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Pollination efficiency of *Apis mellifera* (Hymenoptera: Apidae) on *Helianthus annuus* (Asteraceae) flowers at Dang (Ngaoundéré, Cameroon)

Egono Ndeme Carole Christèle[1*], Kingha Tekombo Bernice Mireille[2], Fameni Tope Sidonie[3], Dounia[4], Tchuenguem Fohouo Fernand-Nestor[5]

[1,2,5]Laboratory of Applied Zoology, Department of Biological Sciences, Faculty of Science, University of Ngaoundéré, P.O. Box 454 Ngaoundéré, Cameroon

[3]Department of Biological Sciences, Faculty of Science, University of Maroua, P.O. Box 814 Maroua, Cameroon

[4]Laboratory of Zoology, Higher Teacher Training College, University of Yaoundé I, P.O. Box 47 Yaoundé, Cameroon

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Abstract

To determine the impact of *Apis mellifera* on fruit and seed yields of *Helianthus annuus* L., foraging and pollination activities of worker bees were studied at Dang, from June to July, in 2016 and 2017. Observations were made on 540 capitula divided in four treatments: two treatments differentiated according to the presence or absence of protection of capitula regarding *A. mellifera* and other insect visits; the third with capitula protected and uncovered when florets where opened, to allow *A. mellifera* visits and the fourth with capitula destined to opening and closing without the visit of insects or any other organism. Workers daily rhythm of activity, their foraging behavior on flowers and their pollination efficiency were evaluated. Results show that, honeybee foraged on sunflower capitula throughout its whole blooming period. This bee intensely harvested nectar and pollen. The greatest number of workers foraging simultaneously per capitulum was 6 and 9 in 2016 and 2017 respectively. The mean foraging speed was 33.95 florets/min in 2016 and 26.03 florets/min in 2017. Through its pollination efficiency on *H. annuus*, *A. mellifera* has increased the fruiting rate by 22.76%, the percentage of fruits with seed by 25.51% and the percentage of normal seeds by 52.93%. Hence, conservation of honeybee colonies close to *H. annuus* fields is recommended to improve pod, seed, pollen as a hive product and honey production in the region.

* **Corresponding Author:** Egono Ndeme Carole Christèle ✉ egonondeme@yahoo.fr

Introduction

It is known that generally anthophilous insects and bees in particular usually increase the fruit and seed yields of many plant species through pollination provision (Keller and Waller 2002; Fluri and Frick, 2005; Sabbahi *et al.*, 2005; Klein *et al.*, 2007; Tchuenguem *et al.*, 2009a).

Helianthus annuus is native of North America (Plant Biosafety Office, 2005). It is cultivated mainly for its seeds, which yield the world's second most important source of edible oil (Plant Biosafety Office, 2005; Dwivedi and Sharma, 2014) which have been used as ant-inflammatory, antipyretic, diuretic, stimulant and vermifuge (Dwivedi and Sharma, 2014). The flowers are yellow (Philippe, 1991) and produce nectar and pollen that attract insects (Tchuenguem *et al.*, 2009a; Abou-Shaara, 2015; Osman and Siham, 2015). Each flower opens in the morning and remains open until it fades 2 to 5 days later (Taissir, 2006).

Despite the high seeds demand in Cameroon, the quantity of *H. annuus* available to consumers is very low (MINADER, 2012). Therefore, it is important to investigate on the possibilities of increasing the production of this Asteraceae in the country. Preliminary studies by Tchuenguem *et al.* (2009a) have shown that in Ngaoundéré many insects including *Apis mellifera* visits *H. annuus* capitula to harvest both nectar and/or pollen and increase the percentage of fruits with seed. Before our work, there has been no previous research reported on the pollination efficiency of *A. mellifera* on *H. annuus*.
The main objective of this research was to contribute to the knowledge of the relationships between honeybees and *H. annuus*, for an optimal management of pollination services. Specific objectives were to: (1) determine the place of *A. mellifera* in the *H. annuus* floral entomofauna; (2) register the activity of *A. mellifera* on flowers; (3) evaluate the apicultural value of this plant; (4) estimate the impact of flowering insects including *A. mellifera* on pollination and fruit and seed yields of this Asteraceae; (5) assess the pollination efficiency of *A. mellifera* on *H. annuus*.

Materials and methods

Study site and biological material

The experiment was carried out from April to August, in 2015 and 2016 at Dang, within the experimental field of the Unit for Apply Apidology (Latitude 7°42.264 N, Longitude 13°53.945 E and altitude 1106 a.s.I.) of the Faculty of Science, University of Ngaoundéré, Adamaoua Region of Cameroon. This Region belongs to the high-altitude Guinean Savannah agro-ecological zone.

The climate is characterized by two seasons: a rainy season (April-October) and a dry season (November-March). The annual rainfall is about 1500 mm. The mean annual temperature is 22°C, while the mean annual relative humidity of 70% (Amougou *et al.*, 2015). The experimental plot measured 437 m2, in which *H. annuus* seeds harvested in of the Unit for Apply Apidology was sown. The animal material included many insect species naturally present in the environment. The number of honeybee colonies located in this area varied from 35 in April 2016 to 72 in July 2017.

The vegetation was represented by crops, ornamental plants, hedge plants and native plants of savannah and gallery forest. During the survey, flowers of surrounding plants species were observed to attract *A. mellifera*. Among these plants were: *Bidens pilosa*, *Cosmos sulphureus* and *Tithonia diversifolia* (Asteraceae); *Cajanus cajan*, *Phaseolus vulgaris* and *Vigna subterranea* (Fabaceae); *Sida rhombifolia* (Malvaceae); *Physalis minima* (Solanaceae); *Lantana camara* (Verbenaceae).

Sowing and weeding

On April 6th 2016 and April 8th 2017, the experimental plot was delimited and divided into 8 subplots, each measuring 8 * 4.5 m2. Four seeds were sown per hole on 9 lines.

