INFLUENCE OF CONTAINER-TYPE AND POSITIONING ON GROWTH OF TOMATO PLANTS AND SUPPRESSION OF *MELOIDOGYNE JAVANICA* EXPOSED TO BIOMUTI AND AFRIKELP

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2019

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DECLARATION

I, Mmagadima Lauraine Sebati, 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.

Candidate: M.L. Sebati — 10 September 2019 Date

Supervisor: Professor P.W. Mashela — 10 September 2019 Date

DEDICATION

To my delightful late mother (Mangoachipa Tryphinah), adorable little sister (Mosima Marlyn), my beloved grandmother (Matlhako Caroline) and the rest of my family.

ACKNOLEDGEMENTS

First and foremost, I would like to thank God for the days of my life, an opportunity granted for me to come to school, His guidance, mercy and His outshining love, thank You Daddy God, the Almighty, the Living God. For a house to be build, a lot has to be done but most importantly foundation must be laid, and that is what was done by my supervisory team, laying a foundation where a mansion is to be build. To my supervisor Professor P.W. Mashela, I thank you for the support, courage and knowledge that you gave to me. You showed and guided me on how to turn dust into mortar, how to make a solid concrete and how to catch a fish, and for all, I thank you! The support and encouragement that I received from my family, I am grateful. I am grateful and thankful to have had my whole family to lean on always. The wall was about to fall and the world had no hope for me; I had my grandmother who had nothing to offer, but prayers and words of wisdom. She taught me how to pray and how to trust in God and to be strong even though everything seemed impossible. She is my pillar of strength. My family alone could not have done it without the help of my extended family members and still I say thank you. To classmates, the general workers and everyone who gave in a helping hand, thank you, the Green Biotechnologies Research Centre of Excellence. Alone, I could not have done it. Again, allow me to go back to the Creator of Heaven and earth, to the One above, the One Who says “talk to Me for I am listening”, the One Who does not sleep nor slumber, Papa God, I thank You for everything. “Kgotso ya Modimo ebe le batho, kea leboga”.

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ABSTRACT

The influence of cultural practices can be modified by environmental conditions such as container-type or positioning. The objective of the study was to determine whether container-type and positioning would have an influence on the growth of tomato plants and suppression of *Meloidogyne javanica* exposed to biomuti (Trial 1) and Afrikelp (Trial 2). Different container-types were filled with approximately 10.4 L growing mixture comprising steam-pasteurised sandy loam soil and Hygromix-T at 3:1 (v/v) ratio. The containers were established in microplots at 0.6 m × 0.6 m spacing, with treatments being brown pot-below; brown pot-above, black pot-below, black pot-above, plastic bag-above and plastic bag-below. Tomato (*Solanum lycopersicum* L.) cv. 'Floradade' seedlings were each transplanted and irrigated with 500 ml chlorine-free tapwater every other day. Seven days after transplanting, each plant was inoculated with 2000 eggs and second-stage juveniles (J2) of *M. javanica*. Biomuti and Afrikelp, obtained from the Agricultural Research Council (ARC)-Vegetable, Ornamentals and Plants (VOP), were applied in separate trials weekly at 2.5%. At 56 days after inoculation, plant growth including selected nutrient elements and nematode variables were measured. Data were subjected to analysis of variance, with separation of means achieved using Fisher's Least Significant Difference test at the probability level of 5%. In the biomuti trial, container-type and positioning had a significant effect on plant height, fruit number, dry root mass, dry shoot mass and fruit mass, contributing 82, 48, 44, 85 and 89% in total treatment variation (TTV) of the respective variables. Relative to brown pot-below; black pot-above, plastic bag-above and brown pot-above reduced plant variables, whereas treatment effects were not significant on nematode variables. In the Afrikelp trial, trends where similar to those in biomuti, treatments had highly significant effects on

plant height, dry root mass, dry shoot mass and gall rating, contributing 91, 88, 66 and 60% in TTV of the respective variables. Relative to brown pot-below; black pot-above, plastic bag-above and brown pot-above reduced the plant variables, but had no significant effects on nematode variables. Generally, plastic bags and polyethylene pots below-ground improved most plant growth variables when compared to those in containers positioned above-ground.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

1.1.1 Description of the research problem

Following the withdrawal of synthetic nematicides from the agrochemical markets, crop yield losses due to nematode damage had since risen to over 37% (Mashela *et al*., 2016), with research and development of various alternative products intended for managing nematodes drastically increasing. Container-size (Landis *et al*., 2010; Tsakaldimi *et al*., 2005) and container-types (Ingram and Ruter, 2015; Markham *et al*., 2011) had been associated with plant growth, particularly under nursery culture. These two factors had been explained on the basis of water absorption, nutrient uptake and breakdown of soil chemicals or bio-remedies (Heller *et al*., 2015; Xu *et al*., 2004), with limited information on positioning of the containers.

1.1.2 Impact of the research problem

Container-size had been associated with root-bound in various crops (Ingram and Ruter, 2015; Landis *et al*., 2010; Ortega *et al*., 2006), with various degrees of deformed root systems (Ortega *et al*., 2006). Container-type could be associated with different degrees of root-zone temperatures, which had been linked to leaf wilting, chlorosis and subsequent poor plant growth (Markham *et al*., 2011). Tomato (*Solanum lycorpesicum* L.) plants grown in black plastic bags on artificial microplot comprising mortar bricks suggested that the performance of cucurbitacin-containing phytonematicides and biomuti intended to suppress population densities of root-knot (*Meloidogyne* species) nematodes lost their efficacies (Nyamandi, 2017).

1.1.3 Possible causes of the research problem

Raising plants for extended periods in containers had been implicated (Marshall and Gilman, 1998), including fluctuation in ambient temperatures that resulted in fluctuating temperatures in the root-zones (Markham *et al*., 2011), which could currently be common due to extremes in temperature, particularly in containers placed above soil surface. Also, the design of the material, for instance, plastic pots or bags, along with the colour of the containers had been implicated in challenges faced by plants raised in containers (Markham *et al*., 2011; O'Connor, 2014).

Under microplot conditions, the containers in nematode trials are sometimes placed below-ground in a way that most of the container is inserted in a hole, with the top being above soil surface to minimise contamination (Lebea, 2017; Maake, 2018; Nyamandi, 2017; Seshweni, 2016; Sithole, 2016). However, in certain reports (Mashitoa, 2016), such containers were left above the soil surface. Generally, the above-ground and below-ground temperatures could differ widely, thereby resulting in different observations. Recently, Nyamandi (2017) observed that cucurbitacin-containing phytonematicides, which had been consistent in suppression of *Meloidogyne* species on plants raised in brown plastic pots (Mashitoa, 2016; Sithole, 2016), failed to supress nematode numbers in black plastic containers placed above the soil surface.

1.1.4 Proposed solutions

The choice of container-type and the placement environment could have potential effects on products used in managing plant-parasitic nematodes. Biomuti is one of the products being tested by the Agricultural Research Council (ARC) as a potential

alternative product for the management of plant-parasitic nematodes. However, in the Nyamadi (2017) trial, this product was one of the failed treatments. Afrikelp is a natural bio-stimulant product containing plant growth regulators such as auxins, extracted from freshly harvested South African giant brown seaweed (Butler and Hunter, 2017), with limited information on how it could perform on nematode suppression.

1.1.5 General focus of the study

The study focused on the influence of container-type and positioning on growth of tomato plants cv. 'Floradade' and suppression of *M. javanica* exposed to biomuti and Afrikelp. Appropriate container-type and positioning would promote plant growth and suppress nematode population densities under artificial microplot conditions.

1.2 Problem statement

Compared with the air, soil is a poor conductor of heat (Markham *et al*., 2011), with the result that root systems could be exposed to stable temperatures under field conditions than when raised in containers placed on soil surface. Root-zone temperatures had been shown to have detrimental effects on plant growth (Markham *et al*., 2011). Under such conditions, the container temperatures could vary drastically, thereby affecting plant growth. The performance of biomuti and Afrikelp could be, just like other soil remedies (Ingram and Ruter, 2015), be affected by container-type and positioning, with plant growth being stimulated, neutral or inhibited. Thus, it is imperative that the influence of container-type and positioning on performance of alternatives to synthetic chemical nematicides be established.

