EFFECTS OF INSECT POLLINATION ON OKRA FRUIT SET AND YIELD ALONG SELECTED HABITAT GRADIENTS AT KILOMBERO DISTRICT, TANZANIA

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EXTENDED ABSTRACT

Pollination is the most important ecosystem services for biodiversity of plants on earth. Insect pollinators are playing an indispensable role in the pollination of agricultural crops such as okra (*A. esculentus*). The main objectives of the study were; to determine the effects of insect pollination on okra (*A. esculentus*) fruit set rate and yield at Kilombero district, Tanzania. The split plot design was used, where okra plots were arranged in a randomized complete block design (RCBD) with two treatments and three replications from the edges of natural forest and sugarcane plantation. GenStat Discovery Edition 4 and Microsoft Excel 2021 computer software programs were used in the data analysis. Results indicated that the dominant okra insect pollinators were *Macrogalea candida*, *Braunsapis bouyssouri*, *Borbo borbonica* and *Apis mellifera*. The insect pollinator species abundance, richness and diversity were higher at the edge of natural forest than sugarcane plantation. The okra plants from the edge of natural forest had significantly higher mean numbers of pods per plant and seed weight per 100 seeds than those from sugarcane plantation. Insect pollination had high significant effects on Okra yield ($P < 0.05$). In addition, there was high significant effects of insect pollination on okra fruit set rate ($P < 0.05$). The research has concluded that insect pollinators have substantial effects on fruit set rate and yield of food crops such as okra (*A. esculentus*). In addition, natural habitats are important. Therefore, conservation of insects' friendly habitats such as natural forests is highly recommended to avoid their decline and consequently declining of pollination services.

DECLARATION

I, **MAGWIRA, JOSEPH AWAMI,** do hereby declare to the senate of Sokoine University of Agriculture that this Dissertation is my own original work done within the period of registration and that it has neither been submitted before nor being concurrently submitted to any other institution.

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DEDICATION

This work is dedicated to my wife, Roda Mwijage and son, Moses Magwira who patiently waited for me during the period I was undertaking this research, for their love, support and encouragement during two years of my study.

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DISSERTATION STRUCTURE

This dissertation consists of four chapters. Chapter one describes background information on insect pollination in okra (*Abelmoschus esculentus*), natural and semi natural habitats influence on insect pollinators, justification and objectives. Chapter two (manuscript one) describes effect of insect pollination on okra (*A. esculentus*) yield along selected habitat gradients at Kilombero district, Morogoro. Chapter three (manuscript two) explains the effect of insect pollination on okra (*A. esculentus*) fruit set rate along selected habitat gradients at Kilombero district, Morogoro. Chapter four contains general conclusions and recommendations from the findings.

ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of Variance
CABI	Centre for Agriculture and Biosciences International
Cm	Centimeters
Df	Degree of freedom
FAO	Food and Agriculture Organization of the United Nations
h	Hours
Ha	Hectare
IPM	Integrated Pest Management
m	Meters
MNRT	Ministry of Natural Resources and Tourism
MRSEP	Morogoro Region Socio Economic Profile
MSc. For	Master of Science in Forestry
NBS	National Bureau of Statistics
NF	Natural forest
NPK	Nitrogen. Phosphorous. Potassium
sp	Unspecified species
SP	Sugarcane plantation
SUA	Sokoine University of Agriculture
TZS	Tanzanian Shillings
URT	United Republic of Tanzania

CHAPTER ONE

1.0 INTRODUCTION

1.1 An overview of flowering plants and insect pollination

Pollination is among the most beneficial ecosystem services for the sustainability of cultivated crops and communities of wild plants on earth. Pollination enables the reproduction of plants, provides fruits, seeds and the leaves that consumed by human throughout the globe (Hein, 2009; Eeraerts *et al.*, 2020). At least 80 percent of overall pollination activity is achieved by insects (Sajid *et al.*, 2020), these includes hymenoptera (e.g. Bees), lepidoptera (e.g. Butterflies and Moths), diptera (Flies) and coleoptera (Beetles). Their ecological task is to ensure fertilization of the ovules in the flower by the male gametes from the pollen grains is successful (Aastha, 2020). The pollination success of flowering plant species depends on the activity of insects that visit and successfully pollinate the flowers (Bashir, 2018). Every insect pollinator has its own pollination efficiency for flowers, based on behavioral features, such as foraging speed, flower constancy, pollen load on the body, pollen collection, and pollen transfer to the stigma (Said *et al.*, 2017; Shaheen *et al.*, 2017; Khan *et al.*, 2012). Majority of these insects especially honeybees and wild bees frequently forage on food crops (Delaplane and Mayer, 2000; Klein *et al.*, 2007), and by doing so, they contribute significantly to the pollination of such crops as coffee Coffea spp. (Klein *et al.*, 2007), tomato *Solanum lycopersicum* (Greenleaf and Kremen, 2006), watermelon (Sawe *et al.*, 2020).

Watermelon (*Citrullus lanatus*) is perhaps among well documented pollinated crop for its dependence on insect pollinators for fruit and seed set due to its monoecious flowering condition of separate staminate (male) and pistillate (female) flowers (Free, 1993; Adlerz, 1996).

Tomato (*Solanum lycopersicon Mill*.) is among the most important vegetable crops produced in Tanzania (Msogoya and Mamiro, 2016). It is among flowering crops that require insect pollination for its substantial yield. For example, Dos Santos *et al.* (2009) found out that insect pollinated tomato plants yielded largest number of fruits with higher weights than self-pollinated plants. Due to this limitation, in the presence of other floral resources, honeybees do not readily visit tomato flowers instead principal visitors to tomato flowers are non-apis bees (Higo *et al.,* 2004).

Nearly all apple (*Malus domestica Borkh*) varieties need to be cross-pollinated with pollen from the flowers of a different apple variety to produce fruit and their main insect pollinators are; Honeybees, mason bees and bumblebees, without an appropriate pollinator partner tree (and bees) a lonely apple tree will produce little to no fruit at all (Watts, 2019).

Pumpkins are found in the family of Cucurbitaceae. They are known to have separate male and female flowers, consequently they require pollination services, particularly from bees in order to have successful fruit set. Therefore, high rate of bee species visits on the female flower of pumpkins is very important,

1.2 Effects of insect pollination on okra

Okra (*Abelmoschus esculentus* (L.) Moench) is found in the Malvaceae or mallow family with its close relative, Cotton (*Gossypium hirsutum* L.), however Okra is known to have much rougher, broader leaves and thicker stem which tends to exhibit growth height of 0.9 to 1.8 m at maturity. Its seed germination occurs between three to six days after being sown, usually the okra seed pods tend to become fibrous and woody, hence in order to get edible pods, must be harvested when immature and eaten as a vegetable or as ingredient to other dishes. It is known as bhindi in India (Tripathi and Ranjin, 2015) and bamia in Tanzania.

In Tanzania, its cultivation is largely by smallholder farmers both for food and for selling to local rural and urban populations, but also it gained attention in urban and peri-urban areas partly because of the introduction of new varieties such as ‘Clemson’s Spineless’ by foreign seed companies (Ndunguru and Rajabu, 2004). Immature okra pods can either be boiled, sliced, fried or added into soups with tasty unique flavor; the ripe okra seeds are roasted, ground and used as a substitute for coffee in some countries (Moekchantuk and Kumar, 2004); its mature fruits and stems containing crude fibre are used in the paper industry (Singh *et al.*, 2014).

Okra *(Abelmoschus esculentus)* is an entomophilous flowering plant. The reproductive system of okra consists of a combination of cross-and self-pollination (Hasnat, 2015). This is because it has large coloured petals and purple coloured base bisexual flowers, additionally, its flowers exhibit characteristics such as extrorse dehiscent anthers, nectar, ornamented sticky pollen grains and colourful stigmata, that okra insect pollinators find very attractive. In okra, as the conditions for self-pollination become favourable, there is also an opportunity for cross-pollination primarily through insects (George, 1989; Al Ghzawi *et al.,* 2003).

Moses (2005) reported that, pollination of okra flowers done by hand and insect received seed setting around 73-84% per fruit, which was higher compared to the 57% seed settings per fruit acquired from the spontaneous self-pollination using bagged flowers. Also, Al-Ghzawi (2003) found that the number of seeds per plant, number of seeds per fruit, seed weight per plant, and fruit weight of okra was greater in plants pollinated by various insects than self-pollinated okra plants. Moreover, Hasnat (2015) reported that insect pollinated okra plants produced a greater number of young fruits and mature fruits due to higher transformation of flowers into tender fruits.

1.3 Influence of natural and semi-natural habitats on insect pollinators

According to Millennium Ecosystem Assessment (2005), insect pollination is an economic benefit that nature provides to people. Sustaining insect pollination services, it requires the conservation and management of natural and semi-natural habitats resources for wild pollinators and managed within agricultural landscapes. A good number of recent studies have examined whether crop pollination services decline with increasing isolation from natural habitats (Ricketts *et al.*, 2008) or clearing of semi-natural habitats. For instance, Ricketts and other researchers (Ricketts 2004; Ricketts *et al.*, 2004) found that bee diversity, visitation rate, pollen deposition rate and fruit set are all significantly greater in crop fields near tropical forest than in fields further away. The natural and semi-natural habitats are ecologically very important since they provide resources such as, suitable nesting habitats for insect pollinators (e.g. suitable soil substrates, tree cavities) but also sufficient floral resources such as pollen and nectar (Kremen *et al.*, 2007) which are vital food sources for insect pollinators.

