2022-03

Effects of selected management practices of gutenbergia cordifolia on insect flower visitation at Mwiba area, Tanzania

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NM-AIST

https://doi.org/10.58694/20.500.12479/1506

EFFECTS OF SELECTED MANAGEMENT PRACTICES OF *Gutenbergia cordifolia* ON INSECT FLOWER VISITATION AT MWIBA AREA, TANZANIA

Mecklina Michael Mbundi

A Dissertation Submitted in Partial Fulfillment of the Requirement for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

March, 2022

ABSTRACT

While only few studies have presented the effect of invasive plant species on insect visitors, even less is documented about how management practices against invasive plants may impact plant-pollinator networks. The study assessed how natural versus chemical-based management practices against the native invasive plant *Gutenbergia cordifolia* affected insect flower visitation in Mwiba area by comparing the number of insect visitors, insect species diversity and richness, the number of flowers visited, flower abundance, and diversity across treatments of *Desmodium uncinatum* crude leaves extract (DUL), the chemical Glyphosate (GLY), and none (Control; CON). After treatments, DUL plots had about one-third higher numbers of insects visitors compared to CON and GLY plots ($F_{2,159} = 9.521$, df = 2, $p = 0.009$), including higher species diversity and richness of bee in DUL than in GLY and CON ($F_{2,12} = 5.497$, df = 2, $p = 0.020$; $F_{2,12} = 21.810$, df = 2, $p < 0.001$ respectively). Further, DUL plots had almost twice as many flowers visited compared to CON and GLY plots ($F_{2,159} = 21.595$, df = 2, $p < 0.001$). Flower abundance was higher in DUL plots compared to CON and GLY as was flower diversity ($\chi^2 = 7.460$, df = 2, $p = 0.024$; $F_{2,12} = 3.963$, df = 2, $p = 0.048$, respectively). Generally, this study discovered that DUL treatment did not disturb insect flower visitation while GLY strongly did; instead, DUL attracted more insect flower visitors. It is hereby concluded that using the natural plant extract treatment is highly preferable to the chemical management of invasive plant *G. cordifolia.*

DECLARATION

I, Mecklina Michael Mbundi do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this Dissertation is my original work and that it has never been submitted for the degree award in any other institution.

Mecklina Michael Mbundi

Name and Signature of the Candidate Date

The above declaration is confirmed by:

Prof. Anna C. Treydte

Treydte

Name and Signature of Supervisor 1 Date

Dr. Issakwisa B. Ngondya

Name and Signature of Supervisor 2 Date

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Senate of the Nelson Mandela African Institution of Science and Technology a dissertation entitled "*Effects of selected management practices of Gutenbergia cordifolia on insect flower visitation at Mwiba Area, Tanzania*" in partial 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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ACKNOWLEDGEMENTS

First and foremost, I must acknowledge the almighty God for giving me the strength and good health to finish this research work.

I wish to express my genuine appreciation to my supervisors, Prof. Anna C. Treydte, and Dr. Issakwisa Ngondya for providing critical comments, guidance, and support throughout the work period. Their valuable supervision and suggestions designed this study making it possible to produce all the results and dissertation in this form. I am grateful to my employer the University of Dar es Salaam, who granted me a study leave.

I would like to acknowledge and thank the African Development Bank (AfDB) and the Rufford Foundation for financial supports, IDEA WILD for research equipment provision, TAWIRI, and COSTECH for issuing research permits, Mwiba Holdings Limited for providing me with accommodation, food, and transport including Mr. Mark Ghaui. I am grateful to my field assistants and all Mwiba staff for their help and kindness. Additional advice was given by Prof. Tamera Minnick on the study design. I acknowledge Dr. Alain Pauly for bee taxa identification. I also appreciate the assistance by Mr. Majaliwa Masolele, Mr. Jofrey Efraim, Mr. Raymond Kisasembe in data analysis, and so many others whom I can't mention all but their assistance is much appreciated.

I acknowledge my family and many friends. A special feeling of gratitude to my loving parents, my father and mother Mr. and Mrs. Michael Mbundi for raising me well, my sister Ms. Serapia Michael for the whole time she spent taking care of my daughter, my daughter Vanessa Bora for being patient with me while I am far away from her studying.

DEDICATION

I dedicate this work to my lovely daughter Vanessa Bora, may almighty God bless her always.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATION AND SYMBOLS

AfDB	African Development Bank
ANOVA	Analysis of Variance
cm	Centimeter
Cmol	Centimole
CON	Control
COSTECH	The Tanzania Commission for Science and Technology
DUL	*Desmodium uncinatum* Leaves Extract
g	Gram
GLY	Glyphosate
ha	Hectare
h	Hours
IPBES	Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services
HSD	Honestly Significant Difference
Kg	Kilogram
km	Kilometer
L	Litre
Ltd	Limited
mm	Millimeter
mL	Milliliter
m^2	Square meter
NCA	Ngorongoro Conservation Area
NISSAP	National Invasive Species Strategy and Action Plan
SE	Standard Error
TAWIRI	Tanzania Wildlife Research Institute
UV-VIS	Ultraviolet-Visible

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

It has been addressed that both native and alien plant invasive species can alter habitat and ecosystem's community composition and services, reduce biodiversity by competition or interbreeding, which may cause ecological and economical damage (Shabani *et al.*, 2020; Stout & Tiedeken, 2017; Culliney, 2005). Ecologically, plants are recognized as the most invaders species due to their capability to convert ecosystem performances, like changing nutrient cycling, plant production, or decomposition (Moron *et al.,* 2018). These invasions within an ecosystem can affect native plant-pollinator interactions (Dietzsch *et al.,* 2011) through competing for pollination services (Tscheulin *et al.,* 2009; Ojija *et al.,* 2019b). Recent studies on the interactions between invasive and pollinator have focused on the influence of invasion on native plant pollination (Hansen *et al.,* 2018; Goodell & Parker, 2017) and some investigated the biological control of invasive plants (Kanagwa *et al.*, 2020; Ojija *et al.*, 2019a; Miller *et al.*, 2018). Whenever plant abundance and diversity are reduced through direct resource competition with invasive plants, this change may be detrimental for arthropods such as pollinators because various species need native floras for food or site for reproduction (Vanbergen *et al.,* 2018; Bartomeus *et al.*, 2016). Hence, management efforts must be aimed at altering this competition to enhance pollinator abundance (Palladini & Maron, 2016). Thus, leaving the indirect impacts of invasive plants on indigenous plant-pollinator interaction without management may continue affecting the native plant-pollinators system, hence reducing the number of insect visitors (White *et al.,* 2006; Bartomeus & Santamaría, 2008).

With the growing understanding of the environmental problems brought by invasive species, ecologists engaged together with other stakeholders in environmental management towards solving these challenges in biodiversity conservation by increasing invasive management intervention (Pyšek & Richardson, 2010). Knowing the impacts and changes brought by invasive species is necessary for decreasing their harmful effects while exploiting their benefits, however, there is limited lack backgrounds connecting theory, management for biological invasions, and impacts towards other parts of biodiversity (Shackleton *et al.*, 2014). Integrated effort that includes science with management and policy on plant invasions is necessary to make a way in developing proper management schemes that will diminish the rate and magnitude effects of invasive species (Foxcroft *et al.,* 2017).

Gutenbergia cordifolia is described as a native invasive weed plant species in various farmland ranges of East Africa (Ngondya *et al.,* 2016). This species was detected to suppress other native plants and dominate most areas in protected land such as the Ngorongoro Crater, reducing pasture accessibility for herbivores (Ngondya *et al.,* 2016). If *G. cordifolia* is not controlled, its invasiveness will negatively impact plant-feeding animals including insect visitors and wildlife (Ngondya *et al.,* 2017). Lately, studies have revealed that a natural crude extract treatment using young fresh leaves crude extract of *Desmodium uncinatum* (DUL) against the invasive *G. cordifolia*, *Tagetes minuta,* and *Parthenium hysterophorus* can be an effective and an ecologically sound and sustainable management choice for treating the three invasive plant species (Ngondya *et al.,* 2016a & b; Ojija *et al.,* 2019). While the efficacy of the *D. uncinatum* crude leaves extract in managing the invasive plants has been well recognized, no studies have yet assessed how DUL affects insect diversity, particularly their flower visitation, after application. On the other hand, the ability of glyphosate in reducing and managing weed and the sub-lethal effects of non-targeted plant and insect pollinators have been documented (Walker & Oliver, 2008; Herbert *et al.,* 2014). Yet, no previous study has evaluated the effect of glyphosate in managing invasive *G. cordifolia* on non-targeted species such as insect visitors, the number of flowers visited, flower abundance, and diversity.

Flower visitation is a significant trait of any insect (Joshi & Joshi, 2010). However, lately, there has been a rapid decrease of insect pollinators universally, which poses a high risk to biodiversity conservation and related pollination services for wild plants and farm crops (Gayer *et al.,* 2021). Among the factors that cause losses of pollinators are pesticides and loss of natural habitat due to plant invasions, which reduce flower abundances and, thus, accessibility of pollinators to food resources (pollen and nectar) (Nicolson & Wright, 2017; Huang & Giray, 2012). Pollinator declines affect plants that depend on animals for pollination, as their capacity to attract insect visitors to their flowers is a critical part of their survival (Conner, 2014). In some of the plant-pollinator networks, optimistic interactions have been confirmed between the number of flowers visited and the insect visitation rate (Szigeti *et al.,* 2017). Moreover, insect visitor diversity and richness generally increase with flower abundance and higher plant diversity (Traveset & Rotllan-puig, 2017). Thus, a better understanding of the connection between flower abundance, diversity, and the number of insect visitation with their diversity and the number of flowers visited is vital for maintaining a functioning ecosystem and its services, particularly after treatment against invasive plants.

Bee species deliver highly quality pollination services and yield for diverse fruit, nut, vegetable, and seed crops, and graded as the most common solitary species of pollinator for natural and agricultural plants globally (Hung *et al.*, 2018; Kremen *et al.*, 2002). Bees and some other insect visitors, pollen, and nectar are the basic parts of food and nutrients especially for larval growth, adult care, and sexual development (Nicholls & Ibarra, 2017). Pollen is the main source of protein for the honey bee's colony, which has numerous quantities of amino acids, lipids, vitamins, and minerals while nectar provides carbohydrates (mainly monosaccharides and oligosaccharides) (Topitzhofer *et al.,* 2019). But, the use of synthetic chemicals affect bees through glyphosate residues on plants as it responds on the shikimic acid pathway, with several events being stimulated can cause plant death (Fuchs *et al.,* 2020; Weidenhamer & Callaway, 2010). Glyphosate disrupts the carbon movement allocation, and minimizing protein synthesis due to the decrease in the concentration of aromatic amino acids, hence negatively affect pollen viability (Brito *et al.,* 2018; Thomas *et al.,* 2004). Herbicides are intended to manage weeds in agroecological areas however, only 1% of the insecticides application arrive at the specified species and the remaining pollute the atmosphere and kill non targeted species including bees (Abraham *et al.,* 2018; Motta *et al.*, 2020; Vázquez *et al.*, 2020; Londo *et al.,* 2014). As of yet, relatively few studies have looked at how bee species diversity and richness could be impacted by the chemical and natural herbicides management against invasive plants.

