

## Foraging Behavior of the Honey Bee (*Apis mellifera* L.) upon the Male and Female Flowers of Squash (*Cucurbita pepo* L.) (Cucurbitaceae) in the Region of Tizi-Ouzou (Algeria)

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Received on 29.06.2019

Accepted on 09.08.2019

### Abstract:

Cucurbitaceae species depend on pollination by bees for fruit production. The objective of this present work was to identify the insect visitors of squash and to study the foraging behavior of the most abundant species in the field. Identification of foraging insects of *Cucurbita pepo* L. (Cucurbitaceae) were performed during 2016 and 2017 flowering periods in the region of Tizi-Ouzou (Algeria). Observations showed that the majority of the insects visiting squash flowers were Apoides Hymenoptera belonging to two families: Apidae and Halictidae. Six species of bees were recorded upon the plant, of which the honey bee *Apis mellifera* L. was the most frequent species. The study of the foraging activity of these insects showed that their visits to squash flowers were more intense at 8am and 9am and were essentially devoted to nectar harvesting. Bee visits proportions were higher on staminate flowers than on pistillate flowers. The duration of visits to flowers was significantly longer in staminate flowers.

### Keywords:

*Apis mellifera*, behavior, *Cucurbita pepo*, foraging insects, honey bees, squash

## INTRODUCTION

The plants belonging to Cucurbitaceae family have been extensively cultivated and used in different ways by humans. They were introduced into cultivation more and more readily due to their dietetic fruit. Squash (*Cucurbita pepo* L.) (Cucurbitaceae) come from America and its cultivation was widespread in Central and South America and worldwide (Hugh et al., 1961). In Algeria, it is one of the most widespread crops thanks to the climatic conditions and the type of soil that are very favorable to it. Currently, its production exceeds 227 000 tones and doubled since 2000 (Sheriff, 2014). Squash is relatively easy to produce but the regularity of the production and the good commercial quality requires a good pollination which is ensured by the contribution of a quantity of pollen sufficient to fertilize the maximum of ovules (Vilain, 1997).

*Cucurbita pepo* is a monoecious and self-compatible plant bearing two types of flowers: male and female. These flowers have a short lifespan and each female flower has a time frame of about 3 hours to be fertilized (Mazollier, 2012). The number of male flowers is higher than that of the female flowers, which is necessary to ensure the fertilization of the latter (Tepedino, 1981). It is pollinated mainly by bees; the pollen grains in *C. pepo* are too large and sticky to be carried by the wind and highly favour insect pollination (Hodges and Baxendale, 1995; Eischen, 2000).

Cucurbitaceae dependence upon entomophilous pollination for fruit production is well established (Mc Gregor, 1976; Free, 1993). Squash plant requires the presence of insects for fruit production (Nevkryta, 1953; Battaglini, 1969; Wolfenbarger, 1962; Wadlow, 1970; Wills and Wearing, 1993; Alen Walters et al., 2006; Benachour, 2008). Thus, in *C. pepo*, the honeybee is the most common visitor to the plant (Avila et al., 1989; Nepi and Pacini, 1993; Greatti et al., 1997; Couto et al., 1999; Benachour, 2008; Das Graças Vidal et al., 2010) and their importance is more in areas where wild bees are absent or their number is unpredictable.

As for all Cucurbitaceae, the success of squash pollination depends on insect visits to staminate and pistillate flowers. Thus, the present work, carried out during two consecutive bloom periods of 2016 and 2017 in the region of Tizi-Ouzou, aims to identify the insect visitors of squash and to study the foraging behavior of the most abundant species in the field.

## MATERIALS AND METHODS

### Study Station

The study was conducted during bloom period of the dry season of 2016 and 2017 in the region of Tizi-Ouzou, located at 100 km East of Algiers (Algeria). The chosen site (90 m<sup>2</sup>) is located in an agricultural fallow at Boukhalfa, at 8 km away from the city of Tizi-Ouzou (36° 43'N 4° 00'E, 180 m). Observations were carried out in a plot of 30 m<sup>2</sup> (10 m long and 3 m wide) planted with variety of squash called verte d'Alger. Seedlings were carried out on 22. IV. 2016 and 18. IV. 2017 in rows spaced 60 cm at the density of 1 plant per m<sup>2</sup>

The study plot is characterized by abundant and diversified herbaceous vegetation. The main melliferous species growing at the edges of the plot were *Urospermum dalechampii* L., *Centaurea pullata* L. (Asteraceae), *Convolvulus tricolor* L. and *Convolvulus arvensis* L. (Convolvulaceae).

