PRODUCTIVITY AND MALNUTRITION ELEMENTS IN LOCAL AND EXOTIC *AMARANTHUS* CULTIVARS

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TABLE OF CONTENTS

DECLARATION

I, Makhado Mashudu Viginia, declare that the dissertation hereby submitted to the University of Limpopo, for the degree Master of Agriculture Management has not been submitted previously by me or anybody for a degree at this or any other University. Also, this is my work in design and in execution and related materials contained herein had been duly acknowledged.

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DEDICATION

To my beloved daughter Worifuna Masindi

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LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Amaranthus species, with their adaptability to grow under various agro-ecologies and soil types, have gained considerable attention in food security due to their high nutritional content. However, various *Amaranthus* species are host to the root-knot (*Meloidogyne* species) nematodes, with limited information on how the pest could affect the nutritional composition of the crop. In other crops, infestation with *Meloidogyne* species have deleterious effects on accumulation of certain essential mineral nutrient elements. However, the influence of *Meloidogyne* species on essential mineral nutrient elements had not been documented on *Amaranthus*. The objectives of the study, therefore, were to determine the influence of infestation by *Meloidogyne* species on growth and accumulation of selected malnutrition elements in four exotic *Amaranthus* relative to the local cultivar under both greenhouse and field conditions. In the greenhouse study, hardened-off seedlings were transplanted into 20-cm-diameter plastic pots containing a steam-pasteurised growing mixture. The 5 × 2 factorial experiments were arranged in a randomised complete block design, with five replications. The first and second factors were five *Amaranthus* cultivars and two *Meloidogyne* species, namely, *M. incognita* and *M. javanica*. At 60 days after inoculation, plant growth and nematode variables were assessed using standard procedures. Roots of all five *Amaranthus* cultivars were heavily galled, with limited cultivar × nematode interactions on plant variables. Additionally, the common factor that influenced either plant or nutrient element variables was the cultivar type, whereas those of nematodes were highly variable, particularly on nutrient elements. Three best performing cultivars were further tested under field conditions under nematode infested and untreated control plots. An exotic cv. 'Tanzania' had higher dry shoot mass, plant height and stem diameter, and accumulated higher Ca and K content than

′Candatus′ and ′Local 33′. Overall, the cultivar × nematode interaction reduced Na content in leaf tissues of *Amaranthus* cultivars under field conditions.

CHAPTER 1

RESEARCH PROBLEM

1.1 Background

1.1.1 Description of the research problem

The genus Amaranthus encompasses approximately 70 species, 40 of which are inherent to the Americas (Ebert, *et al*.,2011). Among the 70 species, 17 are vegetable amaranths with edible leaves, and three are grain amaranths with edible seeds (Ebert, et al.,2011). One of the reasons which led to Amaranthus selection is that it contains most mineral malnutrition elements that are essential for human body are mainly supplied by plants (Mensah *et al*., 2008). Amaranthus, like most indigenous leafy vegetables, contribute significantly to the supply of nutrient elements, which include Fe, Ca, K, Na, and Mg (Gupta *et al*., 2005). This crop is one of the cheapest and most available sources of proteins, vitamins, minerals and essential amino acids (Mensah *et al*., 2008). Mensah *et al.* (2008) reported that Amaranthus could provide ascorbic acid at as high as 408 mg per 100 g cooked material. This, technically called vitamin C, is an important antioxidant, which helps in protecting the body against cancer and other degenerative diseases such as arthritis and type II diabetes mellitus, and strengthens the immune system (Mensah *et al*., 2008). In the vegetable Amaranthus, vitamins C and A are also present in a nutritionally remarkable level, averaging at least 420 ppm vitamin C and 250 ppm beta-carotene (Woulds *et al*., 1984). In addition, minerals such as K, Fe and Mg exist in large concentrations, with average values of 287 ppm Fe, and 2.1 % Ca (Teutonico and Knorr, 1985). The estimates are high for most of the traits, with K and Ca having high values (Shukla *et al*., 2003). There is a pronounced interdependence of roots and shoots for growth regulators (Kramer and

Boyer, 1995). This imply that changes in the root or shoot caused by the occurrence of pests and diseases and/or other environmental factors such as planting conditions may cause changes in growth and nutrients availability. Regardless of its nutritional importance, *Amaranthus* production and consumption is being replaced by exotic vegetables in many rural areas of South Africa (Escudero *et al*., 1999). Due to increased interest in promotion of the consumption of indigenous leafy vegetables in South Africa, *Amaranthus* species are the most preferred vegetables due to their adaptability to a wide range of soils and harsh environmental conditions as well as their remarkable nutritional content (Mziray *et al*., 2000).

1.1.2 Impact of research problem

The ability of a plant to retain adequate nutrients during harvesting is important because most malnutrition elements that are required by the body are supplied by the plants. Inadequate consumption of essential micronutrients contribute to the increasing rates of illness and death from infectious disease and disability such as mental impairment (Black, 2003). Black (2003) continued to report that Fe deficiency, technically referred to as “anaemia”, and is the primary and major cause of early neonatal and maternal mortality. Additionally, Fe deficiency was reported to reduce cognitive development and work performance, with estimated 800 000 deaths annually and 2.4 % of global disability (Black, 2003; Ezzati *et al*., 2002). Also, inadequate Zn supply results in reduced growth rate in children, high rates of diarrhoea, pneumonia and increased child mortality (Sazawal *et al*., 2001).

1.1.3 Possible causes of research problem

A wide range of studies have shown that the nutritional importance of indigenous leafy vegetables cannot continue to be ignored. Most of these vegetables, have been touted as weeds since they predominantly grow in the wild (Mashela and Mollel, 2001). Inherent nutritional benefits in these vegetables are promoting an increased interest in research, seed preservation and cultivation. Cultural practices, soil type, pests and geographical location have influence on the growth, yield and nutritional content of wild indigenous leafy vegetables. Limited water supply and root infection by root-knot (*Meloidogyne* species) nematodes tend to cause changes in root/shoot ratio for different plant species, with a bearing on the productivity of such crops (Mashela and Nthangeni, 2002).Water deficit also have an influence on the nutritional value of selected indigenous leafy vegetables (Luoh *et al*., 2014).

1.1.4 Possible solutions

Variation in geographic locations where plants are growing in terms of rainfall, type of soil, temperature, type of plants and genetics have had enormous influence on growth and nutrient accumulation in various organs of a plant (Barminas *et al*., 1998). In addition, chemical composition of a plant is also reliant on the type of vegetable. Therefore, identifying *Amaranthus* varieties that maintain their optimum growth and nutritional content when under cultivation remains important in resolving nutrition-related challenges.

1.1.5 General focus of the study

The focus of the current study was to assess growth and nutrient accumulation of selected exotic Amaranthus and local cultivars under greenhouse and field conditions. The study aims to assess the accumulation of nutrient and growth at the selected cultivars of *Amaranthus* species which are not yet commercialised but are being consumed and are required by human body.

Problem statement

Different factors can affect the chemical composition of *Amaranthus* species (Chavéz-Servín *et al*., 2017), with such factors including, weather, soil type, infection by nematodes, direct sunlight and many gene-related factors. Accordingly, the geographical location can have direct influence on productivity and nutritional accumulation of a plant species as required by human bodies. A challenge in the cultivation of plants such as *Amaranthus* is to ensure that conditions are modified through appropriate choices of cultural practices that would not compromise the nutritional benefits.

1.3 Rationale of the study

Malnutrition is the condition that develops when the body does not get adequate amount of the vitamins, minerals and other essential substances it needs to maintain healthy tissues and organ functions. The nutritional content of the selected *Amaranthus* species had not been documented. The results of the study would provide information on how growing conditions might influence growth of different *Amaranthus*

cultivars in Limpopo Province. The information would enhance the development of suitable cultural practices and planting conditions in the production of specific *Amaranthus* cultivars that would promote optimum growth and nutrient availability. The ability of *Amaranthus* to accumulate nutrient elements required to fight malnutrition would be of advantage since *Amaranthus* is well adapted to various soils. Commercial cultivation would also enhance the availability of the vegetation to areas where harvesting from wild such as semi-urban and urban areas is not feasible. In addition to improving human health, the livelihood of Amaranthus producers will also improve.

1.4. Purpose of the study

1.4.1 Aim

To compare growth and nutritional composition of selected exotic and indigenous *Amaranthus* cultivars under the root-knot nematode infestations.

1.4.2 Objectives

1. To investigate the differences in growth and accumulation of selected nutritional elements in four exotic *Amaranthus* cultivars and a local cultivar under greenhouse conditions.
2. To determine growth and accumulation of selected nutrient elements in four exotic *Amaranthus* cultivars and a local cultivar under field conditions.

1.4.3 Hypotheses

1. There is difference in growth and accumulation of selected malnutrition elements in four exotic *Amaranthus* cultivars and the local cultivar under greenhouse conditions.
2. There is difference in growth and accumulation of selected nutrient elements in four exotic *Amaranthus* cultivars and the local cultivar under field conditions.

1.5 Reliability, validity and objectivity

For this study, reliability of data were grounded on statistical analysis of data at the probability level of 5%, validity was achieved through repeating the experiments in time, while objectivity was achieved by ensuring that the findings are discussed on the basis of empirical evidence, in order to eliminate all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

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

1.7 Scientific contribution

The study intended to establish specific Amaranthus cultivar that would perform better in the given planting conditions and still accumulate optimum nutrients elements.

1.8 Structure of dissertation

Following the description and outline of the research problem in chapter 1, a review of work done and not yet done on the research problem was undertaken in Chapter 2. Then, each of the two subsequent chapters (Chapter 3 and 4) addressed each of the two objectives, sequentially. In the final chapter (Chapter 5), findings in all chapters were summarised and integrated to provide the significance of the findings and recommendations with respect to future research, culminating in a conclusion which tied together the entire study. The Harvard style of citation and referencing was used as approved by the University of Limpopo Senate.

CHAPTER 2

LITERATURE REVIEW

Amaranthus is one of the leafy vegetables that has been cultivated for over 2000 years ago (Liu and Stutzel, 2004). The plant has been used as grain crop, vegetable or as animal feed (Chavéz-Servín *et al.,* 2017). As vegetable, *Amaranthus* leaves are harvested when they are still young, fresh and succulent (Van Rensburg *et al*., 2007). The economic value decreases with time, with the onset of flowering terminating the vegetative stage. In Africa, the crop is considered as an indigenous vegetable that is cooked on its own or in combination with other leafy vegetables, and consumed with other stable foods such as porridge. However, the current status of suitable cultivars is still low in South Africa as it is traditionally believed that it would grow naturally during the rainy seasons (Van Rensburg *et al.,* 2007). Factors that limit the consumption of Amaranthus species include urbanization and behavioural shift in life style of the rural African population, predominantly the misguided perception of indigenous vegetation is associated with poverty, thus, being avoided by children (Van Rensburg *et al*., 2007). This is a serious challenge because the intake of essential elements among South African children is below two-thirds the recommended dietary allowances (Labadarios *et al*., 2005). This can be addressed by ensuring that households are aware of the nutritive importance of the food that are available at their disposal as it was found on the National Food Consumption survey (Labadarios *et al*., 2005) that most households were food insecure and micronutrient deficiencies were common. As a results, the potential to advance the production and consumption of indigenous vegetables is well observed. *Amaranthus* has a potential to address the issue at hand because it contains more nutrients than most exotic vegetables (Biel *et al*., 2017; Venskutonis and Kraujalis, 2013).

