International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 13, No. 4, p. 22-36, 2018

RESEARCH PAPER OPEN ACCESS

Pollination efficiency of *Braunsapis* sp. (Hymenoptera: Apidae) on *Helianthus annuus* L. (Asteraceae) flowers at Dang (Ngaoundéré, Cameroon)

Faïbawa Esaïe[*1], Dounia[2], Fameni Tope Sidonie[3], Otiobo Atibita Esther Nadine[4], Tchuenguem Fohouo Fernand-Nestor[5]

[1,5]*University of Ngaoundéré, Faculty of Science, Laboratory of Applied Zoology, Ngaoundéré, Cameroon*

[2]*University of Yaoundé I, Higher Teacher Training College, Laboratory of Zoology, Yaounde, Cameroon*

[3]*University of Maroua, Faculty of Science, Department of Biological Sciences, Maroua, Cameroon*

[4]*University of Bamenda, Faculty of Science, Department of Biological Sciences, Bambili, Cameroon*

Article published on October 19, 2018

Key words: *Braunsapis* sp., *Helianthus annuus*, Pollination efficiency, Yields.

Abstract

This research was carried out to evaluate the impact of *Braunsapis* bees on fruit and seed yields of sunflower in an experimental field from June to July in 2016 and 2017 at Dang. Observations were made on 540 capitula divided in four treatments: two treatments differentiated according to the presence or absence of protection of capitula regarding *Braunsapis* sp. and other insect visits; the third with capitula protected and uncovered when florets were opened, to allow *Braunsapis* sp. visits and the fourth with capitula destined to opening and closing without the visit of insects or any other organism. Bee's daily rhythm of activity, its foraging behavior on flowers and its pollination efficiency were evaluated. Results show that, *Braunsapis* sp. foraged on *H. annuus* flowers throughout its whole blooming period. Among 33 insect species recorded on *H. annuus* capitula, *Braunsapis* sp. ranked third accounting for 7.63 % all visits, after *Apis mellifera* (76.06%) and *Ceratina* sp. (10.79%). On florets, individual bees intensely harvested nectar and slightly collected pollen. The mean duration of a visit per floret was 3.79 sec for nectar harvest visits and 9.94 sec for pollen collection visits. For the two years, through its pollination efficiency on *H. annuus*, *Braunsapis* sp. has increased the fruiting rate by 52.67%, the percentage of fruit with seed by 39.50% and the percentage of normal seeds by 73.51%. Hence, conservation of *Braunsapis* sp. nests close to *H. annuus* fields is recommended to improve pod and seed production in the region.

* **Corresponding Author:** Faïbawa Esaïe ✉ faibawaesaie@gmail.com

Introduction

Flowers are the reproductive organs of many plant species where seeds are formed (Abrol, 2012). For the cycle to begin, a pollen grain, which is often carried on an insect, comes in contact with the stigma of the flower of the same plant species (Abrol, 2012). Fruits, vegetables or seed production from 87 of the 115 leading global food crops depends upon animal pollination (Klein *et al.*, 2007). The inseparable relation between flowers and bees has led to the coevolution and diversity of species that we currently know (Benachour, 2008).

Helianthus annuus is native of North America (Plant Biosafety Office, 2005). This crop is ideal for cultivation in any season because of its wider adaptability, drought tolerance, short life cycle, photo and thermal insensitivity characteristics (Krishna *et al.*, 2014). It is cultivated primarily for its seeds, which yield the world's second most important source of edible oil (Plant Biosafety Office, 2005; Dwivedi and Sharma, 2014). The seed oil, shoots, and herb tincture have been employed for anti-inflammatory, antipyretic, diuretic, stimulant and vermifuge purpose (Dwivedi and Sharma, 2014). Florets produce of nectar and pollen and are visited by insects (Vimla *et al.*, 2013). In Kenya, Honeybee pollination increases sunflower seed yield by 30% and oil content by more than 6% (Kasina *et al.*, 2007). They are important flower visitors not only in Kenya (Kasina *et al.*, 2007) but also, in Turkey (Oz *et al.*, 2009), Cameroon (Tchuenguem *et al.*, 2009b), Israël (Pisanty *et al.*, 2013), India (Vimla *et al.*, 2013) and Sudan (Osman and Siham, 2015). Non-*Apis* bees are also known to visit sunflower and have been reported to improve crop yield by enhancing efficiency of *Apis mellifera* (De Grandi and Watkins, 2000; Greenleaf and Kremen, 2006).