1.3 Rationale of the study

Different container-types and positioning have the ability to affect the growth of plants due to the indirect effects on root-zone temperatures (Tsakaldimi *et al*., 2005). Generally, when container-size is kept constant, container-type and positioning could provide information on whether the two factors could be important during the assessment of the efficacy of alternatives to synthetic nematicides under microplot conditions.

1.4 Purpose of the study

1.4.1 Aim

Establishment of the role that container-type and positioning could play in the efficacy bio products of plant growth under microplot conditions.

1.4.2 Objective

To determine whether container-type and positioning would have an influence on the growth of tomato plants and suppression of *M. javanica* exposed to biomuti or Afrikelp.

1.4.3 Hypothesis

Container-type and positioning had an influence on the growth of tomato plants and suppression of *M. javanica* exposed to biomuti or Afrikelp.

1.5 Reliability, validity and objectivity

Reliability of data in this study was based on statistical analysis of data at the probability level of 5%. Validity was achieved through the use of factorial experiment. Objectivity was achieved by ensuring that the results are discussed on the basis of empirical evidence, thereby eliminating all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

Bias was avoided by ensuring that the experimental error was reduced through replications and assigning treatments at random within the replication (Leedy and Ormrod, 2005).

1.7 Structure of the mini-dissertation

Following the detailed outlining of the research problem (Chapter 1), work done on the research problem was reviewed (Chapter 2). Then, Chapters 3 addressed the two components of the objective on empirical-basis. In the final chapter (Chapter 4), the findings from Chapter 3 were summarised and integrated to provide the significance of the findings, with recommendations regarding the areas to be investigated provided, culminating in conclusions that tied the entire study together. The Harvard style of author-alphabet, as approved by the University Senate, was used throughout the mini-dissertation.

CHAPTER 2

LITERATURE REVIEW

2.1 Work done on problem statement

2.1.1 Container-types and plant growth

High surface area to volume ratio of containers and the absorption of direct solar radiation by container surface could expose roots to significant temperature fluctuations and extremes during various seasons (Ingram and Ruter, 2015). Three different containers, namely, paper pot, quick pot and plantek plastic container, under nursery conditions, had significant differences in morphological growth of *Quercus ilex* L. and *Quercus coccifera* L. (Tsakaldimi *et al*., 2005). Ingram (1981) compared the effects of polyethylene bags and black plastic containers on growth of *Cornus florida* L., *Rhododendron ferrugineum* L. and *Pittosporum tobira* (Thunb.) W.T. Aiton and observed that polyethylene bags increased root growth with the increase being species-specific. During production of seedlings in containers, container-size, growing density and design characteristics were shown to be the most important determinants of seedling quality (Tsakaldimi *et al*., 2005).

LeBude *et al*. (2006) studied container-type and volume which were also shown to have high influence on adventitious rooting and subsequent field growth of stem cuttings of Loblolly pine (*Pinus taeda* L.) (LeBude *et al*., 2006), with rigid plastic containers being recommended. During an investigation of the influences of seedling size, container-type and mammal browsing on the establishment of *Eucalyptus globulus* (Labill) in plantation forestry, container-type had no effects on growth of plants (Close *et al*., 2009). Marshall and Gilman (1998) did not observe any effects of container-type on shoot growth, whereas there were significant differences on root

mass of plants that were grown in accelerator® -air root pruning container (ARPC) and standard black plastic containers. According to Ortega *et al*. (2006), field performance of *Pinus radiata* D. Don plants was affected by container-type when four container-types, thermoformed plastic AR260, polyethylene FP200, polyethylene PT270 and polyethylene PF200, were compared. A study conducted at Green Biotechnologies Research Centre demonstrated that black plastic bags had the potential of negatively affecting the performance of the cucurbitacin-containing phytonematicides and biomuti in artificial microplots where containers were placed aboveground (Nyamandi, 2017).

Khurram *et al*. (2017) reported that the container-type on Arizona walnut seedlings had significant effects on dry shoot mass and root volume under nursery culture conditions. Also, Paterson (1996) observed that container-type had significant effects on seedling morphology of black spruce (*Picea mariana* (Mill.) Britton *et al*.) when plants were raised in Multipot #3-96 as depicted by significantly higher plant height, root-collar diameter and total dry biomass than seedlings grown in FH408 paper pot, Multipot #1-67 and Multipot #6-45. Al-Zalzaleh (2013) observed significant effects of container-type on plant height, dry root mass and shoot mass of *Acacia saligna* and *Eucalyptus viminalis*. Al-Zalzaleh and Dcruz (2015) grew *E. viminalis* in conventional pots and spring ring container and found that conventional pots produced plants with maximum height, which did not differ in leaf area and leaf number. Fresh shoot and dry shoot mass were each not significantly different in conventional plastic pots and spring rings. Container-type had no significant effects on root and shoot growth of *Acer rubrum* L., but significantly increased stem diameter (Marshall and Gilman, 1998). Total dry root mass of *Liquidambar* plant was significantly improved when

plants were raised in standard containers than in air-plant containers (Ferrini and Nicese, 2006). Vladan *et al*. (2015) investigated the effects of container-type, namely, bosnaplast 18, bosnaplast 12 and hiko V265, on growth and morphological characteristics of pedunculate oak (*Quercus robur* L.) seedlings under nursery and later field conditions, with significant effects on stem diameter. Seedlings produced in container-type bosnaplast 12, bosnaplast 18 and hiko V265 reached an average stem diameter of 3.22, 3.96 and 4.06 mm, respectively. O'Connor (2014) compared the effects of container-type during nursery production and overwintering on plant growth and survival using two fabric containers and black plastic containers, where seedlings did well in the former. O'Connor (2014) proposed that the use of fabric containers would reduce the severity of root-zone temperature fluctuations relative to black plastic pots, reduce root malformation and/or defects, and enhance tree growth rate.

2.1.2 Container-positioning and plant growth

Martin *et al*. (1999) demonstrated that below-ground positioning of containers with Southwest landscape trees could cause root-zone temperature to be 13°C lower when compared to containers placed above-ground. In contrast, Furuta (1960) observed that there were no significant effects of container positioning on maximum temperature, but there is a slight difference in plant growth of round-leaved holly [*Ilex rotunda* Thunb. (1784)] exposed to three different bases. Ferrini and Nicese (2006) observed that *Acer* and *Liquidambar* species placed above-ground had significantly higher dry root mass, whereas root length was not affected by container positioning. However, there was a slight positive effect of placing plants above-ground on root length in both *Acer* and *Liquidambar* species.

2.1.3 Biomuti and Afrikelp on plant growth and nematode suppression

Root-knot nematodes (*Meloidogyne species*) are the most injurious plant-parasitic pests that reduce plant growth and yield (Jones *et al*., 2013; Pretorius, 2017). Under certain conditions *Meloidogyne species* have the potential to cause as high as 50% reduction in plant growth to total crop failure (Mashela *et al*., 2016). Worldwide, prior to the withdrawal of methyl bromide in 2005, crop losses due to nematode damage were estimated at US$126 billion per annum (Chitwood, 2003). However, eight years after the withdrawal of methyl bromide, yield crop losses due to nematodes were estimated at over US$157 billion (Elling, 2013), with the relative crop yield losses being at over 37% (Mashela *et al*., 2016).

After the withdrawal of methyl-bromide from the agrochemical market, there was a huge void in nematode management options (Tseke and Mashela, 2018). Various alternative management options that could be environment-friendly were researched and developed in various countries. Biomuti, a product from the Agricultural Research Council, was being promoted as a product with the potential to promote plant-growth and reduce plant parasitic pests without harming the environment (Pretorius, 2017). In a preliminary study on suppression of nematodes using biomuti and cucurbitacin-containing phytonematicides as standard, Nyamandi (2017) placed the potted seedlings above-ground and observed that all seedlings, including those of the standard, were heavily galled with high population densities of nematodes.

