POLLINATION BIOLOGY OF *COFFEA KIHANSIENSIS* (RUBIACEAE), AN ENDEMIC WILD COFFEE SPECIES IN KIHANSI GORGE FOREST, TANZANIA

ADILI BUGINGO

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN
ECOSYSTEMS SCIENCE AND MANAGEMENT OF SOKOINE UNIVERSITY
OF AGRICULTURE. MOROGORO, TANZANIA.

2018

ABSTRACT

The Kihansi wild coffee, *Coffea kihansiensis*, is an endemic coffee species found in the understory of the Kihansi gorge forest in Tanzania. In this study, pollination biology of *C. kihansiensis* was assessed. The aim of the study was to understand the relationship between the flower biology of *C. kihansiensis* and its insect pollinators. Specifically, the study investigated flowering patterns, floral dimensions, insect visitors, pollinators, floral rewards and breeding system. A systematic sampling design was employed and three *C. kihansiensis* populations were used as sampling blocks. Data were collected through direct observations, direct counts, measurements and experiments. Flowering in the species exhibited a bing-bang (mast) flowering pattern and was observed to occur from mid-December through to January following short rains. *C. kihansiensis* flowers were visited by a wide range of insects belonging to 44 species. Bees of various species were the most abundant insect visitors on the flowers and appeared to be the most effective pollinators of *C. kihansiensis*. Flowers typically offer pollen and nectar to insect visitors. Insect visitors' frequencies were higher in the morning than in the afternoon and evening, and were associated with the timing of pollen and nectar release. Irrespective of species, insect visitation was higher in morning, at 1100 hr. Fruit set was high in cross pollinated flowers. The low fruit set in the absence of pollinators might be interpreted as an adaptation to low pollinator visitation rate or pollen limitation. The self-incompatibility index (ISI) was estimated to be 0.72. Results from hand-pollination and the ISI value show that *C. kihansiensis* is partially self-incompatible, able to reproduce through both selfing and outcrossing. It showed that pollinators play important role in enhancing the productivity of *C. kihansiensis*. The above findings imply that *in situ* conservation of *C. kihansiensis* must focus on monitoring the species, its insect pollinators and conservation and management of its natural habitat.

DECLARATION

I, **ADILI BUGINGO** do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my original work done within the period of registration and that it has neither been submitted nor being concurrently submitted for a degree award in any other institution.

Bugingo. 18.06.2018.

Adili Bugingo **Date**

(MSc. Candidate)

The above declaration is confirmed by:

18.06.2018

Prof. P. K. T. Munishi **Date**

(Main Supervisor)

18/06/2018

Prof. R. P. C. Temu **Date**

(Co-Supervisor)

COPYRIGHT

ACKNOWLEDGEMENTS

First and foremost, I would like to thank the Almighty God who gave me strength and capabilities to complete this work and whose many blessings have made me who I am today.

Secondly, I am deeply indebted and thankful to my supervisors; Prof. P. K. T. Munishi and Prof. R.P.C. Temu in the Department of Ecosystems and Conservation of the Sokoine University of Agriculture for accepting me as a student, and for their excellent guidance and inspiration starting from the very beginning till the end. I have learned a lot from their rich scientific knowledge and positive thinking. They tended me to grow as a scientist. I appreciate their encouragement, critical comments and above all their kindness and collegial handling. I am also very much grateful for their excellent supervision and continuous technical support throughout the study period. They were always on my side tirelessly reading and commenting on my work for several times. They shared with me their rich experiences in ecological research set up, data collection and analysis. Their critical comments and suggestions were so vital for my work.

Thirdly, I would like to thank Dr. C. J. Kilawe in the same Department for his valuable comments, encouragement, data analysis and other valuable advice during the entire period of my MSc study at the Sokoine University of Agriculture. I greatly appreciate all members of staff in the Department of Ecosystems and Conservation for their contribution and assistance throughout my studies.

Fourthly, I wish to thank my research assistants, Person Kalenga, Bakari Rupia and Lewis Kaguo for their great contribution during the field data collection. I am indebted to Dr. Moses I. Olotu of Mkwawa University College of Education and his team for their help in

insect identification. I also thank the National Environmental Management Council (NEMC) of Tanzania for permission to carry out this study in the threatened Kihansi Gorge Forest.

I am grateful to the Mwalimu Julius K. Nyerere University of Agriculture and Technology (MJNUAT) for sponsoring this research. Finally, to all people who have in different ways contributed towards the accomplishment of this work, I say thank you.

DEDICATION

This work is dedicated to my beloved mother. Wegesa Nyabange, who laid the foundation of my education. I want to extend a special feeling of gratitude to my sisters Violet Mkama and Sarah Bitaliani, my young brother Muhksin Sadick whose words of encouragement led me in this new step. I also dedicate this dissertation to my many friends who have helped me throughout the research process.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND ACRONYMS

a.s.l	above sea level
ANOVA	Analysis of Variance
CP	Cross Pollination by hand
DP	Controlled Open Pollination
GSI	Gametophytic Self-Incompatibility
ha	Hectare
ISI	Index of Self-Incompatibility
IUCN	International Union for Conservation of Nature
LKEMP	Lower Kihansi Environmental Management Project
LWF	Lower Wetland Forest
mm	Millimeter
NAFORMA	National Forestry Resources Monitoring and Assessment of Tanzania
OP	Open Pollination
RSF	Research Station Forest
SC	Self-Compatible
SI	Self-Incompatible
SP	Self Pollination by hand
SS	Control Treatment
UCF	Upper Camp Forest

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Plant pollination is one of the most well-known and important ecosystem services, which is essential for both natural and agro-ecosystems (Garibaldi *et al.*, 2013; Hopwood, 2015). Pollination is important in conservation biology as it facilitates plant fertilization and/or reproduction, thus enhancing self-perpetuation and forest regeneration. This type of ecosystem service is critical to human survival (Kremen *et al.*, 2002; Eardley *et al.*, 2006; Garibaldi *et al.*, 2014).

It is estimated that 85 percent of the world's flowering plants depend on animals, mostly insects for pollination (Ollerton *et al.*, 2011). Pollination by insects is known to occur in about 75% of all cultivated crops (Klein *et al.*, 2007) and 80% of wild plant species (Potts *et al.*, 2003, 2010), and it helps in the production of fruits and seeds. Interactions between pollinators and the host plants have an intrinsic value to the society and contribute to the richness of life on earth. Besides this, these interactions are critically important to fitness, with the majority of species benefiting from transfer of pollen within and between flowers by animal pollinators (Ollerton *et al.*, 2011). Therefore, a decline in pollinator diversity over time threatens plant reproductive success given its reliance on the pollination services provided by the pollinators (Banaszak, 1996; Potts *et al.*, 2010; Melin *et al.*, 2014).

Several studies (Ashman *et al.*, 2004; Knight *et al.*, 2005; Biesmeijer *et al.*, 2006; Damon *et al.*, 2012; Lundgren *et al.*, 2015) have pointed out that deficits in pollinator visitation can limit host plant reproduction, and structure plant population dynamics. The growing evidence of localized declines of pollinators is a cause for concern as the resulting impact on pollinator-dependent flowering plants could be devastating. While pollinator declines are not the sole cause of plant extinctions; and few plant-pollinator systems are absolutely obligate between two species, large-scale losses of either flowering plants or pollinators are likely to result in cascading declines within both groups (Groom, 1998).

According to Pauw (2007), pollinators are predicted to be sensitive to human-caused disruption, and their early loss will trigger a cascade of linked declines among the multiple plant species that they pollinate. One such example of the plant that may be negatively impacted by human activities is the endemic Kihansi wild coffee, *Coffea kihansiensis*, in the Kihansi gorge forest in Tanzania. The species is localized and restricted to an estimated area of about 17 hectares (ha) at altitude of between 775 and 950 m above sea level (Davis and Mvungi, 2004; Rija, 2011), following habitat alteration.

The species is potentially threatened by human disturbances and is considered to be critically endangered species under the IUCN Red List (IUCN, 2001). *C. kihansiensis* is highly vulnerable to local extinction due to changes in its semi-aquatic environment, particularly the river spray on which it appears to depend on (LKEMP, 2004). The on-going destruction of the original habitat may have tremendously effects that can threaten this wild coffee population and the exceptional floristic diversity of these forests. In addition, it can also have a negative impact on pollinator populations, resulting in deficits in pollinator visitation (Biesmeijer *et al.*, 2006; Lundgren *et al.*, 2015). This study therefore aims to investigate the pollination biology of this endemic and critically endangered wild coffee species of Tanzania in the Kihansi gorge forest.

1.2 Problem Statement and Study Justification

Pollination studies contribute useful information on the degree of mutual dependence between species within a community and the reproductive ecology of plant communities.

Pollinators play a crucial role in the out-crossing of and sexual reproduction of many angiosperms, and contribute to the maintenance of biological diversity in natural ecosystems. Studies on the interactions between pollinators and their host plants can provide key insights into the importance of pollination mutualisms for a species' ecology and conservation.

There is enormous knowledge on the ecology and evolution of plant-pollinator interactions (Mitchell *et al.*, 2009; Landry, 2013; Richardson and Irwin, 2015). However, there are many flowering plant species for which their floral biology and floral visitor spectrum remain understudied or unknown. For example, research on the pollination biology of coffee plants were exclusively focused on the traded coffee species such as *Coffea canephora* and *Coffea arabica*, and mostly on the floral biology, flower visitors or fruit set (Klein *et al.*, 2003a; Ngo *et al.*, 2011). Information of the gene pool of wild coffee populations could have high potential for the breeding of new coffee varieties (Hein and Gatzweiler, 2006). One such example is that of the endangered wild coffee species, *Coffea kihansiensis*, in Tanzania.

The Kihansi wild coffee is currently only known from the Kihansi gorge forest in southern Udzungwa Mountains of Tanzania (Davis and Mvungi, 2004). The species occurs naturally at the lower storey of the Kihansi gorge forest, between 775 and 950 m. above sea level (Rija *et al.*, 2014). The species is categorized as critically endangered under the IUCN Red List. This is due to the fact that the species is threatened by human disturbances and habitat change. This may also intensify the pollinator decline due to shrinking natural habitat and food resources.

Information on the flower visitors and potential pollinators for most wild coffee species in general is very limited. For example, there is no information on pollination biology of the wild coffee *C. kihansiensis*. Unavailable information includes that on; flowering pattern, flower biology, flower visitors, floral rewards and periodicity of their release. This research therefore aimed at filling these knowledge gaps.

The findings of this research add knowledge to science on pollination biology of the other wild coffee species of Tanzania. The findings are also important for future research on coffee breeding. In addition, the findings from this study provided information necessary for monitoring population dynamics of the Kihansi wild coffee, and can be used for formulating realistic conservation priorities for conservation of both Kihansi wild coffee, its pollinators, the Kihansi gorge ecosystem and other natural habitats of Tanzania.

1.3 Objectives of the Study

1.3.1 Overall objective

The overall objective of this study was to assess the pollination biology of the endemic wild coffee of Tanzania, *Coffea kihansiensis*, in the Kihansi gorge forest.

1.3.2 Specific objectives

The specific objectives of the study were:

i. To determine the flowering patterns and flower biology of *Coffea kihansiensis* in the Kihansi gorge forest

ii. To determine the diversity and abundance of visitors of the flowers of *C. kihansiensis* in the study area

iii. To determine which are the *de facto* pollinators of *C. kihansiensis* in the study area

iv. To determine *C. kihansiensis* floral rewards to visitors and the periodicity of their release

v. To assess whether pollinators are essential in fruit set in *C. kihansiensis*

1.4 Research Questions

i. Is there any fixed flowering period in *C. kihansiensis*?

ii. Which insects are the primary visitors to *C. kihansiensis* flowers?

iii. Does the flower biology favour the true pollinators as against other visitors?

iv. Which floral rewards are present in *C. kihansiensis* and at what time are they available to flower visitors?

v. Does fruit set in *C. kihansiensis* dependant on pollinators?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview

The genus *Coffea* belongs to the family Rubiaceae. Currently, there are approximately 124 described species of *Coffea* worldwide (Hatanaka *et al.*, 1999; Davis and Rakotonasolo, 2001a, b; Davis *et al.*, 2006; 2007; 2010: 2011). with new species still being discovered (Davis and Rakotonasolo 2001a, b; Stoffelen *et al.*, 2009) and even more varieties being bred (Mazzafera and Carvalho 1991; Nagai *et al.*, 2008). However, 72 of the 124 *Coffea* species, which is about 58%, are threatened with extinction as a result of a combination of decline in quantity and quality of habitats. In Tanzania, there are sixteen described naturally occurring species of *Coffea* (Davis and Mvungi, 2004).

The Afromontane forests of Tanzania which include Kihansi gorge forest, southern Udzungwa Mountains, Milinga Forest Reserve and the Usambara Mountains forests which are found within the Eastern Arc Mountains, constitute the habitats for many species, including wild coffee species (Davis and Mvungi, 2004). One such example of wild coffee is the endemic Kihansi wild coffee, *Coffea kihansiensis*, in the Kihansi gorge forest.

Wild populations of coffee in African forests are genetically diverse and may contribute to the genetic diversification of commercial coffee production worldwide, as they likely possess desirable traits that can be used to improve the cultivated varieties of *Coffea arabica* and *Coffea canephora*, or increase their disease resistance. Nevertheless, like other forests of the world, the Afromontane forests of Africa that harbour the wild coffee gene pools have been under continuous threat due to habitat destruction (Senbeta and

Denich, 2006), forest fragmentation and intensification of agricultural practices (Schmitt *et al.*, 2009; Aerts *et al.*, 2011). Such anthropogenic disturbances are likely affecting the genetic diversity, mating patterns, gene flow, pollinator abundance and diversity, and microclimate; thereby influencing long term viability of the existing coffee populations.

2.2 Flowering Patterns and Flower Biology of Coffee

The flowering period of coffee species vary from region to region. The flowering events of *C. arabica* are in synchronous flushes after a heavy rainfall following a dry period (Vieira, 2008). In one coffee reproductive period of *C. arabica*, there is usually more than one flowering event (Wormer, 1964; Alvim, 1977).

Mature flowers of *C. arabica* are hermaphroditic, white, and have a scent similar to jasmine (Orwa *et al.*, 2009). Flowers open at dawn and pollen starts shedding soon after. At this time, the stigma is receptive and pollination can occur until noon (Alvim, 1985). Flowers of *C. arabica* usually wither in 1 or 2 days after pollination (Free, 1993; Klein *et al.*, 2003a; Aga, 2005), but if not pollinated, they can remain open for up to 5 days (Jiménez-Castano and Castillo-Zapata, 1976).

With other coffee species, anthesis (the flowering period) normally lasts slightly longer, 1–3 days (Wormer, 1964; Alvim, 1977). Upon completion of the flowering period, fruits develop over the next 7–10 months (De Castro and Marraccini, 2006; Eira *et al.*, 2006). Most literature report that the proportion of flowers that eventually develop into mature fruits ranges from 20% to 40% (Free, 1993). Klein *et al.* (2003a) reported that natural fruit set (i.e., the proportion of fruits formed) of *C. arabica* in Costa Rica is 75.2%.Moreover, Garibaldi *et al.* (2013) pointed that wild pollinators enhance fruit set of crops regardless of honey bee abundance.

The structure of the coffee flower is well known (Stoffelen *et al.*, 1997; Davis *et al.*, 2006). Klein *et al.* (2003a) and Orwa *et al.* (2009) summarize coffee flower morphology as follows. Briefly, the flowers are supported by a short pedicel and have a rudimentary five-petal calyx, a white corolla, the lower half of which are fused into a cylindrical, elongated corolla tube, and relatively short anthers (McGregor, 1976; Free, 1993; Klein *et al.*, 2003a; Orwa *et al.* (2009)). *Coffea arabica* and *C. canephora* have five stamens with long anthers and short filaments inserted into the corolla, a long thin style with a two-branched stigma, and an inferior ovary of two chambers each containing one ovule (Klein *et al.*, 2003a). The stigma is receptive when a flower opens at dawn and the anthers dehisce soon afterwards. The disc surrounding the base of the style secretes nectar (Free, 1993).