In Africa in general and in Cameroon in particular, the demand in seed oil of sunflower is very highly whereas its seed yields is weak because notably of the insufficiency of knowledge on the relations between this plant and the anthophilous insects in many agro ecological zones.

Numerous studies to identify pollinating sunflower fauna show that *Apis mellifera* is the main pollinator of this crop (Kasina *et al.*, 2007; Nderitu *et al.*, 2008; Oz *et al.*, 2009; Vimla *et al.* 2013; Pysanty *et al.*, 2013; Osman and Siham, 2015). Other Apoids such as bumblebees visit sunflower flowers and participate in their pollination (Lecomté, 1962).

To our knowledge, the data published after detailed studies on the interactions between insects and *H. annuus* are those of Pham-Delègue *et al.* (1985) in Bulgaria, Ahmed *et al.* (1989) in Soudan, Phillipe (1991) in America, Roubik (2000) in Pakistan, Oz *et al.* (2009) in Turkey, Tchuenguem *et al.* (2009b) in Ngaoudéré (Cameroon), Vimla *et al.* (2013) in India, Pisanty *et al.* (2014) in Israel. In all these investigations, the foraging behavior and pollination activity was carried out in detail only on *A. mellifera*. The flowering entomofauna and the impact of insects on pollination and fruit and/or seed yields of a plant species may vary with the species of insect, time and space (Michener, 2000; Gallai *et al.*, 2009). Hence there is a need of other studies in the Adamaoua region, to supplement existing data. The general objective of this work is to contribute to the understanding of the relationships between *H. annuus* and *Braunsapis* sp., for their optimal management. Specific objectives were to: (a) determine the place of *Braunsapis* sp. in the *H. annuus* floral entomofauana; (b) study the activity of this Apidae on florets of this Asteraceae; (c) evaluate the impact of the flowering insects including *Braunsapis* sp. on pollination and fruit and seed yields of *H. annuus*; (d) estimate the pollination efficiency of *Braunsapis* sp. on this plant species.

Materials and methods

Study site, experimental plot and biological material

The experiment was carried out from April to August, in 2016 and 2017 at Dang within the experimental fields of the Unit for Apply Apidology (latitude: 7°42.264 N; longitude: 13°53.945 E; altitude: 1106 a.s.l.) of the Faculty of Science, University of Ngaoundéré Adamaoua region of Cameroon. This region belongs to the high altitude Guinean savannah

agro-ecological zone (Djoufack *et al.*, 2012). The climate is characterized by a rainy season (April to October) and a dry season (November to March), with an annual rainfall of about 1500 mm. The mean annual temperature is 22°C, while the mean annual relative humidity of 70% (Amougou *et al.*, 2015). The vegetation was represented by crops, ornamental plants, hedge plants and native plants of savanna and gallery forests. The experimental plot was a field of 437 m².

The vegetation near the *H. annuus* field had various unmanaged and cultivated species. The experimental plant material was represented by fruit of *H. annuus* sampled from the surrounding of the Unit for Apply Apidology. The bees *Braunsapis* sp. of the experimental station were recruited among the arthropods naturally present in the environment.

Sowing and weeding

From April to May 2016 and 2017, the experimental plot was delimited, ploughed and divided into 8 subplots, each measuring 8*4.5 m². Four fruits 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

On 25th June 2016, 240 *H. annuus* capitula with florets at bud stage were labelled (15 plants per subplot) among which 120 were left unattended (treatment 1) and 120 were protected using gauze bags net to prevent insect visitors (treatment 2) (Tchuenguem *et al.*, 2001). In similar subplots, on 02sd June 2017, 240 *H. annuus* capitula with florets at bud stage were labelled (15 plants per subplot) among which 120 were left unattended (treatment 3) and 120 were protected using gauze bags net to prevent insect visitors (treatment 4).

In the 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.* (2009a): $Pi = F2/F1$, where *F2* is the number of fruits formed and *F1* the number of viable florets initially set. The allogamy rate (*Alr*) from which derives the autogamy rate (*Atr*) was expressed as the difference in podding indexes between unprotected capitula (treatments 1 and 5) and protected capitula (treatments 2 and 6) (Demarly, 1977): $Alr = [(Pi1 - Pi2)/Pi1]*100$, where *Pi1* and *Pi2* are respectively the podding average indexes in unprotected capitula (treatments 1 or 3) and protected capitula (treatments 2 or 4). $Atr = 100-Alr$.

