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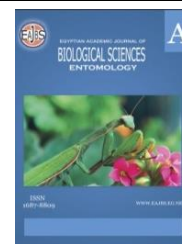
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## Pollen Spectrum and Foraging Plants for the Red Dwarf Bee in Jordan

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### ABSTRACT

Pollen spectrum of honey, and pollen content of the honey sac of the red dwarf honeybee (*Apis florea* F.), which was discovered recently in Jordan, were studied. Pollen diversity and pollen density in these samples, which were collected from two sites in Jordan, were investigated. A total of 4399 pollen grains were counted and classified in the honey of *A. florea*. 2667 pollen grains, or 60.63% of total pollen, were obtained from *Centaurea hylolapis* Bioss., which is considered to be the Dominant Pollen (DP), and the studied honey was monofloral. About 1541 pollen grains, or 35.03% of the total, were collected from Family Caryophyllacea and considered to be a Secondary Pollen (SP) source. *Cardania draba* (L.) was considered a Minor Pollen (MP) source with 2.07% abundance, followed by *Eucalyptus* sp. (0.68%); *Acacia cyanophylla* Lindl. (0.59%); *Leopoldia desirticola* (Rech.f.) Feinbrun (0.48%); *Salvia lonigera* Poir. (0.39%); and *Sambucus racemosa* L. (0.14%). The plant diversity or number of plant species foraged by small bees was eight. While the plant diversity in honey sac content of the small bee from another site, El-Akaba, was nine. *Centaurea* sp., *Echinops* sp. and *Erodium* sp. were considered as Secondary Pollen (SP) sources, with percent abundances of 33.99, 23.60% and 16.23%, respectively. The following Important Minor Pollen sources (IMP) and their (abundance %) and classification were as follows: *Asphodelus* sp. (6.96%); *Phoenix* sp. (5.94%); and *Rubus* sp. (5.66%). Minor Pollen (MP) sources were: *Diplotaxis* sp. (1.6%); *Ecobllium* sp. (1.16%) and *Sencio* sp. (0.16%).

### INTRODUCTION

There are four species of honey bees in the world. Only one species (*Apis mellifera* L) is reared commercially in most countries. Two of the four species, *Apis florea* F., the smallest one, and *Apis dorsata* F., the largest one, build a single comb in an exposed area in the tropics.

*Apis mellifera* was found in Europe and Africa, and in western Iran in the Near East, where it overlaps with *Apis florea*, in Iran and in Oman (Morse and Hooper, 1985). This areal was extended to Sudan (Ali, 1996), and to Iraq (Hussein, 2000). Recently the Red dwarf honeybee (*A. florea*) was found in Jordan (Haddad *et al.* 2008).

The study of the honey pollen spectrum provides ample evidence of the honey bee flora of the locality during the period.

Bee foraging plants and pollen analyses of kinds of honey in Arab countries were studied by Robinson, 1981; Hussein, 1997, 2000; Al-Abd El-Qader, 1998; El-Eid, 1998; Abdilla and Vorwohl, 1998; Al Khalifa and Arify, 1999; Reyahi, 1999; Al-Jabr and Nour, 2001; El-Katheri, 2002; Terrab *et al.*, 2003; Diez *et al.*, 2004; Rateb, 2005 and Damhoureyeh, 2007.

*Apis florea* and *A. dorsata*, build a single comb in an exposed area because they do not nest in cavities and have no way of protecting their nests, they are found only in the tropics. However, the climatic changes in the last decades and the transmission of *A. florea* colonies by man and/or ships, lead to expansion of the areal of the Red dwarf honey bee distribution to subtropical countries, such as Jordan, Palestine, and properly south of Sinai, Egypt.

The work was aimed to study the pollen spectrum of honey from *A. florea*, in Jordan. Studying the pollen diversity and density of the Red dwarf bee honey helps us to understand the possible competition between this species of the honey bee and *Apis mellifera*.

## MATERIALS AND METHODS

The honey samples were brought from Jordan by the Late Prof. Dr. M. H. Hussein during attending a workshop entitled “detecting virus diseases using genetic information” on November 26–30, 2011.

