Sokoine University of Agriculture

MSc Dissertation

Pollination Efficiency of *Apis Mellifera* and *Hypotrigona Gribodoi* on *Capsicum Annuum* Fruit Set and Yield in Morogoro Region, Tanzania

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May 2024

POLLINATION EFFICIENCY OF *APIS MELLIFERA* AND *HYPOTRIGONA GRIBODOI* ON *CAPSICUM ANNUUM* FRUIT SET AND YIELD IN MOROGORO REGION, TANZANIA

Dissertation is Submitted to Sokoine University of Agriculture in Fulfillment of the Requirements for the Master Degree of Science in Forestry to, Morogoro

By

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EXTENDED ABSCTRACT

Pollination by insects accounts for over 70% of global food crops production. Among insect species, bees are one of the most efficient pollinators though their pollination efficiency varies between species. Amidst rising worries about declining pollinator populations due to human activities and changes in climate, comprehending diverse pollinator capabilities is crucial for conservation. While stingless bees show promise as commercial pollinators, their capacities compared to *Apis mellifera* remain insufficiently understood, resulting in limited domestication and thus threatening their population.. This study evaluated the pollination efficiency of *Apis mellifera* and *Hypotrigona gribodoi* on yield and fruit set in *Capsicum annuum*. A randomized complete block design experiment was conducted with three replications and three caged treatmentswhich are *Apis mellifera, Hypotrigona gribodoi*, and self-pollinated plants. Each treatment was replicated three times to assess the effects of pollination on yield and fruit set rates. We made comparison on bees' foraging activities and computed their impact on fruit set. Furthermore, we explored the influence of abiotic factors on bees foraging activities, to a comprehensive analysis of the intricate relationship between pollination, bee behavior, and environmental factors. Analysis of variance (ANOVA) was used to compare the differences in fruit quality, seed quality, and fruit set rate between the two species. Kruskal wallis was used to test for difference among data which are not normally distributed. Tukey's Honestly Significant Difference (HSD) and Dunn tests were used for normally distributed and non-normally distributed data respectively. To compare the difference in bee foraging activity, we use the Wilcoxon rank-sum test. We employed a Generalized Linear Model (GLM) to investigate the relationship between fruit set rate and foraging activity, and a Poisson Regression Model to explore the relationship between foraging activity, abiotic factors, and bee species. The results show that the number of fruits per plant, fruit transverse circumference, fruit vertical circumference, fruit weight, and percentage fruit set rate per plant were higher in the plot pollinated with *Hypotrigona gribodoi*, followed by *Apis mellifera*, and lastly the control plot and the difference was statistically significant ($P < 0.05$). However, there was no significant difference in the number of seeds per fruit and the weight of 100 dry seeds per fruit among the two species ($P>0.05$). *Hypotrigona gribodoi* showed higher flower visits, pollen-carrying workers, and visitors per flower than *Apis mellifera*, with significant differences ($P < 0.001$). Conversely, *Apis mellifera* had more exiting workers ($P < 0.001$). Moreover no difference in time spent on the flower ($P>0.05$). Also the number of workers entering with pollen ($P<0.001$), workers exiting the hive ($P<0.001$), and time spent on the flower ($P<0.05$) have a significant effect on the fruit set rate of *Capsicum annuum*. The number of visitors per flower and the number of flowers visited do not have a significant effect ($P>0.05$). Temperature and humidity significantly affect the number of visitors per flower, the time spent on flower and the number of workers exiting the hives. However, the number of flowers visited and the number of workers entering the hive with pollen were not influenced by temperature and humidity. These findings suggest that *Hypotrigona gribodoi* is a more efficient pollinator of *Capsicum annuum L.* Furthermore, the foraging behavior of *Apis mellifera* and *Hypotrigona gribodoi* is affected by temperature and humidity affecting the fruit set rate of *Capsicum annuum.* More research on the differential pollination efficiency among different bee species in crop production is imperative.

Keywords: Pollination*, Apis mellifera, Hypotrigona gribodoi, Capsicum annuum L.,* yield*,* fruit set rate*,* Foraging behaviour, Abiotic factors (temperature and humidity)

IKISIRI KUU

Uchavushaji unaofanywa na wadudu huchangia zaidi ya 70% ya uzalishaji wa mazao ya chakula duniani. Miongoni mwa aina za wadudu, nyuki ni mojawapo ya wachavushaji bora zaidi ingawa ufanisi wao wa uchavushaji hutofautiana kati ya spishi. Kutokana na kuongezeka kwa wasiwasi kuhusu kupungua kwa idadi ya wachavushaji kutokana na shughuli za binadamu na mabadiliko ya hali ya hewa, kuelewa uwezo mbalimbali wa wachavushaji ni muhimu kwa uhifadhi. Ingawa nyuki wasiouma wanaonyesha ahadi kama wachavushaji kibiashara, uwezo wao ikilinganishwa na nyuki wanaouma (*Apis mellifera)* bado haueleweki vya kutosha, na hivyo kusababisha ufugaji mdogo na hivyo kuhatarisha idadi yao. Utafiti huu ulitathmini ufanisi wa uchavushaji wa nyuki wanaouma (*Apis mellifera)* na nyuki wasiouma (*Hypotrigona gribodoi)* kwenye mazao na seti ya matunda katika Pilipili Hoho (*Capsicum annuum*). Jaribio la usanifu kamili wa bila mpangilio wa bloku tatu na replikesheni tatu ilifanyika yenye nyuki wanaouma (*Apis mellifera*), nyuki wasiouma (*Hypotrigona gribodoi*), na mimea inayochavusha yenyewe. Kila bloku ilirudiwa mara tatu ili kutathmini athari za uchavushaji kwenye viwango vya mazao na viwango vya kuweka matunda. Tulilinganisha shughuli za lishe ya nyuki na kukokotoa athari zao kwenye seti ya matunda. Zaidi ya hayo, tulichunguza athari za hali ya kimazingira kwenye shughuli za nyuki katika kutafuta chakula, kufanya tathmini ya kina juu ya uhusiano kati ya uchavunaji, tabia ya nyuki, na sababu za kimazingira. Uchambuzi wa tofauti (ANOVA) ulitumika kulinganisha tofauti za ubora wa matunda, ubora wa mbegu, na kiwango cha kuweka matunda kati ya aina hizo mbili. Kruskal wallis ilitumika kupima tofauti kati ya data ambayo si kawaida kusambazwa. Majaribio ya Tukey ya Tofauti Muhimu kwa Uaminifu (HSD) na Kruskal-Wallis na Dunn yalitumika kwa data iliyosambazwa na isiyo ya kawaida mtawalia. Ili kulinganisha tofauti katika shughuli ya lishe ya nyuki, tunatumia mtihani wa kiwango cha jumla cha Wilcoxon. Tulitumia Muundo wa Linear wa Jumla (GLM) ili kuchunguza uhusiano kati ya kiwango cha kuweka matunda na shughuli ya kutafuta chakula katika nyuki, na Muundo wa regresheni wa Poisson ili kuchunguza uhusiano kati ya shughuli za kutafuta chakula, sababu za kimazingira na spishi za nyuki. Matokeo yanaonyesha kuwa idadi ya matunda kwa kila mmea, mduara wa matunda, mduara wima wa matunda, uzito wa matunda, na asilimia ya kiwango cha kuweka matunda kwa kila mmea ilikuwa kubwa zaidi katika shamba lililochavushwa na nyuki wasiouma (*Hypotrigona gribodoi*), ikifuatiwa na nyuki wanaouma (*Apis mellifera*), na mwishowe eneo shamba lisilo na wachavushaji, na tofauti ilikuwa muhimu kitakwimu ($P <0.05$). Hata hivyo, hapakuwa na tofauti kubwa katika idadi ya mbegu kwa kila tunda na uzito wa mbegu kavu 100 kwa kila tunda kati ya aina hizo mbili ($P>0.05$). Nyuki wa siouma (*Hypotrigona gribodoi*) ilionyesha ziara za juu katika maua, wafanyikazi wanaobeba chavua, na wageni kwa kila ua kuliko nyuki wanaouma (*Apis mellifera*), na tofauti ilikua muhimu kitakwimu ($P <0.001$). Kinyume chake, nyuki wanaouma (*Apis mellifera*) alikuwa na wafanyikazi wengi wanaoondoka katika mzinga ($P <0.001$). Zaidi ya hayo hakuna tofauti katika muda unaotumika kwenye ua ($P>0.05$) kati ya nyuki wasiouma (*Hypotrigona gribodoi*) na nyuki wanaouma (*Apis mellifera*). Pia idadi ya wafanyakazi wanaoingia na chavua ($P<0.001$), wafanyakazi wanaotoka kwenye mzinga ($P<0.001$), na muda wanaotumia kwenye ua ($P<0.05$) kuwa na athari kubwa kwa kiwango cha kuweka matunda ya Pilipili Hoho (*Capsicum annuum*). Idadi ya wageni kwa kila ua na idadi ya maua yaliyotembelewa hayana athari kubwa ($P> 0.05$) katika kiwango cha kuweka matunda ya Pilipili Hoho (*Capsicum annuum*). Joto na unyevu huathiri kwa kiasi kikubwa idadi ya wageni kwa kila ua, muda unaotumika kwenye maua na idadi ya wafanyakazi wanaotoka kwenye mizinga. Hata hivyo, idadi ya maua yaliyotembelewa na idadi ya wafanyakazi wanaoingia kwenye mzinga na chavua haikuathiriwa na joto na unyevu. Matokeo haya yanapendekeza kwamba nyuki wasiouma (*Hypotrigona gribodoi*) ni wachavushaji bora zaidi wa Pilipili Hoho (*Capsicum annuum L*). Zaidi ya hayo, tabia ya lishe ya nyuki wanaouma (*Apis mellifera*) na nyuki wasiouma (*Hypotrigona gribodoi*) huathiriwa na mambo ya mazingira na kuathiri kiwango cha kuweka matunda cha Pilipili Hoho (*Capsicum annuum*). Utafiti zaidi juu ya tofauti ya ufanisi wa uchavushaji kati ya spishi tofauti za nyuki katika uzalishaji wa mazao ni muhimu.

Maneno muhimu: Uchavushaji, *Apis mellifera, Hypotrigona gribodoi, Capsicum annuum L*., mavuno, kiwango cha kuweka matunda, Tabia ya lishe, Mambo ya Abiotic (joto na unyevunyevu)

DECLARATION

I, **PASCHAL HOTAY MBAZI**, 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 nor being concurrently submitted in any other institution.

HMbazi

15/05/2024

Paschal Hotay Mbazi
(MSc. Candidate)

Date

The above is confirmed by:

15/05/2024

Prof. Pantaleo K.T. Munishi
(Supervisor)

Date

LIST OF MANUSCRIPTS

Manuscript 1: Pollination efficiency of *Apis mellifera* and *Hypotrigona gribodoi* on *Capsicum annuum* yield and fruit set rate.

Manuscript 2: Impact of abiotic factors on the foraging behavior of *Apis mellifera* and *Hypotrigona gribodoi*: implication on *Capsicum annuum* fruit set rate

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I would like to give special thanks to my family, with a special mention of my beloved wife Sophia Boay, my sons Gabriel Mbazi and Gilbert Mbazi, for their prayers and unwavering moral support, which played a crucial role in my academic achievements.

DEDICATION

I dedicate this work to my wife Sophia Boay and my sons, Gabriel Mbazi and Gilbert Mbazi, who patiently waited for me during the period I undertook this research. Their love, support, and encouragement throughout my two years of study have been invaluable.

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LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

ANOVA	Analysis of Variance
DEC	Department of Ecosystem and Conservation
Df	Degree of freedom
GLM	Generalized linear model
h	Hours
m	Meters
MSc. For	Master of Science in Forestry
NBS	National bureau of statistics
RCBD	Randomized complete block design
SUA	Sokoine University of Agriculture
T.C	Transverse circumference
TFS	Tanzania Forest Services Agency
URT	United Republic of Tanzania
V.C	Fruit Vertical circumference
X^2	Chi – square

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background information

Insects provide ecosystem services through pollination and improve yield and quality of crops (Bartomeus *et al.,* 2014). Insect such as stinging bees (*Apis spp*), stingless bees (*Meliponini spp*), and other wild bee play a crucial role in the pollination of food crops and communities of wild plants on earth while searching for pollen and nectar (Musharraf *et al.,* 2021). Different insect species pollinate flowers at different ages when feeding on nectar and pollen (Komamura *et al.,* 2021). Insect pollination efficiency differences are determined by several factors such as the ability of an insect to carry pollen as determined by the structure of insects such as the presence of hair in the abdomen, behavioral and physical characteristics (Society *et al.*, 2008).

