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(RESEARCH ARTICLE)



Impact of the foraging activity of *Apis mellifera* (Hymenoptera: Apidae) on increasing yields of *Gossypium hirsutum* (Malvaceae) in Djoumassi (Garoua, Cameroon)

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Abstract

To evaluate the impact of the foraging behaviour of *Apis mellifera* in increasing boll and seed yields of *Gossypium hirsutum*, its pollination activities were studied in Garoua for two seasons, from September through October 2018 and 2019. Observations were made on 540 flowers divided into four treatments of which two were differentiated according to the presence or absence of flower protection regarding insect visits; the third was made of protected and opened flowers, to allow *Apis mellifera* visits and the fourth made of opened flowers then closed without any visit. The bee's daily rhythm of activity, their foraging and pollination activities were evaluated. Results showed that, on *Gossypium hirsutum* flowers, *Apis mellifera* intensely collected pollen and nectar. Data obtained allow the classification of this Malvaceae as a highly polliniferous and nectariferous bee plant species. Through its pollination efficiency, *Apis mellifera* has increased the fruiting rate by 29.66% and 13.95%, the mean number of seeds per boll by 24.53% and 14.89% and the percentage of normal seeds by 3.89% and 8.87% in 2018 and 2019 respectively. Hence, the conservation of *Apis mellifera* colonies close to *Gossypium hirsutum* fields is recommended to improve its boll and seed yields.

Keywords: Honeybees; Cotton; Flowers; Pollination efficiency; Yields

1. Introduction

Pollination is a vital ecosystem service, an estimated 87.5% of all flowering plants rely on insect pollination [1, 2]. Crop-insect pollinator interactions are very important to maintain pollinator populations, ensure pollination services, increase honey yields and therefore benefit beekeepers [3]. The most important crop pollinator species is the western honeybee (*Apis mellifera* L.), providing roughly 50% of global crop pollination [4]. Worldwide, Honeybees are among the major pollinators of the world's cultivated food and cash crops, such as *G. hirsutum* (Malvaceae) [5, 6].

Gossypium hirsutum is grown for its seeds rich in lipids (36%) and fiber used in textile industry [7]. Cotton leaves are trilobed and flowers are cream and produce approximately 20,000 cream pollen grains per flower and produce low volume of nectar [8, 9, 10]. Considered as a major bee plant species [6], cotton is primarily self-pollinating, although out-crossing can occur [11]. Before our work, the flowering insects of *G. hirsutum* were studied in Cameroon [12, 5, 6] and in Australia [13, 14] where *A. mellifera* was the most frequent visitor on *G. hirsutum* flowers. Near Narrabi in New South Wales, no bee was seen on its flowers, and although small number of wasps and flies were recorded [15]. In USA [8] and in Brazil [16], Bumblebees (*Bombus* sp.) and Apoids are respectively the main cotton pollinators' insects. In Paris, [7] reported that laying *A. mellifera* colonies in cotton plantations leads to increase yields of 5 to 30%.

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Entomofauna of a plant species can vary from one region to another [17], it is therefore, important to deepen the investigations on the relationship between *G. hirsutum* and *A. mellifera* in Cameroon, to supplement the existing data. The main objective of this work was to contribute to the understanding of the relationships between *G. hirsutum* and *A. mellifera*, for their optimal management in Garoua. Specific objectives were to: (a) determine the place of *A. mellifera* in *G. hirsutum* flowering insects; (b) study the activity of this Apidae on cotton flowers; (c) evaluate the impact of flowering insects including *A. mellifera* on bolls and seeds yields; (d) estimate the pollination efficiency of *A. mellifera* on this plant species.

2. Material and methods

2.1. Study site, experimental plot and biological material

The studies were conducted from June to October, in 2018 and 2019, in the locality of Djoumassi (latitude of 9°23'16.512' N, a longitude of 13°23'20.627 E, an altitude 169 m.a.s.l.), Benoue Division, in the city of Garoua, North Region (Cameroon). This region belongs to the Sahel-Sudanian ecological zone (MINEPAT, 2014). It has Sudanian climate type characterized by two annual seasons: a rainy season (April to October) and a dry season (November to March). August is the wettest month of the year [18, 19]. Annual rainfall varies from 750 to 1250 mm and the mean annual temperature is 27 °C [20, 19].

The experimental plot was an area of 437 m². The animal material was mainly represented by insects naturally present in the environment and four and two colonies of *A. mellifera* in 2018 and 2019 respectively, located near the experimental field. The plant material was *G. hirsutum* seeds obtained from the Institute of Research and Agricultural Development (IRAD) of Garoua.

2.2. Preparation of experimental plot, sowing and weeding

From 24th to 29th June 2018 and from 03rd to 06th July 2019, the experimental plot was cleaned and divided into eight subplots, each measuring 8*4.5 m². Four seeds were sown per hole and six lines per subplots were made, each line having six holes. Holes were separated by 50 cm from each other, while lines were 75 cm. Weeding was performed manually during the flowering periods [5].

