}$ | 0.000 | $0^{ns}$ | 415.872 | $27^{ns}$ | 0.607 | $3^{ns}$ |
| M | 1 | 8.791 | $33^{**}$ | 553.878 | $29^{ns}$ | 0.002 | $1^{ns}$ | 389.845 | $25^{*}$ | 3.276 | $17^{ns}$ |
| A × B | 1 | 5.746 | $21^{ns}$ | 595.686 | $31^{ns}$ | 0.110 | $45^{**}$ | 237.808 | $15^{ns}$ | 5.570 | $29^{**}$ |
| A × M | 1 | 0.050 | $0^{ns}$ | 51.040 | $3^{ns}$ | 0.031 | $13^{ns}$ | 26.220 | $2^{ns}$ | 0.055 | $0^{ns}$ |
| B × M | 1 | 3.168 | $12^{ns}$ | 31.626 | $2^{ns}$ | 0.013 | $5^{ns}$ | 118.585 | $8^{ns}$ | 5.570 | $29^{**}$ |
| A × B × M | 1 | 1.031 | $4^{ns}$ | 151.525 | $8^{ns}$ | 0.008 | $3^{ns}$ | 31.001 | $2^{ns}$ | 0.679 | $3^{ns}$ |
| Error | 63 | 2.221 | 8 | 183.707 | 10 | 0.022 | 9 | 132.917 | 9 | 1.171 | 6 |
| Total | 79 | 26.874 | 100 | 1905.326 | 100 | 0.246 | 100 | 1551.59 | 100 | 19.501 | 100 |

$^{ns}$Not significant at P ≤ 0.05, **Significant at P ≤ 0.05, ***Highly significant at P ≤ 0.01.
$^{Z}$Transformation using $\log_{10}(x+1)$.

Table 4.2 Two-way matrix for leaf number and stem diameter as affected by Nemafric-AL (A) × Biomuti (M) interaction under greenhouse conditions in Experiment 2.

| | Biomuti (M) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Leaf number | | | | Stem diameter | | | |
| Nemarioc-AL (A) | $M_0$ | R.I. (%) | $M_1$ | R.I. (%) | $M_0$ | R.I. (%) | $M_1$ | R.I. (%) |
| $B_0$ | 1.456[a] | – | 1.563[a] | 7 | 5.509[a] | – | 6.110[a] | 11[a] |
| $B_1$ | 1.468[a] | 1 | 1.543[a] | 6 | 5.409[a] | –2 | 5.947[a] | 8[a] |

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

Table 4.3 Two-way matrix for stem diameter as affected by Nemafric-BL phytonematicide (B) × Biomuti (M) interaction under field conditions in Experiment 2.

| Biomuti (M) | Nemafric-BL(B) | | | |
|---|---|---|---|---|
| | $B_0$ | R.I. (%) | $B_1$ | R.I. (%) |
| $M_0$ | 5.6335$^{ab}$ | – | 6.0195$^{a}$ | 7 |
| $M_1$ | 5.2835$^{b}$ | –6 | 6.0370$^{a}$ | 7 |

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

Table 4.4 Partitioning mean sum of squares for cucurbitacin-containing phytonematicides (A, B) and Biomuti (M) on calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), zinc (Zn) and phosphorus (P) in leaf tissue of citrus seedlings under field conditions in Experiment 1.

| | | Ca | | K | | Mg | | Na | | Zn | | P | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | DF | MSS | %[e] | MSS | %[e] | MSS | %[e] | MSS | %[e] | MSS | %[e] | MSS | %[e] |
| Experiment 1 | | | | | | | | | | | | | |
| Block | 9 | 380.01 | 7 | 101.58 | 2 | 37.80 | 1 | 3.86 | 27 | 0.27 | 9 | 2.56 | 1 |
| A | 1 | 106.98 | $2^{ns}$ | 966.33 | $16^{***}$ | 1376.02 | $44^{***}$ | 0.40 | $3^{ns}$ | 0.46 | $15^{**}$ | 51.43 | $28^{***}$ |
| B | 1 | 530.71 | $10^{ns}$ | 2517.09 | $40^{***}$ | 331.60 | $10^{***}$ | 2.21 | $15^{ns}$ | 0.00 | $0^{ns}$ | 27.33 | $15^{***}$ |
| M | 1 | 2107.71 | $39^{**}$ | 1644.75 | $26^{***}$ | 55.67 | $2^{ns}$ | 0.77 | $6^{ns}$ | 1.09 | $35^{***}$ | 56.64 | $31^{***}$ |
| A × B | 1 | 184.98 | $4^{ns}$ | 153.57 | $2^{ns}$ | 202.61 | $6^{ns}$ | 5.27 | $37^{ns}$ | 0.01 | $0^{ns}$ | 2.09 | $1^{ns}$ |
| A × M | 1 | 1686.64 | $31^{**}$ | 482.36 | $8^{**}$ | 735.77 | $23^{***}$ | 0.04 | $0^{ns}$ | 0.05 | $2^{ns}$ | 35.01 | $19^{***}$ |
| B × M | 1 | 3.10 | $0^{ns}$ | 1.78 | $0^{ns}$ | 354.63 | $11^{***}$ | 0.32 | $2^{ns}$ | 0.89 | $29^{***}$ | 0.17 | $0^{ns}$ |
| A × B × M | 1 | 9.64 | $0^{ns}$ | 268.06 | $4^{ns}$ | 19.61 | $1^{ns}$ | 0.07 | $0^{ns}$ | 0.08 | $3^{ns}$ | 7.38 | $4^{ns}$ |
| Error | 63 | 362.71 | 7 | 96.31 | 2 | 49.31 | 2 | 1.51 | 10 | 0.22 | 7 | 2.57 | 1 |
| Total | 79 | 5372.57 | 100 | 6231.83 | 100 | 3163.02 | 100 | 14.45 | 100 | 3.07 | 100 | 185.18 | 100 |
| Experiment 2 | | | | | | | | | | | | | |
| Block | 9 | 3020.72 | 19 | 1142.11 | 11 | 16.64 | 19 | 1.70 | 13 | 0.09 | 8 | 2.69 | 16 |
| A | 1 | 451.29 | $3^{ns}$ | 153.68 | $2^{ns}$ | 10.98 | $13^{ns}$ | 0.95 | $7^{ns}$ | 0.32 | $31^{ns}$ | 0.04 | $0^{ns}$ |
| B | 1 | 4182.07 | 26** | 1136.57 | $11^{ns}$ | 26.40 | $31^{ns}$ | 2.91 | $21^{ns}$ | 0.02 | $2^{ns}$ | 1.26 | $7^{ns}$ |
| M | 1 | 956.36 | $6^{ns}$ | 2627.25 | $26^{ns}$ | 0.00 | $0^{ns}$ | 0.85 | $6^{ns}$ | 0.00 | $0^{ns}$ | 0.15 | $1^{ns}$ |
| A × B | 1 | 44.13 | $0^{ns}$ | 794.11 | $7^{ns}$ | 17.94 | $21^{ns}$ | 0.11 | $1^{ns}$ | 0.11 | $10^{ns}$ | 0.02 | $0^{ns}$ |
| A × M | 1 | 31.37 | $0^{ns}$ | 350.43 | $3^{ns}$ | 4.78 | $5^{ns}$ | 1.15 | $8^{ns}$ | 0.15 | $14^{ns}$ | 2.41 | $14^{ns}$ |
| B × M | 1 | 163.83 | $1^{ns}$ | 2206.65 | $22^{ns}$ | 0.15 | $0^{ns}$ | 3.57 | $26^{ns}$ | 0.12 | $11^{ns}$ | 5.79 | $34^{ns}$ |
| A × B × M | 1 | 6331.20 | 39** | 133.49 | $1^{ns}$ | 0.16 | $0^{ns}$ | 0.25 | $2^{ns}$ | 0.15 | $14^{ns}$ | 2.25 | $13^{ns}$ |
| Error | 63 | 1035.35 | 6 | 1695.74 | 17 | 9.36 | 11 | 2.10 | 16 | 0.11 | 10 | 2.55 | 15 |
| Total | 79 | 1035.35 | 100 | 10240.03 | 100 | 86.41 | 100 | 16.54 | 100 | 1.07 | 100 | 17.16 | 100 |

$^{ns}$ Not Significant at $P \leq 0.05$, ** Significant at $P \leq 0.05$ and *** Highly Significant at $P \leq 0.01$.
[e] Total treatment variation.

