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Foraging preferences of honey bees *Apis cerana* in Dakshina Kannada, Karnataka, India

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Abstract: Honey bees visit flowers for collecting nectar and pollen. Pollen serves as a source of protein for the survival of honeybees. To understand the dependence and preference of the honey bee species *Apis cerana* on different pollen resources, we carried out a study to analyze the distribution of different types of pollen in honey in various regions of the coastal plains of the Western Ghats. Fourteen different honey samples from different sites ranging in elevation from 55m to 135m were collected and analyzed. Acetolysis and centrifugation were used for pollen extraction from different honey samples. The extracted pollen was mixed with glycerin jelly and transferred to a glass slide for microscopic analysis. The primary source of pollen as revealed by the current study were found to be *Areca catechu, Cocos nucifera, Ixora coccinea, Mimosa pudica*, and *Psidium guajava*. Morphotype analysis revealed 12 different plants to be the source of the pollen. Each honey sample collected from different locations, however, had only three to six types of pollen indicating that honeybees visit a narrow or a small number of pollen sources. Therefore, based on our study, we conclude that lesser types of pollen in each honey sample indicates that their food resources are getting limited. Therefore, it is necessary to conserve pollen resources for the better survival of honey bees and the environment.

Keywords: *Areca catechu, Cocos nucifera,* coastal plains, *Ixora coccinea,* melissopalynological analysis, *Mimosa pudica*, nectar, pollen, *Psidium guajava*, Western Ghats.

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Author Contribution: AGSK has done all the experiments, AGSK & RKP have written the manuscript.

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INTRODUCTION

Among all insects that pollinate, honeybees are considered to be one of the crucial pollinators (Ballantyne et al. 2017). Animal pollination directly affects about three-quarters of essential crop types, including most fruits, seeds, and nuts (Smith et al. 2015). Honeybees collect different types of pollen and they are added or mixed with honey intentionally or accidentally. Melissopalynological analysis, or studies on pollen, are helpful in determining the geographical and botanical origin of honey (Louveaux et al. 1978; von der Ohe et al. 2004). These studies also evaluate the plant source required for honey production and such knowledge about the pollen resource helps improve beekeeping practices. A few melissopalynological studies were carried out in India, and our study aims to present the foraging preferences of honeybees in the coastal plains of the Western Ghats.

Vegetation of Dakshina Kannada that lies along the Western Ghats expresses itself with thousands of species and several hundred genera of plants. It harbours several endemic and rare plants; this range of diversity is because of the physiological and geographical positioning of Dakshina Kannada along the coastline, exposed to elevations ranging from sea level to about 4,500ft. It consists of the Western Ghats in the east and the seashore in the west. The soil is mostly lateritic and the topography of the district is plain up to 30km inside the coast and changes to undulating hilly terrain sharply towards the east in the Western Ghats (Dakshina Kannada District Profile 2015). Trees such as Bamboo, Rosewood *Dalbergia latifolia*, and Teak *Tectona grandis* are found in the hilly areas towards the east. In rural Dakshina Kannada, houses are situated between farmlands or plantations of Arecanut *Areca catechu* and Coconut *Cocos nucifera* (Anonymous 2015). The earliest research on the pollen present in honey was done by Pfister (1895) by carrying out an analysis of French, Swiss, and European honey. Deodikar et al. (1958) initiated palynological studies in Maharashtra in India. Later, others from different parts of India such as Andhra Pradesh (Ramanujam & Kalpana 1991; Lakshmi & Suryanarayana 2004; Ramakrishna & Swathi 2013; Devender & Ramakrishna 2015), Bihar (Suryanarayana et al. 1992), Himachal Pradesh (Sharma 1970; Sharma & Raj 1985), West Bengal (Bhattacharya et al. 1983; Chakraborti & Bhattacharya 2011; Kamble et al. 2015), Uttarakhand (Garg & Nair 1974), Karnataka (Chauhan & Murthy 2010; Shubharani et al. 2012; Raghunandan & Basavarajappa 2013), Orissa (Upadyay & Bera 2008, 2012, 2014), and Madhya Pradesh (Chauhan & Quamar 2010; Sahney & Seth 2013) conducted studies on these aspects.

