DETERMINING POLLINATORS, FLORAL CALENDAR AND THE EFFECT OF POLLINATION DEFICIT OF AVOCADO *Persea americana* MILL IN KANDARA, MURANG'A COUNTY, KENYA

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DECLARATION

This thesis is my original work and has not been presented for the award of a degree in any other University or any other award

Signature……………………………………………Date……………………….......

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SUPERVISORS' APPROVAL

We confirm that the work reported in this thesis was carried out by the candidate under our supervision and has been submitted with our approval as supervisors

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DEDICATION

I dedicate this thesis to my beloved wife, Petronillah Wambua and our beloved children; Purity Kyambi, Rachael Mwende and Joshua Muuo.

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ABBREVIATIONS AND ACRONYMS

DAFF	Department of Agriculture, Forestry and Fisheries
FAO	Food and Agriculture Organization of the United Nations
HCDA	Horticultural Crops Development Authority
INRA	Institut National de la Recherche Agronomique
KALRO	Kenya Agricultural and Livestock Research Organization
Ksh	Kenya Shillings
MoA	Ministry of Agriculture
MT	Metric Tones
MUFA	Monounsaturated Fatty Acids
NSRC	National Sericulture Research Centre
P	Probability level of significance
UM2	Upper Midland 2

ABSTRACT

Avocado *Persea americana* Mill is an important fruit in Kenya. It is a source of vitamins, oil and income to farmers. However, its optimal production is dependent on insect pollinators. This study was undertaken to determine diversity and abundance of insects visiting avocado flowers, their diurnal visitation counts, the crop floral calendar and the effect of pollination deficit of avocado at Kandara, Murang'a County. The study was carried out in farmers' fields in upper midland 2 agro ecological zone, for three cropping seasons, from August 2015 to March 2017. A total of 60 trees were randomly selected from 12 farms for the study with a minimum distance of 10 m and 200 m between trees and between farms, respectively. Treatments included unlimited access and no access of flowers by pollinators. On each tree, two randomly selected terminal branches with inflorescences of the same age and size were identified, where one was bagged using nylon mosquito nets to deny access by pollinators while the other was left open for unlimited access by pollinators. As soon as flowers wilted, the nets were removed, and fruits on the tagged terminal branches counted. Data collected included identity of the pollinator, number of individuals observed pollinating per species, time of the day, the time (seconds) taken by the pollinator on the flower per visit, the percentage flushing, percentage flower buds, percentage flowering and percentage fruiting. Number of male and female flowers was recorded from 0900 h to 1700 h. Fruit counts were done monthly. At maturity, fruits were harvested and their weight, polar diameter, equatorial diameter, seed weight, ovary polar and equatorial diameters were determined in the laboratory. Pollination deficit data were analyzed using Student's t-test. Findings showed that honey bees *Apis mellifera* were the major flower visitors followed by blow flies *Chrysomya putoria*. Others included hoverflies *Eristalis tenax*, wasps *Polistes* sp., butterflies *Colias electo*, ants *Iridomyrmex reburrus* and beetles *Drypta ruficollis*. Opening and maturity of female and male phase flowers overlapped between 1200 h to 1659 h, with the peak overlap from 1300 h to 1359 h. Fruit set was significantly ($P < 0.001$) higher in flowers where terminal branches were allowed unlimited access to pollinators (19.85±2.00) compared to those denied access (9.38±1.55). Further, a more than two folds increase in final fruit yield was recorded in terminal branches where flowers were exposed to unlimited access (0.46±0.08) by pollinators compared with those denied pollinator access (0.17±0.05). Based on the yield differences, a 64.5% pollination deficit was recorded for avocado. Laboratory results showed existence of significant statistical differences in seed weight ($P=0.001$) with those from terminal branches exposed to unlimited access having 15.41 ±1.75g compared with those denied access (7.13±1.07g). The differences also existed for ovary equatorial diameter ($P=0.001$) and fruit equatorial diameter ($P=0.035$). This study thus confirmed that pollination plays a significant role in avocado production. Farmers are advised to adopt appropriate pollination management plans for their avocado orchards such as provision of honey bee colonies and ensuring presence of diverse community of pollinators, which will enhance pollination provision by honey bees.

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Background

The avocado *Persea americana* Mill, originated in Mexico, Central or South America, and was first cultivated in Mexico as early as 500 BC (Rainey *et al.,* 1994; Duester, 2000; California Avocado Commission, 2012). It is an important crop in many tropical and subtropical regions around the world (Knight, 2002). The world's avocado producing countries, in metric tons (MT) include Mexico - producing 1,520,695 MT, Dominican Republic (428,301 MT), Peru (349,317 MT), Indonesia (307,326 MT), Colombia (288,739 MT), Kenya (218,692 MT), United States (179,124 MT), Rwanda (161,519 MT), Chile (160,000 MT), Brazil (156,699 MT) totaling to over 3.8 million tons annually (FAO, 2014).

Avocado is an important commercial fruit crop grown mainly in Kenya by both small and large scale farmers for fresh fruits and processing into avocado oil. The main avocado varieties grown for export market are Hass, Fuerte and Pinkerton. In addition, three other varieties are traded in the local market: Puebla, Duke and G6. Avocado farming in Kenya has recorded increases in area (13%), production (27%) and value (2.5%) (HCDA, 2016). Yields of 231,453 MT and 246,057 MT in 2015 and 2016, respectively have been recorded (Match Markers Associates, 2017). This has translated to increased demand of avocado for fresh fruit market, pharmaceutics and cosmetic industries, providing a feasible growth and expansion of avocado production.

Kenya has in the past been ranked sixth-largest exporter of avocado to Europe, with a 5-6% share of volume in 2010 (FruiTrop, 2012). It enjoys a competitive advantage over many producers such as Peru, Mexico, Israel and South Africa because of a window of months (October to February) where no other country has avocado fruits (Queensland government, 2009). In Kenya, most of the avocado fruits for export come from Murang'a County, making it an important crop to rural communities and economies, accounting for 12% of the total income and 23% of crop income in Kandara (Cooper *et al.*, 2003; Oduol *et al.*, 2013).

In Kenya, avocado ranks fourth after bananas, pineapples and mangoes in value (HCDA, 2014). In 2014, avocado contributed Ksh. 3.8 billion, accounting for 5% of the total fruits value, and production had increased by 27%, with Nyamira (23.1%), Murang'a (13.1%), Bungoma (8.9%), Kisii (6.9%) and Kiambu (6.1%), which accounted for up to 57% of the volume of avocado produced in Kenya (HCDA, 2014). However, avocado produced in Nyamira and Bungoma are not of the export varieties (HCDA, 2014). Avocado production and marketing in Kandara revolves around two main varieties, namely Fuerte and Hass, which are mainly exported (Oduol *et al.*, 2013).

Avocado production in Kenya faces various challenges such as limited superior varieties or planting materials, poor tree crop management practices, pests and diseases, poor infrastructure, poor pre- and post-harvest handling practices and poor market information (MoA, 2005). In other avocado producing countries such as Israel and New Zealand, the crop is known to suffer from inadequate pollination (Evans *et al.,* 2010). This is occasioned by low pollinator visits and lack of efficient pollinators, among other factors (Afik *et al.,* 2006; Balam *et al.,* 2012).

In Kenya, there is little attention to pollination management by farmers, possibly due to lack of awareness about the role of pollination in crop yields (Kasina *et al.,* 2009a). This lack of awareness is attributed to low knowledge about the pollination service by extension service providers and key decision makers responsible for information packaging and dissemination to farmers (Kasina and Gemmill, 2014). However, it is now globally accepted that pollination is an input of crop production and its proper utilization can drastically enhance crop yields and quality (Kasina *et al.,* 2009a). Hence, there is increasing urgency to package pollination information for farmer utilization in crop productivity.

Pollination is the transfer of pollen grains from anthers (pollen bearing part of a stamen, the male floral organ) to the stigma (receptive surface of the pistil, the female organ of a flower) (Kasina *et al.,* 2009b). High crop yield reductions have been associated with the severe reduction in insect populations leading to pollination deficit. Pollination has also been noted to make a significant contribution to the production of fruits, vegetables, fiber crops and nuts (Levin, 1984; Costanza *et al.,* 1997; Gordon and Davis, 2003).

Pollinators have an economic impact on agricultural output in simple yield measures (numbers, weight and size of fruits and seeds) and this could extend into harvest and post-harvest effects as well (Dag *et al.,* 2007; Gaaliche *et al.*, 2011; Bommarco *et al.,* 2012; Delaplane *et al.,* 2013). Crop yields improvement due to sufficient pollination varies per crop, ranging from over 90% in mango and almonds to between 10 and 20% for peanuts and grape (Morse and Calderone, 2000; Roubik, 2002). Post-harvest effects of pollination include aspects such as fruit sweetness, shape, weight, texture, and other flavor metrics (Gallai *et al.*, 2009).

Fruit quality due to cross-pollination (the transfer of pollen from the male reproductive organ (an anther) of one plant to the female reproductive organ (a stigma) of another plant) and close-pollination (pollination of a flower with pollen of another flower on the same plant) have been noted even in one seeded fruits such as mango (Dag *et al.*, 1999) and avocado (Degani *et al.*, 1990). However, Gallai *et al*. (2009) noted that the production value per unit farming area of insect pollinated crops is four times that of crops that do not need insect pollination. Thus, pollinator-dependent crops could bring more money to the farmers in small units which are more nutritious, giving more health benefits.

In Kenya, insect pollination plays a critical role in increasing crop yields and improving plant hybrid vigour. Kasina *et al*. (2009c) estimated a value of 3.2 million U.S Dollars from the pollination services provided to eight crops grown for vegetables in Kakamega district only. Although some crops were self-pollinating, abundance and visitation by wild bees increased farm yields between 25-99% (Kasina *et al.,* 2009b).

1.2 Statement of the problem

In most developed countries, avocado is known to benefit from flower visitation by a wide range of insects such as stingless bees, honey bees and social wasps (Gazit and Degani, 2002; Can-Alonzo *et al.,* 2005; Afik *et al.,* 2006). Further, it is also reported to suffer from insufficient pollination in commercial orchards, resulting to low fruit yields (Vithanage, 1990; Gazit and Degani, 2002). Thus avocado growers in those countries often use honey bees colonies to provide pollination service.

Avocado flowers are not so attractive to honey bees and when the crop co-flowers with more attractive flowers, honey bee visitation to avocado diminishes (Afik *et al.,* 2006). One of the reasons suspected to make the avocado flowers unattractive is the presence of high mineral concentrations and alcohol perseitol in the flower nectar that repels honey bees (Afik *et al.,* 2011). In contrast, true flies (Dipterans) are reported as efficient pollinators (Can-Alonzo *et al.,* 2005) but a challenge exists in increasing their density for commercial enterprises. Since avocado flower architecture does not suit a single or few arrays of pollinators (Wysoki *et al.,* 2002), it is possible that a diversity of flower visitors can contribute to its pollination success.

There is no study yet conducted in Kenya to investigate the contribution of insect pollinators in avocado yields. In addition, no study has been done to identify the pollinators and their frequency in visiting the flowers at the time of blooming. Lack of these studies impedes management of avocado pollination and therefore the crop is unlikely to reach its full productivity potential. Therefore, this study was carried out in farmers' fields to identify possible avocado pollinators, their abundance, diversity and effects on fruit set and yield. The study also included determination of the crop floral calendar and its interaction with pollinators.

1.3 Justification

In Kenya, avocado is an important fruit tree crop, providing income to many households and foreign exchange to the country. However, the crop still lags behind in production, considering it is ranked 4^{th} in volume among the fruit crops (HCDA, 2016). Kandara in Murang'a County was chosen as the study site due to its high avocado production levels, hence making the area to lead in the export of avocado. Avocado is one of the main sources of livelihoods in the area.

One of the key aspects of avocado production is provision of pollination service, which assures fruit set and hence productivity. There is limited information on importance of pollination in avocado production and majority of farmers in Kenya are not aware of the role pollination plays in crop production (Kasina *et al.*, 2009a).

