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Foraging Insects and Pollinating Efficiency of Bees (Hymenoptera: Apoidea) on the Spring Fababean (*Vicia Faba* L. Variety Minor)

Benachour Karima[1,*], Laadjabi Imène[2], Aguib Sihem[3], Bakiri Esma[3]

[1]Institut of the Nutrition, Food and Agri-Food Technologies, Laboratory of Biosystematics and Ecology of Arthropods, Mentouri Brothers University of Constantine 1, Constantine, DZ-Algeria.
[2]Independent researcher, Constantine, Algeria.
[3]Deparment of Animal Biology, Faculty of Natural and Life Sciences, Laboratory of Biosystematics and Ecology of Arthropods, Mentouri Brothers University of Constantine 1, DZ-Algeria.

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***Corresponding author:** Benachour Karima, Institut of the Nutrition, Food and Agri-Food Technologies, Laboratory of Biosystematics and Ecology of Arthropods, Mentouri Brothers University of Constantine 1, Constantine, DZ-Algeria.
Email: benachour.karima@um.edu.dz

Abstract

The foraging insects of the spring fababean were studied in the Constantine region in the locality of Ibn Ziad (North-west of the Constantine city) during the 2019 flowering period. Apoid Hymenoptera were the most frequent insects on the plant and were mostly represented by the Apidae: the honey bee, *Eucera numida* Lep., *Bombus terrestris* L. and *Xylocopa violacea* L. The wild bee, *E.numida*, always performs a positive foraging on the flowers and all its visits are potentially fertilizing unlike the other three bees which always collect nectar from holes made at the base of the corolla. *Eucera numida* carries more compatible pollen grains on its body; *B.terrestris* and *X.violacea* carry a lot more foreign pollen. The presence of pollinating insects significantly improves the seed yield of fababean; the number of formed pods and seeds, and the seed weight (ten seeds) were significantly higher in open pollination; the pods of the bagged flowers also gave a percentage of aborted and wrinkled seeds higher than that obtained from the free flowers but statistical tests do not show significant difference.

Keywords

Pollinator, Pollinating Efficiency, Seed Yield, Foraging Behavior, *Vicia Faba*

1. Introduction

The faba bean (*Vicia faba* L. minor) is a legume native to southwest Asia (southern Caspian Sea) [1]. It is relatively rich in energy with an interesting protein value and it is mainly cultivated for animal feed (ruminants and poultry) in many parts of the world such as North Africa (Mediterranean Basin), South America, and Western and Northern Europe [2, 3, 4] and the main cultivating countries of the plant are China, Ethiopia, Australia, the United Kingdom and France.

The reproduction mode of *Vicia faba* L. is partially allogamous with allogamy rates varying from 20 to 70% depending on the genotypes, the populations, the abundance of pollinators and climatic conditions [5, 6, 7].

The literature concerning the pollination of *Vicia faba* L. (broad and fababean) is quite abundant; the main agents responsible for fertilizing the plant are bees such as bumblebees, in particular long-tongue species: *Bombus pascuorum* (Scopoli 1763) and *B. hortorum* (L. 1761) which legitimately forage on flowers without perforating the base of the corolla (8, 9, 10, 11]. Species of the genus *Eucera* (*Eucera numida* Lepeletier 1841 and *E. pulveracea* Dours, 1873) also frequent the plant [11, 12, 13]. In Egypt, *Anthophora hispanica* (Fabricius, 1787), *A. aegyptiaca* (Dalla Torre and Friese, 1895) and *Xylocopa pubescens* (Spinola, 1838) (Apidae) are also important foragers of the plant [14].

In Algeria, field bean production remains low; the cultivation of fababean is mainly located in mountainous regions,

in particular Kabylie, where it was used for human and animal food and the only cultivated sub-variety is "sidi Aich" [15]. This species has declined sharply since the development of livestock feed, but from the 1990s, the faba bean was studied for the nutritional value of its seeds with the aim of incorporating them into the diet of domestic animals including broiler chickens [16].