MATERIALS AND METHODS

From February 2016 to April 2016, 14 honey samples were collected from 14 different locations of Dakshina Kannada District, which covers from 12.52^{0}N & 74.53^{0}E to 12.87^{0}N & 74.88^{0}E in the state of Karnataka, India, and has an area of 4,559km^2 (Dakshina Kannada District Profile 2015; Fig. 1). Beekeepers of different areas of Dakshina Kannada were approached and samples were collected from them with the intention of understanding the pollen preferences of honey bees in the district. The geographical position of the honey samples collected was also noted down and all 14 honey samples from different sites ranging in elevation of 55–135 m were sampled (Table 2).

The samples were stored in containers. Honey samples were collected from several apiaries of domesticated *Apis cerana indica* colonies. All honey samples were unprocessed and raw during the time of collection and they were extracted using honey extracting machines from honeycombs by beekeepers. The detailed information on the different honey samples collected is included in Table 1.

To do pollen analysis, 5g of honey sample was dissolved in 5ml of distilled water and centrifuged at 2,500rpm for 10min. After centrifugation, the sediment obtained was treated with 5ml glacial acetic acid. Later, the glacial acetic acid was removed and the material was subjected to acetolysis as per Erdtman (1952). This solution was again centrifuged and the supernatant solution was discarded; the sediment containing pollen was taken and added with glycerin jelly and was transferred to a glass slide. The glass slide was slightly heated to melt the jelly and cover slip was applied. To secure the coverslip, colourless nail polish was applied to its edges (Song et al. 2012; Ponnuchemy et al. 2014). To analyze the pollen present in the honey samples qualitatively and quantitatively, three pollen slides were prepared for each of the samples. Pollen types were identified by comparison with reference slides, which were prepared by collecting pollen directly from the plants in the study area. In addition, palynological literature were also used. For quantification of pollen types, 300 pollen grains were counted from each sample. The percentage frequency of the pollen taxa found in all the samples was calculated. The types of pollen were

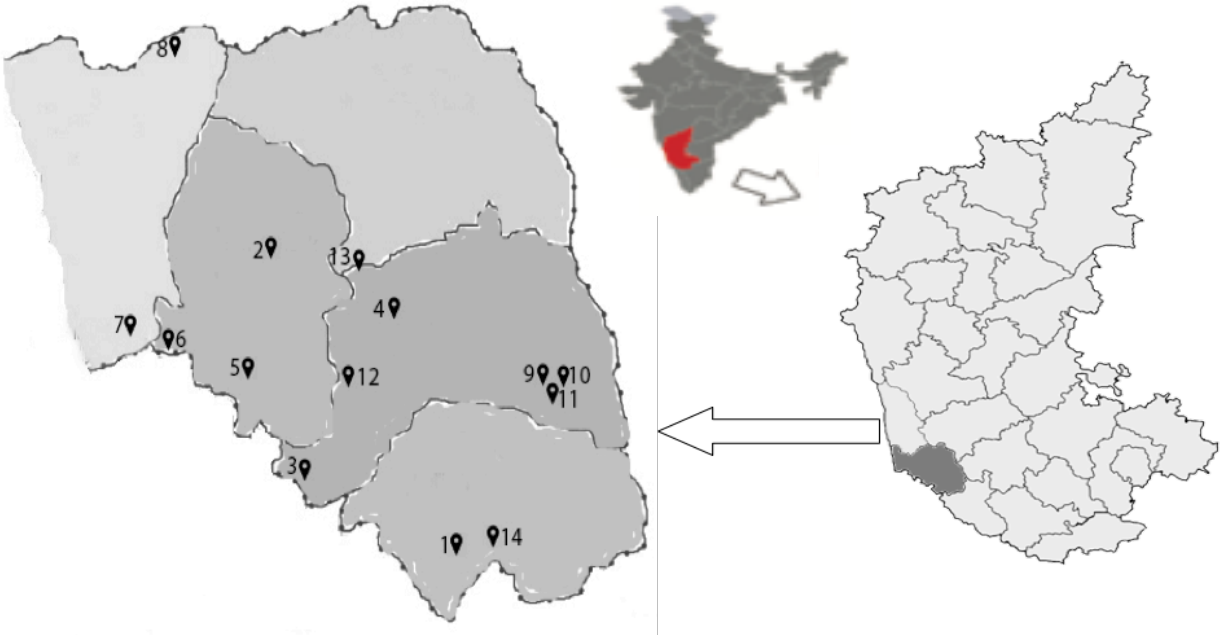

Figure 1. Dakshina Kannada District in Karnataka State, India, showing 14 sites of honey sample collection: 1 - Irde, 2 - Kelinja, 3 - Bettampady, 4 - Parladka, 5 - Mithanadka, 6 - Mudipu, 7 - Derlakatte, 8 - Belvai, 9 - Kadaba, 10 - Deppuni, 11 - Kundadka, 12 - Punacha, 13 - Uppinangady, 14 - Mitoor.