Moreover, bee species especially honey bees are commonly known as central-place flower foragers, meaning that, they tend to return back to their fixed nest sites after they have foraged, due to this, Ricketts *et al.* (2008) reported that proximity of nesting habitats relative to agricultural fields is critical for bee-pollinated crops, including okra. Therefore, it is of great importance to engage in conservation of natural and semi-natural habitats, in return they will enhance insect pollinator diversity and consequently increase pollination success due to functional and temporal complementarity between species (Blüthgen and Klein, 2011; Albrecht *et al.*, 2012). Furthermore, maintaining landscape heterogeneity and improving the quality of semi-natural habitats will increase resource diversity and continuity (Cole *et al.,* 2017).

1.4 Justification

Natural and Semi-natural habitats are integral to most agricultural areas and have the potential to support ecosystem services (Holland, 2017), particularly insect pollination in crop plants like okra (*A. esculentus*) which depend on it for its substantial reproductive performances, therefore, any reduction or rather loss of these habitats will consequently deprive us of these harmless insect pollinators. Furthermore, there have been mounting concerns about possible declines in insect pollinator abundance and diversity caused by anthropogenic disturbances, such as habitat modification and fragmentation (Gemmill-Herren, 2008) and agricultural intensification. Such disturbances, will endanger biodiversity directly, but they may also threaten the productivity, diversity and stability of food production systems by disrupting pollinator communities (Ricketts *et al.,* 2008), particularly of bees since they are principal pollinators of crop plants.

The domesticated honeybees, *Apis mellifera* L. has been utilized to provide managed pollination systems, but for many crops, honeybees are either not effective or are suboptimal pollinators (Westerkamp, 1991). For instance, in Tanzania, as in many other developing countries pollination services is not managed. In such cases, pollination services provided by wild insects, particularly bees may be of key importance (Klein *et al.,* 2007; Kremen *et al.,* 2007) due to the fact that, insect pollinators utilise different habitats to meet their resource requirements throughout the season (Mandelik *et al.*, 2012; Williams *et al.,* 2012). For instance, reports from (Potts *et al.,* 2003; Somme *et al.*, 2015; Richardson *et al.,* 2016) have indicated that, flower species differ in the rewards they offer and pollinator visitation is influenced by the quantity of nectar, pollen and its chemical composition.

In most of the Sub-Saharan African countries, the main approaches to increase crop yields have been to improve soil conditions (fertility and moisture) and to increase the amount of farmland (Perrings and Halkos**,** 2015). Most farmers consider insect pollination as one of nature's many "free services", so taken for granted, rarely considered as an "agricultural input" (Allen *et al*.**,** 1998)**.** Considering insect pollination as a free service, causes ongoing challenges on insect pollinators conservation while maintaining a high and growing food demands to sustain the rapidly growing human population (ca 2.7% per year; OECD, 2016).

Therefore, an alternative way of seeking evidence for pollinator declines is to measure the results of their absence as reductions in fruit or seed production in natural or agricultural ecosystems (Cane and Tepedino, 2001). Hence, the study is designed to document and add to the body of knowledge the importance of ecosystem services, particularly insect pollination using okra as a study crop and give an insight into the okra insect pollinators' biodiversity along selected habitats (natural forest and sugarcane plantation). The findings from this study provide valuable information to the farmers, researchers and decision makers to form a basis for management and conservation policies; and it adds inputs to the pollination biology, especially relationship between okra (*A. esculentus)* and its insect pollinators**.**

1.5 Objectives

1.5.1 Overall objective

To determine the effects of insect pollination on okra fruit set rate and yield along natural forest and sugarcane plantation habitat gradients at Kilombero District.

1.5.2 Specific objectives

i. To determine the effects of insect pollination on okra yield along natural forest and sugarcane plantation habitat gradients.
ii. To evaluate the effects of insect pollination on okra fruit set rate along natural forest and sugarcane plantation habitat gradients.

1.6 Hypotheses

H_{01}: There are no effects of insect pollination on okra yield along natural forest and sugarcane plantation habitat gradients.

H_{02}: There are no effects of insect pollination on okra fruit set rate along natural forest and sugarcane plantation habitat gradients.

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CHAPTER TWO

Manuscript One

2.0 Effects of insect pollination on okra yield along natural forest and sugarcane plantation habitat gradients at Kilombero District, Tanzania

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Abstract

Experiments were done to determine the effects of insect pollination on okra (*Abelmoschus esculentus)* yield along selected habitat gradients. The field experiments were conducted for about four (4) months, starting January 2021 up to early April 2021. Pollination treatments used were control (caging) and open pollination. The number of pods per plant, number of seeds per pod, seed weight per 100 seeds and insect pollinators' abundance and richness were recorded. The okra plants from the edge of natural forest had significantly higher mean numbers of pods per plant and seed weight per 100 seeds than those from sugarcane plantation. Insect pollination had high significant effects on Okra yield. The results revealed that thirty-seven different insect species visited *A. esculentus* flowers. *Macrogalea candida* was the dominant species with (101) individuals, followed by *Braunsapis bouyssouri* (52) and *Borbo borbonica* (22). The insect pollinators species abundance and richness was higher at the edge of natural forest than sugarcane plantation. In order to ensure the sustainability of insect pollination services to our food crops and wild plants, conservation of natural and semi-natural habitats is highly recommended.

Keywords: insect pollinators; okra; pollination; yield; natural forest habitat; sugarcane plantation habitat

2.1 Introduction

Okra (*Abelmoschus esculentus (L.) Moench*) is an important fruit vegetable crop widely cultivated in tropical and sub-tropical regions of the globe**.** It is among family members of Malvaceae family, that includes Hibiscus and Cotton**.** Aladele *et al.* (2008) explained that, Okra was previously included in the genus Hibiscus but later on it was designated to Abelmoschus**.** It has a lot of health benefits such as, it has no cholesterol, helps in ease constipation and due to its high iodine content, it helps to control goitre (Nandhini *et al.* 2018)**.** Hence, it is often recommended by nutritionists in cholesterol controlling, weight reduction programs and to develop immunity (Gemede *et al.*, 2015).

Okra has perfect flowers (both male and female reproductive parts in the same flower) and it is self **–** pollinating crop (Nandhini *et al.*, 2018)**,** it is among monoecious flowering plants. Additionally, okra requires a critical day length of 12.5 hr for better initiation of flowers and ultimately higher fruit yield (Oyolu, 1997). As the okra plant develops, the lowest flower bud on each stem opens soon after sunrise and closes in the middle to late afternoon (Lamont, 1999).

Since okra has attractive golden yellow flowers and presence of nectaries, it is freely visited by honey bees and other insects belonging to different orders during flowering period (Nandhini *et al.*, 2018)**.** Insect pollinators are playing an indispensable role in the pollination of food crops (Akhtar *et al.,* 2018). More than 80 percent of overall pollination activity is achieved by insects (Sajid *et al.,* 2020). The insect pollinator visits not only move outcross pollen among individuals but also increase the total amount of pollen deposited on flower stigmas (Ricketts *et al.,* 2008), consequently will lead to increase of okra crop quantity particularly in terms of yield**.** For instance**,** Moses (2005) reported that pollination of okra flowers done by hand and insect received seed setting around 73-84%

per fruit, which was higher compared to the 57% seed setting per fruit acquired from the spontaneous self - pollination using bagged flowers. In addition, Azo'o *et al.* (2011) found that the mean number of matured seeds is higher in open-pollinated plants and smaller in self-pollinated plants. Moreover, Al Ghzawi *et al.* (2003) found that the weight of seeds per plant, and fruit weight of okra was greater in plants pollinated by various insects than self-pollinated plants. Therefore, loss of insects is certain to have adverse effects on ecosystem functioning (Hallmann *et al.,* 2017) and services particularly pollination of flowering plants such as okra (*Abelmoschus esculentus*). Current data suggest an overall pattern of decline in insect diversity and abundance (Hallmann *et al.*, 2017)**.**

Expansion of agricultural lands, loss of (semi-) natural habitat and associated decline of floral resources have been pointed out as primary factors driving global declines in wild insect pollinators (Baude *et al.,* 2016**;** Vanbergen and Initiative, 2013)**.** Tanzania economy is dependent on agricultural sector, but many farmers still ignore the role of harmless flower visiting insects on pollination of growing plants (Azo'o *et al.*, 2011). Majority of the farmers believes that, adoption of different farming practices such as, fire-prone, crop rotations, organic manures application, and pest control (Tchuenguem *et al.*, 2009) are the only viable options for improved crop yields.