2.3. Determination of the reproduction mode of *Gossypium hirsutum*

On September 25th, 2018, 240 flowers from untreated subplots at the bud stage were labeled among which 120 flowers were left unprotected (treatment 1), while 120 others were bagged using gauze bags nets (treatment 2) to prevent insects' visits (Fig. 1). In similar subplots, on October 04th, 2019, 240 flowers at the bud stage were labeled of which 120 were unprotected (treatment 5), while 120 were bagged (treatment 6). For each cropping year, two weeks after shedding of the last labeled flower, the number of bolls was assessed in each treatment. The fruiting index was then calculated as described by Tchuenguem et al. (2001) [21] : $Pi = Fb/Fa$, where *Fb* is the number of bolls formed and *Fa* the number of viable flowers initially set. The allogamy rate (*TC*) from which derives the autogamy rate (*TA*) was expressed as the difference in fruiting indexes between treatment *X* (unprotected flowers) and treatment *Y* (bagged flowers) [21]. $TC = [(PiX - PiY) / PiX] * 100$, where *PiX* and *PiY* are respectively the mean fruiting indexes of treatment *X* and treatment *Y*; $TA = 100 - TC$.

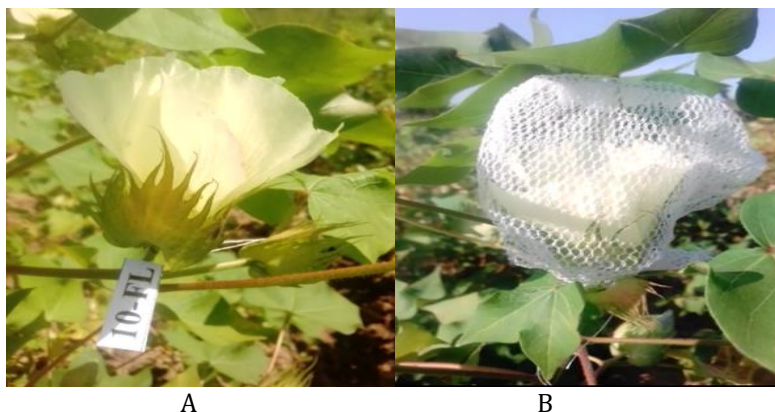


Figure 1 Unprotected (A) and protected (B) flowers of *Gossypium hirsutum* from insects with gauze bag at Djoumassi

2.4. Study of the foraging activity of *Apis mellifera* on *Gossypium hirsutum* flowers

From the opening of the first flower (ocured the 26th September 2018 and 05th October 2019) to the fading of the last flower (02nd October 2018 and 12th October 2019), observations were done on flowers of treatments 1 and 5, according to six daily time frames: 6 - 7 h, 8 - 9 h, 10 - 11 h, 12 - 13 h, 14 - 15 h and 16 - 17 h. Flowering insects that visited *G. hirsutum* flowers were recorded at each daily time frame during the blooming period. All insects encountered on flowers were recorded and the cumulated results expressed as number of visits have been used to determine the relative frequency of *A. mellifera* (F_x) among flowering insects of *G. hirsutum*.

For each year of study, $F_x = [(V_x / V_i) * 100]$, where V_x is the number of visits of *A. mellifera* on flowers of free treatment and, V_i the total number of insect visits on flowers of the same treatment [21].

During our investigations, before starting with the record of visit, the number of opened flowers was counted. The same days as for the registration of frequency of visits, the floral products (nectar and/or pollen) collected by each worker bee were recorded for the same date and daily time frame. The study of this parameter indicates whether *A. mellifera* is strictly polliniferous and / or nectariferous on *G. hirsutum* flowers [22]. This can give an idea of its involvement in the pollination of this plant and honey production. The duration of the individual flower visits was recorded (using a stop watch) according to six daily time frames: 7 - 8 h, 9 - 10 h, 11 - 12 h, 13 - 14 h, 15- 16 h and 17 - 18 h. The foraging speed expressed as the number of flowers visited by a worker bee per minute according to Jacob - Remacle [23] was calculated using the following formula : $V_b = (F_i/d_i) * 60$ where d_i is the time given by the stopwatch and F_i is the number of flowers visited during d_i .

The abundance of foragers (highest number of individuals foraging simultaneously) per flower or per 1000 flowers ($A1000$) were recorded on the same dates and time slots as the duration of visits. Abundance per flower was recorded as a result of direct counting. To determining the abundance per 1000 flowers, some foragers were counted on a known number of opened flowers and $A1000$ was calculated using the following formula: $A1000 = [(A_x / F_x) * 1000]$, where F_x and A_x are respectively the number of flowers and the number of foragers effectively counted on these flowers at time x [24]. The disruption of the activity of foragers by competitors and / or predators and the attractiveness exerted by other plant species on this insect was assessed by direct observations. For the second parameter, the number of times the worker bee went from *G. hirsutum* flowers to another plant species' flowers and vice versa was noted throughout the periods of investigations. During each day, temperature and relative humidity of the station were recorded after every 30 minutes using a mobile thermo-hygrometer (HT-9227) installed in the shade.

2.5. Evaluation of the effect of insects including *Apis mellifera* on *Gossypium hirsutum* yields

The evaluation of the effect of insects including *A. mellifera* on *G. hirsutum* yields was based on the impact of flowering insects on pollination, the impact of pollination on *G. hirsutum* fruiting, and the comparison of fruiting rate, the mean number of seed per boll and the percentage of normal seeds of treatments 1 and 2 (2018) and 5 and 6 (2019). For each year, the fruiting rate due to the foraging insects including *A. mellifera* (Fri) was calculated using the following formula : $Fri = \{[(F_x + E_g) - F_y / (F_x + E_g)] * 100\}$, where F_x and F_y are the fruiting rates in treatment X (flower left in free pollination) and treatment Y (flower protected from all insect visits), and E_g the effect of the gauze bag net who can be calculated using the formula $E_g = F_y - F_z$, where F_z is the fruiting rate in treatment Z (flowers protected then opened and closed without insect or any other organism visit).