There were 20 holes per subplot. Holes were separated 40 cm from each other, while lines were 50 cm apart. Weeding was performed manually as necessary to maintain plots weeds-free.

Determination of the reproduction mode of Helianthus annuus

In June 24th, 2016, 30 capitula of *H. annuus* with florets at the budding stage were labeled on each subplot, giving a total of 240 capitula among which 120 were left unprotected (treatment 1) and 120 were bagged using gauze bags (treatment 2) to prevent insect visitors (Roubik, 1995). On 07th July 2017, the experiment was repeated: 120 capitula were left unprotected (treatment 3) and 120 capitula were bagged using gauze bags (treatment 4) to prevent insect visitors.

In both years, after harvest, the number of fruit formed in each treatment was assessed. For each treatment, the podding index (*Pi*) was then calculated as described by Tchuenguem *et al.* (2001): $Pi = F2 / F1$, where $F2$ is the number of fruits formed and $F1$ the number of viable florets initially set. For each year, the allogamy rate (*TC*) from which derives the autogamy rate (*TA*), was expressed as the difference in podding indexes between unprotected capitula (treatment *a*) and protected capitula (treatment *b*) (Demarly, 1977): $TC = [(Pia - Pib) / Pia] * 100$ where *Pia* and *Pib* are respectively the podding average indexes in treatments *a* and *b*. $TA = 100 - TC$.

Estimation of the frequency of Apis mellifera visits on Helianthus annuus capitula

Observations were conducted on 120 individual opened pollinated capitula of treatments 1 and 3 each day from 25th June to 12th July 2016 and from 08th to 23th July 2017 at 1 h interval from 7h to 18h. In a slow walk along all labeled capitula of treatments 1 and 3, the identity of all insects that visited *H. annuus* florets were recorded. Specimens of all insect taxa were caught using insect net on unlabeled capitula and conserved in 70% ethanol, excluding butterflies that were preserved dry (Borror and White, 1991), for further taxonomy identification. All insects encountered on florets were registered and the cumulated results expressed as the number of visits to determine the relative frequency of *Apis mellifera* in the anthophilous entomofauna of *H. annuus* (Tchuenguem *et al.*, 2009a).

Apis mellifera behavior on Helianthus annuus florets

In addition to the determination of the flower visiting insect frequency, direct observations of the foraging activity of *Apis mellifera* on flowers were made in the experimental field. Workers were categorized based on their specific foraging behavior. Nectar foragers were seen extending their proboscis to the base of the corolla and the stigma, while pollen gatherers scratched the anthers using their mandibles and their legs (Jean-Prost, 1987). During the same time that *A. mellifera* visits on florets were registered, we noted the type of floral products collected by this bee species.

In the morning of each sampling day, the number of opened florets carried by each labeled capitulum was counted. On the same days as for the frequency of visits, the duration of individual flower visits was recorded (using a stopwatch) during six times frames: 6-7h, 8-9h, 10-11h, 12-13h, 14-15h and 16-17h.

Moreover, the number of pollinating visits (the bee came into contact with the stigma (Jacob-Remacle, 1989), the abundance of foragers (the highest number of individuals foraging simultaneously on a floret, a capitulum or on 1000 florets (Tchuenguem *et al.*, 2009a)) and the foraging speed (number of floret visited by an individual bee per minute (Jacob-Remacle, 1989)) were recorded.

The disruption of the activity of foragers by competitors or predators and the attractiveness exerted by other plant species on *A. mellifera* were assessed. During each daily period of investigation, a mobile thermo-hygrometer was used to register the temperature and the relative humidity at the station (Tchuenguem, 2005).

Evaluation of the apicultural value of Helianthus annuus

The apicultural value of *H. annuus* was assessed as in other plant species (Guerriat, 1996; Tchuenguem *et al.*, 2008), using data on flowering intensity and the attractiveness of *A. mellifera* workers with respect to nectar and pollen. The evaluation of the concentration in total sugars of the nectar was recorded using a portable refractometer (0-90% Brix) and a thermometer that gave the ambient temperature. Since the nectar of *H. annuus* is not directly accessible to the investigator, *A. mellifera* workers in full activity of nectar harvest were captured on the florets of this Asteraceae and anesthetized by their introduction into a small bottle containing cotton moistened with chloroform. Then, by small pressures on the bee abdomen placed between the thumb and the forefinger of the experimenter, the nectar of the crop was expelled and its concentration in total sugars (in dry matter g/100g) measured (Tchuenguem *et al.*, 2007).

The collected values were corrected according to the ambient temperature. The data was recorded every day, from 25th June to 12th July (2016) and from the 08th to 23th July (2017) during the same daily periods as for the registration of the abundance of foragers. Five values were registered for each daily time frame according to the bee's rhythm of activity (Tchuenguem *et al.*, 2007).

Evaluation of the impact of flowering insects including Apis mellifera on Helianthus annuus yields

For each investigation year, this evaluation was based on the impact of flowering insects on pollination, the impact of pollination on *H. annuus* fruiting, and the comparison of yields (fruiting rate, percentage of fruits with seed and percentage of normal that is well developed seeds) of treatment a (unprotected capitula) to that of treatment b (protected capitula) (Roubik, 1995). For each observation period, the fruiting rate due to the influence of foraging insects (*Fri*) was assessed using the formula: $Fri = \{[(Fra - Frb) / Fra] * 100\}$, where *Fra* and *Frb* are the fruiting rate in treatment a and treatment b respectively. The fruiting rate of a treatment (*Fr*) is $Fr = [(F2 / F1) * 100]$, where, *F2* is the number of fruits formed and *F1* the number of viable florets initially set (Tchuenguem *et al.*, 2001).

At the maturity, fruits were harvested from each treatment and the numbers of fruits with seed as well as the number of normal seeds were counted.

