



EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
ENTOMOLOGY

A



ISSN
1687-8809

WWW.EAJBS.EG.NET

Vol. 15 No. 4 (2022)



Seasonal Diversity and Species Richness of Pollen Through Body Surface Pollen Analysis of Honey Bees in Two Regions in Upper Egypt

Salah H. Rateb¹, Mahmoud M.H. Kelany² and Eslam M. Omar³

1-Plant Protection Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.

2-Department of Plant Protection, Desert Research Center, Cairo, Egypt.

3-Plant Protection Department, Faculty of Agriculture, Assiut University, Assiut, Egypt

*E-mail: salah.hashem@agr.aun.edu.eg - bahaa800@gmail.com - eslamomar@aun.edu.eg

ARTICLE INFO

Article History

Received:13/11/2022

Accepted:20/12/2022

Available:23/12/2022

Keywords:

Apis mellifera,
foraging bee,
nectariferous,
polleniferous,
pollen diversity.

ABSTRACT

The present study was carried out in two regions of Upper Egypt, (Assiut and Sohag governorates) to verify qualitative and quantitative pollen types on the bodies of honey bee foragers through body surface pollen analysis. It was conducted by taking weekly samples of incoming nectar foragers, collected directly from apiaries, during the blooming season (from March to September). The blooming season was divided into three periods as follows: first period (early spring: March and April), the second period (late spring: May and June), and third period (summer: July to September). The results showed that, in the Assiut area, there were twenty-one plant species belonging to sixteen botanical families, while in the Sohag area, there were 14 plant species belonging to 9 plant families during the blooming season. In the first period, *Foeniculum vulgare* and *Eucalyptus globules* in the Assiut area, and *Brassica kaber* and *Eucalyptus globules* were classified as secondary pollen types and labeled as nectariferous plants, while *Phoenix dactylifera* was labeled as a polleniferous plant in both areas. Thus, the honey yield would be bifloral honey in both regions in this period. While during the second period, the dominant pollen type in both regions belonged to the Egyptian clover (*Trifolium alexandrinum* L.), and the other pollen was labeled as a minor pollen type. Therefore, the type of honey would be monofloral honey (clover honey). In the third period, eucalyptus pollen was the dominant pollen, followed by Alfalfa's pollen as the secondary pollen type in the Assiut area. So, the honey yield would be dominated by eucalyptus nectar. On the contrary, alfalfa's pollen (*Medicago sativa*) was the dominant pollen, and the other pollen was classified as a minor pollen type in the Sohag area. Hence, the honey yield would be dominated by alfalfa nectar. Knowledge about the polleniferous and nectariferous plant sources is essential for bee conservation. as well, as contributing toward the prediction of the expected honey yield type. Consequently, more studies in many areas of Upper Egypt are necessitated.

INTRODUCTION

Plants provide pollen and nectar to honey bees. Honeybees serve as pollinators of agricultural plants which is more valuable than other bee products. A battalion of plants is entomophilous. So, the bees and plants have a co-evolved and interdependent relationship

(Suryanarayan, 1986; Velthius, 1992 and Hargasim, 1974). Honeybees require proteins, carbohydrates, vitamins, minerals, water, and fat for their existence and for forming the body tissues of the bees, particularly through the initial embryonic development as well as the building of a colony (Loper and Berdel, 1980). All these foods come from nectar and pollen. The lack of pollen can disturb the suaviseness of the colony and honey yield (McLellan, 1974; Duff & Furgala, 1986; Nelson, 1987). Therefore, the knowledge of the pollen flora of a region is a fundamental tool for the enhancement and buildup of the beekeeping industry. Also, the information on the pollen flow times could be useful to decide when pollen substitutes should be introduced to colonies. The bee flora can be classified into 3 groups as follows: (i) polleniferous (visited by bees for pollen alone); (ii) nectariferous (visited for nectar alone) (iii) pollen-and-nectariferous (visited for both pollen and nectar).

Pollen load analysis provides us with data about the sources of pollen to honey bees in an area. Likewise, pollen analysis of honey gives us knowledge concerning the plants favored by bees for nectar, as the pollen grains spread in honey. In the case of the plants that are visited for both pollen and nectar, their pollen will be found in honey and pollen loads (Deodikar 1965; Majumdar and Chanda 1984).

Diversified bee flora provides the honey bee colonies with nectars and pollens during the year (Zamarlicki, 1984). Hence, the endurance of honeybees is associated with the abundance of bee floras. The richness of bee plants in an area, containing vegetal and palynological aspects provides evidence of the floral and survival of bees (Sharma, 1972). To study the sources of floras gathered by honey bee worker foragers in any area, bee-gathered pollen can be examined with melissopalynology techniques that are the same as those used to classify pollen in honey to find its plant sources (Jones and Bryant, 2014; Sajwani *et al.*, 2007; Song *et al.*, 2012; Upadhyay and Bera, 2012; Ponnuchamy *et al.*, 2014; Jamil Noor, 2016 and Lau *et al.*, 2019). Nevertheless, pollen foragers do not visit the similar plants that nectar foragers from the same colony do, resulting in differences in the kinds of plants visited by workers according to either nectar or pollen demand (Sajwani *et al.*, 2007 and Sajwani *et al.*, 2014).

Bee foraging plants and honey pollen analyses in Arab countries including Egypt were studied by Robinson, 1981; Hussein, 1997&2000; Al-Abd El-Qader, 1998; El-Eid, 1998; Abdilla and Vorwohl, 1998; Al-Khalifa and Al-Arif, 1999; Reyahi, 1999; Al-Jabr and Nour, 2001; El-Katheri, 2002; Terrab *et al.*, 2003; Díez *et al.*, 2004; Rateb, 2005 and Damhoureyeh, 2007. Meanwhile, as far as known to us there is no studies on the structure of pollen pellets gathered from foragers have been done, accordingly, less knowledge is available about the pollen loads of honey bee worker foragers in urban and suburban areas. Therefore, the objectives of this work are to analyze the pollen loads collected by honey bee worker foragers in Upper Egypt (Assiut and Sohag areas) and to estimate the species richness, evenness, and diversity indices.

