MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF THE INVASIVE PLANT *Gutenbergia cordifolia* TO VARYING BIOPHYSICAL CONDITIONS

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ABSTRACT

This study assessed the effects of varying water stress levels on morphological and physiological traits of an invasive plant *Gutenbergia cordifolia* (*G. cordifolia*) under field and screen house conditions and the responses of these traits on performance *G. cordifolia* under long and short rain seasons in the Ngorongoro Crater, Tanzania. The water stress level assessment was conducted in the screen house at the Nelson Mandela African Institution of Science and Technology (NM-AIST) following a completely randomized design (CRD) while field assessment was conducted in Ngorongoro Conservation Area (NCA). While the maximum and minimum plant heights in the screen house experiment were observed under flood and drought water stress, respectively, the maximum and minimum root collar diameter (RCD) were observed under moderate flood and drought water stress, respectively. Generally, the number of leaves was highest under moderate flood stress and lowest under drought stress. The largest and smallest leaf surface areas (LSA) were observed in flood and drought water stress conditions, respectively. While a decrease in leaf chlorophyll was observed under drought water stress, an increase in leaf anthocyanin levels was observed under flood stress. In comparing field and screen house traits; both maximum *G. cordifolia* height and highest number of leaves were observed under screen house. While the largest and smallest LSA were observed in the field condition and screen house respectively, both the maximum root and shoot fresh and dry weights were observed under field condition. Both leaf anthocyanin and chlorophyll were higher under field compare to screen house. While both the maximum height and the largest RCD were observed during long rain season the highest number of leaves per plant was observed during short rain. Both highest root fresh and dry weight were observed during long rain. The maximum leaf chlorophyll level was observed during short rain season while the minimum was observed during long rain season. The findings from this study indicate that the establishment and spread of *G. cordifolia* is likely to be favoured by projected East African rainfall (2050-2100) as suggested by Platts *et al.* (2015) and inform conservation and management authorities on how growth and performance is likely to be in the future under changing climate and the need of integrating this information in its future management strategies. The study further suggests that efforts to minimize impacts of an invasive *G. cordifolia* in a changing climate must include a good understanding of *G. cordifolia* behaviour under extreme events so as to prepare effective management strategies and actions.

DECLARATION

I, Herieth A. Nyarobi do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my original work and that it has neither been submitted nor being concurrently submitted for the degree award in other institutions.

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CERTIFICATION

The undersigned certify that they have read the dissertation titled "Morphological and physiological responses of the invasive plant *Gutenbergia cordifolia* to varying biophysical conditions" and recommended for examination in fulfillment of the requirements for the degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

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This work is dedicated to my beloved parents, Elizabeth Nyarobi and the late Major Andrew Nyarobi. My Family, Ethan my son and Edmund my husband for his support and courage. May the Almighty God bless them Amen.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
CRBD	Completely Randomized Block Design
CREATES	Centre for Research, Agriculture Advancement, Teaching Excellence and Sustainability
Df	Degrees of Freedom
ECEs	Extreme Climate Events
NCA	Ngorongoro Conservation Area
NM-AIST	Nelson Mandela African Institution of Science and Technology
Per.Obv	Personal Observation
S.E	Standard Error
UNESCO	United Nations Educational, Scientific and Cultural and Organization
π	Pie

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Species shift in range and their expansion has been linked to climate change (Lenoir *et al.*, 2008; Rosenzweig *et al.*, 2008). However, when it comes to developing effective management techniques for rising species populations, such as invasive plants, climate impacts may be underestimated (Genovesi *et al.*, 2017) and therefore have potential negative effects to rangeland productivity affecting nature and nature's contribution to people. Ecosystems are seeing not only slow changes in mean climate conditions, but also huge changes in climate variability and the frequency of extreme weather events (ECEs) like drought, floods, and heat waves are examples of ECEs that vary in frequency, magnitude, timing, and length depending on the region and the specific climate event; the magnitude and frequency of such events is predicted to rise in the future. Climate change can cause ecosystem stress, which can lead to ecological invasion (Masters & Norgrove, 2010). If proper management measures are not taken, the rate of increase of invasive plants as a result of climate change is expected to increase even more in the next decade (Hellmann *et al.*, 2008). If proper management measures are not taken, the rate of increase of invasive plants as a result of climate change is expected to increase even more in the next century (Mainka & Howard, 2010).

Environmental factors that influence plant's growth can potentially affect an invasive plant's ability to exploit the environmental resources for which plants compete (Patterson, 1995). Water stress is a major environmental condition that plants face, and it can significantly affect yield (Barnabás *et al.*, 2008). It is regarded to be one the most important environmental factors influencing plant growth and physiological variation and development (Shakeel *et al.*, 2011). Invasive plants pose a danger to both economic and ecological stability when they display a steady rise in spatial and density in a given area (Wolde & Lal, 2018). Invasive species were identified as the second most important driver of biodiversity and habitat loss after habitat degradation (Millennium Ecosystem Assessment, 2005). Predation, hybridization, and disease transmission all have an impact on biodiversity at the gene, species, and ecosystem levels (Genovesi & Shine, 2004). They are the greatest challenge to conservation because most of them have high fecundity, quickly evolve responses to control efforts alter and responses to community interactions in complex ways. Unfortunately, the existence of most invasive plants is favoured by global climate change, insufficient baseline information for risk assessment, and a lack of public awareness of the problem (Vilà *et al.*, 2010). Invasive plants will likely spread if not managed properly, resulting in a loss of feed and habitat for wildlife and livestock in most rangelands (Ngondya *et al.*, 2016).

In the Ngorongoro Conservation Area (NCA), a multiple land-use area and one of the biodiversity hotspots UNESCO Man and Biosphere (MaB) designated in northern Tanzania. *Gutenbergia cordifolia* is one among the plants species that poses the biggest challenge to conservation of the area (Ngondya & Munishi, 2021). *Gutenbergia cordifolia*, under the family Asteraceae, native to Kenya, Sudan, Democratic Republic of Congo, Rwanda, Burundi, Zambia, Malawi, and Zimbabwe (Ngondya *et al.*, 2016). It is considered a serious problem in most parts of the Serengeti ecosystem and surrounding agricultural area in the Northern part of Tanzania (Ngondya *et al.*, 2016). Besides being unpalatable and toxic to ruminants, this plant suppresses and outcompetes palatable native species thus jeopardizing the ecologically diverse NCA and the entire Serengeti ecosystem. Within NCA, efforts have been made to develop a strategy to control and manage the species.

Changes in broad climatic conditions have been proven to influence the likelihood of species invasions, although the consequences of climate change are anticipated to be diverse and context-dependent, and must be investigated based on the location and potential species to be impacted (Diez *et al.*, 2012). However, the potential for extreme precipitation (drought and flood) has not been carefully assessed, in particular, to promote species invasions. This study used climate projections for the middle and the late 21st century for East Africa following (Platts *et al.*, 2015), to determine the extent to which extreme climate may have an impact on *G. cordifolia* establishment and expansion in East African rangelands. The study assessed the morphological and physiological parameters that are used as evidence for extreme climate impacts on invasion success of the species and its future management implications. By focusing on mechanisms of invasion following extreme rainfall events, this study investigated if generalizations regarding invasion risk are attainable for the species in terms of future establishment trajectories.

Increased non-native species movements and decreased biotic resistance of native communities to invader establishment are expected to become more common and extreme weather events, and their impacts are likely to further facilitate invasions of many species through increased non-native species movements and decreased biotic resistance of native communities to invader establishment (Diez *et al.*, 2012). While future combinations of increased temperature and heavy rainfall could have a dramatic effect on the spread and impact of *G. cordifolia* under climate change, efforts to monitor and mitigate impacts of this and other invasive species in a changing climate must include informed decisions based on a good understanding on how *G. cordifolia* is likely to behave under extreme events. Therefore, understanding *G. cordifolia* morphological and physiological responses to varying water stress and temperature under projected rainfall (2050-2100) as suggested by, how it responds under screen house, field conditions and under differing rain seasons will be useful to inform on future management options as influenced by spatio-temporal changes in rainfall patterns. Moreover, this information will help to understand how *G. cordifolia* is going to behave to the future changing climate and provide potential ways on how to manage and control its impacts.

1.2 Statement of the problem

Gutenbergia cordifolia, an invasive plant that suppresses more palatable indigenous plants, covers more than half of the Ngorongoro crater (Ngondya *et al.*, 2016). *Gutenbergia cordifolia* has been discovered to be harmful to ruminants, endure environmental stress, and maintains green sprouts during dry periods, in addition to being unpleasant (Pers.Obv). As a result, it endangers not only the environmentally diverse NCA, but also the Serengeti ecosystem as a whole. Within NCA, attempts have recently been made to find a means to control and manage the invasive weed *G. cordifolia* (Ngondya *et al.,* 2019). Given the current water and temperature stress circumstances, which are driven by continued climate change, such initiatives require crucial scientific information, notably on *G. cordifolia* invasion success under changing biophysical settings. Understanding its morphological and physiological responses to varying water and temperature stress in a screen house and under a variety of field conditions will be useful in modelling future dynamics of the species as influenced by spatiotemporal changes in climate and other environmental factors, among other things. Thus, this research aims to assess the morphological and physiological changes of *G. cordifolia* underwater stress and temperature variation, in screen house and field conditions and under differing rain seasons. This information will aid in understanding the elements that promote invasion and the forecast of its impending invasion, as well as potential management and control strategies to reduce effects on other native palatable species.

1.3 Rationale of the study

Gutenbergia cordifolia is an invasive plant that dominate almost entire of Ngorongoro crater and spread to some parts Serengeti ecosystem and Mwimba wildlife reserve. Still there have been studies and efforts on how to eliminate this noxious weed *G. cordifolia* and considering biological control actions and the even visitation of pollinator during flowering period. Yet, there is no information that have been documented and considered climate variability's such as temperature, drought and changing in rainfall season can impact to either increase or decrease of *G. cordifolia*. Therefore, through this study will help in management preparedness, control and other management strategies of this invasive weed plant by accounting information generated from these climatic varibles such as rainfall changes and extreme events in order to have a predictive success in managing the invasive species in future under merging global and local climatic and other environmental changes.

1.4 Research objectives

1.4.1 General objective

To evaluate the effects of varying water stress and temperature levels on morphology and physiology of *Gutenbergia cordifolia* under both field and controlled conditions.

1.4.2 Specific objectives

(i) To assess morphological and physiological traits responses of *Gutenbergia cordifolia* to varying water stress under screen house conditions.

(ii) To compare the morphological and physiological traits of *Gutenbergia cordifolia* between field (Ngorongoro crater) and screen house conditions.

(iii) To assess the morphological and physiological traits variations of *Gutenbergia cordifolia* between rain seasons in the Ngorongoro crater.

1.5 Research questions

(i) What are morphological and physiological traits responses of *Gutenbergia cordifolia* to varying water stress under screen house conditions?

(ii) What are the morphological and physiological traits difference of *Gutenbergia cordifolia* between Field (Ngorongoro crater) and Screen house conditions?

(iii) What are rain seasonal variations in morphological and physiological traits of *Gutenbergia cordifolia* in the Ngorongoro crater?

1.6 Significance of the study

This research offer detailed information on the morphological and physiological responses of the unpalatable invasive weed *G. cordifolia* to various environmental stress conditions (such as water stress), paving the way for successful weed management in Tanzania's invaded rangelands and other Ecosystem.

1.7 Delineation of the study

Thus, this study seeks to assess the morphological and physiological changes of *G. cordifolia* under water stress condition, comparing performance of morphological and physiological traits of *G. cordifolia* under field and Screen house conditions and comparing the same traits of *G. cordifolia* under different rain seasons (short and long rain season) at Ngorongoro Conservation area (NCA). The study basically conducted a field survey and screen house experiment to determine morphological and physiological traits of *G. cordifolia* by measuring plant height, root collar diameter, leaf number and surface area, shoot and root weight and leaf pigment (chlorophyll and anthocyanin pigments measurement were taken in the laboratory) at Nelson Mandela African Institution of Science and Technology.