Table 4.5 Two-way matrix for calcium (Ca) and phosphorus (P) as affected by Nemarioc-AL (A) × Biomuti (M) interaction in leaf tissue of citrus seedlings under field conditions in Experiment 1.

| | Biomuti (M) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ca | | | | P | | | |
| Nemarioc-AL (A) | $M_0$ | R.I. (%) | $M_1$ | R.I. (%) | $M_0$ | R.I. (%) | $M_1$ | R.I. (%) |
| $A_0$ | 75.154$^{b}$ | – | 94.603$^{a}$ | 26 | 6.603$^{b}$ | – | 6.962$^{b}$ | 5 |
| $A_1$ | 86.650$^{ab}$ | 15 | 87.732$^{a}$ | 17 | 6.883$^{b}$ | 4 | 9.889$^{a}$ | 50 |

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

Table 4.6 Two-way matrix for magnesium (Mg) and potassium (K) as affected by Nemarioc-AL (A) × Biomuti (M) interaction in leaf tissue of citrus seedlings under field conditions in Experiment 1.

| | Biomuti (M) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mg | | | | K | | | |
| Nemarioc-AL (A) | $M_0$ | R.I. (%) | $M_1$ | R.I. (%) | $M_0$ | R.I. (%) | $M_1$ | R.I. (%) |
| $A_0$ | 20.658[a] | – | 12.924[b] | –37 | 42.053[a] | – | 37.895[a] | –10 |
| $A_1$ | 6.298[c] | –70 | 10.698[bc] | –48 | 40.013[a] | –5 | 26.033[b] | –38 |

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

Table 4.7 Two-way matrix for magnesium (Mg) and zinc (Zn) as affected by Nemafric-BL (B) × Biomuti (M) interaction in leaf tissues of citrus seedlings under field conditions in Experiment 1.

| | Biomuti (M) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mg | | | | Zn | | | |
| Nemafric-BL | $M_0$ | R.I. (%) | $M_1$ | R.I. (%) | $M_0$ | R.I. (%) | $M_1$ | R.I. (%) |
| $B_0$ | 13.409[a] | – | 15.951[a] | 19 | 0.689[a] | – | 0.245[c] | –64 |
| $B_1$ | 13.548[a] | 1 | 7.668[b] | –43 | 0.472[b] | –31 | 0.450[bc] | –35 |

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

Table 4.8 Three-way matrix for calcium as affected by Nemarioc-AL (A) × Nemafric-BL (B) × Biomuti (M) interaction in leaf tissues of citrus seedlings under field conditions in Experiment 2.

| Nemarioc-AL (A) | Biomuti (M) | Nemafric-BL (B) | | | |
|---|---|---|---|---|---|
| | | $B_0$ | R.I. (%) | $B_1$ | R.I. (%) |
| $A_0$ | $M_0$ | $101.26^{c}$ | – | $132.38^{ab}$ | 31 |
| $A_0$ | $M_1$ | $124.54^{abc}$ | 23 | $125.56^{abc}$ | 24 |
| $A_1$ | $M_0$ | $126.74^{abc}$ | 25 | $119.00^{abc}$ | 18 |
| $A_1$ | $M_1$ | $111.63^{abc}$ | 10 | $145.52^{a}$ | 44 |

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

## 4.4 Discussion

### 4.4.1 Interactive effects on plant growth

In the current study, citrus rootstock seedlings tested had varied reactions to the cucurbitacin-containing phytonematicides with or without Biomuti. In the current study, Nemarioc-AL × Nemafric-BL × Biomuti interaction was not significant for all plant growth variables in both experiments. The absence of significant effects on plant variables in the second order interaction could be viewed in terms of saturation as depicted in density-dependent growth (DDG) patterns (Lui *et al.,* 2003). In the current study, the results confirmed observations where the VAM × nematode × Nemarioc-AL interaction had no significant effect on plant growth variables on *Cleome (Cleome gynandra* L.*)* (Rabothata, 2017). Also Mashela and Pofu (2012) observed that the Nematode × *Bradyrhizobium* × *C. myriocarpus* had no significant effect on plant variables of cowpea (*Vigna unguiculata* L.). Under field conditions, Maake (2017) also observed that the interaction of Nemarioc-AL × Nemafric-BL did not have significant effects on plant height, stem diameter, fresh fruit and dry shoot mass of tomato plants. Under similar conditions, Lebea (2017) observed that the cucurbitacin-containing phytonematicides had no significant effect on plant growth variables of butternut squash (*Cucurbita pepo* L.), with a suggestion that the MCSP and ∑k values seemed to be location-specific. Similarly, Nemafric-BL phytonematicide reduced number of galls without any significant effect on plant variables of green bean (*Phaseolus vulgaris* L.).

Nemarioc-AL × Nemafric-BL interaction increased leaf number and stem diameter of the rootstock seedlings in Experiment 2, which contradicted observations where the Nemarioc-AL × Nemafric-BL interaction was not significant for plant height, stem diameter, fresh fruit and dry shoot mass on tomato pants (Maake, 2017). Also, Dube *et al.* (2017) observed that at 3% concentration, Nemarioc-AL × Nemafric-BL interaction did not have significant effects on tomato plants when applied at 14 days interval under field conditions. In contrast, Maake (2017) observed that Nemarioc-AL and Nemafric-BL when applied alone increased stem diameter of tomato plants (Maake, 2017). The fate of many allelochemicals in the soil environment is relatively unknown (Inderjit *et al*., 2005). In pot trials phytonematicide movements are restricted unlike in field trials where the materials have unobstructed movement in the soil (Shadung, 2016). However, the concentration of the phytonematicide within the rhizosphere would be altered by factors such as soil structure, irrigation intensity and slope (Shadung, 2016).

Nemafric-BL × Biomuti interaction increased stem diameter of citrus rootstock seedlings in Experiment 2, which contradicted observations where Nemafric-BL × Biomuti interaction did not affect tomato plant variables, except for reducing dry shoot mas and number of galls (Nyamandi, 2017). Madaure and Mashela (2017) observed that interactions of cucurbitacin-containing phytonematicides and biocontrol agents namely, *Trichoderma harzianum* and *Steinernema feltiae* when combined improved selected plant variables of tomato plants whereas nematode variables were not affected by the combinations of the materials. In contrast, Mashela *et al.* (2007) observed that interaction of *C. myriocarpus*, fever tea (*Lippia javanica L.)* and castor fruit (*Ricinus communis L.)* improved tomato plants and also suppressed nematode numbers, with an implication that the materials served as bionematicide with fertiliser effect using ground leaching technology (GLT). Elongation of the stem diameter could be attributed to accumulation of Ca in leaf tissue of rootstock seedlings, since Ca is required for cell division (Zekri and Obreza, 2013a).

4.4.2 Interactive effects on nutrient elements.

The second order Nemarioc-AL × Nemafric-BL × Biomuti interaction was not significant for all the nutrient elements in Experiment 1. However, the results in the current study confirmed observations in the previous chapter where the three factors did not affect nutrient elements (Chapter 3). In the current study, nutrient elements were not affected by the second order interaction thus, the study suggested that the organs at harvest time were still in the saturation phase (Mashela *et al*., 2015). Mashela *et al.* (2007) observed that in most GLT studies, nutrient concentrations in tomato leaf tissues at the applied concentrations did not support the fertiliser effect concept. Nhlane (2017) also observed similar results when using cucurbitacin-containing phytonematicides on watermelon cultivars. Maake (2017) also observed that the Nemarioc-AL × Nemafric-BL interactions were not significant on tomato plant growth and nutrient elements in leaf tissues of tomato plants. Mashela *et al*. (2015) and Rice (1984) indicated that the allelopathy could be concentration specific, organ-specific and plant-specific.

