EFFICACY OF TWO PHYTONEMATICIDES AS INFLUENCED BY CONTAINER TYPE AND POSITIONING ON GROWTH OF TOMATO PLANTS AND SUPPRESSION OF *MELOIDOGYNE INCOGNITA*

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DECLARATION

I, Tshepho Makwapana [redacted] declare that the Mini-Dissertation hereby submitted to the University of Limpopo, for the degree Master of Agriculture in Plant Protection has not been submitted previously by me or anybody for a degree at this or any other University. Also, this is my work in design and in execution, and related materials contained herein had been duly acknowledged.

Makwapana T (Mr) | Date

DEDICATIONS

To my late parents, Ramatsobane Betty and Tjima Malakia Ramoshu, my daughters, Valentine and Kgethang and my wife, Sharlot Maphaka.

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ABSTRACT

Previously, cucurbitacin-containing phytonematicides that were drench-applied in black plastic containers filled with pasteurised loam soil when placed on the soil surface had no effect on suppression of population densities of root-knot (*Meloidogyne* species) nematodes. The active ingredients of cucurbitacin-containing phytonematicides, namely, the cucurbitacins, had been shown to be thermophilic, with the failure of the products explained from the view of the variability induced by container-type and aboveground positioning. The view was investigated further using Nemarioc-AL and Nemafric-BL phytonematicides as influenced by container-type and positioning on growth of tomato (*Solanum lycopersicum L*.) plants and suppression of *M. incognita* population densities. Tomato cv. ′Floradade′ seedlings were transplanted into 30-cm-diameter brown pot belowground, brown pot aboveground, black pot belowground, black pot aboveground, 5 L polyethylene plastic bag belowground and 5 L polyethylene plastic bag aboveground, each containing 5-dm^3 steam-pasteurised sandy loam soil amended with Hygromix at 3:1 (v/v) ratio. Seedlings were inoculated with 2000 eggs and second-stage juveniles (J2) of *M. incognita* race 2, with Nemarioc-AL and Nemafric-BL phytonematicides applied once 17 days after inoculation in both Experiment 1 and Experiment 2. Also, standard cultural practices were applied throughout the trial. At 56 days after inoculation, container-type and positioning had significant effects on various plant growth and essential nutrient element variables in Experiment 1 and Experiment 2, except that the six treatments did not have significant effects on nutrient elements and nematode population densities in Experiment 2. Relative to brown plastic pot belowground, treatments either increased or decreased plant growth, essential nutrient elements and nematode densities in Experiment 1, with selective similarities

in Experiment 2. Specifically, nematode variables except for J2 in soil and total nematode population densities were significantly affected by the treatments in Experiment 2. Relative to the standard, plastic bag belowground increased J2 in soil and total population in soil by 18%. In conclusion, both container-type and positioning had effects on the efficacy of phytonematicides on plant growth, accumulation of essential nutrient elements and suppression of nematode population densities. Consequently, in trials where cucurbitacin-containing phytonematicides are conducted in microplots, brown plastic pots with the belowground positioning should be used to enhance the efficacy of the phytonematicides in stimulating plant growth and suppression of nematode population densities.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

1.1.1 Description of the research problem

Worldwide, tomato (*Solanum lycopersicum* L.) is one of the most extensively produced and consumed vegetable crop, with increasing tendency to cultivate the crop in containers (Grandillo *et al*., 1999). Root deformation had been shown to be common in plants raised in smooth-sided plastic containers in an extended production cycle (Gilman *et al*., 2003). In such production systems, roots could either circle the container internally or growing vertically from the bottom of the container as opposed to the lateral roots growing horizontally towards the sides of the container (Ruter, 1994). Tsakaldimi *et al*. (2005) demonstrated that root spiralling inside the container is one of the most serious challenges that had the tendency of occurring mostly when round and smooth-walled plastic containers were used. Notwithstanding, such containers are commonly used in trials investigating the efficacy of products under greenhouse and micro-plot conditions.

1.1.2 Impact of the research problem

Container-type and positioning could have influence on root growth in relation to the morphological and physiological features of plants (Kostopoulou *et al.,* 2011). Johnson and Ingram (1984) demonstrated that heat stress directed to roots in container-grown plants could result in a wide range of challenges, with the generated data being not representative of open-field conditions. The exposure of roots in container-grown plants to substrate temperatures above 30^{o}C oftentimes slowed root growth (Johnson and Ingram, 1984). Additionally, such exposure could result in leaf wilting or chlorosis, reduction in flower number and quality, abnormal shoot branching and disturbance of various physiological, biochemical and translocation processes in response to high root zone temperature inside the container (Ingram *et al*., 1989). Container-type and positioning belowground or aboveground could drastically affect the temperature of the growing medium. However, there is limited information on how container-type and positioning belowground or aboveground could affect the efficacy of soil-drenched products such as the cucurbitacin-containing phytonematicides in the management of population densities of root-knot (*Meloidogyne* species) nematodes. In a recent study (Nyamandi, 2017), it was observed that two commonly used cucurbitacin-containing phytonematicides, namely, Nemarioc-AL and Nemafric-BL phytonematicides, failed to suppress population densities of *Meloidogyne* species when the trial was carried out in artificial micro-plots comprising black plastic containers positioned aboveground soil surface. Traditionally, artificial micro-plots comprised brown plastic pots that are positioned below the soil surface.

1.1.3 Possible causes of the research problem

Generally, soil substrate is a poor conductor of heat, with containers inserted in the soil retaining constant heat for extended periods. In contrast, in containers above the soil surface, the temperature of the soil substrate fluctuates with that of ambient temperature, with nocturnal temperatures fluctuating to as low as freezing point. Generally, cucurbitacins are thermophilic (Shadung, 2016), with high decomposition at low temperatures. Consequently, artificial micro-plots with containers placed aboveground would not be suitable for efficacy trials for cucurbitacin-containing phytonematicides, more especially when coupled with the challenges that containers imposed on the root systems of plants Johnson and Ingram (1984), as alluded to earlier.

1.1.4 Proposed solutions

Currently, information on influence of container-type and colour on the efficacy of cucurbitacin-containing phytonematicides is limited. Available containers include polystyrene bags and plastic pots of different sizes and shapes. Generally, brown plastic pots serving as belowground artificial micro-plots are traditionally preferred without any empirical-basis for the preferences. Thus, the gap could be closed by assessing the efficacy cucurbitacin-containing phytonematicides on plant growth and suppression of nematodes in various container-types with various colours and positioning below- and aboveground.

1.1.5 General focus of the study

The study focused on the efficacy of Nemarioc-AL and Nemafric-BL phytonematicides when admixed with pasteurised growing mixture as influenced by container-types of different colours and positioning on growth of tomato plants and suppression of *M. incognita* population densities under micro-plot conditions.

1.2 Problem statement

The colour, type and positioning of containers in artificial micro-plots could have influence on the test plants that could be confounded with the treatment effects. Although much information is available on how containers affect root growth and plant growth, information on how containers and positioning in context of artificial microplots could affect the efficacy of cucurbitacin-containing phytonematicides, is scarce. Since most nematode-phytonematicide trials comprise the use of different containers and positioning, it is imperative that the effects of the two factors on the performance of phytonematicides in relation to plant growth and suppression of nematodes be empirically established.

1.3 Rationale of the study

Most traditional synthetic chemical nematicides had been withdrawn from the agrochemical markets due to their environment-unfriendliness (Mashela *et al*., 2017). Ever since the 2005 cut-off date for the withdrawal of methyl bromide, crop losses due to nematode damage had been increasing, with recent report suggesting yield losses in monetary terms as high as 37% (Mashela *et al*., 2016), thereby necessitating increased agility in research and development of alternative management options (Mashela *et al*., 2016). The cucurbitacin-containing phytonematicides have been in the forefront of research and development of the alternatives for managing nematode populations densities, with trials conducted under greenhouse, micro-plot and field conditions (Mashela and Pofu, 2017). However, in a recent micro-plot study (Nyamandi, 2017), where polystyrene plastic bags were used aboveground, it was observed that Nemarioc-AL and Nemafric-BL phytonematicides failed to suppress population densities of *M. incognita* race 2, with infected roots displaying huge galls. Generally, cucurbitacin A ($C_{32}H_{46}O_9$) and cucurbitacin B ($C_{32}H_{46}O_8$) are active ingredients in Nemarioc-AL and Nemafric-BL phytonematicides, respectively (Mashela and Pofu, 2017), with cucurbitacin A being unstable. However, the previous failure of both phytonematicides could not be explained exclusively through the instability or stability of the two cucurbitacins. In the current study, attempts were made to understand some of the logical reasons for the observed failure.

1.4 Purpose

1.4.1 Aim

Investigation of potential reasons that could lead to failure of cucurbitacin-containing phytonematicides in suppression of *Meloidogyne* species under artificial microplot conditions.

