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NUTRITIONAL VALUE AND CULTIVATION REQUIREMENTS OF *CLEOME GYNANDRA* L.: A WILD VEGETABLE GROWING IN THE EASTERN CAPE PROVINCE, SOUTH AFRICA.

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A thesis submitted in fulfilment of the requirements for

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DECLARATION

I, Linda Ifeanyichukwu Sowunmi, declare that this thesis, submitted to the University of Fort Hare for the degree of Doctor of Philosophy in Ethnobotany in the Faculty of Science and Agriculture, is my original work; and that this work has not been submitted at any other University for the award of any degree.

I also declare that I am fully aware of the University of Fort Hare policy on plagiarism and have taken every precaution to comply with the regulations of the University.

Again, I declare that I am fully aware of the University of Fort Hare policy on research ethics and was cleared to conduct my research.

Signed …… at the University of Fort Hare this …….day of the month of …… year 2015.

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DEDICATION

To my friend, partner and soulmate, Akindayo, a man whose unending love, support and confidence has brought me this far.

and

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TABLE OF CONTENTS

Declaration ii

Acknowledgements iii

Dedication v

Abstract vii

1. General introduction 1
2. Micromorphology of *Cleome gynandra* from the Eastern Cape Province, South Africa 17
3. Mineral composition and proximate analysis of *Cleome gynandra*: an underutilized wild vegetable in the Eastern Cape Province, South Africa 34
4. Phytochemical constituents and antioxidant properties of aqueous and ethanolic extract of *Cleome gynandra* 56
5. Effect of environmental factors and sowing depth on seed germination in *Cleome gynandra* L 80
6. Effects of fertilizers on the growth and physiological response of *Cleome gynandra* 99
7. Effect of inorganic and organic fertilizers on the mineral composition of *Cleome gynandra* cultivated on the field and glasshouse 137
8. Maturity effects on the nutritional composition of *Cleome gynandra* (A wild vegetable)…….184
9. General conclusion and recommendations 212

SUMMARY

South Africa has wide ranges of plants among which are leafy vegetables growing in the wild. These wild vegetables have been reported to have high nutritive and medicinal potentials. Yet, there is still high prevalence of malnutrition and micronutrients deficiencies among the low income bracket of the population. Effective utilization of wild vegetables has been proposed as part of the solutions to address the problem of dietary deficiencies among the populace. Their importance is mainly as relish to accompany and complement starch based diets. They are also known to serve as supplements for food which have the potential to improve the health status of its consumers in many rural communities.

Cleome gynandra L. (Capparaceae) is one of the underutilized species mentioned during asurvey conducted in Nkonkobe municipality of the Eastern Cape Province, South Africa. The plant is an erect annual herb with alternate, palmately compound leaves and its petals are white, pink or lilac. It grows as a weed in this part of the Province and is usually gathered from the wild for food and medicine. It is commonly found on wastes land, road sides and on grass lands. Therefore, this research work was designed to contribute to the possible domestication of this wild vegetable in order to explore the nutritive and therapeutic potentials which would broaden the food base in the Eastern Cape Province.

The ultra-micromorphological features of plants have become an essential tool in proper identification and authentication of several plant species. In the light of this, the micromorphology of *Cleome gynandra* was examined to reveal the micro-morphological characters of the plantand to determine if micro and macro mineral elements are present using energy dispersive x-rays. The present study revealed the epidermal cells of the leaves to be asymmetric in shape with undulating cell walls having four subsidiary cells around the stomata. The leaves have anomocyctic stomata which are more distributed in the abaxial surface than the adaxial surface. The mean length and width of the guard cells in the abaxial surface are 0.09 ± 0.01 mm and 0.08 ± 0.01 mm respectively while that of adaxial surface

are 0.1± 0.01 mm and 0.07 ± 0.01 mm respectively. The energy dispersive x-rays (EDXS) micro-analysis of the leaf revealed the presence of phosphorus, manganese, iron, calcium, sodium, magnesium, potassium and zinc as the major constituents of the crystal deposits present in the stomata pores and the mesophyll.

C. gynandra was collected from the wild and analysed for its mineral and proximatecomposition using standard analytical methods. The plant showed high levels of sodium, iron, zinc, calcium and potassium while many other macro and micro minerals were moderately present. Amongst the proximate factors investigated, moisture content was found to be 82.1% while crude fibre and protein contents were 39.9% and 31.03% respectively. Vitamin C was also high (345.3 mg/100g). The anti-nutrients compositions revealed low concentrations of oxalate (7.4 ± 0.4) and phytate (0.6 ± 0.0) mg/100g. The study revealed high nutritive value of the species growing naturally from the wild.

Interest in the phenolic compounds has greatly increased recently because these compounds have been implicated in suppressing the risk of degenerative diseases in humans. The pharmacological properties of plants may be related to their antioxidant capacities and hence there was need to investigate the antioxidant potential using aqueous, ethanolic and acetone extracts of different parts of *C. gynandra*. The quantities of phenols, flavonoids and flavonols were significantly higher ($p < 0.05$) in both aqueous and ethanol extracts of the leaf while the concentration of proanthocyanidins was higher in the aqueous stem extract compared to other plant parts. The aqueous extract of the different parts exhibited better ABTS, reducing power and NO radical scavenging abilities than ethanol extract. The quantitative estimation of the phytochemical constituents of the crude extracts of the plant further revealed the following concentrations in the acetone leaf extract; total phenolics (126.79 ± 0.55 mg/g), flavonoids (40.58 ± 0.06 mg/g) and flavanols (42.41 ± 0.05 mg/g). The stem extract; proanthocyanidins

(419.01 ± 0.67 mg/g) leaves (403.29 ± 0.89 mg/g) and fruits (107.18 ± 0.59 mg/g). The reducing power of the extracts was significantly higher than that of the standard drugs used in a concentration dependent manner. The activities of the plant extracts against ABTS, DPPH and NO radicals were dose responsive with IC_{50} value of 0.2, 0.1 and 0.03 mg/g respectively. The study indicated that *C. gynandra*possesses high secondary metabolites which accounts for its strong antioxidant activity thus justifying its use as natural occurring antioxidants in folkloric medicine. The finding of the present study encourages regular consumption of this wild vegetable in order to avert oxidative stress related diseases.

Efforts to domesticate and cultivate *C. gynandra* could be hindered by several environmental factors such as temperature and light among others. The viability studies was also determined and the plant was subjected to various treatments such as different temperature and watering regimes, light and dark conditions as well as sowing depths. The average seed weight was 1.2 ± 0.003 mg and the percentage viability in Lot A and B were 22.6 ± 2.3% and 67.3 ± 5.0% respectively. The optimum germination was observed at 30^{o}C for both Lots A and B, when watered bi-weekly at a sowing depth of 0.5 cm. The result also showed that germination was best in the dark (28.7%) for both Lot A and B. Overall, the germination rate under all the conditions was highest in Lot B. it was concluded that *C. gynandra* has the potential of thriving successfully under varied environmental conditions, despite the great fluctuations of high and low temperatures in South Africa.

Field and glasshouse trials were conducted to determine the effect of fertilizers on the growth, physiological response, nutritional and mineral composition of *C. gynandra*. The experiment consisted of three treatments (control, 100 kg N/ha and 8 t goat manure/ha) which were arranged in a randomized complete block design with three replicates. Plant height, total number of leaves, chlorophyll content, moisture, root/shoot ratio, leaf area and stem girth were measured. All parameters measured increased with plant age and significant differences

($p < 0.05$) were observed among the treatments. Generally, fertilizers improved the yield and growth of *C. gynandra*. Application of 100 kg N/ha produced the best plant height, total number of leaves, stem girth and leaf area while 8 t/ha of goat manure boosted the leaf area, chlorophyll contents in the glasshouse trials as well as the moisture contents. Plant root/shoot ratio was significantly higher in the control than the other treatments. These findings showed that both fertilizers increased the growth performance of the plant.

The investigation on the mineral composition of *C. gynandra* revealed significant differences in the uptake of the micro and macro elements. However, the plant had higher concentrations of Fe, Ca, Mn, Mg and Na while K, Cu, Zn and P were moderately present. It also shows that at different growth stages, *C. gynandra* can contribute significantly to the dietary requirements of the people in the Eastern Cape Province, South Africa.

The overall analysis of the proximate parameters analysed on the field and in the glasshouse showed variations in the nutritional composition of *C. gynandra*. 100 kg N/ha influenced more proximate constituents such as vitamin C, protein and ash on the field while 8 t/ha of goat manure increased the crude lipid and phytate contents in the glasshouse. Both fertilizers increased the crude fibre content of the plant on the field. This study further reveals the concentrations of protein, vitamin C, and fibre can be explored better in the 2^{nd}, 6^{th} and 8^{th} weeks respectively. Therefore, it can be concluded that regular consumption of the plant can meet the nutritional requirement to alleviate micronutrient deficiencies.

CHAPTER ONE

General introduction

1.1 What are wild vegetables? 2

1.2 The significance of wild vegetables in human diet..... 2

1.3 The status of wild vegetables in South Africa 3

1.4 The need to popularize wild vegetables in South Africa 4

1.5 The use of fertilizers in vegetable production..... 5

1.6 *Cleome gynandra* L..... 6

1.7 The choice of *Cleome gynandra* for this study.....8

1.8 Objectives of the study..... 9

1.9 The structure of this thesis……10

1.10 References.....11

1.1 What are wild vegetables?

Wild vegetables are edible, mostly herbaceous plants growing in the wild, fallow fields or in cultivated farms as weeds. These weeds are sometimes pulled out or left undisturbed for subsequent use depending on whether that particular species is utilised as a vegetable or not. Wild vegetables are edible portions of a plant which could be consumed totally or in bits as a side or main dish. They are sometimes eaten in raw forms or cooked along with starchy staples. The edible portions includes leaves, flowers, seeds, fruits, stems and roots (Asaolu *et al*., 2012).

Wild vegetables are important dietary and therapeutic components which possess micro and macro nutrients, vitamins and minerals. These nutrients play significant roles in nutrition, food security and serve as supplements in reducing the risk of several diseases (Odhav *et al*., 2007). Some examples of wild vegetables includes; *Momordica balsamina* L., *Bidensbiternata* L., *Corchorus olitorius* L., *Chenopodium album* L., *Spinacia oleracea* L., *Sonchus asper* L. and *Cleome gynandra* L. among others.

1.2 The significance of wild vegetables in human diet

Globally, it has been estimated that 868 million people do not have access to adequate calories (FAO, 2012). About 239 million of the populations in sub-Saharan Africa are affected by chronic under-nutrition (Sasson, 2012). According to Ezzati *et al*. (2002), the low consumption of vegetables and fruits is among the top 10 risk factors for mortality. For instance, about 190 million children and more than 15 million pregnant women are estimated to be vitamin A deficient (WHO, 2009). The diets of the people in both urban and rural areas are mainly cereal-based resulting in poor diets with increased prevalence of nutritional deficiency disorders (Kwapata and Maliro, 1995; Hotz and Gibson, 2007).

In southern Africa, in the year 2000, it was estimated that 11.1 million males and 12.5 million females over 15 years of age had a low intake of vegetables (Schneider *et al*., 2007). It has been reported that daily intake of more than 400 g of vegetables per person can avert diet related diseases (WHO, 2003). However, it is important that wild vegetable species should be incorporated into human diet. Several studies have shown that the nutritional composition of wild plants revealed higher micronutrients levels than those found in most exotic species (Nesamvuni *et al*., 2001; Ohdav *et al*., 2007; Ndlovu and Afolayan, 2008). For example, IPGRI, (2003) reported on the high mineral contents of *Amaranth spp* which contains 57 times more vitamin A than cabbage, 13 times more iron and about 9 times more calcium.

In a similar study, Ndlovu and Afolayan, (2008) reported high contents of crude protein and magnesium of wild okra (*Corchorus olitorius*) when compared with cabbage (*Brassicaoleracea*). According to van Jaarsveld *et al*. (2014) most wild plants are good sources ofmicronutrients and they are able to meet more than 75% of the recommended daily allowance (RDA) in children. Wild vegetables are important in alleviating micronutrient deficiencies since they are the cheapest source of protein, fibres, vitamins, minerals and essential amino acids (Grivetti and Ogle, 2000; Lyimo *et al*., 2003; Afolayan and Jimoh, 2009). These micro nutrients aid in promoting immunity against infections, good health status and providing food security for the people.

1.3 The status of wild vegetables in South Africa

In southern Africa, wild vegetables have been used for decades. For instance the Khoi-San and Bantu tribes relied mostly on wild plants for their survival for centuries (Fox and Norwood Young, 1982; Parsons, 1993; Bundy, 1998; Ntuli *et al*., 2012). In the Eastern Cape Province, 30 plant species were utilized as wild vegetables by the populace (Jaca and

Kambizi, 2010). The knowledge and collection of wild vegetables continues to be a common practice amongst the people in this region especially women and young children (Bhat and Rubuluza, 2002; Jansen van Rensburg *et al*., 2004, Husselman and Sizane, 2006; Modi *et al*., 2006).

Currently, the use of wild vegetables is gradually fading away in most parts of South Africa; particularly those that are still regarded as weeds due to over reliance on exotic varieties and people's perception as a low status food (Shackleton, 2003; Voster and van Rensburg, 2005; Modi *et al*., 2006; Lewu and Afolayan, 2009). Therefore the utilization and availability of wild species have declined severely due to the immense cultivation of field crops. This has resulted in complete eradication of wild plants which are often considered as weeds. Thus, its survival becomes precarious causing these important species to become extinct in future (Odhav *et al*., 2007; Lewu and Mavengahama, 2010). Recently, a resurgence of interest has led to some development of policies and research on wild plant species in order to improve their cultivation, conservation, nutritional and medicinal values (Flyman and Afolayan, 2007; Afolayan and Jimoh, 2009).

1.4 The need to popularize wild vegetables in South Africa

South Africa is rich in biodiversity amongst which are numerous plant species with high nutritional and medicinal potentials. Yet, most of the rural populace are faced with high prevalence of malnutrition, hunger and micronutrients deficiencies. Iron, vitamin A, iodine and zinc have been reported to be the most lacking micronutrients in diets (FAO, 2012). According to Faber *et al*. (2011), 64% of children between the ages of one to nine were shown to be vitamin A deficient, 28% anaemic, 13% have low iron and 45% low zinc levels in South Africa. In the same region, more than 40% of adults are obese due to their unhealthy diet (Njume *et al*., 2014).

A large number of these neglected vegetable species are good dietary sources of micronutrients. Thus, the major approach to manage diet related deficiency in rural communities is to increase the utilization of wild edible herbs in their diet. This can potentially be achieved through exploitation of natural resources. In addition to the nutritional worth, wild species also act as therapeutic agents which improve human health. Venter *et al.* (2007) reported that about 70% of the population in South Africa uses wild vegetables as herbal remedies. These include black nightshade (*Solanum nigrum*) which is used in the treatment of several ailments such as asthma, whooping cough, rabies, conjunctivitis and glaucoma (Ramya *et al*., 2011). *Amaranthus hybridus, Amaranthus dubius, Asystasiagangetica, Galinsoga parviflora, Oxygonum sinuatum, Physalis viscosa* and *Tulbahgia violacea* are used in the treatment of hypertension in KwaZulu-Natal (Mackraj, 2007).Therefore, it is important that wild vegetables should be utilized efficiently in herbal medicine (Sumner, 2000).

1.5 The use of fertilizers in vegetable production

The world's population is increasing every day; it has been estimated that by the year 2050 the population would have risen to 9.4 billion people (USCBIDB, 2012). In order to sustain this increasing population, intensive cultivation is necessary to meet the food needs of the people. This has resulted in enormous cultivation of farmlands without adequately replenishing the soils nutrients. However, during crop production a number of factors are accountable for the low yield in agricultural fields. This includes inappropriate crop nutrient management and poor soil fertility. The use of fertilizer is an essential tool in ameliorating the soil nutrient condition and increasing crop yield (Adamu and Leye, 2012). These are either organic or inorganic sources. Organic fertilizers are natural based materials which could either be of plant or animal origin. Examples include; animal manure, crop residues, household waste, compost and woodland litter. Incorporation of organic fertilizers stimulates

microbial activities in the soil; thus increases the yield of the plant. This phenomenon is attributed to increase in soil organic matter and fertility (Herencia *et al*., 2008; Diacono and Montemurro, 2010). It also plays a pivotal role in plant growth by enhancing the availability of soil macro and micronutrients during mineralization hence improving physical, chemical and microbiological properties of soils (Abbas *et al*., 2013).

In addition, it acts as a centre for cation exchange capacity and buffering agent against undesirable soil pH variation (Olaniyi *et al*., 2010). On the other hand, inorganic fertilizers (synthetic materials) are used in correcting the known nutrient deficiency. This provides the optimum soil fertility conditions thus, improving the quality of the plant. One limiting factor on the use of inorganic fertilizer is its side effects such as influencing soil pH and residual deposition (Nwangburuka *et al*., 2012).

1.6 *Cleome gynandra* L.

Cleome gynandra L. commonly known as spider plant belongs to the Capparaceae family andsubfamily Cleomoideae. This family comprises about 700-800 species, which is divided into 45 genera (Kokwaro, 1994). *C. gynandra* is an erect herbaceous perennial herb that grows up to 1.5 m tall (Fig. 1). This species is thought to have originated in Africa and South East Asia. It is most likely to be a native of Africa and now widely distributed all over the tropical and subtropical regions in the world (Kokwaro, 1976; Chweya and Mnzava, 1997). In the Eastern Cape Province of South Africa, this plant has been found to grow naturally in the wild especially on cultivated farms. It is still regarded as a weed in most parts of the Province. It is a semi cultivated vegetable in the Kentani District of the Eastern Cape and has probably extended its distribution (Van Wyk and Gericke, 2000; Van Rooyen *et al*., 2001).

Figure 1: *Cleome gynandra*: A & B are plants growing in the glasshouse; C & D are plantsgrowing on the farm.

C. gynandra is strongly branched with long taproots, its stems are densely glandular; leavesare alternate, palmately compound. The petals are white, pink or lilac while its capsules are green but turn yellow when ripe. This plant is used primarily as food and medicine in most parts of South Africa. The leaves are widely used as a vegetable where it is consumed as a pot herb or side dish (Chweya and Mnzava, 1997). Besides its culinary value, it has been reported that regular consumption of this vegetable is believed to facilitate childbirth as well as stimulating lactation in women (Onyango and Kunyanga, 2013). The decoction of the leaves is used to treat scurvy, marasmus, epileptic fits and malaria while the seeds and roots are ingested for the removal of roundworms in children (Onyango and Kunyanga, 2013).

Increase in consumption and utilization of this plant should be encouraged, although as earlier mentioned it is gathered from the wild and not formally cultivated in the Eastern Cape Province. This could pose a challenge to its availability and accessibility. Thus, there is need for its domestication in the Province in order to explore its dietary and therapeutic potentials.

1.7 The choice of *Cleome gynandra* for this study

Cleome gynandra is grown in different parts of the world. This includes; India, Nigeria,Kenya, Tanzania, Ghana, Ethopia, Uganda and Botswana (Chweya and Mnzava, 1997). In most African countries, this species has been cultivated, popularized and commercialised except in the Eastern Cape Province, South Africa (Jansen van Rensburg *et al*., 2007). In the Province, it is one of the most popular underutilized species (Bvenura and Afolayan, 2014). The plant is found growing in the wild in Limpopo, North-West, Gauteng, Mpumalanga, KwaZulu-Natal, Free-State and the Northern Provinces (Mishra *et al*., 2011).

According to Njume *et al*. (2014), wild vegetables are neglected and underutilized though they possess high nutritive and medicinal values. At the beginning of this project, very little was known about the agronomic and nutritional requirements of *C. gynandra*. There were many gaps in knowledge with respect to cultivation requirements in the Eastern Cape Province. In view of the above, this study was conducted to determine the effects of organic and inorganic fertilizers on the plant's physiological and nutrient uptake responses. This will hopefully lead to better understanding of the best cultural practices to increase yields and improve its nutritional values.

1.8 Objectives of the study

The overall objective of this study was to investigate the medicinal potential and nutritive value of *Cleome gynandra* growing in the Nkonkobe Municipality in the Eastern Cape, South Africa. This is with a view to domesticating the species as done in other parts of the world.

The specific objectives are:

- Examine the ultrastructure and morphology of this plant with scanning electron microscope.
- Carry out the proximate and mineral analysis of the vegetable
- Investigate its anti-nutrient properties
- Determine the phytochemical analysis of the plant.
- Evaluate out the antioxidant properties of the vegetable.
- Carry out viability and germination tests on *Cleome gynandra* seeds.
- Determine the chemical and physical properties of the soil on which trials will be conducted.
- Determine the chemical properties of the organic manure (goat droppings) to be used in the trials.
- Investigate the effect of fertilizers on growth and physiological response of *Cleome gynandra* cultivated on the field.
- Investigate the effect of fertilizers on growth and physiological response of *Cleome gynandra* cultivated in the glasshouse.
- Investigate the effect of inorganic and organic fertilizers on mineral composition of *Cleome gynandra* cultivated on the field.
- Investigate the effect of fertilizers on proximate composition of *Cleomegynandra* cultivated in the glasshouse.

1.9 The structure of this thesis

This thesis consists of chapters that are in form of reprints, which are already accepted for publication while some chapters are still under review in various accredited Journals. Chapter 1 is the general introduction. Chapter 2 presents the results of the micromorphology of the aerial parts of *Cleome gynandra* while Chapter 3 contains the mineral composition and proximate analysis of the plant collected from the wild. The phytochemical constituents and antioxidant properties of aqueous, acetone and ethanolic extracts of *Cleome gynandra* are reported in Chapter 4. Chapter 5 deals with the effect of environmental factors and sowing depth on seed germination of the species. In Chapter 6 the results of the effect of fertilizers on the growth and physiological response of *Cleomegynandra* cultivated both on the field and in the glasshouse are reported while the effectsof organic and inorganic fertilizers on the mineral uptake of the *C. gynandra* are presented in Chapter 7. Chapter 8 explains the effect of plant age (maturity) on the nutritional composition of the plant in relation to the different growth stages in the glasshouse and on the field. Chapter 9 gives a clearer insight on the conclusions and recommendations of the study.

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CHAPTER 2

MICROMORPHOLOGY OF *CLEOME GYNANDRA* L. FROM THE EASTERN CAPE PROVINCE, SOUTH AFRICA.

CHAPTER TWO

Micromorphology of *Cleome gynandra* L. from the Eastern Cape Province, South Africa.

Abstract 19

Introduction 20

Materials and methods 21

Results 22

Discussion 26

References 31

This chapter has been submitted in this format for publication to the African Journal of Complementary and Alternative Medicine.

Abstract.

Many species in the family Capparaceae are economically important as wild vegetables with medicinal and nutritive values. Among the species is *Cleome gynandra* commonly known as spider plant. Several medicinal uses of the species have been ascribed to its aerial parts. The present study was undertaken to examine the micro morphological features and estimate the elemental composition of the aerial parts of *C. gynandra*. Fresh leaves, stems and roots of the plant were examined for their anatomy and micromorphological characteristics using light and scanning electron microscope. The elemental composition of the aerial parts was also done using energy dispersive x- ray spectroscopy. It was observed that the epidermal cells are asymmetric in shape with undulating cell walls having four subsidiary cells around the stomata. The leaves have anomocyctic stomata which are more distributed in the abaxial surface than the adaxial surface. The mean length and width of the guard cells in the abaxial surface are 0.09 ± 0.01 mm and 0.08 ± 0.01 mm respectively while that of adaxial surface are 0.1± 0.01 mm and 0.07 ± 0.01 mm respectively. The EDXS microanalysis of the leaf revealed the presence of phosphorus, manganese, iron, calcium, sodium, magnesium, potassium and zinc as the major constituents of the crystals present in the stomata pores and the mesophyll. The knowledge of the plant's ultra-morphological characteristics and elemental composition can further help in proper identification to ascertain its use for herbal remedy and consumption.

Key words: Scanning electron microscopy,*Cleome gynandra*, mineral elements, lightmicroscopy, x-ray spectroscopy.

Introduction

The resurgence of interest in wild vegetable species, especially in their ethnopharmacological potentials has become noteworthy. Wild vegetables serve as essential constituents of diets, enriching the body with minerals, essential fatty acids and vitamins. In addition, they have medicinal values such as antibacterial, hepatoprotective and anticarcinogenic properties (Adedapo *et al*., 2011). Based on these, people are becoming aware of the inherent nutritional and medicinal status of wild vegetables, thus, the exploitation of these potentials has increased greatly. Several studies have reported the significance of foliar micromorphological features for taxonomical delimitations of many plants (Dickison, 2000; Sonibare *et al*., 2005; Sonibare *et al*., 2006, Gostin, 2011). Ultra-micromorphological features of plants have become an essential tool in correct identification and authentication of several plant species. These features are also useful in the standardization of herbal products available from various indigenous medicinal plants (Sonibare and Osiyemi, 2012). Micromorphological characters of the leaves that have been used in some studies include epidermal cell types, stomata, trichomes, vascular bundle patterns and arrangements (Bhatt *et al*., 2010; Yasmin *et al*., 2010; Senthamari *et al*., 2011).

Many species in the family Capparaceae are economically important as wild vegetables with medicinal and nutritive values. One important species in this family is *Cleome gynandra* L. commonly known as spider plant. It is a multi-branched herb growing to a height of 1.5 meter with long tap roots and a few secondary roots. Its stems are densely glandular. The leaves are palmately compound with 3-5 leaflets. This plant is native to Africa and widely distributed in South Africa extending from the Limpopo, the North-West, Gauteng, Mpumalanga, KwaZulu-Natal, Free State and the Northern Cape Provinces (Mishra *et al*., 2011). In addition to its edibility as a wild vegetable, the species is used for the treatment of sexual

impotence, epileptic fits, gastro intestinal disorders and inflammatory conditions (Kamatenesi-Mugisha, 2004; Kamatenesi-Mugisha and Oryem-Origa, 2005). These pharmacological values have been ascribed to the property of its leaves, roots and flowers. *C.gynandra* occurs 2400 m above sea level and requires warm conditions that are not below15^{o}C. It is found on a wide range of soils from sandy loam to clay loam provided that the soil is deep and well drained with pH of 5.5-7.0 (Chweya and Mnzava, 1997).

According to Bunanwan *et al*. (2011) the morphology and anatomy of the leaf is the most variable plant organ and micromorphological characters such as the trichomes, which are occasionally specific for species, genera or even families. Today, there is no published literature on the micromorphology of the aerial parts of *C. gynandra*, especially the variety growing in the Eastern Cape Province of South Africa. The present study was undertaken to examine the micro morphological features of the aerial parts of this plant using light and scanning electron microscopes. In addition, the elemental composition of the various parts of the species was estimated using Energy Dispersive X-ray (EDX) analysis. EDX is slowly becoming an essential and strong tool in drug and other plant products standardization.

Materials and methods

Plant material

The leaves, stem and roots of *C. gynandra* were collected from the University of Fort Hare Research Farm in Alice (latitudes 32° 47' 0" South and longitudes 26° 50' 0" East). This plant was identified at the Department of Botany; University of Fort Hare and a voucher specimen (LinMed 2013/01) was deposited in the Giffen's herbarium of the University.

Leaf epidermal studies (Light microscopy)

Leaf, stem and root samples of 1- 3 cm were sectioned using a razor blade and observed under motic light microscope. The microphotographs were taken with a digital camera that was fitted to the microscope.

SEM and Energy Dispersive X-ray Spectroscopy (SEM-EDXS)

Fresh leaves, stems and roots were cut into segments of 4- 6 mm in length and fixed in 6% glutaraldehyde with pH 7.3 for 12 h. Following this, the sections were rinsed with 0.05M sodium cacodylate buffer (pH 7.5). Each sample was later rinsed in distilled water and dehydrated in a graded series of ethanol 10-100% for 20 min per rinse. The sections were dried in a Hitachi HCP-2 critical point dryer and mounted on aluminium stubs with double-sided carbon coated sputter coating with gold palladium (Elko IB-3 Ion Coater). The sectioned parts of the plant were examined at varying magnifications using JEOL (JSM-6390LV) scanning electron microscope (SEM) operated at 10 – 15 kV accelerated voltage. The energy dispersive X- ray spectroscopy (EDXS), involved both fixing and dehydration procedure as in SEM, while the elemental analysis was done using energy dispersive x-ray analyser which was coupled to SEM, manufactured by Thermo Electron Corporation, 6733B-IUUSN, USA. The Noran system six software was used for imaging.

Results

Light and scanning electron microscopy

The leaves of *C. gynandra* were characterized by high distribution of stomata on both sides of the leaf (Fig. 1A and 1B).

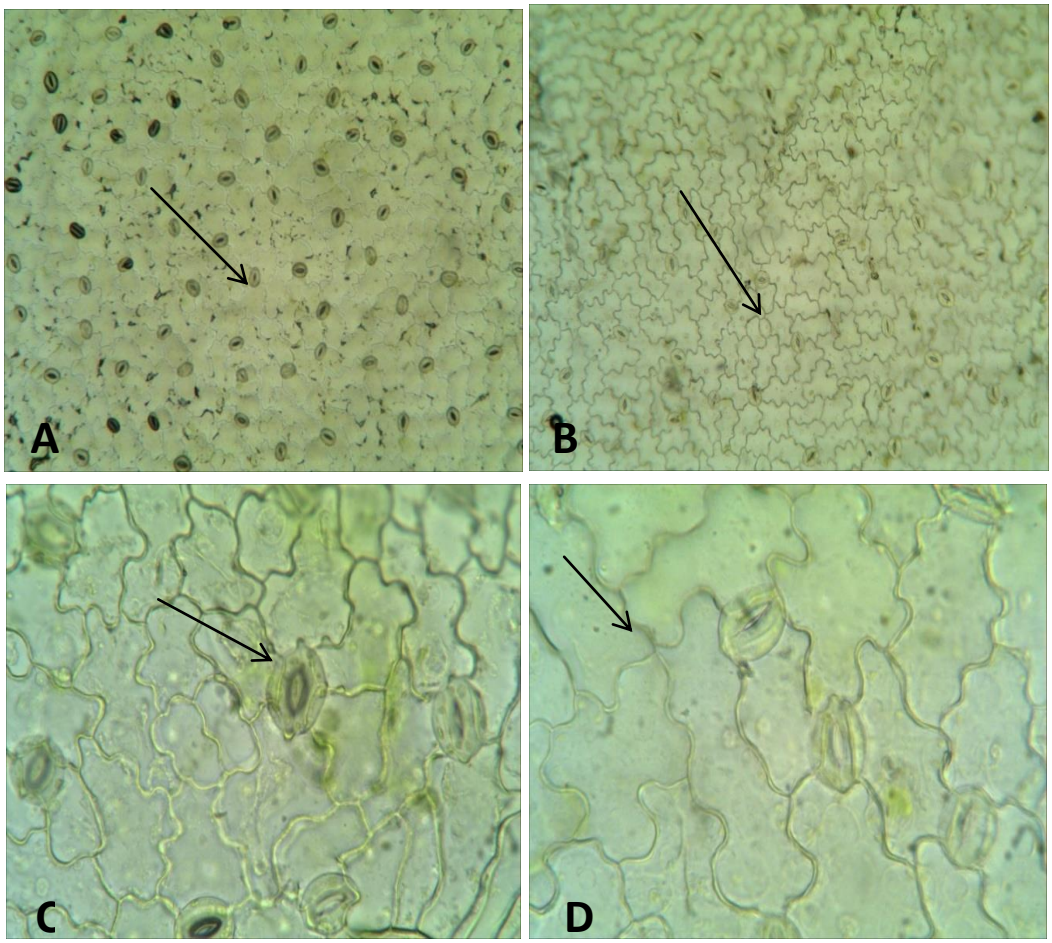


Figure 1: (A) stomata distribution on the abaxial leaf surface (10X); (B) epidermal cells andstomata distribution on the adaxial surface (10X); (C) guard cells and sunken stomata (arrow) in the abaxial surface (40X); (D) epidermal cells (arrow) in adaxial surface (40X).

The mean stomata density on the abaxial and adaxial surfaces was 68.14 ± 0.34 and 43.68 ± 0.21 per mm^2 respectively. The epidermal cells are assymetric in shape with undulating cell walls on both surfaces (Fig 1C & D). The density of the epidermal cells in the lower and upper surfaces was 163.1 ± 0.26 and 124.6 ± 0.32 per mm^2 respectively. The stomata apertures are elliptical (Fig. 2A). The stomata are anomocytic and are embedded within the epidermal layer with four subsidiary cells surrounding each stoma (Fig. 1C & D). The guard cells are banana shaped outlined by a thick inner and thin outer walls which are vertically embedded to the subsidiary cells (Fig. 2B). The guard cell index is 5653.7μm^2 and

5496.7μm^2 in the abaxial and adaxial surfaces respectively. The mean length and width of the guard cells on the abaxial surface are 0.09 ± 0.01 mm and 0.08 ± 0.01 mm respectively while those of the adaxial surface are 0.1± 0.01 mm and 0.07 ±0.01mm respectively.

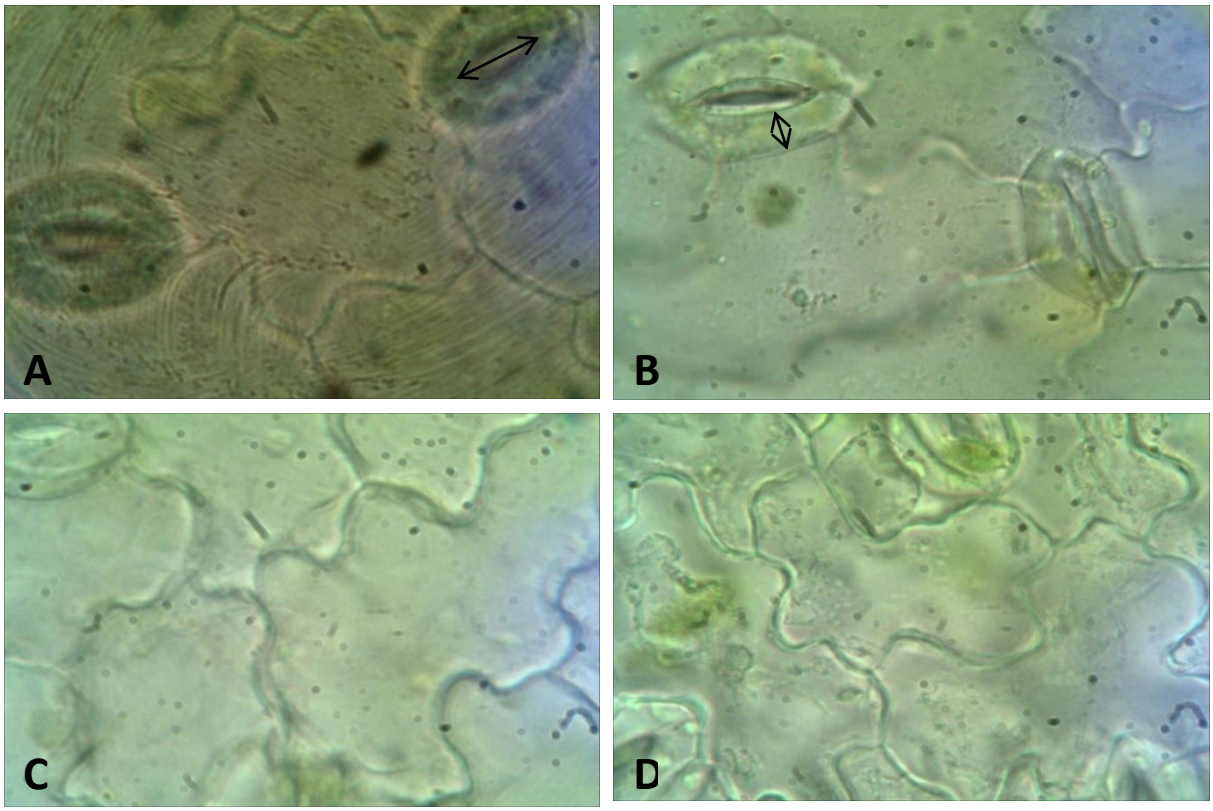


Figure 2: (A) stomata distribution on the abaxial surface (100X); (B) stomata distribution onthe adaxial surface (100X); (C) epidermal cells on the adaxial surface (100X); (D) epidermal cells on abaxial surface (100X).