2.1 Work done on the problem statement

2.1.1 Growth of *Amaranthus* species

The performance of *Amaranthus* grown under greenhouse and field conditions was done for the cv. '*Hypochondriacus*' (Chavéz-Servín *et al*., 2017). Growth biomass and grain yield were better on plants grown in the greenhouse when compared to those that were in the field. However, the opposite was in terms of quality, with the ability to accumulate different nutrients under both environments being affected. In the other study, wild plant species contained a larger proportion of nutrients as compared to those cultivated under controlled environments (Maanda and Bhatt, 2010).

2.1.2 Root-knot nematode status on *Amaranthus* species

Root-knot nematodes are one of the limiting factors in agricultural production and may results in losses of 80 to 100 % on some of the vegetables depending on susceptibility and levels of infestation that are already in the soil (Kimaru *et al*., 2013). Among other nematode species, *Meloidogyne* species are identified as widespread, destructive and most difficult to manage in most leafy vegetables (Nchore *et al.,* 2011; Schippers, 2004). Also, these nematode species were recorded as the most dominant in South Africa. Mashela *et al*. (2013) also reported that *Meloidogyne* species were widespread in various parts of Limpopo Province. *Amaranthus* species, like other leafy vegetables, are host to *Meloidogyne* species (Steyn *et al*., 2012). In support of this view Steyn *et al*. (2012) reported that none of the *Amaranthus* cultivars in South Africa were resistant to *M. incognita* race 2 and *M. javanica.* There was however, a considerable variation among the different varieties with regards to host response to infection (Steyn *et al*.,

2012). In surveys conducted in Pakistan (Ateeq-ur-Rehman, 2009; Zarina and Shahid, 2002), Spain (Steyn *et al.,* 2012) and Fiji (Khurma *et al.,* 2008), it was indicated that *Amaranthus* species were also host to *Meloidogyne* species.

2.1.3 *Amaranthus* species from different regions

Amaranthus species, due to their nutritional value, had since been globalised (Ateeq-ur-Rehman, 2009; Zarina and Shahid, 2002). *Amaranthus* species differ greatly in terms of root/shoot ratio, which could be used as indicator to assess performance of a plant species. The efficiency of root systems for supporting shoot growth and yield and/or a trait for increasing yield is dependent upon the root/shoot ratio (Anderson, 1987)**.** Root quantity that plants produce and the ability of a particular plant to take minerals such as nitrogen is considered to affect competition among different plant species (Kimberly *et al*., 2005). Kimberly *et al.* (2005) further indicated that plants which encounter harsh conditions such as limited supply of nutrients or water are expected to partition more biomass to their roots and less to their shoots. This shows that there is a pronounced interdependence on roots and shoots for growth as controlled by growth regulators (Kramer and Boyer, 1995). Thus, changes in root/shoot ratios caused by the presence of pests and diseases and/or other environmental factors could cause changes in nutrient balances as opposed to when the root/shoo ratio is neutrally favourable for the plant species.

2.1.4 Nutritional status of *Amaranthus* species

Mineral nutrient elements: The nutritional content of *Amaranthus* species can contribute significantly to the nutritional requirements of human beings and has potential to supplement as major source of nutrients (Akubugwo *et al*., 2007). Calcium (Ca) was reported at 2.05 mg per 100 g fresh material (Mensah *et al.,* 2008). Ca is an important mineral required for the formation of strong bones, muscle contraction and relaxation, blood clotting, synaptic transmission and absorption of vitamins. Also, high Mg content in *A. cruentus* (2.53 mg per 100 g cooked was shown to be an important element in lowering blood pressure (Mensah *et al*., 2008). Iron (Fe) is needed for haemoglobin formation (Latunde-Dada, 1990). Therefore, the relatively higher content of Fe in *A. cruentus* makes it an important supplement that can be recommended for anaemia, which is Fe deficiency. However, there is an increasing withdrawal, more especially among young people, from the consumption of this vegetable (Odhav *et al.,* 2007). The decline in the consumption of *Amaranthus* species and other indigenous vegetables by most people has resulted in poor diets and increased occurrences of nutritional deficiency disorders and related diseases in many parts of Africa (Kwapata and Maliro, 1995).

The study conducted by Gupta *et al.* (2005) indicated that *A. tricolor* had high Ca content, which together with Potassium (K) plays an important role in the growth and maintenance of bones, teeth and muscles (Dosunmu, 1997; Turan *et al.,* 2003). In certain *Amaranthus* hybrids Phosphorus (P) content at 34.91 mg/100 g fresh material could equate with that of sweet potato (*Ipomea batatas* L.*)* at 37.28 mg/100 g fresh material (Antia *et al.,* 2006). Cultural practices such as the selection of suitable cultivars could play important roles in ensuring that these the essential macronutrient needed by human body could be available at harvest.

Micronutrient malnutrition substances: There is a high global occurrence of micronutrient malnutrition among the vulnerable sections in the developing countries and thus resulting in increased prevalence of chronic degenerative diseases (Gupta *et al*., 2005). Worldwide, a disease caused by micronutrient deficiencies affects over two billion people (Flyman and Afolayan, 2006). On the National Food Consumption Survey (NFCS, 1999) it was suggested that intake of energy, Ca, Fe, Zinc (Zn), and vitamin A, D, C and E in South African children was below two-thirds the Recommended Dietary Allowances (RDA) (Gupta *et al*., 2005; Labadarios *et al.*, 2005). Using dietary modification strategy as a way of eradicating micronutrient malnutrition has been viewed as important in taking into consideration the locally available leafy vegetables because of their inexpensiveness, along with their easiness to produce and to prepare.

2.2 Work not done on problem statement

Several *Amaranthus* cultivars had been bred and developed overseas, due to the nutritional value of this plant species (Flyman and Afolayan, 2006; Negi and Roy, 2000). The growth and malnutrition status of the exotic *Aramanthus* cultivars and the locally developed *Aramanthus* cultivars had not been compared (Table 2.1).

Table 2.1 *Amaranthus* cultivars that were evaluated for their performance on growth and nutrient elements accumulation in two different growing conditions.

Amaranthus species	Cultivar	Country of origin
Amaranthus species	Local 33[1]	Local
Amaranthus species	Applebosch[1]	Exotic (Unknown)
Amaranthus cruentus	Candatus[2]	Exotic (Unknown)
Amaranthus species	Tanzania[1]	Exotic (Tanzania)

[1] *Amaranthus* species not yet identified.

2 Registered *Amaranthus* species.

CHAPTER 3

COMPARING GROWTH AND SELECTED MALNUTRIENT ELEMENTS OF EXTOTIC AND LOCAL *AMARANTHUS* CULTIVARS UNDER ROOT-KNOT NEMATODE INFESTATIONS IN GREENHOUSE CONDITIONS

3.1 Introduction

Nutrient availability in plants might be influenced by different aspects such as cultivar and environmental conditions, such as soil types, temperature, rainfall, solar radiation, pests and disease, *etc*. (Chavéz-Servín *et al.,* 2017). This implies that the nutritional content of Amaranthus in the wild might differ with the one cultivated in the fields or under controlled environment. It can be hypothesised that *Amaranthus* species would perform differently in different growing conditions. However, the performance of selected cultivars was never investigated and therefore the objective of this study was to compare whether the growth and selected malnutrition elements of some exotic and local *Amaranthus* cultivars under greenhouse conditions were similar. The Null hypothesis was that the growth and selected malnutrition elements of some exotic and local *Amaranthus* cultivars under greenhouse conditions were not similar.

3.2 Materials and methods

3.2.1 Description of study area

The study was conducted under greenhouse conditions at the Green Biotechnologies Research Centre, University of Limpopo, South Africa (23º53'10''S, 29º44'15''E). The first study was conducted in spring (August-October: Experiment 1) 2016 and validated in spring 2017 (Experiment 2) and spring 2018 (Experiment 3). Ambient day

and night temperatures were controlled at about 27^0C using thermostatically activated fans.

3.2.2 Treatments and research design

In all three experiments, the 5 × 2 factorial experiments were laid out in a randomised complete block design (RCBD), with five replications. The first factor comprises *Amaranthus* cultivars, namely, 'Local 33', 'Applebosch', 'Candatus', 'Kobie' and 'Tanzania', whereas the second factor comprise *M. incognita* and *M. javanica*.

3.2.3 Procedures

Planting materials

Seeds of local cultivar and exotic cultivars 'Kobie', ′Applebosch′, ′Candatus′ and 'Tanzania′, were obtained from the Agricultural Research Council- Vegetable and Ornamental Plants, Pretoria. Seeds of each *Amaranthus* cultivar were sown in two seedling trays filled with Hygromix-T (Hygrotech, Pretoria North, South Africa). All seedling trays were irrigated to field capacity and then every other day using tapwater. Four–week-old seedlings were hardened-off outside of the greenhouse through intermittent withdrawal of irrigation water for seven days prior to transplanting. Thereafter, uniform seedlings were grown in 20-cm-diameter plastic pots containing steam-pasteurised loam soil, river sand and Hygromix-T at 2:1:1 (v/v) ratio. Pots were arranged at 0.3 m × 0.25 m spacing on the greenhouse benches. Each seedling was inoculated with 1 000 eggs and second-stage juveniles (J2) of *M. incognita,* previously raised on tomato plants (*Solanum lycopersicon* L.) cv. 'Floradade'. The inocula were placed into 5-cm holes on the cardinal points of seedlings using a 20-ml plastic syringe. Seedlings were irrigated with 500 ml tap water at every other day.

Figure 3.1 Different *Amaranthus* cultivars planted on pots under greenhouse conditions.

3.2.4 Data collection

At 60 days after transplanting, plant height was measured from the soil level to the tip of the flag leaf, chlorophyll content was measured using a chlorophyll meter (MINOLTA, SPAD-502), stem diameter was measured using a digital Vernier calliper and shoots were cut at soil surface and then oven-dried at 60ºC for 72 h for dry mass determination. Oven-dried leaves were ground in a Wiley mill to pass through a 1-mm sieve (Mashela *et al.*, 2017). Each powdered sample was digested in nitric acid at a concentration of 5%, and then mixed using vortex meter. All samples were incubated in a warm water bath at a temperature of 95°C for an hour, left to cool down to room temperature and then filtered and the container covered with foil (SW-846 EPA method

3050B) to minimize direct contact with sunlight. Samples were then subjected to Atomic Absorption Spectrometry (AAS) at Limpopo Agro-food Station (LATS) and analysed for Calcium (Ca), Potassium (K), iron (Fe) and Zinc (Zn). Roots were assayed for nematode infection using root galls and since roots of all cultivars were heavily galled, the nematode numbers were not recorded.

3.2.5 Data analysis

Data for plant growth and nutrient variables were subjected to analysis of variance through the Statistix 10.0 software. Mean separation for significant ($P \leq 0.05$) treatment effects was achieved through the Fisher's Least Significance Difference test at the probability level of 5% only when the interaction effects were significant. The sum of squares were partitioned to estimate the contribution of sources of variation to the total treatment variation (TTV) (Gomez and Gomez, 1984). Unless otherwise stated, treatments were discussed at the probability level of 5%.

3.3 Results

3.3.1 Selected plant growth variables

Dry shoot mass: In all three experiments, nematode effects on dry shoot mass were not significant. The cultivar effects on dry shoot mass were highly significant ($P \leq 0.01$) in Experiment 1 and Experiment 2, contributing 13 and 40% in TTV of the variable, respectively (Table 3.1). In Experiment 3 the cultivar effects on the variable were not significant. Relative to cv. 'Local 33', dry shoot mass in all cultivars did not differ,

except that cv. ‘Kobie’ had significantly higher dry shoot mass than ′Applebosch′, ′Candatus′ and 'Tanzania′ (Table 3.2).