The present work documents for the first time a comprehensive list of insects visiting fababean flowers during the blooming period in the region of Constantine. The foraging behavior and the pollinating efficiency of the main visitors have been studied to determine which ones are most useful for the plant and which can help improve its yield. The effect of the entomophilious pollination on the seed production of the plant was also determined.

2. Material and Mehodes

2.1 Study area

Observations were conducted in a station of the locality of Ibn Ziad at North-west of the Constantine city (36° 22' 45''N, 6° 28' 19'' E, and 468 m). The station is a private plot of approximately two hectares. The plant (sub-variety Sidi Aich) is cultivated in small plots of $1m^2$ each; there are also other cultivated plants such lentils and wheat. Around or in the plots, there is natural herbaceous vegetation composed mainly by different melliferous species such as *Scolymus hispanicus* L., *Galactites tomentosus* L, *Silybum marianum* L. (Asteraceae); *Borago officialis* L., *Echium vulgare* L.,(Boraginaceae); *Hedysarum coronarium* L. (Fabacceae) and *Anethum graveolens* L. (Apiaceae). Peak flowering of these species occurred in late March and early April.

2.2 Count of forager's insects

Observations and measurements of insect flower visitors were made in 2019 during 11 days (24, 28-30 March and 1, 3, 12, 14, 17, 23 and 25 April). The flowering period was characterized by unfavorable weather conditions (rains) and several outings have been canceled (T° = 18.27° ± 2.83°C; HR = 50.54 ± 10.38 %). Counts were made at eight different times during the day (9:00-10:00, 10:00-11:00, 11:00-12:00, 12:00-13:00, 13:00-14:00, 14:00-15:00, 15:00-16:00, 16:00-17:00 ; GMT+1); the observer travelled during each hour the five plots which have been delimited inside the culture and recorded foraging insects present on flowers (one visit was recorded per specimen observed). A measurement of the floral density is also carried out to estimate the forager's density per100 flowers. This measure per unit of flowers is more appropriate in terms of pollination [11]. The collected specimens of bees were identified using keys and reference species.

2.3 Foraging behavior and pollinating efficiency of the bees

In parallel to the counting of foragers, some parameters were obtained for the most abundant species encoutered on the flowers (the honeybee, *Eucera numid*a, *Bombus terrestris* and *Xylocopa violacea*) such as visitation rate: number of flower visited per minute, contact with stigma: whether or not the bee touched the stigma of the visited flower and the purpose of visit: collect nectar, pollen or both rewards.

2.4 Pollen carried on the body of bees

Forager insects vary in the amounts of pollen carried on their bodies [17, 18, 19] or that is deposited on stigmas [20, 21]. In this work, we counted the pollen grains of the fababean transported on the body of the four major bees mentionned above to estimate their fidelity and their potential contribution in pollination of the plant. For pollen extraction, 10 females (pollen foragers for the honeybee) of each species were captured in plastic tube containing filter paper soaked with ethyl acetate to immediately kill insects. The hind legs of bees were removed first to exclude corbicular or scopa loads from the pollen counts because this pollen was not available for pollination [22]. Each individual was placed in a tube containing 70% alcohol completely covering the insect; the tubes were shaken with an agitator for about 15 min to dislodge pollen grains from the body of the insect. Individuals were removed from the tubes and the resulting pollen suspension centrifuged at 10,000 rpm for 15 min. The tubes containing pollen pellets were dried for 24 h at room temperature, and the pollen pellets were incorporated with a clamp into a glycerinated and colored gelatin. Gelatin was deposited in small sections on slides and melted on a hotplate; the slides were covered with coverslips and observed under an optical microscope (Optech, ×200). Slides with fababean pollen are prepared beforehand, and all fababean pollen grains and mixed pollen grains present on the slides were counted.

2.5 Effect of the cross-pollination on the seed yield

To determine the effect of the cross-pollination on the seed production of the plant, flower buds of various inflorescences were covered with tulle bags (1 mm mesh size) to prevent access to foragers. Once the flowers faded completely, the bags are removed and the numbers of pods and aborted pods are measured at the fruit set. The average weight of 10

seeds, the average number of seeds per pod and the numbers of aborted and wricled seeds are also noted.

