# EFFICACY DETERMINATION OF PAINT-BRUSH FLOWER (*KLENIA LONGIFLORA*) ON SUPPRESSION OF *MELOIDOGYNE JAVANICA* AND GROWTH OF TOMATO PLANTS

MAKGOKA GIVEN MOREMI

MINI-DISSERTATION SUBMITTED FOR THE DEGREE MASTER OF AGRICULTURE IN PLANT PROTECTION, DEPARTMENT OF PLANT PRODUCTION, SOIL SCIENCE AND AGRICULTURAL ENGINEERING, SCHOOL OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES, FACULTY OF SCIENCE AND AGRICULTURE, UNIVERSITY OF LIMPOPO, SOUTH AFRICA

SUPERVISOR : DR K.G. SHADUNG

CO-SUPERVISOR : PROFESSOR P.W. MASHELA

2019

TABLE OF CONTENTS

DECLARATION

I, Makgoka Given Moremi, declare that the 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; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

| | | |
|---|---|---|
| Candidate: M.G. Moremi | Signature | Date |
| Supervisor: Dr K.G. Shadung | Signature | Date |
| Co-supervisor: Professor P.W. Mashela | Signature | Date |

DEDICATION

To my parents, Mr Makotonwe Victor and Mrs Emelda Mapula Moremi.

ACKNOWLEDGEMENTS

I would like to thank God the Almighty for His provision of life and strength to carry out this research project. I am grateful for the knowledge, understanding and wisdom (KUW philosophy) that He granted upon me from commencement to the touch line of this research project. I am deeply humbled by the training that my supervisory team, Dr K.G. Shadung and Professor P.W. Mashela, accorded me and for the opportunity to enrol for the postgraduate degree. Their endless support and guidance motivated me to complete this project in record time. Moreover, their research expertise, encouragement, scientific writing skills and constant prodding to work hard, are all highly appreciated. Furthermore, I was accorded an opportunity and sponsored to present my work at the 8th Faculty of Science and Agriculture Research Day, held at Polokwane Bolivia Lodge in 2017, and an oral and poster presentations at the African Combined Congress, held at Ratanga Junction, Cape Town, in 2018. Assistance from Dr Z.P. Dube and Mr P.E. Tseke in disease management and statistical analyses, would forever be greatly appreciated. The technical assistance from Mr M.K. Ralefatana on fertiliser application and pest management could not go unacknowledged. To my fellow MSc students and all the other workers at the Green Biotechnologies Research Centre of Excellence, I had been very humbled by your insightful inputs and suggestions. Being among energetic and eager to work youth like all of you, had been a heart-warming experience. Being in the midst of all of you, I could do it again and again and again! To my lovely parents, Makotonwe and Emelder Moremi, I am grateful for your never-ending support and unconditional love. May God bless you now and always!

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Plant extracts exhibited broad spectrum of activities against root-knot (*Meloidogyne* species) nematodes and had long been considered as an attractive alternative due to their being biodegradable and posing limited risk hazards to the environment, animal and human health. Additionally, the materials had been dubbed as being of low-input costs and had been viewed as being easy to apply in agricultural systems. The objective of the current study was to investigate the efficacy of paint-brush flower (*Kleinia longiflora*) either as fermented or granular formulations on suppression of *M. javanica* and their related effects on growth of tomato (*Solanum lycopersicum*) plants under field and greenhouse conditions. Fermented crude extracts were applied at 0, 2, 4, 8, 16, 32 and 64%, whereas granular materials were applied at 0, 2, 4, 6, 8, 10 and 12 g. Regardless of the product, the treatments were arranged in randomised complete block design (RCBD), with 12 replications. *Kleinia longiflora* plants were collected from the wild, chopped into pieces, oven-dried at 52ºC and fermented in effective microorganisms (EM) for 14 days, whereas the remained were retained for use as granular formulation. Tomato seedlings cv. 'Floradade' were used as test plants inoculated with 2500 eggs and second-stage juveniles (J2) of *M. javanica*. At 56 days after the treatments, nematode and plant variables were collected, prepared using appropriate methodologies and subjected to analysis of variance using Statistix 10.0 software to generate means. Plant variables were subjected to the Curve-fitting Allelochemical Response Data (CARD) computer-based model to generate appropriate biological indices. Nematode and mineral elements variable means were subjected to lines of the best fit. Findings showed second-stage juveniles (J2) in roots, J2 in soil, eggs and Pf under increasing concentration were highly significant and exhibited negative quadratic relationship. The model explained the associations by 82,

81, 74 and 76%, respectively. In granular formulation, the product had no significant effects on nematode population densities. The fermented crude extracts significantly affected and exhibited positive quadratic relations for dry fruit mass, chlorophyll content, dry shoot mass, number of flowers, plant height, number of fruit and stem diameter of tomato plants. The model explained the relationship by 97, 94, 95, 96, 94, 97 and 96%, respectively. In contrast, in granular formulation, the product had significant effects and positive exhibited quadratic relations on Chlorophyll content under field and greenhouse, plant height, dry root mass and dry shoot mass. The model explained the relationships by 52, 45, 56, 47 and 59%, respectively. Plant variables and increasing concentration of the products exhibited density-dependent growth patterns for both formulations, with overall sensitivity (∑k) values of 1 and 11, respectively. In fermented liquid and granular formulations, the Mean Concentration Stimulation Point (MCSP) values were derived at 1.97% and 2.84 g, respectively. The increasing concentration of fermented *K. longiflora* also had significant effects and exhibited negative quadratic relations on the accumulation of K, Na and Zn in leaf tissues of tomato plants. The model explained the associations with 87, 94 and 94%, respectively. In conclusion, the findings in the current study suggested that the nematicidal chemicals in *K. longiflora* could not be released through irrigation water but could be released into solution through microbial degradation. Also, at low concentration suitable for use without inducing phytotoxicity, the products in either formulation could improve the accumulation of certain nutrients in leaf tissues of tomato plants.

# CHAPTER 1
# RESEARCH PROBLEM

## 1.1 Background

Root-knot nematodes *(Meloidogyne* spp.) have the largest number of nematode species, which consist of over 63 species in the genus (De Waele and Elsen, 2007). The species are generally distributed in tropical/subtropical and temperate regions. *Meloidogyne javanica* (Treub.) one of the tropical nematode species, is one of the most aggressive *Meloidogyne* species in South Africa.

### 1.1.1 Description of research problem

The tomato (*Solanum lycopersicum* L.) is one of the most widely-cultivated crops in the world. The tomato fruit is the second most important and popular crop after potatoes in South Africa (Mogala, 2016). However, tomato plants are highly susceptible to *Meloidogyne* species (Mashela, 2002; Seid *et al*., 2015), which could be destructive on roots during reproduction (Seid *et al*., 2015). This nematode genus reproduces at least 350 eggs per cycle and induce root galls, all depending on soil temperature (De Waele and Elsen, 2007). The formation of root galls causes stunted growth, low water uptake and imbalances in essential mineral elements (De Waele and Elsen, 2007; Mashela, 2002).

### 1.1.2 Impact of research problem

Root-knot nematodes are an important group of plant parasites that cause heavy damage to tomato fields, globally estimated at 25% crop losses (Seid *et al*., 2015). Due to the widespread uses of fumigant nematicides such as methyl bromide, the impact of *Meloidogyne* species had been grossly underestimated (Onkendi *et al*.,

2014). However, after the withdrawal of methyl bromide in 2005, reviews had shown that damage due to nematode in plant production systems had since increased by 37% (Mashela *et al*., 2017a).

### 1.1.3 Possible causes of the research problem

Control of plant-parasitic nematodes was largely dependent on effective chemical nematicides until their suspension (Ornat and Sorribas, 2008). Generally, chemical control of nematodes is expensive and now the use of some nematicides has threatened human health and environmentally unfriendly. Conceivably the main concern was that most widely used fumigant nematicides contaminated groundwater and contributed significantly to the degradation of the ozone layer, with increased penetration of ultraviolet rays to the earth (Agbenin, 2011; Mashela *et al*., 2017a). Also, the formation of the greenhouse layer, which is being penetrated by ultraviolet rays, but could not allow the long-rays reflected from the earth to be dissipated into the atmosphere, resulted in global warming (Mashela *et al*., 2017a). The resultant high temperatures had since shortened the life cycles of most *Meloidogyne* species, thereby promoting the economic importance of this genus in various crop systems (Onkendi *et al*., 2014).

### 1.1.4 Possible solutions of the research problem

Historically, management of nematode-induced crop damage had been achieved through the use of plant resistance. However, various studies had since shown that high temperatures (Marques De Carvalho *et al*., 2015), salinity (Bai *et al*., 2018; Mashela *et al*., 1992), cultural practices that in advertently prune roots (Mashela and Nthangeni, 2002) and certain biotic factors such as sucking insects (Pofu *et al*., 2011),

could all break nematode resistance. Two phytonematicides, Nemarioc-AG or AL (G = granular or L = liquid formulation) and Nemafric-BL, were shown to be thermophilic, with their active ingredients, cucurbitacin A and cucurbitacin B, respectively, having excessively high boiling temperatures (Krieger, 2001; Shadung, 2016). However, the same could not be said of plant-based products without known thermophilic status.

## 1.2 Problem statement

Global suspension of environmental unfriendly synthetic nematicides from agrochemical market left a void in the management strategies for nematodes (Mashela *et al*., 2011). However, various botanicals with nematicidal properties are tested to serve as alternatives (Agbenin *et al*., 2005). Studies on determination of nematicidal properties of botanicals had been on the increase as shown by various reviews (Chitwood, 2002; Mashela *et al*., 2015, 2017a).

## 1.3 Rationale

Plant extracts had since exhibited broad spectrum of activities against root-knot nematodes (Agbenin *et al*., 2005). Generally, the products had been shown to be environment-friendly (Mashela *et al*., 2015), with potential application through the Ground Leaching Technology (GLT) (Mashela, 2002) and botinemagation technology (Pelinganga, 2013). In the GLT systems, active ingredients were shown to be released into the rhizosphere by irrigation water, with limited need for microbial degradation (Mashela *et al*., 2015). However, certain plant organs, such as cabbage (*Brassica oleracea* L.) that contain highly toxic chemicals were shown to be devoid of nematicidal properties when exposed to the GLT system (Mashela *et al*., 2012). In contrast, when such products were exposed to EM fermentation, nematicidal properties were

observed (Mashela *et al*., 2012). The plant, paint-brush flower (*Kleinia longiflora* DC.), had been used by local people in Lepelle-Nkumpi Municipality, for managing various pests, without any empirically-related information. In the current investigation, the question of interest is whether active chemicals in *K. longiflora* organs contain nematicidal properties and further, whether they could be released through irrigation water or through fermentation using effective microorganisms (EM).

## 1.4 Purpose of the study

### 1.4.1 Aim

The aim of the study was the development of the nematicidal profile of *K. longiflora* in relation of the release of the active ingredients from harvested plant materials.

### 1.4.2 Objectives

1. To determine the efficacy of increasing concentration of *K. longiflora* in liquid formulation on *M. javanica,* growth of tomato plants and selected mineral elements.
2. To investigate the efficacy of increasing concentration of *K. longiflora* in granular formulation on *M. javanica,* growth of tomato plants and selected mineral elements.

### 1.4.3 Hypotheses

1. Increasing concentration of *K. longiflora* in liquid formulation will supress *M. javanica,* promote growth and accumulation of selected mineral elements of tomato plants.

2. Increasing concentration of *K. longiflora* in granular formulation will supress *M. javanica,* promote growth and accumulation of selected mineral elements of tomato plants.

## 1.5 Reliability, validity and objectivity

Reliability of data was based on statistical analysis of data at the probability level of 5%, while validity was achieved through repeating experiments in different conditions. Objectivity was ensured by discussing results on the basis of empirical evidence (Leedy and Ormrod, 2005).

## 1.6 Bias

Bias was eliminated by using replications and randomisation of the treatments (Leedy and Ormrod, 2005).

## 1.7 Scientific significance of the study

Findings of the research would indicate whether crude extracts of *K. longiflora* with and without EM would be effective in suppression of nematode population densities of *M. javanica* and efficacy on growth of tomato plants. The findings would expand knowledge on botanicals that could potentially be used to suppress nematodes and possibly be commercialised.

## 1.8 Structure of the mini-dissertation

Following detailed and outlining of the research problem (Chapter 1), both work done and work not done were thoroughly outlined as Literature review (Chapter 2). The Objective 1 and 2 were each addressed in Chapter 3 and 4, respectively. The findings

were summarised and integrated to provide the significance of the findings and recommendations with respect to future research, culminating in conclusions that tied the two objectives together in Chapter 5.

# CHAPTER 2
# LITERATURE REVIEW

## 2.1 Introduction

Strategies made available for the management of plant-parasitic nematodes, each has its own precautions and consequences. Although the environmental impact of other tactics could not match those of synthetic chemical nematicides, which lead to their ultimate withdrawal from agrochemical market (Mashela *et al.*, 2015; Pelinganga, 2013). Phytonematicides, as an alternative nematode management strategy, comprised a class of botanicals which could be available for use as granular and liquid formulations (Bajestani *et al*., 2017; Mashela, 2002; Mashela and Mphosi, 2002; Pelinganga, 2013). Generally, phytonematicides could be applied in one of two ways, Ground Leaching Technology (GLT) and botinemagation technology (BT) systems (Mashela *et al*., 2017a; Pelinganga, 2013).

Generally, not all plant species are suitable for use in GLT system. For instance, crude extracts of oleander (*Nerium indicum* L.) leaves, chilli pepper (*Capsicum annuum* L.) fruit and bark of tamboti (*Spirostachys africana* Sond.), regardless of the application method, did not suppress nematode population densities (Thovhakhale, 2005), whereas those of fever tea (*Lippia javanica* F burn.), castor bean (*Ricinus communis* L.) and wild cucumber (*Cucumis myriocarpus* Naude.), consistently reduced nematode population densities (Mashela *et al*., 2017a). Generally, the successful use of phytonematicides could be limited by their phytotoxicities to the protected crops (Mashela *et al*., 2015; Meyer *et al.*, 2008). Generally, as allelochemicals, most crude extracts with credible efficacies for nematode suppression could one way or the other

induce phytotoxicity on crops (Mashela *et al.,* 2015). Therefore, the review was limited on efficacies, application methods and management of phytotoxicities.