A fundamental target of ecology is to recognize changes in species abundance and diversity in communities (Relyea, 2005). This study focused on assessing management impacts of invasive *Gutenbergia cordifolia* using DUL at 100% concentration level and chemical herbicide (glyphosate; GLY) on insect visitors, flowers visited, insect species diversity and richness, bee's diversity, and richness, flower abundance, and diversity in Mwiba area, northern Tanzania. To understand the impacts of management on insect flower visitation, the study experimentally collected information on insect visiting flowers by observation, sweep netting, and through pan traps before and after managing the invasive *Gutenbergia cordifolia* with DUL and GLY.

1.2 Statement of the Problem

Invasive plant species can hinder the establishment and development of native plants which can affect numerous ecosystem supplies including native plant and animal species diversity and abundance, nutrient cycling, fire regimes (Weidlich *et al.*, 2020; Barman, 2019), and plant-pollinator network (Goodell & Parker, 2017). These impacts can be irreversible when appropriate measures are not taken when the invasion occurred (Barman, 2019). The number of studies inspecting the effects of invasive plants on native plants pollination is expanding, but still, there are inadequate studies that have directly managed the existence of the invaders (Gibson *et al.*, 2013), even more, fewer studies have looked on effects of invasive management on insect flower visitation (Hanna *et al.,* 2013; Kaiser-Bunbury, 2019; Macdonald *et al.,* 2019). To current knowledge, effects on insect visitors are facilitated through fluctuations in plant flower diversity and abundance (Robinson *et al.*, 2018). So far ecological researches have mostly been directed on biodiversity changes after the invasion, mainly in declines of species richness and abundance with slight consideration on the effects of invaders on specific ecosystem services (Hulme, 2017). How plant flower diversity and abundance is altered by invasive management and further affects insect flower visitation system and pollination services have not been well studied.

Gutenbergia cordifolia has been present over a long period at the Mwiba area since it is a native plant (pers. comm.). However, within the last two years, *G. cordifolia* had been observed to increase rapidly, to the extent of being regarded as an invasive plant in certain areas of Mwiba and Maswa Game reserve. Yet, its impact on the ecosystem health and the management effort required to halt or reduce the increase of this species have not yet been quantified (pers. comm.). Invasion of *G. cordifolia* in Mwiba area should be a rising concern in wildlife conservation as invasive species are contributing to ecological and socio-economic suffering (Schirmel *et al.*, 2016). While invasive plants including *G. cordifolia* have been well studied and managed through natural and chemical methods, little is known about how these management practices could affect insect flower visitation. This study monitored management effects on insect visitors, flowers visited, insect species diversity and richness, bee species diversity and richness, flower abundance and diversity after spraying the bioherbicide DUL at 100% concentration level, GLY and compared them to an invaded area with no treatment (Control; CON) in Mwiba area, northern Tanzania.

1.3 Rationale of the Study

Management of invasive plant species in the restricted ecosystem has been challenging for years due to increasing failure of some control methods like chemical treatment method, because invasive plants evolve resistance mechanisms; also using chemical herbicides has increased risks on the surroundings (Barman, 2019; Ngondya *et al.,* 2017b). Frequently, synthetic herbicides have been stated as a simple solution, even though they damage the environment and human health

(Ojija *et al.,* 2019a). But, efficient management of a specific invasive plant highly depends on recognizing its history (Ngondya *et al.,* 2016). The challenge is to determine the best method to control these invasive plants and measure the impact of management on biodiversity. Management and elimination of invasive species might cause unexpected changes in plant-pollinator mutualism as invasives can alter the structure and functioning of plant-pollinators interactions in various ways (Hanna *et al.,* 2013).

No earlier studies have yet been considered in Tanzania or somewhere else in the world on whether management of invasive *G. cordifolia* by using DUL treatment as a natural product could have any consequence on insect flower visitation, by assessing the number of insect visitors, the number of flowers visited, flower abundance and diversity, insect species diversity and richness. Therefore, this study aimed at assessing the effects of management practices of *G. cordifolia* on insect flower visitation and diversity and fills the existing gap of information and increases the overall awareness of insect visitors. Besides that, knowing how the management of invasive plants impact insect visitors is necessary for evolving an active strategy on appropriate techniques to regulate invasion while conserving further portions of biodiversity (pollination).

1.4 Research Objectives

1.4.1 General Objective

To evaluate the effect of *G. cordifolia* management practices using natural plant extracts, i.e., *D. uncinatum* crude leaves extract (DUL) at the concentration level of 100% and synthetic herbicide (glyphosate; GLY) on insect flower visitation and their diversity.

1.4.2 Specific Objectives

(i) To determine the effect of *D. uncinatum* crude leaves extract (DUL) and synthetic herbicide (glyphosate; GLY) against *G. cordifolia* on the number of insect visitors after treatments.

(ii) To determine the effect of *D. uncinatum* crude leaves extract (DUL) and synthetic herbicide (glyphosate; GLY) against *G. cordifolia* on insect diversity and species richness after treatments.

(iii) To determine the effect of *D. uncinatum* crude leaves extract (DUL) and synthetic herbicide (glyphosate; GLY) against *G. cordifolia* on flower diversity and abundance after treatments.

1.5 Hypotheses

(i) Insect visitors will be more abundant in DUL treated plots compared to glyphosate and CON plots.

(ii) The number of flowers visited will be higher in DUL treated plots than in glyphosate and CON treated plots.

(iii) The DUL treated plots would have higher diverse and abundant flowers compared to glyphosate, and CON plots.

(iv) Insect diversity and richness will be higher in DUL treated plots than in glyphosate and CON plots.

(v) Bee species diversity and richness will be higher in DUL treated plots than in glyphosate and CON plots.

1.6 Significance of the Study

Both native and alien plant invasive species can alter habitat and ecosystem's community composition and services, reduce biodiversity by competition or interbreeding, which may cause ecological and economical damage (Shabani *et al.,* 2020; Stout & Tiedeken, 2017; Culliney, 2005). Invasions within an ecosystem can affect native plant-pollinator interactions (Dietzsch *et al.,* 2011) through competing for pollination. Hence, management efforts must be aimed at altering this competition to enhance pollinator abundance (Palladini & Maron, 2016). Thus, leaving the indirect impacts of invasive plants on indigenous plant-pollinator interaction without management may continue affecting the native plant-pollinators system, hence reducing the number of insect visitors (White *et al.,* 2006; Bartomeus & Santamaría, 2008). Knowing the impacts and changes brought by invasive species is necessary for decreasing their harmful effects while exploiting their benefits. Flower visitation is a significant trait of any insect (Joshi & Joshi, 2010). A better understanding of the connection between flower abundance, diversity, and the number of insect visitation with their diversity and the number of flowers visited is vital for maintaining a functioning ecosystem and its services, particularly after treatment against invasive plants. With the findings of this study, optimal management practices that will handle *G. cordifolia* invasion could be acclaimed while lessening intimidating effects on pollination services in protected areas. Knowing the impacts of *G. cordifolia* management on native plant-pollinator interactions including their effects on insect diversity and foraging behavior helps to understand how invasive plants affect indigenous plant reproduction.

1.7 Delineation of the Study

This study was carried out to determine how the management of invasive *G. cordifolia* using *D. uncinatum* leaves crude extract (DUL) and glyphosate (GLY) impacted insect flower visitation and diversity in the invaded habitats of Mwiba area across 15 sampling plots. Field surveys were conducted to assess the presence of invasive *G. cordifolia* and native plants in Mwiba area. Information on insect visitors was collected by observation, sweep netting, and pan traps. Further, flowers found within the sampling plots were identified. Insect specimens were taken to the laboratory for identification. Finally, the best management practice was recommended that will reduce plant invasion while minimizing the adverse effects of management on pollination services in protected areas of eastern Africa.

CHAPTER TWO

LITERATURE REVIEW

2.1 Pollination Services and Insect Flower Visitation

Pollination is a delicate ecosystem service, which energies numerous life processes (Mandela *et al.,* 2018). Pollinator's service is an essential process influencing the flowering plant's reproduction while, any alteration in the value of this service distresses the plant seed production (Molina-montenegroet *et al.,* 2008; Goodell & Parker, 2017). Pollination services are generally provided by insects, mainly bees and some flies (O'Connor *et al.,* 2019). Currently, studies of pollinating animals focused on honey bees, while, there is a lack of documentation of universal population trends for other pollinating species e.g., beetles, butterflies, flies, moths... etc (Allen-Wardell *et al.,* 2021). Studies on plant and pollinator networks revealed that insects are important in supporting plant diversity, hence biodiversity conservation (Ojija *et al.,* 2019) (Fig. 1). Their role to plant fitness and the effectiveness in pollinating plants depends on the number of flowers they pollinate (Traveset & Saez, 1997). Plants and insects' interactions have extensive ecological and economic consequences (Denning & Foster, 2017). With more than 85% of flowering plants worldwide are relying on insects visitation for fertilization services which often relates to agricultural intensifications (Menz *et al.,* 2011; Denning & Foster, 2017).

Pollination service is endangered by human activities like land-use modification, pesticides, climate change, pests and disease, and unintentional and intentional introduction of alien plants and pollinators into the natural ecosystem (Memmott & Waser, 2002; Chapman, 2009). These anthropogenic challenges have brought a worldwide concern on the reduction of insect pollinators diversity and distribution and the consequences for pollination services (Chapman, 2009). To date, there is no inclusive evaluation of the status and trends of pollinators and pollination services on the African continent (Gemmill-Herren *et al.,* 2014). Only relying on the global trends supply of information that is relevant in an African context. The present status of nearly all wild pollinator populations (diversity and abundance) in Africa is uncertain and difficult to assess due to the lack of long-term data to assess declines (Melin *et al.,* 2014). At best, global trends can be estimated while acknowledging the large gaps in data (Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services [IPBES], 2016).