The micromorphology of the leaf of *C. gynandra* as seen under SEM showing the distinctive sunken stomata with the presence of mineral crystals positioned in the stomata pores and in the intercellular spaces within the plant surface (Figures 3 A-D).

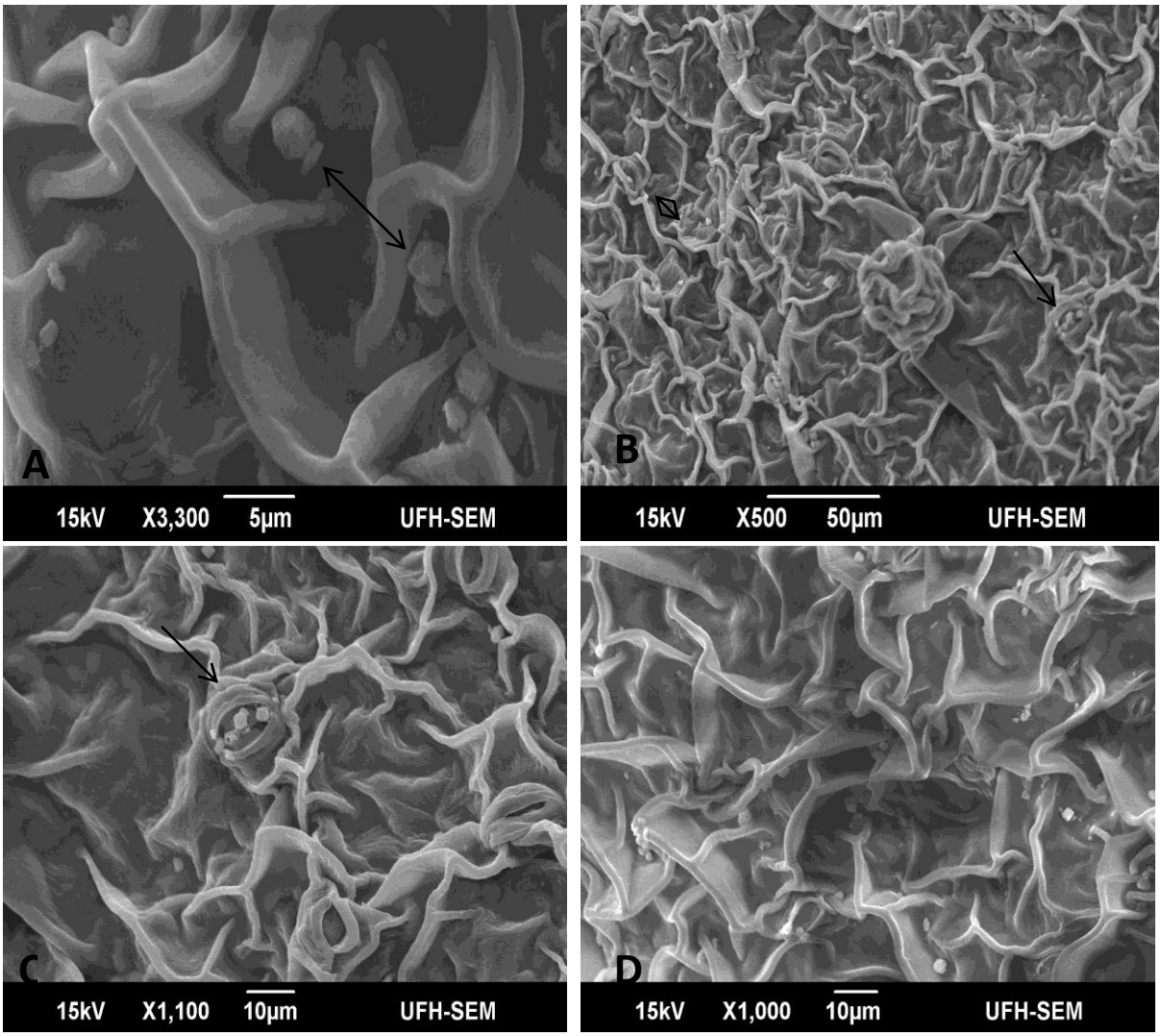


Figure 3: (A) Crystal deposit on the adaxial surface of*Cleome gynandra*leaves (B) sunkenstomata with crystal deposit on the abaxial leaf surface (C) concentrated crystals within the stomata on the abaxial surface (D) fragments of crystals scattered on the abaxial surface.

The micromorphology of the root is presented in Figure 4A showing a cross section of the vascular bundle of the xylem tissue arranged in ringed shape. The structure of the shoot shows the presence of droplets of mineral sediments on the phloem tissue which are

translocated to all parts of the plant (Fig. 4B).

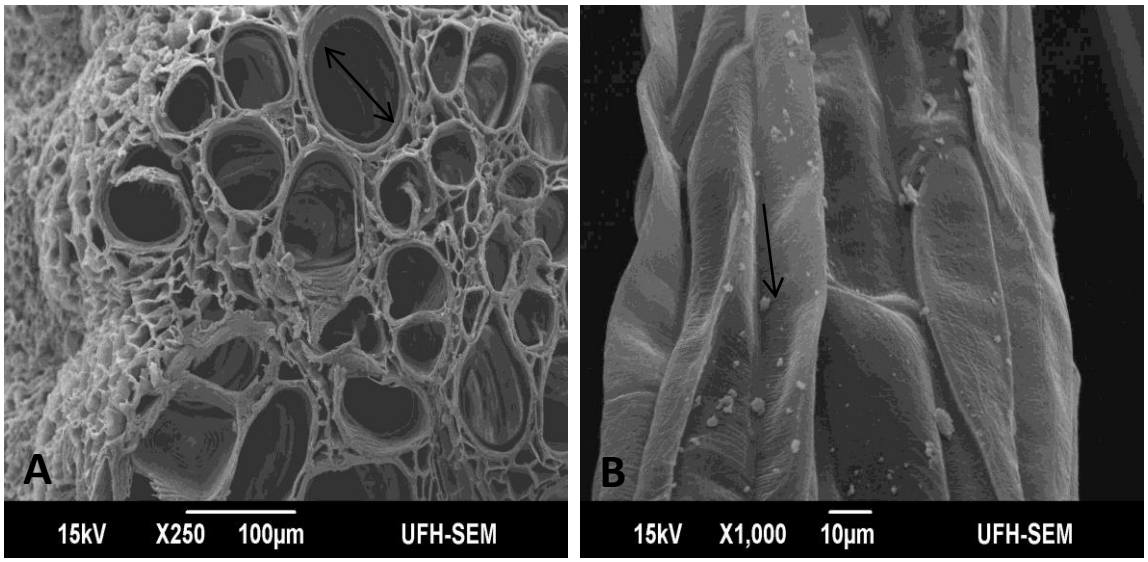


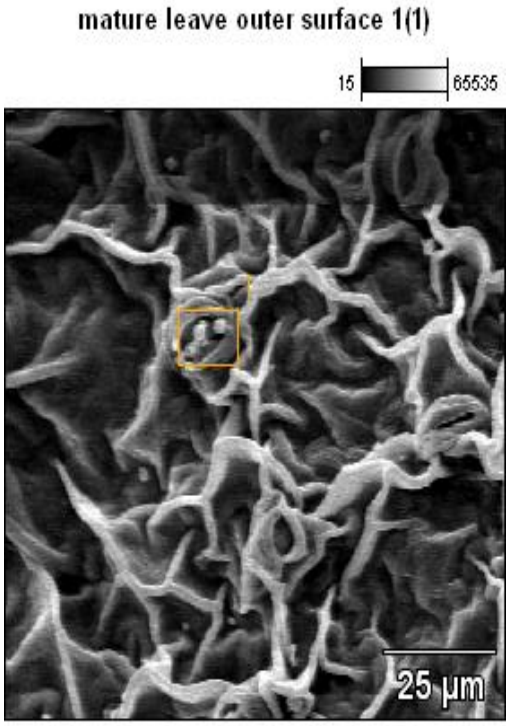
Figure 4: (A) Cross sections of*C. gynandra*root showing the inner ring of xylem tissue and (B) shoot having mineral sediments on the phleom tissue.

Discussion

Different features were observed in this plant. These include type of stomata, stomata density and crystal deposits. These are significant characters in differentiating different species. Other characters are mean guard cell length and width, number of subsidiary cells, number of epidermal cells per unit area and guard cell index in both abaxial and adaxial surfaces. This amphistomatic feature shows the sunken stomata surrounded by the guard cells. The number of epidermal cells and stomata are more in the abaxial surface than the adaxial surface. The xylem tissue serves as a vessel for transporting water, dissolved minerals and fibres which supports the plant.

The X-ray microanalysis of the leaf of *C. gynandra* generated a spectra of the following micro and macro mineral elements: carbon (C), oxygen (O_2), nitrogen (N), iron (Fe), sodium

(Na), magnesium (Mg), phosphorus (P), calcium (Ca), potassium (K), manganese (Mn), zinc (Zn), silicon (Si), sulphur (S) and aluminium (Al) (Fig. 5). Gold (Au) was probably from the spur coater. The mineral constituent of this plant is an indication of its nutritional and ethnopharmacological importance. For instance, the high peaks of carbon, oxygen, nitrogen, phosphorus, manganese, iron and calcium showed the abundance of these elements while sodium, magnesium, potassium and zinc were in moderate amounts. Aluminium, silicon and sulphur were found in small amounts (Table 1). These mineral elements play different metabolic roles in humans. For example, calcium is a vital element known to reduce the risk of various non-communicable diseases including maintenance of bones, teeth and muscles (Vaskonen, 2003; Nieves, 2005). Potassium is involved in regulating acid–base balance and blood pressure (He and MacGregor, 2008). Magnesium on the other hand acts as a co-factor of many enzymes involved in energy metabolism; protein production and maintaining the electrical potential of nervous tissues and cell membranes (Mohammed and Sharif, 2011). Iron prevents anaemia, increases packed cell volume and boosts the immune system (Agunbiade *et al*., 2012). In addition, sulphur plays a significant role in protein synthesis, cell regeneration and blood cleansing (Afolayan and Otunola, 2014). The presence of these elements account for the therapeutic use of *C. gynandra* in gastro-intestinal disorders, anthelmintic, inflammatory, epileptic fits, aches and pruritis ((Mishra *et al*., 2011).



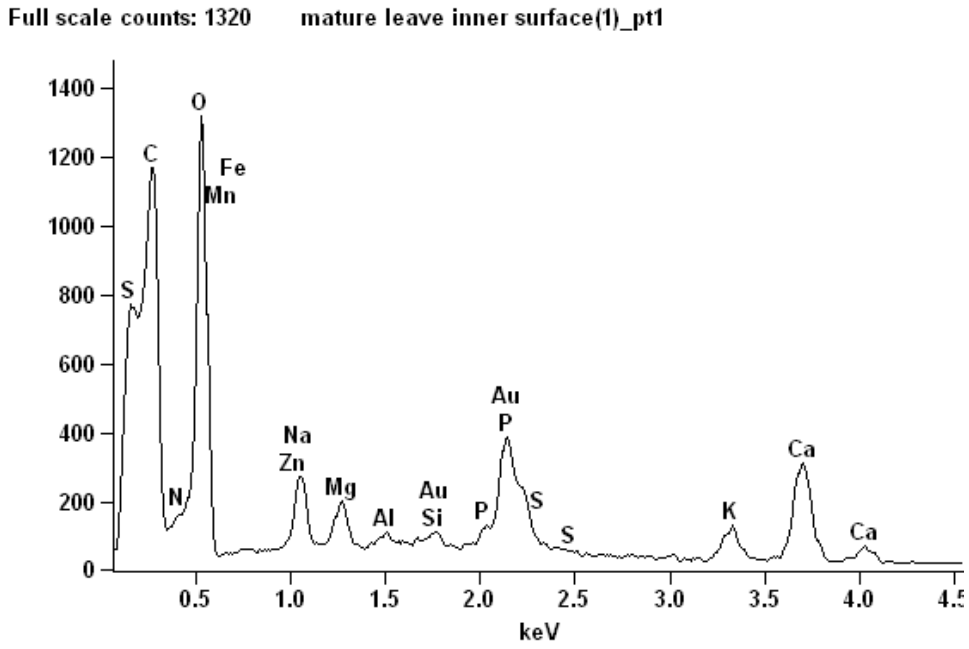


Figure 5: Energy Dispersive X-ray analysis of crystal deposits in the stomata pore of*C. gynandra*; micrograph showing the point of focus of the electron beam.

Table 1: Percentage elemental composition of crystal deposits on*C. gynandra*leaf

Element	**Amount (%)**
Carbon	21.36 ± 0.49
Nitrogen	8.01 ± 1.71
Oxygen	50.08 ± 0.93
Sodium	3.69 ± 0.11
Magnesium	1.89 ± 0.08
Calcium	14.98 ± 0.25
Phosphorus	13.53 ± 0.23
Potassium	2.36 ± 0.10
Aluminium	0.49 ± 0.12
Silicon	0.47 ± 0.07
Sulphur	0.49 ± 0.12
Zinc	3.33 ± 0.11
Manganese	11.24 ± 0.17
Iron	15.78 ± 0.21

Conclusion

The present study reveals the micro-morphological characters of the aerial parts of *C.gynandra* such as anomocytic stomata, amphistomatic epidermal surfaces, asymmetricepidermal cells, crystal deposits and the subsidiary cells surrounding the stomata. It also shows the presence of micro and macro mineral elements which probably account for its high nutritional and ethno-pharmacological importance. The knowledge of this micro-morpholgical characters and elemental compositions will help in proper identification of this

plant in order to ascertain its use for herbal remedy and consumption.

Declaration of interest

The authors declare that they have no competing interest.

Acknowledgements

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CHAPTER 3

MINERAL COMPOSITION AND PROXIMATE ANALYSIS OF *CLEOMEGYNANDRA*: AN UNDERUTILIZED WILD VEGETABLE IN THE EASTERN CAPEPROVINCE, SOUTH AFRICA

CHAPTER THREE

Mineral composition and proximate analysis of *Cleome gynandra* L.: an underutilized wild vegetable in the Eastern Cape Province, South Africa.

Abstract 36

Introduction 37

Methodology 38

Results 42

Discussion 43

References 50

This chapter has been accepted for publication in this format in Icelandic Agricultural Sciences.

Abstract

Cleome gynandra L. is an underutilized vegetable growing in the wild. It is a multipurposevegetable ranging from high level of nutritional content and ethno-pharmacological importance for several ailments. This species showed high levels of sodium, iron, zinc, calcium and potassium while many other macro and micro minerals were moderately present. Surprisingly, aluminium was found in this vegetable. Amongst the proximate factors investigated, moisture content was found to be 82.1% while crude fibre and protein contents were 39.9% and 31.03% respectively. Vitamin C was also high (345.3 mg/100g). The anti-nutrients compositions revealed low concentrations of oxalate (7.4 ± 0.4) and phytate (0.6 ± 0.0) mg/100g. The mineral, proximate and anti-nutritional compositions of *Cleome gynandra* leaves, stem and fruits investigated revealed the suitability of the plant as a vegetable and will possibly enhance the alleviation of diseases associated with dietary malfunction in the Eastern Cape Province.

Keywords:*Cleome gynandra*, macro minerals, anti-nutrients, proximate composition.

Introduction

Sub-Saharan Africa has the highest prevalence of under nutrition in the world, with one in three people being chronically hungry (FAO, 2008), majority of whom live in the rural areas. A large proportion of households in this part of the world are poor and exist on diets composed primarily of staple foods prepared from cereals (maize, millet, sorghum, teff), tubers (cassava, cocoyam, yam) and plantains (Oniang'o *et al*., 2003). These foods are generally low in micronutrients. Many rural households rely mostly on the consumption of wild vegetables to fulfil their daily requirements of micronutrients.

In southern Africa, Wehmeyer and Rose, (1983) identified more than 100 plant species that are consumed as wild vegetables. In the Eastern Cape, however, only 36 wild vegetable species have been reported (Bhat and Rubuluza, 2002). Although the populace especially the elderly women in the Eastern Cape region are aware that wild vegetables are highly nutritious and are also of great medicinal importance (Jaca and Kambizi, 2011). Yet many nutritionally rich species of wild vegetables are sometimes neglected and not utilized (IPGRI, 2000 – 2005). The perception, particularly among young people, that such vegetables are poverty foods, has led to the lack of interest in the cultivation and nutritional knowledge of these plants (Vorster *et al*., 2007). Some of the common wild vegetables found in the Eastern Cape Province are *Amaranthus hybridus, Bidens pilosa, Bidens biternata, Cleome gynandra,Corchorus tridens, Chenopodium album* and *Tribulus terrestris* (Jansen van Resburg *et al*.,2007).

During periods of natural or man –made disasters, humans suffer from severe food shortages; thus, they become reliant on wild vegetables for survival (Leborgne *et al*., 2002). Naturally, these vegetables grow in the wild and are readily available because they do not require any formal cultivation.

Cleome gynandra L. also known as spider plant is a wild vegetable that is rich in nutrientsespecially vitamins A and C, calcium, iron, magnesium and protein (Chweya and Mnzava, 1997). It has long been used as an herbal remedy for a number of ailments including rheumatic and inflammatory conditions (Narendhirakannan *et al*., 2005). The leaves are used in many countries for ear aches, epileptic fits, stomach ache and constipation.

C. gynandra has remained insignificant, unappreciated and hence underutilized in theNkonkobe Municipality of the Eastern Cape, despite its nutritional richness and potential to contribute to healthier diets in the Province. This study was therefore aimed at examining the proximate, anti-nutrient and mineral compositions of the different parts of the species. This information will add to the knowledge of the value of wild vegetables in this Province in particular and Africa in general.

Materials and Methods

Plant collection and Preparation

Samples of *Cleome gynandra* which includes the leaves, fruits and stem were collected fresh from the University of Fort Hare Research farm, Alice, between April and June 2013. The plant was identified at the Department of Botany; University of Fort Hare, and a voucher specimen (LinMed 2013/01) was deposited in the Giffen's herbarium of the University. The plant samples were properly washed with distilled water, oven dried at 40^{o}C to a constant weight, ground to fine powder and stored in airtight bottles which were then kept in the refrigerator at 4^{o}C until needed for the analysis.

Proximate analysis

The ash content of the plant was determined using the method of Antia *et al*. (2006). Briefly, 5 g of the powdered sample was incinerated in an E-Range muffle furnace with TOHO P4

programme at 550^{O}C for 12 h. The final weight of the sample was used to calculate the ash content as follows:

Ash content (%) = (final weight of the sample after incineration (g) /5g) x100

Crude lipid was determined as described by Antia *et al*. (2006). About 5 g of the powdered sample was weighed and dissolved with 100 ml of diethyl ether then covered with aluminium foil and shaken in an orbital shaker for 24 h. It was filtered and the supernatant was decanted. Another 100 ml of diethyl ether was added to the residue and shaken for another 24 h. The residue obtained after filtration was the lipid free sample and it was as:

Crude lipid = Weight of sample after diethyl ether extraction /Initial weight of sample x 100

The crude fibre content of the plant was determined also by the method of Antia *et al*. (2006). About 5 g of the powdered sample was weighed and digested in 100 ml of 1.25% sulphuric acid for 30 min. The acid digested sample was allowed to cool and then filtered. The residue was collected for further digestion with 100 ml of 1.25% sodium hydroxide. The sample was then filtered; the residue dried in an oven at 100^{O}C to a constant weight. The dried residue was incinerated in a muffle furnace for 24 h at 550^{O}C. The crude fibre was obtained from the loss in weight on ignition of dried residue remaining after digestion of fat free samples as:

% fibre = Loss of weight on ignition /Weight of sample used x 100

The vitamin C content of the plant was determined by a modified spectrophotometric method as described by Tahirovic *et al*. (2012). Briefly, 2.5 g of coarsely powdered sample was weighed and 12 ml of glacial acetic acid added. The mixture was stirred for about 20 min and filtered. The filtered solution was made up to 100 ml using distilled water. Fifty microlitres of the sample solution was mixed with 10 µL of methylene blue solution (0.4 mol/l) and diluted

to 10 ml with distilled water. Absorption was measured at 665 nm using a spectrophotometer (AJ-IC03). Stock solution of ascorbic acid (1M) was prepared by dissolving 10 g of ascorbic acid in 56.76 ml of distilled water. The different concentrations were prepared by diluting the stock standard solution in water before use and absorption was also measured at 665 nm. The calibration graph was drawn by plotting the absorbance against concentration of ascorbic acid. The obtained calibration curve was linear in a concentration range of 0.1 to 1M with the linear regression equation as;

$y = 0.01x + 0.041$, and the correlation factor of $r^2 = 0.971$.

The oxalate content was determined using the method of Onwuka, (2005) which involved digestion, oxalate precipitation and permanganate titration. 2 g of finely powdered sample was weighed and 190 ml of distilled water was added; followed by 10 ml of 6 M Hydrochloric acid (HCL) which was digested at $100^{o}C$ for one hour. The digest was left to cool and made up to 250 ml using distilled water. This was filtered and the resulting filtrate was then divided into two equal portions of 125 ml. Four drops of methyl red indicator was added to each portion, followed by the drop wise addition of concentrated ammonia solution until the solution changed from pink to a faint yellow colour. Each solution was then heated to $90^{o}C$, cooled and then filtered to remove the precipitate containing ferrous iron. The filtrate was again heated to $90^{o}C$ and 10 ml of 5% $CaCl_2$ was added with continuous stirring. The solution was cooled and kept in the refrigerator at $5^{o}C$ overnight. The refrigerated solution was centrifuged (Model TJ-6 Centrifuge, Beckman, Great Britain) at 2500 rpm for 5 min. The supernatant was discarded and the precipitate completely dissolved in 10 ml of 20% (v/v) H_2SO_4. The total filtrate was made up to 300 ml. Aliquots of 125 ml of the filtrate was heated to $90^{o}C$ and then titrated against a 0.05 M standardized $KMNO_4$ solution to a faint pink colour which persisted for 5 min.

The phytate content of the plant was determined using the method of Onabanjo and Oguntona, (2003). Briefly, 4 g of powdered sample was soaked in 100 ml of 2% hydrochloric acid for 3 h and then filtered. 25 ml of the filtrate was measured and 5 ml of 0.3% ammonium thiocyanate solution was added, followed by addition of 53.5 ml distilled water. This was titrated against a 1000 ppm standard iron (III) chloride solution until a brownish yellow colour persisted for 5 min. The phytate content was calculated from the iron determinations, assuming a 4:6 iron to phytate molecular ratios and multiplied by a constant of 3.55 (Vijayakumari *et al*., 1997).

The nitrogen content of the plant was determined using the method of Okalebo *et al*. (2002) by means of the Inductively Coupled Plasma - Optical Emission Spectrometer (ICP OES). The crude protein was done using the microkjeldahl nitrogen method which involves the digestion of a given weight of the sample with concentrated Sulphuric acid ($H_2SO_{4)}$ and a catalyst to convert any organic nitrogen to ammonium sulphate in solution, followed by the decomposition of ammonium sulphate with Sodium hydroxide (NaOH). The ammonia liberated was distilled into 5% boric acid. The nitrogen from ammonia was deduced from titration of the trapped ammonia with O.O5N HCl using methylene red and methylene blue indicators. The value of nitrogen obtained was multiplied by 6.25 to give the % crude protein.

The carbohydrate content was determined by subtracting the total crude protein, crude fibre, ash and lipid from the total dry matter. The caloric value estimation was done by summing up the multiplied values for crude protein, fat or lipid and carbohydrate (excluding crude fibre) by their respective factors (4, 9 and 4).

Mineral analysis

The method of Okalebo *et al*. (2002) was adopted to determine mineral elements in plant samples. About 0.5 g of finely ground plant samples was placed in dry, clean digestion tubes

and 5 ml of the digestion mixture comprising 1 part HCIO4 + 2 parts HNO3 added. This mixture was digested at 230^{O}C on a digestion block for 70 min, allowed to cool down and made up to 100 ml volume with distilled water. The macro-minerals (Carbon, Magnesium, Potassium, Sodium and Phosphorus) and micro-minerals (Iron, Zinc, Aluminium, Manganese and Copper) were determined using the Inductively Coupled Plasma - Optical Emission Spectrometer (ICP OES). All analysis was carried out in triplicates.

Data analysis

Data were expressed as mean ± standard deviation. Statistical analysis was done using students' unpaired t- test to compare the data of leaf content with stem and fruit. $p < 0.05$ was considered to be statistically significant. The data was analysed with Graph Pad Prism 4.0V for Windows (Graph Pad Software, San Diego, CA, USA).

Results

The macro minerals that were tested for include; sodium, calcium, potassium, magnesium and phosphorus. Out of these, sodium had the highest concentration in the leaves, stem and fruits (Table 1). It was observed that the second highest mineral in the three plant parts was calcium. On the other hand, the macro mineral with the least concentration in all plant parts was phosphorus. The concentration of potassium was moderately high in all plant parts while magnesium content was low. The micro minerals tested for were iron, aluminium, zinc, manganese and copper. Among these minerals, iron had the highest mean concentration in the three plant parts (Table 1). The second highest mineral in all the plant parts was aluminium while the concentration of manganese was slightly low. The concentration of copper was the least.

From the proximate analysis of *C. gynandra*, the moisture content had the highest concentration (Table 2). The second highest proximate factor was crude fibre. The

concentration of carbohydrate was the least. The leaves had the highest crude ash content in comparison to other parts of the plant. Similarly, crude protein of this species was also high. The calorific value was found to be higher in the fruits than in the leaves and stem. The amount of vitamin C was higher in the leaves compared to other parts of the plant (Table 2). The anti-nutrient composition of this plant revealed high concentration of oxalate in the leaves as compared to other parts of the plant (Table 3).

Discussion

The nutritional importance of a vegetable depends on its mineral constituents. In the current study, the sodium levels in this plant were comparatively high with respect to other reports on some wild leafy vegetables in South Africa (Afolayan and Jimoh, 2009). *C. gynandra* will therefore serve as a good source of sodium. Sodium is involved in the regulation of plasma volume, acid–base balance, nerve and muscle contraction (Akpanyung, 2005). In addition, the levels of sodium in this plant can contribute up to 22.3% of the recommended daily mineral (RDM) of sodium for an adult whose recommended mineral intake is 920 mg (NHMRC, 2005). The concentration of calcium was low when compared to other species of *Cleome* (Pillai and Nair, 2013). However, the low concentration of this element can stillcontribute about 4.75% to the RDM of dietary calcium needed in the body. Calcium is a vital element because it reduces the risk of various non-communicable diseases including the maintenance of bones, teeth and muscles (Vaskonen, 2003; Nieves, 2005).

The potassium content was moderately high in all the plant parts although when compared to other vegetables, it was relatively low (Ng *et al*., 2012). Nevertheless, this low concentration of potassium is still essential to the body since it is involved in regulating acid–base balance and blood pressure (He and MacGregor, 2008). Magnesium is an essential macro mineral which acts as a co-factor of many enzymes involved in energy metabolism, protein production and maintaining the electrical potential of nervous tissues and cell membranes

(Mohammed and Shariff, 2011). The concentration of magnesium was low in the present study when compared with the reported values for *Amaranthus hybridus* (Nwaogu *et al*., 2000). The low levels of the mineral constituents obtained from *C. gynandra* (calcium, potassium and magnesium) could be attributed to the prevailing environmental conditions, rate of mineral uptake by the plant and the age of the plant at harvest. Similar observations have been reported by some workers (Asaolu and Asaolu, 2010; Mohammed and Shariff, 2011). The low concentration of this element was similar to the values reported in the leaves of *Mucuna poggei* (Oko *et al*., 2012). Phosphorus in our diet is believed to prevent osteoporosis, arthritis, pyorrhoea, rickets and tooth decay (Asaolu *et al*., 2012). The iron content obtained in the present study was comparable with the values reported in other vegetables (Afolayan and Jimoh, 2009). The level of iron in this vegetable can meet the recommended daily mineral intake (NHMRC, 2005).

More than 21% of infants in South Africa suffer from anaemia which is due to lack of iron in their diets (van Vuuren, 2006; Ndlovu and Afolayan, 2008). *C. gynandra* could serve as a good source of iron in the diets of the local inhabitants. In addition, iron is an essential element for haemoglobin formation, normal functioning of the central nervous system and in the oxidation of carbohydrates, protein and fats (Adeyeye and Otokiti, 1999; Akubugwo *et al*., 2007). The presence of aluminium in *C. gynandra* is a cause for concern, taking into account that this micro mineral is toxic when consumed in large quantities. This could be postulated to be as a result of high soluble aluminium in the soil which tends to increase as the soil pH falls below 5.0 (Watanabe and Osaki, 2002). It was also observed that the concentration of zinc in this vegetable was high when compared to other species of *Cleome* (Pillai and Nair, 2013). By implication, it shows that this plant will serve as a good source of zinc, hence will play a key role in reproduction processes which is vital for normal sexual maturity specifically for the development of testes and ovaries (Ayoola *et al*., 2010). The

concentrations of copper and manganese have similar values with other wild vegetables **(**Bouba *et al*., 2012). The concentration of copper and magnesium were low when comparedto the acceptable safe level set by the World Health Organisation. However, *C. gynandra* could still serve as good source of micro minerals. The consumption of the vegetable could aid in bone mineralization, protein synthesis, enzyme actions and nerve transmission.

The moisture content obtained in the present study compares favourably with the reported range in five traditional leafy vegetables (Schonfeldt and Pretorius, 2011). The high moisture content will enhance more activity for water soluble enzymes and co-enzymes required for metabolic processes (Iheanacho and Udebuani, 2009). The amount of fibre in this plant was higher in the stem than in the leaves and fruits thus making the whole aerial parts of this vegetable significant (Ndlovu and Afolayan, 2008). This implies that *C. gynandra* could serve as a good source of fibre and low fat diet (Ng *et al*., 2012). Regular intake of leafy vegetables with high crude fibre has been shown to reduce serum cholesterol level, risk of coronary heart diseases and hypertension (Ayo, 2013). The percentage crude protein of *C.gynandra* was high and was comparable with or higher than *Solanum asper* and *Chenopodium album* (Afolayan and Jimoh, 2009). This high protein content in the leaves ofthis plant has revealed the significance of its underutilization. The rural populace in this region and other parts of the world rely mostly on vegetables as sources of protein; thus, this vegetable could play a vital role in providing inexpensive source of protein for its consumers. Similar observation was reported by Afolayan *et al*. (2009) on some wild leafy vegetables in South Africa. The high ash content of the leaves of this plant is also a reflection of its high mineral constituents (Aberoumand and Deokule, 2010). It was also observed that the fruits of this species were high in crude lipid, although this was low when compared with the reported values of some vegetables consumed in West Africa (Akubugwo *et al*., 2007).

According to Antia *et al*. (2006), lipids improve the palatability of food by absorbing and retaining flavours. A diet providing 1 - 2% of its calorific energy as fat is said to be sufficient for humans, as excess fat consumption is implicated in cardiovascular disorders such as atherosclerosis, cancer and aging (Sharma *et al*., 2012). The considerable amount of lipids in *C. gynandra* may therefore improve the palatability and reduce the risk of some diseases. Thecarbohydrate content of the leaves was considerably low compared to other leafy vegetables (Asibey-Berko and Tayie, 1999). This occurrence could be attributed to the high protein content in the vegetable (Asaolu *et al*., 2012). Generally, most leafy vegetables are commonly poor sources of carbohydrates. However, this wild vegetable is a fair source of carbohydrate.

The estimated total energy value was low when compared to the daily energy requirement for adults (Adinortey *et al*., 2012). This low calorific value would be of great benefit to people who are suffering from obesity. A high concentration of vitamin C was recorded in this species. Vitamin C is known to be natural anti-oxidant agents with rich sources of metabolites. Thus, if this vegetable is incorporated into the diet; it could be a vital source of vitamins in the body. The role of the mineral constituents in vegetables could be limited due to the presence of anti-nutrients which makes some of the micro minerals unavailable to human body (Akwaowo *et al*., 2000). The bioavailability of calcium is reduced by high oxalate content. This is because oxalate forms insoluble complexes with calcium, magnesium, zinc and ferrous iron thereby interfering with the management of these mineral elements in the vegetable (Akwaowo *et al*., 2000). The oxalate concentration in this vegetable was below the lethal level for humans. According to Nwinuka *et al*. (2005), oxalate range of 2 – 5 g is injurious to the health of human beings. Akwaowo *et al*. (2000) also reported a daily intake of 450 mg of oxalic acid could hamper the metabolic activities in humans. Phytate has a strong ability to chelate multivalent metal ions, especially Zn, Ca and

Fe in the plant leading to their poor bioavailability (Gupta *et al*., 2006). Generally, the anti-nutrients composition in all parts of *C. gynandra* was low, thus, this vegetable is suitable for consumption in this part of the world and even in the rest of Africa.

In conclusion, the results from this study have revealed that this underutilized wild vegetable is a good source of macro and micro-minerals. It also possesses important dietary components such as crude fibre, lipid, carbohydrates and protein. In addition, this species is rich in vitamin C. This study provides preliminary scientific validation for its use as a vegetable.

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Table 1: Mineral composition (mg/100g) of*Cleome gynandra*L. Data expressed as mean ± SD

Mineral elements	leaf	stem	Fruit
Nitrogen	49.7 ± 0.6^{a}	22.3 ± 1.2^{b}	35.8 ± 0.3^{c}
Calcium	44.5 ± 0.2^{ac}	19.1 ± 1.2^{b}	11.2 ± 0.1^{b}
Magnesium	9.5 ± 0^{a}	4.1 ± 0.1^{b}	3.4 ± 0.0^{bc}
Potassium	29.8 ± 0.2^{ab}	28.5 ± 0.4^{ab}	25.5 ± 0.7^{b}
Sodium	188.7 ± 2.3^{a}	205 ± 0.2^{b}	49.4 ± 1.3^{c}
Zinc	75.3 ± 23.9^{a}	2.9 ± 0.2^{b}	7.1 ± 1.5^{c}
Copper	1.4 ± 0.12^{ac}	0.4 ± 0.03^{b}	0.7 ± 0.03^{b}
Manganese	11.8 ± 0.7^{a}	2.7 ± 0.2^{b}	4.9 ± 0.1^{c}
Iron	100.5 ± 9.8^{a}	18.3 ± 1.5^{b}	31.3 ± 2.1^{c}
Phosphorus	5.9 ± 0.05^{ac}	2.4 ± 0.2^{b}	5.3 ± 0.0^{c}
Aluminium	92.3 ± 12.7^{a}	16.4 ± 2.4^{b}	20.7 ± 1.7^{c}

Different letters along rows represent significant differences at $p < 0.05$. n= 3

Table 2: Proximate composition (mg/100g) in*Cleome gynandra*L. Data expressed as mean ± SD.

Parameters	Leaf	Stem	fruit
Lipids	5.3 ± 0.3^{a}	6.27 ± 0.1^{b}	15.8 ± 0.1^{c}
Crude ash	20.81 ± 0.6^{a}	11.6 ± 0.3^{b}	9.1 ± 0.1^{c}
Crude Fibre	39.9 ± 0.7^{a}	62.9 ± 0.1^{b}	43.42 ± 0.3^{c}
Carbohydrates	1.9 ± 0.2^{a}	5.23 ± 0.1^{b}	8.93 ± 0.1^{c}
Protein	31.03 ± 0.1^{a}	13.8 ± 0.1^{b}	22.2 ± 0.2^{c}
Energy(Kcal)	178.5 ± 0.1^{a}	132.3 ± 0.1^{b}	268.4 ± 0.02^{c}
Vitamin C	345.3 ± 0.47^{a}	242.3 ± 1.69^{b}	288.3 ± 1.69^{c}
Moisture	82.1 ± 0.8	Not evaluated	Not evaluated

Different letters along rows represent significant differences at $p < 0.05$. $n = 3$

Table 3: Anti-nutrient composition (mg/100g) of*Cleome gynandra*L. Data expressed asmean ± SD.

Parameters	Leaf	Stem	fruit
Oxalate	7.4 ± 0.4^{a}	1.4 ± 0.05^{b}	3.4 ± 0.2^{c}
Phytate	0.3 ± 0.04^{ac}	0.5 ± 0.1^{b}	0.6 ± 0.0^{b}

Different letters along rows represent significant differences at $p < 0.05$.; $n = 3$

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CHAPTER 4

PHYTOCHEMICAL SCREENING AND *IN-VITRO* ANTIOXIDANT ACTIVITY OF

CLEOME GYNANDRA (L.): A WILD VEGETABLE GROWING IN THE

EASTERN CAPE, SOUTH AFRICA.