The fruiting rate, the percentage of fruits with seed and the percentage of normal seeds were then calculated for each treatment. The impact of florets visiting insects on seed yields was evaluated using the same method as mentioned above for the fruiting rate.

Assessment of the pollination efficiency of Apis mellifera on Helianthus annuus

In parallel to the constitution of treatments 1, 2, 3 and 4, 600 capitula with florets at bud stage were labelled in 2016 and 2017, and two treatments were formed:

- treatments 5 in 2016 or 7 in 2017: 400 capitula protected using gauze bags to prevent insect visitors and destined exclusively to be visited by *A. mellifera*. As soon as the first floret was opened, each capitulum of treatments 5 and 7 was inspected. Hence, the gauze bag was delicately removed and this capitulum was observed for up to 10 minutes. The floret visited by *A. mellifera* was marked and the capitula were protected once more. Unvisited capitua by this bee were included in treatments 6 or 8;

- treatments 6 in 2016 or 8 in 2017: 200 capitula destined to opening and closing without the visit of insects or any other organism. As soon as the first floret was opened, each capitulum of treatments 6 and 8 was inspected. Hence, the gauze bag was delicately removed and this capitulum was observed for up to 10 minutes, while avoiding its visits by insect or any other organism.

For each observation period, the contribution of *A. mellifera* in the fruiting rate (*Frx*) were calculated using the formula: $Frx = \{[(FrZ - FrY) / FrZ] * 100\}$ where *FrZ* and *FrY* are the fruiting rate in treatments Z (capitula visited exclusively by *A. mellifera*) and Y (bagged capitula opened and closed without visits by insects or any other organism). At the maturity, fruits were harvested and counted from treatments Z and Y. The fruiting rate, the percentage of fruits with seeds and the percentage of normal seeds were then calculated for each treatment.

Data analysis

Data were analyzed 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, chi-square ($\chi 2$) for the comparison of percentages and Microsoft Excel 2010 software.

Results

Reproduction mode of Helianthus annuus

One hundred and twenty capitula were studied for each of the treatments 1, 2, 3 and 4.

The podding indexes were 0.87, 0.17, 0.84 and 0.29 in treatments 1, 2, 3 and 4 respectively. Thus, in 2016, *TC* was 80.46% and *TA* was 19.54%; in 2017, *TC*= 65.48% and *TA* = 34.52%. For the two accumulated years, *TC* was 72.97%, while *TA* was 27.03%. Consequently, the variety of *H. annuus* studied has a mixed reproduction mode (allogamy-autogamy) with the predominance of allogamy over autogamy.

Table 1. Diversity of insects on *Helianthus annuus* capitula in 2016 and 2017 at Dang, number and percentage of visits of different insects.

Insects			2016		2017		Total 2016/2017	
Order	Family	Genus, Species	*n1*	*P1* (%)	*n2*	*P2* (%)	*nT*	*PT* (%)
Hymenoptera	Apidae	*Apis mellifera* (ne, po)	2221	78.15	3587	90.69	5808	81.72
		Ceratina sp. (ne)	49	1.72	31	0.78	80	1.19
		Dactylurina staudingeri (ne, po)	-	-	4	0.10	4	0.05
		Meliponula ferruginea (ne, po)	-	-	208	5.26	208	2.63
		Xylocopa inconstans (ne)	1	0.03	2	0.05	2	0.04
		Xylocopa olivacea (ne)	1	0.03	-	-	1	0.02
	Halictidae	*Lasioglossum* sp. (ne, po)	315	7,00	57	1.44	469	7.46
		Seladonia jucunda (ne, po)	11	0.39	3	0.07	4	0.05
	Megachilidae	*Chalicodoma rufipes* (ne, po)	2	0.07	29	0.73	659	5.52
	Formicidae	(sp. 1) (ne)	412	14.50	1	0.03	1	0.02
		(sp. 2) (ne)	1	0.03	8	0.20	19	0.30
	Vespidae	*Belonogaster juncea* (ne)	-	-	3	0.07	3	0.04
Diptera	Syrphidae	(sp. 1) (po)	9	0.32	14	0.35	23	0.32
		(sp. 2) (ne)	2	0.07	2	0.05	4	0.06
	Tephritidae	*Dacus* sp. (ne)	4	0.14	3	0.07	6	0.08
Hemiptera	Pentatomidae	(sp. 1) (ne)	9	0.32	2	0.05	11	0.17
		(sp. 2) (ne)	7	0.24	-	-	7	0.12
		(sp. 3) (ne)	3	0.10	-	-	3	0.05
	Pyrrhoridae	*Dysdercus voelkeri* (ne)	7	0.24	-	-	7	0.12
Lepidoptera	Pieridae	*Eurema* sp. (ne)	1	0.03	-	-	1	0.02
	Zygenidae	(sp. 1) (ne)	-	-	1	0.03	1	0.02
TOTAL			3053	100	3955	100	7008	100
		21 species	17 species		16 species		21 species	

Comparison of percentages of *Apis mellifera* visits for the two years: $\chi 2$ = 391.07 (df = 1; P < 0.001)

n_1: number of visits on 46405 florets in 18 days, n_2: number of visits on 45725 florets in 16 days, ne: collection of nectar,

po: collection of pollen, p_1 and p_2: percentages of visits, $p_1 = (n_1 / 3053) * 100$, $p_2 = (n_2 / 3955) * 100$.

Concentration in total sugars of Helianthus annuus nectar

The mean concentration in total sugars of *H. annuus* was 35.36% (*n* = 87; *s* = 8.16; *minimum* = 10.68%; *maximum* = 50.81%) in 2016 and 16.55% (*n* = 141; *s* = 7.55; *minimum* = 3.64%; *maximum* = 45.48%) in 2017. The difference between these two means is highly significant (*t* = 129.37; *df* = 226; *P* < 0.001). For the two years, the mean concentration in total sugars of *H. annuus* was 25.95 (*n* = 114; *s* = 7.85).