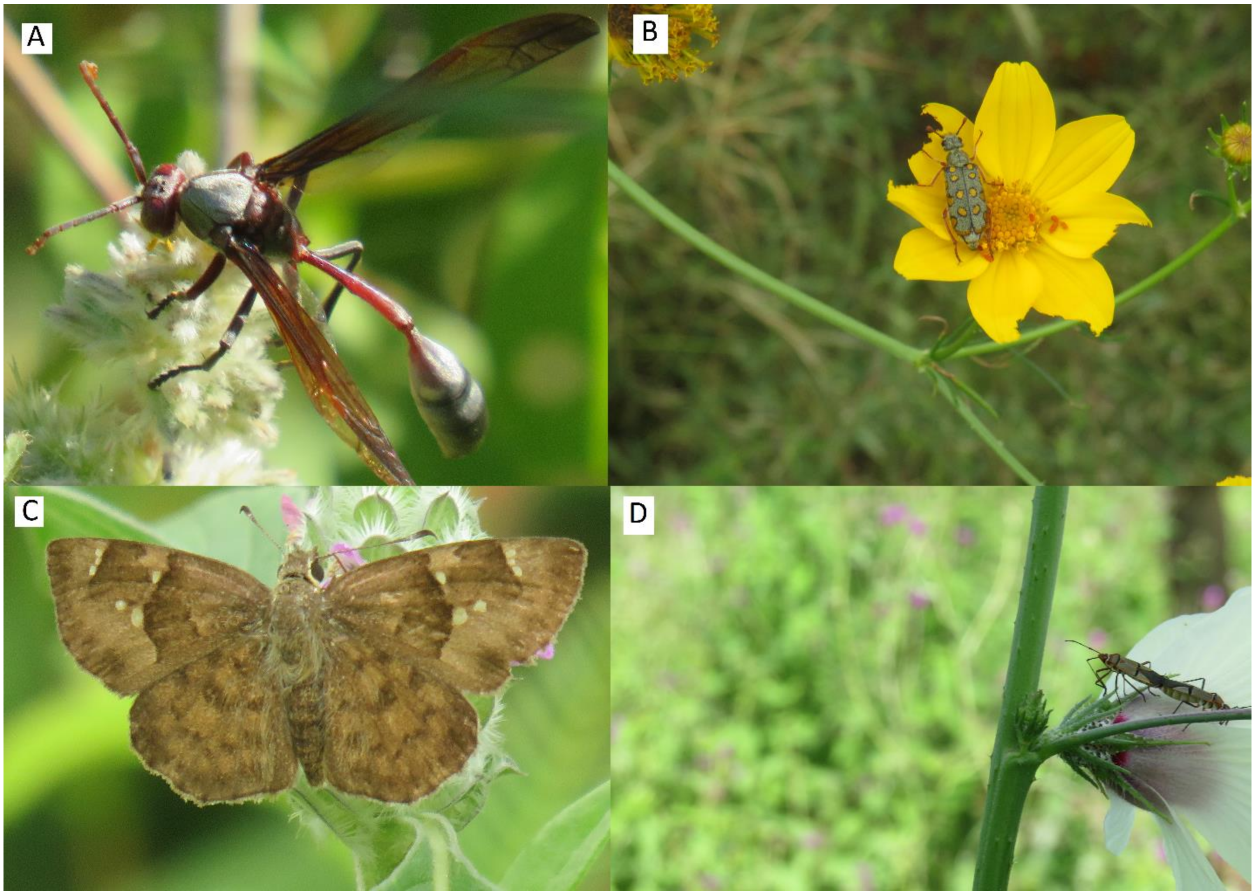

Figure 1: Insect visiting and pollinating flowering plants: (A) Hymenoptera, (B) Coleoptera, (C) Lepidoptera, (D) Hemiptera group (Field observation)

2.2 Invasive and Native Plant-Pollinator Interactions

Plant invasion is recognized as a key threat to biodiversity since they transform numerous processes which are compulsory for ecosystem functioning (Morales & Traveset, 2009; Schuster & Wragg, 2018). Previous studies have exposed that invasive plants can disturb the pollinator–native plant network (Herron-sweet *et al.,* 2016) by dropping visitation rate which could be the cause of the minimization of seed set (Tscheulin & Petanidou, 2013). Invasive plants species are likely to compete with native species for common pollinators; hence in the presence of invaders competitors, pollinator visitation and reproduction achievement of native plants tend to decline (Morales & Traveset, 2009). Invasive plant species such as *Parthenium hysterophorus* have been sharing flower visitors with native flowering plants, which suggests that it can wield negative effects on native plants by attracting flower visitors away from the latter (Ojija *et al.,* 2019b). While evidence is available on how different invasive plants have affected native plant-pollinator interactions, mainly in a negative way (Ngondya *et al.,* 2016), there remains a gap in knowledge about the impact of *G. cordifolia* on pollination services for co-flowering plants.

Most of invasive plants make an impenetrable bushy mono-specific location that produces flowers abundantly, thus initiating a large amount of alien pollen and nectar into the native communities they infest (Larson *et al.,* 2006). With this and several other mechanisms invasive plants could cause harmful effects on native plant reproduction. Through changing plant-pollinator interactions, invasive plants may reduce the frequency of pollinator visits to native species (Brown *et al.,* 2002), also invasive may interfere with native seed production through pollen transfer (Brown & Mitchell, 2001). However, more information on invasive versus native plant pollinators interaction is required to plan and implement actual management practices of invasive plants (Ojija *et al.,* 2019b; Pyšek & Richardson, 2010). There is a growth in the number of studies investigating the impacts of invasive plants on the pollination of native plants, but so far, few studies have directly manipulated the incidence of the invasive (Hanna *et al.,* 2013; Kanagwa, 2020), and even less studies have directly evaluated the impact of invasive manipulation on beneficial insects (Ojija, 2020).

2.3 Invasion of *Gutenbergia cordifolia*

Gutenbergia cordifolia is a weed plant species that grow annually, from family Asteraceae native to Africa (Ngondya, 2017) (Fig. 2a). Its leaves and flowers are allergenic and toxic to animals as they have a chemical sesquiterpene lactone (Fujimoto *et al.,* 1987) which can affect the microbial of the rumen and its entire metabolic efficiency (Ngondya *et al.,* 2016). *Gutenbergia cordifolia* was introduced in many areas of East Africa for medicinal purposes but lately has been dispersing and increasing abundantly and faster than the proportion at which it was intended to be used (Ngondya *et al.,* 2017) and cover most areas of Mwiba Area with wide invasion (Fig. 2b). In 2001 Ngorongoro Conservation Area (NCA) introduced a traditional control method to remove indigenous invasive *G. cordifolia* through mowing followed by burning, however, this approach was reported not to be highly effective control mechanism (Ngondya *et al.,* 2017). Ngondya *et al.* (2016) found that high concentrations of *Desmodium uncinatum* leaf extract (DUL) reduced *G. cordifolia*'s ability to perform photosynthesis and led to stunted growth (Kanagwa, 2020).

Figure 2: (A) *Gutenbergia cordifolia* flower, (B) landscape showing infestation of *Gutenbergia cordifolia* in Mwiba (Field observation)

2.4 Management of Invasive Plants

Worldwide humans have been described as distributors of invasive plants species, both deliberately and accidentally (Vanbergen *et al.*, 2018). Even though, alien invasive plants deliver food resources such as pollen and nectar for pollinator's diet but, can also cause danger for insect visitors safety (Arroyo-correa *et al.,* 2020). Management mechanisms are proceeding in many areas of the world to regulate and reduce the negative costs of invasive plants (Gibson *et al.,* 2013). Controlling invasive plants could be by herbicides, mowing, burning, or labor-intensive practices like slashing or hand-felling (Byun, 2017). Core hindrances to efficient handling of invasive plant species in Eastern Africa are lack of proper strategies and application, inadequate knowledge in identifying and managing invasive plants, insufficient information on the effects of invasive plants on biodiversity, water resources, crop and grassland productivity, human and animal health, financial growth, lastly, lack of reasonable funds to handle the problem at a national or regional level (Ngondya *et al.*, 2017b; Witt *et al.*, 2018). The management of invasive species in Tanzania is ongoing through fragmented efforts by several conservation organizations (Neema, 2019). By the year 2020, different sectoral acts, policies, and guidelines were passed, some comprised the topic of invasive species, however, the outcome of these efforts are difficult to measure due to a lack of a national strategy on invasive species, with the current sectoral approaches being ineffective in managing the ever-increasing invasions (National Invasive Species Strategy and Action Plan [NISSAP], 2015; Ngondya, 2017).

Using allelopathic properties has been highlighted as an active method of controlling invasive weeds (Benchaa & Hazzit, 2018). The term allelopathy was first presented by Molisch in 1937 which generally refers to the detrimental effect of an individual plant species on seed sprouting, growth, and reproduction of another plant species (Kaur *et al.,* 2014). Numerous plants are known to have allelopathic potential in addition many attempts have been done to apply them in managing invasive plants (Mmbone *et al.,* 2014). Due to the allelopathic nature of plants like *Desmodium uncinatum, Tagetes minuta, and Tephrosia vogelii,* these species have been successfully used to handle most weeds and pests in farmlands, promoting high crop harvest (Khan *et al.,* 2008; Makoi *et al.,* 2010; Mmbone *et al.,* 2014). Kaur *et al.* (2014) demonstrates that both root and shoot extracts of three allelopathic leaves of grass species, namely *Dicanthium annulatum*, *Cenchrus pennisetiformis,* and *Sorghum halepense,* reduce germination and eradicate early seedling growth of exotic weed *P. hysterophorus.* The possible application of allelopathy as a natural phenomenon for the control of noxious weeds is promising (Wang *et al.,* 2011).

Synthetic herbicides have been a key component in most weed management strategies, even though they cause damage to the environment and human health (Ojija *et al.,* 2019a). However, in the recent past, some progressive studies have been made using plant-based products as weed control agents (Koul *et al.*, 2009). Numerous other natural products extracted from plants or allelochemicals have displayed a wide range of actions against pests (Suteu *et al.*, 2020). For some time, they have been presented as an outstanding alternative to synthetic chemicals for pest management as they have a small hazard on the atmosphere or human safety (Koul *et al.*, 2009). Moreover, the use of ecological and simply biodegradable plant products has been enhanced in recent years (Moreno *et al.,* 2012; Suteu *et al.,* 2020), through using plant materials with insecticidal ability to kill pest insects without distressing the environment (Kabeh, 2017; Koul *et al.*, 2009). But few studies have assessed and proved the efficacy of managing specific invasive plants by using crude leaves extracted from *Desmodium uncinatum* is a bio-herbicide in managing invasive plants (Ngondya *et al.*, 2016a & b; Ojija *et al.,* 2019). Often, bioherbicide provides selectivity to non-target species, particularly predators and pollinators, in addition to efficacy against insect pests and weed (Moreno *et al.*, 2012).