Phytochemical Screening and *In-vitro* antioxidant activity of*Cleome gynandra* (L.): A wild vegetable growing in theEastern Cape, South Africa.

Abstract 58

Introduction 59

Methodology 60

Results 66

Discussion 67

References 77

This chapter has been submitted in this format for publication to the Pakistan Journal of Botany.

Abstract

Cleome gynandra L. is a well-known nutraceutical plant. Besides its significance as a wildvegetable, its medicinal folkloric uses include the management of inflammatory conditions, cancer and cellular ageing. Total phenolics, flavonoids, flavonols, proanthocyanidins, tannins, saponins and alkaloids were determined using standard methods. The antioxidant activities of aqueous and ethanol extracts of *C. gynandra* were also investigated spectrophotometrically using ferric reducing power, ABTS (2, 2'- azino-bis-3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt, DPPH (1, 1- diphenyl-2-picrylhydrazyl) and NO (nitric oxide) radical scavenging techniques. The quantities of phenols, flavonoids and flavonols were significantly higher ($p < 0.05$) in both aqueous and ethanol extracts of the leaf while the proanthocyanidins were significantly higher in the aqueous stem extract compared to other plant parts. The aqueous extract of the different parts exhibited better ABTS, reducing power and NO radical scavenging abilities than ethanol extract. The study revealed that *C. gynandra* is very rich in secondary metabolites and can serve as natural antioxidant. Our findings also indicate that the aqueous extracts of the plant exhibited significant antioxidant properties which probably accounts for its pharmacological uses in folklore medicine.

Introduction

South Africa has wide ranges of plants among which are leafy vegetables growing in the wild (Lewu *et al*., 2010). These wild vegetables are considered as low status foods; hence they are ignored and underutilized (Jansen van Rensburg *et al*., 2007). Despite the underutilization of wild vegetables in urban centers, the native people rely solely on their use for medicinal purposes in traditional medicine. These medicinal potentials are dependent on the presence of secondary metabolites (phytochemicals) which are stored in various parts of the plant including the leaves, stems, roots, fruits, bark, rhizomes and seeds (Ahmad *et al*., 2011; Arowosegbe *et al*., 2012). Wild vegetables are natural radical scavengers because they exhibit antioxidant properties (Souri *et al*., 2008). Regular consumption of these species has been linked to the mopping up of free radicals including superoxide anion, peroxide, and hydro peroxide in humans.

Cleome gynandra L. is a well-known nutraceutical plant. Besides its significance as a wildvegetable, its medicinal folkoric uses include the management of inflammatory conditions, cancer and cellular ageing (Kumar *et al*., 2012; Meda *et al*., 2013). Despite the wide usage of the plant in traditional medicine, there is a dearth of information on the phytochemical constituents and *in-vitro* antioxidant activity of the species in the Eastern Cape Province of South Africa. Thus, the objective of this study was to investigate quantitatively the phytochemical constituents and antioxidant capacity of the aqueous and ethanol extracts of different parts of *C. gynandra* in order to provide a scientific basis to justify its folkloric therapeutic usage**.**

Materials and methods

Collection of plant materials and preparation of extracts

Fresh leaves, fruits and stems of *C. gynandra* were collected from the University of Fort Hare Research Farm in Alice (latitudes 32° 47' 0" South and longitudes 26° 50' 0" East). This plant was identified at the Department of Botany; University of Fort Hare and a voucher specimen (LinMed 2013/01) were deposited in the Giffen herbarium of the University.

Extraction methods

The collected plant samples were properly washed, oven dried at 40^{o}C to a constant weight, pulverized to fine powder and stored in airtight bottles which were then kept in the refrigerator at 4^{o}C until needed for the analysis. From the powdered samples, 200 g of each plant part was extracted separately in distilled water and ethanol for 48 h under mechanical agitation (Stuart Scientific Orbital Shaker, Essex, UK). The extracts were filtered and the filtrate obtained from distilled water was frozen at -40°C and dried for 48 h using a freeze dryer to yield of 18.5 g, 17.6 g and 21.2 g for leaf, fruit and stem respectively. The ethanol extract was taken to dryness under reduced pressure at 40°C using a rotary evaporator and yielded 16.1 g, 15.3 g and 24.2 g for leaf, fruit and stem respectively. The resulting extracts were reconstituted with ethanol and distilled water to give the required concentrations used in the study.

Chemicals and reagents used

1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS), vanillin, butylated hydroxyl toluene (BHT), rutin, potassium persulphate, sodium nitroprusside ($Na_2[Fe(CN)_5NO]2H_2O$), sulfanilic acid, glacial acetic acid (CH_3COOH), gallic acid, tannic acid, ferric chloride ($FeCl_2$), ascorbic acid, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), aluminium chloride ($AlCl_3$), potassium acetate (CH_3CO_2K), phosphate buffer, potassium ferricyanide[$K_3Fe(CN)_6$], trichloroacetic acid

(TCA) and 2-thiobarbituric acid (TBA). All the chemicals used in this study were of analytical grade and were purchased from Merck, Gauteng, South Africa.

Phytochemical screening

The total phenolic content in the extracts were determined by the modified Folin-Ciocalteu method (Otang *et al*., 2012). An aliquot of 0.5 ml of each part of the plant extract (1 mg/ml) was mixed with 5 ml of Folin- Ciocalteu reagent which was previously diluted with distilled water (1:10 v/v) and 4 ml (75 g/l) of sodium carbonate (Na_2CO_3). The tubes containing the mixtures were vortexed for 15 s and allowed to stand for 30 min at 40°C to develop colour. Absorbance was then read at 765 nm using the AJI- C03 UV- VIS spectrophotometer. Results were expressed as mg/g tannic acid equivalent using the equation based on the calibration curve:

$Y = 0.1216\ x$; $R^2 = 0.9365$, where x is the absorbance and Y is the tannic acid equivalent.

The flavonoid content was determined as described by Otunola and Afolayan, (2013). Briefly 0.5 ml of 2% $AlCl_3$ was prepared in 98ml of ethanol. This was then added to 0.5 ml of the extracts. The mixture was allowed to stand for 60 min at room temperature and the absorbance was read at 420 nm. The extracts were evaluated at a final concentration of 0.1 mg/ml. The result was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve:

$y = 0.0255\ x$, $R^2 = 0.9812$; where x is the absorbance and y is the quercetin equivalent (QE).

The flavonol content was determined based on the method used by Olajuyigbe and Afolayan, (2011). Briefly, 2 ml of each plant extract were mixed with 2 ml of $AlCl_3$ prepared in ethanol. This was followed by adding 3 ml of sodium acetate solution (50 g/l). The mixture was

incubated at 20^{O}C for 2.5 h. Absorbance was measured at 440 nm. The total flavonol content was calculated as quercetin (mg/g) using the following equation based on the calibration curve

$Y=0.0255x$, $R^2=0.9812$, where x is the absorbance and Y is the quercetin equivalent.

The total proanthocyanidin was determined by adopting the procedure of Oyedemi *et al*. (2010). A volume of 0.5 ml of the extract solution was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml hydrochloric acid. The resulting mixture was vortexed and allowed to stand for 15 min at room temperature. The absorbance was then measured at 500 nm. Total proanthocyanidin content was expressed as catechin equivalents (mg/g) using the calibration curve equation:

$Y = 0.5825x$, $R^2 = 0.9277$, where x is the absorbance and Y is the catechin equivalent.

Tannin determination was done as previously described by Mbaebia *et al*. (2012) with some modifications. 0.20 g of plant sample was added to 20 ml of 50% methanol. This was mixed thoroughly and placed in a water bath at 80^{O}C for 60 min. The extract was filtered into a 100 ml volumetric flask; 20 ml of distilled water added, followed by 2.5 ml of Folin-Ciocalteu reagent and 10 ml of 17% Na_2CO_3. This was thoroughly mixed together and made up to 100 ml using distilled water. The mixture was allowed to stand for 20 min. A bluish-green color developed and the mixture of different concentrations ranged from 0-10 ppm. Absorbance of the tannic acid standard solutions and plant samples were measured after color development at 760 nm using the AJI-C03 UV-VIS spectrophotometer.

Results were expressed as mg/g of tannic acid equivalent using the calibration curve:

$Y = 0.0593x - 0.0485$, $R^2 = 0.9826$, where x is the absorbance and Y is tannic acid equivalent.

The alkaloid content was determined as described by Onyilagha and Islam, (2009). About 5 g powdered plant sample was weighed, Two hundred milliliters of 10% acetic acid in ethanol was added. The mixture was covered and allowed to stand for 4 h. It was filtered and the filtrate was concentrated on a water bath to one-fourth of its original volume. Concentrated ammonium hydroxide was added in drops to the extract until precipitation was completed. Thereafter, the whole solution was allowed to settle and the collected precipitates were washed with dilute ammonium hydroxide and then filtered again. The residue collected was dried and weighed. The alkaloid content was determined using this formula:

% alkaloid = (final weight of sample / initial weight of extract) ×100

The saponin content in the plant extracts was determined as previously described by Obadoni and Ochuko, (2001). Briefly, 20 g of the powdered sample was dissolved in 200 ml of 20% ethanol. This was placed on a shaker for 30 min. Afterwards, the plant sample was heated in a water bath at 55°C for 4 h with continuous stirring. The mixture was filtered and the residue was re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over the water bath at 90°C. The concentrated solution obtained was then transferred into a 250 ml separating funnel and extracted twice using 20 ml diethyl ether. The ether layer was discarded, while the aqueous layer was retained and 60 ml *n*-butanol added. The *n*-butanol extracts were washed twice with 10 ml of 5% sodium chloride ($NaCl_2$). The remaining solution was heated in a water bath to evaporate and the samples were oven dried at $40^{O}C$ to a constant weight. The percentage saponin content was calculated using the formula below:

% saponin = (final weight of sample / initial weight of sample) ×100

Antioxidant assay

The reducing power of the extracts was determined as previously described by Wintola and Afolayan, (2011). 1 ml of the extract and standards were prepared in distilled water (0.025-0.5 mg/ml) and mixed thoroughly with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1% w/v). The resulting mixture was incubated at 50^oC for 20 min, followed by adding 2.5 ml of TCA (10% w/v), the mixture was then centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of ferrous chloride (0.1% w/v). The absorbance was read at 700 nm against a blank sample. Increased absorbance of the mixture indicated higher reducing power of the plant extract.

The method described by Oyedemi *et al*. (2010) was used to determine the scavenging activity of different parts of *C. gynandra*. A volume of 2 ml of 10 mM of sodium nitroprusside was prepared in 0.5 mM phosphate buffer saline (pH 7.4). This was then mixed with 0.5 ml of the extract, vitamin C and rutin individually at various concentrations of 0.025 - 0.5 mg/ml. The mixture was incubated at 25^oC for 150 min. An aliquot of 0.5 ml of the solution was mixed with 0.5 ml of Griess reagents [1.0 ml of sulfanilic acid reagent (0.33% prepared in 20% glacial acetic acid) for 5 min with 1 ml of naphthyethylenediamine dichloride (0.1% w/v]. The mixture was incubated at room temperature for 30 min and absorbance was measured at 540 nm. The amount of the nitric oxide radicals inhibited by the extract was calculated using the equation:

> NO radical scavenging activity (%) = [(Abs control- Abs sample) / (Abs control)] ×100, where Abs control is the absorbance of nitric oxide radicals + methanol; Abs sample is the absorbance of nitric oxide radical + sample extract or standard.

The scavenging activities of the different plant extracts against ABTS radical was determined by method described by Adedapo *et al*. (2008). First the working solutions were prepared by mixing two stock solutions of 7 mM ABTS and 2.4 mM potassium persulphate in equal

amounts and allowed to stand for 12 h at room temperature in the dark. The resulting solution was then diluted by mixing 1ml $ABTS^{+}$ solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm after 7 min using the spectrophotometer. The percentage inhibition of $ABTS^{+}$ by the various plant extracts were calculated using the formula:

% inhibition = [(Abs control- Abs sample) / (Abs control)] ×100.

The effects of aqueous and ethanolic extracts on the DPPH free radicals were determined by the method of Duraipandiyan and Lgnacimuthu, (2009). Briefly, 1ml of DPPH prepared in methanol (0.135 mM) was mixed with 1 ml of different concentrations ranging from 0.0025-0.5 mg/ml of various plant extracts of *C. gynandra*. The mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using the spectrophotometer. The scavenging ability of the plant extract on DPPH was calculated using the equation:

DPPH scavenging activity (%) = [(Abs control – Abs sample)] / (Abs control)] ×100,
Where Abs control is the absorbance of DPPH + methanol; Abs sample is the absorbance of DPPH radical + sample (extract/standard).

Statistical analysis

All experiments were done in triplicates and where applicable, the data were subjected to one way analysis of variance (ANOVA) and differences between plant parts were determined by Duncan's Multiple Range Test using the MINITAB program (version 12 for windows). The means were treated as significantly different at $p < 0.05$.

Results

The phytochemical constituents of the ethanolic extract of the different parts of the plant are shown in Figure 1. The phenolic (168.86 mg/g), total flavonoids (40.47 mg/g), flavonols (40.25 mg/g) and proanthocyanidin (209.7 mg/g) contents of the leaf extract was significantly higher than those of the stem and fruit. The alkaloid and saponin contents were appreciably low in all the parts tested when compared to the other phytochemicals and their values did not differ significantly among the plant parts. The aqueous leaf extract had the highest concentration of total phenols (183.77 mg/g), total flavonoids (13.56 mg/g) and flavonols (11.77 mg/g) when compared to that of the stem and fruit extracts (Fig. 2). The proanthocyanidin content in the stem extract (275.33 mg/g) of *C. gynandra* was significantly higher than that of the leaf and fruit. The tannin content was relatively low and similar in all plant parts. A similar trend was also observed in the alkaloid content. The saponin content of the fruit extract (22.35 mg/g) was significantly higher compared with the other plant parts.

The antioxidant potential of *C. gynandra* extracts was evaluated based on their ability to scavenge the ABTS radical (Fig. 3). The fruit and leaf aqueous extracts exhibited a higher radical scavenging ability (73.9% and 72.9% respectively) than that of the stem (55.8%). The aqueous extracts consistently showed stronger inhibition against the ABTS radical compared to the ethanol extract. The scavenging activities of both standards were found to be higher than the plant extracts. The aqueous fruit extract and leaf had IC_{50} values of 0.1 and 0.03 mg/ml followed by aqueous stem, ethanol leaf and ethanol stem at 0.2, 0.23, 0.2 mg/ml respectively. Rutin, ethanol fruit and vitamin C had their activities at 0.3, 0.4 and 0.5 mg/ml (Table 1).

DPPH radical was used to assess the radical scavenging activity of *C. gynandra* extracts (Fig. 4). The ethanolic extract of the stem showed maximum 41.8% inhibition of the DPPH radicals followed by the leaf with 32.4%. The least activity was exhibited by the fruit extract

(19.2%). The ethanolic extracts of the stem and leaf exhibited higher DPPH radical scavenging ability than the aqueous extract. Both standards showed higher scavenging activity than all extracts. The IC_{50} values of the extracts showed the following trend: ethanol stem < ethanol leaf < ethanol fruit < vitamin C < aqueous stem < aqueous fruit < aqueous leaf < rutin (Table 1).

The ferric reducing ability of *C. gynandra* extracts was further assessed by their ability to reduce Fe^{3+} to Fe^{2+} (Fig. 5). It was observed that both aqueous and ethanol extracts of all plant parts exhibited high reducing activities when compared to the standards. The ferric reducing activities of the extracts are shown in the following order: aqueous stem > ethanol fruit > ethanol stem > aqueous leaf > aqueous fruit > ethanol leaf > rutin > vitamin C. There were no significant differences amongst the different plant extracts.

In order to evaluate the antioxidant potency of the different extracts of *C. gynandra* against nitric oxide radical, the percentage inhibition of the radical was examined (Fig. 6). The inhibition of nitric oxide by both extracts was lower when compared to the standards. Among the plant extracts, aqueous leaf (32.9%) was significantly higher than that of the stem (25.3%) and fruit. The minimum inhibitory concentration required to reduce the nitric oxide radicals by 50% were 0.1, 0.2, 0.2 and 0.3 mg/ml for aqueous stem extract, rutin, vitamin C and ethanol stem respectively, while a similar value occurred in the other plant extracts (Table 1).

Discussion

Phytochemical constituents like phenolic compounds are associated with biological activities such as antioxidant and scavenging potentials against free radicals. For instance, phenolics and flavonoids are known to exhibit anti-allergic, anti-inflammatory, anti-microbial and anticancer activities (Ogunmoyole *et al*., 2012). Interestingly, *C. gynandra* extracts showed

that they are rich in polyphenolic compounds, thus, partially justifying this plant's use in folkloric medicine for the management of oxidative stress diseases. In addition, the high concentration of proanthocyanidin in the stem extract of the plant is of great importance because this compound could serve as a potential source of bioactive components in the treatment of cancer and other radical related ailments (Mbaebia *et al*., 2012). Tannins play a major role in the treatment of inflamed tissues, diarrhoea and prevention of cancer (Okwu and Emenike, 2006). The presence of alkaloids in *C. gynandra* extracts shows the potential of the plant to have analgesic, anti-inflammatory and adaptogenic properties (Omololu *et al*., 2011). This may partially justify the traditional use of this species in the treatment of malaria and inflammatory conditions (Bala *et al*., 2010; Kumar *et al*., 2012; Meda *et al*., 2013). On the other hand, saponins are characterized by their bitter taste, foaming properties, cholesterol binding and haemolytic activity on red blood cells. The presence of saponins in the extracts may also boost the antioxidant activities of the plant ranging from antitumor, anti-mutagenic and anti- inflammatory properties (Maisuthiasakul *et al*., 2007). In addition, the low concentration of saponins, alkaloids and tannins observed in this species would suggest low toxicity in the plant.

Proton radical scavenging is an important characteristic of antioxidants. ABTS, a protonated radical, has characteristic absorbance maxima at 734 nm that decreases with the scavenging of the proton radicals (Ashafa *et al*., 2010). When the plant extract reacted with the ABTS radical, the radical cation decolorizes the bluish green chromophore. This decolorization is an indication that the plant extract has the ability to donate electrons freely. The significant ABTS radical scavenging activity of the different plant extracts may however be linked to the presence and structure of hydroxyl groups which are associated with high phenolic compounds. These compounds are responsible in oxidizing the proton radical formed within the species (Oyedemi and Afolayan, 2011). The degree of decolorization of the test solution

is an indication of the scavenging potential of the plant extract in terms of hydrogen donating ability. Thus, the scavenging activity of the plant extracts against DPPH radical indicates their ability in donating hydrogen proton to a free radical in order to eliminate odd electron which causes radical's reactivity. The presence of reductants in *C. gynandra* extracts enhanced the conversion of Fe^{3+} to Fe^{2+}. This was confirmed by the discoloration of the test solution from yellow to green. The reducing power of the plant extracts was potently active compared to the standard drugs. This observation could be ascribed to the high accumulation of flavonoids and phenolics in the plant parts, these constituents are responsible for metal reduction due to their nucleophilic nature which breaks the free radical chain through donating hydrogen atom (Otang *et al*., 2012). This finding corroborates with that of Mbaebia *et al.* (2012) on the aqueous extract of *Schotia latifolia* Jacq. The scavenging activity of the extract was moderately comparable to the standards in inhibiting the formation of nitrite. This observation may be attributed to the presence of flavonoids in the plant extract which competes with oxygen and oxides of nitrogen thereby inhibiting the formation of nitrite. It is well known that nitric oxide has an important role in various inflammatory processes (Gates *et al*., 2008).

Conclusion

The phytochemical screening of the *C. gynandra* showed that the leaf had more phytochemical constituents when compared to the stem and fruit parts. The stem of this species is an excellent source of proanthocyanidin. The plant could serve as an excellent source of natural antioxidants in nutraceutical, food and medicinal industries. Hence, the inclusion of this wild vegetable in the diet is recommended in order to build the immune system against chronic diseases. The result also showed that both the aqueous and ethanol extracts from *C. gynandra* exhibited strong antioxidant activities. Therefore, it would seem likely that both solvents were able to extract the secondary metabolites which are responsible

for the antioxidant activity of the plant. This activity could partly explain the pharmacological use of the plant in the management of diseases in traditional medicine.

Conflicts of interests

The author declares that there is no conflict of interests.

Acknowledgements

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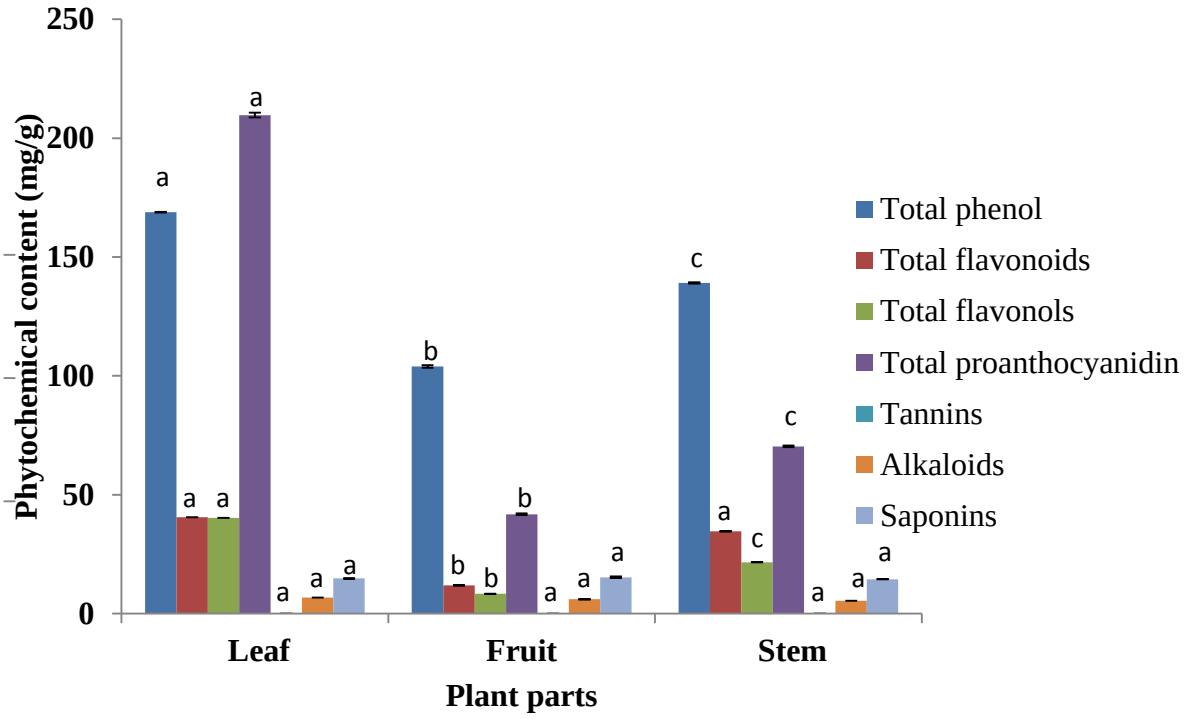

Figure 1: Phytochemical constituents of ethanol extracts of*C. gynandra*. Data are presentedas means ± standard deviation of three replicates. Phytochemicals in the different plant parts having the same letter are not significantly different from each other.

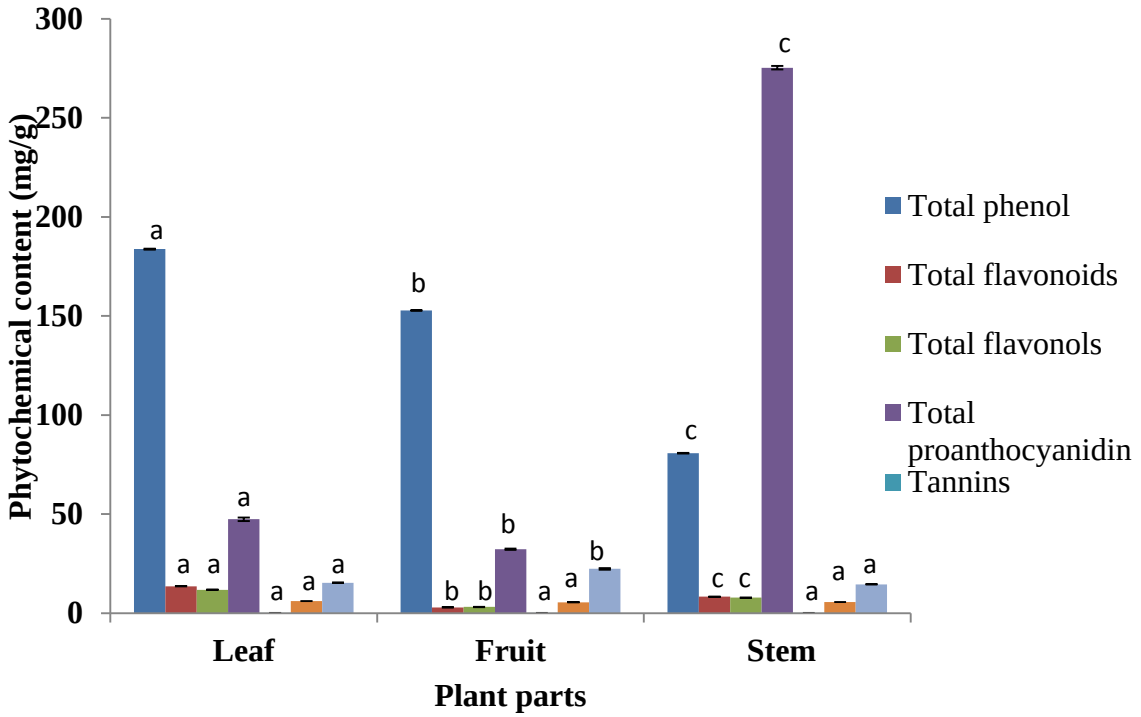

Figure 2: Phytochemical constituents of aqueous extracts of*C. gynandra*. Data are presentedas means ± standard deviation of three replicates. Phytochemicals in the different plant parts having the same letter are not significantly different from each other.

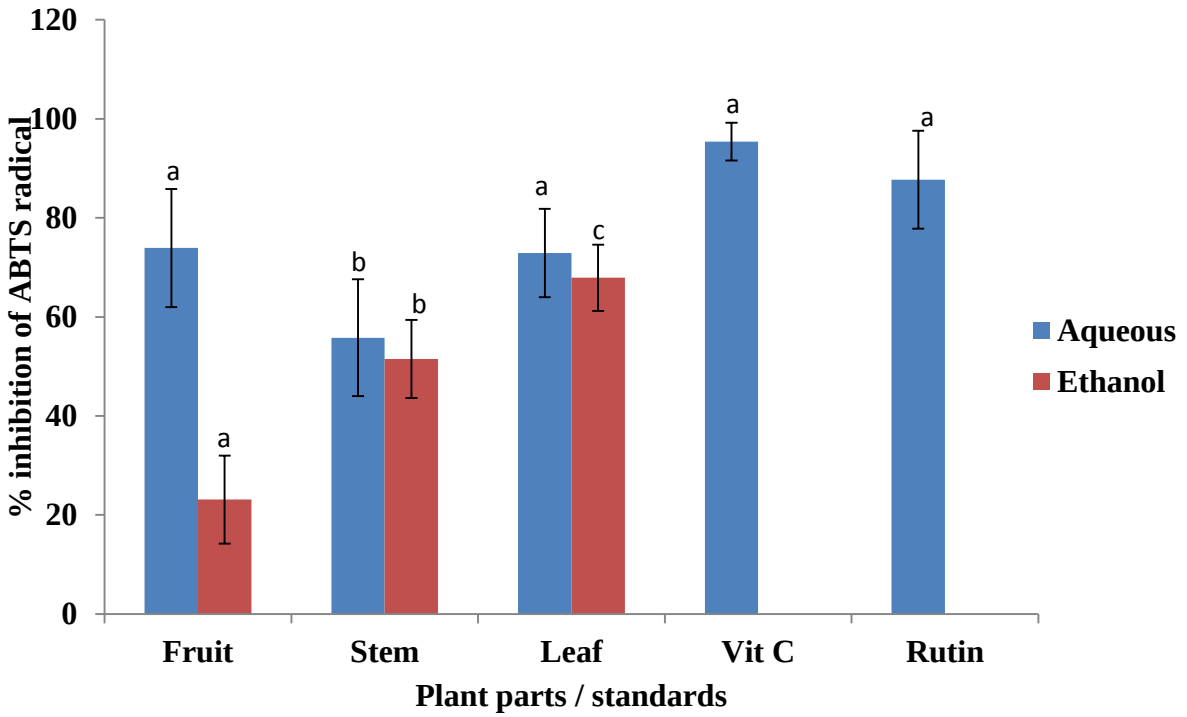

Figure 3: ABTS radical scavenging activities of aqueous and ethanol extracts of*C. gynandra*fruits, stems and leaves. Results are means of 3 replicates. Antioxidants in the different plant parts having the same letter are not significantly different from each other.

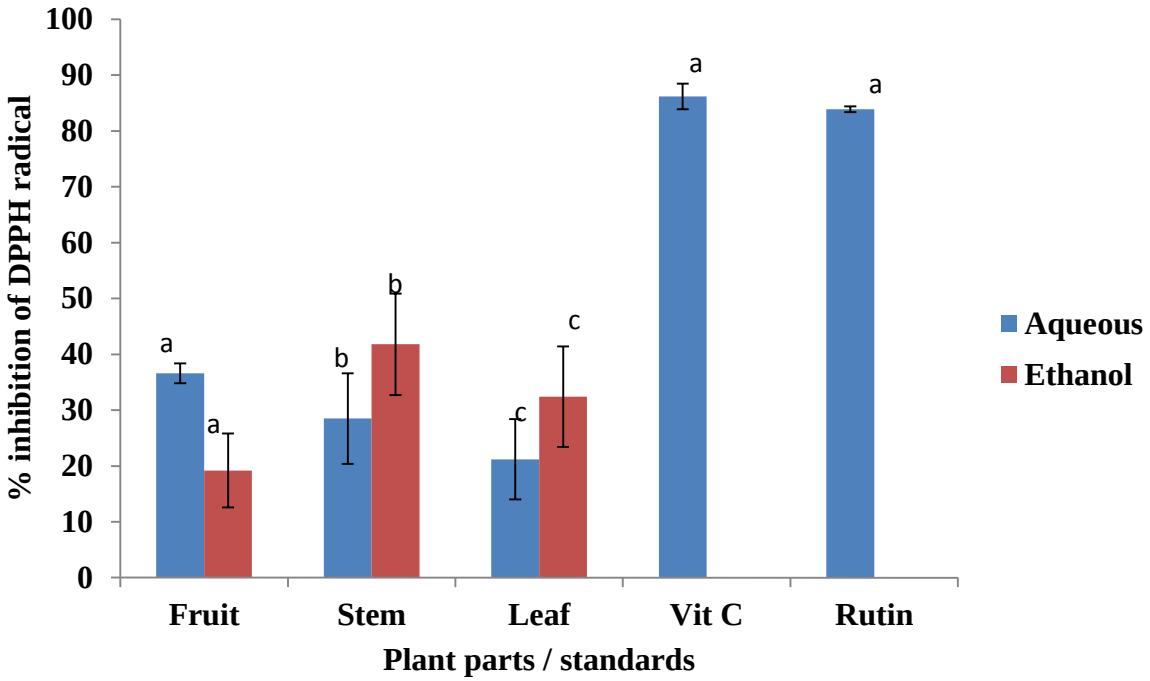

Figure 4: DPPH activities of aqueous and ethanol extracts of*C. gynandra*fruits, stems andleaves. Results are means of 3 replicates. Antioxidants in the different plant parts having the same letter are not significantly different from each other.

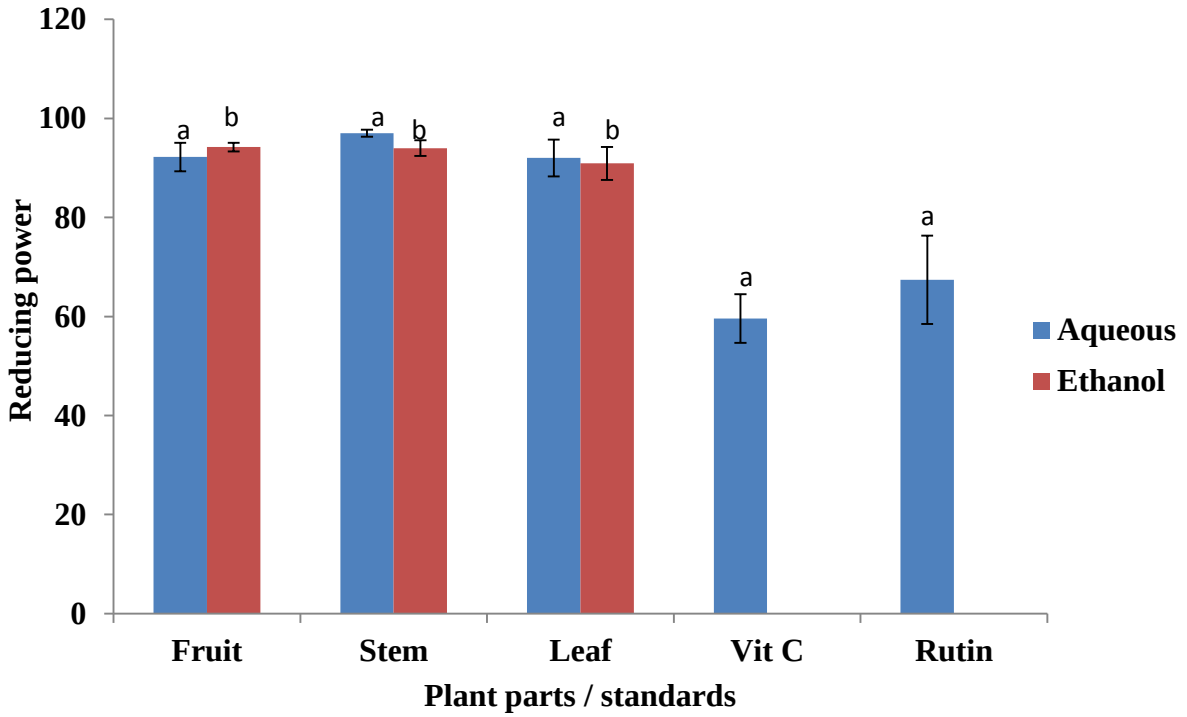

Figure 5: Reducing power activities of aqueous and ethanol extracts of*C. gynandra*fruits,stems and leaves. Results are means of 3 replicates. Antioxidants in the different plant parts having the same letter are not significantly different from each other.

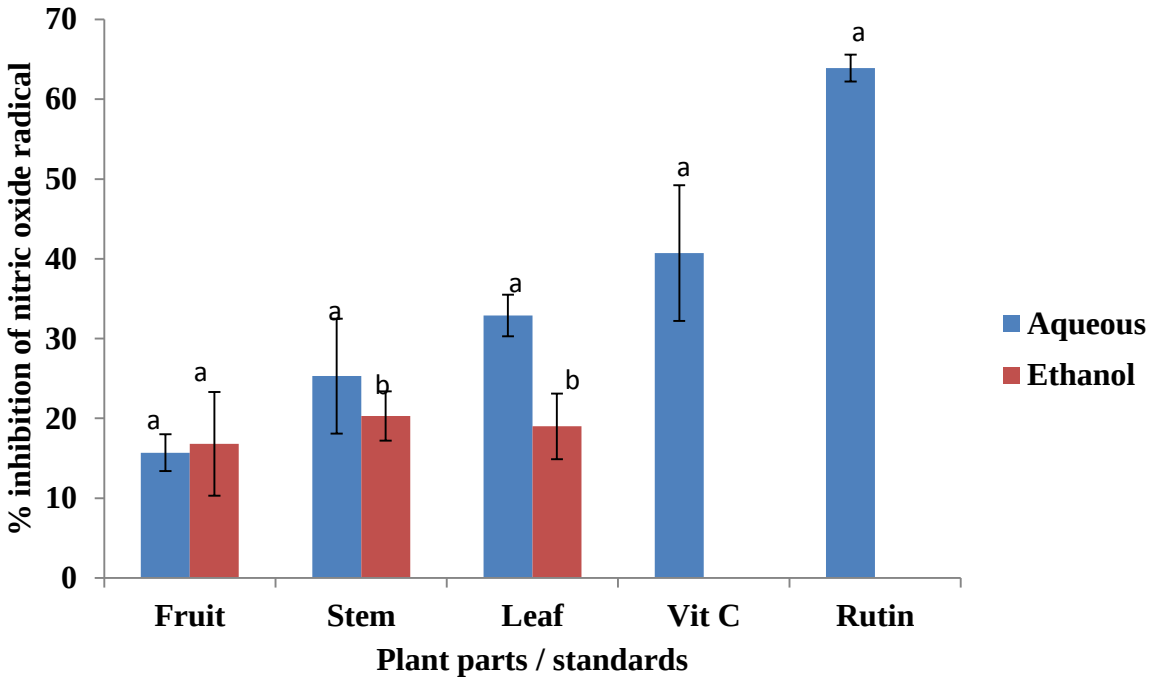

Figure 6: Nitric oxide radical scavenging activities of aqueous and ethanol extracts of*C.gynandra* fruits, stems and leaves. Results are means of 3 replicates. Antioxidants in thedifferent plant parts having the same letter are not significantly different from each other.