The findings of this research**;** add knowledge to science on pollination biology of *Abelmoschus esculentus***,** especially the relationship between *A. esculentus* and its floral entomofauna**;** act as a basis for further research on okra pollination in order to complete the useful available data**;** and it can be used to add useful inputs in conservation policies by conservationists. Therefore, the main objective of this study was to explore the effects of insect pollination on okra yield, particularly number of pods (fruits) per plant, number of seeds per pods and seed weight per 100 seed along two different habitats with two distances from the edges of each habitat.

2.2 Materials and Methods

2.2.1 Description of study sites

A study was conducted in Morogoro region, Kilombero district at Mang’ula A and Msalise villages located at coordinates 7° 49' S, 36° 53' E and 7° 51' S, 36° 57' E respectively (Figure 2.1). Kilombero district is found in Morogoro region, south-western of Tanzania. It is located in immense floodplain, between The Udzungwa Mountains in north-west and the Kilombero river in the south east. The district lies between latitude 8° 13' S to the south of the Equator and longitude 36° 68' E East of the Greenwich, with population of 398,379 (MRSEP, 2006; NBS Census, 2012). The dominant vegetation covers for Kilombero district are miombo woodland, farmland and floodplain grassland. The district receives an annual average rainfall of 900 mm and mean annual temperature ranges between 18℃ on the mountains to 30℃ in river valleys (MRSEP, 2006). The hot months of the region are July, August and September with mean temperature of 29.5℃. The experimental plots established adjacent to; Udzungwa Mountain Forests with a very good conservation status and sugarcane plantation with fairly matured canes.

The meteorological data were collected from two stations in Mang'ula, each station located at respective study site (Mang'ula A and Msalise). The field experiment was conducted from January to early April, 2021, as wetter season in Kilombero district lasts 5 months, from November to April. Tables 2.1 below, shows meteorological data obtained from Mang'ula A and Msalise. The overall climatic conditions during okra cultivation were relative good for its optimum yield performances.

Table 2.1: Study sites meteorological description in Kilombero district

Village	Month	Temperature (^0C)		Relative Humidity (%)		Dew Point	
		Max	Min	Max	Min	Max	Min
Mang'ula A	January	34.6	20.8	100	44.7	25.9	20.2
	February	34.3	21.1	100	46	26.5	20.1
	March	33.6	20.7	100	42.8	25.8	18.2
Msalise	January	32.5	21.4	100	49.9	26.6	19.5
	February	32.3	21.2	100	50.7	27.1	20
	March	35.9	21.2	100	43.9	27.2	17.4

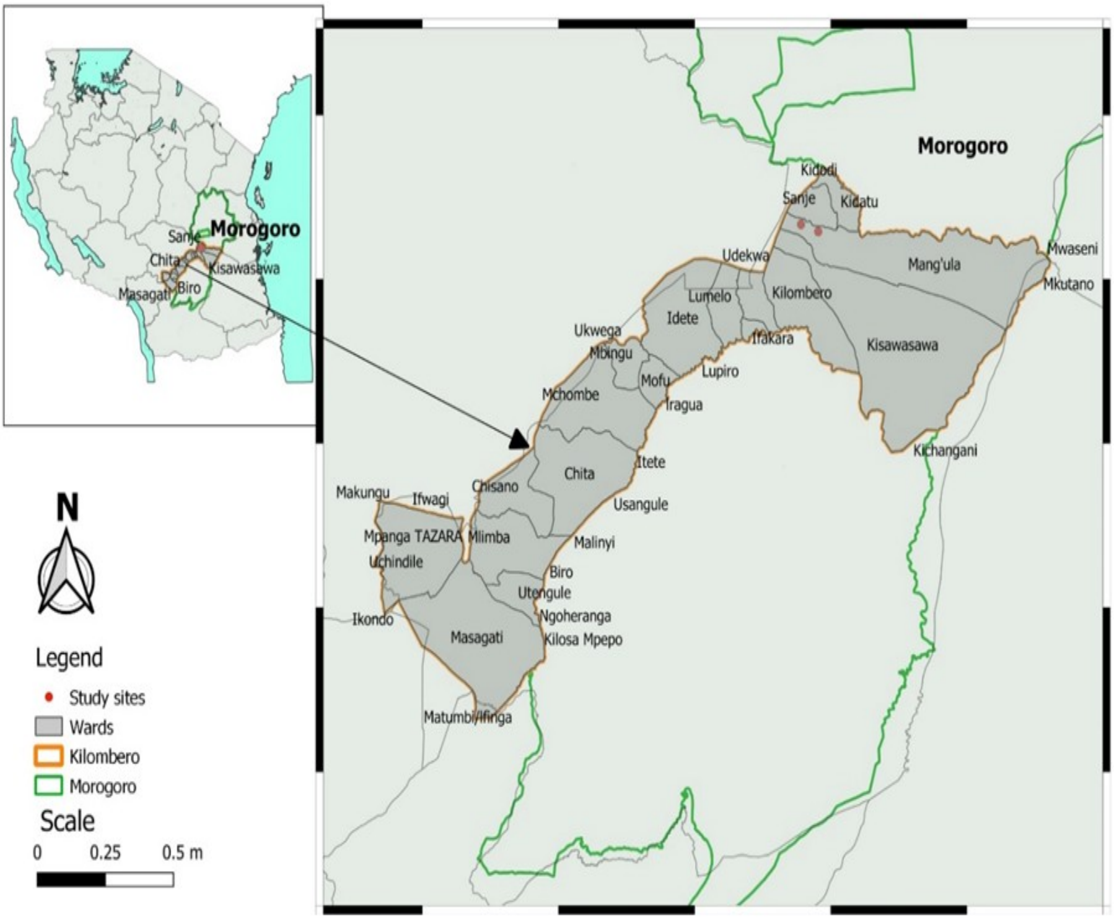

Figure 2.1: Kilombero district map showing location of study sites

2.2 Methodology

2.2.1 Experimental design

The split plot design was used, where experimental plots were arranged in randomized complete block design (RCBD) with two treatments and three replications according to Angbanyere (2012). The experimental plots were established along two different habitats, which were natural forest and sugarcane plantation. The experimental blocks were established at two different sites, site A and site B, with 150m gap between sites, adjacent to each selected habitat so as reduces experimental errors**.** From the edge of each selected habitat**,** there were two experimental blocks, established at distances of 10m and 60m**,** along the same gradients**.** The two treatments used in this study were**;** Control (net covered okra plants with no insect pollinators' accessibility) and Open pollination (okra plants accessible to naturally occurring insect pollinators). The plot size (experimental unit) was 4.26 m^2**,** with each experimental block containing six (6) plots. The distances between plot to plot in each replication, and one replication to another replication within a block, were 1m and 1.5 m respectively**.** Each plot (experimental unit) had 20 okra plants**,** with plant spacing of 71cm x 30cm**.** The okra plants in each plot were arranged in four rows and each row contained five okra plants**.**

2.2.2 Field work

Prior to seed sowing, land preparation involving weed removal, soil digging and shallowing of trenches (harrowing) on areas(blocks) where experimental plots were established was done, using a hand hoe. On 17 January 2021, two Okra seeds of "Clemson spineless" variety were sown in holes of 10 cm of depth. These holes were separated by 30 cm within and 71 cm apart on each row. Two weeks after sowing (31 January 2021), Okra plantlets were thinned to one plant per hole**.** These same days, the first weeding was done when okra plants were approximately 9.5cm in height, followed by pesticide application using Bicron- Profenofos 500 EC and Ivory $M^{TM}{}_{72}$ WP (Fungicide) at the rate of 30 mils and 50 grams per 15 litres of water respectively, to control insect pest such flea beetles (*podagrica spp*.) and blights on okra leaves. Four weeks after sowing (14 February 2021), second weeding and second pesticide application were done. Also on the same days, chemical fertilizer (NPK 17-17-17) was applied around each conserved plant of each hole, at the rate of 233 kghectare^{-1} (Angbanyere, 2012). Six weeks after sowing, pollination n treatment application was applied, where random caging of 24 out of 48 plots was done.

Plate 2.1: Pictures above show; (A) prepared block before setting of the experiment and (B) randomly caged and uncaged of okra experimental plots in a block

2.3 Data collection

To determine the yield data on number of pods per plant, number of seeds per pod and seed weight per 100 seeds and abundance, relative abundance and species richness of insect pollinators visited okra flowers were collected as follow:

The number of pods per plant was determined at the end of harvesting, whereby fruits (pods) grown from treatment group (open pollination) and a control were harvested individually tagged (Azo'o *et al.,* 2011). Harvesting was done four times at interval of six days in order to have an aggregate number of pods for each plot. Mature fruits from five randomly selected and labelled plants of the two middle rows with minimum edge effects in each plot were counted at each harvest (Angbanyere, 2012), starting from eighth week after sowing. Mean number of harvested pods per plant was calculated at the end of harvesting period, by dividing total number of pods harvested over number of plants used in a plot.

The numbers of seeds per pod were determined at the end of harvesting period. Eight dry pods were selected from each plot and the pods were dissected using a knife to extracted seeds from their placenta. The okra seeds from eight (8) dry pods were then counted in the laboratory and their total divided by the number of dry pods used (8) in order to compute mean number of seeds per pod.