MATERIALS AND METHODS

Study Areas:

Field and laboratory work of the present investigation were conducted at the apiary of the Plant Protection Department, Faculty of Agriculture, Assiut University, and a private apiary in the Sohag area during the active season of 2019. Carniolan hybrid honey bee colonies were used.

Collection of Nectar Foragers:

For studying honey bee activities for visiting nectariferous and polleniferous plants in the areas of study, weekly samples from honey bee foragers were collected during the

active season of 2019. Four honey bee hives were selected from each apiary in Assiut and Sohag. Twenty workers from every hive of incoming nectar foragers - without corbicula loads - were captured from the entrance of hives in timeslots (8.00–10.00 AM., 12.00–2.00 PM., and 4.00–6.00 PM.) on a sampling day.

Body Surface Pollen Analysis of Honey Bee Foragers:

Foragers from each sample were stirred with distilled water (about 20 ml distilled water) to dislodge the pollen grains smeared on the bee's body surface and removed the bees from the distilled water. then the solution was filtrated by glass wood to remove any impurities of worker parts. The filtrate of each sample was centrifuged for 20 min at 4500 rpm. The sediment of each sample was smeared in a slide, mounted in Fucsin-glycerin gel, and examined microscopically for pollen analysis, based on the morphology of the pollen types, as described by Nair (1960). Pollen grains were counted and identified per sample and the average number of pollen grains was estimated in ten microscopical fields, with helping of reference slides prepared from local flora during the present study.

Frequency Classes of Pollen Grain in Samples:

Once pollen grains were identified and counted in the samples. Pollen frequency was calculated as the percentage, by dividing the number of pollen grains of a particular taxon, by the total number of pollen types that counted in the sample. Then, each pollen type was assigned to one of the following four pollen frequency classes. Dominant (more than 45%), Secondary (45-16%), Important Minor (3-16%), and Minor pollen types (less than 3%) as described by Louveaux *et al.* (1978).

The expected honey yield was labeled during each period based on the frequency classes of pollen types in samples as a unifloral, bifloral, or polyfloral honey depending on the presence of a predominant pollen type; two secondary pollen types; or more than two secondary pollen types, were recorded, respectively (Ramirez-Arriaga *et al.*, 2011).

Estimation of Diversity:

Floral taxonomic diversity for samples in each month, and then, each season for each region, were calculated to characterize taxonomic richness and evenness according to the Simpson's Index of Diversity (Simpson, 1949).

Simpson's index of diversity was calculated using the equation:

$$\text{Simpson's index of diversity} = (1 - D) = 1 - [\sum(n_i * (n_i - 1)) / (N * (N - 1))]$$

n_i — Number of individuals in the i -th species; and

N — Total number of individuals in the community.

RESULTS AND DISCUSSION

Forager's body surface pollen analysis is a suitable alternative method to the classical methods i.e., pollen spectrum analysis of honey and field monitoring, regarding the accuracy of determining forage plants. Therefore, our study enabled us to establish the important plant sources exploited by honey bee colonies during the activity seasons in two areas of Upper Egypt.

Overall, Pollen Species Diversity:

Pollen samples of 168 microscopic slides were examined. The survey showed that the overall richness of pollen species across both regions (Assiut & Sohag) was twenty-six species. The results revealed higher overall pollen species richness in the Assiut area (21 species, belonging to sixteen families), and lower overall pollen species richness in the Sohag area (14 species, belonging to 9 families). The general mean pollen count during the active season was higher in the Assiut area (5248 pollen grains) than in those from the Sohag area (3601 pollen grains), (Tables 1&2). On the contrary, overall pollen species diversity percentage and species evenness, in examined samples, were higher in the Sohag

area according to Simpson's Diversity Index (85.25 %; 6.78) than in the Assiut area (71.91 %; 3.56), respectively. The observed richness of pollen species number ranged between 5–10 plant species in the Assiut area and between 2–8 plant species in the Sohag area across blooming months (Table 3 and Fig 1). Even though a high richness of pollen types in forager samples, in both Assiut and Sohag regions (14 - 21 taxa) respectively, was encountered. Only six important taxa belonged to native plant species, which were fennel, wild mustard, clover, alfalfa, Eucalyptus, and date palm. These plant taxa have been documented as main pollen sources for native bees in numerous studies, e. g. Hussein (1982) in Assiut; Ghoniemy (1984) in Fayoum, and Abou-Shaara (2015) his review on potential honey bee plants of Egypt.

The numbers of plant species that were shared between the two regions were nine species belonging to eight families as follows: Fabaceae family contained two species (*Trifolium* sp. & *Medicago* sp), while the rest of the families were represented by one species each (Myrtaceae: *Eucalyptus* spp); (Arecaceae: *Phoenix dactylifera*); (Asteraceae: *Helianthus annuus*); (Rosaceae: *Rosa* sp.); (Cucurbitaceae: *Cucumis sativa*); (Brassicaceae: *Brassica rapa*); (Solanaceae: *Solanum* sp.). The existence of the same pollen species in samples of both regions is due to the distribution of the same type of crops in both regions because of the unified governmental agricultural system.

The number of plant species confined to the Assiut area was 12 species belonging to 10 families as follows: Apiaceae and Fabaceae families contained two species (*Daucus carota* & *Foeniculum vulgare*) and (*Mimosa pudica* & *Acacia* spp), respectively, while the rest of the families were represented by one species each, (Amaryllidaceae: *Allium cepa*); Anacardiaceae: *Mangifera indica*); (Brassicaceae: *Brassica napus*); (Convolvulaceae: *Convolvulus* spp.); (Cyperaceous: *Cyperus* sp); (Pedaliaceae: *Sesamum indicum*); (Poaceae: *Zea mays*); (Rutaceae: *Citrus* spp.). While the number of plant species confined to the Sohag area represents by five species belonging to three families as follows: Asteraceae family contained three species (*Lactuca sativa*, *Sonchus* sp & *Helichrysum* sp), while other families were represented by one species each, (Apiaceae: *Coriandrum sativum*); (Brassicaceae: *Brassica kaber*). The existence of these species in one area might be attributed to the farmers of that area and their preference for certain plant species according to their awareness of the requirements for cultivating those plant taxa; as the presence of citrus and mango pollen only in samples examined from the Assiut region could be attributed to the spread of citrus and mango trees in Assiut area, excluding the Sohag region.