Table 1: Scavenging activities of aqueous and ethanolic extracts of *C. gynandra* fruits, stem and leaves.

	Reducing Power		ABTS		DPPH		Nitric oxide	
Sample	IC_{50}	R^2	IC_{50}	R^2	IC_{50}	R^2	IC_{50}	R^2
Aqueous fruit	0.1	98.8	0.1	32.3	0.2	78.5	0.3	22.1
Ethanol fruit	0.2	91.7	0.4	92.3	0.05	14.3	0.3	82.2
Aqueous leaf	0.3	98.4	0.03	6.8	0.3	22.4	0.3	92
Ethanol leaf	0.3	99.5	0.23	55.3	0.2	74.4	0.3	13.9
Aqueous stem	0.05	10.1	0.2	78.7	0.2	69.7	0.1	57.4
Ethanol stem	0.3	99.2	0.2	85.7	0.1	68.4	0.3	97.7
Rutin	0.3	91.3	0.3	98.1	0.5	1.3	0.2	17.4
Vitamin C	0.2	72.6	0.5	13.9	0.2	47.8	0.2	91.2

A: IC_{50} is the concentration (mg/ml) capable to attain 50% of maximum scavenging ability.

B: R^2 is the coefficient of determination; values obtained from regression lines with 95% confidence level.

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CHAPTER 5

EFFECT OF ENVIRONMENTAL FACTORS AND SOWING DEPTH ON SEED GERMINATION IN *CLEOME GYNANDRA* L. (CAPPARACEAE)

CHAPTER FIVE

Effect of environmental factors and sowing depth on seed germination in *Cleomegynandra* L. (Capparaceae)

Abstract 82

Introduction 83

Materials and methods 84

Results 87

Discussion 88

References 95

This chapter has been accepted for publication in Pakistan Journal of Botany in this format.

Abstract

Cleome gynandra is a wild vegetable that is rich in nutrients especially vitamins, mineralelements and protein. It is consumed in most parts of South Africa as a vegetable. The leaves and seeds of this plant are used in folkloric medicine for the treatment of head and stomach aches. Despite its high dietary-medicinal value, the plant is still regarded as a weed in many Provinces of South Africa while the conditions necessary for its optimum growth in the wild are still obscure. Therefore, this study was designed to investigate the effect of various environmental factors and sowing depth on the germination of two types of seeds of *C.gynandra* in the Eastern Cape Province of South Africa. The results show that the averageseed weight was 1.2 ± 0.003 mg and the viability of Lot A and B were 22.6 ± 2.3% and 67.3

± 5.0% respectively. The optimum germination was achieved at 30^{o}C for both Lots A and B when watered bi-weekly at a sowing depth of 0.5 cm. The result also showed that germination was best in the dark (28.7%) for both Lot A and B. In overall, the germination rate under all the conditions was highest in Lot B. This study indicates that *Cleome gynandra* has the potential of thriving successfully under varied environmental conditions despite the great fluctuations of temperatures in South Africa during summer and winter respectively.

Introduction

Traditionally, the use of wild leafy vegetables is an important component of the diet of the people of the Eastern Cape in South Africa. These vegetables are a rich source of micronutrients and vitamins (Nesamvuni *et al*., 2001; Steyn *et al*., 2001). In addition, they play a significant role in nutrition as well as food security and serve as supplements for the management of malnutrition (van Wyk and Gericke, 2000; Steyn *et al*., 2001; Odhav *et al*., 2007; Jansen van Rensbury *et al*., 2007). Some of the most common wild vegetables found in this Province include *Physalis viscosa, Amaranthus paniculatus, Solanum nigrum, Rumexobtusifolius, Physalis peruviana, Sonchus asper*, *Corchorus olitorius* and *Cleome gynandra*

(Rensburg *et al*., 2007).

Cleome gynandra L. is an erect herbaceous annual plant belonging to the Capparaceacefamily. It grows up to 1.5 m in height. The leaves are alternate, palmately compound and its petals are white, pink or lilac. This plant is commonly known as African spider. It grows as a weed in most tropical countries but it is sometimes a semi- cultivated popular tropical leafy vegetable in many parts of sub-Saharan Africa especially in countries such as Botswana, Kenya, Tanzania, Uganda and Zimbabwe (Heever and Venter, 2007). In South Africa, the plant is commonly found in Limpopo, North-West, Gauteng, Mpumalanga, KwaZulu-Natal, Free-State and the Northern Provinces (Smith and Stearn, 1972). *C. gynandra* is a wild vegetable that is rich in nutrients especially vitamins A and C, calcium, iron, magnesium and protein (Chweya and Mnzava, 1997). The succulent shoots are boiled and eaten as a pot herb, stew or side dish in South Africa. In Kenya, several nutritional uses of this plant have been established (Opole *et al*., 1995). These leaves are used, in Thailand, for making a fermented product known as Paksian-dong, (FAO, 1990). Indians use the leaves as a flavoring agent in sauces.

In folkloric medicine, *C. gynandra* leaves and seeds are used, especially, in the treatment of head and stomach aches. Bruised leaves, which are rubefacent and vesicant, are used to treat neuralgia, rheumatism and other localized pains (Chweya and Mnzava, 1997). In India, the sap from the leaves is used as an analgesic for headaches, epileptic fits, ear aches and for the treatment of inflammation (Narendhirakannan *et al*., 2005; Mule *et al*., 2008). Regular consumption of this vegetable has been reported to relieve childbirth complications, to treat scurvy, marasmus and malaria (Onyanyo *et al*., 2013). Despite its ethno-pharmacological importance, this plant is still regarded as a wild vegetable that grows naturally in this part of the world though wild vegetables have been proved to be of potential significance to rural life and development (Flyman and Afolayan, 2006).

Although the natural regeneration of *C. gynandra* is mainly by seed, the seeds of this plant do not germinate readily (Chweya and Mnzava, 1997). To improve its seed germination, pre-treatment methods and alteration of environmental factors have been reported (Ochuodho and Modi, 2005) while no other methods have been devised to promote its proper propagation in South Africa. This study was, therefore, designed to investigate the effect of various environmental factors and sowing depth on germination of the seeds of *C. gynandra* in the Eastern Cape Province of South Africa.

Materials and methods

Seed collection

Mature seeds of *Cleome gynandra* were harvested from the University of Fort Hare research farm in Alice (Latitudes 32° 47' 0" South and Longitudes 26° 50' 0" East). The freshly harvested matured seeds were removed from their capsules, air-dried and used for the germination studies whereas another batch of the seeds were stored in an air tight container at

room temperature (15-25°C) for eight months and later used for various germination trials. For the purpose of this study, they are referred to Lot A seeds and Lot B seeds respectively.

Seed weight determination`

Seed weight was determined by weighing 200 seeds using an analytical balance and the mean weight of one seed was calculated.

Viability test

To ensure that the seeds used for experiments were viable, viability test was carried out using the tetrazolium technique (ISTA, 2003). Four replicates of 50 seeds each were used for each seed Lot. Adopting the procedure of Peters, (2000) the seeds were imbibed for 24 h in water, pierced along the margin without damaging the embryo and soaked in colorless 0.1% solution of 2,3,5 triphenyltetrazolium chloride (TTC) for 16 h at 25°C in the dark. The seeds were then removed from TTC solution, washed with distilled water and soaked in 10 ml of 95% ethyl alcohol to permit direct observation of the embryo. They were then viewed under a light microscope to observe the stained embryos. Embryos of viable seeds appeared bright red in color.

Germination Trial

Seeds were surface sterilized in 0.1 mercuric chloride solution for 60 s to prevent fungal attack before been rinsed in several changes of sterile water. They were then placed in a 9 cm sterile petri dishes lined with two Whatman No.1 filter papers moistened with 3 ml of distilled water. Treatments were arranged in a completely randomized design with three replicates of 50 seeds each. The experiment was repeated thrice and the pooled mean values were separated on the basis of least significant Differences (LSD) at a probability level of 0.05.

Effect of temperature

The effect of temperature on germination was investigated by placing the petri dishes in incubators set at 10, 15, 20, 25, 30 and 35°C. This was observed daily for 14 days. Distilled water was added when necessary.

Effect of light

Seeds were exposed to illuminations produced by sun rays whereas petri dishes were covered with three layers of aluminum foil in the dark treatment (Baskin and Baskin, 1998) and daily observations were assessed in a dark room illuminated with a green light.

Effect of water regimes

The following water treatments were investigated. These include daily, once a week and bi weekly watering. The seeds were examined every 24 h and considered viable when the radicle was observed (Thanos and Rundel, 1995). Germination was recorded daily over a period of 14 days and germinated seeds were removed promptly. At the end of the period, non-germinated seeds were dissected to check for viability (if the embryo and endosperm are intact and not discoloured) (ISTA, 2003). Germination percentage for each treatment was calculated using the formula cited by Czabator, (1962).

Germination (%) = (Total number of seeds germinated /Total number of seeds per replicate) ×100

Effect of sowing depth

Plastic pots were filled with 2 kg of garden soil and five seeds of *C. gynandra* were sown at 11 different depth of 0 (soil surface), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5 cm. The pots were laid out in a completely randomized design with three replicates. Each treatment was irrigated daily with water. Germination counts was measured daily for 14 days. The

germination rate was determined using a modification of Timson's index (Khan and Rizvi, 1994) of germination velocity.

$$\text{Germination velocity} = \sum G/t$$

Where G is the percentage of 2-day- interval germinated seeds and t is the total germination period. The greater the index value, the higher the germination rate.

Statistical analysis

Data were presented as means ± standard deviation of triplicate determination. One way analysis of variance (ANOVA) was used to determine the effect of various factors on germination percentage. Significant level was set at 5% probability while Duncan's multiple range test were used to segregate the treatment means. All analyses were done using MINITAB student version 12 for windows statistical software.

Results

Seed weight and viability

In this study, the average seed weight was 1.2 ± 0.003 mg and the viability of Lots A and B as determined were 22.6 ± 2.3% and 67.3 ± 5.0% respectively. The results revealed that the highest germination was recorded at 30^{o}C, followed by that of 25^{o}C in Lot B while there was no germination recorded at 10^{o}C in Lot A (Fig. 1). The result showed that germination increased with temperature up to 30^{o}C before it began to decrease in both Lots. The result also showed that the germination was significantly affected by varying temperature regimes. Generally, germinated seeds in Lot B were significantly higher than those in Lot A in all temperature treatments.

Watering regime results indicate that the highest germination was recorded at the bi- weekly watering regime in Lot B, followed by that of once a week and the least germination was observed in Lot A. The result of this investigation has shown that the various watering regimes produced varied effects on the germination of *C. gynandra* seeds. Watering once a

week gave intermediate results as shown in Figure 2. Watering of seeds bi-weekly significantly increased the number of seeds with radicle protrusion compared to daily watering. The effect of light and dark conditions on the germination of *C. gynandra* seeds indicated a high number of seeds germinated in the dark when compared to the light condition (Fig. 3). At the end of the experiment, the highest seed germination percentage was observed at a depth of 0.5 cm followed by 1 cm and there was no germination at the soil surface (Fig. 4). This occurrence was also observed in 4 cm, 4.5 cm and 5 cm depths respectively.

Discussion

The low seed viability observed in Lot A could be due to immature embryo of the seeds at harvest, thus, such seeds will require to be stored for ripening processes to occur. This leads to promoting or inhibiting hormones needed for the germination processes. For example, high concentration of Abscisic Acid (ABA) has been shown to delay germination of freshly harvested seeds. The concentration of ABA tends to decrease with time of storage (Rehman and Park, 2000). The viability of seeds in Lot B was found to be much higher than Lot A. This could be ascribed to the presence of gibberellic acid (GA_3) in the seed. The long term storage promotes biosynthesis of this GA_3 in seeds thereby enhancing germination (Finkeltein *et al*., 2008). This finding concur with that of Kamotho, (2004) who reported that dormancy in freshly harvested seeds of this species was broken by storing the seeds from six months to one year. In a similar study, a much higher germination was also reported in seeds stored for one year (Kwack and Kang, 1985). It is, therefore, essential to keep freshly harvested ripe seeds of *C. gynandra* in storage for about six months and up to two years. This will improve the shelf life of the seeds and allow the immature embryos to reach their maturity (Ochuodho, 2005). The rate of germination increases linearly with temperature within a well-defined range (Hegarty, 1977). Hence, the corresponding increase in germination of *C. gynandra* seeds with increasing temperature of 30^{o}C was expected.

Enzymes are known to be affected by temperature. For instance, an increase in temperature of 10^{o}C has been shown to double the rate of enzymatic activity (Mader, 1993). This partially accounts for the increase in germination of *C. gynandra* with increasing temperature up to a threshold of 30^{o}C. However, the germination of seeds at 25^{o}C was still worthwhile and the fact that this seed germination occurred at low temperature of 10^{o}C in seed Lot B suggested that the plant can thrive under varied temperature conditions. Seeds watered daily showed low germination. This was due to the seed coat imbibing excess water which was detrimental to the emergence of the radicle (Sesay, 2009).

C. gynandra seeds are negatively photosensitive when exposed to light for more than 12 h ina day. This response to light exposure could be explained as a survival variation of this species because it is a short day plant requiring at least 12 h of darkness for germination to take place (Ochuodho, 2005). This phenomenon showed that this species has preference for darkness and this should be taken into consideration during field establishment. Gutter *et al.*

(1992) reported similar results on *Amaranthus* species stating that inhibition of seed germination by light seems to be a common phenomenon in wild plants. Seed germination in many plant species is inhibited by continuous white light and such seed germinate well in darkness (Bewley and Black, 1994). In the current study, the highest germination percentage was recorded at a depth of 0.5 cm followed by 1cm while there was no germination recorded at other soil depths. Interestingly, Smith and Fox (1973), reported on the low rate of germination recorded in *Echinochloa colonoa* seeds when sown at higher depths. Generally,the rate of germination observed in this study decreased with increasing depth. While Singh and Achhireddy, (1984) obtained a similar result in *Morrenia odorata* seeds having the highest germination percentage at sowing depth of 0.5 cm to 1.0 cm, Chauhan *et al.* (2006) reported that *Galium tricornutum* seeds showed no germination when placed on the soil

surface. This could be ascribed to some environmental factors such as soil-to-seed contact, light conditions on the soil surface and water availability (Ghorbani *et al*., 1999).

Conclusion

This study has shown that the optimal temperature for germination of this species is between 20^{o}C-30^{o}C. The species also germinates better in dark conditions with bi-weekly watering regime at a sowing depth of 0.5 cm. This study also suggests that *C. gynandra* has the potential of thriving successfully, despite the great fluctuations of high and low temperatures in South Africa, characteristic of summer and winter, respectively.

Declaration of interest

The authors declare that they have no competing interest.

Acknowledgement

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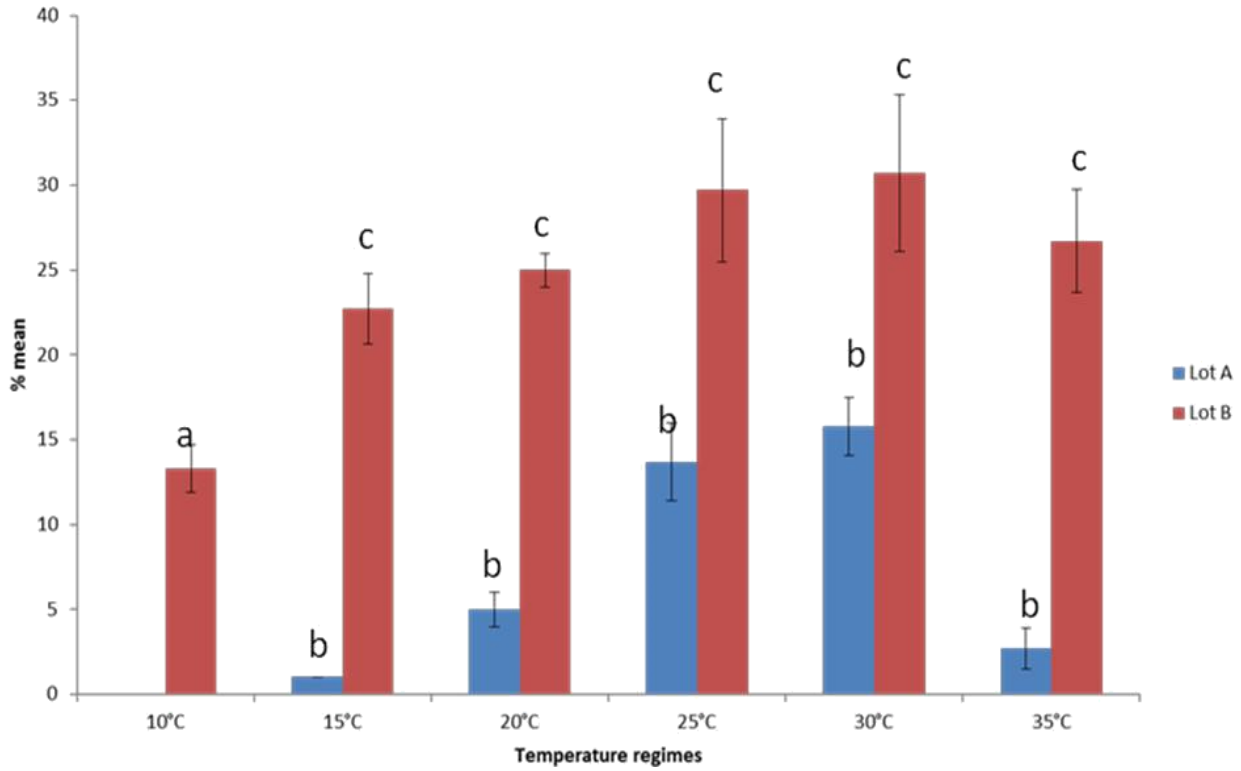

Figure 1: The different temperature regimes showing the percentage mean on*Cleomegynandra*. Data are presented as means ± standard deviation of three replicates withsignificance differences.

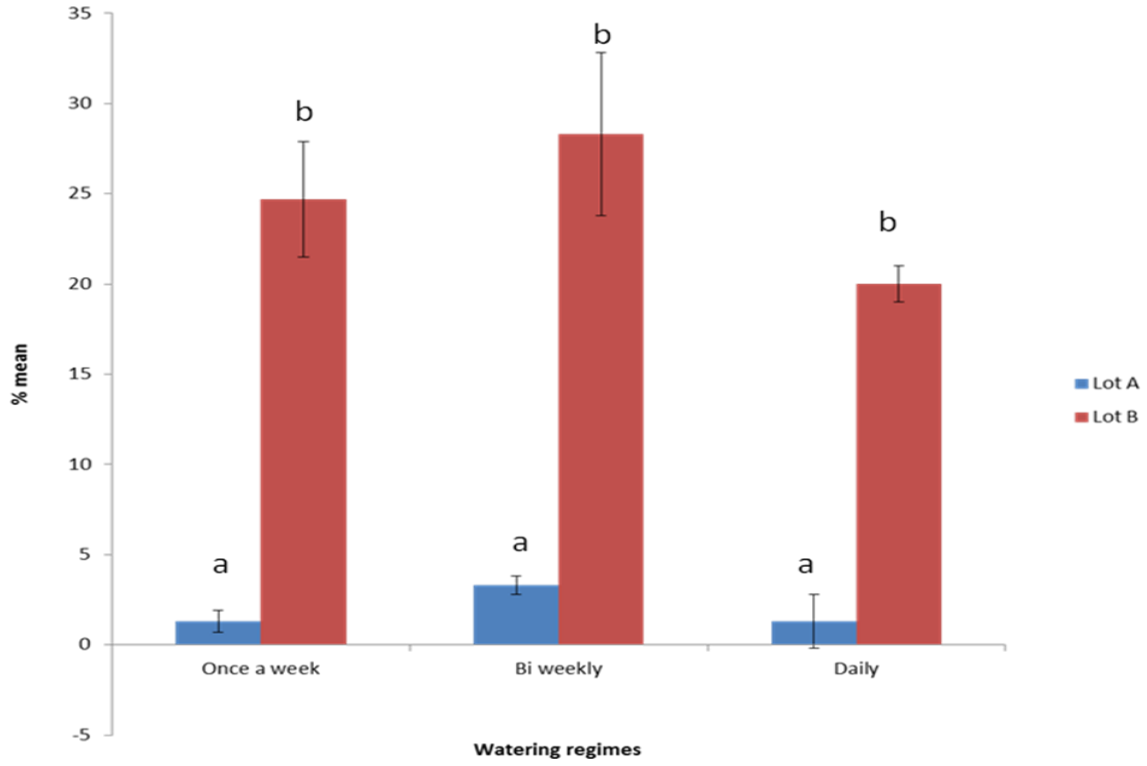

Figure 2: The effect of various watering regimes on seed germination of*C. gynandra*. Dataare presented as means ± standard deviation of three replicates with significance differences.

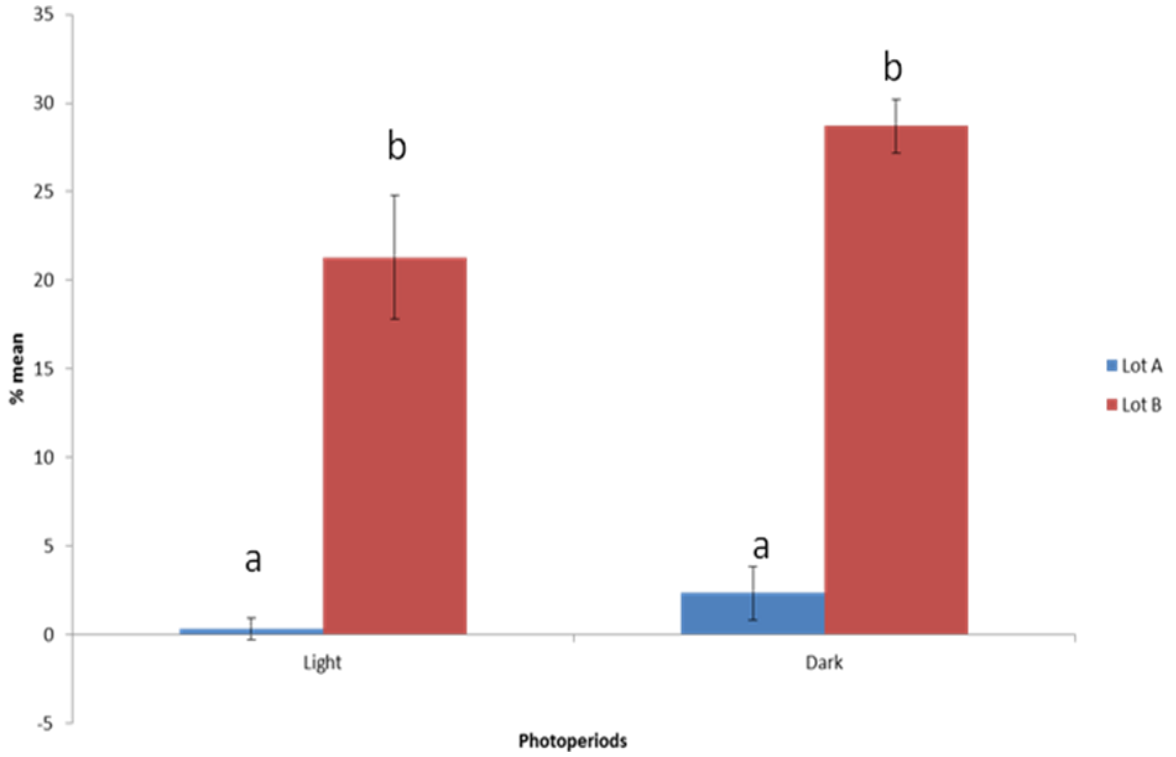

Figure 3: The effect of light and dark conditions on seed germination of*C. gynandra*. Dataare presented as means ± standard deviation of three replicates with significance differences.

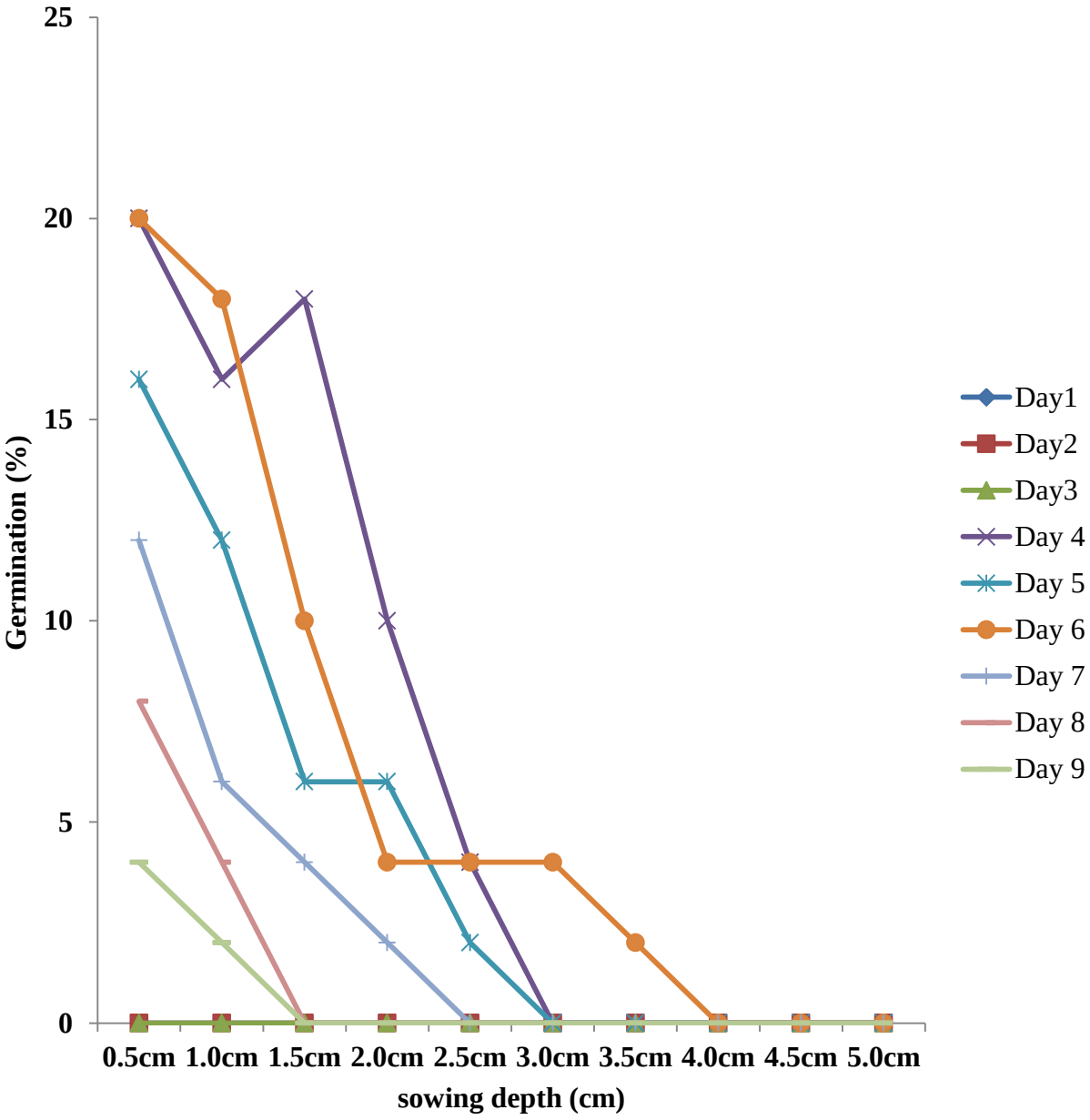

Figure 4: Relationship between germination and depth at different days after sowing.

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CHAPTER 6

EFFECT OF FERTILIZERS ON THE GROWTH AND PHYSIOLOGICAL RESPONSE OF *CLEOME GYNANDRA*

CHAPTER SIX

Effect of fertilizers on the growth and physiological response of *Cleome gynandra* L.: (A wild vegetable growing in the Eastern Cape Province, South Africa)

Abstract 101

Introduction 102

Materials and methods 103

Results 108

Discussion 113

References 132

This chapter has been submitted in this format for publication to the Canadian Journal of Plant Science.

Abstract

Wild vegetables are particularly important as adjunct accompaniment to staples. In order to encourage their productivity and utilization, it is necessary to develop suitable agronomic practices on the response of each species to fertilizers. Field and glasshouse trials were conducted to determine the effect of organic manure (goat droppings) and inorganic fertilizer (NPK) on the growth performance of *Cleome gynandra*. The experiment consisted of three treatments (control, 100 kg N/ha and 8 t goat manure/ha) which were arranged in a randomized complete block design with three replicates. Plant height, total number of leaves, chlorophyll content, moisture, root/shoot ratio, leaf area and stem girth were measured. All parameters measured increased with plant age and significant differences ($p < 0.05$) were observed among the treatments. Generally, fertilizers improved the yield and growth of *C. gynandra*. Application of 100 kg N/ha produced the best plant height, total number of leaves,stem girth and leaf area while the 8 t/ha of goat manure boosted the leaf area, chlorophyll contents in the glasshouse trials as well as the moisture contents. Plant root/shoot ratio was significantly higher in the control than organic and inorganic fertilizers. Therefore, it was deduced that optimal growth performance and better establishment of *C. gynandra* could be obtained with the use of organic and inorganic fertilizers. These findings showed that both inorganic and organic fertilizers increased the growth performance of the species. However, inorganic fertilizer was the most effective.

Keywords: inorganic fertilizers, growth parameters,*Cleome gynandra*,wild vegetables, goat droppings.

Introduction

With increasing human populations and high demand for food, soil nutrients are becoming severely depleted due to intensive cultivation of crops. This however, has been associated with a decline in soil fertility with subsequent reduction in crop yields (Cakmak, 2002; Prasad *et al*., 2015). The soils found in Africa are usually deficient in macro nutrients such as nitrogen and phosphorus while sulphur, manganese, zinc, copper and boron are readily available (Mandiringana *et al*., 2005; Bvenura and Afolayan, 2014). These nutrients have specific functions when absorbed by plants. For instance, nitrogen plays a crucial role in the chlorophyll synthesis of the leaves (Akanbi and Togun, 2002; Olaleye *et al*., 2008). Inadequate supply of nitrogen results in poor growth rate; earlier maturity and shortened vegetative growth phase (Jasso-Chaverria *et al*., 2005).

The use of organic fertilizers such as crop residues, animal manure, woodland litter and household waste has a great potential in ameliorating soil fertility and crop productivity through enhancing the physical, chemical and microbiological properties of the soil as well as nutrient supply (Rajesh *et al*., 2003; Malaiya *et al*.,2004; Adamu and Leye, 2012). The micro and macro nutrients found in organic fertilizer are released more slowly through mineralisation. These nutrients are stored for a longer time in the soil and are made available for plant use thus ensuring higher crop yields (Sharma and Mittra, 1991; Abou El Magd *et al*., 2005; Akande *et al*., 2010). Inorganic fertilizers are often considered a major source of plant nutrients (Naeem *et al*., 2006; Ndaeyo *et al*., 2013). It is usually preferred by farmers because they are readily available to the plant after application; however, its utilization is often restricted due to the negative side effects. These include soil acidity, nutrient imbalance and environmental hazard (Arisha and Bardisi, 1999; Akande *et al*., 2010; Chintala *et al*., 2010).

Cleome gynandra L. (spider plant) is one of the common wild vegetables in South Africa. It

is an erect, annual herb belonging to the Capparaceace family. The leaves of this plant are alternate, palmately compound and its petals are white, pink or lilac. The Plant is highly recognized for its numerous nutritional and medicinal uses. Despite its dietary and therapeutic potentials, formal cultivation of the plant either in home gardens or on the fields is still an uncommon practice. This is because, wild vegetables are gathered mainly by collecting from the wild, fields or emerge naturally as weeds in commercial farms. Thus, the availability and utilization of *C. gynandra* will require suitable agronomic practices to fertilizers with regards to its yield performance. Therefore, the present study was carried out to determine the effect of organic and inorganic fertilizers on the growth and physiological response of *C. gynandra* both on the field and in the glasshouse. This is with the view of domesticating this vegetable.

Materials and Methods

Site description

The experiment was conducted in the glass house and on the field at the University of Fort Hare, Alice campus, South Africa between September and December 2014. The site is within the semi-arid ecological zone with an average annual rainfall of approximately 575 mm in summer; mean daily temperatures of 22.5°C during the day and 18.8°C at night while during the winter the temperature is about 13.6°C during the day and less than 10.3°C at night (Bvenura and Afolayan, 2014). According to the South African system of soil classification, the soils are deep alluvial of the Oakleaf form (Oa) and belong to the Jozini series and texturally sandy loam (Soil Working Group, 1991).

Agronomic practices

Mature seeds of *Cleome gynandra* were harvested between the 6th and 18th of March 2014 from the University of Fort Hare research farm in Alice. Fully dried matured seeds were removed from their capsules, air-dried at room temperature on the laboratory bench for few

hours and stored in a sealed bottle at ambient temperature for further use. For the glasshouse trial, seeds were sown in polystyrene trays filled with Hygromix® medium. Germination occurred after the fourth day of sowing. Seedlings were watered daily and thinned at 3 weeks after sowing. At 4 weeks old, seedlings were transplanted into polythene bags containing 5 kg of soil. Before transplanting, soil samples were taken from a depth of 0- 30 cm from different spots in each plot and bulked together to have a composite sample. The collected samples were thoroughly mixed, air dried and passed through a 2 mm sieve for the determination of physical and chemical properties. For the field trial, the study site was ploughed and harrowed. This was then followed by breaking of the clods in order to attain good tilth for easy establishment of the plant. The seedlings from the glasshouse were later transplanted on the field at 4 weeks old with at least 5 leaves and at a height of about 10 -15 cm. This was done early in the morning onto moist beds to reduce transplanting shock.

The organic fertilizer (goat droppings) used in this experiment was obtained from the University of Fort Hare animal research farm while the inorganic fertilizers were NPK (Nitrogen Phosphorus and Potassium, 2:3:4) and LAN (lime Ammonium Nitrate) were purchased from Umtiza Farmers' Co-operative a local agricultural inputs dealer. The properties of the organic fertilizer (goat droppings) used for the experiment are shown in Table 1.