Plant height: In Experiment 1, the cultivar × nematode interaction had significant effects on plant height, whereas in the other two experiments the interaction effects were not significant. In Experiments 1, 2 and 3 the cultivar effects on plant height were significant, contributing to 31, 72 and 81% in TTV of the variable, respectively, whereas the nematode effects were not significant (Table 3.3). Relative to ′Local 33′, there were no significant differences in plant height for all the cultivars in the presence of nematodes (Table 3.4). Relative to cv. Local 33′, cv. ′Applebosch′, ′Kobie′ and ′Candatus′ had significant differences on plant height, whereas the ′Local 33′, and ′Tanzania′ effects on plant height were not different (Table 3.4).

Table 3.1 Partitioning sum of squares for dry shoot mass of five cultivars of *Amaranthus* under two levels of *Meloidogyne species* under greenhouse conditions at 60 days after transplanting.

		Experiment 1		Experiment 2		Experiment 3	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	9	14.8456	79^{ns}	4.25404	39^{ns}	17.6960	26^{ns}
Nematode (N)	1	0.2237	1^{ns}	0.40960	4^{ns}	12.3201	19^{ns}
Cultivar (C)	4	2.4340	13***	4.36535	40**	8.4952	13^{ns}
N × C	4	0.5362	3^{ns}	0.40035	4^{ns}	14.2863	22^{ns}
Error	81	0.6597	4	1.44879	13	13.2224	20
Total	99	18.6992	100	10.87813	100	66.0216	100

TTV = Total treatment variation.

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

Table 3.2 Mean dry shoot mass of four exotic *Amaranthus* cultivars relative to that of the undescribed 'Local 33' *Amaranthus* cultivar under greenhouse conditions.

	Experiment 1		Experiment 2		Experiment 3	
Cultivar	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
'Local 33'	1.795[ab]	–	4.13[bc]	–	4.82	–
'Applebosch'	1.61[b]	–10	3.98[c]	–4	6.04	25
'Kobie'	2.29[a]	28	4.77[ab]	–4	4.60	–5
'Candatus'	1.73[b]	–4	4.71[abc]	15	4.88	1
'Tanzania'	1.34[b]	–25	5.10[a]	23	4.34	–10
$LSD_{0.05}$	-		-		2.2879	

[y]Column means followed by the same letter were not different according to Fisher's Least Significant Difference test.

[z]R.I. = [(treatment/standard) –1] × 100.

Table 3.3 Partitioning sum of squares for plant height (cm) of five cultivars of Amaranthus under two levels of *Meloidogyne species* under greenhouse conditions at 60 days after transplanting.

		Experiment 1		Experiment 2		Experiment 3	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	9	79.118	14	62.592	13	78.262	8
Nematode (N)	1	46.786	9^{ns}	11.765	2^{ns}	71.572	7^{ns}
Cultivar (C)	4	168.332	31^{**}	340.353	72^{***}	846.288	81^{***}
N × C	4	194.560	36^{**}	7.914	2^{ns}	18.914	2^{ns}
Error	81	53.302	10	49.565	11	23.648	2
Total	99	542.098	100	472.189	100	1038.684	100

TTV = Total treatment variation

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

Table 3.4 Relative impact for plant height as affected by first order interaction of nematodes and Amaranthus cultivars at 60 days after treatment under greenhouse conditions.

	Experiment 1		Experiment 2		Experiment 3	
Cultivar	N_1	R.I. (%)	N_1	R.I. (%)	N_1	R.I. (%)
′Local 33′	26.29[c]	–	48.19[a]	-	55.74[a]	-
′Applebosch′	37.01[a]	41	44.88[abc]	–7	54.27[a]	–8
′Kobie′	38.95[a]	48	40.00[de]	–17	47.47[d]	–15
′Candatus′	38.32[a]	46	39.15[cde]	–19	43.59[bc]	–22
′Tanzania′	30.25[bc]	15	38.58[cde]	–20	38.46[b]	–31

[y]Column means followed by the same letter were not different according to Fisher's Least Significant Difference test.

[z]R.I. = [(treatment/standard) – 1] × 100.

Stem diameter: The cultivar × nematode interaction and nematode effects on stem diameter were not significant in all three experiments. However, the cultivar effects were highly significant in Experiment 1 and Experiment 3, respectively, contributing 68 and 71% in TTV of the variable, respectively (Table 3.5). Relative to ′Local 33′, the stem diameter in all cultivars did not differ in Experiment 3, whereas 'Kobie' and ′Tanzania′ significantly increased (16%) and reduced (35%) stem diameter than the ′Local′ in Experiment 1 (Table 3.6).

Chlorophyll content: In all three experiments, the cultivar × nematode interaction and nematode effects on chlorophyll content were not significant. However, the cultivar effects on chlorophyll content were highly significant in Experiment 3, contributing 67% in TTV of the variable (Table 3.7). Relative to ′Local 33′, the cultivar effects on chlorophyll content on ‘Candatus’ and ′Tanzania′ were different to those of ′Applebosch′, but not different to those of ′Kobie′ in Experiment 2 (Table 3.8). Cultivar ‘Candatus’ had the highest chlorophyll content, followed by ′Tanzania′ and ′Kobie′.

Table 3.5 Partitioning mean sum of squares for stem diameter (mm) of five cultivars of Amaranthus under two levels of *Meloidogyne ~~incognita~~* under greenhouse conditions at 60 days after transplanting.

		Experiment 1		Experiment 2		Experiment 3	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	9	2.607	15^{ns}	1.787	20^{ns}	3.5745	11^{ns}
Nematode (N)	1	0.423	2^{ns}	0.518	6^{ns}	2.9241	9^{ns}
Cultivar (C)	4	12.055	68***	2.256	25^{ns}	23.1803	71***
N × C	4	1.889	11^{ns}	2.437	27^{ns}	1.1329	3^{ns}
Error	81	0.781	4	1.914	22	2.0272	6
Total	99	17.755	100	8.912	100	32.839	100

TTV = Total treatment variation.

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

Table 3.6 Mean stem diameter (mm) of four exotic *Amaranthus* cultivars relative to that of the undescribed 'Local 33' *Amaranthus* cultivar under greenhouse conditions.

	Experiment 1		Experiment 2		Experiment 3	
Cultivar	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
'Local 33'	4.07[b]	–	6.33	–	7.68[a]	–
'Applebosch'	3.52[b]	–14	6.20	–2	6.75[bc]	–12
'Kobie'	4.74[a]	16	6.29	–1	7.38[ab]	–4
'Candatus'	3.54[b]	–13	5.72	–10	5.93[cd]	–23
'Tanzania'	2.63[c]	–35	5.62	–11	5.05[d]	–34
$LSD_{0.05}$	-		0.5505		-	

[y]Column means followed by the same letter were not different according to Fisher's Least Significant Difference test.

[z]R.I. = [(treatment/standard) –1] × 100.

Table 3.7 Partitioning mean sum of squares for chlorophyll content of five cultivars of *Amaranthus* under two levels of *Meloidogyne ~~incognita~~* under greenhouse conditions at 60 days after transplanting.

		Experiment 1		Experiment 2		Experiment 3	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	9	102.416	43^{ns}	96.378	15^{ns}	150.544	18^{ns}
Nematode (N)	1	21.996	9^{ns}	60.528	10^{ns}	78.057	9^{ns}
Cultivar (C)	4	25.747	11^{ns}	365.481	59^{ns}	568.663	67***
N × C	4	46.306	19^{ns}	38.214	6^{ns}	19.193	2^{ns}
Error	81	44.429	18	62.001	10	37.723	4
Total	99	240.894	100	622.602	100	854.18	100

TTV = Total treatment variation

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

Table 3.8 Mean chlorophyll content of four exotic *Amaranthus* cultivars relative to that of the undescribed 'Local 33' *Amaranthus* cultivar under greenhouse conditions.

	Experiment 1		Experiment 2		Experiment 3	
Cultivar	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
'Local 33'	33.67	–	23.58[c]	–	28.15[c]	–
'Applebosch'	34.06	1	23.06[c]	–2	32.47[b]	15
'Kobie'	32.64	–3	26.66[bc]	13	33.30[b]	18
'Candatus'	35.73	6	33.55[a]	42	42.67[a]	52
'Tanzania'	33.51	–4	28.72[ab]	22	35.54[b]	26
$LSD_{0.05}$	4.1939		-		-	

[y]Column means followed by the same letter were not different according to Fisher's Least Significant Difference test.

[z]R.I. = [(treatment/standard) –1] × 100.

3.3.2 Selected malnutrition elements

Iron (Fe): The cultivar × nematode interaction effects on Fe were not significant in Experiment 2 and Experiment 3, but the effects were significant on the variable in Experiment 1, contributing 69% in TTV of the variable (Table 3.9). Relative to the cv. ′Local 33′, Fe content for all nematode-infected cultivars had no significant differences (Table 3.10). However, cv. Tanzania had significantly higher Fe than ′Applebosch′, ′Candatus′ and ′Kobie′. Relative to ′Local 33′, nematode effects on Fe in cv. 'Tanzania' was different to that of 'Applebosch′, ′Candatus′ and ′Kobie′ (Table 3.10). Cultivar Tanzania reduced the concentration of Fe by 78% on treatments with nematodes.

Calcium (Ca): In all three experiments, the interaction effects on Ca were not significant. However, the cultivar and nematode effects on Ca were significant in Experiment 1 and Experiment 3, respectively, contributing 41 and 96% in TTV of the respective experiments (Table 3.11). Relative to *M. incognita*, *M. javanica* had no significant effects on Ca in leaf tissues of *Amaranthus* cultivars in Experiment 1 and Experiment 2, whereas in Experiment 3, *M. javanica* infection reduced Ca by 21% (Table 3.12). Relative to ′Local 33′, the cultivar effects on Ca in leaf tissues had no significant differences in Experiment 2 and Experiment 3. However, relative to ′Local 33′, cv. 'Candatus' had relatively higher Ca content in leaf tissues, whereas its effects on Ca were not different to those induced by ′Kobie′ on the variables (Table 3.13).

Table 3.9 Partitioning mean sum of squares for Fe of five cultivars of *Amaranthus* under two levels of *Meloidogyne* species under greenhouse conditions at 60 days after transplanting.

		Experiment 1		Experiment 2		Experiment 3	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	4	0.098	1	14.95	55	1.738	30
Nematode (N)	1	0.162	1^{ns}	3.40	12^{ns}	0.297	5^{ns}
Cultivar (C)	4	1.085	7^{ns}	2.67	10^{ns}	1.359	24^{ns}
N × C	4	11.198	69^{**}	0.71	3^{ns}	0.724	13^{ns}
Error	36	3.695	22	5.53	20	1.641	28
Total	49	16.238	100	27.26	100	5.759	100

TTV = Total treatment variation

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

Table 3. 10 Relative impact for Fe as affected by first order interaction of *Meloidogyne* species and *Amaranthus* cultivars at 60 days after transplanting under greenhouse conditions.

	Experiment 1		Experiment 2		Experiment 3	
Cultivar	N_0	R.I. (%)	N_1	R.I. (%)	N_1	R.I. (%)
'Local 33'	4.61[a]	–	2.97[a]	–	1.47[a]	–
'Applebosch'	3.44[ab]	–25	2.76[a]	–7.07	1.94[a]	32.00
'Kobie'	4.02[a]	–13	1.51[a]	–49.16	1.50[a]	2.04
'Candatus'	3.17[ab]	–31	1.85[a]	–37.71	1.05[a]	–28.57
'Tanzania'	1.02[b]	–78	2.73[a]	–2.15	0.86[a]	–41.50

Relative impact – R.I. (%) = [(treatment/Control) – 1] × 100.

