ORIGINAL RESEARCH ARTICLE



Abundance and community composition of flower visiting insects of avocado (*Persea Americana* Mill) in the East African region

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Abstract

Pollination of avocado flowers is essential for fruit production and is facilitated by various insect taxa. Currently, there is limited information available on the community structure of the pollinators of avocado crops in East Africa. We duly present the distinct groups of insects visiting avocado flowers across two most important avocado producing countries in the East African region. Insects were swept from avocado flowers along transects and sorted by morphological features that classified them into orders, families, and genera. Five common orders were identified, namely: *Coleoptera, Diptera, Heteroptera, Hymenoptera*, and *Lepidoptera*. The relative abundance of *Hymenoptera* and *Diptera* were highest (49% and 22% respectively) hence we further identified them to the families and genera taxonomic groups. Three *Dipteran* families were found (*Calliphoridae, Muscidae,* and *Syrphidae*) from which we recognized five genera: *Chrysomya, Spilogona, Eristalinus, Phytomia and Syritta. Hymenoptera* on the other hand were placed into two general groups; bees and wasps. For bees, family *Apidae* was represented by *Apis, Ceratina, Meliponula,* and *Xylocopa* whereas seven diverse wasp families (*Braconidae, Philanthidae, Vespidae, Ichneumonidae, Eumeninae, Thynnidae* and *Pompilidae*) were also noted. The occurrence of *Coleoptera* and *Lepidoptera* varied across the three orchards while the rest of the orders remained unaffected. We further recorded the insect's potential pollinator functions and concluded that the main pollinators of avocado flowers in the region are members of *Syrphidae* families. This information serves as a foundation for avocado pollination studies in the East African region that are important for improving pollination services and insect pollinators' conservation.

Keywords Pollination · Morphological features · Insect orders · Genera · Kenya · Tanzania

Introduction

Pollination accounts for the production of estimated 87.5% angiosperm species worldwide (Ollerton et al. 2011), 35% of which directly serves humanity as food sources across the globe (Klein et al. 2007). Insect-mediated pollination has the greatest share in the key ecosystem service accounting for the production of most single crops (Klein et al. 2007). Avocado (*Persea americana* Mill), is a tree crop of the family *Lauraceae* that is recognized globally for its nutritional and cosmetic versatility for human health (Dreher and Davenport 2013; Duarte et al. 2016). In East Africa,

Kenya is the largest producer of the fruit with production of 196,555 metric tons in 2018 alone (FAO 2020). Although avocado production data is currently not available in the FAO database for Tanzania, cultivation and trade of avocados have been reported from the country (Juma et al. 2019).

Using the FAO dataset on cultivated crops, Aizen et al. (2009) found out that avocado is unfortunately one among several crops whose yields have decreased over the years in spite of increased cultivation areas. Their findings proved that yields do not necessarily improve with increased cultivation areas. This fact then begs for new sustainable farming practices that would enhance yields especially for the region that earns a lot of revenue from avocado fruit exports. Despite spirited efforts to eradicate pest and disease and limit post-harvest losses, information is still lacking on how the ecosystem service of pollination could also be included in these efforts to enhance avocado yield for avocado farmers in these two countries.

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One of the major factors that contribute to yield of most angiosperm species is the amount of fruits that set after pollination (Pashte and Kulkarni 2015). A positive correlation between fruit set and pollinator functional group (FG) richness as established by Albrecht et al. (2012) insures plant reproductive success. While conducting their routine foraging for various floral rewards, pollinator insects usually unwittingly pollinate avocados as they transfer pollen stuck on their various body parts from one flower to another (Perez-Balam et al. 2012). These flower foragers include diverse species of bees and wasps (*Hymenoptera*), flies (*Diptera*), and butterflies (*Lepidoptera*) (Mehmood et al. 2015; Pérez-Balam et al. 2012) that exist in wild communities (flies and solitary bees) and managed colonies (honeybees and stingless bees) (Ish-Am and Eisikowitch 1993; Peterson 1955). The diversity of these arthropod pollinators gives the flower a rare advantage of experiencing different pollination behaviour that has a positive net effect on its pollination (Brittain et al. 2013).