Table 1: The chemical properties of the experimental soil (Upper 0-30 cm depth) and organic fertilizer

	Soil	Organic fertilizer
PH	6.45	7.27
Bulk density (g/cm)	1.25	-
EC (µS/cm)	164.35	9.74
CEC sum (meq/100g)	12.31	-
Available P (mg/kg)	74	8350
Exchangeable K (mg/kg)	401	24000
Exchangeable Ca (mg/kg)	1753	27900
Exchangeable Mg (mg/kg)	345	10900
Exchangeable acidity (cmol/L)	0.07	-
Total cations (cmol/L)	12.31	-
Saturated acid (%)	-	-
Zn (mg/kg)	11.6	192
Mn (mg/kg)	22	495
Cu (mg/kg)	7.5	64
Organic C (mg/kg)	11000	-
N (mg/kg)	1600	26400
Clay (%)	16	-
Na (mg/kg)	-	1654
Fe (mg/kg)	-	11784
Al (mg/kg)	-	5435

Experimental design and treatments

The experiments were laid out in a Randomized Complete Block Design (RCBD) with threetreatments and three replicates on the field. The treatments were the control (T1); 8 tmanure/ha (T2) and 100 kg N/ha (T3). Nitrogen was supplied in the form of NPK (nitrogen phosphorus potassium) and LAN (lime ammonium nitrate) fertilizers. Furthermore, goat manure and NPK fertilizers were applied at transplanting while LAN was applied at 4 weeksafter transplanting. This was done by broadcasting at the top of the soil (5-7 cm depth) andthoroughly mixing with a spade in plot size measuring 3 m x 3 m. The experiment in theglasshouse was also laid out in a Randomized Complete Block Design (RCBD) with three treatments. Each treatment had three replicates but each replicate had 20 experimental units to

ensure adequate number of plant samples for the duration of the trial. Since the destructive method of data collection was used. Each replicate consisted of a stand of *Cleome gynandra* in polyethene pot.

Measurements of growth parameters

The growth and yield parameters were assessed. Eight plants per treatment were randomly selected, uprooted and tagged for data collection on the following parameters:

Plant Height and number of leaves

A metre rule was used to measure the distance from the stem base to the tip of the longest shoot (Ng'etich *et al*., 2012). However, the height of the plant was measured before uprooting. Leaves formed were counted from each plant and the average of eight plants determined.

Stem girth

The stem girth was determined by measuring at about 2.5 cm above ground level using a vernier calliper (US EPA, 2001).

Leaf area

Leaf area was determined by the non-destructive length × width method using the relation: LA = 0.75 (length × width), where 0.75 is a constant (Rivera *et al*., 2007).

Chlorophyll content

The non-destructive method was used to determine the chlorophyll content in fresh leaves from the base or apex of the plant using a spectrophotometer (Konica Minolta SPAD -502 PLUS).

Moisture content

The method of Oyelude *et al*. (2012) was adopted. Briefly, 2 g of plant samples were dried to a constant weight in an oven at 110^{O}C in clean and dry porcelain crucibles. Using the final and initial weight of the samples, the percentage content was determined.

Root: Shoot ratio

Roots were separated from the whole plant and dried in the oven at 40^{O}C to a constant weight. The ratio was determined as the dry weight of the roots to the dry weight of the shoot (Muthomi and Musyimi, 2009). This experiment was terminated when the plant reached 100% flowering at 8 weeks after transplanting both in the glass house and on the field. This was done because the vegetative phase of the plant was of utmost importance in the current study and during flowering and fruiting stages; nutrients are transferred from the leaves to the reproductive organs.

Statistical analysis

Data collected were subjected to statistical analysis using MINITAB Release 12. A one way analysis of variance was used to compare the means of various growth parameters among the treatments. A two way analysis of variance was also used to determine interaction between plant age and treatment on various growth parameters. Means were compared using Duncan's multiple range tests. The means were treated as significantly different at $p < 0.05$.

Results

Plant height and number of leaves

a) Field

The effects of fertilizers on plant height and leaf number of *Cleome gynandra* cultivated on the field are shown in Tables 2a and 2b respectively. There were significant differences ($p < 0.05$) among the treatment means. Generally, plant height increased with plant age from 3.1 to 137.3 cm from the time of transplanting until the termination of the experiment. The highest treatment means for the trial period were attained in T3 (58.8 cm) and the lowest in the control T1 (33.3 cm). Similarly, the total number of leaves also increased with increasing plant age from 6 to 140 leaves. The number of leaves differed significantly among the fertilizer treatments where the treatment means with the highest number of leaves was observed in T3 (60) and the lowest was observed in control (38).

The statistical analysis showed an interaction between the plant age and fertilizer treatment on the plant height and number of leaves. A two-way analysis of variance showed an interaction between plant age, the treatment on height and number of leaves with a coefficient of 95% and 96% respectively indicating that plant age had a significant effect on plant height and number of leaves.

b) Glasshouse

In the glasshouse trial, there were no significant differences ($p < 0.05$) among the various treatments on the plant height and number of leaves (Tables 3a and 3b). The height and number of leaves increased significantly with plant age. The highest height amongst the treatments were observed in T3 (57.2 cm) and the lowest in the control T1 (32.2 cm) while the number of leaves were found highest in T3 (59) and lowest in T1 control (49). The analysis showed an interaction between the plant age and fertilizer treatment on the plant height and number of leaves. A two-way analysiof variance showed an interaction between

plant age and the treatment on height and number of leaves with a coefficient of 93% and 95% respectively indicating that plant age had a significant effect on plant height and number of leaves.

Stem girth

a) Field

The effects of organic and inorganic fertilizers on stem girth of *Cleome gynandra* are shown in Table 4a. The treatments varied significantly from each other ($p < 0.05$) in the 3^{rd}, 4^{th} and 8^{th} weeks after transplanting. Although there were no significant difference observed among the treatments at the time of transplanting. The stem girth increased from 0.1 mm to 13.8 mm from the time of transplanting to the 8^{th} week. The treatment means observed in the stem girth were highest in T3 (7.91 mm) and the lowest in the control (6.33 mm). Statistical analysis showed that there an interaction between plant age and fertilizer application on the stem girth of *Cleome gynandra*.

b) Glasshouse

The effects of fertilizers on the stem girth was significantly ($p < 0.05$) influenced by the various treatments (Table 4b). Although no significant difference was recorded during the time of transplanting as the values followed the same trend. The stem girth increased significantly at 2^{nd}, 3^{rd}, 7^{th} and 8^{th} weeks after transplanting. The means for the trial were highest in T3 (6.5 mm) and the lowest in T1 (5.2 mm). A two-way analysis of variance showed that plant age had a significant effect on the stem girth.

Leaf area

a) Field

The leaf area of *Cleome gynandra* differed significantly among the different fertilizer treatments. The leaf area varied from 0.8 cm^2 at the time of transplanting to 56.2 cm^2 in the 5^{th} week in T3 (Table 5a). The highest mean was observed at T3 (26.5 cm^2) followed by T2 (22.3 cm^2) while the least was observed in T1 (21.9 cm^2). The leaf area also increased with plant age at a peak which was observed at the 5^{th} week and started to decrease in the 6^{th}, 7^{th} and 8^{th} week of transplanting in all treatments. However, the analysis of variance showed an interaction between plant age and the fertilizer treatments on the leaf area.

b) Glasshouse

A different trend was observed in the glasshouse trial where the leaf area increased with plant age. The treatment means did not differ significantly from each other but ranged between 0.8 cm^2 to 86.5 cm^2 at the time of transplanting and the 8^{th} week (Table 5b). The means of the treatment for the trial period were highest in T2 (39.4 cm^2) and the least was T1 (34.3cm^2). Although T3 (34.7cm^2) had a close treatment mean value with TI. Statistical analysis showed an interaction between plant age and the fertilizer treatments on the leaf area. The two-way analysis also indicates that treatment means were higher in the glasshouse than on the field.

Chlorophyll content

a) Field

The chlorophyll content increased from the time of transplanting to the 6^{th} week after which it started decreasing in the 7^{th} and 8^{th} weeks (Table 6a). The treatment means were significantly different ($p < 0.05$) from each other where the highest means of the treatments were observed in T3 (60.2 SPAD values) followed by T2 (49.2 SPAD values) and the least T1 (38.7 SPAD values). The chlorophyll content ranged from 11.5 SPAD values to 84.9 SPAD values in the

6^{th} week. There was interaction between plant age and treatment on chlorophyll content indicating that plant age had an effect on chlorophyll content of *Cleome gynandra* leaves.

b) Glasshouse

The effect of organic and inorganic fertilizers on the chlorophyll content of *Cleome gynandra* leaves followed a similar trend as observed on the field trial (Table 6b). There was a sharp increase from the day of transplanting up to the 6^{th} week after which after a decrease was observed in the 7^{th} and 8^{th} weeks. The treatment means were significantly different ($p < 0.05$) from each other where the highest means of the treatments were observed in T2 (47.9 SPAD values) followed by T3 (40.8 SPAD values) and the least T1 (36.9 SPAD values). The organic fertilizer was significant ($p < 0.05$) over inorganic and control treatments.

Moisture content

a) Field

The moisture content varied from 76.9% in the week of transplanting to 94.6% in the 6^{th} week as shown in Table 7a. The treatment means did not differ significantly ($p < 0.05$) among the treatments but remained high throughout the observation period. The mean moisture content of the treatments were 85.08, 87.9 and 85.9% in T1– T3 respectively. Irrespective of no fertilizer application in T1, the moisture content showed an upward trend with plant age having a similar value with T3. The analysis of variation showed that there was an interaction between plant age and treatment on leaf moisture content with a coefficient of 59.7% which also indicated that the plant age had a minimal effect on moisture composition of *Cleome gynandra* leaves.

b) Glasshouse

The moisture content also varied from 76.9% in the week of transplanting and extended to the 8^{th} week (96.7%) as shown in Table 7b. The treatment means differed significantly ($p < 0.05$) among the weeks of transplanting. The means of the treatments were found highest in T2 (87.4%) followed by T3 (84.5%) and least in T1 (82.3%). Statistical analysis showed an interaction between the planting time and the fertilizer treatment on the moisture content.

Root: shoot ratio

a) Field

The results showed the root/shoot ratios to range between 0.23 and 0.17. The ratio decreased from the time of transplanting to the 6^{th} week and began to vary in the control (Table 8a). A different trend was observed in T2 and T3 where it consistently decreased till the 4^{th} week after which it showed an increase in the 5^{th} and 6^{th} weeks. The means for the duration of the trial were highest at T1 (0.15) followed by a tie in T2 and T3 where a similar value occurred. Statistical analysis showed an interaction between plant age and the fertilizer treatment on the root: shoot ratio. The analysis also showed a coefficient of determination (R^2) of 43.3% indicating that plant age had a minimal effect on the root to shoot ratio.

b) Glasshouse

The root: shoot ratio followed a similar trend as observed on the field. However, the treatments decreased with planting time (Table 8b). It was observed in T1 that the root/shoot ratio maintained a decrease throughout the trial while it was different in T2 and T3 where an increase was observed in the 3^{rd} week after which it began to vary. The means for the duration of the trial were highest at T1 (0.18) followed by T3 (0.15) and the least in T2 (0.14). Statistical analysis showed an interaction between plant age and the fertilizer treatment on the root: shoot ratio. The analysis also showed a coefficient of determination (R^2) of 41.3% indicating that plant age had a minimal effect on the root to shoot ratio.

Discussion

Plant height

Plant height is positively correlated with the yield of plants for sustainable crop production (Saeed *et al*., 2007). It is evident that plant height was found highest with inorganic fertilizer (T3). Chweya and Mnzava, (1997) reported a height of 50cm-150cm in naturally growing *Cleome gynandra*. However, in this study, a maximum plant height of 137.3 cm wasobserved on the field and 107.3 cm in the glasshouse. Bvenura and Afolayan, (2014) reported that 100 kg N/ha gave the best height in *Solanum nigrum* experiment and this observation was similar with the results obtained in this trial, where 100 kg N/ha gave the maximum plant height both on the field and in the glasshouse. Also, in another study, Mauyo *et al*. (2008) found a significant increase in the plant height and other growth parameters of *Cleomegynandra* grown in Kenya when different rates of inorganic fertilizer was applied. Thesignificant increase in plant height reflects the positive effect of fertilizer nutrients such as N, P and K. These nutrients are readily available and supplied adequately in more soluble forms to the plant for better vegetative growth. On the other hand, the lowest height and number of leaves were observed in the unfertilized control plots (T1) where some of the plants were stunted in growth as they had to rely on the native soil fertility which, from the result of chemical properties was deficient in some macro nutrients (Table 1).

According to Ng'etich *et al*. (2012) nitrogen fertilization enhanced the vegetative growth of plants thus influencing the yield of most leafy vegetable. This however confirms the vigorous vegetative growth experienced in this study due to the weekly harvesting of the leaves. This frequent harvesting contributed to the increase in partitioning of photosynthates which lead to the formation of new young shoots and production of more leaves (Frankow-Lindberg, 1997).

Stem girth

Stem girth followed a similar trend of response to inorganic fertilizers as observed in plant height and number of leaves. Increase in stem girth is a reflection of appreciable amount of assimilates stored in the stem for leaf production (Law-Ogbomo and Law-Ogbomo, 2009). In this experiment, a positive growth of the stem girth was achieved due to the application of 100 kg N/ha (T3). This conceivably led to the generation of more buds on which the leaf count improved as well as height of the plant. This was followed by the organic fertilizer which also showed a positive trend. This observation corroborates with the findings of Law-Ogbomo and Law-Ogbomo, (2009) who reported a sharp increase in the stem girth of *Zeamays* when NPK fertilizer was applied.

Leaf area

Leaf area is an important factor in assessing the growth and vigour in plants (Gobron, 2009). It is also a vital tool in understanding the water and nutrient use of the plant as well as its growth and yield potential (Pandey and Singh, 2011). In this study, leaf area was significantly varied with different types of fertilizer treatments for the growth and development of *Cleomegynandra* both on the field and in the glasshouse. It was observed that the highest leaf areawas obtained with inorganic fertilizer (T3). A similar work conducted by Ng'etich *et al*. (2012) reported a high (62 cm^2) leaf area in *Cleome gynandra* at 100 Kg N/ha after 100 days of planting. In this study, the highest value (56.2 cm^2) was obtained in the treatment with 100 kg N/ha (T3) on the field. On the other hand, a different trend was observed in the glasshouse where (T2) organic fertilizer produced the widest leaf area over inorganic and control treatments. Increased leaf area implies higher light interception and more dry matter which promotes plant growth (Ofosu-Anim and Leitech, 2009). Trapani *et al*. (1999) showed that the leaf size is very responsive to nitrogen supply due to enhanced cell production and cell expansion, thus its deficiency limits the production of protein and chlorophyll molecules

which are essential for production of new cells, as a result reducing the plants growth (Abukutsa and Karimi, 2007; Ng'etich *et al*., 2012). Generally, organic and inorganic fertilizers significantly affected the leaf area of *C. gynandra*.

Chlorophyll content

In this study, mineral fertilizer enhanced the chlorophyll content of *Cleome gynandra* leaves at the 6^{th} week after transplanting both on the field and glasshouse. 100 kg N/ha favourably increased the chlorophyll content on the field while 8 tonnes of goat manure/ha produced better chlorophyll content in the glasshouse. This observation shows that the use of organic manure and inorganic fertilizer is beneficial on the chlorophyll content of the plant. In a related study, Ng'etich *et al*. (2012) reported values between 28 and 49.7 SPAD units in *Cleome gynandra* cultivated during two seasons at different rates of inorganic fertilizerwhereas in the present study, the range was observed to be between 11.5 and 87.9 SPAD units. This observation is slightly lower than the values obtained in the glasshouse and field trials. This might be attributed to the different methods used during chlorophyll determination which could be interpreted several ways. In addition, the variation could also be attributed to the efficient absorption and assimilation of nitrogen by the plant which serves as a constituent of chlorophyll in the plant tissue. According to the SPAD-502 Plus manual (2009), the SPAD meter measures the greenness of the relative chlorophyll concentration of leaves by measuring the absorbance of the leaf in two wavelengths (400–500 nm and 600–700 nm). Furthermore, plant photosynthetic potential is directly proportional to leaf chlorophyll intensity which is predetermined by nitrogen availability in the soil (Biljana and Aca, 2009). Moreover, nitrogen is the main constituent of all amino acids in proteins and lipids which acts as a structural compound of the chloroplast and determines the rate of photosynthates manufactured through the process of photosynthesis (Badr and Fekry, 1998). This explains the high number of leaves observed in the inorganic fertilizer treatment.

Moisture content

The moisture content ranged between 76.9 and 94.6% with the highest value observed in leaves grown with organic fertilizer (8 t goat manure/ha). This observation is similar to the findings of Bvenura and Afolayan, (2014) who reported the moisture content of *Solanumnigrum* to range between 75.16 and 92.08%. Similarly, Ng *et al*. (2012) also reported themoisture content of six wild vegetables to range between 92.6% and 96.8% and these results are comparable to the present study. Water constitutes about 80-95% of the mass of a growing plant and thus plays an essential role in the lifecycle of plants. Water is needed to maintain physiological processes such as cell enlargement, gas exchange in the leaves, transport in the phloem and various transport processes across membranes (Dainty, 1976; Bvenura and Afolayan, 2014). The results obtained in this study, indicates high moisture content in *Cleome gynandra* leaves which signifies sufficient water for plant growth thus, enhancing more activity for water soluble enzymes and co-enzymes required for metabolic processes (Iheanacho and Udebuani, 2009).

Root and Shoot ratio

It was found that the ratio of the root dry weights to shoot dry weight in the unfertilized control plot was consistently higher when compared to the other treatments. The ratios ranged between 0.17 and 0.23. These values imply that the amount of dry matter incorporated into the roots per plant varies from 17 to 23%. In the current study, the ratio conceivably increased in response to nutrient stress since soil moisture was kept at field capacity throughout the study period. Nutrient depletion in the control treatment apparently led to a high root: shoot ratio while the other treatments lowered the ratio. This could be attributed to NPK being part of the essential macro nutrients required for the production of the meristematic and physiological activities such as leaves, roots, shoots, dry matter production, leading to an

efficient translocation of water and nutrients, interception of solar radiation and carbon dioxide.

Conclusion

The response of this plant to different types of fertilizers is documented in the study. Both inorganic and organic fertilizers have their own roles to play in the growth and performance of *Cleome gynandra*. Growth parameters showed that plant height, no of leaves, stem girth, chlorophyll content were increased by the application of 100 N kg/ha of inorganic fertilizer. However, the application of 8 t/ha of organic manure significantly boosted the moisture content and leaf area of the plant. Both fertilizers increased the growth performance of *C.gynandra* to varying degrees. The treatment with inorganic fertilizer was found to be the mosteffective. Furthermore, due to the low amounts of nitrogen and phosphorus observed in the study site, it is thereby recommended that the incorporation of NPK fertilizers should be encouraged in order to increase the production of vegetables like *C. gynandra*.

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Table 2a: Effect of organic and inorganic fertilizers on plant height (cm) of*Cleome gynandra*L. cultivated on the field

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	3.1 ± 0.23	6.4 ± 0.43[a]	9.6 ± 0.54[a]	16.3 ± 0.54[a]	28 ± 1.66[a]	33 ± 2.05[a]	54.0 ± 3.32[a]	67.7 ± 4.11[a]	81.7 ± 4.64[a]
T2	3.1 ± 0.23	8.5 ± 0.57[a]	13.5 ± 0.62[a]	22 ±0.76[a]	37 ± 2.04[b]	55 ± 3.16[b]	78.3 ± 4.24[b]	84.7 ± 4.84[b]	109.7 ± 7.71[b]
T3	3.1 ± 0.23	10.3 ± 0.53[b]	18.9 ± 0.64[b]	35 ± 1.66[b]	48 ± 2.61[c]	78.7 ± 4.54[c]	92.3 ± 5.82[c]	106.3 ± 7.53[c]	137.3 ± 11.9[c]

Note. 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represent significant differences $p < 0.05$.

Table 2b: Effect of organic and inorganic fertilizers on leaf number of*Cleome gynandra*L. cultivated on the field

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	6 ± 0.51	8 ± 0.82^{a}	15 ± 1.61^{a}	18 ± 1.65^{a}	30 ± 2.22^{a}	40 ± 3.33^{a}	55 ± 6.97^{a}	70 ± 6.57^{a}	98 ± 7.11^{a}
T2	6 ± 0.51	9 ± 0.53^{b}	18 ± 0.53^{a}	32 ± 2.11^{b}	43 ± 2.93^{b}	52 ± 2.92^{b}	75 ± 3.63^{b}	87 ± 5.95^{b}	114 ± 4.53^{b}
T3	6 ± 0.51	10± 0.54^{b}	23 ± 0.84^{b}	38 ± 1.27^{b}	57 ± 2.26^{c}	73 ± 3.35^{c}	89 ± 8.48^{c}	106±10.72^{c}	140 ± 9.52^{c}

Note. 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represent significant differences $p < 0.05$.

Table 3a: Effect of organic and inorganic fertilizers on plant height (cm) of*Cleome gynandra*L. cultivated in the glasshouse

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	3.1 ± 0.22	8.6 ± 0.73^{a}	11.7 ± 1.23^{a}	23.3 ± 1.43^{a}	28.7 ± 1.35^{a}	40.7 ± 1.96^{a}	54.1 ± 2.55^{a}	57.7±2.76^{a}	61.7 ± 2.92^{a}
T2	3.1 ± 0.22	13.6 ± 1.47^{a}	21.9 ± 1.56^{b}	32.1 ± 1.81^{b}	39.7 ± 1.92^{a}	50.2 ± 2.53^{a}	64.7 ± 3.02^{b}	68.7± 3.23^{b}	79.7 ± 3.73^{b}
T3	3.1 ± 0.22	14.9 ± 0.89^{a}	36.3 ± 1.94^{c}	45.6 ± 2.34^{c}	55.7 ± 2.88^{b}	69.3 ± 3.21^{b}	86.7 ± 3.41^{c}	96.3± 4.15^{c}	107.3 ± 4.71^{c}

Note. 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represent significant differences $p < 0.05$.

Table 3b: Effect of organic and inorganic fertilizers on leaf number of*Cleome gynandra*L. cultivated in the glasshouse

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	5 ± 0.52	17 ± 1.63^{a}	25 ± 2.12^{a}	38 ± 2.63^{a}	44 ± 2.82^{a}	61 ± 3.33^{a}	75 ± 4.18^{a}	80 ± 4.56^{a}	98 ± 4.92^{a}
T2	5 ± 0.52	15 ± 1.22^{b}	23 ± 2.14^{a}	31 ± 2.25^{b}	39 ± 2.71^{a}	66 ± 3.47^{a}	80 ± 4.44^{b}	97± 4.78^{b}	104 ± 5.24^{a}
T3	5 ± 0.52	12 ± 0.85^{c}	20 ± 1.66^{b}	35 ± 2.47^{b}	58 ± 3.13^{b}	74 ± 3.93^{b}	98 ±4.97^{c}	106 ± 5.14^{c}	125 ± 5.57^{b}

Note. 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represent significant differences $p < 0.05$.

Table 4a: Effect of organic and inorganic fertilizers on stem girth (mm) of*Cleome gynandra*L. cultivated on the field

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	0.1 ± 0.01	2.8 ± 0.23^{a}	3.3 ± 0.64^{a}	4.6 ± 0.86^{a}	6.4 ± 1.23^{a}	7.4 ± 2.62^{a}	9.3 ± 3.42^{a}	10.9 ± 3.19^{a}	11.9 ± 4.21^{a}
T2	0.1 ± 0.01	2.63 ±0.21^{a}	3.1 ± 0.53^{b}	5.1 ± 0.97^{a}	7.2 ± 2.51^{b}	8.5 ± 2.91^{b}	10.9 ± 3.28^{b}	11.5 ± 3.71^{b}	12.5 ± 4.71^{b}
T3	0.1 ± 0.01	3.1 ± 0.32^{b}	4.4 ± 0.85^{c}	6.8 ± 1.36^{b}	8.2 ± 2.83^{c}	10.7 ± 3.89^{c}	11.4 ± 3.68^{b}	12.7 ± 3.91^{b}	13.8 ± 5.04^{c}

Note. 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represent significant differences $p < 0.05$.

Table 4b: Effect of organic and inorganic fertilizers on stem girth (mm) of*Cleome gynandra*L. cultivated in the glasshouse

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	0.1 ± 0.01	2.6 ± 0.21^{a}	3.0 ± 0.61^{a}	4.4 ± 0.81^{a}	5.5 ± 1.11^{a}	6.1 ± 1.24^{a}	6.4 ± 1.42^{a}	8.9 ± 1.71^{a}	9.5 ± 2.10^{a}
T2	0.1 ± 0.01	2.4 ± 0.22^{a}	2.8 ± 0.31^{a}	4.9 ± 0.95^{a}	6.1 ± 1.25^{b}	6.3 ± 1.32^{a}	7.6 ± 1.51^{b}	7.8 ± 1.53^{b}	10.5 ± 2.61^{b}
T3	0.1 ± 0.01	2.9 ±0.31^{a}	4.7 ± 0.78^{b}	6.1 ± 1.24^{b}	6.9 ± 1.34^{c}	7.2 ± 1.42^{b}	8.8 ± 1.72^{c}	9.7 ± 2.11^{c}	11.8 ± 3.64^{c}

Note. 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represent significant differences $p < 0.05$.

Table 5a: Effect of organic and inorganic fertilizers on leaf area (cm^2) of*Cleome gynandra*L. cultivated on the field

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	0.8 ± 0.04	2.4 ± 0.42^{a}	4.2 ± 0.55^{a}	27.0 ± 3.92^{a}	30.8 ± 3.77^{a}	36.1 ± 6.13^{a}	30.3 ± 5.93^{a}	27.7 ± 0.64^{a}	22.5 ± 0.43^{a}
T2	0.8 ± 0.04	1.9 ± 0.35^{a}	3.5 ± 0.37^{b}	14.3 ± 1.65^{b}	24.9 ± 1.72^{a}	49.9 ±5.45^{b}	45.3 ± 5.03^{b}	29.4 ± 2.21^{a}	21.0 ± 3.08^{a}
T3	0.8 ± 0.04	2.5 ± 0.41^{a}	3.9 ± 0.51^{c}	22.7 ± 0.77^{b}	34.3 ± 6.72^{b}	56.2 ± 11.2^{c}	55.9 ± 9.83^{c}	26.1 ± 9.35^{a}	36.0 ± 0.63^{b}

Note. 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represent significant differences $p < 0.05$.

Table 5b: Effect of organic and inorganic fertilizers on leaf area (cm^2) of*Cleome gynandra*L. cultivated in the glasshouse

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	0.8 ± 0.04	5.7 ± 0.73^{a}	10.6 ± 1.43^{a}	14.3 ± 2.67^{a}	15.3 ± 2.36^{a}	14.2 ± 2.53^{a}	37.8 ± 4.91^{a}	49.4 ± 5.25^{a}	51.2 ± 5.45^{a}
T2	0.8 ± 0.04	3.7 ± 0.46^{b}	8.1 ± 1.17^{b}	27.3 ± 4.91^{b}	29.8 ± 4.72^{b}	36.8 ± 5.16^{b}	79.3 ± 7.13^{b}	82.7 ± 7.93^{b}	86.5 ±7.93^{b}
T3	0.8 ± 0.04	4.9 ± 0.64^{c}	9.7 ± 1.23^{b}	20.7 ± 4.25^{a}	26.6 ± 4.71^{b}	32.5 ± 4.92^{b}	60.2 ± 6.74^{c}	76.1 ± 7.72^{c}	81.0 ± 7.48^{c}

Note. 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represent significant differences $p < 0.05$.

Table 6a: Effect of organic and inorganic fertilizers on chlorophyll (SPAD units) of*Cleome gynandra*L. cultivated on the field

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	11.5 ± 0.93	24.5 ± 1.53^{a}	37.3 ± 1.83^{a}	44.5 ± 2.62^{a}	40.3 ± 2.32^{a}	45.4 ± 2.64^{a}	56.2 ± 3.13^{a}	47.9 ± 2.63^{a}	40.7 ± 2.43^{a}
T2	11.5 ± 0.93	34.5 ±1.82^{b}	46.8 ± 2.72^{a}	57.2 ± 3.23^{b}	53.8 ± 3.14^{b}	56.4 ± 3.22^{b}	66.8 ± 3.77^{a}	63.9 ± 3.42^{b}	51.9 ± 3.14^{a}
T3	11.5 ± 0.93	38.1 ± 1.16^{b}	59.1 ± 3.45^{b}	68.9 ± 3.97^{c}	63.8 ± 3.63^{c}	72.5 ± 4.25^{c}	84.9 ± 5.96^{b}	76.6 ± 4.43^{c}	67. 2 ± 3.84^{b}

Note. 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represent significant differences $p < 0.05$.

Table 6b: Effect of organic and inorganic fertilizers on chlorophyll (SPAD units) of*Cleome gynandra*L. cultivated in the glasshouse

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	11.5 ± 0.93	25.5 ± 1.52^{a}	28.4 ± 1.65^{a}	31.5 ± 1.71^{a}	41.6 ± 2.13^{a}	49.0 ± 2.97^{a}	52.8 ± 3.28^{a}	47.9 ± 2.93^{a}	43.7 ± 2.94^{a}
T2	11.5 ± 0.93	31.7 ± 1.73^{a}	36.9 ± 2.12^{a}	48.2 ± 2.96^{b}	53.2 ± 3.39^{b}	58.8 ± 3.59^{a}	66.7 ± 4.62^{b}	63.9 ± 4.17^{b}	59.9 ± 3.63^{b}
T3	11.5 ± 0.93	33.4 ± 1.97^{a}	38.3 ± 2.15^{b}	44.9 ± 2.92^{b}	48.9 ± 2.72^{b}	49.7 ± 2.76^{a}	53.1 ± 3.39^{a}	46.6 ± 2.83^{a}	41.2 ± 2.13^{a}

Note. 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represent significant differences $p < 0.05$.

Table 7a: Effect of organic and inorganic fertilizers on moisture content (%) of*Cleome gynandra*L. cultivated on the field

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	76.9 ± 0.52	82.6 ± 1.72^{a}	81.7 ± 1.91^{a}	87.5 ± 2.24^{a}	80.9 ± 2.04^{a}	88.6 ± 2.43^{a}	94.6 ± 2.76^{a}	90.6 ± 2.41^{a}	89.2 ± 2.32^{a}
T2	76.9 ± 0.52	78.7 ± 1.65^{a}	88.9 ± 2.42^{b}	89.7 ± 2.64^{a}	88.8 ± 2.53^{b}	92.4 ± 2.88^{b}	93.9 ± 2.45^{a}	91.7 ± 2.52^{b}	90.8 ± 2.42^{b}
T3	76.9 ± 0.52	77.4 ± 1.56^{a}	83.4 ± 1.03^{a}	86.6 ± 2.45^{a}	87.6 ± 2.24^{b}	89.7 ± 2.52^{c}	90.4 ± 2.28^{b}	92.4 ± 2.68^{c}	89.1 ± 2.32^{a}

Note. 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represent significant differences $p < 0.05$.

Table 7b: Effect of organic and inorganic fertilizers on moisture content (%) of*Cleome gynandra*L. cultivated in the glasshouse

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	76.9 ± 0.52	72.6 ± 1.26^{a}	79.7 ± 1.75^{a}	80.5 ± 1.82^{a}	82.3 ± 1.97^{a}	86.2 ± 2.51^{a}	85.9 ± 2.49^{a}	87.6 ± 2.33^{a}	89.2 ± 2.91^{a}
T2	76.9 ± 0.52	74.7 ± 1.35^{a}	76.1 ± 1.63^{a}	89.7 ± 2.25^{b}	91.9 ± 2.73^{b}	93.9 ± 2.87^{b}	92.1 ± 2.71^{b}	94.7 ± 2.95^{b}	96.7 ± 3.16^{b}
T3	76.9 ± 0.52	76.0 ± 1.63^{b}	75.1 ± 1.54^{b}	84.5 ± 2.34^{c}	86.4 ± 2.54^{c}	88.7 ± 2.72^{b}	89.2 ± 2.91^{b}	91.4 ± 2.75^{b}	92.1 ± 2.71^{b}

Note. 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represent significant differences $p < 0.05$.

Table 8a: Effect of organic and inorganic fertilizers on Root: Shoot ratio of*Cleome gynandra*L. cultivated on the field

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	0.23 ± 0.01	0.15 ± 0.01^{a}	0.15 ± 0.02^{a}	0.13 ± 0.02^{a}	0.13 ± 0.02^{a}	0.14 ± 0.02^{a}	0.14 ± 0.03^{a}	0.13 ± 0.01^{a}	0.14 ± 0.02^{a}
T2	0.23 ± 0.01	0.12 ± 0.02^{a}	0.11 ± 0.01^{b}	0.09 ± 0.05^{a}	0.06 ± 0.04^{b}	0.11 ± 0.04^{b}	0.13 ± 0.06^{b}	0.11 ± 0.03^{b}	0.06 ± 0.01^{b}
T3	0.23 ± 0.01	0.11 ± 0.03^{a}	0.10 ± 0.04^{c}	0.10 ± 0.03^{a}	0.08 ± 0.01^{c}	0.12 ± 0.03^{b}	0.12 ± 0.04^{c}	0.10 ± 0.04^{c}	0.09 ± 0.04^{b}

Note. 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represent significant differences $p < 0.05$.

Table 8b: Effect of organic and inorganic fertilizers on Root: Shoot ratio of*Cleome gynandra*L. cultivated in the glasshouse

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	0.23 ± 0.01	0.21 ± 0.01^{a}	0.20 ± 0.01^{a}	0.18 ± 0.02^{a}	0.18 ± 0.02^{a}	0.17 ± 0.02^{a}	0.16 ± 0.03^{a}	0.14 ± 0.03^{a}	0.12 ± 0.04^{a}
T2	0.23 ± 0.01	0.18± 0.02^{a}	0.16 ± 0.02^{b}	0.19 ± 0.01^{a}	0.15 ± 0.01^{b}	0.15 ± 0.04^{b}	0.14 ± 0.01^{b}	0.13 ± 0.01^{b}	0.11 ± 0.07^{b}
T3	0.23 ± 0.01	0.18 ± 0.04^{b}	0.15 ± 0.01^{a}	0.17 ± 0.01^{a}	0.16 ± 0.04^{c}	0.13 ± 0.03^{b}	0.13 ± 0.03^{b}	0.11 ± 0.06^{c}	0.10 ± 0.02^{c}

Note. 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represent significant differences $p < 0.05$.

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CHAPTER 7

MINERAL UPTAKE OF *CLEOME GYNANDRA* L.: A WILD VEGETABLE GROWING IN THE EASTERN CAPE PROVINCE, SOUTH AFRICA.

CHAPTER SEVEN

Mineral uptake of *Cleome gynandra* L.: A wild vegetable growing in the Eastern Cape Province, South Africa

Abstract 139

Introduction 140

Materials and methods 141

Results 142

Discussion 150

References 177

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Abstract

The upsurge of interests in combating dietary deficiencies has resulted in the frequent consumption of wild vegetables for their possible nutritive value in diets. This is particularly true because of the abundance of minerals such as iron, magnesium, potassium, calcium among others. The information on the effect of fertilizers on the mineral uptake on most wild vegetables is still lacking. *C. gynandra* is one of the popular wild vegetables found growing in the Eastern Cape Province, South Africa. Field trial was conducted to determine the effect of fertilizers on the uptake of some micro and macro minerals in relation to their growth stages. Three treatments (control, 100 kg N/ha and 8 t goat manure/ha) were arranged in a Randomized Complete Block Design with three replicates. 100 kg N/ha increased the uptake of more minerals in *C. gynandra* compared to 8 t/ha goat manure and the control treatments. Iron (mg/g) remained high throughout the trial, ranging between 240.7 and 858.7, Potassium remained variably high, ranging between 211.9 and 272.7; Sodium ranged between 59.2 and 164.5; Manganese and Calcium (mg/g) also increased with increasing plant age; Copper also ranged between 15.3-34.9; while Phosphorus and Zinc concentration decreased at the initial stages of growth and ranged between 0.73 and 39.3; 33.7 and 72.7 respectively. The time of harvest has a significant effect on the mineral content of the vegetable.Hence, *C. gynandra* must be harvested at the latter stages of growth preferably the 7^{th} week.

Keywords: Mineral elements,*C. gynandra*, growth stages, wild vegetables, plant age

Introduction

Wild vegetables play a significant role in human existence, because most of them provide essential micro and macro nutrients while others are appreciated for their medicinal worth. These plants constitute a large reservoir of elements which accounts for their high nutritional properties. Inspite of the rich nutrient and mineral worth of these group of plants, they are still despised and neglected (Nnamani *et al*., 2009).

