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Research Article

Distance Effects on Diversity and Abundance of the Flower Visitors of *Ocimum kilimandscharicum* in the Kakamega Forest Ecosystem

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Pollination is an important ecosystem service in the maintenance of biodiversity and most importantly in food production. Pollination is on the decline due to habitat loss, exotic species invasions, pollution, overharvesting, and land use changes. This study analyzed the abundance and diversity of flower visitors' of *Ocimum kilimandscharicum* in Kakamega forest with increasing distance from the forest edge. Data were collected through direct observation and sweep netting. Six study sites were identified along two transects each 2.5 km long and labeled A to F. Distance in metres from the forest edge to each site was A=221, B=72, C=83, D=198, E=113, and F=50. Sampling was done from 7:30 am to 4:00 pm, three days in a week for five months consecutively. Diversity indices of different flower visitors were calculated using the Shannon-Wiener diversity index. One-way analysis of variance was used to compare differences between sites and a two-sample t-test was used to identify mean significant differences in species diversity between the closest and the furthest sites. A total of 645 individuals belonging to 35 species were captured from 4 families. The highest diversity was at site F (H'= 2.38) which was closest to the forest edge and the lowest diversity was from site A (H'=1.44) which was furthest from the forest edge. Distance from the forest edge significantly influenced species diversity ($F_{(3,20)}$=14.67, p=0.024). Distance from the forest edge also significantly influenced species abundance between the furthest sites A, D, and E and the nearest sites F, B, and C to the forest edge (t=4.177; p=0.0312) and species richness (t=3.2893; p=0.0187). This study clearly demonstrates that *Ocimum kilimandscharicum* flower visitors play essential roles in pollination and their higher number of visits translates into higher numbers of seeds set. Many of these pollinators are associated with the forest and hence the need to conserve the Kakamega forest as a source pool for pollinators.

1. Introduction

Pollination is an intangible ecosystem service that drives many life processes [1–3]. Flowering plants depend on pollen vectors that include wind, insects, and birds to transport their pollen. According to Kluser and Peduzzi [4], the current generation is endearing towards the 6th mass extinction with huge losses of biodiversity including pollinators, estimated in the range of 1 – 10% loss per decade as a result of habitat loss and degradation, exotic species invasions, pollution, overharvesting, land use changes such as habitat fragmentation [5, 6] (Potts et al. 2010). According to the Food and Agriculture Organization [7] of the more than 100 crop species that provide 90% of food supply in over 146 countries, 71 are bee pollinated [8]. Insect pollinators have high value and are considered as limited resources [9]. Potts et al. [10] estimated that total economic value of pollination worldwide varies from €30 to € 70 billion annually. Accordingly, any loss of biodiversity should be of great concern as the decreasing pollination trends globally threaten plant reproduction and hence food supply and security [11]. In Europe there have been recent indications of a 70% drop in the United Kingdom

and Netherlands of wild flowers that are insect-pollinated and a shift in pollinator community composition as of 1980's (Biesmeijer and Roberts, 2006). The pollinator decline and loss of pollination services have been linked to habitat destruction and land use intensification [12].

In Africa and Madagascar formation of national parks and reserves may mitigate pollinator decline and loss, but reliance on conserved areas is not sufficient enough to conserve pollinator diversity in the face of increasing land use changes [13]. Moreover, the community structure of forest insect pollinators is related to their host plants [14], meaning a strategic conservation plan should focus on both the insects and their associated floral resources. Gikungu [15] has pointed out *Ocimum kilimandscharicum* as one such favorite floral resource among the insect community in Kakamega forest.

The genus *Ocimum* is wide spread throughout the tropic world of Asia, Africa, and Central and Southern America [13]. Two-thirds of all the known species are reported to originate from West Africa while the remaining one-third originate from Asia and America [16]. Little information is available on the species *Ocimum kilimandscharicum*, leaving a gap that may contribute to the conservation of the genus *Ocimum*. Recent studies show that this plant is economically important and studies by Joshi [17] have revealed that the most abundant compound of this plant is camphor that consists of 45.9 %, 1, 8-cineol at 14.6%, and Limonene at 8.1 %.