## 2.2 Work done on research problem

### 2.2.1 Efficacy of phytonematicides on root-knot nematodes in liquid formulation

Generally, extractions of potent chemicals from plants varies with the nature of the active ingredients. Extraction of potent nematicidal properties from plants depends on the nature of the active ingredient. Therefore, methods of extraction of potent chemicals from plant would vary among botanicals. Cucurbitacin-containing phytonematicides, namely, Nemafric-BL and Nemarioc-AL phytonematicides, had been manufactured using mature fruits collected from wild watermelon (*Cucumis africanus* L.f.) and wild cucumber, respectively (Mashela *et al*., 2015, 2017a). Nemarioc-AL and Nemafric-BL phytonematicides are in liquid formulation as shown by L, whereas A and B are cucurbitacin A ($C_{32}H_{46}O_9$) and cucurbitacin B ($C_{32}H_{48}O_8$), respectively. Cucurbitacin A is an unstable compound which rapidly degrades to cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$), whereas cucurbitacin B is stable (Jeffery, 1978). Cucurbitacin B is insoluble in water and its counterpart cucurbitacin A is water-soluble (Chen *et al*., 2005). Mature fruits are harvested, washed and cut into pieces and dried in an air-forced oven drier at 52ºC for 72 h (Shadung, 2016). Thereafter the material is ground to fine particles that can pass through a 1-mm mesh-sieve. Approximately 80 and 40 g for *C. myriocarpus* and *C. africanus* were fermented using effective microorganisms (EM) to release their respective cucurbitacins into solution (Pelinganga, 2013). The products had been extensively studied and they consistently reduced population densities of plant-parasitic nematodes (Pelinganga and Mashela, 2012; Tseke, 2013). These two products reduced densities of

*Meloidogyne* species by 80-92% and 70-90%, respectively under diverse conditions (Pelinganga, 2013).

Crude extracts from marigolds (*Tagetes* spp.) and other Asteraceae were reported to have nematicidal properties in various formulations including liquid formulation (Slootweg, 1956). Bajestani *et al.* (2017) recently conducted a glasshouse experiment to control *M. javanica* on tomato plants with liquid formulation of marigold, rosemary (*Rosmarinus officinalis* L.) and nigella (*Nigella sativa* L.). Plants were collected from the field, washed, dried, ground and weighed at 100, 200 and 400 g and placed in 1 000 ml flasks, and then covered with distilled water. The flasks were shaken for 24 h at room temperature and the contents passed through a thin gauze muslin to isolate the active ingredients from the debris. Rosemary extract reduced nematode population from 22 to 500, which had the greatest impact on the reduction of nematode populations at 40% concentration as compared to its control. Marigold had the lowest impact on reduction of nematode population densities. However, all the three treatments had significant effect on the reduction of nematode population densities. Leaf extracts of crambe (*Crambe abyssinica* L.E.F.r) had been reported to have nematicidal effects of *M. javanica* in liquid formulation (Coltro-Roncato *et al*., 2016). The crambe leaves were collected at vegetative stage and dried at 45°C for 48 h and then ground. The liquid solution of extract was prepared using hydroalcoholic (70% ethanol and 30% water). The results showed that crambe extracts reduced eggs in roots when applied weekly, with the reduction being at 60% when compared with the untreated control.

### 2.2.2 Efficacy of phytonematicides on plant growth in liquid formulation

Generally, botanicals affect plant growth when applied to protect crops from pests. For instance, Nemarioc-AL and Nemafric-BL phytonematicides affected growth of tomato (Pelinganga, 2013; Tseke and Mashela, 2017). Marigold, rosemary and nigella also affected growth of tomato plants (Bajestani *et al*., 2017). Phytotoxic effects were also reported crops such as eggplant (*Solanum melongena* L.) and pepper (*C. annum*) due to allelochemicals from botanicals (Mafeo, 2012). The effects are such that plant extracts stimulate growth at lower concentration and ultimately inhibit plant growth as concentration increases (Mashela, 2002). The latter was referred to as density-dependent growth (DDG) patterns (Liu *et al*., 2003). However, through a series of studies, Mashela *et al*. (2015) conceptualised the Mean Concentration Stimulation Point (MCSP), which was viewed as an empirically-based concentration that would concurrently and consistently stimulate plant growth and reduce nematode population densities without inducing phytotoxic effects (Mashela *et al.,* 2017a).

### 2.2.3 Efficacy of granular phytonematicides on root-knot nematodes

Conventionally, the use of organic amendments and plant extracts require microbial degradation for the eventual release of active ingredients into soil solutions (Stirling, 2014). Because decomposing plant extracts and organic amendments serve as food source for microorganisms such as fungi, bacteria and actinomycetes, biodegradation could play a significant role in the efficacy of plant-based products. However, the conventional methods of applying organic amendments had drawbacks that included (1) inconsistent results on nematode suppression; (2) large quantities (10-500 t/ha) which were required to achieve nematode suppression; (3) unavailability of the

materials; (4) high transport costs to carry the materials from the production site to that of use (Mashela *et al.,* 2015).

Mashela and Nthageni (2002) argued that in certain plant organs like the castor bean fruit, potent nematicidal chemicals could be leached into the rhizosphere through irrigation water. In such plant organs, microbial degradation was not necessary and the application method was technically referred to as the GLT system (Mashela, 2002). The phytonematicides, Nemafric-BG and Nemarioc-AG (G = granular formulation) phytonematicides, had been successfully used as counterparts of Nemafric-BL and Nemarioc-AL (L = liquid formulation) phytonematicides (Mafeo, 2012; Mashela *et al*., 2011; Mashela *et al*., 2012; Pelinganga, 2013). The products had since been successfully used in GLT system to manage population densities of *Meloidogyne* species on different crops (Mashela 2002; Mashela *et al.*, 2008) and the citrus nematode (*Tylenchulus semipenetrans* Cobb.) in citrus production (Maile, 2013). In the GLT system, mature fruits from *C. myriocarpus* and *C. africanus* are chopped into pieces, oven-dried at 52°C for 72 h and ground in a Wiley mill to pass through a 1-mm-mesh sieve (Mashela, 2002). Quantities as little as 2 g are spread around the base of the stem in a shallow hole, which is then covered with soil (Mashela, 2002; Mashela and Mphosi, 2002). The method was introduced to mitigate the drawbacks of conventional amendments which include negative period which is time to allowed for microbial decomposition (Mashela *et al.*, 2015). The efficacy of Nemarioc-AG phytonematicide on nematode suppression had been comparable to that of aldicarb and fenamiphos (Mashela *et al*., 2008).

### 2.2.4 Efficacy of granular phytonematicides on plant growth

Dried crude extracts from crotalaria (*Crotalaria juncea* L.), mucuna (*Mucuna Pruriens* L.), guandu bean (*Cajanus cajan* L.) and neem (*Azadirachta indica* L.) affected growth of tomato when tested against *M. javanica* (Moreira *et al*., 2015), with most resulting in phytotoxicity to crops protected against nematodes (Mafeo *et al*., 2012). Generally, phytotoxic effects occurred at high concentration, whereas at low concentration the products stimulated plant growth (Moreira *et al*., 2015). Incidentally, *C. myriocarpus* stimulated growth of tomato at lower concentration and the effects were initially referred to as 'fertilizer effect' (Mashela, 2002). However, due to the limited quantities used as granular formulation, treatments did not have significant effects on nutrient elements in leaf tissues of test plants (Mashela, 2002).

### 2.2.5 Managing phytotoxicity

The inception of phytonematicides in management of nematodes has been exciting with regard to environmental safety, consistent nematode suppression, sustainability and human health safety (Mashela *et al*., 2017a). However, efficacy of phytonematicides literally relies on allelochemicals (active ingredients) which are secondary metabolites from botanicals. Normally the allelochemicals are responsible for allelopathy in the plant ecology. Allelopathy is the release of allelochemicals from a plant to supress growth of the adjacent plants (Rice, 1984). However, in context of phytonematicides, the latter is referred as phytotoxicity.

Phytotoxicity is experienced when phytonematicides inhibit growth of crops protected against nematodes (Meyer *et al*., 2008), which had been the greatest drawback of phytonematicides. Mashela *et al*. (2015) solved the challenge by establishing a series

of steps that demonstrated how to successfully use phytonematicides without phytotoxic effects. The steps include development of (1) MCSP, (2) application interval, (3) application frequency and (4) dosage model (Mashela *et al*., 2015, 2017). However, accomplishment of each step is empirically achieved. The MCSP is the first step which is calculated from biological indices developed from the Curve-fitting Allelochemical Response Data (CARD) (Liu *et al*., 2003). The MCSP refers to the concentration that would stimulate growth of protected crop and consistently suppress nematode population densities. The concentration is dependent on crop and phytonematicide. For instance, 2.99 and 2.64% were derived for Nemarioc-AL and Nemafric-BL phytonematicide on tomato (Pelinganga and Mashela, 2012). In *Pelargonium sidoides,* MCSP of 6.18 and 2.87% were derived for Nemarioc-AL and Nemafric-BL phytonematicides (Sithole *et al*., 2016).

### 2.2.6 Effects of phytonematicides on mineral elements

Mashela (2002) reported on negligent effects on the accumulation of essential nutrient elements under various phytonematicides. Additionally, Nemarioc-AL and Nemafric-BL phytonematicides in tomato plants did not have significant effects on Ca, Mg, Mn, Zn, Na and S from leaf tissues (Pelinganga, 2013). During the same period, Khosa (2013) reported that bead bean (*Maerua angolensis* DC.) and *toad tree* (*Tabernaemontana elegans* Stapf.) when used as phytonematicides in granular formulations, the products could affect the accumulation of B, Ca, Cu, Fe, K, Mg, Mn, N, P and Zn in leaf tissues of tomato plants. However, a closer look of the data showed that there were no clear trends in the accumulation of the elements with reference to the untreated control. But recently, Mashela and Pofu (2017) demonstrated that Ca, K, Na and Fe exhibited quadratic relations under the increasing concentration of

Nemarioc-AL and Nemafric-BL phytonematicides in leaves of green bean (*Phaseolus vulgaris* L.).

## 2.3 Work not done on problem statement

The environment-friendly methods of controlling *Meloidogyne* species using botanicals had been widely accepted and advocated as key practice intended to promote climate-smart agriculture due to numerous concerns raised on the use of chemical products. Paint-brush flower (*Kleinia longiflora*) plant had been traditionally known to serve as a biological control tool for various insect pests via intercropping with other crops. However, the efficacy of this plant on suppression of root-knot nematode population densities had not been documented. Additionally, it is not documented whether potent chemicals in dried powder from *K. longiflora* could be released through irrigation water or through microbial degradation.

# CHAPTER 3
# EFFICACY OF FERMENTED *KLEINIA LONGIFLORA* ON *MELOIDOGYNE JAVANICA,* GROWTH AND ACCUMULATION OF SELECTED MINERAL ELEMENTS ON TOMATO PLANTS

## 3.1 Introduction

Root-knot (*Meloidogyne* species) nematodes are amongst the most economically important soil-borne pests which were managed with the use of highly effective chemicals such as methyl bromide and oxamyl, which had been withdrawn from the agrochemical markets due to their being environment-unfriendly (Mashela *et al.,* 2017a; Meyer *et al*., 2008). Growers had been left with limited viable alternatives for use against the root-knot nematodes (Mashela *et al.*, 2017a). Following the failure of various biocontrol strategies, phytochemicals, were shown to contain potent nematicidal chemicals in various countries (Chitwood, 2002; Ntalli and Caboni, 2012; Okwute, 2012).

Botanicals such as neem (*Azadirachta indica* L.) (Meyer, 2008) marigold (*Tagetes* spp. L.) (Tibugari *et al*., 2012) and wild cucumber (*Cucumis myriocarpus* Naud.) (Mashela, 2002), were widely tested for their nematicidal properties. However, the challenge facing the use of botanicals in crop production against plant-parasitic nematodes had been their phytotoxicity to plants being protected against nematode damage (Mashela *et al*., 2015; Meyer *et al.,* 2008; Pelinganga and Mashela 2012; Pelinganga, 2013). However, Mashela *et al*. (2017) adapted the Curve-fitting Allelochemical Response Data (CARD) computer-based model (Liu *et al*., 2003) to empirically solve phytotoxicity of phytonematicides on test crops.

Paint-brush flower (*Kleinia longiflora* DC.), a succulent shrub indigenous to South Africa (Foden and Potter, 2005), had traditionally been associated with serving as an insect pest repellent. However, the status of this plant species as a phytonematicide in liquid formulations had not been reported. The objective of this study was to investigate the efficacy of increasing concentration of fermented *K. longiflora* on *M. javanica,* growth of tomato plants and selected mineral elements.

## 3.2 Materials and methods

### 3.2.1 Description of the study site

Experiments were conducted at the Green Bio-technologies Research Centre of Excellence (GBRCE), University of Limpopo, Limpopo Province, South Africa (23°53′10″S, 29°44′15″E). Field experiment was conducted in autumn (March to May) 2017 (Figure 3.1) and was repeated in the greenhouse during summer November to January (2017/2018) (Figure 3.2).

Field conditions: The site is characterised as Hutton soil form comprising 65% sand, 30% clay, 5% silt and 1.6% organic carbon. Furthermore, the area is generally hot and has a summer day temperature range between 28 and 38°C, with annual rainfall that is less than 500 mm.

Greenhouse conditions: The 100 m × 20 m greenhouse had thermostatically-activated heat-extracting fans and one end opposite to wet wall, which created an internal windy heterogeneous condition. The ambient day/night temperatures averaged 28/21°C and the relative humidity was 70%.

### 3.2.2 Treatments and research design

Two experiments were conducted in field and greenhouse conditions in different seasons arranged in a randomised complete block design with 12 replications. Both experiments comprised seven treatments namely, 0, 2, 4, 8, 16, 32 and 64% of fermented crude extracts of *K. longiflora.*

### 3.2.3 Procedures

Herbaceous material from *K. longiflora* was harvested from Khureng village (24°16'0"S, 29°17'0"E) in Zebediela in autumn (March and April) and summer (September and October) 2017. The material was brought to GBRCE laboratories to be chopped and dried in an air-forced oven at 52°C for 72 hours. The material was ground using Wiley mill to smaller particles to pass through 1 mm mesh sieve. Liquid formulation was prepared by weighing of 40 g *K. longiflora* to ferment in 20 L container with 16 L chlorine free water, 300 ml molasses, 300 ml effective microorganisms (EM) and 100 g sugar (Mashela *et al*., 2015). The solution was allowed to ferment in 28°C for 14 days and acidity reduced to 3.7 units. Three weeks old seedlings cv. 'Floradade' were transplanted under field experiment at spacing of 60 cm for both inter-row and intra-row space, whereas in greenhouse experiment pots were put on benches at spacing 0.25 m intra-row and 0.30 m inter-row. The pots were filled with 2 700 ml steam pasteurised (300°C for 1 hour) loam soil and Hygromix (Hygrotech, Pretoria) at 3:1 (v/v) ratio. Inoculum was prepared by extracting eggs and second-stage juveniles (J2) from roots of greenhouse grown nematode susceptible tomato cv. 'Floradade' in 1% NaOCl solution (Hussey and Barker, 1973). After transplanting each plant was inoculated with 2 500 eggs and second-stage juveniles of *M. javanica*. Plants were fertilised with 3 g NPK 2:3:2 (22) to provide a total of 186 mg N, 126 mg K and 156 mg

P per ml water and 5 g Multifeed (Nulandies, Johannesburg) which provided N, K, P, Mg, Fe, Cu, Zn, B, Mn and Mo per ml water (Mashela, 2002). Irrigation was done to full capacity for an hour morning and afternoon using drip irrigation system in field experiment, whereas 250 ml beaker was used to irrigate greenhouse every other day. The plants were sprayed weekly for disease and pest management with steward, bravo, funginex, and dithane M45, whereas insect pests were scouted and monitored on daily basis.