About 239 million of the populations in sub-Saharan Africa are affected by chronic under-nutrition (Sasson, 2012). According to Ezzati *et al*. (2002), the low consumption of vegetables and fruits is among the top 10 risk factors for mortality. For instance, about 190 million children and more than 15 million pregnant women are estimated to be vitamin A deficient (WHO, 2009). In southern Africa, in the year 2000, it was estimated that 11.1 million males and 12.5 million females over 15 years of age had a low intake of vegetables (Schneider *et al*., 2007). The consumption of wild vegetables has declined greatly due to their association with poverty, low-status food, urbanization and modernization of agriculture (Flyman and Afolayan, 2007; Jansen van Rensberg *et al*., 2007; Bevenura and Afolayan, 2014). This has resulted in poor diets and increased incidences of nutritional deficiencies which affect mostly infants and children, pregnant women and the elderly (Madisa and Tshamekang, 1995; Smith and Ezyaguirre, 2007). Several studies have reported that wild vegetables are good sources of micronutrients and sometimes have superior nutritional qualities when compared with some exotic vegetables. This would suggest that these species have the potential in eradicating micronutrient deficiencies (Nesamvuni *et al*., 2001; Steyn *et al*., 2001; Odhav *et al*., 2007; Ndlovu and Afolayan, 2008; van der Walt *et al*., 2009; Lewu and Mavengahama, 2010). However, the resurgence of interest in wild vegetable has become noteworthy as some species such as *Amaranth spp*, *Solanum nigrum* and *Brassica rapa*

among others are now under cultivation in South Africa (Jansen van Rensberg *et al*., 2007; Bvenura and Afolayan, 2014).

Cleome gynandra L. is an erect herbaceous annual plant with alternate and palmatelycompound leaves. It is commonly known as spider plant. The plant grows to a height of 1.5 meter with long tap roots and a few secondary roots. In South Africa, the plant is still gathered from the wild for food and medicine since it has not been formally cultivated in this region. In other Countries, this species has been reported to be rich in several mineral nutrients such as vitamins A and C, calcium, iron and proteins among others (Ekpong, 2009). Despite the significance of *C. gynandra* as dietary sources of minerals, no reports are available on the effect of fertilizers on its mineral constituents and the optimal harvesting time of the plant in the Eastern Cape. Therefore, the study was carried out to determine the effect of fertilizers on the mineral composition of *Cleome gynandra* at different growth stages when cultivated under field conditions. This is with a view to determine the appropriate time of harvest of the plant in order to explore fully its mineral components in the Eastern Cape Province.

Materials and methods

The experimental site, agronomic practices, experimental design, chemical composition of the experimental soil and organic fertilizer were described in Chapter 6.

Plant and Data collection

The third youngest fully expanded leaves were collected from the shoots by uprooting the whole plant; washed in distilled water to remove sediments and other impurities before drying the samples in a dust free, forced-draft oven at 40^{o}C to a constant weight. The samples were then ground to a powder using a grinder and passed through a 2 mm sieve. The samples were kept in vial bottles and stored in a refrigerator at 4^{o}C until when needed. However,vitamin C was

determined from green freshly harvested plant samples. Data were collected on a weekly basis. The experiment was terminated in the 8^{th} week for the glasshouse and field trials when all plants reached 100% flowering.

Mineral analysis

The concentration of K, Na, P, Mg, Ca, Fe, Mn, Cu and Zn were determined as described in chapter 3.

Statistical analysis

Data of the nutrient concentrations of various treatments were subjected to statistical analysis using MINITAB Release 12. A one way analysis of variance was used to compare the means of various mineral concentrations among the treatments and a two way analysis of variance was used to determine the interaction between plant age (weeks after transplanting) and treatment on the mineral accumulation in the plant. Means were segregated using Duncan's multiple range tests. The means were treated as significantly different at $p < 0.05$.

Results

Potassium (K)

a) Field

Potassium decreased from the time of transplanting to the 3^{rd} week after which it increased in the 4^{th} and 5^{th} weeks and later began to decline in the subsequent weeks (Fig. 1a). There were significant differences ($p < 0.05$) among the treatment means and it ranged between 211.9 and 272.7 mg/100g. The means for the duration of the trial were highest in T3 (254.6 mg/100g) and lowest in T1 (230.8 mg/100g). The concentration of potassium was found at its peak in the 5^{th} week in T3 (272.7 mg/100g). Statistical analysis indicates an interaction between plant age and the fertilizer treatments on the concentration of potassium. The regression analysis with potassium as the dependent variable and time (plant age) as the

regressor showed a coefficient of determination (R^2) of 76.4% indicating that plant age had a significant effect on potassium.

b) Glasshouse

The concentration of potassium ranged between 210.1 and 253.4 mg/100g and generally decreased from the time of transplanting to the 3rd week after which it increased only in the 4th week and later began to decrease (Fig. 1b). The treatment means differed significantly ($p < 0.05$) from each other. The means for the duration of the trial were highest in T3 (236.7 mg/100g) and lowest in T1 (219.7 mg/100g). The concentration of potassium in the glasshouse was found at its peak in T3 (236.7 mg/100g) in the 4th week. Statistical analysis indicates an interaction between plant age and the fertilizer treatments on potassium. The regression analysis with potassium as the dependent variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 64.6% indicating that plant age had a significant effect on potassium.

7.3.2 Sodium (Na)

a) Field

The concentration of sodium ranged between 59.2 and 164.5 mg/100g (Fig. 2a). The treatments generally increased from the time of transplanting to the 4th week after which it began to decrease throughout the trial. The treatment means differed significantly ($p < 0.05$) from each other. The means for the duration of the trial were highest in T3 (142.6 mg/100g) and lowest in T1 (74.04 mg/100g). The concentration of sodium was found at its peak in T3 in the 4th week. Statistical analysis indicates an interaction between plant age and the fertilizer treatments on sodium. The regression analysis with sodium as the dependent variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 65.2% indicating that plant age had a significant effect on sodium.

b) Glasshouse

The effect of fertilizers on the concentration of sodium followed a similar trend as observed on the field. Sodium ranged between 24.7 and 149.7 mg/100g in the glasshouse (Fig. 2b). There were significant differences ($p < 0.05$) among the treatment means. The highest means during the trial were highest in T3 (129.2 mg/100g) and lowest in T1 (61.6 mg/100g). Statistical analysis indicates an interaction between plant age and the fertilizer treatments on the concentration of sodium. The regression analysis with sodium as the dependent variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 55.7% indicating that plant age had a significant effect on sodium.

7.3.3 Phosphorus (P)

a) Field

The concentration of phosphorus increased during the 1st week after transplanting after which it decreased in the 2nd and 3rd weeks (Fig. 3a). Thereafter an increase was observed in the 4th, 5th and 6th weeks. There were significant differences ($p < 0.05$) among the treatment means and the highest mean was found in T2 (24.06 mg/100g) while the least was in T1 (7.9 mg/100g). The range of phosphorus observed on the field was between 0.73 and 39.3 mg/100g. The maximum concentration of phosphorus was observed at the 6th week. Statistical analysis indicates an interaction between plant age and the fertilizer treatments on the concentration of phosphorus. The regression analysis with phosphorus as the dependent variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 54.3% indicating that plant age had a significant effect on phosphorus.

b) Glasshouse

The treatments differed significantly showing an increase from the 1st to 6th weeks after which it decreased in the subsequent weeks (Fig. 3b). The concentration of phosphorus ranged from 1.87 and 31.7 mg/100g and the highest means for the duration of the trial were

highest in T2 (23.72 mg/100g) and lowest in T1 (7.5 mg/100g). The statistical anlysis showed an interaction between plant age and the fertilizer treatments on phosphorus. The regression analysis with phosphorus as the dependent variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 47.8% indicating that plant age had a minimal effect.

7.3.4 Magnesium (Mg)

a) Field

The accumulation of magnesium in the leaves of *Cleome gynandra* ranged from 4.47 to 12.6 mg/100g (Fig. 4a). Magnesium increased from time of transplanting to the 5^{th} week after which it decreased in the 6^{th} week and later showed an increase in the 7^{th} and 8^{th} weeks. The treatments were significantly different ($p < 0.05$) and the means for the duration of the study were highest in T3 (10.08 mg/100g) and lowest in T1 (4.77 mg/100g). The concentration of magnesium was at its peak in the 5^{th} week in T3 (12.6 mg/100g). Statistical analysis indicates an interaction between plant age and the fertilizer treatments on the concentration of magnesium. The regression analysis with magnesium as the dependent variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 57.5% indicating that plant age had a fairly significant effect on magnesium.

b) Glasshouse

Magnesium showed a variable trend where there was more assimilation of minerals between the time of transplanting and the 3^{rd} week (Fig. 4b). The concentration of magnesium ranged between 2.13 and 10.16 mg/100g. A decrease was observed only in T1 in the 4^{th} week after which it began to decrease in all treatments for the subsequent weeks. The treatments were significantly different ($p < 0.05$) and the means for the duration of the study were highest in T3 (8.59 mg/100g) and lowest in T1 (4.20 mg/100g). Comparing the values obtained in the glasshouse with the field trial, a higher concentration of magnesium was recorded on the

field. The regression analysis with magnesium as the dependent variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 61.4% indicating that plant age had a significant effect on magnesium.

7.3.5 Calcium (Ca)

a) Field

Calcium increased linearly between the time of transplanting and the 6^{th} week thereafter it began to decrease till the termination of the experiment (Fig. 5a). The treatments were significantly different ($p < 0.05$) and ranged between 15.0 and 41.3 mg/100g at the time of transplanting and the 8^{th} week respectively. The means for the duration of the trial were highest in T3 (34.59 mg/100g) and lowest in T1 (18.94 mg/100g). Statistical analysis indicates an interaction between plant age and the fertilizer treatments on the concentration of calcium. The regression analysis with calcium as the dependent variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 43.8% indicating that plant age had a significant effect on calcium.

b) Glasshouse

The concentration of calcium increased from time of transplanting to the 2^{nd} week after which it began to decrease only in T1 in the 3^{rd}, 4^{th}, 5^{th} and 6^{th} weeks (Fig. 5b). However, the other treatments showed an increase in the accumulation of calcium in the above weeks and decreased generally in the 7^{th} and 8^{th} weeks. The treatments were significantly different ($p < 0.05$) and ranged between 5.54 and 27.1 mg/100g. The means for the duration of the trial were highest in T3 (22.56 mg/100g) and lowest in T1 (12.73 mg/100g). The concentration of calcium was at its peak in the 6^{th} week in T3 (27.1 mg/100g). Statistical analysis indicates an interaction between plant age and the fertilizer treatments on the concentration of calcium.

The regression analysis with calcium as the dependent variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 53.6% indicating that plant age had a

significant effect on calcium.

7.3.6 Iron (Fe)

a) Field

Iron increased exponentially between the time of transplanting and the 7^{th} week thereafter it decreased slightly in the 8^{th} week (Fig. 6a). Generally, the concentration of iron increased with plant age. The treatments were significantly different ($p < 0.05$) and ranged between 240.7 and 858.7 mg/100g at the time of transplanting. The means for the duration of the trial were highest in T3 (666.9 mg/100g) and lowest in T1 (529.1 mg/100g). The highest concentration of iron was observed in the 7^{th} week in T3. Statistical analysis indicates an interaction between plant age and the fertilizer treatments on the concentration of iron. Regression analysis with iron as the dependent variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 95.4% indicating that plant age had a significant effect on iron.

b) Glasshouse

The concentration of iron ranged between 240.7 and 714.7 mg/100g (Fig. 6b). The means for the duration of the trial were highest in T3 (572.8 mg/100g) and lowest in T1 (399.5 mg/100g). Statistical analysis indicates an interaction between plant age and the fertilizer treatments on the concentration of iron. Regression analysis with iron as the dependent variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 81.6% indicating that plant age had a significant effect on iron.

7.3.7 Manganese (Mn)

a) Field

The concentration of manganese increased with plant age from the time of transplanting to the 6^{th} after which it decreased only in the 7^{th} week, thereafter it increased again in the 8^{th}

(Table 1a). The treatments were significantly different from each other ($p < 0.05$) and ranged

between 45.6 and 87.6 mg/100g at the time of transplanting. The means for the duration of the trial were highest in T3 (75.5 mg/100g) and lowest in T1 (57.2.4 mg/100g). The highest concentration of manganese was observed in the 6^{th} week in T3. Statistical analysis indicates an interaction between plant age and the fertilizer treatments on the concentration of manganese. Regression analysis with manganese as the dependent variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 56.7% indicating that plant age had a significant effect on manganese.

b) Glasshouse

The concentration of manganese increased with from the time of transplanting to the 4^{th} after which it decreased in the 5^{th} week then varied among the various treatments for the subsequent weeks (Table 1b). The means differed significantly ($p < 0.05$) among the treatments and ranged between 34.7 and 77.6 mg/100g. The means for the duration of the trial were highest in T3 (69.6 mg/100g) and lowest in T1 (50.03 mg/100g). The highest concentration of manganese was observed in the 7^{th} week in T3. Statistical analysis indicates an interaction between plant age and the fertilizer treatments on the concentration of manganese. Regression analysis with manganese as the dependent variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 49.4% indicating that plant age had a significant effect on manganese.

7.3.8 Copper (Cu)

a) Field

The concentration of copper ranged between 15.3 and 34.9 mg/100g (Table 2a). Copper increased between the time of transplanting and the 2^{nd} week after which it varied in the 3^{rd}, 4^{th} and 5^{th} weeks, however it began to decrease in the subsequent weeks. The means for the duration of the trial were highest in T3 (27.1 mg/100g) and lowest in T1 (19.2 mg/100g). Statistical analysis indicates an interaction between plant age and the fertilizer treatments on

the concentration of copper. Regression analysis with copper as the dependent variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 37.5% indicating that plant age had a minimal significant effect on copper accumulation.

b) Glasshouse

The concentration of copper ranged between 13.3 and 30.3 mg/100g (Table 2b). The means for the duration of the trial were highest in T3 (25.05 mg/100g) and lowest in T1 (18 mg/100g). The highest concentration of copper was attained in the 5^{th} week in T3. Statistical analysis indicates an interaction between plant age and the fertilizer treatments on the concentration of copper. Regression analysis with copper as the dependent variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 39.4% indicating that plant age had a minimal significant effect on copper.

7.3.9 Zinc (Zn)

a) Field

The effect of fertilizers on the concentration of zinc increased from the time of transplanting to the 3^{rd} week after which it began to vary amongst the treatment (Table 3a). There were significant differences ($p < 0.05$) among the treatment means and ranged between 33.7 and 72.7 mg/100g. The highest means during the trial were highest in T3 (62.7 mg/100g) and lowest in T1 (49.5 mg/100g). The highest concentration of zinc was observed in the 2^{nd} week in T3 after which it began to decrease progressively. Statistical analysis indicates an interaction between plant age and the fertilizer treatments on the concentration of zinc. Regression analysis with zinc as the dependent variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 41.4% indicating that plant age had a significant effect on zinc.

b) Glasshouse

The concentration of zinc ranged between 21.2 and 64.6 mg/100g in the glasshouse (Table

3b). Zinc increased with plant age from the time of transplanting up to the 4th week after which it began to decline in the subsequent weeks. The highest means during the trial were highest in T3 (53.6 mg/100g) and lowest in T1 (40.7 mg/100g). The highest concentration of zinc was observed in the 4th week in T3. Statistical analysis indicates an interaction between plant age and the fertilizer treatments on the concentration of zinc. Regression analysis with zinc as the dependent variable and time (plant age) as the regressor showed a coefficient of determination (R^2) of 35.2% indicating that plant age had a significant effect on zinc.

Discussion

Potassium (K)

The concentration of potassium in the current study is much higher than the values reported by Aberoumand (2009) and Ajiboye *et al*. (2014) in some common vegetables found in Iran, India and South Western Nigeria. However, the range of values of K obtained in both trials was much lower than those of some wild vegetables growing in the Eastern Cape Province of South Africa (Afolayan and Jimoh, 2009). In a similar study, the potassium content in fresh and dried leaves of *Cleome gynandra* was found to be lower than what was obtained in the current study (Abugre *et al*., 2011). The variations in K concentrations are influenced by factors such as soil type, pH, water availability to the plant, climatic conditions, plant age and fertilizer types (Khader and Rama, 2003; Uusiku *et al*., 2010). Potassium is present mostly in inorganic form where the total content may be high in the soil but the amount available may be very small depending on the soil characteristics. Potassium is a multi-functional nutrient which regulates the neurotransmission and acid base balance in the body (Alinnor and Oze, 2011). It also promotes good muscle contraction and increases the availability of iron in the body (Soetan *et al*., 2010; Sodamade *et al*., 2013). According to the New Zealand and Australian Standards, (2005) the daily requirement of potassium is 2000 mg for adults, *C.gynandra* will provide about 13.64% of the recommended daily intake needed.

The result of this study also indicate that harvesting *Cleome gynandra* in the 5th week on the field and 4th week in the glasshouse will contribute to the adequate supply of the mineral to the body per day since the concentration of potassium was at its peak during that time. Furthermore, 100 kg N/ha would be the most favourable fertilizer to enhance the uptake of potassium by the plant.

Sodium (Na)

The trend of sodium accumulation in *C. gynandra* leaves is comparable with the reports by Flyman and Afolayan, (2008). Who stated that sodium concentration in *V. unguiculata* increased up to the 4th week after which it decreased. The concentration of sodium in the current study was found to be lower than the values reported in *Moringa oleifera* and *Phaseolus vulgaris* (Oyelude *et al*., 2012; Sodamade *et al*., 2013). However, results from bothtrials were comparable with those of Hassan *et al*. (2011). They reported a close range of 139.2 mg/100g in *Parkia biglobosa* flowers. Generally, sodium increased with plant age although it decreased after the 4th week where it attained its peak in both trials. Sodium has been shown to be an essential source of electrolytes and aids in the regulation of plasma volume within the body system (Akpanyung, 2005). The uptake of this mineral element can be influenced by the presence of potassium and nitrogen in the soil. Owing to the chemical similarity between sodium and potassium, plant cells accumulate more potassium and exclude sodium. As a result, high potassium-to-sodium ratios come into play where potassium performs more essential functions like cellular metabolism and stomatal movement in plants (Flyman and Afolayan, 2008). This is evident in the current study where *C.gynandra* absorbed more potassium than sodium. The K/Na ratio in the glasshouse rangedbetween 38.43 and 51.73 while on the field it was between 63.75 and 76.29 indicating that the plant is natrophobic in nature. This ability is correlated with the beneficial effect of Na to plant growth (Marschner, 1995; Bvenura and Afolyan, 2014). In view of the recommended daily intake of 500 mg of sodium, *C. gynandra* can contribute 8.23% of the recommended daily

intake (NHMRC, 2005). Furthermore, the highest concentration of sodium could be achieved by harvesting the leaves of *C. gynandra* in the 4th week both on the field and in the glasshouse.

Phosphorus (P)

The phosphorous content was found to be in the range of 0.73 –39.3 mg/100g on the field and 1.87 – 31.7 mg/100g in the glasshouse. These values were similar to those reported by Asaolu *et al*. (2012) on some leafy vegetables found in Nigeria. The reported range in this study compares favorably with values obtained in South Côte d'Ivoire on the seasonal variation in nutritional compositions of spider plant (Agbo *et al*., 2014). However, when compared with the results of K'Opondo *et al.* (2005), the concentration of phosphorus (P) in this study was low. Phosphorus is commonly bound to soil constituents which make it unavailable for plant uptake. This may be accountable for the low concentrations observed in this study. Furthermore, phosphorus is known to have a significant effect in young plant tissues affecting both cell number and cell size. This might explain the high concentration of the mineral element at the early stages of growth (Flyman and Afolayan, 2008). In human physiology, phosphorus plays an important role in the metabolic processes such as bone mineralization and it is also known in preventing osteoporosis, arthristis, pyorrhea and rickets in the body (Abugre *et al*., 2011). However, harvesting the leaves at its initial stages of growth would be appropriate in meeting the required daily intake of phosphorus since it can provide 4.91% (NHMRC, 2005).

Magnesium (Mg)

The values reported in the current study compares favorably with those of *Hensia crinita* found in Akwa Ibom State of Nigeria (Okon and James, 2014). In a similar study, the magnesium content of some common vegetables were found to be lower when compared with the values recorded in the both trials (Ajiboye *et al*., 2014). Emebu and Anyika, (2011)

reported a value which was slightly lower than the magnesium content obtained in this study. However, when compared with other wild vegetables such as *Solanum microcapon* and *Cnidoscolous acinitopholis*, the concentration of Mg was much lower in the present study(Sodamade *et al*., 2013). Also, in Côte d'Ivoire, Agbo *et al*. (2014) reported very high values of magnesium between 7.28 g/100g and 15.08 g/100g in spider plant. According to Khader and Rama, (2003) the increase in the concentration of magnesium in plant may be caused by its ion being in an unfixed form which accumulates with age. In addition, factors such as soil type, available nutrients, rainfall as well as age of the plant at harvest can influence the availability of Mg in plant absorption (Gransee and Fuhrs, 2013; Bvenura and Afolayan, 2014). This probably accounts that the above factors could be responsible for the low uptake of mineral nutrient by the plant. Inspite of this, the concentration of magnesium can still be explored fully in the 5^{th} week after transplanting in both trials. Magnesium functions as a co-factor of many enzymes involved in energy metabolism, protein synthesis and maintenance of the electrical potential of nervous tissues as well as cell membranes (Mohammed and Shariff, 2011). Furthermore, it is also implicated in preventing degenerative diseases such as congenital malformations, immunologic dysfunction and bleeding disorders (Chaturvedi *et al*., 2004; Patricia *et al*., 2014).

Calcium (Ca)

Calcium is an essential mineral component necessary in sustaining healthy bones. It plays a major role in muscle contraction and relaxation, bloodclotting, synaptic transmission and absorption of vitamin B_{12} (Emebu and Anyika, 2011). The calcium content of *C. gynandra* leaves cultivated on the field (15.0 and 41.3 mg/100g) compares favourably with the values obtained in five selected vegetables (Chuku and Ugorji, 2012). On the other hand, the range of values obtained in the glasshouse is similar to 23.3 and 27.9 mg/g in three leafy vegetables reported by Onwordi *et al*. (2009). However, when compared with other wild vegetables

growing in the Eastern Cape Province, the value obtained in both trials were low (Afolayan and Jimoh, 2009). Ca increased sharply from the early stages to the latter stages of growth after which it decreased. Physiologically, Ca is not easily translocated in plant organs due to its immobile nature. During plant growth, flowering are the major sinks, Ca was probably translocated to these sites at the expense of other plant tissues. This is evident as the experiment was terminated when the plants reached 100% flowering in both trials. The results of this study indicate that *C. gynandra* leaves are good sources of calcium. If consumed daily, it will be adequate to provide 24.2% of 1000 mg daily requirement as recommended by New Zealand and Australian Standards, (2005). Furthermore, the maximum concentration of calcium can be obtained by harvesting the leaves in the 6^{th} week.

Iron (Fe)

Cleome gynandra leaves were found to have exceptionally high iron content of 240.7 and858.7 mg/100g. The result compares favorably with the range of values reported by Hussain

et al. (2010) of some selected plant species from Kohat region, Pakistan. The concentration of iron in the current study is 4 times higher than the reported range of values in some wild vegetables growing in the Eastern Cape Province, South Africa (Afolayan and Jimoh, 2009). 100 kg N/ha enhanced high levels of iron uptake by the plant in both trials. This observation is in agreement with the reports of Kebwaro *et al*. (2013). These authors found out that nitrogen fertilizer increased the level of iron with plant maturity. Consequently, the high iron content in *C. gynandra* makes them a potential source of the mineral nutrient for infants, pregnant and lactating mothers. It is also needed by the convalescent and elderly people as well. According to Ndlovu and Afolayan, (2008) 21% of infants suffer from anaemia in South Africa. Furthermore, about 48.2% and 57% in Southeast Asia and Africa are also anaemic (WHO, 2008). The high prevalence of anaemia is accountable for about 800 000 deaths, representing 2.4% of annual global deaths from the disease (Black, 2003). Iron is the most

abundant trace mineral and it is implicated in several biochemical roles in the body such as oxygen binding in hemoglobin and acting as a vital catalytic center in many enzymes as the cytochrome oxidase (Patricia *et al*., 2014). Thus, cultivation of this wild vegetable can be suggested as a food based strategy to alleviate insufficient intake of dietary iron by adolescents. In addition, incorporating this vegetable into the diets of the people will provide 78.06% of the daily requirements needed in the body. Harvesting *C. gynandra* during the 7^{th} week proved to be the most optimum time to derive the maximum concentration of iron on the field and in the glasshouse.

Manganese (Mn)

The concentration of manganese in the present study increased with plant age. The result of this study agrees favorably with the work by Flyman and Afolayan, (2008) who reported an increase in the concentration of manganese in the early stages of growth after which it decreased in the final weeks of planting. The concentration of manganese was within the range of values reported on the mineral composition of common leafy vegetables in Nigeria (Achikanu *et al*., 2013). However, the value obtained in the current study was much lower when compared with the concentration of manganese obtained in *Moringa oleifera* leaf (Sodamade *et al*., 2013). The high rate of decomposition of organic matter releases manganese cations which are made available for plant uptake. This observation could be responsible for the higher concentration of manganese observed on the field than the glasshouse. Manganese is necessary in building the immune system, regulation of blood sugar level and production of energy. They are also involved in bone mineralization, human nutrition, protein synthesis and nerve transmission (Oko *et al*., 2012). The results of this work indicate that *C. gynandra* can contribute to the daily intake of manganese required in the body. It further reveals that harvesting the plant at the final stages of growth will be ideal in obtaining the highest concentration of manganese.

Copper (Cu)

Copper is an important micro nutrient which acts as biocatalysts required for body pigmentation. In this study, the concentrations were higher on the field than the glasshouse. The copper content recorded in *C. gynandra* compares favourably with the range of values reported by Tuncturk *et al*. (2015), on the chemical composition of some edible wild plants grown in Eastern Anatolia. Awol, (2014) reported a lower copper content compared to the range of values obtained in this study. The differences in copper levels could be due to the fact that many soils are geographically deficient in certain minerals and therefore plants cultivated on such soils lack nutrients. A similar problem can be caused by over farming or poor soil management (Nielson, 1996; Mohammed and Shariff, 2011). The results of this study indicate that *C. gynandra* leaves are good sources of copper since it can provide 20.5% of the required daily intake of 1.7 mg needed in a male adult (NHMRC, 2005). However, *C.gynandra* can be harvested in the 5^{th}week under both conditions. This is when the mineralelement reached its peak.

Zinc (Zn)

Zinc is an important mineral element involved in the development of sexual organs such as the testes and ovaries, which are essential for reproduction (Ayoola *et al*., 2010; Mohammed and Shariff, 2011). Adequate intake of vegetables rich in zinc aids in preventing adverse effect of zinc deficiency which results in retarded growth and delayed sexual development. The concentration of zinc in the present study was found to increase from the time of transplanting up to the 4^{th} week after which it decreased as the plant matures. This observation is in line with the report of Atta *et al*. (2010), who reported a similar trend in *Hibiscus sabdariffa*. About 75% of zinc is absorbed by plants and stored in the young shootswhile about 20-30% is transported to the older shoots. This partially explains the decrease in zinc concentration as it approached latter stages of growth in this study (Kabata-

Pendias, 2001). The zinc content recorded in *C. gynandra* compares favourably with the range of values reported by Tuncturk *et al*. (2015). However, when compared with the values reported in *Moringa oleifera* leaves, the value was much lower in this study (Sodamade *et al*., 2013). The minimum concentration of zinc obtained in the glasshouse can contribute 8.11, 4.61 and 10.7% of the required daily intake in women, men and children respectively. Harvesting *C.gynandra* in the $2^{nd}$and 4^{th}weeks proved to be the most optimum time to derive themaximum concentration of zinc on the field and in the glasshouse.

Conclusion

The coefficient of determination on the field decreased in the following order (%): Fe (95.4) > K (76.4) > Na (65.2) > Mg (57.5) > Mn (56.7) > P (54.3) > Ca (43.8) > Zn (41.4) > Cu (37.5), indicating that plant maturity on the field significantly increased the uptake of Fewhile the least effect was on Cu. 100 kgN/ha increased the uptake of more minerals such as K, Na, Mg, Ca, Fe, Mn, Cu and Zn while 8 t/ha goat manure increased only the uptake of phosphorus in the plant. The control treatment did not affect any of the analysed mineral elements. On the field, the maximum concentrations of K, Na, P, Mg, Ca, Fe, Mn, Cu and Zn were as follows 5^{th}, 4^{th}, 6^{th}, 5^{th}, 6^{th}, 7^{th}, 6^{th}, 5^{th} and 2^{nd} weeks after transplanting respectively.

In the glasshouse, coefficient of determination decreased in the order (%): Fe (81.6) > K (64.6) > Mg (61.4) > Na (55.7) > Ca (53.6) > Mn (49.4) > P (47.8) > Cu (39.4) > Zn (35.2). Throughout the experiment, 100 kg N/ha also increased the uptake of more minerals compared to 8 t/ha goat manure and the control treatments. The highest concentrations of the minerals were observed in the following weeks 4^{th}, 4^{th}, 6^{th}, 5^{th}, 6^{th}, 7^{th}, 7^{th}, 5^{th} and 4^{th} for K, Na, P, Mg, Ca, Fe, Mn, Cu and Zn respectively.

This study further revealed that *C. gynandra* though a wild vegetable has enormous nutritional potentials which can be explored better if cultivated and incorporated into the diets of the local inhabitants. In addition, the nutrient values obtained shows the potential of the

plant to contribute greatly towards meeting the recommended dietary intakes of nutrients required in the body. The study also confirmed that the time of harvest has a significant effect on the mineral content of plant. Both fertilizers had significant effects on the mineral composition of the plant although the best fertilizer option and time to harvest the leaves for food may be a challenge as different minerals responded differently to the type of fertilizer used. Thus it is necessary to recommend the best fertilizer and time of harvest based on specific nutritional interventions. In situations where a food-based strategy is sought to address iron deficiencies, *C. gynandra* must be harvested in the latter stages of growth preferably the 7^{th} week.

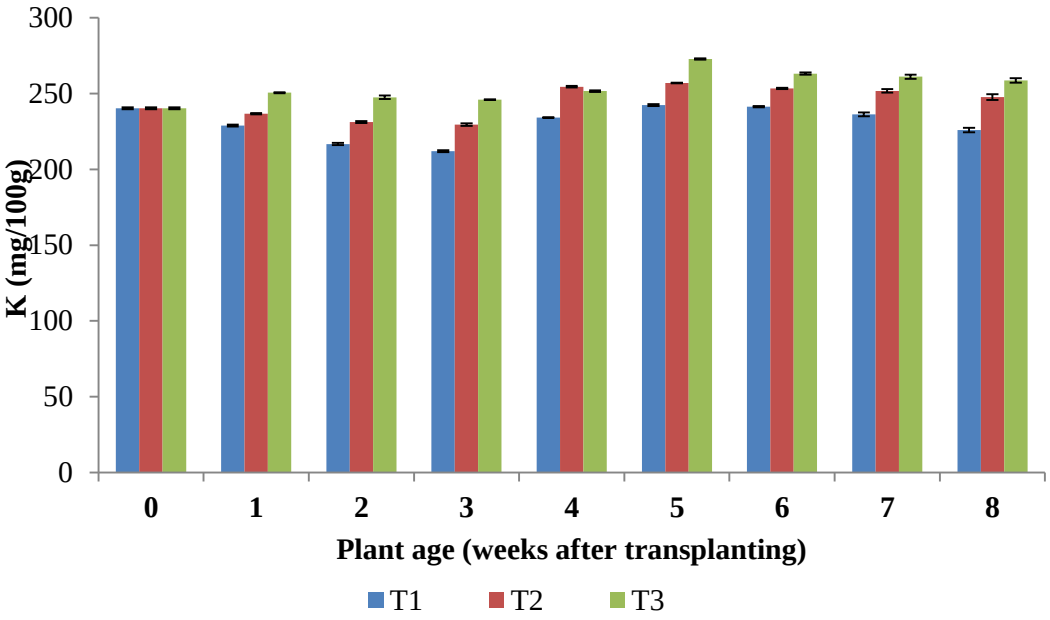

Figure 1a: Effect of organic and inorganic fertilizers on K (mg/100g)*C. gynandra*cultivated on the field.

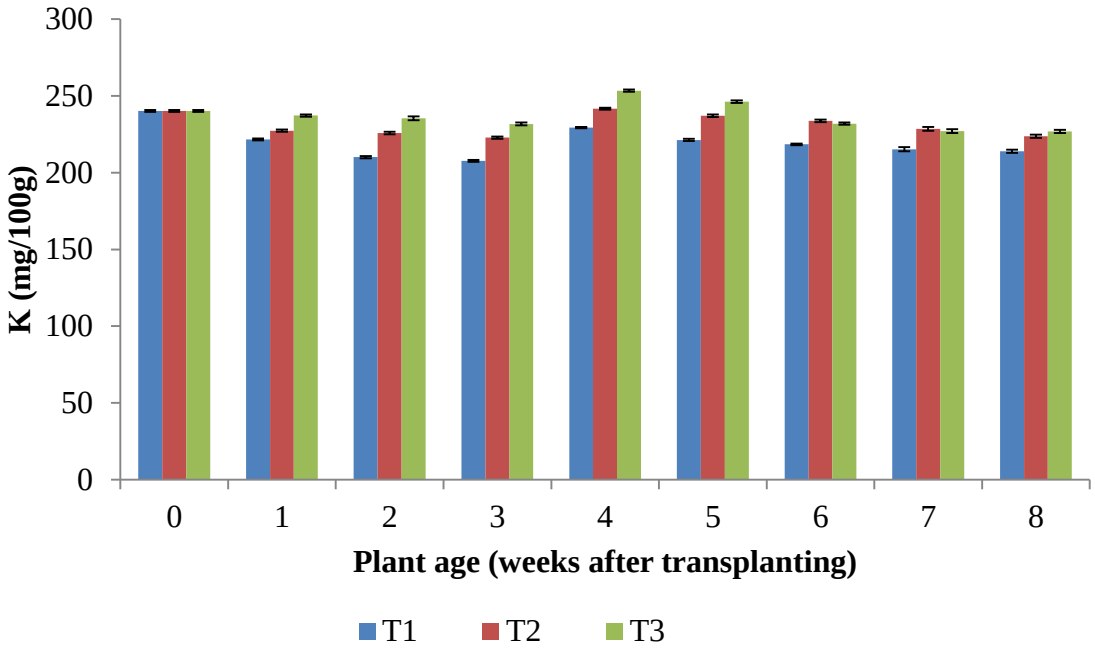

Figure 1b: Effect of organic and inorganic fertilizers on K (mg/100g)*C. gynandra*cultivated in the glasshouse.

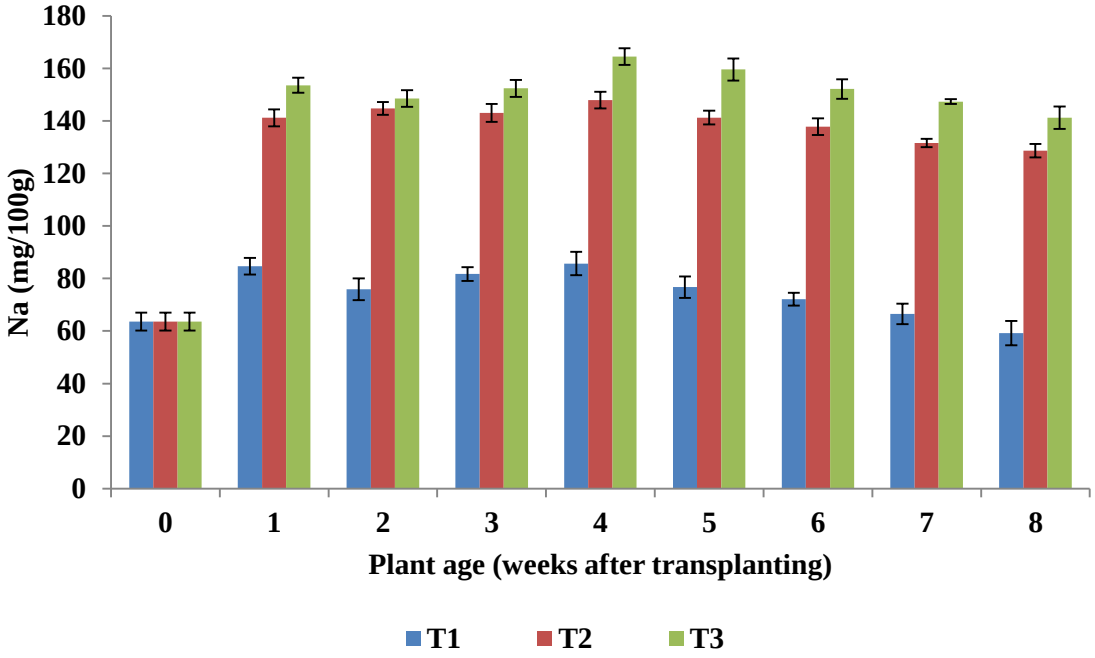

Figure 2a: Effect of organic and inorganic fertilizers on Na (mg/100g)*C. gynandra* cultivated on the field.