The seed weight per 100 seeds were determined as follow; at the end of harvesting period, 8 dry pods were selected from each plot and the pods were dissected using a knife and seeds were extracted from the placenta of the pods. Extracted seeds were then washed with water to remove other pods particles in order to compute an accurate seeds weight. Washed and cleaned okra seeds were spread on butter paper and left for 72 h to dry (Bashir, 2017). Then, 100 seeds from each plot were weighed using an electronic balance, to maintain accuracy, we weighed 100 seeds from 3 replicates for each treatment (caged and open pollination).

Observation of okra insect pollinators was done biweekly (Campos *et al.,* 2000) on open pollinated okra flowers (open pollination treatment), starting from the seventh week up twelfth week after sowing. Direct observations on open pollinated flowers focused on natural population of effective okra insect pollinators during the blooming period at four intervals; 0800 hrs – 0840 hrs, 0900 hrs -0940 hrs, 1000 hrs- 1040 hrs and 1100 hrs-1140 hrs was done. By using electronic stopwatch, we made 40 minutes transect walk along open pollinated plants within a block and captured insects that made effective visits on okra flowers using suction device (an Aspirator) (Azo'o *et al.,* 2011) and plastic tubes. The collected insects were preserved by immersion each in a tube containing 70% of ethanol except for butterflies as they were preserved in special piece of paper to avoid discolouration of their wings. The sampled insect pollinators were then taken to laboratory for identification. Once in the Laboratory, insect specimens were identified with the aid of insect guide books by Kielland (1990), Picker *et al*. (2004), Eardley (2004) and Eardley *et al*. (2010).

2.4 Data analysis

To account for okra yield, the data on pods per plant and seeds per pod were summarized into mean value per treatment. The seed weight per 100 seeds were also summarized into mean values per treatment. GenStat Discovery Edition 4 and Microsoft Excel 2021 computer software programs were used in comparison of means among treatments. One-way analysis of variance (ANOVA) at 5% level of significance was used to statistically test the effect of treatment group on okra yield. For pairwise differences between factor level means, Tukey's 95% confidence interval was used. Insect pollinators were identified into genus/species level and their abundance was given as total number of individuals per species. insect pollinators relative abundance was given as proportions of individuals per species multiplied by one hundred. Insect pollinator species richness was given as number of different species identified.

2.5 Results

2.5.1 Effects of insect pollination on okra yield along selected habitat gradients

The findings show that, the okra plants from the edge of natural forest yielded significantly higher mean numbers of pods per plant and seed weight per 100 seeds than those from sugarcane plantation, while the mean numbers of pods per plant, seeds per pod and seed weight per 100 seeds were significantly higher on open pollinated okra plants than caged okra plants. Moreover, the okra plants at site A, ten meters (10m) and sixty meters (60m) from the edge of selected habitats yielded significantly higher mean numbers of seeds per pod and seed weight per 100 seeds than those at site B, ten meters (10m) and sixty meters (60m) from the edge of selected habitats. Summary of means for yield variables are summarized in Table 2.2**.**

Table 2.1: A summary of mean numbers of pods per plant, seeds per pod and seed weight per 100 seeds for all levels of four main effects at Kilombero District

Treatment effect	Mean number of Pods/Plant	Mean number of seeds/Pod	Seed weight /100 seeds
Natural forest (NF)	8.092 ± 0.09b	67.016 ± 1.24a	5.850 ± 0.08a
Sugarcane plantation (SP)	7.625 ± 0.26a	65.094 ± 0.97a	6.250 ± 0.05b
Site A	7.725 ± 0.15a	67.13 ± 0.39b	6.296 ± 0.06b
Site B	7.992 ± 0.26a	64.979 ± 1.59a	5.804 ± 0.03a
Ten meters (10m)	7.683 ± 0.08a	69.781 ± 0.72b	5.913± 0.04a
Sixty meters (60m)	8.033 ± 0.40a	62.328 ± 1.09a	6.188± 0.07b
Open pollination (OP)	8.45 ± 0.35b	74.630 ± 0.61b	7.508± 0.07b
Control (caged)	7.267 ± 0.05a	57.479 ± 1.85a	4.592 ± 0.08a

Within columns, means with different letters are significantly different (P < 0.05)

The results showed significant difference (P < 0.05) when comparing okra plants from the edges of natural forest and sugarcane plantation in terms of the mean numbers of pods per plant, while there was also significant difference (P < 0.05) in mean numbers of pods per plant between open pollinated okra plants and caged plants (Table 2.3).

Table 2.2: ANOVA of main effects for number of okra (*A. esculentus*) pods per plant

Treatment effect	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Pollination	1	16.8033	16.8033	26.18	<0.001
Habitat	1	2.6133	2.6133	4.07	0.053
Site	1	0.8533	0.8533	1.33	0.258
Distances	1	1.47	1.47	2.29	0.141
Residuals	30	19.2517	0.6417		

The findings revealed significant difference (P < 0.05) in mean numbers of seeds per pod between open pollinated okra plants and caged plants. When comparing the okra plants at site A, ten meters (10m) and sixty meters (60m) from the edge of selected habitats and at site B, ten meters (10m) and sixty (60m) from the edge of selected habitats, the results showed significant difference (P < 0.05) in mean number of seeds per pod (Table 2.4).

Table 2.3: ANOVA table of main effects for number of okra (*A. esculentus*) seeds per pod

Treatment effect	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Pollination	1	3529.9	3529.9	315.85	<0.001
Habitat	1	44.32	44.32	3.97	0.056
Site	1	55.52	55.52	4.97	0.033
Distances	1	666.59	666.59	59.64	<0.001
Residuals	30	335.28	11.18		

There was significant difference (P < 0.05) between okra plants from the edges of natural forest and sugarcane plantation in the mean numbers of seed weight per 100 seeds, while there was also significant difference (P < 0.05) in mean numbers of seeds weight per 100 seeds between open pollinated okra plants and caged plants. When comparing the mean numbers of seed weight per 100 seeds of okra plants at site A, ten meters (10m) and sixty meters (60m) from the edge of selected habitats and at site B, ten meters (10m) and sixty

(60m) from the edge of selected habitats, the results showed significant difference (P < 0.05) as depicted in Table 2.5.

Table 2.4: ANOVA table of main effects for seed weight per 100 seeds.

Treatment effect	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Pollination	1	102.08333	102.08333	1233.43	<.001
Habitat	1	1.92	1.92	23.2	<.001
Site	1	2.90083	2.90083	35.05	<.001
Distances	1	0.9075	0.9075	10.96	0.002
Residuals	30	2.48292	0.08276		

2.5.2 Okra (*A. esculentus*) insect pollinators' abundance and species richness

Two hundred and sixty six (266) effective okra insect pollinators were captured from open pollinated okra plants along natural forest and sugarcane plantation**.** Abundance and species richness of okra dominant insect pollinators were higher on okra plants along natural forest than along sugarcane plantation (Figure 2.2).

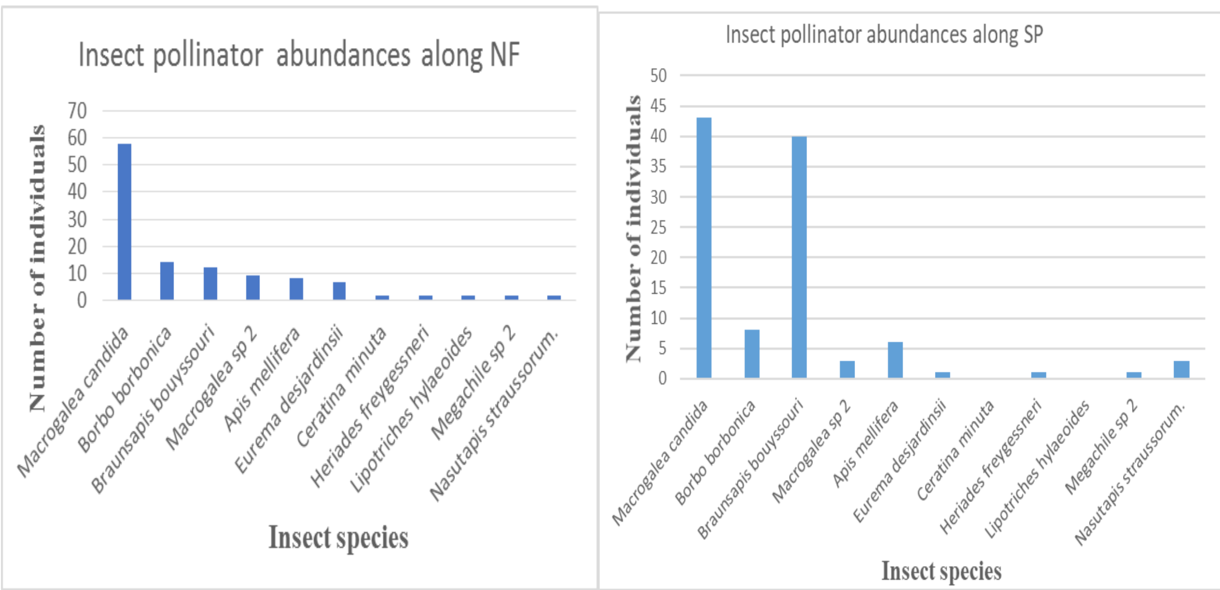

Figure 2.1: Abundance of dominant okra insect pollinators along natural forest (NF) and sugarcane plantation (SP)

2.5.3 Relative abundances of okra insect pollinators captured along natural forest and sugarcane plantation

Okra insect pollinators' relative abundance was higher on okra plants adjacent to the natural forest than on sugarcane plantation. Along natural forest, *Macrogalea candida* (Plate 3.2) had the highest relative abundance of (41.43%) followed by *Borbo borbonica* (10%) and *Braunsapis bouyssouri* (8.57%). Insects such as *Ceratina ericia, Allodapula variegate* and others recorded the lowest relative abundances**.** For those okra plants along sugarcane plantation, *Macrogalea candida also* had the highest relative abundance of (34.13%) followed by *Braunsapis bouyssouri* with (31.75%) and *Borbo borbonica* (6.35%). Insects such *Heriades freygessneri* and *Megachile apiformis* and others recorded the lowest relative abundances.