Table 3.11 Partitioning sum of squares for Ca of five cultivars of *Amaranthus* under two levels of *Meloidogyne* species under greenhouse conditions at 60 days after transplanting.

		Experiment 1		Experiment 2		Experiment 3	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	4	583.96	16	188.32	10	212.22	1
Nematode (N)	1	690.14	19^{ns}	564.60	29^{ns}	21270.09	96**
Cultivar (C)	4	1484.25	41**	192.33	9^{ns}	189.69	1^{ns}
N × C	4	459.84	12^{ns}	286.03	15^{ns}	237.80	1^{ns}
Error	36	438.84	12	716.39	37	270.02	1
Total	49	3657.03	100	1947.66	100	22179.82	100

TTV = Total treatment variation.

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

Table 3.12 Mean Ca content of treatments with *Meloidogyne incognita* relative to those with *M. javanica* under greenhouse conditions.

	Experiment 1		Experiment 2		Experiment 3	
Nematodes	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
M. incognita	58.47	–	41.75	–	61.59[a]	–
M. javanica	51.04	–13	48.73	17	48.41[b]	–21
$LSD_{0.05}$	12.019		16.00		-	

[y]Column means followed by the same letter are not significantly different according to Fisher's Least Significant Difference test.

[z]R.I. = [(treatment/standard) –1] × 100.

Table 3.13 Mean Ca of four exotic *Amaranthus* cultivars relative to that of the undescribed 'Local 33' *Amaranthus* cultivar under greenhouse conditions.

	Experiment 1		Experiment 2		Experiment 3	
Cultivar	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
'Local 33'	42.19[b]	–	42.81	–	51.92	–
'Applebosch'	51.54[b]	22	39.01	–9	60.03	16
'Kobie'	61.09[ab]	45	47.51	11	59.14	14
'Candatus'	72.52[a]	72	50.48	19	50.32	–3
'Tanzania'	46.41[b]	10	46.36	8	53.57	3
$LSD_{0.05}$	-		24.353		14.904	

[y]Column means followed by the same letter were not different according to Fisher's Least Significant Difference test.

[z]R.I. = [(treatment/standard) – 1] × 100.

Potassium (K): The cultivar × nematode interaction effects on K were significant on Experiment 3, contributing 22% in TTV of the variable (Table 3.14). The cultivar effects on K were also significant on Experiment 2, contributing 32% in TTV of the variable. In contrast, nematode effects on K were significant in Experiment 1 and 3, contributing 64 and 51% in TTV of the variable, respectively (Table 3.14). Relative to ′Local 33′, K in ‘Applebosch’ was significantly different, but its effects on ′Kobie′ and ′Candatus′ were not different (Table 3.15).

Table 3.14 Partitioning mean sum of squares for K of five cultivars of *Amaranthus* under two levels of *Meloidogyne incognita* under greenhouse conditions at 60 days after transplanting.

		Experiment 1		Experiment 2		Experiment 3	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	4	323.15	11	1128.39	52	154.98	3
Nematode (N)	1	1898.88	64**	4.45	0ns	2769.17	51**
Cultivar (C)	4	227.90	8ns	685.29	32**	936.48	17ns
N × C	4	285.43	9ns	138.50	6ns	1185.16	22**
Error	36	245.33	8	193.03	10	349.02	7
Total	49	2980.69	100	2149.66	100	5391.81	100

TTV = Total treatment variation.

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

Table 3. 15 Relative impact for K as affected by first order interaction of *Meloidogyne* species and *Amaranthus* cultivars at 60 days after transplanting under greenhouse conditions.

	Nematodes					
	Experiment 1		Experiment 2		Experiment 3	
Cultivars	N_1	R.I. (%)	N_1	R.I. (%)	N_1	R.I. (%)
'Local 33'	46.08[bc]	–	53.46[abc]	–	53.36[abc]	–
'Applebosch'	44.32[bc]	–4	46.92[bc]	–12	48.36[bc]	–9
'Kobie'	38.65[c]	–16	59.03[ab]	10	50.52[bc]	–5
'Candatus'	50.64[abc]	10	55.05a[bc]	3	43.66[c]	–18
'Tanzania'	40.18[c]	–13	43.43[bc]	–19	46.14[c]	–14

[y]Column means followed by the same letter were not different according to Fisher's Least Significant Difference test.

[z]Relative impact – R.I. (%) = [(treatment/Control) – 1] x 100.

3.4 Discussion

3.4.1 Plant growth variables

Dry shoot mass: Cultivar ‘Kobie’ had higher dry shoot mass than the local cultivar, ′Applebosch′, ′Candatus′ and ′Tanzania′ under greenhouse conditions. Subsequently, cv. Kobie was better suited for high temperature and relative humidity under the lowveld conditions and coastal regions of the KwaZulu-Natal Province. Also, the observation on dry shoot mass confirmed the findings that high temperature and relative humidity conditions could favour biomass in some *Amaranthus* cultivars (Chavéz-Servín *et al*., 2017). Reduction in dry shoot mass for cultivars ′Applebosch′, ′Candatus′ and 'Tanzania′ might have been as a result of reduced photosynthesis during the closure of stomata when the high heat inside the greenhouse was combined with the wind streams responsible for extracting hot air. Shading, as observed on other crops, such as purple nutsedge, was instrumental to reduce dry shoot mass (Morales-Payan *et al*., 1997). In the current study, treatments were also blocked for shading in the morning and in the afternoon by the walls of the greenhouse, with substantial degrees of freedom.

Plant height: The interaction of cultivars of *Amaranthus* and nematode species affected plant height in Experiment 1 only, whereas in other experiments there were no significant effects. In cultivars inoculated with nematodes, there was a slight increase in plant height for cv. ‘Tanzania’ when compared with other cultivars that performed better in other variables. The different results could be due to preferential channelling of growth regulators as observed in heights of other *Amaranthus* cultivars (Chavéz-Servín *et al*., 2017).

Stem diameter: The highly significant effects of cultivars on stem diameter in Experiment 1 and Experiment 3 is in line with findings on stem diameter of *Amaranthus* cv. 'Hypochondriacus', which was highly significant at 98 days after transplanting under greenhouse conditions (Chavéz-Servín *et al.,* 2017). Relative to the local, cultivar Tanzania had reduced stem diameter in both Experiment 1 and Experiment 3, which differed with that of 'Kobie' in Experiment 1. Generally, growth conditions play an important role in determining stem diameter in plants. For instance, environmental factors that decrease and increase the sink-status of roots result in increasing and decreasing stem diameter of various plants, respectively (Syvertsen and Levy, 2005). In the current study, it was not obvious which environmental factors could have been in play. Evapotranspiration of the test cultivars could also have contributed to the dryness of the containers, with the degree of drought inducing some effects on the stem diameter (Syvertsen and Levy, 2005). Using various factors to regulate the root/shoot ratio, Mashela and Nthangeni (2002) demonstrated that the direction of the osmolytes played an important role on the degree of the stem diameter. For example, plants with increased sucrose to roots due to natural imbalances, invariably reduce stem diameters in order to serve as physical constrictions for reducing the movement of sucrose to roots, which could otherwise result in osmolysis of cells in roots (Mashela and Nthangeni, 2002).

Chlorophyll content: Chlorophyll content was higher in cv. 'Candatus when compared to other cultivars confirmed those of others when the cultivar was cultivated under shaded conditions (Kosma *et al.,* 2013; Vandana and Bhatt, 1999). The higher chlorophyll content in plants grown under shaded conditions was attributed to the increase in the number and size of chloroplast, with the biosynthesised chlorophyll

being regarded as an adaptive acclimation process to shaded growing conditions (Kosma *et al.,* 2013).

3.4.2 Selected malnutrition elements

Iron (Fe): Cultivars and nematodes each affected the accumulation of Fe in leaf tissues of *Amaranthus* cultivars under given growing conditions. All cultivars had a reduction in Fe leaf tissues when inoculated with nematodes. Generally, Fe as a heavy essential metal, appears to be sensitive to environmental factors that affect the root/shoot ratio. The metal Fe in plant tissues is primarily immobile and its deficiency symptoms occur in young leaves (Mashela *et al*., 1992; Salisbury and Ross, 1992). Also, it had been shown that infection of roots by various nematodes directly affect the availability of Fe in leaf tissues, as reported in various cases that included the citrus nematode, *Tylenchulus semipenetrans* (Mashela *et al*., 1992). Although some cases of Fe deficiency in plants infected with *Meloidogyne* species had been recorded in cleome (Rabothata, 2017) and in tomato plants treated with cucurbitacin-containing phytonematicides (Maake, 2018), the incidents are prominent in soils with low pH (Mashela, 2002). However, in the current study pH was not measured, but it had been shown that infection of plants by *Meloidogyne* species invariably reduce soil pH (Mashela, 2002).

Calcium (Ca): Treatments with nematodes reduced Ca in leaf tissues of cultivars ‘Caudatus’ and ‘Kobie’, the Ca content in the two cultivars was much better than that in the exotic cv. ‘Tanzania’. Reduction of Ca, particularly in Experiment 3 of this study

could also have contributed to the reduction in other cations such as K as clarified elsewhere (Mamphiswana *et al.,* 2011), who argued that the presence of Ca in a plant organ could contribute to an improved accumulation of K, *vice versa*.

Potassium (K): Treatments with nematodes reduced K in leaf tissues of *Amaranthus* by as high as 22 % in Experiment 1. The response of K to nematode infected plants in the current study agreed with that of Mashela *et al*. (2017), where nematodes infection reduced K from 23 to 45% in certain vegetables. Apparently, K, as one of the osmolytes, is very sensitive to nematode infection as reported widely (Duncan *et al*., 1995; Mashela, 2002; Mashela and Nthangeni, 2002; Mashela *et al*., 2017). Reduction in root/shoot ratio of *Amaranthus* in Experiment 3 could be providing some of the reason why there was a reduction on K content as explained elsewhere (Syvertsen and Levy, 2005). In an osmolyte study, Mashela and Nthangeni (2002) demonstrated that reduction in root/shoot ratio invariably provided scientific merit of reduction of K in root and leaf tissues of plants. Further, any factor that results in decreased transportation of K modifies the electro-osmotic potential in sieve tubes of the vascular bundle (Mashela and Nthangeni, 2002).

3.5 Conclusions

Cultivar 'Kobie′ was, in terms of dry shoot mass, better suited for the greenhouse conditions, whereas cv. ‘Candatus’ was having the highest chlorophyll content. These cultivars performed much better in the two attributes than the local and the exotic cultivars. However, all the cultivars were similarly affected by *Meloidogyne* species,

which tended to affect certain nutrient elements, particularly Fe, Ca and K, which are important nutrient elements. Since the listed nutrient elements are important in food security for the rural poor, it remains an important cultural practice that nematodes be managed in *Amaranthus* production.

