

ECOLOGICAL EFFECTS OF SELECTED INVASIVE PLANTS AND THEIR NATURE BASED MANAGEMENT APPROACHES

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ABSTRACT

Biological invasions are one of the major threats to most ecosystems as they often suppress native plant species. This suppressive effect is usually costly and irreversible hence calling for effective management strategies that are ecologically safe and appropriate at local level. This research work aimed at assessing the ecological effects of the invasive weeds *Tagetes minuta* and *Gutenbergia cordifolia* on native vascular plants and development of the nature-based management solutions for the two invasives. To understand if *T. minuta* and *G. cordifolia* have any effects on native plant communities and soil chemistry, the study compared native vascular plant species abundance, richness, cover, height and soil chemistry of the Ngorongoro ecosystem by sampling systematically along a continuum of invasion gradient from uninvaded to highly invaded areas in the Ngorongoro crater. It further identified the most dominant co-existing native vascular plant in the field (*Cynodon dactylon*) that can be facilitated to out-compete the two weeds. The results indicated that the two invasive weeds decreased native species abundance, ground cover and richness while increasing native vegetation height. Soil available Phosphorus, Nitrogen, and Cation Exchange Capacity levels were also influenced by invasions of either and/or both *T. minuta* and *G. cordifolia*. The competitive effects of the observed most dominant native vascular plant (*Cynodon dactylon*) on the growth and development of the two weeds was tested experimentally in both screen house and field plots using a completely randomized and randomized block design respectively in order to identify the most suppressive *C. dactylon* density. It was observed that increasing densities of *C. dactylon* strongly reduced the growth and development of *T. minuta* and *G. cordifolia*. The allelopathic experiments were done in both laboratory and screen house to identify the effective *Desmodium* root/ leaf extract concentration that can impair the growth and germination of *T. minuta* and *G. cordifolia* as a second management approach. Generally, seedlings treated with higher *D. uncinatum* leaf extracts ($\geq 62.5\%$) were half as tall, had one-third the weight and half the leaf chlorophyll content compared to those treated with lower concentrations. Based on the field, screen house and laboratory experiments, the suppressive effects of the two invasives are evident hence necessitating their management. Although needs to be scaled up in the field, this study has devised and tested two novel nature-based management approaches that have proven to be functional.

DECLARATION

I, **ISSAKWISA BERNARD NGONDYA** do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

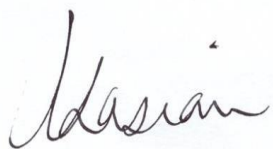


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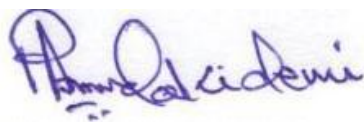
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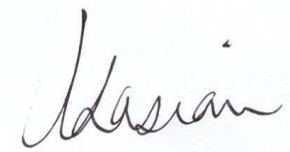
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CERTIFICATION

The undersigned certify that they have read and hereby recommend the dissertation entitled **Ecological effects of selected invasive plants and their nature based management approaches** as a fulfillment of the requirements for the Degree of Doctor of Philosophy in Life Science and Bioengineering (LISBE) at Nelson Mandela African Institution of Science and Technology (NM-AIST).



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DEDICATION

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LIST OF ABBREVIATIONS

GDP - Growth Domestic Product

CBD - Convention on Biological Diversity

UN - United Nations

EIS - Exotic Invasive Species

USAID - United States Agency for International Development

UNESCO - United Nations Educational, Scientific and Cultural Organization

FAO- United Nations Food and Agriculture Organization

IPM - Integrated Pest Management

PRs - Protected Rangelands

NCAA - Ngorongoro Conservation Area Authority

NPK - Nitrogen, Phosphorus, Potassium

Mg - Magnesium

Ca - Calcium

Cu - Copper

Fe- Ferrous / Iron

Zn - Zinc

Mn- Manganese

OC - Organic Carbon

CEC - Cation Exchange Capacity

SNP - Serengeti National Park

CNRIT - Center for Natural Resource Information Technology

ANOVA - Analysis of Variance

Fisher' LSD - Fisher' Least Significant Difference

DuL - *Desmodium uncinatum* leaf extract

DuR - *Desmodium uncinatum* root extract

DiL - *Desmodium intortum* leaf extract

DiR - *Desmodium intortum* root extract

LITI - Livestock Training Institute

DMSO- Dimethyl Sulfoxide

UV/Vis - Ultraviolet visible

ATP- Adenosine triphosphate

NADPH - Nicotinamide Adenine Dinucleotide Phosphate

CDFA- California Department of Food and Agriculture

TPRI -Tropical Pesticides Research Institute

GoT - Government of Tanzania

NM-AIST - Nelson Mandela African Institution of Science and Technology

EIA - Environmental Impact Assessment

CHAPTER ONE

This chapter describe an over view of invasive plants. It mainly focuses on the mechanism of plant invasion, effects of invasive plants on ecosystem, invasive plants and the savanna rangelands, the status of invasive plants in Tanzania, management approaches and challenges, selected invasive plants of the Ngorongoro crater, the way forward for managing invasive plants, problem statement and justification, objectives and research questions of the study.

An over view of invasive plants¹

Abstract

Invasive plants have been exerting negative pressure on native vascular plants and hence devastating the stability and productivity of the receiving ecosystem. They have also been reported to affect soil characteristics therefore further jeopardizing ecosystem sustainability. The effects are usually irreversible if appropriate strategies cannot be taken immediately after invasion, resulting in high cost of managing them both in rangelands and farmlands. With time, the invasive weeds will result in a decreased grazing or browsing area and can lead to population reduction of native plants and animals due to decreased food availability. Management of invasive plants has been challenging over the years as a result of increasing failure of chemical control methods due to evolution of chemical resistant weeds, higher cost of using chemical herbicide and their effects on the environment. Moreover the application of chemical herbicides to some areas for instance in protected areas (PRs) is challenging due to their unpredicted effects on native plants and environment. While traditional methods such as timely uprooting and cutting present an alternative for sustainable invasive plants management they have been associated with promotion of germination of undesired weeds due to soil disturbance. Plant-plant competition and allelopathy therefore, as natural plant life phenomenon presents an opportunity for successful invasive plants management.

Key words: competition, allelopathy, weeds, IPM, control, mowing, abundance, exotic plant

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1.1 General introduction

Invasion of ecosystems by invasive plants are one of the major threats to most ecosystems (Sheil, 2001). Dispersal of invasive plants has been associated with an increased human population and movement patterns (Koutika *et al.*, 2011). While the awareness has increased on the effects of both native and exotic invasive weeds (Gordon, 1998; Hansen and Müller, 2009) there has also been an increasing response by countries in adopting national legislation relevant to the prevention and / or control of invasive alien weeds (McGeoch *et al.*, 2010). It is through this awareness that invasive plants as a subject was included in the Convention on Biological Diversity (CBD), a multilateral treaty of 196 parties with the main goals of conserving biodiversity, sustainable use of its components and fair and equitable sharing of benefits arising from genetic resources. Under Article 8(h), parties of the Convention agree to “prevent the introduction of, eradicate or control those species which threaten species, habitats or ecosystems” (CBD, 1992). However, plant invasive species are still causing serious problems in the ecosystems and ecologists and other stakeholders are posed with challenges of developing appropriate management strategies. This literature review therefore highlights on the mechanism of plant invasion, effects of invasive plants on ecosystem, invasive plants and the savanna rangelands, the status of invasive plants in Tanzania and in the Ngorongoro Conservation Area (NCA), invasive plants management legislations and policies in Tanzania, management approaches and challenges, selected invasive plants of the Ngorongoro crater and the way forward for managing invasive plants.

1.2 Literature review

1.2.1 Mechanism of plant invasion

An invasive plant can either be exotic (a plant living outside its native distribution range) or native to the area. While invasion process of exotic invasive plants comprises of three stages; introduction from a donor region, establishment in the recipient region and range expansion in the recipient region (Simberloff, 2013; Ehrlich, 1986), that of native invasive plants has mainly been looked at from an expansion stage following favorable conditions. More than *ca.* 3000 species, however, are potentially introduced from donor to recipient regions every day by vessels sailing across oceans (Carlton, 1996). Although most introductions of invasive plants are linked

to human activities, some cross barriers and establish in new regions without the help of humans (Ehrlich, 1986). According to Williamson and Fitter (1996), only about 0.1% of all plant species introduced outside their native ranges by humans have actually become invasive. The success of an invasive species can be linked to factors such as low intensities of competition, altered disturbance regimes and low levels of environmental stress, especially high resource availability (Alpert *et al.*, 2000).

1.2.2 Effects of invasive plants on ecosystem

Over decades the effects of invasive plants on an ecosystem have been mainly on ecosystem processes, functioning and ecosystem services. Ecological processes at an ecosystem level normally influence plant and animal productivity that ultimately affects the whole ecosystem functioning. These processes are always linked to the identity of a plant, and or animal forming the community (Naeem *et al.*, 1999). Several studies have attempted to address the effects of invasive species on ecosystem processes (Levine *et al.*, 2003; Dukes and Mooney, 2004). Invasive weeds have the ability to change plant community composition by suppressing the abundance of indigenous species (Vasquez *et al.*, 2008). This suppression often results into local extinction of some native keystone species and a formation of monocultures, which are responsible for further extinction of native plants (Martina and Von Ende, 2008; Cal-IPC, 2016). Local extinction of a particular keystone species normally impacts ecosystem functioning (Mills *et al.*, 1993). Invasion of exotic *Prosopis* spp in the Kalahari Desert for instance, has been associated with increased mortality of a keystone tree species, *Acacia erioloba*. This has further affected bird and insect richness and composition (Shackleton *et al.*, 2015) with the net effect of impaired ecosystem functioning i.e. pollination and dispersal reduction.

Likewise, costs generated by invasive plants on ecosystem processes and functions negatively affect ecosystem services. Ecosystem services are the benefits provided to humans by natural ecosystems (Charles and Dukes, 2008). An exotic invasive weed, Neem tree (*Azadirachta indica*) that has been reported in Saadani National Park (Tanzania) for instance is a threat to native species (Dos Santos and Kiwango, 2010), which eventually reduce income from eco-tourism in Saadani National Park. Similarly, exotic *Eucalyptus* spp plantations have been reported to affect both ground and surface water availability (Bilal *et al.*, 2014), resulting in reduced water

availability for both, household and irrigation. Any modification caused by exotic invasive plants to the plant community, therefore, can impair ecosystems in various ways. Unfortunately, losses of ecosystem processes, functions and services caused by invasive plants are often overlooked.

1.2.3 Invasive plants and the Savanna rangelands

A savanna rangeland is an ecosystem characterized by continuous grass vegetation and scattered trees or shrubs (McPherson, 1997). Savannas occupy nearly a third of the earth's land surface (McPherson, 1997), therefore representing an important resource for the management and conservation of biodiversity. Savannas provide grazing areas to many grazer species (Mott and Groves, 1994) due to their abundance of palatable grasses and shrubs, thus, representing important niches that support a large number of animals including predators. Due to increased grazing pressure, which contributes to removal of possible native weed competitors, most of the savannas have undergone changes in pasture composition by invasive weeds, which are mostly not palatable to grazers (Winter, 1991). Invasive weeds in the savanna ecosystem have been associated with altering the fire regime, increasing grazing pressure on remaining native grasses, competing with native vegetation and occupying previously vacant ecological niches (Scanlan, 1998). In the long run, this can significantly alter the structure, composition and functioning of savannas. The net effect of shifts in native palatable plant composition by unpalatable weeds will, therefore, seriously affect both livestock and wildlife biomass in savanna systems.

1.2.4 The status of invasive plants in Tanzanian rangelands

Little work has been done to document the status and effects of invasive plants in Tanzanian protected areas (Elisante and Patrick, 2014; Namkeleja *et al.*, 2014b) and most research has focused on woody exotic invasive weeds in some selected rangelands (Foxcroft *et al.*, 2006; NCAA, 2011; Obiri, 2011), mainly because of their structural importance. Major invasive weeds affecting most Tanzanian rangelands include woody plants; *Prosopis juliflora*, *Acacia mearnsii*, *Caesalpinia decapetala*, *Eucalyptus camaldulensis*, *Lonicera japonica*, *Psidium guajava*, *Senna spectabilis*, *Acacia farnesiana*, *Acacia polyacantha* (Obiri, 2011) shrubs; *Argemone mexicana*, *Tagetes minuta*, *Datura stramonium*, *Gutierrezia cordifolia*, *Lantana camara*, *Bidens schimperi*, *Opuntia monacantha*, *Opuntia stricta* (Mattay and Lotter, 2005; Foxcroft *et al.*, 2006). Aquatic invasive plants include; *Eichhornia crassipes*, *Typha latifolia*, *Ceratophyllum*

dermesum, *Rapa natas*, *Brasenia sp*, *Cyperus spp*, *Justicia spp*, *Pistia spp* (Ndunguru *et al.*, 2001; Güereña *et al.*, 2015). So far, little is known about invasive herbaceous species, and there is no adequate literature on this plant functional group and its effect on the ecosystems of Tanzania.

1.2.5 Invasive species management legislations and policies in Tanzania

Although Tanzania has many legislations that address various issues on biodiversity conservation and management, there is no legislation and/or policy that specifically address exotic invasive species. The National Environmental Act 2004 (NEMA), which is the main act pertaining to environmental protection, has to some extent managed to address the issue of management and control of invasive weeds, particularly exotic invasives. For instance, sections 1 (a-b) and 2 (a-c) of this act although not directly mentioning invasive species, has strongly recommended an Environmental Impact Assessment (EIA) for projects that are likely to affect ecosystem functioning. Section 12 (f), further insists of the undertaking of EIA for projects in order to protect the productivity, capacity, ecological processes and their maintenance in natural systems. The first schedule (A; page 28) highlights projects that require mandatory EIA, among which are those that require introduction of new/foreign species in agriculture, range management, forestry, fisheries and wildlife. Likewise, both the Wildlife Conservation Act 2009 (Section 35 (3) (b)) and Forest Act 2002 (section 18(2) (c)) insists on undertaking of an environmental impact assessment prior to construction of either road or pipelines in protected areas as these might have negative impacts to native plants including introduction and spread of invasive plants. The main weakness of most Tanzania's biodiversity protection acts regarding invasive species control is that they do not directly mention aspects like procedures for import of foreign species and penalties to be undertaken should there be an unlawful import of exotic invasive species. This shortfall may be responsible for the inadequate attention being given to the management of invasive species and the increasingly introduction and spread of invasive species.

1.2.6 The status of invasive plants in the Ngorongoro Conservation Area (NCA)

The Ngorongoro Conservation Area, together with the Serengeti, Lake Manyara and the Masai Mara National Parks form an ecologically and economically important area and hosts a vast

variety of larger mammalian species. Invasive weeds, however, have been identified as among the main known threats to the biological diversity of these areas (NCAA, 2011; Elisante and Patrick, 2014; Namkeleja *et al.*, 2014b). A total of 139 exotic invasive weeds for instance, have been reported in and around NCA alone, they include *Acacia mearnsii*, *Datura stramonium*, *Lantana camara*, *Leucaena leucocephala*, *Lonicera japonica*, *Tagetes minuta* (Plate 1) etc., (NCAA, 2011). Fortunately, most of these species are still at low numbers, although for some, eradication might already have become impossible (Mattay and Lotter, 2005). Some indigenous invasive plants have also been reported, among them two invasive plant species *Bidens schimperii* and *Gutenbergia cordifolia* (Plate 1), the latter covering approximately 75% of the crater floor (Mattay and Lotter, 2005). As most of these plant species are not palatable, they pose a risk of reducing native plant biomass and, hence, reducing pasture availability for ungulates, which form a large portion of animals in the crater. This might affect herbivore biomass in the long run.



Plate 1: *Tagetes minuta* (with yellow flowers) and *G. cordifolia* (brownish and dry) in the Ngorongoro crater (source: Field survey)

1.2.7 Invasive plant management approaches

Since as early as 19th century, weed management in both farmlands and rangelands have been mainly through chemical herbicides (Bell, 2015). Chemical control of invasive weeds has become a normal practice, which has been associated with a consecutive evolution of resistant weeds and, hence, further proliferated the problem of weed control (Holt and Lebaron, 1990).

Weed resistance to chemical herbicides is becoming a serious and increasingly challenging issue as fueled by heavy reliance on chemical herbicides. Likewise, rising concerns on environmental safety and cost associated with chemical herbicides have made people re-think on the use of chemical herbicides. Although chemicals still manage most weeds other management strategies needs to be adopted to reduce the increasingly weed resistance, environmental pollution and chemical herbicides associated costs.

Traditional methods of weeds control have been reported to include mechanical uprooting, cultivation and burning (FAO, 1982; Altieri and Liebman, 1988). Prior to late 1800's only mechanical control of weeds was used in agriculture (Bell, 2015) but for over years, weed control mechanisms such as biological control and Integrated Pest Management (IPM) have been recommended to complement the traditional control methods for improved performance (Altieri, 1994). Among the goals of biological control of weeds are to improve ecosystems by using living organisms and to manage target weedy plants to lesser competitive intensities so that they do not stress native plant communities or cause damage to livestock (Quimby *et al.*, 1991). According to Quimby *et al.* (1991), livestock's differential grazing habits, preferences and selectiveness for instance, as a biological control, allows for selective grazing pressure on palatable problematic weeds which might result into effective control. While in PRs, maintenance of biodiversity through ecological friendly methods is crucial, IPM might represent an important sustainable weed control tool. In IPM, re-introduction of ecological processes with which species evolved and elimination of processes detrimental to native species has to be ensured (Esparza and McPherson, 2009).

In the NCA the management of invasive weeds has mainly been mechanical removal. In 2000, the first NCA control programme started through mechanical uprooting and cutting invasive weed species at the base (NCAA, 2011). Later in 2001, mowing followed by burning was introduced as a mechanism to facilitate the removal of the indigenous invasive weeds *Bidens schimperi* and *Gutenbergia cordifolia*, accompanied by some trial plots to monitor its effectiveness. Since then, a programme is in place, in which mowing followed by burning or vice versa has been practiced (NCAA, 2011). However, evidence from trial plots suggested that mowing followed by burning or vice versa may not be the most effective control mechanism. Chemical and biological control for invasive plants that pose the biggest threat to NCA have also

been suggested, which include the use of 1 % Tordon super (Picloram 120g/l and Triclopyr 240g/l), 100ml Tordon super in 9.9 liters of diesel (NCAA, 2011). Further, biological control suggestions include using seed feeders like *Melanterius maculates* to control *Acacia mearnsii* (NCAA, 2011). Unfortunately, the current approaches to managing invasive weeds in NCAA seem to be costly, ineffective and unsustainable and that environmentally friendly and nature based approaches for weed control such as plant-plant competition and allelopathy have not yet been tried in NCA.

1.2.8 Invasive plant management challenges

Weed chemical control method has proven to fail both in its final efficiency and economically (O'rourke *et al.*, 1975). Further, chemical herbicides are mostly expensive and can cause bio-accumulation, soil and water pollution (MG van der Werf, 1996), especially when applied to protected rangelands (PRs). Likewise, mechanical control of invasive weeds is costly and time intensive (NCAA, 2011). Further, any soil disturbances that arise from mechanical control promotes germination of undesired weed (Calado *et al.*, 2013) while desired indigenous species may be mistakenly destroyed. The most imminent shortfall of mechanical control of weeds is that, most invasive seeds remains in the soil, hence, posing a risk of future invasions (Personal observation). One of the major challenge over biological control of weeds have been a rising concern on the potential damage to both threatened and endangered native forage plants closely related to a targeted weedy plant (Turner, 1985 in Quimby *et al.*, 1991). Although to date there is no known intentional introduction of weed biological control agent that has caused harm to any known endangered plant (Quimby *et al.*, 1991), the potential is imminent (Turner, 1985 in Quimby *et al.*, 1991). Therefore, care must be observed to introduce biological agents that will provide the greatest benefit with the least risk of harming the receiving community.

1.3 Selected focal invasive plant species of the Ngorongoro Crater

1.3.1. *Tagetes minuta* (Mexican marigold)

The genus *Tagetes* of the family Asteraceae comprises of 56 species that include popular annual plants known as marigolds (Soule, 1993a). Of the 56 species, 29 and 27 are perennials and annuals respectively (Soule, 1993a). While 6 of the 27 annual *Tagetes* are currently cultivated,

only 3 of the 29 perennial *Tagetes* are currently cultivated as horticultural crops (Soule, 1993a). Most of *Tagetes* are native to Mexico (Soule, 1993b; Hulina, 2008) and are cultivated throughout the world as ornamentals, medicinal and as plants for ritual (Nuttall, 1920). While *Tagetes erecta* L., *Tagetes patula* L., *Tagetes lunulata* Ort. and *Tagetes tenuifolia* Cav are four annual *Tagetes* that are commonly cultivated throughout the world as ornamentals (Soule, 1996), *Tagetes filifolia* Lag. and *Tagetes minuta* L. are annuals currently used for essential oil extraction (Lawrence, 1985). These plants are to be used with care as most of them contain strong scented secondary compounds, which might have unpredicted effects to ecosystems. Unfortunately, the abundant annual and perennial *Tagetes* yet to be utilized as horticultural crops if not well managed could escape and become problematic weeds.

Tagetes minuta (Plate 2) has escaped cultivation in most nations and is considered a noxious weed in parts of southern Africa (Wells, 1986) and recently reported as among problematic weeds that have invaded the Ngorongoro Conservation Area (NCAA, 2011). Its seeds produce some secondary products which are used for defense against herbivores and possess hooks that ensures its dispersal (Martinez-Ghersa *et al.*, 2000). This species has been introduced into various areas to the extent that it became a weed in most rangelands and farmlands of Tanzania (USAID-Tanzania, 2012). According to Meissner and Ruth (1986), *T. minuta* roots exudates contain a polyacetylene derivative (thiophene) which delays germination and reduces the yield of crops grown in soil previously infested with the species. Thiophene compounds (Plate 3) have extensive use in both pharmaceuticals and agro-chemicals (Swanston, 2006). They have been proven to kill root knot nematodes (Winoto-Suatmadji, 1969) and hence can possibly affect the growth and development of leguminous pastures that are crucial for improving soil nitrogen. This not only pose a risk of reduced pasture to Ngorongoro crater's herbivores as the weed suppress native pastures but also affect soil available Nitrogen (Chapter three).



Plate 2: *Tagetes minuta* (left) *Gutenbergia cordifolia* (right) (source: screen house experiment).

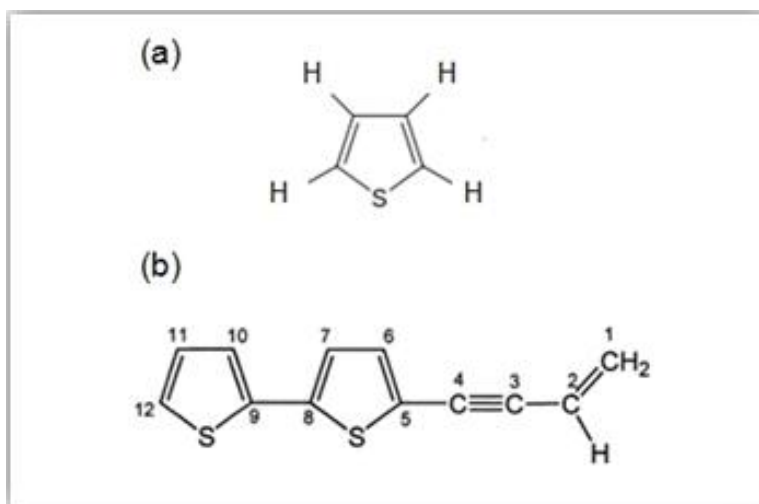


Plate 3: (a) Chemical structure of thiophene ring (b) Chemical structure of 5-(3-buten-1-ynyl)-2, 20-bithiophene (BBT, 1) common in *Tagetes* species (Margl *et al.*, 2001).

1.3.2. *Gutenbergia cordifolia*

Gutenbergia cordifolia (Plate 2) is an annual plant of the family Asteraceae native to Africa. Its leaves and flowers are allergenic and toxic to animals as they contain a chemical sesquiterpene lactone (Zdero and Bohlmann, 1990; Bussmann *et al.*, 2006) (Plate 4). According to Amorim *et al.* (2013), the sesquiterpene lactone alters the microbial composition of the rumen and its overall metabolic functioning. *Gutenbergia cordifolia* has been used extensively for medicinal purposes

(Koch *et al.*, 2005; Ngezahayo *et al.*, 2015), which has led to introduction to various areas to the extent that it might become a weed in most rangelands. In East Africa for instance, particularly in Kenya, the plant has already been reported as an invasive weed in most farmlands (Anderson and Morales, 2005; Gharabadiyan *et al.*, 2012). In Tanzania, the plant is invading and dominating large areas in Ngorongoro Conservation Area (NCA). Recently, the species seems to have invaded and dominated more than half of the entire crater floor (250 km²) (UNESCO, 2001).

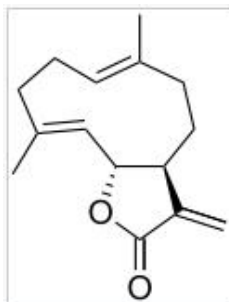


Plate 4: Chemical structure of costunolide a prototypical sesquiterpene lactone (germacranolides) (Fraga, 1988)

1.4 The way forward: Nature-based solutions for managing invasive plants

The use of plant species, particularly those with allelopathic effects, has recently been emphasized as an effective method to suppress invasive weeds (Makoi and Ndakidemi, 2012). Interestingly, plant species like *Desmodium uncinatum* and *Desmodium intortum* have been used to successfully control most weeds in farmlands due to their allelopathic nature and, hence, were able to increase crop yield (Khan *et al.*, 2006; Khanh *et al.*, 2007; Khan *et al.*, 2008; Makoi and Ndakidemi, 2012). Further, *Desmodium* species have been tested and found to be of high agronomic value in a rangeland by having high nutritive value for lactating herbivores (Kimitei, 2015). Moreover, plant-plant competition, being among the factors that shape the plant community, allows for the use of strong competitors that are also palatable to animals as an approach for weeds management (Tilman *et al.*, 1999; Rey Benayas *et al.*, 2007; Makoi and Ndakidemi, 2012; Goss and Wheeler, 2017).

1.4.1 Allelopathic effects of crop plants on invasive plants

Weed-crops interactions have been observed over a long time in agroecosystems. It was observed by Peters (1968), that fields of Kentucky-31 fescue (*Festuca arundinaceae* Schreb.)

were oftenly free of weeds *Brassica nigra* (L.) Koch and *Lotus corniculatus*. With extracts and culture experiments it was later demonstrated that fescue produced toxic materials that inhibited the growth of the two weeds. Dzubenko and Petrenko (1971) reported that root excretions of *Lupinus albus* L. and of Maize (*Zea mays*) inhibited growth of weeds; *Chenopodium album* and *Amaranthus retroflexus*. It was further reported by Prutenskaya (1974) that wheat (*Triticum durum* Desf.), rye and barley strongly inhibits the growth of weedy *Sinapis arvensis* L. Recently, *Desmodium* species as intercrops in maize and sorghum have been reported to successfully inhibit the growth of a noxious weed *Striga hermonthica* (Khan *et al.*, 2008).

1.4.2 Possible use of allelopathy as biological control for invasive plants

The possible use of allelopathy as a natural phenomenon for the control of noxious weeds is increasingly promising (Ndakidemi and Dakora, 2003; Makoi and Patrick, 2011). Putnam and Duke (1974) after screening 526 and 12 accessions of cucumber (*Cumis sativus*) and eight related *Cucumis* species respectively concluded that inducing an allelopathic character into a crop cultivar can improve its competitive advantage over certain noxiuos weeds. Similarly, Fay and Duke (1977) concluded after screening some accessions of *Avena* species that weedy Wild mustard (*Brassica kaber* (DC) L.C. Wheeler var. *Pinnatifida* (Stokes) L.C. Wheeler) grown in close association with the toxic accession exhibited severe chlosis, were stunted and twisting as a result of allelopathy reather than competition. Robson (1977) further suggested that allelopathy could be used as an excellent means of biological control of water weeds. Unfortunately, to-date little have been done on the application of extracts from allelopathic plants to control such problematic weeds as *T. minuta* and *G. cordifolia* despite of few available promising studies on genetic study of allelopathic agents (Panchuk and Prutenskaya, 1973; Grodzinsky and Panchuk, 1974).

1.4.3 Allelopathic effects of *Desmodium* species on invasive plants

Plants of the genus *Desmodium* (Leguminosae) have long been used in traditional Chinese medicine due to their rich possession of phytochemicals (Ma *et al.*, 2011). According to Ma *et al.* (2011), a total of 212 compounds have been isolated from fifteen *Desmodium* species (including *Desmodium uncinatum*), which were characterized mainly as flavonoids (81) and alkaloids (40). The main flavonoids contained in *Desmodium* plants are: flavones, 7,8 prenyl-lactone flavonoids,

flavonols, flavan-3-ols and flavanonols whereas isoflavonoids include isoflavones, isoflavanones, pterocarpans and coumaronochromone (Ma *et al.*, 2011).

Root exudates of *D. uncinatum* have been reported to contain flavonoids, some of which promotes *Striga hermonthica* (African witch-weed) germination while others inhibit seedling development including radical growth (Khan *et al.*, 2008). *Desmodium* species have also been used to control *S. hermonthica* successfully as intercrops in both maize and sorghum. According to Khan *et al.* (2008), *Desmodium* based intercropping represents a practical example of allelopathy at work as more than 10,000 small-scale farmers in Eastern Africa have adopted its use. This, therefore, opens a door for further studies on the use of *Desmodium* species, particularly *D. uncinatum* and *D. intortum* (Plate 5), as potential bio-herbicides in controlling other problematic weeds such as *T. minuta* and *G. cordifolia*.



Plate 5: *Desmodium uncinatum* (left) and *Desmodium intortum* (right) (source: Field survey).

1.4.4 Plant-Plant competition for invasive plant control

Plant-plant competition has received a lot of attention in ecological research (Connell and Slatyer, 1977; Keddy, 1989; Goldberg and Barton, 1992) and was found to directly affect the local distribution of plants in a community (Stoll and Prati, 2001). Plant-Plant competition has well been demonstrated in a range of ecosystems; most vividly in ecosystems where native plants have been exposed to several stresses, for instance water shortage, soil nutrient deprivation and ecological invasion (Daehler, 2003).

As a cheaper and low impact management technique, plant-plant competition has been reported to be effective in restoration projects, for instance in restoration of *Quercus coccoifera* oak forests where competition had a strong impact on oak recruitment (Rey Benayas *et al.*, 2007). Similarly Goss and Wheeler (2017) recommended on the use of most competitive varieties of barley and wheat as an integral part of the integrated weed management strategies and should be considered when planning for weed management. Tilman *et al.* (1999) further insisted that adjustments in resource supply rates is likely to determine the outcome of inter-specific competition thereby allowing desired species to competitively manage weedy species. Therefore, identification and facilitation of the most competitive native plants that can out-compete invasive weeds might present an opportunity for the development of management strategies for some problematic weeds such as *Tagetes minuta* and *Gutenbergia cordifolia*.