CHAPTER TWO

LITERATUE REVIEW

2.1 Invasive plants

Invasive plant species are mostly considered to be non-native (or alien) to the ecosystem whose introduction has negative impacts or likely to cause harm and suppress native plant species. Other studies have termed a plant to be invasive must spread and colonize to be considered invasive (Jones, 2012). To spread, the species must first overcome geological obstacles and second overcome dispersal, germination, spread, and survival hurdles from the original location of establishment. Third, hurdles to generating a self-sustaining population that does not require reintroduction to maintain a population base must be overcome. Finally, a weed is considered invasive if its negative impacts on the environment, economy, or human health exceed any benefits (Richardson *et al.*, 2000). These invasive plant species produce large quantities of seed, thrive on disturbed soil, and have aggressive root systems that spread long distances from a single plant (Vila & Weiner, 2004). Their root systems often grow so densely that they smother the root systems of surrounding vegetation, they also produce chemicals in their leaves or root systems which inhibit the growth of other plants around them (Kariyawasam *et al.*, 2019). Mechanism of distribution Invasive plant seeds are sometimes distributed by birds, wind, or unknowingly by humans by allowing seed to move great distances (Dostál *et al.*, 2013). These plant species are the greatest threats to croplands, rangelands, aquatic areas and wild lands, they degrade the productivity and biological diversity of all ecosystem (Mullin, 2000). Referring to invasive plant like *G. cordifolia* it's a noxious weed and non-native and at first glance they appear pretty, yet invasive but still pose serious environmental threat. According to other reports of invasive species in Tanzania pose substantial threats to agriculture, biodiversity and other ecosystem services (Sorte, 2016). All these information are crucial for listing invasive plants of sub-Saharan Africa. Conservationist together with ecologist put more effort and concern over invasive species due to the negative impacts and damage cause to natural plants communities (Gioria *et al.*, 2019). Understanding the invasion will help in way to prevent while provide insights into ecological and evolutionary processes (Gulezian & Nyberg, 2010).

2.2 Impacts of invasive plants in an ecosystem

According to Richardson and Pyek (2007), a single invasive species in an ecosystem can have major consequences for the native community. Invasive plant species together with habitat fragmentation, destruction, change, and even complete replacement are well-known worldwide to causes ecosystems to fail to function properly (Shrestha, 2021). Invasive plants are the one leading to the disruption of ecosystem services and the loss of biodiversity worldwide (Pyšek & Richardson, 2010; Ricciardi, 2013). These plants reduce the quality of forage by interfering grazing, poisoning animals, impact wildlife and reduce land value, they also affect habitat and forage for wildlife and reduce plant and animal diversity as well (Ditomaso, 2017). In aspect of rangeland ecosystem they have also been observed to have a negative impact by diminishing land productivity and land value, forage loss and value, increased forest fire occurrence, and decreased visual beauty in parks. Other studies have assessed the impacts of invasive plants species in native communities and demonstrated non-linear damages function whereby community components, such as species richness are seemingly unaffected by the presence of an invader until it has attained relatively high levels of abundance, where upon there is a marked decrease with further increases in abundances (Panetta & Gooden, 2017). However, there is growing evidence that such impacts are highly variable among landscape contexts and are modulated by the condition of the recipient native ecosystem (Mason & French, 2007).

Little doubt that widespread and dominant invasive plants can adversely affect natural ecosystem properties when at high abundances, evidence that an invasive plants presence alone causes deleterious changes in the recipient ecosystems condition is less clear (Barney *et al.*, 2013; Hulme *et al.*, 2013). However, there have been high cost and economic efforts of controlling and managing invasive plant species in agriculture and natural ecosystem not only in Tanzania but worldwide (Hoffmann & Broadhurst, 2016). And still there are scarce resources available for control of the invasive plant species in the ecosystems meaning that the likelihood of reducing spread and establishment of invaders will diminish slowly (Panetta, 2007). Given their far reaching impacts, the information on how they respond to various current and future biophysical conditions is of paramount importance if we are to develop effective and sustainable control strategies (Celestine *et al.*, 2004).

2.3 Plants' response to water stress

Water is a paramount factor in determining species distribution and adaptation to different environmental niche. Numerous studies have reported a myriad of changes elicited by water stress, invasive plant responses and adaptations to water stress are critical for their success in any ecosystem (Bradford & Hsiao, 1982). Semi-arid, arid region and savanna ecosystem, the evaporative demand for the atmosphere cause a significant water stress in many plants, which is among of the most severe environmental stress and affect plants functions. Various experimental trials have shown that a short-term increase in water availability can enable ecological invasion in the long run (Milchunas & Lauenroth, 1995). Milchunas and Lauenroth (1995) studied the response of native grass communities in America for more than five years using water, nitrogen, and water combined with nitrogen treatments. Furthermore, long-term observational studies show that increasing yearly precipitation in arid and semi-arid environments promotes the dominance of invasive plants (Dukes & Mooney, 1999). Water stress adversely impacts many aspects of the physiology of *G. cordifolia*, especially photosynthetic capacity (Bhattacharjee & Saha, 2014). Other studies have observed that water stress decreased potential photosynthetic capacity and reduced plant quality by lowering plant height and leaf size (Zhang *et al.*, 2011). If the stress is prolonged, plant growth, and productivity are severely diminished (Osakabe *et al.*, 2014c). It has been discovered that plants adaptive responses to environmental challenges, such as drought, appear to result in enhanced herbicide resistance in weeds (Weller *et al.*, 2019).

2.4 Impact of invasive plant *G. cordifolia* on ecosystems and biodiversity

Gutenbergia cordifolia is a common perennial plant in Africa belonging to family Asteraceae, branching sub shrubby herb up to 2.5 m tall herbaceous. The plant has been utilized for therapeutic purposes, and as a result, it has spread around the world, becoming a weed in most rangelands (Ngondya, 2017). The species appears to have colonized and dominated more than one third the Ngorongoro crater floor (UNESCO, 2001). Due to the presence of a chemical sesquiterpene lactone in its leaves, both leaves and flowers are allergic and harmful to animals (Bussmann *et al.,* 2006; Zdero & Bohlmann, 1990), the plant is disliked by the majority of herbivores (Pers. Obv). These sesquiterpene lactones, according to Amorim *et al.* (2013), affect the rumen's microbial composition and general metabolic performance. As an invasive plant inhibits native pastures, putting herbivores in the invaded ecosystem's food supply in jeopardy and altering the ecosystem's structure, functions, and biodiversity. It has also been suggested that the *G. cordifolia* higher heights and greater densities may interfere with Rhinos feeding behaviour (Brett, 2001). According to other studies reported that *G. cordifolia* are also experiencing changes in phenology and distributions, which may exacerbate the threats to native species like pastures and reduce quality of the rangeland and pasturelands. Most invasive species as expected to *G. cordifolia* have portable traits favouring invasiveness in biodiversity including high tolerance against environmental extremes and greater adaptability in wide range of environmental conditions, high water, light and nutrient use efficiencies, zero or very short dormancy period, high productivity; and high reproductive potential (Singh, 2005). However, their effect on biodiversity has usually been assessed independently, despite good scientific reasons to expect the rate and extent of biological invasions to be influenced by climate change (Hobbs, 2000; Thuiller *et al.*, 2008).

2.5 Morphological and Physiological response of invasive plants in rainfall patterns and season

Changes in rainfall patterns have also been observed, but are more variable than those of temperature (Osakabe *et al.*, 2014b). Even under conservative emission scenarios, future climatic changes are likely to include further increase in rainfall and temperature in some regions and increase the frequency and severity of extreme weather events (Najberek *et al.*, 2017). Increasing extreme events of rainfall, wet and dry season are the cause characteristics of changes in morphology and physiology of most invasive species as a result worsen the problems caused by invasive plants in rangelands and agro-ecosystems at global scale, resulting from their changes in the range and population densities (Kitzberger, 2013). Rain seasons such as short and long rain season induce changes in morphology and physiology of most invasive plant species in different terrestrial ecosystem, increase infestation due to plant ability to adapt with changing climatic condition and cause negative impact to indigenous plant species to their colonizing area (Eskelinen & Harrison, 2014). Changing in rainfall patterns have a direct impact in invasive plant species as a changing physiological constraint by creating adaptive mechanism to a changing climate (Bradley *et al.*, 2010). However, in other perceptions, faster and early germination and plasticity in response to changing precipitations may give an advantage to invasive species compared to native species by enabling them to exploit period of low competition and facilitating their establishment (Bradley *et al.*, 2010; Wainwright & Cleland, 2013). Besides changing precipitation and seasonal rainfall have an indirect effect on invasive species through decreasing the competitive vigor and resilience to non-native invasive plant species (Walther *et al.*, 2009). Most of the available literature on physiology and morphological traits of most plants rather invasive plant species under rainfall season affect the richness, abundance and growth pattern and even time of flowering (Bolat *et al.*, 2014).

2.6 Impact climate change in Invasive plants

Changes in atmosphere and climate induce a wide variety of responses for many invasive plant species. There so many ways that climate change may challenge the way we can perceive and consider invasive species and native species, in such a way that some will change and others will stay steady unaffected and other non-native species to become invasive species, and native species likely to shift their geographical ranges into novel habitats (Bogale & Tolossa, 2021). For a given invasive species in a specific location, the consequences of climate change depends on direct effects of changing climate on individuals, indirect effects that alter availability of resources and interaction with other species and factors such as human influences that may alter the environment for an invasive species (Marambe & Wijesundara, 2021). It was also reported that due to climate change many invasive plants occupy unique phenological niches and track climate change compare to native species (Finch *et al.*, 2021). Where by invasive plants species have flexible phenologies and flower early than native plant species (Sattar *et al.*, 2021). Experiments and field observations provide evidence of the tendency for invasive plant species to outcompete with native plant species in presence of atmospheric seasonal changes (Oduor, 2013). Invasive species have been observed to reduce the resilience of native plants in different ecosystem range to climate change. Conversely, climate change minimize the resilience of habitats to biological invasion and making them more vulnerable to the impacts of climate change (Davidson *et al.*, 2011). For example some of the invasive grasses and trees have become invasive and significantly alter fire regimes, especially in areas that have become drier and warmer and cause other species not sustain and disappear. We may therefore expect that the same traits that make them successful invaders, such as broad environmental tolerances, high phenotypic plasticity, rapid adaptation or acclimatization to the environment, high reproductive output and growth rates, may also be beneficial under a changing climate (Collins *et al.*, 2017). Understanding how these invasive plant species interchange with climate change can be a useful aspect in creating policy, predicting the expected years to come land coverage of these invasive species and strategies of how to eradicate them effectively (Funk & Vitousek, 2007). This study will help to predict accurately and understand how *G. cordifolia* distributions and impacts will change under projected climate scenarios is essential for developing effective preventive, control, and restoration strategies (Gallardo & Aldridge, 2013).

CHAPTER THREE

MATERIALS AND METHOD

3.1 Soils and Climate

The soils of NCA are derived from underlying parent rocks and therefore almost all soils found in the area are basaltic in origin. Three main soil groupings are described as highlands soil type, short grass plains soil, and southwest soil types (NCA GMP, 2010). Ngorongoro's climate is influenced by the season and topography of the area. There are two distinct seasons, wet and dry. The wettest area is the eastern and southern part of the highlands that face the prevailing and moisture-laden winds from the Indian Ocean (Hanby & Bygott, 1998). The driest parts are the plains and Olduvai Gorge, lying at the feet of the mountains on the rain-shadow (Hanby & Bygott, 1998). The mean temperatures are about 10 °C to 38 °C, with higher temperatures occurring in the lower areas, around Masek and Ndutu (NCA GMP, 2010). Overall, the average annual precipitation during the study period in sampling locations (Ndutu, Central, Lerai) within the NCA was 497 mm with their minimum and maximum of 94 mm and 878 mm (Munishi *et al.*, 2020).