Figure 3.1 Layout of tomato cv. 'Floradade' under field conditions.

Figure 3.2 Layout of tomato cv. 'Floradade' under greenhouse conditions.

### 3.2.4 Data collection

At 56 days after initiation of treatments, a ruler was used to measure plant height from the soil surface to the tip of the flag leaf. Flowers and fruits were counted at the day of harvest. Stems were cut off at the soil surface and the stem diameters measured at 5 cm above the severed ends using a digital Vernier caliper. Chlorophyll content was measured on three matured leaves per plant using chlorophyll content meter (Minolta Spad-502). Dry fruit and shoot mass were recorded, where fruits were first cut into pieces, before being oven-dried at 60°C for 72 h and weighed. Root systems were removed from pots, immersed into water to free soil particles.

Nematodes were extracted from total root system/plant by maceration and blending for 60 seconds in 1% NaOCl solution (Hussey and Barker, 1973). The material was passed through nested 75 and 25-µm mesh sieves. The contents left in the 25-µm mesh sieve were collected for further separation of nematodes from debris using the sugar-flotation and centrifugation method (Jenkins, 1964). Soil in each pot was thoroughly mixed and a 500 ml soil sample collected for nematode extraction using the sugar centrifugation and flotation method (Jenkins, 1964). Briefly the sample was put in a bucket filled with water; the solution was stirred and put to settle for 30 seconds. Thereafter, the contents were poured into 25-µm through 75-µm mesh sieve, the contents remained in 25-µm was collected to 50 ml plastic centrifuge tubes. A teaspoon and half of kaolin was added and contents centrifuged at 1 800 rpm for four minutes. The solution was discarded, nematodes settled at the bottom of tubes with soil particles. A 624 g sugar/L was prepared and poured into centrifuge tube and stirred prior to centrifuging for 3 minutes at 1 800 rpm (Marais *et al*., 2017). The aliquot with nematodes in 25-µm was then decanted to 25-µm and sugar was rinsed off and then nematodes washed into 100 ml containers for counting in stereomicroscope. Therefore, soil nematode numbers were converted to 2 700 ml soil per pot, whereas roots nematode numbers were converted per total roots system per plant. Reproductive factors described as final population /initial population numbers were computed.

Oven dried leaves at 60°C, were ground into smaller pieces that will pass 0.75 mm sieve. Weigh of 500 mg of the sample were in digestion vessel, 2 and 3 ml of $HNO_3$ and $H_2O_2$ was added respectively. The mixture was carefully put in the microwave digester and put on the heat for 46 minutes. The vessels were allowed to cool down

for 10 minutes, and then the solution in the vessels was poured in 50 ml tubes containing 40 ml of de-ionised water. Samples were submitted to Limpopo Agro-food Technology Station (LATS) where they were subjected to Inductively Coupled Plasma Emission (ICPE-9000) for determination of sodium K, Na and Zn.

### 3.2.5 Statistical analysis

Data were subjected to analysis of variance using Statistix 10.0 software to generate means. Discrete nematode data were log-transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984), before analysis of variance. Nematode and mineral elements means were subjected to lines of the best fit and optimisation was done using the relation $x = – b_1/2b_2$. Means for plant variables were subjected to CARD model to generate appropriate biological indices and quadratic relations, where $D_m$ and $R_h$, were used to calculate the MCSP value (Liu *et al*., 2003; Mashela *et al*., 2017a). Unless otherwise stated, data were discussed at the probability level of 5%.

## 3.3 Results

### 3.3.1 Nematode variables

Field experiment: Nematode data from field experiment recorded zero number of nematodes and therefore, results are from greenhouse study only.

Greenhouse experiment: Second stage juveniles (J2) in roots, J2 in soil, eggs and Pf under increasing concentration were highly significant and exhibited negative quadratic relationship. The model explained the relationship by 82, 81, 74 and 76%, respectively (Figure 3.3 (a) and Figure 3.3 (b); Figure 3.4 (a) and Figure 3.4 (b)). Using

the relation $x = -b_1/2b_2$ the variables were optimised at 3.11, 3.73, 4.28 and 5.03% phytonematicide (Table 3.1).

### 3.3.2 Plant growth variables

Field experiment: Increasing concentration of fermented *K. longiflora* treatments did not have significant effects ($P \leq 0.05$) on plant height, chlorophyll content, dry shoot mass, dry root mass, and stem diameter under field conditions.

Greenhouse experiment: Dry fruit mass, chlorophyll content, dry shoot mass, number of flowers, plant height, number of fruit and stem diameter under increasing concentration of fermented *K. longiflora* exhibited positive quadratic relations (Figure 3.5). The model explained the relationship by 97 (, 94, 95, 96, 94, 97 and 96%, respectively (Figure 3.5; Figure 3.6). Using the $MCSP = D_m + (R_h/2)$ relation, the MCSP value of fermented *K. longiflora* phytonematicide on tomato was derived at 1.97% (Table 3.2). Dry shoot mass had k value of 1 and other variables had 0 units, whereas overall sensitivity ($\sum k$) was 1 for increasing concentration of fermented *K. longiflora* to tomato.

### 3.3.3 Selected mineral elements

Response of potassium K, Na and Zn under increasing concentration of fermented *K. longiflora* were highly significant and exhibited negative quadratic relations. The model explained the associations with 87, 94 and 94%, respectively (Figure 3.7 (a) and Figure 3.7 (b); Figure 3.8). Using the relation $x = -b_1/2b_2$ the variables were optimised at 11224.39, 504.50 and 125.84% phytonematicide (Table 3.3).

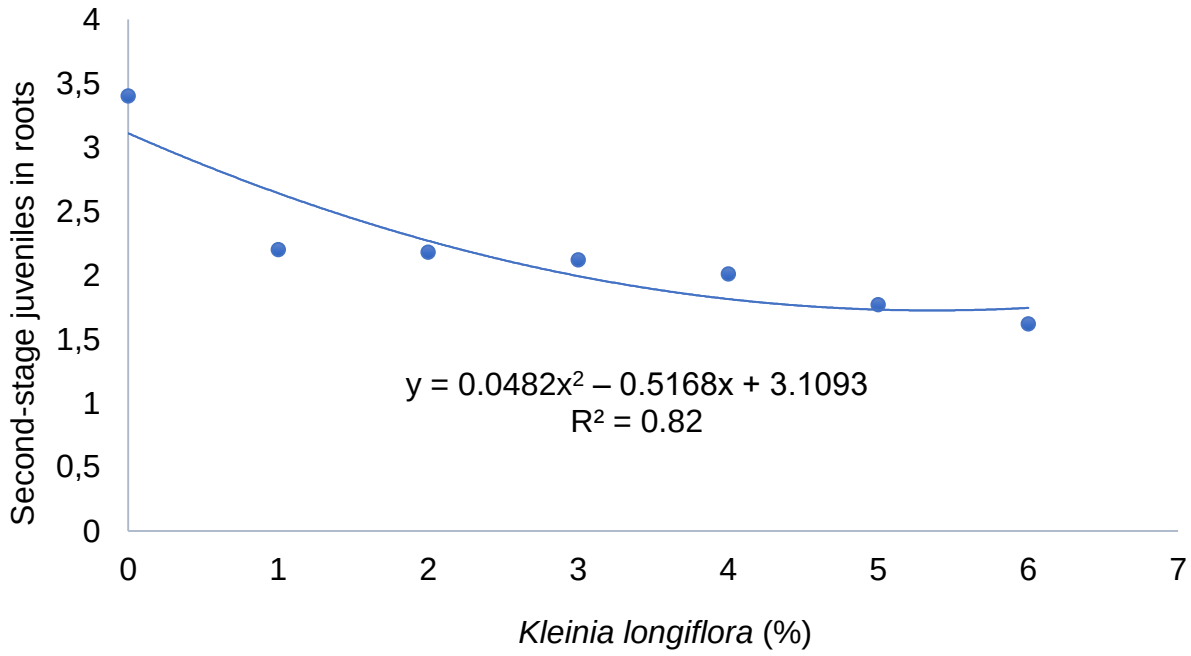


Figure 3.3 (a) Response of second-stage juveniles in roots of *Meloidogyne javanica* to increasing concentration of fermented *Kleinia longiflora* phytonematicide in greenhouse experiment at 56 days after initiation of treatments.

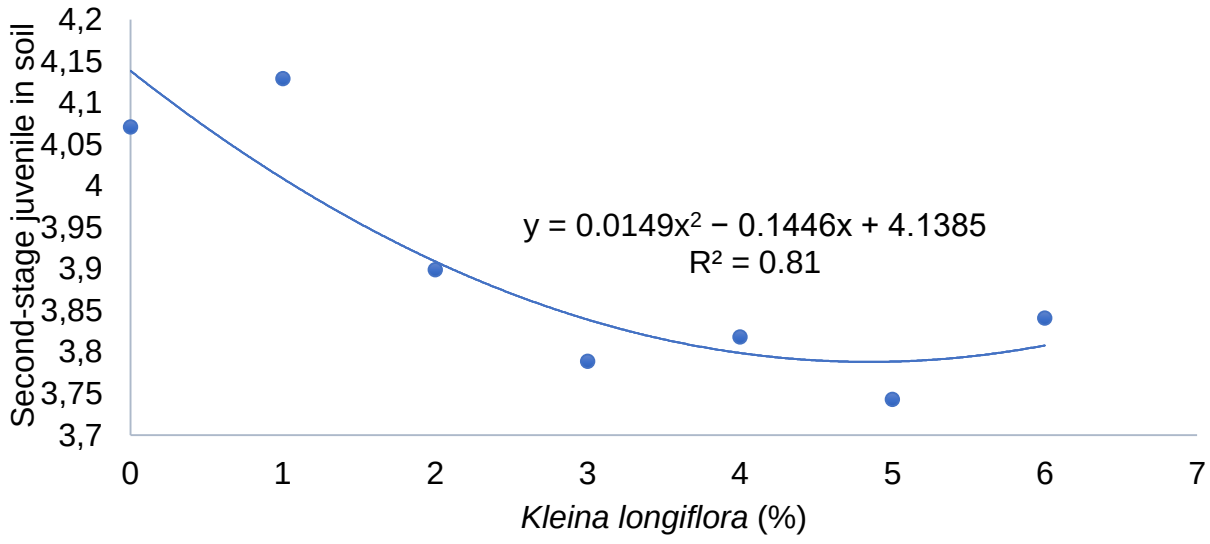


Figure 3.3 (b) Response of second-stage juveniles in soil of *Meloidogyne javanica* to increasing concentration of fermented *Kleinia longiflora* phytonematicide in greenhouse experiment at 56 days after initiation of treatments.

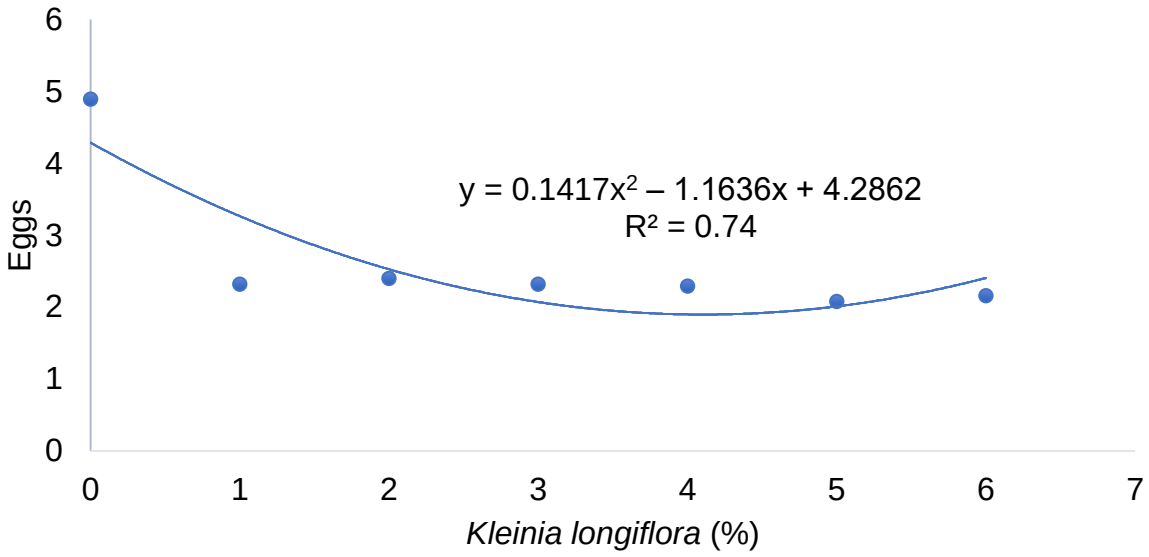


Figure 3.4 (a) Response eggs of *Meloidogyne javanica* to increasing concentration of fermented *Kleinia longiflora* phytonematicide in greenhouse experiment at 56 days after initiation of treatments.