1.4.2 Objective

The objective of the study was to determine whether container-type and positioning would have an effect on the efficacy of Nemarioc-AL and Nemafric-BL phytonematicides each on growth of tomato plants and suppression of *M. incognita* race 2 population densities under artificial micro-plot conditions.

1.4.3 Hypothesis

Container-type and positioning would have an effect on the efficacy of Nemarioc-AL and Nemafric-BL phytonematicides each on growth of tomato plants and suppression of *M. incognita* race 2 population densities under artificial micro-plot 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%. Validity was achieved through repeating proper treatments of the experiments. Objectivity was achieved by ensuring that the findings are discussed on the basis of empirical evidence, thereby eliminating all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

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

1.7 Scientific significance of the study

The findings of this study would provide some empirical evidence on reasons why cucurbitacin-containing phytonematicides could fail when certain containers were used and positioned in a certain manner, particularly on the aboveground surface, where temperature of the growing mixture is a function of ambient temperature. The findings would further demonstrate the need to repeat the greenhouse phytonematicide trials under both micro-plot and field conditions, with the view that if findings could be replicated under all three conditions, the product could be viewed as being stable.

1.8 Structure of Mini-Dissertation

Following the description and detailed outlining of the research problem (Chapter 1), the work done and not yet done on the research problem was reviewed (Chapter 2). The objective was addressed as Chapter 3, followed by Chapter 4 where the findings were summarised and integrated to provide their significance, along with recommendations for future research, culminating in a conclusion that tied together the entire study. The citation and references were done according to the Harvard style of author-alphabet as prescribed by Senate of the University of Limpopo.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The cucurbitacin-containing phytonematicides, namely, Nemarioc-AL and Nemafric-BL phytonematicides, failed to suppress root-knot (*Meloidogyne* species) nematodes when the polystyrene plastic bags used were aboveground (Nyamandi, 2017). The latter suggested that extremes in ambient temperature could have affected the efficacy of the phytonematicides. The review for this study focused on the influence of containers and their positioning in relation to plant growth affected by ambient temperature.

2.2 Work done on the problem statement

2.2.1 Effect of container type on growth of container-grown plants

Heat stress is an important factor in nursery culture, particularly when focused on roots in container-raised plants. Heat stress on roots had been shown to affect aboveground growth in various ways. Increase in temperature in the root-zone could result in the incidence of disease outbreak, plant injury or wilting (Ranney and Peet, 1994; Webber and Ross, 1995). High temperature directed to the root-zone could limit growth, development and reproduction in container-raised plants, along with reduction in root growth, root number and root mass, with the consequent disruption of normal supply of water and uptake of mineral nutrients (Boyer, 1982). The production of plant growth regulators commonly synthesized in roots and transported to shoots could also be disrupted, thereby interfering with sink-source relations between shoots and roots (Foster *et al*., 1991). In addition, challenges facing the production of container-raised plants had not been limited to root deformation, but also to the emergence of root-balls aboveground or above soil surface of container-raised plants (Johnson and Ingram, 1984; Ruter and Ingram, 1992; Yeager *et al*., 1991).

Evans and Hensley (2004) observed that dry shoot mass of tomato plants grown in plastic containers were significantly higher than dry shoot mass of tomato plants grown in either peat or feather containers, whereas the variable did not differ for plants in peat and feather containers. Root deformation tended to be high in plants raised in smooth-sided plastic containers for extended production cycle (Gilman *et al*., 2003; Ruter, 1994), probably because the lateral roots could not extend vertically to the bottom or horizontally sideways, resulting in their forming circle within the containers (Lindström and Rüne, 1999). Container-type could affect plant growth (Nardini *et al*., 2000; Tsakaldimi *et al*., 2005). Plants in paper pots were observed to be significantly taller, with bigger stem diameter, dry shoot biomass, root biomass, shoot/root ratio and root volume than those grown in quick-pot and plantek (Nardini *et al*., 2000).

2.2.2 Efficacy of phytonematicides on growth of container-raised plants

Mashela *et al*. (2010) reported an increase in fruit yield, dry shoot mass, plant height and stem diameter of container-raised tomato plants when treated with ground fever tea (*Lippia javanica* F. Burm.) leaves in ground leaching technology (Mashela, 2002). The application of ground fever tea leaves as a soil-drench had reduced (79-92%) population densities of *M. incognita* in roots of tomato plants raised in brown plastic pots (Mashela *et al*., 2010). A wide range of pot trials with tomato were conducted where Nemarioc-AL and Nemafric-BL phytonematicides successfully suppressed population densities of *Meloidogyne* species under greenhouse (Tseke, 2013, Chokoe, 2017; Malungane, 2014) and micro-plot (Mashela *et al*., 2010; Sithole, 2016; Seshweni, 2017) conditions.

2.2.3 Efficacy of phytonematicides on nematode suppression in container-raised plants

Mashela (2002) reported that in the GLT system fruits of *Cucumis* species consistently suppressed population densities of *Meloidogyne* species by 90% under greenhouse conditions. Also, Pelinganga *et al*. (2011) reported that Nemarioc-AL and Nemafric-BL phytonematicides reduced root-knot nematode population densities by 69% and 89%, respectively.

Pelinganga *et al*. (2013) reported a reduction in nematode population densities in roots of container-grown tomato plants and soil amounting to 94% and 48% when exposed to Nemarioc-AL phytonematicide, respectively. Tseke *et al*. (2013) made similar observations on root-knot nematodes in container-raised tomato plants when exposed to Nemarioc-AL phytonematicide under greenhouse conditions. Also, Tseke *et al*. (2013) reported a constant reduction in population densities of *M. incognita* race 2 in roots of tomato plants and soil amounting to 46 to 92% and 74 to 96%, respectively. Nemafric-BL phytonematicide had been reported to reduce nematode population densities amounting to 85-97%, 45-59% and 78-97% in roots, soil and total nematodes, respectively (Pelinganga *et al*., 2012). Similarly, Mashela (2002); Mofokeng *et al*. (2004) and Shakwane *et al*. (2004) reported nematodes suppression of above 90% in container-raised plants when exposed to Nemarioc-AG phytonematicide. Ghazalbash and Abdollahi (2013) reported nematicidal effect of botanicals and plant materials used to manage nematodes in container-raised tomato plants in all experiments conducted under greenhouse conditions. A reduction of 67 to 90% of root-lesions (*Pratylenchus penetrans* Cobb, 1917) Filipjev and Schuurmans Stekhoven, 1941 and *M. hapla* were observed under 1% neem cake on roots of container-raised tomato plants under greenhouse conditions (Abbasi *et al*., 2009).

2.2.4 Effects of phytonematicides in pot trials under micro-plot conditions

Lebea (2017) observed significant effects on dry shoot mass of squash plants raised in aboveground containers outside the greenhouse, but highly significant effects on the same plant variable when treated with Nemarioc-AL phytonematicide. In addition, Lebea (2017) observed non-significant effects on plant height, stem diameter, chlorophyll content and fresh fruit mass when plants were exposed to increasing concentration of Nemarioc-AL phytonematicide. However, the effects of increasing concentration of Nemarioc-AL phytonematicide on dry shoot mass was inconsistent, resulting in high reduction at lower concentration and an increase in dry shoot mass at lower concentration (Lebea, 2017). Also, Sithole (2016) observed highly significant effects of Nemarioc-AL phytonematicide on plant height, dry root mass and gall rating, but non-significant effects on chlorophyll content, dry shoot mass and dry tuber mass of belowground potted *Pelargonium sidoides* plants. Seshweni (2017) observed non-significant effects on all measured potato plant variables, but there were significant effects on gall rating of belowground potted potato plants. Mashitoa (2017) reported significant effects on fresh bulb mass, dry bulb mass and gall rating but had no effects on fresh root mass, dry shoot mass and leaves of aboveground potted beetroot plants when treated with Nemafric-BL phytonematicide. Under greenhouse conditions, Chokoe (2017) observed significant effects on dry shoot mass of aboveground potted Green bean (*phaseolus vulgaris*) plants when treated with Nemarioc-AL phytonematicide, but were not significant on other measured plant variables.

Lebea (2017) reported highly significant effects on eggs, J2 in roots, J2 in soil and total nematode in aboveground potted squash plants when exposed to increasing concentrations of Nemarioc-AL phytonematicide. Similar trends have been reported by Lebea (2017) on eggs, J2 in roots and total nematodes in aboveground potted squash treated with increasing concentrations of Nemafric-BL phytonematicide. Sithole (2016) reported highly significant effects on eggs and J2s of belowground potted *Pelargonium sidoides* plants treated with Nemarioc-AL phytonemacide, whereas significant effects on eggs and Pf has been observed when belowground potted *P. sidoides* plants were exposed to Nemafric-BL phytonematicide. Mashitoa (2017) reported that there were nematodes in soil and roots samples of aboveground potted beetroot plants treated with both Nemarioc-AL and Nemafric-BL phytonematicides, under microplot conditions. Whereas, Chokoe (2017) observed significant reduction on eggs, J2 in roots, J2 in soil and final nematode population when exposing aboveground container raised green bean plants to Nemarioc-AL phytonematicide, and the same trend had been observed when exposing the same crop to Nemafric-BL phytonematicide.