Biomuti a product consisting of natural soil microorganisms had been reported to improve plant growth (Pretorius, 2017). Biomuti was originally obtained from the extraction of beneficial microbes from virgin soils known to contain suppressive properties to soil-borne diseases. Marketed as a natural organic product, biomuti is viewed as being environment-friendly and it had, on several crops, promoted above-ground plant growth (Pretorius, 2017). The bacteria component of biomuti, are the most effective among the listed micro-organisms, with some having nematicidal or nematostatic properties (Viaene *et al*., 2013). Molecular analyses suggested that the product contains 45 genera of bacterial species, where 49% genera were beneficial bacteria (Pretorius, 2017). Lactic-acid producing bacteria, for instance, was the most abundant during biomuti characterisation. *Bacillus* species is one of the bacteria that were in biomuti with nematicidal properties (Pretorius, 2017). The existence of the high percentage of organisms with beneficial characteristics responsible for plant growth and pest control had been confirmed in biomuti (El-Hadad *et al.*, 2011; Pretorius, 2017).

Freshly harvested South African giant brown seaweed [*Ecklonia maxima* (Osbeck)] is used to produce the effective kelp/seaweed product containing high concentrations of plant growth regulators such as auxins, cytokinins, gibberellins is being marketed as Afrikelp (Butler and Hunter, 2017). Due to the presence of plant growth regulators and lower molecular compounds like polyamines in seaweeds, seaweed extracts had been shown to serve as a plant bio-stimulant (Kannan *et al*., 2015). Plant bio-stimulants do not have direct action or impact against pests, and could therefore not be reported as pesticides (Calvo *et al*., 2014). Plant bio-stimulants could improve efficiency of metabolism in plants and thereby resulting in

yield increase and improved crop quality, increase plant tolerance to abiotic factors and enhance speedy recovery of plants from abiotic stresses, facilitate nutrient assimilation, translocation and use, improve physiological properties of soil and substitute the development of complementary soil micro-organisms (European Biostimulants Industry, 2012).

Afrikelp was shown to have the capability to improve the use of mineral nutrients by plants and also improve soil structure and aeration which stimulate root growth (Calvo *et al*., 2014). Stimulation of mineral nutrient uptake by Afrikelp had been reported in lettuce (*Lactuca sativa* L.), grapes (*Vitis vinifera* L.), soybean [*Glycine max* (L.) Merr], tomato (*Solanum lycopersicum* L.) and winter rapeseed, with substantial accumulation of macro- and micro-nutrients (Calvo *et al*., 2014). Afrikelp application exhibited plant-growth enhancing properties, increased pathogen/pest resistance and tolerance to climatic stresses such as cold or drought, with consequent result of improved yield quality (Arioli *et al*., 2015; Ciepiela *et al*., 2016, Lotze and Hoffman, 2015; Russell, 2002). Other positive responses of Afrikelp include the increased nutrient mobilisation, enhanced chlorophyll content, delayed senescence, improved fruit shelf-life, increased tolerance to frost, pathogen/pest attack and improved tolerance to salt stress (Arthur *et al*., 2013).

2.2 Work not yet done on problem statement

The effect of container-type and positioning on growth of tomato plants cv. 'Floradade' and suppression of *M. javanica* under microplot conditions have not been documented. There is enough literature on effect of container-type on other crops, but less documented information on container-positioning and how containers

influence plant-parasitic pests such as nematodes. Although effects of other synthetic nematicides such as phytonematides is under investigation, products such as biomuti and Afrikelp on suppression of nematodes have not yet been documented.

2.3 Addressing the identified gaps

The effects of container-type and positioning on growth of tomato plants cv. 'Floradade' and suppression of *M. javanica* as exposed to biomuti and Afrikelp would be investigated. Findings of the study would be important on the design of microplot experiments related to research and development of products for use as alternative to methyl bromide.

CHAPTER 3

CONTAINER-TYPE AND POSITIONING ON NEMATODE-INOCULATED TOMATO WITH BIOMUTI AND AFRIKELP

3.1 Introduction

The root-knot (*Meloidogyne* species*)* nematodes are the most injurious pests of tomato (*Solanum lycorpesicum* L.) crops, with potential to cause total crop failure in certain regions (Mashela *et al*., 2013). The withdrawal of methyl-bromide from the agrochemical markets in 2005 and other synthetic nematicides, led to an increase in research and development of environment-friendly alternatives such as phytonematicides, biomuti and Afrikelp. Biomuti also known as SoilBioMuti (SBM) is a natural organic product, therefore it is not harmful to the environment and it exhibit above growth-promoting effects for several crops, including maize (Pretorius, 2017). Afrikelp is being freshly harvested from South African giant brown seaweed (*Ecklonia maxima)* and it contains high concentrations of plant growth regulators (Butler and Hunter, 2017). The efficacy of the cucurbitacin-containing phytonematicides had been shown to be independent of the microbial activities (Mashela, 2002; Mashela and Nthangeni, 2002; Mashela *et al*., 2013*)*.

Recently, Nyamandi (2017) observed that when plastic bags were positioned on the soil surface during microplot trials, cucurbitacin-containing phytonematicides, which had been reported to suppress nematode numbers consistently (Mashela *et al*., 2017), failed to suppress nematode numbers. The failure was explained in terms of changes in ambient temperatures which affected the temperature inside of the containers directly (Nyamandi, 2017). Also, different container-sizes were reported to have the ability to affect plant growth (Tsakaldimi *et al*., 2005), possibly through the

direct effects of root bounding. It was reported that growing plants in containers made of colours lighter than standard black improve root growth (Markham *et al*., 2011). The objective of this study was to determine whether container-type and positioning would have an influence on the growth of tomato plants and suppression of *M. javanica* exposed to biomuti or Afrikelp.

3.2 Materials and methods

3.2.1 Description of the study site

The study was conducted under microplot conditions at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, South Africa (23°53'10"S, 29°44'15"E). The location had an average annual rainfall of less than 500 mm, with the highest distribution being during summer (November-January), with maximum/minimum temperatures of 38/25°C. The study was initiated during autumn (March-May) in 2017 (Experiment 1) and repeated in 2018 (Experiment 2).

3.2.2 Treatments and research design

Parallel trials (Trial 1: biomuti; Trial 2: Afrikelp) each had treatments comprising brown-pot below (20-cm-diameter pot), brown-pot above, black-pot below, black-pot above, plastic-bag below and plastic-bag above (12-L black bags), with below and above being relative to the soil surface. The six treatments were arranged in a randomised complete block design, with five replications (Figure 3.1). Blocking was against heterogeneous conditions such as shade and light during different times of the day caused by the adjacent windbreak trees.

Figure 3.1 Experimental layout showing tomato plants with container-type and positioning.

3.2.3 Procedures

Different container-types were each filled with approximately 10.4 L growing mixture of steam-pasteurised sandy-loam soil (65% sand, 15% clay, 5% silt) and Hygromix-T at 3:1 (v/v) ratio. Soil in each pot was mixed with 5 g NPK 2:3:2 (26), 5% Ca and 0.5% Zn to provide 310 mg N, 210 mg P and 260 mg K prior to transplanting. Fourteen (14) days after transplanting, 5 g NPK 2:3:2 (43) Multifeed (Nulandies, Johannesburg) to provide 0.175 mg N, 0.16 mg K, 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. Uniform seedlings of tomato (*Solanum lycopersicum* L.) cv. 'Floradade' were transplanted into containers and irrigated with 500 ml chlorine-free tapwater every other day. The containers were established in micro-plots with 0.6 m × 0.6 m spacing. Seven days after transplanting, each plant was inoculated with 2 000 eggs and *M. javanica* second-stage juveniles (J2). Biomuti and Afrikelp were each

obtained from Agricultural Research Council-Vegetables, Ornamentals and Plants (ARC-VOP) and applied weekly from inoculation to harvest. Funginex (*a.i*. triforine) was used once monthly at 2.5% to manage powdery mildew.