The genus *Ocimum* has about 200 species of both herbs and shrubs that are known for their many uses, especially, those that enhance human health. It is believed that there are many more health benefits that are yet to be discovered [18]. *Ocimum kilimandscharicum* extracts are used to mitigate many disorders in East Africa comprising remedy of coughs, colds, measles, abdominal pains, diarrhea, and insect repellent, particularly against mosquitoes and storage pest control [19, 20]. The essential oils obtained from this plant act as repellent against nuisance biting insects and malaria vector has been practiced in North-Eastern Tanzania for centuries [21]. Other species that are well-known and studied include *Ocimum basilicum,* also known as sweet basil. It is a culinary herb mostly used in cookery. It is also used as a remedy for several ailments including cancer, convulsion, deafness, diarrhea, epilepsy, gout, hiccup, impotency, insanity, nausea, sore throat, tooth aches, and whooping cough. Basil has been reported in herbal publications as an insect repellent [17, 20], *Ocimum americanum;* this species is characterized by whitish flowers that are used for their medicinal value. It contains flavonoids, tannins, and carbohydrates. It thrives mostly in the native parts of Africa [18], *Ocimum gratissimum,* also known as clove basil. It is known to contain antibacterial and even anticancer components that are yet to be fully exploited and studied especially in the fight against cancer [22, 23]; *Ocimum micranthum* does very well in the South American region. *Ocimum kilimandscharicum* also known as camphor basil is an evergreen shrub of the family Lamiaceae [24, 25]. It grows in tropical areas with warm temperatures, annual rainfall of about 1250 mm, and an altitude of up to 900m with soils that are well drained (Joshi et al. 2010). The leaves contain essential oils that give the plant its worth as a medicinal plant and an extract of these rich oils used to make perfume and ointments [24, 25].

In Kakamega forest *Ocimum kilimandscharicum* is a major plant for insect foraging [14, 26] and it is now being cultivated on individual farmlands for commercial purposes. It is a wild shrub that grows in the forest but the transfer of this species to farmlands leaves a gap that needs to be studied and the effects are identified [15].

Surrounding communities in Kakamega forest area perceive *Ocimum kilimandscharicum* as a wild shrub [17] that grows in the forest or in their farmlands. Some of its important uses to the locals include medicinal purposes as it is used to treat several diseases, e.g., diarrhea and cold [17]. After processing, its remains are used as manure in their farms to increase crop yield and finally it may be used for beautification of their compounds. Given the limited knowledge on *Ocimum kilimandscharicum* this study sought to determine its significance and value in supporting and enhancing pollination services. This information will help reduce knowledge gap on pollination, increase pollination awareness among the local communities, and ultimately increase crop yields [22]. Furthermore, pollinator knowledge will contribute in evaluating the possibility of pollination of more plant species along forest edges and this will be useful in forestry and the breeding of commercial crops [27]. Such knowledge will also help raise awareness on the dependence of human existence on pollinators in line with agricultural practices and food security [28].

This study quantified diversity and abundance of flower visitors of *Ocimum kilimandscharicum*. This study is significant because it has expanded knowledge of pollinators in Kakamega forest that will help in the design and implementation of conservation of plants and their pollinators in the Kakamega forest ecosystem. Emphasis on *Ocimum kilimandscharicum* has helped to create a link between these plant species and pollinators in an effort to improve pollination and the livelihood of the local community through increased crop yields. Consequently, this will drive concerted efforts to conserve the remaining biodiversity in the Kakamega forest. Currently Kakamega forest is undergoing deforestation and forest succession. The current pollination crisis is a major threat facing *Ocimum* genus all over the world [24]. The rapidly diminishing production of *Ocimum* that may be precipitated by anthropogenic activities, forest succession, and inadequate pollination possesses a danger to the livelihood of farmers and the activities of research institutions that depend on *Ocimum* for pharmaceutical purposes and as fertilizers especially around Kakamega forest. Information on *Ocimum kilimandscharicum* propagation and pollinators available is insufficient [24] while the future of *Ocimum kilimandscharicum* production appears grim in light of anthropogenic activities and forest succession [15].

2. Research Methods

2.1. Study Area. Kakamega forest is a tropical rainforest situated in Western Kenya. Its elevation is about 1500 to 1600 m above sea level with an estimated area of about 240km^2 [29]. Kakamega forest is facing major anthropogenic threats

because of overdependence on it by the local population [30] (Vuyiya et al. 2012). The population growth has increased from 406.4 persons per km^2 in 1999 to 521.6 persons per km^2 in 2009 [31].