2.3 Work not done on problem statement

The influence of container-type and positioning on the efficacy of Nemarioc-AL and Nemafric-BL phytonematicides on growth of plants and suppression of nematode population densities had not been documented with the exception of the accidental observation by Nyamandi (2017). Different container-types and positioning, as shown in the above review, could have highly significant effects on plant growth and soil-drenched products, with the major factor being through heat stress on the root-zone.

CHAPTER 3

EFFICACY OF TWO PHYTONEMATICIDES AS INFLUENCED BY CONTAINER-TYPE AND POSITIONING ON GROWTH OF TOMATO PLANTS AND SUPPRESSION OF *MELOIDOGYNE INCOGNITA*

3.1 Introduction

Container-raised seedlings could have various performance-related disadvantages when compared with their bare-root counterparts (Stein *et al*., 1975; Tsakaldimi *et al*., 2005). In containers, root deformation (Gilman *et al*., 2003), root circling (Ruter, 1994) and aboveground shoot deformations (Kostopoulou *et al.,* 2011), could have a confounding effect on the observations.

Ham *et al*. (1993) demonstrated that the use of light coloured containers than standard black containers could improve growth of container-raised plants since light coloured containers have greater albedo than black coloured containers. Nyamandi (2017) accidentally observed that cucurbitacin-containing phytonematicides failed to suppress population densities of nematodes when tomato plants were raised in black polystyrene containers that were placed aboveground surface under artificial micro-plot conditions. The failure was described in terms of the high degree of heat changes on roots during the high day-ambient temperatures and low night-ambient temperatures, which incidentally, affected the efficacy of the phytonematicides as evidenced by high gall ratings. The objective of the study was to determine whether container-type and positioning would have an effect on the efficacy of Nemarioc-AL and Nemafric-BL phytonematicides on growth of tomato plants and suppression of *M. incognita* race 2 population densities under artificial micro-plot conditions.

3.2 Materials and methods

3.2.1 Description of the study site

Two parallel experiments, one for Nemarioc-AL phytonematicide and the other one for Nemafric-BL phytonematicide, were conducted at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, South Africa (23°53′10″S, 29°44′15″E) in autumn (Feb-April) 2017.

3.2.2 Treatments and research design

The 3 × 2 factorial trial, with first and second factors being container-type and positioning. Six treatments, namely, brown plastic-pot belowground (1), brown plastic-pot-above (2), black plastic-pot-below (3), black plastic-pot-above (4), black plastic-bag-below (5) and black plastic-bag-above (6), were arranged in a randomised complete block design, with 10 replicates. Treatments were blocked for shading by the windbreak trees in the morning and late in the afternoon. Three days after transplanting, each plant was fertilised with 3 g NPK 2:3:2 (22) to provide 186 N, 126 K and 156 P, with 2 g NPK 2:1:2 (43) providing 0.35 N, 0.32 K and 0.32 P, 0.9 Mg, 0.75 Fe, 0.075 Cu, 0.35 Zn, 1.0 B, 3.0 Mn and 0.07 Mo mg/ml water (Mashela *et al*., 2015).

Figure 3.1 Tomato plants cv. 'Floradade' planted under microplot conditions.

3.2.3 Procedures

Artificial microplots were established by placing 30-cm-diameter pots and 5-L plastic bags either on the surface or into 20-cm-deep holes. Containers were at 0.60 m × 0.75 m spacing (Figure 3.1). Each container was filled with 5-L steam pasteurized sandy loam amended with Hygromix at 3:1 (v/v) ratio. Hardened-off six-week-old tomato cv. ′Floradade′ seedlings at five-leaf stage were transplanted into the containers. *Meloidogyne incognita* inoculum was prepared by extracting eggs and second-stage juveniles (J2) from roots of greenhouse grown nematode-susceptible tomato plants cv. ′Floradade′ in 1% NaOCl solution that was rinsed off in tapwater (Hussey and Barker, 1973). Seven days after transplanting, each seedling was inoculated by dispensing 2000 eggs and J2 using a 20-ml plastic syringe by placing into 5-cm-deep holes around the cardinal points. Seedlings were irrigated every other day with 500 ml tapwater. Staking of tomato plants grown in various containers with different positioning prevented lodging. Nemarioc-AL and Nemafric-BL phytonematicides each were applied at 3% after every 17 days.

3.2.4 Preparation of phytonematicides

Cucumis africanus and *C. myriocarpus* fruits were collected from locally cultivated plants after fruit maturity, cut into pieces, and dried in air-forced ovens at 52°C for 72 h (Mafeo and Mashela, 2009). Approximately 40 g ground materials of *C. africanus* fruit and 40 g *C. myriocarpus* fruit were each fermented in 20 L air-tight sealed plastic containers, respectively, with 16 L chlorine-free tapwater. An equivalent of 300 g molasses, 100 g brown sugar and 300 g effective microorganisms (EM) were fermented in 20 L container for 14 days at room temperature (Mashela *et al.,* 2015). The EM culture in South Africa comprises strains of yeast, lactic acid bacteria, photosynthetic bacteria, actinomycete bacteria and minor strains of fungi (Mashela *et al.,* 2015). Allowance for the released CO_2 to escape from the container was provided through an air-tight 5-mm-diameter tube with one end glued to a hole on the lid of the 20 L container, with the outlet end being dangled into a 1 L bottle which was half-filled with chlorine-free tapwater. A 20-ml-plastic syringe was used to place 2000 *M. incognita* eggs and J2 into 5-cm-deep holes on the cardinal points of the stem seven days after transplanting. Seven days after inoculation with nematodes, 3% Nemarioc-AL and Nemafric-BL phytonematicides in each experiment was applied at 500 ml solution as substitute for irrigation at 17-days application interval.

3.2.4 Data collection

At 56 days after inoculation plant height was measured from the crown to the tip of the flag leaf, with the stem diameter measured using digital Vernier calliper. Chlorophyll content on three matured healthy leaves per plant was measured using chlorophyll meter (Minolta Spad-502). Shoots were severed from roots and stem diameter measured at 5-cm from the distal end of stem prior to oven drying shoots at 56°C for 72 h and weighed. Matured dried leaves and tomato fruit were finely ground through a Wiley mill to pass through a 1-mm-opening sieve. Root systems per pot were removed from pots, immersed in water to remove soil particles, blotted dry and weighed to facilitate the calculation of nematode number per total root system per plant. Root galls were assessed using the North Carolina Differential Rating Scale of 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls and 5 = >100 galls (Taylor and Sasser, 1978). Nematodes were extracted from the whole root system per plant using the maceration and blending method for 30 seconds in 1% NaOCl (Hussey and Barker, 1973). The material was passed through 75- and 25-µm-nested sieves, with eggs and J2 being collected from the 25-µm mesh sieve. Soil per pot was thoroughly mixed and a 250-ml soil sample collected, with J2 extracted using the sugar-floatation and centrifugation method (Jenkins, 1964). Eggs and J2 from root samples and J2 from soil samples were counted from a 5-ml aliquot of each sample under the stereomicroscope. The J2 from the soil samples were converted to 10 000-ml soils per container and for each treatment combined with eggs and J2 from total root/plant to establish the final nematode population densities (Pf). The Pf was used to compute the reproductive factor (RP = Pf/Pi) (Seinhorst, 1965), which is a proportion of Pf and the initial nematode population densities (Pi).

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

3.2.5 Data analysis

Data for plant and nematode variables were subjected to analysis of variance (ANOVA) through SAS software (SAS Institute, 2008). Discrete nematode data were transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984), but untransformed means were reported. The mean sum of squares were partitioned to determine the contribution of sources of variation to the total treatment variation (TTV) in plant and nematode variables (Gomez and Gomez, 1984). Treatment mean separation was achieved through Fischer's Least Significant Difference test at the probability level of 5%.

3.3 Results

3.3.1 Nemarioc-AL phytonematicide trial

Plant growth variables: In Nemarioc-AL phytonematicide experiment, the treatments had significant increase on plant height, dry root mass, fruit number, dry fruit mass and dry shoot mass, contributing 91, 79, 78, 79 and 86% in TTV of the respective variables (Table 3.1-3.2), but were not significant on chlorophyll content, stem diameter and gall rating. Relative to the control (brown-pot-below), plastic-bag-below increased plant height by 3%, whereas brown-pot-above, black-pot-above and plastic-bag-above reduced plant height by 7, 6 and 4%, respectively (Table 3.3). However, the relative effects of black-pot-below and those of the control on plant height were not different. Relative to brown-pot-below (control), brown-pot-above, black-pot-above and plastic-bag-above increased dry shoot mass by 16, 12 and 19%, respectively, whereas plastic-bag-below reduced the variable by 2%, with black-pot-below having no significant relative effect on the variable. Relative to the control, plastic-bag-above increased the number of fruit by 14%, whereas the black-pot-above and the plastic-bag-above reduced the variable by 17 and 12%, respectively. As in dry root mass, the relative effects of black-pot-below were not different to those of the control on dry shoot mass. Relative to the control, black-pot-below and plastic-bag-below increased dry fruit mass by 8 and 18%, respectively, whereas brown-pot-above, black-pot-above and plastic-bag-above reduced the variable by 9, 13 and 7%, respectively, with black-pot-below having no significant relative effect on the variable.