Pollination efficiency is determined by the match and mismatch between flower morphology and insect morphology (Komamura *et al.*, 2021). The efficiency of various pollinators relies on how the size of flower components compares to the size of the insect, impacting pollination effectiveness (Komamura *et al.,* 2021). The choice for insect-pollinated flowers based on shape is an effect of an evolved preference rather than familiarity with specific flowers (Howard *et al*., 2019). It is important to know the efficiency of different species of bees in pollination so as to uncover their important role in pollination because different species have different abilities for pollination. For example, ability of honey bees to pollinate is limited by diseases, parasites, low efficiency of some crop species due to differences in morphology and size and climatic limitations (Heard and Bees, 2019). Bell pepper (*Capsicum annuum*) will be used as a modal crop to quantify pollination efficiency of stingless (*Hypotrigona gribodoi*) and honey bees (*Apis mellifera)* since efficacy in yield and fruit set differ among bee species (Cruz *et al.*, 2005; Ram, 1993).

1.2 Problem statement and justification

Insect population decline has been a result of anthropogenic activities and their influence on climate and environmental change, habitat degradation, land use change and use of agrochemicals especially insecticides, climate change, and pollution effects (Wagner, 2020). There's a significant gap between the available pollinators and the pollination needs of agriculture (Mashilingi *et al.,* 2021). The demand for honeybee pollination is increasing while their pollination capacity is decreasing globally, a tendency that can be related to their annual population fluctuations (Mashilingi *et al.,* 2022).

Pollination is important services provided by bees and is one of the objectives of Tanzania National Beekeeping Policy Implementation Strategy of 2021-2031 that is to “enhanced beekeeping based national development and poverty alleviation through sustainable supply of bee products and pollination service”. Beekeeping Act Number 15 of 2002 provides a framework for the establishment of Bee Reserves that act as a source of gene pool for both stinging and stingless bees, production of bee products, api-tourism, and enhance biodiversity conservation. Its implementation is indicated in the 2022 guidelines for the establishment and management of Bee Reserves and Apiaries in Tanzania with one of the goals of biodiversity conservation and to be source of package for both stinging and stingless bees (URT, 2022). With the concern of declining naturally occurring pollinator population such as honey bee (*Apis sp*) it is important to consider management of pollination services through conservation of different type of bees to provide pollination services in both agriculture and forest ecosystems.

Increase in human population increases demand for food, forest raw material and thus productivity in agro-ecosystems. Bees play critical role in pollination of food crops and thus, agriculture can benefit from increased yield influenced by increase in bee diversity. Bees are recognized as crucial keystone species, responsible for strengthening food production systems and ecosystem. In order to improve food security, understanding of apiculture and possibilities available for increasing bees populations in agro-ecosystems are important (Riley *et al.,* 2009). Therefore in order to increase production of commercially important crops and improve ecosystem productivity it is important to consider the essential role of bees as pollinators in increasing yields (Bartomeus *et al.,* 2014) as well as their efficiency in the pollination service.

Pollination demand rises as crop diversification rises (Bartomeus *et al.*, 2014), necessitating a better understanding of different species pollination capacity. Among the major insect pollinators for crops are different bee species. Bees play a critical role in pollination by improving ecosystem and production of commercially important crops (Adamidis *et al*., 2019). While bees are significant pollinators in the ecosystem and are composed of different species their capacities and effectiveness in pollination is not well understood and or documented.

Little is known on the effectiveness of different bee species on pollination (per specific plant pollinator dependent species), extent of difference of their contribution to ecosystem and crop yields. Weather pattern such as temperature and humidity and are among abiotic factor that affect bee pollination performance (Tarakini *et al.,* 2021). Foraging activity differ among bee species due to time hours, plant species, weather conditions, and seasons (U. Layek and Karmakar, 2018). Given their capacity for honey production, foraging behavior *Apis mellifera* and *Hypotrigona gribodoi* are among the bee species of importance in crop pollination as foraging behavior determines pollination success and survival of bees as explained by optimum foraging theory that pollinators adopt a foraging strategy that provides the most energy for the lowest cost by maximizing their net energy intake per unit of time spent foraging by choosing where to, when to and what to eat and this lead to pollination success (Pyke, 2016). The quality of the pollination services provided by bee's increases with pollination performance that is measured through forage activity quality (Eeraerts *et al*., 2019).

Understanding the differential pollination efficiency among pollinators and especially bees and their pollination effectiveness is an important aspect of improving the conservation of pollinators, ecosystem productivity and crop production. This knowledge will also assist in taking advantage of the pollination capacity of different pollinators to improve especially agro-ecosystem productivity.

This study aims to assess the differential effectiveness of pollination by two bee species - *Apis mellifera* and *Hypotrigona gribodoi* on fruit set and yield in *Capsicum annuum*. The results from this study will be of significance in conservation of pollinators and management of bee populations and helpful in designing bee farms that can be used to influence positively pollination and crop yields in both agro ecosystems and natural ecosystem. This study will also have an importance in management and conservation of stingless bee as they play role in provision of pollination service and help to improve "2022 Tanzania guideline for management and use of honeybee colonies for pollination services" which mostly consider honey bee as the main pollinator of crops. On other hand information on foraging behavior in relation to environmental condition is important in developing bee's friendly habitat as foraging activity affect pollination efficiency through its direct impact on choice preference made by bees that affect directly pollination success in the plant. Experimental study design will be carried out to determine the differential effectiveness of honey bees *Apis mellifera* and stingless bees *Hypotrigona gribodoi* among other bee species in crop pollination and pollination performance in term of foraging activities of two bees.

1.3 Objectives

1.3.1 Main objective

To assess the differential efficiency of pollination by *Apis mellifera* and *Hypotrigona gribodoi* on fruit set and yield in *Capsicum annuum*.

1.3.2 Specific objective

(i) To determine the influence of *Apis mellifera* and *Hypotrigona gribodoi* pollination on *Capsicum annuum* yield
(ii) To evaluate the effects of *Apis mellifera* and *Hypotrigona gribodoi* pollination on *Capsicum annuum* fruit set rate
(iii) To evaluate effect of environmental temperature and humidity on foraging activity of *Apis mellifera* and *Hypotrigona gribodoi.*

1.3.3 Hypotheses

HO$_1$: There is no difference between *Apis mellifera* and *Hypotrigona gribodoi* pollination on *Capsicum annuum* yield

HO$_2$: There is no difference between *Apis mellifera* and *Hypotrigona gribodoi* pollination on *Capsicum annuum* fruit set rate.

HO3: There no difference in environmental effect on pollination effectiveness of *Apis mellifera* and *Hypotrigona gribodoi* on *Capsicum annuum* yield and fruit set

1.4 Limitations of the study

There were difficulties in maintaining *Apis mellifera* bee colonies for a long period during study.

1.5 Dissertation structure

This dissertation is developed in the format of publishable manuscripts comprising of five main chapters. Chapter one consists of the introduction, which provides background information of the study, problem statement and justification together with study objectives, and research Hypotheses. Chapter two is the first manuscript on Pollination efficiency of *Apis mellifera* and *Hypotrigona gribodoi* on *Capsicum annuum* yield and fruit set rate.

Chapter three is the second manuscript which is about, Impact of abiotic factors on the foraging behaviour of *Apis mellifera* and *Hypotrigona gribodoi*: implication on *Capsicum annuum* fruit set rate. Chapters four and five summarizes the general discussion, conclusions and recommendations on pollination efficiency of *Apis mellifera* and *Hypotrigona gribodoi* on *Capsicum annuum* yield and fruit set rate, their foraging activity, abiotic factor effect on foraging activity and foraging activity implication on fruit set rate.

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CHAPTER TWO

Manuscript One

Pollination Efficiency of *Apis mellifera* and *Hypotrigona gribodoi* on *Capsicum annuum* Fruit Set and Yield

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Abstract

Pollination by insects accounts for over 70% of global food crops production. Among insect species, bees are one of the most efficient pollinators though their pollination efficiency varies between species. Amidst rising worries about declining pollinator populations due to human activities, comprehending diverse pollinator capabilities is crucial for conservation. While stingless bees show promise as commercial pollinators, their capacities compared to *Apis mellifera* remain insufficiently understood. This study evaluated the pollination efficiency of *Apis mellifera* and *Hypotrigona gribodoi* on yield and fruit set in *Capsicum annuum*. A randomized complete block design experiment was conducted with three replications and three caged treatments involving *Apis mellifera, Hypotrigona gribodoi*, and a control plot without pollinators on *Capsicum annum*. Analysis of variance was employed to compare mean differences in fruit quality, seed quality, and fruit set rate among three groups. Tukey's Honestly Significant Difference (HSD) and Kruskal-Wallis and Dunn tests were used for normally distributed and non-normally distributed data respectively. The results show that the number of fruits per plant, fruit transverse circumference, fruit vertical circumference, fruit weight, and percentage fruit set rate per plant were higher in the plot pollinated with *Hypotrigona gribodoi*, followed by *Apis mellifera*, and lastly the control plot and the difference was statistically significant ($P < 0.05$). However, there was no significant difference in the number of seeds in the fruit and the weight of 100 dry seeds per fruit among the two species ($P>0.05$). These findings suggest that *Hypotrigona gribodoi* is efficient pollinator of *Capsicum annuum L.* More research on the differential pollination efficiency among different species in crop production is imperative. Prioritizing the domestication of *Hypotrigona gribodoi* bees will increase their population, resulting in positive pollination impact and enhanced crop productivity and quality.

Keywords: Pollination*, Apis mellifera, Hypotrigona gribodoi, Capsicum annuum L.,* yield, fruit set rate*.*

2.1 Introduction

Pollination is an essential ecosystem service primarily carried out by insects such as bees, butterflies, moths, flies (Elizalde *et al.,* 2020; Rader *et al*., 2016, Bronstein *et al*., 2006 ; Magwira, 2021), wind (Culley *et al*., 2002), birds and bats (Kunz *et al*., 2011 and Ollerton & Coulthard, 2009) and humans through artificial means (Broussard *et al.,* 2023). Pollination is considered as important input to crop production and ecosystem functioning (maintenance of biodiversity and ecosystem resilience) as it improves crop quality and quantity (Alemberhe & Gebremeskel, 2016). It is through pollination that pollen from the male part of a flower (the stamen) is transferred to the female part (the stigma) of the same or a different flower (Asiko, 2012), which results in the fertilization of the ovules and production of seeds (Bronstein *et al*., 2006). Pollination enables transfer of genetic material between plants (Abrol, 2012). Pollination efficiency of an insect refers to the comparative capability of an insect pollinator to effectively pollinate flowers, quantified by the fruit production per a specific unit of measurement (Brunet and Holmquist, 2009 and Keys *et al*., 2008). Over 70% of global food crops depend on pollinators (Ricketts *et al*., 2008) and without them, crop yields would be significantly reduced, impacting food security and the global economy (Gallai *et al*., 2009 and Khanna *et al*., 2021).

Insects particularly bees are most efficient pollinators (Osterman *et al*., 2021; Breeze *et al.*, 2011; Stein *et al.*, 2017). Their remarkable ability to transfer pollen between flowers significantly enhances the yield and fruit set of numerous crops (Dymond *et al*., 2021; Rauf *et al*., 2021; Fijen *et al*., 2018). Their pollination performance is largely attributed to their adeptness in moving from one flower to another (Cheng *et al*., 2016), their wide range availability (Musharraf *et al*., 2021) across different geographical conditions, their foraging behavior and floral constancy (Alemberhe and Gebremeskel, 2016). Furthermore, their propensity to visit a diverse array of plant species further underscores their importance as pollinators (Stanley *et al.,* 2020).

Apis mellifera is a species of bees belonging to the family Apidae and Genus *Apis* (Hilleman, 2009). They live in colonies and known as eusocial insects (Papa *et al*., 2022) with a division of labour among the colony members. Body structure of *Apis mellifera* is well adapted for pollination as it has specialized hairs that allow them to collect pollen (Cheng *et al.,* 2016), long tongues that allow them to reach the nectar at the base of flowers with long corollas (Borrell, 2005) with excellent colour vision that they are attracted to flowers with bright colours (Reser *et al.,* 2012). *Apis mellifera* is a pollinator of *Capsicum annuum* as they enhance productivity in fruit quality and fruit rate set (Dag and Kammer, 2001).

Hypotrigona gribodoi margaretti are small, about 2-3 mm body length (Asiko, 2012), dark-colored, stingless bees found in tropical, subtropical regions of the world and savanna ecosystems (Malovechko *et al.,* 1995). They are eusocial insects (Chakuya *et al.,* 2022) belonging to the family Apidae and subfamily Meliponinae. They forage on nectar and pollen enhancing pollination of more than 60% of different commercial crops (Heard, 1999, Atmowidi *et al*., 2022; Ramalho, 2004). Stingless bees have specialized structures called corbicula on their legs (Asiko, 2012) which enable them to efficiently gather and carry pollen. Their small body size allows them to pollinate small and delicate flowers that larger insects could potentially damage, thus making them important pollinators of many plant species in their natural habitats (Kasiera *et al*., 2022; Wakhungu *et al*., 2022 and Ndungu *et al*., 2019) and valuable contributors to the ecosystem (Slaa *et al*., 2006). *Hypotrigona gribodoi* are pollinators of *Capsicum annuum* as they enhance productivity by increasing quality of fruit and fruit set rate *(Kiatoko et al*., 2014).