1.5 Problem statement and justification

Over decades invasive weeds have been reported to exert negative pressure on native vascular plants (Aguilera *et al.*, 2010; Gichua *et al.*, 2014) and hence devastating the stability and productivity of the receiving ecosystem (Vasquez *et al.*, 2008). They have also been reported to affect soil characteristics therefore further jeopardizing ecosystem sustainability (Strayer *et al.*, 2006; Weidenhamer and Callaway, 2010). These effects are usually irreversible if appropriate strategies cannot be taken immediately after invasion, resulting in high cost of managing them (Pimentel *et al.*, 2005) both in rangelands and farmlands. While it is generally reported that invasive weeds have ecological effects on native plants; less have been studied to prove that invasive weeds *T. minuta* and *G. cordifolia* really have ecological effects on native vascular plants.

Management of invasive weeds has been challenging as a result of increasingly failure of chemical control as a method (O'rourke *et al.*, 1975), due to evolution of resistant weeds (Holt and Lebaron, 1990), higher cost of using chemical herbicide and their effects on the environment (MG van der Werf, 1996). Moreover the application of chemical herbicides to some areas for instance in protected rangelands (PRs) is challenging due to their unpredicted effects on native plants and environment (Kimmins, 1975; Poorter, 2007; Kughur, 2012). While traditional methods such as timely uprooting and cutting presents an alternative for sustainable invasive

weeds management they have been associated with promotion of germination of undesired weeds (Calado *et al.*, 2013) due to soil disturbance. They are also ineffective due to higher costs involved (Personal communication) and thus proving to be unsustainable especially in the face of decline in global economy.

While plant competitive interactions as a low cost and low impact management technique has been recommended for most restoration projects (Tilman *et al.* 1999; Rey Benayas *et al.*, 2007; Goss and Wheeler, 2017) this technique has been less studied. This study, therefore, evaluates the ecological effects of invasive weeds *Tagetes minuta* and *Gutenbergia cordifolia* and explores the competitiveness of native *Cynodon dactylon* species and the allelopathic potential of *Desmodium uncinatum* and *Desmodium intortum* as two nature based approaches for managing the two invasive weeds. The approaches are not only environmentally friendly, cheaper and effective, but they also add value on ecosystem services as both *C. dactylon* and *Desmodium* species are plants of high agronomic value (Heuze *et al.*, 2015; Tropical-forages, 2017).

1.6 Research objectives

1.6.1 General objective

The general objective of this research was to assess the ecological effects of invasive weeds *T. minuta* and *G. cordifolia* and to devise ecological friendly methods for managing the two weeds in Tanzanian rangelands.

1.6.2 Specific objectives

- i. To assess the ecological effects of the invasive plants *T. minuta* and *G. cordifolia* and to identify the most co-existing native plant in the NCA.
- ii. To determine soil chemical properties along invasion gradient of *T. minuta* and or *G. cordifolia* in the NCA
- iii. To determine the competitiveness of a native species *Cynodon dactylon* (the most abundant coexisting species at NCA) grown at different densities against invasive species *T. minuta* and *G. cordifolia*

- iv. To determine the allelopathic effect of *D. uncinatum* and *D. intortum* root and leaf crude extracts on the germination and growth of *T. minuta* and *G. cordifolia*

1.6.3 Research questions

This research work answered the following questions:

- i. How native plant species composition and structure differ along invasion gradient of *T. minuta* and or *G. cordifolia*? and which native species co-exist well with the two invasive weeds in the NCA?
- ii. How soil chemical properties differ along invasion gradient of *T. minuta* and or *G. cordifolia* in the NCA?
- iii. What density of *C. dactylon* is most effective in suppressing *T. minuta* and or *G. cordifolia* invasive plants?
- iv. Under which concentration of *D. uncinatum* and *D. intortum* root and leaf crude extracts is the allelopathic effect most suppressive on the germination and growth of *T. minuta* and *G. cordifolia*?

CHAPTER TWO

*This chapter assessed the ecological effects of invasive weeds *Tagetes minuta* and *Gutenbergia cordifolia* on native plants composition and structure. It further identified the most co-existing native plant that can be facilitated to out-compete the two weeds as a management strategy. The chapter indicated that the two invasive weeds reduced native plants richness, abundance and cover while promoting their height. It further reports a native grass *Cynodon dactylon* as the most co-existing native grass.*

Ecological effects of invasives *Tagetes minuta* L and *Gutenbergia cordifolia* on native plant communities²

Abstract

Understanding the ecological influence of invasive weed species on their native counterparts is important for restoring and managing rangelands sustainably. The study sampled along a continuum of invasion gradient from uninvaded to highly invaded areas to assess the effect of the two invasive plants (*Tagetes minuta* and *Gutenbergia cordifolia*) on native vascular plant species composition and structure in the Ngorongoro Crater, Tanzania. Relative native species abundance decreased along both *T. minuta* and *G. cordifolia* invasion gradients. Native species ground cover was double in uninvaded quadrats than that of moderately and highly invaded quadrats. Average native vegetation height, in contrast, was twice as high in highly invaded areas compared to uninvaded areas. Species richness was three times higher in both *T. minuta* and *G. cordifolia* uninvaded quadrats compared to moderately and highly invaded quadrats. The results highlights how these invasive weeds drastically changed the abundance and richness of the native vascular plant community in a highly protected area. This study further generated a baseline information for long term research to elucidate mechanisms associated with these two invasive plants interactions, while at the same time informing the management authorities on the threats to natives associated with plant invasion.

Keywords: *daisy, herbivore, invasion, marigold, rangeland, weed*

² Under review: *Acta Oecologica*.

2.1 Introduction

Invasive species have recently caught the attention of many ecologists (Moussa *et al.*, 2012; Biware *et al.*, 2013), particularly their effects on native vascular plants. They have been declared as one of the biggest threats to biodiversity (Gichua *et al.*, 2014). These species have been reported not only to affect ecosystems through suppression of local species and, hence, resulting in the extinction of the latter (Clavero and Garcia-Berthou, 2005) but also by altering ecological processes and, therefore, reducing overall ecosystem yields (Vasquez *et al.*, 2008). As human population and people's movement increases, so does dispersal of invasive plants such as *Gutenbergia cordifolia* and *Tagetes minuta* from one locality to another (Boy and Witt, 2013).

Gutenbergia cordifolia and *Tagetes minuta* have been reported as invasive weed species across the tropics, subtropics and several temperate countries (Hulina, 2008; NCAA, 2011; USAID-Tanzania, 2012). In Tanzania, these species have recently invaded the Serengeti National Park (SNP) and the Ngorongoro Conservation Area (NCA) (NCAA, 2011), likely impacting the native plant and herbivore communities in these highly renowned protected areas. Like other invasive species, *G. cordifolia* and *T. minuta* are not edible by animals (Hurst, 1942) and, hence, grow more leaves, roots and flowers than their native counterparts. They, therefore, may have an increased competitive ability to suppress the native plant species (Wolfe *et al.*, 2004). These two species, as most invasives, spread supposedly through a complex interaction of human and animal movement patterns (Dix *et al.*, 2010). However, their ecological effect on native vascular plants has never been quantified in the herbivore-rich NCA ecosystem.

Tagetes minuta has been reported to have a substantial ecological impact in the Serengeti National Park, adjacent to the Ngorongoro Conservation Area (Foxcroft *et al.*, 2013). *Tagetes minuta* can potentially reduce germination and growth of native species (Alhammadi, 2008; López *et al.*, 2008). While ecological impacts of *T. minuta* have been investigated to some extent, not much information is available on those of *G. cordifolia*. There is an urgent need to determine the ecological impacts of these two invasive species, particularly in a fragile ecosystem like NCA, where the survival of animals strongly depends on the vigor of native grasses that might be easily suppressed by the invasives. Identifying the ecological impacts of the two invasive plants will, therefore, contribute to potential intervention mechanisms for

management purposes. This study investigated the effects of these two invasive plant species on species abundance, diversity, richness, vegetation height and ground cover of the native vascular plants.

Invasive weed species can suppress the abundance, diversity and vigor of many native plant species (Aguilera *et al.*, 2010; Gichua *et al.*, 2014). On the other hand, they can encourage native species to invest more in their growth activities as a result of light competition, leading to taller individuals in invaded sites (Martinez-Ghersa *et al.*, 2000; Hejda *et al.*, 2009). The study therefore, hypothesized that invaded plots would have lower native plant species abundance, diversity, richness and percentage ground cover. Further, it was expected that native plants will on average be taller in plots invaded by *T. minuta* and *G. cordifolia* compared to uninvaded plots.

2.2. Material and methods

2.2.1 Study site

Ngorongoro Conservation Area (NCA) is located in the northern part of Tanzania (2°30'-3°30'S, 34°50'-35°55'E), bordering the Serengeti National Park to the West, Lake Eyasi to the South and the Loliondo Game Controlled Area and village lands in the South-East (Niboye, 2010). The NCA covers an area of 8,300 km² with the main crater descending to a depth of 610 m with a basin that covers an area of 260 km². The area receives seasonal rainfall with annual precipitation varying from 500 mm to 1700 mm on arid plains and forested slopes, respectively. Crater slopes are covered by montane forests while the crater floor is mainly grassland composed of various species including *Sporobolus* spp, *Themeda triandra* and *Cynodon dactylon* (Stuart, 2003). The area provides a habitat to over 30 African elephants (*Loxodonta africana*), 7,000 wildebeests (*Connochaetes taurinus*), 4,000 zebras (*Equus quagga*), 3,000 common elands (*Taurotragus oryx*), 4,000 African buffalos (*Syncerus caffer*), and 3,000 Grant's (*Nanger granti*) and Thomson's gazelles (*Eudorcas thomsonii*) (NCAA, 2011).

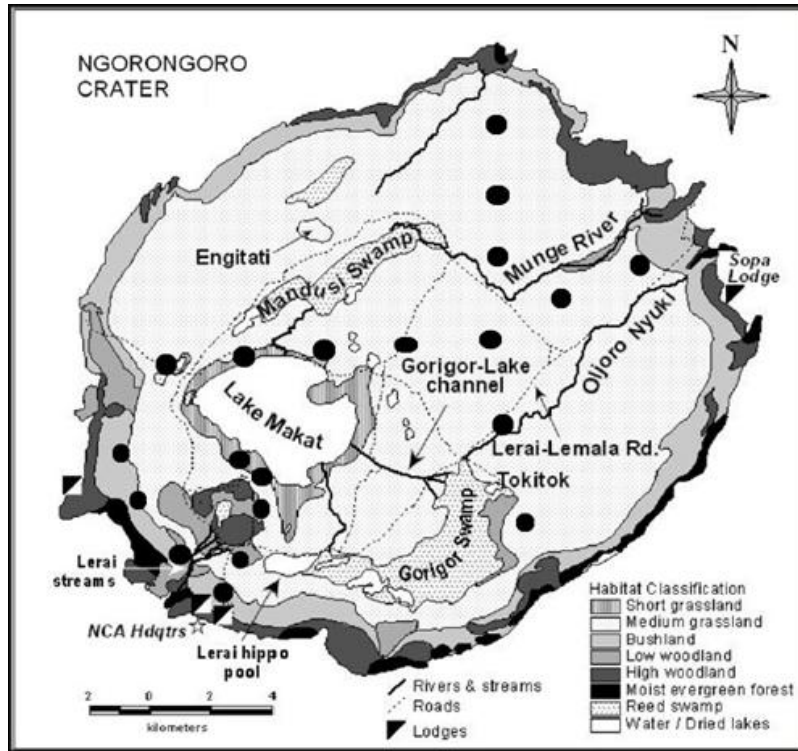


Fig. 1: A map of Ngorongoro Crater (Estes *et al.*, 2006). Circles indicate areas where transects and quadrats were laid.

2.2.2 Sampling design

Quadrats of 1m^2 were established along a continuum of the two species' invasion cover (Terera *et al.*, 2013). Invasion density was grouped based on invasive plant's percentage ground cover at a site (12 sites for each invasion category) into; "uninvaded" (0-24% invasion cover), "moderately invaded" (25-49% invasion cover) and "highly invaded" ($\geq 50\%$ invasion cover) with two replicates (72 quadrats for each species' invasion treatment). The quadrats were randomly laid out and placed at most 20 m apart from each other at a site (Fig.1). For *G. cordifolia*, which was mostly all over the crater floor; each site with six quadrats were placed after every 3 km along two crossing transects running from South to North (18 km long, 0.1 km wide) and East to West (21 km long, 0.1 km wide) of the Ngorongoro Crater. For *T. minuta*, that was covering most of the western parts and the crater rim; each site was placed after every 600 m and 300 m along a 3.6 km long, 0.1 km wide and 2.4 km long, 0.1 km wide transects respectively (Fig.1).

2.2.3 Data collection

The study assessed native vascular plant species richness, ground cover and vegetation height along invasion gradients of *G. cordifolia* and *T. minuta* from early July to late August, 2015. Each native vascular plant species was identified, recorded and counted after consensus by a team of three experts and confirmed identification using literature (Van Wyk and Van Oudtshoorn, 1999). Native vascular plants height was measured in each quadrat at four random points using a ruler. Invasive and native species percentage ground cover was visually estimated (CNRIT, 2014).

2.2.4 Statistical analysis

One way Analysis of Variance (ANOVA) was performed to compare native vegetation height while a Kruskal-Wallis test was performed to compare native species evenness and percentage ground cover along the invasion gradient for both *T. minuta* and *G. cordifolia* separately (Terera *et al.*, 2013). The percentage occurrence of each native grass was calculated to identify the native grass with the highest percentage occurrence along the invasion gradient. Chi-Square Goodness of Fit Test was performed on native species richness and abundance along the invasion gradient for both *T. minuta* and *G. cordifolia* separately. Correlation between the impact of *T. minuta* and *G. cordifolia* invasive plant species on native vascular plant species composition (expressed as Sorensen similarity between invaded and uninvaded plots in a pair) and native species richness were performed using Pearson's product-moment correlation analysis and Spearman's rank-order correlation analysis respectively. The statistical software used was STATISTICA version 8 (StatSoft-Inc., 2007) with the level of significance set at $\alpha < 0.05$.

2.3 Results

2.3.1. Ecological influences of *T. minuta* and *G. Cordifolia*: Differences in native plant species structure under invasion

The total number of native plant species in *T. minuta* uninvaded, moderately and highly invaded quadrats were 41, 21 and 13(S1) respectively while in *G. cordifolia* uninvaded, moderately and highly invaded quadrats were 49, 21 and 15(S2) respectively. Generally, mean native species richness per quadrat was found to be decreasing along the invasion gradient for both *T. minuta*

and *G. cordifolia* (Fig. 2). While species richness differed significantly along the invasion gradient under both *T. minuta* and *G. cordifolia* ($X^2 = 18$, $p = 0.0001$ and $X^2 = 24$, $p < 0.0001$ respectively), species abundance did not ($X^2 = 2.5$, $p = 0.2840$ and $X^2 = 0.2$, $p = 0.8897$, respectively). Uninvaded quadrats had three times higher mean native species richness per quadrat than invaded quadrats (Fig. 2).

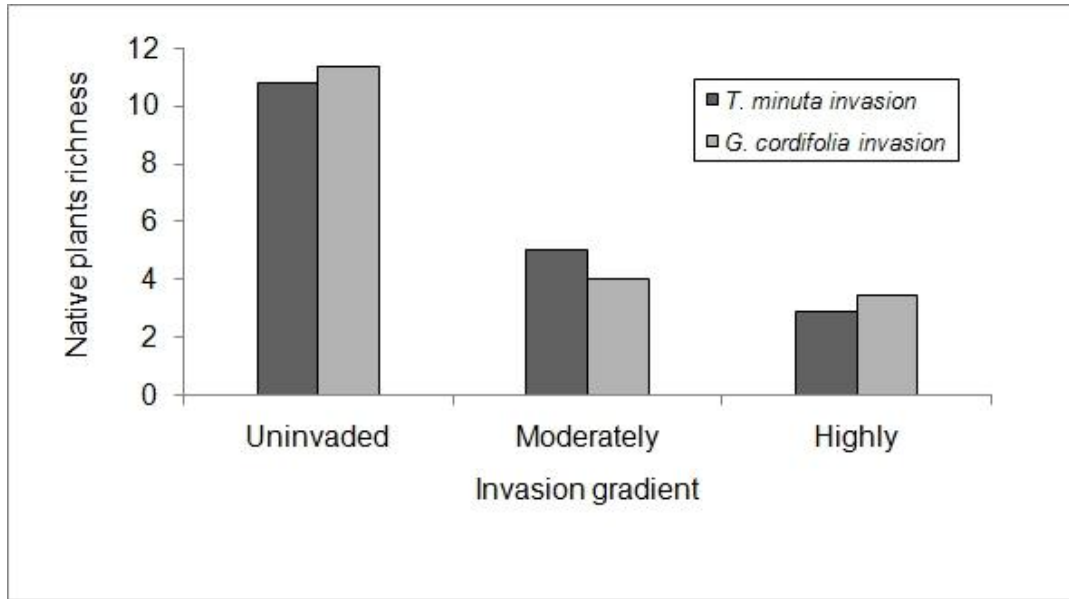


Fig. 2: Native species richness per quadrat under *T. minuta* and *G. cordifolia* invasions

Native vascular plant species composition similarity was negatively correlated with native species richness under both *T. minuta* and *G. cordifolia* invasions ($r = -0.94$, $p = 0.0001$; $\rho = -0.61$, $p < 0.05$, respectively) (Fig. 3).

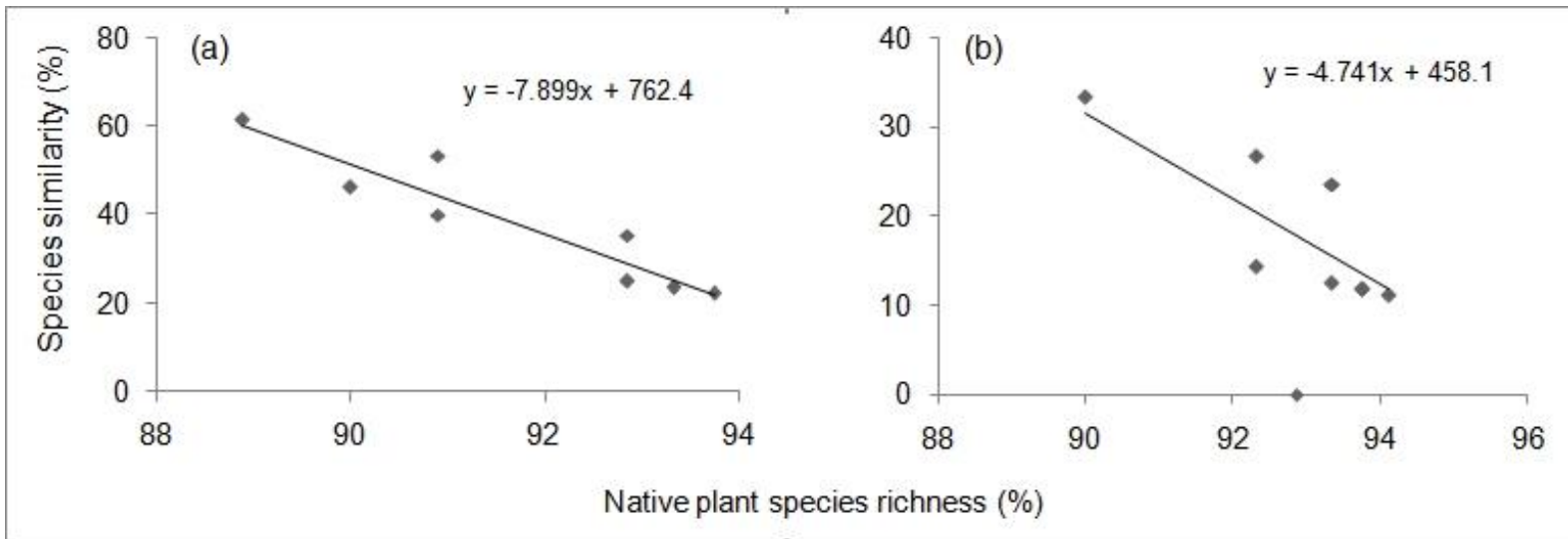


Fig. 3: Correlation between the impact of (a) *T. minuta* and (b) *G. cordifolia* invasive plant species on native vascular plant species composition (expressed as Sorensen similarity between invaded and uninvaded quadrats in a pair) and native species richness.

2.3.2. Ecological influences of *T. minuta* and *G. Cordifolia*: Differences in native plant composition under invasion

Under both *T. minuta* and *G. cordifolia* invasions, the height of native plants in highly invaded quadrats were about double that of in uninvaded quadrats ($p < 0.05$) (Fig. 4).

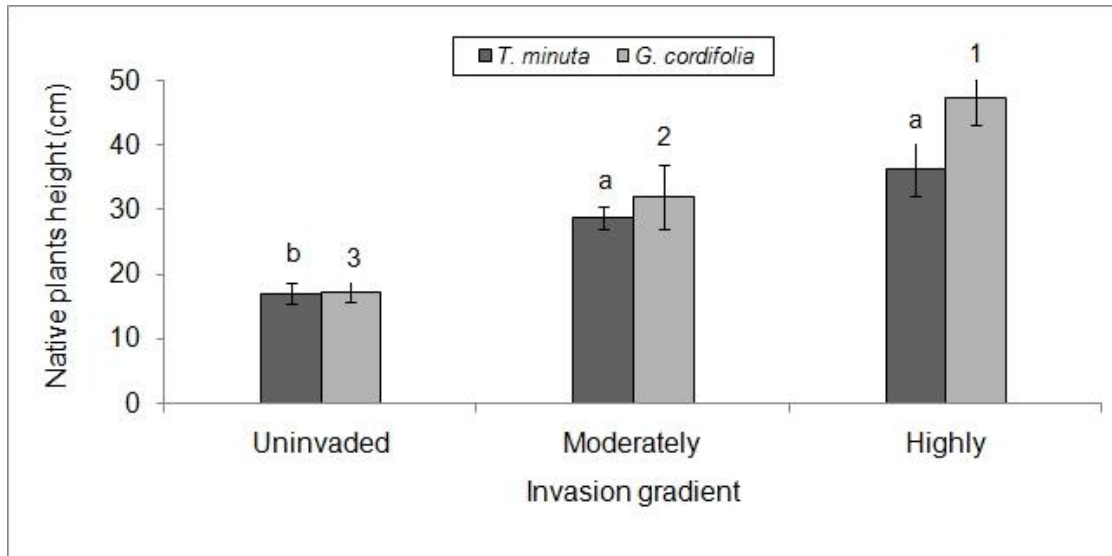


Fig. 4: Average native plants height under *T. minuta* and *G. cordifolia* invasions. Bars with dissimilar letters and or numbers are significantly different by Fisher LSD at $p = 0.05$

Native species percentage ground cover in both *T. minuta* and *G. cordifolia* invasions was twice as high in uninvaded quadrats compared to moderately and highly invaded quadrats ($p < 0.05$) (Table 1 and Fig 5).

Table 1: One-way ANOVA and Kruskal-Wallis test for the three indices; evenness, vegetation height (cm) and ground cover (%) of native plants in both *T. minuta* and *G. cordifolia* quadrats ($H=H_{(2,24)}$, $F=F_{(2,24)}$)

Index	<i>T. minuta</i>				<i>G. cordifolia</i>			
	MS	F	H	P	MS	F	H	P
Evenness (J')	-	-	2.3	0.3177	-	-	3.1	0.2078
Vegetation height	901.2	9.0	-	0.0015	1949.6	24.2	-	< 0.0001
Ground cover	-	-	17.3	0.0002	-	-	19.1	0.0001

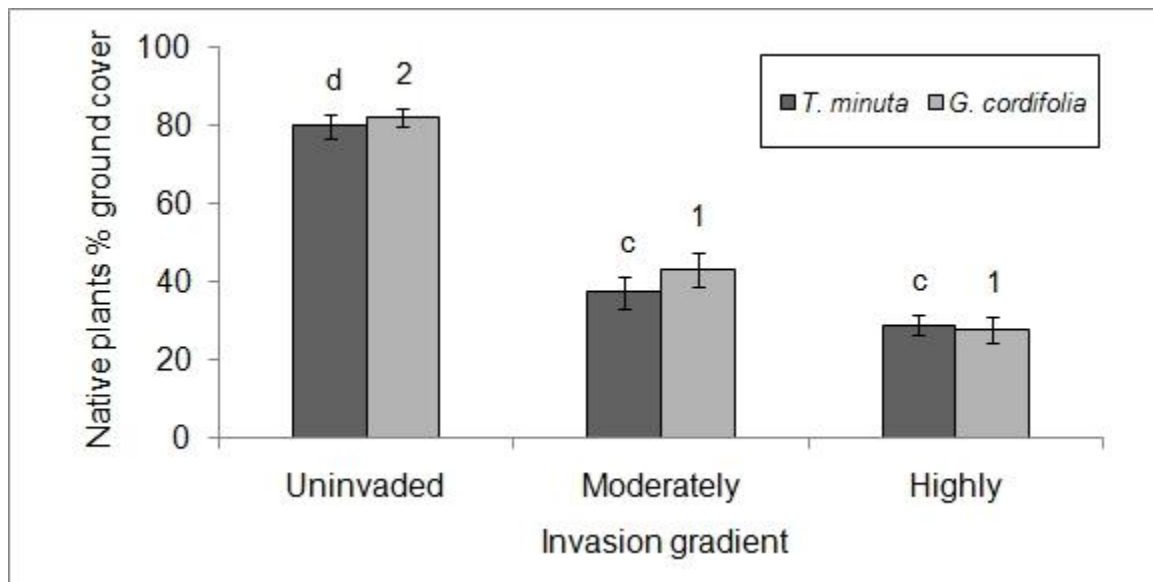


Fig. 5: Native species percentage ground cover under *T. minuta* and *G. cordifolia* invasions. Bars with dissimilar letters and or numbers are significantly different by Fisher LSD at $p = 0.05$

2.4 Most competitive local grass species against invasives

Cynodon dactylon was observed to co-exist often with both *T. minuta* and *G. cordifolia* weeds in both moderately and highly invaded quadrats with an average occurrence of more than 30% and 70% respectively. The mean number of *C. dactylon* individuals in *T. minuta* and *G. cordifolia* highly invaded quadrats were 42 ± 10 and 53 ± 13 , respectively (Table 2). *Chloris pycnothrix* was the second most co-existing local grass species with percentage occurrence of $\geq 17\%$ in both moderately and highly invaded quadrats. The mean number of *C. pycnothrix* individuals in *T. minuta* and *G. cordifolia* highly invaded quadrats were 3 ± 0 and 19 ± 5 , respectively (Table 2).

Table 2: Mean individual number per quadrat (\pm SE) of the common native grass species of occurrence of $\geq 15\%$ in both *T. minuta* and *G. cordifolia* quadrats

Grass species	Measurement	<i>T. minuta</i>			<i>G. cordifolia</i>		
		Uninvaded	Moderately	Highly	Uninvaded	Moderately	Highly
<i>C. pycnothrix</i>	Mean #	0 \pm 0	1 \pm 0	3 \pm 0	111 \pm 4	1 \pm 0	19 \pm 5
	% of appearance	0	17	17	18	17	57
<i>C. dactylon</i>	Mean #	15 \pm 7	53 \pm 17	42 \pm 10	21 \pm 6	45 \pm 8	53 \pm 13
	% of appearance	100	50	89	18	33	71

2.5 Discussion

2.5.1 Ecological influences of invasive weed species *T. minuta* and *G. cordifolia*

Various attempts have been made globally to address the ecological effects of invasives on native ecosystems (Dukes and Mooney, 2004) but the links between these effects and ecosystem responses are largely lacking in the literature. While considerable research has been done on woody invasive weeds (Tyynelä, 2001; Browning *et al.*, 2008; Hasen-Yusuf *et al.*, 2013; Tererai *et al.*, 2013), studies are lacking on herbaceous invasive plant species and their effects in grassland systems. It was observed that the invasion of both *T. minuta* and *G. cordifolia* can be linked to an increase in native vegetation height and a decline in native plant species diversity, richness and cover. These declines are likely due to the high competitive ability of the two invasive plant species and to the latter's strong allelopathic potential (Kil and Shim, 2006). In this study, *T. minuta* and *G. cordifolia* strongly suppressed native plant species diversity but not species evenness. These results agree with other studies on the effects of invasive plants in riparian plant communities (Tererai *et al.*, 2013). Increased competition for both light and nutrients between native species and the two invasive plants could be a reason for a decreased diversity in both moderately and highly invaded areas compared with uninvaded ones. While evidence of the allelopathic potential of *G. cordifolia* is lacking, that of *T. minuta* has been reported to suppress seedling growth of some adjacent plants (Kil and Shim, 2006). Reduced growth of native plants reduces their competitiveness, vigor, and consecutively, their abundance, often leading to a reduction in native species diversity.

The observations that uninvaded quadrats had three times higher native plants species richness than invaded plants is consistent with other previous studies. Alvarez and Cushman (2002) for instance reported that invasive species *Delairea odorata* reduces native species richness and seedling growth in California. Similarly, the invasive plant species *Impatiens glandulifera* reduced native species richness in England (Hulme and Bremner, 2006). These suppressive effects through invasives are likely due to a reduction of reproductive success and pollen deposition of the native species (Martinez-Ghersa *et al.*, 2000). The lower native species richness in invaded quadrats can also be due to higher competitiveness of *T. minuta* and *G. cordifolia*, which is facilitated by a lack of natural enemies such as herbivores as shown by some invasive plants

(Cano *et al.*, 2009). Moreover, *T. minuta* and *G. cordifolia*, tend to produce a large number of seeds per plant (Personal observation), which adds to their success in colonising areas rapidly. The negative correlation between native species similarity and richness could be due to an increase in interspecific competition. As many different species occupy an area, interspecific competition increases and, therefore, the number of similar surviving individual species in the community decreases (mixed composition). In contrast, when few species occupy an area interspecific competition decreases, therefore the number of similar surviving individual species increases (mono-composition).