1.4 Research questions

i. Who are the avocado pollinators in orchards in Kandara, Murang’a County?
ii. What is the preferred diurnal visitation time of the diverse pollinators of avocado trees grown in orchards in Kandara, Murang’a County?
iii. What is the floral calendar of avocado in Kandara, Murang’a County?
iv. What is the effect of avocado pollination deficit in Kandara, Murang’a County?

1.5 Research objectives

1.5.1 General objective

To determine pollinators, floral calendar and the effect of pollination deficit of avocado for enhanced yield and fruit quality in Kandara sub-county, Murang’a County, Kenya.

1.5.2 Specific objectives

i. To evaluate abundance and diversity of insect pollinators of avocado in Kandara, Murang’a County.
ii. To determine diurnal visitation counts of insects pollinating avocado in Kandara, Murang’a County.
iii. To investigate floral calendar of avocado in Kandara, Murang’a County.
iv. To evaluate the effects of avocado pollination deficit in Kandara, Murang’a County

1.6 Hypotheses

i. There is no high abundance and diversity of avocado pollinators in Kandara, Murang'a County.

ii. There is no difference in diurnal visitation counts of insects pollinating avocado in Kandara, Murang'a County.

iii. There are no patterns of avocado flowering in Kandara, Murang'a County.

iv. There are no effects of pollination deficit in avocado in Kandara, Murang'a County.

1.7 Significance of the study

The findings of this study will enhance the understanding of avocado pollination needs and guide in its utilization for productivity of the crop through developing a pollination management plan for the crop in Kenya. In addition, the study findings will provide information that could be used to formulate and improve policies and extension guidance for avocado production. The study will also open more avenues for research leading to more understanding on avocado pollination in Kenya.

CHAPTER TWO: LITERATURE REVIEW

2.1 Taxonomy of avocado and cultivated varieties

Avocado belongs to the Order Laurales, Family Lauraceae and is divided into 3 sub-species, that is, Mexican (sub-tropical), Guatemalan (semi-tropical) and West Indian (tropical). Each sub-species has unique ecological adaptations and identifiable characteristics, with interracial hybrids occurring (Bergh, 1975; Knight Jr, 1980). Several varieties of avocado were introduced in Kenya in the 1930s by the Portuguese (Griesbach, 1985; 2005). These include Bacon, Booth 7, Booth 8, 2 strains of Fuerte from South Africa and the U.S, Hardy, Hass, Maypan, Linda, Lyon, Lula, Nabal, Puebla, Reed, Simmonds, Tonnage and Zutano.

The avocado is a tropical evergreen, upright tree, variable in shape, from tall to widely spreading forms with multiple branches. Fruit bearing starts 3-6 years after transplanting and may continue for 50 years, but the average life span is 35 years. Trees can attain heights of 15–18 m, with manageable height being controlled by pruning (Paull and Duarte, 2011).

In Kenya, avocado production is dominated by smallholders who constitute 85% of total avocado growers in the country (Wasilwa *et al.*, 2007). The domestic market is the largest source of demand for Kenyan avocado, accounting for over 80% of the total production while the rest are exported as fresh fruits or processed and exported as crude oil (Mwangi, 2006).

2.2 Economic importance of avocado

Besides its use as a fresh fruit and in the oil industry, avocado is also useful in the cosmetic, soap and shampoo industry (Maregesi *et al.*, 2014), as well as processed foods derived from it, such as guacamole, frozen products and avocado paste. Avocado farming has improved the livelihoods of the local farmers in Kandara through income and employment opportunities via contract farming, with Hass and Fuerte being the major commercial varieties grown for both local consumption and export (Mwambi *et al.,* 2013).

The avocado is a medium energy dense (1.7 kcal/g) fruit containing about 80% water and dietary fiber (Fulgoni *et al.*, 2013). Unlike other fruits, it is low in sugar and contains 15% monounsaturated fatty acids (MUFA) and rich oil, which helps to increase the bioavailability of carotenoids from salads and recipes, often mixed with avocado during consumption (Fulgoni *et al.*, 2013). The fruits are also rich in vitamins A, B6, C, D, E and K (Pierce, 1959), minerals especially potassium, iron, phosphorus, magnesium, manganese, zinc and copper. They also contain phytochemicals such as lutein, phenolic antioxidants, and phytosterols associated with numerous potential health benefits (Dreher and Davenport, 2013).

2.3 Role of pollination in plants

Pollination is one of the most important processes involved in the maintenance and promotion of biodiversity and, in general plant life on earth. Pollination by animals is being increasingly recognized as an essential ecosystem service, whose sufficient provisioning leads to overall increased and stabilized crop production (Garibaldi *et al.,* 2011; Gemmill - Herren *et al.,* 2014), and therefore sustain income and food security. Almost 90% of the world's flowering plants rely on animal pollination to produce fruits and seeds (Ollerton *et al.,* 2011). Both human and wildlife benefit from fruit and seed bearing plants as products of pollination. A range of studies have shown that pollination makes a very significant contribution to the agricultural production of a broad range of crops, particularly fruits, vegetables, fiber crops and nuts (Gordon and Davis, 2003).

Adequate pollination leads to enhanced quality of the produce such as fruits (Garratt *et al.,* 2014). In addition to the agronomic yield and its many components such as fruit set, seed set, fruit quality (size, sugar content, flavor and nutritional content), pollination affects seed quality (germination rate, oil content), and other characteristics such as earliness to maturity and uniformity of output (Sabbahi *et al*., 2006). While previous research has focused mainly on exploring the effects of pollinators on fruit or seed set (Garibaldi *et al.,* 2011; Garibaldi *et al.,* 2013), which is a more direct measure of plant reproduction, yield measurements have been effectively used to better reflect economic value (Bommarco *et al.,* 2012; Klatt *et al.,* 2014), and hence, farmers' interest.

2.3.1 Pollination provision and requirements in agricultural crops

Approximately three-quarters of food crops worldwide including primarily vitamin and mineral-rich crops like fruits and vegetables, depend on animal pollinators, of which insects comprise the major pollinating group (Klein *et al.,* 2007). Pollination requirements differ within crops, with crop losses ranging from 0 -100% when flowers of different plants are not visited by pollinators. Pollination services in developing countries are feral and management of *Apis mellifera* Linnaeus, 1758 (Hymenoptera: Apidae) colonies for pollination purposes is only found on large plantations (Kasina, 2007).

Use of managed honey bee colonies for crop pollination has been virtually the only practice to increase levels of pollination service to agricultural production in many areas especially in South Africa for the growth of apples (Johannsmeier, 2001). Although honey bees can easily be managed, and their populations increased and moved around to match flowering periods (Rader *et al.,* 2009), they are not always the most optimal pollinators of most crops (Westerkamp, 1991). They are highly susceptible to diseases and degradation of the natural environment (Johannsmeier, 2001) hence the need for non-managed pollinators in crop production.

In both agricultural and natural systems, pollinator limited fruit set has been of concern (Donaldson, 2002). Indirect manipulation of the pollinator fauna is used as the treatment to assess deficits. To control the number of pollinators visiting flowers, screen cages or enclosures are commonly used (Steffan-Dewenter, 2003). A protocol to assess pollination deficits was developed through collaboration between Food and Agriculture Organization (FAO) and the Institut National de la Recherche Agronomique (INRA) in France (Vaissière *et al*., 2011). The protocol was applicable to a range of cropping systems, both extensive and intensive to detect and assess the extent to which insufficient pollination limits crop productivity across crops and across regions (Gemmill-Herren *et al.*, 2014). This is meant to help farmers understand what is lost when pollinators are not well managed.

Insufficient pollination has been suggested as one of the possible limiting factors in commercial avocado production in countries such as Israel, California and South Africa (Ish-Am, 2005; Ish-Am and Lahav, 2011). A mature avocado tree could produce a million flowers of which only 0.3% contributes to fruit set (Dixon and Sher, 2002). Research in New Zealand and Spain has shown that hand pollination can enhance percentage fruit set in avocado (Alcaraz and Hormaza, 2009; Evans *et al.*, 2010). The percent fruit set obtained in hand-pollinated flowers was fifteen times higher than in flowers left to natural pollination. This is an indication that a higher fruit set could be obtained by increasing the supply of pollen to the flower during the female phase (Degani *et al.*, 2003). Therefore, better understanding of the pollination requirements may lead to improved fruit set and increased yield in avocado.

2.3.2 Pollination agents

Several types of vectors may ensure fertilization of a flower by promoting pollination. These include wind, water and animals (Chagnon, 2008). Animal pollination is effected by many different species ranging from vertebrates (bats and birds) to invertebrates such as insects. There is a common misconception that honey bees are the sole organisms responsible for pollination. However, there are over 20,000 recorded species of bees across the globe and understanding the differences between honey bees and other wild pollinators is clearly an important avenue of outreach and education (Wray and Elle, 2015). Insects such as bees and wasps (Hymenoptera), flies (Diptera), moths and butterflies (Lepidoptera) and beetles (Coleoptera) form majority of the plant pollinators (Free, 1993; Roubik, 1995; Klein *et al.*, 2007) of both wild and cultivated plants.

Pollination is a secondary outcome unintended by pollinators. Pollen produced by the plants is for their reproduction and to breed successfully but not indented to supply the flower visitors with food. To realize this, plants need specialized, reliable pollinators and, therefore, often hide their pollen from non-pollinating visitors (International Pollinators Initiative, 1999). Findings by Krakos and Fabricant (2014) indicated that insect pollinators visit flowers to collect resources such as nectar, oils, pollen, scents, among others for their own use and for their young ones. In addition, a flower may be a mating site, shelter or source of construction materials for their nests. Moreover, these visits may not necessarily involve anther or stigma contact hence not contribute to pollination of flowers.

A flower may be visited by many potential pollinators, but critical pollen transfer may be accomplished by few pollinators since not all flower visitors are pollinators (King *et al.*, 2013; Krakos and Fabricant, 2014). In addition, a frequent visitor may carry a small pollen load, while a less frequent visitor may carry a large pollen load (Mayfield *et al.*, 2001). With all this, it has been suggested that visitation is still an accurate measure of pollination because the number of visitors is proportional to the number of actual pollinators (Engel and

Irwin, 2003). Low pollinator activity lowers the pollination rates translating to yields that are significantly lower than average (Papademetriou, 1976). Therefore, visitation rates are useful since there is a general positive relationship between total visitation rates and yield, but not with species richness (Bartomeus *et al.*, 2014).

Insects provide more than 85% of the animal pollination in crops worldwide, of which bees account to more than 90% of the insect pollinators (Kasina, 2007). The importance of honey bees as the best pollinators worldwide has been established. This is due to their foraging behavior and floral constancy (ability to visit flowers of only one plant species on every foraging bout) as they collect pollen and nectar (Gikungu, 2006).

Most farmers understand that bees are vital for the pollination of flowers. However, farmers are not aware that dipterans are among the most important groups of pollinating insects, second only to the Hymenoptera (Kearns, 2001; Ssymank *et al.,* 2008). Although flies are usually regarded as generalist flower visitors (Faegri and Van der Pijl, 1979), they are present in many ecosystems and their role in pollen transfer could be considerable, mainly due to their large numbers (Ssymank *et al*., 2008). When compared to bees, which must supply a nest with floral food, adult flies have low energy requirements and remain active on flowers even at low temperatures.

2.3.3 Avocado pollination and pollinators

A single avocado flower has very low attraction for its potential pollinators. Its attractiveness to a distant pollinator is low, and the amount of reward for its visitors is very little (Ish-Am, 2005). In response to this, large inflorescence, composed of many clustered small flowers offer the visitors a large amount of reward.

Avocado can be pollinated either through cross pollination, self-pollination or close pollination. The flowering behavior of avocado, in which one variety will be in the female stage while the other is in the male stage, seems to promote cross pollination (Ish-Am and Eisikowitch, 1992, 1993; Evans *et al.*, 2010). Cross pollination occurs when pollen transferred from male flowers of a type A cultivar (has female stage flowers in the morning and male stage flowers in the afternoon for example Hass) to female flowers of a type B cultivar (has male stage flowers in the morning and female stage flowers in the afternoon for example Fuerte), and vice versa.

The yield increasing effect is best when A and B type trees are one or two rows away from each other. If the trees are further away there may not be a substantial yield increasing effect. Also, there must be a substantial overlap between the male and female bloom, and bees must be at sufficient density to carry the pollen back and forth from pollinizer trees to the pollinated trees (Ish-Am and Eisikowitch, 1992, 1993; Evans *et al.*, 2010).