2.5 Synthetic Herbicides against Invasive Plants

In most areas, invasive plants and noxious weed management using chemical methods have been efficient in controlling invasive weeds like *Parthenium hysterophorus* (Neema, 2019; Kanagwa, 2020). However, the use of chemical control might not be appropriate for conserved areas due to its non-target effect on some species that belong to an ecosystem with higher biological interaction (Dia & Diagne, 2020). More studies show that herbicides and insecticides can cause dramatic variations in natural communities, yet the understanding of pesticides' effects on natural communities is highly inadequate (Relyea, 2005). Chemical control has been unsuccessful due to weed/invasive resistance, intolerable in environmentally delicate habitats or where people can contact herbicides (Londo *et al.,* 2014). Further, economically chemical control has been unrealistic, due to being expensive (Culliney, 2005) and even being lethal or sub-lethal, direct and/or indirect effects on pollinators, whereas others do not appear to cause any effects (Bohnenblust *et al.,* 2016). Because of the limited information about the effects of herbicides on non-targeted species (Bohnenblust *et al.,* 2016), this study is among the few to report the observation of insect flower visitation reaction under synthetic chemical herbicide treatment against *G. cordifolia*.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Field studies were carried out at Mwiba Wildlife Ranch, formerly known as Makao Open Area located in North-Western Tanzania between 03º22' S to 34º41'E to 34º 53'E (Ngilangwa *et al.*, 2018, Fig. 3) at Meatu District, Simiyu region. Mwiba Wildlife Ranch covers an area of about 19 647 ha and borders the Ngorongoro Conservation Area Authority to the east, Maswa Game Reserve to the North, and Makao Wildlife Management Area to the South West. The average annual temperature ranges between 21 ºC and 27 ºC and precipitation of 750 mm to 915 mm with a bimodal rainfall pattern with short rains in November and December and long rains in March to May (Ngilangwa *et al.*, 2018). The high-water availability from permanent water springs within the Ranch has enabled the establishment of residential wildlife populations (Ngilangwa *et al.*, 2018). *Gutenbergia cordifolia* has recently invaded most of Mwiba Wildlife Ranch area but has not yet been quantified (personal observation). The area is rich in flora abundance (i.e., *Vernonia galamensis*, *Ipomoea purpurea*, *Hibiscus cannabinus*, *Justicia betonica L.*, *Heliotropium steudneri*, etc.,) (Pers. Obser) and fauna (ungulate species includes, African elephant, Zebra, Warthog, Giraffe, bushbuck, dik-dik, hartebeest, waterbuck, African buffalo. Carnivores include lions, leopards, wild dogs, cheetah, and spotted hyenas. High abundance and composition of various bird species (Ngilangwa *et al.*, 2018).

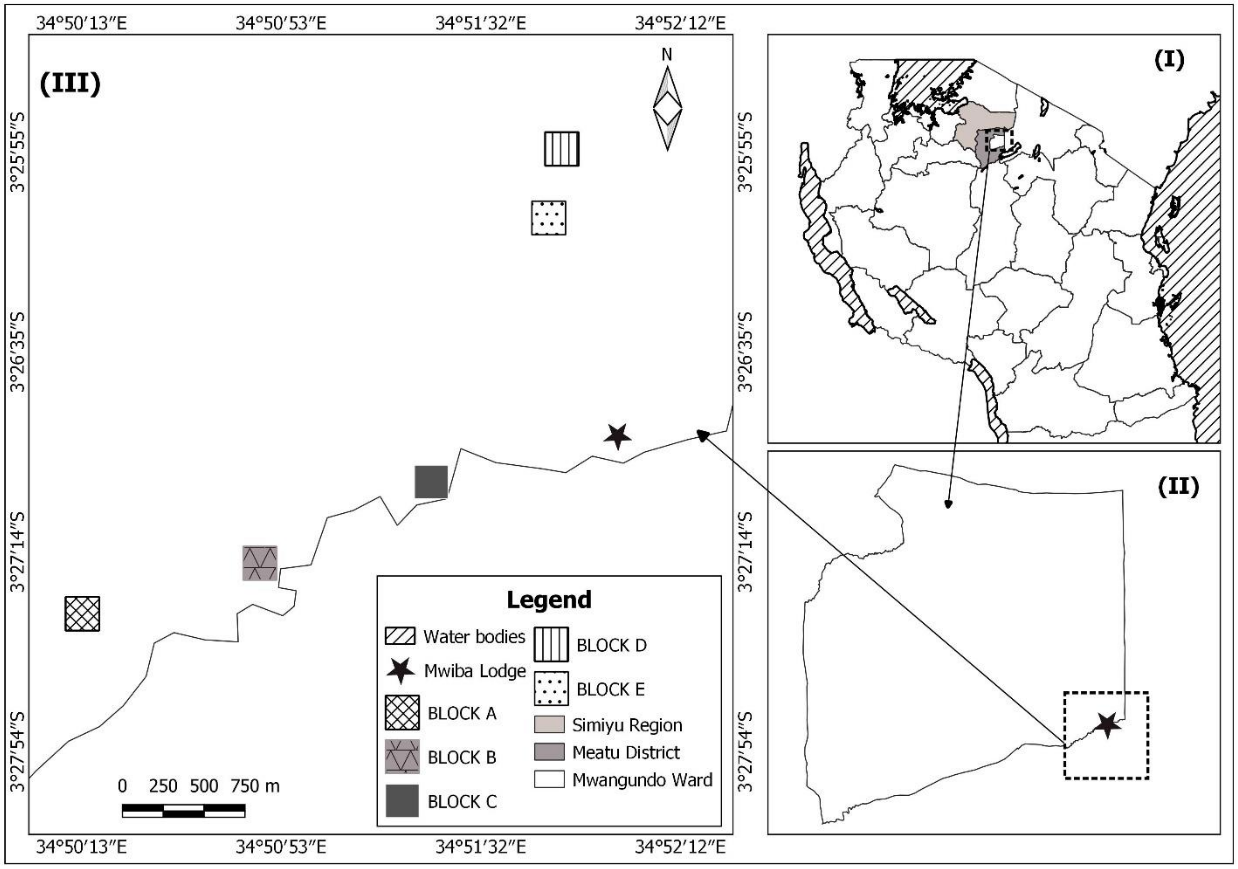

Figure 3: A Map of Mwiba Area in North-Western Tanzania, with the five sampling blocks (A, B, C, D, and E) established at a distance of at least 2 km apart in areas with a similar level of infestation of *Gutenbergia cordifolia* (>75% coverage) and with associated existence of native flowering plants (Field survey)

3.2 Data Collection

3.2.1 Assessing *Gutenbergia cordifolia* Distribution in Mwiba Area

A field reconnaissance survey was done within Mwiba area between January and February 2020 to gather information on the existing distribution data of *G. cordifolia.* While in Mwiba area *G. cordifolia* was invading the area and yet no study has shown the level of its infestation and effects within the area. A survey was conducted inside Mwiba area along roads and within the area by a vehicle and by feet, looking on both sides of the road to observe the occurrence of *G. cordifolia*. Within January and February during the reconnaissance *G. cordifolia* was at a low stage of growth and was difficult to locate their level of infestation within the area, however, the higher infestation was seen in March during data collection. Invaded locations were recorded using Garmin etrex20 GPS. Latitude, longitude, elevation, level of infestation in an area was recorded. The level of infestation was estimated to be high when > 75% of plant covers were invaded with *G. cordifolia* and the rest covered with native herbs and grasses.

3.2.2 Experimental Sampling Design and Treatments

Five blocks of 100 x 100 m2 (Fig. 3) were established at a distance of at least 2 km apart in areas with a similar level of infestation of G. cordifolia (> 75% coverage) and with the associated existence of native flowering plants (Fig. 3). The cover of G. cordifolia was homogeneous across all selected blocks and across all plots that were established before starting the experiment. In each block, three sampling plots of 10 x 10 m2 were randomly established at least 20 m apart from each other. Each sampling plot within one block was then subjected to a management intervention as follows: CON = Control, no treatment, DUL = D. uncinatum crude leaves extract (100% DUL), and GLY = Glyphosate. Each of these management was replicated five times across the blocks within Mwiba Wildlife Ranch, making a total of 15 plots.

3.2.3 *Desmodium uncinatum* Crude Leaves Extract and Chemical (Glyphosate) Preparation and Spraying

Desmodium uncinatum fresh leaves were collected from Nkoaranga village in Meru district between December 2019 and February 2020. Fresh leaves were collected early in the morning to evade feasible deprivation of any allelochemicals (Ojija, 2020). Then, *D. uncinatum* crude leaves extract (DUL) were prepared as described by Ngondya *et al.* (2016). Figure 4a, 100g leaf powder was soaked separately in 1 L of distilled water and left for 72 h, after which the crude extract was filtered to obtain a final volume of 1 L each. The crude extracts were diluted with distilled water in the ratio of 100:0 (extract: water) to 100% concentration level. At each of the five DUL treated plots, 5 L of 100% concentration of *D. uncinatum* crude leaves extract was applied, immediately after the heavy rainy season had ended (end of April 2020). After rain season is a suitable time to spray GLY and DUL as the treatments will not be washed away with rain and also the weather during this time is good for observation of pollinator activity. Studies suggest that weather is one important factor to consider in determining insect visitation rate (Mccall *et al.,* 2012; Fijen & Kleijn, 2017) The chemical herbicide (Glyphosate 360 g/L, registration number HE/0055, Monsanto Kenya Ltd.) was prepared and sprayed as per manufacturers' recommendations. Glyphosate was bought from Arusha market, prepared and sprayed per conditions for use on weed control in which 200 mL/L of water (20% solution of the product in water). The spray tank was filled with one-half of the required amount of clean water and add the proper amount of Glyphosate

360. It was then mixed well before adding the remaining portion of water. Then, five chemical treated plots each received five liters of Glyphosate diluted with water. During dilution and spraying of Glyphosate, a person responsible wore a mask and gloves. However, for *Desmodium uncinatum* crude leaves extract (DUL) preparation and spraying there were no need for PPE due to the safety of the plant to both man and animals (Yadeta & Leta, 2020). The process of spraying Glyphosate was done at the same time as the DUL, to suppress *G. cordifolia* seedlings, flowering, and soil seed banks to allow the re-sprouting of non-invasive native plants. Control plots (CON) with a 75% *G. cordifolia* infestation rate, were left untreated for assessing the outcome of neglected management of invasive plants on insect visitors.

Figure 4: (A) *Desmodium uncinatum* preparation (B) Spraying process of *Desmodium uncinatum* crude leaves extract (DUL), and Glyphosate (GLY) in sampling plots within Mwiba study area at the end of the rainy season (March - June 2020)

3.2.4 Insect Visitors and Flower Sampling

Insect visitors were sampled by observation, sweep net, and pan trap method as followed: In each sampling plot, all insect species visiting flowers were identified and collected both before and after spraying DUL and GLY for three months (March 2020 - June 2020). The aim of collecting data before treatments were to assess whether all plots had the same level of infestation of *G. cordifolia* and the same level of insect visitation, however, data were only analyzed after treatments. Observations were conducted between 0800 h to 1000 h and 1600 h to 1800 h in each plot for a maximum period of 30 minutes (Stubbs *et al.*, 2007) (Fig. 5a). The observer was moving within the plot recording each landing of an insect on flowers. Where possible, unknown observed insect visitors were caught by sweep net (Fig. 5b) and were later identified to species-level following the taxonomic nomenclature under the supervision of insect expert taxonomists from the Department of Zoology and Wildlife Conservation, University of Dar es Salaam (Ojija *et al.,* 2019b). Bees were identified by a bee taxonomist from the Royal Belgian Institute of Natural Sciences. Every visible insect staying for at least 5 seconds on any flower part was recorded and then the visitors were classified into the following functional groups: Hymenoptera (honey bees, wasps, ants, and other bees), Diptera (Syrphidae, Calliphoridae, Asilidae, and other flies), Hemiptera (Scutelleridae, Pentatomidae, and other bugs), Lepidoptera (butterflies and moths), Coleoptera (beetles) and all other insects that did not fit to any of the mentioned collections (Ustinova & Lysenkov, 2020). During the observation, weather conditions, i.e., sunny or overcast, temperature, air pressure, and humidity were recorded. The study regarded a day to be sunny if the cloud cover ranged between 0% and 50% and cloudy when the cloud cover was > 50% (Gaira *et al.*, 2016).

