Detailed Final Report

Title: EFFECT OF GUTENBERGIA CORDIFOLIA MANAGEMENT ON INSECT'S DIVERSITY AND FLOWER VISITATION IN MWIBA WILDLIFE RANCH

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Research project completed

Introduction

Gutenbergia cordifolia has been reported as an indigenous invasive weed in diverse farmland areas of East Africa (Ngondya *et al.,* 2016). This species has been observed to suppress other native plants and dominate large areas of protected land such as the Ngorongoro Crater since 1962, thereby reducing pasture availability for herbivores (Ngondya *et al.,* 2016). If *G. cordifolia* is not managed, its invasiveness will negatively influence forage availability for wild animals including insect visitors (Ngondya *et al.,* 2017). In Tanzania, several Acts regulate biodiversity and controlling of invasive species (National Fisheries Policy, 2015; The Forest Act, 2002; The Plant Protection Act, 1997; The Environmental Management Act, 2005; The Marine Parks and Reserves Act, 1994). Unfortunately, the majority of the existing sectoral policy and legal frameworks are of a long time and have remained almost silent on issues of invasive species. For effective control of invasive species, there is a need to mainstream invasive species in these sectoral policies and legal frameworks.

Studies have shown 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 option for managing 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 three invasive plants has been well recognized, no studies have yet assessed how DUL affects insect flower visitors, particularly their diversity and visitation to flowers after application. On the other hand, the ability of glyphosate (GLY) in reducing and managing weed and the sub-lethal effects on non-targeted plant and insect pollinators have been documented (Walker & Oliver, 2008; Herbert *et al.,* 2014). Yet, no previous study has compared the effect of DUL and GLY in managing invasive *G. cordifolia* on non-targeted species such as insect visitors, the number of inflorescences visited, the inflorescences abundance, and flower diversity.

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 many species require native plants as food or site for reproduction (Vanbergen *et al.,* 2018; Bartomeus *et al.,* 2016). Thus, leaving the indirect effects of invasive plants on native plant-pollinators interaction unmanaged will lead to reduced insect visitation to native flowering plants (White *et al.,* 2006; Bartomeus & Santamaría, 2008). In this study, we investigated the effects of spraying the bioherbicide based on *D. uncinatum* (DUL) at 100% concentration and a chemical herbicide (glyphosate; GLY) as management approaches on the number of insect visitors, the number of inflorescences visited, insect diversity and richness, soil chemical properties, inflorescences abundance

and flower diversity at Mwiba area in the south-western Serengeti ecosystem, northern Tanzania. Specifically, we wanted to address the following research questions: How do DUL and GLY treatments affect the number of insect visitors and the number of flowers visited? How do DUL and GLY treatments affect the insect species richness and diversity? How do DUL and GLY treatments affect inflorescences abundance and flower diversity? How do DUL and GLY treatments affect soil chemical properties?