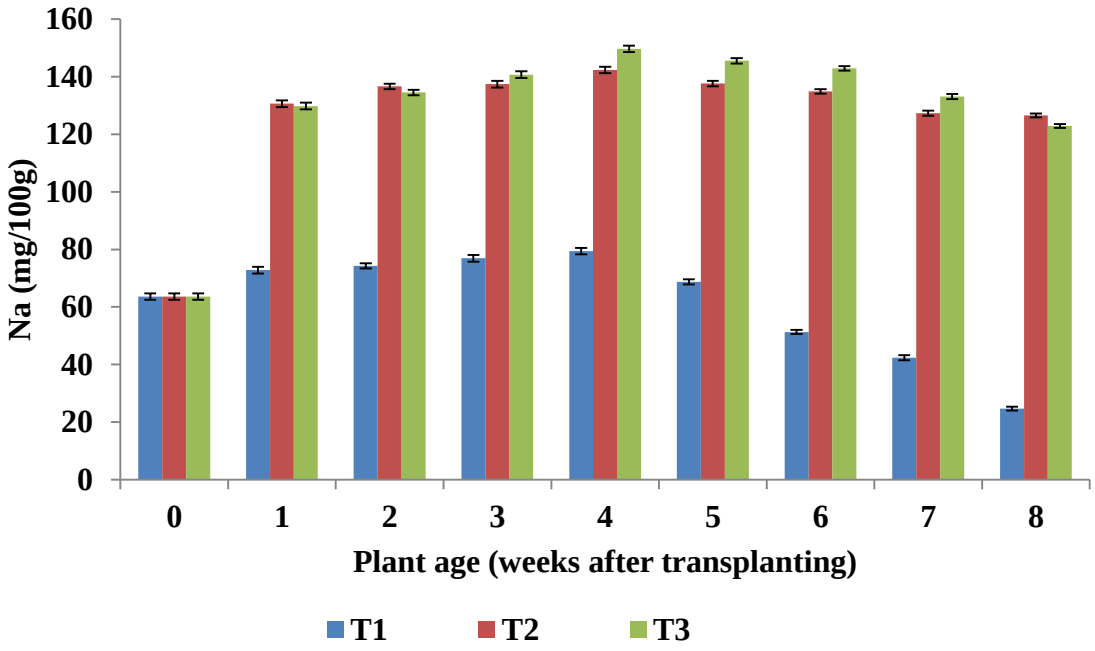

Figure 2b: Effect of organic and inorganic fertilizers on Na (mg/100g)*C. gynandra* cultivated in the glasshouse.

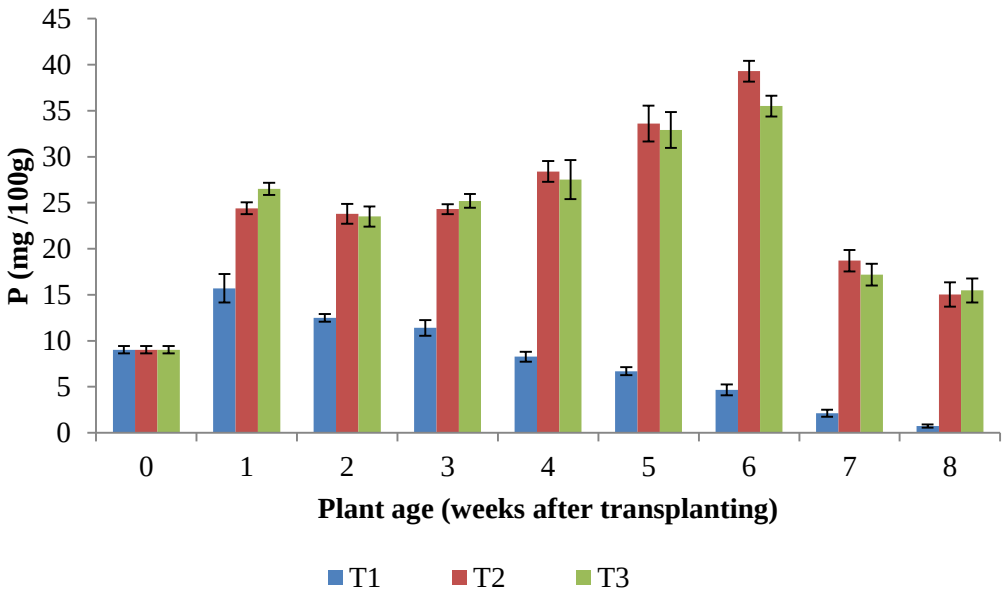

Figure 3a: Effect of organic and inorganic fertilizers on P (mg/100g)*C. gynandra*cultivatedon the field.

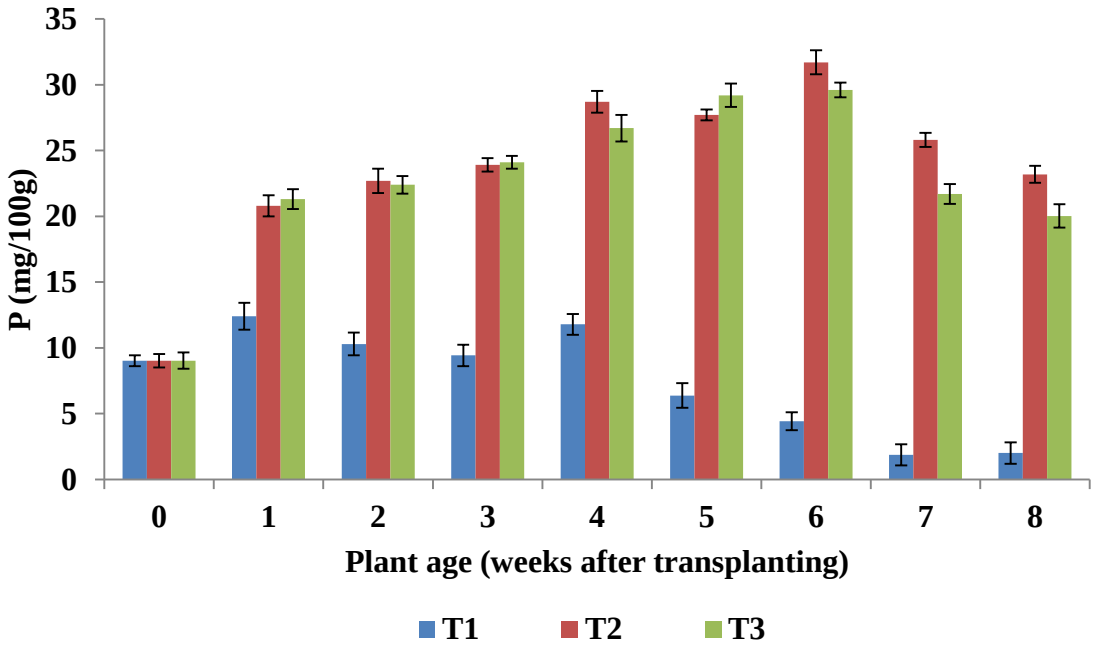

Figure 3b: Effect of organic and inorganic fertilizers on P (mg/100g)*C. gynandra*cultivated in the glasshouse.

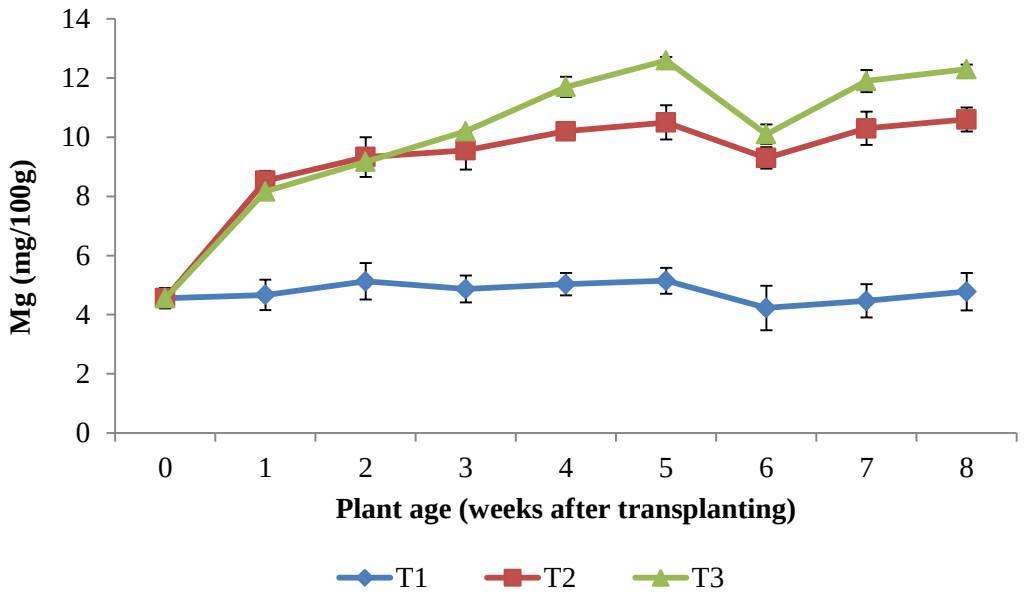

Figure 4a: Effect of organic and inorganic fertilizers on Mg (mg/100g)*C. gynandra*cultivated on the field.

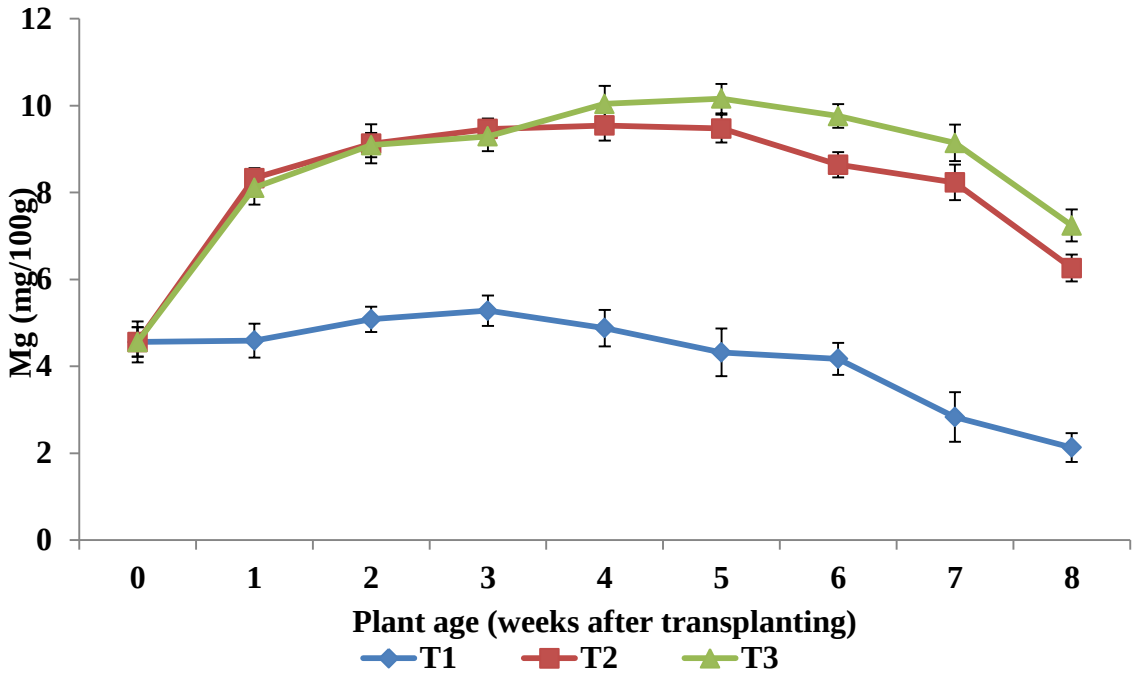

Figure 4b: Effect of organic and inorganic fertilizers on Mg (mg/100g)*C. gynandra* cultivated in the glasshouse.

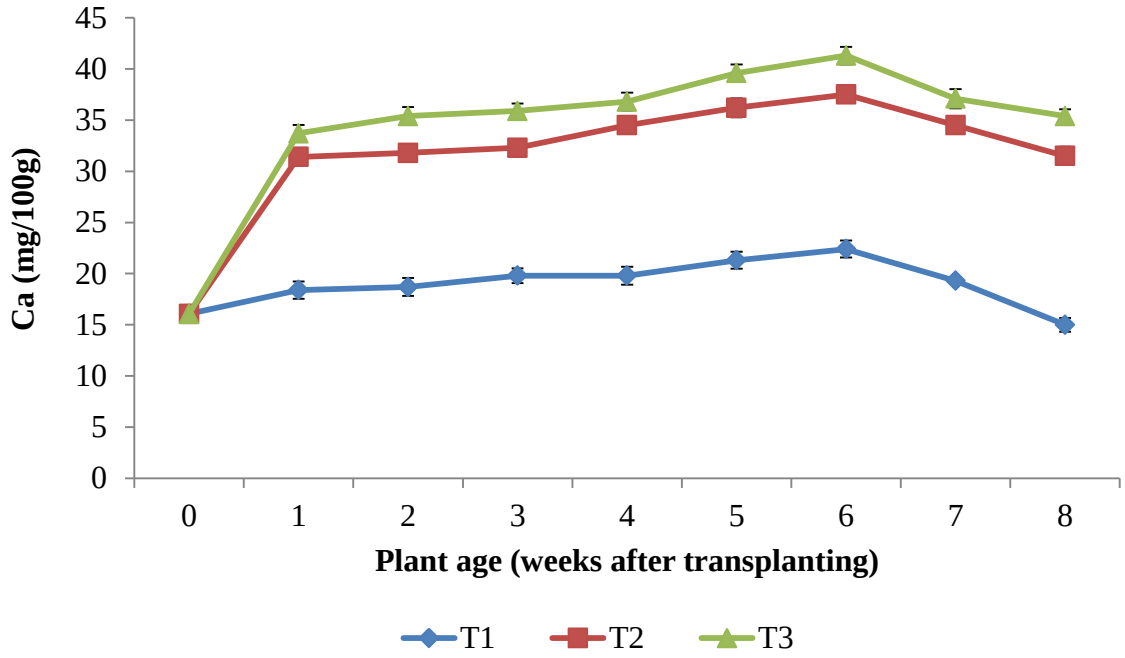

Figure 5a: Effect of organic and inorganic fertilizers on Ca (mg/100g) *C. gynandra*cultivated on the field.

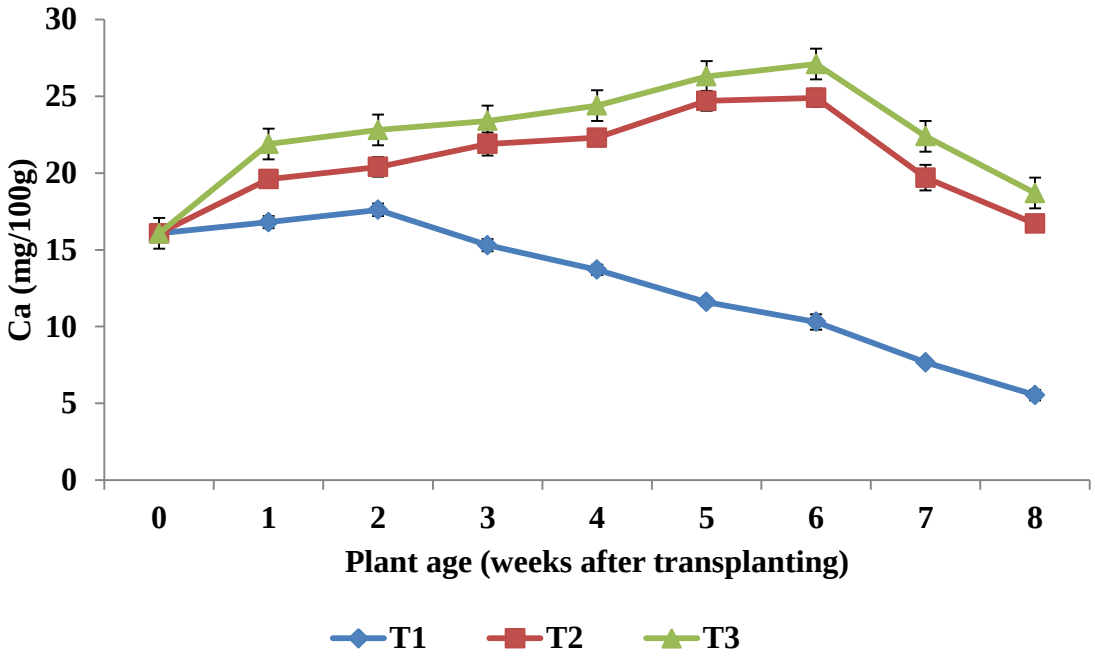

Figure 5b: Effect of organic and inorganic fertilizers on Ca (mg/100g)*C. gynandra* cultivated in the glasshouse.

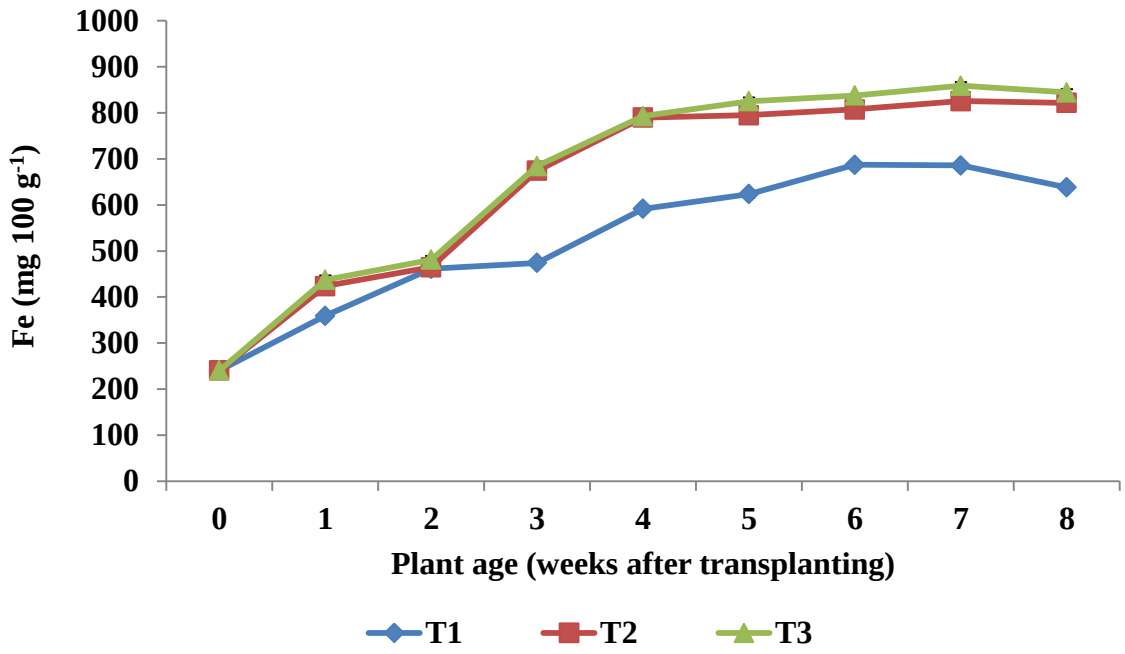

Figure 6a: Effect of organic and inorganic fertilizers on Fe (mg/100g) *C. gynandra*cultivated on the field.

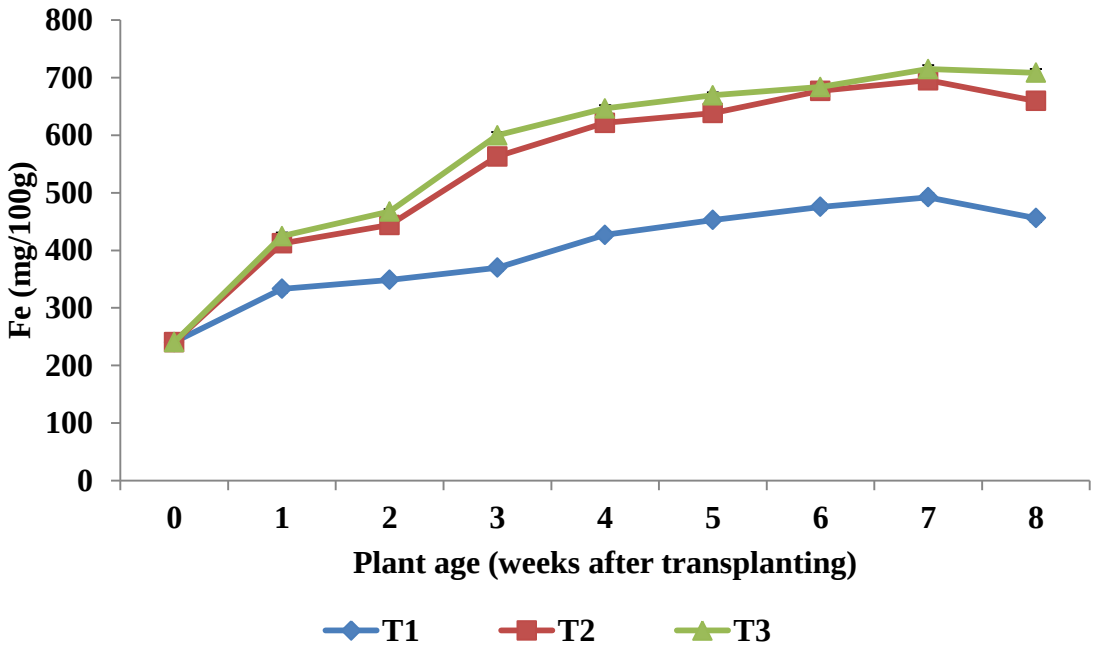

Figure 6b: Effect of organic and inorganic fertilizers on Fe (mg/100g) *C. gynandra*cultivated in the glasshouse.

Table 1a: Effect of organic and inorganic fertilizers on Mn (mg/100g) of*Cleome gynandra*L. cultivated on the field.

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	45.6 ± 0.74	48.4 ± 0.79^{a}	53.7 ± 1.26^{a}	56.3 ± 1.25^{a}	62.8 ± 1.31^{a}	63.3 ± 1.27^{a}	65.3 ± 1.24^{a}	56.7 ± 1.78^{a}	62.3 ± 1.49^{a}
T2	45.6 ± 0.74	61.3 ± 0.84^{b}	66.6 ± 1.37^{b}	68.3 ± 1.48^{b}	74.5 ± 1.41^{b}	76.5 ± 1.48^{b}	79.5 ± 1.41^{b}	74.5 ± 1.48^{b}	77.4 ± 1.43^{b}
T3	45.6 ± 0.74	69.7 ± 0.88^{c}	74.2 ± 1.42^{c}	77.3 ± 1.31^{b}	79.3 ± 1.09^{c}	84.5 ± 1.16^{c}	87.6 ± 1.13^{c}	82.9 ± 1.11^{c}	78.3 ± 1.68^{c}

Notes: 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represents significant differences at $p < 0.05$.

Table 1b: Effect of organic and inorganic fertilizers on Mn (mg/100g) of*Cleome gynandra*L. cultivated in the glasshouse.

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	45.6 ± 0.74	34.7± 0.71^{a}	49.4 ± 1.21^{a}	52.7 ± 1.18^{a}	54.6 ± 1.27^{a}	51.6 ± 1.24^{a}	54.8 ± 1.13^{a}	55.3 ± 1.61^{a}	51.6 ± 1.21^{a}
T2	45.6 ± 0.74	56.6 ± 0.77^{b}	63.8 ± 1.33^{b}	64.2 ± 1.36^{b}	66.3 ± 1.37^{b}	62.4 ± 1.43^{b}	67.4 ± 1.47^{b}	69.1 ± 1.42^{b}	63.4 ± 1.37^{b}
T3	45.6 ± 0.74	62.4 ± 0.83^{c}	70.5 ± 1.46^{c}	72.4 ± 1.43^{b}	73.9 ± 1.01^{c}	74.9 ± 1.23^{c}	76.3 ± 1.26^{c}	77.6 ± 1.37^{c}	72.8 ± 1.51^{c}

Notes: 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represents significant differences at $p < 0.05$.

Table 2a: Effect of organic and inorganic fertilizers on Cu (mg/100g) of*Cleome gynandra*L. cultivated on the field.

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	17.3 ± 0.45	21.9 ± 1.31^{a}	22.6 ± 0.82^{a}	19.7 ± 1.46^{a}	20.4 ± 1.33^{a}	21.1 ± 0.39^{a}	17.2 ± 1.08^{a}	16.3 ± 0.41^{a}	15.5 ± 0.36^{a}
T2	17.3 ± 0.45	24.7 ± 1.45^{a}	26.2 ± 1.59^{b}	25.1 ± 1.13^{b}	26.8 ± 2.69^{b}	28.5 ± 2.04^{b}	22.6 ± 2.07^{b}	21.4 ± 3.30^{b}	20.2 ± 2.12^{b}
T3	17.3 ± 0.45	27.6 ± 1.93^{b}	29.5 ± 0.60^{b}	26.8 ± 1.13^{b}	29.6 ± 1.31^{c}	34.9 ± 1.84^{c}	29.1 ± 1.98^{c}	25.9 ± 2.06^{c}	23.4 ± 1.39^{c}

Notes: 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represents significant differences at $p < 0.05$.

Table 2b: Effect of organic and inorganic fertilizers on Cu (mg/100g) of*Cleome gynandra*L. cultivated in the glasshouse.

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	17.3 ± 0.45	17.5 ± 1.27^{a}	20.1 ± 0.79^{a}	17.3 ± 1.39^{a}	18.9 ± 1.37^{a}	20.6 ± 0.68^{a}	19.2 ± 1.01^{a}	17.8 ± 0.45^{a}	13.3 ± 0.31^{a}
T2	17.3 ± 0.45	22.4 ± 1.41^{b}	23.9 ± 1.52^{b}	22.6 ± 1.25^{b}	24.3 ± 2.34^{b}	27.9 ± 2.44^{b}	25.4 ± 2.03^{b}	23.7 ± 2.24^{b}	18.6 ± 1.26^{b}
T3	17.3 ± 0.45	25.7 ± 1.83^{c}	26.2 ± 0.61^{b}	24.4 ± 1.31^{c}	27.5 ± 1.29^{c}	30.3 ± 2.65^{c}	28.5 ± 1.83^{c}	24.5 ± 1.26^{c}	21.2 ± 1.41^{c}

Notes: 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represents significant differences at $p < 0.05$.

Table 3a: Effect of organic and inorganic fertilizers on Zn (mg/100g) of*Cleome gynandra*L. cultivated on the field.

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	44.2 ± 3.39	49.6 ± 3.27^{a}	54.9 ± 2.05^{a}	57.4 ± 5.72^{a}	63.3 ± 4.49^{a}	52.8 ± 2.49^{a}	47.1 ± 4.99^{a}	42.5 ± 3.29^{a}	33.6 ± 3.56^{a}
T2	44.2 ± 3.39	52.3 ± 3.39^{b}	56.4 ± 2.94^{a}	62.7 ± 6.34^{b}	61.4 ± 6.68^{a}	59.7 ±2.49^{a}	62.3 ± 2.45^{b}	56.7 ± 2.87^{b}	44.3 ± 2.49^{b}
T3	44.2 ± 3.39	63.6 ± 3.68^{c}	72.7 ± 4.49^{b}	72.1 ± 7.48^{c}	67.9 ± 4.89^{b}	65.7 ± 2.49^{a}	57.7 ± 3.39^{c}	64.8 ± 3.74^{c}	55.3 ± 2.05^{b}

Notes: 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represents significant differences at $p < 0.05$.

Table 3b: Effect of organic and inorganic fertilizers on Zn (mg/100g) of*Cleome gynandra*L. cultivated in the glasshouse.

	Plant age (weeks after transplanting)								
	0	1	2	3	4	5	6	7	8
T1	44.2 ± 3.39	42.4 ± 3.15^{a}	49.7 ± 2.01^{a}	51.4 ± 2.71^{a}	55.3 ± 4.36^{a}	39.1 ± 2.93^{a}	33.4 ± 3.17^{a}	29.4 ± 3.13^{a}	21.2 ± 3.08^{a}
T2	44.2 ± 3.39	48.3 ± 3.27^{a}	51.2 ± 2.84^{a}	53.8 ± 2.86^{b}	58.9 ± 4.53^{a}	44.7 ± 2.26^{b}	40.5 ± 2.17^{b}	38.5 ± 2.64^{b}	33.6 ± 2.36^{b}
T3	44.2 ± 3.39	59.1 ± 3.52^{b}	63.2 ± 4.41^{b}	64.1 ± 3.21^{c}	64.6 ± 4.89^{c}	53.6 ± 4.26^{c}	49.8 ± 4.06^{c}	44.5 ± 3.53^{c}	39.5 ± 2.75^{c}

Notes: 0 indicates readings taken at the time of transplanting; values shown are mean ± SD; means with different letters down the same column represents significant differences at $p < 0.05$.

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CHAPTER 8

MATURITY EFFECTS ON THE NUTRITIONAL COMPOSITION OF *CLEOME GYNANDRA* L. (A WILD VEGETABLE)

CHAPTER EIGHT

Maturity effects on the nutritional composition of *Cleome gynandra* L. (A wild vegetable).

Abstract 186

Introduction 187

Materials and methods 188

Results 189

Discussion 192

References 206

This chapter has been submitted for publication to Journal of Soil Science and Plant Nutrition in this format.

Abstract

The increasing demand in exploring the nutritional benefits of wild vegetable species such as *Cleome gynandra* has resulted in the frequent usage of fertilizers to achieve maximumproductivity. However, the impacts of such practices on the nutritional composition in relation to their growth stages have not been fully explored. Thus, *C. gynandra* was cultivated on the field and glasshouse to determine the appropriate time of harvest in order to harness the highest nutritional value from the plant. Three treatments (control, 100 kg N/ha and 8 goat manure t/ha) were arranged in a randomized complete block design in both trials. 100 kg N/ha of inorganic fertilizer influenced more proximate constituents such as vitamin C, protein and ash while 8 t of organic fertilizer increased the crude lipid and phytate contents. The field trial was more favorable compared to the glasshouse. The ash content ranged between 6.49 – 19.64%, fibre 9.62 – 26.13%, lipid 1.07 - 6.94%, vitamin C ranged between 68.33 – 279.7 mg/ 100g, protein 36.55 - 53.82%; phytate 0.74 – 2.91 mg/ 100g; oxalate 1.33 – 2.63 mg/ 100g. It further reveals the concentrations of protein, vitamin C, and fibre can be exploited better in the 2^{nd}, 6^{th} and 8^{th} weeks respectively.

Keywords: nutritional composition, wild vegetables,*Cleome gynandra,* fertilizers

Introduction

Most developing countries depend predominately on starch-based foods as their main staple meal for the supply of both energy and protein. This result in poor diets with increased prevalence of nutritional deficiency among the populace (Akubugwo *et al*., 2007). To address this, interests have been centred on the importance and utilization of food plants, especially vegetables (Dini *et al*., 2005; Hussain *et al*., 2010).

Vegetables are considered one of the sources of mineral elements. These mineral elements are obtained from the soil in which the vegetables are cultivated. The source of the nutrients essential for their growth could either be organic or inorganic. The inorganic sources involve the use of fertilizers which have been reported to increase plant growth to a designated optimum (Stewart *et al*., 2005; Mohammed *et al*., 2008). In addition, the use of organic sources in the form of goat manures and cow dung have been found to complement the use of inorganic fertilizer as both manures aid in improving crop yield (Odiete *et al*., 2005).

Generally, fertilizer affects the productivity and nutrient quality of plants. Weak vegetative growth, poor fruit setting, undesirable fruit quality and low nutritional quality result from inadequate levels of the basic nutrients (Martinetti and Paganini, 2006; Liu *et al*., 2010). In addition, enriched soil nutrients could also improve the quality of vegetables with regards to minerals, vitamins and protein content. However, recently, interest has increased considerably in determining the nutritional quality of wild vegetables after fertilization although this is of great importance for promoting the use of fertilizers in vegetable cultivation (Sossa-Vihotig *et al*., 2013). Hence, adequate fertilizer application has to be encouraged in order to boost the soils in the Eastern Cape Province thereby ensuring sustainable vegetable production with rich mineral nutrients for healthier diets.

Cleome gynandra is a well-known nutraceutical plant which is highly enriched with microand macro nutrients, vitamins, protein and dietary fibres. It is an annual herb belonging to the family Capparaceae and grows up to a height of 150 cm. Consequently, extensive studies have been reported on the nutritional compositions of this species in other countries such as

Côte d'Ivoire, Ghana, Uganda and India among others (Abugre *et al*., 2011; Acipa *et al*., 2013; Agbo *et al*., 2014; Mishra *et al*., 2011). However, there is a lack of information on the effect of fertilizers on the nutritional composition of *C. gynandra* at different growth stages in the Eastern Cape Province, South Africa. Thus, the aim of this study was to determine the effects of fertilizers on the proximate composition of the leaves of *C. gynandra* and the optimum harvesting time for the leaves of this species based on the highest nutrient composition.

Materials and methods

The experimental sites, agronomic practices, experimental design, chemical composition of the experimental soil and organic fertilizer were described in Chapter 6. The plant and data collection was described in Chapter 7.

Proximate analysis

The ash, crude lipid, protein, fibre, vitamin C, phytate and oxalate were determined as described in Chapter 3.

Statistical analysis

Data collected on the proximate parameters of various treatments were subjected to statistical analysis using MINITAB Release 12. A one way analysis of variance was used to compare the means of various proximate constituents among the treatments. Means were compared using Duncan's multiple range tests. The means were treated as significantly different at $p < 0.05$.

Results

Concentration of ash

The ash content ranged from 6.49 to 19.64% on the field and 6.49 to 11.95% in the glasshouse (Fig. 1). The ash content varied significantly ($p < 0.05$) among the various treatments on the field and in the glasshouse where the highest concentration of ash was observed in the inorganic fertilizer treatment on the field (T3F) while the lowest was recorded in the control treatment in the glasshouse (T1G). The maximum concentration of ash in both trials followed the trend: T3F > T2F > T3G > T3G > T1F > T1G. In both trials, the ash content maintained a consistent increase till the termination of the experiment. It further indicates that *Cleome gynandra* cultivated on the field had higher ash content compared to the glasshouse. Statistical analysis showed an interaction between plant age and the fertilizer treatments.

Crude fibre

On the field, the fibre content ranged from 9.62 to 26.13% and 9.62 to 25.93% in the glasshouse (Fig. 2). Fibre content increased from the time of transplanting on the field to the 6^{th} week after which it began to decrease in the 7^{th} and 8^{th} weeks while in the glasshouse it increased positively with plant age from time of transplanting to the 4^{th} week after which it plateaued in the 5^{th} and 6^{th} weeks. The treatment means varied significantly, however the highest mean fibre content was obtained on the field in T3F (18.88%) while the lowest in T1F (17.22%). A different trend was recorded in the glasshouse where the treatment means were highest in T2G (19.50%) and the least was observed in T1G (16%). Generally, the highest concentration of fibre in both trials followed the trend: T2G > T3F > T2F > T1F > T3G > T1G. When comparing both trials, *C. gynandra* cultivated in the glasshouse had more fibre content than the field trial. Statistical analysis showed an interaction between plant age and the fertilizer treatments on the fibre content of this species.