Due to its rich biodiversity and other important ecosystem services Kakamega forest has been ranked as the most sensitive forest ecosystem in Kenya by the International Union of Conservation of Nature [29, 32, 33]. The flora and fauna diversity include 380 plant, 350 bird, 400 butterfly, and 4 primates species, respectively [15, 29, 34].

Kakamega forest receives an average rainfall of 1200 mm–1700 mm annually that is bimodally distributed. Heavy rainfall is experienced between April and May; it gets slightly dry in June and picks up with the short rains between August and September. It is usually dry between December and February [35]. It is located between latitudes 00° 08′ 30.5″ N and 00° 22′ 12.5″ N and longitude 34° 46′ 08.0″ E and 34° 57′ 26.5″ E. Temperature is fairly constant throughout the year, ranging between 20°C and 30°C (Steinbrecher, 2004).

2.2. Data Collection. The focus of the study was on flower visitors of *Ocimum kilimandscharicum*. The main mode of data collection was by observation and experimental where flower visitors were captured using a sweep net and control measures applied to identify the most efficient flower visitor. Two transects of 2.5 Km length were initially randomly established running along the forest edge and farmlands cutting through *Ocimum kilimandscharicum* sites (Winfree et al. 2007). *Ocimum kilimandscharicum* plots, 4mx4m with rich floral patches of either naturally growing or cultivated, were established along the mapped transects. Transect establishment was based on *Ocimum kilimandscharicum* availability and where there was least disturbance from livestock grazing and humans. The general surroundings of the quadrats were noted and considered for any possible external influences.

GPS coordinates of these plots were taken and labeled A (KEEP Shamba), B (Tom's Farm and the Pollinator Garden), C (Kisaina Primary School), D (Cecilia Point), E (Vero's Farm) to F (Morris Farm). Sampling sites were located on these transects as demonstrated in Figure 1. These selected sites were all sampled in similar order in 5 months of collection period for uniformity. Sampling was done for a week in each month and to adequately sample the flower visitors with different diurnal patterns sampling was done within three observation times per day: 7: 30-11:00 am for morning, 12 noon–1 pm, and 2–4 pm for afternoon.

Sites were categorized on the basis of distance from the forest edge. Sites A, 221m, D, 198 m, and E, 113 m, were the furthest while sites F, 50 m, B, 72 m, and C, 83 m, were the closest to the forest edge.

2.3. Determination of Diversity and Abundance of Flower Visitors. All foraging flower visitors in the six study sites along established transects were captured using a sweep net at 60 minutes' intervals in morning, midday, and afternoon sessions. Sweeping for flower visitors was done for one week each month and to achieve randomization, each day was randomly assigned to a particular site. The number of flower visitors collected during each sampling event at a particular site was considered an estimate of the species diversity and abundance [36]. Each collection was labeled with time of collection, date and month of collection, site where it was collected, and collector's name. Further analysis was done at the National Museum of Kenya (Figure 1) and the collection was identified to species level supplemented by studies done by Gikungu (2002 and 2011), Njoroge et al. (2004), and Karanja (2010).



Figure 1: Identified *Ocimum kilimandscharicum* sites.

2.4. Data Analysis. Generally, input of data was done using Microsoft word and management of data was done using Microsoft Excel. The analysis was performed using R 2.10.0 software.

Before input, data was analyzed at the National Museum of Kenya. Identification process of data collected for objective 1 and 2 was done and the data was classified under their family and species name. Shannon diversity profile was used to determine the diversity richness of the flower visitors and the abundance. Shannon diversity profile was preferred as it takes into account the measures of species diversity in a community based on species richness and species abundance meaning the number of individuals per species will be accounted for (Hughes et al. 1978). A habitat with diversity profile starting at a higher level than others would be considered richer. Profiles above others along their range from start to end would indicate a higher diversity or evenness of the habitat [37, 38]. Graphical presentation of data was carried out in the form of bar graphs.