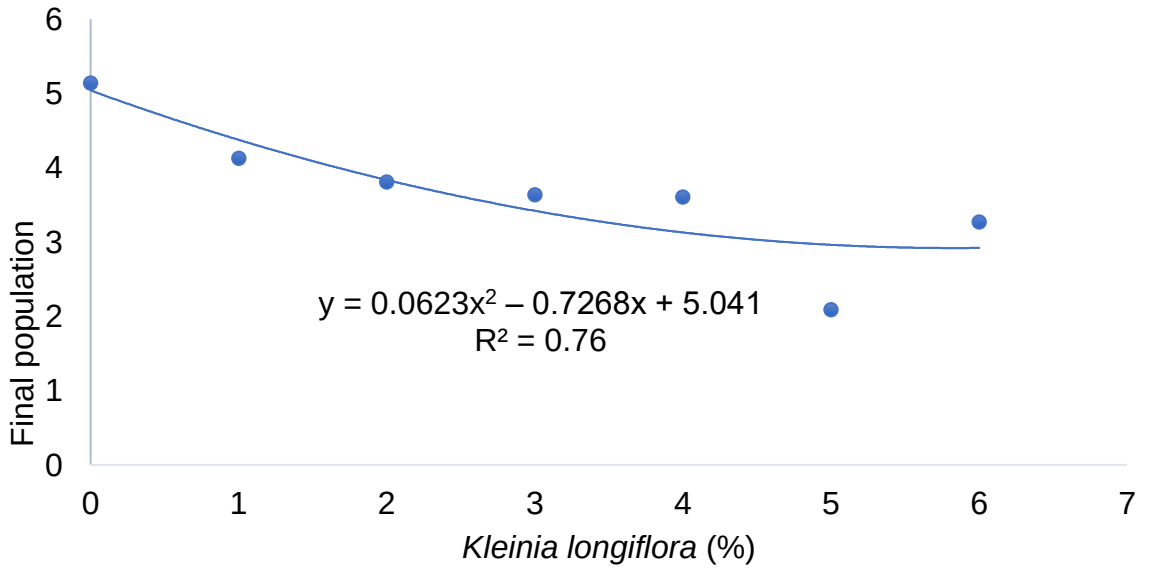


Figure 3.4 (b) Response final population of *Meloidogyne javanica* to increasing concentration of fermented *Kleinia longiflora* phytonematicide in greenhouse experiment at 56 days after initiation of treatments.

Table 3.1 Optimisation models for *Meloidogyne javanica* variables second-stage juveniles (J2) in roots and soil, eggs and final population to increasing concentration of fermented *Kleinia longiflora* at 56 days after initiation of treatments.

| Variable | Model | $R^2$ | X |
|---|---|---|---|
| J2 roots | $Y = 0.048x^2 – 0.516x + 3.1093$ | 0.82 | 3.11 |
| J2 soil | $Y = 0.022x^2 – 0.1875x + 4.1313$ | 0.81 | 3.73 |
| Eggs | $Y = 0.1417x^2 – 1.1636x + 4.2862$ | 0.74 | 4.28 |
| Final population | $Y = 0.0623x^2 – 0.7268x + 5.041$ | 0.76 | 5.03 |

$X = – b_1/2b_2$

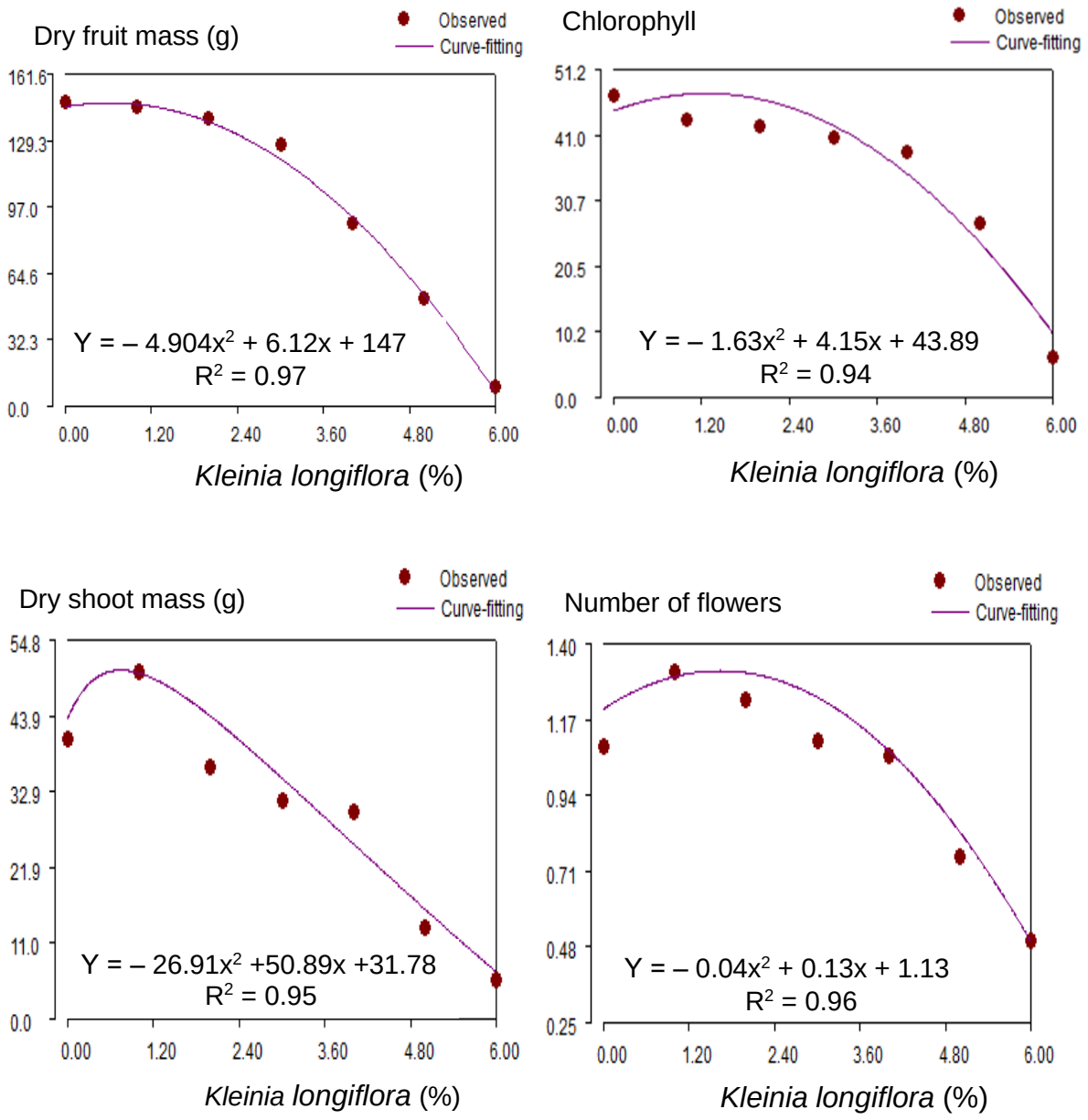


Figure 3.5 Responses of dry fruit mass, dry shoot mass, number of flowers and chlorophyll content of tomato cv. 'Floradade' to increasing concentration of fermented *Kleinia longiflora* phytonematicide in greenhouse experiment at 56 days after initiation of treatments.

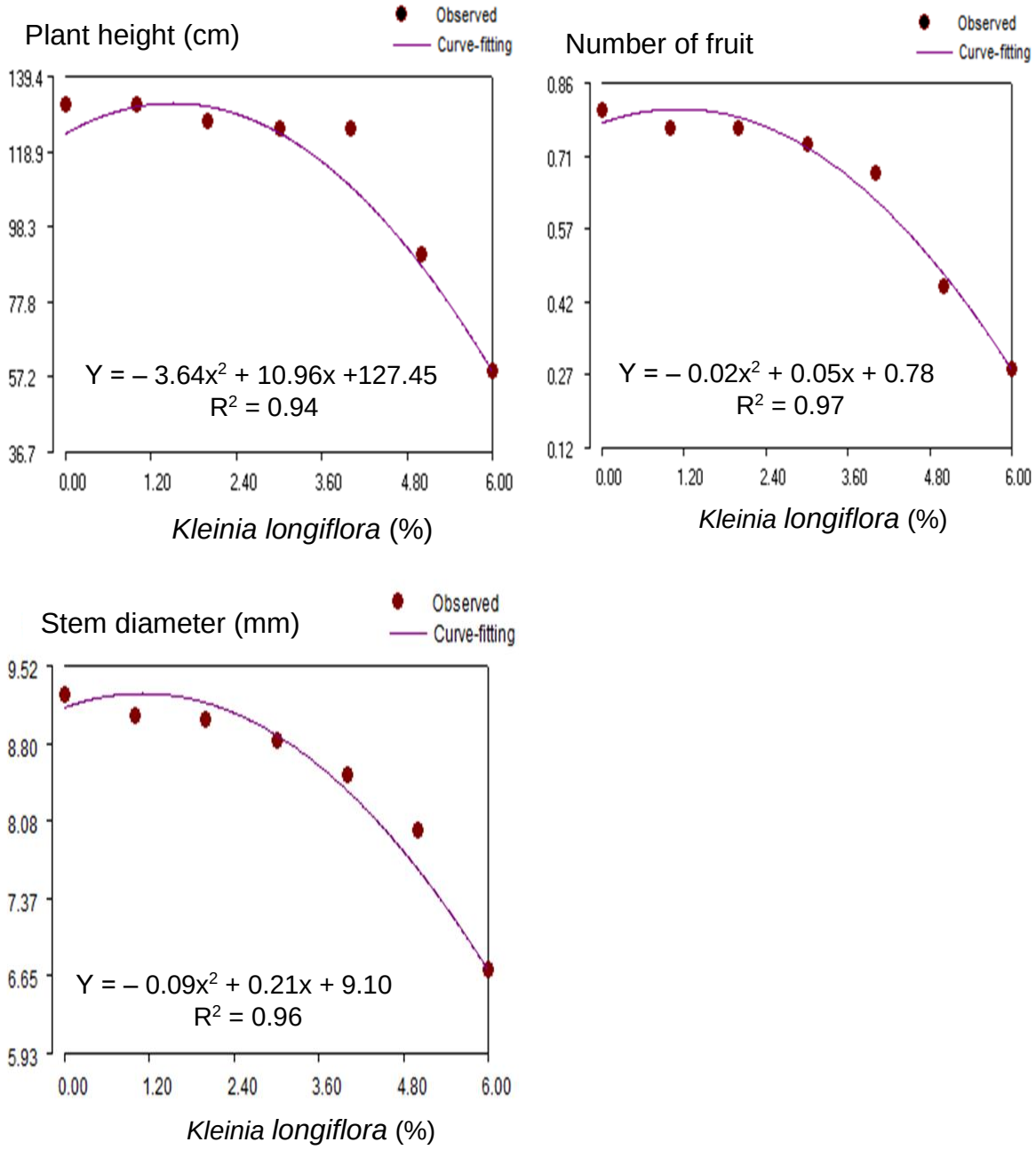


Figure 3.6 Responses of plant height, number of fruit and stem diameter of tomato cv. 'Floradade' to increasing concentration of fermented *Kleinia longiflora* in greenhouse experiment at 56 days after initiation of treatments.

Table 3.2 Biological indices for number of flowers (NFL), dry shoot mass (DSM), chlorophyll content (CHR), plant height (PHT), number of fruit (NFR), stem diameter (STD) and dry fruit mass (DFM) of tomato cv. 'Floradade' to increasing concentration of fermented *Kleinia longiflora* at 56 days after initiation of treatments in greenhouse experiment.

| Biological index | NFL | DSM | CHL | PHT | NFR | STD | DFM | Mean |
|---|---|---|---|---|---|---|---|---|
| Threshold stimulation ($D_m$) | 1.63 | 0.74 | 1.27 | 1.51 | 1.12 | 1.10 | 0.65 | 1.14 |
| Saturation point ($R_h$) | 0.11 | 6.57 | 2.63 | 8.26 | 0.03 | 0.12 | 1.91 | 2.80 |
| 0 % inhibition ($D_0$) | 3.26 | 2.04 | 2.53 | 3.01 | 2.29 | 2.20 | 1.25 | 2.36 |
| 50 % inhibition ($D_{50}$) | 5.74 | 4.38 | 5.14 | 5.95 | 5.48 | 8.04 | 4.55 | 5.61 |
| 100 % inhibition ($D_{100}$) | 7.2 | 6.8 | 6.6 | 7.6 | 7.2 | 10.9 | 6.1 | 7.48 |
| $R^2$ | 0.96 | 0.94 | 0.94 | 0.94 | 0.97 | 0.96 | 0.98 | |
| K-value | 0 | 1 | 0 | 0 | 0 | 0 | 0 | |
| Overall sensitivity ($\sum k$) = 1 | | | | | | | | |
| MCSP = $D_m + (R_h/2)$ = 1.14 + (2.80/2) = 1.97%. | | | | | | | | |

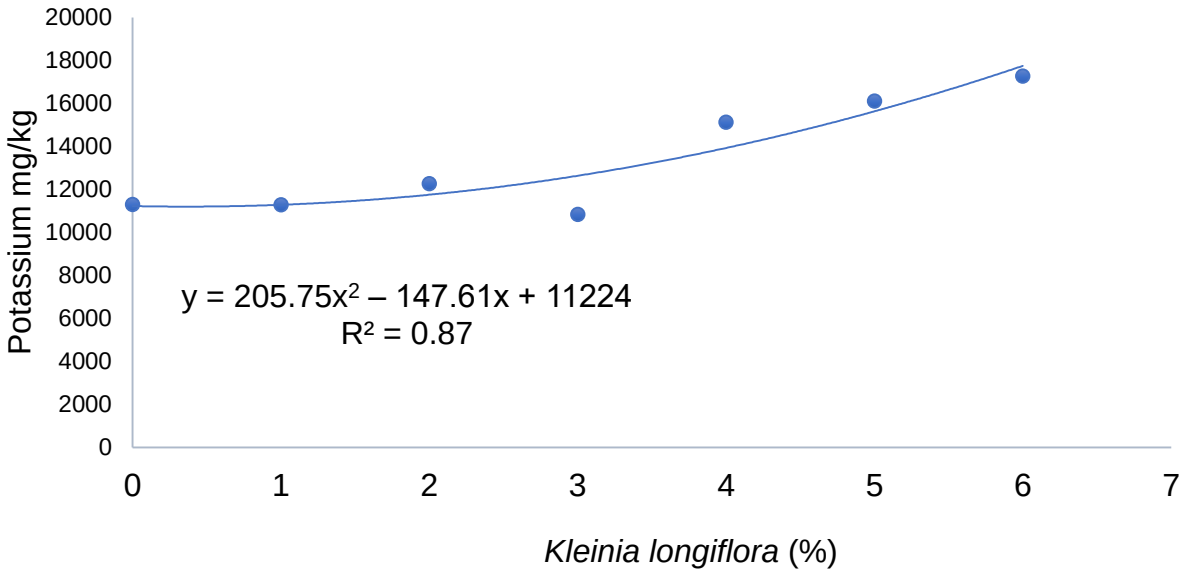


Figure 3.7 (a) Responses of potassium from leaf tissues of tomato cv. 'Floradade' to increasing concentration of fermented *Kleinia longiflora* in greenhouse experiment at 56 days after initiation of treatments.