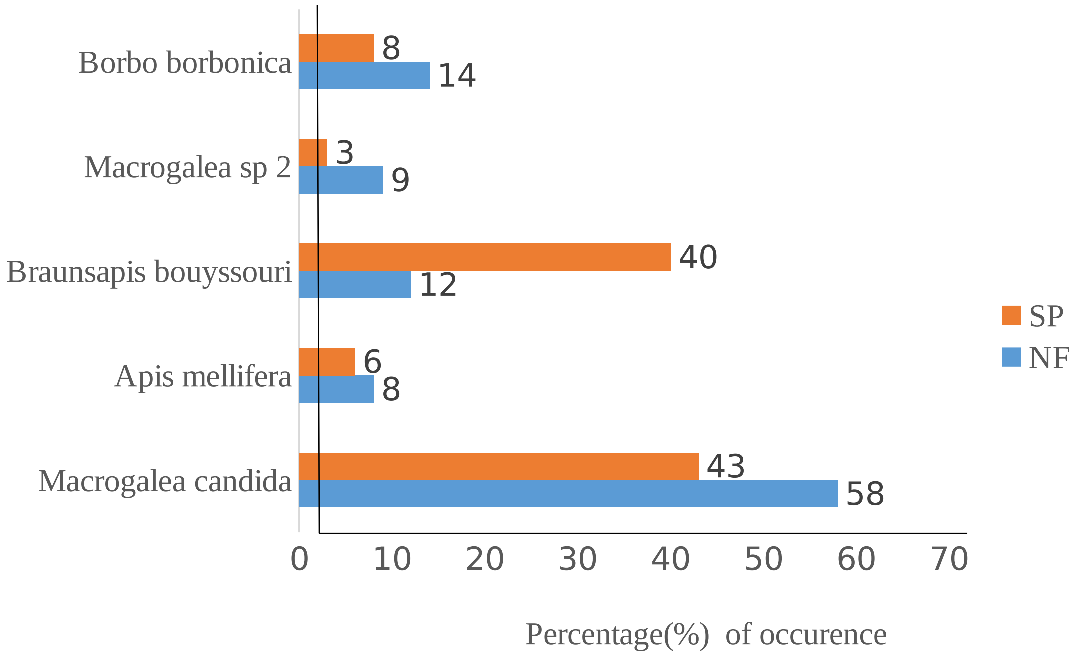

Figure 2.2: Relative abundance (%) of *A. esculentus* dominant insect pollinators captured on experimental plots established along natural forest (NF) and sugarcane plantation (SP)

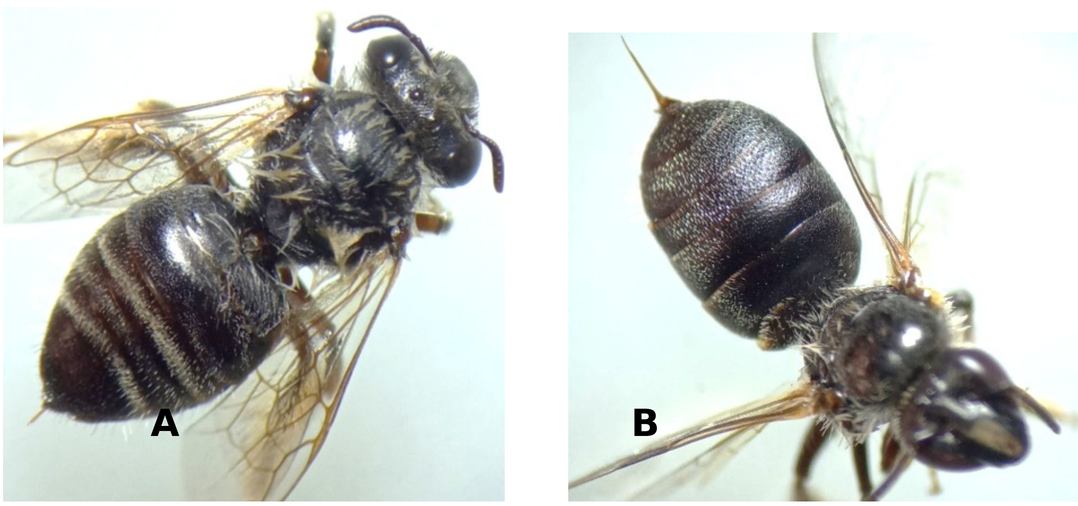

Plate 2.2: Okra (*A. esculentus*) dominant insect pollinators with highest relative abundance; (A) *Macrogalea candida* (B) *Braunsapis bouyssouri*

2.6 Discussion

2.6.1 Effects of insect pollination on Okra yield

The results of mean number of okra pods harvested per plant showed that, higher numbers of pods (fruits) were harvested from open pollinated okra plants than on self-pollinating okra plants in control (cage) plots. Our results are similar to those of Hasnat (2015), who reported that insect pollinated okra plants produced a greater number of young fruits and mature fruits due to higher transformation of flowers into tender fruits. This clearly indicated that, reduction of insect pollinators through anthropogenic activities including; clearance of natural and semi- natural habitats, use of harmful insecticides and expansion of farmlands, play a significant role in decreasing different yield variables, particularly number of fruits harvested. In addition, our results showed that, those okra plots established along natural forest has been influenced to produce higher number of mature okra fruits compared to the plots established along sugarcane plantation in the midst of agro ecosystem. This is perhaps due to the availability of diverse foraging resources from natural forest habitat than in sugarcane plantation that consequently affected abundance and visitation rates of insect pollinators. Additionally, site A along each targeted habitat performed better in terms of okra yield than site B, perhaps due to being further away from external factors, such as excessive use of insecticides from nearby smallholder farmlands, therefore insect pollinators abundance and diversity were higher along site A than site B of selected habitats.

Our findings revealed that, the mean numbers of okra seeds per pod were significantly higher in insect pollinated plots of okra plants than in those okra plants, which were in control (caged) treatment. Perhaps this is due to higher transformation of okra flower ovules into seeds, because of higher visitation rates from insect pollinators, which ensure fertilization of many ovules. These results are similar to those of Al Ghzawi *et al.* (2003), who found that insect pollination increased the number of seeds per mature pods and the development of fertilized ovules into seed. This proved that, the interaction between flowering plants and insect pollinators is necessary for maximum yield according to Bashir (2017). However, our findings are in contrast with Azo'o *et al.* (2011) who stated that, many okra (*A. esculentus*) ovules could be fertilised to assure pod setting without any anthophilous insect visitation. Our results showed significant difference in mean number of seeds per pod harvested at different distances from natural or semi natural habitats. This could be due to fluctuation in pollen deposition on okra female reproductive parts as a result of abundance and diversity of insect pollinators changing. The fact is supported by Ricketts *et al.* (2008), who reported that there are strong exponential declines in insect pollinator richness and their visitation rates, as one moves further away from the edge of natural habitat. Also, the fact is proved by Gemmill-Herren and Ochieng (2008) who revealed that floral visitation rates for all insect species decreased significantly with distance from the forest–field edge.

Our research findings on seed weight (g) per 100 seeds harvested revealed that, seeds obtained from the open pollinated okra pods were heavier in weight as compared to those in control (caged plots). The results are similar to those of Al Ghzawi *et al.* (2003), who reported that the okra plants pollinated by insects produced 7.1 g seed per plant as compared to 3.9 g obtained from non-pollinated plants. This is perhaps due to high deposition of pollen grains, as insect pollinated okra flowers received many visits from their insect pollinators compared to those, which undergo self-pollination. Additionally, factors such as local climate between to selected habitats were more or less the same, and soil nutrients differences were minimized as much as possible by application of constant amount of the chemical fertilizers (NPK).