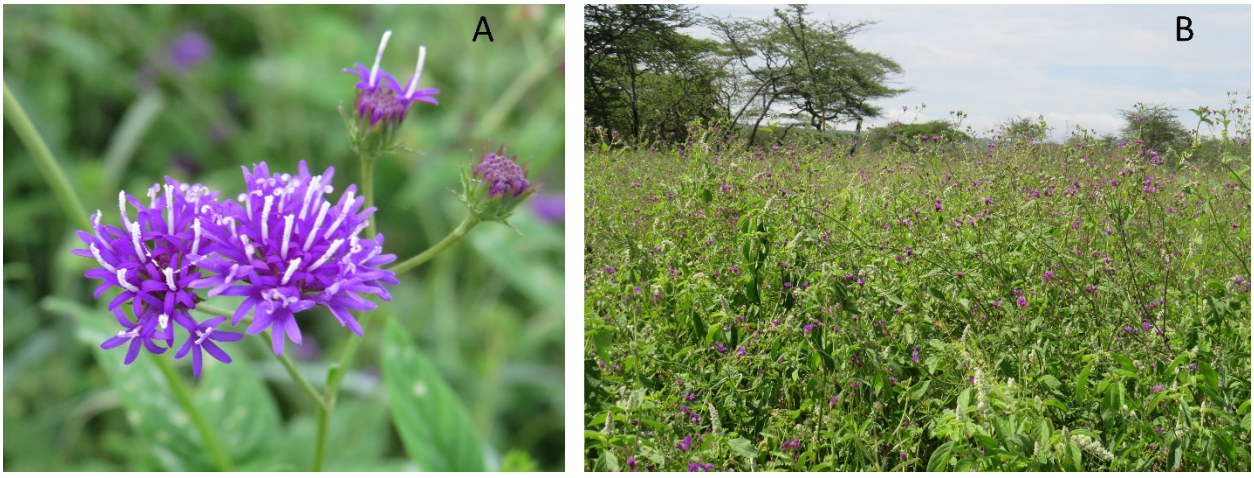

Fig. 1. (A) Flowers of invasive *Gutenbergia cordifolia*, (B) a landscape showing infestation of *G. cordifolia* (> 75% coverage) and with associated existence of native flowering plants in Mwiba area, Tanzania

Study site

Field studies were carried out in the south-western Serengeti ecosystem, at Mwiba area, formerly known as Makao Open Area, located in North-Western Tanzania between 03°22' S to 34°41'E to 34°53'E (Ngilangwa *et al.,*2018, Fig. 1). The Mwiba area covers about 19,647 ha and borders the Ngorongoro Conservation Area to the east, Maswa Game Reserve to the north, and Makao Wildlife Management Area to the southwest. The average annual temperature ranges between 21 °C and 27 °C 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. The high water availability from permanent water springs within Mwiba area has enabled the establishment of residential wildlife populations and it is an important breeding ground for wildebeest (*Connochaetes taurinus*) during their large migration (Ngilangwa *et al.,* 2018). *Gutenbergia cordifolia* has always been at the Mwiba area as a native plant (pers. comm.). In the last two years, *G. cordifolia* was increasingly observed to the extent of being regarded as an invasive plant in certain areas of Mwiba and Maswa Game reserve. However, 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.).