Plant nutrient element variables: In Nemarioc-AL phytonematicide experiment, container-type and positioning treatments had significant effects on Ca, Mg, K, P and

Zn, contributing 68, 42, 53, 62 and 53% in TTV of the respective variables (Table 3.4), without having any significant effect on Fe. The relative effects of brown pot above increased P by 10%, whereas black pot aboveground increased P and Zn by 7% and 4% (Table 3.5). Also, the relative effects of plastic bag below increased Ca by 6%, while plastic bag above reducing Ca by 7% in plastic bag aboveground and increasing potassium and phosphorus by 7% (Table 3.5).

Table 3.1 Partitioning mean sum of squares for plant height (PHT), chlorophyll content (CHC), stem diameter (STD), dry root mass (DRM), fruit number (FTN), dry fruit mass (DFM), dry shoot mass (DSM) and gall rating (GAL) of tomato cv. 'Floradade' as affected by container -type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

		PHT		CHC		STD		DRM		FTN		DFM		DSM		GAL	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Rep	9	0.0024	4	0.0018	53	0.0044	24	0.0202	11	0.038	12	0.0473	13	0.0301	11	0.0103	32
Trt	5	0.0599	91^{***}	0.0006	18^{ns}	0.0076	42^{ns}	0.1505	79^{***}	0.2540	78^{***}	0.2796	79^{***}	0.2385	86^{***}	0.0066	20^{ns}
Error	45	0.0038	5	0.001	29	0.0063	34	0.0191	10	0.0316	10	0.0274	8	0.0094	3	0.0154	48
Total	59	0.0661	100	0.0034	100	0.0183	100	0.1898	100	0.3236	100	0.3543	100	0.278	100	0.0323	100

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

TTV (%) = (Source/Total) × 100

Table 3.2 Effect of container type and positioning on plant height (PHT), dry root mass (DRM), fruit number (FTN), dry fruit mass (DFM) and dry shoot mass (DSM) of tomato cv. 'Floradade' under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

	PHT		DRM		FTN		DFM		DSM	
Treatment	Mean	R.I. (%)	Mean	R.I. (%)	Mean	R.I. (%)	Mean	R.I. (%)	Mean	R.I (%)
Brown pot-below	1.8167^{b}	–	1.3847^{c}	–	1.4210^{b}	–	1.4466^{bc}	–	1.8456^{b}	–
Brown pot-above	1.6838^{c}	–7	1.6105^{a}	16	1.2840^{bc}	–10	1.3227^{cd}	–9	1.5849^{d}	–14
Black pot-below	1.8327^{ab}	1	1.4319^{bc}	3	1.4387^{b}	1	1.5575^{ab}	8	1.8900^{ab}	2
Black pot-above	1.7087^{c}	–6	1.5530^{ab}	12	1.1766^{c}	–17	1.2624^{d}	–13	1.6016^{cd}	–13
Plastic bag-below	1.8787^{a}	3	1.3578^{c}	–2	1.6193^{a}	14	1.7044^{a}	18	1.9449^{a}	5
Plastic bag-above	1.7370^{c}	–4	1.6488^{a}	19	1.2569^{c}	–12	1.3363^{cd}	–7	1.6888^{c}	–8

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

Brown pot below were used as standard for comparison.

Table 3.3 Partitioning mean sum of squares for accumulation of calcium (Ca), magnesium (Mg), potassium (K), iron (Fe), phosphorus (P) and zinc (Zn) in leaves of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

		Ca		Mg		K		Fe		P		Zn	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Rep	9	0.0040	21	0.0007	41	0.0038	32	0.0235	37	0.0017	29	0.0086	28
Trt	5	0.013	68***	0.0007	41**	0.0063	53**	0.0246	39ns	0.0037	63***	0.0162	53**
Error	45	0.0021	11	0.0003	18	0.0018	15	0.0149	24	0.0005	8	0.0059	19
Total	59	0.0191	100	0.0017	100	0.0119	100	0.0630	100	0.0059	100	0.0307	100

nsNot significant at $P \geq 0.05$, **Significant at $P \leq 0.05$, ***Highly significant at $P \leq 0.01$.

Table 3.4 Response of calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P) and zinc (Zn) in leaves of tomato cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

	Ca		Mg		K		P		Zn	
Treatment	Mean	R.I. (%)	Mean	R.I. (%)	Mean	R.I. (%)	Mean	R.I. (%)	Mean	R.I. (%)
[a]Brown pot-below	0.6788^{bc}	–	0.2762^{ab}	–	0.6883^{bc}	–	0.3226^{b}	–	1.8588^{bc}	–
Brown pot-above	0.6382^{cd}	–6	0.2667^{b}	–3	0.7212^{ab}	5	0.3556^{a}	10	1.9168^{ab}	3
Black pot-below	0.7014^{ab}	3	0.2828^{a}	2	0.6908^{bc}	0	0.3130^{b}	–3	1.8724^{abc}	1
Black pot-above	0.6540^{cd}	–4	0.2745^{ab}	–1	0.6975^{abc}	1	0.3445^{a}	7	1.9347^{a}	4
Plastic bag-below	0.7214^{a}	6	0.2876^{a}	4	0.6641^{c}	–4	0.3157^{b}	–2	1.8244^{c}	–2
Plastic bag-above	0.6312^{d}	–7	0.2673^{b}	–3	0.7345^{a}	7	0.3445^{a}	7	1.8955^{ab}	2

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

[a]Brown pot below were used as standard for comparison.

Nematode variables: In Nemarioc-AL phytonematicide experiment, container-type and positioning treatments were not significant on all nematode variables (Table 3.6).

Table 3.5 Partitioning mean sum of squares for juveniles in roots, eggs in roots, total in roots, Juveniles in soil, total in soil, final population and reproductive potential of *Meloidogyne incognita* as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

		J2 in roots		Eggs in roots		Total in roots		J2 in soil		Total in soil		Pf		RP	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Rep	9	0.0259	23	0.0823	53	0.0233	37	0.0517	28	0.0517	28	0.0088	14	0.0001	17
Trt	5	0.0425	25^{ns}	0.0286	18^{ns}	0.0131	21^{ns}	0.0583	32^{ns}	0.0583	32^{ns}	0.0283	46^{ns}	0.0003	50^{ns}
Error	45	0.0433	39	0.0441	28	0.026	42	0.0723	40	0.0723	40	0.0243	40	0.0002	33
Total	59	0.1117	100	0.155	100	0.0624	100	0.1823	100	0.1823	100	0.0614	100	0.0006	100

^{ns}Not significant at P ≥0.05.

3.3.2 Effects of Nemafric-BL phytonematicide as affected by container-type and positioning

Plant growth variables: Nemafric-BL phytonemaicide experiment, container-type and positioning treatments had significant effects on plant height, dry root mass, fruit number, dry fruit mass and dry shoot mass contributing 68, 75, 71, 80 and 92% in TTV of the respective variables, but were not significant on chlorophyll content, stem diameter and gall rating (Table 3.6). Relative to the control, brown pot aboveground decreased fruit number, dry fruit mass and dry shoot mass by 10, 13 and 9%, respectively, while increasing dry root mass by 14%. Relative to the control, black pot aboveground reduced plant height, fruit number, and dry fruit mass and dry shoot mass by 4, 20, 22 and 7%, respectively, but increased dry root mass by 19%. Relative to the control, plastic bag belowground increased dry shoot mass by 3%, whereas, plastic bag aboveground increased dry root mass by 15%, while reducing dry fruit mass and dry shoot mass by 12 and 7%, respectively (Table 3.7).

Nutrient element variables: In Nemafric-BL phytonematicide experiment, container-type and positioning treatments were not significant on Mg, K, Fe and Zn, but were significant on Ca and P contributing 48 and 75% in TTV of the variables, respectively (Table 3.8). Relative to the control, plastic bag-below increased Ca by 7% while reducing P by 13% (Table 3.9).