2.6 Data analysis

Student's t-test was used to compare the average number of seeds per pod and the average weight of 10 seeds in free and self-pollination. The standard-score test (ε) was used to compare the proportions of formed and aborted pods, the proportions of aborted and malformed seeds in presence and absence of pollinators. The Kruskal-Wallis test (H test) and the Mann-Whitney test (test U) (non-parametric tests) were used to compare the numbers of fababean pollen grains carried out on the body of the four abundant bees encountered on the plant.

3. Results

3.1 Plant flowering

The flowering period of the faba bean lasted 36 days, from 20 March to 25 April 2019. Flowering is from bottom to top. On average, a plant has 10 inflorescences and each of which contains an average 5 flowers. The flowering of the plant evolves gradually to reach a peak on April 3 (1000 blooming flowers) then it gradually declines to end on april 25.

3.2 Inventory and density of forager's insects

During the flowering of the plant, various insects belonging to four orders: Hymenoptera, Diptera, Coleoptera and Lepidoptera visited the flowers of the fababean. Hymenoptera are the most abundant with 93% of visits recorded (table 1). This order is represented by the Apoidea with three families (Apidae, Megachilidae and Halictidae) and the vespoidea with a single family (vespidae). Bees are the most common with six observed species; *Apis mellifera* (L.1758), *Eucera numida* (Lepeletier, 1841), *Eucera eucnemidea* (Dours, 1873), *Bombus terrestris* (L. 1758) and *Xylocopa violacea* (L. 1758). The honeybee is the main visitor with 58% of observed visits and an average density / 100 flowers of 16 individuals and the second most abundant species, *E.numida*, recorded a density of 5 individuals/100 flowers (Table 1).

3.3 Foraging behavior and pollinating efficiency of bees

The most abundant bees visiting the fababean flowers collected pollen and nectar at different hours of the day. The two most locally dominant pollinators, *Apis mellifera* and *E.numida* differ in their foraging behavior; *E.numida* always collecs the floral products by the natural opening of the corolla and therefore all its visits can be fertilizing (Table 2). The honey bee collect the nectar through holes mad at the base of the corolla by other bees, so only 42.51% of its visits can bee fertilizing. This foraging behavior is also observed in *Bombus terrestris* and *X.violacea* that harvest the nectar outside the corolla, and only 59% and 64% respectively of their visits can be fertilizing. Concerning the visitation rate on flowers, the visits of the honeybee were slightly less frequent compared to those of the other three bees (Table 2). The visits of the honeybee and *Eucera numid*a were more intense from 11 a.m until 3 p.m and the proportions of pollen and nectar collected by the two bees during the day were almost similar (pollen: 39% and 44%, nectar: 57% and 56% respectively); the pollen was more collected in the morning and the nectar in the afternoon (Figure 1).

3.4 Pollen load carried by bees

The results of counting pollen grains of *Vicia faba* revealed that *E.numida* carried the highest pollen load compared to the other three bees (Figure 2) ($H = 22.81$; $p < 0.01$). The wild bee, transported significantly higher pollen of fababean than the honeybee ($U = 100$; $p = 0.0002$). *B.terrestris* and *X.violacea* carried on their body more mixed pollen than the fababean pollen.

3.5 Effect of the entomophilious pollination on the seed yield

The yield of the plant is higher in the presence than in the absence of pollinators. The percentage of pods formed (69%) from uncovered flowers is greater than that obtained from bagged inflorescences (46%) ($\varepsilon = 2.56$; $p = 0.05$) and the average weight (of 10 seeds) obtained in open pollination (10.10 ± 0.94g) is significantly higher than that obtained in self-pollination (6.29 ± 1.11g) ($t = 8.13$; $p = 0.01$) (Table 3).