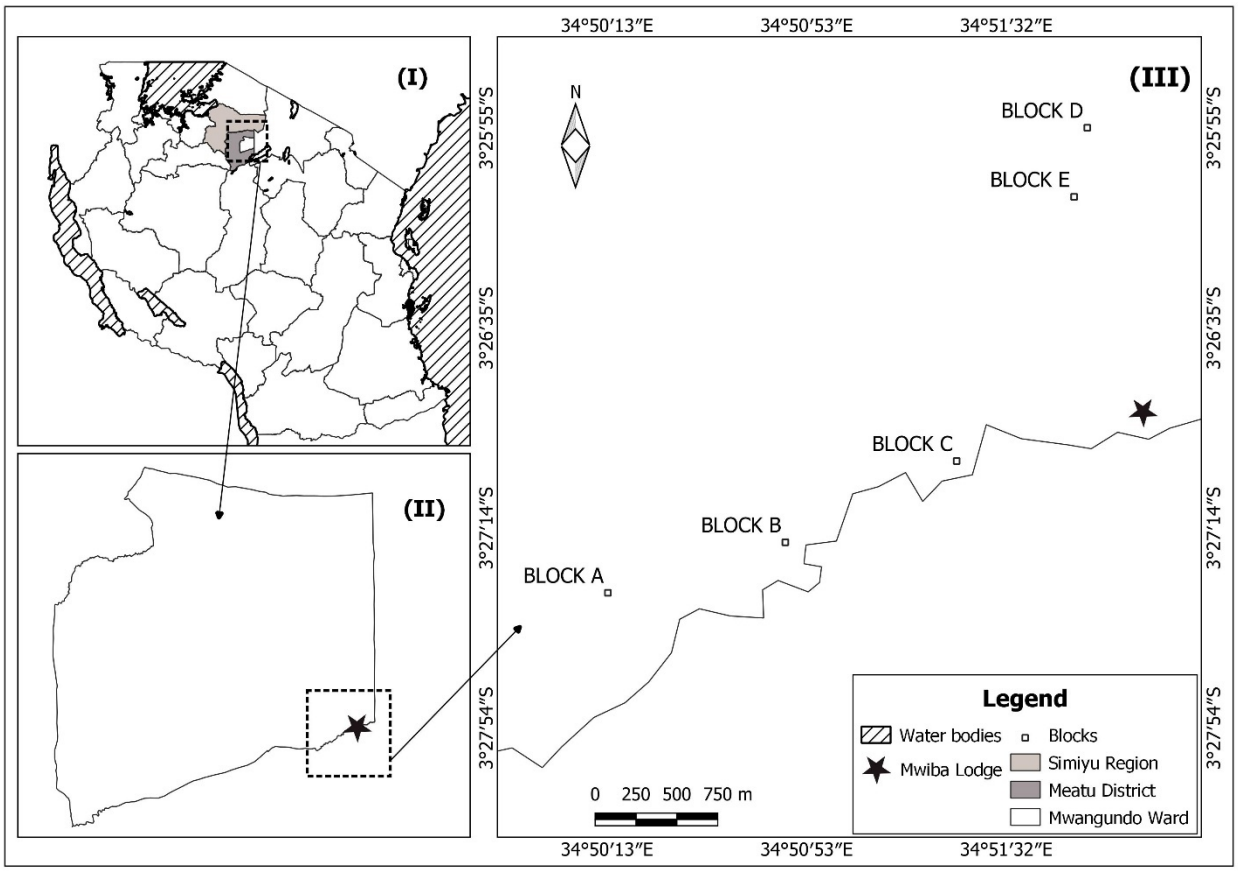

Fig. 2. A map of Mwiba wildlife ranch in North-Western Tanzania, with the five sampling blocks (A, B, C, D, and E) established at a distance of at least 2 km apart.

Assessing *G. cordifolia* distribution in Mwiba wildlife ranch

A field reconnaissance survey was done within Mwiba wildlife ranch between January and February 2020 to gather information on the existing distribution data of *G. cordifolia*. The Mwiba area was selected to be surveyed because it is more susceptible to the invasions as it borders Ngorongoro Conservation area, Serengeti National Park and Maswa Game Reserve where infestation of *G. cordifolia* is highly and well documented (Ngondya *et al.,* 2016). While, in Mwiba Wildlife ranch *G. cordifolia* was invading the area and yet no study has shown on the level of its infestation and effects within the area. I conducted a survey inside Mwiba area along roads by a vehicle, looking on both sides of the road to observe the occurrence of *G. cordifolia*. Within January and February during the reconnaissance *G. cordifolia* were at low stage of growth and was difficult to locate their level of infestation within the area, however, higher infestation was seen in March during data collection. Invaded locations were recorded using Garmin etrex20 GPS. I recorded latitude, longitude, elevation, level of infestation in an area. 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.

Experimental sampling design and treatments

We established five blocks of 100 x 100 m^2 (Fig. 2) 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. 1). The cover of *G. cordifolia* was homogeneous across all selected blocks and across all plots that were established before we started the experiment. In each block, we randomly established three sampling plots of 10 x 10 m^2, at least 20 m apart from each other making a total of 15 plots. 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% concentration; DUL), and GLY = glyphosate.

D*esmodium uncinatum* crude leaves extract and chemical (Glyphosate) preparation and spraying.

D*esmodium uncinatum* fresh leaves were collected from Nkwaranga 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, I prepared *D. uncinatum* crude leaves extract (DUL) as described by (Ngondya *et al.,* 2016) Fig. 4a. At each of the five DUL treated plots, I applied 5 l of 100% concentration of *D. uncinatum* crude leaves extract, immediately after the heavy rainy season had ended (end of April 2020). I also prepared the chemical herbicide (Glyphosate 360 g/l, HE/0055, Monsanto Kenya Ltd.) and sprayed per manufacturer-recommended conditions for use. Five Glyphosate (GLY) treated plots each received 5 l of water containing 20% of Glyphosate solution. 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.

Figure 3: (a) and the photo above is involving the *Desmodium uncinatum* (DUL) preparation (b) Spraying process of *Desmodium uncinatum* crude leaves extract (DUL) in sampling plots within Mwiba study area.

Fig. 4. Preparation and mixing of chemical herbicide Glyphosate with water before spraying.


Fig. 5. Spraying process of Glyphosate (GLY) in sampling plots within Mwiba study area.