Table 2. Fruiting rate, percentage of fruits with seed and percentage of normal seeds according to different treatments of *Helianthus annuus* in 2016 and 2017 at Dang.

Treatments	Years	NC	NFS	TNF	FR (%)	NFS	% FS	NFNS	% FNS
1 (Uc)	2016	115	46405	39930	86.05	33275	83.33	25865	77.73
2 (Pc)		117	39865	6642	16.66	1455	21.91	366	25.15
3 (Uc)	2017	115	45725	38500	84.20	32375	84.09	26520	81.85
4 (Pc)		117	36682	10653	29.04	2959	27.78	547	18.48
5 (Bcva)	2016	68	17680	11575	65.47	5405	46.69	3105	57.45
6 (Bcwv)		88	17425	8050	46.19	2630	32.67	635	24.14
7 (Bcva)	2017	102	27815	16225	58.33	9848	60.70	5961	60.53
8 (Bcwv)		106	27490	13458	48.96	6455	47.96	2037	31.56

Uc: unprotected capitula; Pc: protected capitula; Bcva: bagged capitula exclusively visited by *Apis mellifera*; Bcwv: bagged capitula without the visit of insects or any other organism; NC: number of capitula; NFS: number of florets studies; TNF: total number of fruits; FR: fruiting rate; NFS: number of fruits with seed; % FS: percentage of fruits with seeds; NFNS: number of normal seeds; % FNS: percentage of normal seeds.

Frequency of Apis mellifera visits on Helianthus annuus flowers

Amongst the 3053 and 3955 visits of 17 and 16 insect species recorded on *H. annuus* capitula in 2016 and 2017 respectively, *A. mellifera* was the most represented insect with 2221 visits (72.75%) in 2016 and 3587 visits (90.69%) in 2017 (Table 1). The difference between these two percentages is highly significant ($\chi 2$ = 391.07; *df* = 1; *P* < 0.001).

Activity of Apis mellifera on Helianthus annuus florets

Floral products harvested

From our field observations, *A. mellifera* workers were found to collect pollen (Figure 2) and nectar (Figure 1) on *H. annuus* florets, regulary and intensively. For 825 and 795 visits counted on florets in 2016 and 2017 respectively, 708 (85.82%) and 674 (84.78%) were for nectar collection whereas 117 (14.18%) and 121 (15.22%) were for pollen collection, respectively in 2016 and 2017. For the total of 1620 visits recorded during the two seasons, the number of visits allocated to nectar harvest was 1382 (85.31%) and that for pollen collection was 238 (14.69%).

Rhythm of visits according to the flowering stages

Apis mellifera visits were most numerous in the *H. annuus* field when the number of opened florets was highest (Figure 3). Furthermore, a positive and significant correlation was found between the number of *H. annuus* opened florets and the number of *A. mellifera* visits in 2016 (*r* = 0.65; *df* = 16; *P* < 0.001) as well as in 2016 (*r* = 0.77; *df* = 14; *P* < 0.001).

Daily rhythm of visits

Apis mellifera foraged on *H. annuus* florets throughout the blooming period, with a peak of activity between 9 and 10 am in 2016 and between 11 and 12 am in 2017 (Figure 4). In 2016, the correlation was not significant (*r* = 0.25; *df* = 4; *P* > 0.05) between the number of *A. mellifera* visits on *H. annuus* florets and the temperature, and between the number of visits and relative humidity (*r* = - 0.04; *df* = 4; *P* > 0.05). Equally in 2017, the correlation was not significant (*r* = 0.43; *df* = 4; *P* > 0.05) between the number of *A. mellifera* visits on *H. annuus* florets and the temperature, and between the number of visits and relative humidity (r = - 0.42; df = 4; P > 0.05).

Abundance of Apis mellifera

In 2016, the highest mean number of *A. mellifera* workers simultaneously in activity was 1 per floret (n = 271; s = 0), 2.03 per capitulum (n = 473; s = 0.97; *maximum* = 6) and 6.69 per 1000 florets (n = 479; s = 1.93; *maximum* = 13). In 2017, the corresponding figures were 1per floret (n = 364; s = 0), 2.74 per capitulum (n = 379; s = 1.41; *maximum* = 9) and 6.16 per 1000 florets (n = 368; s = 2.41; *maximum* = 15). The difference between the mean number of foragers per 1000 florets in 2016 and 2017 was highly significant (t = 51.20; df = 845; P < 0.001). For the two cumulated years the mean number of foragers per 1000 florets was 4.01.