allocated to one of four frequency classes: i) predominant pollen type (>45%), ii) secondary pollen type (16–45%), iii) important minor pollen types (3–15 %), and iv) minor pollen types (<3%) (Louveaux et al. 1978; Song et al. 2012). Absolute pollen count in 1g of honey sample was also made. Pollen grains were observed using light microscopy; scanning electron microscopy was also used to observe predominant pollen types.

RESULTS

From the 14 honey samples, 12 pollen morphotypes were identified (Image 1). Eleven samples appeared to be unifloral with predominant pollen and three to be multi-floral. In the samples Irde (DK-Ir-01), Mithanadka (DK-Mi-05), and Mitoor (DK-Mt-14), *Ixora coccinea* was the predominant pollen with pollen ranging from 46% to 60%. In the samples Bettampady (DK-Be-03) and Kadaba (DK-Ka-09), *Cocos nucifera* was the predominant pollen ranging from 50% to 60%. In samples Mudipu (DK-Mu-06), Belvai (DK-Bl-08), Kundadka (DK-Ku-11), it was found that *Psidium guajava* pollen was predominant, ranging from 60% to 68%. In sample Deppuni (DK-Dp-10), *Areca catechu* was predominant with 48% pollen and in samples Derlakatte (DK-De-07) and Uppinangady (DK-Up-13), *Mimosa pudica* was predominant, ranging from 50% to 68% (Table 1). Some of the pollen such as *M. pudica*, *P. guajava*, *A. catechu*, *C. nucifera*, and *I. coccinea* were predominant (Image 2) and can be considered as important source of forage for honey bees in Dakshina Kannada.

Pollen of some species such as *Areca catechu* in samples DK-Ka-09 and DK-Be-03, *Cocos nucifera* in samples DK-Ir-01, DK-Pa-04, DK-Mi-05, DK-Mu-06, DK-Bl-08, DK-Dp-10, DK-Pu-12, DK-Up-13, and DK-Mt-14, *Ixora coccinae* in sample Dk-Pa-04, *Mimosa pudica* in samples DK-Ke-02 and DK-Ku-11, *Moringa olifera* in samples DK-Ir-01 and DK-Pa-04, *Psidium guajava* in samples DK-Pu-12 and DK-Ke-02, and *Syzigium* in sample DK-De-07 were found to be secondary pollen, whereas some others appeared to be minor pollen in specific samples, the details of which are given in Table 1. Geographical position of the honey samples collected from different sites is given in Table 2. Absolute pollen count was measured per gram of honey samples; minimum number of absolute pollen count was found in sample DK-De-07 with 1,740 pollen grains and maximum in sample DK-Mi-05 with 27,300 pollen grains. Percentage of pollen belonging to different plant species present in each of the honey samples were calculated. *Cocos nucifera* found in all honey samples was 30.142%, which was the maximum. The minimum percentage of pollen source was found to be *Acacia* sp., *Mangifera indica*, and *Eucalyptus* sp. (Table 3; Fig. 2). Twelve plant species identified as pollen sources belonged to

Table 1. Frequency class of pollen and data of pollen analysis performed with the honey samples collected from different locations in Dakshina Kannada District, Karnataka, India.