3. Results

3.1. Diversity and Abundance of Ocimum kilimandscharicum Flower Visitors. The species accumulation curve (Figure 2) shows the rate at which new species were found in the study sites which provided an estimate of the species richness of the flower visitors. Sites labeled 1 to 6 corresponded to sites A to F, respectively. Figure 3 clearly demonstrates that there was a significant relationship between sampling effort and species richness. The higher the sampling effort the higher the

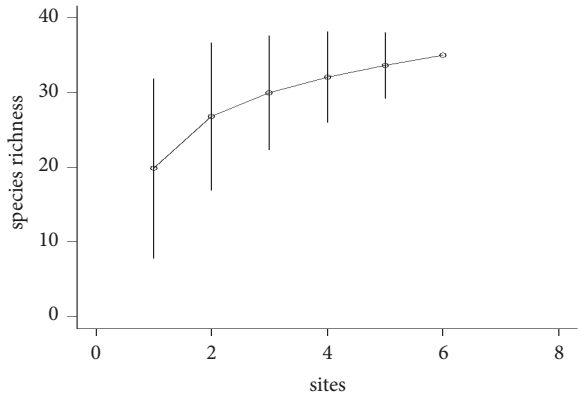


Figure 2: Species accumulation curve.

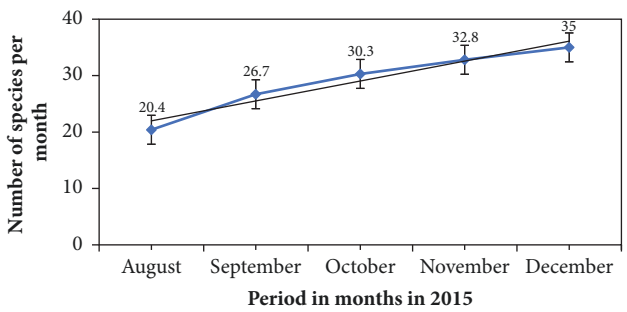


Figure 3: Monthly species richness curve (August to December 2015).

number of species captured. The last site 6 which corresponds to site F shows peak collection with all the species collected being represented from this site.

A total of 645 individual flower visitors belonging to four (4) families and thirty-five (35) species of bees were collected from all the study sites within a period of 5 months (Table 1).

The family Apidae had the highest number of species (17) and individuals (516) followed by Megachilidae with nine (9) species with 60 individuals, Halictidae with eight (8) species with sixty-eight (68) individuals, and a single species from the family Colletidae that had only, with one (1) individual.

A*pis mellifera* was the most abundant species in the family Apidae with 329 individuals followed by *Meliponula* sp. with 44 individuals and the least abundant was the *Flavoruf* a sp. (genus *Collette*, Fam. Colletidae) with one individual. In the family Halictidae the most abundant genus was *Lipotriches* with 18 individuals, followed by *Nomia* sp. with 16 individuals and the least abundant was the *Thrinchostoma*. and the *Pseudapis* spp. each with one (1) individual.

The most dominant species in the family Megachilidae was the species *Megachile* sp. *II* with 18 individuals, followed by *Heriadias* sp. with 11 individuals. The least abundant was the species *Ithanoplectra* sp. with one (1) individual.

3.2. Effect of Distance from the Forest Edge on Flower Visitor Diversity and Abundance. Table 2 provides information on effects of distance from the forest edge on the flower visitors' diversity and abundance. Site F, located 50m from the forest edge, had the highest species richness (n=30; H=2.38)) and

Table 1: Classes and species of flower visitor species collected during the study.

Family	Species	No. Individuals
Apidae	*Apis mellifera*	329
	Allodape sp.	6
	Amegilla sp. I	1
	Amegilla sp. II	6
	Amegilla sp. III	3
	Braunsapis sp.	9
	Ceratina sp. I	25
	Ceratina sp. II	7
	Meliponula sp.	44
	Thyreus sp.	2
	Xylocopacalens	38
	Xylocopa flavorufa	6
	Xylocopa incostans	11
	Xylocopa torridium	2
	Xylocopa (xylomellisosp. I)	8
	Xylocopa (xylomellisosp. II)	18
	Xylocopa sp. III	1
Megachilidae	*Coelioxys* sp	3
	Felinae sp.	6
	Heriadas sp.	11
	Ithanoptera sp.	1
	Megachile sp. II	18
	Megachile sp. III	6
	Megachile sp.VI	10
	Megachile sp. V	1
	Pseudoanthidium sp.	4
Halictidae (8 species)	*Halictinaesp VIII*	13
	Lipotriches sp.	18
	Nomia sp.	16
	Patellapis sp.	7
	Pseudapis sp.	1
	Seladonia sp.	10
	Systropha sp.	2
	Thrinchostoma sp.	1
Colletidae (1 species)	*Collete sp.*	1

was followed by site B (72m) and C (83m) from the forest edge with 23 (H=2.29) and 21 (H=2.05) species, respectively. The site A (221m) had the least number of species (n=11; H=1.44) and was the furthest study site from the forest edge.