Close pollination occur when pollen from male flowers land on stigmas of female flowers during the daily overlap period of male and female stage flowers in the same tree or between neighboring trees in the same cultivar (Ish-Am, 2005; Alcaraz and Hormaza, 2009). Self-pollination occurs in a single flower at the male-stage when a stamen releases pollen that falls into the stigma that is still receptive. An individual flower apparently cannot pollinate itself and subsequently produce a fruit (Gustafson and Bergh, 1966; Alcaraz and Hormaza, 2009), also due to the protogynous nature of anthesis, self-pollination within a single flower may not occur (Ish-Am and Eisikowitch, 1993).

The avocado pollen is coated with a sticky substance, which causes the grains to adhere together and prevents it from being wind borne (Furon, 1963). This firmness also dictates that some pollen transferring agent such as a large, flying insect is required for pollination of avocado flowers. Lammerts (1943) and Peterson (1955) demonstrated the need for pollinating insects with caged avocado trees in southern California.

Effective pollination of subtropical avocado is carried out by the transfer of pollen from a dehisced male stage flower to the receptive stigma of a female stage flower. An overlap between female and male opening occurs most of the time under subtropical climates. This enables extensive close-pollination within the tree and here, pollination is performed almost exclusively by large insects, especially the *Apis mellifera* and large flies are examples (Degani *et al.*, 2003).

The question of whether insects are important as pollinators in avocado has received considerable attention (Lovatt, 1990). It has been confirmed that insect visits significantly increase fruit set in most avocado cultivars worldwide (Gazit and Degani, 2002; Alcaraz and Hormaza, 2009; Balam *et al.,* 2012). Most bee-pollinated flowers have floral rewards usually hidden (Kevan and Baker, 1983), but avocado flowers have exposed nectar and easily collected pollen (Visscher and Sherman, 1998) that attract different insect taxa (Roubik, 1995; Castañeda-Vildózola *et al.,* 1999). Moreover, the avocado flowers morphological features do not seem to fit a specific pollinator (Wysoki *et al.,* 2002), thus, it could be true that different insects could effectively pollinate it.

In the neotropics, where avocado is known to have originated, it is naturally pollinated by a wide range of insects, mainly stingless bees and social wasps (Ish-Am *et al.,* 1999; Can-Alonzo *et al.,* 2005; Afik *et al.,* 2006). In Mexico, Guatemala and Central America, honey bees, flies *Chrysomya megacephala* (Fabricius, 1794), stingless bees (Apidae, Meliponinae) and native wasps *Brachygastra mellifica* (Say, 1837) have been documented on avocado flowers (Can-Alonzo *et al*., 2005; Afik *et al*., 2006; Ish-Am and Lahav, 2011; Balam *et al.,* 2012). In accordance to their visitation rate, behavior and anatomical features, it has been suggested that these insects could be the primary and original avocado pollinators in central and western Mexico (Wysoki *et al.,* 2002).

In some instances, combined pollination effects of avocado by *C. megacephala* and *B. mellifica* are higher compared to the honey bees' (Balam *et al.*, 2012). *Polistes* wasps, mirids *Dagbertus* sp., and flies *Musa domestica* L. have been noted in large numbers visiting avocado flowers than honey bees in South Africa (De Meillon and Wirth, 1979). This is relevant if and when honey bees are attracted to nearby blossoms other than avocado (Visscher and Sherman, 1998).

Insects in the order Hymenoptera, Diptera, Coleoptera, Lepidoptera and Hemiptera have been reported as the primary pollinators of avocado in California (Bergh, 1967), Israel (Bergh, 1975; Gazit and Degani, 2002; Ish-Am, 2008), South Africa (Melin *et al.,* 2014) and in Kenya (Kinuthia *et al.,* 2004; Luvonga, 2015; Odanga *et al.,* 2017). In all the aforementioned studies, honey bees were recorded as the primary pollinators of avocado in the respective avocado production areas of the world. However, the *Bombus* bees have shown some meaningful advantages as avocado pollinator (Ish-Am, 2004). Much pollen is carried by its bigger and more hairy body, in addition to its high visitation frequency of up to 20 flowers per minute compared to the honey bee with visitation frequency of up to 6-9 flowers per minute (Ish-Am, 2004). To effect pollination, the bees must alternately visit both pollen-carrying male flowers and female flowers (Gazit and Degani, 2002).

Ish-Am (2000) found out that most honey bees collect avocado nectar and pollen within a limited area of one to three trees. They often perform cross pollination only between neighboring trees that carry opposite-stage flowers and are at a distance of not more than two rows. Only 2-4% of the foraging honey bees move farther between rows and fields, and may carry avocado pollen for hundreds of yards away from its source. Due to low attractiveness of avocado flowers to the honey bees, pollination is often inadequate (Afik *et al.,* 2006) and the result is negligible fruit set (Gazit and Degani, 2002; Ish-Am, 2005). This makes stingless bees and other flower visitors more economically important as they continue to visit the crop.

Bee activity is strongly influenced by temperature with the warmest days and nights being most favorable to pollination. While cooler temperatures may delay avocado flower opening, this may be a benefit to some degree because overlapping of female and male flowers increases, presumably ensuring pollination in a grove of pure “A” type flowers such as Hass (Peterson, 1955). An overlap of 45 min to 90 min was found to occur in Hass during the midday in Israel conditions (Ish-Am and Eisikowitch, 1989).

Vithanage (1990) suggested that, due to the open structure of the flower and the accessibility of the nectar to insects with lapping mouthparts such as flies, avocado could be a fly-pollinated plant in its natural environment. Flies species in the family Calliphoridae, Muscidae and Syrphidae carry large amounts of avocado pollen and create effective contact with stamens and stigmas, but their pollination efficiency is not high because of low flower visitation rate (Ish-Am *et al.,* 1999; Wysoki *et al.*, 2002).

Thrips are known to be effective pollinators of many plants (Lewis, 1973). Flower thrips *Frankliniella* sp. (Thysanoptera: Thripidae) and ants have been found on avocado flowers, with several thrips in a flower. These can easily transfer pollen grains across the distance from the anther to the stigmatic surface as they constantly wander about the flower (Davenport, 1986). This mechanism could explain why fruit set takes place when few flying insects are visiting flowers.

Davenport *et al.* (1994) concluded that wind is the primary mediator of pollen transfer for tropical avocado cultivars in south Florida. Later, Davenport (2003) and Ying *et al.* (2009) demonstrated how wind is the main avocado pollinator in California and Florida and not honey bees. But according to Hoddle et al. (2010), wind and self-pollination within the flower had no fruits suggesting that it is not important in fruit setting. However, thrips and small insects had a very low level of fruit set, and honey bees had the highest fruit set in California.

The daily honey bee activity on the Type A ‘Hass’ and ‘Reed’ lasts only about 10 hours, starting later in the morning and ending earlier in the afternoon (Ish-Am, 2008). Bee activity on these two cultivars had only one clear daily peak, at around noon (on an average temperature day), that lasted about 2.5 hours and included their self-bisexual overlapping period strengthening close pollination (Ish-Am, 2008). This overlap is important in avocado production especially where pure blocks of a single variety are under cultivation as is the case in most avocado exporting firms. Most of avocado in Kandara are pure

Hass stands and close pollination could be the most important pollination type to study.

2.3.4 Pollinator diversity impact on yields

Diversity is defined as the condition of having or being composed of differing elements (variety) (Merriam-Webster dictionary). Pollinator diversity is then the condition/state of having different or varied pollinators in an area of focus. According to Gikungu (2006), a more diverse pollinator community is very important since pollinators complement each other in effecting pollination. In addition, it provides better pollination service especially in areas where mixed cropping is done, as different pollinators target different flowers. This helps in reducing the risk that may arise due to lack of pollinators during the critical period of crop flowering. For example, honey bees are known to abandon flower patches for more suitable ones and in such a case, having a diverse pollinator community can help offset the lost honey bee function, depending on the crop requirements.

High pollinator diversity has been reported to enhance yield quantity and stability by improving the pollination efficiency of honey bees in other crops (Greenleaf and Kremen, 2006). It also reduces the risk of pollination failure due to climate change (Bartomeus *et al.,* 2013; Rader *et al.,* 2013), or environmental disturbances such as extreme weather events (Brittain *et al.,* 2012).

Pollinator abundance and diversity vary with landscape context in such a way that wild bees populations are generally greater close to or in the natural habitats (Blanche *et al.*, 2006; Chacoff and Aizen, 2006; Ricketts *et al.*, 2008). Proximity of crop fields to semi-native habitats negatively affects pollinator abundance and diversity since loss of native habitats leads to loss of different plant species necessary for different flower visitors who effect pollination on crops.

2.3.5 Pollination management strategy

Gemmill-Herren *et al.* (2014) relates how the increasing demand for farming land, urbanization and other land-use practices due to rapid human population increase could reduce natural habitats. This will put pressure on the ecosystem service delivered by wild pollinators. Besides this, the demand for pollination in agricultural production has increased in order to sustain food production for the growing human population. They also note that there is increase in pollinator-dependent horticultural crops in developing regions of the world compared to developed countries. To respond to this, there is need to start pollination management in the developing countries where pollinators are rarely managed unlike in the developed world.

Approximately 15% of the world's crops are pollinated by a few managed bee species (*A. mellifera* and *Bombus* sp.), the rest are pollinated by un-managed solitary bees and other wildlife (Ingram *et al.*, 1996; Almanza, 2007). Although wild pollinators are known to increase fruit set independent of honey bee visits (Garibaldi *et al.*, 2013), honey bees have the advantage of being less dependent on landscape characteristics and can be managed unlike the wild pollinators, particularly in North America and Europe. They can also be moved around the landscape and this could also help mitigate against wild pollinator loss in more intensively used landscapes where pollination services are degraded.

Research by Button and Elle (2014) and Garibaldi *et al.* (2014) shows that honey bees are not always the most efficient pollinators even in conventional agriculture. However, their contribution to the large-scale commercial agriculture is higher than their overall ecosystem service provision. Interactions between wild pollinators and managed honey bees may lead to a more effective pollination than either alone on most agricultural crops (Greenleaf and Kremen, 2006; Carvalheiro *et al.,* 2011). Therefore, a combination of wild and managed pollinators provides the highest quality of ecosystem services.

A recent global meta-analysis has shown that wild pollinators are more effective crop pollinators than honey bees, enhancing fruit set by twice as much as equivalent levels of visitation by honey bees (Garibaldi *et al.,* 2013). They also contribute to optimal yields of several crops, enhancing the human diet with more nutrients (Chagnon, 2008). On crops where honey bees are not effective pollinators, understanding other species of pollinators is very important. This helps to determine the possible interactions between the pollinating groups (Gemmill-Herren *et al.,* 2014).

Honey bees native to Africa, Western Asia, and Europe have been introduced across the globe for agricultural crop pollination and honey production (Butz-Huryn, 1997). However, wild bees still remain the dominant pollinators in natural habitats and are essential to the maintenance of local biodiversity (Michener, 2000). Research on wild and managed bees as effective pollinators of global crops of economic importance has been documented. However, up to now, the contributions by pollinators other than bees have not been explored well despite their potential to contribute to crop production (Rader *et al.*, 2016).

Non bee insects (flies, beetles, moths, butterflies, wasps, ants, birds, and bats) perform 25–50% of the total number of flower visits, increasing fruit set independently of the honey bee (Rader *et al.,* 2016). Rader *et al.* (2016) strongly suggest that non-bee insect pollinators play a significant role in global crop production by responding differently than bees to landscape structure, probably making their crop pollination services more robust to changes in land use. This service by non-bees is a potential insurance against bee population declines.

Maintaining natural or semi-natural habitats near farms can boost pollinator diversity in agriculture, as well as contribute to higher crop yield (Ricketts, 2004; Ricketts *et al.,* 2008; Kennedy *et al.,* 2013). Several studies have shown that gardens and parks can support a high diversity, abundance, and richness of pollinators in urban areas (Tommasi *et al.,* 2004; McFrederick and LeBuhn, 2006; Wray and Elle, 2015). According to the Avocado Industry Council (2006), avoiding sprays within 10 days before introducing bees to the orchard ensures large numbers of bees being available in avocado orchards to pollinate flowers.