Estimation of the frequency of Braunsapis sp. visits on Helianthus annuus capitula

The frequency of *Braunsapis* sp. visits on *H. annuus* flowers was determined based on observations of capitula of treatments 1 and 5, every day, from 26th June to 16th July 2016 and from 03rd, at 6-7 h, 8-9 h, 10-11 h, 12-13 h, 14-15 h and 16-17 h. In a slow walk along all labelled capitula of treatments 1 and 5, the identity of all insects that visited *H. annuus* florets was recorded (Tchuenguem, 2005). Specimens of all insect taxa were caught using insect net on unlabeled flowers and conserved in 70% ethanol, excluding butterflies that were preserved dry (Borror and White, 1991), for subsequent taxonomic identification. All insects encountered on flowers were registered and the cumulated results expressed as the number of visits to determine the relative frequency of *Braunsapis* sp. in the anthophilous entomofauna of *H. annuus* (Tchuenguem, 2005).

Study of the activity of Braunsapis sp. on Helianthus annuus florets

In addition to the determination of the flower visiting insect frequency, direct observation of the foraging activity of *Braunsapis* sp. on florets was made in the experimental field. The floral products (nectar or pollen) harvested by *Braunsapis* sp. during each floret visit were registered based on its 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 (Borror and White, 1991; Jean-Prost, 1987).

In the morning of each sampling day, the number of opened florets carried by each labelled capitula was counted. During the same days as for the frequency of visits, the duration of individual floret visits was recorded (using a stopwatch) at least six times: 7-8 h, 9-10 h, 11-12 h, 13-14 h, 15-16 h and 17-18 h. Moreover, the number of pollinating visits which was defined as visits with contact between the bees and stigma (Jacob-Remacle, 1989), the abundance of foragers (highest number of individuals foraging simultaneously per floret, per capitula and per 1000 florets) (Tchuenguem *et al*., 2009b) and the foraging speed (number of florets visited by individual bee per minute (Jacob-Remacle, 1989)) were recorded during the same dates and daily periods as the registration of the duration of visits.

The abundance of foragers per floret and per capitula was noted following the direct counting. For the abundance per 1000 florets (A_{1000}), the number of individuals of *Braunsapis* sp. was counted on a known number of florets at the moment *x*. The abundance per 1000 florets is calculated using formula: $A_{1000} = [(A_x / F_x) *1000]$, where F_x and A_x are respectively the number of flourished florets and the numberof browsers actualy to the moment *x* (Tchuenguem, 2005).

The disruption of the activity of foragers by competitors or predators and the attractiveness exerted by other plant species on *Braunsapis* sp. were assessed. During each daily period of investigation, a mobile thermo-hygrometer was used to register the temperature and the relative humidity in the station every 30 min (Tchuenguem, 2005).

Evaluation of the effect of insects including Braunsapis sp. on Helianthus annuus yields

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 unprotected capitula (treatment a) to that of protected capitula (treatment b).

For each observation period, the fruiting rate due to the influence of foraging insects (*Fri*) was calculated using the formula: $Fri = \{[(Fra–Frb)/Fra]*100\}$ where *Fra* and *Frb* are the fruiting rate in treatment a and treatment b. The fruiting rate of a treatment (*Fr*) is: $Fr = [(Fb/ Fa)*100]$, where *Fb* is the number of fruits formed and *Fa* the number of viable capitula initially set (Tchuenguem *et al*., 2001).

At maturity, fruits were harvested from each treatment and the number of fruits with seed as well as the number of normal seeds were counted. The fruiting rate, the percentage of fruits with seeds and the percentage of normal seeds were then calculated for each treatment. The impact of flower visiting insects on seed yields was evaluated using the same method as mentioned above as for the fruiting rate.