Table 1: Pollen analysis presentation (on bee’s body surface) obtained in the Assiut region categorized by season.

Pollen Taxa	Common Name	1 st Period (Early Summer)		2 nd Period (Late Spring)		3 rd Period (Summer)		All Active Season (March to September)		Presence in Samples	
		Monthly Total	Abundance (AC) %	Monthly Total	Abundance (AC) %	Monthly Total	Abundance (AC) %	Monthly Total	Abundance (AC) %	Weeks	Period
<i>Brassica napus</i>	Canola	4	0.50 (MP)	-	-	-	-	4	0.08 (MP)	4	Early Mar to late Mar
<i>Citrus spp</i>	Citrus	31	3.86 (IMP)	-	-	-	-	31	0.59 (MP)	8	Early Mar to late Apr
<i>Foeniculum vulgare</i>	Fennel	297	36.99 (SP)	-	-	-	-	297	5.66 (IMP)	8	Early Mar to late Apr
<i>Mangifera indica</i>	Mango	49	6.10 (IMP)	-	-	-	-	49	0.93 (MP)	4	Late Mar to early Apr
<i>Phoenix dactylifera</i>	Date palm	132	16.44 (SP)	-	-	-	-	132	2.52 (MP)	5	Late Mar to late Apr
<i>Eucalyptus spp</i>	Eucalyptus	172	21.42 (SP)	112	4.47 (IMP)	941	48.53 (DP)	1225	23.34 (SP)	19	Early Apr. to early Jun & late July to late Sept.
<i>Helianthus annuus</i>	Sunflower	-	-	8	0.32 (MP)	30	1.55 (MP)	38	0.72 (MP)	10	Late Jun to early Sep
<i>Trifolium sp</i>	Egyptian clover	118	14.69 (IMP)	2217	88.47 (DP)	58	2.99 (MP)	2393	45.6 (DP)	10	Late Apr to early July
<i>Medicago sp</i>	Alfalfa	-	-	109	4.35 (IMP)	501	25.84 (SP)	610	11.62 (IMP)	20	Early May to late Sep
<i>Mimosa pudica</i>	Sensitive Plant	-	-	4	0.16 (MP)	-	-	4	0.08 (MP)	4	Early May to late May
<i>Rosa spp</i>	Rosa	-	-	17	0.68 (MP)	24	1.24 (MP)	41	0.78 (MP)	12	May & early July to early Sept.
<i>cucumis sativus</i>	Cucumber	-	-	39	1.56 (MP)	20	1.03 (MP)	59	1.12 (MP)	6	Early Jun to early July
<i>Acacia spp</i>	Acacia	-	-	-	-	2	0.1 (MP)	2	0.04 (MP)	2	Early July
<i>Brassica rapa</i>	Turnip	-	-	-	-	54	2.78 (MP)	54	1.03 (MP)	4	Early July to late July
<i>Daucus carota</i>	Carrot	-	-	-	-	109	5.62 (IMP)	109	2.08 (MP)	7	Late July to late Aug & late Sept.
<i>Sesamum indicum</i>	Sesame	-	-	-	-	8	0.41 (MP)	8	0.15 (MP)	4	Late Aug to early Sep
<i>Allium cepa</i>	Onion	-	-	-	-	50	2.58 (MP)	50	0.95 (MP)	3	Late Aug to early Sept.
<i>Convolvulus spp.</i>	Field bindweed	-	-	-	-	22	1.13 (MP)	22	0.42 (MP)	2	Early Aug.
<i>Zea mays</i>	Maize	-	-	-	-	41	2.11 (MP)	41	0.78 (MP)	6	Early Aug to early Sept.
<i>Solanum sp.</i>	Eggplant	-	-	-	-	66	3.4 (IMP)	66	1.26 (MP)	2	Early Sept.
<i>Cyperus Sp</i>	Coco nut grass	-	-	-	-	13	0.67 (MP)	13	0.25 (MP)	2	Late Sept.
Total Average of Pollen types	-	803		2506		1939		5248		-	-
Abundance %	-	15.3		47.75		36.95		-		-	-

Capital letters represent abundance classes (AC) - (MP: Minor Pollen < 3%; IMP: Important Minor Pollen from 3% to 15%; SP: Secondary Pollen from 16% to 45%; DP: Dominant Pollen > 45%).

Table 2: Pollen analysis presentation (on bee’s body surface) obtained in the Sohag region categorized by season.

Pollen Taxa	Common Name	1 st Period (Early Summer)		2 nd Period (Late Spring)		3 rd Period (Summer)		All Active Season (March to September)		Presence in Samples	
		Monthly Total	Abundance (AC) %	Monthly Total	Abundance (AC) %	Monthly Total	Abundance (AC) %	Monthly Total	Abundance (AC) %	Weeks	Period
<i>Coriandrum sativum</i>	Canola	45	3.35 (IMP)	-	-	-	-	45	1.25	2	early Mar only
<i>Phoenix dactylifera</i>	Citrus	260	19.36 (SP)	-	-	-	-	260	7.22 (IMP)	6	early Mar to early Apr
<i>lactuca sativa</i>	Fennel	119	8.86 (IMP)	56	5.48 (IMP)	-	-	175	4.86 (IMP)	9	early Mar to early May
<i>Brassica kaber</i>	Mango	538	40.06 (SP)	92	9.01 (IMP)	-	-	630	17.5 (SP)	10	early Mar to early May
<i>Eucalyptus spp</i>	Date palm	381	28.37 (SP)	48	4.7 (IMP)	-	-	429	11.91 (IMP)	10	early Mar to early May
<i>Trifolium Sp</i>	Eucalyptus	-	-	690	67.58 (DP)	31	2.51 (MP)	721	20.02 (SP)	11	early May to late July
<i>Medicago Sp</i>	Sunflower	-	-	135	13.22 (IMP)	670	54.16 (DP)	805	22.35 (SP)	20	early May to late Sep
<i>Rosa sp</i>	Egyptian clover	-	-	-	-	148	11.96 (IMP)	148	4.11 (IMP)	10	late July to late Sep
<i>Helianthus annuus</i>	Alfalfa	-	-	-	-	152	12.29 (IMP)	152	4.22 (IMP)	6	late July to late Aug
<i>cucumis sativa</i>	Sensitive Plant	-	-	-	-	130	10.51 (IMP)	130	3.61 (IMP)	6	late July to late Aug
<i>Brassica rapa</i>	Rosa	-	-	-	-	59	4.77 (IMP)	59	1.64 (MP)	6	late Aug to late Sep
<i>Solanum sp</i>	Cucumber	-	-	-	-	34	2.75 (MP)	34	0.94 (MP)	3	late Aug to early Sep
<i>Sonchus sp</i>	Acacia	-	-	-	-	11	0.89 (MP)	11	0.31 (MP)	5	late Aug to late Sep
<i>Helichrysum sp</i>	Turnip	-	-	-	-	2	0.16 (MP)	2	0.06 (MP)	2	early Aug.
Total Average of pollen types	-	1343		1021		1237		3601		-	-
Abundance %	-	37.30		28.35		34.35		-		-	-