Finally, $Fri = \{[(F_x - F_z) / (F_x + F_y - F_z)] * 100\}$ [25].

The fruiting rate of a treatment (F) is $F = [(b/a) * 100]$, where b is the number of seeds formed and the number of viable flowers initially set [25].

The impact of flower visiting insects including *A. mellifera* on the number of seeds per boll and the normal seeds were evaluated using the same method as mentioned above for the fruiting rate.

2.6. Evaluation of the pollination efficiency of *Apis mellifera* on *Gossypium hirsutum*

Parallel to the setup of treatments 1, 2, 5 and 6, 600 flowers at bud stage were protected in 2018 and 2019, to form two treatments:

Treatment 3 (2018) or 7 (2019): 400 flowers protected using gauze bag nets to prevent insect or any other organism visits and destined to be visited exclusively by *A. mellifera*. As soon as the first flowers of treatments 3 and 7, the gauze

bag was gently removed and the flower observed for up to 10 minutes; the flower visited once by *A. mellifera* was marked and then protected again ;

Treatment 4 (2018) and 8 (2019): 200 flowers protected using gauze bag nets and destined to be opened then closed without the visit of insects or any other organism. As soon as the first flowers of treatments 4 and 8 were opened, the gauze bag was gently removed and the flower was observed for up to 10 minutes, while avoid insect or any other organism to visit it.

At the maturity, bolls from each treatment were harvested and counted from. The fruiting rate, the number of seeds per boll and the percentage of normal seeds were then calculated for each treatment.

2.7. Statistical analysis

Data were analysed using descriptive statistics, student's *t*-test for the comparison of means of the two samples, Pearson correlation coefficient (*r*) for the study of the association between two variables, and chi-square (χ^2) for the comparison of percentages, Microsoft Excel 2013 software was also used.

3. Results

3.1. Reproduction mode of *Gossypium hirsutum*

The fruiting indexes of *G. hirsutum* were 0.94, 0.88, 0.86 and 0.68 for treatments 1, 2, 5 and 6 respectively. Thus, in 2018, the autogamy rate was 79.07% and the allogamy rate was 20.93%. In 2019, the corresponding figures were 93.62% and 6.38%. For the two cumulative years, the autogamy rate was 86.35% and the allogamy rate was 13.66%. It appears that *G. hirsutum* has a mixed mating system with the predominance of autogamy.

3.2. Activity of *Apis mellifera* on *Gossypium hirsutum* flowers

3.2.1. Frequency of visits

Amongst the 288 and 342 visits of 15 and 21 insect species recorded on its flowers in 2018 and 2019 respectively, *A. mellifera* was the most frequent insect with 139 visits(48.26%) and 178 visits (52.05%) in 2018 and 2019 respectively (Table 1). The difference between these two percentages is not significant ($\chi^2 = 0.89$; df = 1; *P* > 0.05).

3.2.2. Floral products harvested

From our observations and during the two flowering periods, *A. mellifera* were found to harvested intensively pollen and slightly nectar on flowers (Fig. 2).

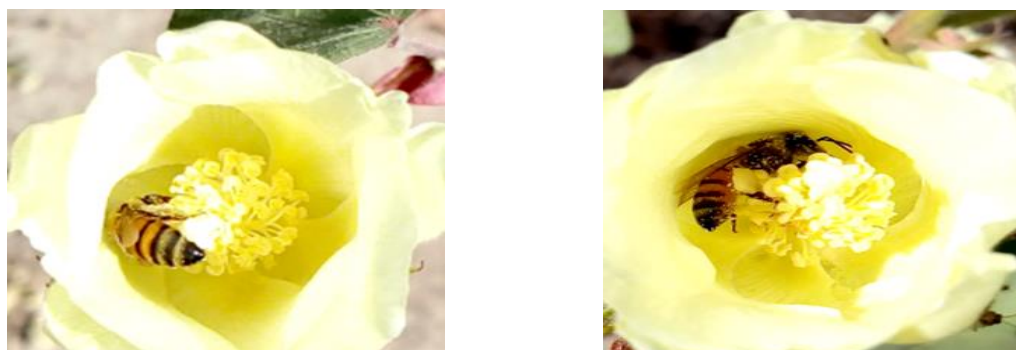


Figure 2 *Apis mellifera* harvesting nectar (A) and pollen (B) on *Gossypium hirsutum* flowers at Djoumassi.

Table 1 Diversity of insects collecting pollen and nectar on *Gossypium hirsutum* flowers in Djoumassi, Cameroon during 2018 and 2019 seasons.