2.5.2 Abundance and relative abundance of okra insect pollinators

The findings revealed that, *Macrogalea candida* (Apidae), *Braunsapis Bouyssouri* (Apidae) and *Borbo borbonica* (Hesperiidae) were the most dominant species of insect pollinators found to visit the experimental plots of okra during the flowering period. Between the two families of insect pollinators, Apidae (Order: hymenoptera) was found to be superior, followed by Hesperiidae (Order: lepidoptera). These findings are in close agreement with Angbanyere (2012), who found that honey bees of family Apidae (order: hymenoptera), were the dominant pollinators of okra (*A. esculentus*) followed by lepidopterans, however in contrast, our findings revealed that *Macrogalea candida* and *Braunsapis bouyssouri* are the dominant okra pollinators in Kilombero and not *Apis mellifera*. Furthermore, our findings confirm other report that Hymenoptera, mainly bees, are the most important foragers for a greatest number of crop plants particularly okra (*A. esculentus*) (Mc Gregor, 1976; Klein *et al.* 2007). *Apis mellifera* was already mentioned as okra dominant pollinator in Cameroon and Ghana (Njoya *et al.* 2005; Angbanyere, 2012). Moreover, the genus Apis with the species cerana was identified as the main *A. esculentus* pollinator in India (Crane, 1991). Insect pollinators abundance and species richness were higher on okra plots along natural forest habitat than sugarcane plantation habitat, perhaps due to the fact that in natural habitat like a natural forest, there are diverse food resources and nesting sites for the insect pollinators and other groups as compared to agroecosystems such as sugarcane plantation habitats.

2.7 Conclusion

Native bee species *Macrogalea candida* and *Braunsapis bouyssouri* are ranked first amongst the insect species found to visit okra (*Abelmoschus esculentus)* flowers in the study area. Their visits primarily concerned with pollen and nectar harvesting. Abundance and species richness of okra insect pollinators is higher along natural forest than along sugarcane plantation. The okra seed formation and number of pods (fruits) per plant is significant in insect pollinated plants and lower in control (caged) plants. It is quite evident that insects help in pollination of our food crops. Insects, particularly insect pollinators depend on natural and semi-natural habitats to meet their nesting and resources requirements. Loss of these habitats, may lead to decline of pollination services offered by many insect pollinators.

2.8 Recommendations

Conservation of natural and semi-natural habitats is of great ecological importance, due to their essential role that they play in conserving insect pollinators and the ecosystem services they provide for human well-being. Mindset change in insect pollinators' importance is needed for their conservation. Formation of appropriate conservation policies is needed especially for natural habitats found with agricultural landscapes.

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CHAPTER THREE

Manuscript Two

3.0 Effects of insect pollination on okra fruit set rate along selected habitat gradients at Kilombero District, Tanzania

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Abstract

Experiments were made to determine the effects of insect pollination on okra (*Abelmoschus esculentus)* fruit set rate (%) along natural forest and sugarcane plantation habitat gradients. Their pollination effects were studied in Kilombero from January 2021 to early April 2021. Pollination treatments included open pollination to all insect pollinators and control (cage). The number of flowers per plant, number of fruit sets per plant and insect pollinator species diversity were recorded. When comparing okra fruit set rate for the two pollination treatments, it appeared that insect pollinators diversity influence the increase in mean numbers of flowers per plant and fruit set rate per plant by 0.93 and 3.6% respectively. The findings showed that thirty-seven different insect species visited *A. esculentus* flowers. Findings indicated that *Macrogalea candida* was the dominant species with (38%), followed by *Braunsapis bouyssouri* (20%) and *Borbo borbonica* (8%). The insect pollinator species diversity was higher at the edge of natural forest than sugarcane plantation. Conservation of natural and semi natural habitats is recommended to avoid decline of insect pollinator species diversity, as they are very important for their pollination services.

Keywords: insect pollinators; okra; natural forest; sugarcane plantation; fruit set rate

3.1 Introduction

Insects, particularly insect pollinators are important because of their diversity, ecological role and influence on agricultural crops (Berenhaum, 1995; Adetundan *et al.* 2005; Premalatha *et al.* 2011). They make up more than 58% of the known global biodiversity (Okrikata and Yusuf, 2019). Insect pollinators help in transferring pollen from anther to stigma within a flower or between flowers of the same plant or different plants of the same species (Amin *et al.* 2019). Pollination by insects is known as entomophily, and it is very important for most of flowering plants, particularly *Abelmoschus esculentus* (okra)

Okra (*Abelmoschus esculentus* (L.) Moench) known in many English-speaking countries as lady's fingers, bhindi in India (Singh *et al.*, 2014) and bamia in Tanzania. It is an annual fruit vegetable, commonly cultivated in tropical, subtropical and warm temperate regions around the world (NAREC, 2008). Okra can be grown on wide range of soils, but well drained fertile soils with adequate organic matter result to high yield (Akinyele and Temikotan, 2007). The optimum weather conditions suitable for better yield and growth of okra indicate that when the temperature reaches 18ºC (minimum) and 35ºC (maximum), respectively, in the tropic region, okra performs best (Ezeakunne, 1984). Okra originated in Africa, as the most literatures suggest that Okra originated somewhere around the Ethiopia, and was cultivated by the ancient Egyptians by the 12th century B.C (Singh *et al.*, 2014). It can be grown on a large commercial farm or as a garden crop (Rubatzky and Yamaguchi, 1997) in your backyard. It produces fibrous fruits or pods containing round, white seeds. Also structurally, it has large leaves, and they alternate, cordate and are divided into three to seven lobes with toothed margins (Nadine *et al.*, 2020). Consumption of young immature okra pods is important as fresh fruits, its fruit is principally consumed fresh or cooked and is a major source of vitamins A, B, C, minerals, Iron and Iodine and important vegetable source of viscous fiber but it is reportedly low in sodium saturated fat and cholesterol (Moaward *et al.* 1984; Kendall and Jenkins, 2004; Adebooye and Oputa, 1996).

Abelmoschus esculentus has large attractive flowers with nectaries, and it tends to open only once in the morning and close after pollination on the same day (Nadine *et al.,* 2020). Since *Abelmoschus esculentus* has bisexual flowers, then structurally, it combines hermaphroditism and self-compatibility (Azo'o *et al.,* 2011). Hence, in okra, as the conditions for selfing become favourable, there is also an opportunity for cross-pollination (George, 2009; Al Ghzawi *et al.,* 2003). However, selfing of the crop in the absence of cross pollination, particularly by insect pollinators such as honey bees, bumble bees, ants, butterflies and other insects belonging to different orders during flowering period as reported by Nandhini *et al.* (2018), generally reduces rate of okra reproductive performances, particularly its rate of fruit setting. For instance, Hasnat *et al.* (2015) found that Insect pollinated plants produced a greater number of young fruits and mature fruits due to higher transformation of flowers into tender fruits.

Many horticultural crops including okra (*Abelmoschus esculentus*) are dependent on insect pollinators for optimal yields (Klein *et al.,* 2007) and fruit setting. However, pollination of crop flowers by wild insects is one such vulnerable ecosystem service (Klein *et al.,* 2007). Due their ecological importance, decline of the insect pollinators is certain to have substantial effects on ecosystem functioning (Hallmann *et al.,* 2017) and services particularly pollination of food crops such as okra. Now, concerns over insect pollinators status have mounted since, Current data point out an overall pattern of decline in insect diversity and abundance (Hallmann *et al.,* 2017). Expansion of agricultural lands, clearance of natural and semi-natural habitats and decline of floral resources have been pointed out as key factors driving global declines in wild insect pollinators (Baude *et* *al.,* 2016; Vanbergen and Initiative, 2013). The outcomes of this research**;** add inputs to the body of science, especially relationship between *A. esculentus* and insect pollinators**;** also acts as a basis for formulation of appropriate conservation policies**;** and provide diversity status of *A. esculentus* insect pollinators in Kilombero. Therefore, the main objective of this study was to determine the effect of insect pollination on Okra (*A. esculentus*) fruit set rate.

3.2 Materials and Methods

3.2.1 Description of study sites

A study was conducted in Morogoro region, Kilombero district at Mang'ula A and Msalise villages located at coordinates 7° 49' S, 36° 53' E and 7° 51' S, 36° 57' E respectively. Kilombero district is found in Morogoro region, south-western of Tanzania. It is located in immense floodplain, between The Udzungwa Mountains in north-west and the Kilombero river in the south east. The district lies between latitude 7° 13' S to the south of the Equator and longitude 36° 68' E East of the Greenwich, with population of 398,379 (MRSEP, 2006; NBS Census, 2012). The dominant vegetation cover for Kilombero district are Miombo woodland, farmland and floodplain grassland. The district receives an annual average rainfall of 900 mm and mean annual temperature ranges between 18℃ on the mountains to 30℃ in river valleys (MRSEP, 2006). The hot months of the region are July, August and September with mean temperature of 29.5℃. The experimental plots established adjacent to; Udzungwa Mountain Forests with a very good conservation status and sugarcane plantation with fairly matured canes.

The meteorological data were collected from two stations in Mang'ula, each station located at respective study site (Mang'ula A and Msalise). The field experiment was conducted from January to March, 2021, as wetter season in Kilombero district lasts 5 months, from November to April. Tables 3.1 below, shows meteorological data obtained from Mang'ula A and Msalise respectively. The overall climatic conditions during okra cultivation were relative good for its optimum reproductive performances.