2.3 Diversity and Abundance of Coffee Flower Visitors and Pollinators

Natural ecosystems and many agro ecosystems depend on pollinator diversity and abundance to maintain overall biological diversity in tropical ecosystems. Pollinators differ from many other providers of essential ecosystem services because they are often part of highly specific pollinator–plant relationships (Eardley *et al.*, 2006). According to Bawa (1990), 89-99% of all flowering plant species of tropical forests are pollinated by animals. Arthropod and pollinator diversity was reported to decline worldwide with increased anthropogenic activities (Klein *et al.*, 2003a). Plants may suffer from reduced pollination because the composition of pollinator assemblages fluctuates temporally (Alarcon *et al.*, 2008; Olesen *et al.*, 2008; Rafferty and Ives, 2012), and not all pollinators are equally effective (Ivey *et al.*, 2003; Sahli and Conner, 2007). The predominant pollinator may change between seasons and between sites. Further, pollinators differ in their efficacy. Data on their relative abundance and diversity gives an indication of pollinator force (Kevan, 1999). There is however limited published data available for sub-

Saharan Africa on ecological importance of pollination services delivered by insect pollinators (Munyuli, 2010). On the other hand, a recent review by Ngo *et al.* (2011) claimed a strong study bias towards Latin America and Asia, with little work from Africa.

There is a growing body of research (e.g. Ricketts. 2004; Klein *et al.*, 2008; Klein, 2009; Jha and Dick, 2010) on the relationships among coffee pollination and biodiversity production in wild adjacent regions and the biodiversity of different types of coffee ecosystems. Some coffee species such as *C. arabica* and *C. canephora* are known to benefit from bee pollination (Klein *et al.*, 2003a), and their fruit set increases markedly with insect pollination (Roubik, 2002a, 2002b; Klein *et al.*, 2003a, 2003b; Ricketts, 2004).

The abundance, diversity and visitation rate of many wild coffee species (e.g. *C. kihansiensis*) by pollinating insects is not documented. Neither the ecological importance nor the economic significance of pollination services delivered by pollinators to coffee had previously been studied in Tanzania.

2.4 Breeding Systems and Fruit Set in Coffee

Plants generally rely on some external agent(s) to transfer pollen and thus effect pollination and reproduction. Although pollinator foraging behaviour may be modified somewhat by flowering patterns or floral specialization, plants have very little direct control over the types of pollen which are transferred to a flower. Thus, many flowers have developed mechanisms to regulate the breeding system, and so give some measure of control over offspring quality. Some species exhibit preferential outcrossing, but are still capable of producing self-fertilized (self-compatibility) seed under certain conditions (Nowak *et al.*, 2011). This implies that if both outcross and self-pollen are received by a plant, some form of pollen competition, or mate choice is operating.

Many bisexual flowering plants avoid the deleterious effects of inbreeding by employing genetically controlled self-incompatibility (SI) mechanisms to ensure outcrossing (Charlesworth and Charlesworth, 1987). SI plays an important role in shaping the spatial and temporal distribution of genetic diversity in plant populations and is thought to influence patterns of lineage diversification in clades within which these mechanisms are utilized (Igić *et al.*, 2008; Goldberg, 2010).

In the genus *Coffea* various adaptations have evolved to enhance outbreeding or attract special pollinators (Nowak *et al.*, 2011). The vast majority of diploid species in the genus *Coffea* utilize a mechanism of gametophytic self-incompatibility (Nowak *et al.*, 2011). The vast majority of *Coffea* species are known to exhibit a strong gametophytic self-incompatibility (GSI) response, but three African species (*C. arabica*, *C. anthonyi* Stoff. and F. Anthony, and *C. heterocalyx* Stoff.) are exceptional for their ability to self-fertilize (i.e. self-compatibility, SC) (Carvalho and Monaco, 1969; Davis *et al.*, 2006; Stoffelen *et al.*, 2009).

C. arabica is widely perceived as a predominantly selfing species with an outcrossing rate of about 10% (e.g. Free, 1993; Anthony *et al.*, 2001; Davis *et al.*, 2006). This knowledge stems from pollination studies in *C. arabica* cultivars which presented outcrossing rates from different coffee producing regions of the world. For instance, outcrossing rates of less than 10% in Colombia (Castillo-Zapata, 1976), 12% in Brazil (Carvalho and Krug, 1949), 7-15% in Kenya (Van der Vossen, 1974), and 40 to 60% in Ethiopia (Meyer, 1965) have been reported, based on pollination studies. Studies on mating patterns in wild Arabica coffee populations in Ethiopia reported a multi locus outcrossing rate of 76%, typical for mixed mating species (Goodwillie *et al.*, 2005; Busch *et al.*, 2010; Zhu and Lou, 2010).

Many bisexual flowering plants avoid the deleterious effects of inbreeding by employing genetically controlled self-incompatibility (SI) mechanisms to ensure outcrossing (Charlesworth and Charlesworth, 1987). SI plays an important role in shaping the spatial and temporal distribution of genetic diversity in plant populations and is thought to influence patterns of lineage diversification in clades within which these mechanisms are utilized (Igić *et al.*, 2008; Goldberg, 2010).

In the genus *Coffea* various adaptations have evolved to enhance outbreeding or attract special pollinators (Nowak *et al.*, 2011). The vast majority of diploid species in the genus *Coffea* utilize a mechanism of gametophytic self-incompatibility (Nowak *et al.*, 2011). The vast majority of *Coffea* species are known to exhibit a strong gametophytic self-incompatibility (GSI) response, but three African species (*C. arabica*, *C. anthonyi* Stoff. and F. Anthony, and *C. heterocalyx* Stoff.) are exceptional for their ability to self-fertilize (i.e. self-compatibility, SC) (Carvalho and Monaco, 1969; Davis *et al.*, 2006; Stoffelen *et al.*, 2009).

C. arabica is widely perceived as a predominantly selfing species with an outcrossing rate of about 10% (e.g. Free, 1993; Anthony *et al.*, 2001; Davis *et al.*, 2006). This knowledge stems from pollination studies in *C. arabica* cultivars which presented outcrossing rates from different coffee producing regions of the world. For instance, outcrossing rates of less than 10% in Colombia (Castillo-Zapata, 1976), 12% in Brazil (Carvalho and Krug, 1949), 7-15% in Kenya (Van der Vossen, 1974), and 40 to 60% in Ethiopia (Meyer, 1965) have been reported, based on pollination studies. Studies on mating patterns in wild Arabica coffee populations in Ethiopia reported a multi locus outcrossing rate of 76%, typical for mixed mating species (Goodwillie *et al.*, 2005; Busch *et al.*, 2010; Zhu and Lou, 2010).

The reproductive success (e.g. fruit set) of animal-pollinated plants primarily depends on flower-pollinator interactions (Newstrom *et al.*, 1994; Ollerton, 2011; Bruckman and Campbell, 2014). Several studies (e.g. Reddy *et al.*, 1988; Klein *et al.*, 2003a; 2003b) on the reproductive biology of the genus *Coffea* have generally focused on important traded species, but wild natural occurring species have received less attention (Campa *et al.*, 2012).

The breeding systems of the genus have also been investigated based on pollination experiments from different species such as *C. canephora* and *C. arabica* (Klein *et al.*, 2003a; 2003b). However, several studies such as Roubik and Degen (2004), Raffetry and Ives (2012) and Scaven and Raffetry (2013); have elucidated the interactions between pollinator behaviour and activities, pollen carryover and floral phenology, and their effects on pollen dispersal and plant breeding systems. Despite a wealth of information on the subject, many species of the genus *Coffea* remain largely unstudied, breeding system data are scarce, and there is virtually no ecological information on factors influencing reproductive success and seed production for the genus.

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 Material

3.1.1 The study area

The study was conducted in Kihansi gorge forest (Plate 1). The forest is located in the Southern part of the Udzungwa Mountains Scarp in the Eastern Arc Mountains (Quinn *et al.*, 2003). Kihansi gorge is found at latitudes 8°35'S and longitudes 35°51'E, covering an area of about 90 ha of closed canopy high forest, most of which consists of mixed tree species (Lovett *et al.*, 1997). Three sampling sites within the recorded range of *C. kihansiensis* along the gorge were used: Kihansi forest at Upper camp (coded UCF), the forest near Kihansi Research Station (coded RSF), and the forest near the Lower spray wetland (coded LWF). Site UCF is located between 875 and 952 m a.s.l. Prior to reduction of the river flow, the area was wet all year round (LKEMP, 2004). It is characterized by stony and shallow soils and increasingly steep slopes towards the Kihansi River valley. Visibility is more than 50%, as it is covered by open forest with a few canopy trees such as *Rinorea ilicifolia* and *Garcinia semeseii*. RSF stretches between 825 and 850 m a.s.l. and is covered by evergreen forests with dense canopy trees at least 10 m high such as *Euclea nobilis* and *Filicium decipiens*. This site slopes gently away from the walking trail, and the soil is black, indicative of high humus content. LWF lies between 800 and 825 m a.s.l. in an area once reached by spray from the waterfall. It has gentle slopes with stony and shallow soils and is dominated by canopy trees such as *Parinari curatellifolia* and *Uapaca kirkiana* (Rija *et al.*, 2011).

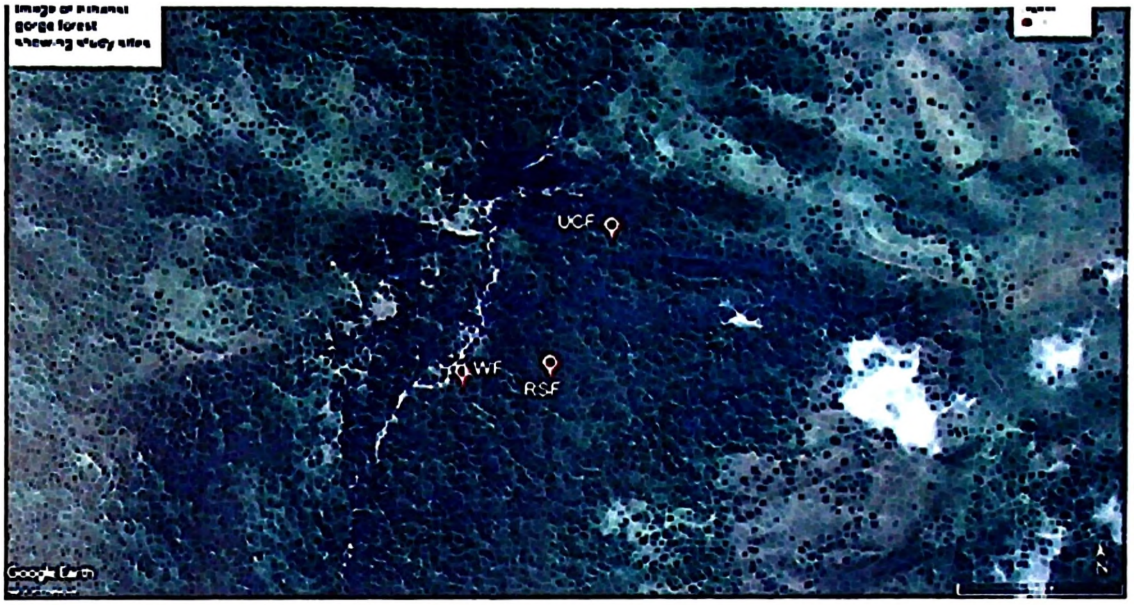

Plate 1: **Kihansi gorge forest in Southern Udzungwa Mountains, Tanzania showing location of the study sites. (Source: Google Earth, 2017)**

3.1.2 Climate

The climate of the study area is tropical with bimodal rainfall seasons. The Kihansi gorge receives an average annual rainfall of about 1800 mm, with mean daily minimum and maximum temperatures of approximately 13°C and 25°C, respectively (Cordeiro *et al.*, 2006).

3.1.3 Vegetation and flora

The Kihansi gorge ecosystem is divided into four major vegetation types. The gorge contains miombo woodland, montane forest, *Filicium* forest, and wetland spray meadow with respective areas of approximately 95 ha, 100 ha, 0.25 ha, and 2.0 ha. More than 23 plant species occur in the Kihansi gorge forest that are either globally threatened or are species having regional and global conservation importance (e.g. *Coffea kihansiensis*). Two unidentified species in the family Acanthaceae and a species in the family Triuridaceae are known only from the Kihansi gorge. The forest harbours montane plant species (e. g. *Aphloia theiformis* and *Olea capensis*) which are found near the waterfall below their normal elevation range (Cordeiro *et al*.. 2006).

3.1.4 Fauna

The Kihansi gorge supports rich faunal communities. Sixteen bird species are reported to occur in the Kihansi gorge that are of conservation concern (Cordeiro *et al*., 2006). Seven of these species are globally or regionally threatened or near-threatened. Initial assessment of bird abundance shows declines in the members of some functional groups, especially the insectivore-frugivore community after severe reduction in moisture after river diversion. These include; *Andropadus virens*, *A. milanjensis* and *A. masukuensis*).Six primate species occur in the Kihansi gorge (Cordeiro *et al*., 2006). These include; the Uhehe Red Colobus monkey (*Piliocolobus gordonorum*), Peters' angolan colobus (*Colobus angolensis palliatus*) and Sykes' monkey (*Cercopithecus mitis monoides*).In addition, the gorge supports an unknown number of herpetofauna including the critically endangered toad, the Kihansi spray toad *Nectophyrinoides asperginis* (Poynton *et al*., 1999). The forest harbours a high diversity and abundance of arthropods that have broad geographic distributions outside of the Kihansi gorge (e.g. Zilihona *et al*., 1998; 2004).

3.2 Methodology

3.2.1 Sampling design

Three naturally occurring populations of *C. kihansiensis* in the Kihansi gorge forest were used as sampling blocks. These blocks are populations at the Upper camp of Kihansi gorge (coded as UCF), population near Kihansi research station (coded RSF), and population in the forest near the Lower spray wetland (coded LWF).

From each block, transects and plots were adopted as sampling units. A systematic sampling design was employed where plots were established systematically along transects at a fixed distance between plots and transects. Within each block, three transects running across the contours were laid at the middle and two edges. The distance between transects was 50m apart. Along each transect five circular plots of 15m radius (adopted from NAFORMA)were laid at a distance of 50 m from each other(Fig. 1) making a total of 45 plots for the entire study area.

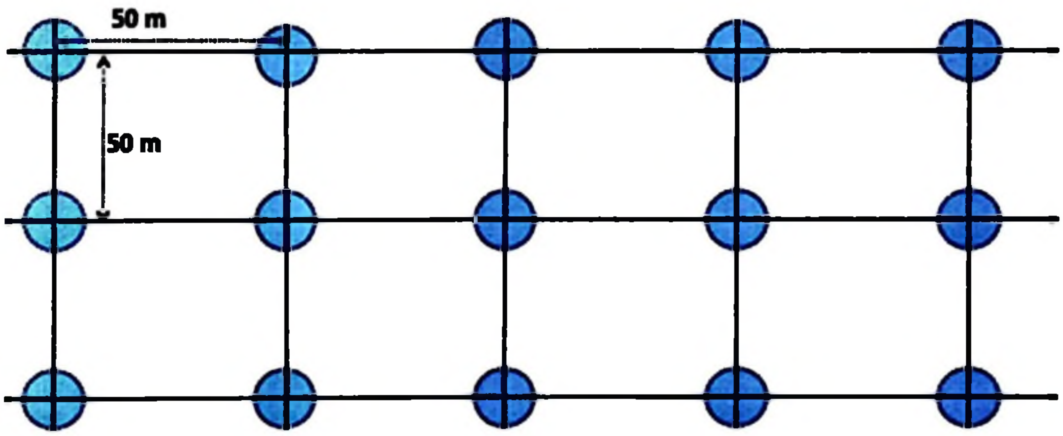

Figure 1: Transects and plots layout

3.2.2 Data collection

The first, second, third and fourth objectives were achieved by direct observations, direct counts and measurements. The fifth objective was achieved by exclusion bagging experiment on randomly selected flowering plants of *C. kihansiensis*.

Objective 1: Flowering patterns and flower biology

Four flowering parameters were observed in individual plants and for entire populations. These were: Onset (flowering start date); flowering duration (date of first and last flower/length of the flowering period); Mean flowering date (peak of flowering): the average of the census dates during which that individual is flowering, with each date valued by the number of flowers in that period: *sensu* Augspurger (1983). This method of studying flowering patterns was adopted from McIntosh (2002).Fifty flowers were randomly collected from the flowering populations for morphological characterization. Two flowers per inflorescence from twenty five individuals in each coffee population were collected for measurements. The floral characters assessed included: Inflorescence type; number of flowers per inflorescence; flower diameter; calyx characteristics (including hypathium, number of sepals): corolla characteristics (number of petals, corolla tube length, corolla lobe length);androecium characteristics (number of stamens, filament length, anther length); and gynoecium characteristics (style length, stigma lobe, ovule numbers).