Capital letters represent abundance classes (AC) - (MP: Minor Pollen < 3%; IMP: Important Minor Pollen from 3% to 15%; SP: Secondary Pollen from 16% to 45%; DP: Dominant Pollen > 45%).

Floral Diversity in The Assiut Region: In Early Spring:

Through surface pollen analysis of forager bodies, the results showed that there were seven plant taxa belonging to 7 botanical families, whose flowers have been visited by foragers. The pollen species diversity percentage calculated according to Simpson's Index of Diversity was 76.44%, and the species evenness was 4.24 (Table 3 & Fig 2). In this period, none of this examined pollen belonged to the predominant category. The pollen samples included secondary and important minor plant taxa. The pollen species from *Foeniculum vulgare*, *Eucalyptus spp.*, and *Phoenix dactylifera* were represented as secondary pollen sources, with abundance percentages of 36.99 %, 21.42 %, and 16.44 %, respectively.

respectively, followed by *Trifolium* sp., *Mangifera indica*, and *Citrus* sp. as important minor pollen from with abundance percentage of 14.69 %, 6.1 %, and 3.86 %, respectively (Table 1).

In Late Spring:

The results indicate that there were 7 plant species belonging to 5 botanical families whose flowers have been visited by foragers. The pollen species diversity percentage on the surface of forager bodies was 21.32%, and the species evenness was 1.27 (Table 3 & Fig.2). During this period, pollen of *Trifolium* sp. was the most “predominant” type, in all examined samples, with abundance percentage of 88.47 %. While other pollen types varied among "important minor or minor" pollen types, and their abundance percentage ranged from 0.16% to 4.47% (Table 1).

In the Summer:

Fifteen plant species, belonging to thirteen botanical families, were identified in the examined samples. The pollen species diversity percentage was 69.03%, and the species evenness was 3.23 (Table 3 & Fig.2). The pollen species that have been identified in the summer period consisted of one predominant and one secondary plant taxa; the predominant pollen was *Eucalyptus* sp. with abundance percentage of 48.53 %, while the secondary pollen, was Alfalfa species with abundance percentage of 25.84 %. While the other pollen types varied among, important minor or minor pollen types and their abundance percentage ranged from 0.10% to 5.62% (Table 1).

Table 3: Summary of Simpson's index of diversity values for both regions categorized by season.

Periods	Regions	Average of pollen types	Species Richness	Species evenness	Simpson's Index of Diversity (1-D) %
1st period (Early Spring)	Assiut	803	7	4.24	76.44
	Sohag	1343	5	3.49	71.31
2nd period (Late Spring)	Assiut	2506	7	1.27	21.32
	Sohag	1021	5	2.05	51.3
3rd period (Summer)	Assiut	1939	15	3.23	69.03
	Sohag	1237	9	2.97	66.3
All active season	Assiut	5248	21	3.56	71.91
	Sohag	3601	14	6.78	85.25

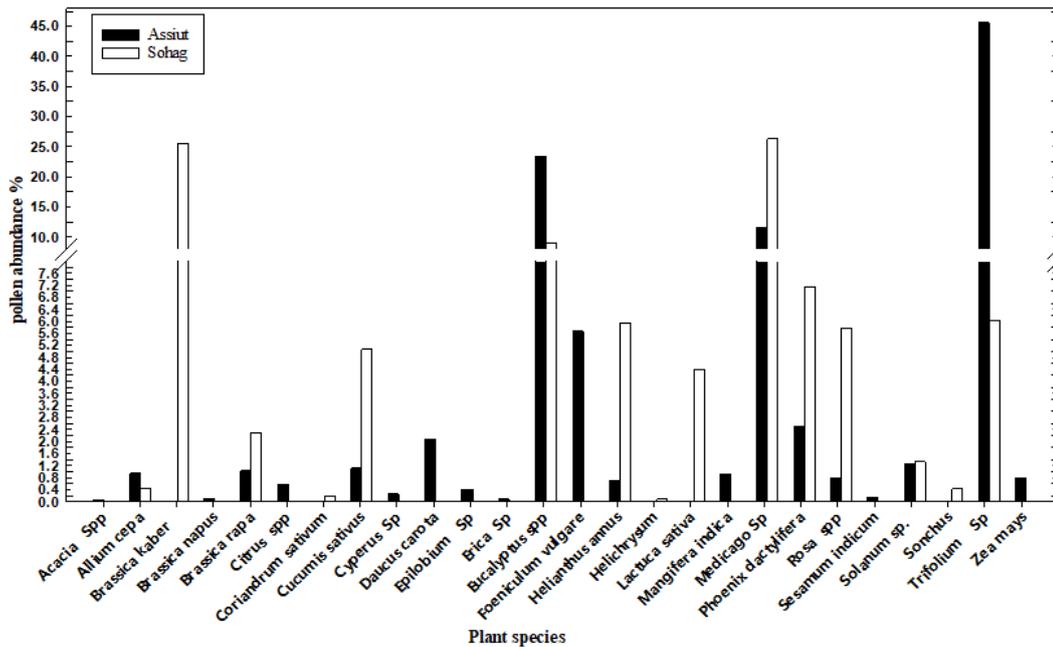


Fig.1: Seasonal-wise pollen types obtained from bee’s body surface pollen analysis in Assiut & Sohag areas during the active season from March to September.