Table 3.6 Partitioning mean sum of squares for plant height (PHT), chlorophyll content (CHC), stem diameter (STD), dry root mass (DRM), fruit number (FTN), dry fruit mass (DFM), dry shoot mass (DSM) and gall rating (GAL) of tomato cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

		PHT		CHC		STD		DRM		FTN		DFM		DSM		GAL	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Rep	9	0.0031	14	0.0003	15	0.0085	45	0.0364	18	0.0360	17	0.0186	6	0.0046	5	0.0081	30
Trt	5	0.0146	68^{***}	0.0011	50^{ns}	0.0056	30^{ns}	0.1547	75^{***}	0.1512	71^{***}	0.2387	80^{***}	0.0817	92^{***}	0.0026	10^{ns}
Error	45	0.0038	18	0.0007	35	0.0048	25	0.0162	8	0.0252	12	0.0417	14	0.0031	3	0.0164	61
Total	59	0.0215	100	0.0021	100	0.0189	100	0.2073	100	0.21245	100	0.2989	100	0.0893	100	0.0271	100

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

Table 3.7 Effect of container type and positioning on plant height (PHT), dry root mass (DRM), fruit number (FTN) and dry shoot mass (DSM) of tomato cv. 'Floradade' under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

	PHT		DRM		FTN		DFM		DSM	
Treatment	Mean	R.I. (%)	Mean	R.I. (%)	Mean	R.I. (%)	Mean	R.I. (%)	Mean	R.I. (%)
[a]Brown pot-below	1.7532[ab]	–	1.3874[b]	–	1.4296[ab]	–	1.8329[a]	–	1.7791[b]	–
Brown pot-above	1.7113[bc]	–2	1.5749[a]	14	1.2832[cd]	–10	1.5999[bc]	–13	1.6195[c]	–9
Black pot-below	1.7778[a]	1	1.3963[b]	1	1.4821[a]	4	1.8250[a]	–0	1.7890[ab]	1
Black pot-above	1.6902[c]	–4	1.6529[a]	19	1.1451[d]	–20	1.4379[c]	–22	1.6463[c]	–7
Plastic bag-below	1.7743[a]	1	1.3700[b]	–1	1.4020[abc]	–2	1.7546[ab]	–4	1.8315[a]	3
Plastic bag-above	1.7033[bc]	–3	1.5904[a]	15	1.2916[bc]	–10	1.6140[bc]	–12	1.6521[c]	–7

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

[a]Brown pot below were used as standard for comparison.

Table 3.8 Partitioning mean sum of squares for accumulation of calcium (Ca), magnesium (Mg), potassium (K), iron (Fe), phosphorus (P) and zinc (Zn) in leaves of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

		Ca		Mg		K		Fe		P		Zn	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Rep	9	0.0027	34	0.0015	60	0.0014	19	0.0228	44	0.0006	8	0.0067	34
Trt	5	0.0038	48**	0.0006	24^{ns}	0.0042	57^{ns}	0.0189	36^{ns}	0.0055	75***	0.0075	39^{ns}
Error	45	0.0015	19	0.0004	16	0.0018	24	0.0105	20	0.0012	16	0.0052	27
Total	59	0.008	100	0.0025	100	0.0074	100	0.0522	100	0.0073	100	0.0194	100

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

Table 3.9 Effect of container-type and positioning on calcium (Ca) and phosphorus (P) on leaves of tomato cv. 'Floradade' under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

	Ca		P	
Treatment	Mean	R.I. (%)	Mean	R.I. (%)
[a]Brown pot below	0.6172[b]	–	0.3416[ab]	–
Brown pot above	0.6159[b]	–0	0.3572[a]	5
Black pot below	0.6296[ab]	2	0.3172[bc]	–7
Black pot above	0.6114[b]	–1	0.3386[ab]	–1
Plastic bag below	0.6580[a]	7	0.2958[c]	–13
Plastic bag above	0.6030[b]	–2	0.3536[a]	4

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

[a]Brown pot below were used as standard for comparison.

Nematode variables: Nemafric-BL phytonematicide experiment, container-type and positioning treatments were not significant on eggs in roots, J2 in roots, total in roots, final population and RP, but were significant to J2 in soil and total population in soil contributing 58% in TTV of the variables, respectively (Table 3.10). Relative to the control, plastic bag below increased J2 in soil and total population in soil by 18% (Table 3.11).

Table 3.10 Partitioning mean sum of squares for juveniles in roots, eggs in roots, total in roots, juveniles in soil, total in soil, final population and reproductive potential of *Meloidogyne incognita* as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

		J2 in roots		Eggs in roots		Total in roots		J2 in soil		Total in soil		Pf		RP	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Rep	9	0.0316	31	0.0582	47	0.0301	41	0.0633	22	0.0633	22	0.0258	30	0.1134	34
Trt	5	0.0321	32^{ns}	0.0376	31^{ns}	0.0239	33^{ns}	0.1662	58**	0.1662	58**	0.0420	49^{ns}	0.1132	34^{ns}
Error	45	0.0382	37	0.0269	22	0.0190	26	0.0579	20	0.0579	20	0.0181	21	0.1106	33
Total	59	0.1019	100	0.1227	100	0.073	100	0.2874	100	0.2874	100	0.0859	100	0.3372	100

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

Table 3.11 Effect of container-type and positioning on J2 in soil and total in soil under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

	J2 in soil		Total in soil	
Treatment	Mean	R.I. (%)	Mean	R.I. (%)
Brown pot below	1.7150^{b}	–	1.7150^{b}	–
Brown pot above	1.7015^{b}	–1	1.7015^{b}	–1
Black pot below	1.8738^{ab}	9	1.8738^{ab}	9
Black pot above	1.7592^{b}	3	1.7592^{b}	3
Plastic bag below	2.0223^{a}	18	2.0223^{a}	18
Plastic bag above	1.7014^{b}	–1	1.7014^{b}	–1

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

Brown pot below were used as standard for comparison.

3.4 Discussion

3.4.1 Nemarioc-AL phytonematicide experiment

<u>Plant growth variables</u>: In Nemarioc-AL phytonematicide experiment, container-type and positioning treatments had significant increase on plant height, dry root mass, fruit number, dry fruit mass and dry shoot mass, but were not significant on chlorophyll content, stem diameter and gall rating. Boyer (1982) and Zinn *et al*. (2010) reported that high temperature resulting from chronic or abrupt heating often affect negatively plant growth, development and reproduction negatively. Under greenhouse conditions, Rizvi *et al*. (2015) observed the significant increase on plant height, fresh and dry weight of nematode-inoculated tomato plants grown in aboveground clay pots when exposed to increasing concentration of neem cake, which confirms the findings of the current study. In addition, the significant increase reported by Maile (2013) on dry shoot mass and dry root mass of aboveground potted rough lemon under greenhouse conditions; confirm the finding of the current study. Similarly, Sithole (2016) reported highly significant increase on plant height and dry root mass of container-grown *P. sidoides* plants when treated with Nemarioc-AL phytonematicide. However, non-significant effect on dry shoot mass and dry tuber mass contradict our findings. Moreover, no-significant effect on chlorophyll content when exposed to the same treatment confirm our findings (Sithole, 2016). Tseke (2013) observed the significant increase on plant height, number of fruit, dry root mass, dry shoot mass, stem diameter and number of flowers of tomato plants grown in green aboveground pots when treated with Nemarioc-AL phytonematicide. Similarly, Deimi and Karami (2017) reported the significant increase in total plant mass and dry shoot mass of eggplant infected with *M. javanica* when treated with rice straw

compost tea and tomato compost tea as a soil drench, under greenhouse trials. Lack of significant effect reported by Rabothata (2017) on all measured plant variables of aboveground brown pots containing *Cleome gynandra* contradicts the findings of the current study. Non-significant effect observed by Seshweni (2017) on chlorophyll content of belowground container-grown potato plants treated with Nemarioc-AL phytonematicide confirms the findings of the current study. In addition, the significant effect reported on dry shoot mass of green bean grown in aboveground brown pots confirms the finding of the current study, under micro-plot conditions (Chokoe, 2017). Under greenhouse, lack of significant effect on measured plant variables reported by Mashitoa (2017) contradicts the findings of the current study. Under micro-plot, non-significant effect reported on all measured plant variables by Mashitoa (2017) contradicts the finding of the current study, except for gall rating. In addition, under micro-plot conditions, non-significant effect reported by Khosa (2013) on tomato plants grown in earthen PVC pipes due to the treatments contradict the findings of the current study. Under greenhouse conditions, Dube (2016) reported the highly significant effects on root gall of nematode-inoculated tomato plants grown in brown aboveground pots when treated with Nemarioc-AL phytonematicide. Gradual increase in growth of honey locust observed by Graves *et al*. (1991) with increasing temperature up to a threshold root zone temperature of 35^{o}C, but also observed reduction in growth of heaven-displayed shoot and root growth at root zone temperature > 30^{o}C which confirms the results of the current study. Relative to the control, brown pot aboveground reduced plant height and dry shoot mass while increasing dry root mass, whereas black pot aboveground reducing plant height, fruit number, dry fruit mass and dry shoot mass, but increased dry root mass. However, plastic bag aboveground-reduced plant height, fruit number and dry shoot mass while increasing dry root mass. There is no a report on the efficacy of phytonematicides as influenced by polyethylene plastic bags and their positioning on growth of nematode-inoculated plants. Relative to the control, plastic bag belowground increased plant height, fruit number, and dry fruit mass and dry shoot mass. The significant increase in plant height and dry shoot mass reported in the current study contradicted those in another study (Maake, 2017) on the same variables when treated with Nemarioc-AL phytonematicide.