Objective 2 and 3: Flower visitors and the *de facto* pollinators

Flower visitors on flowers of *C. kihansiensis* were observed for three consecutive days at each population per week during the peak of flowering period. The type and number of flower visitor species were observed and recorded during the study period. The number of flowers visited, the duration of each visit and flower visitor behaviours were recorded. A Stop watch was used to record time spent by insect visitors on each flower.

All flower visitors were counted and some were collected using sweep net for species identification. The *C. kihansiensis* populations were used for later comparison of the insect visitor and *de facto* pollinator diversity and abundance. Insect identification was done with the aid of insect guide books by Kielland (1990), Picker *et al.* (2004), Eardley (2004) and Eardley *et al.* (2010). The collected voucher specimens were deposited in the Zoology laboratory of the Sokoine University of Agriculture.

Objective 3: To determine which insects are the *de facto* pollinators of *C. kihansiensis*

In order to know the *de facto* pollinators, the behaviour of the visitors as they visited the flowers was observed. These included: their mode of approach, landing, probing behaviour, the type of forage collected, and contact with essential organs as they enter and leave the flowers, and the time spent on the flower. In addition, the number of visited flowers, the duration of each visit and contact with the reproductive parts were recorded. A stop watch was used to record time spent by insect visitors.

Objective 4: Floral rewards to visitors and the periodicity of release

To determine the type of floral rewards collected, periodicity and availability of rewards to visitors, three randomly selected coffee plants were observed for the period between 0700 and 1700 hrs for eleven days. The types of insect floral visitors, purpose of visiting, time interaction with flowers and the foraging activity of visitors were observed. These were observed with reference to the type of forage they collect and contact with essential organs to effect pollination. The rewards obtained were determined through observation. Pollen was observed from insect visitors collecting pollen and carrying pollen loads. Nectar was determined when visitors probe deep in the flowers.

Objective 5: Are pollinators necessary for fruit set and seed production in *Coffea kihansiensis*?

To assess the contribution of pollinators to fruit set in *C. kihansiensis*, pollination experiments were conducted. Five treatments were conducted on twenty randomly selected trees from each site. The five treatments included: open-pollination (pollination by insect and wind), cross-pollination by hand, self-pollination by hand, controlled open pollination (distal end of bag held open allowing insect visitor and wind access to flowers) and control treatment. Cross-pollination was performed by extracting pollen with a paintbrush from an open flower and then placing it on the stigma of a flower of a different individual. On each plant, a set of five flowering branches were used giving a total of 300 branches across the *C. kihansiensis* habitats.

Following Willmer and Stone (1989) and Klein *et al.* (2003a) approaches, bags of very fine nylon mesh gauze (1 mm) were used for the hand, self-pollination and controlled open pollination experiments. The numbers of flowers were counted on the bagged and open branches and the branches tagged. Additionally, to control for the effects of containing the flowers in hand pollination experiments, other control experiments were installed (Coupland *et al.*, 2006). Eight weeks after the end of the major flowering period, the bags were removed and the numbers of green ovules counted.

3.3 Data analysis

3.3.1 Flowering patterns and flower biology of *Coffea kihansiensis*

Descriptive statistics such as number, percentage, frequency, mean and standard deviation were used to analyze flowering phenological and morphological data. Total number of open flowers per branch during the whole reproductive season was averaged for each population and compared among populations with a one-way ANOVA.

3.3.2 Diversity and abundance of visitors of *C. kihansiensis*

3.3.2.1 Species diversity

Shannon – Wiener Index of Diversity, (H') was used to calculate diversity of species in various habitats $H' = -\sum (P_i \ln P_i)$

Simpson index was used to calculate the dominance of species $C = \sum_i Pi^2 = \sum (n^2-n/N^2-N)$

Where,

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

Pi^2=square of Pi

N=total number of all individual

n=number of individual

3.3.2.2 Abundance

Abundance of species in various study sites was calculated as the number of catches of the respective species per site (Total count per site). Insect visitor abundance comparison between *C. kihansiensis* populations was done using analysis of variance (ANOVA). Kruskal-Wallis test was used to compare statistical differences in the number of insect visitor species and their abundance between wild coffee populations.

3.3.3.3. The *de facto* pollinators of *C. kihansiensis*

Descriptive statistics (e.g. frequency, means, percentages etc.), were used in the analysis of frequency of flower visitors and their behaviour. Flower visitors behaviour determined who the true pollinators were.

3.3.3.4 The periodicity and availability of floral rewards to visitors

Descriptive statistics were used in the analysis of frequency of flower visitors.

3.3.3.5 Are pollinators necessary for fruit set and seed production in *C. kihansiensis*?

To compare fruit sets in different coffee populations, multifactor analyses of variance (ANOVA) with three variables (coffee populations, each coffee shrub observed, and pollination treatments) was used. To detect differences in fruit mass among controlled pollination experiments, a Kruskal–Wallis (H) test was used, and a sign test was used to analyze differences among pair-wise comparisons. A quantitative evaluation of plant breeding system was achieved using the index of self-incompatibility (ISI) developed by Zapata and Arroyo (1978). ISI was calculated by dividing the number of fruit set resulting from hand self-pollination by the number of fruit set derived from hand cross-pollination. The values of the ISI reflected the following possibilities (Zapata and Arroyo, 1978): 0 = completely self-incompatible; 0–0.2 = mostly self-incompatible; 0.2–1 = partially self-incompatible and >1 = self-compatible.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

This Chapter deals with the presentation and interpretation of research findings. The results are presented by objective and are presented in sub-chapters.

4.1 Flowering Patterns and Flower Biology

4.1.1 Flowering patterns

Coffea kihansiensis was observed to flower from December to January. Flowering pattern demonstrated for *C. kihansiensis* was a bing-bang flowering (mast flowering), which is a form of mass blooming. The species is probably ephemeral, where flowering is triggered by the first rain showers. Flowers open early in the morning and last for about 2-3 days before withering. The study found that some flower buds remained closed for about 25 days thereafter turning brown and falling off due to absence of sufficient moisture (Plate. 2).

The peak of flowering is in mid-January. Time between onset and peak flowering was 2 days. The total flowering duration in the studied populations was about 11 days and the flowering was synchronized between populations and within individual plants.

Flowering of *C. kihansiensis* varied in the studied population presumably due to variation in the regional climate. Flowering occurs once a year (Davies and Mvungi, 2004). In the present study, the flowering period of this species was shorter and usually terminated before the end of January. Flowering in the species was observed to occur in mid-December through January following light rains, although slight variations in flowering times are evident. In contrast, Rija *et al.* (2011) reported that *C. kihansiensis* flowering started in late September through December, peaking mainly in mid-November. This was presumably due to environmental conditions in the Kihansi gorge. The difference in the flowering period could also be related to the differing rainfall patterns in the study area. Peters and Carroll (2012) noted that, the flowering periods of other traded species of *Coffea* span from February to September. Furthermore, Ngo *et al.* (2010) reported that the flowering period of *Coffea* can vary from region to region. They reported that *C. arabica* generally flowers from January to April, while *C. canephora* flowers from February to March. *C. liberica* var. *liberica* was reported to flower from September to March (Ngo *et al.*, 2011).

The differences in the length of flowering period are probably due to the varied environmental conditions (e.g. soil type and weather conditions), differences among genotypes, or phenotypic plasticity (Elzinga *et al.*, 2007). From the limited data available, it appears that this wild coffee exhibit some differences in its pattern of flowering. The differences are reflected in the commencement, longevity and flowering period.

It is reported that *C. kihansiensis* is mass blooming species. By producing many flowers, the plant experiences regular visiting patterns of local pollinators that will continue to visit despite subsequent decreases in the rate of flower production, providing continuous pollination service (Willmer, 2011). From the observations reported here, it appears that flowering in *C. kihansiensis* is stimulated by rainfall. Similar event was also observed by Vieira (2008) in *C. arabica*. Large scale flowering is triggered by increases in rainfall. Rain showers stimulate bud development and flowers may open a few days after rain. This results in a frequent synchronous pattern of flowering in the genus *Coffea* (Klein *et al.*, 2003a; Ngo *et al.*, 2011). For *C. canephora* and *C. arabica*, there are sufficient data that determine the same flowering patterns. Mass flowering has been recorded in *C. canephora* and *C. arabica* and this is triggered also by rain showers when they are preceded by a dry period (Klein *et al.*, 2003a, b, c; Roubik, 2004; Schroth *et al.*, 2009). Coffee started flowering four days after substantial rainfall and there was synchronized opening of flowers, thus facilitating pollen transfer within individual plants and between plants and populations. Similar trend was observed in *C. canephora* and *C. arabica* (Klein *et al.*, 2003a; b). When compared to other *Coffea* species, the length of the flowering period observed for *C. kihansiensis* in this study was 11 days longer. In the study by Klein et al. (2003a), it was found that the flowering period in *C. canephora* is three days, whereas *C. arabica* had flowering periods of up to seven days.

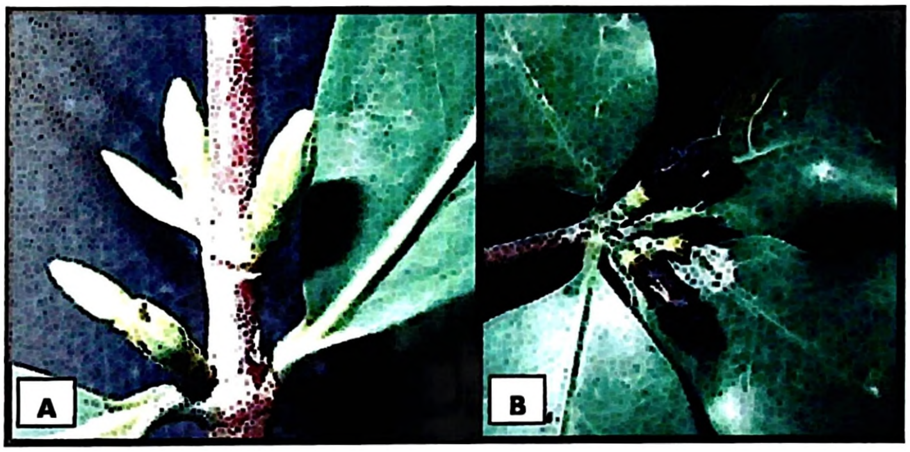

Plate 2: Flowering of *C. kihansiensis* (A) Fresh flower buds (B) Dried flower buds

4.1.2 Flower biology

The inflorescences of *C. kihansiensis* are axillary cymes (Plate 3). The inflorescence axes have a mean length of 6.78 (±0.30) mm. Each inflorescence has 1 or 2 flowers per inflorescence. The inflorescence and floral dimensions are presented in Table 1. There were variations in the number of calyx, petals and stamens. They vary from four to six, but the most frequent number was five. The stigma is presented above the level of the anthers (herkogamy).

Plate 3: ***C. kihansiensis*** **at anthesis**

Table 1: Dimensions (mm) of the inflorescence and floral parts in *C. kihansiensis*

Flower Character (mm)	Mean	SD	CV (%)	Range
Inflorescence length (IL)	6.78	0.3	4.47	6.07-7.23
Flower diameter (FD)	19.3	0.86	4.46	16.8-20
Calyx length (CL)	1.88	0.07	3.78	1.7-2.0
Petal/Corolla length (PL)	9.76	0.37	3.84	8.5-10.7
Corolla tube length (CTL)	4.4	0.13	2.88	4.13-4.5
Corolla lobe length (CLL)	7.43	0.38	5.1	6.17-8.13
Corolla lobe width (CLW)	1.83	0.13	7.26	1.57-2.07
Filament length (FL)	0.56	0.09	16.24	0.47-0.80
Anther length (AL)	4.96	0.27	5.38	4.33-6.0
Style length (SL)	9.05	0.84	9.3	7.96-10.1
Stigma lobe (STL)	1.6	0.12	7.78	1.5-1.9

The floral dimensions such as the length of the calyx, corolla and corolla tube did not vary greatly, as was the length of the filament, length and width of the corolla lobe and the floral diameter. Moreover, the anther, style and stigma length varied constantly, as were the floral primary sexual structures that are important for the precision of the pollination mechanism.

The flowers are bisexual and actinomorphic and have five-lobed calyx. Flowers have five white petals, the lower half of which are fused into a cylindrical corolla tube. The petals are elliptic or oblong, and valvate. Corolla tube is shorter than corolla lobes. Corolla lobes are oblong or oblong-elliptic with an obtuse to rounded apex. There are five stamens with long anthers and short filaments which are epipetalous (Plate 4). There is a long thin style with a two-branched stigma and an inferior ovary of two chambers each containing one ovule.

Plate 4: Floral parts of *C. kihansiensis*

Display character in flower is vital to attracting adequate insect visits. *C. kihansiensis* exhibits floral characteristics that are adapted to insect pollination. The floral traits such as petals as landing sites, herkogamy and colour are all helpful for insect-pollination (Mayer *et al.*, 2011). The presentation of stigma above the level of the anthers indicates that the species is promoting outcrossing. Studies by Webb and Lloyd (1986) and Luo and Widmer (2013) pointed out that, in self-incompatible species, herkogamy may function primarily to reduce sexual interference, whereas in self-compatible plants, it is usually considered an adaptive character that decreases the likelihood of self-pollination and increases the opportunity for outcrossing.

Floral biology of the *C. kihansiensis* is generally similar to that of *C. arabica* and *C. canephora*, with synchronized, ephemeral, diurnal anthesis and fragrance (Klein *et al.*, 2003a, b; Davis and Mvungi, 2004; Ngo *et al.*, 2010). Variations are on the size of the flower and number of flowers per axils. Free (1993) and (Klein *et al.*, 2003a), pointed out that, the flowers of *C. canephora* are bigger and there are more flowers in the axils of leaves, on average 8–20 flowers per axil for *C. canephora* and 2–12 for *C. arabica.*

It was observed that, some flower buds drop off (aborted) in studied populations, probably due to climatic factors, and/or by plant factors (e.g., levels of stored resources) that change as the season progresses. According to Damatta *et al.* (2007) coffee flowering embraces a complex sequence of biochemical, physiological and morphological events which are affected by several factors such as temperature, light as well as soil and air water availability which could also probably be reasons for flower buds abortion in *C. kihansiensis.* The flowers are produced in one season depending on the condition of heat and moisture that prevail in the particular season. Contrary to (Damatta and Ramalho, 2006), when rainfall is perennial, blooming in *C. arabica* continues practically all the year. Flowering pattern in coffee can be controlled by abiotic environmental conditions such as precipitation, temperature, humidity and photoperiodicity, as well as biotic factors including intraspecific and interspecific competition for resources (Peters and Carroll, 2012). Drought is considered the major environmental stress affecting coffee production in most coffee-growing countries (Damatta and Ramalho, 2006). Both flowering pattern and flower biology may affect pollinator behavior, in approaching the flower, whilst they forage within a plant, and interplant movement.

4.2 Flower visitors of *C. kihansiensis*

A total of 1 938 individuals of different insect species were observed visiting the flowers of *C. kihansiensis* (Appendix 1). Forty four different species belonging to 14 different families and mainly in the orders; Coleoptera, Hemiptera, Hymenoptera and Lepidoptera were recorded.

4.2.1 Species richness, diversity and abundance of insect visitors on different coffee populations

There was variation in insect visitation as per the different wild coffee populations of the Kihansi Gorge Forest. The coffee population at the Upper Camp Forest (UCF) had more species visitation when compared to the other populations. Species richness and number of individuals were higher in the population at Upper Camp Forest (UCF) site, followed by the population at Lower Wetland Forest (LWF) and at Research Station Forest (RSF) sites respectively (Table 2). Species richness differed significantly across the surveyed coffee populations ($H = 8.061$; $d.f = 2$; $P = 0.0178$).

Table 2: Summary of species richness and abundance of flower visitors of the three populations of *C. kihansiensis* of the Kihansi gorge forest

	Coffee population		
	UCF	LWF	RSF
Species richness	37	34	25
Abundance	862	813	263

Upper Camp Forest site had the highest species diversity ($H' = 2.74$) than Lower Wetland Forest ($H' = 2.635$) and Research Station Forest ($H' = 2.122$) and the difference was not significant ($H = 2.046$; $d.f = 2$; $P = 0.3596$). Moreover, species evenness was greater at UCF ($E = 0.4185$) and LWF ($E = 0.4102$) and lowest in RSF ($E = 0.3338$), (Table 3). Most flower visitor species were "rare", reflected by the fact that 17 of 44 insect species observed on flowers were represented by one, two or three individuals.