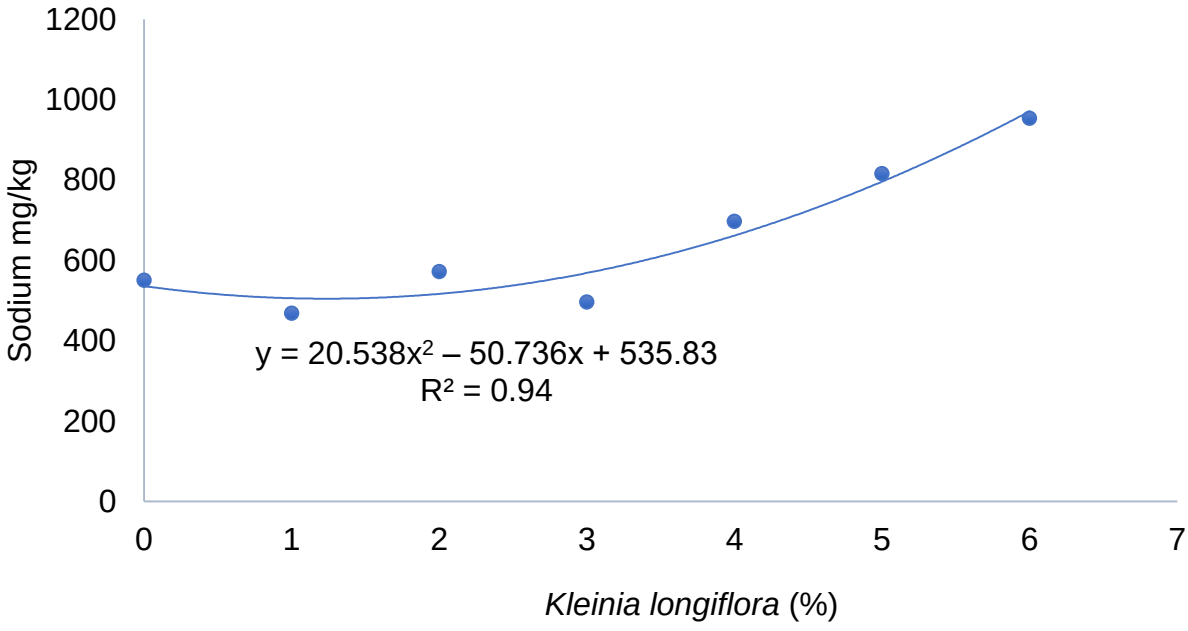


Figure 3.7 (b) Responses of sodium from leaf tissues of tomato cv. 'Floradade' to increasing concentration of fermented *Kleinia longiflora* in greenhouse experiment at 56 days after initiation of treatments.

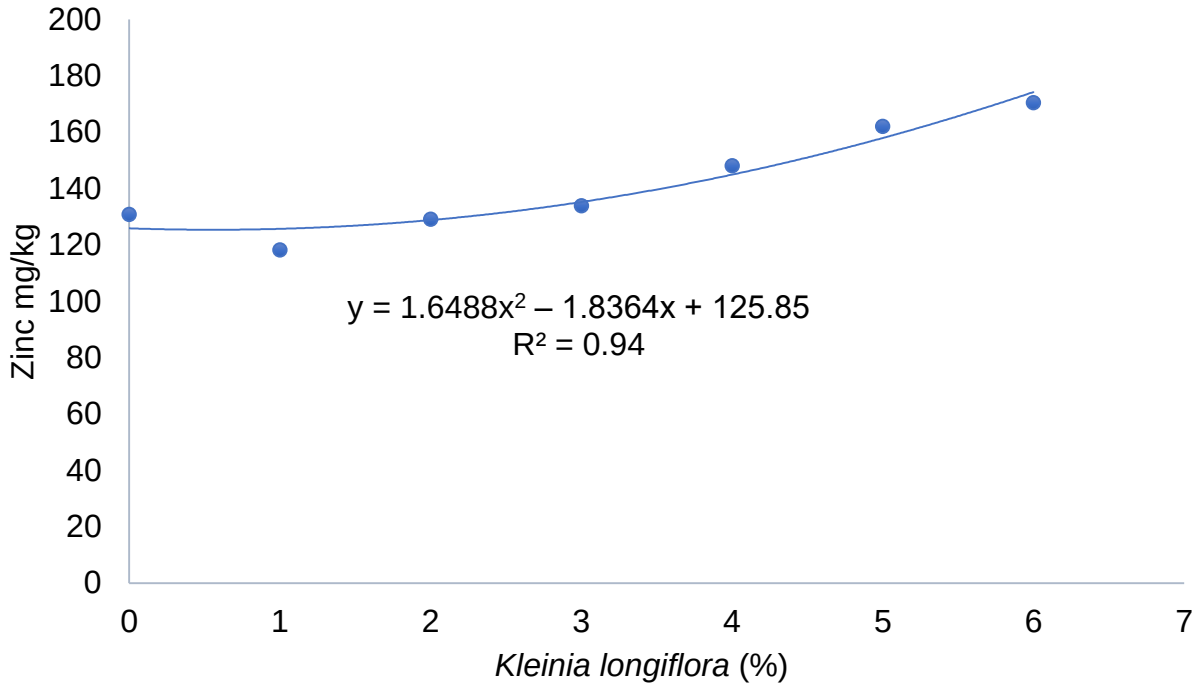


Figure 3.8 Responses of zinc from leaf tissues of tomato cv. 'Floradade' to increasing concentration of fermented *Kleinia longiflora* in greenhouse experiment at 56 days after initiation of treatments.

Table 3.3 Optimisation models for potassium, sodium and zinc to increasing concentration of fermented *Kleinia longiflora* at 56 days after initiation of treatments.

| Variable | Model | $R^2$ | X |
|---|---|---|---|
| Potassium | $Y = 205.75x^2 – 147.6x + 11224$ | 0.87 | 11224.39 |
| Sodium | $Y = 20.538x^2 – 50.736x + 535.83$ | 0.94 | 504.50 |
| Zinc | $Y = 1.6488x^2 – 1.83.64x + 125.85$ | 0.94 | 125.84 |

$X = – b_1/2b_2$

## 3.4 Discussion

### 3.4.1 Nematode variables

Response of J2 in roots, J2 in soil, eggs and Pf under increasing concentration of fermented *K. longiflora* were significant and exhibited negative quadratic relation. These results suggested that the effective microorganisms were successful in releasing the potent chemical with bioactivities to the soil rhizosphere. Similar observations were made when *M. javanica* were exposed to increasing Nemarioc-AL and Nemafric-BL phytonematicides on tomato (Pelinganga, 2013; Tseke, 2013) watermelon (Nhlane, 2017), citrus (Maile, 2013) and *Cucurbita pepo* (Lebea, 2017). Also, leaf extracts of crambe (*Crambe abyssinica* R.E.Fr.) affected nematode population density on tomato (Coltro-Roncato *et al*., 2016), although the active ingredient was extracted through hydroalcoholic (70% ethanol and 30% water) solution. Marigold leaves and flowers, castor beans (*Ricinus communis* L.) and garlic (*Allium sativum* L.) also affected *M. javanica* in tomato plants (Mashela and Nthageni, 2002; Tibugari *et al*., 2012).

Furthermore, liquid extracted from seeds of tobacco (*Nicotiana tabacum* L.), clove (*Syzygium aromaticum* L.), betelvine (*Piper betle* L.) affected *M. incognita* population density on chilli pepper (*Capsicum annuum* L.) (Wiratno *et al*., 2009). The trend of the effects was that increasing concentration of fermented *K. longiflora* reduced nematode population in the phase referred as inhibition in the context of density-dependent growth (DDG) patterns. Based on DDG patterns, the concentration may either stimulate, neutral or inhibit nematode population density. However, the observations in the current study contradicted with that of Dube (2016), where increasing concentration of Nemarioc-AL at lower concentration stimulated and only inhibited at

higher concentration. The efficacy of plant extracts is based on allelochemicals contained (Mashela *et al*., 2017b). Perhaps the difference between the allelochemicals of the two plant extracts is the source of the contradiction (Mashela *et al*., 2018b).

Generally, the efficacy of phytonematicides is dependent on allelochemicals and the level toxicity in controlling nematodes (Chitwood, 2002; Mashela *et al.,* 2017a; Ntalli and Caboni, 2012). For instance, cucurbitacin A and cucurbitacin B are active ingredients extracted from Nemarioc-AL and Nemafric-BL phytonematicides, respectively (Chen *et al*., 2005; Mashela *et al*., 2017a). Garlic has allicin as its active ingredient and it is lethal to various microorganisms including nematodes (Chitwood, 2002), whereas alpha-terthienyl is believed to be the main active ingredient in marigold responsible for nematode suppression (Marahatta *et al*., 2012). Neem has azadirachtin as active ingredient (Meyer *et al.,* 2008). Consequently, the efficacy of these materials depends on their level of toxicity and concentration used during experiments (Boyd *et al*., 2006; Meyer *et al.,* 2008). Phytonematicides developed from botanicals have high variability in the active ingredients (Weaver and Subramanyam, 2000). For instance, cucurbitacin A of Nemarioc-AL phytonematicide is concentrated in fruits of *C. myriocarpus*, whereas cucurbitacin B of Nemafric-BL phytonematicide is concentrated at every part of the *C. africanus* crop (Shadung and Mashela, 2016).

### 3.4.2 Plant growth variables

In field experiment, increasing concentration of fermented *K. longiflora* treatments did not have significant effects on tomato growth variables. Similar results were observed when beetroot (*Beta vulgaris* L.) was exposed to increasing concentration of Nemafric-BL phytonematicide (Mashitoa, 2017). Sithole (2016) tested Nemarioc-AL

phytonematicide on wild geranium (*Pelargonium sidoides* DC.) which also did not affect plant variables such as chlorophyll content, dry shoot mass and tuber mass under microplot conditions. However, in other studies the results were not consistent, Shadung (2016) reported that Nemarioc-AL and Nemafric-BL phytonematicides did not affect fruit number, plant height, stem diameter and dry shoot mass except for chlorophyll content of tomato 'Rodade' under field conditions.

In greenhouse conditions fermented crude extracts from *K. longiflora* were highly significant and exhibited positive quadratic relations to measured plant variables. Similar observations were made, when cucumber (*Cucumis sativus* L.), muskmelon (*Cucumis melo* L.), pepper (*C. annuum*) and tomato (*Solanum lycopersicum* L.) seedlings were exposed to increasing concentration of *S. aromaticum* (Meyer *et al.*, 2008). Marigold (*T. patula*.), rosemary (*R. officinalis*) and nigella (*N. sativa*) also confirmed the effects of increasing concentration of fermented *K. longiflora* on tomato plants (Bajestani *et al*., 2017). The increasing concentration of fermented *K. longiflora* inhibited plant growth at higher concentration in chlorophyll content, stem diameter and plant height, whereas number of flowers, dry fruit mass and dry shoot mass were stimulated at lower concentration. Pelinganga (2013), also reported similar trend where Nemarioc-AL phytonematicide stimulated and inhibited plant variables such as stem diameter, plant height and dry shoot mass of tomato. Chukwuka *et al*. (2014) also noted that the stimulation and inhibition of crude extracts from bitter leaf (*Vernonis amygdalina* Del.) on maize seedlings were dosage-dependent. Generally, it is postulated that at lower concentration phytonematicides increase plant growth, whereas at higher concentration they inhibit plant growth (Mashela *et al*., 2017a; Pelinganga, 2013). However, these tenets are referred as DDG patterns (Mashela *et*

*al.,* 2017a). These DDG patterns are characterised by three distinct growth phases namely stimulation, neutral and inhibition (Liu *et al*., 2003; Salisbury and Ross 2005). The Curve-fitting Allelochemical Response Data (CARD) model in this study confirmed DDG patterns on the quadratic relations. Incidentally these DDG patterns suggest that increasing concentration used in field study were in neutral phase of DDG patterns, hence the recorded plant variables were not affected (Mashela *et al*., 2017a; Sithole, 2016). The CARD model is a computer-based model that is used to quantify concentration of allelochemicals which lead to the three phases namely, stimulation, neutral and inhibition that characterise DDG patterns for various organisms (Mashela *et al.,* 2017a).

The MCSP value for *K. longiflora* increasing concentration on tomato was derived to be 1.97%, which have significant difference to that of Nemarioc-AL phytonematicide 2.63% which was also derived for tomato (Pelinganga and Mashela, 2012). The ∑k was derived at 1 in the current study which was different to Pelinganga and Mashela (2012) (∑k = 3) who described it as moderate sensitivity. Generally, the lower the ∑k index, the higher is the sensitivity of the plant to the tested phytonematicide and vice versa (Mashela *et al*., 2017a). Therefore, the observed ∑k suggest that tomato is very sensitive to increasing concentration of fermented *K. longiflora*.

### 3.4.3 Selected mineral elements

Response of K, Na and Zn under increasing concentration of fermented *K. longiflora* were highly significant and exhibited negative quadratic relations. Khosa (2013) also supported the observations over several plant extracts. Furthermore, Nemarioc-AL

and Nemafric-BL also affected foliar nutrient elements under field condition (Pelinganga, 2013; Shadung, 2016).

Tomato plants absorb high amounts of potassium from the soil during growth. It has significant contribution in growth of tomato plants that include photosynthesis, enzyme activation, cell turgor maintenance and ion homeostasis. Additionally, it is also involved in the enhancement of lycopene content of tomato fruit through synthesis of pigments or carotenoids (Alia *et al*., 2015). Zinc is also one of the important nutrient elements for tomato, it is essential for synthesis of auxins in tomato. Thus, its deficiency results in shortened internodes and eventual stunted growth. Furthermore, it improves flowering of tomato plants (Alia *et al*., 2015; Botrini *et al*., 2000). Normally, phytonematicide do not show a clear tendency on nutrient elements that follows order of treatments. For instance, Nemarioc-AL and Nemafric-BL increased Ca, P, Mg, Na and P, whereas Fe was reduced in leaf tissues of tomato under field conditions (Shadung, 2016). The unexplainable stimulation effects by cucurbitacin-containing phytonematicides were referred as “fertilizer effect” (Mashela, 2002). However, the existence of DDG patterns has since given much insight of the efficacy of plant extracts on nematodes, plant variable and mineral elements (Mashela and Pofu, 2017). The increasing concentration of fermented *K. longiflora* effects on K, Na and Zn were on the stimulation phase of the negative quadratic relations.

Sodium is highly toxic element to physiology of tomato plants and toxicity appears as necrosis or scorching of the leaf tips and margins (Botrini *et al*., 2000). However, it is used in small amount in tomato plants because it improves the organoleptic

characteristics of the fruit. The increasing concentration affected the accumulation of Na in the negative quadratic characteristic as explained earlier.