For effective pollination management, the floral calendar of the crop needs to be understood. Possible pollinators' abundance and diversity needs to be evaluated in addition to the losses that could occur when pollinators of the specific crop are missing. In crops like avocado which have a complex flowering behavior, there is need to assess the diurnal visitation counts of its insect pollinators.

2.3.6 Threats to pollination provision

The demand for pollination service is rising while pollinator abundance and diversity are declining. This has happened at a time when cultivation of pollinator-dependent crops has expanded faster than that of non-dependent crops in the world (Aizen *et al.,* 2009). Therefore, this highlights the need for the conservation of most pollinators other than the few managed and domesticated bee species (honey bees, *Bombus* bees and stingless bees). Domestication of pollinators has been a big challenge since most of them such as wasps, flies, butterflies and beetles are solitary.

A decline in the population of pollinators has been caused by a number of factors which include agricultural intensification, use of pesticides, loss of natural habitats, diseases and parasites killing bees, among other causes. Pesticides use is a key negative predictor of pollinator abundance (Otieno *et al.,* 2011). Lack of knowledge in management of pollinators is a challenge in pollinators' provision. In most cases the general public is unaware that wild pollinators even exist (Kasina *et al.*, 2009b).

2.4 Avocado vegetative growth and flowering

Growth of avocado branches takes place in distinctive vegetative flushes, which generally occur two or three times throughout the year and do not necessarily include the whole tree (Davenport, 1982; Scholefield *et al.*, 1985). Avocado vegetative buds' differentiation to reproductive buds takes place at the end of shoot expansion, usually in August in California (Salazar-Garcia *et al.*, 1999; California Avocado Commission, 2012). In late September to early October on Florida grown avocado cultivars, the last vegetative flush prior to inflorescence development has been reported (Davenport, 1982).

Results by the California Avocado Commission (2012) indicate that the bulk of flower opening occur usually over a six week period from late March to early May in the 'Hass' cultivar in San Diego County. However, this could change in some years with flower opening beginning as early as late January with timing varying considerably in a given location. Nevertheless, 'Hass' in the more northern counties may have a flowering period of about two to four weeks later than San Diego. Similarly, findings by Dirou (2003) indicate that avocado flowering normally lasts for three to four weeks, though it's longer in cooler growing areas.

Avocado flowering in Nelspruit, South Africa was reported to take place as from June to October (Robertson, 1969). In 2012, the Department of

Agriculture, Forestry and Fisheries (DAFF) of the republic of South Africa noted that avocado flowers appear in January to March before the first seasonal growth. They appear in terminal panicles of 200 to 300 small yellow-green blooms. Additionally, each panicle could produce only one to three fruits.

Avocado flowers are small (1 cm diameter) and open in shape. Each flower is bisexual, opening twice with an intermediate closing. The first opening is in the female stage and the second, usually on the following day, is in the pollen-releasing male stage (Peterson, 1955; Ish-Am and Lahav, 2011). This opening and closing of the daily female and male flower populations is synchronized within the tree (and cultivar) (Ish-Am and Lahav, 2011).

Individual avocado flowers do not open and close simultaneously but rather one after the other, over a 2 to 3-hour period, which establishes a regular overlapping of subsequent flower stages on the tree, and enables extended pollen release (Ish-Am, 2008). This unique flowering behavior is known as diurnally synchronous dichogamous protogyny, with intermediate closing (Gazit and Degani, 2002; Ish-Am, 2005; Alcaraz and Hormaza, 2009; Ish-Am and Lahav, 2011).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study site

This study was carried out in farmers' fields in Ng'araria location situated between 37°0'0''E; 37°1'12"E and 0°54'36"S; 0°55'48"S in the Upper midland 2 (UM2) agro ecological zone at Kandara sub county, Murang'a County, Kenya (Figure 3.1), where the export variety 'Hass' is mainly grown. Kandara experiences a bimodal rainfall pattern, with long rainy season between March to May and short rainy season from October to December. The average annual rainfall varies from 1,400 to 2,000 mm (Jaetzold *et al.*, 2006). The mean annual temperature is between 18 and 21^{0}C; Soils are deep, well drained, weathered humic nitisols with moderate to high inherent fertility (Jaetzold *et al.*, 2006).

3.2 Experimental design

The studies were conducted during three cropping seasons (August 2015 - July 2016; April - October 2016 and August 2016 - March 2017) in UM2 agro ecological zone. A total of 60 Hass trees were randomly selected for the study and maintained throughout the three seasons. Twelve farms were selected and from each farm, five trees with good canopy were identified for the study. Each avocado tree was treated as a plot and only fully matured avocado trees of not less than five years of age were used in all the studies since they had stabilized in fruit production (Griesbach, 2005). A minimum distance of about 10 m and 200 m was maintained between trees and between farms, respectively.

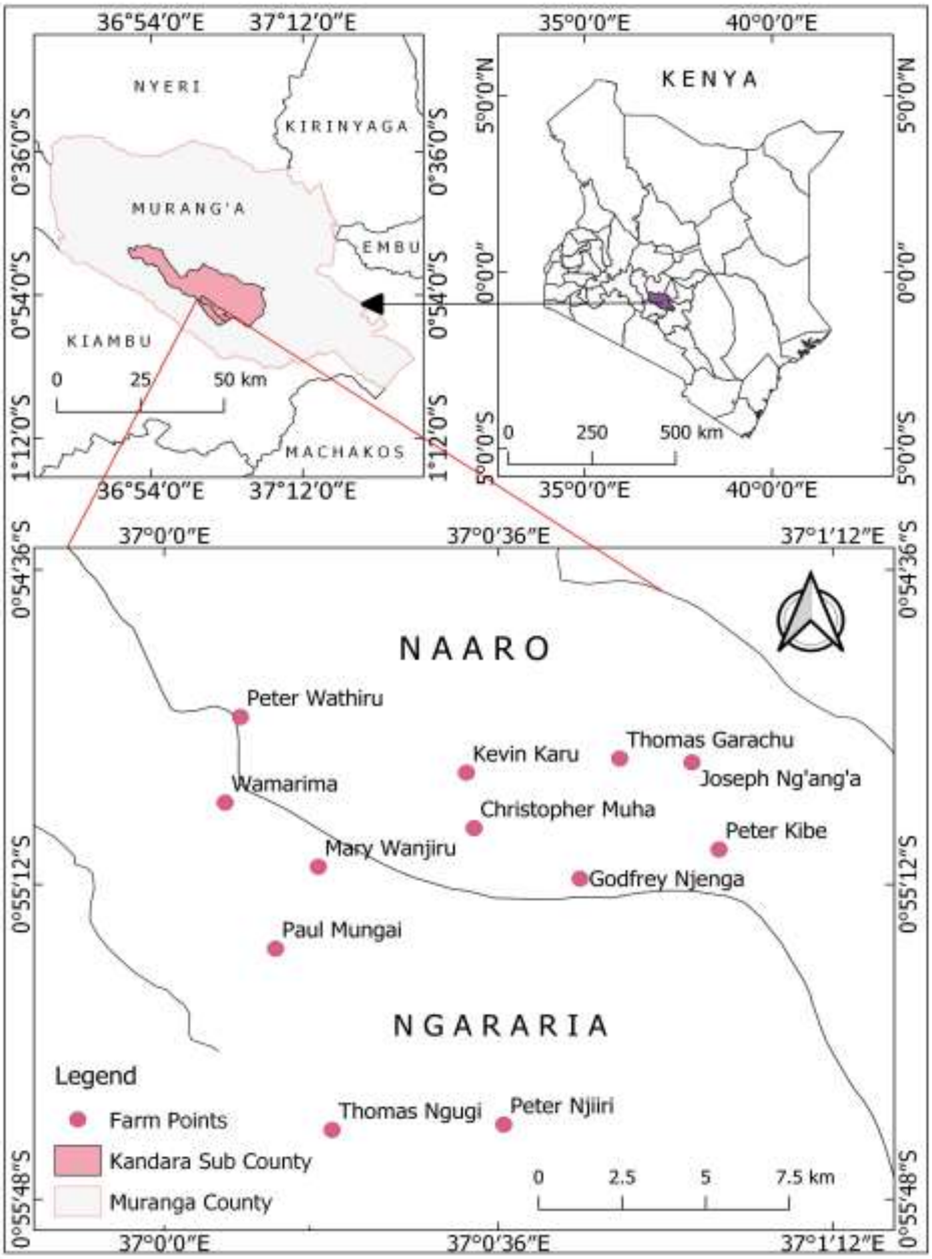

Figure 3.1 Map of the study site in Kandara, Murang'a County
(Source: Mutungi R. M., 2019)

3.3 Evaluating abundance and diversity of avocado flower insect pollinators in Kandara, Murang'a County

On each tree, observations were done on the lower canopy to detect avocado flower pollinators for about 10 minutes in every farm, weekly throughout the blooming period from 0900 h to 1700 h. Each sampling started from a different farm to ensure unbiased observations. It was assumed that the number of pollinators landing on each tree was equivalent to the number of pollinators exiting in the environment and the same assumption was used to compensate for the possibility of one flower visitor being counted twice.

Observations were done from the onset of the blooming period, especially when 10% or more of the plants had started to bloom. In addition, the temperature was warm, low wind speed, no rain, and dry vegetation following Westphal *et al.* (2008) guidelines; with at least three samplings per season. The flower pollinators' identity and number of insects visiting per species were recorded. Insects were collected using sweep nets for three flowering seasons (September 2015- October 2015; March 2016 – May 2016 and August 2016 – October 2016), preserved in 70% alcohol and taken to KALRO – Kabete Entomology laboratory for identification.

3.4 Determining diurnal visitation counts of insects pollinating avocado flowers in Kandara, Murang'a County

In addition to the procedures in 3.3, time of the day and the time (seconds) taken by each pollinator on a flower were recorded by use of a stop watch. This was aimed at recording the insect pollinators with the highest visitation counts during an overlap between the male and female avocado flowers. This is the possible time for close pollination to occur especially under Kandara conditions where avocados are grown purely as Hass blocks.

3.5 Investigating the floral calendar of avocado in Kandara, Murang'a County

The observations included the percent flushing, flower buds, flowering and fruiting from September 2015 to October 2016 (Figure 3.2). During the flowering period, the number of male and female phase flowers were recorded in eight randomly selected inflorescences per tree from 0900 h to 1700 h. In addition, the number of aborted flowers was also determined by counting the remaining flower stalks after flower drop.

3.2 A

3.2 B

3.2 C

3.2 D

Figure 3.2: Avocado flower formation
3.2 A – Flushing; 3.2 B – Flower buds; 3.2 C – Female flower phase; 3.2 D – Male flower phase

3.6 Evaluating the effect of avocado pollination deficit in Kandara, Murang'a County

A total of 95 trees were used in the three seasons' period (September 2015 - July 2016; April 2016 – December 2016 and August 2016 – March 2017). On each tree, two randomly selected terminal branches with inflorescences of the same age and size were identified, one was bagged to deny pollinator visits and another left open for unlimited access by pollinators.

The un-bagged terminal branches were open pollinated with all the flowers of each accessible to autonomous self-, wind- and insect-pollination. In the bagged terminal branches, all flowers were enclosed in nylon nets commercially used for household management of mosquito bites of an appropriate size to cover the whole terminal branch. Thus, in the bagged terminal branches, all flowers were exposed to self-pollination and limited wind pollination effects, but not to insect pollination. Net placement was done carefully and in most cases before anthesis to avoid increased levels of self-pollination (nets were put over the flower buds before the onset of flowering). As soon as flowers had wilted, nets were removed, and the fruits on the tagged terminal branches counted for fruit set data and left to develop until harvest. Fruit counts were done every month to monitor fruit drops during their development.

After five to nine months, the mature fruits from the bagged and un-bagged terminal branches were harvested and the numbers recorded in each season. Fruit and seed weights were measured in grams (g) while fruit polar diameter, fruit equatorial diameter, ovary polar and equatorial diameters were measured in millimeters (mm) and recorded (Figure 3.3).