As expected, the results strongly suggest that invasion of *T. minuta* and *G. cordifolia* species facilitated an increase in native plant species height and reduced their stem strength. This reduction in stem strength is probably due to an increased competition for light due to shading through the invasives as reported by (Hager *et al.*, 1998) for crop species. Invasive weeds have been shown to encourage native species to invest more in their growth (elongation) activities as a result of light competition, leading to taller individuals in invaded sites (Martinez-Ghersa *et al.*, 2000; Hejda *et al.*, 2009). Overall, native vegetation height and ground cover in this study provide evidence that *T. minuta* and *G. cordifolia* invasion can result in a pasture with reduced biomass due to competition (Levine *et al.*, 2003) and, hence, less productive forage for inhabiting ungulates. Moreover, invaded areas are likely to be avoided by most ungulates as they are possible hideouts for predators due to taller vegetation (Ripple and Beschta, 2003). Most prey species have been reported to prefer predator-free areas and, hence, spend more time in uninvaded but nutrient poor areas (Lima and Dill, 1990; Cresswell, 2008).

A percentage ground cover twice as high in uninvaded quadrats compared to invaded ones is likely due to the absence of suppressive effects of invasive species such as allelopathy and competition. Absence of suppression in turn results in healthier native species with higher biomass and horizontal growth (i.e., more ground cover) (Nagashima and Hikosaka, 2011). Moreover, reduced reproductive success and pollen deposition of the native species in invaded sites can further lead to reduced ground cover (Martinez-Ghersa *et al.*, 2000).

2.5.2 Most co-existive native grass species

Among the common grass species (Poaceae) surveyed, *Cynodon dactylon* was found to be the most successful grass species that co-existed well with *T. minuta* and *G. cordifolia*. Although *C. dactylon* abundance was lower in invaded quadrats compared with uninvaded ones, its percentage occurrence was higher than other surveyed grasses. The high occurrence of *C. dactylon* could be due to its physiological advantages. For example, with its stoloniferous ability, *C. dactylon* is able to escape from invasion by forming roots away from invaded areas (Horowitz, 1973) and, hence, will access soil nutrients and light that would have been competed for with *T. minuta* and or *G. cordifolia*. Moreover, *C. dactylon* possesses a deep root system, which in drought situations and under penetrable soil can grow to over 2 m deep (Horowitz, 1973). Moreover, the colonization ability of *C. dactylon* has been reported to be larger than that of other species such as *Sorghum halepense* (Horowitz, 1973). Its drought tolerance tendency as reported by (Zhao *et al.*, 2005; Shi *et al.*, 2012) can be another reason for its successful competition with *T. minuta* and or *G. cordifolia*. Further, *C. dactylon* can grow on soils with a wide range of pH and survive flooding (Burton and Hanna, 1985). Similarly, Horowitz (1973) reported that submerging *C. dactylon* rhizome for more than a week under water had no effect on its sprouting ability. When grown with *Acacia smallii* in mixed and monocultures, with and without added fertilizer, *C. dactylon* was observed to grow over twice as large in mixed cultures than in monoculture (Cohn *et al.*, 1989). Its competitiveness is thought to be due to its ability to reduce the level of nutrients to below the necessary amount needed by *Acacia smallii* (Cohn *et al.*, 1989).

2.6 Conclusion

As invasive weeds pose significant impacts to native plants, their impacts can result in an overall change of not only plant community structure and composition but also associated herbivore community structure and composition (Gan *et al.*, 2007; Johnson and Cushman, 2007). It is now evident through this study that the invasives *T. minuta* and *G. cordifolia* consecutively result in a reduction of the Ngorongoro crater's herbivore biomass as a consequence of decreased native forage plants. This in turn might result in a reduction of Ngorongoro crater's attractiveness as a tourist destination, which might result in income loss for government, park authorities and local

people who are either directly or indirectly interacting with tourists. This study generated the information that contributes to the understanding of the ecological influence of invasive plant species and is important for incorporating into the management plans of rangelands. Generally, the two invasive plants, changed the ecology of the native plant communities, particularly the native species richness, cover and height. As most rangelands are increasingly threatened by these invasive plant species, this study is therefore timely and has generated a baseline information for long term research to elucidate mechanisms associated with these two invasive plants interactions with natives, while also informing the management authorities on the threats to native plants and animals associated with invasion of the two plant species.

CHAPTER THREE

*The information presented in this chapter describes the effects of the invasive weeds *Tagetes minuta* and *Gutenbergia cordifolia* on the Ngorongoro crater's soil chemistry. Generally, available P, N, and CEC levels were influenced by invasions of either or both *T. minuta* and *G. cordifolia*.*

Assessment of Ngorongoro crater's soil chemistry along invasion gradients of *Tagetes minuta* and *Gutenbergia cordifolia*

Abstract

Plant invasions affect both ecosystem functioning and structure, though little is known on the effects of invasives *Tagetes minuta* and *Gutenbergia cordifolia* on soil chemistry. Sampling was done along a continuum of invasion gradient from uninvaded to highly invaded areas in the Ngorongoro crater, Tanzania to assess the relationship between the invasive plants *T. minuta* and *G. cordifolia* and soil pH, available P and N, exchangeable bases (Ca, Mg, K, Na), Cation Exchange Capacity (CEC) and available Cu, Fe, Mn and Zn. Available N was four times higher at *G. cordifolia* highly invaded areas compared with uninvaded areas. On the contrary, *T. minuta*'s uninvaded areas had four times higher available N compared with highly invaded areas. A significant positive correlation between available N and invasion status was observed under *G. cordifolia* invasion while a significant negative correlation was observed under *T. minuta* invasion. There were significant differences in available P along invasion gradients of both *G. cordifolia* and *T. minuta*. While available P was reduced with increasing invasion status for *G. cordifolia*, it was positively related with increasing *T. minuta* invasion. CEC was three times higher in *G. cordifolia* highly invaded areas than uninvaded areas while under *T. minuta* invasion, uninvaded areas had five times higher CEC compared with highly invaded areas. Generally, available P, N, and CEC levels were influenced by invasions of either or both *T. minuta* and *G. cordifolia*.

Keywords: Daisy, soil nutrients, Tanzania, Marigold, Weed, Rangeland management

3.1 Introduction

Plants invasions have been known for their effects on both ecosystem functioning and structure. Invasion of *Phragmites australis* in salt marsh ecosystems of New England, for instance, have been associated with alteration of trophic structure, nutrient cycling and habitat use by both birds and fish (Meyerson *et al.*, 2000; Wainright *et al.*, 2000 in Talley and Levin, 2001). Recently, attention on the effects of invasive plants on soil physical and chemical properties, litter communities and soil microfauna has risen (Talley and Levin, 2001; Motard *et al.*, 2015). It has been established that invasive plants, particularly alien species, are highly competitive compared with their native counterparts (Vila and Weiner, 2004). In contrast, little is known about the available soil nutrients under associated plant invasions (Eckert and Kinsinger, 1960; Ehrenfeld, 2003). Potential shifts in soil nutrients through invasions might be responsible for native plant extinction and formation of invasion monocultures (Talley and Levin, 2001).

Soil fertility of an area is normally judged by the availability of sufficient nutrients to sustain plant growth and development (FAO, 2016). Low soil fertility is responsible for the decline of an area's productivity (Chakraborty and Mistri, 2015) and, therefore, overall reduction of both fauna and flora. The consequences of invasive plants on the ecosystem may include changes in the soil, which may impact the invasibility of ecosystems and invasive plant success (Ehrenfeld, 2003). Although newly introduced species have been reported to affect soil nutrient cycling these effects depend on how different the new species is compared with the existing natives (Ehrenfeld, 2003). It remains debatable whether the introduction of new species is causing changes in soil properties with some studies showing some effects (Aguiar *et al.*, 1996; Gill and Burke, 1999), while others did not (McCarron and Knapp, 2001).

In the Ngorongoro crater of the Ngorongoro Conservation Area (NCA), a total of 139 exotic invasive plants have so far been reported (NCAA, 2011) including *Tagetes minuta*. Most of these plants have been associated with a replacement of native perennials (Chapter two), which might result in the loss of genetic and species diversity, reduced forage production, decreased soil and food web stability, increased fire frequencies and decreased mineral cycling (Duda *et al.*, 2003). The exotic and native invasive plants *T. minuta* and *G. cordifolia*, respectively, have been reported to affect native species abundance and richness in Ngorongoro crater (Chapter two),

thereby posing a risk for local extinction of some native plants. However, little is known on the influence of these invasive plants particularly *G. cordifolia* on soil chemistry. The mechanisms behind their successful suppression of native plants may be due to their high competitive ability, which involves a formation of large numbers of seeds per plant (Westbrooks, 1998), and the production of allelo-chemicals that suppress nearby plants (Arora *et al.*, 2015). As grassland productivity depends mostly on soil nutrients (Hopkins *et al.*, 1994), ruminants also have nutrients requirements that are directly linked to the available nutrients in the soil (Hopkins *et al.*, 1994). Assessing deficiencies of nutrients in the soil, therefore, is crucial in a highly diverse mammalian herbivore community, as is found in Ngorongoro crater. Hence, how strongly invasives influence soil nutrients is of high importance for a sustainable rangeland management in order to meet a ruminant's dietary requirements and to sustain a biodiverse fauna and flora. This study therefore, tested soil pH, available P and N, exchangeable bases (Ca, Mg, K, Na), Cation Exchange Capacity (CEC) and available Cu, Fe, Mn and Zn along a continuum of *T. minuta* and *G. cordifolia* invasion gradient from uninvaded to highly invaded areas in Ngorongoro crater, a protected area rich in wild mammalian herbivores in Tanzania.

3.2 Materials and methods

3.2.1 Study site

Ngorongoro crater's soil is characterized by volcanic soil (Anderson and Herlocker, 1973) developed five to seven million years ago (Pickering, 1968). Colluvial deposits cover most of south, east and northern parts of the crater, red-brown and yellow pumiceous tuffs outcrop covers western parts (Anderson and Herlocker, 1973). Lacustrine deposits dominate around lake Makat while, mounds as a results of volcanic mud flow dominates the south-western wall (Anderson and Herlocker, 1973). The area receives seasonal rainfall with annual precipitation varying from 500 mm to 1700 mm on arid plains and forested slopes, respectively.

3.2.2 Sampling design

Sampling was performed as described in Chapter two. One composite soil sample was collected per quadrat (six composite soil samples per site) at a depth of 20cm using soil auger from early July to late August, 2015. The total sample size per species was 72 composite soil samples.

3.2.3 Determination of chemical soil properties

Soil pH was determined by pH meter (Peech, 1965) as follows: 10 g of soil sample was soaked in 25 ml of distilled water. A ratio of soil weight to water of 1:2.5 was maintained. Samples were shaken with a mechanical shaker for 30 min at 220 rpm and left to settle for 10 min. pH of supernatant was determined using a calibrated pH meter. Available P was extracted following the Bray I procedure (Bray and Kurtz, 1945), and determined by ascorbic acid-molybdate blue method (Murphy and Riley, 1962). Available N was determined following the Kjeldal method (Bremner, 1965) as follows: 1 g of catalyst mixture (CuSO_4 , Selenium powder, $\text{K}_2(\text{SO}_4)_3$) and 10 ml of concentrated Sulphuric Acid (H_2SO_4) were added on 1 g of soil sample sieved using 0.5 mm sieve. The mixture was heated at 300°C for about 2 h, and then 50 ml of 32% NaOH was added. Distillation of the sample was performed and 35 ml of the distillate was collected with 20 ml of Methylated Boric Acid. Back titration of the distillate was performed by adding 0.01 M (2 Normal) H_2SO_4 . Available Nitrogen (N) was calculated as follows:

$$\text{N} = \frac{\text{Vol. of acid used for back titration} \times \text{Normality of the acid} \times 1.4}{\text{Sample weight (g)}}$$

Exchangeable bases and available Cu, Fe, Mn and Zn were determined by atomic absorption spectrophotometer (Hesse, 1971) as follows: 35 ml of Ammonium acetate ($\text{CH}_3\text{COONH}_4$) was added into 10 g of soil sample and the mixture was left overnight. The mixture was then filtered using Watsman filter paper number 40 by adding $\text{CH}_3\text{COONH}_4$ to a final filtrate volume of 100 ml. Exchangeable bases and available Cu, Fe, Mn and Zn in the filtrate were determined using Atomic Absorption Spectrophotometer (AAS). Exchangeable Na (%) was calculated according to Robbins (1984) as follows:

$$\text{Exchangeable Na (\%)} = (\text{exchangeable Na} / \text{CEC}) 100\%$$

The CEC was determined by the Ammonium saturation method (Chapman, 1965) as follows: 35 ml of Ammonium acetate ($\text{CH}_3\text{COO NH}_4$) was added into 10 g of soil sample and the mixture was left overnight. The mixture was then filtered using Watsman filter paper number 40 by adding $\text{CH}_3\text{COO NH}_4$. The remaining soil in the filter paper was further leached using 80% Ethanol to remove excess Ammonia (washing) and the Ethanol filtrate was discarded. The

remaining soil was further leached with 1M KCl (1 Normality) to a volume of 100 ml. 25 ml of the filtrate were collected and 40 ml of 32% NaOH was added to make it alkaline, distillation of the sample was performed and the 35 ml of the distillate was collected with 20 ml of Methylated Bolic Acid. Back titration of the distillate was performed by adding 0.01M (2 Normal) H₂SO₄. CEC was calculated as follows:

$$\text{CEC} = \frac{(\text{Vol. of acid used for back titration} \times \text{Normality of the acid}) \times 100}{\text{Sample weight (g)}}$$

3.2.4 Statistical analysis

Shapiro-Wilk test for normality was performed on soil pH, available P and N, CEC and available Cu, Fe, Mn and Zn. For all data that passed normality test, one-way analysis of variance (ANOVA) was carried out (Tererai *et al.*, 2013) whilst for non-normally distributed data a Kruskal–Wallis test was performed (Tererai *et al.*, 2013). For both invasive weed species, Kruskal-Wallis test was performed on soil pH, available N, CEC and available Cu, Fe and Mn. One-way ANOVA was carried out on available P and Zn. Pearson's Product Moment and Spearman correlations were also performed on normally and non-normally distributed data respectively. The statistical software used was STATISTICA version 8 (StatSoft-Inc., 2007) and the level of significance was set at $p < 0.05$.

3.3 Results

3.3.1 Soil pH, available P and available N

No significant differences were observed for soil pH under both *T. minuta* and *G. cordifolia* invasion gradients (Table 3).

Table 3: Mean \pm S.E. of chemical properties of soil collected along a continuum of invasion of *T. minuta* and *G. cordifolia* in Ngorongoro Crater of the Ngorongoro Conservation Area and recommended critical levels for proper pasture growth. Statistical *p* values are shown.

Parameter	<i>T. minuta</i>				<i>G. cordifolia</i>				Critical levels	Reference
	Uninvaded	Lightly	Highly	<i>p</i>	Uninvaded	Lightly	Highly	<i>p</i>		
pH	6.8 \pm 0.1	7.1 \pm 0.3	7.5 \pm 0.4	0.20	7.3 \pm 0.3	6.8 \pm 0.3	6.6 \pm 0.3	0.50	6.0-7.0	Barnhart, 2010
Ca:Mg	14.1 \pm 2.1	11.6 \pm 2.3	11.1 \pm 3.1	-	20.3 \pm 1.2	17.4 \pm 2.6	20.3 \pm 2.0	-	3:1-8:1	Allan <i>et al.</i> 1997
Mg:K	1.1 \pm 0.1	1.3 \pm 0.2	1.1 \pm 0.0	-	1.2 \pm 0.2	1.1 \pm 0.1	1.2 \pm 0.2	-	\geq 1.5	CCMA, 2016
K/(Ca+Mg)	0.1 \pm 0.0	0.1 \pm 0.0	0.1 \pm 0.0	-	0.1 \pm 0.0	0.1 \pm 0.0	0.1 \pm 0.0	-	-	-
Cu	1.6 \pm 0.1	1.2 \pm 0.1	1.4 \pm 0.1	0.70	1.5 \pm 0.2	1.5 \pm 0.2	1.8 \pm 0.3	0.80	>12mg/kg	DiaryNZ, 2016
Fe	25.9 \pm 2.5	22.2 \pm 2.7	22.2 \pm 2.3	0.60	23.5 \pm 3.7	25.9 \pm 4.1	24.6 \pm 2.8	0.70	>4.5mg/kg	DiaryNZ, 2016
Zn	0.3 \pm 0.0	0.2 \pm 0.0	0.2 \pm 0.0	0.90	0.4 \pm 0.1	0.8 \pm 0.3	0.4 \pm 0.1	0.70	>10mg/kg	DiaryNZ, 2016
Mn	25.6 \pm 4.3	24.3 \pm 7.6	20.1 \pm 2.3	0.80	23.7 \pm 2.5	32.5 \pm 3.3	29.6 \pm 2.8	0.10	>500mg/kg	DiaryNZ, 2016
CEC	51.3 \pm 3.8	29.7 \pm 3.1	12.4 \pm 0.8	0.02	18.1 \pm 1.7	30.5 \pm 4.4	56.6 \pm 4.2	0.01	>12cmol _c /kg	NSS,1990

Significant available *P* differences were observed along both *G. cordifolia* and *T. minuta* invasion gradient ($H_{(2,24)} = 7.95, p = 0.002$; $F_{(2,24)} = 9.40, p = 0.0012$ respectively) (Fig. 6).

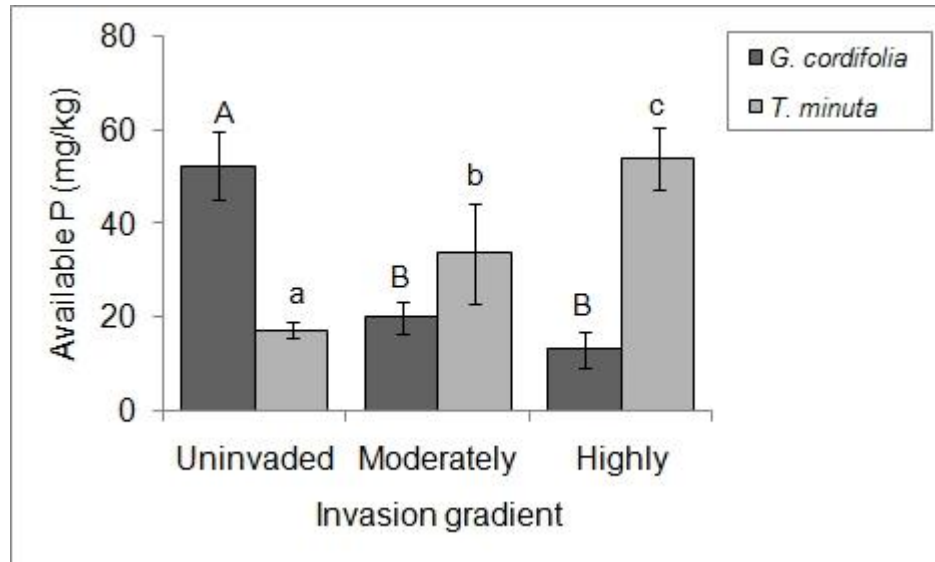


Fig. 6: Available *P* of soil collected along a continuum of invasion of *T. minuta* and *G. cordifolia* in Ngorongoro Crater of the Ngorongoro Conservation Area. Bars with dissimilar letters are significantly different by Fisher LSD at $p = 0.05$

While significant negative correlation between available *P* and invasion status was observed under *G. cordifolia* invasion ($\rho = -0.58, p < 0.05$) (Fig. 7a), a significant positive correlation was observed under *T. minuta* invasion ($r = 0.69, p = 0.0002$) (Fig. 7b).

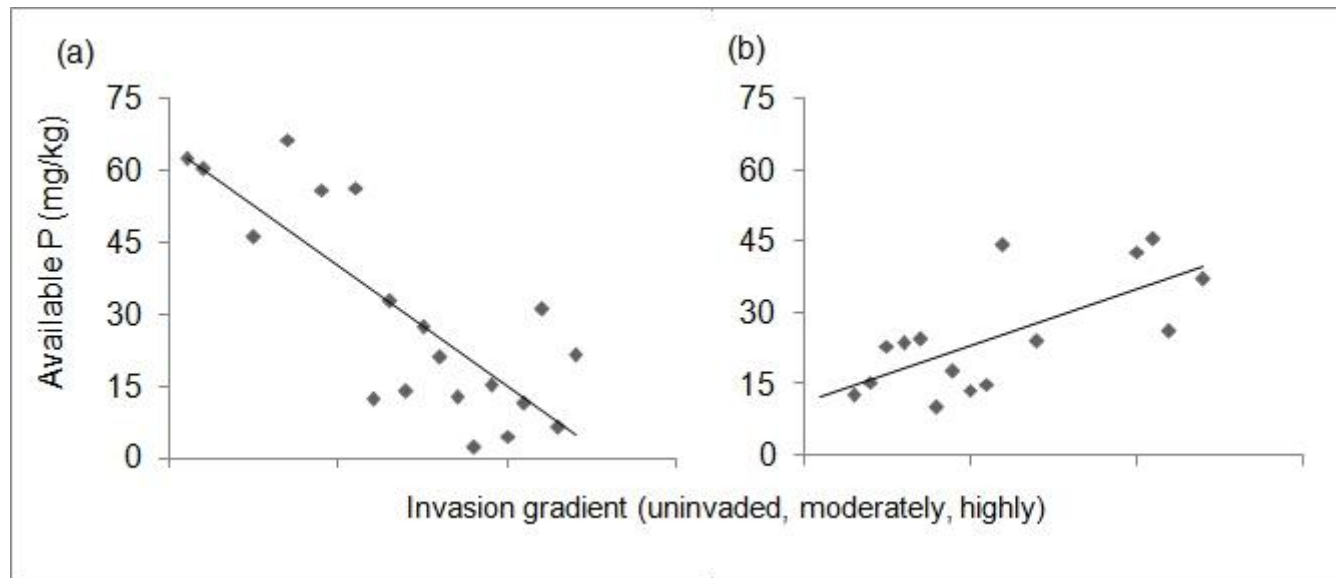


Fig. 7: Correlation between available P and (a) *G. cordifolia* and (b) *T. minuta* invasion status in Ngorongoro Crater of the Ngorongoro Conservation Area.

There were four times higher available N on *G. cordifolia* highly invaded areas compared to uninvaded areas ($H_{(2,24)} = 15.62$, $p = 0.0004$). On the contrary, *T. minuta*'s uninvaded areas had four times higher available N compared to highly invaded areas ($H_{(2,24)} = 19.16$, $p = 0.0001$) (Fig. 8).

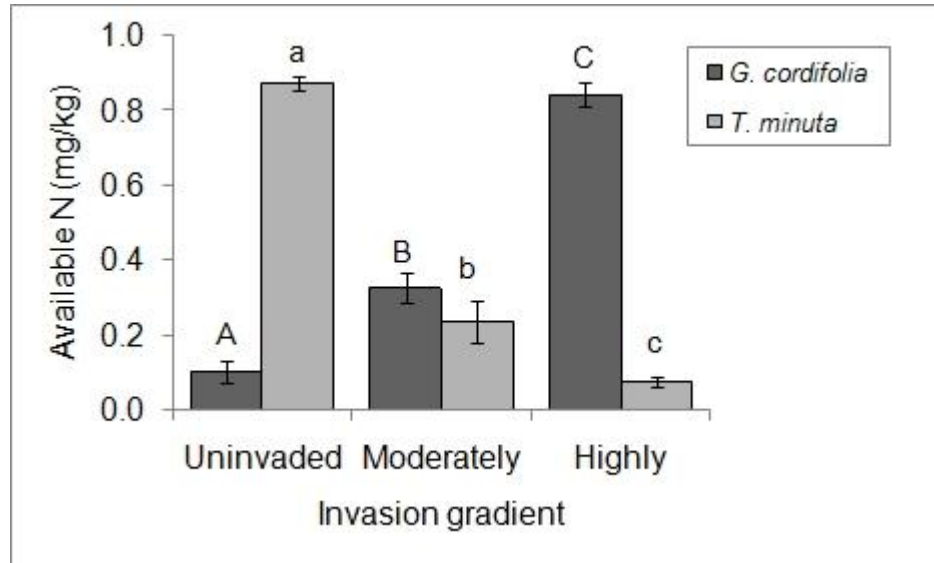


Fig. 8: Available N of soil collected along a continuum of invasion of *T. minuta* and *G. cordifolia* in Ngorongoro Crater of the Ngorongoro Conservation Area. Bars with dissimilar letters are significantly different by Fisher LSD at $p = 0.05$

A significant positive correlation between available N and invasion status was observed under *G. cordifolia* invasion ($\rho = 0.62$, $p < 0.05$) (Fig. 9a) while a significant negative correlation was observed under *T. minuta* invasion ($\rho = -0.61$, $p < 0.05$) (Fig. 9b).

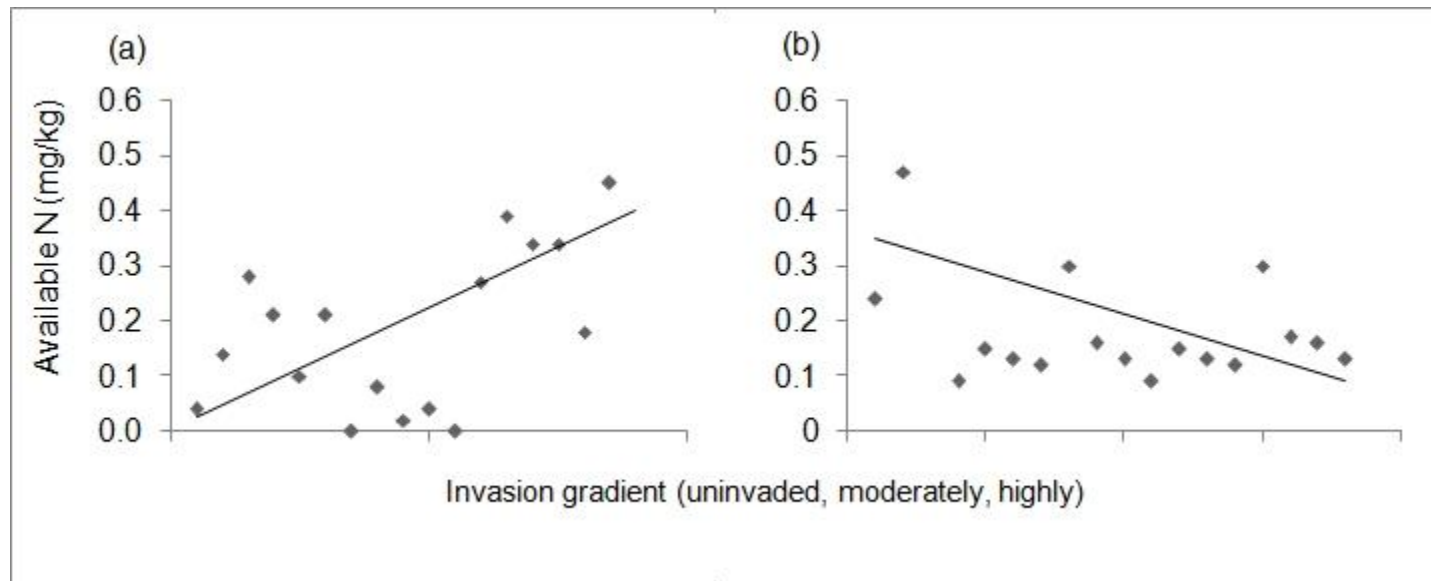


Fig. 9: Correlation between available N and *G. cordifolia* (a) and *T. minuta* (b) invasion status in Ngorongoro Crater of the Ngorongoro Conservation Area.

3.3.2 Exchangeable cations in soil

Exchangeable soil cations did not differ significantly along invasion gradients of both *T. minuta* and *G. cordifolia*, only Ca: Mg ratio was decreasing along the invasion gradient under *T. minuta* invasion while remaining unchanged under *G. cordifolia* (Table 3), Mg: K ratio was averaged at 1.15 ± 0.11 and 1.17 ± 0.13 under *T. minuta* and *G. cordifolia* invasions respectively. $K / (Ca + Mg)$ was slightly increasing along the invasion gradient under *T. minuta* invasion while remaining unchanged under *G. cordifolia* (Table 3). Exchangeable Na (%) was decreasing along the invasion gradient under both *T. minuta* and *G. cordifolia* invasions (Fig. 10).

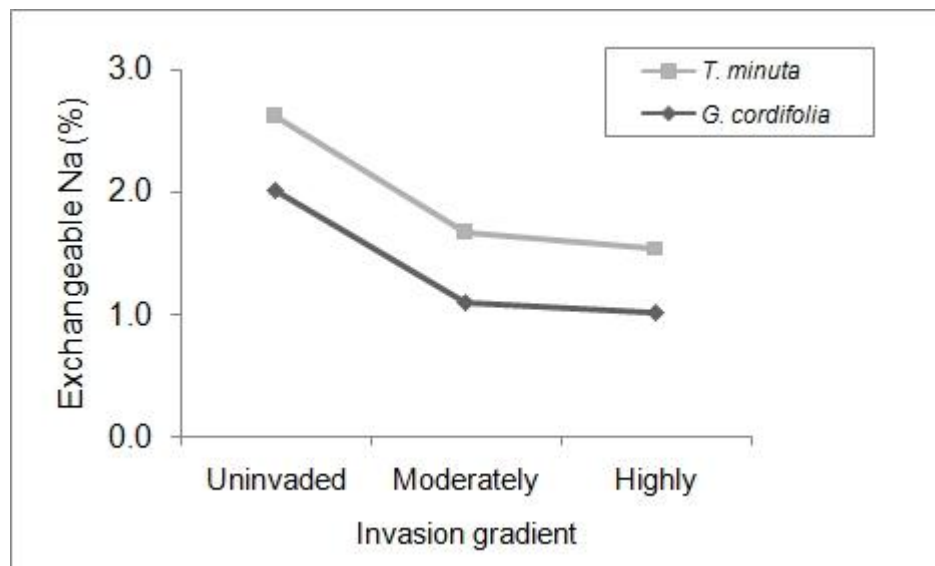


Fig. 10: Exchangeable Na percentage of soil collected along a continuum of invasion of *T. minuta* and *G. cordifolia* in Ngorongoro Crater of the Ngorongoro Conservation Area.

CEC was three times higher in *G. cordifolia* highly invaded areas than uninvaded areas ($H_{(2,24)} = 15.63$, $p = 0.0004$) while under *T. minuta* invasion, uninvaded areas had five times higher CEC compared with highly invaded areas ($H_{(2,24)} = 19.30$, $p = 0.0001$) (Fig. 11).