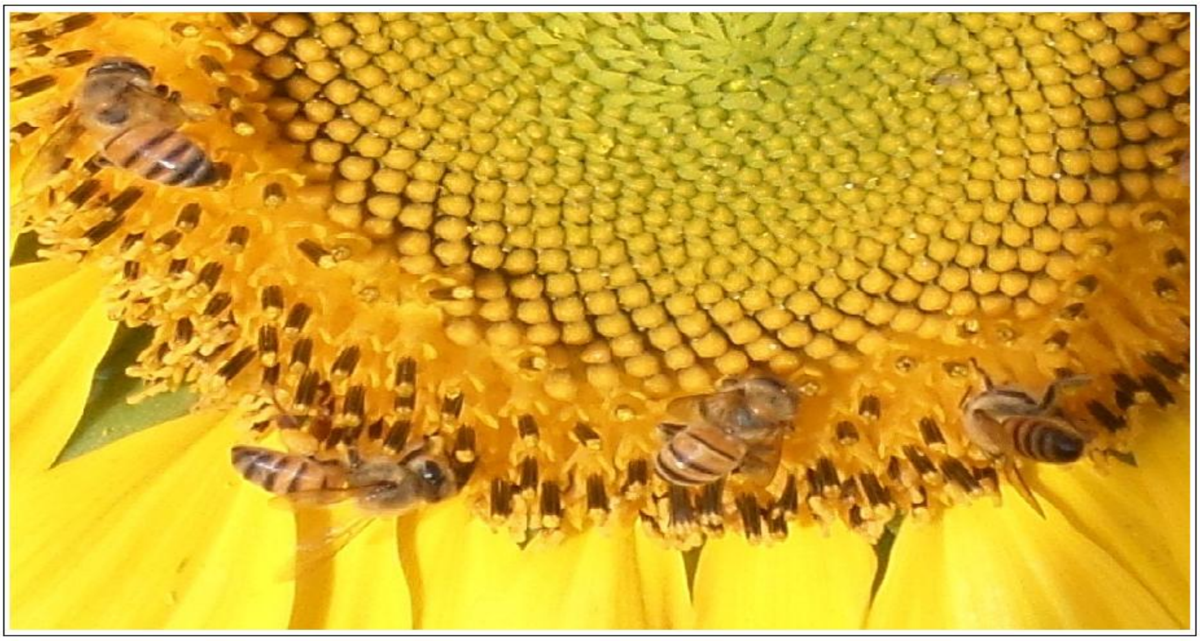

Fig. 1. *Apis mellifera* workers collecting nectar in florets of *Helianthus annuus* at Dang in 2017.

Duration of visits per floret

The mean duration of one *A. mellifera* visit per *H. annuus* floret varied significantly according to the type of food harvested. In 2016, the mean duration of a visit was 2.09 sec (n = 708; s = 1.26; *maximum* = 14) for nectar collection, against 1.21 sec (n = 117; s = 0.49; *maximum* = 4) for pollen harvest. In 2017, the corresponding figures were 2.16 sec (n = 674; s = 1.44; *maximum* = 17) for nectar, against 1.78 sec (n = 121; s = 1.20; *maximum* = 8) for pollen.

The difference between the duration of a visit for nectar harvest in 2016 and 2017 is highly significant (t = 17.88; df = 1380; P < 0.001), as well as the difference between the duration of a visit for pollen collection in 2016 and 2017 (t = 36.62; df = 236; P < 0.001). For the two cumulated years, the mean duration of a floret visit was 2.12 sec (n = 691; s = 1.35) for nectar collection and 1.49 sec (n = 119; s = 0.84) for pollen harvest. The difference between these two latter means is highly significant (t = 49.60; df = 808; P < 0.001).

Foraging speed of Apis mellifera on Helianthus annuus florets

In the experimental field, *A. mellifera* visited between 1 and 240 florets per minute in 2016 and between 5 and 84 florets per minute in 2017.

The mean foraging speed was 33.95 florets per minute (n = 492; s = 23.14) in 2016 and 26.03 florets per minute (n = 488; s = 10.14) in 2017. The difference between these two means is highly significant (t = 108.35, df = 978; P < 0.001). For the two cumulated years, the mean foraging speed was 29.99 florets per minute (n = 490; s = 16.64).

Influence of the fauna

Workers of *A. mellifera* were disturbed in their foraging activity by other foragers of the same species which were the competitor for *H. annuus* nectar and/or pollen or by the wind. In 2016, for 825 visits of *A. mellifera*, 15 (1.81%) were interrupted by *A. mellifera* and 2 (0.24%) by the wind, whereas in 2017, for 795 visits, 48 (6.03%) were interrupted by *A. mellifera* and 4 (0.50%) by the wind. In order to obtain their nectar or pollen load, individuals of *A. mellifera* who suffered such disturbances were forced to visit more florets and/or capitula during the corresponding foraging trip. In pollen foragers, these disturbances resulted in partial loss of carried pollen.

Fig. 2. *Apis mellifera* workers collecting pollen on florets of *Helianthus annuus* at Dang in 2017.

Influence of the neighboring flora

During the observation period, flowers of many other plant specie growing in the study area of *H. annuus* were visited by *A. mellifera*, for nectar (ne) or pollen (po). Among these plants were *Bidens pilosa* (ne and po), *Cajanus cajan* (ne), *Cosmos sulphureus* (ne and po), *Lantana camara* (po), *Phaseolus vulgaris* (ne), *Physalis minima* (ne and po), *Sida rhombifolia* (ne), *Tithonia diversifolia* (ne) and *Vigna subterranea* (ne). During the two years of study, we observed no passage of *A. mellifera* from *H. annuus* florets to flowers of another plants species.

Apicultural value of Helianthus annuus

During the observation periods of *H. annuus* flowering, a well elaborated activity of *A. mellifera* workers was registered on its flowers. In particular, there were good daily and seasonal frequency of visits, high density of workers per capitulum, very good nectar and pollen harvest, fidelity of the workers to the florets and high concentration in total sugars. Moreover, each capitulum of *H. annuus* could produce 100 to more than 1500 florets. Each floret of *H. annuus* produces nectar that is rich in sugars and easy for honeybees to harvest. These data highlight the good attractiveness of *H. annuus* nectar and pollen to *A. mellifera*. Therefore, *H. annuus* is a highly nectariferous and polliniferous bee plant.

Impact of anthophilous insects including Apis mellifera on Helianthus annuus yields

During nectar or pollen harvest on *H. annuus*, foraging insects always shook flowers and regularly contacted anthers and stigma, increasing self-pollination and/or cross-pollination possibilities of *H. annuus*. The comparison of the fruiting rate (Table 2) showed that the differences observed were highly significant between treatments 1 and 2 ($\chi 2$ =

41558.58; $df = 1$; $P < 0.001$) and treatments 3 and 4 ($\chi2 = 25726.84$; $df = 1$; $P < 0.001$). Consequently, in 2016 and 2017, the fruiting rate of exposed capitula (treatments 1 and 3) was higher than that of capitula bagged during their flowering period (treatments 2 and 4).

The comparison of the percentage of fruits with seed (Table 2) showed that the difference observed were highly significant between treatments 1 and 2 ($\chi2 = 11332.24$; $df = 1$; $P < 0.001$) and treatments 3 and 4 ($\chi2 = 13093.53$; $df = 1$; $P < 0.001$). As a matter of fact, in 2016 and 2017, the percentage of fruits with seed of exposed capitula was higher than that of capitula bagged during their flowering period.