Assessment of the pollination efficiency of Braunsapis sp. on Helianthus annuus

In parallel to the constitution of treatments 1, 2, 3 and 4, 600 capitula with florets at bud stage were protected in 2016 and 2017 and two treatments were formed: treatments 5 in 2016 or 7 in 2017: 400 capitula protected using gauze bags nets to prevent insect visitors and destined exclusively to be visited by *Braunsapis* sp. As soon as the first floret was opened, each capitulum of treatments 3 and 7 was inspected. Hence, the gauze bag was delicately removed and this capitulum was observed for up to 10 minutes; the capitula visited by *Braunsapis* sp. was marked and then protected once more;-treatments 6 in 2016 and 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 4 and 8 was inspected. Hence, the gauze bag was delicately removed and this capitulum was observed for up to 10 minutes to avoiding the insect visit and any other organism.

For each observation period, the contribution of *Braunsapis* sp. on the fruiting rate, the percentage of fruits with seed and the percentage of normal seeds were calculated using data of treatment 3 or 7 and those of treatment 4 or 8.

For the each observation year, the contribution of *Braunsapis* sp. in the fruiting (*Frb*) rate was calculated using the formula: *Frb* = {[(*FrX*-*FrY*)/*FrX*]*100}, where *FrX* and *FrY* are fruiting rate in treatment *X* (capitula visited exclusively by *Braunsapis* sp.) and treatments *Y* (bagged capitula opened and closed without insect visit or other organism visits).

At the maturity, fruits were harvested and counted from each treatment. The fruiting rate, the percentage of fruits with seed 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 two samples, Pearson correlation coefficient (*r*) for the study of the association between two variables, chi-square (χ^2) for the comparison of two percentages, using Microsoft Excel 2010 software and R commander, version i386 3.2.0.

Results

Reproduction mode of *Helianthus annuus*

The podding indexes were 0.86, 0.08, 0.92 and 0.07, in treatments 1, 2, 3 and 4 respectively (Table 1).

Hence, *Alr* and *Atr* were respectively 90.17% and 9.83% in 2016 against 92.12% and 7.88% in 2017. For the two accumulated years, *Alr* rate was 91.14%, while *Atr* was 9.86%. Thus the variety of *H. annuus* studied had a mix reproduction system (allogamy-autogamy) with the predominance of allogamy.

Table 1. Reproduction mode of *Helianthus annuus*.

Treatments	Years	Number of florets	Number of fruits	Fruiting idex	Allogamy rate	Autogamy rate
1 (Uc)	2016	71378	61492	0.86	90.17	9.83
2 (Pc)		78101	6617	0.08		
3 (Uc)	2017	55706	51116	0.92	92.12	7.88
4 (Pc)		46339	3349	0.07		

Uc: unprotected capitula; Pc: protected capitula.

Frequency of Braunsapis sp. visits on Helianthus annuus flowers

Among the 2744 and 8756 visits of 21 and 35 insect species recorded on *H. annuus* flowers in 2016 and 2017 respectively, *Braunsapis* sp. ranked third accounting for 9.96% and 6.95% of all visits. The first place was occupied by *Apis mellifera* in 2016 (74.06% and 8.12%) and 2017 (76.53% and 11.60) respectively (Table 2).

Table 2. Diversity of floral insects on *Helianthus annuus* in 2016 and 2017, number and percentage of visits of different insects.

Insects			2016		2017		Total	
Order	Family	Genus and Species	*n1*	*P1* (%)	*n2*	*P2* (%)	*Nt*	*Pt* (%)
Diptera	Calliphoridae	*Calliphora* sp. (ne)	102	3.72	62	0.71	164	1.43
	Sargophagidae	*Sarcophaga* sp. (ne)	4	0.15	5	0.06	9	0.08
		(sp. 1) (ne)	4	0.15	21	0.24	25	0.22
		(sp. 2) (ne)	-	-	18	0.21	18	0.16
		(sp 3.) (ne)	25	0,91	26	0.30	51	0.44
	Syrphidae	(sp. 4) (ne)	3	0.11	9	0.10	12	0.10
		(sp. 5) (ne)	-	-	2	0.02	2	0.02
	Muscidae	*Musca domestica* (ne)	-	-	1	0.01	1	0.01
Coleoptera		(sp. 1) (ne)	9	0.33	3	0.03	12	0.10
Hymenoptera	Apidae	*Apis mellifera* (ne, po)	2033	74.06	6714	76.53	8747	76.06