Nutrient element variables: In Nemarioc-AL phytonematicide experiment, container-type and positioning treatments had significant increase on Ca, Mg, K, P and Zn, but were not significant on iron. Cassen and Barber (1976) reported that nutrients absorption and assimilation is dependent of factors such as nutrients supply, root metabolic activities, growth rate of the root system, root morphology and nutrients absorption characteristics of the root system. The significant increase reported by Maake (2017) on Ca and P in leaf tissues of tomato plants grown in belowground plastic bag treated with Nemarioc-AL phytonematicide confirms the findings of the current study. In addition, Nyamandi (2017) observed significant reduction on K in leaf tissues of tomato plants grown in belowground plastic bags when exposed to Nemarioc-AL phytonematicide. Lack of significant effects on K and Zn in leaves of *Cleome gynandra* (Rabothata, 2017), contradicted the findings in the current study on similar foliar nutrient elements, except for Fe. Under greenhouse, the significant increase in P and K reported by Rizvi *et al*. (2015) in leaves of tomato plants treated with neem cake and *G. fasciculatum* individually and in combination, confirmed the findings in the current study. Lingle and Davis (1959) demonstrated that extreme root zone temperature often results in decreased root metabolism, which result in decreased root growth and amounts of energy required for absorption, assimilation and translocation of nutrients. This hypothesis demonstrated the contradictions and agreement between the results of the current researcher with the work of other researchers cited above. Cooper (1973) stated that there was a general relationship in most plant species between root-zone temperature and mineral content of the plant tissues.

Relative to the control, brown pot aboveground increased P, however, black pot above increased P and Zn. In addition, plastic bag belowground increased Ca, whereas plastic bag aboveground increased K and P, but reduced Ca. There is no report on the efficacy of phytonematicides as influenced by polyethylene plastic bags and their positioning on accumulation of essential nutrient elements in the leaves of nematode-inoculated plants.

Nematode variables: In Nemarioc-AL phytonematicide experiment, container-type and positioning treatments were not significant on all measured nematode variables. Under greenhouse conditions, other findings (Chokoe, 2017) on nematode variables in nematode-inoculated aboveground potted tomato plants confirms the findings of the current study except for J2 in roots. Under microplot, findings in (Chokoe, 2017) on J2 in soil, J2 in roots, eggs in roots of green bean and final nematode population contradict the findings of the current study. The significant effect reported by Lebea (2017) on eggs, J2 in roots, J2 in soil and total nematode due to increasing concentration of Nemarioc-AL phytonematicide, contradicted the findings of the current study. The significant effects observed due to increasing concentrations of Nemarioc-AL phytonematicide on J2 in roots of tomato plants and final nematode population confirm findings of the current study, but the significant effect on J2 in soil (Tseke, 2013) contradict our findings. Under greenhouse, Deimi and Karami (2017) reported the significant reduction in nematode population in soil of container-grown eggplant when compost of various botanicals was applied as a soil drench. Similarly, Khosa (2013) reported the significant reduction of J2 and eggs in roots of container-grown tomato plants treated with crude plant-meal oils, under greenhouse conditions. The significant reduction in eggs/egg masses reported by Rizvi *et al*. (2015) contradict the findings of the current study except for nematodes in soil.

3.4.2 Nemafric-BL phytonematicide experiment

<u>Plant growth variables</u>: In Nemafric-BL phytonematicide experiment, container-type and positioning treatments had significant increase on plant height, dry root mass, fruit number, dry fruit mass and dry shoot mass, but were not significant on chlorophyll content, stem diameter and gall rating. Similarly, Malungane (2014) reported significant increase on fresh root mass, fresh shoot mass and dry shoot mass except for fruit yield of tomato plants grown in brown pots with aboveground positioning when treated with the crude extracts of *Tulbaghia violacea* (wild garlic). Under greenhouse, Parihar *et al*. (2015) observed the significant enhancement of all measured plant variables of eggplant grown in pots when treated with oil cakes. Tseke (2013) made similar

observations under greenhouse conditions on plant height, number of fruit, dry root mass, dry shoot mass, stem diameter and number of flowers of tomato plants grown in green aboveground pots when treated with Nemafric-BL phytonematicide. However, the significant effect observed by Tseke (2013) on stem diameter contradicts the finding of the current study. Under microplot conditions, Maake (2017) reported a significant effect on plant height of belowground container-grown tomato plants treated with Nemarioc-AL phytonematicide. The significant effect reported by Malungane (2014) on root galling contradicts the findings of the current study. Similarly, under greenhouse conditions, non-significant effect reported by Rabothata (2017) on stem diameter and chlorophyll content of *Cleome gynandra* grown in aboveground brown pots agrees with findings of the current study except for other measured plant variables. Under greenhouse conditions, non-significant effect of Nemafric-BL phytonematicide reported by Lebea (2017) on chlorophyll content and stem diameter of butternut squash agree with the findings of the current study except for dry shoot mass, plant height and leaf number. The findings of the study by Lebea (2017) on dry fruit mass and fruit number confirms the results of the current study on the same variables. In addition, the significant increase in plant height of belowground potted nematode-inoculated tomato plants treated with fever tea agree with the findings of the current study (Mashela *et al*., 2010).The significant increase observed on measured plant variables by Mashitoa (2017) when exposed to Nemafric-BL phytonematicide confirm the finding of the current study, but the significant effect on gall rating and non-significant effect on fresh root mass, dry shoot mass and number of leaves contradict our findings. Under greenhouse conditions, Dube (2016) reported the highly significant effects on root gall of nematode-inoculated tomato plants grown in brown aboveground pots when treated with Nemafric-BL phytonematicide, which disagree with results of the current study. Under greenhouse conditions, Pelinganga and Mashela (2012) observed a significant effect on plant height, dry shoot mass and dry root mass of aboveground potted tomato plants when exposed to allelochemicals from the crude extracts of *Cucumis africanus*, which confirms the findings of the current study on similar variables. However, the significant effect reported on stem diameter under the treatment with allelochemicals from the crude extracts of *C. africanus* disagree with the findings of the current study on the same variable. Hagan (1952) and Nielsen (1974) demonstrated that a decreased root growth and maturation that is due to extreme root zone temperature often result in reductions shoot growth and development. This hypothesis justifies the difference between the results of the current study with those of other researchers cited.

Relative to the control, brown pot aboveground decreased fruit number, dry fruit mass and dry shoot mass while increasing dry root mass. Chokoe (2017) observed a reduction in dry shoot mass of green bean plants grown in brown pot with aboveground positioning when exposed to medium concentration of Nemafric-BL phytonematicide. However, an increase in dry shoot mass reported at low concentration contradicts the findings of the current study. In addition, Mashela *et al*. (2010) observed a significant increase on dry shoot mass of belowground potted nematode-inoculated tomato plants when treated with fever tea, which contradicts the findings of the current study. Relative to the control, black pot aboveground reduced plant height, fruit number, dry fruit mass and dry shoot mass, while increasing dry root mass, whereas, plastic bag below increased dry shoot mass. Lack of significant effect on all measured plant variables reported by Chokoe (2017) on green beans grown in aboveground brown pots contradicts the findings of the current study. Relative to the control, plastic bag above increased dry root mass, while reducing dry fruit mass and dry shoot mass. The significant reduction reported by Lebea (2017) on dry fruit mass of butternut squash grown in aboveground plastic bags when treated with Nemafric-BL phytonematicide under greenhouse, confirms the findings of the current study.

<u>Plant nutrient element variables</u>: In Nemafric-BL phytonematicide experiment, container-type and positioning treatments were not significant on all measured nutrient element except for Ca and P. Lack of significant effect on K, Fe and Zn in leaves of *Cleome gynandra* in the study conducted by Rabothata (2017) confirms the findings of the current study on similar foliar nutrient element. The findings of the current study confirm the findings by Nyamandi (2017) on foliar Ca and P of nematode-inoculated tomato plants grown in belowground plastic pots when treated with Nemafric-BL phytonematicide. Khosa (2013) reported that *E. ingens* and *S. cupulare* at 5g and 10g significantly lowered N, P and K in leaves of tomato plants, which contradict our findings. In addition, phosphorus and potassium of *C. myriocarpus* treated tomato plants was significantly lower than the control, which contradict the findings of the current study. However, observation made by Gosselin and Trudel (1986) demonstrate the increase and decrease in foliar nutrients in the current study. Gosselin and Trudel (1986) observed an increase in concentration of N, P and K in leaves of greenhouse pepper with increasing root zone temperature from 12 to 36^{o}C, as well as a decrease in

concentration of Mg and Ca in leaves of the same greenhouse pepper due to increasing root zone temperature. Relative to the control, plastic bag below increased calcium while reducing phosphorus, which contradicts an increase in P observed by Maake (2017) on nematode-inoculated tomato plants grown in belowground plastic bags treated with Nemafric-BL phytonematicide.