## 3.5 Conclusion

Increasing concentration from fermented *K. longiflora* effectively reduced population densities of *M. javanica*, whereas growth of tomato plants was highly compromised. Ultimately, crude extracts from *K. longiflora* in liquid formulation are highly phytotoxic. However, non-phytotoxic derived concentration of *K. longiflora* phytonematicide on tomato was 1.97%. Therefore, fermented crude extracts of *K. longiflora* portray some nematicidal properties which need to be validated under diverse conditions before being recommended suitable to use in tomato production.

# CHAPTER 4
# EFFICACY OF DRIED CRUDE EXTRACTS OF *KLEINIA LONGIFLORA* ON SUPPRESSION OF *MELOIDOGYNE JAVANICA*, GROWTH AND ACCUMULATION OF SELECTED ELEMENTS IN TOMATO PLANTS

## 4.1 Introduction

The active ingredients from plants are by-products of the plant metabolism, ideally, synthesized during catabolism of carbohydrates in the respiration pathway, which have been collectively referred to as secondary metabolites (Chitwood, 2002). These metabolites may either be stored in vesicles as part of compartmentalisation or are exuded through different plant organs. Generally, most of the secondary metabolites are released after plant organs have withered through leaching (Mashela and Nthangeni, 2002; Thovhakhale, 2005). The release of certain active ingredients with nematicidal properties from plant extracts is dependent upon microbial activities in certain plants such as chilli pepper (*Capsicum annuum* L.) tamboti (*Spirostanchys africana* Sond.) and cabbage (*Brassica oleracea* L.) (Mashela *et al.,* 2012; McSorley and Gallaher, 1995; Stirling, 2014; Thovhakhale, 2005). Therefore, after phasing out of nematicides from the market, organic amendments were regarded as potential alternatives in managing root-knot nematodes (*Meloidogyne* species) (Chitwood, 2002; Mashela, 2002; Stirling, 2014).

Mashela and Nthangeni (2002) introduced a classical method called Ground Leaching Technology (GLT) which postulate that dried and ground crude extracts are applied on shallow hole around the base of the plant. The potent chemicals are leached to soil rhizosphere through irrigation water. It was also discovered that the method depends on solubility of the dried crude extracts of particular botanical into water rather than biodegradation (Mashela *et al*., 2017a). The method has since been used successfully

on botanicals such as wild cucumber (*Cucumis myriocarpus* Naud.), castor bean (*Ricinus communis* L.) and fever tea (*Lippia javanica* F. Burm.) (Mashela, 2002; Mashela and Nthangeni 2002; Mashela *et al.,* 2007). The objective of this study was to investigate the efficacy of increasing concentration of *K. longiflora* in granular formulation on *M. javanica,* growth of tomato plants and selected mineral elements.

## 4.2 Materials and methods

### 4.2.1 Description of study site

The study was conducted at Green Bio-technologies Research Centre of Excellence, University of Limpopo, Limpopo Province, South Africa (23°53′10″S, 29°44′15″E). Both field and greenhouse studies were conducted concurrently with experiments previously described (Chapter 3).

### 4.2.2 Treatments and research design

Both trials were conducted in autumn (March-May) 2017 and summer (November-January) 2017/2018 for field and green house as outlined (Chapter 3). The treatments ranged from 0, 2, 4, 6, 8, 10 and 12 g of granular formulation of *K. longiflora*. Treatments were arranged in randomised complete block design (RCBD) with 12 replications.

### 4.2.3 Procedures

Herbaceous materials from *K. longiflora* were harvested from Khureng village and prepared to the granular stage as described previously (Chapter 3). However, in this study the materials were not fermented, but were applied as granular formulation in 5-cm deep holes at 3-cm away from the base of each tomato stem (Mashela *et al*., 2012).

Three-week-old tomato seedlings cv. 'Floradade' were transplanted for the field trial during mid-autumn to early winter (March-May) 2017, at the 0.60 m spacing for both inter-row and intra-row spaces. In summer (November-January) 2017/2018 the greenhouse trial was conducted using 20-cm-diameter plastic pots, at 0.25-m intra-row and 0.30-m inter-row spacing filled with 2 700 ml steam-pasteurised (300°C for 1 hour) loam soil and Hygromix (Hygrotech, Pretoria) at 3:1 (v/v) ratio. Inoculum was prepared by extracting eggs and second-stage juveniles (J2) from roots of tomato cv. 'Floradade' as described previously (Chapter 3).

### 4.2.4 Data collection

At 56 days after initiating treatments, nematode variables, plant variables and selected mineral elements were collected as described previously (Chapter 3).

### 4.2.5 Statistical analysis

Nematode and mineral elements data were subjected to analysis of variance using Statistix 10.0. Discrete nematode data was log-transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984), before analysis of variance. Plant variables were subjected to CARD model to generate appropriate biological indices and quadratic relations (Liu *et al*., 2003; Mashela *et al*., 2017a). Using the $MCSP = D_m + (R_h/2)$ relation (Mashela *et al*., 2017a), the MCSP value of *K. longiflora* phytonematicide on tomato was derived. Unless otherwise stated, the data were discussed at the probability level of 5%.

## 4.3 Results

### 4.3.1 Nematode variables

Nematodes were detected under greenhouse and not under field conditions. However, the product had no significant effects on eggs and J2 of *M. javanica* in both root and soil samples under greenhouse conditions (Table 4.1) (Appendix 4.1 to 4.4).

### 4.3.2 Plant growth variables

Chlorophyll content (field), chlorophyll content (greenhouse), plant height, dry root mass and dry shoot mass under increasing concentration of dried crude extracts of *K. longiflora* had significant effects and exhibited positive quadratic relations (Figure 4.1). The model explained the relationships by 52, 45, 56, 47 and 59%, respectively (Figure 4.1; Figure 4.2). Using the MCSP = $D_m + (R_h/2)$ relation (Mashela *et al*., 2017a), the MCSP value of *K. longiflora* phytonematicide on tomato was 2.84 g (Table 4.3). Under field conditions, plant height, chlorophyll content, dry shoot mass and dry root mass had sensitivity values of 3, 0, 2 and 5, respectively, whereas under the greenhouse conditions chlorophyll content had sensitivity of 1. Overall sensitivity ($\sum k$) under field and greenhouse conditions was 11.

### 4.3.3 Selected mineral elements

Increasing concentration of dried crude extracts from *K. longiflora* did not have significant effect on K, Na and Zn from leaf tissues of tomato cv. 'Floradade' (Table 4.2) (Appendix 4.5 to 4.7).

Table 4.1 Partitioning mean sum of squares of *Meloidogyne javanica* eggs, second stage juveniles (J2) roots, J2 soil and final population (Pf) affected by increasing concentration of dried crude extracts from *Kleinia longiflora* in 56 days after initiation of treatments.

| | | Eggs | | J2 roots | | J2 soil | | Pf | |
|---|---|---|---|---|---|---|---|---|---|
| Source | DF | MSS | TTV (%) | MSS | TTV (%) | MSS | TTV (%) | MSS | TTV (%) |
| Block | 11 | 0.33 | 45 | 0.13 | 25 | 0.19 | 40 | 0.40 | 46 |
| Treatment | 6 | 0.25 | 34$^{ns}$ | 0.15 | 28$^{ns}$ | 0.16 | 34$^{ns}$ | 0.17 | 20$^{ns}$ |
| Error | 66 | 0.15 | 21 | 0.25 | 47 | 0.12 | 26 | 0.29 | 34 |
| Total | 83 | 0.73 | 100 | 0.53 | 100 | 0.47 | 100 | 0.86 | 100 |

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

Table 4.2 Partitioning mean sum of squares for potassium, sodium and zinc on tomato cv. 'Floradade' leaves affected by increasing concentration of dried crude extracts from *Kleinia longiflora* at 56 days after initiation of treatments.

| | | Potassium (mg/kg) | | Sodium (mg/kg) | | Zinc (mg/kg) | |
|---|---|---|---|---|---|---|---|
| Source | DF | MSS | TTV (%) | MSS | TTV (%) | MSS | TTV (%) |
| Block | 11 | 1.62 | 32 | 31510.2 | 44 | 246.19 | 40 |
| Treatment | 6 | 1.57 | 31$^{ns}$ | 17597.8 | 26$^{ns}$ | 167.19 | 27$^{ns}$ |
| Error | 66 | 1.92 | 37 | 22700.4 | 32 | 208.58 | 33 |
| Total | 83 | 5.11 | 100 | 71808.4 | 100 | 621.77 | 100 |

$^{ns}$Not significant at P ≤ 0.05.

Table 4.3 Biological indices for field experiment plant height (PHT), chlorophyll content (CHR), dry shoot mass and dry root mass (DRM) and greenhouse chlorophyll content of tomato cv. 'Floradade' to increasing concentration of dried crude extracts of *Kleinia longiflora* at 56 days after initiation of treatments.

| | Field | | | | Greenhouse | |
|---|---|---|---|---|---|---|
| Biological indices | PHT | CHR | DSM | DRM | CHR | Mean |
| Threshold stimulation ($D_m$) | 0.42 | 0.663 | 0.54 | 0.21 | 6.533 | 1.67 |
| Saturation point ($R_h$) | 6.52 | 1.72 | 2.03 | 0.29 | 1.16 | 2.34 |
| 0 % inhibition ($D_0$) | 1.67 | 0 | 1.84 | 0.73 | 3.68 | 1.58 |
| 50 % inhibition ($D_{50}$) | 22.18 | 12.12 | 5.10 | 82.83 | 8.92 | 26.23 |
| 100 % inhibition ($D_{100}$) | – | 17.8 | 10.8 | – | 15.2 | 14.6 |
| $R^2$ | 0.93 | 0.64 | 0.88 | 0.78 | 0.90 | – |
| Sensitivity (k) | 3 | 0 | 2 | 5 | 1 | |
| Overall sensitivity ($\sum k$) = 11 | | | | | | |

MCSP = $D_m + (R_h/2)$ = 1.67 + (2.34/2) = 2.84 g.

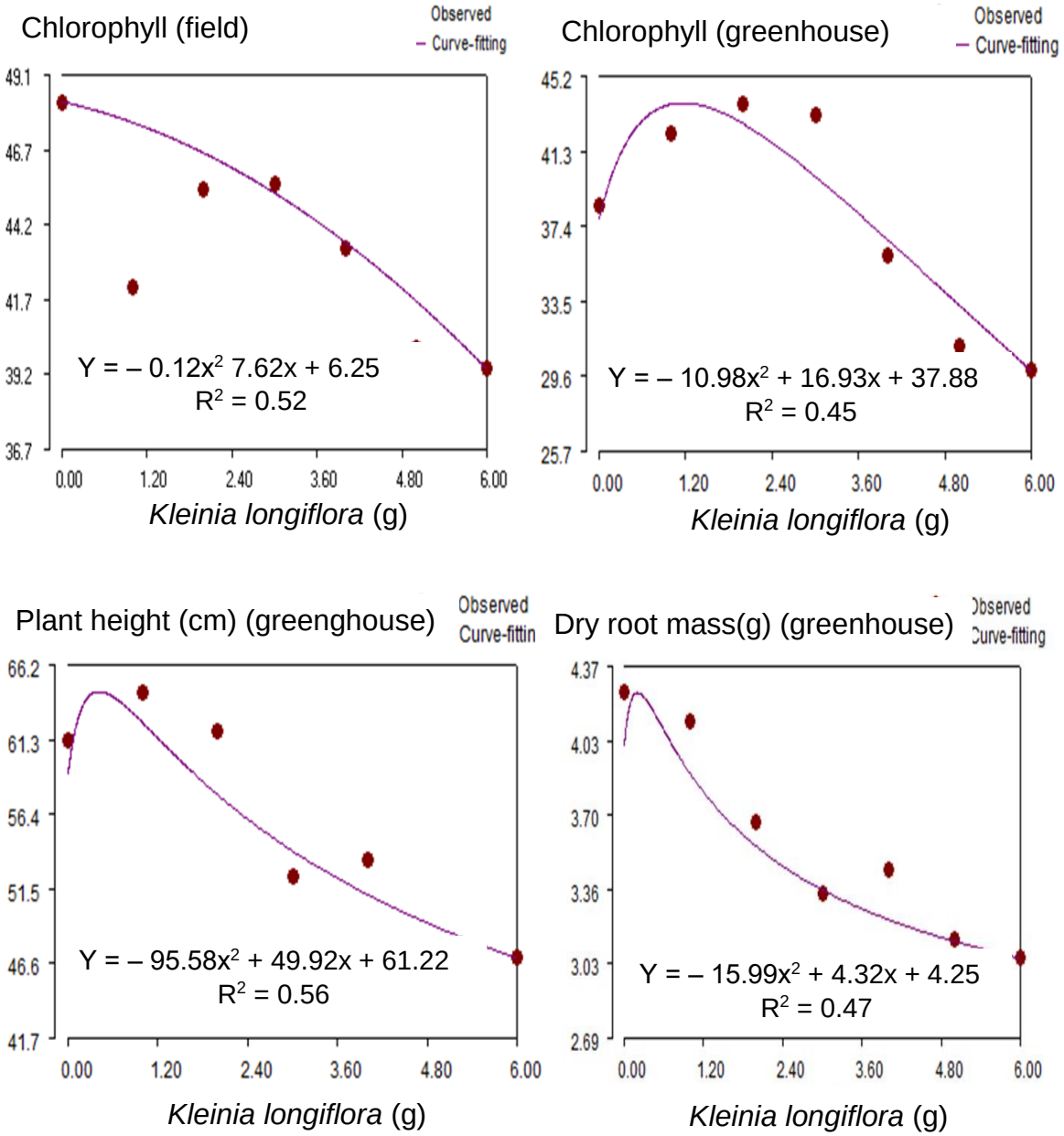


Figure 4.1 Responses of field and greenhouse chlorophyll content, plant height and dry root mass of tomato cv. 'Floradade' to concentration of *Kleinia longiflora* phytonematicide in greenhouse experiment at 56 days after initiation of treatments.

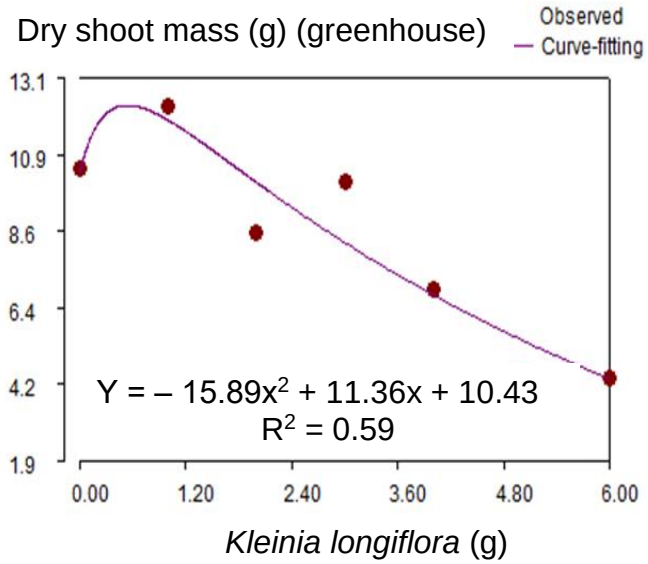


Figure 4.2 Responses of dry shoot mass of tomato 'Floradade' to increasing concentration of dried crude extracts of *Kleinia longiflora* phytonematicide in greenhouse experiment at 56 days after initiation of treatments.