Insects			2018		2019		Total		
Order	Family	Genus and species	n_1	$P_1(\%)$	n_2	$P_2(\%)$	n_T	$P_T(\%)$	
Coleoptera	Coccinellidae	<i>Cheilomenes lunata</i> (Po)	6	2.08	10	2.92	16	2.5	
	Meloidae	<i>Coryna</i> sp. (Po)	2	0.69	3	0.88	5	0.79	
Diptera	Calliphoridae	(1 sp.) (Ne)	4	1.39	2	0.58	6	0.99	
	Muscidae	<i>Musca domestica</i> (Ne)	5	1.74	7	2.05	12	1.9	
	Syrphidae	<i>Episyrphus</i> sp. (Ne)	8	2.78	5	1.46	13	2.12	
		(1 sp.) (Ne)	1	0.35	3	0.88	4	0.62	
Heteroptera	Pentatomidae	(1 sp.) (Po)	3	1.04	2	0.58	5	0.81	
Hymenoptera	Apidae	<i>Amegilla</i> sp. (Ne, Po)	96	33.33	84	24.56	180	28.95	
		<i>Apis mellifera</i> (Ne, Po)	139	48.26	178	52.05	317	50.16	
	Formicidae	<i>Camponotus</i> sp. (Ne)	-	-	5	1.46	5	0.73	
		<i>Myrmecaria opaciventris</i> (Ne)	3	1.04	1	0.29	4	0.67	
	Halictidae	<i>Macronomia vulpina</i> (Ne, Po)	8	2.78	10	2.92	18	2.85	
	Vespidae	<i>Belonogaster juncea</i> (Ne)	5	1.74	3	0.88	8	1.31	
			(1 sp. 1) (Ne)	-	-	2	0.58	2	0.29
			(1 sp. 2) (Ne)	3	1.04	4	1.17	7	1.11
Lepidoptera	Nymphalidae	(1 sp.) (Ne)	-	-	3	0.88	3	0.44	
	Pieridae	<i>Eurema</i> sp. (Ne)	4	1.39	2	0.58	6	0.29	
		<i>Papilio demodocus</i>	-	-	11	3.22	11	1.61	
			(1 sp.) (Ne)	-	-	3	0.88	3	0.44
Orthoptera	Acridae	<i>Tettigonia viridissima</i> (Po)	-	-	2	0.58	2	0.29	
			(1 sp.) (Po)	1	0.35	2	0.58	3	0.47
Total			288	100	342	100	630	100	
			15		21		21		

number of visits on 120 flowers in seven days; n_2 : number of visits on 120 flowers in eight days; percentage of visits $p_1 = (n_1 / 288) * 100$; $p_2 = (n_2 / 342) * 100$; Comparison of percentages of *Apis mellifera* visits (2018/2019): $\chi^2 = 0.89$; $df = 1$; $P > 0.05$; Po: collection of pollen; Ne: collection of nectar; sp.: unidentified species.

3.2.3. Relationship between visits and flowering stages

The visits of *A. mellifera* were more numerous on treatment 1 and 3 when the number of opened flowers was high (Fig. 3A and B). The correlation was highly significant between the number of *G. hirsutum* opened flowers and the number of *A. mellifera* visits in 2018 ($r = 0.94$; $df = 5$; $P < 0.01$) as well as in 2019 ($r = 0.95$; $df = 6$; $P < 0.001$).