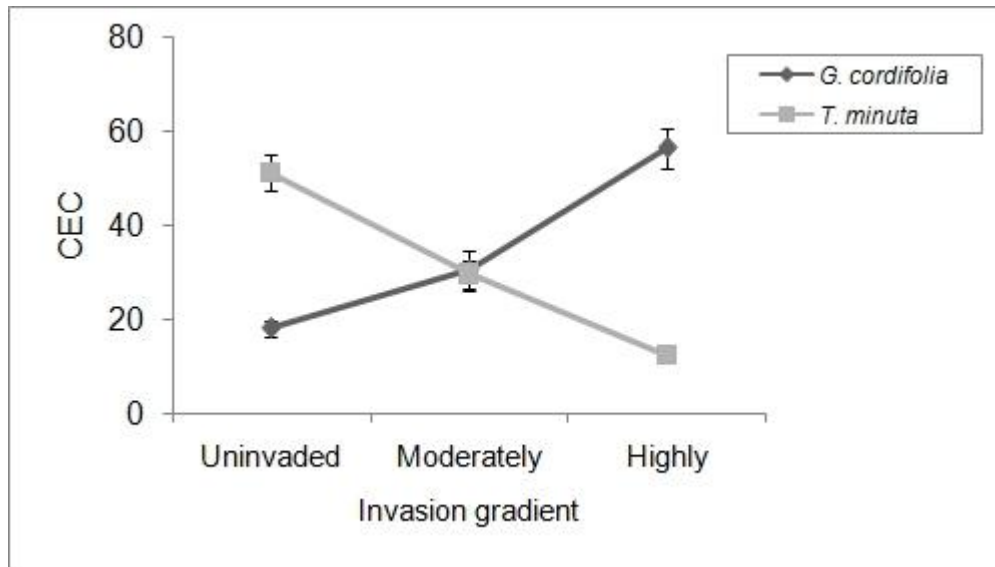


Fig. 11: CEC of soil collected along a continuum of invasion of *T. minuta* and *G. cordifolia* in Ngorongoro Crater of the Ngorongoro Conservation Area.

3.3.3 Available Cu, Fe, Mn and Zn

Available Cu remained unchanged along the invasion gradient under both *T. minuta* and *G. cordifolia*. On the contrary, available Fe decreased along invasion gradient under *T. minuta* and remained unchanged under *G. cordifolia* invasion (Table 3). Available Mn decreased along the invasion gradient under *T. minuta* invasion while increased under *G. cordifolia* invasion. Available Zn remained unchanged along invasion gradient under both *T. minuta* and *G. cordifolia* invasions (Table 3). In all surveyed areas Zn, Cu and Mn were below the proposed deficiency level for pastures (Table 3).

3.4 Discussion

3.4.1 Soil pH, available P and available N

Results from this study indicated that *T. minuta* highly invaded areas had slightly alkaline soils (pH>7.0) (Koenig and Kuhns, 2010), hence, posing a high risk for iron deficiency (iron chlorosis) (Egesi *et al.*, 2015) in the pastures of Ngorongoro crater. This implies that invasion of *T. minuta* induces increased soil pH, a condition that might affect Ngorongoro crater's pasture quality and overall herbivores health. The higher pH levels under *T. minuta* invasion could be

due to a high accumulation of Calcium Carbonate, making iron unavailable for plants, this might result in impaired physiological processes such as photosynthesis due to reduced chlorophyll in leaves (Lohry, 2007; Egesi *et al.*, 2015). On the other hand, a decrease in pH along the invasion gradient of *G. cordifolia* could be favorable for native pasture grass growth as the pH falls within the recommended range for grass growth (Barnhart, 2010), (Table 3). This implies that the survival chance for grasses in *G. cordifolia* invaded areas might not be strongly impaired and, hence, presents an opportunity for successful management of *G. cordifolia* using native grasses like *C. dactylon*.

Available P and N were both positively and negatively associated with invasion of *T. minuta* and *G. cordifolia*. While *T. minuta* invaded areas had higher available P and lower available N compared with uninvaded areas, those under *G. cordifolia* showed the opposite tendency. This difference could be due to the fact that *G. cordifolia* is a native plant that has become invasive through intensive grazing (Berendse, 1985) while *T. minuta* is an exotic invasive plant originating from South America (Hulina, 2008). The net effect therefore will be on the entire ecosystem processes and functioning. High available P and low available N under *T. minuta* highly invaded areas showed that either *T. minuta* prefer areas of high P/low N or increases P/reduces N in soils once it has established. Phenolics released by *T. minuta* roots and leaves into the rhizosphere could be responsible for this impact (Meissner and Ruth, 1986). As phosphate insolubility hinders its availability to plants, phenolics have been reported to solubilize and accumulate inorganic P in the soil rhizosphere (Makoi and Ndakidemi, 2007), hence making it available to plants. On the contrary, the decreased available N in soil could be due to the effects of phenolics released that delays organic matter decomposition and mineralization and its alteration on microbial activities (Makoi and Ndakidemi, 2007). Although an increase in available P in *T. minuta* invaded areas might be beneficial to native plant species, negative effects will include increased dominance of the invasive plant, *T. minuta* and potential eutrophication in the nearby water bodies (Beegle and Durst, 2002).

Available P and N under both *T. minuta* and *G. cordifolia* can be managed to facilitate for proper growth of native forage grasses such as *Cynodon dactylon*. While therefore, low available P/N levels in soils under invasions necessitates supplying P/N, intercropping of native (*C. dactylon*) that co-exist well with *T. minuta*/*G. cordifolia* (Chapter Four) might be effective management

option for the utilization of higher available P/N in invaded areas. In small scale rangelands cheap locally available P and N sources could be used as intercropping plant species, such as *Vernonia subligera* O.Hoffn (Ndakidemi, 2007), and a native forage grass such as *C. dactylon* might keep the abundance of *G. cordifolia* and *T. minuta* low.

3.4.2 Cation Exchange Capacity (CEC)

Generally, the results indicated that the Ngorongoro crater's soil is fertile as only one out of 72 *T. minuta* (1.4%) and one out of 72 *G. cordifolia* (1.4%) surveyed quadrats had CEC below the recommended critical level (Table 3) and values did not change much. It is generally accepted that fertile soil has higher CEC and hence has higher yield potential compared to those with lower CEC (Asadu *et al.*, 1997). The trends of high and low CEC under *G. cordifolia* and *T. minuta* highly invaded areas, respectively, showed that either *G. cordifolia* seeks out areas of high CEC or increases CEC while the opposite is true for *T. minuta*. The two scenarios can affect native plant abundance negatively as they result in either increased dominance of the invasive plants, *G. cordifolia* and *T. minuta*, or in decreased yield of native forage due to decreased soil fertility (Asadu *et al.*, 1997). Low CEC under *T. minuta* could be due to phenolics released by both *T. minuta*'s roots and leaves (Meissner and Ruth, 1986), altering soil microbial activities, thereby delaying organic matter decomposition and mineralization (Makoi and Ndakidemi, 2007). In contrast, high CEC under *G. cordifolia* could be due to high *G. cordifolia* biomass accumulation in the crater as it dominates over half of the whole crater floor (UNESCO, 2001). It is evident, therefore, that the Ngorongoro crater soil can be utilized by local plants that are beneficial to wild animals as they provide pasture to wildlife if *T. minuta* and *G. cordifolia* are managed appropriately.

3.4.3 Exchangeable bases (Ca, Mg and K)

The Ca: Mg ratio under *T. minuta* and *G. cordifolia* invasion being above the recommended level for pastures (Table 3) poses a risk of Mg deficiency to Ngorongoro crater's pastures. According to Allan *et al.* (1997), Ca: Mg > 8.1 causes Mg deficiency in pastures which can induce grass tetany (hypomagnesaemia), a metabolic disease in ruminants, associated with low levels of Mg. High amounts of nitrogen or potassium supplied by herbivore's dung (Treydte *et al.*, 2009) could have caused the low soil Mg levels in the crater (Elliott, 2016). The observed lower than

recommended Mg: K ratio (Table 3) along invasion gradients of both *T. minuta* and *G. cordifolia* (Table 3) also can induce grass tetany (CCMA, 2016). Although Mg deficiency observed in this study seems not to either favor or unfavour invasion of both *T. minuta* and *G. cordifolia*, it necessitates Mg supplementation for both improving pasture and hypomagnesaemia free wildlife.

3.4.4 Available Cu, Fe, Mn and Zn

Amongst the micro nutrients Cu, Mn and Zn, the latter is most commonly in short supply (Lohry, 2007). In all surveyed areas lower Zn, Cu and Mn were observed than proposed deficiency levels for pastures (Table 3). While Zn deficiency in plants has been reported to affect the rate of protein synthesis, RNA polymerase activity and ribosome's structural integrity, Mn acts as an activator for enzymes in plant growth processes and assists iron in chlorophyll formation (Lohry, 2007; Egesi *et al.*, 2015). Likewise, as Cu acts as an activator of several enzymes and also functions in electron transport and energy capture by oxidative proteins and enzymes (Lohry, 2007) its deficiency effects in pastures cannot be underestimated. Hence, the already micro-nutrient deprived soils of Ngorongoro crater highlight that management of any invasive plants impacting the soils is highly important, as well as using beneficial enrichment characteristics of native plants to improve the soil quality.

Although the available Fe was adequate for plant growth under both invasions as it was above the recommended level (Table 3), it decreased along invasion gradient under *T. minuta* and remained unchanged under *G. cordifolia* invasion. While the decrease in available Fe under *T. minuta* invasion could be due to an increase in pH level and decreased biomass under *T. minuta* invasion (Personal observation), the unaffected available Fe under *G. cordifolia* invasion could be due to the decrease in pH and an increase in biomass as *G. cordifolia* dominated the area over a long period (UNESCO, 2001). Soil organic matter content and pH have been reported to have strongest influence on iron levels (Egesi *et al.*, 2015). This necessitates initiatives for the management of *T. minuta* to avoid future problems such as iron chlorosis that might affect pasture availability for wild animals.

3.5 Conclusion

It has been shown that some soil nutrient characteristics are associated with the invasion of *T. minuta* and *G. cordifolia*, highlighting the importance of appropriate management of these two invasive species. This study also highlighted that the status of the Ngorongoro crater soil is fairly fertile although soil micro-nutrient values were below recommended levels for pastures. The results further suggest that available P, N, Fe and soil CEC levels were influenced by invasions of either and or both *T. minuta* and *G. cordifolia*. While therefore, low available P and N in soils under *G. cordifolia* and *T. minuta* invasions respectively necessitates supplying P/N, intercropping of native forages such as *C. dactylon* that have been observed to co-exist well with *T. minuta*/*G. cordifolia* presents effective management option for the utilization of higher available P and N in *T. minuta* and *G. cordifolia* invaded areas respectively. Moreover, improved soil Ca, Mg, K, Zn, Cu and Mn levels up to or above the recommended minimum levels can help to improve Ngorongoro crater's soil pasture and hence provide better feed to wild animals. Therefore, invasive unpalatable weeds like *T. minuta* and *G. cordifolia* that consume soil nutrients should be managed to allow for better quality native grasses to grow. This call for environmentally friendly soil management actions such as the use of forage leguminous plants like *Desmodium* species that can act as both organic soil amendments as soil N- fixers and food to wild animals. This study highlighted that there is a relationship between the two invasive plants and soil fertility, which might strongly influence their future invasion success and competitive ability with native plant species.

CHAPTER FOUR

*This chapter describes how increasing density of *Cynodon dactylon* in an intercropping system affects the growth and development of invasive weeds *Tagetes minuta* and *Gutenbergia cordifolia*. Generally, increasing the density of *C. dactylon* strongly affected the two weed's growth and development. Further this section provides information about effective *C. dactylon* density that can be used to out-compete the two weeds as a management strategy.*

Can *Cynodon dactylon* be used to suppress invasive weeds? The effects of density-dependency on the growth and development of *Tagetes minuta* and *Gutenbergia cordifolia*³

Abstract

Plant-Plant competitive interactions have been reported to be among the forces that shape plant community structure. This study assessed the effects of varying the density of *C. dactylon* on the growth and development of *T. minuta* and *G. cordifolia* in screen house and field plot experiments following a completely randomized design. Various growth parameters and leaf pigmentations of *T. minuta* and *G. cordifolia* were determined. *Tagetes minuta* seedlings were half as tall both in screen house and in the field when under *C. dactylon* density of more than 8 per pot/plot compared to those in pots/plots without *C. dactylon*. Significant results were also revealed under *C. dactylon* density of more than 8 per pot/plot with *Tagetes minuta* and *G. cordifolia* having half shoot diameter and were twice as light compared to those in pots/plots without *C. dactylon*. The total leaf chlorophyll for the two invasives consistently decreased with the increased density of *C. dactylon* in both screen house and field plots respectively, whereas the total leaf anthocyanins was twice as high under *C. dactylon* density of more than 8 per pot/plot in screen house and field plots respectively compared to control treatments. Generally, increasing the density of *C. dactylon* strongly affected the two weed's growth and development. This study suggests that, a potential exists for *C. dactylon* to manage the two invasive plants thus, improving forage production and biomass in affected rangelands.

Keywords: *association, couch grass, invasion, management, rangeland management, Eastern Africa, Mexican marigold*

³Under second review: *International Journal of Pest Management*

4.1 Introduction

The mechanism by which plants influence the structure and composition of their surrounding plant community has been investigated since the 19th century. Competition has since then received a lot of attention in ecological research (Connell and Slatyer, 1977; Connell, 1983; Schoener, 1983; Keddy, 1989; Goldberg and Barton, 1992) and was found to directly affect the local distribution of plants in a community (Stoll and Prati, 2001). Plant-Plant competition has well been demonstrated in a range of ecosystems; most vividly in ecosystems where native plants have been exposed to several stresses, for instance water shortage, soil nutrient deprivation and ecological invasion (Daehler, 2003). The most competitive plant always dominates the ecosystem and hence, poses a risk for local extinction of some associated flora and fauna.

Besides the direct competition for nutrients, water and light, the allelopathic nature of some native grass species such as *Cynodon dactylon* is likely to contribute to their competitiveness (Auclair and Dupraz, 2013). *Cynodon dactylon* has been reported to successfully escape from stresses like invasion and drought by creeping away from invaded areas through stolones and by developing a deep root system (Horowitz, 1973; Shi *et al.*, 2012). Further, *C. dactylon* can grow on soils with a wide range of pH, can survive flooding (Burton and Hanna, 1985) and can grow over twice as large in mixed cultures than in monoculture (Cohn *et al.*, 1989). Moreover, *C. dactylon* has been reported to be highly competitive over most crops (Juraimi *et al.*, 2005), which highlights its importance as a potential fodder grass for management of invasive weeds.

Management of invasive weeds in protected ecosystems poses great challenges. Invasive weed species generally spread fast and herbicides often have quite strong negative effects on other native flora and fauna should they be chosen for management purposes (Poorter, 2007). Although alternative management options such as uprooting and mowing are normally opted for, they are labor-demanding and only a short term remedy as many invasive plant seeds remain in the soil seed bank. Therefore, if utilized well, plant density dependent competitive interactions might present an opportunity for developing management strategies for some problematic weeds such as *Tagetes minuta* and *Gutenbergia cordifolia*, thereby helping restoration of previously invaded ecosystems. As a low-cost, low impact management technique, plant-plant competition has been reported to be effective in restoration projects, for instance in restoration of *Quercus*

coccifera oak forests where competition had a strong impact on oak recruitment (Rey Benayas *et al.*, 2007).

This study utilized *C. dactylon* as a competitor due to its agronomic value as a grazing species (Heshmati and Pessarakli, 2011). Also this species was found in a previous study to be the most dominant coexisting native grass species to *Tagetes minuta* and *Gutenbergia cordifolia* (Chapter One), its ability to form deep roots (Horowitz, 1973; Shi *et al.*, 2012) and as it can grow on soils with a wide range of pH (Burton and Hanna, 1985). The density dependent competitive effects of *C. dactylon* on growth parameters and leaf pigments of *T. minuta* and *G. cordifolia* were therefore assessed in this study in both screen house and field plot experiments by varying *C. dactylon* densities. The study hypothesized that *C. dactylon* will suppress the two weeds and, therefore, reduce their growth and development through suppression of the studied parameters. This study paved a way for the application of *C. dactylon* as a management strategy against the invasive plants *T. minuta* and *G. cordifolia*.

4.2 Materials and Methods

4.2.1 Experimental design

Tagetes minuta and *G. cordifolia* seeds were sown separately in pots (screen house) and in plots (field), in combination (mixed) with *C. dactylon* and separately (monoculture) following a simple additive design (Kelty and Cameron, 1995). The two invasive species were referred to as “Weed species (W)” i.e. *Wt* and *Wg* for *T. minuta* and *G. cordifolia*, respectively while *C. dactylon* was referred to as “species (*Cd*)”. Weed species were grown separately and in combination with *Cd* in 0.25m² (6 liters) pots / 0.50 m x 0.50 m plots. Density proportions of *Cd* versus *W* were as follows: *W2:Cd0*, *W2:Cd4*, *W2:Cd6*, *W2:Cd8* and *W2:Cd10*, while those for monocultures were *W2:Cd0*, *W4:Cd0*, *W6:Cd0*, *W8:Cd0* and *W10:Cd0*. *W2:Cd0* was used as control and each treatment was replicated three times. A total of 60 pots/plots were used in this study both mixed and monocultures. The interaction between *Wt*, *Wg* and *Cd* under uniform conditions (space, moisture and nutrients) was studied using a completely randomized design. Seeds of both *Cd* and *W* were sown at a spacing of ≥ 2 cm apart. During the first two weeks, pots / plots were irrigated with water ad-libitum to ensure establishment, after which plants in all pots / plots were irrigated with 0.5 liters of water daily.

4.2.2 Measured parameters

For the number of vegetative branches, number of leaves, seedling height and shoot diameter, the number of plants per pot was considered as population in which > 30% was sampled (three plants per pot). The number of vegetative branches and leaves were counted for each plant. Seedling height was measured using a meter ruler while shoot diameter was measured using vernier calipers at a height of 5 cm from the ground. The total number of leaves in all pots / plots under the same treatment was considered as a population; over 30% of leaves were randomly sampled for leaf area determination. Leaf areas were determined using Image J (Schneider *et al.*, 2012). *Tagetes minuta* and *G. cordifolia* root lengths were measured using a meter ruler. Young leaves from the top-most part of the seedling were sampled randomly per pot/plot for chlorophyll determination while mature leaves were randomly sampled for anthocyanin level determination.

Tagetes minuta and *G. cordifolia* were harvested (uprooted), placed into paper bags and dried at 60°C for 2 days (Makoi *et al.*, 2010). Shoot and root material was separated and weighed to obtain total above/below ground dry biomass (SERAS, 1994).

4.2.3 Leaf pigments measurement

Chlorophyll of *T. minuta* and *G. cordifolia* seedlings was extracted according to Hiscox and Israelstam (1979) with some modifications: 50 mg of fresh leaves of 2.25 cm² were immersed in 4 ml of Dimethyl Sulfoxide (DMSO) and incubated at 65°C for 12 h. The extract was transferred to glass cuvettes for absorbance determination. The absorbance of blank liquid (DMSO) and samples were determined under 2000 UV/VIS spectrophotometer (UNICO®) at 663 nm and 645 nm (Hiscox and Israelstam, 1979) and the total leaf chlorophyll (total Chl) calculated according to Arnon (1949) using the following equation:

$$\text{Total Chl} = 0.0202A_{663} + 0.00802A_{645}$$

Where A_{663} and A_{645} are absorbance readings at 663 nm and 645 nm respectively

Bioassay of levels of anthocyanins in leaves of *T. minuta* and *G. cordifolia* were performed as described by Makoi *et al.* (2010). Leaves of *T. minuta* and *G. cordifolia* were oven-dried at 60°C for 48 h, weighed, ground into a fine powder. Then, 0.10 g of well-ground leaf material was

weighed and mixed with 10 ml of acidified methanol prepared from a ratio of 79:20:1 MeOH:H₂O:HCl. The mixture was incubated for 72 h in darkness for auto-extraction and filtered through Whatman paper number 2. The extract was transferred to glass cuvettes for absorbance determination. The absorbance of acidified methanol as standard and that of samples were determined under a 2000 UV/VIS spectrophotometer (UNICO®) at 530 nm and 657 nm and expressed as Abs g.DM⁻¹ Mirecki and Teramura, 1984 in (Makoi *et al.*, 2010). Anthocyanin concentration in leaf extracts was measured as $A_{530} - 1/3A_{657}$ (Lindoo and Caldwell 1978 in Makoi *et al.*, 2010) where A_{530} and A_{657} are absorbance readings at 530 nm and 657nm, respectively.

4.2.4 Statistical analysis

Shapiro-Wilk test for normality was performed on the number of vegetative branches, leaves, seedling height, shoot diameter, root length, leaf area, leaf total chlorophyll content, leaf anthocyanin concentration, shoot and root biomass of weed species. For all data that passed normality test, one-way analysis of variance (ANOVA) was carried out whilst for non-normally distributed data a Kruskal–Wallis test was performed (Kelty and Cameron, 1995). For both invasive weed species, one-way ANOVA was performed on the number of vegetative branches, leaf area, shoot diameter, leaf total chlorophyll and leaf anthocyanin concentration versus varying density of *C. dactylon*. Kruskal-Wallis test was carried out on the number of leaves, plant height, root biomass, shoot biomass and root length per plant. Pearson's Product Moment and Spearman correlations were also performed on normally and non-normally distributed data respectively. The statistical software used was STATISTICA version 8 (StatSoft-Inc., 2007) and the level of significance was set at $p < 0.05$.

4.3 Results

4.3.1 Density dependent competitive effects on *Tagetes minuta* and *Gutenbergia cordifolia*:

General effect

While no significant differences were observed in monocultures of both *T. minuta* and *G. cordifolia*, higher *C. dactylon* density affected the overall growth and development of both *T. minuta* and *G. cordifolia* seedlings in mixed pots / plots (Plates 6 and 7).



Plate 6: *Tagetes minuta* seedlings vigor under different *C. dactylon* density treatments. C= *C. dactylon* and T= *T. minuta*, numbers indicates the number of individuals per pot.



Plate 7: *G. cordifolia* seedlings vigor under different *C. dactylon* density treatments. C= *C. dactylon* and G= *G. cordifolia*, numbers indicates the number of individuals per pot.

4.3.2 Density dependent competitive effects on *Tagetes minuta* and *Gutenbergia cordifolia*:

Plant growth parameters

The number of vegetative branches per plant and mean leaf area per plant of both *T. minuta* and *G. cordifolia* species differed significantly across the five *C. dactylon* treatments (Tables 4 and 5) and was over five times higher in control pots/plots than in pots/plots with *C. dactylon* density ≥ 8 per pot/plot.

Table 4: Parameters for two *T. minuta* and *G. cordifolia* seedlings intercropped with increasing density (0, 4, 6, 8 and 10) of *C. dactylon* separately in a screen house experiment (d.f =4).

Parameters	<i>T. minuta</i>				<i>G. cordifolia</i>			
	MS	<i>F</i>	<i>H</i>	<i>p</i>	MS	<i>F</i>	<i>H</i>	<i>p</i>
Veg. branches per/plant	-	-	10.0	0.03	46	4.4	-	0.02
Leaves/plant	162	23.9	-	<0.01	12	1.3	-	0.33
Leaf area (mm ²)	-	-	12.2	0.01	1213	6.6	-	0.01
Plant height (cm)	1732	32.2	-	<0.01	452	1.9	-	0.18
Shoot diameter (mm)	19	21.6	-	0.01	8	8.2	-	<0.01
Chl	0.03	116.6	-	<0.01	-	-	13.2	0.01
Root biomass (g)	-	-	12.2	0.01	-	-	1.3	0.86
Shoot biomass (g)	-	-	11.7	0.02	-	-	11.1	0.02
Root length (cm)	142	5.2	-	0.02	44	1.9	-	0.17
Anthocyanins (Abs g.DM ⁻¹)	-	-	11.7	0.02	0.01	2.5	-	0.11

Table 5: Parameters for two *T. minuta* and *G. cordifolia* seedlings intercropped with increasing density (0, 4, 6, 8 and 10) of *C. dactylon* separately in field plot experiment (d.f=4).

Parameters	<i>T. minuta</i>				<i>G. cordifolia</i>			
	MS	F	H	P	MS	F	H	P
Veg. branches per/plant	379	65.1	-	<0.01	184	14.3	-	<0.01
Leaves/plant	206	17.4	-	<0.01	67	7.9	-	0.01
Leaf area (mm ²)	43487	31.4	-	<0.01	-	-	12.3	0.02
Plant height (cm)	1414	13.3	-	0.01	480	1.7	-	0.22
Shoot diameter (mm)	35	38.7	-	<0.01	9.7	32.8	-	<0.01
Chl	-	-	13.0	0.01	-	-	13.5	0.01
Root biomass (g)	-	-	13.2	0.01	-	-	7.0	0.13
Shoot biomass (g)	-	-	13.5	0.01	-	-	10.5	0.03
Root length (cm)	250	13.8	-	<0.01	54.8	2.8	-	0.08
Anthocyanins (Abs g.DM ⁻¹)	0.0676	28.7	-	<0.01	0.0244	12.5	-	0.01

The number of leaves and seedling height per plant differed significantly in *T. minuta* ($p < 0.05$), being half as many and shorter in pots/plots with ≥ 8 *C. dactylon* per pot/plot as those in control treatment but no difference was observed for *G. cordifolia* ($p > 0.05$) (Figs. 12 and 13).

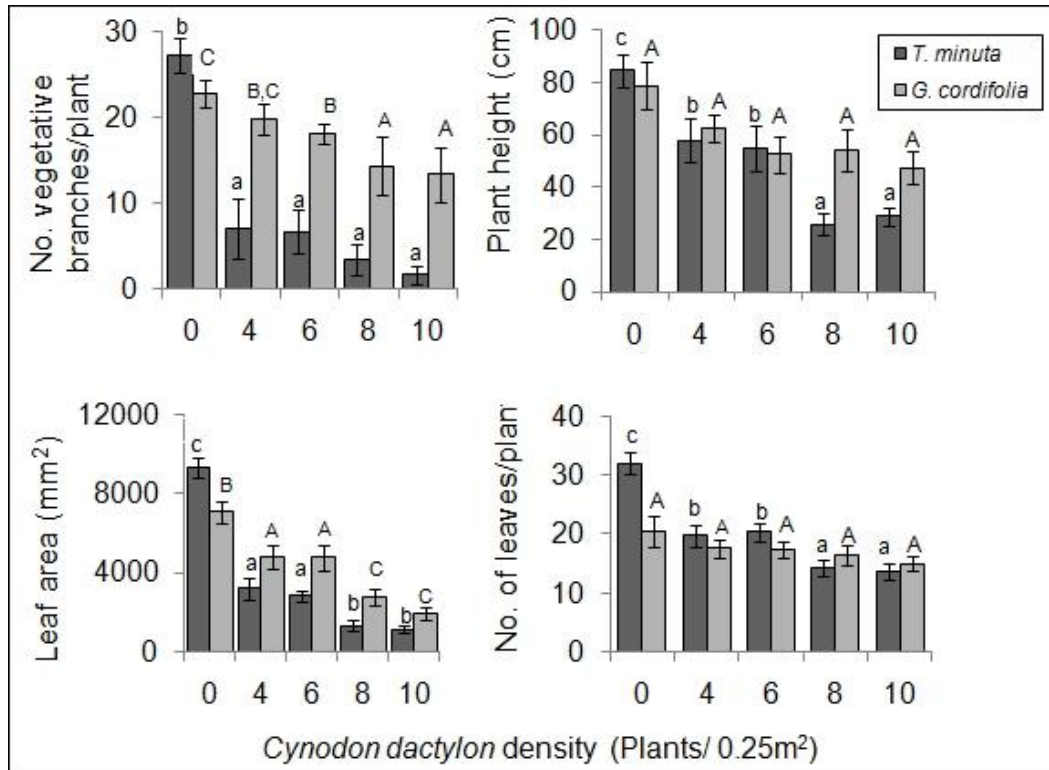


Fig. 12: *Cynodon dactylon* varying density effects on the number of vegetative branches per plant, number of leaves per plant, seedling height and leaf area per plant of *T. minuta* and *G. cordifolia* in a screen house experiment. Bars with dissimilar letters are significantly different by Fisher LSD at $p = 0.05$

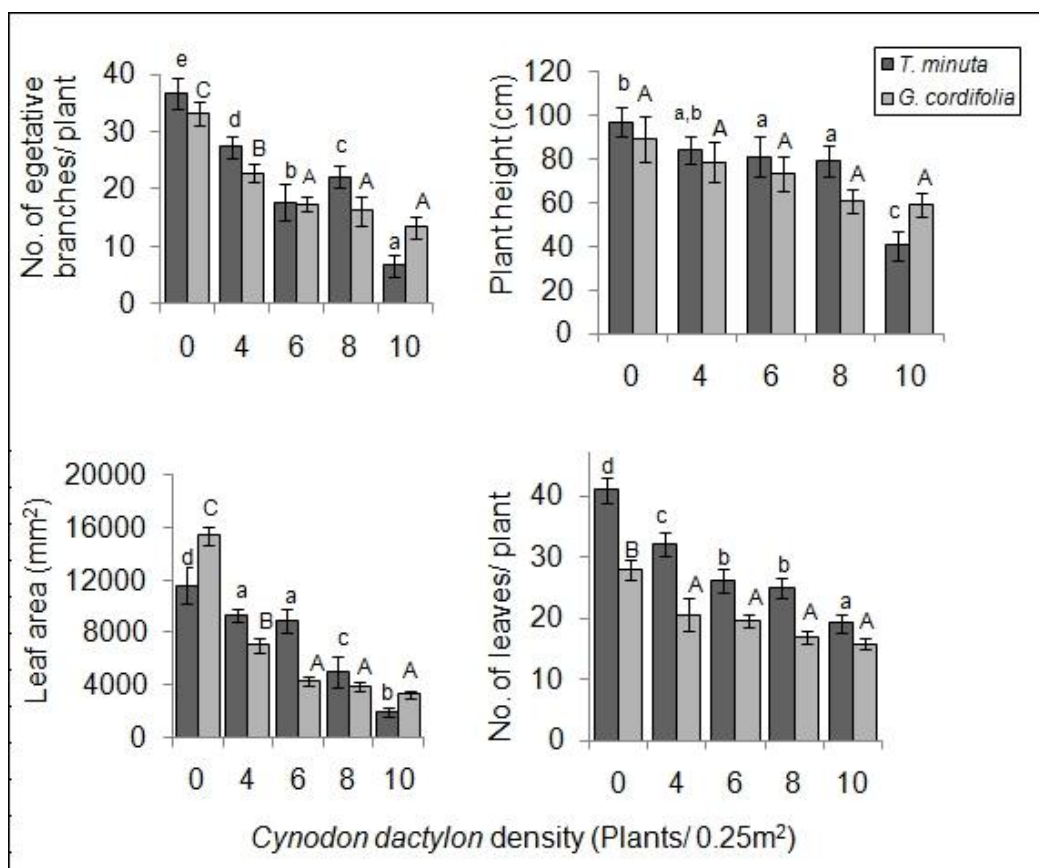


Fig. 13: *Cynodon dactylon* varying density effects on the number of vegetative branches per plant, number of leaves per plant, seedling height and leaf area per plant of *T. minuta* and *G. cordifolia* in field experiment. Bars with dissimilar letters are significantly different by Fisher LSD at $p = 0.05$

Shoot diameter and shoot biomass differed significantly across the five *C. dactylon* densities in both *T. minuta* and *G. cordifolia* ($p < 0.05$). *Tagetes minuta* and *G. cordifolia* in pots/plots with *C. dactylon* density ≥ 8 per pot or plot had half the diameter and were twice as light as *T. minuta* and *G. cordifolia* contained in control pots/plots. Mean root biomass and root length differed significantly only in *T. minuta* ($p < 0.05$) but not in *G. cordifolia* ($p > 0.05$) (Figs. 14 and 15). *Tagetes minuta* in pots/plots with *C. dactylon* density ≥ 8 per pot or plot had roots with over four times lighter weight and half the length of roots of *T. minuta* in control pots/plots (Tables 4 and 5).