		Braunsapis sp. (ne, po)	268	9.76	610	695	878	7.63
		Ceratina sp. 1 (ne, po)	223	8.12	1018	11.60	1241	10.79
		Xylocopa olivacea (ne)	13	0.47	31	0.36	44	0.39
		Xylocopa inconstans (ne)	-	-	8	0.09	8	0.07
	Formicidae	(sp. 1) (ne)	14	0.51	18	0.2	32	0.28
		(sp. 2) (ne)	2	0.07	4	0.05	6	0.05
	Halictidae	*Lasioglossum* sp. 1 (ne, po)	9	0.33	26	0.30	35	0.30
Hymenotera	Halictidae	*Lasioglossum* sp. 2 (ne, po)	3	0.11	30	0.34	33	0.29
	Megachilidae	*Chalicodoma refupes* (ne)	10	0.36	40	0.46	50	0.43
		Chalicodoma cincta (ne)	7	0.26	6	0.07	13	0.11
		Megachille sp. 1 (ne)	-	-	29	0.33	29	0.25
	Vespidae	(sp. 1) (ne)	-	-	22	0.25	22	0.19
		(sp. 3) (ne)	-	-	2	0.02	2	0.02
		(sp. 4) (ne)	-	-	2	0.02	2	0.02
		Philanthus trangulum (ne, pr)	1	0.04	-	-	1	0.01
Himiptera		(sp. 1) (ne)	7	0.26	8	0.09	15	0.13
		(sp. 2) (ne)	-	-	4	0.05	4	0.03
		(sp. 4) (ne)	-	-	1	0.01	1	0.01
Lepidoptera	Nymphalidae	*Precis* sp. (ne)	1	0.04	25	0.28	26	0.23
	Acraeidae	*Acraea* sp. (ne)	-	-	5	0.06	5	0.04
		(sp. 1) (ne)	1	0.04	2	0.02	3	0.03
		(sp. 2) (ne)	-	-	2	0.02	2	0.02
Orthoptera	(Ensifera)*	(sp.) (po)	5	0.18	2	0.02	7	0.06
Total		33 species	2744	100.00	8756	100.00	11500	100.00

The difference between the percentages of *Braunsapis* sp. visits in 2016 as well as in 2017 is highly significant (χ^2 = 23.23; *df* = 1; *P* < 0.001).

Activity of Braunsapis sp. on Helianthus annuus florets

Floral products harvested

Individuals of *Braunsapis* sp. were seen collecting pollen (Fig. 1) and nectar (Fig. 2) on *H. annuus* florets. Nectar collection was regular and intensive whereas pollen collection was less intensive. On 780 visits recorded in 2016, 683 (87.56%) were devoted to exclusive nectar harvest and 97 (12.44%) to pollen harvest; in 2017, from 626 visits, 392 (62.62%) were devoted to exclusive nectar harvest and 234 (37.38%) to pollen collection. For the two cumulated years on 1406 visits recorded, 1075 (76.46%) were devoted to exclusive nectar harvest and 331 (23.54%) to pollen harvest. Nectar and pollen were harvested during all scheduled observation time frames.

Table 3. Abondance of *Braunsapis* sp. on *Helianthus annuus* florets in 2016 and 2017 at Dang.

Years	*Abondance*									
	Per capitulum					Per 1000 florets (*A1000*)				
	n	m	s	mini	maxi	n	m	s	min	max
2016	563	2.04	1	1	6	78	40.60	28.44	10	200
2017	302	1.87	0.85	1	7	265	22.74	16.29	5.10	200
Total 2016/2017	865	1.96	0.93	1	7	343	31.67	22.37	5.10	200

Rhythm of visits according to the flowering stages

Braunsapis sp. visits were numerous in the *H. annuus* field when the number of opened florets was highest (Fig. 3).

Furthermore, we found a positive and significant correlation between the number of *Braunsapis* sp. visits and the number of *H. annuus* opened florets in 2016 (*r* = 0.45; *df* = 21; *P* < 0.05). In 2017 this correlation was not significant (*r* = 0.27; *df* = 14; *P* > 0.05).

Daily rhythm of visits

Braunsapis sp. was active on *H. annuus* florets from 8 am to 5 pm in 2016 and in 2017, with a peak of visits between 12 and 13 pm in 2016 as well as in 2017 (Fig. 4). In 2016, the correlation was not significant between the number of *Braunsapis* sp. visits and the temperature (*r* = 0.76; *df* = 4; *P* > 0.05) and between

the number of visits and relative humidity (r = -0.63; df = 4; P > 0.05) (Figure 4A). In 2017, the correlation was significant between the number of *Braunsapis* sp. visits and the temperature (r = 0.83; df = 4; P < 0.05) and between the number of these visits and relative humidity (r = -0.83; df = 4; P < 0.05) (Fig. 4B).