Capsicum annuum belong to genus *Capsicum* and family *Solanaceae* (Pandey *et al*., 2012). It is a widely cultivated vegetable crop in different tropical and subtropical parts of the world (Pandey *et al*., 2012). Their flowers do not have poricidal anthers thus they do not require buzz pollination (Slaa *et al*,. 2006). *Capsicum annuum* flowers produce both nectar and pollen (Greco *et al.,* 2011) as flower reward to pollinators (Simpson and Neff, 1981). While the plant is capable of self-pollination, insect pollination can significantly enhance its productivity (Cruz *et al.,* 2005; Greco *et al.,* 2011).

An increase in agricultural activity leads to an increased demand for pollination services while activities associated with agriculture leads to pollinator decline (Aizen *et al.,* 2009) thus creating gap between pollination service demand and pollinator available to provide the services. Given the global concern over declining pollinator populations (Villanueva-G *et al.,* 2005; Steffan-Dewenter *et al.,* 2005) due to anthropogenic activities, including deforestation, habitat fragmentation, the use of pesticides (Chacoff, 2006; Li, 2019) and land use intensification and the expanding human population (Picanço *et al.,* 2017), there is a growing global concern regarding the continuity of essential pollination services by insects (Allen-Wardell *et al.,* 1998). As results, understanding the varying capacities of different pollinators has become imperative in order to effectively prioritize their conservation efforts.

The ecological role of stingless bees as pollinators for various plants (Norowi and Fahimie, 2010) highlights their potential as promising alternatives for commercial crop pollination, emphasizing their importance (Slaa, 2006). However, there is limited understanding of the pollination capacities of different bee species, particularly stingless bees *Hypotrigona gribodoi*, has led to limited domestication, threatening their population. While stingless bees show promise as commercial pollinators, their capacities compared to *Apis mellifera* remain insufficiently understood. This study aims to assess the differential efficiency of *Apis mellifera* and *Hypotrigona* *gribodoi* in pollinating *Capsicum annuum* and their impact on fruit set and yield. Finding from this study will have significance in improving the conservation of stingless bees, ecosystem productivity, and crop production.

2.2 Materials and methods

2.2.1 Description of study site

A pollination control experiment was conducted at the Crop Museum Organic Experimental Farm, located in the main campus of Sokoine University of Agriculture (SUA) in Morogoro municipality. Morogoro municipal is situated approximately 200 km west of Dar es-salaam, the major business town of Tanzania. The farm is positioned at latitude 6° 45' south and longitude 37° 40' east, with an elevation of 525 meters above sea level. The average temperature in the district ranges from 21.9 °C to 27.3°C. Additionally, Morogoro experiences an average annual rainfall of around 900 mm to 1000 mm (NBS Census, 2012).

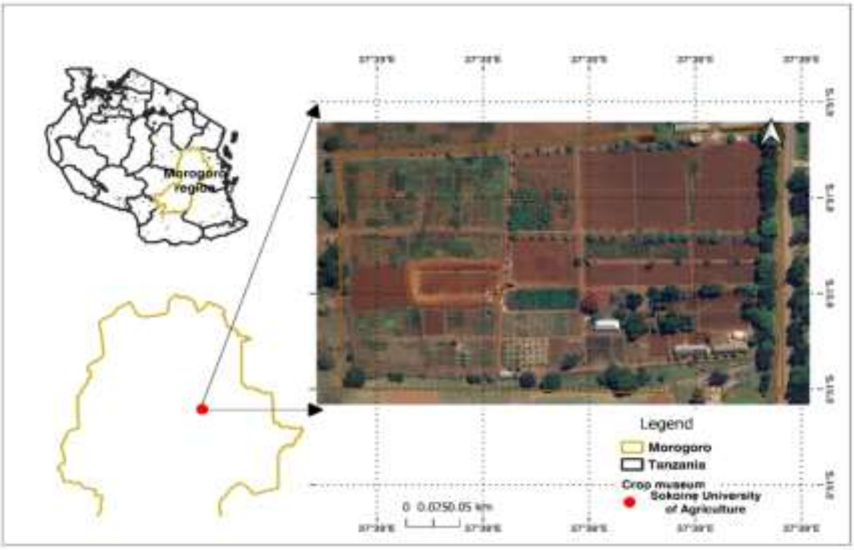

Figure 2.1: Morogoro urban map showing study area location

2.2.2 Experimental design

Experimental plots were arranged in randomized complete block design (RCBD) with three treatments and three replications. Experimental area is 56 m^2 with 4 m^2 (2 m x 2 m) plots. Plots were 0.5 m apart in replication and replication are 1M apart. Each plot was planted with 20 *Capsicum annuum* plant in five columns and four rows with plant spacing of 0.5 m between plants and rows are 0.6 m apart. Two week after sowing we cover plots with Mosquito net then we randomize three treatments which are *Hypotrigona gribodoi, Apis mellifera* and caged control plot in three replication.

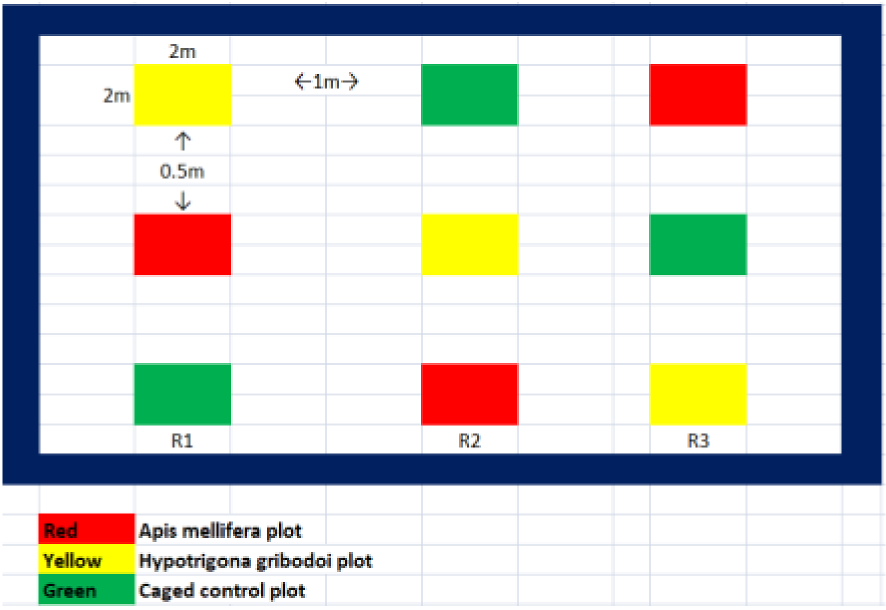

Figure 2.2: Field plot layout for the study of the difference in pollination efficiency between *A. melifera* and *H. gribodoi*

Plate 2.1: Experimental setup for studying the difference in pollination efficiency of *Apis melifera* and *Hypotrigona gribodoi* (A) prepared block with (B) Apis mellifera (C) Control caged with mosquito net

Each plot was planted with 20 *Capsicum annuum* seedlings in a spacing of 0.5m between plants where rows are 0.6 m apart. Weeding was done three times and mosquito nets were placed on plots before the initiation of flowering. Bee colonies were introduced to the plots immediately after installing the mosquito nets. Bee colonies were fed with 1.5L (50% w/v) sugar syrup solution every morning to ensure their survival as nectar available on pepper is not enough for their survival (Kwon and Saeed, 2003). First and second harvests of mature fruits were done 57 and 71 days after initial planting. Yield measurements were conducted in a laboratory.

Plate 2.2: (A) Field preparation (B) planting of *Capsicum annuum* seedling

2.3 Data collection

The number of flowers that emerged throughout the experiment from the six middle plants was counted, and after the fruit set, the total number of fruits from the same plant in each plot was counted. The fruit transverse circumference, vertical circumference and weight of ten sample fruits from each plot were measured. Additionally, seeds from ten sample fruits were extracted and counted, oven dried overnight at103^{0}C.The weight of 100 dry seeds per fruit and fruit weight was measured using an electronic balance (Sawe *et al*., 2020) with precision of 0.0005 g. Samples were taken from fruits of six middle plants with a minimum edge effect, and harvesting was done two times to aggregate the number of fruits from each plot at interval of 15 days.

2.4 Data analysis

The fruit set rate per plant, expressed in percentage, was calculated by dividing the mean number of fruits per plant harvested by the mean number of flowers per plant and then multiplied by hundred (Magwira, 2021). Pollination efficiency of each treatment was computed by dividing the total number of flowers that produced fruits by the total number of observed flowers (Putra *et al*., 2014).

$$\text{P. E} = \frac{\text{Total numbers of flowers that produce fruits}}{\text{Total number of observed flowers.}} \times 100$$

P.E represents treatment pollination efficiency

The contribution of *Apis mellifera* and *Hypotrigona gribodoi* pollination to *Capsicum annuum* yield was calculated as a percentage by comparing the mean number of fruit per plant, fruit transverse circumference, fruit vertical circumference (cm),seed dry weight, fruit weight and the number of seed per fruit between the treatment caged with bee species and the treatment caged without pollinator (Morse and Calderone, 2000; Tonola *et al.,* 2023)

$$D = \frac{\text{Yield from } \textit{Apis mellifera} \text{ pollination} - \text{Yield from control}}{\text{Yield from } \textit{Apis mellifera} \text{ pollination}} \times 100$$

$$D = \frac{\text{Yield from } \textit{H. gribodoi} \text{ pollination} - \text{Yield from caged control}}{\text{Yield from } \textit{H. gribodoi} \text{ pollination}} \times 100$$

D represents dependence of *Capsicum annuum* yield on bee pollination

Data were tested for normality using Shapiro–Wilk test. For normally distributed data, one-way Analysis of Variance (ANOVA) was employed to determine the difference between mean among three group followed by a post hoc test using Turkey HSD (honestly significant difference) for pair-wise comparison. On the other hand, Kruskal-Wallis ranks test was employed far data that were not normally distributed to compare mean among three group, and post hoc pair wise analysis was conducted using Dunn's test using Bonferroni correction. We use the trim function in R to perform Winsorization on our data to handle outliers. All analyses were performed using Microsoft excel and R Version 4.2.3 for windows (R Core Team 2023) computer software.

2.5 Results

2.5.1 Effect of *Hypotrigona gribodoi* and *Apis mellifera* on the *Capsicum annuum* Yield.

The number of fruits per plant was higher in treatments pollinated by *Hypotrigona gribodoi* (21.39±0.8) followed by *Apis mellifera* (15.94±0.5) and lastly control plot (13.5±0.7) (Table 2.2). ANOVA results reveal that number of fruits per plant was significantly different among treatments (P<0.001) (Table 2.1). The Turkey post hoc results showed that the higher difference was between plot pollinated by *Hypotrigona gribodoi* and control plot without pollinator (P= 0.0000000), followed by *Hypotrigona gribodoi* and *Apis mellifera* (P= 0.0000024), and lastly, between *Apis mellifera* and control treatments without pollinator (P= 0.03889) (Figure 1A).

Fruit transverse circumference was higher in treatments pollinated by *Hypotrigona gribodoi* (2.49±0.5 cm), followed by *Apis mellifera* (19.54±0.5 cm) and control plot (18.07±0.5 cm) (Table 2.2). ANOVA results revealed that fruit transverse circumference was significantly different among treatments (P<0.001) (Table 2.1). The Turkey post hoc results showed that the most significant difference was between the treatments pollinated by *Hypotrigona gribodoi* and the control plot without a pollinator (P= 0.0000485), followed by *Hypotrigona gribodoi* and *Apis mellifera* (P = 0.0293909). There was no significant difference between *Apis mellifera* and the control treatments without a pollinator P= 0.1268336 (Figure 2.1B).

Fruit vertical circumference was higher in treatments pollinated by *Hypotrigona gribodoi* (21.49±3.5 cm), followed by *Apis mellifera* (18.96±0.5 cm), and the control plot (17.93±0.5 cm) (Table 2.2). ANOVA results reveal that fruit vertical circumference was significantly different among treatments ($P<0.001$) (Table 2.1). The Turkey post hoc results showed that most significant difference was between the plot pollinated by *Hypotrigona gribodoi* and the control plot without a pollinator ($P= 0.0010029$), followed by *Hypotrigona gribodoi and Apis mellifera* ($P= 0.0385193$). While no significant different was observed between *Apis mellifera* and control treatments without a pollinator $P= 0.4368058$ (Figure 2.1C).

The weight of fruit per plant was higher in treatments pollinated by *Hypotrigona gribodoi* (80.32±5.6 g), followed by *Apis mellifera* (58.05±5.5 g), and lastly the control plot (50.80±4.5 g) (Table 2.2). Kruskal-Wallis results revealed that the number of fruits per plant was significantly different among the treatments ($X^2= 16.065$, df = 2, $P=0.0003247$). The Dunn's post hoc results showed that the most difference was between the plot pollinated by *Hypotrigona gribodoi* and the control plot without a pollinator ($P= 0.0002$), followed by *Hypotrigona gribodoi* and *Apis mellifera* ($P= 0.0044$). While no significant different was observed between *Apis mellifera* and control plots without a pollinator $P= 0.6055$ (Figure 2.1D).