### Inventory and density of pollinating fauna

The quadrat method was used for observation and counting of pollinators and floral density (Sonnet and Jacob-Remacle, 1987; Abrol, 1988). Nine squares (quadrats) of 1 m<sup>2</sup> each were delimited by means of wires and piles in the first two rows from the edge of the crop. The distance between two quadrates was 60 cm long. All quadrats were exposed to the sun and benefited from the same watering performed every three days.

Every two days and from the flowering, the observer travels the quadrats from 7 am until 11 am (Gmt + 1). For duration of 7 to 8 minutes, foraging insects were counted in each quadrat. Specimens of each species encountered were captured for laboratory identification and constitution of reference material.

Parallel to counting, a measure of the flowers density was performed in each quadrat. The density of insects was thus estimated by relating the number of insects counted to a number of 100 flowers. This method is currently the most frequently used to compare pollinator densities in crops (Pierre et al., 1997, 1999).

### Apoids foraging behavior

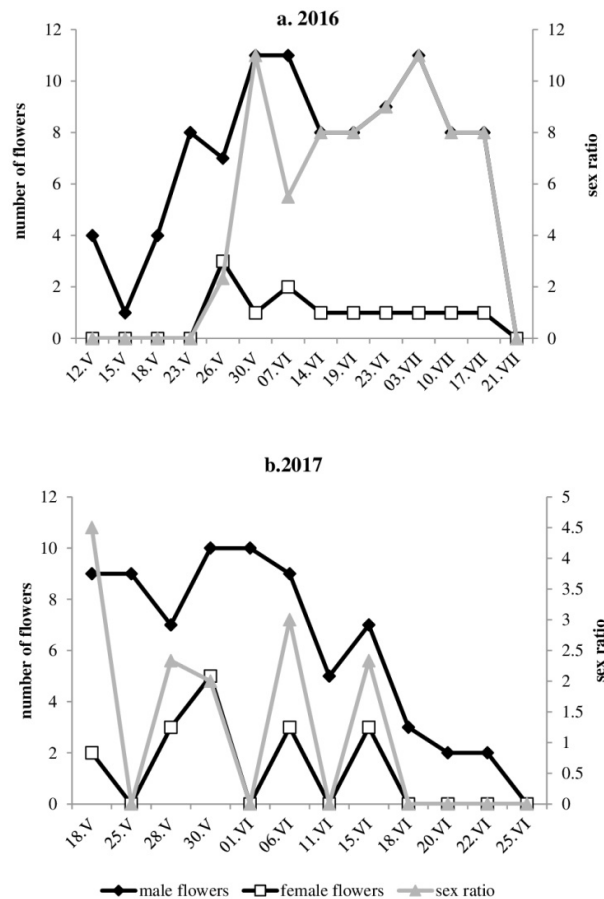
In order to assess the pollinating efficiency of the most frequent bee species during both flowering periods of 2016 and 2017 (in 2017, the behavior of wild bees on flowers was not studied because the number of visits was very low), observations of foraging behavior were conducted to determine the nature of the visits (positive or negative by noting whether the browsing insect touches or not the

stigma of the flower). Visits showing stigma-insect contact were described as pollinating or positive visits (Jacobe-Remacle, 1989) as opposed to negative visits which do not allow the flower fertilization. Simultaneously, the dietary purpose of the visits made by the various pollinators (nectar or pollen) was noted. Another parameter for determining pollinator efficacy was considered. It consists to determine the number of staminate and pistillate flowers visited by the honey bee and to measure the time spent by this species on each type of flower during the flowering season of 2016 and 2017.