	Honey sample	Type of pollen			
		Predominant pollen (>45%)	**Secondary pollen (16–45 %)**	**Important minor pollen (3–15 %)**	**Minor pollen (<3%)**
1.	DK-Ir-01	*Ixora coccinea* (46%)	*Cocos nucifera* (30%), *Moringa olifera* (20%)	*Areca catechu* (3%)	*Mimosa pudica*
2.	DK-Ke-02	Nil	*Mimosa pudica* (42%), *Psidium guajava* (41%)	*Cocos nucifera* (15%)	Asteraceae
3.	DK-Be-03	*Cocos nucifera* (50%)	*Areca catechu* (45%)	*Psidiumguajava* (5%)	Nil
4.	DK-Pa-04	Nil	*Moringa olifera* (40%), *Ixora coccinea* (31%), *Cocos nucifera* (21%)	*Mimosa pudica* (7%)	*Mangifera indica*
5.	DK-Mi-05	*Ixora coccinea* (60%)	*Cocos nucifera* (30%)	*Mimosa pudica* (10%)	Nil
6.	DK-Mu-06	*Psidium guajava* (60%)	*Cocos nucifera* (26%)	*Areca catechu* (12%)	*Acacia* sp., *Eucalyptus sp.*
7.	DK-De-07	*Mimosa pudica* (50%)	*Syzygium* sp. (35%)	*Cocos nucifera* (15%)	Nil
8.	DK-Bl-08	*Psidium guajava* (68%)	*Cocos nucifera* (32%)	Nil	Nil
9.	DK-Ka-09	*Cocos nucifera* (60%)	*Areca catechu* (30%)	*Mimosa pudica* (9%)	Asteraceae
10.	DK-Dp-10	*Areca catechu* (48%)	*Cocos nucifera* (35%)	*Mimosa pudica* (15%)	*Psidium guajava*
11.	DK-Ku-11	*Psidium guajava* (60%)	*Mimosa pudica* (23%)	*Cocos nucifera* (7%), *Syzygium* sp. (6%)	*Areca catechu*, *Moringa olifera*
12.	DK-Pu-12	Nil	*Psidium guajava* (41%), *Cocos nucifera* (35%)	*Areca catechu* (10%), *Mimosa pudica* (10%)	Arecaceae, *Syzygium* sp.
13.	DK-Up-13	*Mimosa pudica* (68%)	*Cocos nucifera* (26%)	*Psidium guajava* (4%)	Arecaceae, *Moringa olifera*
14.	DK-Mt-14	*Ixora coccinea* (48%)	*Cocos nucifera* (40%)	*Moringa olifera* (10%)	*Mimosa pudica*

Irde (DK-Ir-01), Kelinja (DK-Ke-02), Bettampady (DK-Be-03), Parladka (DK-Pa-04), Mithanadka (DK-Mi-05), Mudipu (DK-Mu-06), Derlakatte (DK-De-07), Belvai (DK-Bl-08), Kadaba (DK-Ka-09), Deppuni (DK-Dp-10), Kundadka (DK-Ku-11), Punacha (DK-Pu-12), Uppinangady (DK-Up-13), Mitoor (DK-Mt-14).