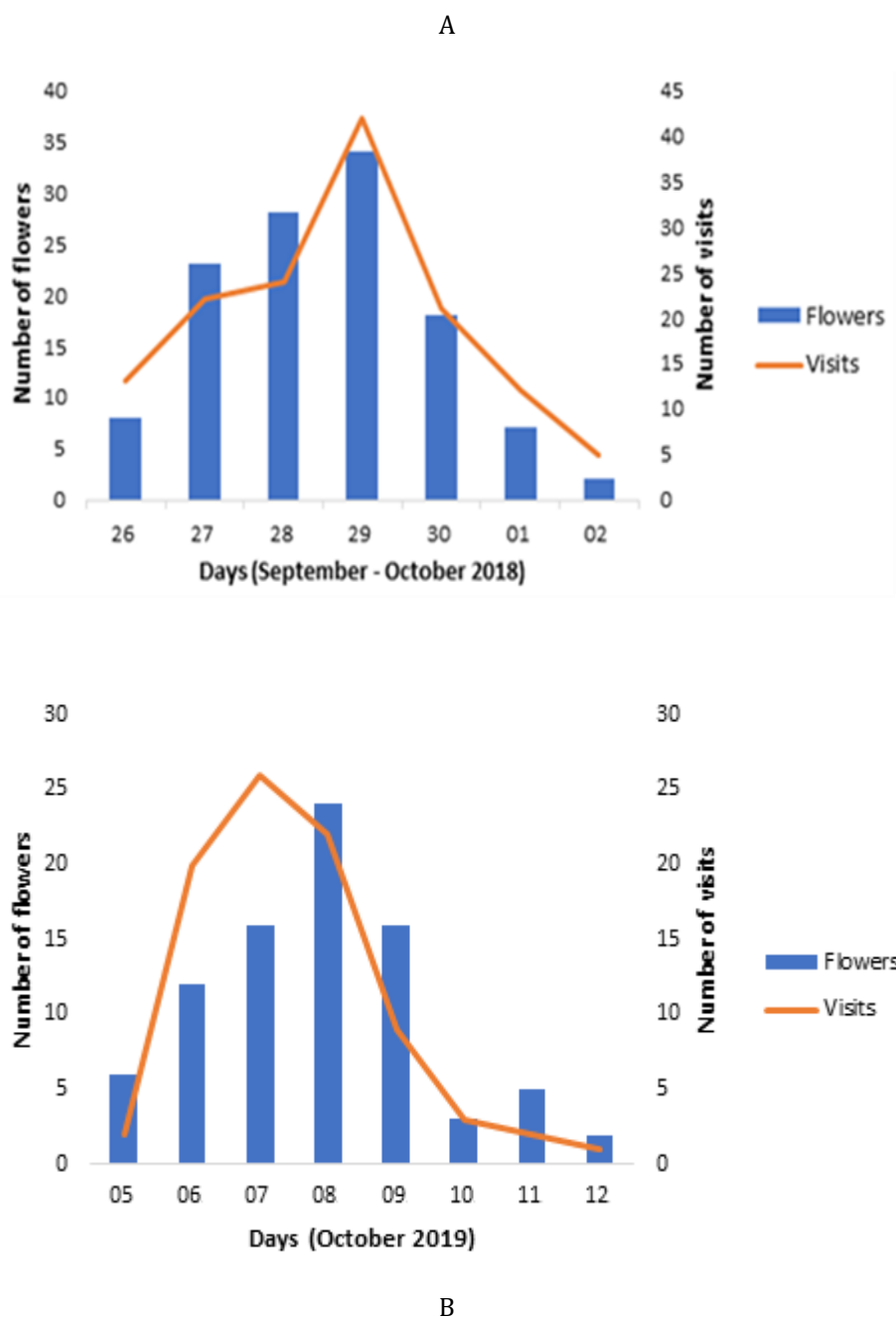


Figure 3 Seasonal variation of the number of *Gossypium hirsutum* opened flowers and the number of *Apis mellifera* visits in 2018 (A) and 2019 (B) at Djoumassi

3.2.4. Daily visits

The worker bee was active on *G. hirsutum* flowers throughout the day. The correlation was significant between the number of *A. mellifera* visits and relative humidity in 2018 ($r = 0.83$; $df = 4$; $P < 0.05$) and was not significant in 2019 ($r = 0.79$; $df = 4$; $P > 0.05$) (Fig. 4A and B). The correlation was significant between the number of *A. mellifera* visits and the temperature in 2018 ($r = 0.87$; $df = 4$; $P < 0.05$) and was not significant in 2019 ($r = 0.28$; $df = 4$; $P > 0.05$).

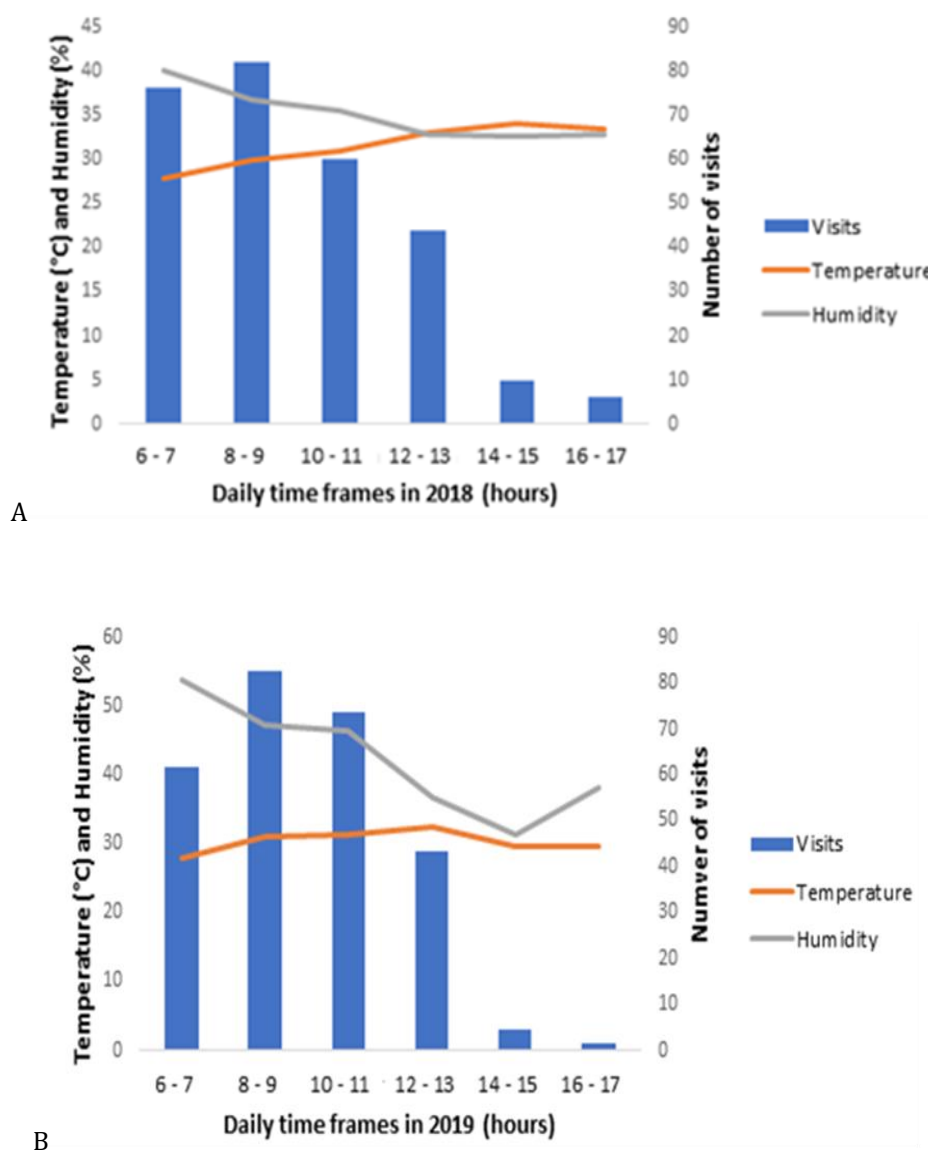


Figure 4 Daily distribution of *Apis mellifera* visits on *Gossypium hirsutum* flowers over seven days in 2018 (A) and eight days in 2019 (B), mean temperature and mean humidity of the study site.