Insect visitors and flower sampling

Insect visitors were sampled by observation, sweep net and pan trap method as followed; In each sampling plot, I identified all insect species visiting flowers and collected specimens both before and after spraying DUL and GLY over a period of three months (March 2020 - June 2020). The aim of collecting data before treatments was to assess whether all plots had the same level of infestation of *G. cordifolia* and the same level of insect visitation, however, I analyzed only data after treatments. I conducted observations between 0800 hrs. and 1000 hrs. and 1600hrs to1800 hrs. in each plot for a maximum time span of 30 min (Constance *et al.,* 2007) (Fig. 9a). 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. 9b) and identified later to species-level following the taxonomic nomenclature under the supervision of insect expert taxonomists from the University of Dar es Salaam, under Department of Zoology and Wildlife Conservation (Ojija *et al.,* 2019b). Bees were identified by a bee taxonomist, Royal Belgian Institute of Natural Sciences. I recorded every visible insect staying for at least 5 sec. on any flower part and then classified the visitor 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 was recorded. I 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. 4c). Total of 90 yellow pan traps were evenly distributed throughout the sampling blocks per day, and within each of the sampling plot 30 traps were placed above ground on the level of vegetation at a standing pole. After 10 hours during the evening all traps placed for that day were collected, but I only sorted out bees as efficient visitors of flowers (Etanidou *et al.,* 2008). I did not leave my traps for more than 24hrs as in other studies, to evade losing specimens to bird eating (Pardo *et al.,* 2020).
I assessed the abundance of flowers within each of my sampling plot and recorded the number of flowers that were visited by particular insect visitors during the sampling periods. I identified and counted all flowers within the plots, with stigmas and anthers measured as individual flowers (Blaauw & Isaacs, 2014). Every observation consisted of 10 min. period of watching flowers, where I defined a visitation when an insect touched the flower part.

Fig. 6. A photo of a box with collected insects from Mwiba area pinned for identifications

Fig. 7. A photo of project leader pinning insects collected from Mwiba study area for identification.


Fig. 8. A box with identified insects collected from Mwiba area.

Soil sampling and analyses

Soil samples from the depth of 5 cm were collected from each sampling plot however, CON plot of each sampling block was used as our baseline point for comparison on the effect of each management on soil after treatment. Thus, 15 sampling plots from 5 sampling blocks in total 30 sampling soil were taken into account. Prior to collection of soil samples, the litter layer was removed and one soil samples, from the center of each sampling plot were collected using garden trowel. The samples were separately placed into zip-lock plastic bags and transported to the laboratory at the Sokoine University of Agriculture (SUA) for analysis. Each sample of soil taken for analysis weighed 500g. Soil samples were cautiously washed of plant debris to evade contamination by organic matter during analysis (Wietrzyk *et al.,* 2018). The samples were air-dried softly crushed and filtered through a 2-mm mesh. All laboratory analyses were done on flame photometer, UV-VIS spectrophotometer & pH meter machine and the method that used was 1:2.5 w/v Bray 1 & Olsen & Ammonium Acetate pH 7. The soil samples were then analysed for chemical properties i.e., soil pH (1:2.5) (in H_2O) means pH used water as media in a ratio of 1 to 2.5 (soil to water) 10g of the soil weighed and added 25 mls of distilled water, EC which is a short form of Electrical conductivity and its unit is MicroSiemens per Centimeter, we also analyzed Exch. Bases (Cmol/Kg) Potassium which is one of the exchangeable bases and it is presented by the unit Centimol per Kg, TN-Kjeld means Total Nitrogen was analysed using Kjeldehl Method, OC-BLKW means

Organic Carbon was analysed using method Walkley- Black, while, P Olsen means Extractable Phosphorus was analysed using Olsen Method.

Fig. 9: Insect sampling by (a) observation (b) Sweep net (c) pan traps (d) sorting of insect specimen for species identification in Mwiba study area.