In the current study, Nemarioc-AL × Biomuti interaction incresed Ca and P of citrus rootstock seedlings, which contradicted other observation where the Nemarioc-AL × Biomuti interaction did not have significant effects on all nutrients elements in leaf tissues of tomato. Calcium is an essential nutrient element which is required for growth and development, especially root and shoot tip (Tuteja and Mahajan, 2007). It is also responsible for structural role in the cell wall and membranes where it exist as Ca pectate. Calcium deficiency could be due to inadequate supply of Ca requirements to rapidly growing tissue (Tuteja and Mahajan, 2007).

Increase of P is crucial in citrus rootstock seedlings since nutrient element plays a major role in organization and the transfer of heredity traits (Zekri and Obreza, 2013b). Worldwide, most soils face crisis of low P availability (Abelson, 1999). Generally, low accumulation of P in plant tissues reduces the development of new tissues (McCauley *et al.*, 2009). Moreover, in a situation where P is deficient in plants, it could also be explained as when phosphorus react with clay, iron, aluminium or calcium in the soil the element becomes less mobile (Zekri and Obreza, 2013a), and result in plants that are susceptible to stress (Tuteja and Mahajan, 2007).

Nemarioc-AL × Biomuti interaction reduced Mg and K in leaf tissues of citrus rootstock seedlings, which contradicted other observations where Nemarioc-AL × Biomuti interaction did not have significant effects on foliar nutrient elements of tomato plants under microplot and field conditions (Nyamandi, 2017). Hydrolyses of sucrose into glucose and fructose requires activation of starch synthase by K (Mashela and Nthangeni, 2002b). Potassium is indispensable for a number of physiological functions, such as, sugars and starch metabolism, synthesis of proteins, normal cell division, growth and neutralisation of organic acids (Abbas and Fares, 2008). Deficiency of K result in retarded Growth and also the plants become more sensitive to biotic and abiotic stresses (Marchner, 1995).

Tang and Luan (2017) reported antagonistic interactions between Ca and Mg, however, Guoa *et al.* (2016) demonstrated that plant cells compensate for low Ca by increasing Mg transporters. Decrease of Mg in leaf tissues of citrus rootstock seedlings could be due to poor synthesis of chlorophyll molecules in green tissues (Shaul, 2002). Homeostatic balance of Ca and Mg could play a significant role in optimum growth and development of plants (Tang and Luan, 2017).

Nemafric-BL × Biomuti interaction matrix demonstrated a reduction in Mg and Zn in leaf tissues of the citrus rootstock seedlings in Experiment 1. The results in the current study contradicted observations where Nemafric-BL × Biomuti interaction did not affect foliar nutrient elements, except for decreasing P (Nyamandi, 2017). Recently, Zn and P deficiencies have become a worldwide concern in crop yield (Bouain *et al*., 2014). However, the cause of interveinal necrosis and reduction of biomass could be attributed to Zn deficiency (Bouain *et al*., 2014). The availability of Zn to plants is generally inadequate due to its immobile nature and the adsorptive properties of soil (Sharma *et al.,* 2012). Growth and development of plants depend on a homeostatic balance between Ca and Mg, since both elements have antagonistic interactions in plant cells (Tang and Luan, 2017). Magnesium is required for synthesis of chlorophyll (Shaul, 2002).

Nemarioc-AL × Nemafric-BL × Biomuti interaction increased Ca, which is required mainly for cell division and chromosome stability (Zekri and Obreza, 2013a). The results in the current study contradicted observations in the previous chapter where the three factors did not affect nutrient elements, except for decreasing Mg (Chapter 3). This is the first observation where these three factors are interacted to improve Ca in leaf tissues of citrus rootstock seedlings. Tang and Luan (2017) reported that Ca accounts for about 0.1 to 5% on plant dry shoot mass. Sufficient supply of Ca allows plants to be able to resist stress, neutralize vacuolar anions and strengthen cell walls (Tang and Luan, 2017). Homeostatic balance of Ca and Mg could enhance optimum growth and development of plants as described previously (Tang and Luan, 2017).

In the current study, plant growth variables and foliar nutrient elements responded to cucurbitacin-containing phytonematicides and Biomuti in accordance to the DDG patterns, depicted by the increased and reduced relation, whereas non-significant variables were operating within the neutral phase (Mashela *et al*., 2015). The detailed analysis of nutrient elements in leaf tissue of citrus rootstock seedlings did not support the stimulation effect on plant variables. The latter agreed with most of the findings (Mashela, 2002; Mashela *et al*., 2007 and Nhlane, 2017).

## 4.5 Conclusion

All second order Nemarioc-AL × Nemafric-BL × Biomyuti and first order Nemarioc-AL × Nemafric-BL, Nemarioc-AL × Biomuti, Nemafric-BL × Biomuti interactions had no significant effect on plant growth variables such as chlorophyll, plant height and dry shoot mass. First order Nemarioc-AL × Nemafric-BL interaction increased leaf number, stem diameter and Nemafric-BL × Biomuti interaction also increased stem diameter of citrus rootstock seedlings. Second order interaction Nemarioc-AL × Nemafric-BL × Biomuti increased Ca, whereas first order Nemarioc-AL × Biomuti interaction increased Ca and P in leaf tissues of citrus rootstock seedlings. These data led us to approach the conclusion that, the integration of sustainable products specifically Nemarioc-AL, Nemafric-BL phytonematicides and Biomuti could be adopted in in low-input agricultural systems as growth is invariably stimulated by these products, whereas accumulation of nutrient elements in general did not support the stimulation effect, which suggest that the products should be applied separately.

# CHAPTER 5

# SUMMARY, SIGNIFICANCE OF THE FINDINGS, FUTURE RECOMMENDATIONS AND CONCLUSIONS

## 5.1 Summary

Management approaches had been focusing on environment-friendly nematode management tactics, due to the withdrawal of synthetic nematicides from the agrochemical markets (Ujvary, 2002). Nemarioc-AL and Nemafric-BL phytonematicides and Biomuti-produced in South Africa are sustainable products that could be used in climate-smart agriculture for sustainable nematode management. Under greenhouse conditions, interactions of phytonematicides and Biomuti had some effects on plant growth and accumulation of nutrient elements in citrus rootstock seedlings. Second order interactions had no significant effects on all plant growth variables. Nutrient elements were generally reduced by all second order interactions and selected first order interactions except for the Nemarioc-AL × Biomuti interaction that increased Ca in leaf tissues of citrus rootstock seedlings.

Under field conditions, second order and first order interactions had no significant effects on plant growth variables such as chlorophyll, plant height and dry shoot mass. The first order Nemarioc-AL × Nemafric-BL interaction increased leaf number, stem diameter, along with Nemafric-BL × Biomuti interaction which increased stem diameter of citrus rootstock seedlings. The second order interaction increased Ca, along with the first order Nemarioc-AL × Biomuti interaction that increased Ca and P in leaf tissues of citrus rootstock seedlings.

## 5.2 Significance of the findings

The current research demonstrated that selected combinations of Nemarioc-AL and Nemafric-BL phytonematicides and Biomuti could have beneficial effects on plant growth of citrus rootstock seedlings under both greenhouse and field conditions. Also, certain combinations had beneficial effects on accumulation of foliar nutrient elements in *P. trifoliata* rootstock seedlings. The different active ingredients contained in the products appear to have antagonistic and/ or synergistic effects depending on the variable. The significance of the findings in the current study suggested that the three locally produced products with different active ingredients should be applied combined or separately, depending on the variable of interest.

## 5.3 Future recommendations

In the current study the cucurbitacin-containing phytonematicides and Biomuti served mainly as biostimulants on *P. trifoliata* rootstock seedlings, without information on nematode suppression. Since the products were not phytotoxic to *P. trifoliata* rootstock seedlings, certain combinations should be tested on suppression of *T. semipenetrans* population densities.