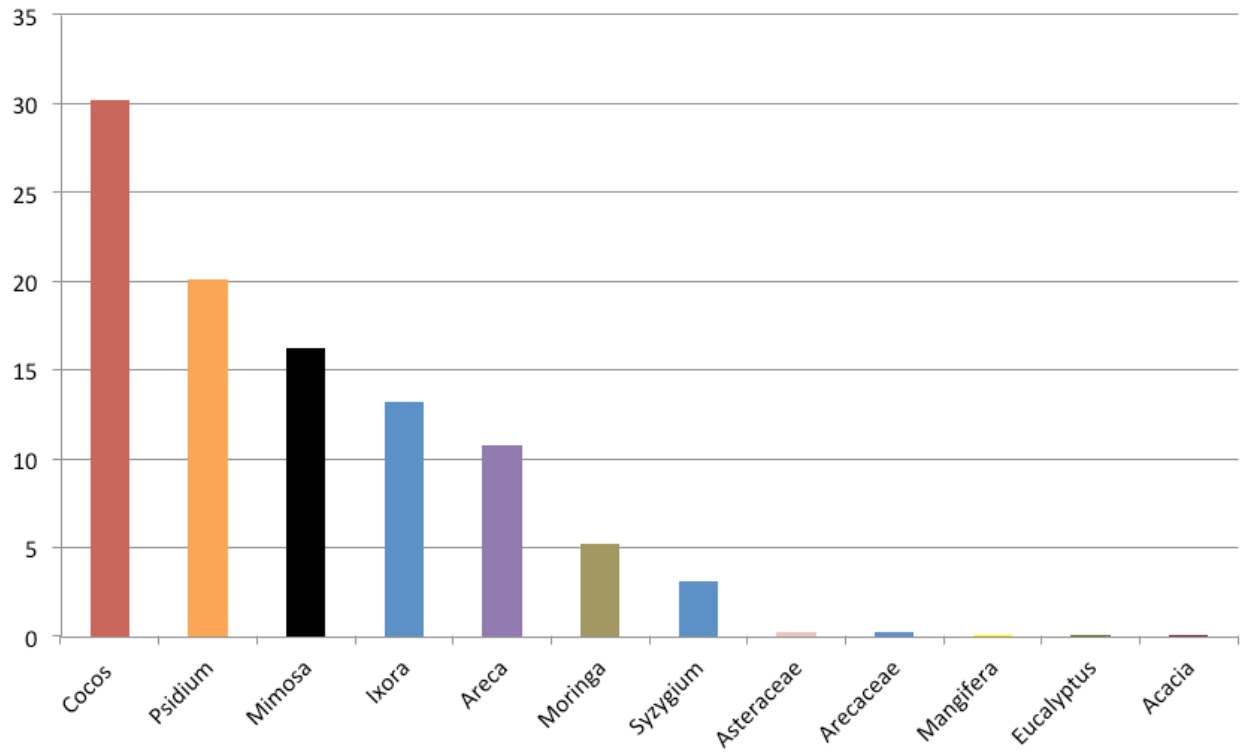

Figure 2. Total percentage of pollen sources found in all 14 sites of Dakshina Kannada District, Karnataka, India.

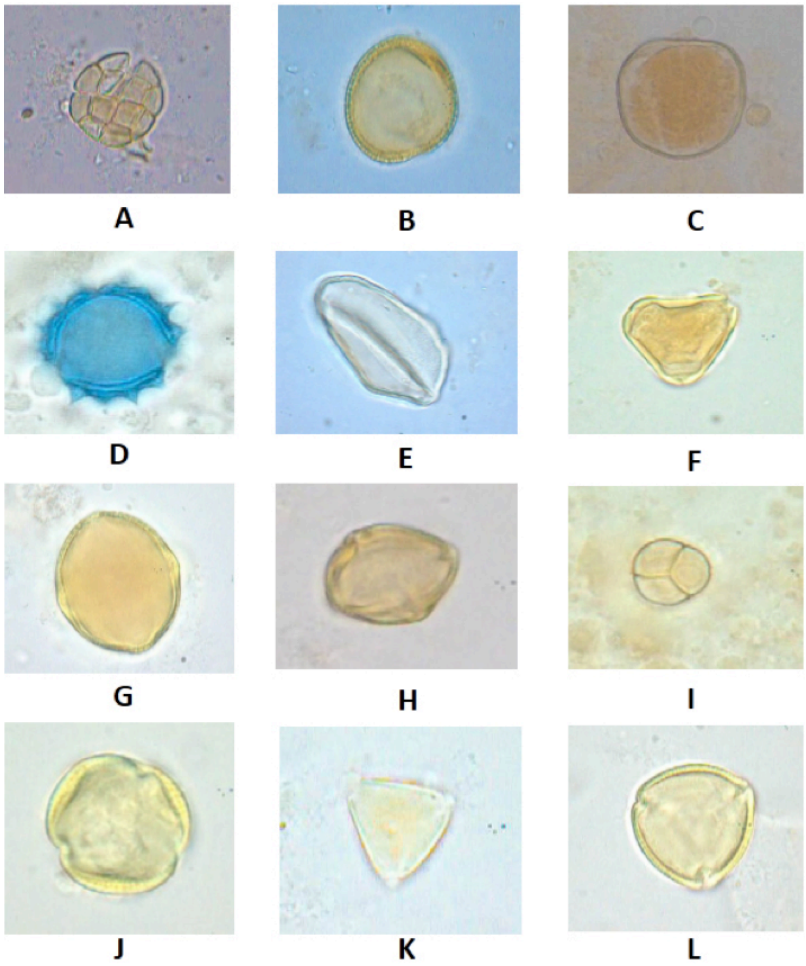

Image 1. Microscopic images of pollen grains found in honey samples: a - *Acacia* | b - *Areca catechu* | c - Arecaceae | d - Asteraceae | e - *Cocos nucifera* | f - *Eucalyptus* sp. | g - *Ixora coccinea* | h - *Mangifera indica* | i - *Mimosa pudica* | j - *Moringa olifera* | k - *Psidium guajava* | l - *Syzygium* sp.

seven families. Three species belonged to Arecaceae, three to Myrtaceae, and two to Fabaceae; Rubiaceae, Anacardiaceae, Asteraceae, and Moringaceae had one species each (Table 4). Pollen count of each of the pollen sources found in all the samples was also made (Table 5). *Cocos nucifera* was found in honey samples collected from all 14 sites and therefore can be considered as one of the most important pollen sources in Dakshina Kannada.