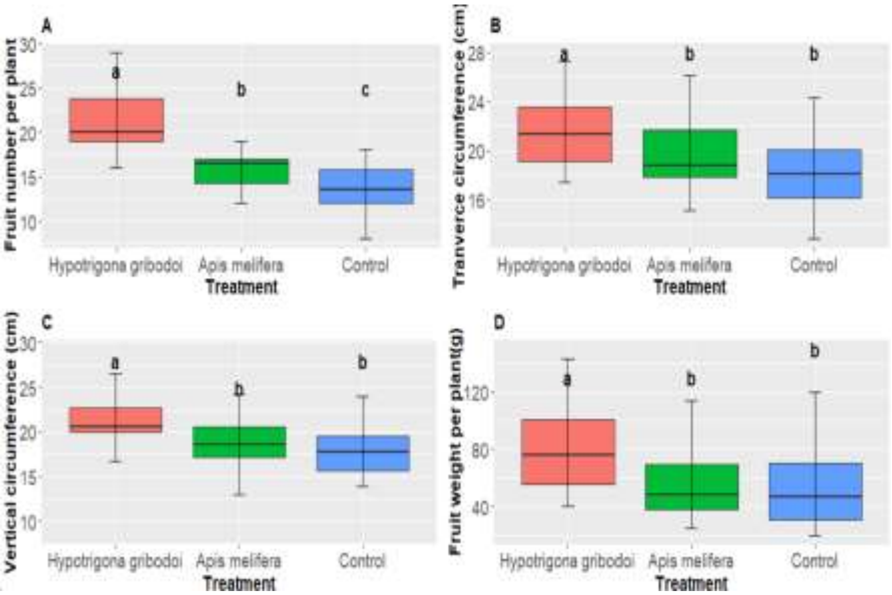

Figure 2.3: **Boxplot showing differences fruit quality (A) fruit number per plant (B) transverse circumference (C) vertical circumference and (D) fruit weight per plant in *Capsicum annum* when pollinated by *Hypotrigona gribodoi*, *Apis mellifera* and caged control plot without pollinator. The horizontal line across the box represents the median, the box represents the 25th and 75th percentiles, the vertical line outside the box represent the minimum and maximum values, and letters (a, b, and c) indicate statistical significance among treatment groups.**

Table 2.1: Analysis of Variance on the pollination effect of *Capsicum annuum*, including the number of fruits per plant, fruit transverse circumference (cm) and fruit vertical circumference (cm)

Parameter	Response	Df	Sum Sq	Mean Sq	F Value	Pr(>F)
Number of fruits per plant	Treatment	2	587.11	293.556	34.678	3.098e-10 ***
	Residuals	51	431.72	8.465		
Fruit Transverse circumference (cm)	Treatment	2	176.57	88.283	10.479	8.366e-05 ***
	Residuals	87	732.95	8.425		
Fruit Vertical circumference (cm)	Treatment	2	150.59	75.297	7.1993	0.001279 **
	Residuals	87	909.93	10.459		

Table 2.2: Summary of mean numbers of fruit per plant, seeds per fruit and seed weight per 100 seeds for all three level of treatments. Means with different letters are significantly different (P < 0.05)

Treatment effect	Number of fruit /plants	Percent age fruit set rate per plant	Fruit T.C (cm)	Fruit V.C (cm)	Seed weight /fruit	fruit weight	Number of seed/ fruits
C.C.P	13.5±0.7^{c}	45.93±1.1^{c}	18.1±0.5^{b}	17.9±0.5^{b}	1.01±0.09^{a}	50.8±4.5^{b}	189.5±13.42^{a}
H. gribodoi	21.4±0.8^{a}	72.89±1.4^{a}	21.5±0.5^{a}	24.4±3.5^{a}	2.22±0.19^{a}	80.3±5.6^{a}	153.8±11.94^{a}
A. mellifera	15.9±0.5^{b}	65.33±0.9^{b}	19.5±0.5^{b}	18.9±0.5^{b}	0.966±0.1^{a}	58.1±5.5^{b}	189.73±18.14^{a}

T.C , V.C and C.C.P are fruit transverce circumference, fruit vertical circummference and Caged control plot without pollinator repectively.

The number of seeds per fruit was higher in treatments pollinated by *Apis mellifera* (189.73±18.14), followed by the control plot without a pollinator (189.53±13.42) and lastly, *Hypotrigona gribodoi* (153.8±11.94) (Table 2.2). Kruskal-Wallis results reveal that the difference in the number of seeds per fruit was statistically insignificant among treatments (X^2= 2.2744, df = 2, P=0.3207) (Figure 2.2A).

The weight of the dry seeds per plant was higher in treatments pollinated by *Hypotrigona gribodoi* (2.22±0.19 g), followed by the control plot without a pollinator (1.01±0.09 g), and lastly *Apis mellifera* (0.966±0.1 g) (Table 2.2). Kruskal-Wallis results reveal that 100 dry seed weight per plant difference was statistically insignificant among treatments (X^2 =0.66743, *df* = 2, P = 0.7163) (Figure 2.4B).

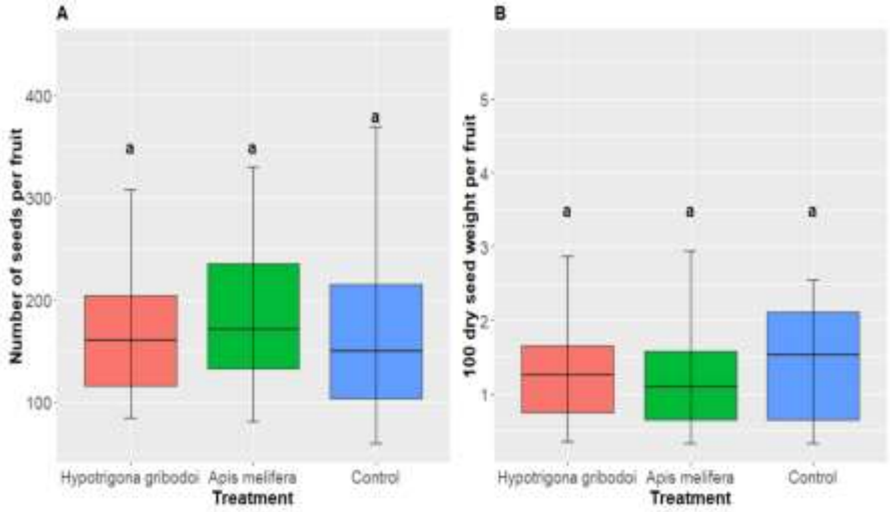

Figure 2.4: **Boxplot showing (A) The number of seeds per fruit (B) weight of the dry seeds per plant difference in *Capsicum annum* pollinated by *Hypotrigona gribodoi*, *Apis mellifera* and the caged control plot without a pollinator. The horizontal line across the box shows the median, the box represents 25th and 75th percentiles, the vertical line outside the box represents the minimum and maximum values, and letters (a, b and c) indicates statistical significance among the treatment groups.**

2.5.2 Effect of *Apis mellifera* and *Hypotrigona gribodoi* Pollination on *Capsicum annuum* fruit set rate

The fruit set rate per plant was higher in treatments pollinated by *Hypotrigona gribodoi* (72.89±1.4), followed by *Apis mellifera* (65.33± 0.9), and lastly, the control plot without a pollinator (45.93±1.1) (Table 2.2). Kruskal-Wallis results revealed that the Percentage of fruit set rate per plant was significantly different among treatments ($X^2 = 43.03$, $df = 2$ and $P = 4.53e-10$) (Figure 2.3C).The Dunn's post hoc results showed that the most significant difference was between the plot pollinated by *Hypotrigona gribodoi* and the control plot without a pollinator (P= 0.0000), followed by the caged control plot and *Apis mellifera* (P=0.0002), and lastly, between *Hypotrigona gribodoi* and *Apis mellifera* treatments (P=0.0085) (Figure 2.2C).

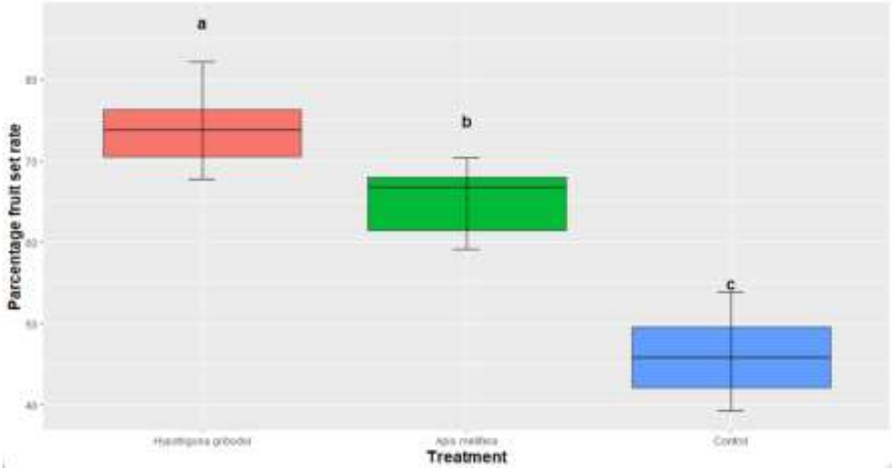

Figure 2.5: **Boxplot showing fruit set rate difference in *Capsicum annum* pollinated by *Hypotrigona gribodoi*, *Apis mellifera* and the caged control plot without a pollinator. The horizontal line across the box shows the median, the box represents 25th and 75th percentiles, the vertical line outside the box represents the minimum and maximum values, and letters (a, b and c) indicates statistical significance among the treatment groups.**

2.5.3 Pollination efficiency and contribution of *Hpotrigona gribodoi* and *Apis mellifera* on *Capsicum annuum* Yield

The pollination efficiency was higher in the plot pollinated by Hypotrigona *gribodoi* (72.78%), followed by *Apis mellifera* (65.52%), and lastly, the caged control without a pollinator (45.76%) (Figure 2.6). The yield of *Capsicum annuum* was observed to increase by 36.9%, 15.8%, 26.6%, 54.5%, and 36.7% in the number of fruits per plant, fruit transverse circumference, fruit vertical circumference,dry seed weight per fruit, and fruit weight, respectively, while the number of seeds per fruit was observed to decrease by 23.3% in *Hypotrigona gribodoi* Pollinated treatments.

On the other hand, in *Apis mellifera* pollinated treatments, the yield of *Capsicum annuum* showed a 15.1% increase in the number of fruits per plant, 7.1% increase in fruit transverse circumference, 5.2% increase in fruit vertical circumference, 12.5% increase in fruit weight, and a 0.12% increase in the number of seeds per fruit; however, the 100 dry seed weight per fruit decreased by 4.55%.

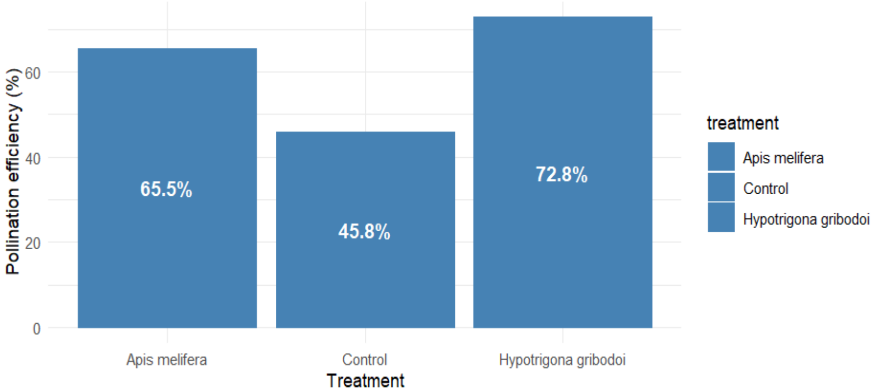

Figure 2.6: Pollination efficiency under treatments pollinated by *Hypotrigona gribodoi, Apis mellifera,* and the control group (without a pollinator)

2.6 Discussion

2.6.1 Effect of *Hypotrigona gribodoi* and *Apis mellifera* on the *Capsicum annuum* Yield (fruit and seed quality)

The findings show that treatments pollinated by *Hypotrigona gribodoi* have higher mean number of fruits, fruit transverse circumference, fruit vertical circumference, and weight of fruit. These results signify the successful pollination of *Capsicum annuum* by stingless bees such as *Hypotrigona gribodoi*, resulting in the production of a greater quantity of larger and heavier fruits. Our findings are also similar to study by Putra *et al.* (2016) on stingless bees where *Trigona minangkabau* and *Tetragonula leaviceps* increases in *Capsicum annuum L* number of fruits by 29.31% and 25.06% and fruit weight per plant 66.46% and 49.75%, respectively. Atmowidi *et al.* (2022) found stingless bee *Heterotrigona itama* increase in number of fruits in melon. Cruz *et al*. (2005) found that sweet pepper pollination by *Melipona subnitida* produce heavier, wider, and higher quality fruits with a lower percentage of malformed fruits compared to self-pollinated sweet pepper. Stingless bee *Melipona Fasciculate* improves fruit yield and quality of *Solanum melongena L* (Nunes-Silva *et al.,* 2013). Stingless bees *Trigona carbonaria*, are efficient pollinator of *macadamia* compared to *Apis mellifera* due to the fact that they mainly collect pollen, resulting in intimate contact with the stigma while honey bees like *Apis mellifera* mainly collect nectar, resulting in less frequent contact with the stigma (Heard, 2016). These outcomes collectively indicate that effective pollination by stingless bees contributes to a reduction in fruit malformation, as supported by (Cruz *et al*., 2005). This improved pollination is crucial for raising crop quality standards as similar findings in passion fruit were found in other studies (Shahidah, 2018; Wietzke *et al*., 2018).