The increasing global threats to pollinators such as pests and diseases, pesticides, land use intensification and invasive species (Dicks et al. 2020) have led to declines in pollinator populations (Vanbergen et al. 2013). An example is in a UK study where Biesmeijer et al. (2006) documented declines in bee and hoverfly pollinator species that was succeeded by parallel declines in out-crossing plant species of Britain and Netherlands that depended entirely on these insects for pollination.

As an outcrossing species, avocado is at a high risk of suffering insufficient pollination owing to the diminishing pollinator communities. Pollination of avocado flowers by insects is well studied in other regions of avocado mass production like Mexico (Perez-Balam et al. 2012), Spain (Perez-Balam et al. 2012), California (Davenport 2019), Israel (Perez-Balam et al. 2012) and South Africa (Toit and Swart 1993). The most abundant pollinator is the Western honeybee, Apis mellifera, followed by stingless bees and a range of dipteran flies, but with quite regionspecific flower visitor communities. While the honeybee is globally distributed, many other species have a limited range of distribution and might not occur in Africa, while related species of the same group do occur. This could be true for stingless bees which are frequently found visiting avocado flowers in South and Central America (Can-Alonzo et al. 2005) however, related species do occur in Africa (Ndungu et al. 2017).

The lack of sufficient knowledge on pollination systems in avocado orchards in East Africa therefore presents a huge gap in understanding the identity and impact of pollinator communities on avocado production for this region. In order to improve pollination services it is required to know the identity of those communities. We present an inventory of insects, their abundance and diversities in three avocado production sites of Kenya and Tanzania in order to highlight the insect groups that could be paramount for avocado pollination in these countries. Our intention is to use this knowledge to build a foundation for further research on pollinators of avocados and how the ecosystem service may be manipulated for increased production of avocados in the East Africa region.

Materials and methods

Sampling locations

Sample collection was conducted in three major avocado growing sites of Murang'a in Kenya, Siha, and Lushoto districts of the United Republic of Tanzania. Murang'a County lies at an elevation of 1,267 m above sea level and experiences a tropical climate with average temperature and precipitation at 20.0°C and 1,195 mm respectively (Climatedata.org). Siha District is elevated at 1,115 m above sea level; the region experiences a warm and temperate climate and with annual temperature and precipitation at 20.5°C and 1,189 mm respectively (Climate-data.org). Lushoto district, on the other hand, sits at an elevation of 1,378 m above sea level, experiences a warm and temperate climate with annual temperature and precipitation averages of 17.3°C and 1,074 mm respectively (Climate-data.org). The farms at were located at 0°55'4.71°S, 37°10'22.00°E; 3°9'56.35"S, 37°4'25.22"E; and 4°35'42.26°S, 38°18'58.97°E for Murang'a, Siha and Lushoto sites respectively.

Insects were sampled from two large scale production zones in Murang'a and Tanzania and a smallholder farming plot in the district of Lushoto. The two large-scale sites were supplemented with at least two honeybee hives per farming plot while four stingless bee hives were observed in the Lushoto farm. While the orchard setting in Murang'a comprised of numerous avocado plots (2.1 ha each) bordering one another with intervals of hedges and tall trees, Siha consisted of fewer plots that were at least three times larger than Murang'a plots. Moreover, Siha farms bordered the great Kilimanjaro forest which is a wealthy ecosystem harboring diverse animal species. We chose 13 plots in the Murang'a orchard with average measurements of 400 m \times 600 m and eight plots in the Siha orchard with average sizes of 400 m×900 m. The farm in Lushoto was approximately 400 m² tucked within the Usambara mountains. The farm entailed an intercropped system of mango and avocado trees where we counted a total of 35 avocado trees. Other avocado trees were also sighted within a 2 km range from the sampling site. Sample collection occurred during the major avocado flowering season between September and November 2018.