3.2.5. Duration of a visit per flower

In 2018, the mean duration of *A. mellifera* was 38.21 sec ($n=57$; $s=27.67$; $\text{maxi}=105$ sec) and 36.06 sec ($n=62$; $s=23.18$; $\text{maxi}=109$) for pollen and nectar collections respectively. In 2019, the corresponding figures were 34.87 sec ($n=45$; $s=27.39$; $\text{maxi}=98$ sec) and 33.98 sec ($n=52$; $s=21.73$; $\text{maxi}=91$ sec) for pollen and nectar collection respectively. The difference between the duration of the visit to harvest pollen for the cropping season is not significant ($t=0.12$, $df=100$, $P>0.05$). Also, the difference between the duration of visit for nectar in 2018 and 2019 is not significant ($t=0.09$, $df=112$, $P>0.05$).

For the two cumulated years, the mean duration visit for pollen harvest was 36.54 sec and 35.02 sec for nectar.

3.2.6. Abundance of *Apis mellifera* foragers

In 2018, the highest mean number of *A. mellifera* simultaneously active was one per flower ($n=54$, $s=0$) and 403.70 per 1000 flowers ($n=54$; $s=196.15$; $\text{maxi}=800$). In 2019, the corresponding figures were 1.05 per flower ($n=75$; $s=0.23$) and 456 per 1000 flowers ($n=75$; $s=186.17$, $\text{maxi}=800$). The difference between the mean number of *A. mellifera* per 1000 flowers in 2018 and that in 2019 was not significant ($t=0.27$; $P>0.05$).

3.2.7. Foraging speed of *Apis mellifera* on *Gossypium hirsutum* flowers

During our observations, *A. mellifera* visited between 0.64 and 15 flowers/min in 2018 and between 0.52 and 16.67 flowers/min in 2019. The mean foraging speed was 4.31 flowers/min ($n = 92$, $s = 2.14$) in 2018 and 3.91 flowers/min ($n = 77$, $s = 2.42$) in 2019. The difference between these means is not significant ($t = 0.16$; $df = 167$; $P > 0.05$). For the two cumulative years, the mean foraging speed was 4.11 flowers/min.

3.3. Influence of neighboring floral

During each observation periods, flowers of many other plant species surrounding the study area were visited by *A. mellifera*, for nectar and / or for pollen. Among these plants were: *Bidens pilosa* (Ne, Po), *Mangifera indica* (Ne), *Zea mays* (Po), *Vigna subterranea* (Ne), *Vigna unguiculata* (Ne, Po), *Ceratotheca sesamoides* (Ne, Po) and *Commelina diffusa* (Ne, Po).

3.4. Influence of wildlife

The foragers of *A. mellifera* were disturbed in their foraging activity by biotic factors such as other arthropods that were either by competitors for nectar and/or pollen and abiotic factors like wind, rain and temperature. These disturbances resulted in the interruption of some visits. In 2018, for 119 visits, two (1.68%) were interrupted by wind, 11 (9.24%) by other individuals of *A. mellifera* and 33 (27.73%) by *Cheilomenes lunata*. While in 2019 for 136, 12 (8.82%) were interrupted by wind, eight (5.88%) by other individuals of *A. mellifera* and 17 (12.5%) by *Cheilomenes lunata*. For their load of floral products, some individuals of *A. mellifera* who suffered such disturbances were forced to visit more flowers during the corresponding foraging trip.

3.5. Impact of flowering insects including *Apis mellifera* on *Gossypium hirsutum* yields

During nectar or pollen harvest on *G. hirsutum*, foragers always shook flowers and regularly contacted anthers and stigma, increasing self - pollination and/or cross - pollination possibilities of this plant species.

Table 2 Gives the fruiting rate, the mean number of seeds per boll and the percentage of normal seeds in different treatments. It appears from this table that:

- The fruiting rates were 86.66%, 68.33%, 94.17% and 87.5% in treatments 1, 2, 5 and 6 respectively. The differences between these four percentages are highly significant ($\chi^2 = 33.14$; $df = 3$; $P < 0.001$). Two - to - two comparisons showed that the difference observed is highly significant between treatments 1 and 2 ($\chi^2 = 11.57$; $df = 1$; $P < 0.001$) and not significant between treatments 5 and 6 ($\chi^2 = 3.20$; $df = 1$; $P > 0.05$). Consequently, in 2018, the fruiting rate of exposed flowers (treatment 1) was higher than that of flowers bagged during their flowering period (treatment 2).