DISCUSSION

The protein that pollen provides is essential for brood production and development of young honey bees. Pollen is also the primary source of protein to honey bees. Stored pollen grains along with glandular secretions containing enzymes and acids is called bee bread. Larval honey bees feed on bee bread and brood food mixture. Enzymes and acids are added to the pollen by the bees so that it can be preserved for long term (Ellis et al. 2010). In order to complete the development of the body, newly emerged bees also feed on bee bread. The requirement of bee bread by a single worker bee larva is estimated to be about 124–145 mg; this consists of about 30mg of protein. A diet of high protein pollen increases the longevity of worker bees and also improves brood rearing; brood rearing gets reduced when supported by pollen grains low in protein. It is observed that honey bees choose pollen based on the physical configuration and odour of the pollen rather than based on their nutritive value. On average, 15–30 % of the colony's forager bees collect pollen, and a single bee can carry and bring back pollen load that weighs about 35% of the bee's body weight. Bees carry pollen on their hind legs, on specialized structures commonly

Table 2. Geographical position of samples collected and absolute pollen count of the honey samples from Dakshina Kannada District, Karnataka, India.

	Honey sample	Geographical position	Elevation (m)	Absolute pollen count (APC)
1.	DK-Ir-01	12.672°N, 75.200°E	84.5	1780
2.	DK-Ke-02	12.802°N, 75.087°E	84.6	2520
3.	DK-Be-03	12.662°N, 75.199°E	112.2	16260
4.	DK-Pa-04	12.749°N, 75.204°E	110.6	4800
5.	DK-Mi-05	12.732°N, 75.022°E	91.1	27300
6.	DK-Mu-06	12.795°N, 74.970°E	122.8	3660
7.	DK-De-07	12.809°N, 74.883°E	55.5	1740
8.	DK-Bl-08	13.125°N, 74.997°E	126.2	2640
9.	DK-Ka-09	12.731°N, 75.471°E	107.2	1900
10.	DK-Dp-10	12.722°N, 75.499°E	130.6	2960
11.	DK-Ku-11	12.711°N, 75.470°E	104.0	3720
12.	DK-Pu-12	12.723°N, 75.148°E	103.7	1920
13.	DK-Up-13	12.838°N, 75.255°E	40.8	15840
14.	DK-Mt-14	12.556°N, 75.428°E	135.3	1790

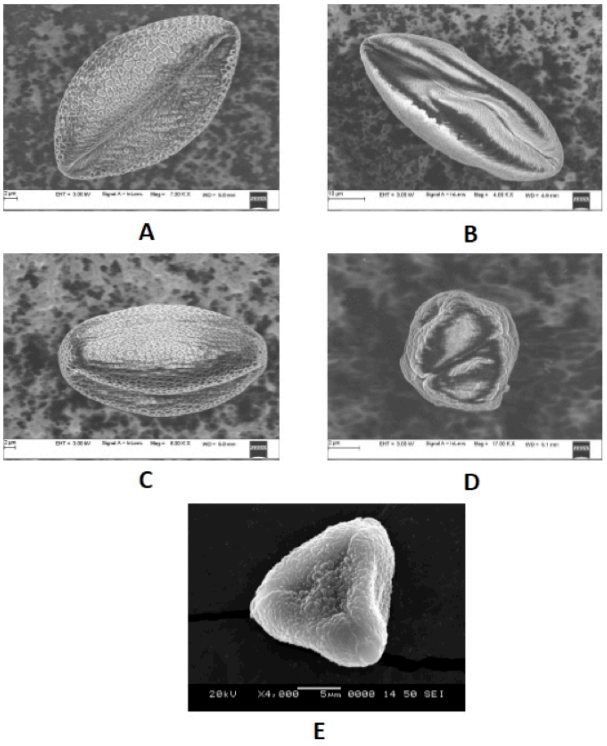

Image 2. Scanning electron microscope images of predominant pollen grains observed: a - *Areca catechu* | b - *Cocos nucifera* | c - *Ixora coccinea* | d - *Mimosa pudica* | e - *Psidium guajava*.

called pollen baskets, also known as corbicula (Ellis et al. 2010).