Sampling technique

Line transects were place along the width of individual plots at intervals of 200 m in Murang'a and Siha locations thus resulting into three and five transects respectively per farming plot. Random scan sampling technique was employed in Lushoto, where a third of the number of trees per farm was used for sampling hence 15 trees were sampled from a total of 35 trees. Each farming plot was sampled twice for all the three sites; in the morning from 9 am to 12 pm and afternoon from 2 to 5 pm in order to accommodate insects that visit at different times of day. Murang'a and Siha farms were each sampled for five days while the Lushoto farm was sampled in two days. Collection time per tree was limited to 10 min. Insects were directly obtained from flowers by the use of sweep nets and sweeping out insects that were sighted visiting the avocado inflorescences. The specimens were immobilised by exposing them to cotton swabs soaked in absolute ethanol (98%) followed by immediate storage in absolute ethanol in collection tubes.

Sample processing

Specimens were removed from ethanol and set at a sterilized work surface and left to dry for 15 min to facilitate accurate identification. Identification was done using a Zeiss® light microscope (Carl Zeiss GmBH, Germany) attached with a digital camera. Digital images of the samples were captured using the ZEN image suite (Jena, Germany). The two most abundant insect orders across the three sites were subjected to further identification up to the genus level. Identification

Fig. 1 Relative frequencies of the six insect groups sampled across three regions in Kenya and Tanzania. Lushoto had the highest frequency for Hymenoptera while Siha and Murang'a had the highest relative frequencies for Diptera and Lepidoptera respectively. The most common insects across the three regions are Hymenoptera, Diptera and Lepidoptera

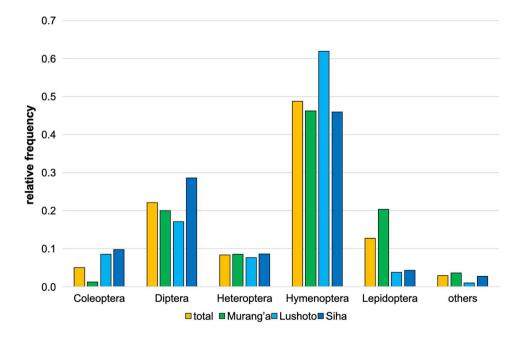
Data analysis

Tabular data were tailored and loaded into R studio for analyses. Abundance of specimens in each order per site were plotted in a bar chart using the ggplot2 package in R. Shannon–Weaver diversity indices (SWDI) for the orders from each site were also calculated using the Microsoft Excel spreadsheets then jack-knifed over orders. The SWDI dataset was also loaded in R studio where we applied a oneway ANOVA along with an LSD post hoc test (package *DescTools*, Signorelli et al. 2020) conducted to query for the effect of geographical location and farm sizes on insect order diversity.

The distribution of the insect orders across the three regions was tested using Monte Carlo statistics for RxC tables (http://rxc.sys-bio.net, Blüthgen et al. 2006). We used the row- and column-wise Kullback–Leibler-distance to infer regions and orders that contributed to the overall diversity.

Results

A total of 619 individual insects were collected in the sampling points (Murang'a n=329; Lushoto n=105; Siha n=185). These were grouped into the five distinct orders of *Coleoptera*, *Diptera*, *Heteroptera*, *Hymenoptera*, and *Lepidoptera* as illustrated in Fig. 1 and Table 1. depicting



Site	Coleoptera	Diptera	Heteroptera	Hymenoptera	Lepidoptera	Others	Total
Murang'a	4	66	28	152	67	12	329
	(0.01)	(0.20)	(0.09)	(0.46)	(0.20)	(0.04)	
Lushoto	9	18	8	65	4	1	105
	(0.09)	(0.17)	(0.08)	(0.62)	(0.04)	(0.01)	
Siha	18	53	16	85	8	5	185
	(0.10)	(0.29)	(0.09)	(0.46)	(0.04)	(0.03)	

 Table 1
 Insects collected from avocado fields of Kenya and Tanzania grouped by orders. Under Others, we grouped a range of different orders (e.g. Odonata, Orthoptera) which overall contribute less than
 5% of the total insects. Values indicate the abundance while in brackets are the relative frequencies per region

abundance of each insect order in the three respective sites. SWDI values showed that the abundance of insects per order was significantly different across the three sites (ANOVA, F = 3.88, $p \le 0.05$). Murang'a and Siha showed an SWDI of 0.77 and were not significantly different in an LSD post hoc test, while Lushoto had an SWDI of 0.66 and differed remarkably from the other regions (LSD post hoc test, Murang'a-Lushoto: $p \le 0.05$; Siha-Lushoto: 0.03). Of all the five orders represented, orders *Diptera* and *Hymenoptera* had the highest abundance while *Coleoptera* and *Lepidoptera* were the least abundant across the three sites.