Abundance of Braunsapis sp.

In 2016, the highest mean number of *Braunsapis* sp. individuals simultaneous in activity was 1 per floret and 19.02 per 1000 florets (n = 206; s = 17.81). In 2017, the corresponding figures where 1 per floret and 22.74 per 1000 florets (n = 265; s = 16.29) (Table 3).

Table 4. Duration visits of *Braunsapis* sp. on *Helianthus annuus* florets in 2016 and 2017 at Dang.

Years	Harvested products	Duration visits per floret (sec)					Comparison of means		
		n	m	s	mini	maxi	t-value	df	p-value
2016	Nectar	683	3.89	1.99	1	10	17.47	778	< 0.001 VHS
	Pollen	97	9.89	7.64	1	37			
2017	Nectar	533	4.01	2.44	1	17	16.72	617	< 0.001 VHS
	Pollen	86	10.19	4.07	3	19			
$Total_{2016/2017}$	Nectar	1216	3.95	2.22	1	17	329.05	1417	< 0.001 VHS
	Pollen	183	10.04	5.84	1	37			

For the two cumulated years the mean number of individuals of *Braunsapis* sp. was 20.88 per 1000 florets. The difference between the mean number of this bee per 1000 florets in 2016 and 2017 was highly significant (t = 25.35; df = 469; P < 0.001).

Duration of visits per floret

The mean duration of a *Braunsapis* sp. visit per *H. annuus* floret varied significantly according to floral product harvested (Table 4). In 2016, the mean duration of a floret visit for nectar harvest was 3.89 sec (n = 683; s = 1.99) and that for pollen collection was 9.89 sec (n = 97; s = 7.60); in 2017, the corresponding figures were 4.01 sec (n = 553; s = 2.44) for nectar and 10.19 sec (n = 86; s = 4.07) for pollen. For the two cumulated years, the mean duration of a floret visit was 3.95 sec (n = 1236; s = 2.22) for nectar collection and 10.04 sec (n = 183; s = 5.84) for pollen harvest.

The difference between these two letter means was highly significant (t = 329.05; df = 1417; P < 0.001).

Table 5. Foraging speed of *Braunsapis* sp. on *Helianthus annuus* florets in 2016 and 2017 at Dang.

Years	Number of florets / minute					Comparison of means
	n	*m*	*s*	*mini*	*maxi*	
2016	442	12,72	5,15	3	28,19	t = 533,50; = 1157; P < 0.001 VHS)
2017	717	27,82	8,57	9,60	85,71	
$Total_{2016/2017}$	1159	20,27	6,86	3	85,71	

Foraging speed of Braunsapis sp. *on Helianthus annuus florets*

In the *H. annuus* field an individual of *Braunsapis* sp. visited between 3 and 28 florets per minute in 2016 and between 2 and 29 florets per minute in 2016 (Table 5). The mean foraging speed was 12.72 florets/min (n = 442; s = 5.15) in 2016 and 13.11 florets per minute (n = 717; s = 8.57) in 2017. The difference between these two means is highly significant (t = 553.50; df = 1157; P < 0.001).

Influence of fauna

Individuals of *Braunsapis* sp. were disturbed in their foraging activity by other individuals of the same species or those from other species, which were the competitor for *H. annuus* nectar and/or pollen.

In 2016, for 838 visits, 10 (1.19%) was interrupted by *A. mellifera* and 2 (0.24%) by individuals of *Braunsapis* sp. whereas in 2017, for 619 visits, 7 (1.13%) was interrupted by *A. mellifera* and 1 (0.16%) by individuals of *Braunsapis* sp. (Table 6) In order to obtain their nectar or pollen load, individuals of *Braunsapis* sp.

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.

Table 6. Interrupted frequency visits of *Braunsapis* sp. on *Helianthus* flowers in 2016 and 2017 at Dang.