## 5.4 Conclusions

The results of the study demonstrated that Nemarioc-AL and Nemafric-BL phytonematicides and Biomuti interactions had stimulation and neutral effects on plant growth and selected nutrient elements in *P. trifoliata* rootstock seedlings. The sustainable products could be applied using certain combinations to improve growth of citrus rootstock seedlings.

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# APPENDICES

Appendix 3.1 Analysis of variance for dry shoot mass of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 26.656 | 2.96176 | | |
| Nemarioc-AL (A) | 1 | 2.861 | 2.86146 | 2.64 | 0.1091 |
| Nemafric-BL (B) | 1 | 1.386 | 1.38601 | 1.28 | 0.2622 |
| Biomuti (M) | 1 | 3.515 | 3.51541 | 3.25 | 0.0764 |
| A × B | 1 | 0.888 | 0.88831 | 0.82 | 0.3686 |
| A × M | 1 | 2.839 | 2.83881 | 2.62 | 0.1104 |
| B × M | 1 | 0.352 | 0.35245 | 0.33 | 0.5704 |
| A × B × M | 1 | 0.476 | 0.47586 | 0.44 | 0.5098 |
| Error | 63 | 68.228 | 1.08299 | | |
| Total | 79 | 107.202 | 16.36306 | | |

Appendix 3.2 Analysis of variance for plant height of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 3219.82 | 357.758 | | |
| Nemarioc-AL (A) | 1 | 0.21 | 0.210 | 0.01 | 0.9132 |
| Nemafric-BL (B) | 1 | 5.25 | 5.253 | 0.30 | 0.5863 |
| Biomuti (M) | 1 | 36.59 | 36.585 | 2.08 | 0.1538 |
| A × B | 1 | 9.87 | 9.870 | 0.56 | 0.4562 |
| A × M | 1 | 0.11 | 0.105 | 0.01 | 0.9386 |
| B × M | 1 | 0.53 | 0.528 | 0.03 | 0.8629 |
| A × B × M | 1 | 0.70 | 0.703 | 0.04 | 0.8420 |
| Error | 63 | 1106.02 | 17.556 | | |
| Total | 79 | 4379.10 | 428.568 | | |

Appendix 3.3 Analysis of variance for leaf number of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 0.28945 | 0.03216 | | |
| Nemarioc-AL (A) | 1 | 0.05610 | 0.05610 | 2.41 | 0.1256 |
| Nemafric-BL (B) | 1 | 0.00191 | 0.00191 | 0.08 | 0.7757 |
| Biomuti (M) | 1 | 0.01926 | 0.01926 | 0.83 | 0.3667 |
| A × B | 1 | 0.00165 | 0.00165 | 0.07 | 0.7913 |
| A × M | 1 | 0.03458 | 0.03458 | 1.48 | 0.2276 |
| B × M | 1 | 0.00143 | 0.00143 | 0.06 | 0.8054 |
| A × B × M | 1 | 0.03980 | 0.03980 | 1.71 | 0.1959 |
| Error | 63 | 1.46718 | 0.02329 | | |
| Total | 79 | 1.91135 | 0.21018 | | |

Appendix 3.4 Analysis of variance for chlorophyll of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 965.91 | 107.323 | | |
| Nemarioc-AL (A) | 1 | 106.95 | 106.953 | 4.12 | 0.0466 |
| Nemafric-BL (B) | 1 | 83.44 | 83.436 | 3.21 | 0.0778 |
| Biomuti (M) | 1 | 0.70 | 0.703 | 0.03 | 0.8698 |
| A × B | 1 | 9.45 | 9.453 | 0.36 | 0.5484 |
| A × M | 1 | 57.97 | 57.970 | 2.23 | 0.1401 |
| B × M | 1 | 10.44 | 10.440 | 0.40 | 0.5283 |
| A × B × M | 1 | 95.27 | 95.266 | 3.67 | 0.0600 |
| Error | 63 | 1635.70 | 25.964 | | |
| Total | 79 | 2965.83 | 497.508 | | |

Appendix 3.5 Analysis of variance for stem diameter of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 16.1665 | 1.79627 | | |
| Nemarioc-AL (A) | 1 | 1.0557 | 1.05570 | 2.42 | 0.1246 |
| Nemafric-BL (B) | 1 | 0.2195 | 0.21945 | 0.50 | 0.4805 |
| Biomuti (M) | 1 | 0.5528 | 0.55278 | 1.27 | 0.2643 |
| A × B | 1 | 0.0466 | 0.04656 | 0.11 | 0.7448 |
| A × M | 1 | 4.5078 | 4.50775 | 10.34 | 0.0021 |
| B × M | 1 | 0.0189 | 0.01891 | 0.04 | 0.8357 |
| A × B × M | 1 | 0.0839 | 0.08385 | 0.19 | 0.6624 |
| Error | 63 | 27.4543 | 0.43578 | | |
| Total | 79 | 50.1057 | 8.71705 | | |

Appendix 3.6 Analysis of variance for calcium of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 4161.8 | 462.42 | | |
| Nemarioc-AL (A) | 1 | 532.0 | 532.05 | 0.86 | 0.3581 |
| Nemafric-BL (B) | 1 | 443.4 | 443.35 | 0.71 | 0.4012 |
| Biomuti (M) | 1 | 380.7 | 380.67 | 0.61 | 0.4365 |
| A × B | 1 | 121.0 | 120.96 | 0.19 | 0.6604 |
| A × M | 1 | 6395.1 | 6395.14 | 10.30 | 0.0021 |
| B × M | 1 | 291.2 | 291.20 | 0.47 | 0.4959 |
| A × B × M | 1 | 1675.6 | 1675.64 | 2.70 | 0.1054 |
| Error | 63 | 39106.7 | 620.74 | | |
| Total | 79 | 53107.4 | 10922.17 | | |

Appendix 3.7 Analysis of variance for potassium of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 13729 | 1525.41 | | |
| Nemarioc-AL (A) | 1 | 8933 | 8933.13 | 5.95 | 0.0175 |
| Nemafric-BL (B) | 1 | 3790 | 3789.92 | 2.52 | 0.1171 |
| Biomuti (M) | 1 | 868 | 867.71 | 0.58 | 0.4500 |
| A × B | 1 | 1245 | 1245.28 | 0.83 | 0.3659 |
| A × M | 1 | 2052 | 2051.83 | 1.37 | 0.2468 |
| B × M | 1 | 1493 | 1492.56 | 0.99 | 0.3226 |
| A × B × M | 1 | 126 | 126.48 | 0.08 | 0.7726 |
| Error | 63 | 94592 | 1501.45 | | |
| Total | 79 | 126827 | 21533.77 | | |

Appendix 3.8 Analysis of variance for magnesium of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 100.21 | 11.134 | | |
| Nemarioc-AL (A) | 1 | 15.92 | 15.924 | 1.37 | 0.2462 |
| Nemafric-BL (B) | 1 | 282.45 | 282.451 | 24.30 | 0.0000 |
| Biomuti (M) | 1 | 107.73 | 107.732 | 9.27 | 0.0034 |
| A × B | 1 | 117.80 | 117.797 | 10.13 | 0.0023 |
| A × M | 1 | 60.48 | 60.482 | 5.20 | 0.0259 |
| B × M | 1 | 684.61 | 684.614 | 58.90 | 0.0000 |
| A × B × M | 1 | 79.55 | 79.533 | 6.84 | 0.0111 |
| Error | 63 | 732.24 | 11.623 | | |
| Total | 79 | 2181.00 | 1371.29 | | |

Appendix 3.9 Analysis of variance for sodium of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 1521.6 | 169.067 | | |
| Nemarioc-AL (A) | 1 | 463.2 | 463.203 | 2.35 | 0.1305 |
| Nemafric-BL (B) | 1 | 521.3 | 512.323 | 2.64 | 0.1090 |
| Biomuti (M) | 1 | 5.2 | 5.233 | 0.03 | 0.8711 |
| A × B | 1 | 363.7 | 363.719 | 1.84 | 0.1794 |
| A × M | 1 | 4.0 | 4.023 | 0.02 | 0.8869 |
| B × M | 1 | 0.9 | 0.937 | 0.00 | 0.9453 |
| A × B × M | 1 | 2.0 | 1.978 | 0.01 | 0.9206 |
| Error | 63 | 12428.7 | 197.281 | | |
| Total | 79 | 15310.7 | 1717.764 | | |