Pan trap surveys were carried out twice per sampling block before and after spraying DUL and GLY (Fig. 5c). A total of 90 yellow pan traps were evenly distributed throughout the sampling blocks per day, and within each of the sampling plots, 30 traps were placed above ground on the level of vegetation at a standing pole. The method is adopted from Pardo *et al.* (2020), whereby the yellow pan traps were evenly distributed throughout the sampling area to sample insects as the trap's color supposed to reflect the color of flowers which could be the reason why traps are usually evenly distributed. After 10 h during the evening, all traps placed for that day were collected, but only bees were sorted out as efficient visitors of flowers (Etanidou *et al.*, 2008). Traps were not left for more than 24 h as in other studies to evade losing specimens to other predators such as birds (Pardo *et al.,* 2020).

The abundance of the flower was assessed within each of the sampling plots, and the number of flowers that were visited by particular insect visitors was recorded during the sampling period. All flowers within the plots were identified and counted with stigmas and anthers measured as individual flowers (Blaauw & Isaacs, 2014). Every observation consisted of 10 minutes period of watching flowers, whereby visitation was defined when an insect touched the flower part.

Figure 5: Insect sampling by (A) observation (B) sweep net (C) pan traps (D) sorting of insect specimen for species identification in Mwiba study area at the end of the rainy season (March - June 2020)

3.3 Statistical Analysis

The generalized linear model was used to evaluate the mean number of insect visitors from the observation method and the mean number of flowers visited across CON, DUL, and GLY treatments. Poisson distribution model was fitted with treatment type, the number of insect visitors, and the number of flowers visited. The count data model was over-dispersed and, therefore, a negative binomial model was used (Tanis *et al.,* 2020). The likelihood ratio test/ANOVA was used to compare the significant difference in the mean number of insect visitors and flowers visited across CON, DUL, and GLY treatments. One-way ANOVA was used to compare means of bee species and richness and diversity of flowers across CON, DUL, and GLY after treatment. For post-hoc analysis, Tukey's Honestly Significant Difference (HSD) test to compare mean of insect visitors, flowers visited, bee diversity and richness, and flower diversity between treatments. After homogeneity of variance (Levene's) and normality (Shapiro-Wilk) tests, the Kruskal-Wallis test was used to compare significant differences in the mean number of insect diversity and richness and flower abundance. Wilcoxon signed-rank test was used to compare the mean of flower abundance between treatments. The Shannon-Wiener Diversity Index was calculated for insect visitors and flowers across CON, DUL, and GLY. For insect species richness, a dataset was built covering the number of species found per sampling plot across each treatment. Data were analyzed using R software (version 4.0.3) with the level of statistical significance set at $p < 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Comparing the Effects of *Desmodium uncinatum* Leaves Extract and Glyphosate Treatments on the Number of Insect Visitors

A total number of 1660 individual insect visitors from 30 families and 74 species were recorded visiting flowers within the surveyed plots only after treatments. Seven groups of insect visitors observed were; bees and wasps (Hymenoptera were 25%), flies (Diptera 12%), butterflies (Lepidoptera 21%), beetles (Coleoptera 6%), bugs (Hemiptera 26%), grasshoppers, and bush-crickets Orthoptera 10%) and dragonfly (Odonata 0%). More than half of the insect visitors observed were found visiting flowering plants in DUL plots with 55%, CON followed with 26%, and GLY plots with 19%. Significant differences were found in mean number of insect visitors across DUL, CON and GLY treatments ($F_{2,159}$ = 9.521, df = 2, p = 0.009), with DUL plots having almost twice as many visitors compared to CON (p = 0.029) and GLY (p = 0.029), while CON and GLY did not differ (p = 0.975), Fig. 6A.

4.1.2 Comparing the Effects of *Desmodium uncinatum* Leaves Extract and Glyphosate Treatments on the Number of Flowers Visited

The total number of flowers visited after treatments were 2378. Twice as many flowers were visited in DUL plots with 59%, CON followed with 25%, and GLY plots with 16% of flowers visited. The mean number of flowers visited across DUL, CON and GLY treatments differed significantly ($F_{2,159}$ = 21.595, df = 2, $p < 0.001$), with DUL having over One-third more flowers visited compared to CON (p = 0.002) and GLY ($p < 0.001$) while no significant difference was observed between CON and GLY (p = 0.582), (Fig. 6B).

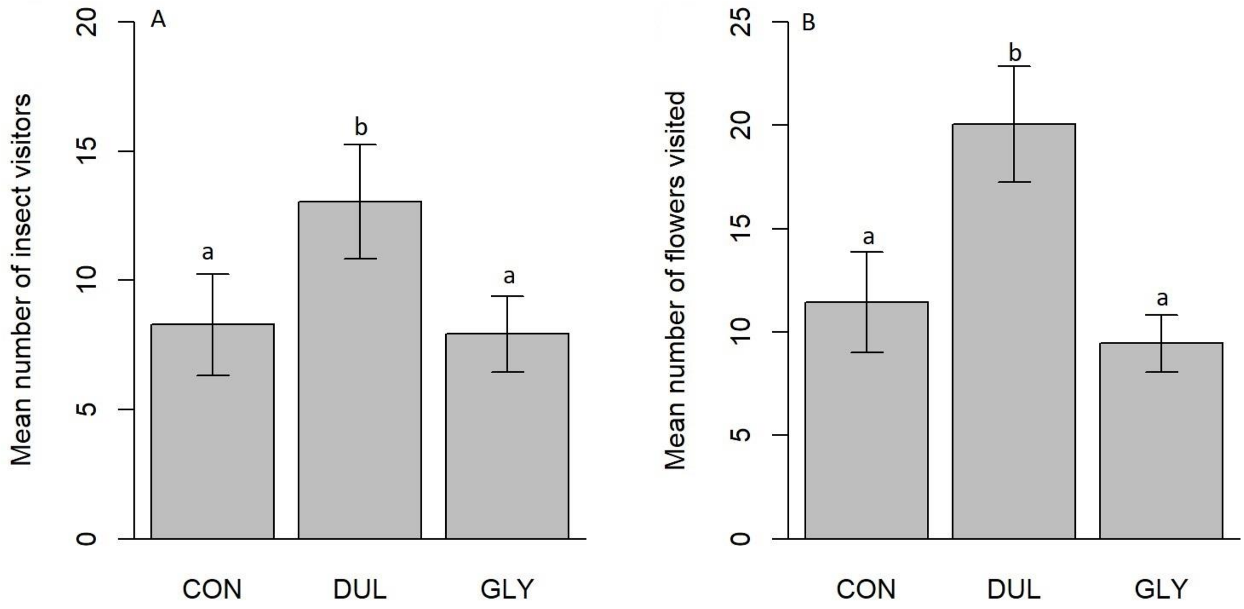

Figure 6: Mean number (±SE) of (A) insect visitors and (B) flowers visited across Control (CON), *Desmodium uncinatum* crude leaves extract (DUL), and Glyphosate (GLY) sampling plots after treatments in Mwiba study area at the end of the rainy season (March - June 2020). Different letters above bars show significant differences across treatments at *p* < 0.05 based on Tukey's HSD test

4.1.3 Comparing the Effects of *Desmodium uncinatum* Leaves Extract and Glyphosate Treatments on Insect Diversity and Richness

The study found that insect diversity across CON, DUL, and GLY treatments showed no significant difference (χ^2 = 3.38, df = 2, p = 0.18) but was slightly higher in DUL compared to CON and GLY after treatments (Fig. 7A). The same trend was visible for insect species richness across CON, DUL and GLY treatments (χ^2 = 3.38, df = 2, p = 0.18) (Fig. 7B).

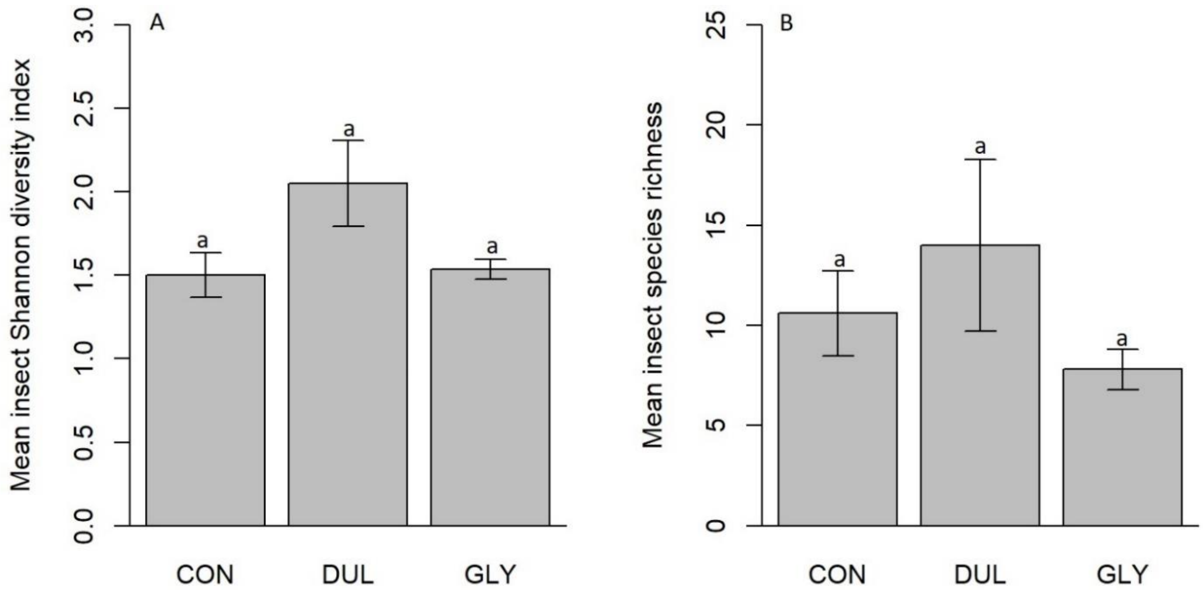

Figure 7: **Mean (±SE) of (A) Shannon diversity index of insect visitors and (B) insect species richness across control (CON), *Desmodium uncinatum* crude leaves extract (DUL) and Glyphosate (GLY) sampling plots after treatments in Mwiba area at the end of the rainy season (March - June 2020). The same letters above bars showed no significant difference across treatments *p* > 0.05 based on Kruskal-Wallis test**

4.1.4 Comparing the Effects of *Desmodium uncinatum* Leaves Extract and Glyphosate Treatments on Bee Diversity and Species Richness

The number of bee individuals collected by the pan traps method was 401 with 59 species from two families (Apoidea and Halictidae). Bee diversity across CON, DUL and GLY were significantly different after treatments ($F_{2,12}$ = 5.497, df = 2, p = 0.020), with higher mean diversity in DUL than in GLY (p < 0.001) and CON (p = 0.051), not significantly but higher between CON and GLY (p = 0. 146), Fig. 8A. Bee species richness across CON, DUL and GLY experiments showed significance of difference after treatment ($F_{2,12}$ = 21.810, df = 2, p < 0.001), with DUL having significance higher mean bee species richness more than CON (p = 0.003), and GLY (p < 0.001) and between CON and GLY (p = 0.051), (Fig. 8B).