The average number of seeds/ pod (3) in open pollination was a little higher than that obtained in self-pollination (2) (Table 3) but the statistical test (Student's test) indicates a significant difference ($t = 7.63$; $p < 0.01$). The average number of seeds per inflorescence was also significantly higher in open pollination (8.40 ± 1.8) than in self-pollination (2.8 ± 3.86) ($t = 6.58$; $p = 0.0001$). The percentage of dropped pods (54%) was also higher in self-pollination compared to open pollination (31%) ($\varepsilon = 1.95$; $p < 0.05$). The pods of the bagged flowers gave a percentage (6%) of aborted and wrinkled (14%) seeds higher than that of the pods from the free flowers (respectively 1.36% and 3.18%) but statistical tests do not show significant difference.

Table 1. Densities of foragers insects encountered on fababean (*Vicia faba* L. minor) in the locality of Ibn Ziad (2019 flowering period)

Foraging insects	Num.of specimens	% of visits	Avg dens/100 flow
1-Hymenoptera			
a-Apoïdea			
*Apidae			
Apis mellifera (L. 1758)	1026	58.33	15.86
Eucera numida (L. 1841)	352	20.01	5.44
Eucera eucnemidea (Dours. 1873)	21	1.19	0.32
Bombus terrestris (L.1758)	124	7.05	1.92
Xylocopa violacea (L. 1758)	80	4.55	1.23
* Megachilidae			
Megachile ericetorum (L. 1841)	7	0.40	0.11
Megachile parietina (Geoffroy. 1785)	5	0.28	0.08
Rhodanthidium siculum (Spinosa, 1838)	4	0.23	0.06
* Halictidae			
Lasioglossum discum (Smith, 1853)	4	0.23	0.06
b-Vespoidea			
*Vespidae (gn.sps indt)	18	1.02	0.28
Total	1641	93.29	25.36
2- Lepidoptera			
*Pieridae			
Pieris brassicae (L. 1758)	36	2.05	0.56
Pieris rapae (L. 1758)	21	1.19	0.32
Pyronia tithonus (L. 1758)	14	0.79	0.22
Total	71	4.03	1.1
3-Diptera			
*Syrphidae			
Eristalis tenax (Latreille, 1804)	3	0.18	0.04
Eristalis sp	5	0.28	0.08
Sphaerophoria sp	4	0.23	0.06
*Tabanidae			
Tabanus spp.	24	1.36	0,38
Total	36	2.05	0.56
4-Coleoptera			
*Coccinellidae			
Coccinella septempunctata (L. 1758)	6	0.34	0.09
Adalia bipunctata (L. 1758)	5	0.29	0.08
Total	11	0.63	0.17
Final total	**1759**	**100**	**27.19**

Table 2. Havested floral products, proportion of fertilizing visits and visitation rate of the four main bees encountred on faba bean flowers during flowering period of 2019 (N = number of observed specimens; P = pollen; N = nectar; (+) = positif; (-) = negative)

Species	*A.mellifera*		*E.numida*		*B.terrestris*		*X.violacea*	
Rewards	N = 661		N = 224		N = 96		N = 62	
P^+	257	38.88%	98	43.75%	57	59%	40	64%
N^+	00	00%	126	56.25%	00	00%	00	00
N^-	380	57.48%	00	00%	39	41%	22	36%
$(P + N)^+$	24	3.63%	00	00%	00	00%	00	00
% of positiv visits	42.51%		100%		59%		64%	
N	50		50		32		35	
Visitation rate	4.66 ± 0.97		5.74 ± 1.29		6.50 ± 0.80		5.68 ± 0.36	