Appendix 3.10 Analysis of variance for zinc of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 0.31943 | 0.03549 | | |
| Nemarioc-AL (A) | 1 | 0.00916 | 0.00916 | 0.32 | 0.5717 |
| Nemafric-BL (B) | 1 | 0.01513 | 0.01513 | 0.53 | 0.4677 |
| Biomuti (M) | 1 | 0.00087 | 0.00087 | 0.03 | 0.8614 |
| A × B | 1 | 0.00065 | 0.00065 | 0.02 | 0.8801 |
| A × M | 1 | 0.03200 | 0.03200 | 1.13 | 0.2919 |
| B × M | 1 | 0.07638 | 0.07638 | 2.70 | 0.1056 |
| A × B × M | 1 | 0.02152 | 0.02152 | 0.76 | 0.3868 |
| Error | 63 | 1.78494 | 0.02833 | | |
| Total | 79 | 2.26008 | 0.21953 | | |

Appendix 3.11 Analysis of variance for phosphorus of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 11.590 | 1.2878 | | |
| Nemarioc-AL (A) | 1 | 52.100 | 52.0999 | 10.92 | 0.0016 |
| Nemafric-BL (B) | 1 | 43.601 | 43.6010 | 9.14 | 0.0036 |
| Biomuti (M) | 1 | 1.496 | 1.4960 | 0.31 | 0.5775 |
| A × B | 1 | 3.562 | 3.5617 | 0.75 | 0.3908 |
| A × M | 1 | 14.689 | 14.6890 | 3.08 | 0.0842 |
| B × M | 1 | 32.896 | 32.8961 | 6.90 | 0.0108 |
| A × B × M | 1 | 3.105 | 3.1047 | 0.65 | 0.4229 |
| Error | 63 | 300.565 | 4.7709 | | |
| Total | 79 | 463.604 | 157.5071 | | |

Appendix 3.12 Analysis of variance for dry shoot mass of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 154.573 | 17.1748 | | |
| Nemarioc-AL (A) | 1 | 4.622 | 4.6224 | 2.01 | 0.1612 |
| Nemafric-BL (B) | 1 | 0.621 | 0.6213 | 0.27 | 0.6051 |
| Biomuti (M) | 1 | 0.234 | 0.2344 | 0.10 | 0.7506 |
| A × B | 1 | 4.985 | 4.9850 | 2.17 | 0.1460 |
| A × M | 1 | 15.940 | 15.940 | 6.93 | 0.0107 |
| B × M | 1 | 0.597 | 0.5969 | 0.26 | 0.6123 |
| A × B × M | 1 | 2.142 | 2.1419 | 0.93 | 0.3383 |
| Error | 63 | 144.921 | 2.3003 | | |
| Total | 79 | 328.638 | 50.7589 | | |

Appendix 3.13 Analysis of variance for plant height of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 12038.9 | 1337.66 | | |
| Nemarioc-AL (A) | 1 | 49.9 | 49.93 | 0.86 | 0.3562 |
| Nemafric-BL (B) | 1 | 380.2 | 380.19 | 6.58 | 0.0127 |
| Biomuti (M) | 1 | 58.1 | 58.14 | 1.01 | 0.3197 |
| A × B | 1 | 162.5 | 162.45 | 2.81 | 0.0986 |
| A × M | 1 | 6.2 | 6.16 | 0.11 | 0.7451 |
| B × M | 1 | 45.9 | 45.90 | 0.79 | 0.3762 |
| A × B × M | 1 | 74.9 | 74.88 | 1.30 | 0.2593 |
| Error | 63 | 3640.7 | 57.79 | | |
| Total | 79 | 16457.2 | 2173.1 | | |

Appendix 3.14 Analysis of variance for leaf number of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 0.021232 | 0.02359 | | |
| Nemarioc-AL (A) | 1 | 0.00075 | 0.00075 | 0.05 | 0.8193 |
| Nemafric-BL (B) | 1 | 0.00656 | 0.00656 | 0.46 | 0.5008 |
| Biomuti (M) | 1 | 0.05478 | 0.05478 | 3.83 | 0.0549 |
| A × B | 1 | 0.02036 | 0.02036 | 1.42 | 0.2375 |
| A × M | 1 | 0.04938 | 0.04938 | 3.45 | 0.0679 |
| B × M | 1 | 0.01213 | 0.01213 | 0.85 | 0.3608 |
| A × B × M | 1 | 0.00165 | 0.00165 | 0.12 | 0.7351 |
| Error | 63 | 0.90177 | 0.01431 | | |
| Total | 79 | 1.25971 | 0.18351 | | |

Appendix 3.15 Analysis of variance for chlorophyll of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 273.18 | 30.353 | | |
| Nemarioc-AL (A) | 1 | 43.81 | 43.808 | 1.09 | 0.3004 |
| Nemafric-BL (B) | 1 | 144.18 | 144.184 | 3.59 | 0.0628 |
| Biomuti (M) | 1 | 0.68 | 0.685 | 0.02 | 0.8966 |
| A × B | 1 | 24.86 | 24.865 | 0.62 | 0.4344 |
| A × M | 1 | 117.61 | 117.613 | 2.93 | 0.0920 |
| B × M | 1 | 0.20 | 0.200 | 0.00 | 0.9440 |
| A × B × M | 1 | 2.59 | 2.592 | 0.06 | 0.8003 |
| Error | 63 | 2531.18 | 40.177 | | |
| Total | 79 | 3138.30 | 404.477 | | |

Appendix 3.16 Analysis of variance for stem diameter of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 16.8060 | 1.86733 | | |
| Nemarioc-AL (A) | 1 | 0.8080 | 0.80802 | 1.48 | 0.2286 |
| Nemafric-BL (B) | 1 | 0.0072 | 0.00722 | 0.01 | 0.9089 |
| Biomuti (M) | 1 | 0.0858 | 0.08581 | 0.16 | 0.6933 |
| A × B | 1 | 0.4440 | 0.44402 | 0.81 | 0.3709 |
| A × M | 1 | 0.5544 | 0.55445 | 1.01 | 0.3177 |
| B × M | 1 | 0.0004 | 0.00040 | 0.00 | 0.9784 |
| A × B × M | 1 | 0.0490 | 0.04901 | 0.09 | 0.7656 |
| Error | 63 | 34.4403 | 0.54667 | | |
| Total | 79 | 53.1952 | 4.36293 | | |

Appendix 3.17 Analysis of variance for calcium of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 14271 | 1585.7 | | |
| Nemarioc-AL (A) | 1 | 444 | 444.4 | 0.20 | 0.6546 |
| Nemafric-BL (B) | 1 | 5550 | 5550.4 | 2.52 | 0.1172 |
| Biomuti (M) | 1 | 107 | 107.1 | 0.05 | 0.8261 |
| A × B | 1 | 2 | 2.2 | 0.00 | 0.9747 |
| A × M | 1 | 856 | 855.7 | 0.39 | 0.5351 |
| B × M | 1 | 10147 | 10146.6 | 4.61 | 0.0356 |
| A × B × M | 1 | 3677 | 3676.9 | 1.67 | 0.2008 |
| Error | 63 | 138574 | 2199.6 | | |
| Total | 79 | 173629 | 24568.6 | | |

Appendix 3.18 Analysis of variance for potassium of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 25720 | 2857.72 | | |
| Nemarioc-AL (A) | 1 | 455 | 455.25 | 0.20 | 0.6584 |
| Nemafric-BL (B) | 1 | 1329 | 1328.78 | 0.58 | 0.4507 |
| Biomuti (M) | 1 | 9613 | 9613.24 | 4.17 | 0.0454 |
| A × B | 1 | 1590 | 1589.90 | 0.69 | 0.4096 |
| A × M | 1 | 122 | 121.92 | 0.05 | 0.8189 |
| B × M | 1 | 1381 | 1380.79 | 0.60 | 0.4421 |
| A × B × M | 1 | 5220 | 5220.33 | 2.26 | 0.1375 |
| Error | 63 | 145353 | 2307.19 | | |
| Total | 79 | 190783 | 24875.12 | | |