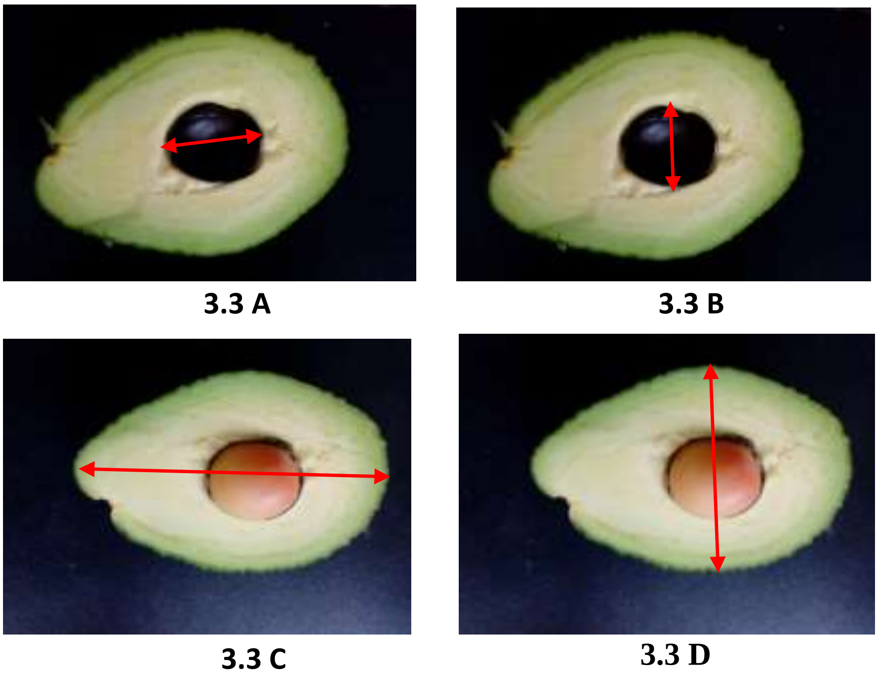

Figure 3.3: Avocado fruit dimensions

3.3 A – Ovary polar diameter; 3.3 B – Ovary equatorial diameter; 3.3 C- Fruit polar diameter; 3.3 D – Fruit equatorial diameter

3.7 Data analysis

The number of pollinators per species on avocado flowers and time of the day they visited were entered into excel data sheets for analysis. Species richness, diversity and species evenness were analyzed using Shannon Weiner Index (H) = (- [∑ Pi ln Pi]) (Shannon and Weaver 1949), where P is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), ln is the natural log, Σ is the sum of the calculations. The diversity index is the proportion of species (Pi) relative to the total number of species (S) summed up and multiplied by natural logarithm of this proportion (ln Pi) (Magurran, 1988). The product is summed across species and multiplied by (-1) (Rosenzweig, 1995). Evenness was calculated by dividing H' by ln S, where S is the number of species.

Data on percent flushing, flower buds, flowering and fruiting were entered in excel data sheets and analyzed using line graphs. Female and male flowers opening were recorded during the day and bar graphs were plotted. Diurnal visitation rates for each pollinator were constructed using bar graphs and the highest times of the day for each pollinator documented for the three seasons separately (September-October 2015; March-May 2016 and August-October 2016). Fruit set, yield and diurnal visitation rates counts were $\log_{10}$ (n+1) transformed before analysis to minimize skewness. Fruit set, yield and characteristics values from bagged and un-bagged terminal branches were analyzed for significance using student's t-test at 0.05 level of significance in Genstat 10th Edition.

CHAPTER FOUR: RESULTS

4.1 Abundance of avocado flower pollinators in Kandara, Murang'a County

In season one, the pollinators recorded belonged to two orders namely, Hymenoptera and Diptera. Hymenoptera had four species whose abundances were as follows: *Apis mellifera* (Family: Apidae) at 92.8%, *Polistes* sp. Latreille, 1802 (Family: Vespidae) at 2.0% and both *Iridomyrmex reburrus* Shattuck, 1993 (Family: Formicidae) and *Meliponula ferruginea* Lepeletier, 1841 (Family: Apidae) at 0.25%. Diptera had two species with abundances of 2.8% for *Eristalis tenax* Linnaeus, 1758 (Family: Syrphidae) and 1.9% for *Chrysomya putoria* Wiedemann, 1818 (Family: Calliphoridae) (Table 4.1).

Abundances of 35.5%, 32.8%, 12.9%, 7.5%, 5.9%, 2.2%, 1.6%, 1.1% and 0.5% for *A. mellifera*, *C. putoria*, *Polistes* sp., *E. tenax*, *I. reburrus*, *M. ferruginea*, *Colias electo* Linnaeus, 1763 (Family: Pieridae), *Halictus species* Latreille, 1804 (Family: Halictidae) and *Drypta ruficollis* Dejean, 1831 (Family: Carabidae) were recorded in season two, respectively (Table 4.2). In season three, *A. mellifera* had an abundance of 93.95%, followed by *C. putoria* and *E. tenax* with 2.27% each. *Polistes* sp. was fourth with abundance of only 1.01%, while *M. ferruginea* and *Halictus* sp. had abundances of 0.25% each (Table 4.3).

Combined abundances for the three seasons showed that nine insect species in four orders (Hymenoptera, Diptera, Lepidoptera and Coleoptera) were observed. The major avocado flower pollinators were *A. mellifera* (87.3%), *C. putoria* (5.3%), *E. tenax* (3.1%) and *Polistes* sp. (2.7%). Other rare pollinators of avocado flowers recorded were *I. reburrus* (0.73%), *M. ferruginea* (0.45%), *Halictus* sp. (0.23%), *C. electo* (0.17%) and *D. ruficollis* (0.06%) (Table 4.4).

Table 4.1: Abundance of avocado pollinators during season one (September – October 2015) in Kandara, Murang'a County

Order	Species	Frequency	% abundance
Hymenoptera	*Apis mellifera*	736	92.81
	Polistes sp.	16	2.02
	Iridomyrmex reburrus	2	0.25
	Meliponula ferruginea	2	0.25
Diptera	*Eristalis tenax*	22	2.77
	Chrysomya putoria	15	1.89
Total		**793**	**100**

Table 4.2: Abundance of avocado pollinators during season two (March-May 2016) in Kandara, Murang'a County

Order	Species	Frequency	% abundance
Hymenoptera	*Apis mellifera*	66	35.48
	Polistes sp.	24	12.90
	Iridomyrmex reburrus	11	5.91
	Meliponula ferruginea	4	2.15
	Halictus species	2	1.08
Diptera	*Chrysomya putoria*	61	32.80
	Eristalis tenax	14	7.53
Lepidoptera	*Colias electo*	3	1.61
Coleoptera	*Drypta ruficollis*	1	0.54
Total		**186**	**100**

Table 4.3: Abundance of avocado pollinators during season three (September – October 2016) in Kandara, Murang'a County

Order	Species	Frequency	% abundance
Hymenoptera	*Apis mellifera*	746	93.96
	Polistes sp.	8	1.01
	Meliponula ferruginea	2	0.25
	Halictus species	2	0.25
Diptera	*Chrysomya putoria*	18	2.27
	Eristalis tenax	18	2.27
Total		**794**	**100**

Table 4.4: Abundance of avocado pollinators for the three seasons combined (September - October 2015; March - May 2016 and September - October 2016) in Kandara, Murang'a County

Order	Species	Frequency	% abundance
Hymenoptera	*Apis mellifera*	1,548	87.31
	Polistes sp.	48	2.71
	Iridomyrmex reburrus	13	0.73
	Meliponula ferruginea	8	0.45
	Halictus speices.	4	0.23
Diptera	*Chrysomya putoria*	94	5.30
	Eristalis tenax	54	3.05
Lepidoptera	*Colias electo*	3	0.17
Coleoptera	*Drypta ruficollis*	1	0.06
Total		**1,773**	**100**

4.2 Diversity of avocado pollinators in Kandara, Murang'a County

Six insect species; *A. mellifera, E. tenax, Polistes* sp., *C. putoria, I. reburrus and M. ferruginea* in two orders (Hymenoptera and Diptera) were observed pollinating avocado flowers in season one. In season two, nine insect species: *A. mellifera, E. tenax, Polistes* sp., *C. putoria, I. reburrus, M. ferruginea, D. ruficollis, Halictus* sp. and *C. electo* in four orders (Hymenoptera, Diptera, Lepidoptera and Coleoptera) were observed on avocado flowers. Six insect species: *A. mellifera, E. tenax, Polistes* sp., *C. putoria, M. ferruginea* and *Halictus* sp. in two orders (Hymenoptera and Diptera) were observed on avocado flowers in season three (Table 4.5).

A diversity index of 0.35 was recorded, translating to an evenness of 0.2 in season one. In season two, the diversity index was 1.59, with evenness of 0.72. This evenness in season two implies that the percent abundances of most insect pollinators were close (Table 4.5). A diversity index of 0.31 and an evenness of 0.17 were recorded in season three. This was due to the varied frequency of the insects pollinating avocado flowers (Table 4.5).

The pollinator diversity index and evenness of avocado pollinators in Kandara for the three seasons were very low (Table 4.5). There were nine species with diversity index of 0.25 and ln(9) of 2.20. The evenness was 0.11 and this was very low for the insect pollinators of avocado based on known normal evenness of one when all pollinators have equal abundances (Table 4.5).

Table 4.5: Diversity of avocado pollinators during the three seasons (September - October 2015; March - May 2016 and September - October 2016) in Kandara, Murang'a County

Pollinator	**Frequency Season 1**	**Frequency Season 2**	**Frequency Season 3**	**Frequency three seasons**
Apis mellifera (Linnaeus, 1758)	736	66	746	1,548
Meliponula ferruginea (Lepeletier, 1841)	2	4	2	8
Halictus sp. (Latreille, 1804)	-	2	2	4
Iridomyrmex reburrus (Shattuck, 1993)	2	11	-	13
Polistes sp. (Latreille, 1802)	16	24	8	48
Chrysomya putoria (Wiedemann, 1818)	15	61	18	94
Eristalis tenax (Linnaeus, 1758)	22	14	18	54
Colias electo (Linnaeus, 1763)	-	3	-	3
Drypta ruficollis (Dejean, 1831)	-	1	-	1
Species richness	6	9	6	9
Species evenness	0.2	0.72	0.17	0.11
Diversity index	0.35	1.59	0.31	0.25
n	**791**	**186**	**794**	**1,773**

4.3 Diurnal visitation counts of avocado insect pollinators at Kandara, Murang'a County

In season one, avocado flower visitation by insects started later in the morning from 1000 h where *A. mellifera* and *E. tenax* were the earliest. *Apis mellifera* visitation increased gradually throughout the day, peaking from 1500 h to 1559 h with 309 visits and recording a reduction in avocado flower visitations up to beyond 1600 h. Among the Dipterans, *E. tenax* visits on avocado flowers were observed earlier, from 1000 h while *C. putoria* observations were made from 1100 h. However, peak visitations for both were recorded from 1100 h to 1159 h with seven and four visits, respectively. *Chrysomya putoria* were observed visiting avocado flowers beyond 1600 h while *E. tenax* visits were up to 1559 h. *Polistes* sp. visitation started from 1100 h, gradually increasing through the day, registering highest counts from 1400 h to 1459 h with seven visits but no observations made beyond 1559 h (Figure 4.1).