CHAPTER 4

COMPARING GROWTH AND SELECTED NUTRIENT ELEMENTS OF EXTOTIC AND LOCAL *AMARANTHUS* CULTIVARS UNDER FIELD CONDITIONS

4.1 Introduction

The five test *Amaranthus* cultivars responded differently under greenhouse conditions. However, greenhouse results do not always translate to those under field conditions (Mamphiswana *et al.*, 2011; Moraghan, 1993). Although the variability under field conditions could be due to the continuous variability of the environment, variation on plant growth could also be due to nutrient element accumulation capabilities of plants, which could differ from plant to plant (Bowen, 1976; Moraghan, 1993; Mamphiswana *et al*., 2011). Under the greenhouse conditions, root-knot nematodes appeared to have played a major role on some of the observed variability in growth of the *Amaranthus* cultivars (Chavéz-Servín *et al*., 2017). Most soils in Limpopo Province are predominantly infested with root-knot nematodes (Mashela *et al*., 2013). Although certain *Amaranthus* species (*A. dubius, A. cruentus, A. hypochondrius*) were shown to have some degree of resistance to nematodes, the tested *Amaranthus* cultivars were shown to be highly susceptible to *M. incognita* under greenhouse conditions (Nchore *et al*., 2013). The objective of this study was to determine whether growth and selected malnutrition elements of three greenhouse-tested *Amaranthus* cultivars infected with and without *Meloidogyne* species under field conditions would be similar. The Null hypothesis suggested that growth and selected malnutrition elements of three

greenhouse-tested *Amaranthus* cultivars infected with and without *Meloidogyne* species under field conditions would not be similar.

4.2 Materials and methods

4.2.1 Description of study area

The field study was conducted at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, South Africa (23º53'10'' S, 29º44'15''E). The study was initiated in summer (November-January) 2016 (Experiment 1) and repeated in summer 2017 (Experiment 2) and summer 2018 (Experiment 3). The location has hot and dry summers, with daily maximum temperature being from 28 to 38°C. The average annual rainfall is less than 500 mm and occurs mainly in summer. Soil at the location was Hutton sandy loam (65% sand, 30% clay, 5% silt), with organic C = 1.6%, EC = 0.148 dS/m and pH (H_2O) = 6.5.

4.2.2 Treatments and research design

Under field conditions, a 3 × 2 factorial experiment was laid-out in randomised complete block design, with 10 replications. The first factor was three *Amaranthus* cultivars, namely, 'Local 33', 'Applebosch' and 'Candatus', whereas the second factor was with and without *Meloidogyne* species.

4.2.3 Procedures

The *Amaranthus* seeds were obtained from the Agricultural Research Council - Vegetable and Ornamental Plants. Seedlings of each cultivar were raised in seedling trays containing Hygromix-T (Hygrotech, Pretoria North, South Africa) growing

mixture. Soon after sowing, all seedling trays were irrigated to field capacity and then when necessary. Four weeks old seedlings were hardened-off outside of the greenhouse through intermittent withdrawal of irrigation water and when at least 50% seedlings had collapsed, the trays were taken to the shade and irrigated. After full recovery, the trays were returned to the sun. After seven days, the seedlings were ready for transplanting. Uniform seedlings were grown in 0.3 m × 0.5 m spacing, with the three cultivars randomised within the three rows, with border rows comprising the local cultivar (Figure 4.1). Seedlings for nematode treatments were inoculated with 1000 eggs and second-stage juveniles using mixture of *M. incognita* and *M. javanica,* previously raised on kenaf, using the syringe method. Plots of untreated control seedlings were treated with Velum synthetic nematicide. Seedlings were irrigated using 500 ml tapwater per plant every other day interval. Plants were fertilised once using 5 g 2:3:2 (26) NPK fertiliser mixture/plant, which provided a total of 155 mg N, 105 mg P and 130 mg K per ml water and Multifeed (Nulandies, Johannesburg) at 1 g 2:1:2 (43) which provided a total of 0.175 mg N, 0.16 mg K and 0.16 mg P, 0.45 mg, 0.378 mg Fe, 0.0375 mg Cu, 0.175 mg Zn, 0.5 mg B, 1.5 mg Mn and 0.035 mg Mo per ml water. Pests, were monitored daily, were not observed and therefore were not managed.

Figure 4.1 Establishment of three *Amaranthus* cultivars under field conditions.

4.2.4 Data collection

At 60 days after transplanting, plant height was measured from the soil level to the tip of the flag leaf, chlorophyll content was measured using a chlorophyll meter (MINOLTA, SPAD-502), stem diameter was measured using a digital Vernier calliper and shoots were cut at the soil surface and then oven-dried at 60ºC for 72 h for dry matter determination. Oven-dried leaves were ground in a Wiley mill to pass through a 1-mm sieve (Mashela *et al.*, 2017). Each powdered sample was digested in nitric acid at a concentration of 5%, and then mixed using vortex meter. All samples were incubated in a warm water bath at 95°C for an hour. The samples were left to cool down to room temperature, thereafter filtered and each container covered with foil (SW-846 EPA Method 3050B) to minimise direct contact with light. Samples were subjected to Atomic Absorption Spectrometry (AAS) at Limpopo Agro-food Technology Station (LATS) and analysed for Calcium (Ca), Iron (Fe), Potassium (K) and Zinc (Zn). Root samples were collected and assayed for plant-parasitic

nematodes using the local extraction methods. Nematodes were not counted since roots were all galled.

4.2.5 Data analysis

Data for nutrient variables were subjected to analysis of variance through the Statistix 10.0 software. Mean separation for significant ($P \leq 0.05$) treatment effects was achieved through the Fisher's Least Significance Difference test at the probability level of 5%. Unless otherwise stated, treatments were discussed at the probability level of 5%.

4. 3 Results

4.3.1 Selected plant growth variables

Dry shoot mass: The interaction did not have significant effects on dry shoot mass, whereas nematode and cultivar effects on dry shoot mass were significant in Experiment 1 and Experiment 2, contributing 38% and 76% in TTV of the variable in respective experiments (Table 4.1). Relative to cv. 'Local 33', cv. 'Tanzania' had 54% and 95% dry shoot mass in Experiment 1 and Experiment 2, whereas in Experiment 3 cv. 'Candatus' had the lowest dry shoot mass (Table 4.2). In Experiment 3, nematodes reduced dry shoot mass by 27% (Table 4.3).

Plant height: The interaction and nematodes each had no significant effects on plant height, whereas the cultivar effects were significant and highly significant on the variable in Experiment 1 and Experiment 2, respectively, contributing 49% and 53% in TTV of the variable (Table 4.4). Relative to cv. 'Local 33', plant height in cv. 'Tanzania'

was increased by 21% and 25% in Experiment 1 and Experiment 2, respectively (Table 4.5).

Table 4.1 Partitioning mean sum of squares for dry shoot mass of three cultivars of *Amaranthus* on plots with and without *Meloidogyne* species under field conditions at 60 days after transplanting.

		Experiment 1		Experiment 2		Experiment 3	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	9	63.762	16	16.463	10	17.637	10
Nematode (N)	1	147.267	38**	0.060	0^{ns}	80.968	48^{ns}
Cultivar (C)	2	122.950	31^{ns}	130.158	76***	49.464	29^{ns}
N × C	2	18.653	5^{ns}	12.063	7^{ns}	3.313	2^{ns}
Error	45	39.824	10	11.465	7	18.589	11
Total	59	392.456	100	170.209	100	169.971	100

TTV = Total treatment variation.

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

Table 4.2 Mean dry shoot mass (g) of two exotic *Amaranthus* cultivars relative to that of the undescribed 'Local 33' *Amaranthus* cultivar under field conditions.

	Experiment 1		Experiment 2		Experiment 3	
Cultivar	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
'Local 33'	7.13[ab]	–	5.10[b]	–	7.10[ab]	–
'Candatus'	6.33[b]	–11	6.19[b]	21	6.20[b]	–13
'Tanzania'	10.97[a]	54	9.96[a]	95	9.26[a]	30

[y]Column means followed by the same letter were not different according to Fisher's Least Significant Difference test. [z]R.I. = [(treatment/standard) – 1] × 100.

Table 4.3 Mean dry shoot mass content of treatments with nematodes (N_1) relative to those without nematodes (N_0) under field conditions.

	Experiment 1		Experiment 2		Experiment 3	
Nematodes	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
N_0	9.71	–	7.11	–	8.68[a]	
N_1	6.57	–32	7.05	–1	6.35[b]	–27
$LSD_{0.05}$	4.02		2.16		–	

[y]Column means followed by the same letter were not different according to Fisher's Least Significant Difference test. [z]R.I. = [(treatment/standard) – 1] × 100.

Table 4.4 Partitioning mean sum of squares for plant height of three cultivars of *Amaranthus* on plots with and without *Meloidogyne* species under field conditions at 60 days after transplanting.

		Experiment 1		Experiment 2		Experiment 3	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	9	108.983	10	136.938	20	76.268	11
Nematode (N)	1	231.909	22^{ns}	5.017	1^{ns}	285.580	42^{ns}
Cultivar (C)	2	506.525	49**	367.383	53***	15.842	2^{ns}
N × C	2	96.361	9^{ns}	120.187	17^{ns}	218.155	32^{ns}
Error	45	114.582	11	66.028	9	81.701	13
Total	59	1058.36	100	695.533	100	677.546	100

TTV = Total treatment variation.

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

Table 4.5 Mean plant height (cm) of two exotic *Amaranthus* cultivars relative to that of the undescribed ′Local 33′ *Amaranthus* cultivar under field conditions.

	Experiment 1		Experiment 2		Experiment 3	
Cultivar	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
′Local 33′	36.45[b]	–	33.68[b]	–	38.49	–
′Candatus′	34.77[b]	–4	38.56[ab]	14	36.75	–6
′Tanzania′	44.20[a]	21	42.22[a]	25	37.94	–1

[y]Column means followed by the same letter were not different according to Fisher's Least Significant Difference test. [z]R.I. = [(treatment/standard) – 1] × 100.

Stem diameter: In all three experiments, the interaction and nematode effects on stem diameter were not significant. However, the cultivar treatment was significant on stem diameter in Experiment 2, contributing 59% in TTV of the variable (Table 4.6). Relative to cv. ′Local 33′, 'Tanzania' had 54% bigger stem diameter, whereas those of the local and 'Candatus' were not different.

Chlorophyll content: In all three experiments, the interaction and cultivar effects on chlorophyll content were not significant. In contrast, the nematode effects on chlorophyll content were significant in Experiment 1, contributing 55% in TTV of the variable (Table 4.8). Relative to the untreated control, chlorophyll content for plots with nematodes was reduced by 8% (Table 4.9).

Table 4.6 Partitioning mean sum of squares for stem diameter of three cultivars of *Amaranthus* on plots with and without *Meloidogyne* species under field conditions at 60 days after transplanting.

		Experiment 1		Experiment 2		Experiment 3	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	9	13.002	11	22.259	11	13.215	21
Nematode (N)	1	52.267	46^{ns}	18.481	9^{ns}	23.877	37^{ns}
Cultivar (C)	2	31.781	28^{ns}	120.622	59**	10.359	16^{ns}
N × C	2	2.541	2^{ns}	9.999	5^{ns}	2.240	3^{ns}
Error	45	14.929	13	31.842	16	14.637	23
Total	59	114.52	100	203.203	100	64.328	100

TTV = Total treatment variation

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

Table 4.7 Mean stem diameter (mm) of two exotic *Amaranthus* cultivars relative to that of the undescribed 'Local 33' *Amaranthus* cultivar under field conditions.

	Experiment 1		Experiment 2		Experiment 3	
Cultivar	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
'Local 33'	10.105	–	8.97[b]	–	10.490	–
'Candatus'	9.415	–7	10.970[ab]	22	9.835	–6
'Tanzania'	11.860	17	13.855[a]	54	11.272	7
$LSD_{0.05}$	2.2461		–		18.893	

[y]Column means followed by the same letter were not different according to Fisher's Least Significant Difference test. [z]R.I. = [(treatment/standard) – 1] × 100.

Table 4.8 Partitioning mean sum of squares for chlorophyll content of three cultivars of *Amaranthus* on plots with and without *Meloidogyne* species under field conditions at 60 days after transplanting.