Table 3: Summary of diversity indices of flower visitors of the three populations of *C. kihansiensis* of the Kihansi gorge forest

	Coffee population		
Diversity Indices	**UCF**	**LWF**	**RSF**
Shannon's Index (H')	2.74	2.635	2.122
Simpson's Index (1-D)	0.9108	0.8981	0.7965
Evenness (E)	0.4185	0.4102	0.3338

Flower visitors observed in studied populations included various insect species. Bee species (Plate 5) were the most diverse and dominant flower visitors when compared to other insect species (Plate 6).

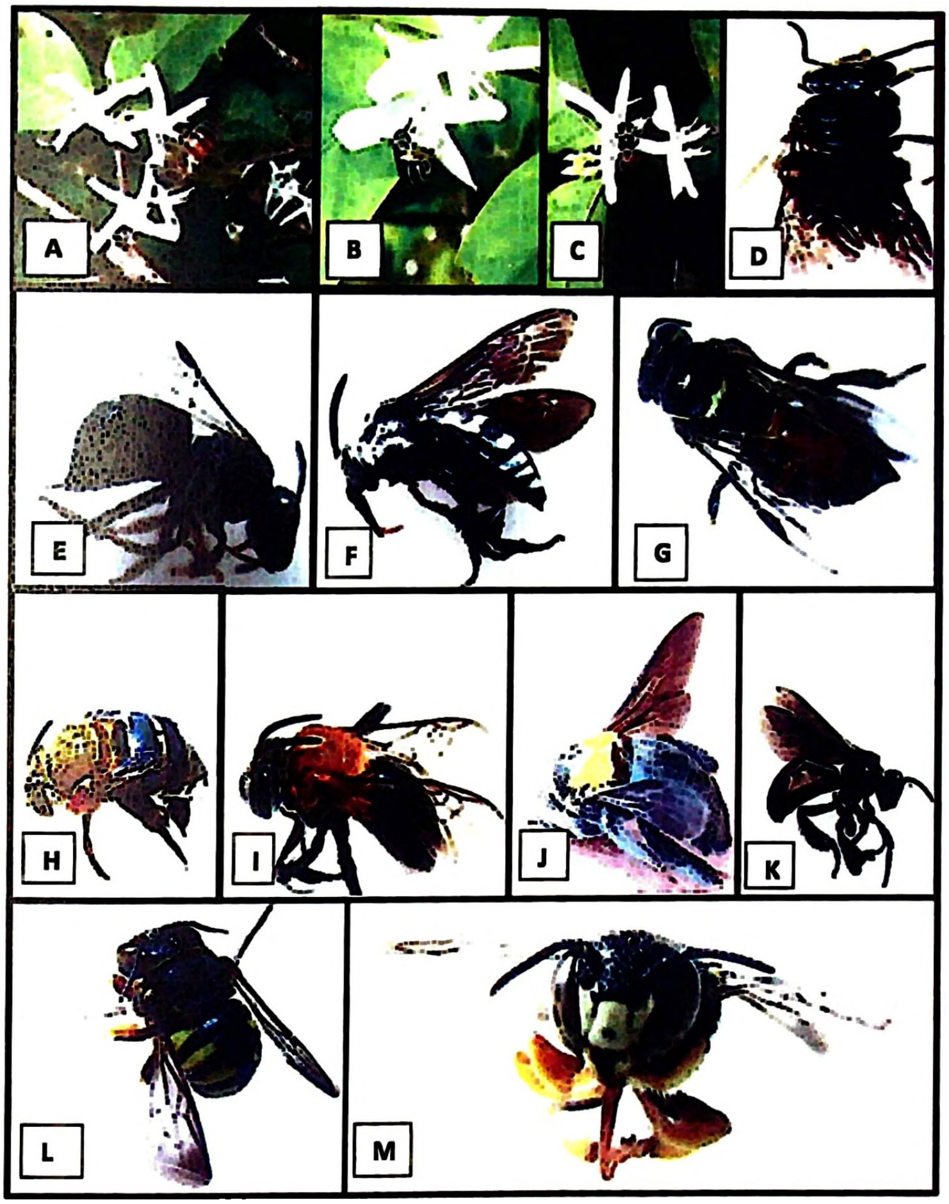

Plate 5: Bee species visiting flower of *C. kihansiensis* (A) *Apis mellifera* (B) *Halictus* sp. (C) *Sphecodes* sp. (D) *Ceratina* sp. 1 (E) *Allodapula* sp. (F) *Braunsapis* sp. (G) *Nomia chandleri* (H) *Amegilla atrocincta* (I) *Xylocopa scioensis* (J) *Xylocopa flavorufa* (K) *Dactylurina schmidti* (L & M) *Anthidium* sp.

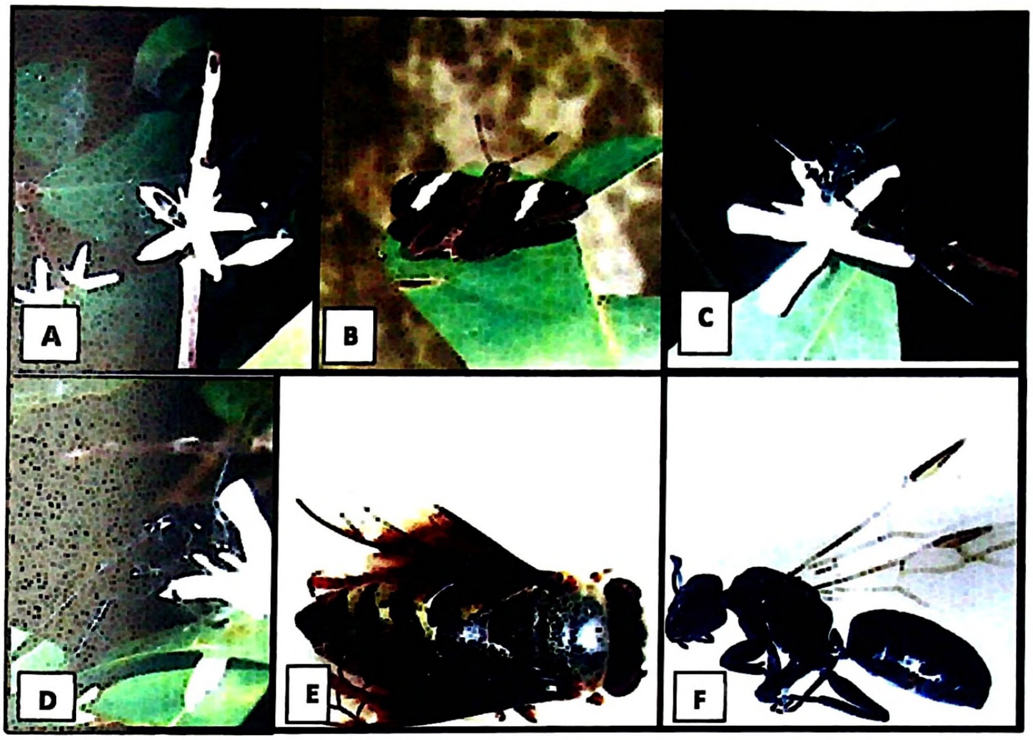

Plate 6: Other visitors of *C. kihansiensis* flowers (A) *Sphenoptera* sp.(B) *Celaenorrhinus sanjeensis* (C) *Promeces longipes* (D) *Anterhynchium fallax* (E) *Tiphiid* sp. (F) *Camponotus* sp.

Among the visitors of *C. kihansiensis*, species in the families, Apidae were dominant, accounting for 34.09% (n = 15) of the total observed species, while species in Andrenidae, Buprestidae, Cerambycidae, Chrysomelidae, Pentatomidae, Pompilidae, Sphingidae, Tiphiidaeand Vespidae were the least dominant, 2.27% (n=1), (Table 4).Similar trend was recorded on the three populations where Apidae, ranked the first in terms of species richness as follows; UCF (13 species), LWF (14 species) and RSF (11 species).The family Halictidae, ranked the second on the study sites. Considering the number of insect visitors across the entire study habitats, family Apidae scored the highest with 60.63% (n = 1175), followed by Halictidae 28.53% (n = 553), Formicidae 7.69% (n = 149) and Megachilidae 1.14% (n = 22). Families with few individuals were Andrenidae, Buprestidae, Cerambycidae, Chrysomelidae, Hesperiidae, Pentatomidae, Pompilidae, Sphingidae Tiphiidae and Vespidae; accounting for less than 1%.

Table 4: Families of insect visitors on the flowers of *Coffea kihansiensis* in the Kihansi gorge forest

S/N	Family	Species	Number of individuals encountered
1	Andrenidae	*Melliturgula* sp.	2
2	Apidae	*Allodapula* sp.	53
		Amegilla atrocincta	8
		Amegilla sp. 2	3
		Apis mellifera	347
		Braunsapis sp.	105
		Ceratina sp. 1	361
		Ceratina sp. 2	78
		Ceratina sp. 3	54
		Ceratina sp. 4	60
		Dactylurina schmidti	34
		Macrogalea sp.	38
		Thyreus sp.	1
		Xylocopa flavorufa	11
		Xylocopa scioensis	16
		Xylocopa sp. 3	6
3	Buprestidae	*Sphenoptera* sp.	2
4	Cerambycidae	*Promeces longipes*	9
5	Chrysomelidae	Leaf beetle (Unidentified species)	2
6	Formicidae	*Camponotus* sp.	18
		Ant (Unidentified species)	129
7	Halictidae	*Halictus* sp. 1	165
		Halictus sp. 2	113
		Lasioglossum sp.	30
		Lipotriches sp. 1	178
		Lipotriches sp. 2	53
		Nomia sp.	1
		Nomia chandleri	3
		Nomia sp. 2	1
		Patellapis sp.	1
		Pseudapis sp.	2
		Spatunomia sp.	1
		Sphecodes sp.	3
		Sweat bee(Unidentified species)	2
8	Hesperiidae	*Celaenorrhinus sanjeensis*	1
		Celaenorrhinus sp. 2	1
9	Megachilidae	*Anthidium* sp.	1
		Heriades sp.	10
		Megachille	11
10	Pentatomidae	*Bagrada* sp	2
11	Pompilidae	*Cyphononyx anguliferus*	10
12	Sphingidae	Hawk moth (Unidentified species)	1
13	Tiphiidae	Tiphiid wasp (Unidentified species)	9
14	Vespidae	*Anterhynchium fallax*	2

The numbers of flower visitors were higher in Upper Camp Forest (UCF) site (862 insect visitors), followed by Lower Wetland Forest (LWF) site (813 insect visitors) and then by Research Station Forest (RSF) site (263 insect visitors). Flower visitors' abundance differed significantly across the surveyed forest sites (H = 11.83; $d.f$ = 2; P = 0.0027). Dunn's multiple comparison tests reveal no significant difference between two coffee populations when UCF and LWF compared (P> 0.05). The difference was revealed between UCF and RSF (P< 0.01) and between LWF and RSF (P< 0.05). Relative abundance of flower visitors differed significantly between study sites (H=33.37; $d.f$=2; P< 0.0001).

Bees were dominant and more abundant than the rest of flower visitors. When the abundance data were pooled together, the most abundant species was unidentified species of *Ceratina* (*Ceratina*sp. 1) with an index of relative abundance (IRA) of 18.63%. Nine species had lowest index of relative abundance (0.052%) each appearing as single individual. The most abundant species at the UCF site was *Ceratina* sp. 1 with the highest index of relative abundance (IRA) of 16.47% and was closely followed by *Apis mellifera* with IRA of 14.97%. Eleven species had the lowest IRA of 0.12% each in this study site. Similarly, the most abundant species at the RSF site was *Ceratina* sp. 1 with the highest index of relative abundance (IRA) of 37.26%. At LWF site the most abundant species was the honey bee (*Apis mellifera*) with a relative abundance of 19.93%, eight species in this study site each had the lowest relative abundance of 0.12%.There was a significant difference in the relative abundance between the surveyed coffee populations (H = 33.37; $d.f$= 2; P<0.0001). This difference was revealed between UCF and LWF sites as well as between UCF and RSF sites (Dunn' test: P<0.001).

This study provided important information about the *C. kihansiensis* flower visitor species richness, diversity and abundance. The study did not find significant differences in the flower visitor diversity among the coffee populations sampled. There are possible explanations for no differences in diversity. First, it could be possible that differences do not exist in flower visitor diversity across the coffee populations. The geographic location of all the populations was the same and the diversity of flower visitors could be consistent over this area. Second, the coffee populations may not have been large and distant enough to show differences in flower visitor diversity. The populations were relatively small and close when compared to the foraging range of some flower visitor e.g. solitary bee from a nesting site. The foraging radius of flower visitor especially bee genus could have overlapped coffee populations. According to Abrol (2012; 2015), bees are able to visit the flowering resources in more than one plant population on a foraging trip creating overlap and even bee diversity across the population. Also, the flower visitor diversity may have been off-set by the availability of nesting sites.

Insect visitors' richness and diversity were higher in populations at UCF and LWF than in population at RSF (Table 3). These suggest that insect visitor' richness and diversity related to flower density (Scriven *et al.*, 2013). There were significantly more flowers in these coffee populations, a greater availability of nesting habitat and proximity to forested edges. This study suggests that more nesting opportunities for bees and floral resources were available to accommodate a greater diversity of flower visitor species. Results from this study affirms the suggestion that flower visitors are likely to visit dense patches of flowering species to minimize on the foraging costs (Hegland and Boeke, 2006; Karanja *et al.*, 2010). Veddeler *et al.* (2006) argued that flower visitor species richness is positively affected by high resource availability in a given population. This is consistent with the study findings.

In contrast to UCF and LWF coffee populations, low richness and diversity of insect visitors in coffee population at RSF might be because flower density, as food resources was lower. Similar results were reported by Klein *et al.* (2003b) where the richness and diversity of insect visitors in *C. arabica* and *C. canephora* decrease with decreased food resources.

The abundance of *C. kihansiensis* floral visitors in the study area varied from one coffee population to another due to availability of food resources. Specifically, higher flower visitor abundance was found in coffee populations with higher flower density. Flower visitor abundance corresponds to coffee populations with high flower density. The abundance of insects visiting coffee flowers was higher in the populations at UCF and LWF than the population at RSF as presented in Table 3. This maybe so because coffee is a mass flowering plant that produces high amounts of nectar and pollen grains (Klein *et al.*, 2002; Klein *et al.*, 2008). The abundance of flower visitors seemed to increase proportionally with increasing resource availability. This is in accordance with a study by Klein *et al.* (2002) that found an increase in the abundance of flower visitors with increasing flower density in coffee populations. Several studies have also shown that flower visitor abundance and species richness is correlated with flower density (Klein *et al.*, 2003a, b, c; 2005; Hegland and Boeke, 2006; Ebeling *et al.*, 2008; Essenberg, 2013; Saturni *et al.*, 2016).

4.3 Pollinators of *Coffeakihansiensis*

Bees comprised 90.4% of all 1 938 individual insect visitors encountered. Bee species visiting flowers of *C. kihansiensis* are appended in Appendix 2. Other visitors which included coleopterans, hemipterans, hymenopterans and lepidopterans accounted for

9.6%.Their role in pollination was considered insignificant due to their infrequent visitation and rarely was observed touching the stigma or anthers.

Two visitors, *Cyphononyx unguliferus* (Pompilidae) and *Anterhynchium fallax* (Vespidae) were observed (Plate 7), visiting *C. kihansiensis* on five and three occasions respectively. Also, one small, black unidentified species of ant (Formicidae) was frequently observed walking along the branches and inflorescence of *C. kihansiensis* collecting nectar (Plate 7 c).They were clearly attracted to the flower as they flew straight toward it. However, they never made contact with reproductive parts, but instead walked on the leaves and petals, as if exploring, before leaving the patch. Two Lepidoptera species were attracted to and visited *C. kihansiensis*. None of them made contact with anthers and stigma; instead they all landed on the petals and feeding on nectar. All of these non-bee visitors are considered as "ecological noise" because they visit flowers and were never reported as efficient pollinators.

Plate 7: Ocassional flower visitors of*Coffea kihansiensis*(A) *Cyphononyx optimus* (B) *Anterhynchium fallax* (C) Unidentified ant

Among the bees, *Apis mellifera*, *Ceratina* sp. 1, *Lipotriches* sp. 1, and *Halictus* sp. 1 were the most abundant, with *Anthidium* sp., *Heriades* sp., *Nomia* sp., *Nomia* sp. 2, *Patellapis* sp. And *Thyreus* sp. being occasional visitors.

Based on a combination of abundance, insect visitors' behaviour, the type of forage collected, contact with essential organs (anther and stigma), entering and leaving the flower, the time spent on the flower and the presence of pollen on their bodies, the potential pollinators of *C. kihansiensis* were identified as 32 bee species (Appendix 2). While approaching the flower and collecting nectar or both pollen and nectar from the flowers, they contact the stamens and stigma of the same or different flowers with their wings, thorax, head and abdomen always and they were observed actively flying between flowers and this foraging behavior contribute to effecting pollination.