Table 3.1: Study sites meteorological data (January- March 2021)

Village	Month	Temperature(^{0}C)		Relative Humidity (%)		Dew Point	
		Max	Min	Max	Min	Max	Min
	January	34.6	20.8	100	44.7	25.9	20.2
Mang'ula A	February	34.3	21.1	100	46	26.5	20.1
	March	33.6	20.7	100	42.8	25.8	18.2
	January	32.5	21.4	100	49.9	26.6	19.5
Msalise	February	32.3	21.2	100	50.7	27.1	20
	March	35.9	21.2	100	43.9	27.2	17.4

3.3 Methodology

3.3.1 Experimental design

The split plot design was used, where experimental plots were arranged in randomized complete block design (RCBD) with two treatments and three replications according to Angbanyere (2012). The experimental plots were established along two different habitats, which were natural forest and sugarcane plantation. The experimental blocks were established at two different sites, site A and site B, with 150m gap between sites, adjacent to each selected habitat to reduces experimental errors**.** From the edge of each selected habitat**,** there were two experimental blocks, established at distances of 10m and 60m**,** along the same gradients**.** The two treatments used in this study were**;** Control (net covered okra plants with no insect pollinators accessibility) and Open pollination (okra plants accessible to naturally occurring insect pollinators). The plot size (experimental unit) was 4.26 m^2**,** with each experimental block containing six (6) plots. The distances between plot to plot in each replication, and one replication to another replication within a block, were 1m and 1.5 m respectively**.** Each plot (experimental unit) had 20 okra plants**,** with plant spacing of 71cm x 30cm**.** The okra plants in each plot were arranged in four rows and each row contained five okra plants**.**

3.3.2 Field work

Prior to seed sowing, land preparation involving weed removal, soil digging and shallowing of trenches (harrowing) on areas (blocks) where experimental plots were established was done, using a hand hoe. On 17 January 2021, two Okra seeds of “Clemson spineless" variety were sown in holes of 10 cm of depth. These holes were separated by 30 cm within and 71 cm apart on each rows. Two weeks after sowing (31 January 2021), Okra plantlets were thinned to one plant per hole**.** These same days, the first weeding was done when okra plants were approximately 9.5cm in height, followed by pesticide application using Bicron- Profenofos 500 EC and Ivory $M^{TM}{}_{72}$ $_{WP}$ (Fungicide) at the rate of 30 mils and 50 grams per 15 litres of water respectively, to control insect pest such flea beetles (*podagrica spp*.) and blights on okra leaves. Four weeks after sowing (14 February 2021), second weeding and second pesticide application were done. Also on the same days, chemical fertilizer (NPK 17-17-17) was applied around each conserved plant of each hole, at the rate of 233 $kghectare^{-1}$ (Angbanyere, 2012). Six weeks after sowing, pollination treatment application was applied, where random caging of 24 out of 48 plots was done.

Plate 3.1: Pictures above show; (A) prepared land (block) before setting of the experiment along sugarcane plantation habitat and (B) randomly caged and uncaged experimental plots in a block along natural forest

3.4 Data collection

To determine the number of fruit set rate (%) using number of flowers per plant (formed) and fruit sets per plant, and determination of diversity of okra insect pollinators. By using a systematic sampling, two middle rows were used as sampling unit to reduce edge effects for all okra experimental plots. Five (5) okra plants from the two middle rows of each plot were randomly chosen, labelled and their flowers counted and monitored over a period of four-week. Counting started from 8th week after germination. The aggregate number of flowers from five (5) okra plants for each exper imental plot was then divided by the total number of plants used in a plot to compute mean number of flowers per plant.

At the end of harvesting period, the mean number of pods per plant from five (5) randomly selected and labeled plants, in which their flowers were counted and monitored, was calculated for each plot. Also, the mean number of flowers formed from those five (5) randomly selected and labeled plants was calculated for each plot. The fruit set rate per plant (%) was computed by dividing mean number of pods per plant harvested over mean number of flowers per plant, and the answer is multiplied by a hundred to get its percentages (%).

Observation of okra insect pollinators was done biweekly (Campos *et al.*, 2000) on open pollinated okra flowers (open pollination treatment), starting from the seventh week up twelfth week after sowing. Direct observations on open pollinated flowers focused on natural population of effective okra insect pollinators during the blooming period at four intervals; 0800 hrs – 0840 hrs 0900 hrs -0940 hrs, 1000 hrs - 1040 hrs and 1100 hrs -1140 hrs was done. By using electronic stopwatch, we made 40 minutes transect walk along open pollinated plants within a block and captured insects that visited okra flowers using suction device (an Aspirator) (Azo'o *et al.*, 2011) and plastic tubes. The collected insects were preserved by immersion each in a tube containing 70% of ethanol except for butterflies as they were preserved in special piece of paper to avoid discolouration of their wings. The sampled insect pollinators were then taken to laboratory for identification. Once in the Laboratory, insect specimens were identified with the aid of insect guide books by Kielland (1990), Picker *et al*. (2004), Eardley (2004) and Eardley *et al*. (2010).

3.5 Data analysis

To account for rate of okra flowers transformation into fruits (pods), the data on number of flowers per plant and fruit set rate per plant were summarized into mean value per treatment. GenStat Discovery Edition 4 and Microsoft Excel 2021 computer software programs were used in comparison of means among treatments. One-way analysis of variance (ANOVA) at 5% level of significance was used to statistically test the effect of treatments on okra fruit set rate (%). For pairwise differences between factor level means, Tukey's 95% confidence interval was used. The Shannon Diversity Index, (H′) was used to calculate diversity of okra insect pollinators on okra plants along selected habitats

$H' = -\sum (P_i \ln P_i)$

Where**;**

Pi=proportional of individual in a species given as Pi=n/N

Pi^2=square of Pi

N=total number of all individual

n=number of individual

3.6 Results

3.6.1 Effects of insect pollination on Okra fruit set rate

3.6.1.1 Mean numbers of okra flowers per plant and fruit set rate (%) per plant

The findings showed that, the mean numbers of flowers per plant and fruit set rate per plant were significantly higher on open pollinated okra plants than caged okra plants. A summary of means is summarized in Table 3.2.

Table 3.2: A summary of mean numbers of flowers per plant and fruit set rate (%) per plant for all levels of four main effects

Treatment effects	Fruit set rate per plant (%)	Mean number of flowers per plant

Natural forest (NF)	95.053 ± 1.16a	8.500 ± 0.18a
Sugarcane plantation (SP)	94.713 ± 0.07a	8.050 ± 0.28a
Site A	95.220 ± 1.06a	8.108 ± 0.13a
Site B	94.546 ± 1.42a	8.442 ± 0.59a
Ten meters (10m)	95.001 ± 0.89a	8.075 ± 0.05a
Sixty meters (60m)	94.765 ± 0.79a	8.475 ± 0.46a
Open pollination (OP)	96.682 ± 1.39b	8.742 ± 0.49b
Control (caged)	93.083 ± 0.99a	7.808 ± 0.04a

Within columns, means with different letters are significantly different (P < 0.05)

The findings revealed significant difference (P < 0.05) in mean numbers of flowers per plant between open pollinated okra plants and caged plants as shown in Table 8.

Table 3.3: ANOVA table of main effects for okra (A. esculentus) mean number of flowers per plant.

Treatment effect	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Pollination	1	10.4533	10.4533	15.03	<.001
Habitat	1	2.43	2.43	3.49	0.071
Site	1	1.3333	1.3333	1.92	0.176
Distances	1	1.92	1.92	2.76	0.107
Residuals	30	20.8717	0.6957		

The results showed that, there was significant difference (P < 0.05) in mean numbers of fruit set rate per plant between open pollinated okra plants and caged plants as shown in Table 3.4.

Table 3.4: ANOVA table of main effects for A. esculentus fruit set rate per plant

Treatment effect	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Pollination	1	155.43	155.43	13.84	<0.001
Habitat	1	1.38	1.38	0.12	0.728

Site	1	5.44	5.44	0.48	0.492
Distances	1	0.67	0.67	0.06	0.809
Residuals	30	336.91	11.23		

3.6.1.2 Diversity of okra (*Abelmoschus esculentus*) insect pollinators along selected habitats

On open pollinated okra plants along two selected habitats (natural forest and sugarcane plantation)**,** 266 insect pollinators were captured while visiting *A. esculentus* flowers. They belonged to 7 families and 37 genus /species.

Diversity indices showed that insect species captured on okra (*A. esculentus)* plants along natural forest are more diverse with higher equitability (0.69), than those captured along sugarcane plantation Overall, *A. esculentus* experimental blocks established 10m from the edge of natural forest (NF10) had the highest diversity (H' = 2.52) and equitability of insect pollinators species. The least diverse plots, were those established 60m from the edge of sugarcane plantation (SP60) with diversity index (H' = 1.86). A summary of diversity indices for three main effects are depicted in a Table 3.5.