Concentration of crude lipid

The lipid content varied significantly among the various fertilizer treatments in both trials (Fig. 3). Lipids increased from the time of transplanting up till the 3rd week after which it decreased in the 4th week in T2F and T3F on the field. A different trend was observed in the 5th week where an increase was recorded amongst all the treatments after which it maintained a decrease throughout the trial. The treatment means were highest in T2F (4.88%) and the least was observed in T1F (4.35%). On the other hand, the lipid content increased from time of transplanting to the 3rd week after which it decreased in the subsequent weeks in the glasshouse. The treatment means during this trial were highest in T2G (4.79%) and the least was observed in T1G (2.89%). The lipid content was therefore at its peak in the 5th and 3rd weeks on the field and in the glasshouse respectively. Furthermore, the composition of lipids was found higher on the field than the glasshouse.

Vitamin C concentration

The vitamin C content differed significantly ($p < 0.05$) among the various treatments in both trials (Fig. 4). The concentration of vitamin C cultivated under field conditions showed an increase from the time of transplanting to the 6th week where the highest value was obtained in T3F (279.7 mg/100g) after which it decreased. The treatment means were highest in T3F (194.14 mg/100g) and the least was observed in T1F (132.9 mg/100g). On the other hand, vitamin C composition in the glasshouse showed a similar trend as obtained on the field. The maximum concentration was obtained in the 6th week (256.3 mg/100g) after which it began to decrease in the 7th and 8th weeks. The treatment means during this trial were highest in T3G (185.5 mg/100g) and the least was observed in T1G (128.2 mg/100g). The concentration of vitamin C decreased as follows: T3F > T3G > T2F > T2G > T1F > T1G. Furthermore, plant age had a significant effect on the vitamin C composition in both trials.

Protein concentration

Protein increased sharply from the time of transplanting to the 2nd week after which it decreased slightly in the 3rd and began to vary amongst the treatments from the 4th week to the 8th week on the field (Fig. 5). The treatment means for the duration of the trial were highest in T3F (50.69%) and the lowest T1F (41.36%). However, the highest concentration of protein was observed in week 2. In the glasshouse, the treatment means varied significantly from each other ($p < 0.05$) in the 6th, 7th and 8th weeks. The maximum concentration of protein was also observed in the 2nd week. The treatment means were highest in T3G (50.54%) and the lowest in T1G (41.12%). However, the concentration of protein was in close range when compared with the values obtained on the field. In both trials, it was also observed that the protein concentrations in T3G, T3F, T2F and T2G maintained a progressive trend in most of the weeks after transplanting. Statistical analysis showed an interaction between plant age and the fertilizer treatment on the protein content.

Phytate content

The concentration of phytate in both trials ranged from 1.18 to 2.87 mg/100g and 0.74 to 2.91 mg/100g in respect of field and glasshouse (Fig. 6). The phytate content on the field decreased from the 1st week to the 5th week after which it increased only in the 6th week and then began to show a decline again throughout the trial. However, the highest concentration of phytate was observed in the 6th week. On the other hand, the phytate content in the glasshouse decreased from the time of transplanting to the 7th week after which it increased in the 8th week. The maximum concentration of phytate was observed in the 8th week. Generally, phytate content decreased with plant age in both trials.

Oxalate

The concentration of oxalate in both trials ranged from 1.33 to 2.63 mg/100g and 1.25 to 2.57

mg/100g in respect of the field and glasshouse (Fig. 7). Oxalate content decreased from the time of transplanting to the 4th week after which it began to increase in the 5th and 6th weeks and then varied throughout the experiment on the field. The treatment means were found to be highest in T3F (2.09 mg/100g) and the lowest in T2F (1.97 mg/100g). In the glasshouse trial, the oxalate content showed a different trend where it decreased from the time of transplanting to the 3rd week after which it increased in the 4th week in T2G and T3G (Fig.7). This increase continued in all treatments in the 5th and 6th weeks after which it began to decrease. The treatment means during this trial were highest in T3G (2.02 mg/100g) and the least was observed in T2G (1.89 mg/100g). However, the highest concentration of oxalate was observed in the 6th week for both trials. Statistical analysis showed an interaction between plant age and the fertilizer treatment on the oxalate content.

Discussion

Ash

The values reported in the current study are comparable with or slightly higher than those of other wild vegetable species found in the Eastern Cape Province (Jimoh *et al*., 2011). Similar results have been reported in Nigeria where the ash content of some wild vegetables were observed to range between 19.3 and 21.20% (Akindahunsi and Salawu, 2005; Onwordi *et al*., 2009). Furthermore, Cakilcioglu and Khatun, (2011) also reported a close range of values between 7.50 and 18.50% in 16 wild vegetables in Turkey. The high ash content obtained in the species is a measure of the mineral constituents stored in the plant. These minerals act as inorganic cofactors which are required for proper physiological functioning in the body (Iheanacho and Udebuani, 2009). In the absence of these inorganic cofactors, there could be impaired metabolism. The results of this study indicate a high deposit of mineral elements in the leaves of *C. gynandra* on the 8th week after transplanting both in the glasshouse and on the field. Therefore, this suggests that delayed harvest of *Cleome gynandra* could result in

accumulation of more minerals which may be of benefit to the people who consume them.

Crude Fibre

The fibre content in this study is much higher than what was reported by Hussain *et al*. (2009), Oduro *et al*. (2008) as well as Adeniyi *et al*. (2012). These authors reported 6.66, 9.25 and 0.33% fibre in *Chenopodium album*, *Moringa spp* and *Corchorus olitorius* respectively. In a similar study, Jimoh *et al*. (2011) also reported a fibre content of 15.25 and 18.33% in *Sonchus oleraceus* and *Sonchus asper* which, when compared to the present study was low.In contrast, the fibre content in *Cordiamyxa roxb* was found to be in close range with the values obtained in this species (Aberoumand, 2009). In another related study, Iheanacho and Udebuani, (2009) reported high fibre content in *Gnetum africana*. The variations in the composition of fibre in leafy vegetables could be influenced by farming practices and prevailing environmental conditions. Often, sources of differences could be attributed to the plant age at harvest as well as application of organic and inorganic fertilizer (Gupta *et al*., 2005). Adequate intake of dietary fibre plays a crucial role in preventing the absorption of excess cholesterol, risk of coronary heart disease, constipation, gastrointestinal disorder and breast cancer in humans. Fibre enhances the regulation of intestinal transit, increases dietary bulk thereby reducing the intake of excess starchy food which is the characteristics of the diet of the people in the region of the current study. On the other hand, low dietary fibre intake has been shown to be responsible for the increasing incidence of diverticulosis which is associated with cancer of the colon (Aberoumand, 2009). According to the National Health and Medical Research Council of Australia (NHMRC), (2005), a plant rich in fibre must provide between 10-15% of required intake. The required daily allowance (RDA) of fibre for children, adults, pregnant and lactating mothers are 19-25, 21-38, 28 and 29 g, respectively

(Adedapo *et al*., 2011). Thus, the substantial amount of fibre present in *C. gynandra* cultivated both in the glasshouse and on the field indicates the potential of this species to enhance the proper functioning of the digestive system in the human body. The plant should be harvested during the 6th week. This is when the highest fibre content was observed on the field whereas the 4th week would be ideal in the glasshouse.

Crude lipid

The crude lipid content of *Cleome gynandra* leaves compares favorably with the previous reports of Adedapo *et al*. (2011) as well as Iheanacho and Udebuani (2009). The values obtained in the present study show a much higher lipid content than *Amaranthus cruentus* (0.45%), *Corchorus argenta* (0.21%) and *Corchorus olitorius* (0.32%) consumed in West Africa (Onwordi *et al*., 2009). Lola, (2009) and Oduse *et al*. (2012) also reported low crude lipid content in *Solanum nigrum* harvested from the wild. However, the crude lipid content in this species was fairly low when compared to those of *Gongronema latifolium*, *Amaranthushybridus* and *Solanum indicum* (Aberoumand, 2009; Asaolu *et al*., 2012).

The lipid content in the current study confirmed the findings of several authors which showed that leafy vegetables are fair sources of lipids (Akubugwo, 2007; Patricia *et al*., 2014).

However, it's worth noting that a diet providing 1 – 2% of its caloric energy as fat is said to be adequate to humans, as excess fat consumption is implicated in cardiovascular disorders such as atherosclerosis, cancer and aging (Sodamade *et al*., 2013). Lipids are essential fats that play a very vital role in human physiology. It aids in proper functioning of the brain and joints mobilization. They also help the body to absorb fat-soluble vitamins such as vitamins A and E which are necessary for normal growth and metabolic activities (Adeniyi *et al*., 2012). In order to achieve a diet with moderate lipid content, it is advised to harvest the plant in the 5th week on the field while the 3rd week would be ideal in the glasshouse due to the high concentration of lipids observed during these periods.

Vitamin C

The use of fertilizer, particularly inorganic sources increased the vitamin C content both on the field and in the glasshouse. The highest concentration of vitamin C obtained in both trials fell within the reported range of values for the same species (Chweya, 1995). The vitamin C content in this study was found to be higher than what was reported by Mkandawire and Masamba, (2014). In a similar study, Mibei and Ojijo, (2011) also reported a lower concentration of vitamin C in the same plant. Comparing with other wild vegetables, Lyimo *et al*. (2003) revealed a range of values between 7.7 and 266 mg/100g. Gupta *et al*. (2005) reported that *Delonix elata* leaves contained 295 mg/100 g vitamin C which was slightly higher than what was obtained in the current study. The variations in the concentration of vitamin C in plants have been attributed to several factors such as the genotypic differences, pre-harvest climatic conditions, cultural practices, maturity and harvesting methods and postharvest handling procedures (Lee and Kader, 2000). In addition, plants exposed to more sunlight contain more concentration of vitamin C. This probably accounts for the slight variations between the vitamin C content obtained on the field and in the glasshouse. Vitamin C is a water-soluble antioxidant that promotes absorption of dietary iron where Fe is absorbed as Fe^{2+} rather than Fe^{3+} (Okwu and Emenike, 2006). According to Lui *et al*. (2008), high concentration of vitamin C in plant samples are linked with free radical scavenging ability and health benefits like anti-carcinogenic and anti-atherogenic properties. A report by Nestle Food and Nutrition Communication (NFNC), (2007) stated that about 400 mg of vitamin C can be consumed from food sources; however, doses from 400 mg to 1 g are still regarded safe. Results from the present study indicate that harvesting *C. gynandra* in the 6^{th} week both on the field and in the glasshouse gives the highest vitamin C concentrations.

Protein

The highest protein content in the present study is 53.59% higher than what was reported by

Pillai *et al*. (2013), for *Cleome viscosa* and *Cleome burmanni*. On the other hand, the amounts of protein in the current study were slightly lower than those reported in some Nigerian leafy vegetables (Onwordi *et al*., 2009). Although when compared to other wild vegetables such as *Momordica balsamina* (11.29%), *Moringa oleifera* (20.72%), *Lesianthera africiana* leaves (13.10-14.90%) and *Leptadenia hastate* (19.10%), the protein content obtained in the current study was higher(Asaolu *et al*., 2012). Therefore, the leaves of *C. gynandra* are considered good sources of protein since they can provide 4 times more (300 g) dietary protein requirement for children, pregnant and lactating mothers. Generally, vegetables grown with inorganic fertilizer have higher nitrate content than organically produced vegetables (Siderer *et al*., 2005). According to Worthington (2001), nitrogen from inorganic fertilizer affects the amounts of vitamin C and nitrates as well as the quantity and quality of protein produced by plants. Protein is an essential component of diet that supplies adequate amounts of amino acids for the building and repairing of body tissues, regulation of body processes and formation of enzymes (Pugalenthi *et al*., 2004). Proteins are also known to aid in the formation of antibodies that enable the body to fight infection and diseases. The results of this study showed that adequate protein is present in *C. gynandra*. This could play a significant role in providing a cheap source of protein for the local inhabitants. Furthermore, the highest concentration of protein could be achieved by harvesting the leaves of *C. gynandra* in the 2nd week after transplanting both on the field and in the glasshouse.

Phytate

The phytate content obtained in both trials of the present study were higher than what was reported by Khan *et al*. (2015). However, when compared with the phytate content reported in *Sonchus asper* and *Sonchus oleraceus*, the value was lower (Jimoh *et al*., 2011). Uusiku *et al*. (2010) also reported a range of values in some African leafy vegetables to fall between 0.1 mg/100g and 481 mg/100g. Phytate is an organic form of phosphorus in plants. It has 12 replaceable hydrogen atoms and therefore could form insoluble salts with Ca, Fe, Zn, Mg and

P, thus, preventing the bioavailability and utilization of these minerals (Gupta *et al*., 2006; Salna *et al*., 2011). According to Hurrel *et al*. (1992), a phytate intake of 4-9 mg/100g reduces iron absorption in humans. Above this range, phytate could be of nutritional danger resulting in the reduction of essential dietary nutrients. However, a high concentration of phytate in vegetables can be eliminated through various processes such as boiling, frying and soaking (Akwaowo *et al*., 2000). Inspite of the harmful effect of anti-nutrients when found in high doses, some positive significance have been attributed to its ability in scavenging free radicals and their anti-carcinogenic properties (Jenab and Thompson, 2002). The incorporation of *C. gynandra* in the diet may provide better bioavailability of minerals thereby averting nutrient deficiencies such as rickets and osteroporosis. In addition, the lowest concentrations of phytate can be obtained in the 5^{th} and 6^{th} weeks both on the field and in the glasshouse.

Oxalate

The oxalate content in this study is comparable to the values reported in *Phaseolus vulgaris* (Oyelude *et al*., 2012). A review of the concentration of oxalates in some selected African leafy vegetables was reported to range between 1 mg/100g and 1115 mg/100g (Uusiku *et al*., 2010). In addition, the reported oxalate content in *Ipomea batatas* was far above the reported range in the present study (Aregheore, 2012). Generally, oxalates in food may be either soluble or insoluble and according to Gupta *et al*. (2005), soluble oxalates form strong chelates with dietary calcium, which results in the formation of calcium oxalate crystals. These crystals are deposited as urinary stones which are linked with the blockage of renal tubules in the body. The oxalate content obtained in the present study was far below the toxic range of 2-5 g/day (Hassan and Umar, 2004). Therefore consumption of *C. gynandra* is unlikely to have adverse effects in the body. Furthermore, the lowest concentrations of oxalate can be obtained in the 8^{th} week both on the field and in the glasshouse. This was

when the lowest concentration of oxalate was observed.

Conclusion

The overall analysis of the proximate parameters analysed on the field and in the glasshouse showed variations in the nutritional composition of *Cleome gynandra*. Generally, inorganic fertilizer influenced more proximate constituents such as vitamin C, protein and ash on the field while organic fertilizer increased the crude lipid and phytate content in the glasshouse. Both fertilizers increased the crude fibre content of the plant on the field. Thus, fertilizers should be applied when cultivating *Cleome gynandra*. The anti-nutritional analysis revealed that plant samples contained phytate and oxalate. However, values obtained were appreciably low such that the available minerals can be utilized effectively in the body. The highest concentrations of protein, vitamin C, and crude fibre can be explored better in the 2^{nd}, 6^{th} and 8^{th} weeks after transplanting. Furthermore, the proximate constituents revealed in this study could serve as supplements for food which can contribute significantly to the daily requirement for normal growth and also aid in averting diseases associated with malnutrition. Therefore, their cultivation and consumption should be encouraged.

Acknowledgements

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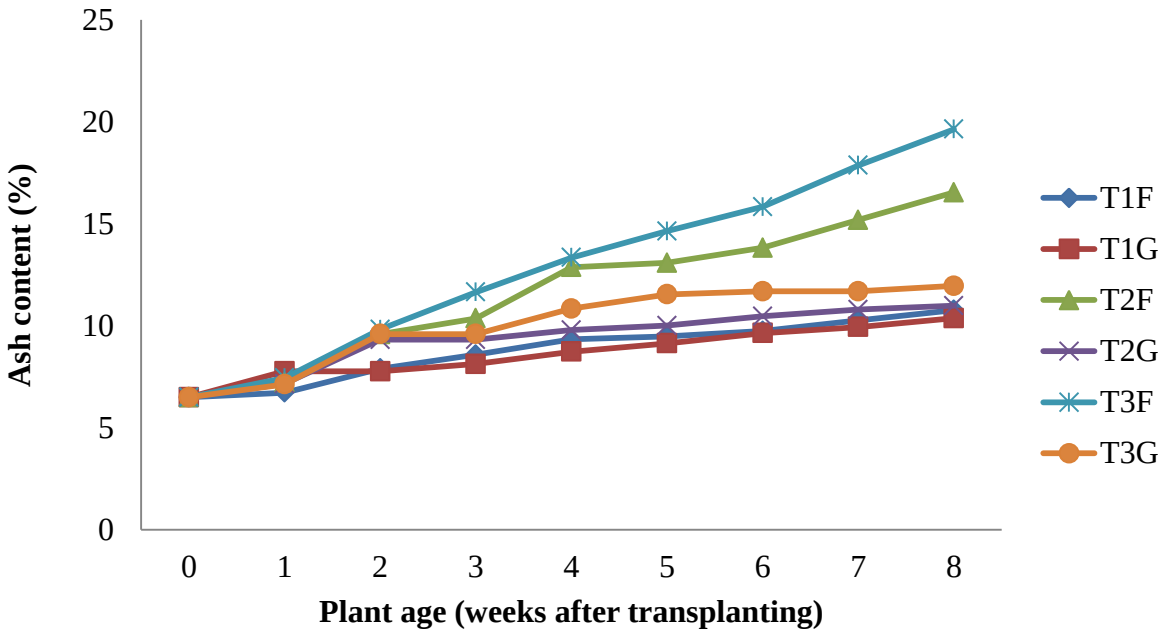
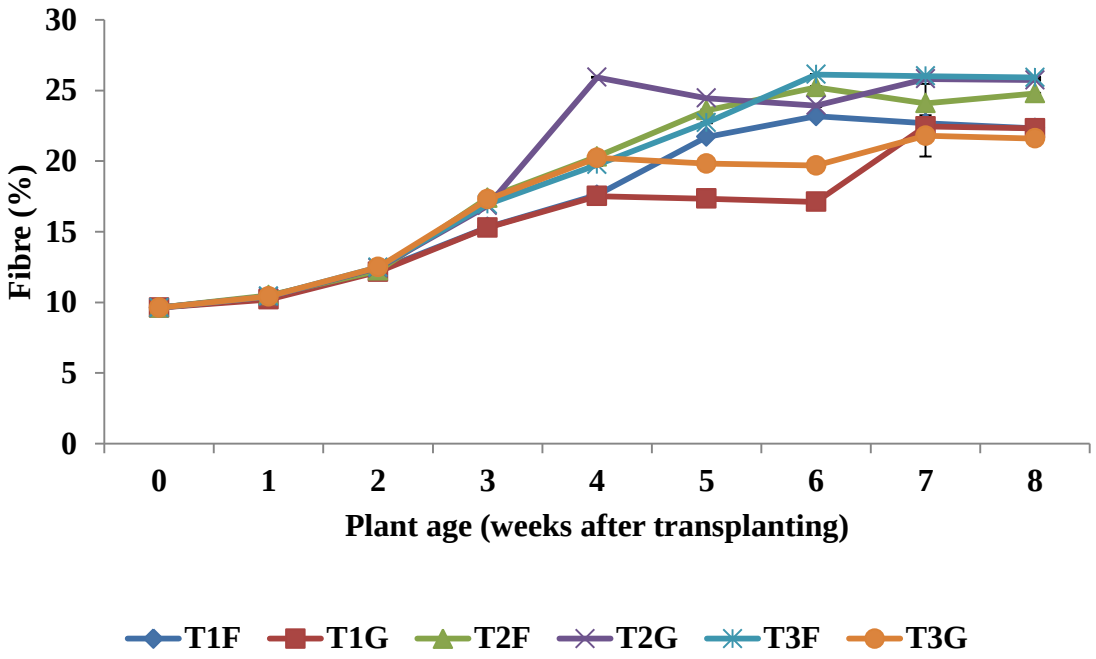


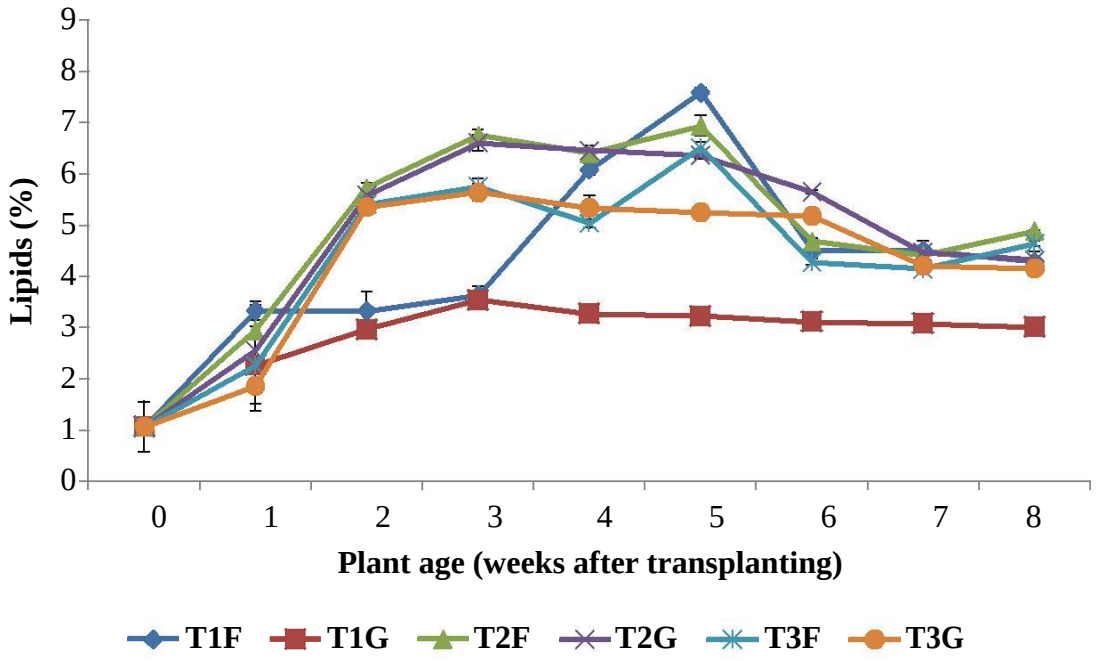
Notes: T1F: control on the field; T1G: control in the glasshouse; T2F: 8 t manure/ha of the field; T2G: 8 t manure/ha in the glasshouse; T3F: 100 kg N/ha on the field; T3G: 100 kg N/ha in the glasshouse

Figure 1: Effect of organic and inorganic fertilizers on the ash content (%) of*Cleomegynandra* cultivated on the field and in the glasshouse.



Notes: T1F: control on the field; T1G: control in the glasshouse; T2F: 8 t manure/ha of the field; T2G: 8 t manure/ha in the glasshouse; T3F: 100 kg N/ha on the field; T3G: 100 kg N/ha in the glasshouse

Figure 2: Effect of organic and inorganic fertilizers on the fibre content (%) of*C. gynandra*cultivated on the field and in the glasshouse.



Notes: T1F: control on the field; T1G: control in the glasshouse; T2F: 8 t manure/ha of the field; T2G: 8 t manure/ha in the glasshouse; T3F: 100 kg N/ha on the field; T3G: 100 kg N/ha in the glasshouse.

Figure 3: Effect of organic and inorganic fertilizers on the lipid content (%) of*C. gynandra* cultivated on the field and in the glasshouse.

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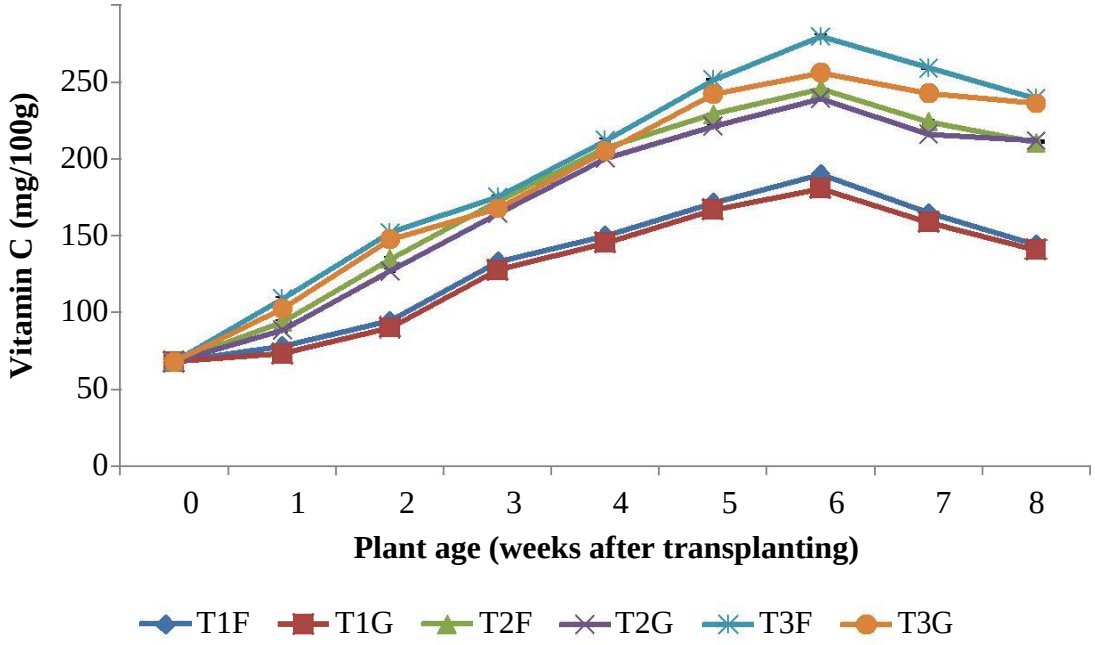
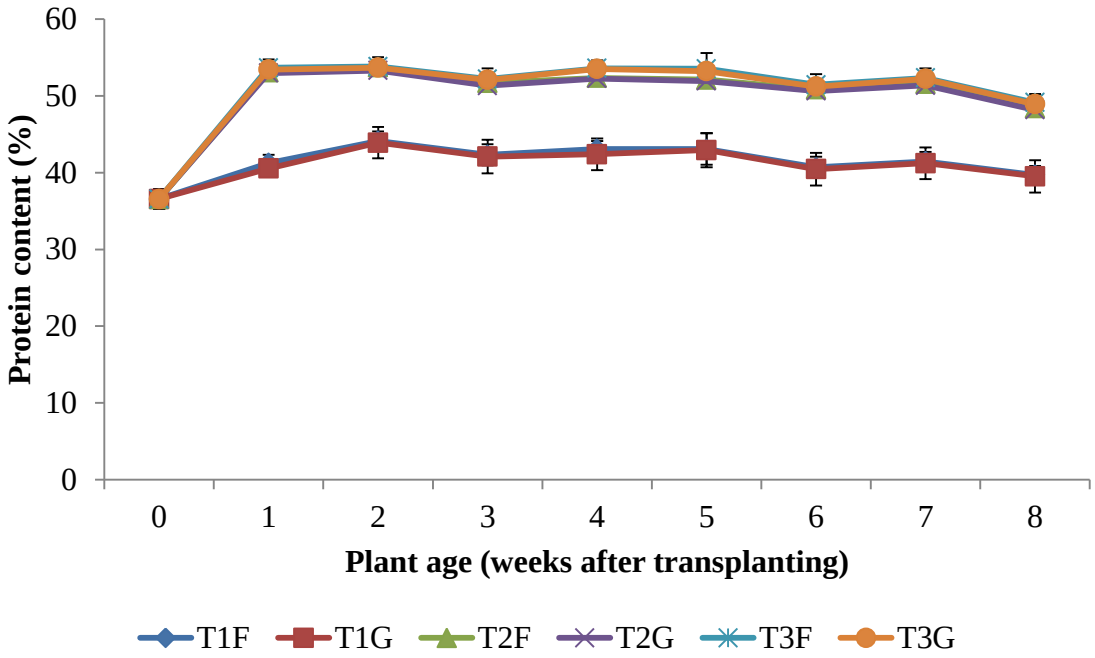


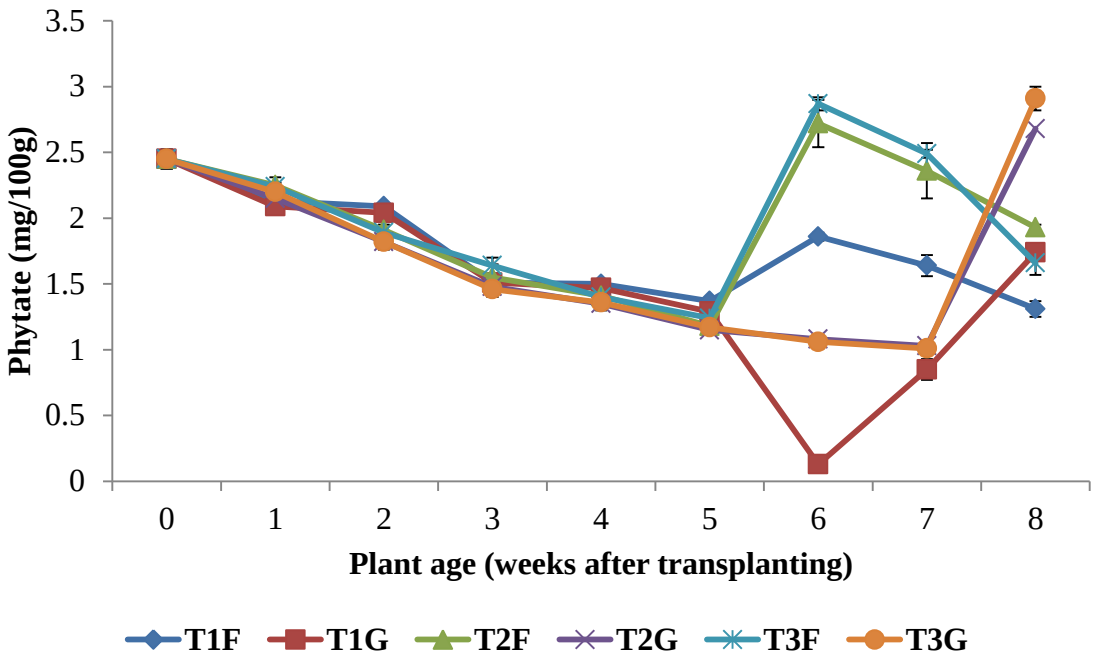
Notes: T1F: control on the field; T1G: control in the glasshouse; T2F: 8 t manure/ha of the field; T2G: 8 t manure/ha in the glasshouse; T3F: 100 kg N/ha on the field; T3G: 100 kg N/ha in the glasshouse

Figure 4: Effect of organic and inorganic fertilizers on vitamin C (mg/100g) of*C. gynandra*cultivated on the field and in the glasshouse.



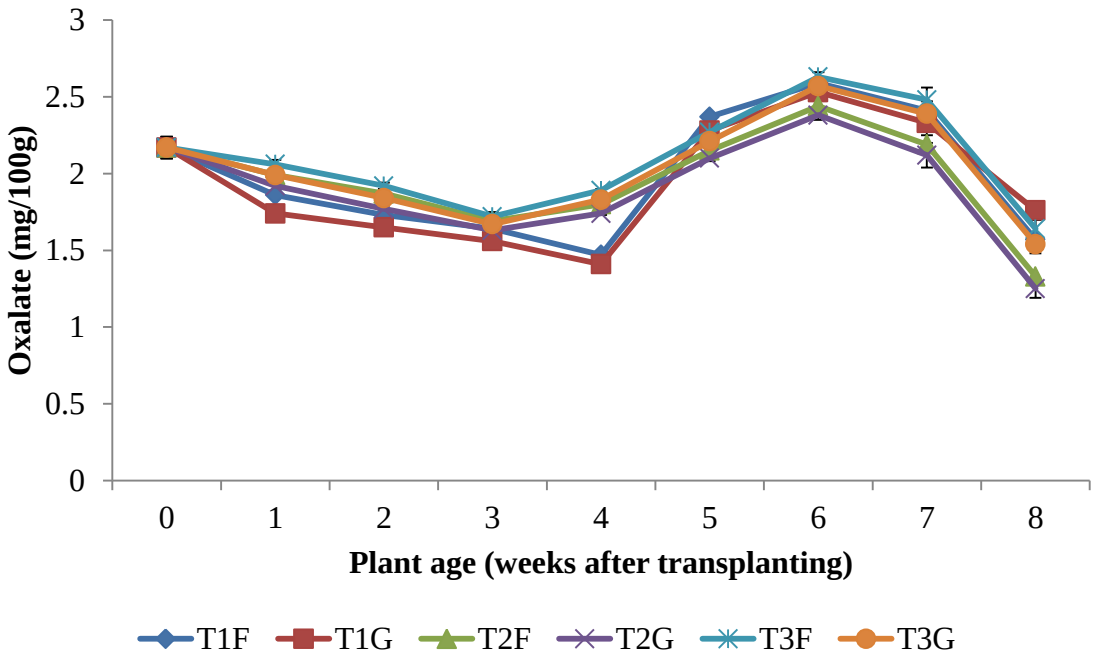
Notes: T1F: control on the field; T1G: control in the glasshouse; T2F: 8 t manure/ha of the field; T2G: 8 t manure/ha in the glasshouse; T3F: 100 kg N/ha on the field; T3G: 100 kg N/ha in the glasshouse.

Figure 5: Effect of organic and inorganic fertilizers on the protein content (%) of *C. gynandra* cultivated on the field and in the glasshouse.



Notes: T1F: control on the field; T1G: control in the glasshouse; T2F: 8 t manure/ha of the field; T2G: 8 t manure/ha in the glasshouse; T3F: 100 kg N/ha on the field; T3G: 100 kg N/ha in the glasshouse.

Figure 6: Effect of organic and inorganic fertilizers on the phytate content (mg/100g) of*C.gynandra* cultivated on the field and in the glasshouse.



Notes: T1F: control on the field; T1G: control in the glasshouse; T2F: 8 t manure/ha of the field; T2G: 8 t manure/ha in the glasshouse; T3F: 100 kg N/ha on the field; T3G: 100 kg N/ha in the glasshouse.

Figure 7: Effect of organic and inorganic fertilizers on the oxalate content (mg/100g) of*C.gynandra* cultivated on the field and in the glasshouse.

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CHAPTER 9

GENERAL CONCLUSION AND RECOMMEDATIONS

9.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

The following are the main findings and conclusions from this work:

- The micro-morphological structures of *C. gynandra* are revealed for the first time. These might be responsible for the production and storage of some secondary metabolites (Chapter 2).
- The phytochemical screening revealed the presence of phenols, flavonoids, flavonols, and proanthocyandins. It also revealed appreciable concentration of saponins, alkaloids and tannins, especially the stem of this plant is an excellent source of proanthocyanidins (Chapter 4).
- *C. gynandra* possesses strong antioxidant properties which are implicated in reducingthe risk of degenerative diseases. This property might justify its use as natural occurring antioxidants in folkoric medicine (Chapter 4).
- Seed germination is erractic in *C. gynandra*. However, high viability can be achieved by storing seeds for 6 – 12 months. In addition, optimum germination can be achieved at 30^{o}C with biweekly watering regime at a sowing depth of 0.5 cm under dark conditions as discussed in Chapter 5.
- The growth parameters of *C. gynandra*measured in this study improved significantly with the application of organic and inorganic fertilizers. However, inorganic fertilizer was the most effective in growing this species both on the field and in the glasshouse (Chapter 6).
- The effect of fertilizers on the mineral uptake in *C. gynandra* indicated that soil amendments increased the concentrations of micro and macro minerals in the leaves of the plant. The concentration of minerals in the species was observed to be higher on the field than in the glasshouse. This could be attributed to some factors such as high amounts of soil organic matter on the field, adequate sunlight for photosynthetic

activities and larger surface area for root expansion (Chapter 7).

- The proximate parameters analysed showed variations in the nutritional composition of *C. gynandra*. Inorganic fertilizer influenced more proximate constituents when compared to the organic and unfertilized (control) treatments. However, plant age has significant effect on the nutritional composition in the species. The highest concentration of protein, vitamin C and crude fibre were observed to be better in the 2^{nd}, 6^{th} and 8^{th} weeks after transplanting both on the field and in the glasshouse. Thus, *C. gynandra* can contribute significantly to the dietary requirements of the people at different growth stages of the plant (Chapter 8).
- Cultivated *C. gynandra* is highly nutritious when compared with the species growing naturally in the wild (Chapter 3).

These findings have enhanced our knowledge of the nutritive and therapeutic value of *C. gynandra* in the Eastern Cape Province, South Africa.