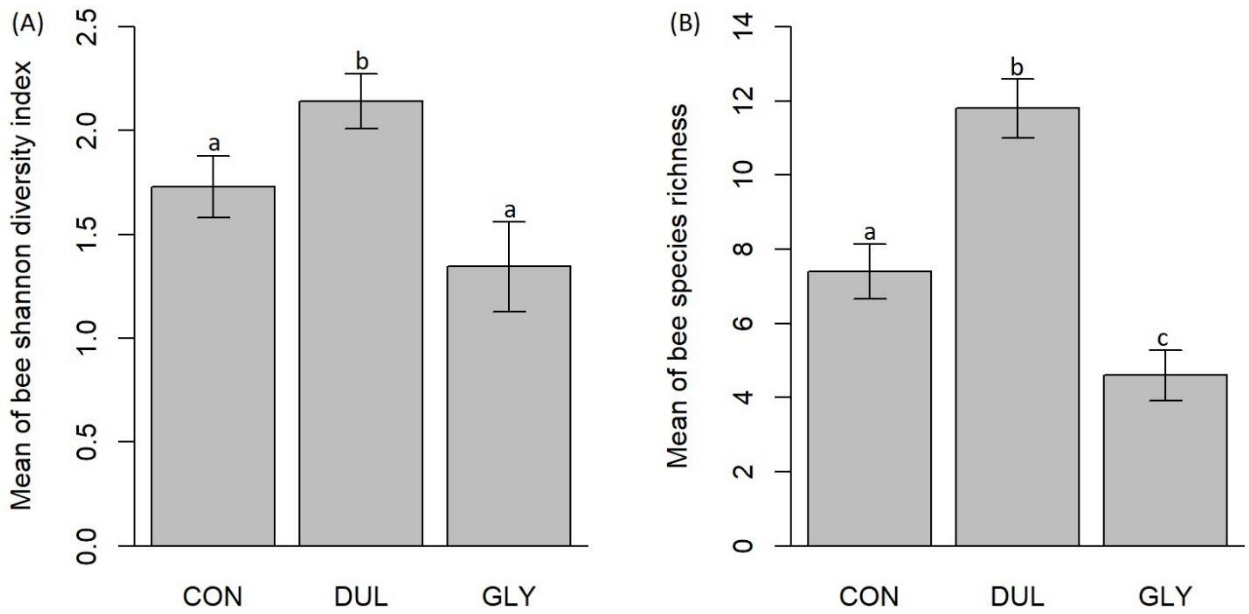

Figure 8: Mean number + SE of bees (A) Shannon diversity Index between (B) species richness, across Control (CON), *Desmodium uncinatum* crude leaves extract (DUL), and Glyphosate (GLY) sampling plots after treatments in Mwiba study area at the end of the rainy season (March - June 2020). Different letters above bars show significant differences across treatments at $p < 0.05$ based on Tukey's HSD test

4.1.5 Comparing the Effects of *Desmodium uncinatum* Leaves Extract and Glyphosate Treatments on Flower Diversity and Flower Abundance

In total, the study found 2957 flowers after treatments, with the most abundant flowering plants observed receiving insect visitation was *Vernonia galamensis* (Asteraceae, 20% of all flower abundance), *Justicia betonica* (Acanthaceae, 12%), *Cyathula orthacantha* (Amaranthaceae, 13%) as well as *Hibiscus cannabinus* (Malvaceae, 10%). The DUL plots had with more than half of the flowers (55%) the highest flower abundance, followed by 25% in CON and 20% in GLY plots. The difference in flower relative abundance across CON, DUL and GLY was significant (χ^2 = 7.460, df = 2, p = 0.024), as was that between GLY and DUL (p = 0.048) but not between CON and DUL (p = 0.167) and between GLY and CON (p = 0.929), (Fig. 9A). Flower diversity differed across treatments ($F_{2,12}$ = 3.963, df = 2, p = 0.048), with DUL having slightly higher diversity than GLY (p = 0.081) and CON (p = 0.069) but no significance difference was observed between GLY and CON (p = 0.996), (Fig. 9B).

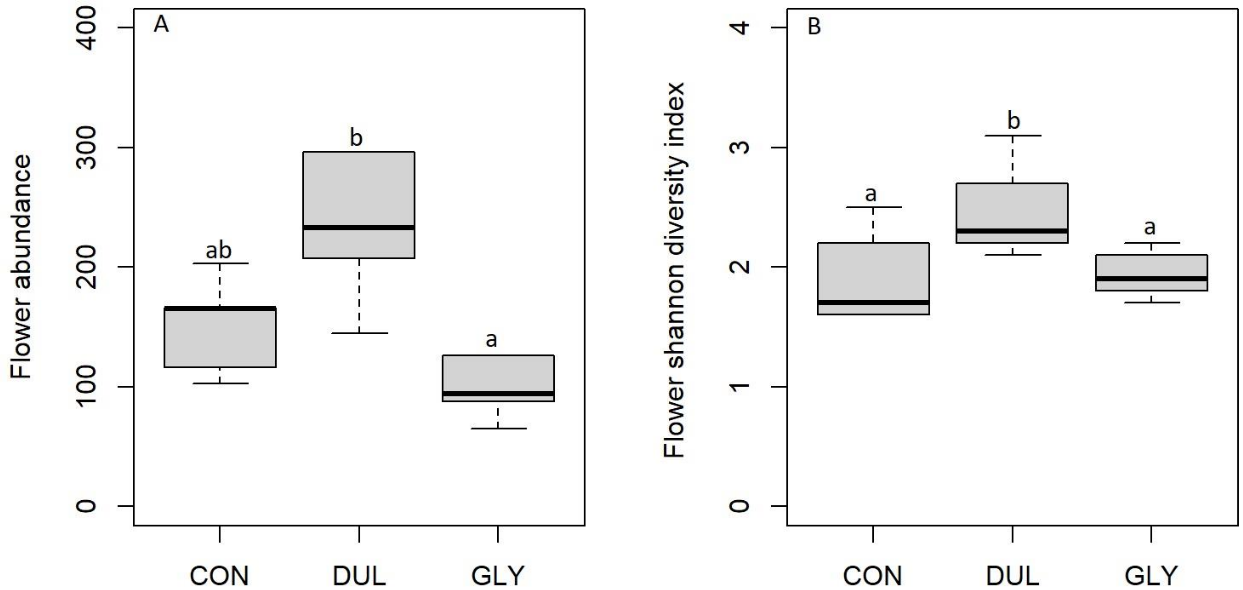

Figure 9: (A) Number of individual flowers per plant species, (B) flower Shannon diversity index across Control (CON), *Desmodium uncinatum* crude leaves extract (DUL), and Glyphosate (GLY) sampling plots after treatments in Mwiba area at the end of the rainy season (March - June 2020). Different letters above bars showed significant differences across treatments $p < 0.05$ based on Wilcoxon signed-rank test and Tukey's HSD test

4.2 Discussion

4.2.1 Comparing the Effects of *Desmodium uncinatum* Leaves Extract and Glyphosate Treatments on the Number of Insect Visitors

As hypothesized, the study observed that the number of insects visiting flowers in plots treated with GLY highly decreased. The GLY effects on floral resources foraged by insects could have indirectly affected insect visitor's survival rates, and reduced their number; previous studies indicate that honey bee survival could be reduced by exposure to glyphosate when gathering contaminated nectar, pollen, and water sources (Motta *et al.*, 2020). However, several scientific studies have also revealed that declines in bee colonies and fluctuations in honey bee behavior are the results of contacting chemical pesticides (Kumar *et al.*, 2018; Herbert *et al.*, 2014). It has been further established that pollinator's gut microbiota can be disturbed by glyphosate, which could cause higher susceptibility to diseases and malnutrition (Farina *et al.*, 2019; Motta *et al.*, 2018) thus, glyphosate can further affect and even kill non-targeted pollinators (Abraham *et al.*, 2018). Hence, the findings of this study highlight that the use of glyphosate could reduce insect visitors, particularly that of pollinators, thereby threatening the overall ecosystem health in a Tanzania ecosystem.

While the effectiveness of DUL treatment in managing invasive plants *Tagetes minuta, G. cordifolia*, and *Parthenium hysterophorus* has been well established (Ngondya *et al.,* 2016 a & b; Ojija *et al.,* 2019), the results of this study indicate that the application of DUL as a bioherbicide can also attract more insect visitors when applied to manage *G. cordifolia*. As hypothesized, the study found one-third more insect visitors at flowering plants in DUL plots than in GLY plots after treatment, showing that DUL is a relatively target-specific plant-based extract (Ngondya *et al.*, 2016 a & b) had no detrimental effects on insect visitors (Moreno *et al.,* 2012). So far, research efforts have been paying attention to the bioactivity, application approaches, cost-effectiveness, and reasonable use of botanical pesticides on insect pests (Mmbone *et al.,* 2014). This study ignites a discussion as to whether management of invasive *G. cordifolia* using plant extract (DUL) affects insect visitors. The study emphasizes that DUL not only manages an invasive *G. cordifolia* but also increases the number of insect visitors, and, therefore, stands out as a highly effective approach to be used in controlling *G. cordifolia,* especially in protected areas where chemical herbicides are not recommended.