3.2 Vegetation

The vegetation type identified in NCA includes scrub heath, montane long grasslands, high open moorland and the remains of dense evergreen montane forests covering the steep slopes. The crater floor is mainly open short grass plains with two patches of Acacia woodland: Lerai forest, with co-dominants yellow fever tree (*Vachellia xanthophloea*) and *Rauvolfia caffra*. The undulating plains to the west are grass-covered with occasional umbrella acacia *Vachellia tortilis* and *Commiphora africana* trees (Berry, 2009). Within undulating Savannah plain are occasional Umbrella Acacia trees thus, giving way to the more open areas and dry shrubs regenerating following disturbance; the dominant shrubs recorded on the site include *Grewiabicolour, Grewia smiles, Combretum sp., Vachellia brevispica, Maerua angorensis,* and *Vachellia senegal*. The ground cover is mainly grassy with some forbs and herbs. The characteristic grass species are *Andropogon greenwayi, Themeda triandra, Digitaria scalarum,* and *Aristida* species. Herb layer is characterized by *Helichrysum schimperi, Lippia javanica, Lupinus princeii, Hypericum revolutum,* and *Crotalaria* species.

3.3 Seedling and soil sample collection

The field site where field data were collected was in the Ngorongoro Conservation Area (NCA). The NCA is a multiple land use protected area, located in northern Tanzania (20^0 30`- 30 30'S, 340, 50'-350 55'E) and covering an area of 8283 km2 (Fig. 1). It borders Loliondo Game Controlled Area (LGCA) to the North, Serengeti National park to the west, Lake Eyasi to the south and agricultural communities on the border of southern east (Niboye, 2010). Ngorongoro Crater is considered as the world's leading intact, inactive caldera occupying approximately 300 km^2 about four percent of total NCA. The rim is 2200 m above sea level and the crater floor (250 km^2) is about 600 m below, composed predominantly of grassland composed of small patches of grasses and Acacia riverine forest (Fyumagwa *et al.*, 2007).

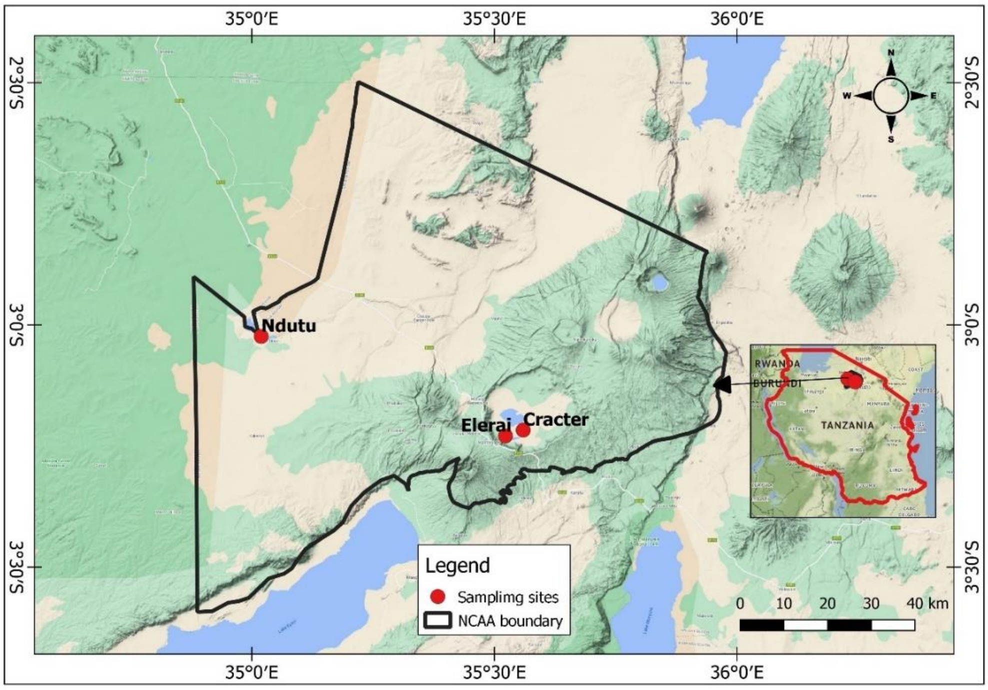

Figure 1: A map of Ngorongoro Conservation Area showing areas where *Gutenbergia cordifolia* seedlings and soil samples were collected

3.4 Experimental Design

3.4.1 Seedlings and soil sampling for screen house experiment

Seedlings and soil sample that were used for screen house-controlled study were collected from areas that have been highly invaded by *G. cordifolia* in different sites with different biophysical conditions within the Ngorongoro Conservation Area and this includes; Ndutu area (03º01' S, 03º01' E), Crater Centre (03º13' S, 035º31' E) and Lerai forest (03º01'S 035º31' E) within the

Ngorongoro Conservation Area located in northern Tanzania (Fig. 1). Seedlings collected from the three sites were mixed to form one composite sample that was later exposed under different irrigation treatments in the screen house experiment. The same was performed for the collected soil samples. An ecological control comprising *G. cordifolia* seedlings of the same cohort (estimated one-month age) were collected in the field during short rain (2019 November-January 2020) and soil samples were sorted according to sites prior to experimentation (Plates 1 and 2). Screen house experiment for *G. cordifolia* seedlings that were collected from the field were performed to assess their morphological and physiological responses to varying water levels (irrigation regimes).

Plate 1: *Gutenbergia cordifolia* seedling collection from different sites within Ngorongoro Conservation Area

Plate 2: Soil sample collection in different invaded site with *Gutenbergia cordifolia* in Ngorongoro Conservation Area

3.4.2 Screen house study design (effects of water stress)

In the screen house, the effects of water stress on *G. cordifolia* morphological traits (height, root collar diameter, leaf size, leaves number biomass (fresh and dry weight), root length, and physiological traits (leaf chlorophyll and leaf anthocyanin content) were studied. Four (4) seedlings of *G. cordifolia* were planted in 3 kg pots each filled with soil that were collected from Ngorongoro Conservation Area then acclimatized for seven (7) days in the screen house to adapt with screen house conditions. There after two seedlings were uprooted from each pot for initial morphological and physiological measurements. The remaining two (2) seedlings per pot were then exposed under different water stress levels (irrigation regimes) at the same growth phase and vigor. The following treatments (irrigation regimes) were applied: V_0 (control) = 350 ml/ 1.5 kg soil sample, V_1 = 500 mls /1.5 kg soil sample, V_2 = 800 ml/1.5 kg sample, V_3 = 150 ml /1.5 kg soil sample and V_4 = 150 mls /1.5 kg soil sample. The first three treatments (V_0, V_1 and V_2) were reflecting flood extremes and were irrigated 48 times per month while the rest (V_3 and V_4) reflected the drought extreme and were irrigated once and thrice per month respectively. All plants in screen house were randomized weekly to avoid any positional effects.

3.4.3 Field data collection (effects under field conditions)

The project comprised a field reconnaissance assessment on the 22nd of December 2019 within the Ngorongoro Conservation Area to identify prospective sites for measuring *G. cordifolia* morphological and physiological features under field circumstances within the NCA. Then in early January 2020, physiological and morphological data were collected in three different sites with high infestations of *G. cordifolia*: Ndutu, Lerai woodland, and the Center within the Ngorongoro conservation area.

Plate 3: Measurement of morphological parameter under field condition

3.4.4 Field data collection (effects under different rainfall season)

Data collection was done in two rain season during short rain and long rain season. During short rain season data's were collected on early of January 2020 while long rain season data's were taken on late of May. Data collected were Morphological traits (Plant height, root collar diameter, relative growth rates, leaf surface area, number of leaves, root and shoot biomass (fresh and dry weight), and physiological parameters and physiological traits (leaf chlorophyll and leaf anthocyanin content). At least 5% of individual stands in each site were assessed in a systematic/purposive sampling of Gutenbergia cordifolia strongly invaded locations (Ndutu, Lerai, and Center in Ngorongoro crater (three sites each of 0.5 acre) for measuring both morphological and physiological features of *G. cordifolia*.

3.5 Measurement of morphological traits

Gutenbergia cordifolia morphological traits (height, leaf length and basal radius, root collar diameter, number of leaves, root and shoot weight) were measured after 12 weeks under screen house condition and in each field visit under short and long rain season. A 1-meter ruler and a digital calliper were used to measure the height of seedlings and the diameter of the root collar at 5 cm above the ground (Ngondya, 2017). A population was defined as the total number of leaves in all pots that were subjected to the same treatment. The total number of leaves on each plant was counted, and over 30% of leaves were randomly sampled to determine leaf area. Leaf surface area was determined by measuring leaf basal radius and leaf length using a meter ruler (Tabot and Adams 2012a). Leaf area was calculated geometrically from the leaf basal radius and length using area equations based on the conical shape of the leaf (Tabot and Adams 2012b).

$$(\boldsymbol{S}) = \boldsymbol{\pi r^2} + \boldsymbol{\pi}\mathbf{rh} \quad \textbf{………………….Equation 1}$$

Where S = leaf surface area, π = 3.142, r = leaf basal radius and h = leaf length

For biomass determination, shoot and root material were separated and weighed using a digital weigh balance to obtain total above/below ground fresh and dry biomass (SERAS, 1994).The whole *G. cordifolia* plant were harvested (uprooted), washed, and dried at 60 ^{o}C for 2 days (Makoi *et al.*, 2010) prior to measurement to obtain shoot and root dry weight.

3.6 Measurement of physiological traits

According to Ngondya (2017), the total chlorophyll in the leaves of *G. cordifolia* seedlings was extracted as follows, 50 mg of fresh leaves with a surface area of 2.25 cm2 were immersed in 4 ml of Dimethyl Sulfoxide (DMSO) and incubated at 65 °C for 12 hours. To determine absorbance, the extract was put to glass cuvettes. The absorbance of blank liquid (DMSO) and samples was measured at 663 nm and 645 nm using a 2000 UV/VIS spectrophotometer (UNICO®) (Hiscox and Israelstam 1979), and the total leaf chlorophyll (total Chl) was computed using Arnon's (1949) equation:

$$\textbf{Total Chlorophyll content} = 0.0202A_{663} + 0.00802A_{645} \textbf{ …………..Equation 2}$$

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

Leaf anthocyanin's of *G. cordifolia* were extracted as described by Makoi *et al.* (2010). Leaves of *G. cordifolia* were oven-dried at 60 °C for 48 hrs. Weighed, ground into a fine powder. Then, 0.10 g of well-ground leaf material were weighed and mixed with 10 ml of acidified methanol prepared from a ratio of 79:20:1 MeOH: H_2O: HCl. The mixture was incubated for 72 h in darkness for auto-extraction and filtered through Whitman paper number 2. The extract were transported to glass cuvette for determination of absorbance The absorbance of acidified methanol as standard and that of samples were subjected under a 2000UV/VIS Spectrophotometer (UNICO®)at 530 nm and 657 nm and expressed as Abs $g.DM^{-1}$ (Makoi *et al.*, 2010). Anthocyanin concentration in leaf extracts were measured as follows:

Anthocyanin concentration $A_{530} - \frac{1}{3}A_{657}$ **(Makoi *et al.*, 2010)…………Equation 3**

Where $\mathbf{A_{530}}$ and $\mathbf{A_{657}}$ are absorbance readings at 530 nm and 657 nm, respectively.