3.2.4 Data collection

Plant variables: At 56 days after inoculation with nematodes, plant height was measured from the crown to the terminal end of the flag leaf, stems were cut at the crown and stem diameters measured at 3 cm above the cut ends using a digital Vernier calliper (DC – 515, Bangkok, Thailand) (Figure 3.2A). Chlorophyll content was measured on the leaf blade using the chlorophyll meter (Minolta SPAD – 502, Hangzhou, China) (Figure 3.2A). Fruit number and mass were determined. Shoots were oven-dried at 52°C for 72 h and weighed. Root systems were removed from pots, immersed in water to remove soil particles (Figure 3.2B), blotted dry and weighed. Root galls (Figure 3.2C, D) were assessed using the North Carolina Differential Rating Scale at 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls and 5 ≥ 100 galls per root system (Taylor and Sasser, 1978).

Figure 3.2 Recording of plant variables during harvest time (A), collection of root system and soil samples (B), root system of tomato plants with few root galls under performance of Afrikelp (C) and heavily infested with galls under performance of biomuti (D).

Nutrient analysis: Approximately 0.5 g ground leaf materials were digested in 75 ml vessel with 2 ml nitric acid (HNO_3) and 3 ml of hydrogen peroxide (H_2O_2). The mixture was then vortexed for 2 minutes at least 10 minutes before the vessels were closed. Samples were inserted in a microwave digester (Perkin Eimer, Totan MPS) to run for 46 minutes under temperature ranging up to 260°C, thereafter, vessels

were allowed to cool down at room temperature for 20 minutes. Samples were decanted into 50 ml tubes and stored in cold room to avoid evaporation prior to analytical process. K, Na, Zn and Fe content in leaf tissues were determined using Inductively Coupled Plasma Optical Emission Spectrometry (Shimadzu, ICPE-9000).

Nematode variables: Nematodes were extracted from whole root system per plant using maceration and blending method (Hussey and Barker, 1973). Soil per pot was thoroughly mixed and 250 ml soil sample were collected, with nematodes extracted using the sugar-floatation and centrifugation method (Jenkins, 1964). Eggs and J2 from root and J2 from soil were counted from a 10 ml aliquot of each sample with the use of a stereomicroscope (Zeiss Stemi 508, Suzhou, China). Final nematode population density (Pf) was determined by adding total eggs and J2 in root system to total J2 in soil. Reproductive potential (RP = eggs + J2/g root), which is the capability of nematode to reproduce in a given plant per unit gram of root was also determined.

3.2.5 Data analysis

Collected data were subjected to analysis of variance (ANOVA) through the SAS software (SAS Institute, 2008). Separation of means was achieved using Fisher's Least Significant Difference test at a probability level of 5%. Nematode data were transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984). The degrees of freedom and their associated mean sum of squares were partitioned to provide the total treatment variation (TTV) for different sources of variation. Unless stated otherwise, treatment means were discussed at the probability level of 5%.

3.3 Results

In each trial (biomuti or Afrikelp), the seasonal interaction was not significant and therefore data were pooled (n = 60) and re-analyzed.

3.3.1 Treatment effects on plants with biomuti

Plant growth variables: Treatments had highly significant effects on plant height, fruit number, dry root mass, dry shoot mass and fruit mass, contributing 82, 48, 44, 85 and 85% in TTV of the respective variables (Table 3.1), whereas there were no significant effects on stem diameter, chlorophyll content and root gall rating (Appendix 3.1, 3.3, 3.7). Relative to brown-pot-below soil surface, plastic-bag-above, brown-pot-above and black-pot-above reduced plant height by 16, 19 and 22%, respectively, but black-pot-below and plastic-bag-below had no significant effect on the variable (Table 3.2). Relative to brown-pot-below soil surface, fruit numbers were reduced by 8, 28, 24 and 29% in black-pot-below plastic-bag-above, brown-pot-above and black-pot-above, respectively, whereas plastic-bag-below had no significant effect on the variable (Table 3.2).

Black-pot-below, plastic-bag-above, brown-pot-above and black-pot-above decreased dry root mass by 7, 23, 25 and 30%, respectively. However, plastic-bag-below had no significant effect on the variable (Table 3.2). Dry shoot mass in plastic-bag-above, brown-pot-above and black-pot-above was reduced by 38, 25 and 32%, respectively, whereas black-pot-below and plastic-bag-below showed no significant effect on the variable (Table 3.2). Relative to brown-pot-below; plastic-bag-above, brown-pot-above and black-pot-above increased dry fruit mass by 24, 52 and 57%,

respectively, whereas black-pot-below and plastic-bag-below had no significant effect on the variable (Table 3.2).

Nutrient element analysis: All analysed elements showed no significant response to the treatment (Table 3.3-3.4, Appendix 3.9–3.12).

Nematode variables: Treatments had no significant effects on nematode variables (Table 3.5-3.6, Appendix 3.13–3.18).

Table 3.1 Partitioning mean sum of squares for plant height (PLH), stem diameter (STD), chlorophyll content (CHL), fruit number (FTN), dry root mass (DRM), dry shoot mass (DSM), gall rating (GLR) and fruit mass (FTM) for tomato cv. 'Floradade' in biomuti-drenched soil in response to container-type and positioning at 56 days after treatment initiation.

Source	DF	PLH		STD		CHL		FTN	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	9	44.84	8	4.82	34	13.91	26	180.82	40
Treatment	5	449.27	82***	5.39	39^{ns}	21.23	40^{ns}	214.75	48***
Error	45	54.37	10	3.79	27	18.25	34	53.68	12
Total	59	584.48	100	14	100	53.39	100	449.25	100
Source	DF	DRM		DSM		GLR		FTM	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	9	123.41	40	84.21	7	0.82	41	617.30	6
Treatment	5	135.31	44**	951.54	85***	0.70	35^{ns}	9880.88	89***
Error	45	48.29	16	87.08	8	0.47	24	580.12	5
Total	59	307.01	100	1122.83	100	1.99	100	11078.30	100

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

Table 3.2 Effect of container-type and positioning on plant height (PLH), fruit number (FTN), dry root mass (DRM), dry shoot mass (DSM) and fruit mass (FTM) for tomato cv. 'Floradade' in biomuti-drenched soil at 56 days after treatment initiation.

Treatment	PLH		FTN		DRM		DSM		FTM	
	Mean[y]	R.I. (%)[z]	Mean	R.I. (%)	Mean	R.I. (%)	Mean	R.I. (%)	Mean	R.I. (%)
Brown-pot-B	69.32[a]	−	38.80[a]	−	25.45[a]	−	54.02[a]	−	102.28[c]	−
Black-pot-B	68.92[a]	−1	35.70[b]	−8	23.69[b]	−7	55.49[a]	3	92.38[c]	−10
Plastic-bag-B	66.20[a]	−5	34.90[ab]	−10	26.51[a]	4	52.59[a]	−3	90.08[c]	−12
Plastic-bag-A	58.29[b]	−16	28.00[c]	−28	19.51[bc]	−23	33.49[b]	−38	127.17[b]	24
Brown-pot-A	56.29[b]	−19	29.60[bc]	−24	18.98[c]	−25	40.59[b]	−25	155.29[a]	52
Black-pot-A	54.29[b]	−22	27.70[c]	−29	17.85[c]	−30	36.49[b]	−32	161.02[a]	57

A = above soil surface, B = below soil surface.

[y]Column means with same letter were not significantly different at P ≤ 0.05 according to Fisher's Least Significant Difference (LSD) test.

[z]Impact (%) = [(Treatment/Control − 1) × 100], Brown-pot-below ground used as a standard for comparison.

Table 3.3 Partitioning mean sum of squares for iron (Fe), potassium (K), sodium (Na) and zinc (Zn) in biomuti-drenched soil for tomato cv. 'Floradade' in response to container-type and positioning at 56 days after treatment initiation.