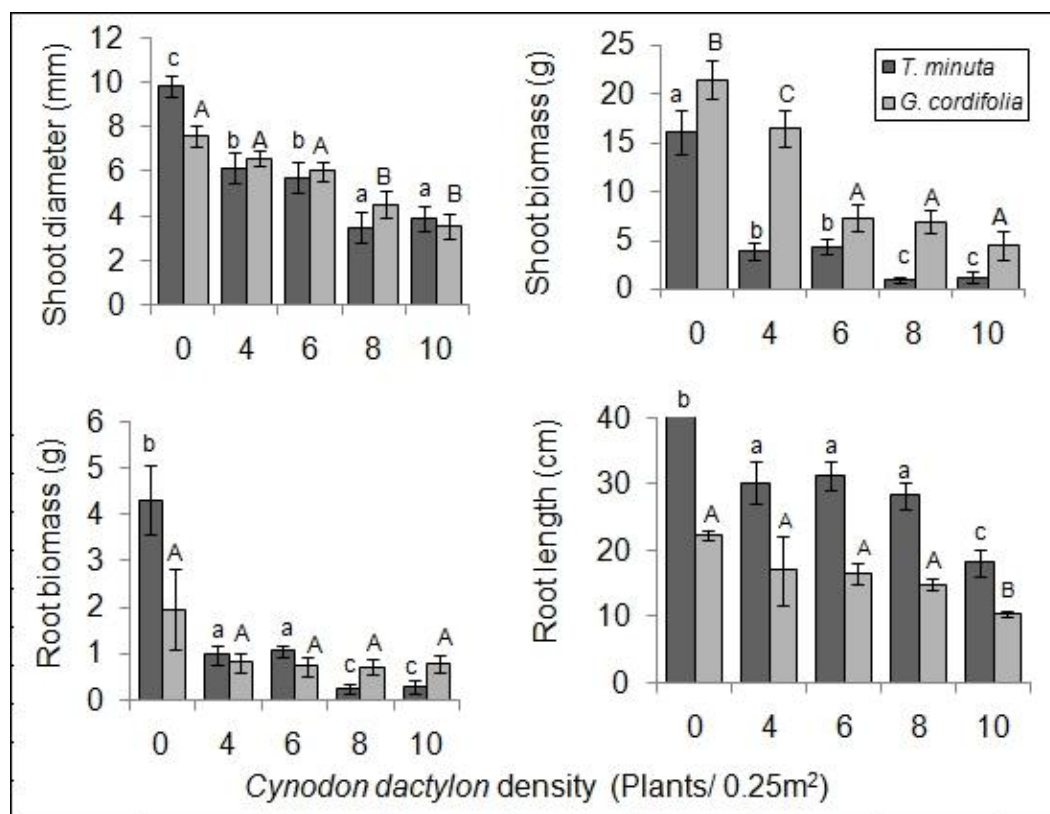


Fig. 14: *Cynodon dactylon* varying density effects on mean shoot diameter, root biomass, shoot biomass and root length of *T. minuta* and *G. cordifolia* intercrops in a screen house experiment. Bars with dissimilar letters are significantly different by Fisher LSD at $p = 0.05$

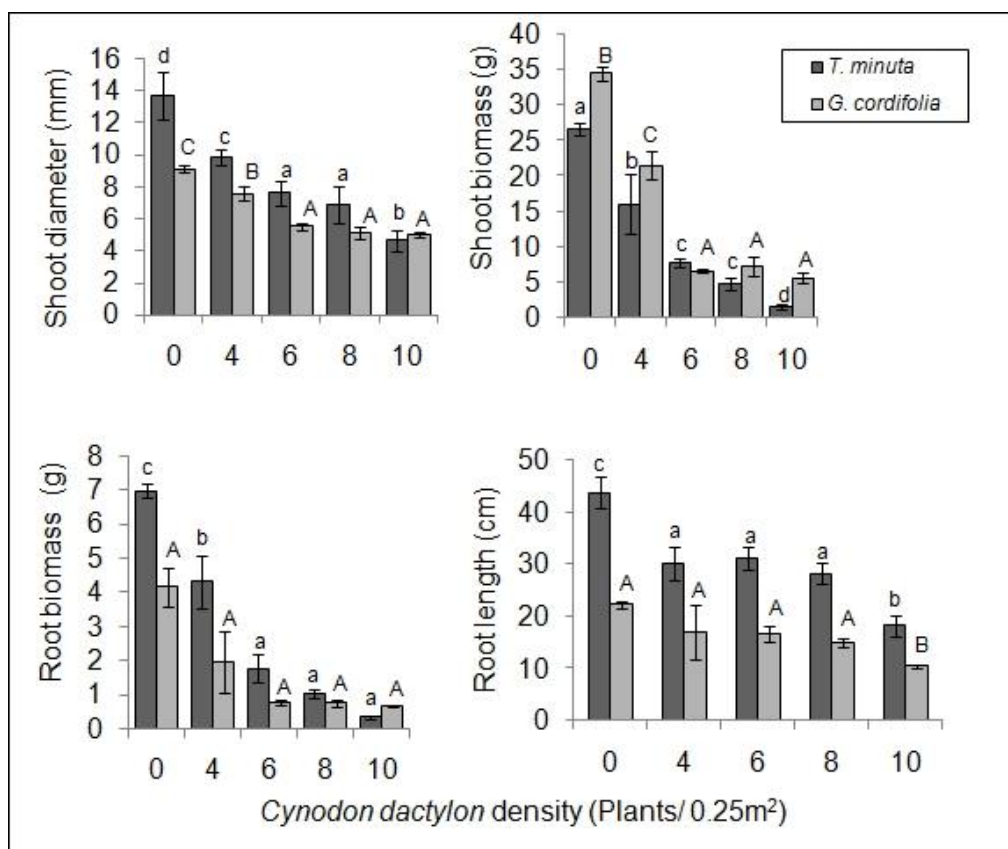


Fig. 15: *Cynodon dactylon* varying density effects on mean shoot diameter, root biomass, shoot biomass and root length of *T. minuta* and *G. cordifolia* intercrops in field plot experiment. Bars with dissimilar letters are significantly different by Fisher LSD at $p = 0.05$

4.3.3 Density dependent competitive effects on *Tagetes minuta* and *Gutenbergia cordifolia*: Leaf pigmentation

In both *T. minuta* and *G. cordifolia*, total leaf chlorophyll content differed significantly across the five *C. dactylon* treatments that were planted separately ($p < 0.05$) (Fig. 16; Tables 4 and 5). *Tagetes minuta* and *G. cordifolia* in control pots/plots had three times total leaf chlorophyll than *T. minuta* and *G. cordifolia* contained in pots/plots with *C. dactylon* density ≥ 8 per pot or plot.

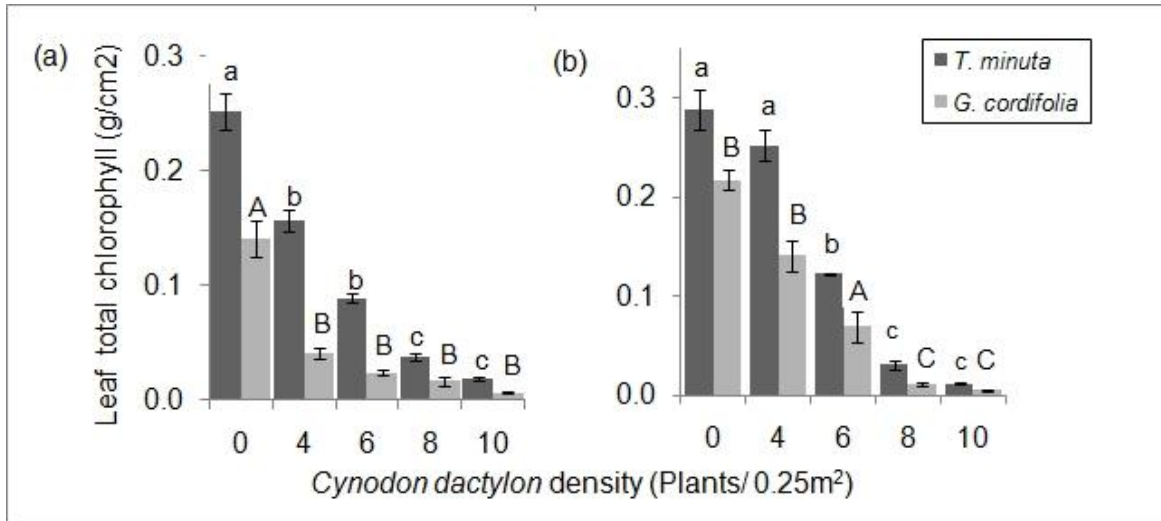


Fig. 16: Total leaf chlorophyll content of *T. minuta* and *G. cordifolia* planted with various *C. dactylon* densities (a) in screen house and (b) field plot experiments. Bars with dissimilar letters are significantly different by Fisher LSD at $p = 0.05$

Leaf anthocyanin concentrations differed significantly across the five *C. dactylon* treatments in both *T. minuta* and *G. cordifolia* ($p < 0.05$) (Fig. 17; Tables 4 and 5). *Tagetes minuta* and *G. cordifolia* contained in pots/plots with *C. dactylon* density ≥ 8 per pot or plot had twice the total leaf anthocyanin than *T. minuta* and *G. cordifolia* in control pots/plots.

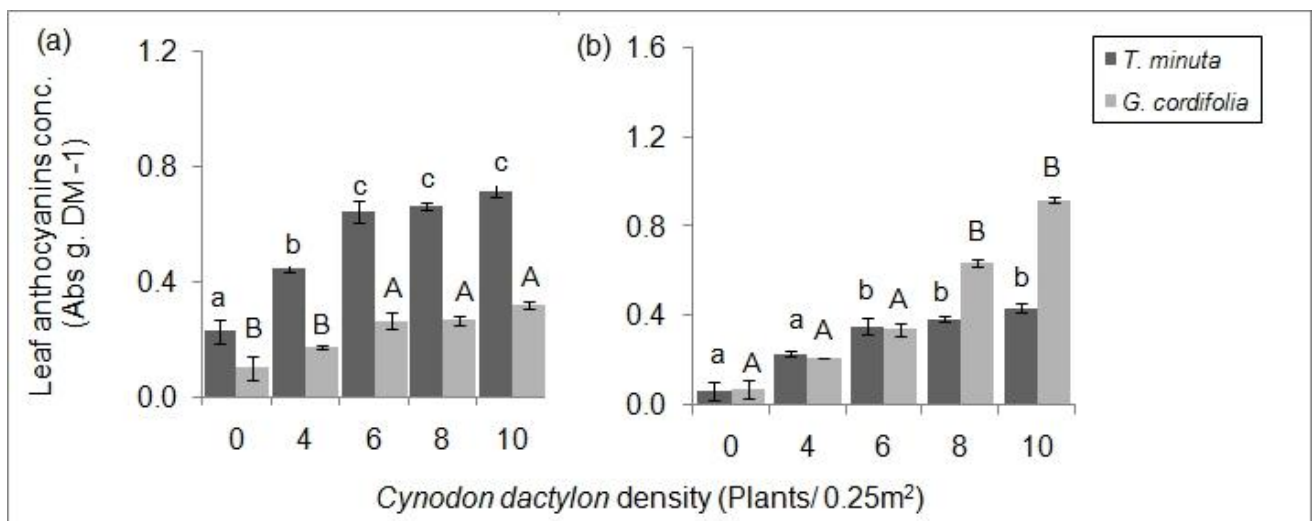


Fig. 17: Total leaf anthocyanins of *T. minuta* and *G. cordifolia* planted with various *C. dactylon* densities (a) in screen house and (b) field plot experiments.

While the mean number of vegetative branches per plant, leaves per plant, seedling height, mean leaf area per plant, shoot diameter, shoot biomass, root length and leaf total chlorophyll content of both *T. minuta* and *G. cordifolia* species were strongly negatively correlated with increasing density of *C. dactylon*; leaf anthocyanin content was strongly positively correlated with increasing density of *C. dactylon* (Figs. 18, 19, 20 and 21).

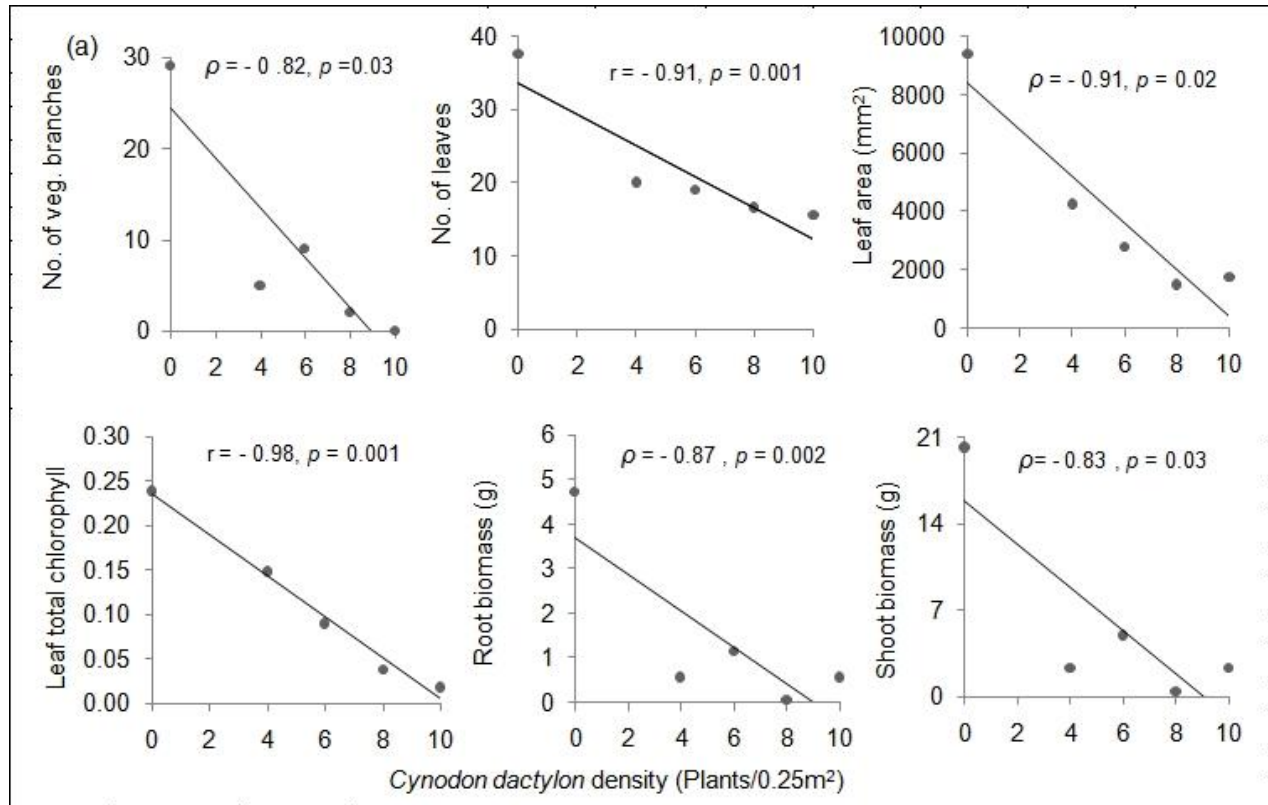


Fig. 18(a): Correlation between the density of *C. dactylon* per pot and *T. minuta*' number of vegetative branches, leaves per plant, leaf area per plant, leaf total chlorophyll content, root biomass and shoot biomass in a screen house experiment.

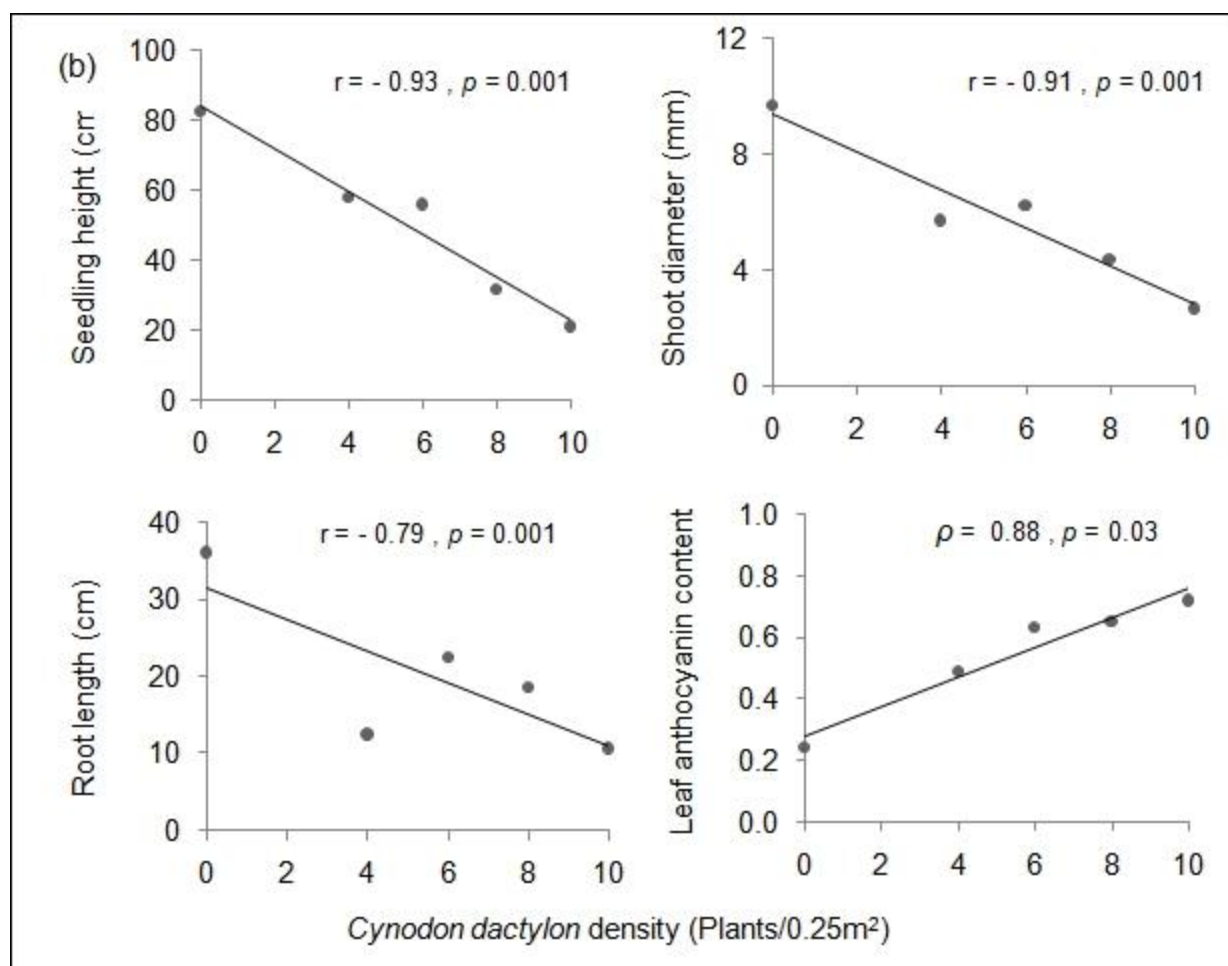


Fig. 18(b): Correlation between the density of *C. dactylon* per pot and *T. minuta*' seedling height, shoot diameter, root length, and leaf anthocyanins content in a screen house experiment.

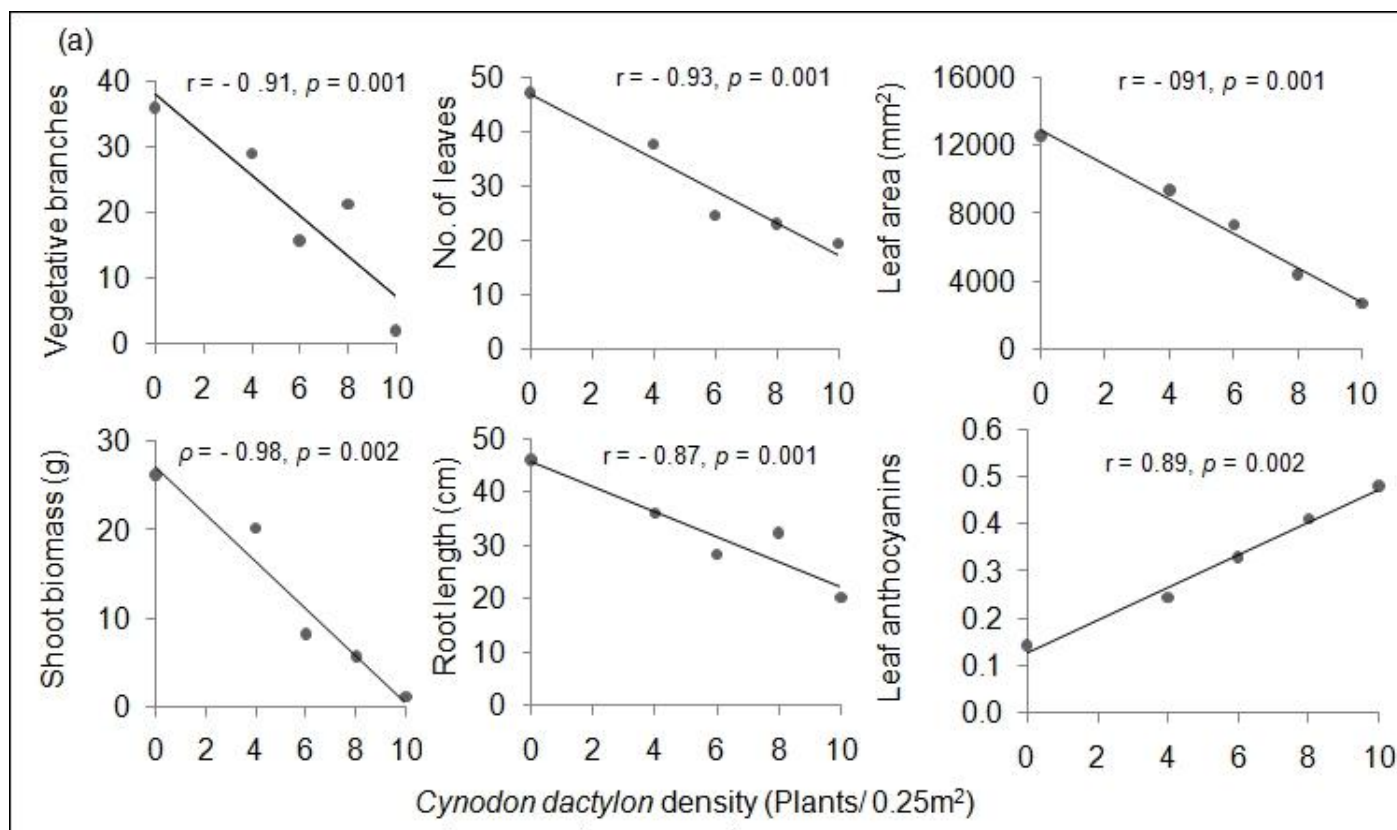


Fig. 19(a): Correlation between the density of *C. dactylon* per plot and *T. minuta*' number of vegetative branches, number of leaves per plant, leaf area per plant, shoot biomass, root length, and leaf anthocyanins content in the field experiment.

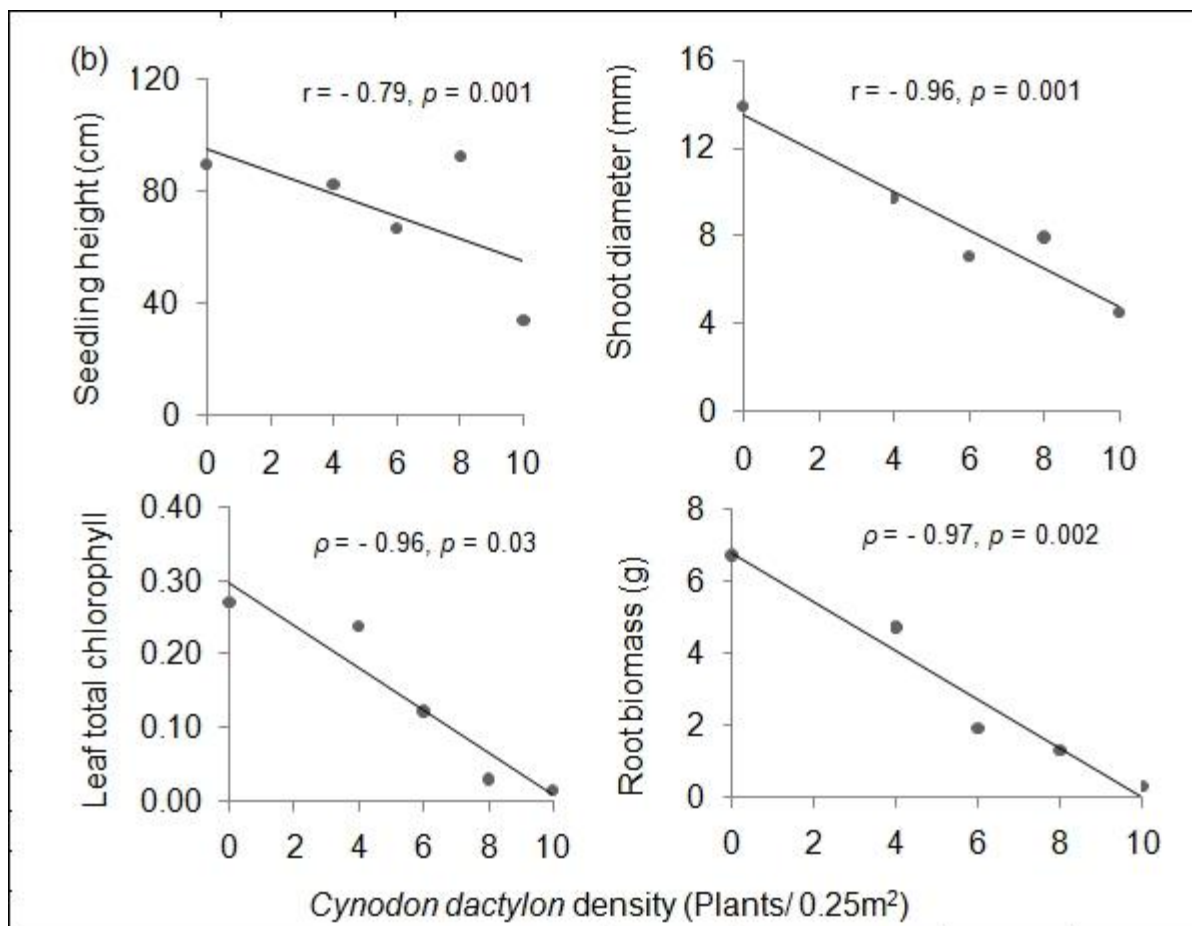


Fig. 19(b): Correlation between the density of *C. dactylon* per plot and *T. minuta*' seedling height, shoot diameter, leaf total chlorophyll content and root biomass in the field experiment.

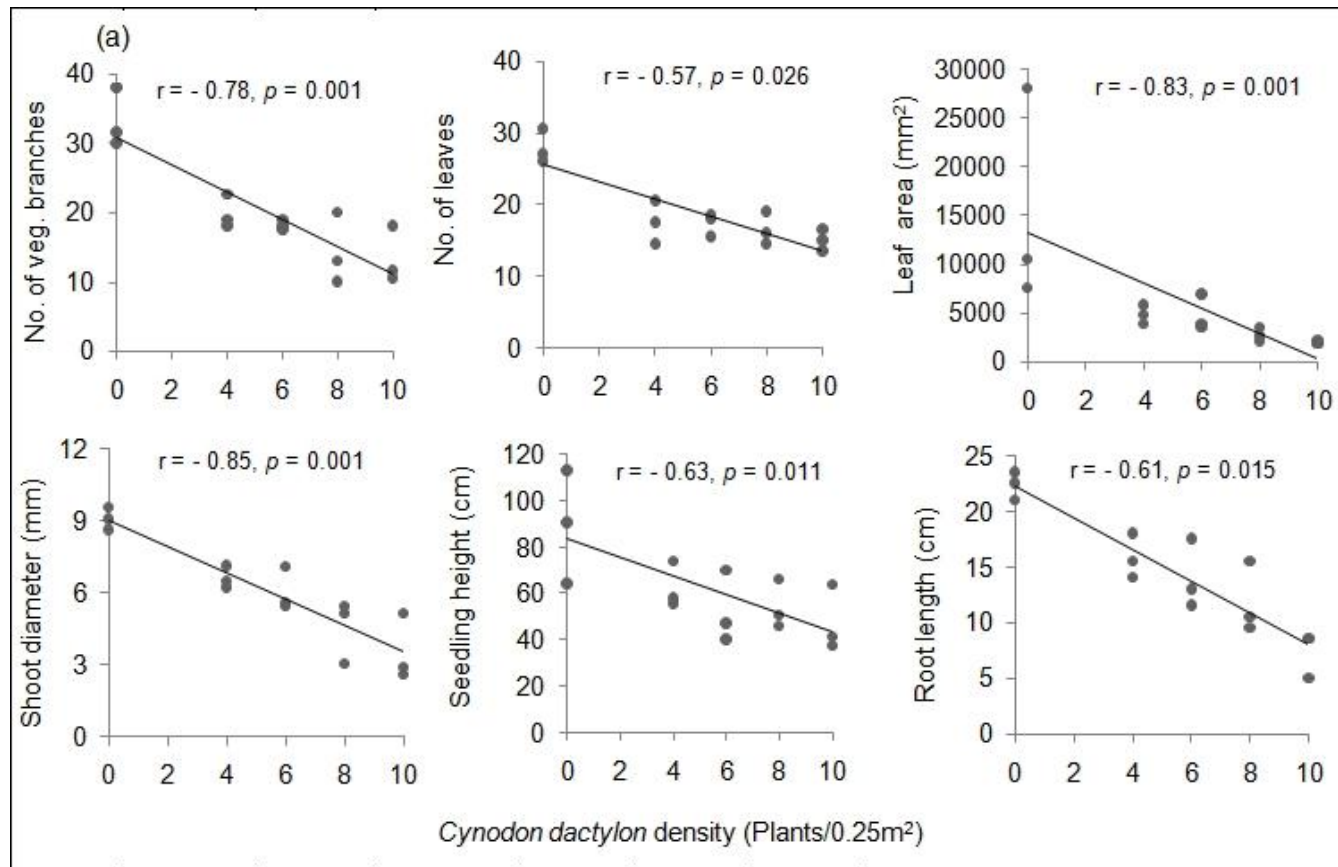


Fig. 20(a): Correlation between the density of *C. dactylon* per pot and *G. cordifolia*' number of vegetative branches, number of leaves per plant, leaf area per plant, shoot diameter, seedling height and root length in a screen house experiment.