The most frequent flower visitors were thought to have a potentially high contribution to pollination of flowers. The most important pollinators for *C. kihansiensis*, in order of importance were *Ceratina* sp., *Apis mellifera*, *Lipotriches* sp., *Halictus* sp., *Braunsapis* sp., *Allodapula* sp. and *Macrogalea* sp. The length of visits varied very much, from a few seconds up to three minutes, as was the frequency of visits during the day with a peak in the morning.

Among the identified potential pollinators of wild *C. kihansiensis* in this study, bees were the most common. This study has clearly demonstrated Apis and non-Apisbee species often visit flowers of *C. kihansiensis* throughout its flowering period and they are potential pollinators. Honey bees, *A. mellifera* and *Ceratina* species were found to be the most dominant and frequent visitors in all the coffee populations studied, accounting for over 90% of the individuals netted on the flowering shrubs. This dominance corroborates earlier studies on coffee plant-pollinators in other tropical regions (Roubik, 2002a; Ricketts, 2004; Vergara and Badano, 2009).

Bee species have shown to be positively contributing to fruit and seed set in *Coffea* species in several studies (Steffan- Dewenter and Tschamtke, 1997; Klein *et al.*, 2003a, b, c; Hoehn *et al.*, 2008); similar results were found in the present study. More abundant pollinator species found to effect pollination in *C. kihansiensis* include *Ceratina* sp. 1 followed by *Apis mellifera.* Pollinator abundance has been shown to enhance pollination in other coffee plants such as *C. canephora* and *C. arabica* (Garibaldi *et al.*, 2013; Munyuli, 2014). Moreover, the abundance of honey bees (*Apis mellifera*) could be attributed to the fact that they are obligate florivores, and both larvae and adult stages feed on floral products (Winfree *et al.*, 2011), unlike other pollinators.

Coffeaarabica and *Coffea canephora* in several studies were reported to be visited mostly by small to medium sized bees (e.g. Klein *et al.*, 2003a; Roubik, 2004). In these studies, solitary and social bees were regularly observed on flowers of genus *Coffea* collecting pollen and nectarwhile pollinating flowers. Roubik (2004) in Panama, observed 22 flower-visiting bee species pollinating *C. arabica.* Willmer and Stone (1989) in Papua New Guinea observed four bee genera, *Apis*, *Trigona*, *Creightonella*, and *Amegilla*, which were effective pollinators for *C. canephora.* The present study reports two (*Apis* and *Amegilla*) out of four genera, but also several other genera capable of effectively pollinating *C. kihansiensis.*

There is extensive evidence showing that honeybees (*A. mellifera*) are important coffee pollinators (Roubik, 2002; Ngo *et al.*, 2011; Giannini *et al.*, 2015). The success of *A. mellifera* pollination may come from its generally slower nectar intake rates, which might favour pollen transfer during floral visits (Fidalgo and Kleinert, 2010). Additionally, it was reported that *A. mellifera* visit more flowers during a foraging trip than individuals of other bee species (Roubik and Buchmann, 1984). However, non-*Apis* bee pollination have been found to enhance higher coffee fruit set in some regions (Daly *et al.*, 2012; Garibaldi *et al.*, 2013). Indeed, solitary bees had similar effect on the fruit set. Results showed that non-*Apis* bees enhance fruit set despite *A. mellifera* abundance (Garibaldi *et al.*, 2013) and that the presence of non-*Apis* bees could guarantee the stability of the pollination service in coffee populations (Garibaldi *et al.*, 2011). Klein *et al.* (2003) indicated that coffee species, although they differ in pollination biology, significantly profit from pollination through natural pollinators. Raw and Free (1977) observed that coffee species are pollinated by both *Apis* bees and non-*Apis* bees in coffee fields during the flowering period. Other studies indicated that, non-*Apis* bees are the more effective pollinators (Willmer and Stone, 1989; Klein *et al.*, 2002). In comparison, solitary bees was found to be the main pollinators of *C. arabica* in Indonesia (Klein *et al.*, 2003b), while social native bees are the major pollinators of *C. canephora* in Uganda (Munyuli, 2014). A recent revision confirms that crop pollination services are mainly provided by a minority of common bee species (Klein *et al.*, 2008; Winfree, 2008).

4.4 Floral Rewards to Visitors and the Periodicity of Release

Flower visiting insects in *C. kihansiensis* populations comprised a diversity of species with varying floral resource requirements. *C. kihansiensis* attracted insect visitors by offering nectar and pollen rewards. Bees were observed to collect pollen from flowers of *C. kihansiensis* (Plate 8). As observed by Masierowska (2003), bees feed almost exclusively on pollen and nectar and need to visit a great number of flowers in order to satisfy the colony's needs (Corbet *et al.*, 1991). Hawk moth, ants, *Bagrada* sp., *Promeces longipes*, *Sphenoptera* sp., *A. fallax*, unidentified species of leaf beetle and the *Celaenorrhinus* species (Plate 9) were observed to collect only nectar. Among the bees, *Xylocopa* species were observed exclusively forage on nectar. However, Keasar (2010) pointed out that, carpenter bees (*Xylocopa* sp.) in natural habitats are generalist nectar and pollen foragers. The nectar and both pollen and nectar foragers are presented in Table 5 and Table 6 respectively.

Table 5: Nectar foragers on *C. kihansiensis* in the Kihansi gorge forest

S/N	Species	Family
1	*Anterhynchium fallax*	Vespidae
2	*Bagrada* sp.	Pentatomidae
3	*Camponotus* sp.	Formicidae
4	*Celaenorrhinus sanjeensis*	Hesperiidae
5	*Celaenorrhinus* sp. 2	Hesperiidae
6	*Cyphononyx anguliferus*	Pompilidae
7	*Promeces longipes*	Cerambycidae
8	*Sphenoptera* sp.	Buprestidae
9	Unidentified Tiphiid wasp	Tiphiidae
10	Unidentified ant species.	Formicidae
11	Unidentified Hawk moth	Sphingidae
12	Unidentified Leaf beetle	Chrysomelidae
13	*Xylocopa flavorufa*	Apidae
14	*Xylocopa scioensis*	Apidae
15	*Xylocopa* sp. 3	Apidae

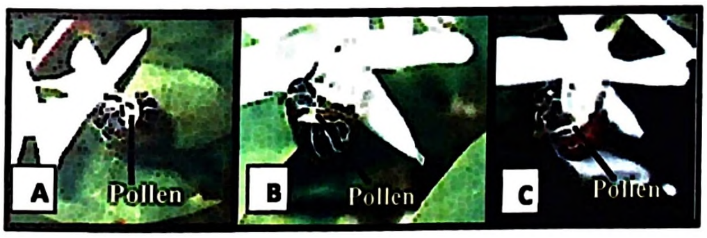

Plate 8: Pollen collectors on *C. kihansiensis*flower (A & B) *Halictus* sp.1 (C) *Sphecodes* sp.

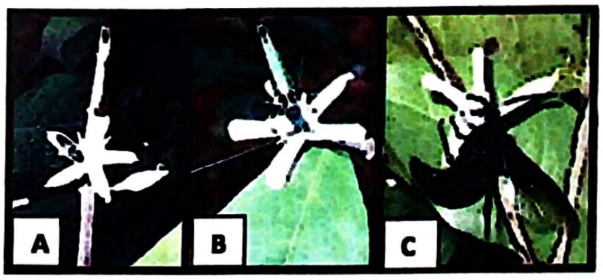

Plate 9: Nectar collectors on *C. kihansiensis* flower (a) *Sphenoptera* sp. (b) *Promeces longipes* (c) *Anterhynchium fallax*

Table 6: Pollen and nectar foragers on *C. kihansiensis*

S/N	Flower visitor	Family
1	*Allodapula* sp.	Apidae
2	*Amegilla atrocincta*	Apidae
3	*Amegilla* sp. 2	Apidae
4	*Anthidium* sp.	Megachilidae
5	*Apis mellifera*	Apidae
6	*Braunsapis* sp.	Apidae
7	*Ceratina* sp. 1	Apidae
8	*Ceratina* sp. 2	Apidae
9	*Ceratina* sp. 3	Apidae
10	*Ceratina* sp. 4	Apidae
11	*Dactylurina schmidti*	Apidae
12	*Halictus* sp. 1	Halictidae
13	*Halictus* sp. 2	Halictidae
14	*Heriades* sp.	Megachilidae
15	*Lasioglossum* sp.	Halictidae
16	*Lipotriches* sp. 1	Halictidae
17	*Lipotriches* sp. 2	Halictidae
18	*Macrogalea* sp.	Apidae
19	*Megachilles* sp.	Megachilidae
20	*Meliturgula* sp	Andrenidae
21	*Nomia chandleri*	Halictidae
22	*Nomia* sp. 2	Halictidae
23	*Nomia* sp. 3	Halictidae
24	*Patellapis* sp.	Halictidae
25	*Pseudapis* sp.	Halictidae
26	*Spatunomia* sp.	Halictidae
27	*Sphecodes* sp.	Halictidae
28	*Thyreus* sp.	Apidae
29	Unidentified sweat bee	Halictidae

The peak pollen release and nectar secretion time for *C. kihansiensis* was observed at 1100 hours (Fig. 2). A relatively higher number of insects visiting flowers of *C. kihansiensis* were observed at 1100 hours, which is more or less aligned with the species' pollen release times and nectar secretion dynamics. This trend was consistently observed in all three coffee populations on all days (Fig. 2). Although the peak pollen release and nectar secretion times occurred in the morning, the insect visitations continued into the afternoon indicating the nectar secretions in *C. kihansiensis* flowers decrease with time. There was a general noticeable decrease in the total insect activity in the area after 1500 p.m. Visitations decreased with respect to the time of the day where by lowest number of flower visitors were observed between 1500-1700 hours.

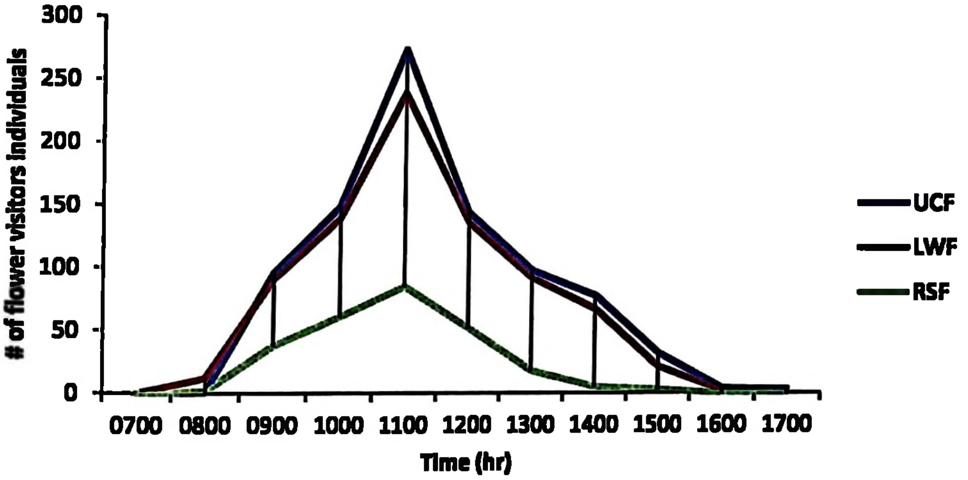

Figure 2: Number of visitors on flowers of *C. kihansiensis* in relation to time

Flower visitors' frequencies were not restricted to the pattern of rewards release. Visiting insects frequencies were higher in the morning than in the afternoon and evening. This was associated with the timing of pollen and nectar availability. In addition to nectar and pollen, the flowers of *C. kihansiensis* also produce a strong fragrance in the morning. This fragrance may be associated with the peak period of nectar secretion. Waller *et al.* (2007) reported that coffee flowers are extremely attractive to a number of insects searching for nectar or pollen which are related to its fragrance (Emura *et al.*, 1997). The pattern of insect visitation differed significantly with the time of the day ($P < 0.0001$), but not with coffee populations (F=0.6744; *d.f* =2; P=0.5278), possibly due to the reduction in floral rewards production.

Most plants use colour, odour, pollen and nectar to attract their visitors and pollinators. Flowers of *C. kihansiensis* show a typical entomophilous character offering both nectar and pollen as a rewards to flower visitors. Nectar is the most sought reward by flower visitors (Willmer, 2011), many of whom depend on the energy it provides for flight, and often has other nutrition benefits such as amino acid content. Nectar with high sugar concentration in the flowers of the genus *Coffea* is an adaptation for pollination by bees (Willmer and Stone, 1989; Roubik, 2002). The present study showed that bees visit the flowers, collect nectar, pollen or both, and effect pollination. These findingsare consistent with those of Waller (2007) who reported that bees collected both nectar and pollen as the principal protein source for their own food from coffee plants.

The availability of floral rewards determines the visiting pattern of insects on flowers. The peak visitation of the flower visitors and the anthesis of the flower took place together in the morning. Thus, it might be assumed that the amount of nectar and pollen were highest in the morning, leading to high visitations at that time period. Floral reward structure, as the ultimate attractant of flower visitors, might have influenced foraging behaviour of bees. Flower visitors are known to prefer high quality nectar (Willmer, 2011; Woodcock *et al.*, 2014). Social bees (*Apis mellifera*) have high resource demands to support large colonies and are likely to benefit more from a mass flowering resource such as coffee (Boreux *et al.*, 2013).

The nectar of the members of the family Rubiaceae, which are pollinated by bees, is known to be hexose dominant to sucrose rich (Wolff, 2006; Raju *et al.*, 2011). Study by Galetto *et al.* (1998) reported that nectar concentration being 48 % for melittophilous Rubiaceae. Nectar is the most sought after resource by flower visitors (Kevan and Baker, 1983), whereas the pollen is eaten by a diversity of insects, and can provide a balanced diet when compared to plant tissues or nectar (Willmer, 2011). Bees prefer very concentrated nectar to guarantee energetically profitable foraging (Wollf, 2006). The nectar of *C. kihansiensis* may also provide a few or some essential and non-essential amino acids for the flower visitor; however these warrant further studies on nectar analysis. This study finds that floral rewards release started in the mid-morning. The similar case was observed in the study by Willmer and Stone (1989), they observed that, at 0900 hrs pollen and nectar release is the highest for *C. canephora*. However, flowers of *C. Arabica* are reported to open at dawn and pollen starts shedding soon after (Ngo *et al*., 2010). At this time, the stigma is receptive and pollination can occur until noon (Alvim, 1985). It was found that there are two distinct groups associated with the different floral rewards. Based on the floral rewards, feeding specialization of the two flower visitor groups were nectar foragers that appear to be relatively specialist species and both pollen and nectar foragers that appear to be generalists.

4.5 Breeding System and Fruit set in *C. kihansiensis*

Fruit set for *C. kihansiensis* was 73.21% through open pollination, 77.87% when flowers were hand cross pollinated (with pollen of another plant) and 61.24% in hand self-pollination. Fruit set from spontaneous selfing was 15.07% (Fig. 4). There were no significant differences between hand pollination with pollen of another plant and open pollination by insects and wind. However, self-pollination (geitonogamy) and controlled open pollination (DP-wind and animal controlled) resulted in a significantly reduced fruit set by61.24% and 49.7% respectively. Accordingly, bee pollination caused an increase of 11.97% in fruit set compared to self-pollination by hand and spontaneous selfing (control treatment). Cross pollination resulted in a 62.8% higher fruit set than in the control, and open pollination in a 58.14% higher fruit set than the control (Fig. 4). Fruit set differed significantly between pollination treatments (*F*= 21.51; *df*= 4; P< 0.0001) and coffee populations (*F*= 13.01; *df*= 2; P< 0.0001) but not between coffee shrubs observed (*F*= 1.24; *df*= 19; P= 0.224).