In Early Spring:

Through surface pollen analysis of forager bodies, the results show that there were five plant taxa belonging to five botanical families have been visited by foragers. The pollen species diversity percentage according to Simpson's Diversity Index was 71.31%, and the species evenness was 3.49 (Table 3 & Fig.2). None of the examined pollen belonged to the predominant category; similarly, to Assiut region in the early spring. The pollen samples included secondary and important minor plant taxa. The pollen species of *Brassica kaber*, *Eucalyptus spp.*, and *Phoenix dactylifera* were represented as a secondary pollen source with abundance percentages of 40.06 %, 28.37 %, and 19.36 %, respectively, followed by *Lactuca sativa*, and *Coriandrum sativum* as an important minor pollen source with abundance percentage of 8.86 % and 3.35 %, respectively (Table 2).

In Late Spring:

The results indicate that there were five plant species belonging to four botanical families whose flowers have been visited by foragers. The pollen species diversity percentage was 51.30%, and the species evenness was 2.05 (Table 3 & Fig.2). During this period, pollen of *Trifolium sp.* was the most predominant type in all examined samples with an abundance percentage of 88.47 %, like the results of Assiut region. While other pollen types were recorded as an important minor pollen source, and their abundance percentage ranged from 4.70% to 13.22% (Table 2).

In Summer:

Nine plant species belonging to six botanical families were identified in the examined samples. The pollen species diversity percentage was 66.30%, and the species evenness was 2.97 (Table 3 & Fig. 2). The pollen species that have been recorded consisted of one predominant and a few "important minor and minor" taxa. The dominant pollen belonged to *Alfalfa* taxa with an abundance percentage of 54.16 %, while the important minor pollen belonged to *Helianthus annus*, *Rosa sp.*, *Cucumis sativa*, and *Brassica rapa* with an abundance percentage of 12.29%, 11.96%, 10.51%, and 4.77%, respectively. Other pollen types were recorded as minor pollen sources and their abundance percentage ranged from 0.16% to 2.75% (Table 2).

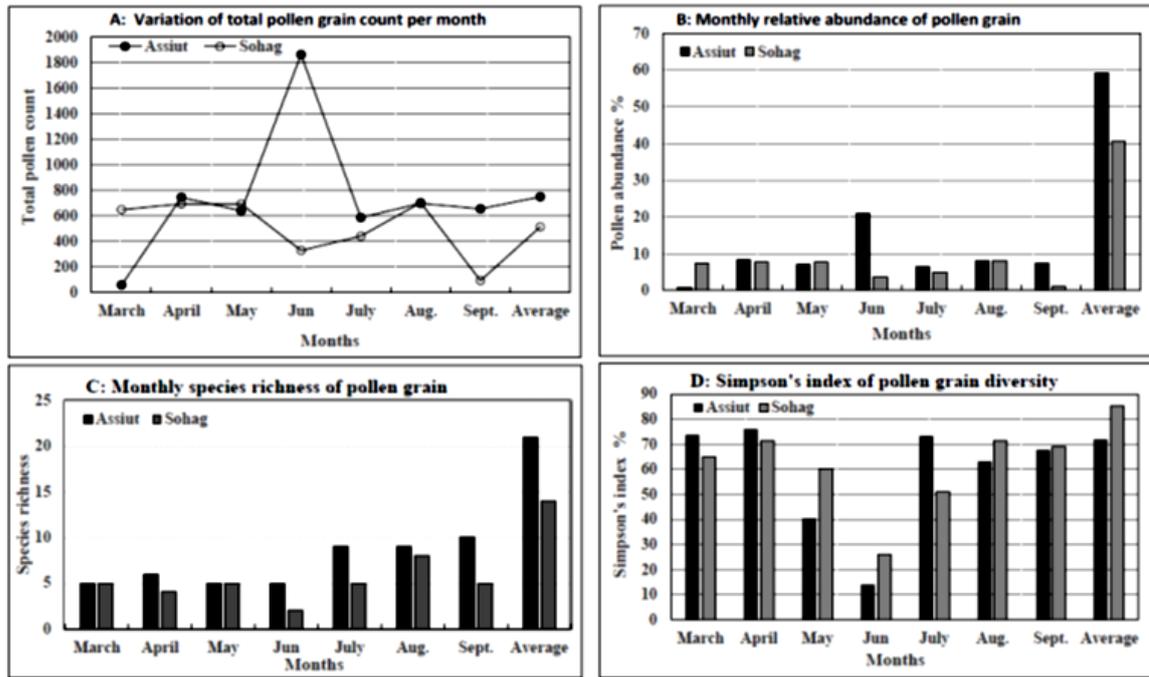


Fig. 2: Pollen analysis presentation (on bee's body surface) obtained in Assiut & Sohag areas during the active season from March to September.

Comparison of Floral Diversity of Both Regions:

Surface pollen analysis of forager bodies enabled us to observe the following: Assiut was the area with the highest number of visited plant taxa ($n = 21$ over activity seasons) compared to the Sohag area ($n = 14$). Despite this taxonomic abundance, the Simpson's Index of Diversity suggests that there was low species evenness over every sampling period in each sample (3.56 plant taxa) compared to the Sohag area (6.76 plant taxa). This indicates that the foragers did not collect pollen evenly during a sampling period, and instead, preferred to collect pollen from a few pollen resources in each collection. These pollen resources were represented by clover, Eucalyptus, and Alfalfa plants, with an abundance percentage of 45.6%, 23.34%, and 11.62%, respectively. It is clear that samples from the Sohag area were characterized by less numerous plant taxa (Richness of pollen species), compared to the Assiut area. This reflects that bee foragers in the Assiut area visited more plant species, compared to bee foragers in the Sohag area (Table 1 & 2). The differences observed in pollen counts and species between both regions might be due to the relative change in the composition of the crops and the areas planted of those crops, in the tested areas. Senapathi *et al.* (2015) explained that the difference in the cultivated areas around the bee nests influenced the availability of nectar and pollen sources relatively, in addition to the weather factors which directly affect their foraging activity. Also, long-term impacts of anthropogenic activities such as unrestricted urban sprawl through agricultural land for building houses and shopping centers. Undoubtedly, these activities are major drivers of biodiversity loss, which strongly affect the composition and diversity of available plants and food sources for honey bee colonies (Parreño *et al.*, 2022).