Appendix 3.19 Analysis of variance for magnesium of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 91.650 | 10.1833 | | |
| Nemarioc-AL (A) | 1 | 15.304 | 15.3038 | 1.60 | 0.2104 |
| Nemafric-BL (B) | 1 | 29.149 | 29.1491 | 3.05 | 0.0856 |
| Biomuti (M) | 1 | 29.149 | 29.1491 | 3.05 | 0.0856 |
| A × B | 1 | 10.089 | 10.0891 | 1.06 | 0.3082 |
| A × M | 1 | 13.886 | 13.8861 | 1.45 | 0.2326 |
| B × M | 1 | 69.546 | 69.5459 | 7.28 | 0.0090 |
| A × B × M | 1 | 1.827 | 1.8271 | 0.19 | 0.6635 |
| Error | 63 | 602.186 | 9.5585 | | |
| Total | 79 | 862.786 | 188.692 | | |

Appendix 3.20 Analysis of variance for sodium of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 102.956 | 11.4396 | | |
| Nemarioc-AL (A) | 1 | 14.669 | 14.6693 | 1.41 | 0.2388 |
| Nemafric-BL (B) | 1 | 0.42 | 0.4616 | 0.04 | 0.8336 |
| Biomuti (M) | 1 | 8.896 | 8.8958 | 0.86 | 0.3579 |
| A × B | 1 | 3.361 | 3.3608 | 0.32 | 0.5712 |
| A × M | 1 | 0.014 | 0.0136 | 0.00 | 0.9712 |
| B × M | 1 | 6.905 | 6.9049 | 0.67 | 0.4176 |
| A × B × M | 1 | 11.293 | 11.2928 | 1.09 | 0.3007 |
| Error | 63 | 653.365 | 10.3709 | | |
| Total | 79 | 801.920 | 67.4093 | | |

Appendix 3.21 Analysis of variance for zinc of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 8.2189 | 0.91321 | | |
| Nemarioc-AL (A) | 1 | 0.8798 | 0.87984 | 1.08 | 0.3026 |
| Nemafric-BL (B) | 1 | 0.5935 | 0.59352 | 0.73 | 0.3965 |
| Biomuti (M) | 1 | 1.5634 | 1.56338 | 1.92 | 0.1708 |
| A × B | 1 | 0.5896 | 0.58960 | 0.72 | 0.3980 |
| A × M | 1 | 0.5967 | 0.59670 | 0.73 | 0.3952 |
| B × M | 1 | 1.2125 | 1.21251 | 1.49 | 0.2269 |
| A × B × M | 1 | 0.3884 | 0.38844 | 0.48 | 0.4923 |
| Error | 63 | 51.3017 | 0.81431 | | |
| Total | 79 | 65.3447 | 7.55151 | | |

Appendix 3.22 Analysis of variance for phosphorus of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under greenhouse conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 43970 | 4885.58 | | |
| Nemarioc-AL (A) | 1 | 4904 | 4903.72 | 1.01 | 0.3183 |
| Nemafric-BL (B) | 1 | 4767 | 4766.73 | 0.98 | 0.3251 |
| Biomuti (M) | 1 | 4335 | 4335.29 | 0.89 | 0.3478 |
| A × B | 1 | 4443 | 4443.40 | 0.92 | 0.3419 |
| A × M | 1 | 4891 | 4890.74 | 1.01 | 0.3189 |
| B × M | 1 | 4843 | 4843.08 | 1.00 | 0.3213 |
| A × B × M | 1 | 3884 | 4883.91 | 1.01 | 0.3193 |
| Error | 63 | 300357 | 4844.47 | | |
| Total | 79 | 376394 | 42796.92 | | |

Appendix 4.1 Analysis of variance for dry shoot mass of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 27.207 | 3.02300 | | |
| Nemarioc-AL (A) | 1 | 2.798 | 2.79752 | 1.26 | 0.2660 |
| Nemafric-BL (B) | 1 | 0.046 | 0.04608 | 0.02 | 0.8859 |
| Biomuti (M) | 1 | 8.791 | 8.79138 | 3.96 | 0.0510 |
| A × B | 1 | 5.746 | 5.74592 | 2.59 | 0.1127 |
| A × M | 1 | 0.050 | 0.50000 | 0.02 | 0.8812 |
| B × M | 1 | 3.168 | 3.16808 | 1.43 | 0.2368 |
| A × B × M | 1 | 1.031 | 1.03058 | 0.46 | 0.4983 |
| Error | 63 | 139.929 | 2.22109 | | |
| Total | 79 | 188.765 | 27.323869 | | |

Appendix 4.2 Analysis of variance for plant height of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions n in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 1883.0 | 209.217 | | |
| Nemarioc-AL (A) | 1 | 126.8 | 126.756 | 0.69 | 0.4093 |
| Nemafric-BL (B) | 1 | 1.9 | 1.891 | 0.01 | 0.9195 |
| Biomuti (M) | 1 | 553.9 | 553.878 | 3.02 | 0.0874 |
| A × B | 1 | 595.7 | 595.686 | 3.24 | 0.0765 |
| A × M | 1 | 51.0 | 51.040 | 0.28 | 0.6000 |
| B × M | 1 | 31.6 | 31.626 | 0.17 | 0.6796 |
| A × B × M | 1 | 151.5 | 151.525 | 0.82 | 0.3672 |
| Error | 63 | 11573.3 | 183.707 | | |
| Total | 79 | 14968.9 | 1905.328 | | |

Appendix 4.3 Analysis of variance for leaf number of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 0.20482 | 0.02276 | | |
| Nemarioc-AL (A) | 1 | 0.03732 | 0.03732 | 1.73 | 0.1929 |
| Nemafric-BL (B) | 1 | 0.00037 | 0.00037 | 0.02 | 0.8968 |
| Biomuti (M) | 1 | 0.00171 | 0.00171 | 0.08 | 0.7792 |
| A × B | 1 | 0.10991 | 0.10991 | 5.10 | 0.0274 |
| A × M | 1 | 0.03133 | 0.03133 | 1.45 | 0.2324 |
| B × M | 1 | 0.01335 | 0.01335 | 0.62 | 0.4342 |
| A × B × M | 1 | 0.00817 | 0.00817 | 0.38 | 0.5404 |
| Error | 63 | 1.35752 | 0.02155 | | |
| Total | 79 | 1.76449 | 0.24647 | | |

Appendix 4.4 Analysis of variance for chlorophyll of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 1558.2 | 173.138 | | |
| Nemarioc-AL (A) | 1 | 16.2 | 16.200 | 0.12 | 0.7282 |
| Nemafric-BL (B) | 1 | 415.9 | 415.872 | 3.13 | 0.0818 |
| Biomuti (M) | 1 | 389.8 | 389.845 | 2.93 | 0.0917 |
| A × B | 1 | 247.8 | 247.808 | 1.86 | 0.1770 |
| A × M | 1 | 26.2 | 26.220 | 0.20 | 0.6585 |
| B × M | 1 | 118.6 | 118.585 | 0.89 | 0.3485 |
| A × B × M | 1 | 31.0 | 31.001 | 0.23 | 0.6308 |
| Error | 63 | 8373.8 | 132.917 | | |
| Total | 79 | 11177.6 | 1551.586 | | |