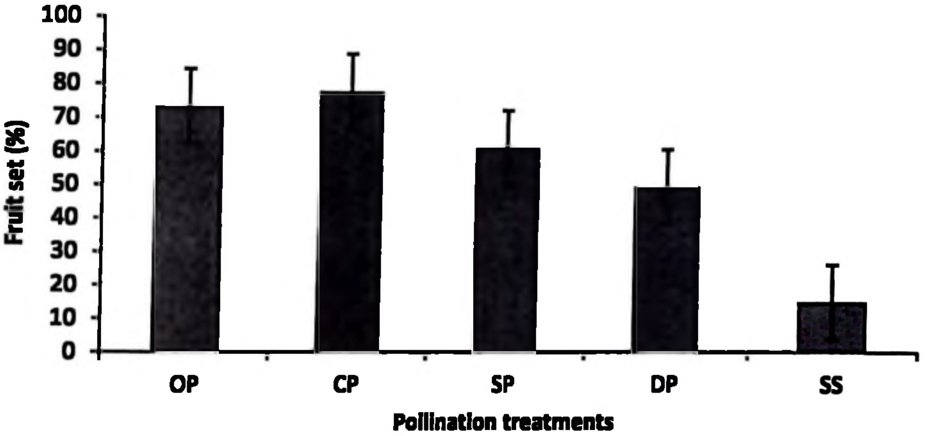

Figure 3: Fruit set of *C. kihansiensis* after different pollination treatments. OP, open pollination (insects and wind); CP, cross pollination by hand (between-plants); SP, self-pollination by hand (within-plant); DP, controlled open pollination; SS, no manipulation (control or selfing)

When coffee populations were compared, the population at LWF had highest fruit set. Fruit set at UCF was 76.08% in open pollination (OP), 80.12% in cross pollination (CP), 62.21% in self-pollination (SP) and 50.06% in controlled open pollination (DP). Fruit set at LWF was 78.69% in open pollination, 81.06% in cross pollination, 64.47% in self-pollination and 52.08% in controlled open pollination. Fruit set at RSF was 64.86% in open pollination, 72.42% in cross pollination, 57.04% in self-pollination and 46.95% in controlled open pollination. Control treatment (SS) resulted in lower fruit set compared to the rest of the treatments in both three coffee populations (Fig. 5).

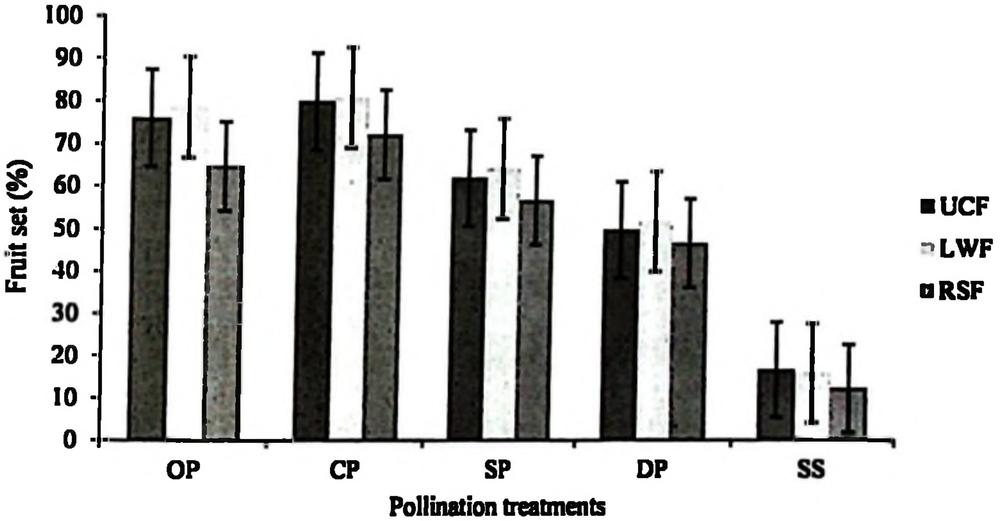

Figure 4: **Fruit set of *C. kihansiensis* at three populations after different pollination treatments**

Result showed that, the index of self-incompatibility (ISI) in *C. kihansiensis* was 0.72, which indicates partial self-incompatibility (*fide* Zapata and Arroyo, 1978). The ISI of *C. kihansiensis* at UCF, LWF, and RSF was 0.72, 0.70, and 0.75 respectively. The reproductive efficacy (RE) of *C. kihansiensis* in the Kihansi gorge forest was 0.98. The RE was high in LWF (1.4), followed by RSF (0.94) and then UCF with RE of 0.61, (Table 7). Natural fruit set was low, while fruit set from cross pollination was higher in both coffee populations, leading to low reproductive efficacy.

Table 7: **Fruit set (%) after different pollination treatments in three different populations of *C. kihansiensis* in the Kihansi gorge forest**

Population	Pollination treatments				
	OP	CP	SP	DP	SS
UCF	76.08	80.12	62.21	50.06	16.81
LWF	78.69	81.06	64.47	52.08	16.02
RSF	64.86	72.42	57.04	46.95	12.39
Mean	73.21	77.87	61.24	49.7	15.07

It was found that; *C. kihansiensis* is partially self-incompatible species. This can be interpreted as an intermediate stage between self-incompatibility and self-compatibility, commonly known as mixed-mating system, which is considered an optimal and evolutionary stable mating strategy by several authors (Goodwillie *et al*., 2005; Harder *et al*., 2007; Ramírez and Nassar, 2016). The breeding experiments demonstrated that *C. kihansiensis* reproduce through both selfing and out-crossing. High level of fruit set depended on pollen getting deposited on stigma by pollinators. In the absence of pollen vectors (control treatment); fewer fruits were produced, indicating that while they can self, the frequency of this in the field is probably low without pollinators. Mixed mating systems are the most prevalent in animal pollinated plants and considered the best because they are able to outcross and self-pollinate (Goodwillie, 2005). Autogamy could provide reproductive assurance when pollinators are scarce (Brys and Jacquemyn, 2011), but could have costs due to gamete and pollen discounting and inbreeding depression (Harder and Aizen, 2010). The breeding experiment showed the control treatment produce few fruits compared to treatments with pollen being transferred by hand. The breeding system of *C. kihansiensis* favors pollinators depositing pollen, but some fruit set is possible without the pollinators in the wild coffee populations.

The breeding system of *C. kihansiensis* contrasts with that of most studied coffee species (i.e. *C. canephora* and *C. arabica*). Davis*et al*. (2006) and Stoffelen *et al*. (2009) reported that, the vast majority of *Coffea* species are known to exhibit a strong Self Incompatibility (SI) response, but three African species (*C. arabica*, *C. anthonyi*, and *C. heterocalyx*) are exceptional for their ability to self-fertilize (i.e. self-compatibility, SC). It is reported that some SI species or populations of plants with functional SI systems show plasticity or variation in the strength of SI (Good-Avila *et al*., 2001). In contrast, a recent study suggests that partial self-incompatibility in plants is a transitional stage along an irreversible evolutionary path toward self-compatibility or extinction (Raduski *et al*., 2012). However, partial self-incompatibility in *C. kihansiensis* species recorded in our study can be interpreted as evidence of the high level of reproductive success associated with mixed-mating under the current scenario of pollination service in natural ecosystems. Besides, Partial self-incompatibility might be a mechanism in *C. kihansiensis* to ensure its survival and perpetuation in its natural habitat.

It was found that, the species is mostly an out-breeder; due to the fact that, outcrossed flowers produced more fruits than self-pollinated ones. As reported by Razanajatovo *et al.* (2016) and Raduski *et al.* (2012), species classified as self-compatible might not be fully self-compatible, and those classified as self-incompatible might be partly self-incompatible. Cross pollination appears to be generally important in the genus *Coffea*. It was observed that out-crossing predominate when pollinator activity was great and flowers were manipulated. However, in the control treatment some flowers produced fruits even in the absence of pollinators. This indicates that some *C. kihansiensis* flowers need cross pollination, whereas others develop fruits even after spontaneous self-pollination. The similar result was reported by Raw and Free (1977), Roubik (1995) and Klein *et al.* (2003a) in *C. arabica*. They observed that some *C. arabica* flowers produced fruits in the absence of any external pollen vector and concluded that *C. arabica* may be amphicarpic. Selfing can be particularly important for plant species whose population sizes are often small (Mena-Ali and Stephenson, 2007), like that of *C. kihansiensis*.

The results from this study showed that *C. kihansiensis* profit from cross pollination by its potential pollinators. The fruit set of *C. kihansiensis*, following cross pollination by hand and by open pollination (insect and wind pollination), was significantly higher than fruit set of manually self-pollinated flowers and the control (selfing).

Results indicate that the open pollinated treatment (73.21%) and the out-crossed by manual treatment (77.87%) were not distinctly different and had the highest values for all five treatment types. Specifically, the presence of pollinators resulted in an increase of 11.97% in fruit set compared to self-pollination by hand and selfing. Correspondingly, pollinator exclusion significantly reduced seed set for *C. kihansiensis*. This indicates that *C. kihansiensis* is dependent on insect pollinators to outcross with other individuals within its population. This finding is consistent with previous results which showed that cross pollination on coffee species increases the amount of fruit set (Klein *et al*., 2003a, 2003b; Roubik, 2004; De Marco and Coelho, 2004; Klein *et al*., 2008; Bravo-Monroy *et al*., 2015; Saturni *et al*., 2016). In contrast, physiological studies argue that coffee fruit set is not affected by the degree of pollination (DaMatta *et al*., 2007). When coffee populations where compared, population at RSF had lower fruit set. There were fewer pollinator visits overall in this population presumably due to environmental factors (e.g. light or temperature) interfered with reproduction in this population.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

This study dealt with the flowering patterns, flower biology, flower visitors, pollinators, floral rewards and their release time, fruit set and breeding system of *Coffea kihansiensis* in Kihansi gorge forest. In this concluding chapter the major findings of the research are briefly presented and practical recommendations based on the study findings are drawn. The chapter concludes with a range of suggestions for further research towards the *in situ* conservation of Kihansi wild coffee.

5.1 Conclusions

The following conclusions are made based on this study.

This study provides a basic understanding of the pollination biology of a wild coffee endemic to the Kihansi gorge forest for which very little information is available. Insect visitation varied between the three studied coffee populations. The flower visitor species diversity was higher in coffee population at UCF. In addition, the coffee population at UCF ranked the highest in abundance of flower visitor species and coffee population at RSF ranked the lowest. The insect families Apidae and Halictidae were noted to be the most diverse and dominant in the area. Floral visitors of *C. kihansiensis* are diverse; some considered as potential pollinators due to their actively foraging behavior while othersmay be considered just flower visitors due to their insignificant foraging behaviour and low visit frequencies.

Floral morphology, flower visitors, pollinators, floral rewards and mating system suggest that *C. kihansiensis* is a bee-pollinated species though fruit set can occur in the absence of pollinators. The pollination strategy of *C. kihansiensis* involves flowers offering two different rewards, pollen and nectar though some taxa could not remove pollen from the anthers and thus visited flowers solely for nectar without pollinating the flower.

This study has provided the first formal breeding system analysis of *C. kihansiensis* in its natural habitat, and report partial self-incompatibility for Kihansi wild coffee. Generally *C. kihansiensis*, significantly profit from pollination through natural pollinators and insect pollination in *C. kihansiensis* clearly contributes to increased coffee fruit which is of conservation importance. Enhancement of bee populations as part of *insitu* wild coffee conservation is foreseen.

5.2 Recommendations

In view of the above discussions and conclusions and to ensure the *in situ* conservation of Kihansi wild coffee in Kihansi gorge, this study recommends the following:

(i) Need for sensitizing policy-makers, land-use planners, forest managers about importance of protecting pollinators and their habitats for plants productivity and ecosystem health enhancements.

(ii) Advocating for policy-changes and for development of national policy that address properly the issue of sustainable conservation of pollinators in both natural and agricultural landscapes taking into account pollination as an ecosystem service that has a wide contribution on productivity at a landscape scale.

(iii) Building taxonomic capacity and develop a good monitoring plan as well as developing good communication strategy to disseminate information on pollination as an ecosystem service to the public.

(iv) There is a need to attract public interests and attach economic values to pollination services. Such information will help governments, policy makers, researchers and the general public to contribute to mitigate the impacts of loss of pollinators.

(v) Further studies and continued monitoring of the *C. kihansiensis* species are needed, with emphasis on understanding basic species biology and population trends.

REFERENCES

Abrol, D. P. (2012). *Pollination biology: Biodiversity Conservation and Agricultural Production*. Springer Dordrecht Heidelberg London, New York. 792pp.

Abrol, D. P. (2015). *Pollination Biology. Pests and Pollinators of Fruit Crops, vol. 1*. Springer, New York. 452pp.

Aerts, R., Hundera, K., Berecha, G., Gijbels. P., Baeten, M., Van Mechelen, M., Hermy, M., Muys, B. and Honnay, O. (2011).Semi-forest coffee cultivation and the conservation of Ethiopian Afromontane rainforest fragments. *Forest Ecology and Management* 261: 1034-1041.

Aga, E., Bekele, E. and Bryngelsson, T. (2005). Inter-simple sequence repeat (ISSR) variation in forest coffee trees (*Coffea arabica* L.) populations from Ethiopia. *Genetica* 124: 213-221.

Alarcón, R., Waser, N. M. and Ollerton, J. (2008). Year-to-year variation in the topology of a plant–pollinator interaction network. *Oikos* 117: 1796–1807.

Alvim, P. T. (1977). Factors affecting flowering of coffee. *Journal of Coffee Research* 7: 15–25.

Alvim, P. T. (1985). *Coffea*. In CRC handbook of flowering. Vol. 2. Edited by A. H. Halevy. CRC Press, Boca Raton, Fla. pp. 308–316.

Ashman, T. L., Knight, T. M., Steets, J. A., Amarasekare, P., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mazer, S. J., Mitchell, R. J., Morgan, M. T. and Wilson W. G. (2004). Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology* 85: 2408–2421.

Augspurger. C. K. (1983). Phenology. flowering synchrony, and fruit set of six neotropical shrubs. *Biotropica* 15(4): 257–267.

Bailey. S., Requier. F, Nusillard, B, Roberts. S. P. M.. Potts. S. G. and Bouget. C. (2014). Distance from forest edge affects bee pollinators in oilseed rape fields. *Ecology and Evolution* 4(4): 370–380.

Banaszak. J. (1996). Ecological bases of conservation of wild bees: The conservation of bees. *Linnaean Society Symposium Sseries* 18: 55-62.

Bawa, K. S. (1990). Plant-pollinator interactions in tropical rain forests. *Annual Review of Ecology and* Systematics 21: 399 – 422.

Biesmeijer, J. C., Roberts, S. P. M., Reemer. M.. Ohlemueller, R., Edwards, M., Peeters, T., Schaffers, A. P., Potts. S. G., Kleukers, R., Thomas, C. D., Settele, J. and Kunin, W. E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* 313: 351–354.

Boreux, V., Kushalappa, C. G., Vaast, P. and Ghazoul, J. (2013). Interactive effects among ecosystem services and management practices on crop production: Pollination in coffee agroforestry systems. *Proceedings of the National Academy of the Science* 110 (21) 8387-8392.

Bravo-Monroy, L., Tzanopoulos, J. and Potts, S. G. (2015). Ecological and social drivers of coffee pollination in Santander. Colombia. *Agriculture, Ecosystems and Environment* 211: 145–154.

Bruckman, D. and Campbell, D. R. (2014). Floral neighborhood influences pollinator assemblages and effective pollination in a native plant. *Oecologia* 176: 465.

Brys, R. and Jacquemyn, H. (2012). Effects of human-mediated pollinator impoverishment on floral traits and mating patterns in a short-lived herb: an experimental approach. *Functional Ecology* 26: 189-197.

Campa, C., Mondolot, L., Rakotondravao. A., Bidel, L. P. R., Gargadennec. A., Couturon, E., Fisca, P. L., Rakotomalala, J., Jay-Allemand, C. and Davi A. P. (2012). A survey of mangiferin and hydroxycinnamic acid ester accumulation in coffee (*Coffea*) leaves: biological implications and uses. *Annals of Botany* 110: 595–613.

Carvalho, A. and Monaco, L. C. (1969). *The breeding of arabica coffee*. In: Fewerda, F. P. and Wit, F. (eds), Outlines of perennial crop breeding in the tropics. Misc. Pap. Agric. Univ. *Wageningen* 4: 189-241.

Charlesworth, D. and Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18: 237–268.

Corbet, S. A., Williams, I. H. and Osborne, J. L. (1991). Bees and the pollination of crops and wild flowers in the European Community. *Bee World* 72: 47-59.

Cordeiro, N. J., Lovett, J. C., Mulungu. E., Maina, G. G. and Gerstle, J. H. (2006). Initial trendsof bird assemblages before and after river diversion in an endemic-rich African forest. *Biodiversity and Conservation* 15: 971– 983.

Coupland. G. T., Paling, E. I. and McGuinness, K. A. (2006). Floral abortion and pollination in four species of tropical mangroves from northern Australia. *Aquatic Botany* 84: 151–157.