Our study concluded that the early spring provided the highest diversity of forage plant taxa in the Assiut, and Sohag regions compared to other seasons with a diversity percentage of 76.44% and 71.31%, respectively. The species evenness was 4.24 plant taxa in the Assiut area, and the pollens come from Fennel, Eucalyptus, clover, and Date palm. While it was 3.49 plant taxa in the Sohag area and the pollens came from Wild mustard,

Eucalyptus, and Date palm (Table 3).

In the late spring, low plant diversity was observed in forager body samples in both regions (21.32 % in Assiut & 51.30 % in Sohag) and also, low species evenness in both regions (1.27 in Assiut & 2.05 in Sohag). This denotes that foragers did not collect pollen equally from various plant species, and instead, preferred collecting pollen from a few floral resources in each collection. This is evidenced by the abundance of plants grouped in the Fabaceae family, especially in the *Trifolium* and *Medicago* genera. Also, most of the pollen found on the forager bodies in each region came from clover plants with an abundance percentage of 88.47% in Assiut and 67.58% in Sohag compared to other plant taxa. Subsequently, the clover plant was the most “predominant” type and reflects the importance of the clover plant as a honey bee forage (Table 3). This is inconsistent with previous literature, which reported that even in cases when there is a great diversity of plant resources obtainable, the foragers tend to focus their nectar and pollen-gathering efforts on a few plant species, this is due to, the preferred plant resources are more abundant than other species, or because they provide specific nutrients that honey bee colonies lack during activity seasons (Baum *et al.*, 2004; Smart *et al.*, 2017 and Richardson *et al.*, 2015). Also, Amaya-Marquez (2009) stated that a colony’s temporary specialization and the floral constancy of foragers in collecting pollen from a specific source. The summer provided the highest richness of plant pollen species (15 plant taxa in Assiut & nine plant taxa in Sohag) compared to other seasons with diversity percentage and evenness values mediated between the values of the early spring and late spring (Table 3). The foragers preferred collecting pollen from a few floral resources in each collection, The collected pollens come from Eucalyptus and *Medicago* in the Assiut area and *Medicago* sp., *Helianthus annuus*, and *Rosa* sp. in the Sohag area.

Prediction of Honey Type Using Surface Pollen Analysis of Forager Bodies:

Pollen analysis of forager bodies enabled us to identify the important nectariferous and polleniferous plant sources visited by bees during the principal honey harvest periods. It also enabled us to record the chronologies of plant species blooming in the two areas in Upper Egypt (Table 1 & 2). In both regions, several various genera were identified as predominant and secondary plant taxon belonging to the family Fabaceae (*Trifolium* sp. and *Medicago* sp.), family Apiaceae (*Foeniculum vulgare*), and family Brassicaceae (*Brassica kaber*). There was also a large representation of pollen from trees of *Phoenix dactylifera* (Arecaceae) and *Eucalyptus* sp. (Myrtaceae) in both regions.

Using these obtained results, it is possible to predict the honey yield type from the honey bee colonies. That is by calculating the density of pollen grains for each plant taxon on forager bodies during the three blooming periods the extent of potential participation of each plant taxon in honey production. The results show that three types of honey were expected, Over the three periods in both study areas.

Accordingly in the early spring, the Fennel and Eucalyptus were the nectariferous plants, and the Date palm was the polleniferous plant in the Assiut area (Table 1). While, In the Sohag area, the wild mustard and Eucalyptus were the nectariferous plants, and the Date palm was the polleniferous plant (Table 2). So, the expected honey would be bifloral honey in each region. In the late spring, the dominant pollen type in both regions were belonging to Egyptian clover in both areas (Table 1 & 2) and other pollen types varied from important minor to minor pollen types. Therefore, the type of honey produced during this period would be monofloral honey (clover honey) in both regions. Our results here agree with El-Metwally (2015) who examined Egyptian bee honey samples and classified fourteen types of pollen. Clover pollen was found in a high percentage (30.2%) while date palm and umbellifers pollen were recorded in considerable percentages at 13.20 and 9.39%, respectively. Also, the results of our study are consistent with previous work where clover

has been considered as the main nectar resource, and the main type of honey produced in Upper Egypt comes from the clover plant (Nour, 1988; Nour *et al.*, 1991; Rateb, 2005; El Metwally, 2015). In addition, Nour (1988) analyzed sixty Egyptian honey samples and found that the main pollen sources of Egyptian honey were clover (*Trifolium alexandrinum* L.), Eucalyptus (*Eucalyptus* spp.), Citrus sp. Date palm (*Phoenix dactylifera* L.), Maize (*Zea mays* L.), Sunflower (*Helianthus annuus* L.) and Faba bean (*Vicia faba* L.).

In Summer, in the Assiut area, Eucalyptus pollens were the dominant pollen, followed by Alfalfa's pollen as secondary pollen types, with abundance percentages of 48.53 %, and 25.84 %, respectively (Table 1). This reflects that honey yield would be dominated by Eucalyptus nectar. On the contrary, in the Sohag area, alfalfa's pollen (*Medicago sativa*) was the dominant pollen, with an abundance percentage of 54.16 %, and the other pollen types varied between important minor or minor pollen types (Table 2). Therefore, the honey yield would be dominated by alfalfa nectar. Our results here also agree with Nour (1988) who examined sixty Egyptian honey samples and found that the second main source of Egyptian honey was Eucalyptus sp. Also, Andrada *et al.* (1998) mentioned that the predominant pollen type was Eucalyptus sp. in Argentine. In addition, our results are in full agreement with the previous works which showed the main types of honey produced in Upper Egypt (Hussein, 1982&2001; Hussein and Abdel-Aal, 1982; Hussein *et al.*, 1992; Nour, 1988; Farag, 2007; Abou-Shaara, 2015; Karabournioti & Karabagias, 2017 and El-Sofany *et al.*, 2018). In the current study in Assiut and Sohag regions, the use of bee's body surface pollen analysis exhibited fewer differences in the pollen spectrum between the two regions. As Bogdanov (2007) reported that if the geographical zones are closer, differences between types of honey samples are more difficult to differentiate.