## RESULTS AND DISCUSSION

### Plant flowering

The bloom of *C. pepo* started on May 12 for the year 2016 and May 18 for 2017 and was spread over 70 and 38 days, respectively. Plant flowering was less important in 2017 due to a drier winter compared to 2016. The total production of staminate flowers (males) and pistillate flowers (females) during both blooms is shown in Figure 1.



**Figure 1: Production of male and female flowers and floral sex ratio (FSR: ♂ / ♀) during 2016 (a) and 2017 (b) blooms of *Cucurbita pepo* plants observed in the region of Tizi-Ouzou**

In 2016, the beginning of flowering was characterized by an increase in the number of male flowers that stabilized in mid-season and decreased at the end of flowering. Female flowers appeared only on

the fourth day of observation and their number was almost stable throughout the season (Figure 1 a). Whereas in 2017, male and female flowers appeared at the same time and their number varied constantly throughout the flowering period. At the end of the season, the pistillate flowers disappeared before the staminate flowers (Figure 1 b).

The sex ratio of the two populations also varied over time showing average values of 7 ♂ / 1 ♀ in 2016 and 6 ♂ / 1 ♀ in 2017.

### Pollinator diversity and density

The observations conducted during the bloom period of squash in 2016 and 2017 showed that the insects foraging *C. pepo* flowers were mainly Apidae Hymenoptera belonging to two families: Apidae and Halictidae. Six species of bees were recorded on the plant (Table 1). The species *Apis mellifera*, *Bombus terrestris* (Apidae) and *Lasioglossum villosulum* (Halictidae) were present during both flowering periods. The honeybee recorded the largest number of visits. Several investigations conducted in Algeria (Benachour, 2008), Italy (Nepi and Pacini, 1993), Brazil (Krug et al., 2010) and United States of America (Esther Julier and Roulston, 2009) have also found that *A. mellifera* is the most frequent visitor on the squash. In India, Devika Rani et al., (2017) revealed the presence of four *Apis* species on *C. pepo* while, in the United States, Cane et al., (2010) recorded *Peponapis pruinosa* (Apidae) as the most abundant species on the plant.

The densities of the bees per 100 flowers are however overestimated because the foragers were dispersed upon only a few flowers. The Apidae, *Eucera eucnemidea* was observed only during the bloom period of 2016, while both Halictidae *Lasioglossum malachurum* and *Lasioglossum glabriusculum* were observed only during the bloom period of 2017.

**Table 1: Densities per 100 flowers of *Cucurbita pepo* pollinators during 2016 and 2017 flowering seasons in the region of Tizi-Ouzou (absence)**

Family	Species	2016	2017
Apidae	<i>Apis mellifera</i> L. 1758	37	73
	<i>Bombus terrestris</i> L. 1758	1	1
	<i>Eucera eucnemidea</i> Dours, 1873	-	1
Halictidae	<i>L. (Evylaeus) malachurum</i> Kirby 1802	5	-
	<i>L. (Evylaeus) villosulum</i> Kirby 1802	5	3
	<i>L. (Evylaeus) glabriusculum</i> Morawitz, 1872	1	-

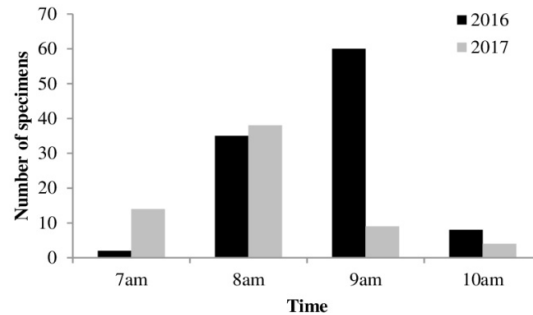
### Foraging behavior and pollinator efficiency

#### Foraging behavior

Squash flowers have a short lifespan and each female flower has a short duration of about 2 to 3 hours to be fertilized.

The honey bee visits to the flowers were more frequent during the second and third observation periods (8am and 9am) when a maximum of flowers blossomed. However, in 2017, *A. mellifera* recorded a maximum of visits during the first and second observation period (7am and 8am). From 10 am, bees visits decreased because many flowers began to close (Figure 2). These results are similar to those of Sanduleac (1959), Benachour (2008) and Mudssar et al. (2014) who reported intense bee visits between 8am and 9am. According to Tepedino (1981), squash flowers usually open shortly after dawn and close permanently at noon or shortly thereafter, often receiving early and frequent morning pollinator visits. Indeed, this period corresponds to the maximum production of pollen and nectar resulting in intense bee activity. This important activity of the bees ensures an adequate pollination of

the plant since the flowers, like those of other Cucurbitaceae, remain open only one day and for only a few hours.