Contrary to our findings, stingless bees *Nannotrigona perilampoides* have similar *Lycopersicon esculentum* fruit weight as control plots without pollinators (Cauich *et al.*, 2004), while research by Kendall *et al.* (2022) demonstrates similar blueberry fruit weights between plots visited by honey bees and stingless bees; furthermore, studies reveal that the stingless bee *Trigona iridipennis* and honey bee *Apis cerana* exhibit similar efficacy in yielding tomato pollination (Putra & Kinasih, 2013).

Findings from this study show that *Hypotrigona gribodoi* and *Apis mellifera* have similar effect on number of seeds per fruit but seed weight was higher in plot pollinated by *Hypotrigona gribodoi*. Despite insignificant difference observed in this study, dry seed weight were slightly higher in plot pollinated by *Hypotrigona gribodoi* compare to *Apis mellifera* and caged control plot, indicating successful fertilization and resulting in improved reproductive fitness through the quantity of pollen deposited by the pollinator on the stigma of the flower (Dogterom *et al*., 2000). Stingless bees primarily carry pollen from flowers (Puteri *et al., 2022)*, thereby increasing the chances of successful pollination for the plants.

Contrary to our findings, study conducted by Kiatoko *et al.* (2014) reported that *Hypotrigona gribodoi* produce *Capsicum annuum* fruit with higher seed quality in terms of number and weight compared to self-pollination and unmanaged pollination (by feral insects). Aditionaly stingless bees *Trigona minangkabau* and *Tetragonula leaviceps* increase *Capsicum annuum L*. number of seeds by 56.36% and 45.91%, respectively compared to wind pollination (Putra *et al.,* 2016). Futhermore, *Lycopersicon esculentum* number of seed were different in plot pollinated by stingless bee *Nannotrigona perilampoides*, plot without pollinator and mechanical vibration plot (Cauich *et al.,* 2004).

2.6.2 *Hypotrigona gribodoi* and *Apis mellifera* pollination impact on *Capsicum annuum* fruit set rate

Our finding shows that fruit set rate was higher in plots pollinated by *Hypotrigona gribodoi* compared to *Apis mellifera* and the caged control plot. This indicates that *Hypotrigona gribodoi* is more efficient pollinator of *Capsicum annuum* compared to *Apis mellifera*. Our findings are similar to those of Putra *et al.* (2016), where stingless bees *Trigona minangkabau* and *Tetragonula leaviceps* increased the fruit set rate of *Capsicum annuum L*. by 12.32% and 9.66% respectively. Cauich *et al.* (2004) finds that *Nannotrigona perilampoides* yielded a higher fruit set in the pollination of tomato *Lycopersicon esculentum* while Atmowidi *et al.* (2022) report that *Tetragonula laeviceps* increased strawberry fruit set rates and reduced abnormal fruits. Additionally, stingless bees *Lepidotrigona terminate* were reported to have increased the fruit set rate of *Coffee arabica and* Coffea by 80% and 84%, among coffee varieties (Slaa, 2006; Klein, 2013). Successful pollination of flowers by *Hypotrigona gribodoi* increases the fruit set rate. The ability of insect pollinators to deliver pollen to flower stigmas (Vit *et al*., 2018) is influenced by morphological and behavioural traits, such as larger body length, increased hairiness, and longer visits durations *(Phillips et al*., 2018). *Hypotrigona gribodi*, being smaller in size (Eardley, 2004; Kajobe, 2007), is associated with a higher pollen carrying capacity (Ramalho *et al.,* 1998; Mayes *et al.,* 2019), enabling them to efficiently navigate and access flowers compared to *Apis mellifera.* This size difference could be one of the determinants of successful pollination, as suggested by (Kiatoko *et al*., 2022), that body size is a factor that influences pollination. Their ability to adapt to environmental stress allows them to thrive and perform well even in challenging conditions, enhancing their suitability as pollinators in diverse agricultural environments (Atmowidi *et al.*, 2022).

2.6.3 *Apis mellifera* and *Hypotrigona gribodoi* Pollination efficiency and percentage contribution in yield of *Capsicum annuum*

Based on this study, *Hypotigona gribodoi* has higher pollination efficiency than *Apis mellifera*. The Percentage contribution to *Capsicum annuum* yield in term of the number of fruits per plant, fruit transverse circumference, fruit vertical circumference, 100 dry seed weight per fruit and fruit weight was higher in the *Hypotrigona gribodoi* plot compare to *Apis mellifera* plot, while the number of seed was higher in the plot pollinated by *Apis mellifera*. Asiko (2012) report that, *Hypotrigona gribodoi* has a similar effect to *Apis mellifera* on the pollination of strawberries. Compared to *Apis mellifera*, the smaller size *of Hypotrigona gribodoi* contribute to their enhanced effectiveness as pollinators for *Capsicum annuum* flowers, as result of their specific behaviours or strategies, such as flight patterns or pollen transfer mechanisms, as suggested by other studies, including their ability to forage effectively in enclosed conditions (Slaa, 2006). Results show that *Hypotrigona gribodoi* and *Apis mellifera* increase *Capsicum annuum* pollination compared to caged control plot without pollinator Bobadoye (2023) has reported that, stingless bees foragers are capable of detecting and differentiating scent trails left by foragers workers of both the same and foreign species. This allows them to avoid visiting food sources, such as flowers, that have already been visited by foragers from different species to avoid competition and conflict due their less aggressive behaviour and increase their chances of survival. Thus, they tend to enhance pollination success by pollinating flowers that have not yet been pollinated by foragers from different pollinator species, increasing the likelihood of pollinating all the flowers within a tree.

2.7 Conclusion

Apis mellifera and *Hypotrigona gribodoi* enhance the pollination of *Capsicum annuum*. There is variation in the level of pollination between the two bee species. *Hypotrigona gribodoi* exhibits higher yields, particularly in terms of fruit quality and fruit set, for *Capsicum annuum*. The number of seeds and dry seed weight per fruit are similar between *Hypotrigona gribodoi* and *Apis mellifera*. Our findings suggest that *Hypotrigona gribodoi* is a more effective pollinator of *Capsicum annuum* compared to *Apis mellifera*. Moreover, the findings suggest that *Hypotrigona gribodoi* and *Apis mellifera* are crucial for cross-pollination. The study underscores the importance of enhanced pollination, primarily facilitated by the stingless bee *Hypotrigona gribodoi*. This underscores their role in elevating crop quality standards, as evidenced by increased fruit production, larger and heavier fruits, and improved seed quality.

Stingless bees, such as *Hypotrigona gribodoi* play an important role in ecological balance as pollinators of various wild plant species and also emerge as strong contenders for providing commercial pollination services (Slaa *et al*., 2006). Considering the importance of pollination services provided by bees, which enhance both the quality and quantity of crops and have a direct positive impact on the global economy and dietary outcomes (Musharraf *et al*., 2021), as well as their contribution to maintain plant species diversity, ensuring ecosystem resilience (Senapathi *et al.,* 2015) and as outlined in the objectives of the Tanzania National Beekeeping Policy Implementation Strategy of 2021-2030 (URT, 2021), it is crucial to adopt conservation strategies that go beyond solely focusing on *Apis mellifera* as the only potential pollinator species. Therefore, it is imperative to include stingless bee species, such as *Hypotrigona gribodoi*, in these conservation efforts.

2.8 Recommendation

- More research is needed to understand the biology, behaviour, and ecological needs of stingless bees *Hypotrigona gribodoi.* Conducting research on the diversity and distribution of stingless bees, their role in pollination, and their response to different management practices can help inform conservation efforts.

- Conservation policies aimed at protecting pollinators should consider stingless bees *Hypotrigona gribodoi* as important contributors to improving commercial crop yields, enhancing food security, and maintaining ecosystem health.

- Raise awareness among people about the value of stingless bees in pollination and promote their conservation. Creating a favourable environment for the conservation of stingless bees can be achieved by educating farmers, decision-makers, and the general public about the importance of these insects. Furthermore, conserving natural habitats such as forests, mangroves, and wetlands will provide a safe habitat for these bees to thrive.

Prioritizing the domestication of *Hypotrigona gribodoi* bees will increase their population, resulting in positive pollination impact and enhanced crop productivity and quality.

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Declarations

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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CHAPTER THREE

Manuscript Two

Impact of Abiotic factors on the foraging behaviour of *Apis mellifera* and *Hypotrigona gribodoi*: implication on *Capsicum annuum* fruit set rate.

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Abstract

Bees are potential pollinators of crops, but changes in weather are possible causes of their decline. Weather changes directly affect their behaviour, specifically their ability to forage, which, in turn, affects their pollination performance. Thus, this study was conducted to understand the effect of weather on the foraging behaviour of *Apis mellifera and Hypotrigona gribodoi* and its implication for *Capsicum annuum* fruit set rate. Two treatments applied, where plants were caged with *Apis mellifera* and *Hypotrigona gribodoi* using mosquito nets. To compare difference in bee foraging activity, we use the Wilcoxon rank-sum test. We employed a Generalized Linear Model (GLM) to investigate the relationship between fruit set rate and foraging activity, and a Poisson regression model to explore the relationship between foraging activity, abiotic factors, and bee species. *Hypotrigona gribodoi* showed higher flower visits, pollen-carrying workers, and visitors per flower than *Apis mellifera*, with significant differences ($P < 0.001$). Conversely, *Apis mellifera* had more exiting workers ($P < 0.001$).Moreover, no difference in time spent on the flower ($P>0.05$). Also the number of workers entering with pollen ($P<0.001$), workers exiting the hive ($P<0.001$), and time spent on the flower ($P<0.05$) have a significant effect on the fruit set rate of *Capsicum annuum*. The number of visitors per flower and the number of flowers visited do not have a significant effect ($P>0.05$). Temperature and humidity have a significant impact on both the number of bee visitors per flower and the duration they spend on each flower. Additionally, temperature affects the number of bee workers leaving the hives. However, the number of flowers visited and the number of workers entering the hive with pollen were not influenced by temperature and humidity. These findings suggest that the foraging behaviour of *Apis mellifera* and *Hypotrigona gribodoi* is affected by environmental factors, influencing the fruit set rate of *Capsicum annuum*, with *Hypotrigona gribodoi* being identified as effective pollinators for this plant.

Keywords: *Apis mellifera, Hypotrigona gribodoi, Capsicum annuum,* Fruit set rate, Foraging behaviour, Pollination and Abiotic factors (temperature and humidity)

3.1 Introduction

Pollination is one of the ecosystem services that ensure survival of plant species by improving their productivity in both crop and agro-ecosystem (Duque-Trujillo *et al.,* 2023). Approximately 75% of plant species worldwide depend on insect pollinators for production of quality fruits and seeds, thereby increasing food production (Bartomeus *et al.,* 2014 and Klein *et al.*, 2007). Considering the importance of efficient and effective pollinators in maintaining ecosystem stability (Crenna *et al.,* 2017) and crop productivithy, the Ministry of Natural Resources in Tanzania recognizes bees as crucial pollinators and actively works towards conserving them to ensure the provision of pollination services for crop plants and enhance the resilience of the ecosystem (URT., 1998). Foraging activity determines the pollination performance of bee species, as the higher the frequency of flower visits, the greater the chance for the species to come into contact with the stigma of the flower (Eeraerts et al., 2019)**.** Bees forage on the flowers of different plants for nectar and pollen (Silva *et al.,* 2013), unintentionally transferring pollen from the male parts of one flower to the female parts of another flower of the same species (Galen *et al.,* 2017). This fertilizes the flower, ensuring its reproduction and allowing it to produce high quality fruit and seeds (Karbassioon *et al.,* 2023). Foraging activity differ among insects leading to variation in pollen removal from anthers and pollen deposition on stigmas (Hasegawa *et al.,* 2015), resulting to variations in pollination performance in terms of fruit quality and fruit set rates due to variations in the interaction between flowers and pollinators (Baranzelli *et al.,* 2020). Extreme weather conditions, including temperature, humidity, and rainfall, can disrupt the foraging activity of pollinator insects (Uthoff & Ruxton, 2022), leading to unfavourable effects on plant-pollinator interactions and resulting in significant ecological and economic consequences (Lawson & Rands, 2019 and Hegland *et al.,* 2009). Tolerance levels to weather vary among different insects (Uthoff & Ruxton, 2022).