		Experiment 1		Experiment 2		Experiment 3	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	9	55.467	22	43.530	17	32.936	17
Nematode (N)	1	141.988	55**	58.608	23^{ns}	0.000	0^{ns}
Cultivar (C)	2	5.348	2^{ns}	96.019	38^{ns}	83.909	44^{ns}
N × C	2	19.527	8^{ns}	17.333	7^{ns}	36.611	19^{ns}
Error	45	34.560	13	34.582	14	35.413	20
Total	59	256.890	100	250.069	100	188.869	100

TTV = Total treatment variation

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

Table 4.9 Mean chlorophyll content of treatments with nematodes (N_1) relative to untreated control under field conditions.

	Experiment 1		Experiment 2		Experiment 3	
Nematodes	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
N_0	53.32[a]	–	51.88	–	50.44	–
N_1	49.24[b]	–8	49.91	–4	50.44	0
$LSD_{0.05}$	-		3.0582		3.0947	

[y]Column means followed by the same letter were not different according to Fisher's Least Significant Difference test.

[z]R.I. = [(treatment/standard) – 1] × 100.

4.3.2 Selected malnutrition elements

Iron (Fe): The interaction effects on Fe were not significant in Experiment 1 and Experiment 2, but were significant in Experiment 3, contributing 26 % in TTV of the variable (Table 4.10). Also, the cultivar effects on Fe were significant in Experiment 3, contributing 30% TTV of the variable (Table 4.10). Relative to cv. 'Local 33', cv. ′Candatus′ accumulated Fe by 48% and 26% in Experiment 1 and Experiment 2, respectively (Table 4.11).

Calcium (Ca): The interaction effects on Ca were not significant in Experiment 1 and Experiment 3, but were significant in Experiment 2, contributing 28% in TTV of the

variable (Table 4.12). The cultivar and nematode effects on Ca were significant in Experiment 1 and Experiment 2, respectively, contributing 68% and 53% in TTV of the variable, respectively (Table 4.12). Relative to cv. 'Local 33', cv. 'Tanzania' reduced Ca by 34%, whereas Ca in 'Candatus' was not different to those in either cultivar (Table 4.13).

Table 4.10 Partitioning mean sum of squares for Fe of three cultivars of *Amaranthus* on plots with and without *Meloidogyne* species under field conditions at 60 days after transplanting.

		Experiment 1		Experiment 2		Experiment 3	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	4	4.181	15	9.521	17	0.854	16
Nematode (N)	1	5.852	20^{ns}	26.219	48^{ns}	1.153	21^{ns}
Cultivar (C)	2	5.892	21^{ns}	8.176	15^{ns}	1.626	30**
N × C	2	7.559	26^{ns}	4.957	9^{ns}	1.436	26**
Error	20	5.210	18	5.808	11	0.370	7
Total	29	28.694	100	54.681	100	5.439	100

TTV = Total treatment variation.

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

Table 4.11 Relative impact for Fe as affected by first order interaction of nematodes and *Amaranthus* cultivars at 60 days after treatment under field conditions.

Cultivar	Nematode					
	Experiment 1		Experiment 2		Experiment 3	
	N_1	R.I. (%)	N_1	R.I. (%)	N_1	R.I. (%)
Local 33′	1.75[ab]	–	5.07[ab]	–	1.51	–
′Candatus′	2.59[a]	48	6.30[a]	24	1.37	–9
′Tanzania′	2.44[ab]	39	3.64[ab]	–28.01	1.18	–22
$LSD_{0.05}$	–		–		12.610	

[y]Column means followed by the same letter were not different according to Fisher's Least Significant Difference test. [z]R.I. = [(treatment/standard) – 1] × 100.

Table 4.12 Partitioning mean sum of squares for Ca of three cultivars of *Amaranthus* on plots with and without *Meloidogyne* species under field conditions at 60 days after transplanting.

		Experiment 1		Experiment 2		Experiment 3	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	4	370.37	14	806.01	10	681.475	33
Nematode (N)	1	29.40	1^{ns}	4478.14	53^{***}	178.657	9^{ns}
Cultivar (C)	2	1757.77	68^{**}	462.81	5^{ns}	629.553	31^{ns}
N × C	2	34.43	13^{ns}	2360.08	28^{**}	44.441	2^{ns}
Error	20	395.56	15	406.14	5	520.108	25
Total	29	2587.53	111	8513.18	101	2054.234	100

TTV = Total treatment variation.

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

Table 4.13 Two-way table for Ca as affected by first order interaction of nematode and *Amaranthus* cultivars at 60 days after treatment under greenhouse conditions (Experiment 2).

	Nematode			
Cultivar	N_0[y]	R.I. (%)	N_1	R.I. (%)
'Local 33'	78.36[a]	–	35.09[cd]	–
'Candatus'	65.66[ab]	–16	24.60[d]	–30
'Tanzania'	51.58[bc]	–34	62.60[ab]	78

[y]Column means followed by the same letter were not different according to Fisher's Least Significant Difference test. [z]R.I. = [(treatment/standard) – 1] × 100.

Potassium (K): In all three experiments, the interaction and nematode effects on dry shoot mass were each not significant. However, foliar K was significantly affected by cultivar effects in Experiment 1, nematode effects in Experiment 3, contributing 12 and 78% in TTV of the variable in the respective experiments (Table 4.14). Relative to cv. 'Local 33', cv. 'Tanzania' reduced leaf K by 22%, but the former and cv. 'Candatus' contained similar K levels in Experiment 1 (Table 4.15). In contrast, relative to cv. 'Local 33' cv. 'Tanzania' had higher K in leaf tissues in Experiment 2 and Experiment 3, whereas that in 'Candatus' was similar to that of the standard in both experiments. Relative to nematode-free plots, nematode reduced foliar K by 30% in Experiment 3 (Table 4.16).

Sodium (Na): In all three experiments, the interaction and cultivar effects on foliar Na were not significant. Nematode effects on foliar Na were significant in Experiment 3, contributing 71% in TTV of the variable (Table 4.17). Relative to plots without nematodes, nematodes reduced foliar Na by 41% in Experiment 3 (Table 4.18).

Table 4.14 Partitioning mean sum of squares for K of three cultivars of *Amaranthus* on plot with and without *Meloidogyne* species under field conditions at 60 days after transplanting.

		Experiment 1		Experiment 2		Experiment 3	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	4	1324.30	32	447.60	23	58.72	2
Nematode (N)	1	337.1	8^{ns}	2.39	0^{ns}	2233.79	78**
Cultivar (C)	2	479.52	12**	1149.86	59^{ns}	74.97	3^{ns}
N × C	2	1012.55	25^{ns}	98.89	5^{ns}	216.02	8^{ns}
Error	20	959.0	23	246.14	13	274.07	10
Total	29	4113.08	100	1944.88	100	2857.57	101

TTV = Total treatment variation.

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

Table 4.15 Mean K of three exotic *Amaranthus* cultivars relative to that of the undescribed 'Local 33' *Amaranthus* cultivar under field conditions.

	Experiment 1		Experiment 2		Experiment 3	
Cultivar	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
'Local 33'	59.23[a]	–	64.76[ab]	–	46.63[b]	–
'Candatus'	58.43[a]	–1	50.91[b]	–21	47.52[b]	2
'Tanzania'	46.86[b]	–22	72.01[a]	11	51.75[a]	11

[y]Column means followed by the same letter were not different according to Fisher's Least Significant Difference test.

[z]R.I. = [(treatment/standard) – 1] × 100.

Table 4.16 Mean K content of *Amaranthus* with nematodes (N_1) relative to those without nematodes (N_0) under field conditions.

	Experiment 1		Experiment 2		Experiment 3	
Nematode	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
N_0	51.48	–	62.28	–	57.26[a]	–
N_1	58.19	13	62.84	1	40.00[b]	–30
$LSD_{0.05}$	23.593		11.950		-	

[y]Column means followed by the same letter were not different according to Fisher's Least Significant Difference test.

[z]R.I. = [(treatment/standard) –1] × 100.

Table 4.17 Partitioning mean sum of squares for Na of three cultivars of *Amaranthus* on plots with and without *Meloidogyne* species under field conditions at 60 days after transplanting.

		Experiment 1		Experiment 2		Experiment 3	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	4	0.823	7	2.139	29	0.267	9
Nematode (N)	1	0.605	5^{ns}	0.245	3^{ns}	2.187	71**
Cultivar (C)	2	1.113	9^{ns}	0.897	12^{ns}	0.152	5^{ns}
N × C	2	4.390	37^{ns}	2.248	30^{ns}	0.171	6^{ns}
Error	20	5.044	42	1.877	25	0.321	9
Total	29	11.975	100	7.406	100	3.098	100

TTV = Total treatment variation.

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

Table 4.18 Mean Na content of treatments with nematodes (N_1) relative to those without nematodes (N_0) under field conditions.

	Experiment 1		Experiment 2		Experiment 3	
Nematode	Variable	R.I. (%)	Variable	R.I. (%)	Variable[y]	R.I. (%)
N_0	2.82	–	2.50	–	1.34[a]	–
N_1	3.10	10	2.32	–7	0.79[b]	–41
$LSD_{0.05}$	1.7106		1.0436		-	

[y]Column means followed by the same letter were not different according to Fisher's Least Significant Difference test.

[z]R.I. = [(treatment/standard) – 1] × 100.

4.4 Discussion

4.4.1 Selected plant growth variables

Dry shoot mass: The N × C interaction did not have significant effects on dry shoot mass, suggesting that the null hypothesis, which hypothesizes that there was no relationship between the measured variables should be accepted (Salisbury and Ross, 1992). Generally, a data set is typically deemed to be statistically significant if the probability of the phenomenon being random is less than 1/20, resulting in a p-value of 5% (Little and Hills, 1978). In a number of measured variables, there were no relationships between *Amaranthus* cultivars and nematodes. Notwithstanding, occasionally each of the factors had significant effects on the variables. For instance,

nematodes had effects on dry shoot mass, which confirmed Ntidi *et al*. (2012) that most *Amaranthus* cultivars were highly susceptible to nematodes, especially *Meloidogyne* species. However, recent work demonstrated that there were ARC *Amaranthus* cultivars that were resistant to various tropical root-knot nematodes, namely, *M. incognita* race 2, *M. incognita* race 4 and *M. javanica* (Steyn *et al*., 2012). In two of the current experiments, cv. ′Tanzania′ outperformed the other two cultivars, suggesting that it might be suited for high temperatures that are common in Limpopo Province, due to its tropical origin.

Plant height: The interaction and nematodes each had no significant effects on plant height, whereas the cultivar had significant effects. As observed in dry shoot mass, cv. ′Tanzania′ outperformed the other two cultivars, thereby having a competitive edge in intercepting solar radiation and therefore being a better competitor to shorter plants (Mashela and Nchabeleng, 2000).

Stem diameter: The interaction and nematode effects on stem diameter were not significant. Generally, plants that are heavily infected by root-knot nematodes have smaller stem diameters (Mashela and Nthangeni, 2002), which was explained elsewhere as being important in ensuring that photosynthate movement towards the root systems is restricted as a measure to regulate turgor pressure (Mashela and Nthangeni, 2002). Apparently, plants with smaller stem diameters could be suitable for drought tolerance as explained in fruit trees (Syvertsen and Levy, 2005). In the present study, cv. 'Tanzania' had the largest (54%) stem diameters, an attribute which must be taken into consideration when making selections for Limpopo Province, which

is generally an arid region, with most regions having less than 500 mm rainfall per annum (Mzezewa *et al*., 2010).

Chlorophyll content: All factors except nematodes had no effects on chlorophyll of Amaranthus, whereas nematodes reduced the variable. In another study where Amaranthus-nematode relations were assessed (Ntidi *et al*., 2012). Generally, nematodes affect chlorophyll by inducing imbalances in essential nutrient elements (Mashela *et al*., 1991), as it was confirmed below.