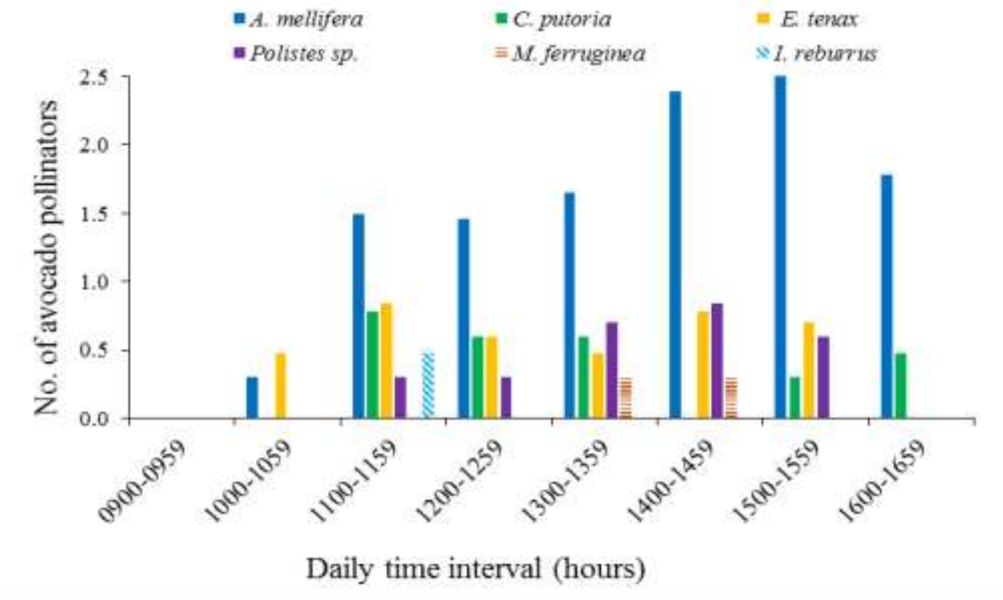

Figure 4.1: Avocado insect pollinators' diurnal visitation counts in Kandara, Murang'a County during season one (September – October 2015)

During season two, insect visits to avocado flowers were observed as early as from 0900 h. This early visitation was by Hymenopterans (*Polistes* sp. and *Halictus* sp.), Dipteran (*C. putoria*) and Lepidoptera (*C. electo*). *Apis mellifera* visitation started from 1100 h and peak visitation counts were recorded between 1200 h to 1259 h with 36 visits. Other avocado flower visitors with similar peak visitation time as *A. mellifera* were *E. tenax* and *Polistes* sp. with eight, and 14 visits, respectively. Highest visits of 21 for *C. putoria* were recorded between 1300 h and 1359 h. There were no visits recorded on avocado flowers beyond 1459 h by *A. mellifera*, *E. tenax* and *Polistes* sp. However, *C. putoria* and *M. ferruginea* were observed on avocado flowers beyond 1500 h (Figure 4.2).

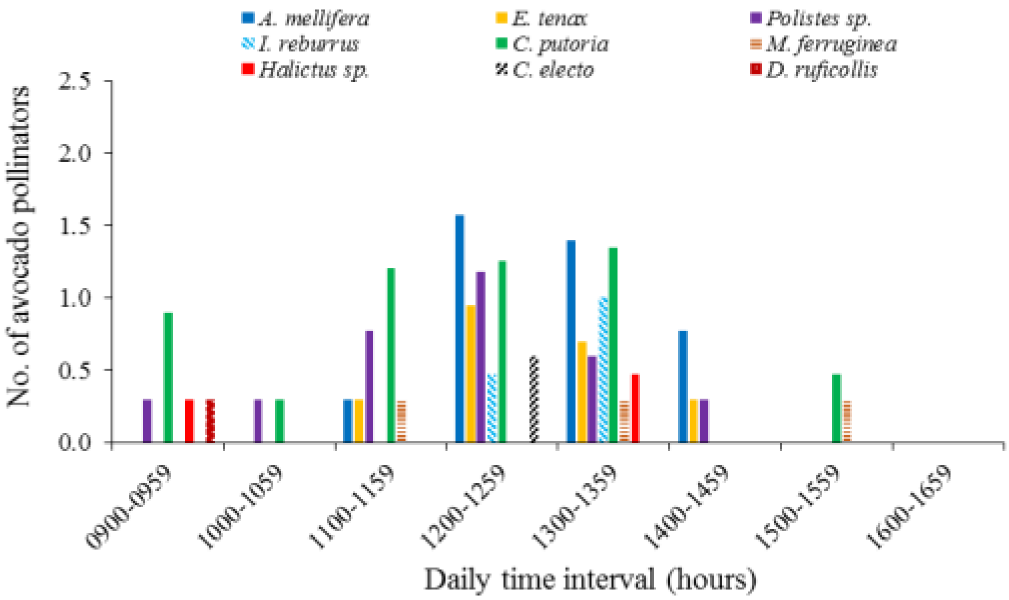

Figure 4.2: Avocado insect pollinators' diurnal visitation counts in Kandara, Muranga County during season two (March – May 2016)

Avocado flower visitation in season three started from 0900 h with *A. mellifera*, *E.tenax* and *C. putoria* as the early visitors. Results showed highest visitation counts of 202, eight and seven for *A. mellifera*, *C. putoria* and *E. tenax*, respectively between 1000 h and 1059 h. During the same period, *Polistes* sp. showed four visits as the highest between 1100 h and 1159 h. *Apis mellifera* abundances were the highest among the pollinators throughout the day in season three with no observations recorded after 1559 h (Figure 4.3).

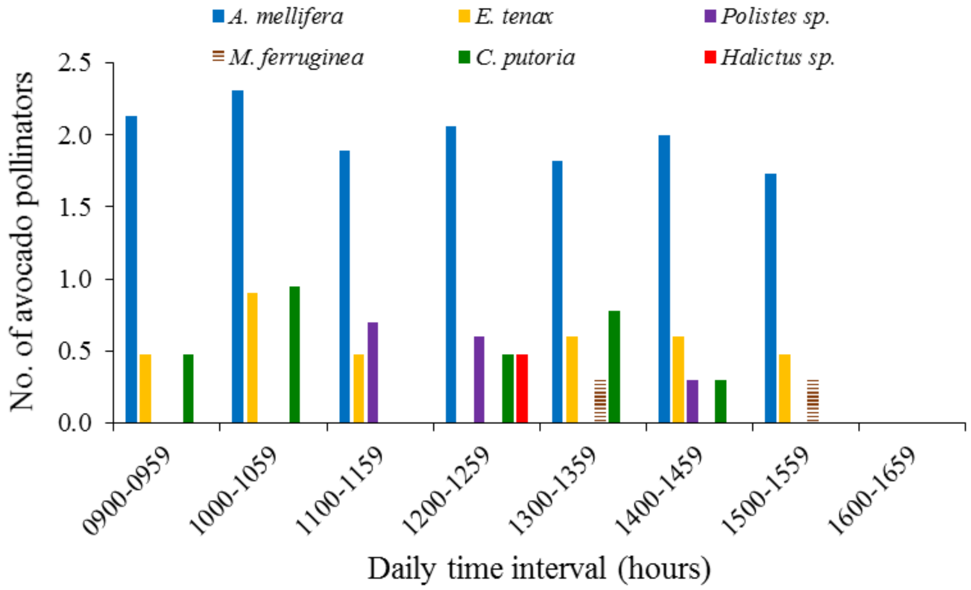

Figure 4.3: Avocado insect pollinators' diurnal visitation counts in Kandara, Murang'a County during season three (September – October 2016)

4.4 Floral calendar of avocado in Kandara, Murang'a County

The results showed that there were two avocado flowering seasons in a year at Kandara. One was from August to October and the other from February to May. March and September were the peak blooming months. The results have shown that when fruiting and flushing were at their peaks (October to January and April to July), flowering and flower buds were at their lowest and vice versa (Figure 4.4). Peak flushing and fruiting were in May and November, thus indicating two seasons of either annually. The study also showed that avocado trees had fruits throughout the year and also vegetative growth was continuous (Figure 4.4).

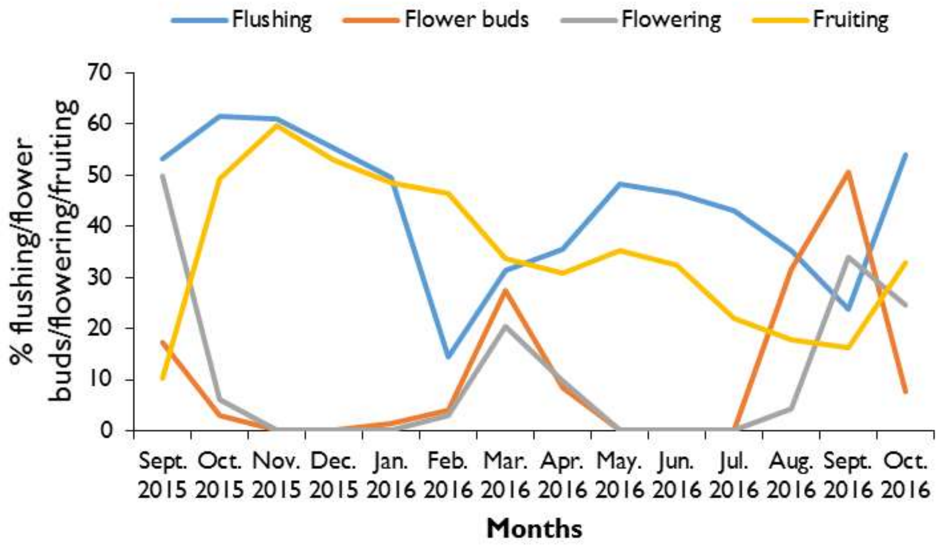

Figure 4.4: Percent flushing, flower buds, flowering and fruiting of avocado in Kandara, Murang'a County for three seasons (September 2015 - October 2016)

Female flowers' phase was observed during morning hours from 0900 h to 1159 h until male flowers started to open at around 1200 h. An overlap period was noted from 1200 h to 1500 h lasting three hours, with the peak overlap at 1300 h to 1400 h (Figure 4.5). Highest peaks were between 1000 h to 1300 h and 1500 h to 1700 h for female and male flowers, respectively, in season one.

In season two, female phase flowers opening started from 0900 h to 1259 h. The male phase flowers opened later from 1100 h to 1659 h. This flower opening behavior introduced an overlap of both flower types from 1100 h to 1259 h lasting 2 hours. Peak flower opening were between 1000 h to 1059 h and 1300 h to 1359 h for female and male phase flowers, respectively (Figure 4.5).

Female phase flowers' opening started from 0900 h to 1559 h with a peak opening between 1200 h and 1259 h in season three. The male phase flowers opened gradually from 1100 h to 1559 h reaching peak populations between 1400 h and 1459 h (Figure 4.5). The male and female phase flower overlap started between 1100 h to 1559 h lasting three hours, with peaks between 1300 h to 1559 h.