Table 3.5: Diversity indices on insect pollinators captured on plots at different habitats, sites and varying distances

Treatment effect	Treatment levels	Shannon Index (H')	Equitability (J)
Habitat	NF	2.36	0.69
	SP	2.00	0.66
Sites	NF1	2.57	0.82
	NF2	1.80	0.66

	SP1	1.96	0.76
	SP2	1.87	0.68
	NF10	2.52	0.84
Distances	NF60	2.08	0.67
	SP10	1.88	0.69
	SP60	1.86	0.73

NF1 and NF2 – All plots at site 1 and 2 along natural forest respectively

SP1 and SP2 – All plots at site 1 and 2 along sugarcane plantation respectively

NF10 and NF60 – All plots established 10m and 60m from the edge of natural forest

SP10 and SP60 – All plots established 10m and 60m from the edge of sugarcane plantation

NF – Natural forest habitat

SP – Sugarcane plantation habitat

3.7 Discussion

3.7.1 Effects of insect pollination on okra fruit set rate

3.7.1.1 Effects of insect pollination on mean numbers of flowers per plant and fruit set rate per plant (%)

Our findings showed that, open pollinated okra plants developed a greater number of flowers while self-pollinated (caged) okra plants recorded fewer numbers of flowers. This could be due to high transformation of flowers into young pods as result of higher visitation rates, the insect pollinated okra plants received. Our results are similar to those reported by Al Ghzawi *et al.* (2011). However, contrasting results from Ghana (Angbanyere (2012), showed that, there is no significant difference between number of okra flowers on open pollinated plots to those self-pollinating plots of okra (caged).

Our study results on the fruit set rate per plant (%) showed that, there was higher rate of fruit setting from those okra plants which were open pollinated as compared to those which where control (caged). This means that insect pollinators ensured fertilization and rapid conversion of flowers into okra pods. These findings are similar to those of Al

Ghzawi *et al.* (2003) who reported that Insect-pollinated plants developed a greater percentage of flowers into young pods than selfed plants.

3.7.1.2 Diversity of insect pollinators along selected habitats

A total of seven (7) families and thirty-seven (37) insect species were found on open pollinated okra plants along selected habitats. 266 individual insect species were captured during the experiment. Wild bee species *Macrogalea candida* and *Braunsapis bouyssouri* were ranked first amongst the insect species found to visit okra (*Abelmoschus esculentus)* flowers during the experiment, this is similar to Azo'o *et al.* (2011) who also found "wild bees" *Eucara macrognatha* and *Tetralonia fraternal* as the dominant insect pollinators of okra in his study area. However, our findings disagree with Angbanyere (2012) who found that Honeybees (*Apis Mellifera*) were the dominant insect pollinators of okra (*A. esculentus*). Moreover, those okra plots established along natural forest were more diverse in terms of insect pollinator species captured as compared to those along sugarcane plantation. In addition, the distances affected the species diversity of okra insect pollinators, as those insect species collected 10m from natural forest were the most diverse. This could be due to sufficient resources found within natural forest habitat, but when moving further away from natural forest the availability of these resources declining, thus insect species also declining.

3.8 Conclusion

In Kilombero district, Tanzania, thirty-seven (37) species of insect pollinators of okra distributed in seven (7) families found to visit okra (*A. esculentus*) flowers purposely for nectar and/ or pollen harvesting, but in doing so they pollinated okra flowers. Bees species from apidae family were dominant. *Macrogalea candida* was the dominant insect pollinator of okra, followed by *Braunsapis bouyssouri*. Insect pollination is evidently an important ecosystem service for optimum okra (*A. esculentus*) reproductive performances and other food crops. The data on okra fruit set rate revealed that, insect pollinators diversity increased the number of flowers per plant that are transformed into okra fruits. Also the influence of natural and semi natural habitats and distances, were observed in number of different insect pollinators found to visit okra flowers.

3.9 Recommendations

Conservation of natural and semi natural habitats is very important for provisioning of nesting sites of the wild bees and other beneficial insect pollinators. This will ensure continuity of ecosystem services they provide for human wellbeing and ecosystems in general. Further researches are necessary to determine the effect of insect pollinators on food crops and floral plant genetic makeup.

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CHAPTER FOUR

4.0 CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

Based on the findings from this study the following conclusions are made;

The study has found significant effect of insect pollination on okra fruit set rate and yield.

The mean numbers of flowers per plant, fruit set rate per plant, pods per plant, seeds per pod and seed weight per 100 seeds were higher in insect pollinated okra plants than in control (caged) okra plants hence, indicated that insect pollination played key role in okra reproductive performances.

The study has shown that natural or semi- natural habitats played a vital role in ecologically supporting insect pollinators, as they influenced insect diversity, abundance and species richness.

The study has shown that insect pollination is affected by distance from the edge of natural habitat (e.g. natural forest).

4.2 Recommendations

Based on the findings from this research and literatures from other studies, it is recommended that;

i. Farmers live or do farming activities nearby natural forests, should take measure to continue conserve them, as they inhabit enormous number of insect pollinators and other important living organisms for ecosystem stability.

ii. Since, due to time constraint this research was not able to assess the temporal nature of insect pollination, particularly fluctuation in insect pollinators visitation rates in a day, further researches are recommended on this.

APPENDICES

Appendix 1: Abundance of okra (*Abelmoschus esculentus*) insect pollinators

S/N	Family	Genus/species	NF	SP	Total
1	Apidae	*Macrogalea candida*	58	43	101
		Ceratina ericia	1	0	1
		Ceratina (Copo ceratina) sp.	0	1	1
		Ceratina minuta	2	0	2
		Braunsapis facialis	0	2	2
		Apis mellifera	8	6	14
		Braunsapis sp 2	0	5	5
		Braunsapis bouyssouri	12	40	52
		Braunsapis foveata	1	1	2
		Allodapula variegate	1	0	1
		Allodape punctate	1	0	1
		Macrogalea sp 3	0	1	1
		Macrogalea sp 2	9	3	12
		Nasutapis straussorum.	2	3	5
		Macrogalea sp.	1	0	1
	Halictidae	*Lipotriches sp 1*	1	3	4
		Lipotriches sp 2	1	0	1
		Lipotriches hylaeoides	2	0	2
		Lasioglossum (Sellalictus) sp.	1	0	1
		Lipotriches sp 3	1	0	1
		Seladonia jucunda	2	0	2
	Megachilidae	*Megachile sp 1*	1	1	2
		Megachile sp 2	2	1	3
		Megachile sp 3	1	0	1
		Othinosmia sp	1	0	1
		Heriades (Pachyheriades) sp	1	0	1
		Wainia elizabethae	2	0	2
		Heriades freygessneri	2	1	3
		Megachile apiformis	1	1	2
		Pseudoheriades sp	1	1	2
		Megachile pyrrhithorax	1	0	1
	Hesperiidae	*Borbo borbonica*	14	8	22
	Pieridae	*Eurema desjardinsii*	7	1	8
	Colletidae	*Hylaeus (Deranchylaeus) sp.*	0	1	1
	Syrphidae	*Syrphus sp*	1	0	1
		Total	**139**	**123**	**266**

Appendix 2: Relative abundance of okra (*A. esculentus*) insect pollinators

S/N	Family	Genus/species	Relative Abundance (%) for NF	Relative Abundance (%) for SP
1	Apidae	*Macrogalea candida*	41.43	34.13
		Ceratina ericia	0.71	0.00
		Ceratina (Copo ceratina) sp.	0.00	0.79
		Ceratina minuta	1.43	0.00
		Ceratina (Macroceratina)	0.00	0.79
		Ceratina nasalis	0.71	1.59
		Braunsapis facialis	0.00	1.59
		Apis mellifera	5.71	4.76
		Braunsapis sp 2	0.00	3.97
		Braunsapis bouyssouri	8.57	31.75
		Braunsapis foveata	0.71	0.79
		Allodapula variegate	0.71	0.00
		Allodape punctate	0.71	0.00
		Macrogalea sp 3	0.00	0.79
		Macrogalea sp 2	6.43	2.38
		Nasutapis straussorum.	1.43	2.38
		Macrogalea sp.	0.71	0.00
2	Halictidae	*Lipotriches sp 1*	0.71	2.38
		Lipotriches sp 2	0.71	0.00
		Lipotriches hylaeoides	1.43	0.00
		Lasioglossum (Sellalictus) sp.	0.71	0.00
		Lipotriches sp 3	0.71	0.00
		Seladonia jucunda	1.43	0.00
3	Megachilidae	*Megachile sp 1*	0.71	0.79
		Megachile sp 2	1.43	0.79
		Megachile sp 3	0.71	0.00
		Othinosmia sp	0.71	0.00
		Heriades (Pachyheriades) sp	0.71	0.00
		Wainia elizabethae	1.43	0.00
		Heriades freygessneri	1.43	0.79
		Megachile apiformis	0.71	0.79
		Pseudoheriades sp	0.71	0.79
		Megachile pyrrhithorax	0.71	0.00
4	Hesperiidae	*Borbo borbonica*	10	6.35
5	Pieridae	*Eurema desjardinsii*	5	0.79
6	Colletidae	*Hylaeus (Deranchylaeus) sp.*	0	0.79
7	Syrphidae	*Syrphus spp.*	0.71	0

Appendix 3: Field plot layout

Key:

Red colour boxes: All plots of okra established 10m from selected habitat

Blue colour boxes: All pl ots of okra established 60m from selected habitat

NF: Natural forest habitat

SP: Sugarcane plantation habitat