The bee foragers utilized more diverse floral resources in the late spring and summer seasons where pollen choice was more restricted. In those seasons, the bees were dependent on the crops cultivated by humans in both study areas in Upper Egypt.

REFERENCES

- Abdilla, F.S. and Vorwohl, G. (1998): Microscopical data of six sidr comb honeys from Yemen. *Proceeding of the 2nd Arab Conference Jordan*, 22-25.
- Abou-Shaara, H. F. (2015): potential honey bee plants of Egypt. *Cercetări Agronomice în Moldova, XLVIII (2)*: 99-108.
- Al-Abd El-Qader, M. (1998): Characterization of some local and imported honey using pollen grains and identification of the main honey plants in Jordan. *M.Sc. Thesis, Univ. of Amman, Jordan*.
- Al-Jabr, A.M. and Nour, M.E. (2001): Content of some Saudi honeys of pollen spectrum and sucrose. *Journal of Agricultural Science, Mansoura Univ.*, 26 (6): 4000-4014.
- Al-Khalifa, A. and Al-Arif, J. (1999): Physicochemical characterization and pollen spectrum of some Saudi honeys. *Food Chemistry*, 67: 21-25.
- Amaya-Marquez, M. (2009): Floral constancy in bees: a revision of theories and a comparison with other pollinators. *Revista Colombiana de Entomologia*, 35: 206–216.
- Andrada, A.; Valle, A.; Aramayo, E.; Lamberto, S. and Cantamutto, M. (1998): Pollen analysis of honeys from the Austral Mountains, Buenos Aires province, Argentine. *Investigation Agraria, Production Protection – Vegetables*, 13 (3): 265-275.
- Baum, K.A.; Rubink, W.L.; Coulson, R.N. and Bryant, V.M. Jr. (2004): Pollen Selection by Feral Honey Bee (Hymenoptera: Apidae) Colonies in a Coastal Prairie Landscape. *Environmental Entomology*, 33 (3): 727–739

- Bogdanov, S. (2007): Authenticity of Honey and other bee products: state of the art. *Bulletin USAMV-CN*, 63-64.
- Damhoureyeh, S.A. (2007): Herbaceous vegetation cover analysis of selected sites in Jordan. *Dirasat Pure Science*, 34 (1): 34-42.
- Deodikar, G B (1965): Melittopalynology. *Indian Bee Journal*, 27: 59-69
- Díez, M.J.; Andrés, C. and Terrab, A. (2004): Physicochemical parameters and pollen analysis of Moroccan honeydew honeys. *International Journal of Food Science and Technology*, 39: 167–17.
- Duff, S. R. & Furgala, B. (1986). Pollen trapping in honey bee colonies in Minnesota, Part II: Effect on foraging activity, honey production, honey moisture content, and nitrogen content of adult workers. *American Bee Journal*, 126, 755–758.
- El-Eid, R. (1998): Physicochemical and pollen analysis of Algerian honeys. *Second Arab Congress, Jordan*, 97-99.
- El-Katheri, G. (2002): Bee forage plants and their flowering seasons in Hadrammout Valley, Yemen. *Symposium on Honey Bees and Sidr Trees, Yemen*, 2-9.
- El-Metwally, S.E.A. (2015): Factors affecting the physical and chemical of characteristic of Egyptian bee honey. *Ph.D. Thesis, Cairo Univ., Egypt*. 333pp.
- El-Sofany, A.; Al Naggar, Y.; Naiem, E. and Seif, A. (2018): Characterization of *Apis mellifera* honey from different botanical and geographical origins in Egypt. *The Egyptian Journal of Experimental Biology (Zoology)*, 14: 75–84.
- Farag, R.M.A. (2007): Studies on Egyptian honeys. *M.Sc. Thesis, Fac. Agric., Cairo Univi.*, 183pp.
- Feller-Demalsy, J.; Parent, J. and Strachan, A.A. (1987): Microscopic analysis of honeys from Alberta, Canada. *Journal of Apicultural Research*, 27(2): 123–132.
- Ghoniemy, A.H., (1984): Studies of some activities of the honey bee colonies under the environmental conditions of Fayoum region. *M.Sc. Thesis, Faculty of Agriculture, Cairo University, Egypt*.
- Hargasim, O. (1974). Bee as pollinators of entomophilous crops. *Bee world*, 55 (4): 137 – 140.
- Hussein, M.H (1982): The pollen flora of Assiut Governorate, Egypt. *Assiut Journal of Agricultural Sciences*, 13 (6): 173 – 184.
- Hussein, M.H. and Abdel-Aal, S.A. (1982): Wild and honey bees as pollinators of 10 plant species in Assiut area, Egypt. *Zeitschrift für Angewandte Entomologie*, 93: 342-346.
- Hussein, M.H.; Mannaa, S.H.; Omar, M.O. and Mostafa, A.M. (1992): Species composition of collected pollen loads by honeybee (*Apis mellifera* L.), pollen flora and floral calendar of Assiut region. *Proc. 4th Nat. Conf. Pests Dis. Veg. and Fruits, Egypt*, 177-195.
- Hussein, M.H. (1997): Beekeeping in Sultanate of Oman. *Proceedings of the 35th Apimondia Congress, Belgium, Antwerp*, 452 -472.
- Hussein, M.H. (2000): A review of beekeeping in Arab Countries. *Bee World*, 81 (2): 56-71.
- Hussein M.H. (2001): Beekeeping in Africa: I - north, east, north-east and west African countries. *Proceedings of 37th International Apicultural Congress of Apimondia, Durban, South Africa*. 28 October-1 November.
- Jamil Noor, M.; Ahmad, M.; Ashraf, M.A.; Zafar, M.; and Sultana, S. (2016): A review of the pollen analysis of South Asian honey to identify the bee floras of the region. *Palynology*, 40 (1): 54–65.
- Jones, G.D. and Bryant, V.M. (2014): Pollen studies of East Texas honey. *Palynology*, 38 (2): 242–258.