4.2.2 Comparing the Effects of *Desmodium uncinatum* Leaves Extract and Glyphosate Treatments on the Number of Flowers Visited

As hypothesized, GLY treatment negatively impacted the number of flowers visited, by reducing the floral resources that attract insects visiting flowers (Lazaro *et al.,* 2020). This result indicates that glyphosate as a non-selective synthetic herbicide could negatively affect plant development and caused a rather non-specific plant death (Zhang, 2020). The low plant diversity due to chemicals leads to floral resource reduction (Muratet & Fontaine, 2015; Aniko *et al.,* 2017), and thereby reduced food resources (i.e., nectar and pollen), which usually attract insects to flowers (Siregar *et al.,* 2016). Hence, the chemical treatment not only directly kills insects but also indirectly reduces their numbers by suppressing the growth of insect-attractive flowers (Carpenter *et al.,* 2020). Normally, the number of flowers visited by insects determines the number of events, in which pollen will be deposited for plant fertilization (Novella-fernandez *et al.,* 2019). This study showed that GLY treatment inhibited these services, in contrast to DUL, and thereby threatened ecosystem functions.

Further, in agreement with the hypothesis, the study found that DUL treatment had no negative impact on the native flowering plant resources, which allowed more visitations of insects on DUL-treated flowers. This study is among the very few studies to indicate/highlight the bio-safety of DUL treatment against invasive *G. cordifolia* as it increases flower abundance and diversity, which led to twice as many flowers being visited by diverse insects compared to GLY plots. The higher pollinator numbers assure a higher reproduction of plants and availability of food resources, not only to pollinators but also to herbivorous insect species, that mutually depend on each other for the survival and balancing of the whole ecosystem (Menz *et al.*, 2011).

4.2.3 Comparing the Effects of *Desmodium uncinatum* and Glyphosate Treatments on Insect Diversity and Richness

Despite variation in flower abundance and diversity, still, the results were contradicting the predictions, as, the study did not find any significant difference in insect species diversity and richness across DUL, CON, and GLY plots. However, the study observed a small increase of insect diversity and species richness after treatment within DUL plots, suggesting that floral resources and, thus, pollinator networks, might have been at least enhanced (Siregar *et al.*, 2016). It was found that DUL bio-herbicide did not interrupt non-targeted flower abundance and diversity, thus assuring the accessibility of resources that are critical for sustaining various insect visitors (Hegland & Boeke, 2006).

Studies explain that insect richness and diversity will be higher in areas with abundant floral resources (Blaauw & Isaacs, 2014). Additionally, studies display that flower diversity is more important than the nature of the area on maximizing insect flower visitors diversity, suggesting that management should be introduced to support insect diversity (Scriven *et al.*, 2013). Other studies similar to this have shown that GLY treatment on weeds can have non-targeted negative effects on the richness and diversity of insects within a short or long time (Sharma *et al.*, 2018), causing insect death, alteration in reproduction rates, and population size of numerous insect taxonomic group, including Hymenoptera, Coleoptera, and Orthoptera (Lins *et al.*, 2007). In this study, results showed that GLY reduced insect diversity, which could negatively impact the ecological services as the productivity of > 88% of flowering plant species depend on diverse pollinators for sexual reproduction (Devkota *et al.*, 2020). It can, therefore, be claimed that DUL can support insect diversity and richness and preserve a healthy ecosystem, while it ensures plant fertilization and maximizes plant genetic diversity (Siregar *et al.*,2016). Therefore, in an environmental context, DUL assures insect diversity and richness in natural as well as agricultural areas, which can have important implications for plant reproductivity, plant productivity, and pasture availability after invasive management (Sajjad *et al.*, 2012).

4.2.4 Comparing the Effects of *Desmodium uncinatum* and Glyphosate Treatments on Bees' Diversity and Species Richness

As predicted, results showed that bee's diversity and richness were high in DUL plots, highlighting that DUL had no negative impact on efficient insect visitors, thus ensuring pollination and fertilization of both crops and wild flowering plants. For years bees conservation efforts have focused on restoring flowering plant resources by improving and increasing territory for nesting and foraging resources (Buckles *et al.*, 2019; Tonietto & Larkin, 2018). This result is on track with other growing studies that show natural products extracted from plants had no adverse impacts on non-targeted species including bees. Bees as significant insect visitors are critically important in supporting biodiversity worldwide and they need to be preserved for the majority of pollination in agricultural and natural systems (Lu *et al.*, 2020; Siregar *et al.*, 2016; Tonietto & Larkin, 2018). Insecticides (even at low concentrations) have been observed to cause direct bee's mortality, disrupt the social structure, trigger loss of reproductive ability and cause the local decrease of wild bee diversity and abundance, or even affect bees indirectly by decreasing flora resource accessibility (Decourtye *et al.*, 2019; O'brien, 2017)

4.2.5 Comparing the Effects of *Desmodium uncinatum* and Glyphosate Treatments on Flower Diversity and Abundance

As hypothesized, the study found that DUL treatment increased flower abundance and diversity, which has been shown to directly determine pollinator communities (Tonietto & Larkin, 2018). This is likely due to its targeting capacity on suppressing the invasive *G. cordifolia* only, without further affecting non-targeted flowering plants. Supporting the current results, glyphosate treatment on weed can have further effects on flower abundance and diversity of other plants by changing their biomass, flowering phenology, reproduction ability, and flowering period (Londo *et al.*, 2014). Other studies contradict the findings of this study as their results showed that glyphosate disturbance is within the normal variations of plant species diversity and large animals in terrestrial ecosystems, suggesting that glyphosate could be appropriate for managing vegetation and biodiversity (Sullivan & Sullivan, 2003). However, their results required data on how glyphosate affects pollinator species diversity, enhances plant diversity and ecosystem output (Robinson *et al.*, 2018). Thus, this study delivers important evidence on the adverse impact of GLY on flowering plants and insects in Mwiba Ranch within the Serengeti ecosystem which is the eastern African savanna systems. The study identifies that DUL sustained the mutualistic interaction among insect visitors and flowers visited, highlighting the reliability of natural bioherbicide for the conservating the ecosystem services delivery inside and outside of protected areas.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study pinpoint that using a 100% concentration level of *Desmodium uncinatum* crude leaves extract (DUL) as a management option against the invasive plant *G. cordifolia* is the best approach as it did not negatively affect insect flower visitors and flowers in contrast to glyphosate (GLY). Results exhibited that applied plant extracts (bioherbicide) had the potential in attracting more insect visitors, therefore, the study acclaim that natural DUL herbicide as a highly ideal substitute to GLY for controlling *G. cordifolia* in eastern African savanna systems, and particularly inside of protected areas, where chemical herbicides are not recommended. The natural herbicide is not only promising for environmental health but also does not affect biodiversity.

5.2 Recommendations

(i) This study highlighted species-specific effects of invasive *G. cordifolia* management by DUL and GLY on insect visitor communities in invaded sites of Mwiba area. Hence, it is recommended for management programs to prioritize DUL for current and future control and eradication of the tested invasive species. Invasive *G. cordifolia* inside and outside of wildlife habitat are likely to present ecological and economic problems for wildlife conservation and the communities surrounding protected areas. With the rising infestation of *G. cordifolia*, in the protected areas, the cost for their management also increases. Therefore, this study recommends the following suitable and appropriate actions for future studies: Before deciding on any type of management practices, this study recommends conducting a consistent and comprehensive field survey to evaluate the infestation rate of *G. cordifolia* to know its impact on the native plant-pollinator interaction, and prepare an effective invasive control plan without adversely impacting biodiversity and ecosystem services.

(ii) The use of naturally extracted products for the management of invasive plants is highly recommended; as this study suggested the importance of DUL treatment against invasive *G. cordifolia* on the environment. This treatment strongly enhanced insect visitors, and flowers. But further studies should be done on the bio-safety of this bio-herbicide on other insect groups, birds, animals, human health, and on the environment in general.

(iii) The natural herbicide supported environmental health in this study. However, the study also recommends more long-term studies and monitoring on the impacts of invasive plant management options on insect visitors and other wild animals for sustainable rangelands conservation.

(iv) Further this study show that no management (CON) resulted in insect visitors and flower abundances as low as that of chemical treatment (GLY), recommending the urgency of developing environmental-friendly management technologies against invasive plant species in the Mwiba area.

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APPENDIX

Appendix 1: R-Statistical Functions and Packages used during Statistical Analysis

Load package

```
require(MASS)
require(multcomp)
```

Load data

```
insectvisito <- read.csv("D:/all csv/insect flowers.csv")

insectvisito$Experiment<-ifelse(insectvisito$Experiment=="Control","CON",
                    ifelse(insectvisito$Experiment=="DuL","DUL",
                        ifelse(insectvisito$Experiment=="Glyphosate","GLY","CON")))
```

1. fitting model for insect visitors and flowers visited

```
# convert to factor
insectvisito$Experiment<-as.factor(insectvisito$Experiment)

# Fitting model
m2<-glm.nb(insect.visitors~Experiment,data=insectvisito)

summary(m2)
##
## Call:
## glm.nb(formula = insect.visitors ~ Experiment, data = insectvisito,
##     init.theta = 1.181863229, link = log)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -1.4748  -1.0041  -0.5800  -0.0843   3.9781
##
## Coefficients:
```

```
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)    2.11421    0.13506  15.653   <2e-16 ***
## ExperimentDUL  0.45294    0.17727   2.555   0.0106 *
## ExperimentGLY -0.04443    0.20778  -0.214   0.8307
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for Negative Binomial(1.1819) family taken to be 1)
##
##     Null deviance: 180.76  on 161  degrees of freedom
## Residual deviance: 171.23  on 159  degrees of freedom
## AIC: 1090.9
##
## Number of Fisher Scoring iterations: 1
##
##
##               Theta:  1.182
##           Std. Err.:  0.133
##
##  2 x log-likelihood:  -1082.925
```

```
# Multiple wise comparison
c2<-glht(m2,linfct = mcp(Experiment="Tukey"))

summary(c2)
```

```
##
##   Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: Tukey Contrasts
##
##
## Fit: glm.nb(formula = insect.visitors ~ Experiment, data = insectvisito,
##     init.theta = 1.181863229, link = log)
```

```
##
## Linear Hypotheses:
##                  Estimate Std. Error z value Pr(>|z|)
## DUL - CON == 0  0.45294    0.17727   2.555   0.0285 *
## GLY - CON == 0 -0.04443    0.20778  -0.214   0.9750
## GLY - DUL == 0 -0.49737    0.19523  -2.548   0.0290 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