Ten grams from samples of the honey of the Red dwarf bee, which were collected in Jordan, were mixed with 10 ml hot distilled water, then centrifuged for 20 min at 4500 rpm. The sediment was smeared on two slides and was mounted in Fucsin-glycerine gel and was examined microscopically for pollen analysis. Pollen types were identified with the help of a reference slide collection of the local flora and relevant literature. The pollen types were identified as far as possible to the genus or species, but sometimes identification was possible only to family. Pollen types that could not be assigned to even a family were placed in the category “others”. Pollen spectra were constructed for each honey sample based on their pollen frequencies (Louveaux *et al.*, 1978).

The total number of pollen grains in ten microscopic fields, five fields in each slide, was counted and the mean number of pollen grains/field was calculated as an indicator of pollen grains density. The number of pollen types was counted in each sample and their percentage of abundance was calculated. Dominant pollen (DP) comprises more than 45% of the total number of pollens, Secondary pollen (SP), from 16% to 45%, Important Minor Pollen (IMP), from 3% to 15%, and Minor Pollen (MP), less than 3% (Ricciardelli D'Albore G.; and D'Ambrosio M. 1973). The number of pollen types was counted and considered as pollen diversity. Microphotographs of different types of pollen in the tested honey sample were prepared (Fig. 1).

Workers of the Red dwarf bees (25 individuals) were homogenized with 25 ml hot distilled water and were filtered with glass wool. The filtrate was centrifuged at 4500 rpm for 20 minutes and the sediment was smeared and mounted as described above.

## RESULTS AND DISCUSSION

The total number of pollen grains in ten microscopic fields from prepared slides of pollens from the Red dwarf honeybee samples from Jordan was 4399 pollen grain, from which 2667 pollen grains were obtained from *Centaurea hylolapis* Boiss. (Family

Asteraceae), with pollen density of 266.7 pollen grain/field, or 60.63% of all counted pollen. It means that *Centaurea* is a Dominant Pollen (DP), and this honey sample is unifloral or monofloral honey (Table 1 and Fig. 1).

Genus *Centaurea* (Family: Asteraceae) was predominant during this work. The same was noticed by Barth and Daluz, (1998) in Brazil; Reyahi (1999) in the West Bank, Palestine; Andrade *et al.* (1998); Valle *et al.* (2000), and Malacalza *et al.* (2007) in Argentina. Also, Bhusari *et al.* (2005) in India reported that foraging workers of *Apis florea* bee preferred the taxa of Asteraceae as many Asteraceae species produce nectar and according to our results the taxa of this family are of great importance both as nectar and pollen source in the area of study, and particularly in the winter season.

In addition, the different species of Asteraceae had a high percentage (78%) of pollen in the honey collected during winter and can be ranked first as bee forage plants in the Saoner region, India (Bhusari *et al.*, 2005).

A plant species from the family Caryophyllaceae comes next with a pollen density of 154.1 pollen/field, or 35.03%. This source is considered to be Secondary pollen (SP). In the same line, Sajjad *et al.* (2017) studied the yearlong association of *Apis dorsata* and *A. florea* with 49 plant species at Multan, Pakistan. They found that *A. florea* visited *Spergula arvensis* L. (Caryophyllaceae) only 5 times per month during its flowering period (March-April).

Pollen grains of *Cardaria draba* (L.) Desv. comprises 2.07% of total pollen, or Minor Pollen (MP) source. The following pollen sources (and their abundance) were also Minor Pollen (MP): *Eucalyptus* sp. (0.68%); *Acacia cyanophylla* Lindl. (0.59%); *Leopoldia deserticola* (Rech.f.) Feinbrun (0.48%); *Salvia lanigera* Poir. (0.39%); and *Sambucus racemosa* L. (0.14%).

The minor pollen in our study probably due to the reason different flora nectar constitutions are favored by different species (Abrol, 2011); e. g. *A. florea* favors flowers with low caloric rewards (Sihag & Rathi, 1992) whereas *A. dorsata* favors flowers with high caloric rewards.

Pollen spectrum of the Red dwarf bee (*A. florea*) honey contains eight sources; thus, pollen diversity reflects the foraging activity of honeybees and the availability of forage plants during this work.