The foraging behaviour of honey bees (*Apis mellifera)* is influenced by the interaction between the bee colony and ambient environmental conditions, such as temperature, solar radiation, humidity, wind speed, flower nectar quantity, and plant flowering rhythm (Larke & Obert, 2018, Djonwangwe *et al.,* 2011, Di Trani *et al.,* 2022,Cui and Corlett, 2016, Larke and Obert, 2018 ,Basari *et al.,* 2018 and Farooqi *et al*., 2021), which affects their frequency of flower visits (Primack & Inouye, 1993). Several studies have reported higher pollen collection by honey bees in the early morning, lower amounts in the afternoon, and a peak foraging period observed between 11:00 to 12:00h (Abou-Shaara, 2014)*.* The colony's response to environmental conditions, coupled with the availability of floral resources, determines the timing and intensity of honey bee foraging activity (Pernal & Currie, 2001 and Honey *et al.,* 2021). Higher temperature tend to increase flower visitor activity, while high relative humidity tend to suppresses bee activity (Puentes *et al.,* 2019).

Stingless bees (Meliponines) are social bees, and their foraging activity is also affected by environmental condition such as temperature and humidity (Dantas, 2016, Asma *et al.,* 2019 and Palma *et al.,* 2008). They work together to maintain a constant temperature because variations in ambient temperature affect their activity (Dantas, 2016).

Capsicum annuum, unlike other Solanaceae species, lacks poricidal anthers and, therefore, does not rely on buzz pollination (Slaa., *et al* 2006). Instead, their flowers undergo self-pollination, although insect pollination enhance fruit formation, size, seed count, and shape (Slaa., *et al* 2006). The Pollination efficiency of *Capsicum annuum*, as one of angiosperm plant, is related to strategies developed to attract pollinators (Puentes *et al*., 2019), which represents a revolutionary relationship between plant and its pollinators. Pollination of *Capsicum annuum* flowers by insects has a positive effect on enhancing fruit quality (Reichenbach *et al.,* 2019, Nyamasyo *et al*., 2020 and Kiatoko *et al.,* 2014).

The role of bees in pollination of different crop and ecosystem resilience is well known (Alemberhe and Gebremeskel, 2016, Boruff *et al.,* 2021 and Huang *et al.,* 2021). However they face challenges from biotic and abiotic factor, particularly fluctuations in level of environmental temperature and humidity (Owayss & Basuny, 2017 and Wu *et al.,* 2022). These factors directly affect their foraging activity and consequently, their impact on pollination performance (Farooqi *et al*., 2021). Understanding the foraging behaviour of bees in search of food is crucial for maintaining effective pollination services. This study aims to investigate foraging behaviour of *Apis mellifera* and *Hypotrigona gribodoi*, and their impact on the fruit set rate of *Capsicum annuum*. Additionally, the study aims to explore the influence of temperature and humidity on the foraging behaviour of *Apis mellifera* and *Hypotrigona gribodoi*. Understanding these dynamics is essential for studying bee behaviour and their ecological role as pollinators.

3.2 Material and methods

3.2.1 Description of the study site

A pollination control experiment was conducted at the Crop Museum Organic Experimental Farm, which is located on the main campus of Sokoine University of Agriculture (SUA) in Morogoro municipal. Morogoro municipal is situated approximately 200 km west of Dar es-salaam, the major business town of Tanzania. The farm is positioned at latitude 6° 45' south and longitude 37° 40' east, with an elevation of 525 meters above sea level in Morogoro Region. The average temperature in the district ranges from 21.9°C to 27.3°C. Additionally, Morogoro experiences an average annual rainfall of around 900 mm to 1000 mm (NBS Census, 2012).

3.2.2 Experimental design

The experimental plots were arranged in a randomized complete block design (RCBD) consisting of two treatments and three replications. The experimental area covered 36 square meters, with each individual plot measuring 4 square meters (2 meters by 2 meters). The plots within each replication were spaced 0.5 meters apart, and the replications were one meter apart. In each plot, 20 *Capsicum annuum* plants were planted in a grid pattern, with five columns and four rows. The spacing between plants within a row was 0.5 meters, and the rows were 0.6 meters apart. To protect the plants, the plots were covered with a mosquito net.

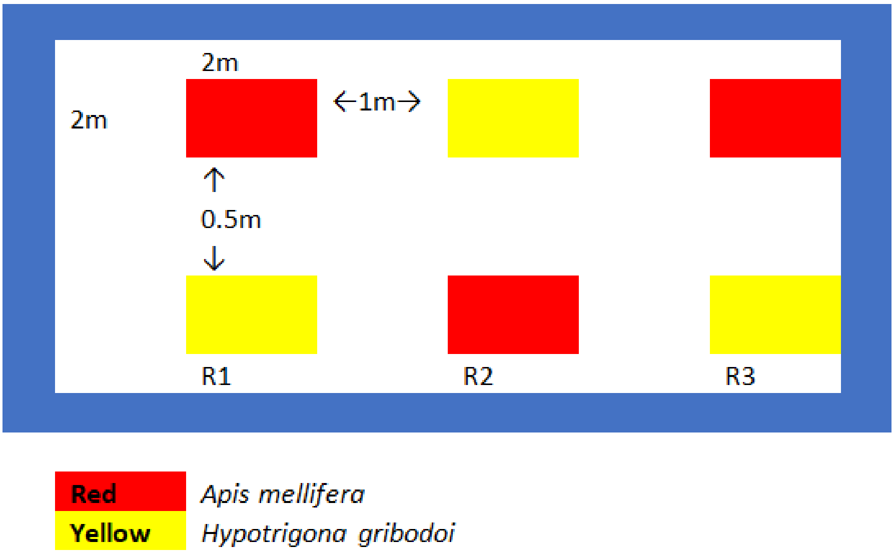

Figure 3.1: Field plot layout for the study of the difference in foraging activities in *Apis melifera* and *Hypotrigona gribodoi*

3.2.3 Field work

On December 14, 2022, "Hercules F1 *Capsicum annuum*" seeds from Seed Co were planted in a nursery bed located within a greenhouse. Horticultural substrates (potting soil) from Kekkila Oy, Ratatie 11, FI-01300 Vantaa, were used in the nursery. Organic fertilizers (Bio-genic fertilizer) were applied. Land preparation took place on January 2, 2023, involving the removal of weeds and oil excavation the designated areas for the experimental plots. The land preparation was carried out manually using a hand hoe. On 16th January 2023, the seedlings were planted in the experimental plots. Each plot was planted with 20 *Capsicum annuum* seedlings, with a spacing of 0.5m between plants, and rows were 0.6m apart. The first weeding was conducted on January 31, 2023, followed by subsequent weed removal on February 15, 2023, and March 1, 2023. On January 20, 2023, mosquito nets were placed over the plots, and on 30th January, bee colonies were introduced to the plots. The bee colony was fed with 1.5 litres 50% w/v sugar syrup solution every morning to ensure their survival, as the nectar available on pepper plants is not sufficient for their survival (Kwon & Saeed, 2003). Data collection began on 28nd February 2023 to 13, March.

Plate 3.1: (A) plot with *Apis mellifera* hive (B) plot with *Hypotrigona gribodoi* hive and (C) bee visiting *Capsicum annuum* flower

3.2.4 Data collection

To determine how weather conditions affect *Apis mellifera* and *Hapotrigina gribodoi* flower visits, we acquire weather data on various temporal scales. We used weather logger, Maxime Integrated iButton Hydrochron device with registration number (5B00000021AC3841), to record real time temperature and humidity. These measurements were taken at three-time interval namely morning (0800 to 0900 hrs), noon (1200 to 1300) and afternoon (1600 to 1700 hrs) (Nielsen *et al.,* 2017). Data recorder was set to take measurement of real time temperature and humidity data at interval of ten minute, six times at each time interval while observing foraging behaviour data. The data loggers were elevated 2.5 meters above the ground on wooden platforms at the edge of a plot's net cover. Observations on the number of visitors per flower, the number of workers exiting colonies, the number of workers entering with pollen, time spent per flower using stopwatch with defined time in seconds and number of open flowers were recorded three times a day (morning, noon and afternoon) (Tarakini *et al*., 2021). Six observers were assigned to make observation at each plot and at each time interval, observer make observation at interval of ten minute and recorded the date to avoid confusion in taking recordings. Observers shifted plot to plot each day to minimize biases associated with recoding data. Bee foraging activity data and weather data recording were conducted simultaneously for a period of fourteen (14) days, from February 28, 2023, to March 13, 2023.To determine the fruit set rate, we recorded the number of flowers that emerged and the number of fruits that reached maturity. To avoid edge effect, we collected data on the six middle plants.

3.2.5 Data analysis

Preliminary exploratory analyses were conducted on the dataset to look for outliers and investigate relationships between response variables and explanatory variables, following the methods outlined by (Zuur *et al.,* 2010, Tranmer *et al.,* 2020 and Sawe *et al.,* 2020). The Shapiro-Wilk test was used to assess the normality of the data, and the Wilcoxon rank-sum test was employed to compare foraging activity of *Apis mellifera* and *Hypotrigona gribodoi*. Furthermore, a generalized linear model (GLM) with a Gaussian family (Equation 1) was used to predict the effect of bee foraging activities and species type (*Apis mellifera* and *Hypotrigona gribodoi)* on *Capsicum annuum* fruit set rates. The fruit set data was scaled to range from 0 to 1 by dividing each value by 100 in order to fit the model.

$\boldsymbol{g}(\mu\boldsymbol{i}) = \boldsymbol{\alpha} + \boldsymbol{\beta_1 x_{i1}} + \boldsymbol{\beta_2 x_{i2}} + \boldsymbol{\varepsilon}$ …… (Equation 1)

g(μi), α , x_{i1}, x_{i2} represents *Capsicum annuum* fruit rate set, intercept, independent variable bee foraging activity and independent variable bee species respectively where $\boldsymbol{\beta_1}$ and $\boldsymbol{\beta_2}$ are coefficients associated with the independent variables x_{i1}, x_{i2} respectively. ε is error term accounting for variability in the dependent variable.

On the other hand, effects of temperature, humidity, and bee species on bees foraging activities was performed using Poisson distribution with log link function (Equation 2). The response variables (bee foraging activities) are the number of visitors per flower, the number of workers exiting the hive, the number of workers entering the hives with pollen, time spent on flower in seconds and the number of flowers visited.

$\boldsymbol{g}(\mu\boldsymbol{i}) = \boldsymbol{\alpha} + \boldsymbol{\beta_1 x_{i1}} + \boldsymbol{\beta_2 x_{i2}} + \boldsymbol{\beta_3 x_{i3}} + \boldsymbol{\varepsilon}$ …… (Equation 2)

g(μi), α , x_{i1}, x_{i2}, x_{i3} represents bee foraging activities, intercept, independent variable bee temperature, independent variable humidity, independent variable bee species respectively where β_1, β_2 and β_3 are coefficients associated with the independent variables x_{i1}, x_{i2} and x_{i3} respectively. ε is error term accounting for variability in the dependent variable.

All analysis was conducted by using R Version 4.2.3 for windows (R Core Team 2023) computer software.

3.5 Results

3.5.1 Foraging activity of *Apis mellifera* and *Hypotrigona gribodoi* in pollination of *Capsicum annuum*

A total of 756 observations were collected over 14 days for each foraging activity variable within each bee species. The number of flowers visited, workers entering the hive with pollen, and the numbers of visitors per flower were higher in *Hypotrigona gribodoi* than in *Apis mellifera*, and the differences were statistically significant (P < 0.001). Conversely, the number of workers exiting the colony was higher in *Apis mellifera* compared to *Hypotrigona gribodoi*, and this difference was also statistically significant (P < 0.001) (Figure 3.2). On the other hand, the number of workers exiting the hive was higher in *Apis mellifera* (W = 11856, P=1.281e-11). Furthermore, the time spent on flowers was higher in *Apis mellifera* compared to *Hypotrigona gribodoi*, however, the difference was statistical insignificance (W = 8430.5, P=0.3951) (Table 3.1).

Table 3.1: A summary of the mean number of Foraging Activities of *Apis mellirefa* and *Hypotrigina gribodi* in *Capsicum annuum* pollination

Treatment	Number of flowers visited	Number of workers entering with pollen	Number of workers exiting colon	Time spent on flower (sec)	Number of visitors per flower
A mellifera	7.77 ± 0.256^{a}	13.52 ± 0.95^{a}	177.63 ± 8.31^{a}	180.89 ± 10.14^{a}	2.67 ± 0.13^{a}
H gribodoi	9.92 ± 0.36^{b}	19.404 ± 1.35^{b}	103.33 ± 6.304^{b}	160.67 ± 8.89^{a}	3.17 ± 0.12^{b}

3.5.2 Effect of *Apis mellifera* and *Hypotrigona gribodoi* Foraging Activities on *Capsicum annuum* fruit set rate

The fruit set rate of *Capsicum annuum* plants is influenced by the foraging activity of *Apis mellifera* and *Hypotrigona gribodoi*. There is a positive correlation between the *Capsicum annuum* fruit set rate and the number of workers entering with pollen (P<0.001), the number of workers exiting the hive (P<0.001), and the time spent on flowers (P<0.05) by *Hypotrigona gribodoi* and *Apis mellifera*. On the other hand, the number of visitors per flower and the number of flowers visited exhibit insignificant correlation with the *Capsicum annuum* fruit set rate (P>0.05) (Table 3.2). Additionally, *Hypotrigona gribodoi* has a significant positive influence on *Capsicum annuum* fruit set (P < 0.001) (Table 3.2).