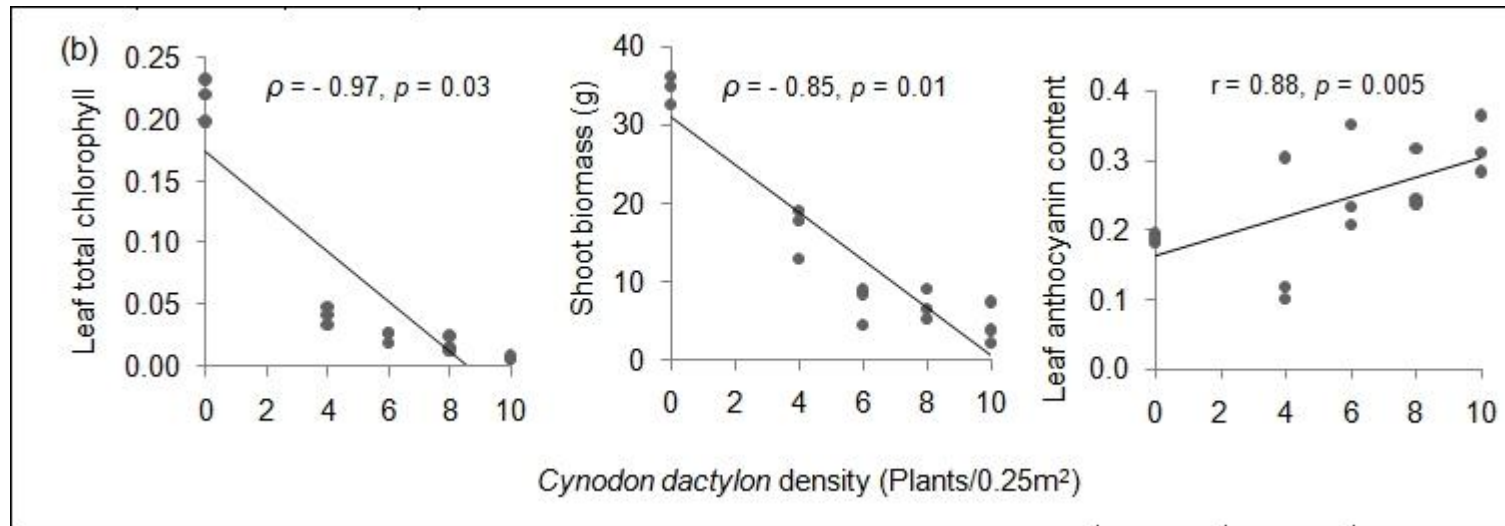


Fig. 20(b): Correlation between the density of *C. dactylon* per pot and *G. cordifolia*' leaf total chlorophyll content, shoot biomass and leaf anthocyanins content in a screen house experiment.

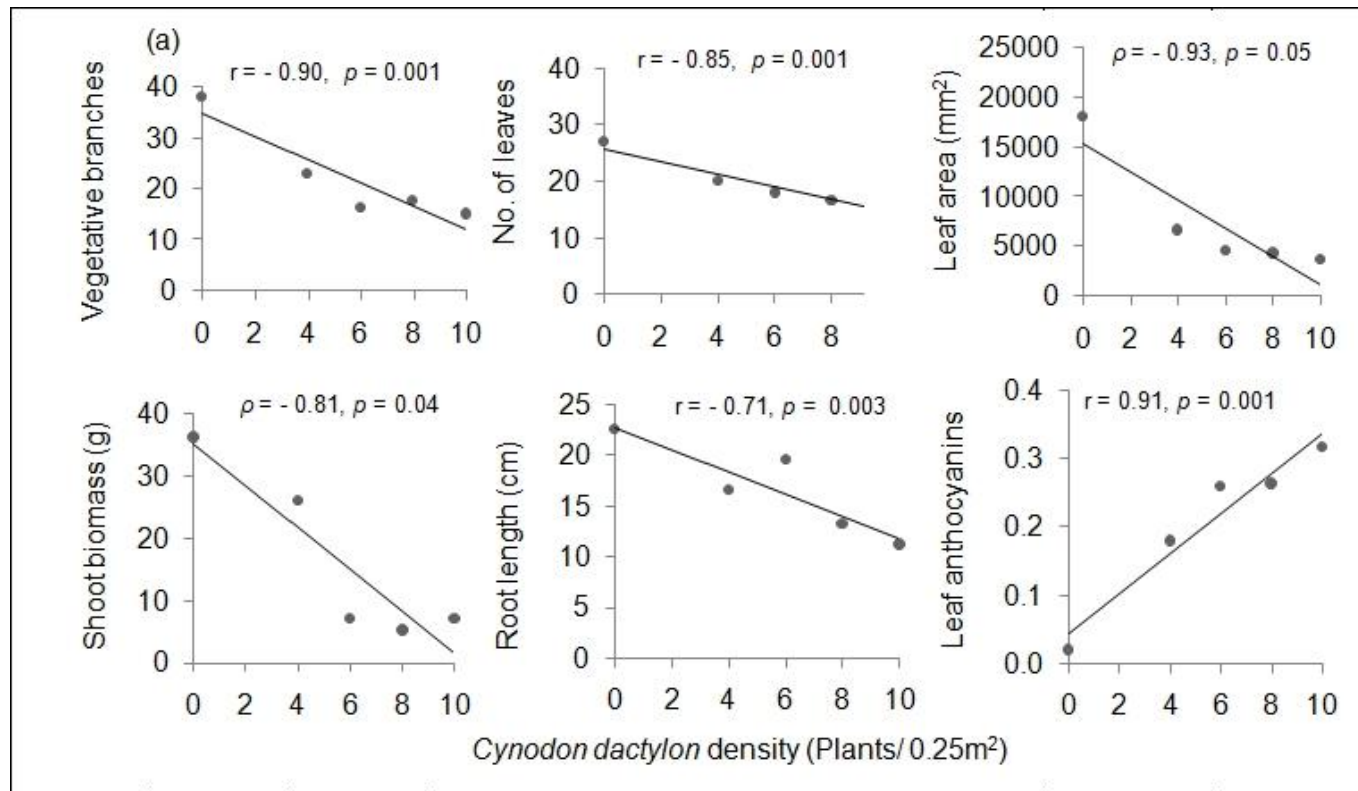


Fig. 21(a): Correlation between the density of *C. dactylon* per plot and *G. cordifolia*' number of vegetative branches, leaves per plant, leaf area per plant, shoot biomass, root length and leaf anthocyanins content in field experiment.

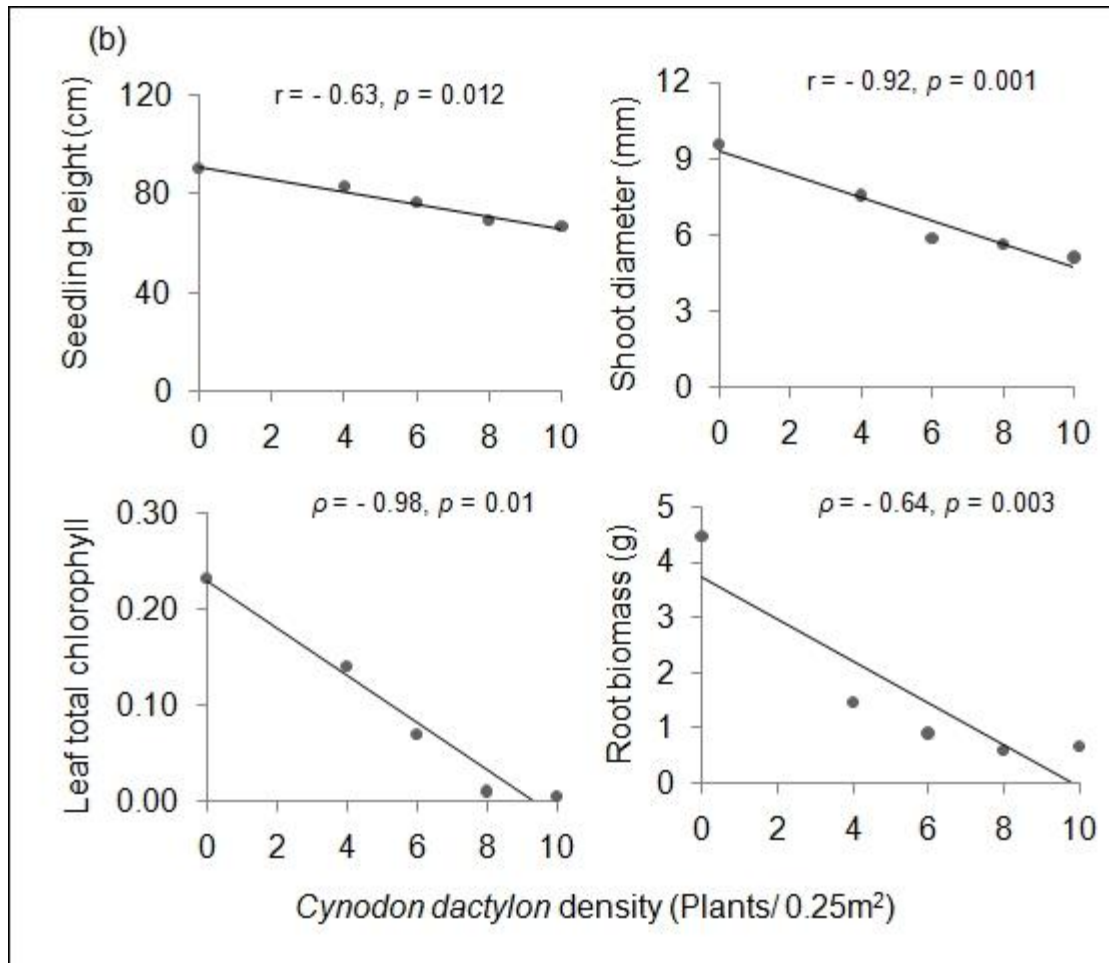


Fig. 21(b): Correlation between the density of *C. dactylon* per plot and *G. cordifolia*' seedling height, shoot diameter, leaf total chlorophyll content and root biomass in field experiment.

4.4 Discussion

4.4.1 *Cynodon dactylon* density dependent competitive effects on *Tagetes minuta* and *Gutenbergia cordifolia* growth parameters

While increasing densities of *C. dactylon* decreased seedling height, shoot diameter, shoot biomass, leaf area, root biomass, the number of vegetative branches, leaves and root length of *T. minuta*, only shoot diameter, shoot biomass, the number of vegetative branches and leaf area were affected in *G. cordifolia*. Plant height, leaf area and number of leaves have ensured plants to intercept up to a recommended 95% of the incoming solar radiation for photosynthesis (Falster and Westoby, 2003; Brougham, 1956) while shoot diameter and biomass aid in overcoming stresses such as trampling by animals and wind destruction (Personal observation). The higher negative competitive effects of *T. minuta* versus *G. cordifolia* can be associated with the former species' root morphology, which was shorter and lighter compared to those of the latter species. Interestingly, *G. cordifolia* root weight and length were observed to be heavy and long, respectively, and, hence, render this species less prone to be suppressed by competition. As predicted, the negative competitive effects were more pronounced with increasing densities of *C. dactylon*, likely due to increased competition for available nutrients and space, in which *C. dactylon* out-competed the two invasive weeds. Competitiveness of *C. dactylon* has been associated with its stoloniferous nature and an ability to develop deep roots (Horowitz, 1973) that easily escape the effects of inter-specific competition. While monocultures from invasive plants have been reported to be suppressive to native species (Martina and Von Ende, 2008) intercrops were shown to be mostly of interspecific facilitative nature, especially in maize-legume combinations (Zhang and Li, 2003; Wang *et al.*, 2014). In contrast, in this study particularly the *C. dactylon* density dependent competition resulting from inter-planting *C. dactylon* / *T. minuta* and or *C. dactylon* / *G. cordifolia* can potentially reduce invasiveness of the two weeds as weed' growth parameters that are crucial for plant fitness were significantly reduced.

4.4.2 *Cynodon dactylon* density dependent competitive effects on *Tagetes minuta* and *Gutierrezia cordifolia* leaf pigmentations

A drop in leaf total chlorophyll content was observed in both *T. minuta* and *G. cordifolia* when inter-cropped with *C. dactylon*, which could be due to the weed species' reduced access to available limited resources (water, nutrients and space). As Nitrogen, for example, becomes less available to a particular plant, its chlorophyll production is reduced (Zhao *et al.*, 2005; Boussadia *et al.*, 2010) and, consecutively, its leaf chlorophyll content. The results of this study imply that an increase in *C. dactylon* density has a potential of exerting enough stress to affect the two weeds' chlorophyll productivity. Leaf chlorophyll content has been linked to plant health status (Pavlovik, 2014) as it is associated with energy production and, hence, important for other metabolic activities (Pavlovik, 2014). Plants with reduced chlorophyll amount and, thereby, reduced photosynthetic capacity (Boussadia *et al.*, 2010) also possess flowers with accelerated abscission (Saifuddin *et al.*, 2010), which reduces chances of dispersal by pollinators. Reduced dispersal of the two weeds will reduce the chance for weed's monoculture formation, which have been proven to be devastating in an invaded ecosystem (Cannell, 1999; Martina and Von Ende, 2008). Increasing density of *C. dactylon* in *T. minuta* and or *G. cordifolia* invaded areas therefore, can potentially be used as an environmentally friendly invasive species management approach.

The study observed an increasing anthocyanin concentration in *T. minuta* and *G. cordifolia* with increasing numbers of *C. dactylon*. Anthocyanins, which are a small group of pigments within flavonoids, form red-blue coloration in most plants (Neill, 2002). The increase of anthocyanin levels in plant leaves under increasing *C. dactylon* densities in this study can be linked to the increasing level of competition, specifically for nutrients and space due to increasing density of *C. dactylon*. This is in line with Chalker-Scott (1999) who argued that anthocyanin induction and / or accumulation in a plant tissue can be associated with nitrogen and / or phosphorus deficiency. Allelopathic effects posed by *C. dactylon* as a competition strategy (Mahmoodzadeh and Mahmoodzadeh, 2013) could be another possible cause of increased anthocyanins in leaves of both *T. minuta* and *G. cordifolia* exposed to increasing density of *C. dactylon*. Generally, the presence of these pigmentations in leaves is normally associated with stressors (Neill, 2002). In this study, the stressors that have possibly induced increased anthocyanins in leaves of both *T.*

minuta and *G. cordifolia* were probably allelopathic effects of *C. dactylon* and competition for the available nutrients. It is a known fact that the rate of photosynthesis is directly proportional to plant's chlorophyll content (Emerson, 1929) intercepting solar radiation. Anthocyanin pigments reduce a plant's chlorophyll content, thereby negatively affecting photosynthesis. Therefore, the study suggest that treating the two invasive plants with increasing densities of *C. dactylon* can be used to biologically manage them efficiently.

4.5 Conclusion

In this study, shorter seedling height, smaller shoot diameter, smaller leaf area and lower shoot biomass of *T. minuta* and *G. cordifolia* under higher *C. dactylon* densities reduces both *T. minuta* and *G. cordifolia* fitness. Moreover, reduced leaf total chlorophyll and increased anthocyanin levels in leaves affects the photosynthetic ability of both invasives *T. minuta* and *G. cordifolia*. The net effect, therefore, is the development of weaker *T. minuta* and *G. cordifolia* plants that are easily affected by other stressors such as animal trampling and, thus, can be managed accordingly. Based on results of this study, therefore, treating *T. minuta* and *G. cordifolia* with *C. dactylon* density ≥ 8 per 0.25 m^2 negatively affects growth and vigor of the two invasive plants. This potentially provides an environmentally friendly management option in areas where manual weeding or herbicide use is not feasible. The experimental setup in both the screen house and in the field highlighted the critical density of *C. dactylon* that should be used to suppress invasives and highlights the option of finding alternative ways to suppress weeds, particularly in rangelands or protected areas.

CHAPTER FIVE

This chapter demonstrates the effects of crude extracts of Desmodium spp on the growth and development of invasive weeds Tagetes minuta and Gutenbergia cordifolia. Generally, higher concentration ($\geq 62.5\%$) of Desmodium uncinatum leaf extract strongly affected the two weed's growth and development, therefore can be used as a management strategy for the two weeds.

Demonstrative effects of crude extracts of *Desmodium* spp. to fight against the invasive weed species *Tagetes minuta*⁴ and *Gutenbergia cordifolia*⁵

Abstract

Methods that are both effective and ecologically safe to suppress invasives are urgently needed but have rarely been used, thus, highlighting the need to devise and test ones. In this study the effects of two *Desmodium* spp extracts on *T. minuta* and *G. cordifolia* was tested and germination rate, height, fresh weight and leaf total chlorophyll content of *T. minuta* and *G. cordifolia* seedlings after thirty days in both laboratory and screen house separately was assessed to understand *T. minuta* and *G. cordifolia* vigor after treatment. Seedling germination rate was halved only by *Desmodium uncinatum* leaf extract (DuL), with higher concentrations ($\geq 62.5\%$) rather than lower concentrations ($< 62.5\%$). Likewise, in both laboratory and screen house experiments, germination rate under DuL treatments was observed to decline with increasing concentrations. Seedling height, fresh weight and leaf total chlorophyll content (Chl) were also observed to be affected mostly by DuL treatments rather than *D. uncinatum* root extract (DuR), *Desmodium intortum* leaf extract (DiL) and *D. intortum* root extract (DiR) treatments. Generally, seedlings treated with higher DuL treatments were half as tall, had one-third the weight and half the leaf Chl content compared to those treated with lower concentrations. This study shows a novel tool to be applied where *T. minuta* and *G. cordifolia* may be driving native flora and fauna to local extinction.

Keywords: *alien species, germination, biomass, chlorophyll, management, seedling, bio-herbicide, black mint, allelopathy, ecological invasions.*

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⁵Published by Springer International Publishing AG, Journal: SpringerPlus, DOI 10.1186/s40064-016-3480-y

5.1 Introduction

The management of invasive plant species in protected areas has posed a lot of challenges to both managers and conservationists, due to possible effects of herbicides on other native plant species and environment (Kimmins, 1975; Poorter, 2007; Kughur, 2012). While methods such as mechanical uprooting can be applied, they are only a short term remedy as a lot of invasive plant seeds remain in the soil seed bank. Moreover, this method often results in high operational costs and is, therefore, economically unfeasible. Both *T. minuta* and *G. cordifolia* are unpalatable and toxic invasive plants to human and animals, *G. cordifolia* for instance, can result in both allergies and toxicity to animals due to presence of germacranolides (Zdero and Bohlmann, 1990; Bussmann *et al.*, 2006). In Kenya, *T. minuta* was first recorded as an exotic weed during the 1920s, originally restricted to higher altitudes but increasingly spreading to lower ones (Stadler *et al.*, 1998). *G. cordifolia* on the other hand was observed to be dominant over several square miles of the Ngorongoro Crater (Beentje, 2000) in the Ngorongoro Conservation Area (NCA) in 1962. Recently, the two species have been reported to invade most of the Serengeti ecosystem and, particularly *G. cordifolia* dominates almost over a half of the entire Tanzania's Ngorongoro crater floor (250 km²) (UNESCO, 2001).

As most invasive species do, *T. minuta* and *G. cordifolia* roots produce allelo-chemicals which delays germination, suppresses native plants and, thus, reduces pasture area (Li *et al.*, 2010). Allelopathic plants have various effects from inhibition of nodulation in legumes (Murthy and Ravindra, 1974; Rice, 1979) to changing the amount of chlorophyll produced and respiratory inhibition on nearby native plant species (Reza, 2016). Considerably less research has been done on allelopathic effects of herbaceous angiosperms compared to woody plants in forests (Rice, 1979). Mechanical removal, traditional and chemical controls have been recommended to be applied whenever an invasive weed invades an area (Mada *et al.*, 2013). However, as weed control using chemical herbicides is often not advised in natural ecosystems including protected rangelands, less ecologically intrusive management options must be adopted. Fortunately, the possibility of using native plants with allelopathic properties as a potential bio-herbicide has recently been reported (Khanh *et al.*, 2007; Khan *et al.*, 2008; Sodaieizadeh and Hosseini, 2012).

Successful management of a particular weed strongly depends on the available knowledge of its life history. While the medicinal use of both *T. minuta* and *G. cordifolia* have been well studied (Koch *et al.*, 2005; Sadia *et al.*, 2013; Ngezahayo *et al.*, 2015) little is known about their germination and growth characteristics as a pre-caution for their control once they escape as weeds, especially in protected areas such as NCA. The study therefore, chose to investigate the allelopathic effects of leaf and root crude extracts of two commonly known allelopathic *Desmodium* species (*Desmodium uncinatum* and *Desmodium intortum*) (Reinhardt and Tesfamichael, 2012; Pickett *et al.*, 2013) on the germination and growth characteristics of *T. minuta* and *G. cordifolia*. The two species are generally preferred by herbivores (Heuze V *et al.*, 2015; Heuze *et al.*, 2015) and can be inter-planted in invaded areas to potentially suppress *T. minuta* and *G. cordifolia*. While providing feed to animals, particularly *D. uncinatum* is known to successfully control some very problematic weeds such as *Striga* species (Khan *et al.*, 2006; Pickett *et al.*, 2013). The mechanism behind *D. uncinatum* allelopathic nature is reportedly due to its possession of higher iso-flavonoids particularly uncinanone C and di-C- glycosylflavone, which have recently been reported to inhibit the growth of other plants (Khan *et al.*, 2008). However, little have been done to study the effects of *D. uncinatum* and *D. intortum* root and leaf crude extracts on germination and growth of *T. minuta* and *G. cordifolia*, which is of particular interest in devising a management option to suppress the two invasive plants in protected areas. Thus, using extracts from the two *Desmodium* species might be a highly successful, eco-friendly and cheap *T. minuta* and *G. cordifolia* management tool. Therefore, the study assessed the effects of root and leaf crude extracts of *D. uncinatum* and *D. intortum* on the seed germination, seedling height, seedling fresh weight and leaf total chlorophyll content of *T. minuta* and *G. cordifolia*. It was hypothesized that, the crude extracts will reduce germination rate and seedling height of the two invasive plants, which are important measures of seedling vigor, and that it will also reduce seedling biomass and leaf chlorophyll content. It was further expected that higher concentrations of the extracts will lead to stronger reduction of the four parameters and *D. uncinatum* and *D. intortum* extracts will have similar effects.

5.2 Material and methods

5.2.1 Laboratory study design

The effects of *D. uncinatum* and *D. intortum* leaf and root crude extracts on the seed germination, seedling height, leaf chlorophyll content and fresh weight (biomass) of *T. minuta* and *G. cordifolia* were studied using a completely randomized design from October to November, 2015. Ten seeds of each *T. minuta* and *G. cordifolia* were placed separately in each of six petri dishes (70.84 cm² surface area) lined with cotton wool, and subjected to six different concentration treatments, each treatment was replicated three times, which summed up to 144 (72 each) samples overall. Distilled water was added ad libitum to moisten the seeds. Seeds were observed every day and the number of germinated seeds were recorded and counted for 30 days. After 30 days, seedlings were harvested and fresh weight, seedling height, leaf total chlorophyll content and root length were determined for each germinated seedling.

5.2.2 Screen house study design

The effects of leaf and root crude extracts of *D. uncinatum* and *D. intortum* on the seed germination, seedling height, leaf chlorophyll content, root length and fresh weight of *T. minuta* and *G. cordifolia* were studied using a completely randomized design in a screen house from June to July and October to November, 2015 respectively. 10 seeds of each *T. minuta* and *G. cordifolia* were placed separately in each of six pots (763.82 cm² surface area) under six different concentration treatments. Each treatment was replicated three times (n = 144). Normal tap water was added ad libitum. Seeds were observed every day and the number of germinated seeds were recorded and counted for 30 days. After 30 days, seedlings were harvested and fresh weight, seedling height, root length and leaf total chlorophyll content was determined for each germinated seedling.

5.2.3 Root and leaf crude extract preparation

Fresh roots and leaves from young *D. uncinatum* and *D. intortum* were collected from the Livestock Training Institute (LITI), Tengeru demonstration plots, in early January 2015. Roots and leaves were air dried under room temperature for fourteen days, ground into powder and stored in sealed plastic bags prior to experiment. Extracts were prepared according to Namkeleja

et al. (2014a) as follows: for each species, 100 g of root and leaf powder were soaked separately in 1 l of distilled water and left for 72 h, after which the crude extracts were filtered using Watsman filter paper No. 1 to obtain a final volume of 1l each. Both crude extracts (ml) were diluted with distilled water (ml) in the ratio of; 0:100, 12.5:87.5, 25:75, 37.5:62.5, 50:50, 62.5:37.5, 75:25 and 100:0 (extract: distilled water) to obtain different treatment concentrations (100ml each) of 0%, 12.5%, 25%, 37.5%, 50%, 62.5%, 75% and 100%. The diluted extracts were stored at 4°C prior to experiment.

5.2.4 *T. minuta* and *G. cordifolia* seed preparation and treatment

Seeds of *T. minuta* and *G. cordifolia* were collected from Gomba Estate farms in Arusha in late August 2014 and Ngorongoro Crater of the Ngorongoro Conservation Area Authority in late August 2015 respectively. Prior to the experiment, the seeds were air dried and stored in plastic bags. *T. minuta* and *G. cordifolia* seed viability was determined by germination testing (Wildfong, 2014), in which all fifteen seeds (100%) from each *T. minuta* and *G. cordifolia*, were selected randomly from a seed stock and planted separately in two petri dishes lined with cotton wool in early May and September 2015 respectively, germinated. Seeds were washed using tap water and sterilized with 5 % NaOCl for 2 minutes, then rinsed with distilled water before planting. Each petri dish / pot was irrigated once with 10 ml/100ml respectively, of the different solution treatments. The seeds that were treated with distilled water ($T_0 = 0\%$) were taken as a control.

5.2.5 Leaf total chlorophyll determination

Chlorophyll of *T. minuta* and *G. cordifolia* seedlings was extracted according to Hiscox and Israelstam (1979) with some modifications: 50 mg of fresh leaves of *G. cordifolia* of 2.25 cm² were immersed in 4 ml of Dimethyl Sulfoxide (DMSO) and incubated at 65 °C for 12 h. The extract was transferred to glass cuvettes for absorbance determination. The absorbance of blank liquid (DMSO) and samples were determined under 2000 UV/Vis spectrophotometer (UNICO®) at 645 and 663 nm (Hiscox and Israelstam, 1979) and the leaf total chlorophyll (Chl) calculated according to Arnon (1949) using the following equation:

$$Total\ Chl = 0.0202 A_{663} + 0.00802 A_{645}$$

Where: A_{663} and A_{645} are absorbance readings at 663 nm and 645 nm, respectively.

5.2.6 Statistical analysis

Shapiro-Wilk test for normality was performed on seedling height, fresh weight and leaf total Chl contents of *T. minuta* and *G. cordifolia* under all treatment types and levels (DuL, DuR, DiL and DiR) in both, laboratory and screen house experiments. Also, percentage germination data were arcsine transformed and tested for normality. For all data that passed normality test, one-way analysis of variance (ANOVA) test was carried out whilst those data which did not met the normal distribution test criteria, a Kruskal-Wallis test was performed (Alhammadi, 2008). Therefore one-way ANOVA was performed on seedling's height and fresh weight in DuL, DuR, DiL and DiR, and leaf total chlorophyll contents in DuR, DiL and DiR, while Kruskal –Wallis test was performed on *G. cordifolia* seedling germination under DuL, DuR, DiL and DiR treatments and leaf total Chl contents under DuL; and on *T. minuta* seedling's height, fresh weight and leaf total Chl contents under both *D. uncinatum* and *D. intortum* root and leaf crude extracts. The statistical software used was STATISTICA version 8 (StatSoft-Inc., 2007), the resulting means were separated by the Fisher's Least Significant Difference (LSD) test at $p = 0.05$.

5.3 Results

5.3.1 Allelopathic effects on seed germination

Generally, higher concentrations ($\geq 62.5\%$) of *D. uncinatum* leaf treatments in both laboratory and screen house experiments were effective in suppressing *T. minuta* and *G. cordifolia* seed germination ($p < 0.05$) (Plate 8).

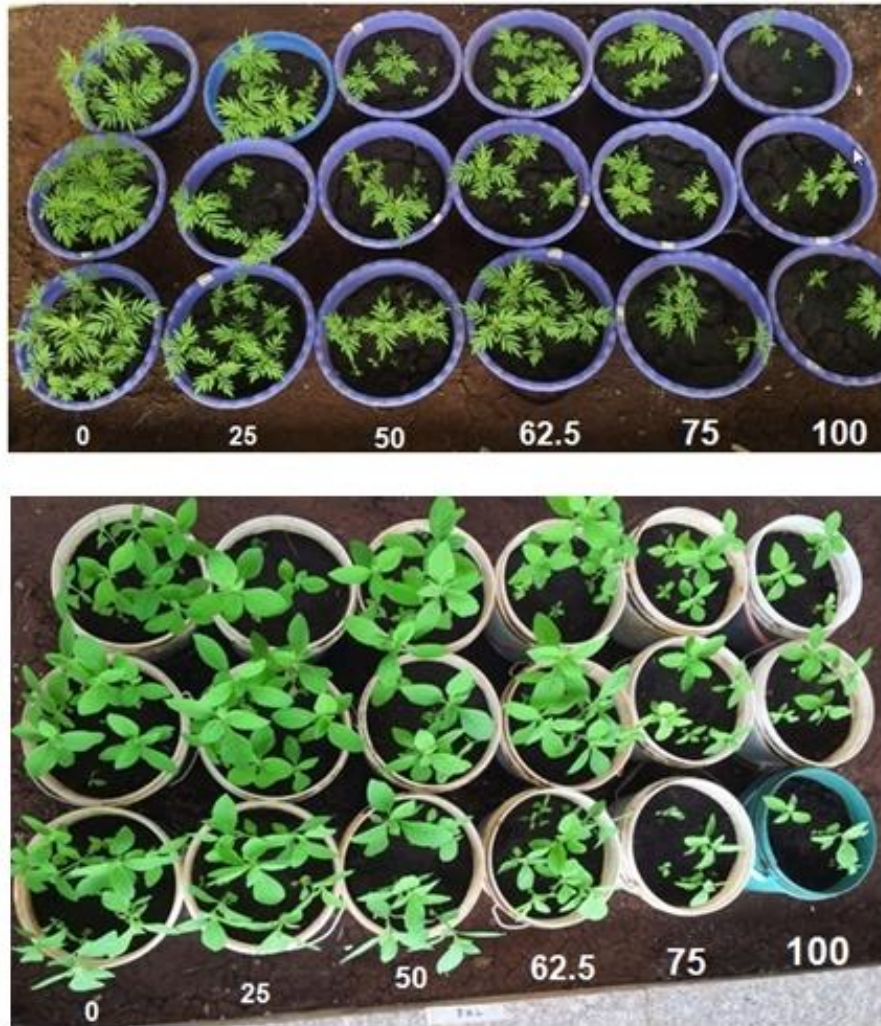


Plate 8: Seedling germination patterns in *T. minuta* (top) and *G. cordifolia* (bottom) experiments under DuL treatment (numbers represents extract concentration in percentage).