Fig. 10. A photo of our research team during data collection in the field area of Mwiba wildlife ranch

Results

DUL enhanced while GLY did not affect the number of insect visitors and the number of inflorescences visited

A total number of 1,660 individual insect visitors from 30 families and 74 species were recorded visiting flowers within the surveyed plots after treatments. The seven groups of insect visitors observed in our study were bees and wasps (Hymenoptera), flies (Diptera), butterflies (Lepidoptera), beetles (Coleoptera), bugs (Hemiptera), grasshoppers, and bush-crickets (Orthoptera), and dragonfly (Odonata). More than half (55%) of the insect visitors observed were found visiting flowering plants in DUL plots, CON followed with 26%, and GLY plots with 19%. We found a significant difference in the number of insect visitors, with DUL plots having almost twice as many visitors compared to CON ($\beta = 0.453 \pm 0.177$, $p = 0.011$), while the GLY treatment did not differ from CON ($\beta = -0.307 \pm 0.291$, $p = 0.831$; Fig.11A). In total, 2,378 inflorescences were visited after treatments. Twice as many inflorescences were visited in DUL plots with 59%, compared to CON with 25%, and GLY plots with 16%. The number of inflorescences visited differed significantly between DUL and CON treatments ($\beta = 0.561 \pm 0.162$, $p < 0.001$), with DUL having over 1/3rd more inflorescences visited compared to CON and GLY while no significant difference was observed between CON and GLY ($\beta = -0.189 \pm 0.191$, $p = 0.322$; Fig. 11B).

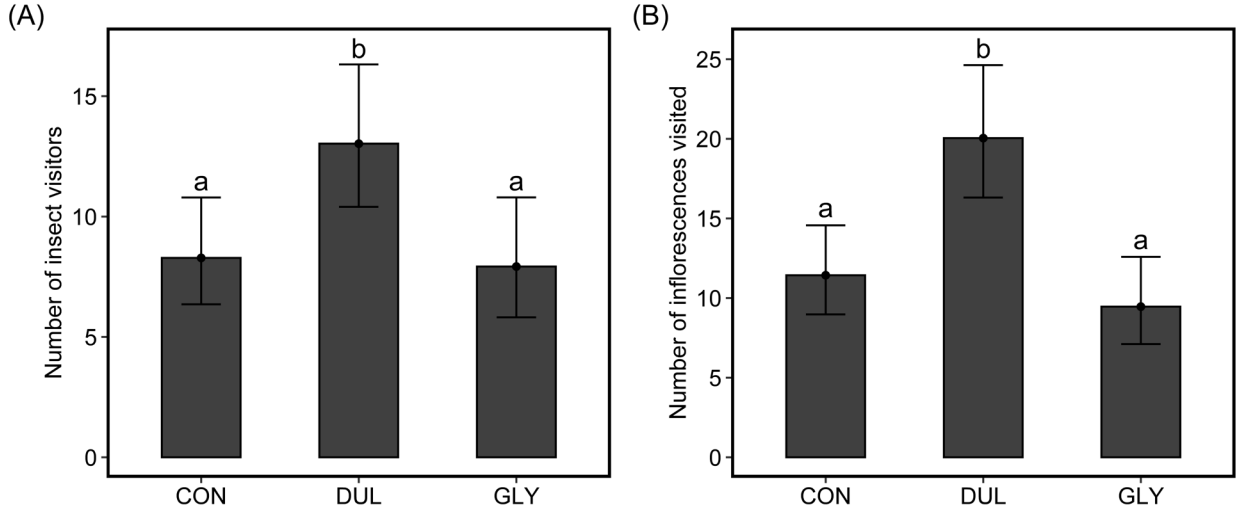

Fig. 11: (A) Mean (±95%CI) number of insect inflorescences visitors and (B) number of inflorescences visited across control (CON), *Desmodium uncinatum* crude leaves extract (DUL), and glyphosate (GLY) sampling plots in Mwiba area, Tanzania. Different letters above bars show significant differences at $p < 0.05$ based on Tukey Post-hoc Test.

DUL and GLY did not affect insect species diversity and richness

We found no significant differences between DUL and CON ($\beta = 0.279 \pm 0.270$, $p = 0.303$), however, DUL was slightly higher in insect species richness than CON plots. There was also no significant difference between GLY and CON ($\beta = -0.307 \pm 0.290$, $p = 0.291$; Fig.12A), but GLY treated plots had slightly lower insect species richness compared to CON. We found that treatments (CON, DUL, and GLY) had no significant effect on insect species diversity ($F_{2,12} = 21.595$, $p < 0.076$), however, insect species diversity was slightly higher in DUL than in CON plots ($\beta = 0.550 \pm 0.243$, $p = 0.043$). While, on the other side there was no significant difference between GLY and CON ($\beta = 0.034 \pm 0.243$, $p = 0.891$; Fig.12B).