		Fe ppm		K %		Na ppm		Zn ppm	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	9	0.009169	36	0.01229	25	0.05053	17	0.01264	51
Treatment	5	0.007013	28^{ns}	0.02623	53^{ns}	0.16630	57^{ns}	0.00752	30^{ns}
Error	45	0.009176	36	0.01131	22	0.07546	26	0.00470	19
Total	59	0.025358	100	0.04983	100	0.29229	100	0.02486	100

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

Table 3.4 Means for iron (Fe), potassium (K), sodium (Na) and zinc (Zn) in biomuti-drenched soil for tomato cv. 'Floradade' in response to container-type and positioning at 56 days after treatment initiation.

Treatment	Fe (ppm)	K (%)	Na (ppm)	Zn (ppm)
Brown-pot-B	2.94	4.26	3.13	1.98
Black-pot-B	2.91	4.19	2.96	2.04
Plastic-bag-B	2.92	4.17	2.99	1.99
Plastic-bag-A	2.94	4.22	3.03	2.01
Brown-pot-A	2.96	4.14	2.78	1.99
Black-pot-A	2.98	4.27	3.13	2.04
$LSD_{0.05}$	Ns	Ns	Ns	Ns

A = above soil surface, B = below soil surface.

Table 3.5 Partitioning mean sum of squares for eggs and second-stage juveniles (J2) in roots, juveniles (J2) in soil, final population of nematodes (Pf) and reproductive potential (RP) for tomato cv. 'Floradade' in biomuti-drenched soil in response to container-type and positioning at 56 days after treatment initiation.

		Eggs + J2 in root		J2 in soil		Pf		RP	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	9	0.26	47	0.13	42	0.10	48	11.99	61
Treatment	5	0.04	7^{ns}	0.05	16^{ns}	0.04	19^{ns}	1.51	8^{ns}
Error	45	0.25	46	0.13	42	0.07	33	6.25	31
Total	59	0.20	100	0.31	100	0.21	100	19.75	100

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

Table 3.6 Means for total second-stage juveniles (J2) and eggs in roots, J2 in soil, final population of nematodes (Pf) and reproductive potential (RP) for tomato cv. 'Floradade' in biomuti-drenched soil in response to container-type and positioning at 56 days after treatment initiation.

Treatment	J2 and eggs in roots	J2 in soil	Pf	RP
Brown-pot-B	3.19	2.08	3.86	4.06
Black-pot-B	3.11	2.22	4.01	5.28
Plastic-bag-B	3.25	2.19	3.94	4.80
Plastic-bag-A	3.27	2.07	3.88	4.68
Brown-pot-A	3.18	2.07	3.88	4.71
Black-pot-A	3.14	2.12	3.86	4.74
$LSD_{0.05}$	Ns	Ns	Ns	Ns

A = above soil surface, B = below soil surface.

3.3.2 Treatment effects on plants with Afrikelp

Plant growth variables: Treatment effects were highly significant on plant height, dry root mass, dry shoot mass and root gall rating, contributing 91, 88, 66 and 60% in TTV of the respective variables (Table 3.7). Treatments had a significant effect on fruit mass, contributing 43% in TTV of the variable (Table 3.7), whereas no significant effects were detected on stem diameter, chlorophyll content and fruit number (Appendix 3.20-3.22). Relative to brown-pot-below, plastic-bag-above, brown-pot-above and black-pot-above reduced plant height by 21, 25 and 26%, respectively, whereas brown-pot-below and plastic-bag-below had no significant effect on the variable (Table 3.8). Plastic-bag-above, brown-pot-above and black-pot-above increased dry root mass by 82, 105 and 96%, respectively, but black-pot-

below and plastic-bag-below showed no significant effect on the variable (Table 3.8). Dry shoot mass in plastic-bag-above, brown-pot-above and black-pot-above was reduced by 42, 38 and 37%, respectively, with no significant effects of black-pot-below and plastic-bag-below on the variables detected (Table 3.8). Fruit mass in plastic-bag-above and black-pot-above was reduced by 36 and 31%, respectively, although black-pot-below, plastic-bag-below and brown-pot-above had no significant effects on the variable (Table 3.8). Relative to brown-pot-below, plastic-bag-above and black-pot-above increased gall rating by both 18%, whereas black-pot-below, plastic-bag-below and brown-pot-above had no significant effects on the variable (Table 3.8).

Nutrient element analysis: Treatments did not have significant effects on all nutrient elements (Table 3.9, Appendix 3.27–3.30).

Nematode variables: Treatment effects were not significant on all nematode variables, although nematode variables were reported (Table 3.10-3.12, Appendix 3.31–3.36).

Table 3.7 Partitioning mean sum of squares for plant height (PLH), stem diameter (STD), chlorophyll content (CHL), fruit number (FTN), dry root mass (DRM), dry shoot mass (DSM), gall rating (GLR) and fruit mass (FTM) for tomato cv. 'Floradade' in Afrikelp-drenched soil in response to container-type and positioning at 56 days after treatment initiation.

Source	DF	PLH		STD		CHL		FTN	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	9	53.00	4	16.18	39	49.38	45	231.34	60
Treatment	5	1088.94	91***	14.85	36^{ns}	34.62	32^{ns}	100.86	26^{ns}
Error	45	58.07	5	10.30	25	24.55	23	52.98	14
Total	59	1200.01	100	41.33	100	108.55	100	385.18	100
Source	DF	DRM		DSM		GLR		FTM	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	9	2.48	8	657.90	29	0.71	29	57193.10	42
Treatment	5	27.60	88***	1493.89	66***	1.47	60***	58079.20	43**
Error	45	1.30	4	121.60	5	0.27	11	20333.40	15
Total	59	31.38	100	2273.39	100	2.45	100	135605.70	100

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

Table 3.8 Effect of container-type and positioning on plant height (PLH), dry root mass (DRM), dry shoot mass (DSM), fruit mass (FTM) and gall rating (GLR) for tomato cv. 'Floradade' in Afrikelp-drenched soil at 56 days after treatment initiation.

	PLH		DRM		DSM		FTM		GLR	
Treatment	Mean[y]	R.I. (%)[z]	Mean	R.I. (%)	Mean	R.I. (%)	Mean	R.I. (%)	Mean	R.I. (%)
Brown-pot-B	70.29[a]	−	3.18[b]	−	49.58[a]	−	517.03[a]	−	4.00[bc]	−
Black-pot-B	73.97[a]	5	3.07[b]	−3	56.06[a]	13	494.28[a]	−4	3.90[c]	−3
Plastic-bag-B	72.71[a]	3	3.30[b]	4	50.86[a]	3	453.17[ab]	−12	3.90[c]	−3
Plastic-bag-A	55.51[b]	−21	5.80[a]	82	28.79[b]	−42	329.75[b]	−36	4.70[a]	18
Brown-pot-A	53.04[b]	−25	6.51[a]	105	30.81[b]	−38	392.35[ab]	−24	4.40[ab]	10
Black-pot-A	51.98[b]	−26	6.24[a]	96	31.17[b]	−37	355.37[b]	−31	4.70[a]	18

A = above soil surface, B = below soil surface.

[y]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference (LSD) test.

[z]Impact (%) = [(Treatment/Control − 1) × 100], Brown pot below ground used as a standard for comparison.

Table 3.9 Partitioning mean sum of squares for iron (Fe), potassium (K), sodium (Na) and zinc (Zn) to container-type and positioning in Afrikelp-drenched soil for tomato cv. 'Floradade' at 56 days after treatment initiation.

		Fe ppm		K %		Na ppm		Zn ppm	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	9	0.00911	13	0.00763	28	0.06818	25	0.18587	33
Treatment	5	0.03512	51^{ns}	0.01059	38^{ns}	0.10629	38^{ns}	0.21571	39^{ns}
Error	45	0.02460	36	0.00942	34	0.10165	37	0.15703	28
Total	59	0.06883	100	0.02764	100	0.27612	100	0.55861	100

^{ns}Not significant at P ≤ 0.05

Table 3.10 Means for iron (Fe), potassium (K), sodium (Na) and zinc (Zn) in Afrikelp-drenched soil for tomato cv. 'Floradade' in response to container-type and positioning at 56 days after treatment initiation.