The competition between *A. cerana japonica* (Radoszkowski) and *A. mellifera ligustica* Spinola was noticed in Japan (Nagamitsu and Inoue, 1999). Some observations on the competition between *A. florea* and *A. mellifera jemenitica* Ruttner was reported by Hussein (1997), and between *A. florea* and *A. mellifera*, in Sudan, by Ali (1996).

In India, pollen analyses of ten samples of *A. florea* honey showed that *Prosopis* pollen was dominant, (DP) and its content ranged from 52% to 95% of the total. This pollen and nectar source for both *A. florea* and *A. cerana* reflects the possible competition between these two species (Kalpana and Ramaniam, 1990). Also, it was noticed in the field that many of the taxa providing forage to *Apis florea* bees are also visited by *Apis cerana* and *A. dorsata* Fabr. which suggests a high degree of imbrication in the foraging behavior of these three species of honey bees (Kalpana *et al.*, 1990; Suryanarayana *et al.*, 1992; Ramanujam *et al.*, 1992 and Bhusari *et al.*, 2005).

**Table 1.** Pollen spectrum of honey from the Red dwarf bee, *Apis florea*, in Jordan.

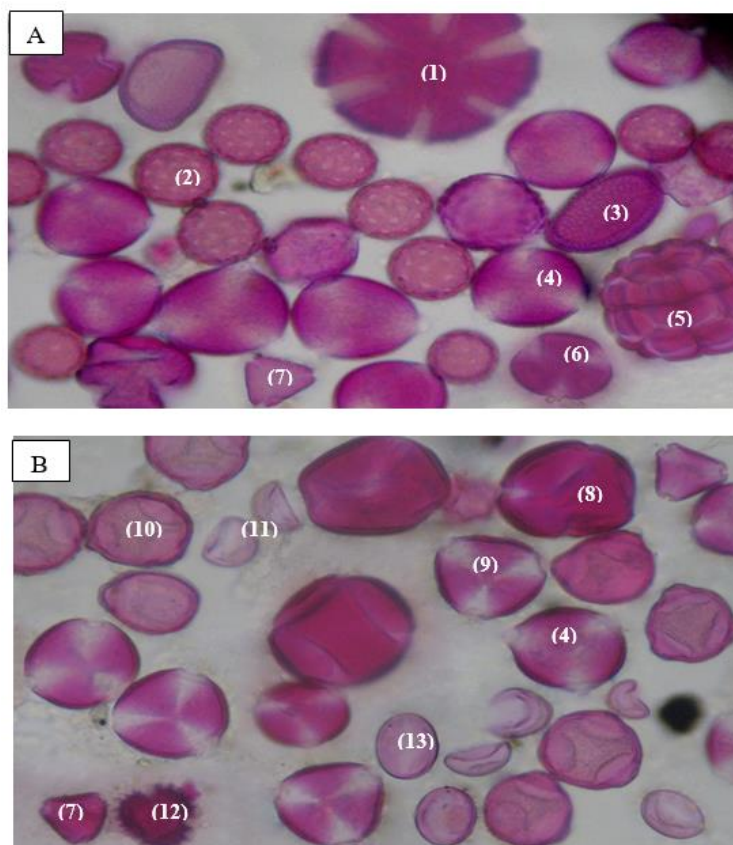
Honeybee forage plants	Total number of counted pollens	Mean number of pollen/field (Pollen Density)	%	Classification
1- <i>Centaurea hylolopsis</i> Fam. Asteraceae	2667	266.7	60.63	Dominant Pollen (DP)
2- Fam. Caryophyllaceae	1541	154.1	35.03	Secondary pollen (SP)
3- <i>Cardaria draba</i>	91	9.1	2.07	Minor pollen (MP)
4- <i>Eucalyptus</i> sp. Fam. Myrtaceae	30	3	0.68	Minor pollen (MP)
5- <i>Acacia cyanophylla</i> Fam. Mimosaceae	26	2.6	0.59	Minor pollen (MP)
6- <i>Leopoldia deserticola</i>	21	2.1	0.48	Minor pollen (MP)
7- <i>Salvia lanigera</i> Fam. Labiatae	17	1.7	0.39	Minor pollen (MP)
8- <i>Sambucus racemosa</i>	6	0.6	0.14	Minor pollen (MP)
<b>Grand total</b>	<b>4399</b>			