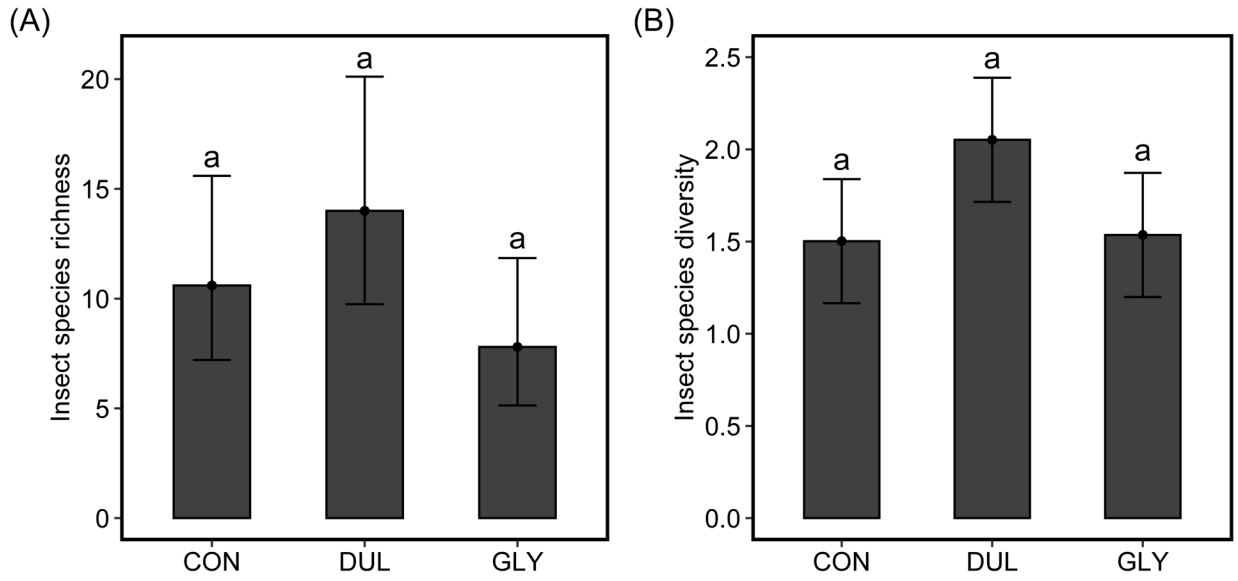

Fig. 12: (A) Mean (±95%CI) of insect species richness (B) insect species diversity across control (CON), *Desmodium uncinatum* crude leaves extract (DUL), and glyphosate (GLY) sampling plots in Mwiba area. The same letters above bars on insect species diversity and richness graphs showed no significant differences.

DUL enhanced while GLY did not affect flower diversity and inflorescences abundance

In total, we found 2,957 inflorescences after treatments, with the most abundant flowering plants that received insect visitation being *Vernonia galamensis* (Asteraceae), *Justicia betonica* (Acanthaceae), *Cyathula orthacantha* (Amaranthaceae) as well as *Hibiscus cannabinus* (Malvaceae). The DUL plots had more than half of the inflorescence's abundance (55%), followed by 25% in CON, and 20% in GLY plots. The number of inflorescences was about twice as high in DUL compared to CON (β = 0.770 ± 0.271, p = 0.005) but there was no difference between GLY and CON (β = -0.265 ± 0.273, p = 0.333; Fig. 13A). Treatments had a significant effect on flower diversity ($F_{2,12}$ = 3.963, p = 0.048), with DUL having slightly higher flower diversity than CON (β = 0.560 ± 0.226, p = 0.029) while no significant difference was observed between GLY and CON (β = 0.020 ± 0.226, p = 0.931; Fig. 13B).