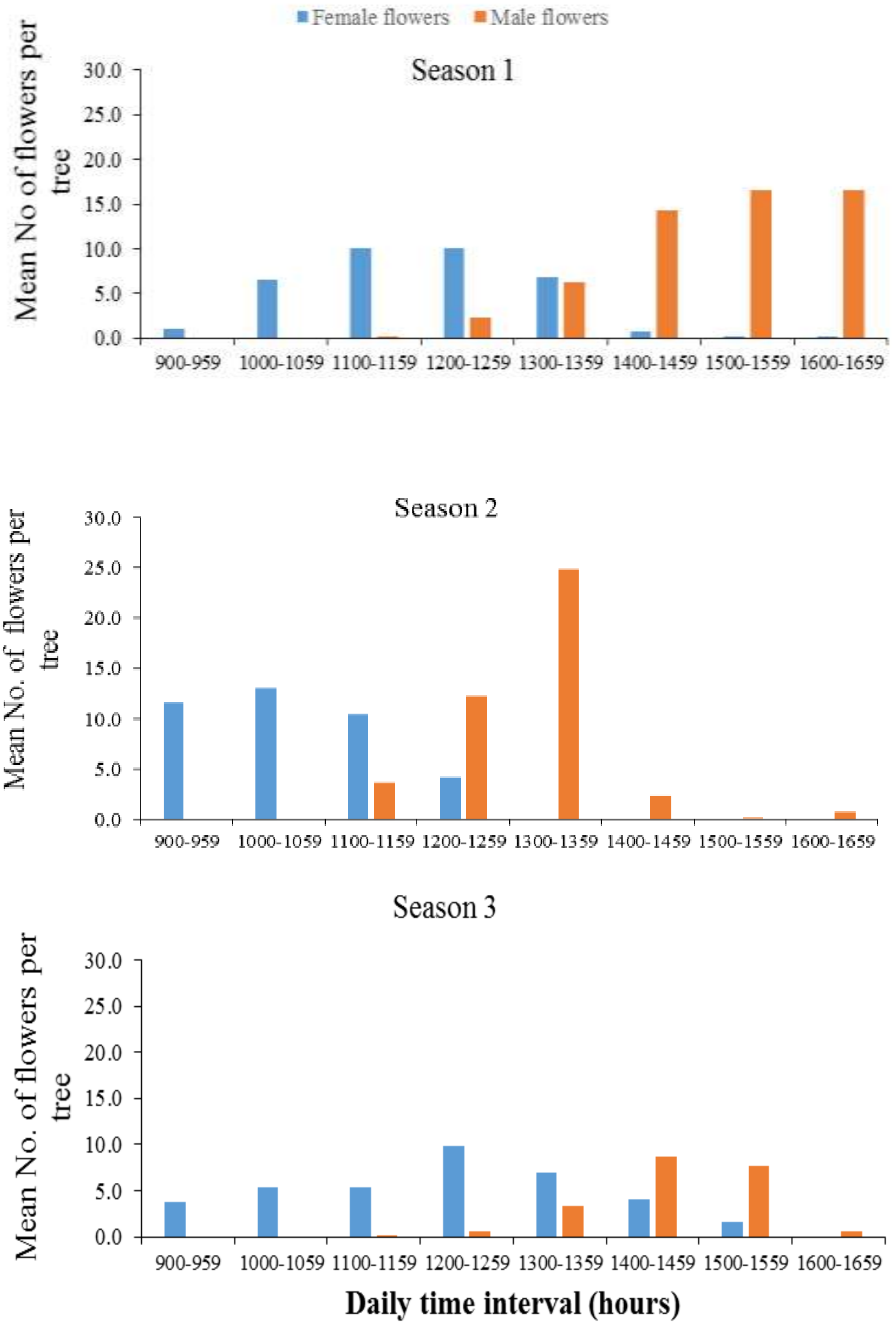

Figure 4.5: Diurnal opening of male and female phase avocado flowers in Kandara, Murang'a County in season one, two and three (September - October 2015; March - May 2016 and September - October 2016)

4.5 Evaluating the effect of avocado pollination deficit in Kandara, Murang'a County

In season one, 303 fruitlets were set in bagged terminal branches while 925 fruitlets were set in the un-bagged terminal branches. There was highly significant ($P<0.001$) differences in fruit set between the bagged (6.9±1.43) and un-bagged (21.0±2.76) per terminal branch. Yield from bagged terminal branches was six fruits while 20 fruits were harvested from un-bagged terminal branches. This showed a significant ($P=0.024$) difference in fruit yield harvested from bagged (0.1±0.07) and un-bagged (0.4±0.12) per terminal branch. Fruit drop was very high at 98.1% and 97.9% in both bagged and un-bagged terminal branches, respectively. The fruit set increased by 305.1%, yields increased by 333.4% and the pollination deficit was 70% when unlimited pollination was allowed through un-bagged terminal branches (Table 4.6).

A total of 24 fruitlets set in the bagged terminal braches as compared to 46 fruitlets from the un-bagged terminal branches in season two. There was no significant ($P=0.076$) difference in fruit set between bagged (2.2±0.9) and un-bagged (4.2±0.9) per terminal branch. Three fruits were harvested from bagged terminal branches and 11 from un-bagged terminal branches. There were no significant ($P=0.111$) differences in fruit yield between bagged (0.3±0.14) and un-bagged (1.0±0.40) per terminal branch. However, fruit drops of 87.5% and 76.1% were recorded on bagged and un-bagged terminal branches, respectively. There was also 191.7% increase in fruit set, 366.7% increase in fruit yield and a pollination deficit of 72.7% when avocado flowers were bagged to exclude avocado pollinators (Table 4.7).

Results in season three showed a total of 564 and 915 fruitlets set on the bagged and un-bagged terminal branches, respectively. There was a highly significant (P=0.002) difference in fruit set in bagged (14.1±3.28) and un-bagged (22.9±3.28) per terminal branch. Seven fruits were harvested from the bagged terminal branches while 14 fruits were harvested from the un-bagged treatment. This revealed a significant (P=0.014) difference in fruit yield from bagged (0.2±0.08) and un-bagged (0.4±0.08) per terminal branch. A 98.8% and 98.5% in fruit drop was noted from bagged and un-bagged terminal branches, respectively during the fruit growth and development period. There was 162.3% increase in fruit set, 200% increase in fruit yield and a pollination deficit of 50% between bagged and un-bagged terminal branches (Table 4.8).

Table 4.6: Mean avocado fruits set and yield ($\log_{10}$ (n+1)) in bagged and un-bagged terminal branches in Kandara, Murang'a County in season one (August 2015 - July 2016)

Treatment	Fruit set per terminal branch	Fruit yield per terminal branch	% fruit drop	% fruit set increase	% yield increase	% deficit
Bagged	0.68±0.07[b]	0.03±0.02[b]	98.1			70.0
Un-bagged	1.17±0.07[a]	0.11±0.03[a]	97.9	305.1	333.4	
t-value	-5.2	-2.3				
df	86	86				
p-value	<0.001	0.024				

Means ±SE followed by the same letter(s) within the same column are not significantly different at P≤0.05 (Student's t-test).

Table 4.7: Mean avocado fruit set and yield (log_{10} (n+1)) in bagged and un-bagged terminal branches in Kandara, Murang'a County in season two (April- October 2016)

Treatment	Fruit set per terminal branch	Yield per terminal branch	% fruit drop	% fruit set increase	% yield increase	% deficit
Bagged	0.86±0.25[a]	0.19±0.10[a]	87.5			72.7
Un-bagged	1.46±0.20[a]	0.52±0.18[a]	76.1	191.7	366.7	
t-value	-1.87	-1.67				
df	20	20				
p-value	0.076	0.111				

Means ±SE followed by the same letter(s) within the same column are not significantly different at P≤0.05 (Student's t-test).

Table 4.8: Mean avocado fruit set and yield (log_{10} (n+1)) in bagged and un-bagged terminal branches in Kandara, Murang'a County in season three (August 2016 – March 2017)

Treatment	Fruit set per terminal branch	Yield per terminal branch	% fruit drop	% fruit set increase	% yield increase	% deficit
Bagged	2.22±0.16[b]	0.05±0.02[b]	98.8			50.0
Un-bagged	2.86±0.12[a]	0.10±0.03[a]	98.5	162.3	200	_
t-value	-3.24	-1.5				
df	78	78				
p-value	0.002	0.014				

Means ±SE followed by the same letter(s) within the same column are not significantly different at P≤0.05 (Student's t-test).

Combined data for the three seasons recorded a total of 891 fruitlets set on the bagged terminal branches while un-bagged terminal branches set 1886 fruitlets. This was a 211.6% increase in fruit set after exposure to flower visitors (Table 4.9). A highly significant (P <0.001) difference in fruit set between the bagged (9.38±1.55) and un-bagged (19.85±2.00) was recorded across the three seasons (Table 4.9). Total fruit yield was 45 and 16 from the un-bagged and bagged terminal branches, respectively. This translated to a 281.3% increase in fruit yield, considering fruit abortion of 98.2% in bagged and 97.7% in un-bagged terminal branches. There was a significant (P= 0.002) difference in fruit yield between the bagged (0.17±0.05) and un-bagged (0.46±0.08) fruits per terminal branch in the three seasons. This translated to 64.5% pollination deficit when avocado flowers were denied pollination from possible insect pollinators through bagging (Table 4.9).

Table 4.9: Mean avocado fruit set and yield (log_{10} (n+1)) in bagged and un-bagged terminal branches in Kandara, Murang'a County in three seasons (August 2015 - July 2016; April - October 2016 and August 2016 - March 2017)

Treatment	Fruits set per terminal branch	Yield per terminal branch	% fruit drop	% fruit set increase	% yield increase	% deficit
Bagged	1.76±0.11[b]	0.05±0.01[b]	98.2			64.5
Un-bagged	2.62±0.10[a]	0.12±0.02[a]	97.7	211.6	281.3	
t-value	-5.78	-3.16				
df	188	188				
p-value	<0.001	0.002				

Means ±SE followed by the same letter(s) within the same column are not significantly different at P≤0.05 (Student's t-test).

Results showed significant differences in fruit weight (P=0.002), fruit equatorial diameter (P=0.04), seed weight (P<0.001), ovary equatorial diameter (P<0.001) and ovary polar diameter (P=0.028) in season one; but no significant (P=0.238) difference in fruit polar diameter between fruits harvested from bagged and un-bagged terminal branches (Table 4.10). In season two, there was highly significant (P=0.002; P=0.001) differences in ovary equatorial diameter and seed weight, respectively, between fruits harvested from bagged and un-bagged terminal branches. Additionally, there was significant (P=0.043) differences in fruit equatorial diameter and ovary polar diameter (P=0.016) between fruits harvested from bagged and un-bagged terminal branches. However, there were no significant difference (P=0.368) in fruit polar diameter and in fruit weight (P=0.098) between the fruits harvested from the bagged and un-bagged terminal branches in season two (Table 4.11).

There was a significant difference in seed weight (P=0.009) in season three. However, there were no significant differences in fruit equatorial diameter (P=0.253), fruit polar diameter (P=0.105), fruit weight (P=0.062), ovary equatorial diameter (P=0.263) and ovary polar diameter (P=0.06) between the fruits harvested from bagged and un-bagged terminal branches (Table 4.12).

On fruit parameters for the three seasons, there were highly significant differences in ovary equatorial diameter (P<0.001) and in seed weight (P<0.001) between fruits from the bagged and un-bagged terminal branches. There was also significant difference in fruit equatorial diameter (P=0.035). However, there were no significant difference in fruit polar diameter (P=0.947), in fruit weight (P= 0.055) and in ovary polar diameter (P=0.136) between fruits harvested from bagged and un-bagged terminal branches (Table 4.13).

Table 4.10: Mean yield parameters for avocado fruits harvested from bagged and un-bagged terminal branches in Kandara, Murang'a County during season one (August 2015 - July 2016)

Treatment	Fruit weight (g)	Fruit equatorial diameter (mm)	Fruit polar diameter (mm)	Seed weight (g)	Ovary equatorial diameter (mm)	Ovary polar diameter (mm)
Bagged	121.6±6.85[b]	55.83±1.17[b]	75.5±2.06[a]	4.82±0.77[b]	21.17±0.54[b]	26.17±1.2[b]
Un-bagged	165±10.52[a]	62.1±1.52[a]	79.7±1.78[a]	18.09±3.05[a]	29.25±1.64[a]	32.15±1.34[a]
t-value	-3.46	-2.17	-1.21	-4.22	-4.68	-2.34
df	23	24	24	21	22	24
p-value	0.002	0.04	0.238	<0.001	<0.001	0.028

Means ±SE followed by the same letter(s) within the same column are not significantly different at P≤0.05 (Student's T-test).

Table 4.11: Mean yield parameters for avocado fruits harvested from bagged and un-bagged terminal branches in Kandara, Murang'a County during season two (April - October 2016)

Treatment	Fruit weight (g)	Fruit equatorial diameter (mm)	Fruit polar diameter (mm)	Seed weight (g)	Ovary equatorial diameter (mm)	Ovary polar diameter (mm)
Bagged	99.3±19.18[a]	52.67±3.33[b]	68±5.29[a]	6.3±3.09[b]	22.67±1.86[b]	27.67±2.33[b]
Un-bagged	137.3±9.79[a]	59.73±1.38[a]	72.55±2.13[a]	22.58±1.83[a]	32.73±1.18[a]	33.73±0.95[a]
t-value	-1.79	-2.27	-0.94	-4.21	-4.07	-2.82
df	12	12	12	12	12	12
p-value	0.098	0.043	0.368	0.001	0.002	0.016

Means ±SE followed by the same letter(s) within the same column are not significantly different at P≤0.05 (Student's T-test).