Nematode variables: In Nemafric-BL phytonematicide experiment, container-type and positioning treatments were not significant on all measured nematode variables except for J2 in soil and total population in soil. Similarly, Seshweni (2017) reported a significant effect on J2 in soil of nematode-inoculated potato plants grown in earthern brown pots when treated with Nemafric-BL phytonematicide. The significant effect reported by Chokoe (2017) on J2 in roots, nematode eggs in roots and final nematode population of aboveground container-grown *Phaseolus vulgaris* cv 'Tahoe' when treated with Nemafric-BL phytonematicide, contradict the findings of the current study. Non-significant effect observed on J2 in roots of belowground container-grown potato plants by Seshweni (2017) confirms the findings of the current study. Lack of significant effect on all measured nematode variables reported in the current study contradicts the findings of Malungane (2014) on final population of *M. incognita* race-2 when treated with the crude extracts of *T. violaceae*. Also, Lebea (2017) observed significant effect on J2 in soil and total nematode which confirms our results, but the significant effect on eggs and J2 in roots contradicts our findings. Under greenhouse conditions, Tseke (2013) observed the significant effect on J2 in soil of nematode-inoculated tomato plants grown in aboveground pots when exposed to Nemafric-BL phytonematicide. However, the significant effect reported by Tseke (2013) on eggs in roots, J2 in roots and final population of *Meloidogyne incognita* contradict the findings of the current study. The significant reduction reported by Khosa (2013) on eggs and J2 in roots of tomato plants grown in earthen pipes when treated with extract of *T. elegans* and *C. cactiformis* contradict the findings of the current study. The significant effect reported by Pelinganga and Mashela (2012) on J2 in soil and total nematodes in soil of container-grown tomato plants, confirms the findings of the current study on the same variables. However, the significant effect on nematodes in roots of aboveground potted tomato plants contradicted the results of the current study on the same variable (Pelinganga and Mashela, 2012). Under greenhouse conditions, Moosavi (2012) observed the significant reduction in J2 in soil of aboveground potted tomato plants when treated with herbal extracts. The significant effect reported by Maile (2013) on J2 in soil of aboveground potted rough lemon when treated with the crude extracts of *Cucumis myriocarpus* confirms our results, under greenhouse conditions. Under greenhouse and microplot conditions, Malatji (2017) reported the significant effect on nematodes number in roots, soil and final population of container-grown tomato plants when treated with plant extracts of *Lantana camara*. However, the significant effect reported by Malatji (2017) on nematodes number in roots and final population disagree with our findings. Relative to the control, plastic bag below increased J2 in soil and total population in soil. The significant reduction of eggs in roots, J2 in roots and final nematodes population reported by Lebea (2017) contradict the finding of the current study. In addition, the significant reduction of eggs in roots, J2 in roots, J2 in soil and final nematode population reported by Tseke (2013) disagree with the findings of the current study.

Moreover, the significant reduction of J2 and total nematodes in soil reported by Pelinganga and Mashela (2012) on aboveground potted tomato plants when treated with the crude extracts of *C. africanus*, contradict the findings of the current study on the same variables.

3.5 Conclusion

There was a reduction in measured plant variables of container raised tomato plants with aboveground positioning, and that was due to temperature fluctuations more especially during the night. Moreover, high reduction in measured plant parameter have been observed in black plastic pots and polyethylene plastic bags with aboveground positioning. Nematodes did not reproduce in the root system of all plants grown in various containers with different positioning. On ground container positioning increased nutrients assimilation. The below ground treatment had moderating effects on the variability of the treatments and should therefore be prepared in micro-plot studies.

CHAPTER 4

SUMMARY OF FINDINGS, SIGNIFICANCE, RECOMMENDATIONS AND CONCLUSIONS

4.1 Summary of the findings

The study was carried out to determine the efficacy of Nemarioc-AL and Nemafric-BL phytonematicides as influenced by container type and positioning on growth of nematode-inoculated tomato plants under microplot conditions. At 56 days after the initiation and application of treatments, 3% concentration of Nemarioc-AL and Nemafric-BL phytonematicides as influenced by container type and positioning significantly increased plant height, dry root mass, fruit number, dry fruit mass and dry shoot mass, but the same treatments were not significant on chlorophyll content, stem diameter and gall rating. In Nemarioc-AL phytonematicide, the treatment had a significant increase on Ca, Mg, K, P and Zn, but non-significant effect on Fe. However, Nemafric-BL phytonematicide treatment was not significant on all measured nutrient element except for Ca and P. In Nemarioc-AL phytonematicide, the treatments were not significant on all measured nematode variables. In Nemafric-BL phytonematicide, the treatments were not significant on all measured nematode variables except for J2 in soil and total population in soil.

4.2 Significance

The findings of this study provide empirically evidence on the effects of container-type and positioning on the efficacy of 3% Nemarioc-AL and Nemafric-BL phytonematicides. Furthermore, this provides much needed information on the choice of container type and positioning for use in container-grown plants under microplot conditions. In this study black plastic bags with belowground positioning significantly increased all measured plant variables and certain nutrient elements while suppressing nematodes in soil. The findings of the current study should be validated under various conditions which include greenhouse conditions.

4.3 Recommendations

In the current study, temperature of the growing medium was not measured and if fluctuations of ambient temperature have to be used to explain the findings, it would be essential that temperature of the growing medium and ambient temperature be measured during the trials.

4.4 Conclusions

There was a reduction in measured plant variables of container raised tomato plants with aboveground positioning, and that was due to temperature fluctuations more especially during the night. Moreover, high reduction in measured plant variables has been observed in black plastic pots and polyethylene plastic bags with aboveground. Nematodes did not reproduce in the root system of all plants grown in various containers with different positions. Aground container positioning increased nutrients assimilation. The below ground treatment had moderating effects on the variability of the treatments and should therefore be prepared in microplot studies.

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APPENDICES

Appendix 3.1 Analysis of variance (ANOVA) for plant height (PHT) of tomato cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.02170	0.00241		
Treatment	5	0.29968	0.05994	15.78	0.01
Error	45	0.17094	0.00380		
Total	59	0.49232	0.06615		

Appendix 3.2 Analysis of variance (ANOVA) for chlorophyll content (CHC) in leaves of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.01623	0.001803		
Treatment	5	0.00309	0.0006180	0.63	0.6805
Error	45	0.04440	0.0009867		
Total	59	0.06372	0.0034077		

Appendix 3.3 Analysis of variance (ANOVA) for stem diameter (STD) of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.03966	0.004406		
Treatment	5	0.03795	0.007590	1.21	0.3214
Error	45	0.28300	0.006289		
Total	59	0.36060	0.018285		

Appendix 3.4 Analysis of variance (ANOVA) for dry root mass (DRM) of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.18216	0.02024		
Treatment	5	0.75269	0.15054	7.90	0.01
Error	45	0.85793	0.01907		
Total	59	1.79278	0.18981		

Appendix 3.5 Analysis of variance (ANOVA) for dry fruit mass (DFM) of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.42522	0.04725		
Treatment	5	1.39810	0.27962	10.20	0.01
Error	45	1.23329	0.02741		
Total	59	3.05661	0.35428		

Appendix 3.6 Analysis of variance (ANOVA) for fruit number (FTN) of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.34180	0.03798		
Treatment	5	1.27010	0.25402	8.04	0.01
Error	45	1.42241	0.03161		
Total	59	3.03430	0.32361		

Appendix 3.7 Analysis of variance (ANOVA) for dry shoot mass (DSM) of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.27068	0.03008		
Treatment	5	1.19256	0.23851	25.28	0.01
Error	45	0.42449	0.00943		
Total	59	1.88773	0.27802		

Appendix 3.8 Analysis of variance (ANOVA) for gall formation (GAL) of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.09227	0.01025		
Treatment	5	0.03293	0.00659	0.43	0.8259
Error	45	0.69061	0.01535		
Total	59	0.81582	0.03219		

Appendix 3.9 Analysis of variance (ANOVA) for eggs of *Meloidogyne incognita* in roots of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.74083	0.08231		
Treatment	5	0.14290	0.02858	0.65	0.6642
Error	45	1.98372	0.04408		
Total	59	2.86745	0.15497		

Appendix 3.10 Analysis of variance (ANOVA) for second stage juveniles of *Meloidogyne incognita* in roots of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.23280	0.02587		
Treatment	5	0.21271	0.04254	0.98	0.4392
Error	45	1.94932	0.04332		
Total	59	2.39484	0.11173		