Appendix 4.5 Analysis of variance for stem diameter of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 10.206 | 1.13395 | | |
| Nemarioc-AL (A) | 1 | 1.439 | 1.43916 | 1.23 | 0.2718 |
| Nemafric-BL (B) | 1 | 0.607 | 0.60726 | 0.52 | 0.4741 |
| Biomuti (M) | 1 | 3.276 | 3.27645 | 2.80 | 0.0993 |
| A × B | 1 | 5.570 | 5.57040 | 4.76 | 0.0329 |
| A × M | 1 | 0.055 | 0.05460 | 0.05 | 0.8297 |
| B × M | 1 | 5.570 | 5.57040 | 4.76 | 0.0329 |
| A × B × M | 1 | 0.679 | 0.67896 | 0.58 | 0.4492 |
| Error | 63 | 73.774 | 1.17102 | | |
| Total | 79 | 101.177 | 19.5022 | | |

Appendix 4.6 Analysis of variance for calcium of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 3420.0 | 3420.1 | | |
| Nemarioc-AL (A) | 1 | 107.0 | 106.98 | 0.29 | 0.5890 |
| Nemafric-BL (B) | 1 | 530.7 | 530.71 | 1.46 | 0.2309 |
| Biomuti (M) | 1 | 2107.7 | 2107.71 | 5.81 | 0.0189 |
| A × B | 1 | 185.0 | 184.98 | 0.51 | 0.4778 |
| A × M | 1 | 1686.6 | 1686.64 | 4.65 | 0.0349 |
| B × M | 1 | 3.1 | 3.10 | 0.01 | 0.9266 |
| A × B × M | 1 | 9.6 | 9.64 | 0.03 | 0.8710 |
| Error | 63 | 22850 | 362.71 | | |
| Total | 79 | 30900.4 | 8412.57 | | |

Appendix 4.7 Analysis of variance for potassium of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 914.2 | 101.58 | | |
| Nemarioc-AL (A) | 1 | 966.3 | 966.33 | 10.03 | 0.0024 |
| Nemafric-BL (B) | 1 | 2517.1 | 2517.09 | 26.14 | 0.0000 |
| Biomuti (M) | 1 | 1644.8 | 1644.75 | 17.08 | 0.0001 |
| A × B | 1 | 153.6 | 153.57 | 1.59 | 0.2113 |
| A × M | 1 | 482.4 | 482.36 | 5.01 | 0.0288 |
| B × M | 1 | 1.8 | 1.78 | 0.02 | 0.8922 |
| A × B × M | 1 | 268.1 | 268.06 | 2.78 | 0.1002 |
| Error | 63 | 6067.6 | 96.31 | | |
| Total | 79 | 13015.7 | 6231.83 | | |

Appendix 4.8 Analysis of variance for magnesium of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 340.18 | 37.80 | | |
| Nemarioc-AL (A) | 1 | 1376.02 | 1376.02 | 27.90 | 0.0000 |
| Nemafric-BL (B) | 1 | 331.60 | 331.60 | 6.72 | 0.0118 |
| Biomuti (M) | 1 | 55.67 | 55.67 | 1.13 | 0.2921 |
| A × B | 1 | 202.61 | 202.61 | 4.11 | 0.0469 |
| A × M | 1 | 735.77 | 735.77 | 14.92 | 0.0003 |
| B × M | 1 | 354.63 | 354.63 | 7.19 | 0.0093 |
| A × B × M | 1 | 19.61 | 19.61 | 0.40 | 0.5306 |
| Error | 63 | 3106.79 | 49.31 | | |
| Total | 79 | 6522.88 | 3163.02 | | |

Appendix 4.9 Analysis of variance for sodium of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 34.760 | 3.86221 | 3.86221 | |
| Nemarioc-AL (A) | 1 | 0.396 | 0.39621 | 0.39621 | 0.26 |
| Nemafric-BL (B) | 1 | 2.208 | 2.20780 | 2.20780 | 1.46 |
| Biomuti (M) | 1 | 0.766 | 0.76636 | 0.76636 | 0.51 |
| A × B | 1 | 5.269 | 5.26851 | 5.26851 | 3.49 |
| A × M | 1 | 0.037 | 0.03741 | 0.03741 | 0.02 |
| B × M | 1 | 0.324 | 0.32385 | 0.32385 | 0.21 |
| A × B × M | 1 | 0.070 | 0.07021 | 0.07021 | 0.05 |
| Error | 63 | 94.987 | 1.50774 | 1.50774 | |
| Total | 79 | 138.818 | 14.4403 | | |

Appendix 4.10 Analysis of variance for zinc of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 2.4176 | 0.26862 | | |
| Nemarioc-AL (A) | 1 | 0.4628 | 0.46276 | 4.19 | 0.0448 |
| Nemafric-BL (B) | 1 | 0.0007 | 0.00071 | 0.01 | 0.9366 |
| Biomuti (M) | 1 | 1.0878 | 1.08781 | 9.86 | 0.0026 |
| A × B | 1 | 0.0081 | 0.00809 | 0.07 | 0.7875 |
| A × M | 1 | 0.0469 | 0.04689 | 0.42 | 0.5169 |
| B × M | 1 | 0.8872 | 0.88715 | 8.04 | 0.0062 |
| A × B × M | 1 | 0.0811 | 0.08110 | 0.73 | 0.3946 |
| Error | 63 | 6.9538 | 0.11038 | | |
| Total | 79 | 11.9459 | 2.68489 | | |

Appendix 4.11 Analysis of variance for phosphorus of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 1.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 23.077 | 2.5642 | | |
| Nemarioc-AL (A) | 1 | 51.427 | 51.4275 | 20.03 | 0.0000 |
| Nemafric-BL (B) | 1 | 27.329 | 27.3289 | 10.65 | 0.0018 |
| Biomuti (M) | 1 | 56.646 | 56.6464 | 22.07 | 0.0000 |
| A × B | 1 | 2.087 | 2.0872 | 0.81 | 0.3706 |
| A × M | 1 | 35.009 | 35.0092 | 13.64 | 0.0005 |
| B × M | 1 | 0.175 | 0.1747 | 0.07 | 0.7951 |
| A × B × M | 1 | 7.380 | 7.3799 | 2.87 | 0.0949 |
| Error | 63 | 161.723 | 2.5670 | | |
| Total | 79 | 364.854 | 185.185 | | |

Appendix 4.12 Analysis of variance for dry shoot mass of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 57.399 | 6.3776 | | |
| Nemarioc-AL (A) | 1 | 0.343 | 0.3432 | 0.08 | 0.7767 |
| Nemafric-BL (B) | 1 | 25.696 | 25.6964 | 6.08 | 0.0164 |
| Biomuti (M) | 1 | 0.007 | 0.0068 | 0.00 | 0.9680 |
| A × B | 1 | 0.023 | 0.0231 | 0.01 | 0.9413 |
| A × M | 1 | 2.949 | 2.9491 | 0.70 | 0.4068 |
| B × M | 1 | 0.1566 | 0.1566 | 0.04 | 0.8480 |
| A × B × M | 1 | 2.7232 | 2.7232 | 0.64 | 0.4253 |
| Error | 63 | 266.411 | 4.2287 | | |
| Total | 79 | 355.708 | 42.5047 | | |

Appendix 4.13 Analysis of variance for plant height of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 2486.3 | 276.256 | | |
| Nemarioc-AL (A) | 1 | 79.4 | 79.401 | 0.40 | 0.5317 |
| Nemafric-BL (B) | 1 | 785.6 | 785.631 | 3.91 | 0.0523 |
| Biomuti (M) | 1 | 533.0 | 533.028 | 2.66 | 0.1082 |
| A × B | 1 | 20.1 | 20.100 | 0.10 | 0.7527 |
| A × M | 1 | 68.6 | 68.635 | 0.34 | 0.5608 |
| B × M | 1 | 40.2 | 40.186 | 0.20 | 0.6561 |
| A × B × M | 1 | 1.3 | 1.275 | 0.01 | 0.9367 |
| Error | 63 | 12647.6 | 200.756 | | |
| Total | 79 | 16662.2 | 2005.268 | | |