The comparison of the percentage of normal seeds (Table 2) showed that the difference observed were highly significant between treatments 1 and 2 ($\chi2 = 2084.90$; $df = 1$; $P < 0.001$) and treatments 3 and 4 ($\chi2 = 6086.06$; $df = 1$; $P < 0.001$). Hence, in 2016 and 2017, the percentage of normal seeds of exposed capitula was higher than that of capitula bagged during their flowering period.

In 2016, the numeric contribution of anthophilous insects in the fruiting rate, the percentage of fruits with seed and the percentage of normal seeds were 86.64%, 73.71% and 67.64% respectively. In 2017, the corresponding figures were 65.51%, 66.96% and 77.44%. For the two cumulated years, the numeric contributions of flowering insects were 76.08%, 70.34% and 72.54% for the fruiting rate, the percentage of fruits with seed and the percentage of normal seeds, respectively.

Pollination efficiency of Apis mellifera on Helianthus annuus

During nectar and pollen harvest on florets, foragers regularly contacted anthers and carried pollen. The percentage of the number of visits during which *A. mellifera* came into contact with the anthers of the visited florets was 100% in 2016 as well as in 2017. Consequently this bee increased possibilities of the pollination of *H. annuus* capitula.

The comparison of the fruiting rate (Table 2) showed that the differences observed were highly significant between treatments 5 and 6 ($\chi2 = 1322.13$; $df = 1$; $P < 0.001$) and treatments 7 and 8 ($\chi2 = 488.79$; $df = 1$; $P < 0.001$). Hence, in 2016 and 2017, the fruiting rate of capitula protected and visited exclusively by *A. mellifera* was higher than that of capitula protected, opened and closed without the visit of insects or any other organisms.

The comparison of the percentage of fruits with seed (Table 2) showed that the difference observed were highly significant between treatments 5 and 6 ($\chi2 = 386.23$; $df = 1$; $P < 0.001$) and treatments 7 and 8 ($\chi2 = 481.69$; $df = 1$; $P < 0.001$). For the two years, the difference was highly significant between the yields of capitula protected and visited exclusively by *A. mellifera* and those of capitula protected, then opened and closed without any visit.

The comparison of the percentage of normal seeds (Table 2) showed that the difference observed were highly significant between treatments 5 and 6 ($\chi2 = 788.59$; $df = 1$; $P < 0.001$) and treatments 7 and 8 ($\chi2 = 1309.73$; $df = 1$; $P < 0.001$). Our observations pointed out that capitula visited by *A. mellifera* have the highest number of normal seeds compare to those protected then opened and closed without the visit of insects or any other organisms.

In 2016, the numeric contribution of *A. mellifera* on the fruiting rate, the percentage of fruits with seed and the percentage of normal seeds were 29.45%, 30.02% and 57.98% respectively. In 2017, the corresponding figures were 16.08%, 20.99% and 47.88% respectively. For the two cumulated years, the corresponding figures were 22.76%, 25.51% and 52.93% respectively.

Discussion

Activity of Apis mellifera on Helianthus annuus florets

Results obtained from these experiments indicated that *Apis mellifera* was the main floral insect visitor of *H. annuus*. In a related study conducted in the same site at Dang, *A. mellifera* was also ranked first in terms of flowering insects of this Asteraceae (Tchuenguem *et al.*, 2009a). The same result was found in Soudan (Ahmed *et al.*, 1989; Osman and Sihan, 2015), in Europe and United State of America (Philippe, 1991) and in Pakistan (Roubik, 2000c) on *H. annuus* flowers. The significant difference between the frequencies of *A. mellifera* visits and those of other insects can be explained by the strategies adopted by this bee that consist of recruiting a great number of workers for the exploitation of an interesting nutritional source (von Frisch, 1969; Louveaux, 1984; Kajobe, 2006). Consequently, there may be a limitation of the number of visits of other insect species due to the occupation of the majority of open florets by *A. mellifera* workers.