**Figure 2: Numbers of *Apis mellifera* visits recorded on *Cucurbita pepo* at different observation times during flowering 2016 and 2017.**

The majority of honey bee visits involved the nectar harvest recording a percentage of 81% in 2016 and 86% in 2017. The percentages of 19% and 14%, respectively in 2016 and 2017, were devoted to pollen harvesting. Most of wild bee visits, of about 93%, were also devoted to nectar harvesting. Although female flowers produce more nectar than male flowers, bees prefer that of staminate flowers (Table 2). Our results are consistent with those of Benachour (2008) who pointed out that honey bee floral visits concern the exclusive harvesting of nectar which is secreted in large quantities by the flowers (Tepedino, 1981; Nepi and Pacini, 1993). On the other hand, pollen is little harvested by the bee and staminate flowers do not produce much pollen which is mostly abundant during the early hours of anthesis (Nepi and Pacini, 1993). Similar results found by Michelbacher *et al.* (1964) and Philippe (1991) noted also that this product is rarely harvested by bees. However, Devika Rani *et al.* (2017) revealed that more than 47% of honey bee visits are devoted to pollen harvesting and 19% to nectar harvesting.

On the female flowers and before entering the bottom of the corolla to collect the nectar, the honey bee first lands on the stigma on which it moves. Sometimes it makes these movements before leaving the flower. Thus, honey bee visits on pistillate flowers are all potentially pollinating.

On the male flowers, the bee lands on the anthers and scrapes with its forelegs to collect pollen. To collect the nectar, the insect lands near the edge of the corolla and goes to the bottom of it. When leaving the flower, the body of the bee is completely covered with pollen. It spends long moments to brush itself and the pollen often falls in small piles.

#### ***Pollinator efficiency***

The pollinating efficacy of bees on squash, which is a monoecious plant, was estimated by the number of visits to male and female flowers (Table 2).

The proportion of male (staminate) flowers visited by the honeybee was higher than that of female flowers in 2016. In 2017, 72% of bee visits were also made to staminate flowers (Table 2). This results can be explained by their number which was higher compared to that of the female flowers. Our results corroborate those obtained by Benachour (2008) and Mudssar *et al.* (2014) who commented that male flowers are more visited because the sugar concentration of the nectar produced by these flowers is higher. However, other investigations (Tepedino, 1981; Couto *et al.*, 1990; Philippe, 1991; Nepi and Pacini, 1993) demonstrated that female flowers produce more nectar than male flowers and are more frequently visited by bees.

**Table 2: Visits distribution (%) of *Apis mellifera* and wild bees on *Cucurbita pepo* according to the floral product harvested during 2016 and 2017 in the region of Tizi-Ouzou.**

Flowering	2016								2017			
Species	Honey bees				Wild bees				Honey bees			
Harvested product	n		%		n		%		n		%	
N+ (female)	22		21		18		32		22		28	
N (male)	63	83*	60	79*	34	38*	61	68*	46	55*	60	72*
P (male)	20		19		4		7		9		12	
Total number of visits	105		100		56		100		77		100	

N = nectar; P = pollen; +: pollinating visit; n= number of specimens observed;

\*= total number of visits on male flowers.

### Duration of visit on the flowers

The pollinating efficiency of bees on flowers also depends on the time the insect spends on both types of flowers. If the visit time of a species is long, the rate of its visits would be so low that the number of pollinating visits is low. We have, therefore, measured the time spent by the honeybee on both types of flowers.

The results (Table 3) revealed that during the bloom of 2016 and 2017, the bee spent significantly more time on pistillate flowers than on staminate flowers (Mann Whitney test (U) = 5, p <0.05). Similarly, Tepedino (1981), Nepi and pacini (1993) and Benachour (2008) noted that the bees spent more time on pistillate flowers because of the high amount of nectar and nectary position.