Appendix 3.11 Analysis of variance (ANOVA) for total population of *Meloidogyne incognita* in roots of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.20922	0.02325		
Treatment	5	0.06525	0.01305	0.50	0.7728
Error	45	1.16896	0.02598		
Total	59	1.44343	0.06228		

Appendix 3.12 Analysis of variance (ANOVA) for second stage juveniles of *Meloidogyne incognita* in soil as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.46550	0.05172		
Treatment	5	0.29167	0.05833	0.81	0.5508
Error	45	3.25284	0.07229		
Total	59	4.01000	0.18234		

Appendix 3.13 Analysis of variance (ANOVA) for total population of *Meloidogyne incognita* in soil as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.46550	0.05172		
Treatment	5	0.29167	0.05833	0.81	0.5508
Error	45	3.25284	0.07229		
Total	59	4.01000	0.18234		

Appendix 3.14 Analysis of variance (ANOVA) for final population of *Meloidogyne incognita* as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.07881	0.00876		
Treatment	5	0.14161	0.02832	1.17	0.3403
Error	45	1.09232	0.02427		
Total	59	1.31274	0.06135		

Appendix 3.15 Analysis of variance (ANOVA) for reproductive potential of *Meloidogyne incognita* in roots of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.00090	0.00009989		
Treatment	5	0.00129	0.0002579	1.09	0.3772
Error	45	0.01061	0.0002358		
Total	59	0.01280	0.00059359		

Appendix 3.16 Analysis of variance (ANOVA) for calcium (Ca) in leaves of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.03639	0.00404		
Treatment	5	0.06477	0.01295	6.26	0.01
Error	45	0.09314	0.00207		
Total	59	0.19430	0.01906		

Appendix 3.17 Analysis of variance (ANOVA) for magnesium (Mg) in leaves of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.00605	0.0006724		
Treatment	5	0.00345	0.0006897	2.52	0.05
Error	45	0.01232	0.0002737		
Total	59	0.02182	0.0016358		

Appendix 3.18 Analysis of variance (ANOVA) for potassium (K) in leaves of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.03432	0.003814		
Treatment	5	0.03152	0.006304	3.55	0.01
Error	45	0.07994	0.001776		
Total	59	0.14578	0.011894		

Appendix 3.19 Analysis of variance (ANOVA) for iron (Fe) in leaves of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.21184	0.02354		
Treatment	5	0.12310	0.02462	1.66	0.1646
Error	45	0.66863	0.01486		
Total	59	1.00357	0.06302		

Appendix 3.20 Analysis of variance (ANOVA) for phosphorus (P) in leaves of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.01564	0.001737		
Treatment	5	0.01840	0.003680	7.73	0.01
Error	45	0.02141	0.0004757		
Total	59	0.05544	0.0058927		

Appendix 3.21 Analysis of variance (ANOVA) for zinc (Zn) in leaves of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemarioc-AL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.07701	0.00856		
Treatment	5	0.08105	0.01621	2.75	0.05
Error	45	0.26545	0.00590		
Total	59	0.42352	0.03067		

Appendix 3.22 Analysis of variance (ANOVA) for height (PHT) of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.02797	0.00311		
Treatment	5	0.07281	0.01456	3.83	0.01
Error	45	0.17096	0.00380		
Total	59	0.27174	0.02147		

Appendix 3.23 Analysis of variance (ANOVA) for chlorophyll content (CHC) of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.00286	0.0003180		
Treatment	5	0.00525	0.001050	1.40	0.2410
Error	45	0.03364	0.0007476		
Total	59	0.04175	0.0021156		

Appendix 3.24 Analysis of variance (ANOVA) for stem diameter (STD) of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.07688	0.008542		
Treatment	5	0.02805	0.005610	1.17	0.3379
Error	45	0.21544	0.004788		
Total	59	0.32036	0.01894		

Appendix 3.25 Analysis of variance (ANOVA) for dry root mass (DRM) of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.32734	0.03637		
Treatment	5	0.77359	0.15472	9.53	0.01
Error	45	0.73032	0.01623		
Total	59	1.83125	0.20732		

Appendix 3.26 Analysis of variance (ANOVA) for dry fruit mass (DFM) of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.16706	0.01856		
Treatment	5	1.19331	0.23866	5.72	0.01
Error	45	1.87696	0.04171		
Total	59	3.23733	0.29893		

Appendix 3.27 Analysis of variance (ANOVA) for fruit number (FTN) of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.32423	0.03603		
Treatment	5	0.75604	0.15121	5.99	0.01
Error	45	1.13546	0.02523		
Total	59	2.21573	0.21247		

Appendix 3.28 Analysis of variance (ANOVA) for dry shoot mass (DSM) of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.04094	0.00455		
Treatment	5	0.40836	0.08167	26.67	0.01
Error	45	0.13781	0.00306		
Total	59	0.58711	0.08928		

Appendix 3.29 Analysis of variance (ANOVA) for gall formation (GAL) of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.07288	0.00810		
Treatment	5	0.01302	0.00260	0.16	0.9762
Error	45	0.73842	0.01641		
Total	59	0.82432	0.02711		

Appendix 3.30 Analysis of variance (ANOVA) for eggs of *Meloidogyne incognita* in roots of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.52403	0.05823		
Treatment	5	0.18807	0.03761	1.40	0.2424
Error	45	1.20875	0.02686		
Total	59	1.92086	0.1227		

Appendix 3.31 Analysis of variance (ANOVA) for second stage juveniles of *Meloidogyne incognita* in roots of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.28469	0.03163		
Treatment	5	0.16032	0.03206	0.84	0.5288
Error	45	1.71852	0.03819		
Total	59	2.16353	0.10188		

Appendix 3.32 Analysis of variance (ANOVA) for total population of *Meloidogyne incognita* in roots of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.27069	0.03008		
Treatment	5	0.11937	0.02387	1.26	0.2995
Error	45	0.85542	0.01901		
Total	59	1.24548	0.07296		

Appendix 3.33 Analysis of variance (ANOVA) for second stage juveniles of *Meloidogyne incognita* in soil as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.56937	0.06326		
Treatment	5	0.83075	0.16615	2.87	0.05
Error	45	2.60732	0.05794		
Total	59	4.00743	0.28735		

Appendix 3.34 Analysis of variance (ANOVA) for total population of *Meloidogyne incognita* in soil as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.56937	0.06326		
Treatment	5	0.83075	0.16615	2.87	0.05
Error	45	2.60732	0.05794		
Total	59	4.00743	0.28735		

Appendix 3.35 Analysis of variance (ANOVA) for final population of *Meloidogyne incognita* as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.23240	0.02582		
Treatment	5	0.20998	0.04200	2.32	0.0583
Error	45	0.81324	0.01807		
Total	59	1.25562	0.08589		

Appendix 3.36 Analysis of variance (ANOVA) for reproductive potential of *Meloidogyne incognita* in roots of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	1.02062	0.11340		
Treatment	5	0.56602	0.11320	1.02	0.4152
Error	45	4.97628	0.11058		
Total	59	6.56292	0.33718		

Appendix 3.37 Analysis of variance (ANOVA) for calcium (Ca) in leaves of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.02355	0.002617		
Treatment	5	0.01884	0.003768	2.56	0.05
Error	45	0.06629	0.001473		
Total	59	0.10868	0.007858		

Appendix 3.38 Analysis of variance (ANOVA) for magnesium (Mg) in leaves of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.01274	0.001416		
Treatment	5	0.00260	0.0005192	1.43	0.2330
Error	45	0.01637	0.0003639		
Total	59	0.03171	0.0022991		

Appendix 3.39 Analysis of variance (ANOVA) for potassium (K) in leaves of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.01296	0.001440		
Treatment	5	0.02120	0.004240	2.32	0.0586
Error	45	0.08219	0.001827		
Total	59	0.11635	0.007507		

Appendix 3.40 Analysis of variance (ANOVA) for iron (Fe) in leaves of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.20511	0.02279		
Treatment	5	0.09472	0.01894	1.81	0.1308
Error	45	0.47195	0.01049		
Total	59	0.77179	0.05222		

Appendix 3.41 Analysis of variance (ANOVA) for phosphorus (P) in leaves of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.00503	0.0005591		
Treatment	5	0.02739	0.005479	4.46	0.05
Error	45	0.05531	0.001229		
Total	59	0.08774	0.007276		

Appendix 3.42 Analysis of variance (ANOVA) for zinc (Zn) in leaves of tomato plants cv. 'Floradade' as affected by container-type and positioning under Nemafric-BL phytonematicide at 56 days after initiation of treatments (n = 60).

Source	DF	SS	MS	F	P
Replication	9	0.06020	0.006689		
Treatment	5	0.03761	0.007522	1.44	0.2299
Error	45	0.23574	0.005239		
Total	59	0.33355	0.01945		