Although the orders *Coleoptera* and *Lepidoptera* had the least representation across the three sites, their presence in the orchards had the greatest significant effect on the overall diversity (Monte Carlo statistics for RxC matrices, p < 0.0001) with an overall frequency of 5 and 12%, respectively as represented in Table S1.

Hymenoptera were further classified into two generalized groups: the wasps and bees. Wasps had a total of 6 families scattered across the regions while the bees identified belonged to the Apidae family. In Apidae, we managed to recognize four genera and it is only the genus *Apis* that was represented across the three studied sites, *Ceratina* was found only in Murang'a while *Xylocopa* and *Meliponula* were only observed in Lushoto (Table 1). Classification of the Diptera resulted in three families; the *Calliphoridae*, *Muscidae* and *Syrphidae* from which we extracted six genera (Table 2). We additionally provided morphological evidence for the relevance of each of these genera in pollination such as presence of body hairs for trapping pollen particles (Table 3).

Discussion

Our results reveal that five major insect orders visited flowers in the sampled avocado orchards of Kenya and Tanzania. Whereas all the orders were represented, Diptera and Hymenoptera had the highest representation across the three orchards. We also noted that the abundance at Lushoto was significantly lower than in Murang'a and Siha, probably due to the orchard size and farming practice differences. According to Carvalheiro et al. (2010), farming practices had a remarkable effect on pollinator populations occurrence and densities in semi-natural habitats of Serbia during summer. In the study, tillage improved insect pollinator densities while proximity to natural habitats and places of high biodiversity affected pollinator species occurrence. Shuler et al. (2005) also reported effect of tillage on pollinator density in curcubit farms though other practices such as pesticide use or supplementation of farms with honeybees did nothing to affect the same.

The results of the Shannon–Weaver diversity analysis also showed that diversity of insects in Murang'a and Siha are

Table 2 (Classification of	of Diptera and H	ymenoptera into	families and genera.	For wasps only f	families were identified

	Diptera			Hymenoptera		
	Calliphoridae	Muscidae	Syrphidae	Bees (Apidae)	Wasps	
Murang'a	Chrysomya	Spilogona	Eristalinus	Apis	Braconidae	
-	Hemipyrellia		Syritta Phytomia	Ĉeratina	Eumeninae Ichneumonidae Philan- thidae Pompilidae Thynnidae Vespidae	
Lushoto	Hemipyrellia		Eristalinus	Apis Meliponula Xylocopa	Vespidae	
Siha	Chrysomya Hemipyrellia	Spilogona	Eristalinus	Apis	Vespidae Philanthidae	

Table 3 Overview of flower visitors at avocado (<i>Persea</i>)	Order	Family	Species	Function	Supporting evidence
<i>americana</i>) in East Africa including their inferred function	Coleoptera	diverse	diverse	`Mess-and-soil pollinators '	Hairy body Dense setae and pubescence
and evidence for it	Diptera	Calliphoridae	Hemipyrellia sp.	pollinators	Hairy body
			Chrysomya sp.	pollinators	Hairy body
		Muscidae	Spilogona sp.		
		Syrphidae	Eristalinus sp.	pollinator	hairy body
			Phytomia sp.	pollinator	hairy body
			Syritta sp.	pollinator	hairy body
	Heteroptera	diverse	diverse	herbivore	
	Hymenoptera	Apidae	Apis mellifera	pollinator	hairy body, known role as pollinator
			Ceratina sp.	pollinator	hairy body
			Meliponula sp.	pollinator	hairy body, known role as pollinator
			Xylocopa sp.	pollinator	hairy body
		Braconidae	Zelodia sp.	parasitoid	none
		Philanthidae	Philanthus sp.	predator	none
		Ichneumonidae	Charops sp.	parasitoid	none
		Pompilidae	Ageniella sp.	predators of spiders	none
		Thynnidae	Methocha sp.	parasitoid	none
		Vespidae	Eumenes sp., Belonogaster sp.	predator	none
	Lepidoptera	diverse	diverse	Pollinators	Hairy body
	others	diverse	diverse	Several predators and herbivores	none