```
# likelihood ratio test
anova(m2,test="LRT")
```

```
## Warning in anova.negbin(m2, test = "LRT"): tests made without re-estimating
## 'theta'
## Analysis of Deviance Table
##
## Model: Negative Binomial(1.1819), link: log
##
## Response: insect.visitors
##
## Terms added sequentially (first to last)
##
##
##            Df Deviance Resid. Df Resid. Dev Pr(>Chi)
## NULL                         161     180.75
## Experiment  2   9.5211       159     171.23 0.008561 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# anova test
# Fitting model of flower visited
m3<-glm.nb(Flower.visited~Experiment,data=insectvisito)

summary(m3)
```

```
##
## Call:
## glm.nb(formula = Flower.visited ~ Experiment, data = insectvisito,
##     init.theta = 1.383577998, link = log)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -1.6518  -0.9684  -0.6195   0.0542   3.5184
##
## Coefficients:
##               Estimate Std. Error z value Pr(>|z|)
## (Intercept)     2.4366     0.1236  19.707  < 2e-16 ***
## ExperimentDUL   0.5613     0.1623   3.459 0.000541 ***
## ExperimentGLY  -0.1894     0.1911  -0.991 0.321824
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for Negative Binomial(1.3836) family taken to be 1)
##
##     Null deviance: 193.24  on 161  degrees of freedom
## Residual deviance: 171.64  on 159  degrees of freedom
## AIC: 1189.1
##
## Number of Fisher Scoring iterations: 1
##
##
##               Theta:  1.384
##           Std. Err.:  0.155
##
##  2 x log-likelihood:  -1181.146
```

```
# Multiple wise comparison
c3<-glht(m3,linfct = mcp(Experiment="Tukey"))
```

```
summary(c3)
```

```
##
##   Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: Tukey Contrasts
##
##
## Fit: glm.nb(formula = Flower.visited ~ Experiment, data = insectvisito,
##     init.theta = 1.383577998, link = log)
##
## Linear Hypotheses:
##                Estimate Std. Error z value Pr(>|z|)
## DUL - CON == 0   0.5613     0.1623   3.459  0.00159 **
## GLY - CON == 0  -0.1894     0.1911  -0.991  0.58151
## GLY - DUL == 0  -0.7506     0.1797  -4.178  < 0.001 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

```
# Likelihood ratio test
anova(m3,test = "LRT")
```

```
## Warning in anova.negbin(m3, test = "LRT"): tests made without re-estimating
## 'theta'
## Analysis of Deviance Table
##
## Model: Negative Binomial(1.3836), link: log
##
## Response: Flower.visited
##
## Terms added sequentially (first to last)
##
##
```

```
##            Df Deviance Resid. Df Resid. Dev  Pr(>Chi)
## NULL                         161     193.24
## Experiment  2   21.595       159     171.64 2.045e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2. Testing flower abundance and diversity

```
## Loading required package: ggplot2
## The following objects are masked from DV (pos = 4):
##
##     Flower.Abundance, Flower.diversity, Treatment
## [1] "Treatment"        "Flower.diversity" "Flower.Abundance"
## 'data.frame':    15 obs. of  3 variables:
##  $ Treatment       : chr  "CON" "CON" "CON" "CON" ...
##  $ Flower.diversity: num  2.2 1.6 1.6 1.7 2.5 2.7 2.2 2.1 2.3 3.1 ...
##  $ Flower.Abundance: int  116 165 103 203 166 145 233 296 207 745 ...
##
##  Shapiro-Wilk normality test
##
## data:  Flower.diversity
## W = 0.92663, p-value = 0.2428
##
##  Shapiro-Wilk normality test
##
## data:  Flower.Abundance
## W = 0.64508, p-value = 6.814e-05
##
##  Kruskal-Wallis rank sum test
##
## data:  Flower.Abundance by Treatment
## Kruskal-Wallis chi-squared = 7.46, df = 2, p-value = 0.02399
##
```

```
##  Kruskal-Wallis rank sum test
##
## data:  Flower.Abundance by Treatment
## Kruskal-Wallis chi-squared = 3.9382, df = 1, p-value = 0.0472
##
##  Kruskal-Wallis rank sum test
##
## data:  Flower.Abundance by Treatment
## Kruskal-Wallis chi-squared = 1.32, df = 1, p-value = 0.2506
##
##  Kruskal-Wallis rank sum test
##
## data:  Flower.Abundance by Treatment
## Kruskal-Wallis chi-squared = 5.7709, df = 1, p-value = 0.01629
##             Df Sum Sq Mean Sq F value Pr(>F)
## Treatment    2  1.009  0.5047   3.963 0.0477 *
## Residuals   12  1.528  0.1273
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
##  One-way analysis of means (not assuming equal variances)
##
## data:  Flower.diversity and Treatment
## F = 4.6254, num df = 1.0000, denom df = 7.9983, p-value = 0.06372
##
##  One-way analysis of means (not assuming equal variances)
##
## data:  Flower.diversity and Treatment
## F = 0.0095238, num df = 1.0000, denom df = 5.9318, p-value = 0.9255
##
##  One-way analysis of means (not assuming equal variances)
##
```

```
## data:  Flower.diversity and Treatment
## F = 6.7814, num df = 1.0000, denom df = 5.8824, p-value = 0.04118
```

3. Testin insect visitors diversity and richness

```
4.  ##
5.  ##  Shapiro-Wilk normality test
6.  ##
7.  ## data:  richness
8.  ## W = 0.65172, p-value = 7.921e-05
9.  ##
10. ##  Shapiro-Wilk normality test
11. ##
12. ## data:  diversity
13. ## W = 0.87574, p-value = 0.041
14. ##
15. ##  Kruskal-Wallis rank sum test
16. ##
17. ## data:  diversity by Experiment
18. ## Kruskal-Wallis chi-squared = 3.38, df = 2, p-value = 0.1845
19. ##
20. ##  Kruskal-Wallis rank sum test
21. ##
22. ## data:  richness by Experiment
23. ## Kruskal-Wallis chi-squared = 3.3815, df = 2, p-value = 0.1844
```

4. Testing bee diversity and richness

```
# load data
beediv <- read.csv("D:/bees.csv")


beediv$Experiment<-ifelse(beediv$Experiment=="Control","CON",
                        ifelse(beediv$Experiment=="DuL","DUL",
                            ifelse(beediv$Experiment=="Glyphosate","GLY","CON")))
require(ggplot2)


# Attach data
attach(beediv)
## The following objects are masked from beediv (pos = 3):
##
##     Blocks, Diversity, Experiment, Richness, Treatment
names(beediv)
```

```
## [1] "Treatment"  "Blocks"     "Experiment" "Diversity"  "Richness"
str(beediv)
## 'data.frame':    15 obs. of  5 variables:
##  $ Treatment : chr  "After" "After" "After" "After" ...
##  $ Blocks    : chr  "A" "B" "C" "D" ...
##  $ Experiment: chr  "CON" "CON" "CON" "CON" ...
##  $ Diversity : num  1.68 1.54 1.95 1.32 2.15 ...
##  $ Richness  : int  6 8 7 6 10 11 9 13 13 13 ...
# Normality test
shapiro.test(Diversity)
##
##  Shapiro-Wilk normality test
##
## data:  Diversity
## W = 0.93606, p-value = 0.3354
shapiro.test(Richness)
##
##  Shapiro-Wilk normality test
##
## data:  Richness
## W = 0.92091, p-value = 0.1989
oneway.test<-aov(Diversity~Experiment,data=beediv)
summary(oneway.test)
##             Df Sum Sq Mean Sq F value Pr(>F)
## Experiment   2  1.579  0.7897   5.497 0.0202 *
## Residuals   12  1.724  0.1437
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
TukeyHSD(oneway.test)
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
```

```
## Fit: aov(formula = Diversity ~ Experiment, data = beediv)
##
## $Experiment
##               diff        lwr        upr     p adj
## DUL-CON  0.4123882 -0.2271544  1.0519308 0.2378450
## GLY-CON -0.3822713 -1.0218139  0.2572713 0.2852627
## GLY-DUL -0.7946595 -1.4342021 -0.1551169 0.0157523
```

```
# CON and DUL
candD<-subset(beediv,Experiment=="CON"|Experiment=="DUL")
oneway.test(Diversity~Experiment,data=candD)
```

```
##
##  One-way analysis of means (not assuming equal variances)
##
## data:  Diversity and Experiment
## F = 4.3152, num df = 1.0000, denom df = 7.8981, p-value = 0.07186
```

```
# CON and GLY
candG<-subset(beediv,Experiment=="CON"|Experiment=="GLY")
oneway.test(Diversity~Experiment,data=candG)
```

```
##
##  One-way analysis of means (not assuming equal variances)
##
## data:  Diversity and Experiment
## F = 2.1261, num df = 1.0000, denom df = 7.0755, p-value = 0.1877
```

```
# DUL and GLY
DandG<-subset(beediv,Experiment=="DUL"|Experiment=="GLY")
oneway.test(Diversity~Experiment,data=DandG)
```

```
##
##  One-way analysis of means (not assuming equal variances)
##
## data:  Diversity and Experiment
## F = 9.8277, num df = 1.0000, denom df = 6.6212, p-value = 0.01774
```

```
oneway.test<-aov(Richness~Experiment,data=beediv)
```

```
summary(oneway.test)
##            Df Sum Sq Mean Sq F value   Pr(>F)
## Experiment   2  131.7   65.87   23.81 6.65e-05 ***
## Residuals   12   33.2    2.77
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
TukeyHSD(oneway.test)
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = Richness ~ Experiment, data = beediv)
##
## $Experiment
##         diff        lwr          upr     p adj
## DUL-CON  4.4   1.593455  7.206545223 0.0033619
## GLY-CON -2.8  -5.606545  0.006545223 0.0505498
## GLY-DUL -7.2 -10.006545 -4.393454777 0.0000492
# CON and DUL
candD<-subset(beediv,Experiment=="CON"|Experiment=="DUL")
oneway.test(Richness~Experiment,data=candD)
##
##  One-way analysis of means (not assuming equal variances)
##
## data:  Richness and Experiment
## F = 16.133, num df = 1.0000, denom df = 7.9646, p-value = 0.003895
# CON and GLY
candG<-subset(beediv,Experiment=="CON"|Experiment=="GLY")
oneway.test(Richness~Experiment,data=candG)
##
##  One-way analysis of means (not assuming equal variances)
##
## data:  Richness and Experiment
```

```
## F = 7.6863, num df = 1.0000, denom df = 7.9238, p-value = 0.02443
```

```r
# DUL and GLY
DandG<-subset(beediv,Experiment=="DUL"|Experiment=="GLY")
oneway.test(Richness~Experiment,data=DandG)
```

```
##
##  One-way analysis of means (not assuming equal variances)
##
## data:  Richness and Experiment
## F = 47.127, num df = 1.0000, denom df = 7.7914, p-value = 0.000146
```

RESEARCH OUTPUTS

(i) Publication

Mecklina, M. M., Issakwisa, B. N., Mark, G., & Anna, C. T. (2021). Comparison of the effects of a broad-spectrum herbicide and a bio-herbicide on insect flower visitation in the Serengeti ecosystem, Tanzania. *Journal for Nature Conservation*, 64, 1-8.

(ii) Poster Presentation

Mecklina, M. M. (2021). *Comparison of the effects of a broad-spectrum herbicide and a bio-herbicide on insect flower visitation in the Serengeti ecosystem, Tanzania*. [Poster Presentation]. Nelson Mandela African Institution of Science and Technology, Arusha.