Treatment	Fe	K	Na	Zn
Brown-pot-B	2.88	4.06	2.56	1.95
Black-pot-B	2.92	4.02	2.48	1.90
Plastic-bag-B	2.86	4.11	2.72	2.25
Plastic-bag-A	2.79	4.07	2.73	1.88
Brown-pot-A	2.79	4.07	2.60	1.85
Black-pot-A	2.77	4.09	2.71	1.89
$LSD_{0.05}$	Ns	Ns	Ns	Ns

A = above soil surface, B = below soil surface.

Table 3.11 Partitioning mean sum of squares for total second-stage juveniles and eggs in roots, juveniles (J2) in soil, final population of nematodes (Pf) and reproductive potential (RP) for tomato cv. 'Floradade' at 56 days after treatment initiation.

		J2 and eggs in root		J2 in soil		Pf		RP	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	9	0.06	19	0.25	57	0.12	46	0.07	41
Treatment	5	0.18	56[ns]	0.03	7[ns]	0.05	19[ns]	0.04	24[ns]
Error	45	0.08	25	0.16	36	0.09	35	0.06	35
Total	59	0.32	100	0.44	100	0.26	100	0.17	100

[ns]Not significant at $P \leq 0.05$

Table 3.12 Means for total second-stage juveniles (J2) and eggs in roots, J2 in soil, final population of nematodes (Pf) and reproductive potential (RP) for tomato cv. 'Floradade' in Afrikelp-drenched soil in response to container-type and positioning at 56 days after treatment initiation.

Treatment	J2 + eggs in root	J2 in soil	Pf	RP
Brown-pot-B	3.15	2.15	3.92	0.73
Black-pot-B	2.92	2.12	3.81	0.64
Plastic-bag-B	3.24	2.09	3.89	0.71
Plastic-bag-A	2.92	2.04	3.76	0.61
Brown-pot-A	3.17	2.20	3.96	0.76
Black-pot-A	3.04	2.09	3.86	0.68
$LSD_{0.05}$	Ns	Ns	Ns	Ns

A = above soil surface, B = below soil surface.

3.4 Discussion

3.4.1 Treatment effects on plants with biomuti

Plant growth variables with biomuti: Treatments (3 container-types, 2 positions) had highly significant effects on plant variables with biomuti serving as a bio-stimulant, without any significant effects on stem diameter, chlorophyll content and gall rating. The uniqueness of the findings in the current study were that container-type and positioning had never before been conducted and compared as simultaneous treatments. However, container-type studies (Al–Zalzaleh, 2013; Fitzpatrick *et al*., 1994; Ingram, 1981; Khurram *et al*., 2017; LeBude *et al*., 2006; Ortega *et al*., 2006; Paterson, 1996; Tsakaldimi *et al*., 2005; Vladan *et al*., 2015) and positioning studies (Ferrini and Nicese, 2006; Furuta, 1960; Martin *et al*., 1999) had separately demonstrated that the treatments each could significantly affect plant growth and development. However, in other studies (Al-Zalzaleh and Dcruz, 2015; Close *et al*., 2009; Ferrini and Nicese, 2006; Marshall and Gilman, 1998), container-type had no significant effects on plant growth variables.

In agreement with observations in the current study, container-type and positioning were observed to have significant effects on most plant growth variables (Ferrini and Nicese, 2006; Klooster *et al*., 2010; Martin *et al*., 1999; Self and Ward, 1965), whereas in other studies (Bartos, 1960; Furuta, 1960; Jansen and Bopp, 1965) the treatments did not have significant effects on certain plant growth variables as observed in the current study. Also, in the current study, the combination of the two treatments, along with treating plants with biomuti or Afrikelp, could have contributed to a large number of plant variables that responded differently to the treatments in other studies. However, the nature of the experimental design in the current study

did not allow inferences to be made about biomuti and Afrikelp since they were not treatments.

Relative to brown-pot-below, plastic-bag-above, brown-pot-above and black-pot-above reduced tomato plant height, but black-pot-below and plastic-bag-below had no significant effects on the variable. Under greenhouse conditions, Khurram *et al*. (2017) observed an increase in plant height of Arizona walnut that was grown in green containers when compared to seedlings in black standard containers. Paterson (1999) observed an increase in plant height of black spruce (*Picea mariana* (Mill.) Britton, Sterns and Poggenburg) in multi-pot #3-96, whereas multi-pot #6-45, multi-pot #1-67 and FH408 paperpot had no significant effects on the variable. Martin *et al*. (1999), observed that plant height where containers were placed above the ground also reduced plant height of sweet acacia (*Vachellia farnesiana* L.). The decrease was suggested to have resulted from high root-zone temperatures in above-ground containers as compared to below-ground containers (Martin *et al*., 1999). In contrast, Hubbel *et al*. (2018) observed an increase in plant height of *Pinus occidentalis* Sw. raised in polybags than those raised in D40 containers. Also, Al-Zalzaleh (2013) reported an increase in plant height of *Acacia saligna* (Labill.) H.L. Wendl., raised in conventional plastic bags than of plants raised in root trainers and spring rings.

Relative to brown-pot-below, fruit numbers were reduced in black-pot-below, plastic-bag-above, brown-pot-above and black-pot-above, whereas plastic-bag-below had no significant effect on the variable. In contrast, Hassan *et al*. (2011) observed black increased fruit number of strawberry (*Fragaria x ananassa* Duchesne) in black bags

than those that were in white containers. Generally, most of the studies in this area were on non-fruiting plants and could, therefore, not be compared. However, in Nyamandi (2017) study, where only above-ground containers were studied, the results suggested that black plastic bags had no effect on tomato fruit number. In other studies (Arancon *et al*., 2010; Massetani *et al*., 2017; Stevenson and Fisher, 1975) various treatments below-ground increased fruit numbers.

Relative to brown-pot-below, plastic-bag-above, brown-pot-above and black-pot-above increased fruit mass, whereas black-pot-below and plastic-bag-below had no significant effect on the variable. In contrast, (Hassan *et al*., 2011; Markham *et al*., 2011) observed that there were no effects of black and silver containers on fruit mass of strawberry (*Fragaria x ananassa* Duchesne) and bush beans (*Phaseolus vulgaris* L.), respectively. The observed increase in fruit mass in the current study could also be attributed to the stimulation effects of biomuti, which had been shown to promote above-ground plant growth in several crops (Pretorius, 2017).

Relative to brown-pot-below, black-pot-below, plastic-bag-above, brown-pot-above and black-pot-above decreased dry root mass, whereas plastic-bag-below had no significant effects on the variable. Container-type appeared to play an overriding role in root growth than the actual positioning of the containers. In agreement with this observation, containers placed below-ground were shown to increase dry root mass of sweet acacia (*Vachellia farnesiana* L.) when compared to those above-ground positioning (Martin *et al*., 1999). Also, Ferrini and Nicese (2006) observed an increase in dry root mass of Liquidambar plants in standard containers compared to plants grown in air-plant container in above- and below-ground positioning, whereas

dry root mass of *Acer platanoides* seedlings in containers placed above-ground were observed to increase. Also, Ingram (1981) observed an increase in dry root mass of *Cornus florida* L., rhododendron (*Rhododendron simsii* Planch. 'Formosa') and Japanese pittosporum (*Pittosporum tobira* Banks), when grown in black plastic containers when compared to those in polyethylene bags. In contrast, dry root mass of *Pinus occidentalis* Sw. raised in polybags were increased than of plants raised in D40 containers (Hubbel *et al*., 2018). Roots in containers above-ground were more susceptible to injury by supra-optimal root-zone temperatures (Martin *et al*., 1999). *Viburnum odoratissimum* (Ker-gawl.) plants grown in white Multipot Box System (MPBS) had increased dry root mass than those that were raised in black MPBS, which was further explained by lower root-zone temperatures (26.4-38.7°C) in white MPBS compared to black MPBS with temperatures (27.7-41.1°C) (Irmak *et al*., 2005).