Plate 4: Grounded leaves of *Gutenbergia cordifolia* for Chlorophyll and Anthocyanin content for analysis

3.7 Statistical data analysis

Shapiro-Wilk test was used to test for normality of *G. cordifolia* physiological and morphological traits data under different water stress levels. For normally and non-normally distributed data, One-way analysis of variance (ANOVA) and the Kruskal–Wallis test were used. The Fisher's Least Significant Difference (LSD) was used to separate the resulting means for normally-distributed and Wilcoxon test with Bonferroni correction was used to test for significant differences in mean values for non-normally distributed data. Paired sample T-test was used to compare the effect of rain season (wet and dry) and comparison of field and screen house condition on *G. cordifolia*.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Effects of water stress on *G. cordifolia* height and stem diameter under field and screen house condition

Gutenbergia cordifolia height differed significantly between treatments ($F_{(4, 19.4)}$ = 15.2, *p* = <0.001). The maximum *G. cordifolia* height of 159 cm was observed under flood irrigation while the minimum height of 9 cm was observed under drought irrigation (Fig. 2). Significant change in root collar diameter of *G. cordifolia* was observed across treatments ($\chi^2_{(4,N=5)}$ = 29.1, *p* = 0.001) with the largest and smallest *G. cordifolia* root collar diameter of 5 mm and 1.24 mm observed under moderate flood and drought irrigation regimes respectively (Table 1).

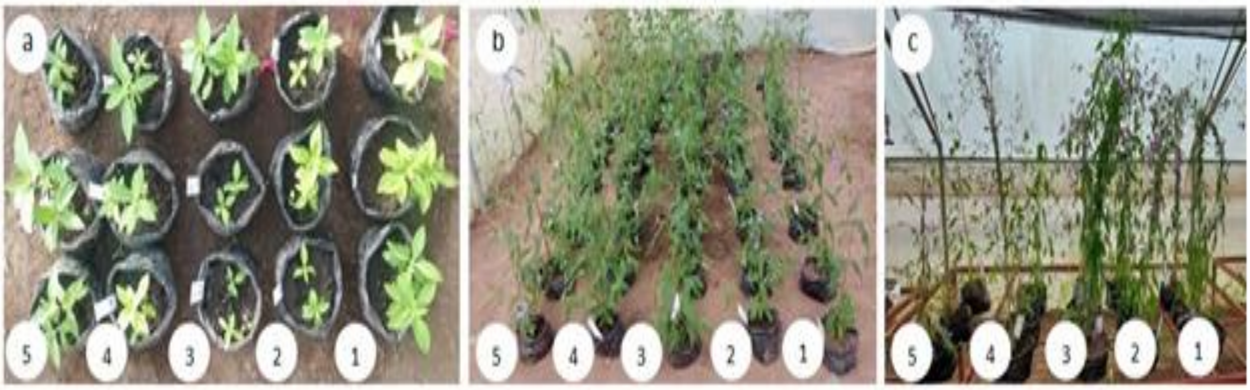

Plate 5: *Gutenbergia cordifolia* mortality and vigor at age of (a) two weeks (b) six weeks and (c) twelve weeks under varying water stress levels (1 = normal, 2 = moderate flood, 3 = flood, 4 = moderate drought and 5 = drought)

Table 1: *Gutenbergia cordifolia* mean (± S.E) height and root collar diameter per treatment

Treatments	**Height (cm)**		**RCD (mm)**	
	Range	**Mean**(± S.E)	**Range**	**Mean**(± S.E)
Control (350 ml)	60.00 -110.60	90.01±5.80[a]	2.83-5.00	3.65±0.25[a]
Moderate flood (500 ml)	76.25-126.46	95.20±7.24[a]	3.17-5.00	4.09±0.26[ab]
Flood (800 ml)	70.00 -159.00	103.63±9.73[a]	4.10-4.80	4.20±0.13 [b]
Moderate drought (150 mlx3)	39.40-82.00	53.49±3.98[b]	2.25-2.60	2.64±0.11 [c]
Drought (150 mlx1)	9.00-65.50	39.54±8.19[cb]	1.24-3.57	2.21±0.35 [c]
F-statistics ** *p*-value**	$\mathbf{F_{(4,19.4)} = 15.2}$ ***p* = <0.001**		$\boldsymbol{\chi^2_{(4,N=5)}} = \mathbf{29.1}$ ***p* = <0.001**	

S. E = Standard Error, RCD = root collar diameter, means with the same letter are not significant at p< 0.05

4.1.2 Effects of water stress on *G. cordifolia* leaf characteristics under field and Screen house condition

A significant change in the number of leaves per plant were observed ($\chi^2_{(4, N=45)} = 26.6$, $p = <0.001$, Table 2, Fig. 2) across water treatment levels. Generally, the number of leaves was highest under moderate flood and flood treatments, 143 and 194 leaves/plant respectively, while the lowest number of leaves (13 leaves/plant) were observed under drought treatment (Table 2). Different water stress levels caused a significant decrease in leaf surface area per plant ($\chi^2_{(4, N=45)} = 17.3$, $p = 0.002$). Largest leaf surface area was observed under flood treatment (9234.04 mm^2) while the smaller leaf surface area of 362.59 mm^2 was observed under drought irrigation (Table 2).

Table 2: *Gutenbergia cordifolia* mean leaf surface area (±S. E) and number of leaves per treatment

Treatments	No. of leaves		LSA (mm^2)	
	Range	**Mean** (±S. E.)	**Range**	**Mean** (±S. E.)
Normal (350 ml)	38.00-106.00	83.00±7.30[a]	2377.51-4683.94	3384.3±313[a]
Moderate flood (500 ml)	69.00-143.00	98.00±8.80[ab]	1649.55-4565.62	3247.9±268[a]
Flood (800 ml)	60.00-194.00	99.00±18.10[ab]	1239.83-9234.04	3458.4±869[a]
Moderate drought (150 mlx3)	43.00-95.00	60.00±5.20[bc]	1079.57-4450.84	2465.7± 384[a]
Drought (150 mlx1)	13.00-52.00	41.00±6.84[d]	362.59-3120.98	1108.9±323[b]
H-statistics ***p*-value**	$\chi^2_{(4,N=45)}$ **= 26.6** ***p = <0.001***		$\chi^2_{(4,N=45)}$ **= 17.3** ***p = <0.001***	

S.E = Standard Error, L.S.A = Leaf surface area, means with the same letter are not significant at p< 0.05

4.1.3 Effects of water stress on *G. cordifolia* shoot biomass under field and screen house condition

While generally there were no significant change in shoot fresh weight per treatment ($F_{(4,19.5)}$ = 2.04, p = 0.128) a significant increase in shoot dry weight was observed ($F_{(4,19.5)}$ = 6.29, p = 0.002) particularly under flood treatment (Fig. 3). Highest shoot fresh and dry weight of 69 mg and 23.38 mg respectively were observed under flood treatment and the lowest shoot fresh and dry weights of 12 mg and 2.56 mg was observed under moderate drought and drought treatments respectively (Fig. 3).

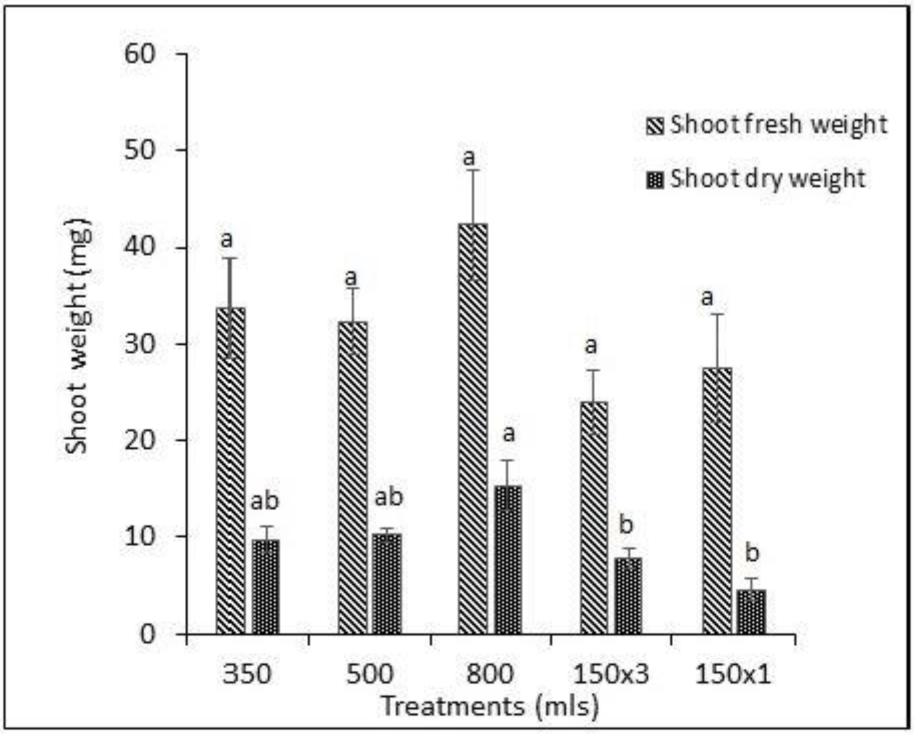

Figure 2: Effect of water stress on *Gutenbergia cordifolia* shoot fresh weight and dry weight

4.1.4 Effects of water stress on *G. cordifolia* root biomass under field and Screen house condition

Generally, water stress caused a significant decrease in both *G. cordifolia* root fresh and dry weight ($\chi^2_{(4, N=45)}$ = 11.7, p = 0.020 and $\chi^2_{(4, N=45)}$ = 26.7, p = 0.001 respectively). The highest both root fresh and dry weight of 6.00 mg and 2.00 mg respectively were observed under normal treatment, and the lowest root dry and fresh weight of 0.13 mg and 2.00 mg were observed under moderate drought and drought treatments respectively (Fig. 4).

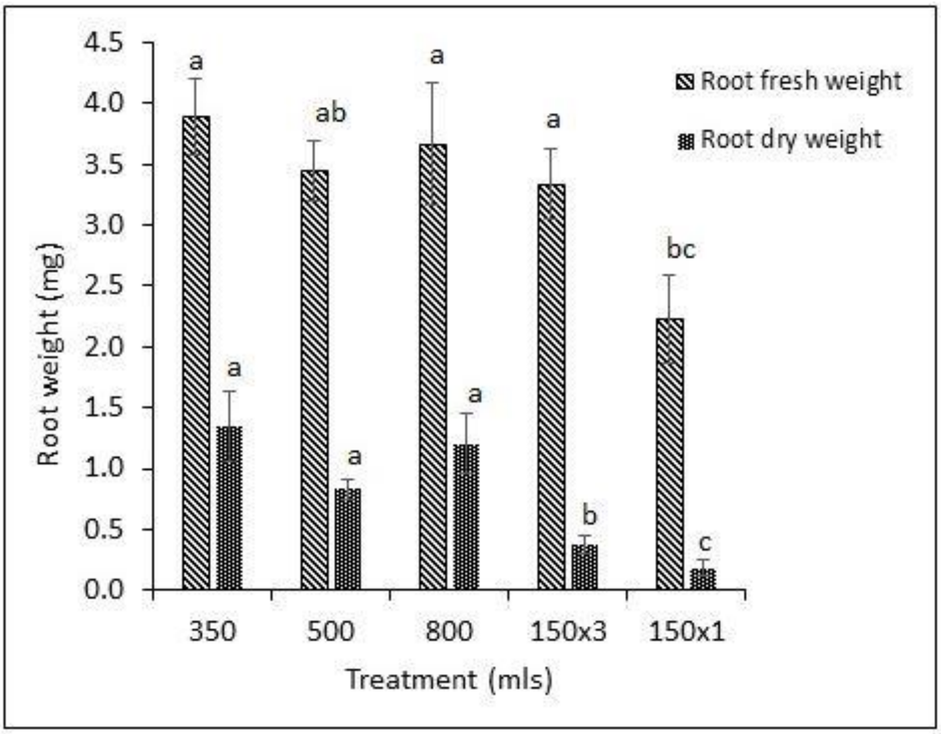

Figure 3: Effect of water stress on *G. cordifolia* root fresh and dry weight

4.1.5 Effects of water stress on *G. cordifolia* leaf pigmentation under Field and Screen house condition

Water stress significantly decreased leaf anthocyanin level ($F_{(4, 18.2)} = 16.61$, $p = 0.001$). The maximum mean anthocyanin pigment level was 0.298 Abs $g.DM^{-1}$ under flood treatment and the lowest anthocyanin pigment was 0.08 Abs $g.DM^{-1}$ under drought treatment. A further significant decrease in leaf chlorophyll was observed ($\chi^2_{(4, N=45)}$) = 35.4, $p = 0.001$). The maximum and minimum leaf chlorophyll level of 0.057 and 0.018 were observed under moderate flood and drought treatments respectively (Fig. 5).