**Table 3: Visit duration of *Apis mellifera* on *Cucurbita pepo* flowers during the bloom of 2016 and 2017.**

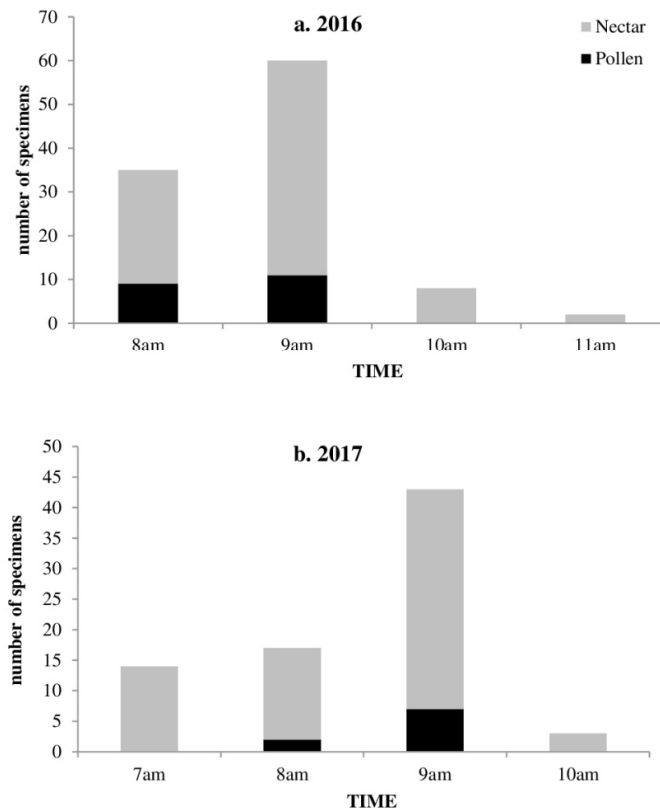
Flowering	Duration of visit on the male flowers (second)	Duration of visit on the female flowers (second)	U	P
2016	26.5 ±10.67	46.66 ± 15.61	140	0
2017	26 ± 10.61	50 ± 14.38	95	0

±: standard error; n =30: number of specimens observed.

Mann Whitney test (U), p <0.05.

### Food research of the honeybee

The major of the honey bee floral visits during the two blooms concerned the harvest of nectar (Figure 3) and the collection proportions were higher from 8am to 9am in 2016 and at 9am in 2017. The pollen was slightly harvested at 9am during the two blooms. In India, Devika et al. (2017) noted that pollen was collected mainly from 8am to 10 am. As for the nectar, it was harvested in excess of 10 am to 12 am. Quant au nectar, il a été récolté plus de 10 heures à midi. According to Vidal et al. (2006), the production of nectar by the male flowers is very small at 7 o'clock and it increases as temperatures and solar radiation increase. However, pollen is collected in abundance during the early hours of the day (Vidal et al., 2010).



**Figure 3: Food research of *Apis mellifera* on *Cucurbita pepo* at observation times during the 2016 and 2017 flowering periods.**

## CONCLUSION

In the region of Tizi-Ouzou as in Constantine (Algeria), Italy, Brazil and the United States of America, *A. mellifera* remains the main pollinator of *C. pepo* with a foraging which could be fruitful in 100% of visits and a number of visits that represents more than 76% of the total number of visits observed. Two species of Halictidae namely *L. malachurum* and *L. villosulum* were also very effective for squash pollination.

Because of the relative abundance of the honey bee on *C. pepo* flowers and its potentially fertilizing visits to pistillate flowers, this species can be considered as the main pollinator of *C. pepo* in the region. In the absence of wild pollinators, and in order to ensure adequate pollination of crops, the introduction of sufficient colonies in the fields is thus recommended.

It is also suggested to conserve and protect nesting sites in neighboring areas of crops and to avoid unjustified use of pesticides during the flowering period. These apoids can play a significant role in the pollination of crops where their density is quite high affecting thus squashes production. Indeed, the pollination carried out by these insects allows obtaining fruits of good commercial quality with important yields.

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