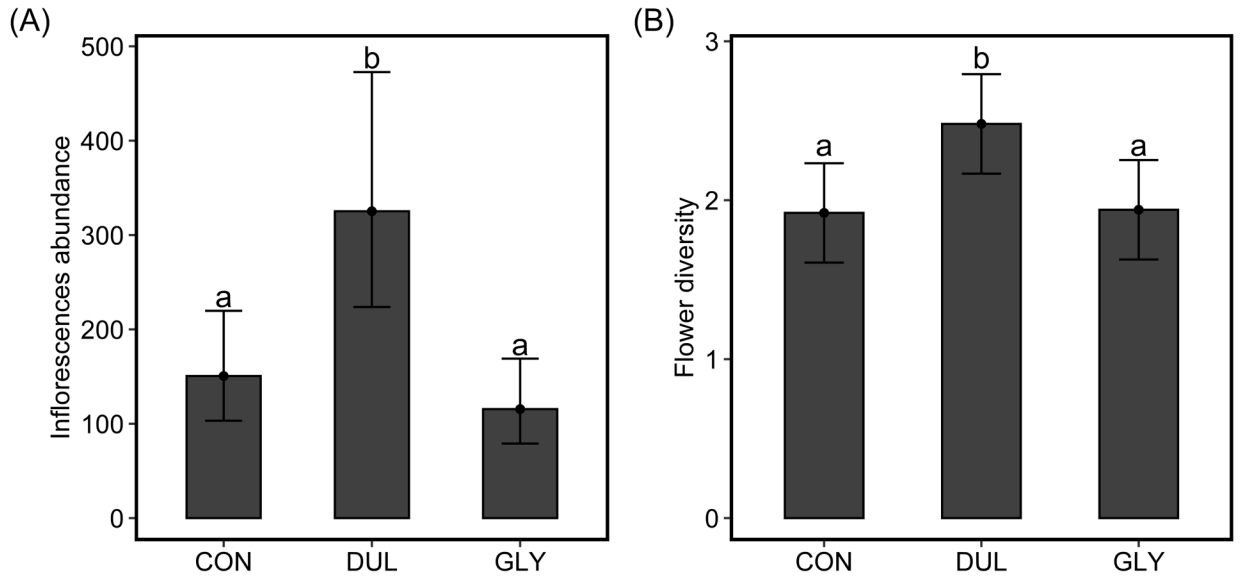

Fig. 13: (A) Mean (±95%CI) of inflorescences abundance, (B) flower diversity across control (CON), *Desmodium uncinatum* crude leaves extract (DUL), and glyphosate (GLY) sampling plots after treatments in Mwiba area. Different letters above bars on the flower abundance graph showed significant differences across treatments $p < 0.05$ based on Tukey Post-hoc Test.

DUL and GLY did not significantly affected soil chemical properties

We found no significant differences in all of our soil chemical properties across CON, DUL and GLY before and after treatments ($p > 0.05$). However, mean of the soil pH was slightly higher in DUL plots followed by CON and GLY. While Means of the EC, were slightly higher in GLY followed with DUL and CON. Means of TN-Kjeld were slightly higher in DUL and GLY and lower in CON. Means of OC-BlkW were slightly higher in DUL followed by GLY and lower in CON. Means of Ext.P. and Exchng. Bases were the same across CON DUL and GLY.

Conclusion

In this study, we highlighted that *Desmodium uncinatum* leaves extract (DUL) is an effective management option against the invasive plant *G. cordifolia* as it did not negatively affect insect flower visitation. While our study focused on insect flower visitors only, we recommend that more studies should be done on the biosafety of this bio-herbicide on other insects' groups, birds, animals, human health, and on the environment in general. The natural herbicide still seems to be not only good for environmental health but also does not impact wild animals. Our results suggest that DUL is a potential alternative to chemical herbicides for controlling invasive plants in African savanna ecosystems, and particularly inside protected areas, where chemical herbicides are not recommended. We further show that no management (CON) resulted in flower visitors and inflorescences abundances as low as that of chemical treatment (GLY), highlighting the urgency of developing environmental-friendly management technologies against invasive plant species in the Mwiba area.

Acknowledgement

I wish to express my genuine appreciation to my supervisors, Prof Anna C. Treydte, Dr. Issakwisa Ngondya and Mark Ghaui 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 the progress report 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 thank the Rufford Foundation for financial supports, TAWIRI and COSTECH for issuing research permits, Mwiba Holdings Limited for hosting me during data collection. I am grateful to my field assistants and Mwiba staff for their help and kindness. Additional advice was given by T. Minnick on the study design. I acknowledge A. Pauly for insect taxa identification. I also appreciate the assistance of M. Masolele in data analysis.