- The means number of seeds per boll were 24.29, 18.5, 25.02 and 20.15 in treatments 1, 2, 5 and 6 respectively. The differences between these four means are highly significant ($F = 285.71$; $df_1 = 3$; $df_2 = 400$; $P < 0.001$). Two - to - two comparisons showed that the difference observed was highly significant between treatments 1 and 2 ($t = 36.24$; $df = 184$; $P < 0.001$) as well as between treatments 5 and 6 ($t = 6.03$; $df = 216$; $P < 0.001$). Hence, in 2018 and 2019, the mean number of seeds per boll from exposed flowers was higher than that from flowers bagged during their flowering period.

- The percentages of normal seeds were 91.61%, 81.34%, 92.95% and 82.31% in treatments 1, 2, 5 and 6 respectively. The differences between these four percentages are highly significant ($\chi^2 = 229.26$; $df = 3$; $P < 0.001$). Two - to - two comparisons showed that the difference observed was highly significant between treatments 1 and 2 ($\chi^2 = 94.99$; $df = 1$; $P < 0.001$) as well as between treatments 5 and 6 ($\chi^2 = 124.42$; $df = 1$; $P < 0.001$). Hence, in 2018 and 2019, the percentage of normal seeds from exposed flowers was higher than that from flowers bagged during their flowering period.

In 2018, the contribution of anthophilous insects including *A. mellifera* in the fruiting rate, the mean number of seeds per boll and the percentage of normal seeds were 34.29%, 18.82% and 8.91% respectively. In 2019, the corresponding figures were 18.73%, 19.94% and 11.55% respectively. For the cumulative years, the contributions of flowering insects were 26.51%, 19.38% and 10.23% for the fruiting rate, the mean number of seeds per boll and the percentage of normal seeds respectively.

The insect pollinators played a significant role in increasing bolls and seeds yields of *G. hirsutum*.

Table 2 Fruiting rate, number of seeds per boll and percentage of normal seeds according to different treatments of *Gossypium hirsutum* in 2018 and 2019 at Djoumassi.

Years	Treatments	NF	NFF	FrR (%)	Seeds/boll		TNS	NS	%NS
					<i>m</i>	<i>sd</i>			
2018	1 (Uf)	120	104	86.66	24.29	1.41	2478	2270	91.61
	2 (Pf)	120	82	68.33	18.5	0.71	1635	1330	81.34
	5 (Uf)	120	113	94.17	25.02	6.17	2727	2535	92.96
2019	6 (Pf)	120	105	87.5	20.15	5.70	1864	1526	82.31

Uf: Unprotected flowers; Pf: Protected flowers; NF: Number of flowers; NFF: Number of formed fruits; FrR: Fruiting rate; TNS: Total number of seeds; NS: Normal seeds; % NS: Percentage of normal seeds; m: mean; sd: standard deviation.

3.6. Pollination efficiency of *Apis mellifera* on *Gossypium hirsutum*

During pollen harvest on cotton flowers, workers of *A. mellifera* always contact anthers and stigma, thereby increased the pollination possibilities of *G. hirsutum* as they frequently flew from flowers to flowers on the same plants and / or on other flowers of the neighboring plants of cotton. Table 3 reveals that:

- The fruiting rates were 72.5%, 51%, 86% and 74% in treatments 3, 4, 7 and 8 respectively. The differences between these four percentages are highly significant ($\chi^2 = 48.28$ $df = 3$; $P < 0.001$). The difference was highly significant between treatments 3 and 4 ($\chi^2 = 15.71$; $df = 1$; $P < 0.001$) and significant between treatments 7 and 8 ($\chi^2 = 6.87$; $df = 1$; $P < 0.05$). Hence, in 2018 and 2019, the fruiting rate of flowers protected and visited exclusively by *A. mellifera* was higher than those protected, opened and closed without insect or any other organism visit.

- The means number of seeds per boll were 26.5, 20, 23.5 and 20 in treatments 3, 4, 7 and 8 respectively. The differences between these four means are highly significant ($F = 922.57$; $df_1 = 3$; $df_2 = 438$; $P < 0.001$). The difference was highly significant between treatments 3 and 4 ($t = 9.31$; $df = 194$; $P < 0.001$) as well as between treatments 7 and 8 ($t = 5.06$; $df = 244$; $P < 0.001$). These results pointed out that flowers visited by *A. mellifera* have the highest number of normal seeds compare to those protected then opened and closed without the visit of insect or any other organism.

- The percentages of normal seeds were 87.04%, 83.65%, 90.21% and 82.21% in treatments 3, 4, 7 and 8 respectively. The differences between these four percentages are highly significant ($\chi^2 = 67.83$; $df = 3$; $P < 0.001$). The difference was significant between treatments 3 and 4 ($\chi^2 = 6.59$; $df = 1$; $P < 0.05$) and highly significant between treatments 7 and 8 ($\chi^2 = 61.67$; $df = 1$; $P < 0.001$). These results pointed out that flowers visited by *A. mellifera* have the highest number of normal seeds compare to those protected then opened and closed without the visit of insect or any other organism.

In 2018, the contribution of *A. mellifera* via a single flower visit were 29.66% for the fruiting rate, 24.53% for the mean number of seeds per boll and 3.89% for the percentage of normal seeds. In 2019, the corresponding figures were 13.95%, 14.89% and 8.87% respectively. For the two cumulate years, the contribution of *A. mellifera* on the fruiting rate the mean number of seeds per boll and the percentage of normal seeds were 21.81%, 19.71% and 6.38% respectively.