A 100% DuL extract was observed to be the most effective, with as much as three times the suppressive effect of DuR, DiL and DiR (Tables 6 - 9).

Table 6: Mean percentage germination of *T. minuta* seeds per treatment of *D. uncinatum* and *D. intortum* leaf and root extracts after 14 days of treatment in a laboratory experiment ($F = F_{(7,24)}$).

Concentration (%)	<i>D. uncinatum</i>		<i>D. intortum</i>	
	leaves	Roots	Leaves	roots
0.0	91±5c	80±1c	88±4a	82±5
12.5	60±1b	80±3c	84±5a	75±1
25.0	60±3b	64±2abc	73±3abc	60±1
37.5	44.80b	55±5ab	71±2abc	62±9
50.0	60±7b	55±8ab	68±1abc	57±5
62.5	28±5a	73±6bc	62±8ab	64±8
75.0	31±9a	62±5abc	80±1a	51±8
100.0	28±8a	51±5a	53±3b	62±8
Statistics	$F=7.96^{**}$	$F=2.93^*$	$F=2.76^*$	$F=1^*$

* $P \leq 0.05$, ** $P \leq 0.003$ Values represent mean \pm SE, values with dissimilar letter(s) in a column are significant by Fisher LSD at $p=0.05$

Table 7: Mean percentage germination of *G. cordifolia* seeds after treatment with *D. uncinatum* and *D. intortum* leaf and root extracts in different concentrations after 30 days of treatment in the laboratory ($F = F_{(5,18)}$).

Concentration (%)	<i>D. uncinatum</i>		<i>D. intortum</i>	
	Leaves	Roots	Leaves	Roots
0.0	87 ± 3b	83 ± 9a	73 ± 2a	83 ± 9a
25.0	83 ± 9b	80 ± 10a	76 ± 3a	77 ± 3a
50.0	57 ± 4ab	77 ± 3a	73 ± 3a	70 ± 9a
62.5	67 ± 9ab	77 ± 9a	83 ± 7a	60 ± 9a
75.0	40 ± 0a	70 ± 5a	70 ± 0a	80 ± 9a
100.0	33 ± 3a	63 ± 7a	67 ± 7a	73 ± 3a
Statistics	$F = 3.3^*$	$F = 0.8^n$	$F = 0.9^n$	$H = 3.9^n$

* $p \leq 0.05$, $^n p > 0.05$. Values represents Mean ± SE, values with dissimilar letter(s) in a column are significant by Fisher LSD at $p = 0.05$

Table 8: Mean percentage germination of *T. minuta* seeds per treatment of *D. uncinatum* and *D. intortum* leaf and root extracts after 30 days of treatment in the screen house experiment ($F = F_{(5,18)}$ and $H = H_{(5,18)}$).

Concentration (%)	<i>D. uncinatum</i>		<i>D. intortum</i>	
	leaves	Roots	Leaves	roots
0.0	87±0.2c	77±0.2	93±0.2	83±0.2
25.0	73±0.2ac	77±0.2	87±0.2	87±0.2
50.0	63±0.1a	73±0.2	77±0.2	73±0.2
62.5	60±0.2a	70±0.2	67±0.2	80±0.2
75.0	37±0.1b	67±0.2	73±0.2	80±0.2
100.0	27±0.1b	60±0.1	83±0.2	87±0.2
Statistics	$F = 19.49^*$	$H = 8.5^n$	$H = 7.2^n$	$H = 7.5^n$

* $p \leq 0.00002$, $^n p \geq 0.1$. Values represent mean ± SE, values with dissimilar letter(s) in a column are significant by Fisher' LSD at $p = 0.05$

Table 9: Mean percentage germination of *G. cordifolia* seeds per treatment of *D. uncinatum* and *D. intortum* leaf and root extracts in different concentrations after 30 days of treatment in the screen house experiment ($H = H_{(5,18)}$).

Concentration (%)	<i>D. uncinatum</i>		<i>D. intortum</i>	
	Leaves	roots	Leaves	Roots
0.0	97 ± 0.2c	90 ± 0.2a	92 ± 0.2c	82 ± 0.2a
25.0	63 ± 0.2b	80 ± 0.2a	86 ± 0.2c	88 ± 0.2a
50.0	60 ± 0.1b	90 ± 0.2a	75 ± 0.2ab	72 ± 0.2a
62.5	57 ± 0.1b	87 ± 0.2a	66 ± 0.2b	81 ± 0.2a
75.0	50 ± 0.1b	73 ± 0.2a	72 ± 0.2ab	80 ± 0.2a
100.0	40 ± 0.1a	76 ± 0.2a	82 ± 0.2c	86 ± 0.2a
Statistics	$H = 12.4^*$	$H = 10.0^n$	$H = 11.8^*$	$H = 4.7^n$

* $p \leq 0.05$, $^n p > 0.05$. Values represents Mean ± SE, values with dissimilar letter(s) in a column are significant by Fisher' LSD at $p = 0.05$

5.3.2 Allelopathic effects on seedling height

In laboratory experimentation, *T. minuta* seedling height differed significantly across all four groups ($p < 0.05$) (Table 10) while *G. cordifolia* seedling's height differed significantly in DuL, DuR and DiL treatments ($p < 0.05$) but not in DiR (Table 11). For both species' seedling height in screen house experimentation, differed significantly only in DuL and DiL treatments ($P < 0.05$) (Tables 12 & 13). Seedlings treated with higher concentrations ($\geq 75\%$) of DuL in both laboratory and screen house experiments were twice as short as those of higher concentrations of DuR, DiL and DiR (Figures 22 & 23).

Table 10: Kruskal Wallis and One-way ANOVA test of *T. minuta* seedling parameters (seedling fresh weight, height and chlorophyll content) per treatment after 14 days of treatment in a laboratory experiment ($H=H_{(7,24)}$).

Parameters	<i>D. uncinatum</i>		<i>D. intortum</i>	
	Leaves	Roots	Leaves	Roots
Seedlings height	$H=22.18^{**}$	$F= 6.38^{**}$	$H= 21.69^{**}$	$F= 143.79^{****}$
Fresh weight	$H=21.86^{**}$	$H= 22.30^{**}$	$H=22.88^{**}$	$H= 22.60^{**}$
Chl content	$H= 22.79^{**}$	$H= 22.58^{**}$	$H= 22.58^{**}$	$H= 22.58^{**}$

$*p\leq 0.05$, $**p\leq 0.003$, $***p\leq 0.0003$, $****p\leq 0$

Table 11: Kruskal-Wallis and One-way ANOVA test of *G. cordifolia* seedling parameters (seedling fresh weight, height and leaf total chlorophyll content) per treatment after 30 days of treatment in a laboratory experiment ($H = H_{(5)}$ and $F = F_{(5,18)}$).

Parameters	<i>D. uncinatum</i>		<i>D. intortum</i>	
	Leaves	Roots	Leaves	Roots
Seedlings height	$F = 9.40^{***}$	$F = 4.52^*$	$F = 6.66^{**}$	$F = 1.82$
Fresh weight	$F = 12.97^{***}$	$H = 3.43$	$F = 4.75^*$	$F = 1.84$
Chl content	$F = 44.38^{****}$	$H = 9.02$	$F = 1.72$	$F = 11.45^{***}$

$*P < 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, $****P \leq 0.0001$

Table 12: Kruskal-Wallis rank sum and One-way ANOVA test of *T. minuta* seedling parameters (height, fresh weight and chlorophyll content) per treatment after 30 days of treatment in a screen house experiment ($H=H(5, N=18)$ and $F=F_{(5,18)}$).

Parameters	<i>D. uncinatum</i>		<i>D. intortum</i>	
	Leaves	Roots	Leaves	Roots
Seedlings height	$F= 11.13^{***}$	$F= 2.29$	$F= 7.89^{**}$	$F= 1.74$
Fresh weight	$F =7.31^{**}$	$F = 1.54$	$F=2.74$	$F= 2.48$
Chl content	$F= 16.39^{****}$	$F= 2.92$	$F= 1.23$	$F= 8.31^{**}$

$*p<0.05$, $**p\leq 0.008$, $***p\leq 0.0003$, $****p\leq 0.00005$

Table 13: Kruskal-Wallis and One-way ANOVA test of *G. cordifolia* seedling parameters (height, fresh weight and leaf total chlorophyll content) per treatment after 30 days of treatment in a screen house experiment ($H = H_{(5,18)}$ and $F = F_{(5,18)}$).

Parameters	D. uncinatum		D. intortum	
	Leaves	Roots	Leaves	Roots
Seedlings height	$F = 22.21^{****}$	$F = 2.22$	$F = 6.17^{**}$	$F = 0.15$
Fresh weight	$F = 11.56^{***}$	$F = 0.97$	$F = 1.21$	$F = 5.35^{**}$
Chl content	$H = 13.96^*$	$F = 7.66^{**}$	$F = 1.58$	$F = 12.52^{***}$

$*p < 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, $****p \leq 0.0001$

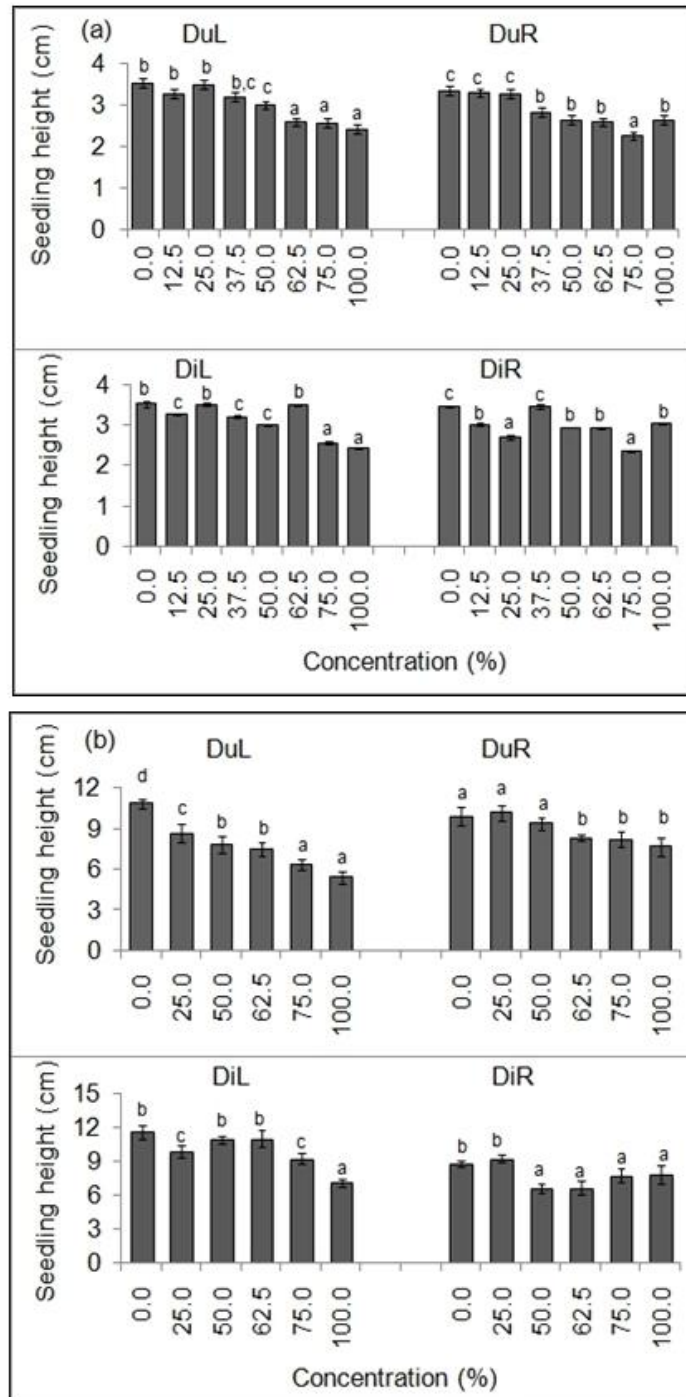


Fig. 22: Mean *T. minuta* seedling heights (\pm S.E) of germinated seeds in all groups (a) after 14 days in laboratory and (b) after 30 days in a screen house. Bars with dissimilar letter(s) are significant by Fisher LSD at $p = 0.05$; DuL (*D. uncinatum* leaf), DuR (*D. uncinatum* root), DiL (*D. intortum* leaf) and DiR (*D. intortum* root).

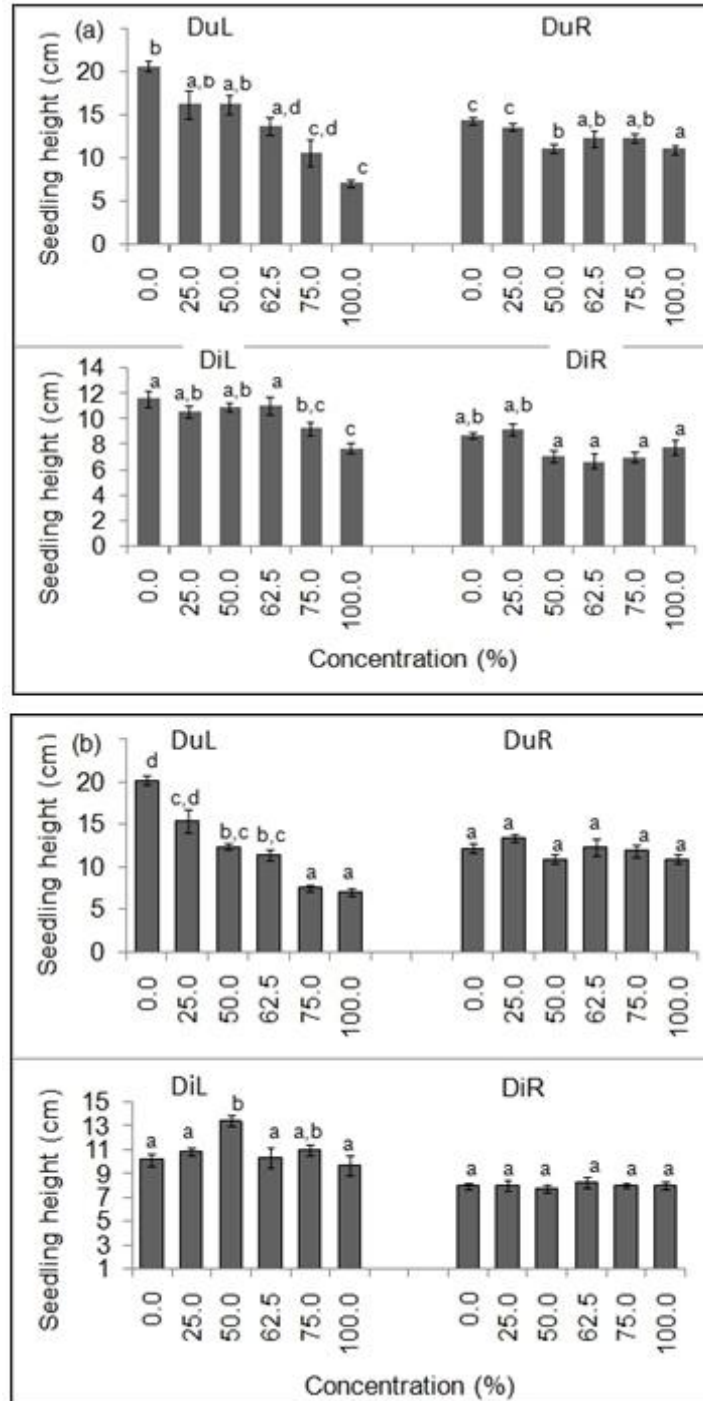


Fig. 23: Mean *G. cordifolia* seedling heights (\pm S.E) of germinated seeds in all groups (a) after 30 days in laboratory and (b) after 30 days in a screen house. Bars with dissimilar letter(s) are significant by Fisher LSD at $p = 0.05$; DuL (*D. uncinatum* leaf), DuR (*D. uncinatum* root), DiL (*D. intortum* leaf) and DiR (*D. intortum* root).

5.3.3 Allelopathic effects on seedling fresh weight

DuL treatments had significant effects on both *T. minuta* and *G. cordifolia* seedling fresh weight in the laboratory and screen house experiments ($p < 0.05$) (Tables 10 - 13). Seedlings treated with higher concentrations of DuL had three times lower fresh weight than those treated with lower concentrations (Fig 24 & 25).

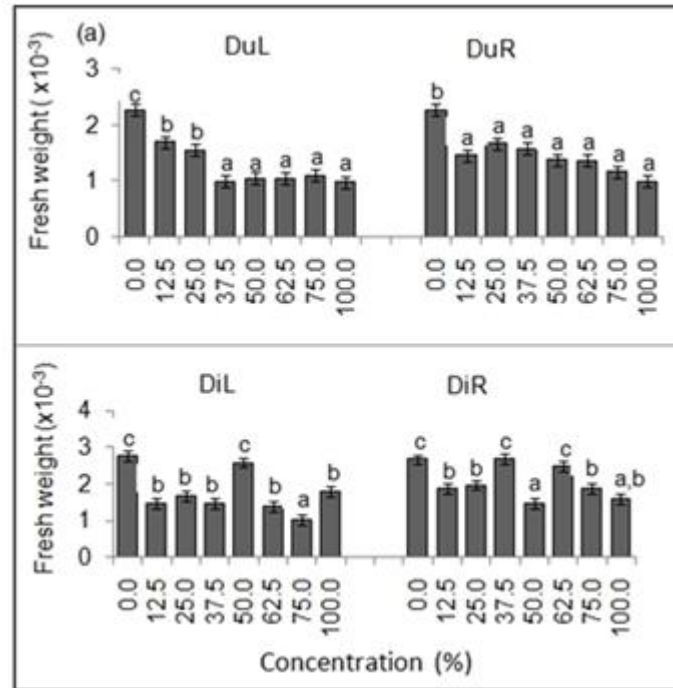


Fig. 24(a): Mean *T. minuta* seedling fresh weight (\pm S.E) of germinated seeds in all groups after 14 days in laboratory. Bars with dissimilar letter(s) are significant by Fisher LSD at $p = 0.05$; DuL (*D. uncinatum* leaf), DuR (*D. uncinatum* root), DiL (*D. intortum* leaf) and DiR (*D. intortum* root).

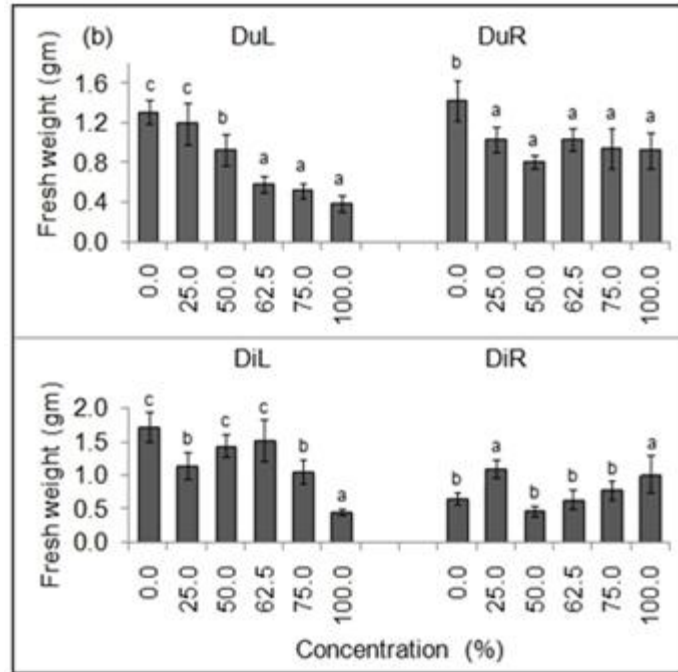


Fig. 24(b): Mean *T. minuta* seedling fresh weight (\pm S.E) of germinated seeds in all groups after 30 days in a screen house. Bars with dissimilar letter(s) are significant by Fisher LSD at $p = 0.05$; DuL (*D. uncinatum* leaf), DuR (*D. uncinatum* root), DiL (*D. intortum* leaf) and DiR (*D. intortum* root).

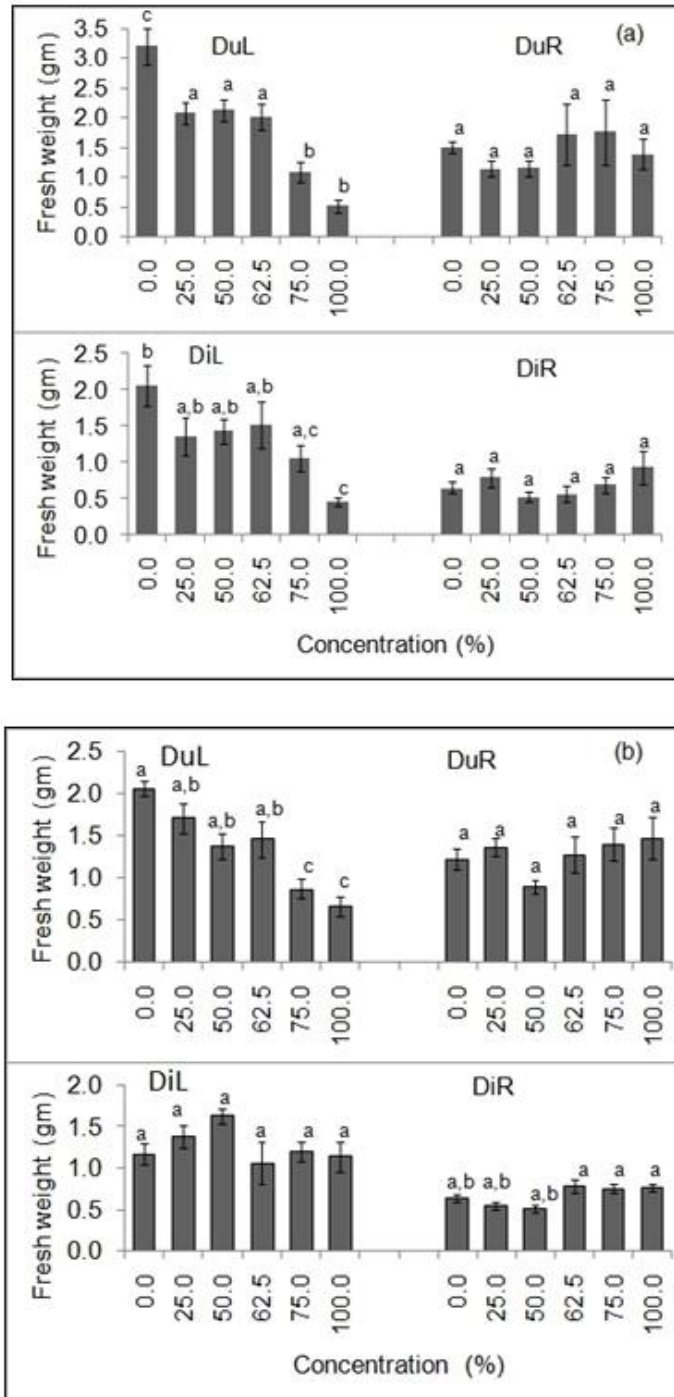


Fig. 25: Mean *G. cordifolia* seedling fresh weight (\pm S.E) of germinated seeds in all groups (a) after 30 days in laboratory and (b) after 30 days in a screen house. Bars with dissimilar letter(s) are significant by Fisher LSD at $p = 0.05$; DuL (*D. uncinatum* leaf), DuR (*D. uncinatum* root), DiL (*D. intortum* leaf) and DiR (*D. intortum* root).

5.3.4 Allelopathic effects on seedling leaf total chlorophyll content

Seedling leaf total chlorophyll (Chl) differed significantly across DuL treatments in both laboratory and screen house experiments ($p < 0.05$) (Tables 10 - 13). Leaves of seedlings that were treated with higher concentrations of DuL had over three times lower chlorophyll than those treated with lower concentrations (Fig 26 & 27).

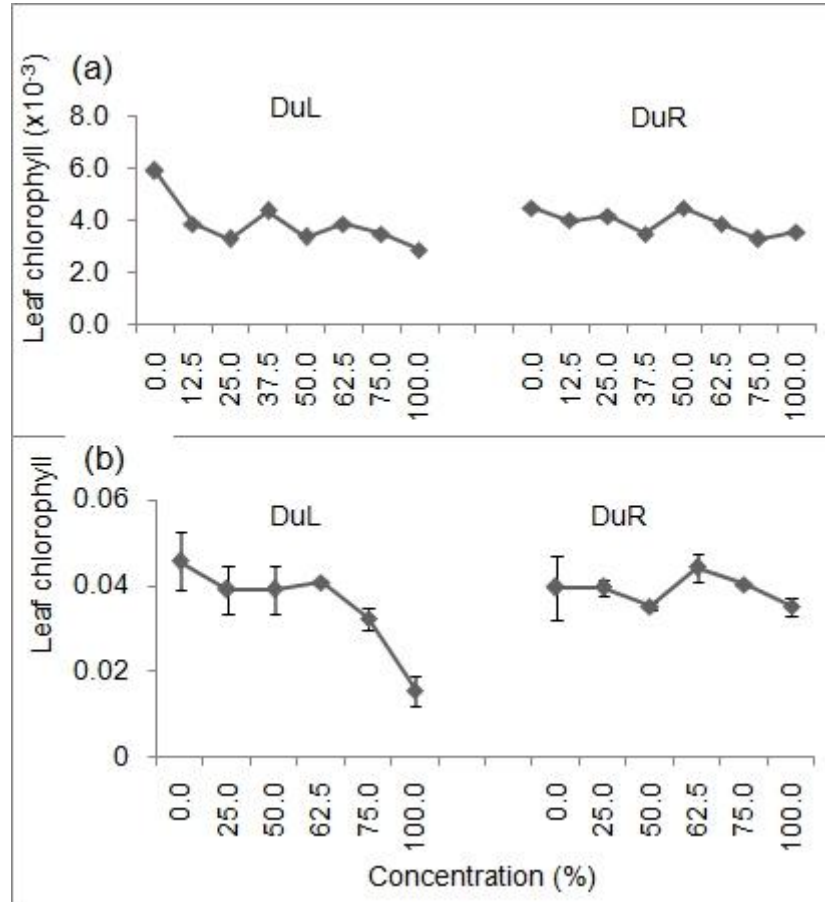


Fig. 26: Mean *T. minuta* seedling leaf chlorophyll content (\pm S.E) (a) after 14 days in laboratory and (b) after 30 days in a screen house, DuL (*D. uncinatum* leaf), DuR (*D. uncinatum* root) treatments.

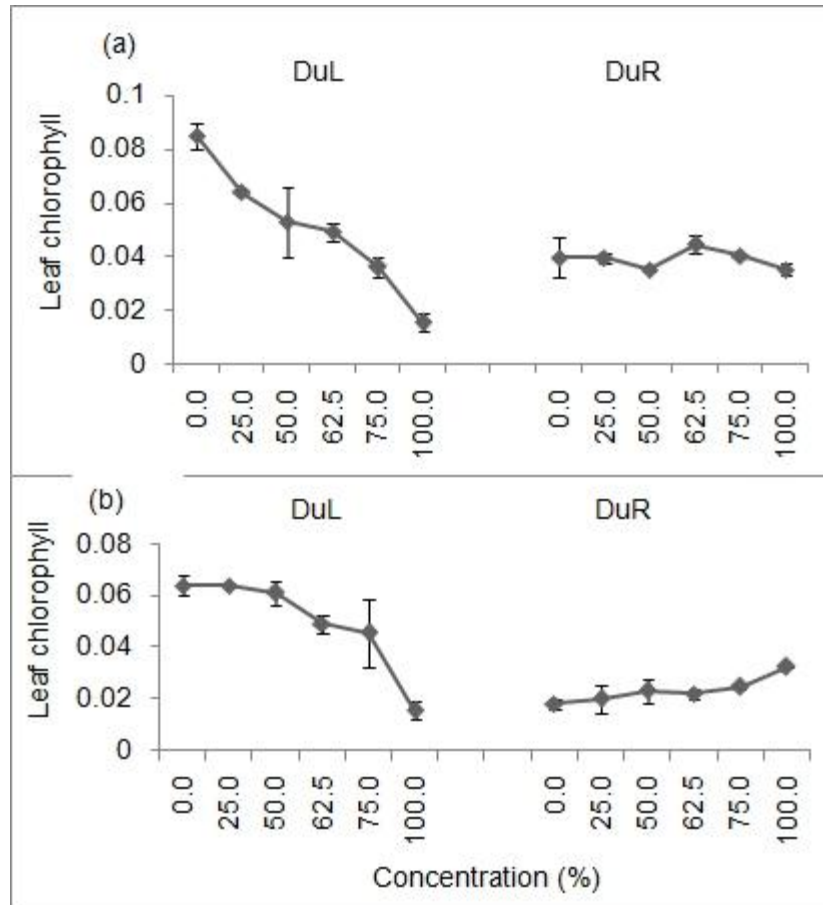


Fig. 27: Mean *G. cordifolia* seedling leaf chlorophyll content (\pm S.E) (a) after 30 days in laboratory and (b) after 30 days in a screen house, DuL (*D. uncinatum* leaf), DuR (*D. uncinatum* root) treatments.