4.4.2 Selected malnutrition elements

Iron (Fe): The interaction effects on Fe were not significant in other experiments except in Experiment 3. In contrast to the explanation above (4.4.1), a significant interaction implies that there is a relation between the factors in relation to the test variable. The major contributing factor was cultivar differences, whereas the effects of nematode infection on Fe were less clear. Generally, Fe is responsible for the formation of chlorophyll in plants (Cohen *et al*., 1998) and it is required in small quantities. Due to the immobility of Fe in plants, deficiency symptoms occur in young leaves (Cohen *et al*., 1998) but in the current study, only mature healthy leaves was sampled. Iron is an important element in biofortification of crops (Pofu *et al*., 2016) and in humans it is essential for blood production (Latunde-Dada, 1990).

Calcium (Ca): The interaction for Ca was significant in Experiment 2 and this element is usually responsible for holding together cell walls and improves water penetration

in plants (Christiansen and Foy, 1979). In the current study, cv. 'Tanzania' was the poorest bio-accumulator of Ca, which should be taken into consideration when attempting to adopt it. In humans, Ca is essential for bone formation (Dosunmu, 1997).

Potassium (K): The major factor in K accumulation was cultivar differences, without any effect of nematodes or the interactions. Potassium, along with Na and Cl, are technically referred to as osmoticum ions (Mashela and Nthangeni, 2002), which play important roles in osmoregulation in roots (Mashela and Nthangeni, 2002). Generally, in plants that are susceptible to nematodes, Na and Cl accumulate in leaf tissues and decrease in root tissues, whereas K is reduced in both leaf and root tissues (Mashela and Nthangeni, 2002). As observed in other nutrient elements, K was poorly accumulated in leaf tissues of cv. 'Tanzania'. In addition to osmoregulation in plants, K is also responsible for the opening and closing of stomata and therefore regulates carbon dioxide uptake in plants (Madaure *et al*., 2017). Incidentally, in humans, K and Na play a role in hypertension (Turan *et al*., 2003), with a preferable balance occurring when K is higher than Na in urine (Yatabe *et al*., 2017) Consequently, cv Tanzania would not be suitable for playing the roles related to lowering the hypertension risks.

Sodium (Na): Nematode effects on Na were significant in Experiment 3, with the factor reducing the variable by as high as 41% in other trials. As indicated earlier, this observation did not support others under conditions infested with nematodes. Generally, Na is required in C_4 plants for metabolism (Brownell and Crossland, 1971). In exceptional cases, it is also required for improving fruit quality in tomato plants (Idowu and Aduayi, 2008). In *Amaranthus* species, the lowering of Na under conditions

with nematodes is important since this would ensure that the Na:K ratio in urine is always less than unity and therefore, ameliorating incidents of hypertension in humans.

4.5 Conclusions

Cultivar Tanzania can be better suited in Limpopo province with an interest of growth and yield. The plant height and stem diameter for cv Tanzania was much better in terms of plant height and stem diameter which makes it most preferred in Limpopo province for better yield. However, in the presence of nematodes all cultivars had reduced chlorophyll content. There was a clear relationship between nematodes and nutrient variables tested, and as such cultivars responded differently. Calcium accumulation in cv. ‘Tanzania’ was affected by the presence of nematodes and therefore proper management should be taken into consideration when planting this cultivar in Limpopo Province as many soils are infested with nematodes.

CHAPTER 5

SUMMARY OF FINDINGS, SIGNIFICANCE, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary of findings

Greenhouse experiments: Dry shoot mass was significant on Experiment 1 and 2, but their effects were not different. However, cv. 'Kobie' had significant higher dry shoot mass than ′Applebosch′, ′Candatus′ and 'Tanzania′. Plant height was affected by the interaction of nematodes and cultivars in Experiment 1, wherein in the absence of nematodes there was no variation in plant height. Cultivars 'Applebosch', ′Kobie′ and ′Candatus′ had significant differences on plant height for nematodes treatments. In contrast, their impact was different to that of ′Local 33′, which was not different to that of ′Tanzania′. *Amaranthus* cultivars were highly significant on experiment 1 and 3. Cultivar Kobie slightly increased stem diameter by 16%, meanwhile, cv. 'Tanzania' reduced it by 35%. This implies that cv. ′Tanzania′ has significantly reduced stem diameter followed by ′Candatus′ and then ′Applebosch′. The cultivar effects on chlorophyll content were highly significant in Experiment 3. Relative to ′Local 33′, the cultivar effects on chlorophyll content on cv. 'Candatus' and ′Tanzania′ were different to those of ′Applebosch′ which were not different to those of ′Kobie′ in Experiment 2. Cultivar 'Candatus' had more chlorophyll content followed by ′Tanzania′ and ′Kobie′.

The interaction of nematodes and *Amaranthus* cultivars were significant on Experiment 1. Relative to the cv. 'Local 33', Fe content for all cultivars without nematode were not different. However, cv. 'Tanzania' had more effects on Fe than

′Applebosch′, ′Candatus′ and ′Kobie′. In other words, cv. ‘Tanzania’ highly reduced the concentration of Fe by 78% on treatments with nematodes. Cultivar and nematode effects on Ca were significant in Experiment 1 and 3, respectively. Compared to treatment without, nematode effects on Ca were not having a significant difference in Experiment 1 and 2. Meanwhile, on Experiment 3, nematode effects were different to those without nematodes. Therefore, nematode treatments had less Ca concentration compared to those without nematodes. Relative to ′Local 33′, Ca on cultivar effects had no significant difference in Experiment 2 and 3, respectively. However, relative to ′Local 33′, cv. ‘Candatus’ had a relatively higher Ca content and its effects were not different to those of ′Kobie’. The interaction effects on K was significant on Experiment 3, meanwhile, the cultivar effects on K were significant on Experiment 2. In contrast, Nematode effects on K were significant in Experiment 1 and Experiment 2. Relative to ′Local 33′, treatment with nematode effects on K had no significant difference. Relative to ′Local 33′, cv. ‘Applebosch’ was significantly different but its effects were not different to those of ′Kobie′, ′Kobie′ and ′Candatus′

Field experiments: the cultivar effects on dry shoot mass were highly significant in Experiment 2. Meanwhile, the nematode effects on dry soot mass were significant in Experiment 1, relatively, dry shoot mass for all cultivars was not having significant differences in Experiment 1 and 3, respectively. However, cv. ′Tanzania′ had significant effects on dry shoot mass, which was lower than ′Local 33′. The cultivar effects on plant height were significant in Experiment 1 and Experiment 2. Relative to the cv. ′Local 33′, plant height for all cultivars was not having significant differences on Experiment 1 and 3, respectively. However, cv. ′Tanzania′ had different effects, accordingly, it had relatively higher plant height than ′Candatus′. However, the cultivar

effects on stem diameter were significant in Experiment 2 with no significant differences. However, cv. ′Tanzania′ had higher stem diameter compared to ′Candatus′. Nematode effects on chlorophyll content was significant in Experiment 1, while chlorophyll content for all nematode treatments were not having significant differences in Experiment 2 and 3, respectively. However, treatments with nematodes reduced chlorophyll content and its effect were relatively different to those with nematodes.

The interaction effects on Fe were significant on Experiment 3. Meanwhile, the cultivar effects on Fe were only significant on Experiment 3. The effects of Fe for all cultivars were not having significant differences. However, relative to cv. ′Local 33′ without nematodes, Fe for ′Candatus′ was having significant differences. Cultivar ‘Candatus’ was having a relatively higher Fe content than ′Tanzania′. The interaction effects on Ca were significant on Experiment 2. The cultivar and nematode effects on Ca was significant on Experiment 1 and Experiment 2. ′Tanzania′ was having significant effects on Ca content, however, its effects were not different to those of ′Candatus′. In contrary, cv. Tanzania was having a relatively higher Ca content. The cultivar effects on K were significant on Experiment 1, meanwhile, nematode effects on K were significant on Experiment 3. K for all cultivars was not having significant differences. However, cv. Tanzania had significant higher K than ′Candatus′. Treatments with nematodes had no significant differences in Experiment 1 and 2. However, in Experiment 3, relative to treatments without nematodes, treatments with nematodes on K had different effects. Nematode effects on Na were significant on Experiment 3.

Only Na in treatments with nematodes was having significant difference. Therefore, nematodes treatments had reduced Na content than in those without nematodes.

5.2 Significance

The current study demonstrated that nutrient accumulation is influenced by plating conditions and also crop cultivar. Therefore, this implies that choosing a suitable condition to grow *Amaranthus* is influenced by nutrient of interest. This research further provides a baseline for choosing a specific cultivar that may yield better results.

5.3 Recommendations

There is a need to investigate the influence of growing conditions on the growth and nutrient accumulation of similar *Amaranthus* cultivars tested. There are other locally available *Amaranthus* accessions that are of importance to consumers and are currently consumed which also needs to be tested to their adaptability to different growing conditions and how they retain those important nutrients.

5.4 Conclusions

The study results revealed that cultivar Kobie performed better in the greenhouse in terms of dry shoot mass and stem diameter under the greenhouse conditions. Meanwhile, cv. ‘Tanzania’ reduced stem diameter under such given conditions. Furthermore, ′Candatus′ has more chlorophyll content than ′Kobie′, ′Candatus′ and ′Tanzania′. Both Kobie′ and ′Candatus′ increased the accumulation of Ca under

greenhouse conditions. In the interaction of *Amaranthus* cultivars and nematodes, treatments with nematodes had less Ca concentration. Under field conditions an exotic cultivar 'Tanzania' had higher dry shoot mass, plant height, stem diameter, Ca and K as compared to 'Candatus' and 'Local 33'. However, cv. 'Candatus' had more Ca content in the field as well. The interaction of *Amaranthus* cultivars and nematodes resulted in the reduction in Na content under field conditions.

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APPENDICES

Appendix 3.1 Analysis of variance for dry shoot mass of five cultivars of *Amaranthus* inoculated with and without *Meloidogyne* species under greenhouse conditions at 60 days after transplanting (Experiment 1).

Source	DF	SS	MS	F	P ≤
Replication	9	133.611	14.8456		
Nematode (N)	1	0.224	0.2237	0.34	0.5619
Cultivar (C)	4	9.736	2.4340	3.69	0.0082
N × C	4	2.145	0.5362	0.81	0.5206
Error	81	53.434	0.6597		
Total	99	199.149			

Appendix 3.2 Analysis of variance for dry shoot mass of five cultivars of *Amaranthus* inoculated with *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions at 60 days after transplanting (Experiment 2).

Source	DF	SS	MS	F	P ≤
Replication	9	38.286	4.25404		
Nematode (N)	1	0.410	0.40960	0.28	0.5964
Cultivar (C)	4	17.461	4.36535	3.01	0.0227
N × C	4	1.601	0.40035	0.28	0.8925
Error	81	117.352	1.44879		
Total	99	175.110			

Appendix 3.3 Analysis of variance for plant height of five cultivars of *Amaranthus* inoculated with *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions at 60 days after transplanting (Experiment 1).

Source	DF	SS	MS	F	P ≤
Replication	9	712.06	79.118		
Nematode (N)	1	46.79	46.786	0.88	0.3516
Cultivar (C)	4	673.33	168.332	3.16	0.0183
N × C	4	778.24	194.560	3.65	0.0087
Error	81	4317.48	53.302		
Total	99	6527.89			

Appendix 3.4 Analysis of variance for plant height of five cultivars of *Amaranthus* inoculated with *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions at 60 days after transplanting (Experiment 2).