Table 3 Fruiting rate, number of seeds per boll and percentage of normal seeds according to different treatments of *Gossypium hirsutum* in 2018 and 2019 at Djoumassi.

Years	Treatments	NF	NFF	FrR (%)	Seeds/boll		TNS	NS	%NS
					<i>m</i>	<i>sd</i>			
2018	3 (FvA)	200	145	72.5	26.5	5.92	3111	2708	87.04
	4 (Fbwv)	100	51	51	20	3.49	838	701	83.65
	7 (Fva)	200	172	86	23.5	5.74	3361	3032	90.21
2019	8 (Fbwv)	100	74	74	20	4.57	1400	1151	82.21

FvA: Flowers bagged then opened and exclusively visited by *Apis mellifera*; Fbwv: Flowers bagged then closed and opened without insect or any other organism visit; NF: Number of flowers; NFF: Number of formed fruits; FrR: Fruiting rate; TNS: Total number of seeds; NS: Normal seeds; % NS: Percentage of normal seeds; m: mean; sd: standard deviation.

4. Discussion

Apis mellifera was the most frequent insect visitor on *G. hirsutum* flowers during the observation periods. This result confirms those reported by Mazi et al. (2013) [5] at Dang and Dounia and Tchuenguem [12] at Maroua in Cameroon. According to Pires et al. (2014) [16] in Brazil, Apidae are the important flower visitor of cotton. The peak activity of *A. mellifera* was observed in the morning, between 8:00 and 9:00 am. This peak could be linked to the period of the highest availability of nectar and pollen in the flower of this Malvaceae [12].

The high abundance of *A. mellifera* per 1000 flowers and the positive and significant correlation between the number of *G. hirsutum* opened flowers and the number of honey bee visits underscore the attractiveness of *G. hirsutum* nectar and pollen to *A. mellifera*. The high density of workers per 1000 flowers is due to the natural faculty of honeybees to recruit a high number of workers to exploit an interesting resource of food [26]. Honeybees can smell or detect pollen or nectar odors using sensory receptors located on the flagellum of their antennae [27]. Worker communicate the location of an important resource of food to the rest of hivesmates through a special dance inside the hive. The dance informs the distance and the direction from the hive to the food source [28]. The abundance per 1000 flowers was higher (429 workers for the two cumulated cropping years) than that recorded at Dang (313.9 workers for the two cumulated cropping years) by Mazi et al. [5]. This difference could be explained by the high availability of honeybee colonies on the study site in Djoumassi compare to that in Dang.

Significant differences observed between the duration of pollen harvest visit and that of nectar harvest could be explained by the accessibility of each of these floral products.

As a highly nectariferous and polliniferous bee plant with the flowering period located in the rainy season, *G. hirsutum* could be cultivated and protected to strengthen *A. mellifera* colonies. The significant difference observed between the duration of visits in 2018 and 2019 could be explained by the biotic (flower robbers and competitors for nectar and/or pollen of this Malvaceae) and abiotic (wind, rain and temperature) factors of the study area. Interruptions of bee visits took place when there was a heavy wind, collisions between visitors, visitor capture attempts by a predator or approach of flower already occupied by a first visitor. Similar observations have been made by Dounia and Tchuenguem [12] and by Mazi et al. [5] in Dang and in Maroua respectively.

The present study revealed that during one foraging trip, an individual bee foraging on a given plant species scarcely visited another plant species. This result indicates that *A. mellifera* showed flower constancy [29] of *G. hirsutum*.

During the collection of nectar and/or pollen on each flower, *A. mellifera* workers regularly come into contact with the stigma and anthers. They could thus enhance self-pollination, which has been demonstrated in the past [12, 5]. *Apis mellifera* could provoke cross-pollination through carrying of pollen with their furs, legs and mouth accessories, and put them onto another flowers belonging to different plant of the same species.

The significant contribution of anthophilous insects including *A. mellifera* in the number of seeds per boll of *G. hirsutum* is similar to the findings of Dounia and Tchuenguem [12] and Basga et al. [6] at Maroua and Djamboutou respectively which showed that cotton produce less seeds per boll in the absence of efficient pollinators.

The positive and significant contribution of *A. mellifera* alone in boll and seed yields of this Malvaceae could be justified by its pollination possibilities. The workers have facilitated the liberation of pollen from anthers for optimal occupation of the stigma, thus increasing pollination possibilities. The flowers that were exposed to pollinators provided more seeds than protected flowers. Our findings agree those of Dounia and Tchuenguem [12], Mazi et al. [5] and Basga et al. [6] respectively at Dang, Maroua and Djamboutou on the same plant species.

5. Conclusion

From our observations, *G. hirsutum* is a plant species that highly benefits from pollination by insects among which *A. mellifera* is one of the most important and harvested pollen and nectar. Data on the foraging activity of *A. mellifera* enable the classification of *G. hirsutum* among the highly polliniferous and nectariferous stingless bee plant. Thus, this plant could be grown or protected to increase pollen and nectar production as bee hive product and stabilize *A. mellifera* colonies.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors Mazi Sanda, Bernice Mireille Kingha Tekombo, Moïse Adamou and Clément Minéo Yatahaï declare that they have no conflict of interest.

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