The site with most abundant species was C, 83m from the forest edge (N=147; n=21; H=2.05), and least species abundant site associated with *Ocimum kilimandscharicum* flower visitors was A, 221m from the forest edge (N=64; n=11; H=1.44) which was the furthest from the forest edge.

Determination of the differences in the effects of distance from the forest edge on species diversity and abundance was tested using a two-sample t-test, after clustering the sites according to distance from the forest edge, with sites F, B, and C being closest to the forest and A and D and E being furthest from the forest edge, respectively. At a

Table 2: Distance of sites from forest edge, abundance, species richness, and diversity of bee flowers visitors from the selected study sites.

Site	Distance from the forest edge (m)	Total abundance (N)	Species richness (n)	Shannon-Weiner Diversity Index (H)
A	221	64	11	1.44
B	72	77	23	2.29
C	83	147	21	2.05
D	198	135	15	1.54
E	113	102	19	1.94
F	50	120	30	2.38

significant level of 0.05, distance had a significant effect on species abundance (t=4.177; p=0.0312) and species richness (t=3.2893; p=0.0187). One-way analysis of variance on the differences in the variation in flower visitor composition among the study sites differences yielded $F_{(3,20} = 14.47$, p= 0.024, indicating that, between sites A to site F, distance had a significant effect the flower visitors' composition.

Figure 3 shows the monthly species richness curve of the flower visitors by month from August to December 2015. Flower visitor species increased steadily from August to December then leveled off.

Figure 4 shows the species rank abundance curve of bee flower visitors associated with *Ocimum kilimandscharicum*. The species rank abundance conformed to lognormal distribution of Preston (1948).

3.3. Frequency Visits of Individual Flower Visitors by Family/Species in Selected Sites. Figure 5 shows the frequency of visits of flower visitors at the three selected study sites; red for Morris', yellow for Vero's, and blue for the Pollinator Garden.

Results clearly show that the rate at which all flower visitors foraged in the selected *Ocimum* plant differed. *Apis mellifera* was the most frequent visitor of flowers in all the three sites with a frequency of 30, resulting in the highest number of seed set produced from *Apis mellifera* in all the sites. This was followed by *Meliponula* sp. *Ceratina* sp. *1*, and *Megachilidae* with a frequency of 7 while the lowest frequency was recorded from *Allodape, Amegilla, Ceratina* sp. *II, and Heriadias* sp. with a frequency of 3 (Figure 5).

4. Discussion and Conclusions

This study has clearly demonstrated that *Ocimum kilimandscharicum* flower visitors play essential roles in pollination. Many of these pollinators are associated with the forest, which add to reasons as to why Kakamega forest needs to be conserved. In addition, the study has also shown that the number of visits by flower visitors has implications on the number of seeds realized by the plants. The study also demonstrated that prevailing temperature tends not to have significant impacts on the flower visitors' activity, possibly because these parameters do not show extreme fluctuations in Kakamega forest [30].

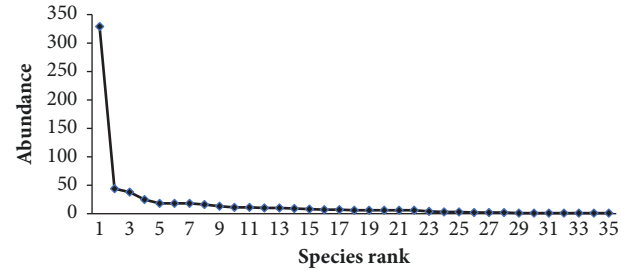


Figure 4: Species rank abundance of bee flower visitors on Ocimum kilimandscharicum.