Source	DF	SS	MS	F	P ≤
Replication	9	563.33	62.592		
Nematode (N)	1	11.76	11.765	0.24	0.6274
Cultivar (C)	4	1361.41	340.353	6.87	0.0001
N × C	4	31.66	7.914	0.16	0.9581
Error	81	4014.78	49.565		
Total	99	5982.94			

Appendix 3.5 Analysis of variance for stem diameter of five cultivars of *Amaranthus* inoculated with *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions at 60 days after transplanting (Experiment 1).

Source	DF	SS	MS	F	P ≤
Replication	9	23.467	2.6074		
Nematode (N)	1	0.422	0.4225	0.54	0.4642
Cultivar (C)	4	48.220	12.0551	15.43	0.0000
N × C	4	7.554	1.8885	2.42	0.0552
Error	81	63.266	0.7811		
Total	99	142.930			

Appendix 3.6 Analysis of variance for stem diameter of five cultivars of *Amaranthus* inoculated with *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions at 60 days after transplanting (Experiment 3).

Source	DF	SS	MS	F	P ≤
Replication	9	32.170	3.5745		
Nematode (N)	1	2.924	2.9241	1.44	0.2332
Cultivar (C)	4	92.721	23.1803	11.43	0.0000
N × C	4	4.531	1.1329	0.56	0.6932
Error	81	164.200	2.0272		
Total	99	296.548			

Appendix 3.7 Analysis of variance for chlorophyll content of five cultivars of *Amaranthus* inoculated with *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions at 60 days after transplanting (Experiment 3).

Source	DF	SS	MS	F	P ≤
Replication	9	1354.90	150.544		
Nematode (N)	1	78.06	78.057	2.07	0.1542
Cultivar (C)	4	2274.65	568.663	15.07	0.0000
N × C	4	76.77	19.193	0.51	0.7294
Error	81	3055.59	37.723		
Total	99	6839.97			

Appendix 3.8 Analysis of variance for Fe in leaf tissues of five cultivars of *Amaranthus* inoculated with *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions at 60 days after transplanting (Experiment 1).

Source	DF	SS	MS	F	P ≤
Replication	9	3.908	0.9770		
Nematode (N)	1	0.162	0.1624	0.04	0.8351
Cultivar (C)	4	4.341	1.0851	0.29	0.8802
N × C	4	44.790	11.1976	3.03	0.0298
Error	81	133.036	3.6954		
Total	99	186.237			

Appendix 3.9 Analysis of variance for Ca in leaf tissues of five cultivars of *Amaranthus* inoculated with *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions at 60 days after transplanting (Experiment 1).

Source	DF	SS	MS	F	P ≤
Replication	9	2335.8	583.95		
Nematode (N)	1	690.1	690.14	1.57	0.2179
Cultivar (C)	4	5937.0	1484.25	3.38	0.0190
N × C	4	1839.3	459.84	1.05	0.3963
Error	81	15798.1	438.84		
Total	99	26600.3			

Appendix 3.10 Analysis of variance for Ca in leaf tissues of five cultivars of *Amaranthus* inoculated with *Meloidogyne incognita* under and *Meloidogyne javanica* greenhouse conditions at 60 days after transplanting (Experiment 3).

Source	DF	SS	MS	F	P ≤
Replication	9	848.9	212.22		
Nematode (N)	1	2170.1	2170.09	8.04	0.0075
Cultivar (C)	4	758.7	189.69	0.70	0.5954
N × C	4	951.2	237.80	0.88	0.4852
Error	81	9720.9	270.02		
Total	99	14449.8			

Appendix 3.11 Analysis of variance for K in leaf tissues of five cultivars of *Amaranthus* inoculated with *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions at 60 days after transplanting (Experiment 3).

Source	DF	SS	MS	F	P ≤
Replication	9	619.9	154.98		
Nematode (N)	1	2769.2	2769.17	7.93	0.0078
Cultivar (C)	4	3745.9	936.48	2.68	0.0468
N × C	4	4740.6	1185.16	3.40	0.0186
Error	81	12564.6	349.02		
Total	99	24440.2			

Appendix 3.12 Analysis of variance for K in leaf tissues of five cultivars of *Amaranthus* inoculated with *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions at 60 days after transplanting (Experiment 2).

Source	DF	SS	MS	F	P ≤
Replication	9	4513.58	1128,39		
Nematode (N)	1	4.45	4.45	0.02	0.8803
Cultivar (C)	4	2741.14	685.29	3.55	0.0163
N × C	4	554.00	138.50	0.72	0.5861
Error	81	6370.05	193.03		
Total	99	4513.58	1128.39		

Appendix 3.13 Analysis of variance for K in leaf tissues of five cultivars of *Amaranthus* inoculated with *Meloidogyne incognita* and *Meloidogyne javanica* under greenhouse conditions at 60 days after transplanting (Experiment 1).

Source	DF	SS	MS	F	P ≤
Replication	9	1292.6	323.15		
Nematode (N)	1	1898.9	1898.88	7.74	0.0085
Cultivar (C)	4	911.6	227.90	0.93	0.4580
N × C	4	1141.7	285.43	1.16	0.3431
Error	81	8831.7	245.33		
Total	99	14076.5			

Appendix 4.1 Analysis of variance for dry shoot mass of three cultivars of *Amaranthus* inoculated with and without *Meloidogyne* species under field conditions at 60 days after transplanting (Experiment 2).

Source	DF	SS	MS	F	P ≤
Replication	9	148.165	16.463		
Nematode (N)	1	0.060	0.060	0.01	0.9426
Cultivar (C)	2	260.316	130.158	11.35	0.0001
N × C	2	24.126	12.063	1.05	0.3576
Error	45	515.922	11.465		
Total	59	948.590			

Appendix 4.2 Analysis of variance for dry shoot mass of three cultivars of *Amaranthus* inoculated with and without *Meloidogyne* species under field conditions at 60 days after transplanting (Experiment 1).

Source	DF	SS	MS	F	P ≤
Replication	9	573.85	63.762		
Nematode (N)	1	147.27	147.267	3.70	0.0608
Cultivar (C)	2	245.90	122.950	3.09	0.0554
N × C	2	37.31	18.653	0.47	0.6290
Error	45	1792.10	39.824		
Total	59	2796.42			

Appendix 4.3 Analysis of variance for plant height of three cultivars of *Amaranthus* inoculated with and without *Meloidogyne* species under field conditions at 60 days after transplanting (Experiment 1).

Source	DF	SS	MS	F	P ≤
Replication	9	980.85	108.983		
Nematode (N)	1	231.91	231.909	2.02	0.1617
Cultivar (C)	2	1013.05	506.525	4.42	0.0177
N × C	2	192.72	96.361	0.84	0.4380
Error	45	5156.17	114.582		
Total	59	7574.70			

Appendix 4.4 Analysis of variance for plant height of three cultivars of *Amaranthus* inoculated with and without *Meloidogyne* species under field conditions at 60 days after transplanting (Experiment 2).

Source	DF	SS	MS	F	P ≤
Replication	9	1232.45	136.938		
Nematode (N)	1	5.02	5.017	0.08	0.7841
Cultivar (C)	2	734.77	367.383	5.56	0.0069
N × C	2	240.37	120.187	1.82	0.1737
Error	45	2971.28	66.028		
Total	59	5183.88			

Appendix 4.5 Analysis of variance for stem diameter of three cultivars of *Amaranthus* inoculated with and without *Meloidogyne* species under field conditions at 60 days after transplanting (Experiment 2).

Source	DF	SS	MS	F	P ≤
Replication	9	200.33	22.259		
Nematode (N)	1	18.48	18.481	0.58	0.4501
Cultivar (C)	2	241.24	120.622	3.79	0.0302
N × C	2	20.00	9.999	0.31	0.7321
Error	45	1432.90	31.842		
Total	59	1912.96			

Appendix 4.6 Analysis of variance for chlorophyll content of three cultivars of *Amaranthus* inoculated with and without *Meloidogyne* species under field conditions at 60 days after transplanting (Experiment 1).

Source	DF	SS	MS	F	P ≤
Replication	9	499.20	55.467		
Nematode (N)	1	141.99	141.988	4.11	0.0486
Cultivar (C)	2	10.70	5.348	0.15	0.8571
N × C	2	39.05	19.527	0.57	0.5723
Error	45	1555.18	34.560		
Total	59	2246.12			

Appendix 4.7 Analysis of variance for Fe of three cultivars of *Amaranthus* inoculated with and without *Meloidogyne* species under field conditions at 60 days after transplanting (Experiment 3).

Source	DF	SS	MS	F	P ≤
Replication	9	3.4143	0.85359		
Nematode (N)	1	1.1533	1.15326	3.12	0.0926
Cultivar (C)	2	3.2523	1.62613	4.40	0.0261
N × C	2	2.8713	1.43566	3.88	0.0376
Error	45	7.3928	0.36964		
Total	59	18.0840			

Appendix 4.8 Analysis of variance for Ca of three cultivars of *Amaranthus* inoculated with and without *Meloidogyne* species under field conditions at 60 days after transplanting (Experiment 1).

Source	DF	SS	MS	F	P ≤
Replication	9	1481.5	370.37		
Nematode (N)	1	29.4	29.40	0.07	0.7879
Cultivar (C)	2	3515.5	1757.77	4.44	0.0253
N × C	2	68.9	34.43	0.09	0.9170
Error	45	7911.1	395.56		
Total	59	13006.4			

Appendix 4.9 Analysis of variance for Ca of three cultivars of *Amaranthus* inoculated with and without *Meloidogyne* species under field conditions at 60 days after transplanting (Experiment 2).

Source	DF	SS	MS	F	P ≤
Replication	9	3224.1	806.01		
Nematode (N)	1	4478.1	4478.14	11.03	0.0034
Cultivar (C)	2	925.6	462.81	1.14	0.3399
N × C	2	4720.2	2360.08	5.81	0.0102
Error	45	8122.8	406.14		
Total	59	21470.8			

Appendix 4.10 Analysis of variance for K of three cultivars of *Amaranthus* inoculated with and without *Meloidogyne* species under field conditions at 60 days after transplanting (Experiment 2).

Source	DF	SS	MS	F	P ≤
Replication	9	1790.38	447.60		
Nematode (N)	1	2.39	2.39	0.01	0.9225
Cultivar (C)	2	2299.71	1149.86	4.67	0.0216
N × C	2	197.77	98.89	0.40	0.6744
Error	45	4922.81	246.14		
Total	59	9213.08			

Appendix 4.11 Analysis of variance for K of five cultivars of three cultivars of *Amaranthus* inoculated with and without *Meloidogyne* species under field conditions at 60 days after transplanting (Experiment 3).

Source	DF	SS	MS	F	P ≤
Replication	9	234.90	58.72		
Nematode (N)	1	2233.79	2233.79	8.15	0.0098
Cultivar (C)	2	149.95	74.97	0.27	0.7635
N × C	2	432.04	216.02	0.79	0.4683
Error	45	5481.44	274.07		
Total	59	8532.11			

Appendix 4.12 Analysis of variance for Na of three cultivars of *Amaranthus* inoculated with and without *Meloidogyne* species under field conditions at 60 days after transplanting (Experiment 3).

Source	DF	SS	MS	F	P ≤
Replication	9	1.06675	0.26669		
Nematode (N)	1	2.18725	2.18725	6.81	0.0173
Cultivar (C)	2	0.30385	0.15193	0.47	0.6304
N × C	2	0.34217	0.17109	0.53	0.5957
Error	45	6.10632	0.32139		
Total	59	1.06675	0.26669		