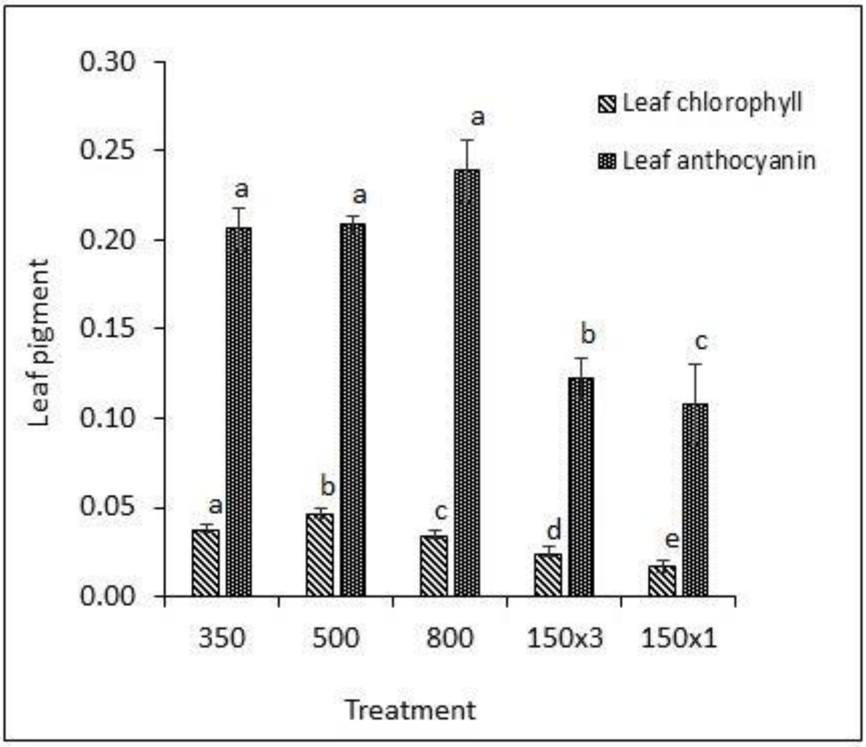

Figure 4: Effect of water stress on *G.cordifolia* leaf anthocyanin and chlorophyll

4.2 Response of physiological and morphological traits of *G. cordifolia* under Field and Screen house condition

4.2.1 Effect of *G. cordifolia* height and diameter under field and screen house condition

A significant difference in *G. cordifolia* height was observed between field and screen house observation ($t_{(44)} = 7.52$, p<0.001). The maximum height of 246 cm was observed in the NCA, while a maximum height of 159.4 cm was observed under screen house conditions. While a significant change in *G. cordifolia* root collar diameter was observed between field and screen house conditions ($t_{(44)} = 4.39$, $p<0.001$), the highest and lowest root collar diameter of 10.1 cm and 1.24 cm were observed under field and screen house conditions, respectively (Table 3).

Table 3: The mean (± S.E.) height and root collar diameter *for G. cordifolia*

Site	Height(cm)		Diameter(cm)	
	Range	**Mean** (± S.E.)	**Range**	**Mean** (± S.E.)
Field	51-246	142.64±5.62[a]	2.39-10.10	6.34±0.27[a]
Screen house	39.4-159	99.23±4.31[a]	1.24-5.00	3.36±0.16[b]
t-statistics	**$t_{(44)} = -7.52$, *$p<0.001$***		**$t_{(44)} = 4.39$ *$p<0.022$***	

S.E = Standard Error, RCD = root collar diameter, means with the same letter are not significant at p< 0.05

4.2.2 Effects of *G. cordifolia* leaf number and surface area under field and screen house conditions

There were no significant difference in the number of leaves per plant ($t_{(44)} = -0.38$, $p<0.71$) for *G. cordifolia* between field and screen house conditions (Table 4). Overall, the highest number of leaves (340 leaves/plant) was observed in screen house conditions and the lowest (27 leaves/plant) was observed under field conditions. Likewise, no significant effect in *G. cordifolia* leaf surface area ($t_{(44)} = -0.89$ $p<0.37$) was observed between screen house and field conditions. The largest leaf surface area was observed under field condition (18 503.54 mm^2) while the smaller leaf surface area (2 377.51 mm^2) was observed under screen house condition (Table 4).

Table 4: Observed *G. cordifolia* mean (±S. E.) leaf surface area and number of leaves under field and screen house conditions

Site	No of leaves		LSA (mm^2)	
	Range	**Mean** (±S. E.)	**Range**	**Mean** (±S. E.)
Field	282-27	94.00±7.90[a]	18503.00-2383.26	11018.54±537.91[a]
Screen house	340-35	99.00±9.13[a]	10074.04-2377.51	4795.66±782.426[a]
t-statistics	**$t_{(44)} = -0.38$, $p<0.71$**		**$t_{(44)} = -0.89$ $p = <0.37$**	

S. E = Standard Error, L.S.A = Leaf surface area, means with the same letter are not significant at p< 0.05

4.2.3 Effects of *G. cordifolia* root biomass under field and screen house conditions

Generally, significant change in root biomass between screen house and field were observed. While significant change in root fresh weight ($t_{(44)} = 3.94$, $p = 0.001$) were observed between screen house and field condition. While significance root dry weight observed was ($t_{(44)}$ 4.5, $p = 0.001$) and the maximum (4.84 mg) and minimum (0.014 mg) root dry weights were observed under field and screen house conditions respectively (Fig. 6). On the other hand, the highest root fresh weight (12 mg) and lowest root fresh weights (2 mg) were observed under screen house and field condition respectively.

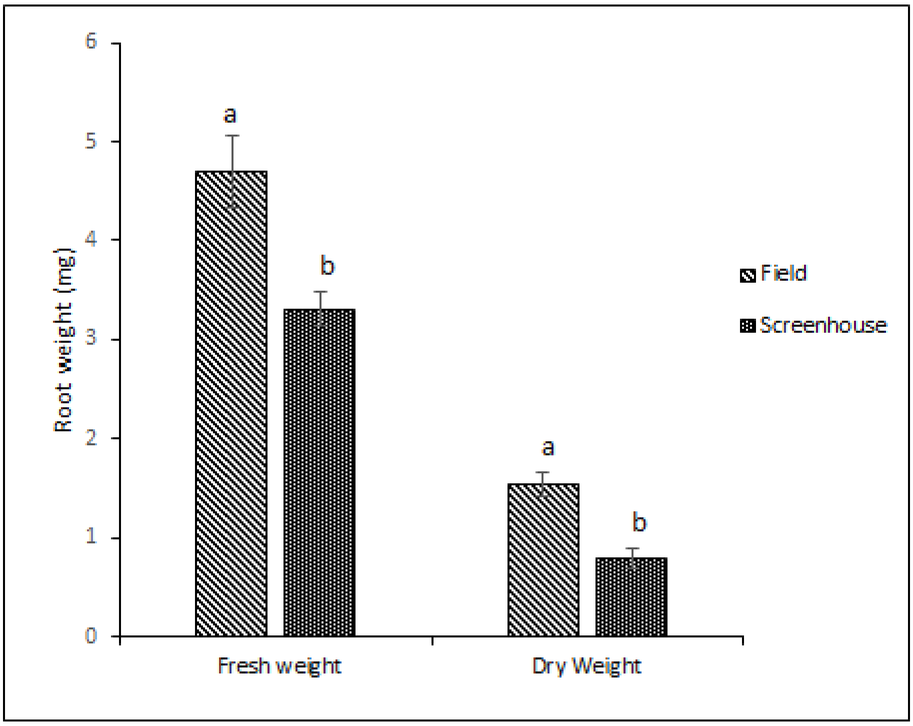

Figure 5: Effects on *G. cordifolia* root biomass under field and screen house conditions

4.2.4 Effects of *G. cordifolia* shoot fresh biomass under field and screen house conditions

Generally, a significant difference in shoot fresh weight were observed between screen house and field conditions ($t_{(44)} = 3.94$, $p = 0.001$) while no significant difference in shoot dry weight was observed ($t_{(44)} = -0.17$, $p = 0.863$). The highest and lowest shoot fresh weights of 152 mg and 10 mg were observed under field condition, similarly, the highest and lowest dry weight of 40.32 mg and 1.88 mg were observed under field conditions (Fig. 7).

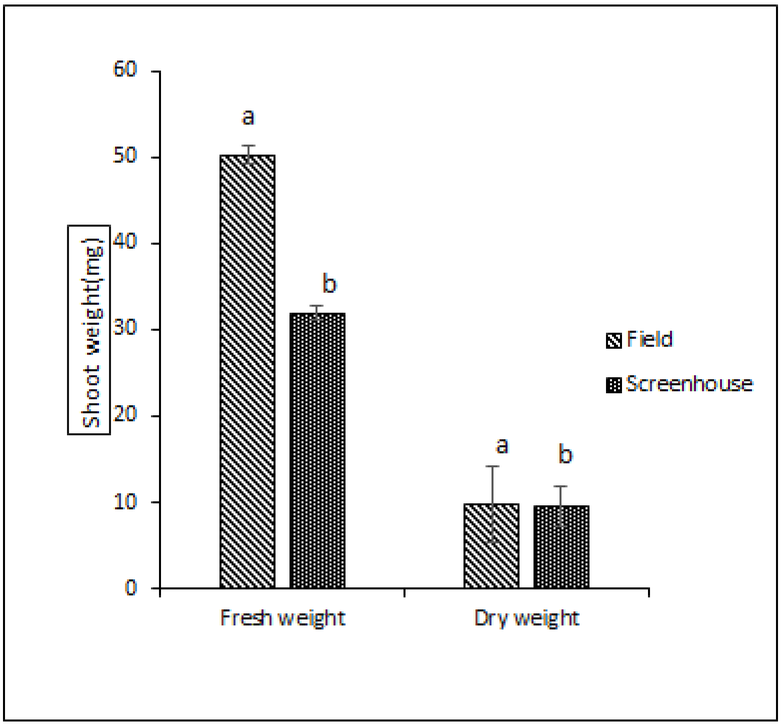

Figure 6: Effects on *G. cordifolia* shoot biomass under field and screen house condition

4.2.5 Effects of *G. cordifolia* leaf pigment under field and screen house condition

No significant effect was observed of leaf anthocyanin level ($t_{(17)} = -1.83$, $p = 0.085$) on field and screen house condition during this study. The mean anthocyanin pigment level of 0.576 Abs $g.DM^{-1}$ was highest under field and the lowest mean anthocyanin pigment of 0.16 Abs $g.DM^{-1}$ was observed under screen house conditions. A further significant difference in leaf chlorophyll content was observed between field and screen house conditions (w = (17) p = 0.002), with the maximum and minimum leaf chlorophyll levels of 0.0673 and 0.023, respectively (Fig. 8).