It is assumed that pollen found in honey sediment falls into the nectar while in the flower, which is then transported in the bee's honey stomach to the hive. This pollen will be present during the ripening process, and also appear finally in ripe honey (Maurizio 1951).

Our study reveals the variation in the amount and type of pollen present in the samples. Several uncontrollable factors influence the presence of pollen types in honey samples; some of them are the availability of pollen, unbalance in the abundance of flowers among plant species, and the honey bee's collection preferences. In fact, there is a necessity of more studies addressing these questions more specifically with controlled experiments (Oliveira et al. 2010). The presence of pollen types in honey samples indicates the botanical origin of the honey to that region. Battesti & Goevry (1992) verified that climatic conditions influence the metalliferous and polleniferous potential of the local flora. Pollen richness in the honey sample depends on the pollen production by the parent plant, climatic conditions, the distance from the beehive to the flower field, filtering of pollen by the bees' proventriculus, the diameter of the pollen grain, and method of extraction of honey (von der Ohe 1994). Significant variation in pollen content is caused due to some factors like i) the amount of pollen filtered out in the bee's honey sac (Maurizio 1975) and ii) the bee taking only pollen without the nectar (Anklam 1998). Chaturvadi (1983) verified that the pollen spectra of honey are also influenced by the crops which are cultivated in the localities. The pollen types and percentage of pollen in honey does not represent genuinely the same proportion of quantity of nectar gathered from these plants because some pollen types are over-represented and some underrepresented; however, from the pollen spectra, it is apparent that honey bees eagerly visit the honey sources for nectar and pollen to form honey (Maurizio 1975). When the bees collect and transfer nectar to their honey stomach, the amount of pollen in the nectar get reduced. The honey stomach is connected to the ventriculus by the proventriculus; the valves of the proventriculus remain closed during foraging and opens only when the bee feeds for itself. This valve in its closed position can abstract small solid particles from the full honey stomach and pass them into the ventriculus. While doing so, the volume of the fluid does not get reduced (Whitcomb &

Table 3. Plant species and the percentage of pollen found in honey samples obtained from different sites in Dakshina Kannada District, Karnataka, India.

	Plant species	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12	Site 13	Site 14	Total %
1.	*Acacia* sp.	-	-	-	-	-	1%	-	-	-	-	-	-	-	-	0.071
2.	*Areca catechu*	3%	-	45%	-	-	12%	-	-	30%	48%	2%	10%	-	-	10.714
3.	Arecaceae	-	-	-	-	-	-	-	-	-	-	-	2%	1%	-	0.214
4.	Asteraceae	-	2%	-	-	-	-	-	-	1%	-	-	-	-	-	0.214
5.	*Cocos nucifera*	30%	15%	50%	21%	30%	26%	15%	32%	60%	35%	7%	35%	26%	40%	30.142
6.	*Eucalyptus* sp.	-	-	-	-	-	1%	-	-	-	-	-	-	-	-	0.071
7.	*Ixora coccinea*	46%	-	-	31%	60%	-	-	-	-	-	-	-	-	48%	13.214
8.	*Mangifera indica*	-	-	-	1%	-	-	-	-	-	-	-	-	-	-	0.071
9.	*Mimosa pudica*	1%	42%	-	7%	-	-	50%	-	9%	15%	23%	10%	68%	2%	16.214
10.	*Moringa olifera*	20%	-	-	40%	-	-	-	-	-	-	2%	-	1%	10%	5.214
11.	*Psidium guajava*	-	41%	5%	-	-	60%	-	68%	-	2%	60%	41%	4%	-	20.071
12.	*Syzygium* sp.	-	-	-	-	-	-	35%	-	-	-	6%	2%	-	-	3.071

Table 4. Plant species as pollen sources, their families, habit, and number of sites in which they are found in Dakshina Kannada District, Karnataka, India.