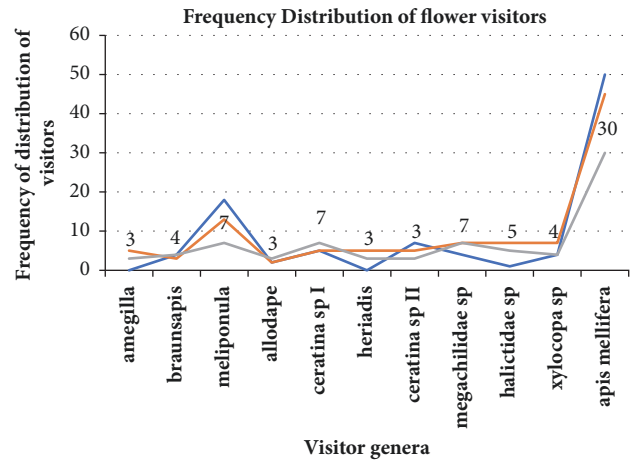


Figure 5: Frequency distribution of flower visitors along respective sites.

The study has clearly shown that the most common flower visitors are members of the bee families Apidae, Megachilidae, Halictidae, and Colletidae. Apidae was the dominant bee family throughout the study period and at all the study sites. These finding are in line with the findings of Chiawo (2011) whereby he found that the family Apidae dominated most of the composition of flower visitors in fallow farmlands [26]. In a study by Potts et al. [14] on pollinator decline, Apidae was also the most dominant family and consequently experienced the highest decline in abundance from disturbances.

Of the six study sites, site F had the highest abundance and diversity of *Ocimum kilimandscharicum* flowers visitors followed by site B and C, respectively. This may be explained on the basis of their close proximity to the forest edge. Site F demonstrated the foraging range of forest flower visitors and their ability to forage on plants on the forest edge. These findings are in agreement with those of Potts et al. [14] in Mt Carmel in which it was reported that the most dominant pollinator species were collected in grass and shrub like areas near the forest. Steffan-Dewenter and Westphal [12] demonstrated that habitats undergoing ecological successions were more effective in enhancing pollinator diversity and abundance. These findings support the importance and value of Kakamega forest and identifies the forest as a key sink of pollinator communities, significantly, bee species.

Overall abundance was high in site C. The site was located in an open farmland where there were various farming activities that enhanced the growth of different kinds of plants. These findings support studies by (Gikungu et al. (2011)) and Steffan-Dewenter and Westphal [12] where it was shown that bee abundance was high in open farmlands. The rank abundance curve conformed to Lognormal of Preston (1948) whereby he argued that species abundance follows a normal distribution and so the abundance distribution is lognormal. This indicated that an increase in the number of sites visited increased the probability of collecting different flower visitor species and so this can support the recommendation of carrying out this study with a longer study period.

Distance had significant effects on the composition of flower visitors, with sites closest to the forest being more diverse and with higher abundance than those sites located far. These findings conform to those of Zurbuchen (2010), who reported that whenever distance between nesting stands and experimental sites was increased, the duration of foraging bouts consistently increased and bee species composition deferred too.

Graph of species richness against sampling duration (months) indicates that increase in sampling duration increases the likelihood of collecting rare species and hence causes an increase in their abundance and the normal distribution becomes more visible. The pattern indicates that, as more time was spent in this study, more flower visitor species were captured and identified. The abundance also increased and so the normal distribution became more evident as sample duration increased.

In conclusion, fragmentation and habitat alteration and disturbances in Kakamega forest will have irreversible effect on the fauna including a wide range of bee flower visitors that play a major role in pollination and are attracted by the *Ocimum kilimandscharicum* plants. These flower visitors as shown in this study are diverse and are dependent on the forest as they forage along the forest for resources. Consequently, by planting *Ocimum* which attracts many diversified pollinators, local communities can benefit from their pollination services to increase their crop yields. While the findings of this study may be inconclusive, because of its short duration, the information obtained forms a foundation and offer baseline data for future studies on pollinators of the *Ocimum* family.

The diversity and abundance of pollinators observed on *Ocimum kilimandscharicum* flowers clearly show that this plant species can be used to attract pollinators in the larger farmlands that carry out commercial farming. There is need for studies to be carried out to identify the significance of *Ocimum kilimandscharicum* flower visitors in the crop yields of the surrounding communities. This study also recommends that more detailed studies on effects of precipitation, temperature, and other weather parameters on *Ocimum kilimandscharicum* flower visitors with longer study period in Kakamega forest be thoroughly investigated in view of the anticipated climate change. Further recommendations are made for those who domesticate bee for honey production; they can plant *Ocimum kilimandscharicum* as the study has shown that it is a favorite forage plant among bees including the stingless bees.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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