similar, a factor that may be attributed to similar farming practices and the presence of beehives in each farming plot in both orchards. While the importance of honeybees to avocado pollination is quite undisputed, the contribution of specific fly families and genera to the same still remains subject to discussion (Eardley and Mansell 1996). Although the Diptera have previously been associated with erratic visitation rates, they are still great in pollinating avocados especially in the absence of honeybees or in farms where honeybee abundance is low (Eardley and Mansell 1996; Ish-Am et al. 1999). The most common Diptera foraging on avocado belong to the family Syrphidae (hoverflies) though other flies have been recorded in Pakistan (Mehmood et al. 2015). Ssymank et al. (2008) identified syrphid Diptera as the most important fly pollinators of avocado with admirable qualities such as flower constancy and predation on plant pests such as aphids in a UK study on temperate ecosystems. Orford et al. (2015) additionally established insignificant differences in pollen loads by syrphid and non-syrphid Diptera, ascertaining that other Diptera are equally as important to the avocado in matters of pollination. Ish-Am et al. (1999) also observed that honeybees foraged less on avocado flowers in the vicinity of other more attractive flowers in the orchards of Mexico. Similar observations have likewise been noted for South Africa (Eardley and Mansell 1996) and this probably agrees with Peña and Carabalí (2018) since the overall effectiveness of the honeybee for pollination of avocado flowers is tied to foraging activity.

Stingless bees, on the other hand compete with honeybees for forage and the dominance of one group in a particular habitat diminishes the other (Roubik 1978). This is a likely explanation for the presence of stingless bees in Lushoto, a region that had fewer honeybees compared to Murang'a and Siha in our study. We also attribute this phenomenon to the stingless bee husbandry that we found in progress at our site in Lushoto. Pollination of avocado by wild bees in the region unfortunately remains largely unexplored, and presently only Luvonga (2015) observed the presence of Ceratina in a farm in Taita, Kenya, and their utility in the pollination of the avocado could be the subject of more research. Ceratina was absent in the Tanzanian farms and this could be as a result of the higher elevations of the two sites that may not favour their habitation although more research needs to be done to ascertain this.

While studying the California almond, Brittain et al. (2013) observed farms that hosted non-*Apis* bees present

experienced more visits by honeybees, a behaviour that culminated into effective pollination of the crop. It is likely therefore, that the presence of Ceratina within Murang'a farms could be beneficial in the overall pollination of avocados in the vicinity. Although wasps were also identified from avocado farms, their potential as efficient pollinators was ruled out due to their inability to trap and ferry pollen on their bald bodies (Ish-Am et al. 1999; Pérez-Balam et al. 2012). Moreover, most of the wasp families identified predate on smaller insects and their presence within the farms could negatively affect pollinator availability. Although the functionality of honeybees for pollination of avocado flowers is a known subject, other insects that also perform this ecosystem service have relatively been ignored. Our study testifies that pollinating insects that frequently visit avocado blooms are mainly of orders Diptera and Hymenoptera that are present in diverse genera and species. The global decline of honeybee colonies in the northern hemisphere serves as a trigger for the identification and conservation of alternative pollinator insects for pollination sustainability (Ellis et al. 2010; Neumann and Carreck 2010). We, therefore, encourage further studies to decipher optimal farm conditions that would enable the thriving of pollinators in our avocado farming systems.

Conclusion

The value of insect pollinators mainly of the orders Diptera and Hymenoptera cannot be overlooked especially in the East African region where there is potential for increased production of this crop for subsistence and export. From the study we conclude that honeybees are the dominant pollinators of avocados since they occur across the three regions and in high abundance. Ceratina was the only pollinator unique to Murang'a while Xylocopa dominated at Lushoto. Both genera play their unique roles in facilitating pollination and its therefore imperative to quantify their effectiveness in pollinating avocados. We also noted the genera Eristalinus (Syrphidae) and Chrysomya (Calliphoridae) in all the regions and conclude that the avocado farming ecosystem could be a strong enabler for their survival, a state that should be preserved in order to encourage habitation by other Dipteran pollinators.

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Declarations

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

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