Da Matta, F. M. and Ramalho, J. D. C. (2006). Impacts of drought and temperature stress on coffee physiology and production: a review. *Brazilian Journal of Plant Physiology* 18(1): 55-81.

Da Matta, F. M., Ronchi, C. P., Maestri, M. and Barros, R. S. (2007). Ecophysiology of coffee growth and production. *Brazilian Journal of Plant Physiology* 19(4): 485-510.

Daly, Z., Melhim, A. and Weersink, A. (2012). Characteristics of honey bee and non-*Apis* bee (Hymenoptera) farms in Canada. *Journal of Economic Entomology* 105(4): 1130-1133.

Damon, A., Hernández-Ramírez, F., Riggi, L., Verspoor, R., Bertolini, V., Lennartz-Walker, M., Wiles, A. and Burns, A. (2012). Pollination of euglossinophylic epiphytic orchids in agro-ecosystems and forest fragments in southeast Mexico. *European Journal of Environmental Sciences* (2)1: 5–14.

Davis, A. P. and Mvungi, E. (2004). Two new and endangered species of *Coffea* (Rubiaceae) from the Eastern Arc Mountains (Tanzania) and notes on associated conservation issues. *Botanical Journal of the Linnean Society* 146: 237 – 245.

Davis, A. P. and Rakotonasolo, F. (2001a). Three new species of *Coffea* L. (Rubiaceae) from North East Madagascar. *Adansonia* 23: 137– 146.

Davis, A. P. and Rakotonasolo, R. (2001b). Two new species of *Coffea* L. (Rubiaceae) from northern Madagascar. *Adansonia* 23: 337–345.

Davis, A. P., Chester, M., Maurin, O. and Fay, M. F. (2007). Searching for the relatives of *Coffea* (Rubiaceae, Ixoroideae). The circumscriptions and phylogeny of *Coffea* based on plasmid sequence data and morphology. *American Journal of Botany* 94: 313-329.

Davis, A. P., Govaerts, R., Bridson. D. M. and Stoffeelen, P. (2006). An annotated taxonomic conspectus of the genus *Coffea* (Rubiaceae). *Botanical Journal of the Linnean Society* 152: 465-512.

Davis, A. P., Tosh, J., Ruch, N. and Fay, M. F. (2011). Growing coffee: *Psilanthus* (Rubiaceae) subsumed on the basis of molecular and morphological data; implications for the size, morphology, distribution and evolutionary history of *Coffea*. *Botanical Journal of the Linnean Society* 167: 357-377.

Davis, A. P., Rakotonasolo, F. and De Block, P. (2010). *Coffea toshii*sp.nov. (Rubiaceae) from Madagascar. *Nordic Journal of Botany* 28: 1341-1336.

De Castro, R. D. and Marraccini, P. (2006). Cytology biochemistry and molecular changes during coffee fruit development. *Brazilian Journal of Plant Physiology* 18(1): 175–199.

De Marco, P. and Coelho, F. M. (2004). Services performed by the ecosystem: forest remnants influence agricultural cultures' pollination and production. *Biodiversity Conservation* 13: 1245-1255.

Eardley, C. D. (2004). Taxonomic revision of the African stingless bees (Apoidea: Apidae: Apinae: Meliponini). *African Plant Protection* 10(2): 63–96.

Eardley, C., Kuhlmann, M. and Pauly, A. (2010). The Bee Genera and Subgenera of sub-Saharan Africa. *Abc Taxa* vol 7: i-vi, 138 pp.

Eardley, C., Roth, D., Clarke, J., Buchmann, S. and Gemmill, B. (2006). *Pollinators and Pollination*: a resource book for policy and practice. African Pollinator Initiative, African Research Council, Plant Protection Research Institute, Pretoria, South Africa. 77pp.

Eira, M. T. S., Amaral da Silva, E. A., De Castro, R. D., Dussert, S., Walters, C., Bewley, J. D. and Hilhorst, H. W. M. (2006). Coffee seed physiology. *Brazilian Journal of Plant Physiology* 18(1): 149–163.

Elzinga, J. A., Atlan, A., Biere, A., Gigord, L., Weis, A. E. and Bernasconi, G. (2007). Time after time: flowering phenology and biotic interactions. *Trends in Ecology & Evolution* 22(8): 432-439.

Emura, M., Nohara, I., Toyoda, T. and Kanisawa, T. (1997). The volatile constituents of the coffee flower (*Coffea arabica* L.). *Flavour and Fragrance Journal* 12: 9–13.

Essenberg, C. J. (2013). Explaining the effects of floral density on flower visitor species composition. *The American Naturalist* 181(3).

Fidalgo, A. O. and Kleinert, A. M. P. (2010). Floral preferences and climate influence in nectar and pollen foraging by *Melipona rufiventris* Lepeletier (Hymenoptera: Meliponini) in Ubatuba, São Paulo state, Brazil. *Neotropical Entomology*, 39(6): 879-884.

Free. J. B. (1993). *Rubiaceae. Insect Pollination of Crops* (2nd Edition). Academic Press, London, UK. pp 475-478.

Galetto, L., Bernadello, G. and Sosa, C. A. (1998). The relationship between floral nectar composition and visitors in *Lycium* (Solanaceae) from Argentina and Chile: what does it reflect? *Flora* 193: 303–314.

Garibaldi. L. A., Bartomeus, I., Bommarco. R., Klein. A. M., Cunningham. S. A., Aizen, M. A., Boreux, V., Garratt, M. P. D., Carvalheiro, L. G., Kremen, C., Morales, C. L., Schüepp, C., Chacoff, N. P., Freitas, B. M., Gagic, V., Holzschuh, A., Klatt, B. K., Krewenka, K. M., Krishnan, S., Mayfield, M. M., Motzke, I., Otieno, M., Petersen, J., Potts, S. G., Ricketts. T. H., Rundlöf, M., Sciligo, A., Sinu, P. A., Steffan-Dewenter, I., Taki, H., Tscharntke, T., Vergara, C. H., Viana, B. F. and Woyciechowski, M. (2015). Trait matching of flower visitors and crops predicts fruit set better than trait diversity. *Journal of Applied Ecology* 52(6): 1436-1444.

Garibaldi, L. A., Carvalheiro, L. G., Leonhardt, S. D., Aizen, M. A., Blaauw, B. R., Isaacs, R., Kuhlmann, M., Kleijn, D., Klein, A. M., Kremen, C., Morandin, L., Scheper, J. and Winfree. R. (2014). From research to action: enhancing cropyield through wild pollinators. *Frontiers in Ecology and the Environment* 12(8): 439–447.

Garibaldi, L. A., Steffan-Dewenter, I., Kremen, C., Morales, J. M., Bommarco, R., Cunningham, S. A., Carvalheiro, L. G., Chacoff, N. P., Dudenhöffer, J. H., Greenleaf, S. S., Holzschuh, A., Isaacs, R., Krewenka, K., Mandelik, Y., Mayfield, M. M., Morandin, L. A., Potts, S. G., Ricketts, T. H., Szentgyörgyi, H., Viana, B. F., Westphal, C., Winfree, R. and Klein, A. M. (2011). Stability of pollination services decreases with isolation from natural areas despite honey bee visits. *Ecology Letters* 14:1062-1072.

Garibaldi, L. A., Steffan-Dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R. S., Cunningham, A. and Kremen, C. (2013). Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science* 339(6127): 1608-1611.

Giannini, T. C., Boff, S., Cordeiro, G. D., Cartolano, E. A., Veiga, A. K., Imperatriz-Fonseca, V. L. and Saraiva, A. M. (2015). Crop pollinators in Brazil: a review of reported interactions. *Apidologie* 46(2): 209–223.

Goldberg, E. E., Kohn, J. R., Lande, R., Robertson, K. A., Smith, S. A. and Igić, B. (2010). Species selection maintains self-incompatibility. *Science* 330(6003): 493-495.

Good-Avila, S. V., Frey, F. and Stephenson, A. G. (2001). The effect of partial self-incompatibility on the breeding system of *Campanula rapunculoides* L. (Campanulaceae) under conditions of natural pollination. *International Journal of Plant Sciences* 162: 1081–1087.

Goodwillie, C., Kalisz, S. and Eckert, C. G. (2005). The evolutionary enigma of mixed mating systems in plants: occurrence theoretical explanations and empirical evidence. *Annual Review of Ecology Evolution and* Systematics 36: 47-79.

Groom, M. J. (1998). Allee effects limit population viability of an annual plant. *The American Naturalist* 151: 487-96.

Harder, L. D. and Aizen, M. A. (2010). Floral adaptation and diversification under pollen limitation. *Proceeding of the Royal Society of London B: Biological Science* 365: 529-543.

Harder, L. D., Richards, S. A. and Routley, M. B. (2007). Effect of reproductive compensation, gamete discounting and reproductive assurance on mating-system diversity in hermaphrodites. *Evolution* 62: 157–172

Hatanaka, T., Choi, Y. E., Kusano, T. and Sano, H. (1999). Transgenic plants of coffee Coffea canephora from embryogenic callus via *Agrobacterium tumefaciens*-mediated transformation. *Plant Cell Reports* 19(2): 106–110.

Hegland, S. J. and Boeke, L. (2006). Relationships between the density and diversity of floral resources and flower visitor activity in a temperate grassland community. *Ecological Entomology* 31(5): 532-538.

Hein, L. and Gatzweiler, F. (2006).The economic value of coffee (*Coffea arabica*) genetic resources. *Ecological Economics* 60:176–185.

Herrera, C. M. (1995). Floral biology, microclimate, and pollination by ectothermic bees in an early blooming herb. *Ecology* 76: 218-228.

Hoehn, P., Tscharntke, T., Tylianakis, J. M. and Steffan-Dewenter, I. (2008). Functional group diversity of bee pollinators increases crop yield. *Proceedings of the Royal Society B: Biological Science* 275: 2283-2291.

Hopwood, J., Hoffman, B., Lee-Mäder, E., Charlap, A., Preston, R., Mozumder, K. and Fleury, S. (2015). Literature Review: Pollinator Habitat Enhancement and

Best Management Practices in Highway Rights-of-Way. The Xerces Society for Invertebrate Conservation and ICF International. Federal Highway Administration, Washington DC. 68pp.

Igic, B., Kohn, J. R. and Lande, R. (2008). Loss of self-incompatibility and its evolutionary consequences. *International Journal of Plant Sciences* 169: 93–104.

IUCN (2001). *IUCN Red List categories v.3.1.* Gland, Switzerland: IUCN Species Survival Commission. [http://www.iucnredlist.org/static/categories_ criteria _3_1] site accessed on 18th April 2018.

Ivey, C. T., Martinez, P. and Wyatt, R. (2003). Variation in pollinator effectiveness in swamp milkweed, *Asclepia sincarnata* (Apocynaceae). *American Journal of Botany* 90: 214–225.

Jha, S. and Dick, C. W. (2010). Native bees mediate long-distance pollen dispersal in a shade coffee landscape mosaic. *Proceedings of the National Academy of Sciences* 107: 13760-13764.

Jiménez-Castano, W. and Castillo-Zapata, J. (1976). Observations on *Coffea arabica* L. pollination in the central coffee zone of Colombia. *Cenicafe* 27: 51–66.

Karanja, R. H., Njoroge, G. N., Gikungu, M. W. and Newton, L. E. (2010). Bee interactions with wild flora around organic and conventional coffee farms in Kiambu District, Central. *Journal of Pollination Ecology* 2(2): 7-12.

Keasar. T. (2010). Large carpenter bees as agricultural pollinators. *Psyche: A Journal of Entomology* 2010: 1-7.

Kevan. P. G. (1999). Pollinators as bio-indicators of state of the environment: species, activity and diversity. *Agriculture, Ecosystems and Environment* 74: 373-393.

Kevan. P. G. and Baker, H. G. (1983). Insects as flower visitors and pollinators. *Annual Review of Entomology* 28: 407–453.

Klein, A. M. (2009). Nearby rain forest promotes coffee pollination by increasing spatio-temporal stability in bee species. *Forest Ecology and Management* 258: 1838-1845.

Klein, A. M., Cunningham, S. A., Bos, M. and Steffan-Dewenter, I. (2008). Advances in pollination ecology from tropical plantation crops. *Ecology* 89(4): 935–943.

Klein, A. M., Steffan-Dewenter, I. and Tscharntke. T. (2003a). Bee pollination and fruit set of Coffea arabica and C. canephora (Rubiaceae). *American Journal of Botany* 90(1): 153–157.

Klein, A. M., Steffan-Dewenter, I. and Tscharntke. T. (2003b). Fruit set of highland coffee increases with the diversity of pollinating bees. *Proceeding of the Royal Society of London B: Biological Sciences* 270(1518): 955–961.

Klein, A. M., Steffan-Dewenter, I. and Tscharntke, T. (2003c). Pollination of *Coffea canephora* in relation to local and regional agroforestry management. *Journal of Applied Ecology* 40: 837-845.

Klein, A. M., Steffan-Dewenter, I., Buchori, D. and Tscharntke, T. (2002). Effects of land use intensity in tropical agroforestry systems on coffee flower-visiting and trap-nesting bees and wasps. *Conservation Biology* 16(4): 1003–1014

Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C. and Tscharntke, T. (2007) Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences* 274: 303-313.

Knight, T. M., Steets, J. A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mitchell, R. J. and Ashman, T. L. (2005). Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology, Evolution, and Systematics* 36:467–497.

Kremen, C., Williams, N. M. and Thorp, R. W. (2002). Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of Sciences of the USA* 99: 16812–16816.

Krug, C. A. (1935). Hybridization of coffee, a preliminary study of flowering habits and methods of crossing. *Journal of Heredity* 26: 325–330.

Landry, C. L. (2013). Pollinator-mediated competition between two co-flowering Neotropical mangrove species, *Avicennia germinans* (Avicenniaceae) and *Laguncularia racemosa* (Combretaceae). *Annals of Botany* 111: 207–214.

LKEMP (2004). *Lower Kihansi Hydropower project: immediate rescue and emergency measures. Final Specialist report: amphibian studies*: report produced for

Tanzania Electric Supply Company Ltd (TANESCO), Dar es Salaam, Tanzania.

Lovett, J. C., Hatton, J., Mwasumbi, L. B. and Derstle, J. H. (1997). Assessment of the Lower Kihansi Hydropower Project on the forests of Kihansi Gorge, Tanzania. *Biodiversity and Conservation* 6: 915-933.

Lundgren, R., Lázaro, A. and Totland, Ø. (2015). Effects of experimentally simulated pollinator decline on recruitment in two European herbs. *Journal of Ecology* 103:328–337.

Luo, Y. and Widmer, A. (2013). Herkogamy and Its Effects on Mating Patterns in *Arabidopsis thaliana*. *PLoS ONE* 8(2): e57902.

Masierowska, M. L. (2003). Floral nectaries and nectar production in brown mustard (*Brassica juncea*) and white mustard (*Sinapis alba*) (Brassicaceae). *Plant Systematics and Evolution* 238: 97–107.

Mayer, C., Adler, L., Armbruster, W.S., Dafni, A., Eardley, C., Huang, S.Q., Kevan, P., Ollerton, J., Packer, L., Ssymank, A. and Stout, J. C. (2011). Pollination ecology in the 21st century: key questions for future research. *Journal of Pollination Ecology*, 3(2): 8-23.

Mazzafera, P. and Carvalho, A. (1991). Breeding for low seed caffeine content of coffee (*Coffea* L.) by interspecific hybridization. *Euphytica* 59: 55–60.

McGregor, S. E. (1976). *Insect Pollination of Cultivated Crop Plants*. U.S. Department of Agriculture, Washington, D.C. 849pp.

McIntosh, M. E. (2002). Flowering phenology and reproductive output in two sister species of *Ferocactus* (Cactaceae). *Plant Ecology* 159(1): 1–13.

Melin, A., Rouget, M., Midgley, J. J. and Donaldson, J. S. (2014). Pollination ecosystem services in South African agricultural systems. *South African Journal of Science* 110(11): 1–9.

Mena-Ali, J. I. and Stephenson, A. G. (2007). Segregation analyses of partial self-incompatibility in self and cross progeny of *Solanum carolinense* reveal a leaky S-allele. *Genetics* 177: 501–510.

Michener, C. D. (2007). *The Bees of the World*, 2nd Edition. The John Hopkins University Press, Baltimore, MD. 952 pp.

Mitchell, R. J., Irwin, R. E., Flanagan, R. J. and Karron J. D. (2009). Ecology and evolution of plant–pollinator interactions. *Annals of Botany* 103:1355–1363.

Munyuli, T. (2010). Pollinator biodiversity and economics of pollination services in Uganda. Dissertation for Award of PhD Degree at Makerere University, Kampala, Uganda. 451pp.