Appendix 4.14 Analysis of variance for leaf number of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 0.33208 | 0.33208 | | |
| Nemarioc-AL (A) | 1 | 0.00766 | 0.00766 | 0.27 | 0.6034 |
| Nemafric-BL (B) | 1 | 0.09262 | 0.09262 | 3.30 | 0.0742 |
| Biomuti (M) | 1 | 0.01412 | 0.01412 | 0.50 | 0.4809 |
| A × B | 1 | 0.00110 | 0.00110 | 0.04 | 0.8435 |
| A × M | 1 | 0.07401 | 0.07401 | 2.63 | 0.1096 |
| B × M | 1 | 0.03741 | 0.03741 | 1.33 | 0.2529 |
| A × B × M | 1 | 0.09470 | 0.09470 | 3.37 | 0.0711 |
| Error | 63 | 1.76995 | 0.02809 | | |
| Total | 79 | 2.42365 | 0.68179 | | |

Appendix 4.15 Analysis of variance for chlorophyll of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 1212.54 | 134.727 | | |
| Nemarioc-AL (A) | 1 | 233.59 | 233.586 | 2.58 | 0.1135 |
| Nemafric-BL (B) | 1 | 12.40 | 12.403 | 0.14 | 0.7127 |
| Biomuti (M) | 1 | 80.60 | 80.601 | 0.89 | 0.3494 |
| A × B | 1 | 92.24 | 92.235 | 1.02 | 0.3170 |
| A × M | 1 | 21.32 | 21.321 | 0.24 | 0.6294 |
| B × M | 1 | 25.88 | 25.878 | 0.29 | 0.5951 |
| A × B × M | 1 | 190.65 | 190.653 | 2.10 | 0.1520 |
| Error | 63 | 5712.49 | 90.674 | | |
| Total | 79 | 7581.71 | 882.078 | | |

Appendix 4.16 Analysis of variance for stem diameter of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 12.5122 | 1.39024 | | |
| Nemarioc-AL (A) | 1 | 0.3472 | 0.34716 | 0.28 | 0.5992 |
| Nemafric-BL (B) | 1 | 6.4923 | 6.49230 | 5.2 | 0.0257 |
| Biomuti (M) | 1 | 0.5528 | 0.55278 | 0.44 | 0.5075 |
| A × B | 1 | 0.0202 | 0.02016 | 0.02 | 0.8991 |
| A × M | 1 | 0.0053 | 0.00528 | 0.00 | 0.9483 |
| B × M | 1 | 0.6753 | 0.67528 | 0.54 | 0.4641 |
| A × B × M | 1 | 0.5729 | 0.57291 | 0.46 | 0.4999 |
| Error | 63 | 78.3955 | 1.24437 | | |
| Total | 79 | 99.5736 | 11.30048 | | |

Appendix 4.17 Analysis of variance for calcium of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 27186.4 | 3020.72 | | |
| Nemarioc-AL (A) | 1 | 451.3 | 451.29 | 0.44 | 0.5116 |
| Nemafric-BL (B) | 1 | 4182.1 | 4182.07 | 4.04 | 0.0488 |
| Biomuti (M) | 1 | 956.4 | 956.36 | 0.92 | 0.3402 |
| A × B | 1 | 44.1 | 44.13 | 0.04 | 0.8371 |
| A × M | 1 | 31.4 | 31.37 | 0.03 | 0.8624 |
| B × M | 1 | 163.8 | 163.83 | 0.16 | 0.6922 |
| A × B × M | 1 | 6331.2 | 6331.20 | 6.12 | 0.0162 |
| Error | 63 | 64191.6 | 1035.35 | | |
| Total | 79 | 103538.3 | 16216.32 | | |

Appendix 4.18 Analysis of variance for potassium of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 10279 | 1142.11 | | |
| Nemarioc-AL (A) | 1 | 154 | 153.68 | 0.09 | 0.7644 |
| Nemafric-BL (B) | 1 | 1137 | 1136.57 | 0.67 | 0.4161 |
| Biomuti (M) | 1 | 2627 | 2627.25 | 1.55 | 0.2179 |
| A × B | 1 | 794 | 794.11 | 0.47 | 0.4963 |
| A × M | 1 | 350 | 350.43 | 0.21 | 0.6510 |
| B × M | 1 | 2207 | 2206.65 | 1.30 | 0.2584 |
| A × B × M | 1 | 133 | 133.49 | 0.08 | 0.7800 |
| Error | 63 | 105136 | 1695.74 | | |
| Total | 79 | 122817 | 10240.03 | | |

Appendix 4.19 Analysis of variance for magnesium of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 149.724 | 16.6360 | | |
| Nemarioc-AL (A) | 1 | 10.983 | 10.9825 | 1.17 | 0.2830 |
| Nemafric-BL (B) | 1 | 26.395 | 26.3952 | 2.82 | 0.0982 |
| Biomuti (M) | 1 | 0.04 | 0.0044 | 0.00 | 0.9828 |
| A × B | 1 | 17.941 | 17.9409 | 1.92 | 0.1712 |
| A × M | 1 | 4.77 | 4.7775 | 0.51 | 0.4777 |
| B × M | 1 | 0.147 | 0.1469 | 0.02 | 0.9007 |
| A × B × M | 1 | 0.156 | 0.1563 | 0.02 | 0.8976 |
| Error | 63 | 580.469 | 9.3624 | | |
| Total | 79 | 790.625 | 86.4021 | | |

Appendix 4.20 Analysis of variance for sodium of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 15.274 | 1.69712 | | |
| Nemarioc-AL (A) | 1 | 0.955 | 0.95466 | 0.46 | 0.5022 |
| Nemafric-BL (B) | 1 | 2.914 | 2.91373 | 1.36 | 0.2428 |
| Biomuti (M) | 1 | 0.854 | 0.85417 | 0.41 | 0.5255 |
| A × B | 1 | 0.110 | 0.11013 | 0.05 | 0.8194 |
| A × M | 1 | 1.155 | 1.15495 | 0.55 | 0.4606 |
| B × M | 1 | 3.568 | 3.56788 | 1.70 | 0.1968 |
| A × B × M | 1 | 0.250 | 0.25047 | 0.12 | 0.7307 |
| Error | 63 | 129.913 | 2.09538 | | |
| Total | 79 | 154.993 | 13.59849 | | |

Appendix 4.21 Analysis of variance for zinc of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 0.85467 | 0.09496 | | |
| Nemarioc-AL (A) | 1 | 0.32232 | 0.32232 | 2.99 | 0.0889 |
| Nemafric-BL (B) | 1 | 0.01941 | 0.01941 | 0.18 | 0.6730 |
| Biomuti (M) | 1 | 0.00111 | 0.00111 | 0.01 | 0.9194 |
| A × B | 1 | 0.11444 | 0.11444 | 1.06 | 0.3071 |
| A × M | 1 | 0.14894 | 0.14894 | 1.38 | 0.2446 |
| B × M | 1 | 0.11979 | 0.11979 | 1.11 | 0.2962 |
| A × B × M | 1 | 0.15428 | 0.15428 | 1.43 | 0.2364 |
| Error | 63 | 6.69072 | 0.10791 | | |
| Total | 79 | 8.42568 | 1.08316 | | |

Appendix 4.22 Analysis of variance for phosphorus of citrus seedlings to cucurbitacin-containing phytonematicides and Biomuti alone and combined under field conditions in Experiment 2.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 9 | 24.210 | 2.68996 | | |
| Nemarioc-AL (A) | 1 | 0.40 | 0.03960 | 0.02 | 0.9012 |
| Nemafric-BL (B) | 1 | 1.264 | 1.26366 | 0.50 | 0.4840 |
| Biomuti (M) | 1 | 0.155 | 0.15472 | 0.06 | 0.8062 |
| A × B | 1 | 0.016 | 0.01616 | 0.01 | 0.9368 |
| A × M | 1 | 2.407 | 2.40691 | 0.94 | 0.3350 |
| B × M | 1 | 5.787 | 5.78670 | 2.27 | 0.1370 |
| A × B × M | 1 | 2.247 | 2.24717 | 0.88 | 0.3514 |
| Error | 63 | 158.032 | 2.54891 | | |
| Total | 79 | 194.518 | 17.15379 | | |