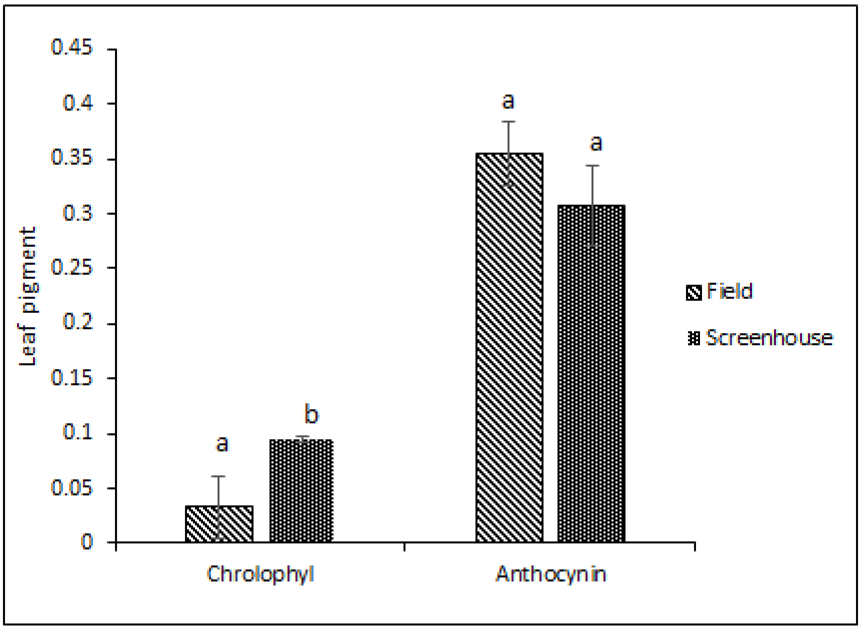

Figure 7: Effects on *G. cordifolia* leaf pigment under field and screen house condition

4.3 Effect of rain seasonal variations on morphological and physiological traits of *G. cordifolia* under different field site

4.3.1 Effect of rain season on *G. cordifolia* height and root collar diameter under different field site

A significant change in height per plant were observed per rain season $t_{(9)} = 10.108$, $p = <0.002$ and $t_{(24)} = 8.68$, $p = 0.001$ (Table 5) within Center and Lerai site respectively. Generally, the maximum and minimum heights of 213 cm and 51 cm were observed in Ndutu and center sites respectively during long rain season. During short rain season, the maximum and minimum heights of 198 and 67.4 cm were observed in Lerai and the Center sites, respectively. Significant difference in root collar diameter of *G. cordifolia* was observed between short and long rain season ($t_{(24)} = 3.21$, $p = 0.004$; $t_{(9)} = -3.23$ $p = 0.01$) in the Center and Ndutu sites, respectively. During long rain season, the largest and smallest root collar diameters of 10.1 cm and 4.02 cm were observed under Lerai and the Center sites, respectively. While during short rain season the largest and smallest root collar diameters of 9.53 mm and 4.31 mm were observed in within the two different sites within the center (Table 5).

Table 5: Observed *G. cordifolia* mean (±S. E.) height and root collar diameter (RCD) under field and screen house conditions

Site	Height (Short-rain)		Height (Long-rain)		RCD (Short rain)		RCD (Long rain)	
	Range (cm)	Mean	Range (cm)	Mean	Range (mm)	Mean	Range (mm)	Mean
Center	95.3-51	69.6±4.7 [a]	135-103	119.5±3.1 [b]	9.53-4.31 [a]	5.95±0.57	9.24±5.12	7.79-0.41 [b]
t-statistic	**$t_{(9)}$ = 10.108 *p* = <0.002**				**$t_{(9)}$ = -3.23 *p* = <0.01**			
Ndutu	181-61	111.76±4. 59[a]	213-119	157.92±5.36[a]	8.65-5.04	6.65±0.21[a]	8.22-4.02	5.6±0.25[b]
t-statistic	**$t_{(24)}$ = 1.61 *p* = <0.12**				**$t_{(24)}$ = 3.21 *p* = <0.004**			
Lerai	198-111	158.8±5.57[a]	116-67.4	90±3.58 [b]	7.89-4.7	6.27±0.25[a]	10.1-4.74	6.54±0.36[a]
t-statistic	**$t_{(14)}$ = 8.68, P<0.001**				**$t_{(14)}$ = 0.65 p=0.523**			

S. E = Standard Error, R.C.D = Root Collar Diameter, means with the same letter are not significant at p< 0.05

4.3.2 Effect of Rain season on *G. cordifolia* leaf characteristics in different areas in the NCA

A significant effect in the number of leaves per plant during short rain and long rain season were observed ($t_{(9)} = 10.11$, $p = <0.002$; $t_{(24)} = 3.6$, $p = 0.001$ and $t_{(14)} = 5.8$, $p = 0.001$) in the Center, Ndutu and Lerai sites, respectively. Generally, the number of leaves was highest (340 leaves/plant) in Lerai site and 35 leaves/plant in Ndutu site in short rain season, while in long rain season the highest and lowest number of leaves 282 and 20 (leaves/plant) were observed in Ndutu site (Table 6) respectively. Short and long rain seasons caused a significant effect in leaf surface area per plant ($t_{(9)} = 3.23$, $p = 0.01$; $t_{(24)} = 8.74$, p = <0.001) in the Center and Ndutu site respectively, while no significant effects were observed in Ndutu area during the short rain seasons ($t_{(14)} = 2.07$, $p = <0.057$). During long rain season largest leaf surface area was 26 083 mm^2, while the smallest leaf surface area of 983.0 mm^2 was observed in Lerai site during short rain season. While in short rain season highest leaf surface area was 26 005 mm^2 under Lerai site and the lowest leaf surface area of 2 307.9 mm^2 was observed under Ndutu site (Table 6).

Table 6: Observed *G. cordifolia* mean (±S. E) leaf surface area and number of leaves under short rain and long rain season

Site	No_of leaves Short rain		No_of leaves Long rain		LSA Short rain		LSA Long rain	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Center	162-41	73.4±11.24[a]	154-72	105.4-7.81[b]	12481.5-4312.8	8130.01-1001.2[a]	1447.5-3838.3	8290.2±1094.92[b]
t-statistic	**$t_{(9)}$ = 10.11, *P* = <0.002**				**$t_{(9)}$ = -3.23, *p* = <0.01**			
Ndutu	282-20	78±14[b]	340-65	147±13[a]	14243.04-2307.9	8906.8±585.36[a]	7472.57-983.03	3235.9±379.68[b]
t-statistic	**$t_{(24)}$ = 3.67, *p* = 0.001**				**$t_{(24)}$ = 8.74, *p* = <0.001**			
Lerai	146-35	95.4 ±8.41[a]	198-111	158.8±8.41[a]	26005-4001	13717±1586[b]	26083.27-4233.8	10751±1452[a]
t-statistic	**$t_{(24)}$ = 5.8, *p* = 0.001**				**$t_{(24)}$ = 2.07, *p* = <0.057**			

S. E = Standard Error, L.S.A = Leaf surface area, means with the same letter are not significant at p< 0.05.

4.3.3 Effect of Rainfall on *G.cordifolia* shoot weight in different areas in the NCA

Significant difference in shoot fresh weight ($t_{(24)} = -5.58$, $p<0.001$) were observed under both long rain season and short rain season. Whereby maximum and minimum shoot fresh weight of 112 mg was observed in Ndutu site and 10 mg under Lerai and Center sites in Short rain season respectively. In contrast significant increase in shoot dry weight was observed ($t_{(24)} = 3.67$, $p = 0.001$) particularly both short rain and long rain season. Highest Shoot dry weight 46.55 mg under Lerai site and lowest root fresh weight of 2 mg observed under Ndutu site in Long rain season, while in short rain season maximum and minimum shoot dry weight was 26.44 mg and 1.88 mg both in in center site (Fig. 9).

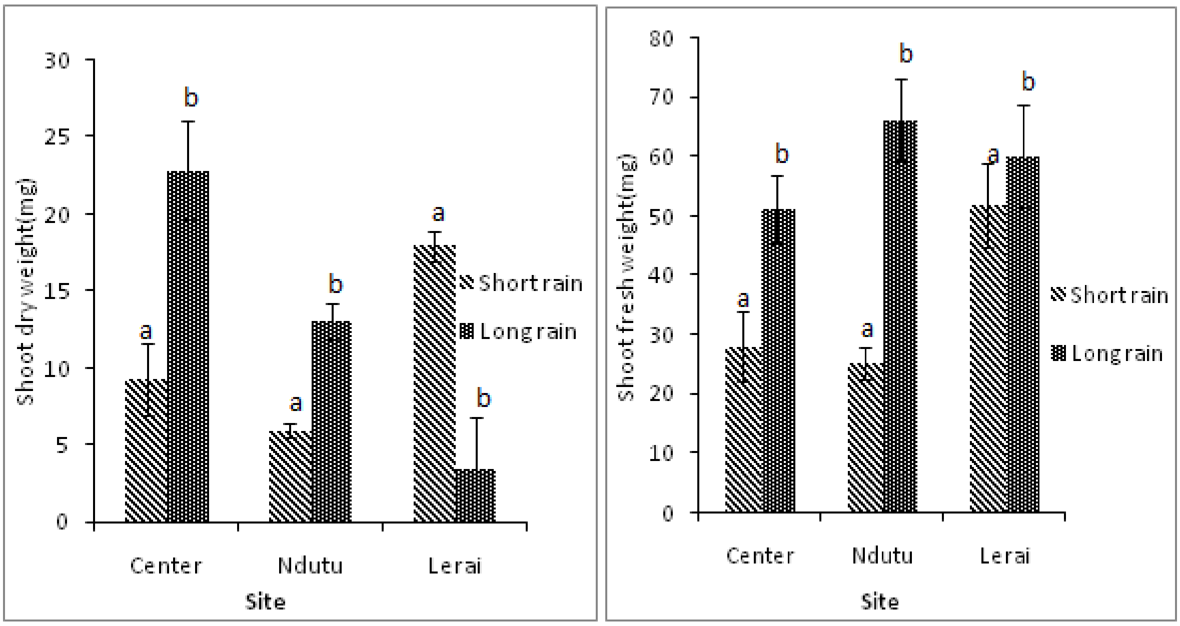

Figure 8: Effects on short rain and long rain season on *Gutenbergia cordifolia* shoot fresh and dry weight

4.3.4 Effect of Rain season on *G. cordifolia* root weight in the field sites

No Significant effects in root fresh weight ($t_{(24)} = 0.26$, $p = 0.75$) were observed under short and long rain season. The highest and lowest root fresh weight of 46.55 mg and 2 mg in Lerai and Ndutu site was observed in long rain season, respectively. Highest root fresh weight of 26.44 mg and the lowest root fresh weight of 1.88 mg were observed during the short rain season in the main Center site. Similarly no significant difference in root dry weight was observed ($t_{(24)} = -0.63$, $p = 0.53$) particularly between short and long rain season. Whereby maximum and minimum height of 5.01 and 0.09 mg was observed in Ndutu and Lerai site respectively, in short rain season the maximum of 6 mg and minimum of 0.37 mg root dry weight were observed in the Center site in long rain season (Fig. 10).