Table 3.2: Generalized linear model Summary on relationship between *Capsicum annuum* fruit set rate and *Apis mellifera* and *Hypotrigona gribodoi* foraging activity

Models	Explanatory variable	Estimate	Std. Error	t value	Pr(>|t|)
1	Intercept	0.6542397	0.0029360	222.835	<2e-16 ***
	Visitor per flower	0.0002795	0.0008854	-0.316	0.753
	H. gribodoi	0.0755546	0.0025074	30.133	<2e-16 ***
2	Intercept	6.491e-01	2.115e-03	306.987	<2e-16 ***
	workers entering with pollen	3.224e-04	9.259e-05	3.482	0.000587 ***
	H. gribodoi	7.352e-02	2.471e-03	29.747	<2e-16 ***
3	Intercept	6.390e-01	2.983e-03	214.174	<2e16 ***
	worker exiting hive	8.167e-05	1.404e-05	5.817	1.83e-08 ***
	H. gribodoi	8.148e-02	2.540e-03	32.077	<2e-16 ***
4	Intercept	6.488e-01	2.698e-03	240.448	<2e16 ***
	time spent on flower	2.608e-05	1.146e-05	2.276	0.0237 *
	H. gribodoi	7.594e-02	2.454e-03	30.944	<2e-16 ***
	Intercept	0.6500007	0.0032577	199.526	<2e-16 ***
	Number of flowers visited	0.0004496	0.0003545	1.268	0.206
5	*H. gribodoi*	0.0744477	0.0025759	28.901	<2e-16 ***

P-values, represented by apostrophes, indicate the significance levels of relationships, with the number of apostrophes corresponding to the significance of the relationship ‘***’ P<0.001 ‘**’ P< 0.01 ‘*’ P< 0.05

3.5.3 Effect of temperature and humidity on the foraging activities of *Apis melifera* and *Hypotrigona gribodoi* during pollination of *Capsicum annuum*.

At the time of data collection, the minimum and maximum temperatures were 25.88°C and 37.11°C, respectively, with a mean of 32.99°C ± 0.2382. The minimum and maximum humidity levels were 31.17% and 77.68%, respectively, with a mean of 52.17% ± 0.8157.The foraging activities of *Hypotrigona gribodoi* and *Apis mellifera* are influenced by weather conditions. There is a positive correlation between temperature with the number of visitors per flower ($P<0.05$), workers entering the hives with pollen ($P<0.001$), and workers exiting the hives ($P<0.001$). On the other hand, temperature and humidity show a negative correlation with the time spent on the flower ($P<0.001$). The number of flowers visited shows no correlation with temperature and humidity ($P>0.05$). *Hypotrigona gribodoi* is positively correlated with the number of visitor per flower ($P<0.05$), the number of workers entering the hive with pollen ($P<0.001$), workers exiting the hives ($P<0.001$), the number of flowers visited ($P<0.001$) and negatively correlated with the time spent on the flower ($P<0.001$).

Table 3.3: Summary of Generalized linear model relationship between *Capsicum annuum* flower visitation and temperature, humidity, and bee species

Response variable	Explanatory variable	Estimate	Std. Error	z value	Pr(>|z|)
Number of visitor per flower	Intercept	-2.18445	1.38105	-1.582	0.1137
	Temperature	0.06192	0.02897	2.137	0.0326 *
	Humidity	0.02141	0.00840	2.549	0.0108 *
	H. gribodoi	0.17185	0.07404	2.321	0.0203 *
Number of workers entering the hive with pollen	Intercept	0.390758	0.570544	0.685	0.493416
	Temperature	0.045387	0.011980	3.789	0.000151 ***
	H. gribodoi	0.36165	0.03156	11.458	< 2e-16 ***
	Humidity	0.013676	0.003478	3.932	8.41e-05 ***
Number of workers exiting the hives	Intercept	4.327904	0.189638	22.822	< 2e-16 ***
	Temperature	0.020278	0.003988	5.085	3.68e-07 ***
	H. gribodoi	-0.541803	0.011022	-49.155	< 2e-16 ***
	Humidity	0.003487	0.001161	3.004	0.00267 **
Time spent on flower	Intercept	6.8095663	0.1604313	42.445	< 2e-16 ***
	Temperature	-0.039245	0.0033756	-11.626	< 2e-16 ***
	Humidity	-0.006132	0.0009901	-6.194	5.87e-10 ***
	H. gribodoi	-0.118502	0.0096576	-12.270	< 2e-16 ***
Number of flowers visited	Intercept	1.233214	0.753441	1.637	0.102
	Temperature	0.016915	0.015834	1.068	0.285
	Humidity	0.004958	0.004612	1.075	0.282
	H. gribodoi	0.244367	0.042678	5.726	1.03e-08 ***

P-values, represented by apostrophes, indicate the significance levels of relationships, with the number of apostrophes corresponding to the significance of the relationship ‘***’ P<0.001 ‘**’ P< 0.01 ‘*’ P< 0.05

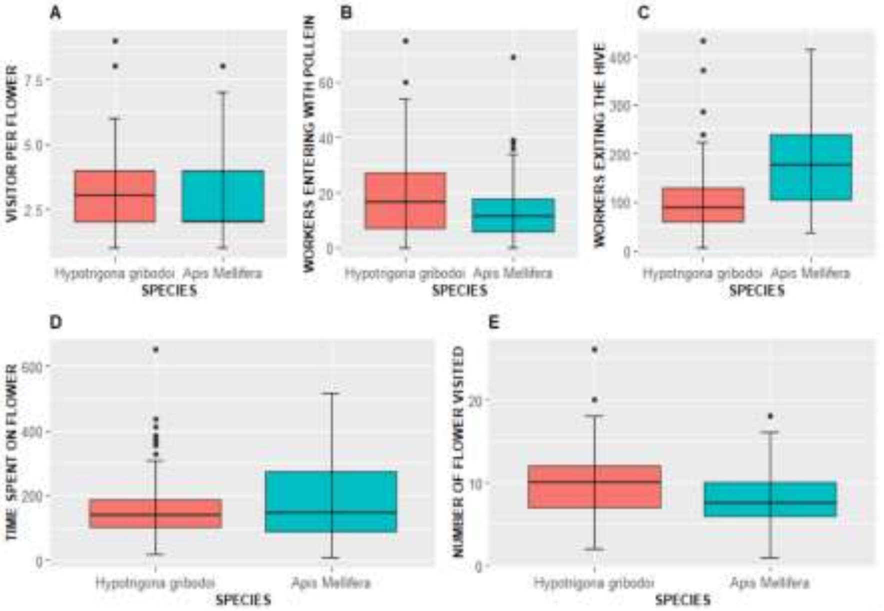

Figure 3.2 Boxplot showing the number of visitors per flower (A), the number of workers entering the hives with pollen(B), the number of workers exiting the hive (C), time spent on flower in seconds (D), and the number of flowers visited (E) by *Apis mellifera*, and *Hypotrigona gribodoi*. The horizontal line across the box shows the median, the box represents 25th and 75th percentiles, and the vertical line outside the box represents the minimum and maximum values.

3.6 Discussion

3.6.1 Foraging activity of *Apis mellifera* and *Hypotrigona gribodoi* in pollination of *Capsicum annuum*

Our findings show that show that *Apis mellifera* and *Hypotrigona gribodoi* differ in their foraging behaviour. *Hypotrigona gribodoi* visits more flowers, has more workers entering with pollen, and has a number of visitors per flower, while *Apis mellifera* has a higher number of workers exiting the hive and spends more time on each flower.

This indicates that *Hypotrigona gribodoi* has more contact with flower's reproductive part of plant which may have implications for enhancing the pollination of *Capsicum annuum* compared to *Apis mellifera. Hypotrigona gribodoi* transfers pollen from the male part of the flower to the female part, potentially resulting in a higher frequency of contact with the stigma. With a high number of workers returning with pollen to the hive, *Hypotrigona gribodoi* appears to collect more pollen than *Apis mellifera* from *Capsicum annuum* flower, increasing the probability of transferring pollen to flower stigma. This increase chance of making successful pollination results in a higher fruit set rate for *Capsicum annuum* plants in plots pollinated by *Hypotrigona gribodoi* compared to *Apis mellifera*.

On the other hand, *Hypotrigona gribodoi* has been observed visiting flowers more frequently than *Apis mellifera*, indicating their contribution in *Capsicum annuum* flower pollination. This is consistent with the study conducted by Tarakini, *et al.*, (2021) where *Apis mellifera scutellata* was compare to *Hypotrigona gribodoi* on flowers of butternut, dry bean and mustard in monocultures and polyculture systems. It highlights the importance of stingless bee as beneficial pollinator for various crops. However, contrasting results were observed by Putra *et al*. (2014), where similar foraging activity (visitation rate) in honey bee (*Apis cerana*) and Stingless Bees (*Trigona laeviceps*) visiting *Capsicum annuum L* flowers. The foraging activity of *Apis mellifera* depends to the amount of glucose, fructose, and sucrose in the nectar, and they prefer flowers with a higher quantity of sugar, as their activity is found to be correlated with the sugar quantity in nectar (Seo *et al.,* 2019). *Capsicum annuum* flowers are found to secret nectar (Dag, 2009). Contrasting results were observed in a study conducted by Kendall *et al.,* (2022), indicating that stingless bees visit blueberry flowers for a longer duration compared to honey bees.

3.6.2 Effect of *Apis mellifera* and *Hypotrigona gribodoi* foraging activities on *Capsicum annuum* fruit set rate

The findings of this study indicate that the number of workers entering with pollen, the time spent on a flower, and number of workers exiting the hive show a statistically significant effect on fruit set rate of *Capsicum annuum*. This suggesting an increase in the number of workers entering with pollen, the time spent on a flower, and number of workers exiting the hive associated with an increase in fruit formation. However, the number of visitors per flower and the number of flowers visited by *Apis mellifera and Hypotrigona gribodoi* do not have a significant effect on the fruit set rate of *Capsicum annuum*. Our study results on the number of visitors per flower and the number of flowers visited effect on *Capsicum annuum* fruit set rate are similar to the findings of (Nunes-Silva *et al.,* 2013) that indicate no relation between the number of flower visits and the amount of pollen transferred by stingless bee *Melipona Fasciculate* on *Solanum melongena L.* Our findings indicate that the presence of the pollinator species *Hypotrigona gribodi* has a significant impact on the fruit set rate of *Capsicum annuum*. They are associated with the highest increase in the fruit set rate compared to the reference level, which is *Apis mellifera.* Due to the small body size of *Hypotrigona gribodoi,* they may have a higher likelihood of coming into contact with female flower part (stigma) compared to *Apis mellifera*. The number of visitors per flower does not have a significant effect, but the numbers of workers entering the hive with pollen and the time spent on the flower have a significant impact on fruit set rates. Our study yielded similar results to a previous study on stingless bees, *Tetragonula laeviceps* and *Heterotrigona itama,* visiting strawberries and melons, respectively, and it demonstrated enhancement in fruit formation while simultaneously reducing the occurrence of abnormalities (Atmowidi *et al.,* 2022).

3.6.3 Effect of temperature and humidity on foraging activities of *Apis melifera* and *Hypotrigona gribodoi* in pollination of *Capsicum annuum*

Our findings indicate that the number of visitors per flower increases for both *Apis mellifera* and *Hypotrigona gribodoi* with rising temperature and decreasing humidity. The number of workers entering the hive with pollen remains unaffected by temperature and humidity. Additionally, the number of workers exiting the hives increases with higher temperature, while the time spent on flowers decreases with an increase in temperature and humidity. Furthermore, the number of flowers visited remains unaffected by temperature and humidity.

Similar to our findings, *Tarakini et al.* (2021) reported that the foraging time of *Apis mellifera scutelate* and *Hypotrigona gribodoi* decreases with an increase in temperature. (Kajobe & Echazarreta, 2005) have observed similar patterns for stingless bee species (*Meliponula ferruginea and Meliponula nebulata)* and honey bees (*Apis mellifera*). Farooqi *et al.* (2021) reported that, abiotic factors such as ambient temperature and humidity have an effect on the foraging activity of *Apis dorsata*, with their study revealing that higher temperatures correlate with an increase in the number of existing workers, whereas elevated humidity levels are associated with a decrease in their numbers. Palma *et al.,* (2008) observe similar findings on the foraging activity of *Nannotrigona perilampoides and Bombus impatiens* with correlations between environmental variables and the number of bees entering the hive and the number of bees on flowers). It was found that the activities of *Bombus impatiens* had a negative correlation with environmental temperature, whereas the stingless bees *Nannotrigona perilampoides* exhibited a highly positive correlation with light intensity (Palma *et al.,* 2008). Our results align with the findings of Maeda *et al*. (2023), suggesting that weather conditions, particularly temperature, have an impact on pollinator activity, fruit set, and crop yield, as observed in Japanese apricots. Unfavorable weather conditions can restrict pollinator activity, resulting in a reduction in the fruit set rate and overall plant yield.