5.4 Discussion

5.4.1 Allelopathic effects of *Desmodium* species on *T. minuta* and *G. cordifolia* germination

Although *G. cordifolia* is a native species, its invasiveness should not be ignored. The sesquiterpene lactone contained in its leaves is an antimicrobial agent that might alter the microbial composition of the ruminants' rumen and thereby affect its overall metabolic functioning (Amorim *et al.*, 2013). It was found that high treatment concentrations of DuL were effective in suppressing the germination and seedling vigor of both *T. minuta* and *G. cordifolia* (Tables 6 - 9). Higher concentration of DuL extract suppressed the *T. minuta* and *G. cordifolia* germination rate by over 50% compared to DuR, DiL and DiR. Similarly, Khan *et al.* (2008)

found that the successful control of *Striga hermonthica* under maize field intercropped with *Desmodium* species was due to the strong allelopathic effects displayed by *D. uncinatum*. Therefore, *D. uncinatum* may have probably developed a competitive mechanism that accumulates high amounts of allelo-chemicals in its leaves (Khan *et al.*, 2008), which, when grown with other plants or as litter, suppress and outcompete other nearby growing plants. Suppression of under storey plants by allelopathic litter as a competition strategy has previously been reported (McPherson and Thompson, 1972). The efficiency of any herbicide has been reported to depend mostly on its dosage (Khaliq *et al.*, 2011). In some instances, there may be an unintentional overestimation of the dose (Zhang *et al.*, 2000), which might cause serious ecological and environmental problems such as weed resistance and health hazards (Heap, 2016). Hence, the use of plants such as *D. uncinatum*, which are both allelopathic and with high agronomic (nutritive and palatable) values, could be a better and effective alternative as a potential biological herbicide (Sodaeizadeh and Hosseini, 2012). Allelopathic plants have been successfully used for inhibiting the emergence of some weeds and pathogens in rice fields (Khanh *et al.*, 2007). Interestingly, rice itself was reported to be allelopathic to both monocot and dicot weeds including *Echinochloa crusgalli* P. Beauv. Var. *oryzi-cola* Ohwi (barnyard grass), an associated weed of paddy rice (Bhadoria, 2011). This further supports the idea for the potential of employing indigenous plants with allelopathy as a new source of natural herbicides and may help to reduce the present dependency for synthetic herbicides while at the same time aiding in the development of biological herbicides.

5.4.2 Allelopathic effects of *Desmodium* species on *T. minuta* and *G. cordifolia* seedling length

Seedling vigor among other factors is represented by the seedling length (Islam *et al.*, 2000; Taye *et al.*, 2013). Taller plants ensure their leaves' ability to intercept up to a recommended 95% of the incoming solar radiation (Brougham, 1956) and, hence, achieve an effective photosynthesis as a requirement for maximum growth. Among the benefits of taller plants in a plant community is an ensured access to light (Falster and Westoby, 2003), although this competitive advantage depends much on relative rather than absolute height. Shading of *Bougainville* species, for instance, increased flowering time, reduced number of flowers and leaf chlorophyll (Saifuddin *et al.*, 2010). The net effect therefore, will always be that taller plants will

have stronger shoots compared to their counterparts as they invest much in stems and vasculature for support, which in turn ensures their ability to withstand stresses such as animal trampling. Shorter plants, on the other hand, will suffer from a reduced number of flowers (fewer seed formation) which might result into limited dispersal to stunted growth due to reduced photosynthesis. A significant decrease in seedling length from lower to higher extract concentrations was observed with $\geq 62.5\%$ DuL being the most effective concentration that reduced the seedling length by half compared to those of DuR, DiL and DiR (Fig 22 & 23). This can be a milestone to the successful management of *T. minuta* and *G. cordifolia* as affecting *T. minuta* and *G. cordifolia*'s seedling length will not only result in seedling's reduced ability to perform photosynthesis but also the development of seedlings with weaker shoots that are susceptible to trampling by animals. In the long run, an emergence of native palatable plants such as *Cynodon dactylon*, *Chloris pycnothrix*, *Chloris gayana*, *Digitaria abyssinica* and *Pennisetum clandestinum* that have been observed to co-exist with invasive weeds *T. minuta* and *G. cordifolia* (Chapter Two) might arise and, hence, provide sufficient food to herbivores.

5.4.3 Allelopathic effects of *Desmodium* species on *T. minuta* and *G. cordifolia* seedling fresh weight

It was further observed that higher DuL concentration successfully suppressed seedling fresh weight in both laboratory and screen house experiments (Fig. 24 & 25). Krishnamurthy *et al.* (2011), suggested that low seedling fresh weights will render them more susceptible to pathogens as well as abiotic stresses. As previously reported by Chaturvedi *et al.* (1995) in Oladokun and Ennos (2006), lodging-tolerant submerged lowland rice had more vascular bundles and hence higher fresh weight than flood susceptible varieties. This could imply that rice with low fresh weight is more susceptible to the effects of flood within its environment and, hence, its destruction. The study, therefore, propose that affecting seedling stability by suppressing its fresh weight will be an added advantage to the management of the undesired invasive weeds such as *T. minuta* and *G. cordifolia*. As deposition of seeds by animals normally occurs in areas where they spend most of their time (Dennis *et al.*, 2007) and as these areas will be more trampled, spraying of $\geq 62.5\%$ DuL extract will affect *T. minuta* and *G. cordifolia* seedlings fresh weight and, hence, make them more susceptible to animal trampling effects.

5.4.4 Allelopathic effects of *Desmodium* species on *T. minuta* and *G. cordifolia* seedling chlorophyll contents

As expected, seedling chlorophyll contents decreased with increasing treatment concentration, particularly under DuL treatments. Plant growth, development and adaptation to various different environmental conditions depend strongly on its leaf's photosynthetic efficiency, associated with chlorophyll content (Beltramin da Fonseca *et al.*, 2013). Leaf chlorophyll, therefore, is essential in the conversion of the solar radiation into chemical energy (ATP and NADPH) and, thus, for plant growth and development (Araújo *et al.*, 2013). While leaves that have higher chlorophyll show a better photosynthesis performance than their counterparts (Gabrielsen, 1948), those with low chlorophyll content have been associated with low competitive ability for light and, thus, to survive (Krause and Weis, 1991). Moreover, plants with reduced leaf chlorophyll content are likely to produce flowers with accelerated abscission (Saifuddin *et al.*, 2010), which might reduce nectar availability and consequently low seed dispersal through pollinators. It is therefore, speculated that affecting *T. minuta* and *G. cordifolia* seedling chlorophyll content with higher concentration of DuL ($\geq 62.5\%$) will add to the effort towards successful management of these invasive plants.

5.5 Conclusion

While the four discussed parameters in most invasive plants can be efficiently suppressed through chemical herbicides (CDFA, 2014), this study suggest a novel way of using natural components leading to the same effect but with less harm to the environment. The spraying of extracts as biological herbicides has proved to be a successful weed management tool without affecting productivity in cotton, soybean, wheat and rice (Soltys *et al.*, 2013). Based on results from this study, a suppression of more than 70% of *T. minuta* and *G. cordifolia* seedlings in affected areas with a single spray application of 100 ml ($\geq 62.5\%$) of *D. uncinatum* leaf extract per 0.03 m² is expected. Currently, timed-mowing and uprooting of *T. minuta* and *G. cordifolia* before anthesis and controlled burning are among the management options that are utilized in Ngorongoro Crater (Personal observation). These three strategies are only short-term solutions, long-term solutions such as the use of *D. uncinatum* extract that not only affect individual *T. minuta* and *G. cordifolia* but also reduce their soil seed bank and, hence, reduces their chance of

germinating in the future, are needed. Moreover, *D. uncinatum* is readily available as it can be grown easily, thereby providing a possibility for future development of a biological herbicide that can help in invasive species management.

This study has shown that a natural extract can offer remedies to the negative impacts of invasive plant species in rangelands and is especially applicable in areas sensitive/limited to the use of synthetic herbicides for sustainable rangeland management. Based on findings from this study, spraying the landscape infested with seeds or seedlings of *T. minuta* and *G. cordifolia* with approximately 100ml of $\geq 62.5\%$ DuL extract per 0.03 m^2 will keep the abundance of these non-palatable invasive plant low. This treatment will limit *T. minuta* and *G. cordifolia*' growth and prevent future spread in our rangelands. In the long run, using a biological herbicide might provide a beneficial management approach to suppress aggressive invasive species such as *T. minuta* and *G. cordifolia*, which might invade various rangelands inside and outside of most protected areas. Further research though is needed to identify the mechanisms responsible for *Desmodium spp* in reducing the germination and growth of *T. minuta* and *G. cordifolia*.

CHAPTER SIX

*This chapter highlights the general introduction, discussion, conclusion and recommendations of the study and discusses the major findings of chapters two, three, four and five. The chapter explores the ecological effects of weeds and potential of utilizing a native and most co-existing forage grass *Cynodon dactylon* and *Desmodium uncinatum* leaf extract as two nature based approaches for managing invasive weeds *T. minuta* and *G. cordifolia* for sustainable rangeland management.*

General introduction, Discussion, Conclusion and Recommendations

6.1 General introduction

This research work has dealt with the challenges of invasive species *Tagetes minuta* and *Gutierrezia cordifolia* in the Ngorongoro crater of Tanzania. The Ngorongoro crater is a protected rangeland and a World heritage site, therefore the research provides a potential solution to dealing with an imminent challenge in a World- renowned site. Likewise, the research is highly commendable as it deals with a key ecological challenge of invasive weeds facing most protected rangelands. The work has the potential of being commercialized to produce environmentally friendly and selective bio-herbicide in the future. By affecting Ngorongoro crater's native plants that are source of food for the crater's inhabitants, the two invasive weeds pose a threat not only to crater's herbivores and carnivores but also to the entire crater ecosystem. This will further reduce crater's aesthetic value which consequently might hurt the Tourism industry at large and thereby reduce government's revenue. This research therefore assessed the ecological effects of the invasive weeds *T. minuta* and *G. cordifolia* on native vascular plants; it has further developed nature-based management solutions for the two invasives based on *Cynodon dactylon* competitiveness and allelopathic effects of *Desmodium uncinatum*.

6.2 General discussion

6.2.1 Ecological effects of invasive plants

As most of alien invasive weeds lack natural enemies/pests in their new habitats (US-Forest-Service, 2015), they tend to spread faster and hence with time, they result in a decreased grazing or browsing area and can lead to local extinction of native plants and herbivorous animals due to decreased food availability. Understanding ecological effects of invasive weeds based on scientific research findings rather than traditional hear-say is crucial for wise use of available resources for their management. Recently, there have been an increasing concern on the ecological effects of invasive weeds on native plant species (Aguilera *et al.*, 2010; Gichua *et al.*, 2014). The effects have been associated with reduced agro-ecosystems stability and productivity (Vasquez *et al.*, 2008). They have also been reported to affect soil characteristics therefore further jeopardizing ecosystem sustainability (Strayer *et al.*, 2006; Weidenhamer and Callaway, 2010). Results from this research study on the ecological effects of invasive weeds (*T. minuta* and *G. cordifolia*), based on the field, screen house, plot and laboratory experiments, shows that the ecological effects of the two invasives are real hence necessitating immediate management. The decrease in for instance; native plants richness and cover (chapter two) and the edaphic changes (chapter three) within the Ngorongoro crater not only jeopardize the existence of herbivores but also carnivores whose food is mainly herbivores. If not managed, the two weeds will in a long run put the entire Ngorongoro crater ecosystem at risk.

6.2.2 Management of invasive plants

Management of invasive weeds has been challenging as a result of increasingly failure of chemical control as a method (O'rourke *et al.*, 1975), due to evolution of resistant weeds (Holt and Lebaron, 1990), higher cost of using chemical herbicide and their effects on the environment (MG van der Werf, 1996). Likewise, mechanical control of invasive plants have proved to be costly and time consuming (NCAA, 2011). Further, any soil disturbance that arise from mechanical control promotes germination of undesired weed (Calado *et al.*, 2013) while desired indigenous species may be mistakenly destroyed. The most imminent shortfall of mechanical control of weeds is that, most invasive seeds remains in the soil, hence, posing a risk of future invasions. Invasive weeds have been reported as a problem in most rangelands and farm lands, fortunately, in farmlands farmers are advised to use chemical herbicides such as Roundup

(Monsanto), Cimarron Plus 63DF (DuPont), Cimarron Max (DuPont), Aim (FMC) e.t.c (Murphy, 2011), which some seems to work well although some are failing due to weeds' resistance (Holt and Lebaron, 1990). Unfortunately, the use of such herbicides in protected rangelands poses a great challenge due to their unpredicted future negative impacts to the entire rangeland ecosystem (Kimmins, 1975; Poorter, 2007; Kughur, 2012), thus a need arise for the use of biological based approaches. Besides the fact that biological control is safe to ecosystem and environment, care must be observed to utilize biological agents that will provide the greatest benefit with the least risk of harming the receiving community. This motivated the idea to research on utilization of *C. dactylon* , a native palatable forage as an intercrop and extracts from allelopathic plants such as *D. uncinatum*, a readily available and palatable forage as potential bio-herbicide to manage *T. minuta* and *G. cordifolia* invasive weeds particularly in rangelands / protected areas where chemical herbicides are not recommended.

As invasive plants normally affect native plants (Chapter one) that are the major source of food for rangeland inhabitants and the fact that chemical and traditional methods for invasive weed management are increasing failing (O'rourke *et al.*, 1975; Calado *et al.*, 2013) their management using environmentally friendly methods such as allelopathy (chapter five) and plant-plant competition (chapter four) might be advocated. Interestingly, these approaches are environmentally friendly with minimum impacts to the environment, they pose no risk of developing weed's resistances, they have low cost of use, they are readily locally available and they are not like synthetic chemical herbicides which when decomposed they yield metabolites each with different chemical properties including adsorption capacity and resistance to degradation (Custer-National-Forest, 2006) that are toxic to both plants and animals.

6.2.3 Management of invasive plants *T. minuta* and *G. cordifolia*: The way forward

The spread of *T. minuta* and *G. cordifolia* in the Ngorongoro Conservation area is increasing (Personal observation), and their management approach is not only ineffective but also expensive as it includes mowing and mechanical uprooting that involves the use of hired laborers (Personal communication). While these methods can be applied as an alternative to chemical control during invasion of *T. minuta* and *G. cordifolia*, they are only short-term remedies as many *T. minuta* and *G. cordifolia* invasive plant seeds remain in the soil seed bank. Recently, there have

been an increasing concern on the use of weed control mechanisms such as biological control and Integrated Pest Management (IPM) as complements to the traditional control methods for improved performance (Altieri, 1994). Although biological control of weeds have raised a concern due to its potential damage to untargeted native forage plants and plants closely related to a targeted weedy plant (Turner, 1985 in Quimby *et al.*, 1991), to date there is no known intentional introduction of weed biological control agent that has caused harm to any known native forage plant (Quimby *et al.*, 1991). Therefore utilizing two forage plants; *Cynodon dactylon* and *Desmodium* species presents environmentally friendly methods for the management of both *T. minuta* and *G. cordifolia*.

While *C. dactylon*, was found to co-exist well with the two weeds (Chapter two) hence providing an opportunity to utilize it for the management of the two invasive plants through competitive facilitation, *Desmodium uncinatum*'s leaf crude extract was observed to negatively affect the growth of the two plants and therefore act as a promising *T. minuta* and *G. cordifolia* management approach through allelopathy (Chapter five). The use of *C. dactylon* competitiveness and *D. uncinatum* leaf extract as management approaches for the management of *T. minuta* and *G. cordifolia* not only present two environmentally friendly methods but also ensures continued stoppage of germination of the two invasive plant's seeds in soil seed bank hence preventing future invasion which cannot be achieved using the current available management approaches (chemical and traditional control methods). Moreover, both *C. dactylon* and *D. uncinatum* are of high agronomic value as they are preferred by most herbivores (Heuze *et al.*, 2015; Tropical-forages, 2017), therefore intercropping of for example *C. dactylon* while controlling the two invasives will also ensure food availability to herbivores. Interestingly therefore, as the two weeds are controlled using these two approaches, food availability for the crater's herbivores and carnivores will be ensured which might increase Ngorongoro crater's and other Tanzanian rangelands attractiveness as Tourist destinations. This will not only increase Government's revenue but will also improve Tanzanian rangelands' surrounding communities livelihood whose revenue depend mostly on the flow of Tourists in their nearby rangelands.

Performed activities and results obtained from this study are summarized in figure 28.

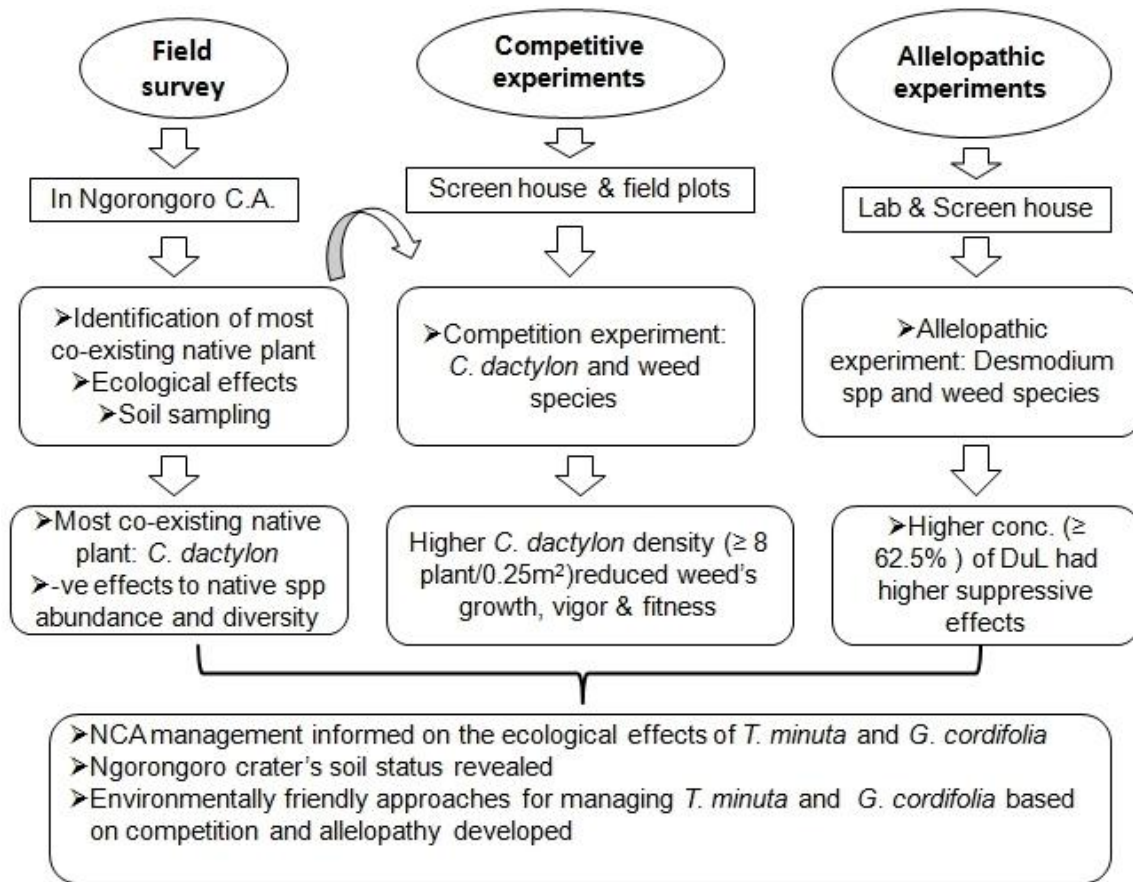


Fig. 28: Summarized performed activities and results obtained from this study

6.3 General conclusion

It has been proven that the two invasive weeds *T. minuta* and *G. cordifolia* negatively affect species composition, structure and soil chemical properties of the Ngorongoro Crater. The two invasive weeds therefore, present a significant threat not only to the Ngorongoro crater's native plants that are the main source of food to wild animals but also to the entire crater's ecosystem. Likewise, the two invasive weeds are likely to indirectly reduce Ngorongoro crater's aesthetic value as a Tourist destination which might result into decrease in the number of Tourists who are willing to visit the crater. This will ultimately decrease revenue and collapse businesses whose existence depends much on the presence of both native plants and wild animals within the crater. Immediately management of the two invasive weeds is therefore crucial.

To manage invasive plants *T. minuta* and *G. cordifolia*, it was demonstrated that competitive facilitation of native plants and allelopathy successfully suppressed the two invasive weed's seed germination and seedling vigor. *Cynodon dactylon*, a native plant that co-existed well with the two invasive weeds presents a potential management tool in controlling the two weeds through competition. Moreover, as inter-cropping *T. minuta* and *G. cordifolia* with high densities of *C. dactylon* (≥ 8 plants per 0.25 m^2) negatively affected the growth and vigor of the two invasive weeds, this also potentially provides an environmentally friendly *T. minuta* and *G. cordifolia* management option, which is particularly useful in rangeland or protected areas where mammalian herbivores can simultaneously use the *C. dactylon* plants in their diet. Likewise, spraying the landscape infested with seeds or seedlings of *T. minuta* and *G. cordifolia* with 100ml of $\geq 62.5\%$ *Desmodium uncinatum* leaf (DuL) extract per 0.03 m^2 will limit *T. minuta* and *G. cordifolia* growth and prevent future spread in our rangelands or protected areas. As *D. uncinatum* is abundant in some rangelands of Tanzania and as producing an extract of its leaves is rather easy, this plant is a cheap and highly environmentally friendly alternative to chemical herbicides for the management of both *T. minuta* and *G. cordifolia*. With this research work not only that two novel nature-based management approaches that have proven to be functional have been devised and tested but also the society have been informed on the ecological effects of the two invasive weeds. These approaches though need to be scaled up in the field at the same time characterization of DuL extract to further elucidate its potential for commercial development of this technique is crucial.

6.4 Recommendations

Findings from this study not only lead to the development of two nature based approaches for managing the invasive weeds *T. minuta* and *G. cordifolia*, but also brought into attention about their effects to the ecology of an area where they invade. These findings can be demonstrated in fields invaded by either *T. minuta* or *G. cordifolia* at a small scale before embarking into scaling up to restore the invaded areas. They also open doors for new areas of researches; these areas can be pioneered in an effort to achieve invasive weeds free rangelands for sustainable rangelands management. These new areas of research that could be dealt with may include but not limited to:

- i. Researching on and developing pro-active measures to ensure no new introduction of invasive weeds to protected rangelands.
- ii. Studying on the use of competitive interaction between native and exotic invasive weeds and or development of bio-herbicides from such plants as *D. uncinatum* as an alternative to chemical herbicides to manage problematic invasive weeds such as *T. minuta* and *G. cordifolia*.
- iii. Modelling of possible future spread of the invasive weeds including *T. minuta* and *G. cordifolia* to new areas to prepare for future management actions.
- iv. Detailed field assessment and monitoring to identify the most likely invasion hotspots so that weed reduction activities can be concentrated on these areas.
- v. In the field (in invaded rangelands) demonstration of the effectiveness of *C. dactylon* and allelopathic effects of *D. uncinatum* leaf extract in managing the invasive weeds *T. minuta* and *G. cordifolia* should be done as, thus far, our study has conducted experiments in the controlled environments of laboratory and screen house first.
- vi. Assessing wheather or not *D. uncinatum* leaf crude extract affects under and above ground micro communities, insects and birds.
- vii. Researching on *D. uncinatum* leaf crude extract shelf life and establishing the longevity of the extract.
- viii. Assessing the potential for utilizing *D. uncinatum* leaf crude extract in farmlands

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APPENDICES

S.1 Surveyed plant species checklist for *T. minuta* uninvaded, moderately and highly invaded quadrats

S/N	Species name		
	Uninvaded	Moderately	Highly
1.	<i>Acacia lahai</i>	<i>Achyranthes aspera</i>	<i>Alchimella johnstonii</i>
2.	<i>Aerva lanata</i>	<i>Alchimella johnstonii</i>	<i>Bidens pilosa</i>
3.	<i>Alchimella johnstonii</i>	<i>Chloris gayana</i>	<i>Chloris pycnothrix</i>
4.	<i>Bidens pilosa</i>	<i>Chloris pycnothrix</i>	<i>Cyathula orthacantha</i>
5.	<i>Bothriochloa radicans</i>	<i>Commelina benghalensis</i>	<i>Cynodon dactylon</i>
6.	<i>Bothriocline longipes</i>	<i>Crotalaria barkae</i>	<i>Cynoglossum amplifolium</i>
7.	<i>Commelina benghalensis</i>	<i>Cyathula cylindrica</i>	<i>Cyperus esculentus</i>
8.	<i>Conyza steudelii</i>	<i>Cynodon dactylon</i>	<i>Cyperus rigidifolius</i>
9.	<i>Cyathula cylindrica</i>	<i>Cynodon plectostachyus</i>	<i>Digitaria velutina</i>
10.	<i>Cynodon dactylon</i>	<i>Cyperus rigidifolius</i>	<i>Pennisetum clandestinum</i>
11.	<i>Cynoglossum coeruleum</i>	<i>Cyperus rotundus</i>	<i>Setaria sphacelata</i>
12.	<i>Cynoglossum amplifolium</i>	<i>Eleusine jaegeri</i>	<i>Solanum incanum</i>
13.	<i>Cyperus distans</i>	<i>Euphorbia inaequilatera</i>	<i>Urtica massaica</i>
14.	<i>Cyperus molipes</i>	<i>Hypoestes forskalii</i>	-
15.	<i>Cyperus rotundus</i>	<i>Justicia diclipteroides</i>	-
16.	<i>Dactyloctenium aegyptium</i>	<i>Panicum maximum</i>	-
17.	<i>Eragrostis aspera</i>	<i>Pennisetum clandestinum</i>	-
18.	<i>Eragrostis racemosa</i>	<i>Setaria verticillata</i>	-
19.	<i>Eragrostis superba</i>	<i>Solanum incanum</i>	-
20.	<i>Euphorbia inaequilatera</i>	<i>Sonchus oleraceus</i>	-
21.	<i>Hibiscus diversifolius</i>	<i>Trifolium burchellianum</i>	-
22.	<i>Hypoestes aristata</i>	-	-
23.	<i>Hypoestes forskalii</i>	-	-
24.	<i>Indigofera spicata</i>	-	-
25.	<i>Justicia diclipteroides</i>	-	-

26.	<i>Kalanchoe densiflora</i>	-	-
27.	<i>Launaea cornuta</i>	-	-
28.	<i>Microchloa kunthii</i>	-	-
29.	<i>Oplismenus hirtellus</i>	-	-
30.	<i>Oxalis corniculata</i>	-	-
31.	<i>Panicum maximum</i>	-	-
32.	<i>Pavonia urens</i>	-	-
33.	<i>Pennisetum clandestinum</i>	-	-
34.	<i>Setaria sphacelata</i>	-	-
35.	<i>Solanum incanum</i>	-	-
36.	<i>Sporobolus africanus</i>	-	-
37.	<i>Sporobolus ioclodus</i>	-	-
38.	<i>Trifolium burchellianum</i>	-	-
39.	<i>Urtica massaica</i>	-	-
40.	<i>Vernonia galamensis</i>	-	-
41.	<i>Viola abyssinica</i>	-	-

S.2 Surveyed plant species checklist for *G. cordifolia* uninvaded, moderately and highly invaded quadrats

S/N	Species name		
	Uninvaded	Moderately	Highly
1.	<i>Chloris gayana</i>	<i>Abutilon mauritianum</i>	<i>Amaranthus graecizans</i>
2.	<i>Acacia hockii</i>	<i>Bidens schimperii</i>	<i>Chloris gayana</i>
3.	<i>Aerva lanata</i>	<i>Cetalia pumila</i>	<i>Chloris pycnothrix</i>
4.	<i>Alectra spp</i>	<i>Chloris gayana</i>	<i>Commelina imberbis</i>
5.	<i>Andropogon greenway</i>	<i>Chloris pycnothrix</i>	<i>Cymbopogon exacavatus</i>
6.	<i>Bidens schimperii</i>	<i>Commelina benghalensis</i>	<i>Cynodon dactylon</i>
7.	<i>Brachiaria eruciformis</i>	<i>Conyza bonariensis</i>	<i>Digitalia abysinica</i>
8.	<i>Chloris pycnothrix</i>	<i>Cynodon dactylon</i>	<i>Elangia alternifolia</i>
9.	<i>Chloris virgata</i>	<i>Cynodon nlemfuensis</i>	<i>Hibiscus aponeurus</i>

10.	<i>Commelina benghalensis</i>	<i>Dicanthium anulatum</i>	<i>Justicia matamensis</i>
11.	<i>Ctenium somalense</i>	<i>Digitalia abyssinica</i>	<i>Pennisetum mezianum</i>
12.	<i>Cynodon dactylon</i>	<i>Digitalia scaralum</i>	<i>Pennisetum spacelata</i>
13.	<i>Cynodon nlemfuensis</i>	<i>Elangia alternifolia</i>	<i>Sida rhombifolia</i>
14.	<i>Cyperus basiloides</i>	<i>Hibiscus diversifolius</i>	<i>Sporobolus spicatus</i>
15.	<i>Cyperus bulbosus</i>	<i>Ischaemum afrum</i>	<i>Themeda triandra</i>
16.	<i>Cyperus rotundus</i>	<i>Leucas mollis</i>	-
17.	<i>Cyperus scuarosa</i>	<i>Microchloa kunthii</i>	-
18.	<i>Dactyloctenium aegyptiaca</i>	<i>Pennisetum mezianum</i>	-
19.	<i>Dichanthium annulatum</i>	<i>Sida ovate</i>	-
20.	<i>Digitaria abyssinica</i>	<i>Themeda triandra</i>	-
21.	<i>Digitaria mycoblephalis</i>	<i>Trifolium rueppelianum</i>	-
22.	<i>Digitaria scaralum</i>	-	-
23.	<i>Digitaria abyssinica</i>	-	-
24.	<i>Eragrostis caespitosa</i>	-	-
25.	<i>Euphorbia inaequilatera</i>	-	-
26.	<i>Heterochloea mozambiensis</i>	-	-
27.	<i>Heteropogon contortus</i>	-	-
28.	<i>Hibiscus diversifolius</i>	-	-
29.	<i>Hypoestes aristata</i>	-	-
30.	<i>Indigofera arecta</i>	-	-
31.	<i>Justicia debile</i>	-	-
32.	<i>Justicia matamensis</i>	-	-
33.	<i>Kyllinga odorata</i>	-	-
34.	<i>Lippia javanica</i>	-	-
35.	<i>Melhanian ovata</i>	-	-
36.	<i>Microchloa kunthii</i>	-	-
37.	<i>Monsonia angustifolia</i>	-	-
38.	<i>Pennisetum cephalcelata</i>	-	-
39.	<i>Pennisetum mezianum</i>	-	-

40.	<i>Psilolema jaegerii</i>	-	-
41.	<i>Sida ovata</i>	-	-
42.	<i>Solanum incunum</i>	-	-
43.	<i>Sporobolus ioclodus</i>	-	-
44.	<i>Sporobolus spicatus</i>	-	-
45.	<i>Themeda triandra</i>	-	-
46.	<i>Trifolia rueppelianum</i>	-	-
47.	<i>Urochloa panicoides</i>	-	-
48.	<i>Vigna luteola</i>	-	-
49.	<i>Vigna oblongifolia</i>	-	-