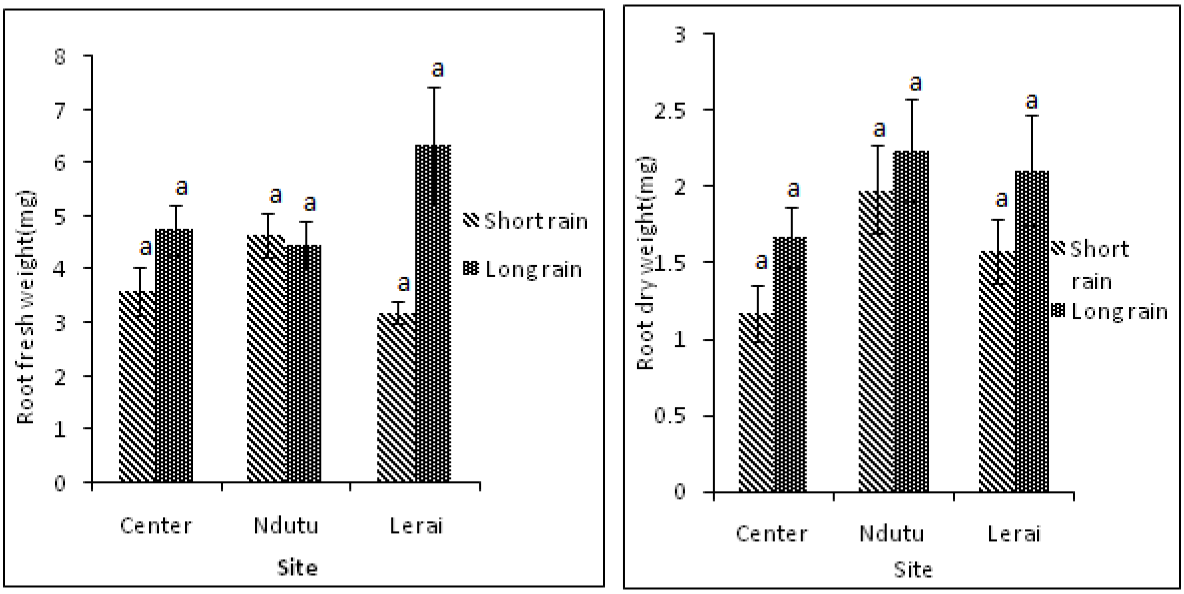

Figure 9: Effects on short rain and Long rain season on *Gutenbergia cordifolia* root fresh and dry weight

4.3.5 Effect of rain season on *G. cordifolia* leaf pigment in different site

No observed significant difference in *G. cordifolia* leaf anthocyanin level ($t_{(8)} = 0.248$, $p = 0.058$) between the field sites. The maximum and minimum anthocyanin pigment level of 0.19 Abs $g.DM^{-1}$ and 0.06 Abs $g.DM^{-1}$ was observed in Lerai and the Center site under short rain season, respectively. While highest and lowest anthocyanin pigment of 0.25 Abs $g.DM^{-1}$ and 0.05 Abs $g.DM^{-1}$ in Lerai and Ndutu site during long rain season. A further significant difference in leaf chlorophyll was observed ($t_{(8)}) = -2.65$, $p = 0.05$). The maximum and minimum leaf chlorophyll level of 0.06 and 0.024 were observed in the Center site during short rain season and while maximum and minimum leaf chlorophyll 0.041 and 0.019 in Center and Lerai site under Long rain season respectively (Fig. 11).

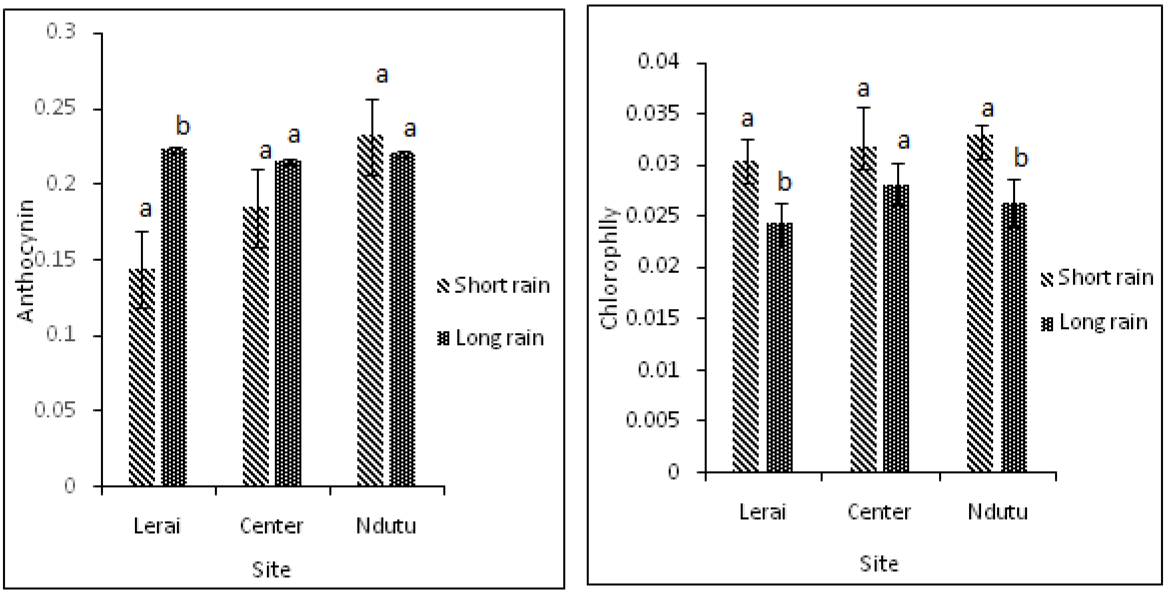

Figure 10: Effects on short and long rain season on *Gutenbergia cordifolia* leaf pigment

4.4 Discussion

4.4.1 Effects of water stress on *G. cordifolia* height and root collar diameter

In this study *G. cordifolia* height and root collar diameter were significantly small in water stress. Short height and root collar diameter was observed under drought treatment this might have been due to increasing drought stress, as previously reported by Nezami *et al*. (2008). Most plants exposed under stress environment normally invest much on ensuring reproduction success rather than growth (Davis, 2020). Drought stress might have brought down the water potential of stem cells to a lower level needed for cell elongation and, as a result, shorter internodes, small root collar diameter and stem height (Chaves *et al.*, 2002). Shortage of water to ensure elongation and girth growth of *G. cordifolia* ultimately resulted into reduced height and root collar diameter. On the other hand, the observed increase in *G. cordifolia*'s height and root collar diameter under both flood and moderate flood treatments was likely due to the ability of a plant to adapt to different environmental conditions. Based on these observations it is of interest that with changing climate as forecasted by the increase in rainfall intensity all over east Africa will have tremendous both negative and positive effects to the proliferation of *G. cordifolia.* Although this is not necessarirly implying that *G. cordifolia* will be eliminated but it will likely reduce its soil seedbank particularly in low raifall areas (Eslami *et al.*, 2010). Interestingly, as water stress tends to reduce the level of seed dormancyof some plant species (Probert *et al.,* 2009), it is therefore expect that most of the East African regions that will be facing drought conditions to have significant reduction of *G. cordifolia*. This information is important to understand *G. cordifolia's* physiological mechanisms involved in water stress tolerance or susceptibility, which will help to predict their future productive potential under predicted rainfall regimes in East Africa (Platts *et al., 2015*) and ultimately, provide the needed information to manage the plant so that to ensure pastures availability in East African Rangelands.

4.4.2 Effects of water stress on *G. cordifolia* Leaf characteristics

Significant decrease in *G. cordifolia's* number of leaves was observed in drought and moderate drought, while increased number of leaves were observed in flood and moderately flood conditions. This shows that water deficit is common environmental stress experienced by plants and strongly impairs production of leaves, increased drought therefore can severely affect *G. cordifolia* fitness which might in turn affect its seed production ability. The ultimate effect therefore is a reduced both abundance and seed bank of *G. cordifolia* in the future should there be drought. The observed increase in leaf surface area from control, moderate flood to flood and the decrease from drought to severe drought can similarly be explained by the tendency of plants to regulate the required amount of water through evapo-transpiration under excessive water availability While in an opposite way, plant have a tendency to retain water when exposed under water stress by ensuring there is a reduced surface area through which water from the plant can be lost (Chen *et al.*, 2020). *Gutenbergia cordifolia* exposed under no stress (control), moderate flood and flood had excessive water so to reduce this, plant had to have leaves with larger surface area to allow for more water loss through evapo-transpiration due to increase in number of chloroplast and vice versa. Severe drought conditions tend to have water deficit so to retain water, plants have to form leaves with smaller surface areas so as to reduce water loss through evapo-transpiration. Leaf surface area reduction is due to water stress increments where leaf became spindle and remained in a stunted state (Riaz *et al.*, 2013). Although *G. cordifolia* seem to adapt to changing water stress levels, it is interesting that in the event of future drought we are likely to have stunted *G. cordifolia* which are not likely to add much seeds to the soil and therefore the overall outcome will be reduced abundance of this plant. The reduced abundance of *G. cordifolia* in rangelands will ensure availability of pasture and hence healthier ecosystems.

4.4.3 Effects of water stress on *G. cordifolia* shoot biomass

Shoot fresh weight was observed to be higher in flood irrigation and low in drought irrigation, and this was due to water deficit under drought conditions. Similar observation was reported by Yu *et al.* (2010) where root and shoot biomass ratios of both drained and flooded plants were higher than in control plants.The higher fresh weight under flood irrigations could be due to *G. cordifolia* investing much in shoot growth so that to support the entire *G. cordifolia* growth performance. It is clear that in the presence of enough resources (water) plants invest much in growth (Shi *et al.*, 2019). The findings suggest that in the event of increased future rainfall, most likely, invasion of *G. cordifolia* will have increased seed production and increase plant vigor, hence increased abundance and invasion success. Except healthier and not easily trampled by animals *G. cordifolia* which are likely to cover larger areas of the rangelands. The overall impact therefore will likely to reduce palatable plant species and pasture shortage to herbivores. However, as the results have clearly demonstrated that *G. cordifolia* shoot weight increase under flood condition, it shows how *G. cordifolia* can invade more under areas that are likely to have higher rainfall therefore, much efforts should be directed on how to manage the weed by predicting the future rainfall intensity in different biomes.

4.4.4 Effects of water stress on *G. cordifolia* root biomass

No significant effect in root fresh weight was observed but a significant change in root dry weight under control irrigation. The lowest root dry weight was observed within the drought irrigation regime. The effect of drought stress in root weight was much more significant than flood condition announced due to changes in environmental conditions. In drought stress condition *G. cordifolia* root fresh weight increase was due to its struggle to survive as it was straddling searching for survival resources (Nejad, 2011). As it was further observed by Boutraa *et al.* (2010) there was an increase in plant root dry weight under mild water deficit and no change under severe water deficit. Moreover, according to Zhang *et al.* (2017) plants invest more biomass to root when water is scarce. Effect of flooding may inhibit root elongation and branching, but not in flood-tolerant weed plant species (Sakazono *et al.*, 2014). Therefore, Water deficiencies lowered leaf, stem, and total dry weight, indicating that biomass was redistributed towards the roots, increasing the root to shoot ratio (Luo *et al.*, 2020). Flood and drought are anticipated to become more often and severe in the future due to increase in frequency and severity and may have impact in changing root elongation as a result reduce and increase root weight, according to future climate estimates in East Africa (Platt’s *et al.*, 2021).

Therefore ecological consequences of flooding and drought conditions are to be taken into consideration so to come up with effective strategies on how to manage the invasive plant *G. cordifolia* so as to overcome possible loss of forage for wild animals due to its invasion success.

4.4.5 Effects of water stress on *G. cordifolia* leaf pigmentation

Chlorophyll is one of the major chloroplast components for photosynthesis responsible for capturing energy from sunlight, convert it and store it in energy storage molecules (Rahdari *et al.,* 2012). In this study the maximum chlorophyll and anthocyanin levels were observed under moderate flood the minimum levels of chlorophyll and anthocyanin were observed under drought irrigation. While the observed small chlorophyll content under drought stress has been considered a typical symptom of pigment photo-oxidation. Decrease in anthocyanin indicates that drought has less severe impacts to leaf anthocyanin levels compared to chlorophyll level (Anjum *et al.*, 2011). Similar studies have reported that drought stress reduces the plant growth by influencing various physiological as well as biochemical functions such as chlorophyll synthesis (Hussain *et al.,* 2018). While on the other hand an increase in leaf chlorophyll under moderate flood shows that flood has no severe impacts on leaf chlorophyll (Rahdari *et al.,* 2012b) as compared to leaf anthocyanin. It has also been established that if stress (flood) is prolonged, plant growth and productivity are severely diminished and level of anthocyanin in leaves increase that indicate that a plant is stressed (Osakabe *et al.*, 2014a). The maximum chlorophyll and anthocyanin levels under moderate flood irigation further indicates that *G. cordifolia* is able to survive under flood condition compared to drought as the localization of anthocyanin in leaf tissues have been reported to allow plants to develop resistance to several environmental stresses. Based on the importance of chlorophyll in photosynthesis and therefore overall plant health; the reduction in leaf chlorophyll in drought stressed *G. cordifolia* therefore, presents an opportunity for reduced abundance of *G. cordifolia* in East African rangelands due to impaired photosynthesis in the future in an event where there will be decreased rainfall. The opposite is true then in an even where the future will be characterized by increased rainfall. These observation therefore informs conservationist on how the weed is likely to behave in the future so that they can prepare to effectively manage it for improved pasture in east African rangelands.