Table 4.12: Mean yield parameters for avocado harvested from bagged and un-bagged terminal branches in Kandara, Murang'a County during season three (August 2016 - March 2017)

Treatment	Fruit weight (g)	Fruit equatorial diameter (mm)	Fruit polar diameter (mm)	Seed weight (g)	Ovary equatorial diameter (mm)	Ovary polar diameter (mm)
Bagged	110.59±13.09[a]	50.86±2.34[a]	75.43±5.22[a]	11.06±0.85[a]	22.43±1.78[a]	29.57±2.14[a]
Un-bagged	83.88±6.97[a]	47.79±1.43[a]	66.57±2.62[a]	6.96±0.88[b]	20.21±1.03[a]	25.29±1.09[a]
t-value	1.99	1.18	1.7	2.95	1.15	2
df	19	19	19	16	19	19
p-value	0.062	0.253	0.105	0.009	0.263	0.06

Means ±SE followed by the same letter(s) within the same column are not significantly different at P≤0.05 (Student's T-test).

Table 4.13: Mean yield parameters for avocado harvested from bagged and un-bagged terminal branches in Kandara, Murang'a County for three seasons (August 2015 - July 2016; April - October 2016 and August 2016 - March 2017)

Treatment	Fruit weight (g)	Fruit equatorial diameter (mm)	Fruit polar diameter (mm)	Seed weight (g)	Ovary equatorial diameter (mm)	Ovary polar diameter (mm)
Bagged	112.6±7.03[a]	53.06±1.32[b]	74.06±2.56[a]	7.13±1.07[b]	22±0.84[b]	27.94±1.13[a]
Un-bagged	133±7.65[a]	57.07±1.28[a]	73.87±1.49[a]	15.41±1.75[a]	27.29±1.12[a]	30.4±0.88[a]
t- value	-1.96	-2.18	0.07	-4.04	-3.78	-1.51
df	48	43	59	59	56	59
p-value	0.055	0.035	0.947	<0.001	<0.001	0.136

Means ±SE followed by the same letter(s) within the same column are not significantly different at P≤0.05 (Student's T-test).

CHAPTER FIVE: DISCUSSIONS

5.1 Abundance and diversity of avocado pollinators in Kandara, Murang'a County

Season one and three had insect pollinator diversity of six species each with similar trends in insect pollinators' abundances being noted. High abundances for *A. mellifera* were documented, with the rest of the pollinators having very low occurrence. This could be associated to the warm and dry weather conditions in the locality during the months of September and October since it is postulated that the density and abundance of honey bees is likely to be high in warmer conditions (Bergh, 1967; Eardley and Mansell, 1996; Florence-Dem, 2011). These two seasons lead to the off season avocado crop that had low yields compared to season two that produced the on-season avocado crop with higher yields.

In season two, nine species of pollinators were recorded on avocado flowers with a decrease in *A. mellifera* populations. This reduction in honey bee observations on avocado flowers could be associated with the wet weather in the months of March to May. Honey bees are known to be less active during rain seasons (Vaughan and Black, 2006; Puškadija *et al.*, 2007). Additionally, low temperatures keep honey bees in hives as they feed on honey accumulated during warmer periods thereby limiting their foraging activity (Conte and Navajas, 2008; Needham, 2010).

There was an increase in the abundances of *C. putoria, Polistes* sp., *E. tenax, I. reburrus* and *M. ferruginea* hence higher species richness. This could have been due to the warm and dry weather conditions experienced in the months of January and February which promotes increased insect populations. This is supported by findings by Silva et al. (2011) that highest abundance of most arthropods is noted in the transition period from the end of the dry season to the start of the rainy one. This increase in species richness and evenness could have led to the higher fruit set and fruit yield per terminal branch recorded in season two as compared to season one and three which had low species richness (six species) and lower species evenness of 0.2.

The higher abundances of *A. mellifera* noted in season one and three as compared to season two could have been due to the warm and dry weather conditions experienced in this area during the months of September. Dry and warm weather is known to promote foraging by *A. mellifera*. This is based on the assumption that the density and population of honey bees is likely to be high in warmer conditions (Bergh, 1967; Eardley and Mansell, 1996; Florence-Dem, 2011).

Hymenoptera and Diptera orders contributed to more than 99% of the avocado flower pollinators recorded during the study period. Two species, *C. putoria* and *E. tenax* in the order Diptera and five species (*I. reburrus*, *A. mellifera*, *Polistes* sp., *M. ferruginea* and *Halictus species*) in the order Hymenoptera were also recorded. Although, *A. mellifera* had the highest visitation counts in most times during the day for the three flowering seasons' period, abundances of *C. putoria*, *E. tenax* and *Polistes* sp. were higher on avocado flowers than the other pollinators during the study period. These were possibly the leading avocado pollinators in Kandara. In addition to honey bees, other bee species (*M. ferruginea* and *Halictus species*) were observed visiting avocado flowers but in low abundances.

Combined observations for Hymenopterans showed that they were the leading pollinator insect group with 91.43%, Dipterans (8.35%), Coleopterans (0.17%) and Lepidopterans (0.06%) of the total visits. These findings are in agreement with findings reported by Kearns (2001) and Ssymank *et al.* (2008) that dipterans are second in importance after the hymenopterans as pollinators in most crops.

5.2 Diurnal visitation counts of insect pollinating avocado in Kandara, Murang'a County

Observations for each avocado pollinator for the three seasons showed that *A. mellifera* had the highest visits at all times of the day with 1548 observations. Second were *C. putoria* with 94 observations while *E. tenax* and *Polistes* sp. had 54 and 48 observations, respectively throughout the study period.

Avocado flower visitation by insect pollinators started from 0900 h to 1600 h in Kandara. Dipterans were observed throughout the day in the three seasons with *E. tenax* and *C. putoria* as the earliest avocado flower pollinators. Ssymank (2008) noted similar results showing that flies visited flowers earlier than bees. He attributed this to low energy requirements by the flies in addition to not provisioning a brood and being able to depend on less rewarding flowers. In addition, many Dipterans are active at low temperatures that prevail mostly in the morning and are known to dominate high altitude and high latitude plant-flower visitor systems (Elberling and Olesen, 1999).

As compared to Dipterans, *A. mellifera* were observed later in the morning but their numbers increased significantly throughout the day. Similar results have been noted by Ish-Am (2005, 2008) that honey bees visited both male and female stage flowers in avocado, throughout the day. In this study, short and long visitation patterns were noted in different days. This could have been due to differences in daily temperatures. California Avocado Commission (2012) made similar observations that during cool days, *A. mellifera* delayed in visiting flowers leading to less pollination and during warm days, they could be noted earlier on flowers. Bees are sensitive to weather conditions (Lesley and Bringhurst, 1951; Peterson, 1955; Bergh, 1967) and one may observe high levels of bee activity on warm days, but cold or cloudy, rainy weather markedly reduces their activity (Davenport, 1986).

5.3 Floral calendar of avocado in Kandara, Murang'a County

Avocado flowering in Kandara was noted in two seasons per year, February to May and August to October with highest peaks in March and September, respectively. This has been reported in other parts of the world (Davenport, 1982; Salaar-Garcia *et al.*, 1999 and California Avocado Commission, 2012).

Female and male flower phases overlap was observed for two hours in season one and two, but for three hours in season three, with extended female phase and a delay in male phase flowers during cool weather. Overlaps of half an hour to one and half hours was noted by Ish-Am and Eisikowitch (1989) and Ish-Am (2008) between male and female phase avocado flowers in a day in Israel. Also, Davenport (1986) found out that due to the shifts that occur in avocado flowering behavior during periods of cool weather, both functional male and female phase flowers may be present at the same time on individual trees resulting to close-pollination.

The unique flowering behavior known as diurnally synchronous dichogamous protogyny, with intermediate closing was also noted during this study. Similar results were recorded by Peterson (1955); Ish-Am and Eisikowitch (1991, 1992); Bergh and Lahav (1996); Gazit and Degani (2002); Alcaraz and Hormaza (2009) and Ish-Am and Lahav (2011). Female phase flower opening takes place in the morning hours up to the afternoon while the male phase flowers start opening during the afternoon through evening.

5.4 Effect of pollination deficit on avocado fruit production in Kandara, Murang’a County

The study showed that pollinators significantly affected fruit yield (quantity) and seed quality (weight and size), with the latter being of importance during seedling growth. Results showed that pollination was a limiting factor in avocado production. This is in support of similar findings by Gazit and Degani (2002), Degani *et al.* (2003) and Ish-Am (2005) who reported that insects are very important as avocado flower visitors facilitating pollination. The findings affirm the importance of pollination management in avocado and calls for utilization of pollinators for increased productivity and quality of fruits.

Bagging terminal branches prevented avocado flower pollinators by creating a physical barrier. However, there were fruit set in the bagged inflorescences during the study and similar results were reported by Lewis (1973); Davenport (1986) and Hoddle *et al.* (2010). They concluded that the fruit set in the bagged inflorescences could have been due to airborne pollen or small insects like thrips inside the flowers that effect self-pollination.

Pierre *et al.* (2011) noted a yield increase on oilseed rape in experimental plots with net bagged flowers in sites with more pollinators. They related this to other factors correlated with insect visitation such as the release of airborne pollen by foraging bees. It has been concluded that the difference between yields from such treatments represents the contribution from insects' pollination (Sacchi and Price, 1988; Wragg and Johnson, 2011). In this study, treatments had significant effects on avocado seed weight and ovary equatorial diameters. This indicated that insect pollination could affect the quality of the fruits.

When Dipteran (blow flies and hoverflies) abundance on avocado flowers increased in season two, fruit yield per terminal branch increased significantly; this is in agreement with Garibaldi *et al.* (2013) that an increase in the abundance of wild pollinators' increases fruit set by twice as much of the same increase in *A. mellifera*. This increase in the number of fruits per terminal branch in season two could be associated with the increased diversity and higher evenness of the avocado flower pollinators. This was also in agreement with findings from Greenleaf and Kremen (2006) who noted that an increase in diversity increases fruit set.

Non-honey bee avocado flower pollinators were not observed in all farms under study. This could suggest that they are not readily available for avocado flowers' visitation across farms leading to pollination. This could be due to the effect of landscape composition which is known to affect non-bee pollinators and stingless bees more than the *A. mellifera* (Zou, 2017). Any management practice that could enhance the populations of non-bee species is of importance to boost avocado pollination and hence increase production.

High fruit abortion was noted during this study and this could be associated to different plant responses including lack of enough soil water, inadequate nutrients, pests and disease attacks, heavy rains as well as high wind speed. Management of these possible causes of fruit abortion is of importance and could include minimizing water stress through provision of irrigation, pests and disease management as well as providing wind breaks.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- Honey bees *A. mellifera* were noted to be the most abundant and frequent insect flower pollinators of avocado in almost every time of the day and in each farm.

- Blow flies *C. putoria*, hoverflies *E. tenax* and wasps *Polistes* sp., could also be effective pollinators, especially when honey bees' visitations are low.

- Avocado flower opening in the female phase was in the morning hours while the male phase was recorded later in the day.

- An overlap between the male and female phase flowers lasting for 2-3 hours in all the three seasons was noted. This was observed as early as 1100 h and delayed up to 1600 h in some days.

- Most avocado pollinators were active during the female and male flower phase overlap period. This confirms their ability to pollinate pure stands of Hass variety.

- Avocado pollinators increased fruit set leading to increased fruit production. Additionally, seed weight and size were higher for fruits resulting from insect pollination.

6.2 Recommendations

- Beehives could be placed in avocado orchards to increase honey bees' abundance for increased avocado yields.
- In addition to honey bee hives, stingless bees could be domesticated and their hives brought into the farms for complementing the honey bees when they are unable to pollinate avocado flowers.
- Establishment and protection of natural habitats/patches of shrubs and bushes in portions of the farms to increase pollinators' abundance and diversity. These could also act as pollinators' nesting sites hence a possibility of increased avocado pollination from insects translating to increased yields.

6.3 Suggestions for further studies

- Determination of the pollen load for each insect visiting avocado flowers is necessary since some visitors may have low visitation rates but very high pollen loads hence making them efficient pollinators.
- Pollinator management practices to conserve and maintain the available avocado flower pollinators.
- Analysis of quality attributes of the avocado fruits such as nutrient content, oil levels as well as viability of seeds and growth rate of seedlings from bagged and un-bagged treatments to determine the influence by insect pollinators.

- Long term monitoring of flower visitors' populations could be established as an ecosystem health measure in the area and other parts of Kenya relative to the yield responses.
- Determination of possible causes of fruit abortion and how to manage it.

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