Data of the content of pollen grains in workers of *A. florea* are tabulated in Table 2 and Figure 1. *Centaurea* sp., *Echinops* sp. and *Erodium* sp. were recorded as Secondary Pollen sources (SP) with percent abundance of 33.99%, 23.60% and 16.23%, while *Asphodelus* sp., *Phoenix* sp. and *Rubus* sp. were recorded as Important Minor Pollen sources (IMP), with percent abundance of 6.96%, 5.94% and 5.66%, respectively. The following plants *Diplotaxis* sp. (1.60%), *Ecobllium* sp. (1.16%), and *Sencio* sp. (0.16%) were considered as Minor Pollen (MP) sources. Pollen diversity of the red dwarf honeybee workers was nine as noticed during this work in Jordan. Most of the forage plants of the red dwarf bees were herbaceous, and these plants are also visited by the Western honeybee. The pollen diversity of bees particularly the red dwarf bees differ from one place to another e. g. Adikmet area, Hyderabadin, India, it was 24 pollen types and 20 families; also, (Kalpana *et al.*, 1990); Saoner, India, it was 39 pollen types and 21 families (Bhusari *et al.*, 2005 and in Muscat and the Al Batinah region, Oman, it was 94 pollen types and 39 families (Sajwani *et al.* 2014). The difference is due to the difference in plant cover and species, the morphology of flowers, and also the area and sampling numbers. In addition, the foraging behavior of bees is a complicated phenomenon that relies on several factors. Likewise, the physical characteristics of flowers such as color, shape and scent, the variety of the environmental factors such as temperature, humidity, light, solar radiation, time of the day and nectar flow determine the behavior ways of pollinating insects (Visscher and Seeley, 1982; Corbet *et al.*, 1993).

The possibility of competition between *A. mellifera syriaca* and *A. florea* in Jordan and adjacent countries, Egypt, Saudi Arabia and Palestine needs more studies on pollen spectrum of kinds of honey and pollen content of honey stomachs of these two species, where they were scrounging in the same locality.

**Table 2.** Pollen content of the Red dwarf honey bees, *A. florae* from Akaba, Jordan.

Honeybee forage plant	Total number of counted pollens	%	Classification
1- <i>Centurea</i> sp. (F. Asteraceae)	1466	33.99	Secondary Pollen (SP)
2- <i>Echinops polyceras</i> Boiss. (F. Compositae)	1018	23.60	Secondary Pollen (SP)
3- <i>Erodium cicutarium</i> (L.) L'Hér. ex Aiton (F. Geraniaceae)	700	16.23	Secondary Pollen (SP)
4- <i>Asphodelus estivus</i> Brot. (F. Liliaceae)	300	6.96	Important Minor Pollen (IMP)
5- <i>Phoenix</i> sp. (F. Palmae)	256	5.94	Important Minor Pollen (IMP)
6- <i>Rubus sangunieneas</i> Friv. (F. Rosaceae)	244	5.66	Important Minor Pollen (IMP)
7- <i>Diploaxis ereciodes</i> (L.) DC. (F. Crucifera)	69	1.60	Minor Pollen (MP)
8- <i>Ecobllium elatirum</i> (L.) A.Rich. (F. Cucurbitaceae)	50	1.16	Minor Pollen (MP)
9- <i>Senecio verualis</i> Waldst. & Kit. (F. Compositae)	7	0.16	Minor Pollen (MP)
<b>Grand total</b>	<b>4313</b>		



**Fig. 1.** Pollen picture from the honey (A) and the sac of the Red dwarf honey bee (B). 1-*Salvia lonigera*, 2-Fam. Caryophyllaceae, 3-*Leopoldia deserticola*, 4-*Centurea hylolopsis*, 5-*Acacia cyanophylla*, 6-*Cardaria draba*, 7-*Eucalyptus* sp., 8-*Erodium cicutarium*, 9-*Rubus sangunieneas*, 10-*Echinops polyceras*, 11-*Phoenix* sp., 12-*Senecio verualis*, 13-*Asphodelus estivus*

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