Munyuli, T. (2014). Influence of functional traits on foraging behaviour and pollination efficiency of wild social and solitary bees visiting coffee (*Coffea canephora*) flowers in Uganda, *Grana* 53(1): 69.

NAFORMA (2010). National Forestry Resources Monitoring and Assessment of Tanzania. *Field Manual, Biophysical Survey*. Dar es Salaam: Ministry of Natural Resources and Tourism, Forestry and Beekeeping Division. 96pp.

Nagai, C., Rakotomalala, J. J., Katahira, R., Li, Y., Yamagata, K. and Ashihara, H. (2008). Production of new low-caffeine hybrid coffee and the biochemical mechanism of low caffeine accumulation. *Euphytica* 164(1): 133–142.

Newstrom, L. E., Frankie, G. W., Baker, H. G. and Colwell, R. K. (1994). "*Diversity of long-termflowering patterns*" in *La Selva: Ecologyand Natural History of a Neotropical Rain Forest*, L. A. McDade, K. S. Bawa, H. A. Hespenheide, and G. S. Hartshorn, (Eds) University of Chicago Press, Chicago, Ill, USA. 142–160 pp.

Ngo, H. T., Mojica, A. C. and Packer, L. (2011). Coffee plant-pollinator interactions: a review. *Canadian Journal of Zoology* 89: 647-660.

Nowak, M. D., Davis, A. P., Anthony, F. and Yoder, A. D. (2011). Expression and Trans-Specific Polymorphism of Self-Incompatibility RNases in *Coffea* (Rubiaceae). *PLoS ONE* 6(6): e21019. https:// doi. org/ 10.1371/ journal.pone.0021019

Olesen, J. M., Bascompte, J., Elberling, H. and Jordano, P. (2008). Temporal dynamics in a pollination network. *Ecology* 89: 1573–1582.

Ollerton, J., Winfree, R. and Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos* 120: 321-326.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Simons, A. (2009). Agroforestree Database: A Tree Reference and Selection Guide, version 4.0. World Agroforestry Centre ICRAF, Nairobi, KE.

Peters, V. E. and Carroll, C. R. (2012). Temporal variation in coffee flowering may influence the effects of bee species richness and abundance on coffee production. *Agroforestry Systems* 85(1): 95-103.

Picker, M., Griffith, C. and Weaving, A. (2004). *Field Guide to Insects of South Africa.* Craft Print International Ltd, Cape Town. 444pp.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O. and Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology and Evolution* 25: 345-353.

Potts, S. G., Vulliamy, B., Dafni, A., Ne'eman, G. and Willmer, P. (2003). Linking bees and flowers: How do floral communities structure pollinator communities? *Ecology* 84: 2628-2642.

Poynton, J. C., Howell, K. M., Clarke, B. T. and Lovett, J. C. (1999). A critically endangered new species of *Nectophrynoides* (Anura:Bufonidae) from the Kihansi Gorge, Udzungwa Mountains, Tanzania. *African Journal of Herpetology* 47: 59-67.

Quinn, C. H., Ndangalasi, H. J, Gerstle, J. and Lovett, J. C. (2003). Effect of the Lower Kihansi Hydropower Project and post-project mitigation measures on wetland vegetation in Kihansi Gorge, Tanzania. *Biodiversity and Conservation* 14: 297-308.

Raduski, A. R., Haney, E. B. and Igic, B. (2012). The expression of self-incompatibility in Angiosperms is bimodal. *Evolution* 66:1275–1283.

Rafferty, N. E. and Ives, A. R. (2012). Pollinator effectiveness varies with experimental shifts in flowering time. *Ecology* 93: 803–814.

Raju, A. J. S., Ramana, K. V. and Lakshmi, P. V. (2011). *Wendlandia tinctoria*(Roxb.) DC. (Rubiaceae), a key nectar source for butterflies during the summer season in the southern Eastern Ghats, Andhra Pradesh, India. *Journal of Threatened Taxa* 3(3): 1594-1600.

Razanajatovo, M., Maurel, N., Dawson, W., Essl, F., Kreft, H., Pergl, J., Pyšek, P., Weigelt, P., Winter, M. and van Kleunen, M. (2016). Plants capable of selfing are more likely to become naturalized. *Nature Communications* 7: 13313.

Reddy, A. G. S., Raju, K. V. V. S. N. and Dhannaraj, P. S. (1988). Pollination in cultivars of *Coffea arabica* L. *Journal of Coffee Research* 18: 78–84.

Richardson, L. L. and Irwin, R. E. (2015). Pollination Ecology and Floral Visitor Spectrum of Turtlehead (*Chelone glabra* L.; Plantaginaceae). *Journal of Pollination Ecology* 17(20): 132-144.

Ricketts, T. H. (2004). Tropical forest fragments enhance pollinator activity in nearby coffee crops. *Conservation Biology* 18: 1262- 1271.

Rija, A. A. (2014). Seed Predation and Plant Recruitment in an Endangered *Coffea Kihansiensis*. Project report submitted to the Rufford Small Grant Foundation, UK. 22pp.

Rija, A. A., Mwamende, K. A. and Hassan, S. N. (2011). The aftermath of environmental disturbance on the critically endangered *Coffea kihansiensis* in the Southern Udzungwa Mountains, Tanzania. *Tropical Conservation Science* 4(3): 359–372.

Roubik, D. W. (1989). *Ecology and Natural History of Tropical Bees.* Cambridge University Press. 514 pp.

Roubik, D. W. (2002a). African Honeybees Augment Neotropical Coffee Yield. In: *Pollinating Bees: The Conservation Link Between Agriculture And Nature.* Edited by Kevan, P. G. and Imperatriz- Fonseca, V. L. Ministry of Environment, Brasilia, Brazil. pp. 255–266.

Roubik, D. W. (2002b). The value of bees to the coffee harvest, (London). *Nature* 417(6890): 708.

Roubik, D. W. and Buchmann, S. L. (1984). Nectar selection by *Melipona* and *Apis mellifera* (Hymenoptera: Apidae) and the ecology of nectar intake by bee colonies in a tropical forest. *Oecologia* 61: 1-10.

Roubik, D. W. and Degen, B. (2004). Effects of animal pollination on pollen dispersal, selfing, and effective population size of tropical trees: a simulation study. *Biotropica* 36: 165-179.

Sahli, H. F. and Conner, J. K. (2007). Visitation, effectiveness, and efficiency of 15 genera of visitors to wild radish, *Raphanusraphanistrum* (Brassicaceae). *American Journal of Botany* 94(2): 203-9.

Sakai, S. (2002). General flowering in lowland mixed dipterocarp forests of South-east Asia. *Biological Journal of the Linnean Society* 75: 233–247.

Saturni. F. T., Jaffé. R. and Metzger. J. P. (2016). Landscape structure influences bee community and coffee pollination at different spatial scales. *Agriculture, Ecosystems and Environment* 235: 1–12.

Schmitt, C. B., Senbeta, F., Denich, M., Preisinger, H. and Boehmer, H. J. (2009). Wild coffee management and plant diversity in the montane rainforest of southwestern Ethiopia. *African Journal of Ecology* 48: 78-86.

Schroth, G., Laderach, P., Dempewolf, J., Philpott, S., Haggar, J., Eakin, H., Castillejos, T., Moreno, J. G., Pinto, L. S. Hernandez, R.and Eitzinger, A. (2009). Towards a climate change adaptation strategy for coffee communities and ecosystems in the Sierra Madre de Chiapas, Mexico. *Mitigation and Adaptation Strategies for Global Change* 14(7): 605-625.

Scriven, L. A., Sweet, M. J. and Port, G. R. (2013). Flower density is more important than habitat type for increasing flower visiting insect diversity. *International Journal of Ecology* 2013: 1–12.

Senbeta, F. (2006). Biodiversity and ecology of Afromontane rainforests with wild *Coffea arabica*populations in Ethiopia. *Ecology and Development Series* No. 38.

Steffan-Dewenter, I. and Tscharntke, T. (1997). Bee diversity and seed set in fragmented habitats. *Acta Horticulturae* 437: 231–234.

Stoffelen, P., Noirot, M., Couturon, E., Bontems, S., De Block, P. and François, A. (2009). *Coffea anthonyi*, a new self-compatible central African coffee species, closely related to an ancestor of *Coffea arabica*. *Taxon*, 58: 133–140.

Saturni. F. T.. Jaffé. R. and [illegible] and community and coffee [illegible] *Ecosystems and Environment* [illegible] of bee

Schmitt. C. B.. Senbeta. F.. Denich [illegible] : 594- coffee management and [illegible] southwestern Ethiopia. [illegible] production in

Schroth. G.. Laderach. P.. Dempewolf [illegible] management systems. T.. Moreno. J. G.. Pinto. L. [illegible] a climate change adaptation [illegible] cultivation. In Plant-parasitic the Sierra Madre de Chiapas [illegible] Springer Science. Dordrecht. *for Global Change* 14 [illegible]

Scriven. L. A.. Sweet. M. [illegible] (2007). *Coffee pests. diseases and their* habitat type [illegible] pp. *Journal* [illegible]

Senbeta. F. (2006) [illegible] The avoidance of interference between the [illegible] angiosperms. II. Herkogamy. *New* [illegible] 163 [illegible]

Steffan [illegible] and floral ecology. Princeton University Press. [illegible] pp.

Stout [illegible] pollination of [illegible] bees in Papua New [illegible]

Winfree, R., Aguilar, R., Vázquez, D. P., LeBuhn, G. and Aizen, M. A. (2009). A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology* 90: 2068–2076.

Winfree, R., Gross, B. J. and Kremen, C. (2011). Valuing pollination services to agriculture. *Ecological Economics* 71: 80–88

Winfree, R., N. M., Williams, H., Gaines, J., Ascher, S. and Kremen, C. (2008). Wild bee pollinators provide the majority of crop visitation across land-use gradients in New Jersey and Pennsylvania, USA. *Journal of Applied Ecology* 45(3): 794–802.

Wolff, D. (2006). Nectar Sugar Composition and Volumes of 47 Species of Gentianales from a Southern Ecuadorian Montane Forest. *Annals of Botany*, 97(5): 767–777.

Woodcock, T. S., Larson B. M. H., Kevan P. J., Inouye, D. W. and Lunau, K. (2014). Flies and flowers II: Floral attractants and rewards. *Journal of Pollination Ecology* 12(8): 63–94.

Wormer, T. M. (1964). The growth of the coffee berry. *Annals of Botany* 28: 47–55.

Zapata, T. R. and. Arroyo, M. T. K. (1978). Plant reproductive ecology of a secondary deciduous tropical forest in Vanezeula. *Biotropica* 10: 221-230.

Zilihona, I., Heinonen, J. and Nummelin, M. (1998). Arthropod diversity and abundance along the Kihansi Gorge (Kihansi River) in the Southern Udzungwa Mountains, Tanzania. *Journal of East African Natural History* 87: 233–240.

Zilihona, I., Niemala, J. and Nummelin, M. (2004). Effects of a hydropower plant on Coleopteran diversity and abundance in the Udzungwa Mountains, Tanzania. *Biodiversity and Conservation* 13: 1453 –1464.

APPENDICES

Appendix 1: Insect visitor species on C. kihansiensis in the Kihansi gorge forest

			Coffee populations			
S/N	Scientific name	Family	UCF	LWF	RSF	Σ ind.
1	*Allodapula* sp.	Apidae	31	22	0	53
2	*Amegilla atrocincta*	Apidae	4	3	1	8
3	*Amegilla* sp. 2	Apidae	0	2	1	3
4	*Anterhynchium fallax*	Vespidae	1	0	1	2
5	*Anthidium* sp.	Megachilidae	0	1	0	1
6	*Apis mellifera*	Apidae	129	162	56	347
7	*Bagrada* sp.	Pentatomidae	1	0	1	2
8	*Braunsapis* sp.	Apidae	48	57	0	105
9	*Camponotus* sp.	Formicidae	14	3	1	18
10	*Celaenorrhinus sanjeensis*	Hesperiidae	0	1	0	1
11	*Celaenorrhinus* sp. 2	Hesperiidae	0	1	0	1
12	*Ceratina* sp. 1	Apidae	142	121	98	361
13	*Ceratina* sp. 2	Apidae	33	18	27	78
14	*Ceratina* sp. 3	Apidae	25	17	12	54
15	*Ceratina* sp. 4	Apidae	14	35	11	60
16	*Cyphononyx anguliferus*	Pompilidae	2	7	1	10
17	*Dactylurina schmidti*	Apidae	18	11	5	34
18	*Halictus* sp. 1	Halictidae	72	87	6	165
19	*Halictus* sp. 2	Halictidae	63	49	1	113
20	*Heriades* sp.	Megachilidae	9	1	0	10
21	*Lasioglossum* sp.	Halictidae	14	11	5	30
22	*Lipotriches* sp. 1	Halictidae	78	89	11	178
23	*Lipotriches* sp. 2	Halictidae	22	31	0	53
24	*Macrogalea* sp.	Apidae	21	17	0	38

	25	*Megachile* sp.	Megachilidae	1	8	2	11
	26	*Meliturgula* sp.	Andrenidae	1	1	0	2
	27	*Nomia chandleri*	Halictidae	3	0	0	3
	28	*Nomia* sp. 2	Halictidae	1	0	0	1
	29	*Nomia* sp. 3	Halictidae	0	1	0	1
	30	*Patellapis* sp.	Halictidae	1	0	0	1
	31	*Promeces longipes*	Cerambycidae	3	5	1	9
	32	*Pseudapis* sp.	Halictidae	1	1	0	2
	33	*Spatunomia* sp.	Halictidae	1	0	0	1
	34	*Sphecodes* sp.	Halictidae	1	2	0	3
	35	*Sphenoptera* sp.	Buprestidae	1	0	1	2
	36	*Thyreus* sp.	Apidae	0	1	0	1
	37	*Tiphiid* sp.	Tiphiidae	3	5	1	9
	38	Unidentified ant sp. 1	Formicidae	85	36	8	129
	39	Unidentified sp. (Sweat bee)	Halictidae	2	0	0	2
	40	Unidentified sp. (Hawk moth)	Sphingidae	0	1	0	1
	41	Unidentified sp. (Leaf beetle)	Chrysomelidae	1	0	1	2
	42	*Xylocopa flavorufa*	Apidae	5	2	4	11
	43	*Xylocopa scioensis*	Apidae	6	4	6	16
	44	*Xylocopa* sp. 3	Apidae	5	0	1	6
Total				862	813	263	1938

Appendix 2: Bee species visiting the flowers of *C. kihansiensis*in the Kihansi gorge forest

S/N	Family	Bee species	Σ ind.
1	Apidae	*Allodapula* sp.	53
2	Apidae	*Amegilla atrocincta*	8
3	Apidae	*Amegilla* sp 2	3
4	Megachilidae	*Anthidium* sp	1
5	Apidae	*Apis mellifera*	347
6	Apidae	*Braunsapis* sp.	105
7	Apidae	*Ceratina* sp. 1	361
8	Apidae	*Ceratina* sp. 2	78
9	Apidae	*Ceratina* sp. 3	54
10	Apidae	*Ceratina* sp. 4	60
11	Apidae	*Dactylurina schmidti*	34
12	Halictidae	*Halictus* sp 1	165
13	Halictidae	*Halictus* sp 2	113
14	Megachilidae	*Heriades* sp.	10
15	Halictidae	*Lasioglossum* sp.	30
16	Halictidae	*Lipotriches* sp. 1	178
17	Halictidae	*Lipotriches* sp. 2	53
18	Apidae	*Macrogalea* sp	38
19	Megachilidae	*Megachile* sp	11
20	Andrenidae	*Meliturgula* sp	2
21	Halictidae	*Nomia chandleri*	3
22	Halictidae	*Nomia sp. 2*	1
23	Halictidae	*Nomia* sp. 3	1
24	Halictidae	*Patellapis* sp.	1
25	Halictidae	*Pseudapis* sp	2
26	Halictidae	*Spatunomia* sp	1
27	Halictidae	*Sphecodes* sp	3
28	Apidae	*Thyreus* sp.	1

29	Halictidae	Unidentified sp (Sweat bee)	2
30	Apidae	*Xylocopa flavorufa*	11
31	Apidae	*Xylocopa scioensis*	16
32	Apidae	*Xylocopa* sp. 3	6
33	Total		1752

29	Halictidae	Unidentified sp (Sweat bee)	2
30	Apidae	*Xylocopa flavorufa*	11
31	Apidae	*Xylocopa scioensis*	16
32	Apidae	*Xylocopa* sp. 3	6
33	**Total**		**1752**