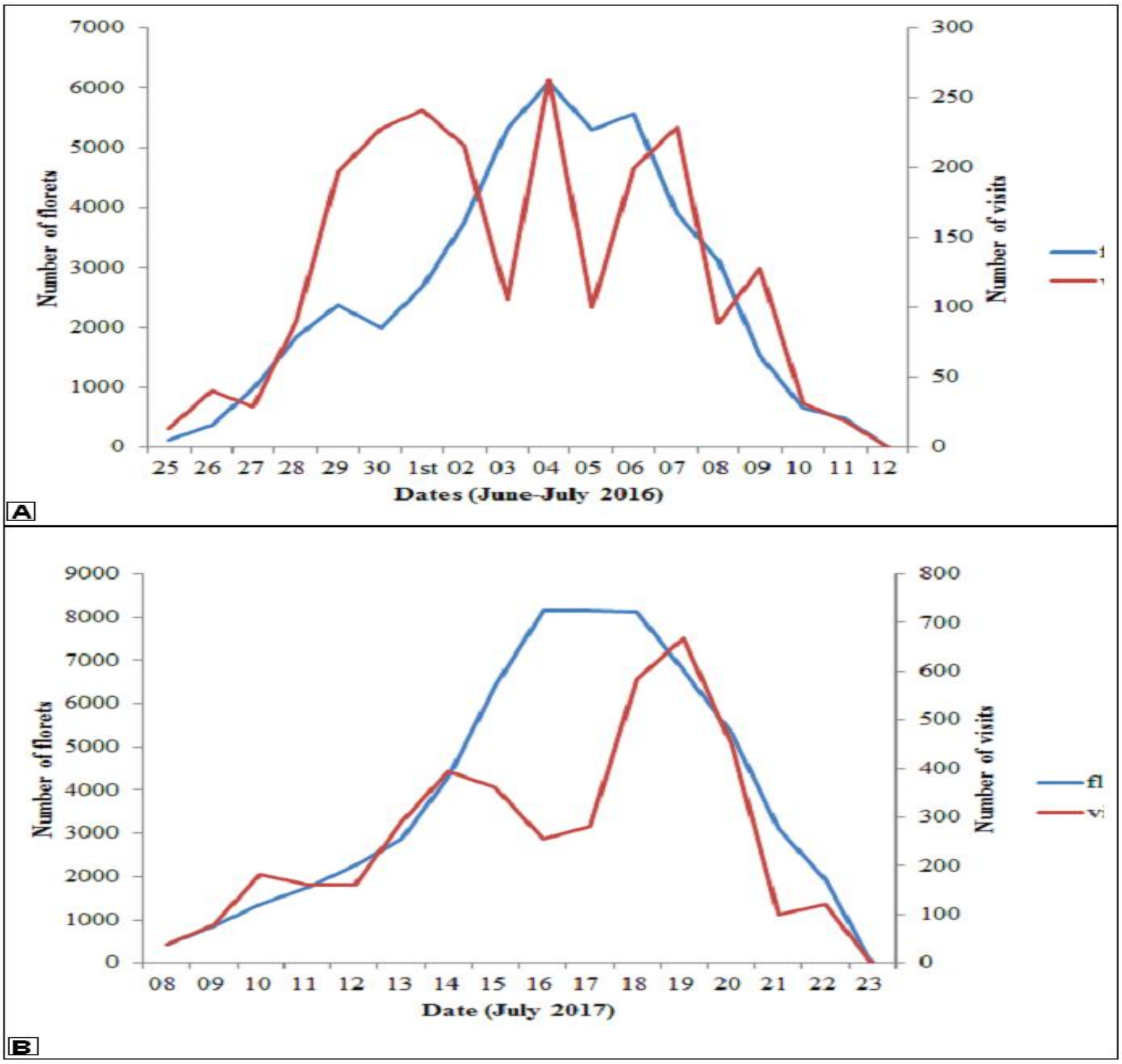

Fig. 3. Seasonal variation of the number of *Helianthus annuus* opened florets and the number of *Apis mellifera* visits in 2016 (A) and 2017 (B) at Dang.

In Dang, Tchuenguem *et al.* (2009a) also registered a high nectar harvest and a low pollen collection on *H. annuus* flowers by *A. mellifera*. These authors found that 61.46% of *A. mellifera* visits were devoted to nectar collection and 7.85% to pollen collection. The pollen collection occurred only in the morning whereas nectar collection occurred throughout the day is in agreement with the observations of Tchuenguem *et al.* (1997, 2004). The peak of activity of *A. mellifera* has been observed on *H. annuus* flowers in the morning between 9 and 10h and 11 and 12h.

This peak could be linked to the period of the highest availability of nectar or pollen on this Asteraceae. Our results are not agreed with those obtained in the same site by Tchuenguem *et al.* (2009a) who found two peaks, one in the morning (9 and 10h) and another in the afternoon (15 and 16h). The high abundance of workers per 1000 florets and the positive and significant correlation between the number of *H. annuus* florets and the number of honey bee visits, underscore the attractiveness of *H. annuus* nectar and or pollen for *A. mellifera*. This attractiveness could be explained by the highest availability and accessibility of these products and the high concentration in total sugars of *H. annuus* nectar, considering the range of 15 to 75% for several plant species (Proctor *et al.*, 1996; Thomson *et al.*, 2012).