- Karabournioti, S. and Karabagias, I. K. (2017): Pollen characteristics of Egyptian clover honey. *Journal of Botanical Sciences*, 6: 32–34.
- Lau, P.; Bryant, V.; Ellis, J.D.; Huang, Z.Y.; Sullivan, J.; Schmehl, D.R.; Cabrera, A. R. and Rangel, J. (2019). Seasonal variation of pollen collected by honey bees (*Apis mellifera*) in developed areas across four regions in the United States. *PLoS ONE*, 14 (6): e0217294.
- Loper, G. M. and Berdel, R.L. (1980): A nutritional bioassay of honeybee brood – rearing potential. *Apidologie* 11: 181 – 189.
- Louveaux, J.; Maurizio, A. and Vorwohl, G. (1978) Methods of melissopalynology. *Bee World* 59: 139–157.
- Majumdar, J. and Chanda, S. (1984): Bees, pollination and Melissopalynology. *Science Culture*, 50: 216-220.
- McLellan, A. R. (1974): Some effects of pollen traps on colonies of honeybees. *Journal of Apicultural Research*, 13: 143–148.
- Nair, P.K.K. (1960). A modification in the method of pollen preparation. *Journal of Scientific and Industrial Research*, 19c (1): 26-27.
- Nelson, D. L. (1987): The effect of continuous pollen trapping on sealed brood, honey production and cross income in Northern Alberta. *American Bee Journal*, 127: 648–650.
- Nour, M.E. (1988). Some factors affecting quality of Egyptian honey. *PhD thesis, Faculty of Agriculture, Cairo University*. 252 pp.
- Nour, M. E.; Abou Zaid and Selim, H.A. (1991): Effect of long-term storage on Egyptian honey quality. *Journal of Agricultural Science, Mansoura University*, 16: 2691-2698.
- Parreño, M.A., Alaux, C.; Brunet, J-L.; Buydens, L.; Filipiak, M. et al. (2022): Critical links between biodiversity and health in wild bee conservation. *Trends in Ecology & Evolution*, 37 (4): 309-321.
- Ponnuchamy, R.; Bonhomme, V.; Prasad, S.; Das, L.; Patel, P.; Gaucherel, C.; et al. (2014): Honey pollen: using melissopalynology to understand foraging preferences of bees in tropical South India. *PLoS ONE*, 9 (7): e101618.
- Ramirez-Arriaga, E.; Navarro-Calvo, L.A. and Diaz-Carbajal, E. (2011): Botanical characterisation of Mexican honeys from a subtropical region (Oaxaca) based on pollen analysis. *Grana*, 50: 40–54.
- Rateb, S.H. (2005): Studies on pollen spectrum chemical and physical characters of some types of honeys. *Ph.D. Thesis, Assiut Univ.*, Egypt, 330 pp.
- Reyahi B.A. (1999): Melliferous flora of Palestine: some important species with potential for introduction to other regions of the world. *Proceedings of 36th Apimondia Congress, Vancouver, Canada*, 12–17 Sep.1999; p. 269.
- Richardson, R.T.; Lin C-H.; Quijia, J.O.; Riusech, N.S.; Goodell, K. and Johnson, R.M. (2015): Rank-based characterization of pollen assemblages collected by honey bees using a multi-locus metabarcoding approach. *Applications in Plant Sciences*, 3 (11): doi: 10.3732/apps.1500043
- Robinson, W. (1981): Beekeeping in Jordan. *Bee World*, 62 (3): 91-97.
- Sajwani, A.; Farooq, S.A.; Patzelt, A.; Eltayeb, E.A and Bryant, V.M. (2007): Melissopalynological studies from Oman. *Palynology*, 31 (1): 63–79.
- Sajwani, A.; Farooq, S.A. and Bryant, V.M. (2014): Studies of bee foraging plants and analysis of pollen pellets from hives in Oman. *Palynology*, 38 (2): 207–223.
- Senapathi, D.; Carvalheiro, L.G.; Biesmeijer, J.C.; Dodson, C-A.; Evans, R. L.; McKerchar L.; Morton, R.D.; Moss, E.D.; Roberts, S.P.M.; Kunin, W.E. and Potts, S.G. (2015): The impact of over 80 years of land cover changes on bee and wasp pollinator

- communities in England. *Proceedings of the Royal Society B: Biological Sciences* 282 (1806). <http://dx.doi.org/10.1098/rspb.2015.0294>
- Sharma, M. (1972): Studies in the flower of *Datura stramonium* Linn. In relation to bee botany. *Journal of palynology*, 8: 17-21.
- Simpson, E. H. (1949): Measurement of diversity. *Nature*. 163 (4148): 688.
- Smart, M.; Cornman, R.S.; Iwanowicz, D.; McDermott-Kubeczko, M.; Pettis, J.S.; Spivak, M.S. and Otto, C.R.V. (2017): A comparison of honey bee-collected pollen from working agricultural lands using light microscopy and ITS metabarcoding. *Environmental Entomology*, 46 (1): 38–49.
- Song, X-Y.; Yao, Y-F. and Yang, W-D. (2012): Pollen analysis of natural honeys from the central region of Shanxi, North China. *PLoS ONE*, 7 (11): e49545.
- Suryanarayan, M.C. (1986): Honeybee – flower relationship. *Bulletin of Botanical survey of India*, 28 (1-4): 55-62.
- Terrab, A.; Valdés, B. and Díez, M. J. (2003): Pollen analysis of honeys from the Mamora forest region (NW Morocco). *Grana*, 42: 47–54.
- Upadhyay, D. and Bera, S. (2012): Pollen spectra of natural honey samples from a coastal district of Orissa, India. *Journal of Apicultural Research*, 51 (1): 10–22.
- Velthuis, H.H.W. (1992): Pollen digestion and the evolution of sociality in bees. *Bee world*, 73 (2): 77 – 89.
- Zamarlicki, C.C. (1984): Evaluation of honeybee plants in Burma – a Case study. *Proceeding of the FAO (UN) expert committee*, 57-76 pp.