## 4.4 Discussion

### 4.4.1 Nematodes variables

Dried crude extracts from *K. longiflora*, as opposed to the cucurbitacin-containing phytonematicides, did not affect nematodes. Similar results were observed when dried crude extracts of hot chilli (*Capsicum frutescence* L.) fruits, highly toxic *S. Africana* bark and cabbage (*Brassica oleracea* L.) leaves were not suitable for use in nematode management through GLT system since they did not contain potent nematicidal compounds (Mashela *et al*., 2012; Thovhakhale *et al.,* 2006). Mashela *et al*. (2012) demonstrated that not all plant extracts are suitable to be used in the GLT low-input farming system since the active ingredients were released through irrigation water. The interaction of *Bacillus* and *B. oleracea* reduced nematode, whereas *B. oleracea* alone neither affected nor reduced *M. incognita* (Mashela *et al*., 2012). Thus, suggested that microbial degradation was necessary for the efficacy of the product to

be realised in nematode suppression. Mashela (2002) further suggested that the ability of water to leach out potent chemicals in the GLT system was dependent upon the solubility of the target active ingredients. Pasteurisation of growing media also renders the ineffective of plant extracts because it certainly kills the soil actinomycetes, bacteria and fungi responsible for decomposition of plant extracts (Stirling, 2014).

In reality, for nematodes to be affected by any botanical product there should be active ingredients that have nematicidal properties, with Mashela (2002) further adding that organs with oily properties, were more effective in nematode suppression should a suitable active ingredient exists. For instance, *R. communis* fruits contain a ricin which is highly toxic to nematodes, whereas *L. javanica* leaves have volatile oily properties and linalool, ipsodienone, iridoid glycosides and toxic triterpernoids (Chitwood, 2002; Van Wyk *et al*., 1997). Contrary to the observations in the study, the cucurbitacin-containing phytonematicides are also suitable and successfully used in granular formulation through GLT system (Mashela *et al*., 2012). Furthermore, *A. indica* also affected and reduced population densities of root-knot nematodes (Thoden *et al*., 2011; Youssef and Lashein, 2013).

### 4.4.2 Plant growth variables

Dried crude extracts form leaves of spear mint (*Mentha spicate* L.) and sage (*Salvia officialis* L.) affected plant height, dry shoot mass and fresh root mass of cowpea (Wafaa *et al*., 2016). Dried crude extracts of lantana (*Lantana camara* L.) and guava (*Psidium guava* L.) also affected growth cowpea on similar plant variables (Wafaa *et al.,* 2016). The efficacy of ground *R. communis* fruit also affected growth of tomato (Mashela and Nthangeni, 2002).

Generally, at low concentration plant crude extracts stimulate growth of crops protected against nematodes (Mafeo *et al*., 2012; Mashela, 2002; Mashela *et al*., 2011). Hence, allelopathic effects were from plant extracts were also reported over the years (Mafeo, 2012). The unexplainable stimulation of plant growth by low dosages of dried crude extracts of many botanicals which was not supported by foliar nutrients might be due to density-dependent growth (DDG) pattern, which was characterised by stimulated, saturated and inhibited growth patterns as dosages of crude extracts increased. Liu *et al*. (2003) hypothesised that all biological entities would display a DDG response when exposed to increasing concentration of allelochemicals. Generally, DDG patterns demonstrate plant response to allelochemicals is concentration dependent that there was, stimulation, saturation/neutral and then inhibition growth range (Liu *et al*., 2003; Mashela *et al*., 2015; Rice, 1984; Salisbury and Ross, 2005).

The MCSP value for dried crude extracts of *K. longiflora* phytonematicide on tomato was derived at 2.84 g, which seemed to have been similar to that derived for cucurbitacin-containing phytonematicides 2.64 and 2.99 g of Nemafric-BG and Nemarioc-AG phytonematicides respectively, which was also applied on tomato (Pelinganga, 2013). The MCSP is generally allelochemical dependent because crops generally react different to allelochemicals (Mafeo, 2012). The concentration will be non-phytotoxic to tomato crop. The ∑k was 11 which demonstrated that the plant was less sensitive to the dried crude extracts of *K. longiflora* because generally the closer the value of ∑k of plant to zero, the higher the sensitivity of the plant to crude extracts applied (Mashela *et al.,* 2017a). It is a biological indicator for the degree of sensitivity to an extrinsic or intrinsic factor to the variable measured (Liu *et al*., 2003). It is

generally dependent on organ and crop. For instance, the observed results demonstrate that dry root mass was less sensitive whereas, chlorophyll content was very sensitive to dried crude extracts of *K. longiflora*. In other studies, (Mafeo, 2012) Nemarioc-AG phytonematicide on three crops from Gramineae family, had the overall sensitivities of the variables with the comparisons being that millet was most sensitive, whereas sorghum and maize followed in that order.

### 4.4.3 Selected mineral elements

In the current study the material had no significant effects on nutrient elements. Mashela (2002) reported on negligible effects from Nemarioc-AG phytonematicide on nutrient elements from leaves of tomato. Potassium is the most absorbed essential element that is very significant to yield and fruit quality of tomato for plant growth (Subbarao *et al*., 2003). Generally, sodium is not an essential element for plants but is used in small quantities, comparable to micronutrients, to aid in metabolism and synthesis of chlorophyll content making it one of the important element's plants. Zinc is an important element of tomato for development and function of growth regulators such as auxin that influence internode elongation. It is also involved in chloroplast development (Subbarao *et al*., 2003).

## 4.5 Conclusion

Dried crude extracts of *K. longiflora* did not affect population density of *M. javanica*, whereas the crude extracts appeared to be phytotoxic to growth of tomato. The non-phytotoxic concentration was derived at 2.84 g but would not be useful for use as a product for managing nematodes, since the product was not effective in nematode

management. However, further tests are recommended to validate the results of the present study.

# CHAPTER 5
# SUMMARY, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

## 5.1 Summary

After phasing out of synthetic nematicides, botanicals have been studied and researched to fill the gaps opened by synthetic nematicides on suppression of root-knot nematodes (*Meloidogyne* spp.) in particular. In this study paint-brush flower (*Kleinia longiflora* DC.) was researched in two formulations namely fermented liquid and granules. The results demonstrated that increasing concentration from fermented *K. longiflora* affected and reduced population eggs J2 in roots, J2 in soil and final population densities of *M. javanica* (Chapter 3). Contrary to fermented extracts, dried crude extracts (granular formulation) did not affect nematode population densities at all levels (Chapter 4). The formulations affected plant growth variables which demonstrated that the effects were concentration dependent. The latter is generally referred as density-dependent growth (DDG) patterns. Furthermore, the increasing concentration from fermented *K. longiflora* affected the accumulation of selected nutrient elements (K, Na and Zn) the effects are such that accumulation of nutrient elements was in stimulation phase of the negative quadratic relations as concentration increased, whereas there were no effects in the granular formulation. Overall, the study demonstrated that not all botanicals are suitable to be used in GLT agricultural low input system.

## 5.2 Significance of findings

The efficacy of increasing concentration of fermented *K. longiflora* demonstrated to have potential nematicidal properties. In contrast, the granular formulation in increasing concentration did not affect the nematodes at all level through application of GLT method. The contradiction confirmed the hypothesis that not all plant extracts are suitable to be used in the GLT agricultural low-input system. Therefore, microbial degradation is necessary for release of potent chemical containing nematicidal properties from plant parts of *K. longiflora* to soil rhizosphere. Furthermore, the exhibition of quadratic relations on the efficacy of increasing concentration of *K. longiflora* in two formulations confirmed the existence of DDG patterns with reference to allelochemicals used from other botanicals. Additionally, the increasing concentration affected selected nutrient elements, and the effects are such that the accumulation of nutrient elements were in stimulation phase of the negative quadratic relations.

## 5.3 Recommendations

Further studies are for the determination of active ingredients with potential nematicidal properties is necessary through discipline such as molecular approaches. The study on the interaction between dried crude extracts of *K. longiflora* and biodegradation organism such *Bacillus thuringiensis* on the suppression of nematodes is necessary. The outcome will validate the outcome of this study that dried crude extracts of *K. longiflora* cannot be used in the GLT agricultural low input system.

## 5.4 Conclusions

In conclusion, increasing concentration of fermented *K. longiflora* showed to contain potential nematicidal properties. Therefore, the study demonstrated that nematicidal

properties contained in *K. longiflora* can only be released through microbial degradation. Thus, there was no significant effect when the dried crude extracts of *K. longiflora* were applied in GLT method. The study also demonstrated that accumulation of selected nutrients affected by plant extracts conforms to the DDG patterns.

REFERENCES

AGBENIN, N.O. 2011. Biological control of plant parasitic nematodes: Prospects and challenges for the poor Africa farmer. *Plant Protection Science* 47:62-67.

AGBENIN, N.O., EMECHEBE, A.M., MARLEY, P.S. and A.D. AKPA. 2005. Evaluation of nematicidal action of some botanicals on *Meloidogyne incognita In-vivo* and *In-vitro. Journal of Agriculture and Rural Development in the Tropics and Subtropics* 1:29-39.

ALIA, M., MEHRAJB, H. and A. JAMAL-UDDINC. 2015. Effects of foliar application of zinc and boron on growth and yield of summer tomato. *Journal of Bioscience and Agriculture Research* 6:512-517.

BAI, Y., KISSOUDIS, C., YAN, Z., RICHARD, G. and G. Van Der LINDEN. 2018. Plant behaviour under combined stress: Tomato responses to combined salinity and pathogen stress. *The Plant Journal* 93:781-793.

BAJESTANI, M., DOLATABADI, K. and E. MAHDIKHANI-MOGHADAM. 2017. Effect of medicinal plant extracts on inoculated *Meloidogyne javanica* in tomato. *Pakistan Journal of Nematology* 35:73-78.

BOTRINI, L., LIPUCCIDI-PAOLA, M. and A. GRAIFENBERG. 2000. Potassium affects sodium content in tomato plants grown in hydroponic cultivation under saline-sodic stress. *Horticultural Science* 36:1220-1222.

BOYD, N.S., BRENNAN, E.B. and S.A. FENNIMORE. 2006. Stable seedbed techniques for or organic vegetable production. *Weed Technology* 20:1052-1057.

CHEN, J.C., CHIU, M.H., NIE, R.L., CORDELL, G.A. and S.X. QIU. 2005. Cucurbitacin and cucurbitane glycosides: Structures and biological activities. *Nature Product Reports* 22:386-399.

CHITWOOD, D.J. 2002. Phytochemical-based strategies for nematode control. *Annual Review of Phytopathology* 40:221-249.

CHUKWUKA, K.S., OBIAKARA, M.C. and I.A. OGUNSUMI. 2014. Effects of aqueous plant extracts and inorganic fertilizer on germination, growth and development of maize (*Zea mays*). *Journal of Agricultural Sciences* 3:243-254.

COLTRO-RONCATO, R.S., STANGARLIN, J.R., ODAIR, J.K., VALENTINA, E.D., GONÇALVES, O.D., FORLIN D.R., RISSATO. B.B., *et al.*, 2016. Nematicidal effect of *Crambe abyssinica* leaf extracts to *Meloidogyne javanica* on tomato. *African Journal of Agricultural Research* 11:3004-3011.

DE WAELE, D. and E. ELSEN. 2007. Challenges in tropical plant nematology. *Annual Review Phytopathology* 45:457-485.

DUBE, Z.P. 2016. Nemarioc-AL and Nemafric-BL phytonematicides: Bioactivities in *Meloidogyne incognita*, tomato crop, soil type and organic matter. PhD Thesis. University of Limpopo, Sovenga, South Africa.

FODEN, W. and L. POTTER. 2005. Red List of South African Plants Version: A National Assessment. South African National Biodiversity Institute (SANBI): Pretoria, South Africa.

GOMEZ, K.A. and A.A. GOMEZ. 1984. Statistical Procedures for Agricultural Research. Wiley: New York, USA.

HUSSEY, R.S. and K.R. BARKER. 1973. A comparison of methods of collecting inoculation of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter* 57:1025-1028.

JEFFERY, C. 1978. Cucurbitaceae. *In*: Heywood, V.H. (ed.). Flowering Plants of the World. Oxford University Press: Oxford, UK.

JENKINS, W.R. 1964. A rapid centrifugal-floatation technique for separating nematodes from soil. *Plant Disease Reporter* 48:692.

KHOSA, M.C. 2013. An investigation into the potential of crude extracts and partially separated material of selected non-crop plant species plant species as control agents of root-knot nematodes (*Meloidogyne incognita*) in tomato. PhD Thesis. North-West University, Potchefstroom, South Africa.

KRIEGER, R. 2001. Handbook of Pesticides Toxicology. Academic Press: San Diego, USA.

LEBEA, M.P. 2017. Mean concentration stimulation point of Nemarioc-AL and Nemafric-BL phytonematicides on *Cucurbita pepo* cultivar 'Caserta'. MSc dissertation. University of Limpopo, Sovenga, South Africa.

LEEDY, P.D. and J.E. ORMROD. 2005. Practical Research: Planning and Design. Prentice Hall: Upper Saddle River, USA.

LIU, D.L., JOHNSON, I.R. and J.V. LOVETT. 2003. Mathematical modelling of allelopathy. III. A model for Curve-fitting allelochemical dose responses. *Non-linearity in Biology, Toxicology and Medicine* 1:37-50.

MAFEO, T.P. 2012. Responses of economically important crops to crude extracts of *Cucumis* fruit when used a pre-emergent bio-nematicides. PhD Thesis. University of Limpopo, Sovenga, South Africa.

MAFEO, T.P., MASHELA, P.W. and M.S. MPHOSI. 2012. Allelopathic responses of various seeds to crude extracts from *Cucumis myriocarpus* fruits when used at pre-emergent bio-nematicide. *Acta Horticulturae* 938:409-414.

MAILE, K.D. 2013. Responses of *Tylenchulus semipenetrans* to crude extracts of indigenous *Cucumis* fruits with and without effective micro-organisms in citrus production. Masters of Agricultural Management dissertation. University of Limpopo, Sovenga, South Africa.

MARAHATTA, S.P., WANG, K.H., SIPES, B.S. and R.R. CERRUTI. 2012. Effects of *Tagetes patula* on active and inactive stages of root-knot nematodes. *Journal of Nematology* 1:26-30.