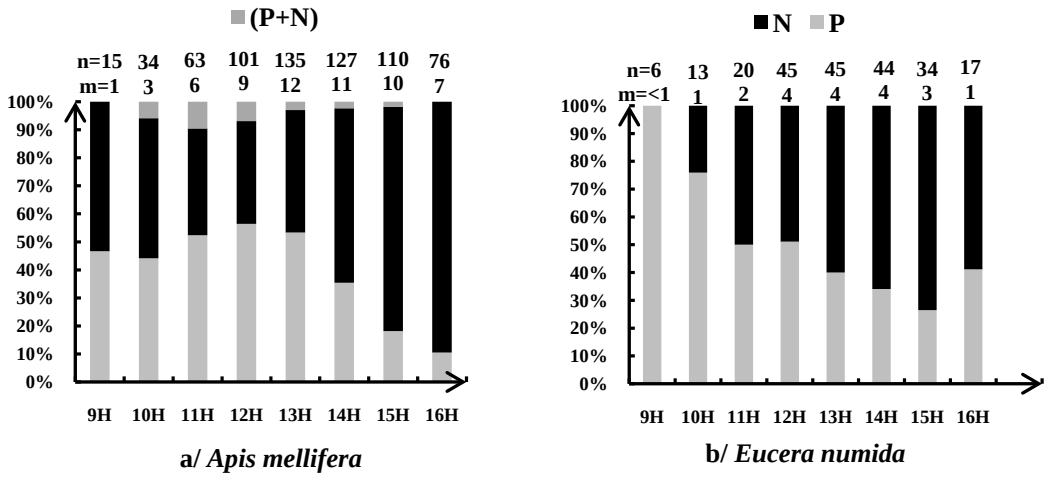

Figure 1. Percentages of collected rewards by *Apis mellifera* (a) and *Eucera numida* (b) on Faba bean during different hours of the day (flowering period of 2019) (n= total number of observed specimens; m= average number of observed specimens, N= nectar; P= Pollen).

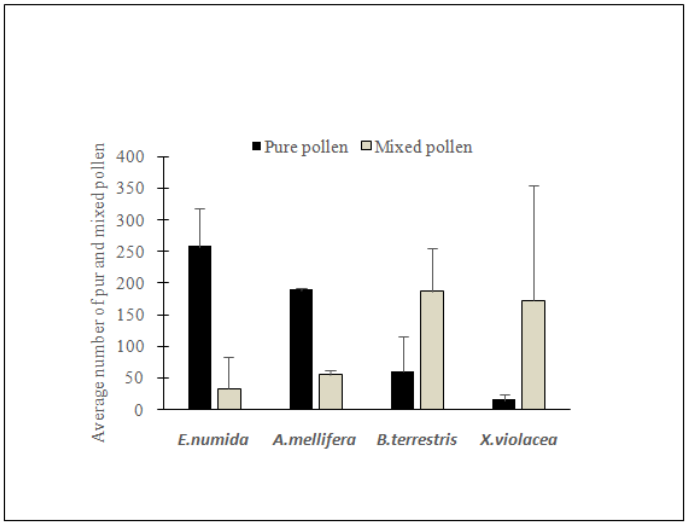

Figure 2. Average number (± ET) of pur and mixed pollen carried on the body of the bees during the flowering period of the fababean (2019) (number of sampled females = 10).

Table 3. Yield's parameters of spring Fababean in self and free pollination during flowering period of 2019 (ε = Standard-score test; t= Student test; S = significant; NS = No significant)

Treatments	Self Pollinisation		Free Pollinisation		Statistical Test
Number of flowers (25 infl)	122		121		
Number of formed pods	35	46.05%	68	68.69%	ε =2.56; p=0.05 (S)
Average weihgt of 10 seeds	6.29 ± 1.11g		10.10 ± 0.94g		t=8.13; p=0.01 (S)
Average number of seeds/pod	2.00 ± 0.64		3.09 ± 0.71		t=7.63; p<0.01 (S)
Average number of seeds/Inf	2.8 ± 3.86		8.40 ± 1.78		t=6.58; p=0.0001 (S)
Number of aborted pods	41	53.95%	31	31.31%	ε = 1.95; p<0.05 (S)
Number of wrincled seeds	12	13.95%	7	3.18%	ε = 0.31; p>0.05 (NS)
Number of aborted seeds	5	5.81%	3	1.36%	ε = 0.76; p>0.05 (NS)