Contrary to our study (Uthoff and Ruxton, 2022) reported a correlation between higher temperatures and a decrease in the number of flower visits per minute. Additionally, the number of workers exiting the hive in stingless bees *(Melipona asilvai)* is not affected by temperature and humidity (Do Nascimento, 2012*)*.

3.7 Conclusion

The foraging activities of *Apis mellifera* and *Hypotrigona gribodoi* in the pollination of *Capsicum annuum* vary. *Hypotrigona gribodoi* has been found to visit more flowers, more workers entering with pollen, and higher number of visitors per flower compared to *Apis mellifera.* However*, Apis mellifera* was found to spend more time on flowers compared to *Hypotrigona gribodoi*. The number of workers entering with pollen, the time spent on a flower, and the number of workers exiting the hive of *Apis mellifera* and *Hypotrigona gribodoi* has been found to have a significant effect on the fruit set rate of *Capsicum annuum.* However, the number of visitors per flower and the number of flowers visited by both bee species do not significantly impact the fruit set rate. The presence of *Hypotrigona gribodoi* as a pollinator species has a significant positive impact on the fruit set rate compared to *Apis mellifera*. This suggests that their specific foraging behaviour and characteristics contribute to improved fruit formation. Stingless bees of the species *Hypotrigona gribodoi* exhibit a higher contact frequency with the reproductive parts of the flower and increased pollen collection, making them beneficial pollinators for enhancing various crops. This indicates that bee foraging activity enhances pollination in *Capsicum annuum*. On the othrer hand, *Apis mellifera* and *Hypotrigona gribodoi* show an increase in the number of visitors per flower with higher temperature and humidity. Their number of workers exiting the hive increases with higher temperature. However, the time spent on a flower decreases with increased temperature and humidity.

The obtained results challenge the prevailing belief that honey bees are the primary pollinators in agricultural environments (Gross, 2001), which is consistent with the findings reported by (Tarakini, 2021), suggesting that managing stingless bee colonies could potentially have a significant impact on crop pollination (Slaa, 2006) and enhance ecosystem resilience. Therefore, it is concluded that the foraging behaviours of *Hypotrigona gribodoi*, and environmental factors, optimize fruit set rates and improve agricultural productivity. The findings of this study emphasize the importance of considering bee species diversity by the conservation of stingless bees and their habitat. This is due to their significant role in enhancing crop pollination and ecosystem resilience as a results of their foraging behaviours. These considerations can help optimize fruit set rates and improve agricultural productivity.

3.8 Recommendation

Based on these findings, we recommend considering not only the use of honey bees *Apis mellifera* but also stingless bees in pollination. When planning pollination strategies for crops, it is important to take into account the specific foraging behavior and characteristics of different bee species. Additionally, further studies on impacts of environmental factors such as wind speed, light intensity, temperature and humidity on foraging activity is crucial to optimize the management and environmental conditions for different bee species, thereby enhancing their pollination efficiency.

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On behalf of all authors, the corresponding author states that there is no conflict of interest.

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CHAPTER FOUR

4.0 GENERAL DISCUSSION

4.1 Effect of *Hypotrigona gribodoi* and *Apis mellifera* on the *Capsicum annuum* Yield.

The study reveals that plots pollinated by *Hypotrigona gribodoi* yield more fruits on average, with larger transverse and vertical fruit circumferences and heavier fruits. Similar to our study, previous research has shown that stingless bees such as *Trigona minangkabau* and *Tetragonula leaviceps (*Putra *et al.* 2016) and *Melipona subnitida (*Cruz *et al*., 2005) increase yield in *Capsicum annuum*. Also similar to our finding on stingless bee *Hypotrigona gribodoi* in improving pollination of *Capsicum annuum*, *Heterotrigona itama* increases melon yield (Atmowidi *et al.*, 2022), while *Melipona Fasciculate* increase *Solanum melongena* yield (Nunes-silva *et al.,* 2015). Conversely, in contrast to our findings regarding stingless bees, there is evidence that another stingless bee, *Trigona iridipennis* yielded similar to the honey bee *Apis cerana* in tomato pollination (Putra & Kinasih, 2013) and *Nannotrigona perilampoides* did not increase the yield in *Lycopersicon esculentum* (Cauich *et al.*, 2004).

Hypotrigona gribodoi and *Apis mellifera* have similar effect on the number of seeds per fruit and seed weight. Despite insignificant differences observed in seed quality, dry seed weights were slightly higher in plots pollinated by *Hypotrigona gribodoi* compared to *Apis mellifera* and caged control plots. contrary to our findings, stingless bee *Hypotrigona gribodoi, Trigona minangkabau* and *Tetragonula laeviceps* were observed to increase seed quality in *Capsicum annuum L (*Kiatoko *et al.,* 2014; Putra *et al.,* 2016).

4.2 *Hypotrigona gribodoi* and *Apis mellifera* pollination impact on *Capsicum annuum* fruit set rate

Our findings indicate that the fruit set rate was significantly higher in plots pollinated by *Hypotrigona gribodoi* when compared to those pollinated by *Apis mellifera* and the caged control plot. This suggests that *Hypotrigona gribodoi* is a more efficient pollinator of *Capsicum annuum* compared to *Apis mellifera*. This observation aligns with previous research, which has also demonstrated the positive impact of stingless bees on fruit set rates (Witter *et al.,* 2015). Similar to our study, previous research has shown that stingless bees, such as *Trigona minangkabau* and *Tetragonula laeviceps (*Putra *et al.,* 2016), increase the fruit set rate of *Capsicum annuum. Nannotrigona perilampoides* increase the fruit set rate of *Lycopersicon esculentum (*Cauich *et al.,* 2004), *Tetragonula laeviceps* increase strawberry fruit set while reducing fruit abnormalities (Atmowidi *et al.,* 2022), and *Lepidotrigona terminate* increase the fruit set rate in *Coffee arabica* (Slaa, 2006; Klein, 2012).

4.3 Foraging activity of *Apis mellifera* and *Hypotrigona gribodoi* in pollination of *Capsicum annuum*

Based on the findings of this study, *Apis mellifera* and *Hypotrigona gribodoi* differ in their foraging behaviour. *Hypotrigona gribodoi* visits more flowers, has more workers entering the hives with pollen, and has a number of visitors per flower, while *Apis mellifera* has a higher number of workers exiting the hive and spends more time on each flower. This study aligns with the findings of Tarakini, *et al.* (2021), which indicate that the stingless bee *Hypotrigona gribodoi* exhibits a higher frequency of flower visits compared to *Apis mellifera scutellata*. These results underscore the significance of stingless bees as beneficial pollinators for crops, as previously reported by (Tarakini, 2021; Slaa, 2006). Furthermore, Putra *et al*. (2014), also observed similar foraging activities between the honey bee *Apis cerana* and Stingless Bees *Trigona laeviceps* when visiting *Capsicum annuum L* flowers. However, Kendall *et al.,* (2022) present contrasting findings, suggesting that stingless bees tend to spend more time visiting blueberry flowers compared to honey bees.

4.4 Effect of *Apis mellifera* and *Hypotrigona gribodoi* foraging activities on *Capsicum annuum* fruit set rate

The findings of this study reveal that the number of workers entering with pollen, the time spent on a flower, and the number of workers exiting the hive show a statistically significant effect on the fruit set rate of *Capsicum annuum*. This suggests an increase in the number of workers entering with pollen, the time spent on a flower, and the number of workers exiting the hive associated with an increase in fruit formation. However, the number of visitors per flower and the number of flowers visited by *Apis mellifera* and *Hypotrigona gribodoi* do not exert a significant effect on the fruit set rate of *Capsicum annuum*.

Our study findings concerning the influence of the number of visitors per flower and the number of flowers visited on the fruit set rate of *Capsicum annuum* align with the results reported by Nunes-Silva *et al.* (2013) indicating that no relation between the number of flowers visits and the amount of pollen transferred by stingless bee *Melipona Fasciculate* on *Solanum melongena L.*

Our findings indicate that the presence of the pollinator species *Hypotrigona gribodi* has a significant impact on the fruit set rate of *Capsicum annuum*. Notably, their presence is associated with the highest increase in the fruit set rate compared to the reference species, *Apis mellifera.* This could be attributed to the smaller body size of *Hypotrigona gribodoi*, which may increase their likelihood of coming into contact with the female flower part (stigma), compared to *Apis mellifera*.

The number of visitors per flower does not have a significant effect on the fruit set rate of *Capsicum annuum*, but the number of workers entering the hive with pollen and the time spent on the flower have a significant impact on fruit set rates of *Capsicum annuum*. This finding aligns with those of a previous study involving stingless bees, *Tetragonula laeviceps* and *Heterotrigona itama,* visiting strawberries and melons, respectively. In that study by Atmowidi *et al.* (2022), similar enhancements in fruit formation were observed, while simultaneously reducing the occurrence of abnormalities.

4.5 Effect of temperature and humidity on foraging activity of *Apis mellifera* and *Hypotrigona gribodoi* in pollination of *Capsicum annuum*

Our findings indicate that the number of visitors per flower increases for both *Apis mellifera* and *Hypotrigona gribodoi* with rising temperature and decreasing humidity. The number of workers entering the hive with pollen remains unaffected by temperature and humidity. Additionally, the number of workers exiting the hives increases with higher temperatures, while the time spent on flowers decreases with an increase in temperature and humidity. Furthermore, the number of flowers visited remains unaffected by temperature and humidity.

In line with previous research, *Tarakini, et al.* (2021) found that *Apis mellifera scutelate* and *Hypotrigona gribodoi* decreases foraging time as temperatures rise. Similar trends were observed by Kajobe and Echazarreta, (2005) stingless bee species (*Meliponula ferruginea and Meliponula nebulata)* and honey bees *Apis mellifera*. Farooqi *et al.,* (2021) revealed that *Apis dorsata* foraging is influenced by temperature and humidity, with higher temperatures increasing worker numbers and elevated humidity decreasing them. Palma *et al.,* (2008) observed similar effects on *Nannotrigona perilampoides and Bombus impatiens*. However, *Bombus impatiens* showed a negative temperature correlation, while *Nannotrigona perilampoides* had a positive light intensity correlation (Palma *et al.,* 2008). Our finding align with findings of Maeda *et al*. (2023), highlighting the weather's impact on pollinator activity and crop yield. Conversely, Uthoff & Ruxton (2022) reported temperature related decreases in flower visits, and Do Nascimento, (2012*)* found number of workers exiting the hive in stingless bees *(Melipona asilvai)* are not affected by temperature and humidity.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Our findings suggest that *Hypotrigona gribodoi* outperforms *Apis mellifera* in pollination of *Capsicum annuum*, resulting in higher fruit quality and fruit set, while both bee species yield similar *Capsicum annuum* seed quality. The study emphasizes that both *Hypotrigona gribodoi* and *Apis mellifera* play a crucial role in enhancing crop quality through increased fruit production, larger and heavier fruits, and improved seed quality, highlighting their significance in cross-pollination. Our findings also indicate that *Apis mellifera* and *Hypotrigona gribodoi* exhibit differences in foraging behaviour, with *Hypotrigona gribodoi* visiting more flowers, having more workers entering the hives with pollen, and having a higher number of visitors per flower than *Apis mellifera*, although *Apis mellifera* spends more time on individual flowers. Moreover, the study found that *Hypotrigona gribodoi* and *Apis mellifera* foraging activities, including the number of workers entering the hive with pollen, the time spent on a flower, and the number of workers exiting the hive have an impact on *Capsicum annuum* fruit set rate. In contrast, the number of visitors per flower and the number of flowers visited have insignificant impact on the *Capsicum annuum* fruit set rate. Their foraging activities are affected by environmental temperature and humidity, with both *Apis mellifera* and *Hypotrigona gribodoi* experiencing an increase in the number of visitors per flower, workers entering the hives with pollen and workers exiting the hives increase as temperature increases. On the other hand, their time spent on the flowers decreases with an increase in temperature and humidity, while the number of flowers visited remains unaffected by temperature and humidity.

The study challenges the prevailing idea that honey bees are the primary pollinators in agricultural environments, consistent with findings by (Tarakini, 2021), suggesting that stingless bees have a significant impact on crop pollination and enhance ecosystem resilience. Considering the importance of pollination services provided by stingless bee *Hypotrigona gribodoi*, it is crucial to adopt conservation strategies that go beyond solely focusing on *Apis mellifera* as the only potential pollinator species. Therefore, it is imperative to include stingless bee species, such as *Hypotrigona gribodoi*, in these conservation efforts.

5.2 Recommendation

1. More research is essential to understand stingless bees' biology, behaviour, and ecological needs. They play a vital role in pollination, which benefits crop yields, food security, and ecosystem health. Raising awareness and educating farmers and the public is crucial for their conservation.

2. Preserving natural habitats is key to their thriving through including stingless bees within conservation efforts.

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