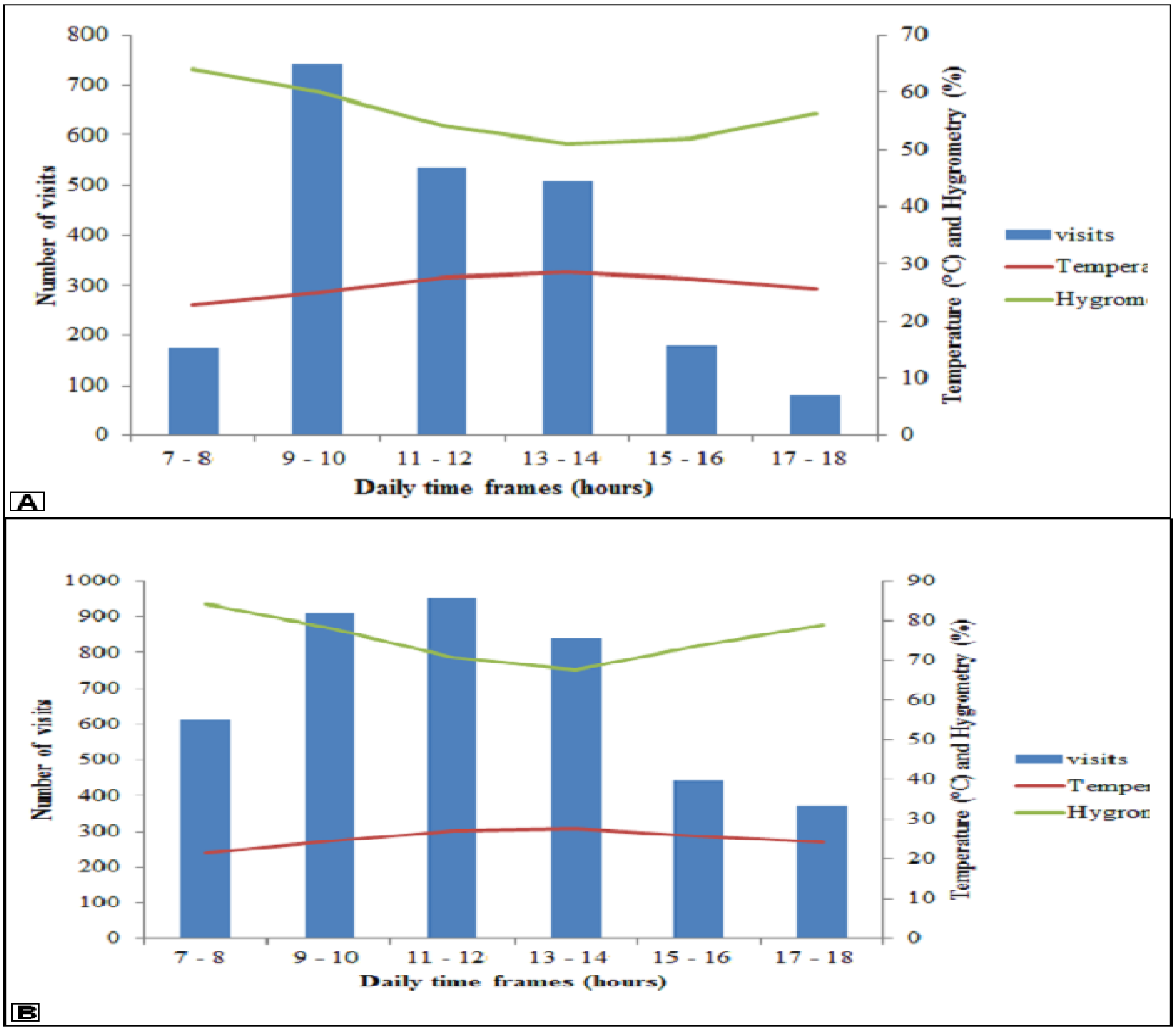

Fig. 4. Daily variation of *Apis mellifera* visits on *Helianthus annuus* florets, mean temperature and mean hygrometry of the study site in 2016 (A) and 2017 (B) at Dang.

The high densities of workers per 1000 florets and per capitula were due to the natural faculty of honeybees to recruit a high number of workers to exploit an interesting food source (Louveaux, 1984). Honeybees can smell or detect pollen or nectar odors (Free, 1970) using sensory receptors located on the flagellum of their antennae. In fact, worker honeybees dance inside the nest after a successful foraging trip in other to communicate to their nest mates information about the food odor, the distance and the direction from the hive to the food source (Frisch, 1967).

The round dance is performed when the resource is within 50 meters from the hive, while the wagging dance takes place for the resource located 100 meters away from the hive (Frisch, 1967). There was a high number of honeybee colonies close to the experimental field.

The significant difference observed between the mean duration of a pollen harvests and that of nectar collection could be explained by the accessibility and disponibility of each of these floral products. Pollen is produced by the anthers, which are on the top of the stamen and are thus easily accessible to *A. mellifera* whereas nectar is between the base of style and stamens. Under these conditions, an individual bee must spend much more time on a floret to obtain its pollen load, compared with the time needed for the collection of nectar (Tchuenguem *et al.*, 2009a).

The disruption of visits by other insects reduced the duration of certain *A. mellifera* visits. This obliged some workers of *A. mellifera* to visit more florets during a foraging trip to maximize their pollen or nectar loads. Similar observations have been made in Dang by Tchuenguem *et al.*, 2009a.

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 (Basualdo *et al.*, 2000) on *H. annuus*. This flower constancy of *A. mellifera* could be partially due to the high sugar content of the nectar of this Asteraceae.

Sunflower is a highly nectariferous and polliniferous bee plant with the flowering period located in the rainy season. The visit of sunflower as a source of nectar and pollen by *A. mellifera* has also been show in Arizona (De Grandi and Watkins, 2000) and in Brazil (Paiva *et al.*, 2002). *Helianthus annuus* could be cultivated and protected to strengthen *A. mellifera* colonies, to improve pollen production as a hive product and to increase honey yield in the region (Tchuenguem *et al.*, 2009a).

Impact of Apis mellifera activity on the pollination and yields of Helianthus annuus

During the collection of nectar or pollen on each flower, *A. mellifera* workers regularly come into contact with the stigma and anthers. They could thus enhance self-pollination by applying pollen of one flower on its own stigma. *Apis mellifera* could provide allogamous pollination through carrying of pollen within their hairs, silk, legs, mouthparts, thorax and abdomen, which is then deposited on flowers belonging to a different plant of the same species (geitogamy) (Philippe, 1991; Roubik, 1995; Abrol, 2012).

The intervention of *A. mellifera* in the pollination of *H. annuus* is especially probable since their density per 1000 florets and their foraging speed were high.

The positive and significant contribution of *A. mellifera* in the fruiting rate, the percentage of fruit with seed and the percentage of normal seeds of *H. annuus* is justified by the action of this bee on the pollination of visited florets. The numeric contribution of *A. mellifera* to the yields of *H. annuus* through its pollination efficiency was significantly higher than that of all insects on the exposed flowers. This shows that *A. mellifera* is one of the main insect pollinators of *H. annuus*.

Conclusion

At Dang, *H. annuus* is a plant species that benefits highly from pollination by insects, among which *Apis mellifera* is the most important and harvests nectar and pollen. The comparison of fruit and seed yields of unprotected capitula visited exclusively by *Apis mellifera* with the capitula protected from insects then opened and close without the visit of insects or any organism, underscores the value of this bee in increasing fruit and seed yields as well as seed quality. As a very highly polliniferous and highly nectariferous apicultural plant, *Helianthus annuus* could be cultivated and protected to increase honey production and to strengthen *Apis mellifera* colonies. The installation and/or the kept of *Apis mellifera* hive at the vicinity of *H. annuus* plant is recommended for

Cameroonian farmers to increase fruit and seed yields. *Helianthus annuus* should be cultivated more in Cameroon to contribute to the economy of the country and to favor populations of *Apis mellifera*. Furthermore, insecticide and or herbicide treatments should be avoided during the flowering period of *H. annuus*. If these treatments are necessary, the choice of the insecticides that are less toxic for bees or the integrated pest control should be recommended to protect pollinating insects such as *Apis mellifera*.

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