MARAIS, M., SWART, A., FOURIE, H., BERRY, S.D., KNOETZE, R. and P.M. MALAN. 2017. Techniques and Procedures. *In*: Fourie, H., Spaull, V.W., Jones, R., Daneel, M.S. and D. De Waele. (eds.). Nematology in South Africa: A View from the 21st Century. Springer International Publishing: Heidelberg, Switzerland.

MARQUES De CARVALHO, L., BENDA, N.D., VAUGHAN, M.M., CABRERA, A.R., HUNG, K., COX, T., *et al*., 2015. Mi-1-mediated nematode resistance in tomatoes is broken by short-term heat stress but recovers over time. *Journal of Nematology* 47:133-140.

MASHELA, P., DE WAELE, D., DUBE Z., KHOSA, C., POFU, K., TEFU, G., *et al*., 2017a. Alternative Nematode Management Strategies. *In*: Fourie, H., Spaull,

V.W., Jones, R., Daneel, M.S. and D. De Waele. (eds.). Nematology in South Africa: A View from the 21st Century. Springer International Publishing: Heidelberg, Switzerland.

MASHELA, P., POFU, K. and B. NZANZA. 2012. Suitability of *Brassica oleracea* leaves in managing *Meloidogyne incognita* through the g round leaching technology system under microplot conditions*. Acta Agriculturae Scandivanica, Section B–Soil and Plant Science* 63:19-24.

MASHELA, P.W., DUNCAN, L.W., GRAHAM, J.H. and R. McSORLEY. Leaching soluble salts increases population densities of *Tylenchulus semipenetrans*. *Journal of Nematology* 1:103.

MASHELA, P.W. 2002. Ground wild cucumber fruits suppress numbers of *Meloidogyne incognita* on tomato in microplots. *Nematropica* 32:13-19.

MASHELA, P.W. and K.M. POFU. 2017. Influence of cucurbitacin-containing phytonematicides on selected nutrient elements in leaf tissues of green bean under greenhouse conditions. *Acta Agriculturae Scandivanica, Section B–Soil and Plant Science* 67:743-747.

MASHELA, P.W. and M.E. NTHANGENI. 2002. Efficacy of *Ricinus communis* fruit meal with and without *Bacillus* species on suppression of *Meloidogyne incognita* and growth of tomato. *Phytopathology* 150:399-402.

MASHELA, P.W. and M.S. MPHOSI. 2002. Wild cucumber fruit residues reduce population densities of *Meloidogyne incognita* on tomato plants. *African Plant Protection* 10:125-142.

MASHELA, P.W., DE WAELE, D. and K.M. POFU. 2011. Use of indigenous *Cucumis* technologies as alternative to synthetic nematicides in management of root-knot nematodes in low-input agricultural farming systems: A review. *Scientific Research Essay* 6:6762-6768.

MASHELA, P.W., DUBE, Z.P. and K.M. POFU. 2015. Managing the Phytotoxicity and Inconsistent Nematode Suppression in Soil Amended with Phytonematicides. *In*: Meghvansi, M.K. and A. Vorma (eds.). Organic Amendments and Soil Suppressiveness in Plant Disease Management, Soil Biology 46. Springer International Publishers: Heildelberg, Switzerland.

MASHELA, P.W., POFU, K.M. and J.T. MADAURE. 2017b. Responses of citrus nematode population density post-application of Nemarioc-AL phytonematicide and aldicarb synthetic nematicide. *Research on Crops* 3:523-327.

MASHELA, P.W., SHIMELIS, H.A. and F.N. MUDAU. 2008. Comparison of the efficacy of ground wild cucumber fruits, aldicarb and fenamiphos on suppression of the root-knot nematode in tomato. *Journal of Phytopathology* 156:264-267.

MASHITOA, M.F. 2017. Development of non-phytotoxic concentration of Nemarioc-AL and Nemafric-BL phytonematicides on beetroot (*Beta vulgaris*) cultivar 'Detroit dark red'. MSc dissertation. University of Limpopo, Sovenga, South Africa.

McSORLEY, R. and R.N. GALLAHER. 1995. Cultural practices improve crop tolerance to nematodes. *Journal of Tropical and Subtropical* 25:53-60.

MEYER, S.L., LAKSHMAN, D.K., ZASADA, I.A., VINYARD B.T. and D.J. CHITWOOD. 2008. Phytotoxicity of clove oil to vegetable crop seedlings and nematotoxicity to root-knot nematodes. *Horticultural Technology* 18:631-638.

MOGALA, M. 2016. A profile of the South African tomato market value chain. Department of Agriculture, Forestry and Fisheries: Pretoria, South Africa.

MOREIRA, J.C., DA SILVA, C.B., RODRIGUES, A.A. and K.N. TAVARES. 2015. Alternative control of root-knot nematodes (*Meloidogyne javanica* and *M. enterolobii*) using antagonists. *International Journal of Agronomy and Agricultural Research* 72:121-129.

NHLANE, R.N. 2017. Influence of cucurbitacin-containing phytonematicides on growth, yield and foliar nutrient elements in watermelon production. Masters of Agricultural Management dissertation. University of Limpopo, Sovenga, South Africa.

NTALLI, N.G. and P. CABONI. 2012. Botanical nematicides in the Mediterranean basin. *Phytochemical Review* 11:351-359.

OKWUTE, S.K. 2012. Plants as Potential Sources of Pesticidal Agents: A Review. *In*: Soundararajan, R.P. (ed.). Pesticides: Advances in Chemical and Botanical Pesticides. IntechOpen: London, United Kingdom.

ONKENDI, M.E., KARIUKI, G.M., MARAIS, D.M. and L. MOLELEKI. 2014. A threat of root-knot nematodes (*Meloidogyne* spp.) in Africa: A review. *Plant Pathology* 4:12202.

ORNAT, C.T. and F.J. SORRIBAS. 2008. Integrated management of root-knot nematodes in Mediterranean horticultural crops. *Nematropica* 27:85-90.

PELINGANGA, O.M. 2013. Developing phytonematicides using indigenous *Cucumis africanus* and *Cucumis myriocarpus* fruits for tomato production Systems. PhD Thesis. University of Limpopo, Sovenga, South Africa.

PELINGANGA, O.M. and P.W. MASHELA. 2012. Mean dosage stimulation range of allelochemicals from crude extracts of *Cucumis africanus* fruit for improving growth of tomato plant and suppressing *Meloidogyne incognita* numbers. *Journal of Agricultural Science* 4:8-12.

POFU, K.M., MASHELA, P.W., MOKGALONG, N.M. and D. DE WAELE. 2011. Greenhouse whitefly infection on wild watermelon break resistance to the root-knot nematode. 10th African Crop Science Conference Proceedings. Abstract No. 239.

RICE, J.1984. Allelopathy. Academic Press: New York, USA.

SALISBURY, F.B. and C.W. ROSS. 2005. Plant Physiology. Wiley: New York, USA.

SEID, A., FININSA, C., MEKETE, T., DECRAEMER, W. and W.M.A. WESEMAEL. 2015. Tomato (*Solanum lycopersicum*) and root-knot nematodes (*Meloidogyne* spp.): A century-old battle. *Nematology* 17:995-1009.

SHADUNG, K.G. and P.W. MASHELA. 2016. Suitable organ(s) in wild Cucumis africanus for the production of nemafric-BL phytonematicide. *Acta Agriculturae Scandivanica, Section B–Soil and Plant Science* 66:381-383.

SHADUNG, K.G. 2016. Quality protocols for Nemarioc-AL and Nemafric-BL phytonematicides and potential chemical residues in tomato fruits. PhD Thesis. University of Limpopo, Sovenga, South Africa.

SITHOLE, N.T. 2016. Mean concentration stimulation point and overall sensitivity of Nemarioc-AL and Nemafric-BL phytonematicides on *Pelargonium sidoides*: An indigenous future cultigen. MSc dissertation. University of Limpopo, Sovenga, South Africa.

SITHOLE, N.T., POFU, K.M., MASHELA, P.W., DUBE, Z.P. and H. ARAYA. 2016. Overall sensitivity of *Pelargonium sidoides* and root-knot nematodes to Nemarioc-AL phytonematicide. *Transylvanian Review* 24:2996-3001.

SLOOTWEG, A.F.G. 1956. Root rot of bulbs caused by *Pratylenchus* and *Hoplolaimus* sp. *Nematologica* 1:192-201.

STIRLING, G.R. 2014. Biological Control of Plant-parasitic Nematodes: Soil Ecosystem Management in Sustainable Agriculture. CAB International: Wallingford.

SUBBARAO, G.V., ITO, O., BERRY, W. L. and R. M. WHEELER. 2003. Sodium—A Functional Plant Nutrient. *Critical Reviews in Plant Sciences* 22:391-416.

THODEN, T.C., KORTHALS, G.W. and A.J. TERMORSHUIZEN. 2011. Organic amendments and their influences on plant-parasitic and free-living nematodes: A promising method for nematode management. *Nematology* 13:133-153.

THOVHAKHALE, L.P. 2005. Responses of root-knot nematode densities to aqueous extracts of chilli and tamboti. Magister Technologiae in Agriculture dissertation. Central University of Technology, Bloemfontein, South Africa.

THOVHAKHALE, L.P., MASHELA, P.W. and C. VAN DER WESTHUIZEN. 2006. Responses of the root-knot nematode densities to aqueous extracts of chilli and tamboti. *Journal of Elsenburg* 2:10-12.

TIBUGARI, H., MOMBESHORA, D., MANDUMBU, R., KARAVINA, C. and C. PARWADA. 2012. A comparison of the effectiveness of the aqueous extracts of garlic, castor beans and marigold in the biocontrol of root-knot nematode in tomato. *Journal of Agricultural Technology* 8:479-492.

TSEKE, P.E. 2013. Responses of tomato plant growth and root-knot nematodes to phytonematicides from fermented fresh fruits of two indigenous *Cucumis* species. MSc dissertation. University of Limpopo, Sovenga, South Africa.

TSEKE, P.E. and P.W. MASHELA. 2017. Efficacy of fresh fruit from *Cucumis myriocarpus* as Nemarioc-AL phytonematicide on suppression of root-knot nematodes in tomato plant production. *Acta Agriculturae Scandivanica, Section B–Soil and Plant Science* 68:161-165.

VAN WYK, B.E., VAN OUDSHOORN, B. and N. GERICKE. 1997. Medicinal Plants of South Africa. Briza Publications: Pretoria, South Africa.

WAFAA, M.A., YOUSSEF, M.M. and K.G. EL-ROKIEK. 2016. Allelopathic effect of dry leaves of lantana and guava for controlling root knot nematode, *Meloidogyne incognita* on cowpea and some associated weeds. *International Journal of Chemical Technology Research* 9:55-62.

WEAVER, D.K. and B. SUBRAMANYAM. 2000. Botanicals. *In*: Subramanyam, B. and D.W. Hagstrum. (eds.). Alternative to Pesticides in Stored Products IPM. Kluwer Academic Publisher: Dordrecht, Netherlands.

WIRATNO, D., TANIWIRYONOC, H., VAN DEN BERGB, J., RIKSEND, I., RIETJENSB, S., DJIWANTIA, J., *et al*., 2009. Nematicidal activity of plant extracts against the root-knot nematode (*Meloidogyne incognita*). *The Open Natural Products Journal* 2:77-85.

YOUSSEF, M.M.A. and A.M.S. LASHEIN. 2013. Efficacy of Different medicinal plants as green and dry leaves on root knot nematode, *Meloidogyne incognita* infecting eggplant. *American-Eurasian Journal of Agricultural and Environmental Sciences* 2:10-14.

# APPENDICES

Appendix 4.1 Analysis of variance for *Meloidogyne javanica* second-stage juveniles in roots of tomato cv. 'Floradade' with increasing concentration of dried crude extracts of *Kleinia longiflora* under greenhouse conditions.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 11 | 0.9779 | 0.10865 | | |
| Treatment | 6 | 0.5133 | 0.08556 | 0.46 | 0.83 |
| Error | 66 | 10.0998 | 0.18703 | | |
| Total | 83 | 11.5910 | | | |

Appendix 4.2 Analysis of variance for *Meloidogyne javanica* eggs with increasing concentration crude extracts of *Kleinia longiflora* under greenhouse conditions.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 11 | 2.9994 | 0.33326 | | |
| Treatment | 6 | 1.5283 | 0.25472 | 1.69 | 0.14 |
| Error | 66 | 8.1525 | 0.15097 | | |
| Total | 83 | 12.6801 | | | |

Appendix 4.3 Analysis of variance for *Meloidogyne javanica* Pf in tomato cv. 'Floradade' with increasing concentration of dried crude extracts of *Kleinia longiflora* under greenhouse conditions.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Block | 11 | 3.6285 | 0.40317 | | |
| Treatment | 6 | 1.0529 | 0.17548 | 0.59 | 0.73 |
| Error | 66 | 16.1375 | 0.29884 | | |
| Total | 83 | 20.8189 | | | |

Appendix 4.4 Analysis of variance for *Meloidogyne javanica* J2 in soil for tomato cv. 'Floradade' with increasing concentration of dried crude extracts *Kleinia longiflora* under greenhouse conditions.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Block | 11 | 1.73455 | 0.19273 | | |
| Treatment | 6 | 0.97177 | 0.16196 | 1.28 | 0.28 |
| Error | 66 | 6.32173 | 0.12643 | | |
| Total | 83 | 9.9776 | | | |

Appendix 4.5 Analysis of variance for Na with increasing concentration dried crude extracts of *Kleinia longiflora* under greenhouse conditions.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 11 | 341231 | 31021.0 | | |
| Treatment | 6 | 211058 | 35176.3 | 1.53 | 0.18 |
| Error | 66 | 1519451 | 23022.0 | | |
| Total | 83 | 2071739 | | | |

Appendix 4.6 Analysis of variance for K with increasing concentration dried crude extracts of *Kleinia longiflora* under greenhouse conditions.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 11 | 0.19947 | 0.01813 | | |
| Treatment | 6 | 0.10985 | 0.01831 | 1.21 | 0.31 |
| Error | 66 | 0.98330 | 0.01513 | | |
| Total | 83 | 1.67554 | | | |

Appendix 4.7 Analysis of variance for Zn with increasing concentration dried crude extracts of *Kleinia longiflora* under greenhouse conditions.

| Source | DF | SS | MSS | F | P |
|---|---|---|---|---|---|
| Block | 11 | 3242.4 | 294.762 | | |
| Treatment | 6 | 1197.2 | 199.540 | 0.97 | 0.45 |
| Error | 66 | 13587.6 | 205.873 | | |
| Total | 83 | 18027.2 | | | |