	Plant species	Family	Habit	Number of sites
1	*Acacia*	Fabaceae	Tree	1
2	*Areca catechu*	Arecaceae	Tree	7
3	Arecaceae	Arecaceae	Tree	2
4	Asteraceae	Asteraceae	Creeper	2
5	*Cocos nucifera*	Arecaceae	Tree	14
6	*Eucalyptus* sp.	Myrtaceae	Tree	1
7	*Ixora coccinea*	Rubiaceae	Shrub	4
8	*Mangifera indica*	Anacardiaceae	Tree	1
9	*Mimosa pudica*	Fabaceae	Herb	11
10	*Moringa olifera*	Moringaceae	Tree	5
11	*Psidium guajava*	Myrtaceae	Tree	8
12	*Syzygium* sp.	Myrtaceae	Tree	3

Wilson 1929; Todd & Vansell 1942). For example, if the bees feed on syrup containing pollen, the pollen content of the honey stomach is reduced to one-half or one-third in the first 15–30 min after feeding. Therefore, it must be reckoned that the amount of pollen in the honey is dependent on the time taken by the bee to return from the source of forage to the hive. So a question arises whether a selection process of pollen grains according to their size takes place during the transfer of pollen through the proventriculus, which would alter the relative proportions of different pollen grains (Whitcomb & Wilson 1929; Todd & Vansell 1942). An experiment conducted by Maurizio (1949), however, verified that neither the size of the pollen nor the concentration of the syrup nor the original pollen content of the syrup significantly affected the rate of pollen disappearing from the honey stomach. Therefore, the amount of pollen is indeed reduced but no selection process is known that would alter the proportions of different types of pollen present in the syrup.

From the above observations, it is clear that all these species contribute as pollen sources for honey bees and some of the predominant pollen sources are very important for the endurance and survival of honey bee colonies and also for the development of apiculture industry in the study area. For the survival of honey bee colonies and the improvement of apiculture, knowledge of honey plants and pollen sources is of utmost importance. The study of honey bees that are excellent pollinators also contribute to the increased pollination and production of food crops, economically important plants, and medicinal plants, which in turn improve the economy of forest and rural areas. In horticulture, bee pollination leads to higher number of fruits, berries, or seeds. It also gives better quality products. Some crops have flowers that are pollinated during a short period. If such crops are not pollinated during the limited time interval, flowers will fall and no seeds, fruits, or berries will develop. There should be a sufficient number of bees in order to pollinate crops. Based on all the above observations, conservation of honey plants are crucial and much necessary. Depletion of honey plants will directly affect the number of honey bee colonies, causing their respective decline, which

Table 5. Pollen sources and their pollen count in different sites in Dakshina Kannada District, Karnataka, India.

Site	*Acacia*	*Areca*	Arecacea	Asteracea	*Cocos*	*Eucalyptus*	*Ixora*	*Mangifera*	*Mimosa*	*Moringa*	*Psidium*	*Syzygium*
1	-	53	-	-	534	-	818	-	17	356	-	-
2	-	-	-	50	378	-	-	-	1,058	-	1,033	-
3	-	7,317	-	-	8,130	-	-	-	-	-	813	-
4	-	-	-	-	1,008	-	1,488	48	336	1,920	-	-
5	-	-	-	-	8,190	-	16,380	-	2,730	-	-	-
6	36	432	-	-	936	36	-	-	-	-	2160	-
7	-	-	-	-	261	-	-	-	870	-	-	609
8	-	-	-	-	844	-	-	-	-	-	1795	-
9	-	570	-	-	1,140	-	19	-	171	-	-	-
10	-	1,420	-	-	1,036	-	-	-	444	-	59	-
11	-	74	-	-	260	-	-	-	855	74	2,232	223
12	-	192	38	-	672	-	-	-	192	-	787	38
13	-	-	158	-	4118	-	-	-	10771	158	633	-
14	-	716	859	35	-	-	179	-	-	-	-	-

1 - Irde, 2 - Kelinja, 3 - Bettampady, 4 - Parladka, 5 - Mithanadka, 6 - Mudipu, 7 - Derlakatte, 8 - Belvai, 9 - Kadaba, 10 - Deppuni, 11 - Kundadka, 12 - Punacha, 13 - Uppinangady, 14 - Mitoor.

will lead to the downfall of agriculture and economy of the area. Artificial pollination is unimaginable and is practically very difficult. Therefore, honey plants which are essential pollen sources must be protected and conserved well.

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