4.4.6 *Gutenbergia cordifolia* response on morphological traits under screen house and field conditions

The study observed higher *Gutenbergia cordifolia* height and root collar diameter under field condition compared to screen house condition. The observed increase in both height and root collar diameter of *G. cordifolia* under field condition compared to screenhouse.This may be due to increased nutrients and favourable seasonal factors such as rain fall and temperature as many plant traits have been reported to be sensitive to climate change variability (Gloser, 2004). As previously reported by Chauhan and Johnson (2010), jungle rice's height was reduced with increasing stress condition (space, nutrients availability and light intensity) under screen house condition. Increase in growth of *G. cordifolia* observed under field condition may be due to its potential to regulate ecosystem process such as carbon (C), nitrogen (N) and water cycles (Ehrenfeld, 2003). Furthermore, observed differences in *G. cordifolia* height and root collar diameter between field and screen house conditions can be attributed with decreased soil moisture (Santos *et al.*, 2017) as field sampling area were characterized with high ground cover which reduce the rate of evaporation (Ward *et al.*, 2012). Furthermore this study indicated that *G. cordifolia* is likely to perform better in the future as majority of East African rangelands are likely to have high rainfall and high soil moisture, which calls for the conservation and management authorities to put strategies in place that will likely be needed to control the species in potential areas for its establishment.

No significant change in number of leaves and leaf surface area under field and screen house conditions. But Leaf surface area and number of leaves were observed to increase under screen house condition compared to field condition. Variation in the number of leaves and leaf surface area of *G. Cordifolia* between field and screen house may be due to reduced competition for water (Galmés *et al.*, 2005). Likewise the variation might be due to variation in light intensity and nutrients availability in which *G. cordifolia* under screen house condition are likely to have been exposed under reduced competition for both light and nutrients. Moreover, the reduced number of *G. cordifolia* leaves in the field may be due to most plant's tendency to shed leaves so that to avoid water loss due to environmental stresses such as long dry periods (Ward *et al.*, 2012).

It was observed that *G. cordifolia* shoot and root biomasses were higher in the field compared to screen house. The observed higher shoot and root biomass under field condition compared to screen house may be attributed to increased soil nutrients enrichment from both wildlife and livestock droppings (Razaq *et al.,* 2017; Han *et al.,* 2016) and decaying organic matters. Likewise an increased ground cover in the field might have aided in ensuring sufficient soil moisture compared to screen house where the pot's soil were exposed to direct sunlight. As reported by Zhang *et al.* (2019), plants that are exposed under areas with sufficient nutrients, and soil moisture tends to invest more in shoot and root growth to ensure perpetuation. Unlike *G. cordifolia* that were grown in the screen house and that were sufficiently irrigated with no competition from other plants, the higher root and shoot biomass of *G. cordifolia* in the field can also be due to increased competition for growth resources as *G. cordifolia* competes with other wild plants. In so doing, *G. cordifolia* in the field invest more in root formation to maximize the available resources (Doerner & Tian, 2013). The higher *G. cordifolia* shoot and root biomass in the field indicates that *G. cordifolia* is a highly competitive plant whose management needs to be well planned to ensure successful management. Its effective management therefore as suggested by Ngondya *et al.* (2019) needs to benefit from an in depth assessment of native varieties that can successfully outcompete *G. cordifolia.*

4.4.7 *Gutenebrgia cordifolia* response on physiological traits under screen house and field condition

Pigments can provide useful insight into the physiological performance of leaves and ecosystem production, hence determining photosynthetic pigments is one of the most common analyses in plant ecology and physiology (Marchiori *et al.,* 2019*).* The leaf of *G. cordifolia* was observed to have an increase in anthocyanin pigment and Chlorophyll content under field conditions. During stress periods and soil with high saline sodic soil *G. cordifolia* was observed to increase production of leaf anthocyanin content (Mbarki *et al.,* 2018) and whereby under favourable condition such as light intensity and soil nutrient cycling favors production of Leaf Chlorophyll content. Production of chlorophyll and anthocyanin pigments play an important role in plant defence against abiotic stress (Pérez *et al.,* 2019). However field stresses can directly or indirectly affect the physiology status of *G. cordifolia* by changing its metabolism, growth and development result increase in production of anthocyanin and chlorophyll (Naing & Kim, 2021). Increase in chlorophyll and anthocyanin content level under field condition this indicate that *G. cordifolia* able to survive on exposure of environmental stress or abiotic stress, these process of response include alteration in photosynthetic rates, assimilate translocation, nutrient uptake and translocation, changes in water uptake, and evapotranspiration. Therefore production of these leaf pigments in *G. cordifolia* is very important since plant create ability to sustain abiotic stress and colonize wide range of NCA site. Understanding response of physiological traits of *G. cordifolia* will predict their future productive potential in the ramge lands and how to control them to ensure availability of posture for wild animals.

4.4.8 Effect of short and long rain season condition on *G. cordifolia* morphological traits

Climate change may directly or indirectly influence the change in morphology of *G. cordifolia*, climate change cause shifting in amount of frequency of precipitation in different sites which is expected to have implications in *G. cordifolia* performance (Didiano *et al.*, 2016). This study showed increase in stem height of *G. cordifolia* under short rain period in Ndutu site. According to climatic data of NCA, Ndutu is the site that experiences more rain fall compare to other area in the park (Prins & Loth, 1988) this might be the reason as to why *G. cordifolia* had good growth performance. According to other studies reported that availability of soil moisture due to annual precipitation are major factor of plant height and root collar diameter growth (Zhang *et al.,* 2015a). Moreover increased precipitation promotes increase in root collar diameter (Chen *et al.,* 2019). Understanding growth performance of *G. cordifolia* will have a good implication on how to control invasion, management and prediction of its invasion in the future.

However, increase in number of leaves and large leaf surface area were observed in long rain season compared to short rain season. During the vegetative growth stage of *G. cordifolia* and increased precipitation can promote the production of new leaves and branches (Zhang *et al.*, 2015b) and increase the number of leaves and flowers (Liu *et al.*, 2012). Studies focusing on interspecific patterns between plant traits and climatic factors have identified a correlation between leaf area and mean annual precipitation (Wolfe & Liston, 1998). Variation in leaf size and number of leaves has been shown to be correlated with climatic condition. In addition, other environmental factors, such as light intensity and nutrient availability, can influence leaf size and surface area (Royer *et al.,* 2008) therefore from these result it could be easy to understand how seasonal factor can facilitate growth and reproduction performance of *G. cordifolia* in different site and arrange proper time for control so as to overcome impacts of its invasion in the future and foster production of fodder and quality pasture for wild animals in East African range lands.

Shoot and root biomass of *G. cordifolia* was observed to increase during short rain and long rain respectively. Increase in root biomass is due to the ability to invest in growth of root rather that stem growth during water scarcity (García *et al.*, 2008). For instance, less frequency but larger rainfall events cause a decrease in above ground biomass of *G. cordifolia* rather than increase in root biomass (Hossain & Beierkuhnlein, 2018). When the precipitation event is large, but the dry interval is prolonged, rapid biomass production is initiated and continues until when the soil water is in deficit (Didiano *et al.*, 2016). However, long dry intervals may also down regulate plant activity or cause mortality of *G. cordifolia*. In long rain season *G. cordifolia* will increase vigor and maintain sustainability otherwise during dry period this plant will be stressed and mature early so as to produce more seed that will be able to sprout during the coming rain season. Therefore, to come up with good control practice of this noxious weed it is good to know their life cycle according to rain season so as to remove them completely and reduce competition with other plant species which are source of food to wild animal and their potential role in Ngorongoro ecosystem.

4.4.9 Effect of short rain and long rain season condition on *G. cordifolia* physiological traits

There was no observed significant change in *G. cordifolia* leaf anthocyanin level. The maximum and minimum anthocyanin pigment level were observed in Lerai and Centre site under short rain season respectively. Increase in anthocyanin content is due to stress factors such as soil moisture content, disturbance done with wild animal especially in Lerai site most of big animal like elephants were found around the site. In Center plot near Ziwani inside the crater, soil was observed to be saline sodic; this cause *G. cordifolia to* create resistance mechanism and produce more anthocyanin content. Therefore, due to these stress factors, Lerai and Center site were observed to have maximum anthocyanin. Minimum anthocyanin is due to some areas under the site tend to have more nutrients and more soil moisture and other environmental factors. While no significant change in leaf chlorophyll was observed under long rain and short rain season. Leaf pigment contents have ability to respond according to rainfall season (Li *et al.*, 2018). During short rain season *G. cordifolia* experience minimum rainfall which result to decrease in chlorophyll content and increase in anthocyanin pigments due to water deficit. Under long rain season *G. cordifolia* increase production of leaves and chlorophyll content cause an increase ability to invade large range of NCA (Bajwa *et al.*, 2017).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study investigated the change of the morphological and physiological characteristics of an invasive plant *G. codifolia* in response to varied water stress levels estimated from the projected East African annual rainfall of over 100 years, seasonal rainfall of NCA and comparing response under field and screenhouse conditions. The study adressed three objectives whose findings indicated that, *G. cordifolia* can respond differently and in a significant way in varying water stress environment (varying future East African rainfall). The study also looked on the response of *G. cordifolia* under long rain and short rain season and on performance of *G. cordifolia* under screen house and field conditions.

Generally both drought and flood stress had a significant effects on morphology and physiology of the plant indicating that the projected East African intense rains for both mid century (2041-2070) and late century (2071-2100) are likely to favor the spread of *G. cordifolia* in most East African Rangelands and hence may hinder other potentially palatable plants to germinate and grow well. This projected colonization of *G. cordifolia* might therefore, jeopardize pasture availability for herbivores, thus, altering the ecosystem structure, functions and ultimately lead to loss of biodiversity.In addition seasonal raifall that are available in Ngorongoro Conservation Area favors the spread and increase in *G. cordifolia* invasion. The study indicated that during long rain season *G. cordifolia* is capable of being stressed but still able to increase in abundance and therefore likely to colonize in new areas. Also, from this study *G. cordifolia* has been observed to perform well both in field and screen house condition highlighting its invasion potential. *Gutenebrgia cordifolia* is capable of sustaining environmental stress (extremes) therefore much effort and a good understanding of its responses to environmental stresses is needed to be accounted during formulation of its control strategies so as to improve healthy grazing areas for both wildlife and livestock.

5.2 Recommendations

Based on findings from this study, it is recommended that:

(i) Preparing a management plan and policy which are sustainable in eradication of any invasive spcies apart from *G. cordifolia* should consider behaviour, adaptation strtegies of these these invasive species with changing climate by considering the variation in temperature and rainfall so as to come up with effect control and removing menthods.

(ii) Since extreme climatic events, such as floods are expected to increase, these will facilitate *G. cordifolia* invasions leading to decreased biotic resistance of native communities. Efforts to minimize impacts of *G. cordifolia* in a changing climate must should include among others, preparation of strategies and action plans like national action plans of Invasive alien species in Tanzania, that account for extreme events such as flood and drought.

(iii) Development of proper *G. cordifolia* control action plans should consider variability in seasonal rainfall so as to ensure effectiveness of control efforts in areas invaded by *G. cordifolia* in rangelands, including the NCA.

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RESEARCH OUTPUTS

(i) Publication

Nyarobi, H. A., Ngondya, I. B., & Munishi, L. K. (2022). The effects of extreme climate on the invasive plant Gutenbergia cordifolia: Implications for its future management in savannah ecosystems. *Heliyon*, *8*(3), 1-7. https://doi.org/10.1016/j.heliyon.2022 .e09172

(ii) Poster Presentation

Herieth, A. N. (2022). *Morphological and physiological responses of the invasive plant Gutenbergia cordifolia to varying biophysical conditions*, Tanzania [Poster Presentation]. Nelson Mandela African Institution of Science and Technology, Arusha.