Relative to brown pot-below, plastic-bag-above, brown-pot-above and black-pot-above reduced dry shoot mass of tomato plants, whereas black-pot-below and plastic-bag-below did not have significant effects on the variable. Fitzpatrick *et al*. (1994) observed no difference in dry shoot mass of *Swietenra mahagon* L. when the plant was raised in black plastic and air root pruning containers. Markham *et al*. (2011) illustrated that black container reduced dry shoot mass of bush bean (*Phaseolus vulgaris* L.) seedlings when compared to light-coloured containers, but without considering container positioning. In agreement with the results in the current study, containers positioned below-ground had a significant increase on dry shoot mass of blue palo verde (*Parkinsonia florida* [Benth. ex. A. Gray] S. Wats) when compared with those of in containers in the above-ground positioning, whereas in

sweet acacia (*Vachellia farnesiana* L) the below-ground positioning increased dry shoot mass when compared with those at the above-ground positioning (Martin *et al*., 1999). According to Hubbel *et al*. (2018), dry shoot mass of *Pinus occidentalis* Sw. raised in polybags increased compared to those raised in D40 containers, without considering the container positioning.

Nutrient elements in plants with biomuti: Treatment effects did not have significant effects on Fe, K, Na and Zn in leaf tissues of tomato plants, which confirmed results in the previous study (Nyamandi, 2017). However, Nyamandi (2017) observed when biomuti interacted with Nemarioc-AL phytonematicide and Mycoroot, Ca in leaf tissues of tomato plants were reduced. Literature is replete with information on effects of phytonematicides on foliar nutrient elements (Maake, 2018; Mashela *et al*., 2017, Shadung, 2016). Also, Hassan *et al*. (2011) observed that there were no effects of container-type on foliar K of strawberry (*Fragaria x ananassa* Duchesne). Although, Klooster *et al*. (2010) observed an increase in foliar K in *Pinus strobus* L. and *Abies fraseri* (Pursh.) Poir. in containers placed below-ground. In contrast, polybags increased foliar K and Zn of *Pinus occidentalis* Sw. as compared to D40 containers. The contradictions might be explained by the difference in growth media that were used both studies.

Nematode variables on plants with biomuti: Although all plants were heavily galled and have high population densities of nematodes, treatment effects had no significant effects on all nematode variables. Effective microorganisms in biomuti comprise bacterial strains with capabilities to suppress nematode population densities (Pretorius, 2017). In contrast, Nyamandi (2017) also observed that biomuti

in plastic bags placed above-ground had no significant effects on nematode population densities, which confirmed the results in the current study.

3.4.2 Treatment effects on plants with Afrikelp

Plant growth variables with Afrikelp: Treatments had highly significant effects on plant height, dry root mass, dry shoot mass, fruit mass and gall rating, but had no significant effects on stem diameter, chlorophyll content and fruit number. Relative to brown-pot-below, plastic-bag-above, brown-pot-above and black-pot-above reduced plant height, and brown-pot-below and plastic-bag-below had no significant effect on the variable. Also, relative to brown-pot-below, dry shoot mass of tomato plants grown in plastic-bag-above, brown-pot-above and black-pot-above was reduced, with no significant effect in black-pot-below and plastic-bag-below. As observed in the biomuti trial, both plant height and dry shoot mass were reduced, showing the influence of container-type and positioning. In contrast, relative to brown-pot-below, plastic-bag-above and black-pot-above increased gall rating, whereas black-pot-below, plastic-bag-below and brown-pot-above had no significant effect on the variable. The observation suggested that the treatment promoted growth of nematode population densities, and that Afrikelp was not able to suppress nematodes as shown in another organo-nematode study (Nyamandi, 2017).

Relative to brown pot-below, plastic-bag-above, brown-pot-above and black-pot-above increased dry root mass and black-pot-below and plastic-bag-below showed no significant effect on the variable. The increase in dry root mass agreed with those in other studies of Afrikelp (Calvo *et al*., 2014; European Biostimulants Industry, 2012; Ferrini and Nicese, 2006). However, in the current study, since roots were heavily infected by nematodes as shown by the root galls, the latter could have

contributed to increased root mass, as is usually the case in plants with root galls (Nyamandi, 2017).

Nutrient elements in plants with Afrikelp: Treatment effects did not have significant effects on foliar Fe, K, Na and Zn in the six treatments where the growing mixture was drenched with Afrikelp. The observations in the study contradicted those of Calvo *et al*. (2014), who showed that Afrikelp stimulated nutrient uptake in various crops, including tomato plants. However, it should be emphasised that the design of the current study was not conducive to inconclusively make a ruling on the effects of the organic products including Afrikelp.

Nematode variables on plants with Afrikelp: Treatment effects had no significant difference on all nematode variables; however, all plants were heavily galled and have high population densities of nematodes. The observation here, although it could not have support from literature, was a further indication that Afrikelp did not have nematicidal properties.

3.5 Conclusions

Apparently, the brown-pot-below standard along with black-pot-below and plastic-bag-below under microplot conditions would each be suitable for use in microplot studies, since the treatment improved the test plant variables in relation to the above-ground treatments, regardless of the container-type. In the current study, temperature was not measured and inferences as made in most literature that heat in the root-zone was responsible for significant differences in below- and above-ground containers could not be made. In conclusion, positioning of containers,

regardless of container-type, should not be done in trials conducted under microplot conditions due to high variation in ambient temperature.

CHAPTER 4

SUMMARY OF FINDINGS, SIGNIFICANCE, RECOMMENDATIONS AND CONCLUSIONS

4.1 Summary of findings

Regardless of whether the growing mixture was treated with biomuti or Afrikelp, the three container-type and two positioning treatments had significant effects on most plant growth variables. However, the treatments did not have significant effects on nutrient elements in leaf tissues and nematode variables. Since relative to the standard, namely, brown plastic-pot below, most plant growth variables were reduced, suggested that this standard should be retained for use in microplot trials. Similarly, those container-types which were not significantly different relative to the standard could also be used under microplot conditions.

4.2 Significance

Container-type and positioning trials were previously conducted independent of each other. However, in the current study the two factors were assessed simultaneously and it was clear that they could have a significant bearing on a wide range of plant growth variables. The observation that brown-pot below the soil surface was the best for trials under microplot conditions in terms of improving different plant growth variables in tomato plants was important because it would ensure that the experimental data under the stated conditions were not confounded with the container-type and positioning. Thereby improving the comparisons of the findings from different trials.

4.3 Recommendations

In the current study, it was not feasible to single out the effects of biomuti and Afrikelp on plant growth and nematode variable. Since the current study had shown that brown-pot below was the preferred container-type and positioning, an experiment should be done using this standard and biomuti or Afrikelp in a 2 × 2 factorial setup, where the first factor is the positioning and the second factor is biomuti or Afrikelp, with the focus being on the interactive effects on plant and nematode variables. In such as study, it would be imperative to measure soil minimum and maximum temperatures in order to have an idea why there were significant differences, should there be any.

4.4 Conclusions

Container-type and positioning had significant effects on various plant growth variables under microplot conditions. However, the treatments did not have significant effects on nutrient elements or nematode population densities. The major finding in the current study was that brown-pot below was the best container-type and positioning for tomato plant trials under microplot conditions.