4. Discussion

The observations carried out during the flowering of the fababean in 2019 showed that several insects represented by Lepidoptera, Coleoptera and Diptera frequent this forage plant but hymenoptera were more abundant. These latter are represented by the honey bee and the solitary bee, *E.numida*, which recorded the highest densities (respectively 16 and 6 individuals/100 flowers). The densities of *Bombus terrestris* and *X.violacea* were much lower. In France, [11] found that the honeybee was the most common visitor on different faba bean lines but the *Eucera* bees seem to have a preference for *Vicia faba* flowers. In the south of Spain, *E.numida* was the most frequent visitor represented 89% of pollinating insects of the plant. In the region of Constantine and during the period from 2000 to 2002, this bee was the main pollinator of broad bean with a proportion of visits of 70% [12] while in the region of Tizi-Ouzou (mid Algeria), an another *Eucera* (*Eucera pulveracea* Dours) was the most abundant insect on the flowers [13].

Several works have also mentioned the presence on the plant of bumblebees (*Bombus hortorum* L, 1761) *B.ruderatus* (Fabricius,1775), *B.pascuorum* (Scopoli, 1763)*; B.agrorum* (Fabricius, 1787)*; B.lucorum* (L,1761) and *B.terrestris* L.). Carpenter bees (*Xylocopa violacea* L., *Xylocopa aestuans* (L, 1758) also frequent the flowers [23, 24, 11, 12, 25]. In Egypt, [14] also noted the presence of different species on Broad bean, including *Andrena ovatula* (Kirby, 1802) (Andrenidae), *Chalicodoma siculum* (Rossi, 1792) (Megachilidae), *Anthophora hispanica* (Fabricius, 1787) and *Xylocopa bubescens* (Spinola, 1838) (Apidae).

Bee visits are synchronized with nectar secretion and pollen production during the day [26, 27, 28, 29]; the visits of the honey bee and *E.numida* were more frequent in the morning from 11h and in the afternoon until 15h. During this period, more nectar and pollen are producted since all new flower buds open in the afternoon [23, 30, 31, 26].

The type of foraging of a bee depends on the floral morphology; long-tongued bees can reach the nectar of flowers whose corolla is deep, this is the case of the wild bee *E.numida* that exhibited legitimate flower visits because it collects nectar by the opening of the corolla while the honey bee performs a non-fertilizing foraging when it collects this product from holes made by bumblebees at the base of the corolla ("nectar robbers") and only 42% of its visits can be fertilizing. This foraging behavior is also observed on the plant by several authors [23, 31, 32, 11, 12, 25]. The two other bees, *B.terrestris* and *X.violacea* also mostly robbed nectar and did not provide efficient pollination service. [13] and [25] also noted that these two bees practice negative foraging on flowers when they collect nectar.

Analysis of the pollen load of the four bees showed that *Eucera numida* carried a greater quantity of *Vicia faba* pollen on its body, which indicates that the bee was more constant in its foraging on the plant; moreover, all its visits are made through the opening of the corolla, which allows its body to always be in contact with the anthers. [12] found this bee showed high plant fidelity since it did not visit wildflowers around the plant throughout the flowering period. The honeybee does not seem as well adapted to the flower morphology compared to the eucera bee since very often it fails to open the closed corollas. This behavior was also observed by [12].

Insect pollination is essential to improve bean seed production. In presence of pollinators, the numbers of formed pods and seeds were significantly higher than those obtained without insects; also the seeds were larger and better formed. Similar results were obtained by several authors who found that the yield of the bean is always improved in the presence of insects [33, 34, 32, 35].

In Conclusion, *Eucera numida* is certainly the best pollinator of the plant in the region despite its low abundance. Unfortunately, the populations of this species have greatly declined; the observations made on the plant during the last five years have shown a clear decline in the populations of this bee (Benachour et al., Unp data). The honey bee, although less effective and less faithful to the plant, remains a very useful pollinator and the presence of sufficient colonies in flowering fields ensures adequate pollination of the plant.

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