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APPENDICES

Appendix 3.1 Analysis of variance for plant height under biomuti-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	403.51	44.835		
Treatment	5	2246.35	449.270	8.26	0.0000
Error	45	2446.42	54.365		
Total	59	5096.29			

Appendix 3.2 Analysis of variance for stem diameter under biomuti-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	43.349	4.81653		
Treatment	5	26.975	5.39497	1.42	0.2348
Error	45	170.768	3.79484		
Total	59	241.092			

Appendix 3.3 Analysis of variance for chlorophyll content under biomuti-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	125.18	3.9090		
Treatment	5	106.17	21.2346	1.16	0.3419
Error	45	821.41	18.2536		
Total	59	1052.77			

Appendix 3.4 Analysis of variance for fruit number under biomuti-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	1627.35	180.817		
Treatment	5	1073.75	214.750	4.00	0.0044
Error	45	2415.75	53.683		
Total	59	5116.85			

Appendix 3.5 Analysis of variance for fruit mass under biomuti-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	5555.7	617.30		
Treatment	5	49404.4	9880.88	17.03	0.0000
Error	45	26105.3	580.12		
Total	59	81065.4			

Appendix 3.6 Analysis of variance for dry shoot mass under biomuti-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	757.89	84.209		
Treatment	5	4757.71	951.543	10.93	0.0000
Error	45	3918.60	87.080		
Total	59	9434.19			

Appendix 3.7 Analysis of variance for gall rating under biomuti-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	7.3500	0.81667		
Treatment	5	3.4833	0.69667	1.47	0.2190
Error	45	21.3500	0.47444		
Total	59	32.1833			

Appendix 3.8 Analysis of variance for dry root mass under biomuti-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	1110.71	123.412		
Treatment	5	676.54	135.308	2.80	0.0275
Error	45	2173.02	48.289		
Total	59	3960.27			

Appendix 3.9 Analysis of variance for iron (Fe) under biomuti-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	0.08252	0.009169		
Treatment	5	0.03515	0.007031	0.77	0.5790
Error	45	0.41293	0.009176		
Total	59	0.53061			

Appendix 3.10 Analysis of variance for potassium (K) under biomuti-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	0.11064	0.01229		
Treatment	5	0.13116	0.02623	2.32	0.0587
Error	45	0.50880	0.01131		
Total	59	0.75060			

Appendix 3.11 Analysis of variance for sodium (Na) under biomuti-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	0.45478	0.05053		
Treatment	5	0.83149	0.16630	2.20	0.0704
Error	45	3.39556	0.07546		
Total	59	4.68193			

Appendix 3.12 Analysis of variance for zinc (Zn) under biomuti-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	0.45478	0.05053		
Treatment	5	0.83149	0.16630	2.20	0.0704
Error	45	3.39556	0.07546		
Total	59	4.68193			

Appendix 3.13 Analysis of variance for second-stage juveniles (J2) in roots under biomuti-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	0.53960	0.05996		
Treatment	5	0.57732	0.11546	0.88	0.4990
Error	45	5.87119	0.13047		
Total	59	6.98812			

Appendix 3.14 Analysis of variance for eggs in roots under biomuti-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	5.2601	0.58445		
Treatment	5	1.5550	0.31100	1.05	0.3999
Error	45	13.3144	0.29587		
Total	59	20.1295			

Appendix 3.15 Analysis of variance for J2 and eggs in roots under biomuti-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	2.3136	0.25707		
Treatment	5	0.1858	0.03716	0.15	0.9797
Error	45	11.3224	0.25161		
Total	59	13.8214			

Appendix 3.16 Analysis of variance for juveniles in soil under biomuti-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	1.09561	0.12173		
Treatment	5	0.21802	0.04360	0.35	0.8808
Error	45	5.63751	0.12528		
Total	59	6.95113			

Appendix 3.17 Analysis of variance for final nematode population under biomuti-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	0.89215	0.9913		
Treatment	5	0.19519	0.03904	0.56	0.7279
Error	45	3.12141	0.06936		
Total	59	4.20875			

Appendix 3.18 Analysis of variance for reproductive potential under biomuti-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	107.997	11.9997		
Treatment	5	7.550	1.5101	0.24	0.9418
Error	45	281.216	6.2493		
Total	59	396.764			

Appendix 3.19 Analysis of variance for plant height under Afrikelp-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	477.02	53.00		
Treatment	5	5444.70	1088.94	18.75	0.0000
Error	45	2613.34	58.07		
Total	59	8535.06			

Appendix 3.20 Analysis of variance for stem diameter under Afrikelp-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	145.649	16.1832		
Treatment	5	74.231	14.8462	1.44	0.2281
Error	45	463.545	10.3010		
Total	59	683.425			

Appendix 3.21 Analysis of variance for chlorophyll content under Afrikelp-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	444.44	49.3820		
Treatment	5	173.10	34.6203	1.41	0.2388
Error	45	1104.62	24.5470		
Total	59	1722.16			

Appendix 3.22 Analysis of variance for fruit number under Afrikelp-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	2082.02	231.335		
Treatment	5	504.28	100.857	1.90	0.1125
Error	45	2383.88	52.975		
Total	59	4970.18			

Appendix 3.23 Analysis of variance for fruit mass under Afrikelp-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	514738	57193.1		
Treatment	5	290396	58079.2	2.86	0.0253
Error	45	915005	20333.4		
Total	59	1720138			

Appendix 3.24 Analysis of variance for dry shoot mass under Afrikelp-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	5921.1	657.90		
Treatment	5	7469.5	1493.89	12.29	0.0000
Error	45	5471.9	121.60		
Total	59	18862.4			

Appendix 3.25 Analysis of variance for gall rating under Afrikelp-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	6.4000	0.71111		
Treatment	5	7.3333	1.46667	5.50	0.0005
Error	45	12.0000	0.26667		
Total	59	25.7333			

Appendix 3.26 Analysis of variance for dry root mass under Afrikelp-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	22.350	2.4833		
Treatment	5	138.015	27.6029	21.17	0.0000
Error	45	58.668	1.3037		
Total	59	219.032			

Appendix 3.27 Analysis of variance for iron (Fe) under Afrikelp-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	0.08196	0.00911		
Treatment	5	0.17559	0.03512	1.43	0.2327
Error	45	1.10688	0.02460		
Total	59	1.36444			

Appendix 3.28 Analysis of variance for potassium (K) in roots under Afrikelp-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	0.06863	0.00763		
Treatment	5	0.05293	0.01059	1.12	0.3613
Error	45	0.42373	0.00942		
Total	59	0.54529			

Appendix 3.29 Analysis of variance for sodium (Na) in roots under Afrikelp-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	0.61359	0.06818		
Treatment	5	0.53144	0.10629	1.05	0.4029
Error	45	4.57404	0.10165		
Total	59	5.71907			

Appendix 3.30 Analysis of variance for zinc (Zn) in roots under Afrikelp-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	1.67286	0.18587		
Treatment	5	1.07556	0.21571	1.37	0.2521
Error	45	7.06638	0.15703		
Total	59	9.81780			

Appendix 3.31 Analysis of variance for second-stage juveniles in roots under Afrikelp-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	2.79046	0.31005		
Treatment	5	0.51522	0.10304	0.74	0.5986
Error	45	6.27860	0.13952		
Total	59	9.58428			

Appendix 3.32 Analysis of variance for eggs in roots under Afrikelp-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	2.2977	0.25530		
Treatment	5	2.9076	0.58151	1.94	0.1069
Error	45	13.5114	0.30025		
Total	59	18.7166			

Appendix 3.33 Analysis of variance for J2 and eggs in roots under Afrikelp-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	0.53950	0.05994		
Treatment	5	0.89582	0.17916	2.17	0.0742
Error	45	3.71480	0.08255		
Total	59	5.15011			

Appendix 3.34 Analysis of variance for juveniles in soil under Afrikelp-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	2.19362	0.24374		
Treatment	5	0.15722	0.03144	0.20	0.9603
Error	45	7.03277	0.15628		
Total	59	9.38362			

Appendix 3.35 Analysis of variance for final nematode population under Afrikelp-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	1.07161	0.11907		
Treatment	5	0.27425	0.05485	0.60	0.6972
Error	45	4.08706	0.09082		
Total	59	5.43292			

Appendix 3.36 Analysis of variance for reproductive potential under Afrikelp-drenched soil.

Source	DF	SS	MS	F	P
Replication	9	0.64687	0.07187		
Treatment	5	0.17672	0.03534	0.61	0.6932
Error	45	2.61054	0.05801		
Total	59	3.43412			