Years	NSV	N	P (%)	Percentages of flowering insects responsible of the interrupted visit (%)	
2016	838	12	1.43	*Apis mellifera* = 1.19	*Braunsapis* sp. = 0.24
2017	619	8	1.29	*Apis mellifera* = 1.13	*Braunsapis* sp. = 0.16
$Total_{2016/2017}$	1457	20	1.37	*Apis mellifera* = 1.67	*Braunsapis* sp. = 0.21

NSV: Number of studied visits; N: Number of interrupted visits; *P* = (n/NSV)*100: Percentage.

Table 7. Floral products harvested by *Braunsapis* sp. on plant species flowers surrounding the experimental in 2015 and 2016 at Dang.

Plants species	Floral products harvested	
	Nectar	Pollen
Bidens pilosa	+++	+++
Cosmos sulphureus	+++	+++
Sida rhombifolia	+++	+
Stachytarpheta cayennensis	+++	+
Stachytarpheta indica	++	+
Tithonia diversifolia	+++	++
Waltheria indica	+++	+

+ = law harvest; ++ = higher harvest; +++ = very higher harvest.

Influence of neighboring flora

During the observation periods, flowers of many other plant species growing around the experimental field were visited by *Braunsapis* sp., for either nectar or pollen (Table 7). During the two years of study, we observed no passage of *Braunsapis* sp. from *H. annuus* florets to flowers of another plants species.

Table 8. Fruiting rate, percentage of fruits with seed and percentage of normal seeds according to different treatments of *Helianthus annuus* in 2015 and 2016 at Dang.

Treatments	Years	NCS	NFS	TNFr	FR (%)	NFrS	% FrS	NNS	% FNS
1 (Uc)	2016	113	71378	61492	86.15	48214	78.41	35869	74.40
2 (Pc)		119	78101	6617	8.47	928	14.02	43	4.63
3 (Uc)	2017	98	55706	51116	91.76	43671	85.44	34119	78.13
4 (Pc)		101	46339	3349	7.23	2466	73.63	101	4.10
5 (Bcvb)	2016	61	38515	8950	23.24	2542	28.40	975	38.36
6 (Bcwv)		72	55014	6435	11.70	1237	19.22	76	6.14
7 (Bcvb)	2017	127	69528	17594	25.30	9641	54.80	1594	16.53
8 (Bcwv)		103	63922	7168	11.21	2094	29.21	128	6.11

Uc: unprotected capitula; Pc: protected capitula; Bcvb: bagged capitula and exclusively visited by *Brausapis* sp.; Bcwv: bagged capitula, without the visit of insects or any other organism; NC: number of capitula studies; NFS: number of florets studies; TNFr: total number of fruits; FR: fruiting rate; NFrS: number of fruits with seed; NNS: number of normal seeds; **%** FrS: percentage of fruits with seed; % FNS: percentage of normal seeds.

Impact of flowering insects including Braunsapis sp. 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 8) showed that the differences observed were highly significant between treatments 1 and 2 (χ^2 = 90723.97; *df* = 1; *P* < 0.0001) and treatments 3 and 4 (χ^2 = 72636.08; *df* = 1; *P* < 0.0001). Consequently, in 2016 and 2017, the fruiting rate of exposed flowers (treatments 1 and 3) was higher than that of flowers bagged during their flowering period (treatments 2 and 4). The comparison of the percentage of fruits with seed (Table 8) showed that the difference observed were highly significant between treatments 1 and 2 (χ^2 = 12324.46; *df* = 1; *P* < 0.0001) and treatments 3 and 4 (χ^2 = 337.95; *df* = 1; *P* < 0.0001). As a matter of fact, in 2016 and 2017, the percentage of fruits with seeds of exposed flowers was higher than that of flowers bagged during their flowering period.

Fig. 1. *Braunsapis* sp. collecting pollen on a floret of *Helianthus annuus* at Dang in 2016.

The comparison of the percentage of normal seeds (Table 8) showed that the difference observed were highly significant between treatments 1 and 2 (χ^2 = 2 2 5 2 .71; *df* = 1; *P* < 0.0001) and treatments 3 and 4 (χ^2 = 6677.83; *df* = 1; *P* < 0.0001). Hence, in 2016 and 2017, the percentage of normal seeds of exposed flowers was higher than that of flowers bagged during their flowering period.

In 2016, the numeric contribution of anthophilous insects on the fruiting rate, the percentage of fruits with seed and the percentage of normal seeds were 90.16%, 82.20% and 93.78% respectively. In the 2017, the corresponding figures were 92.12%, 13.82% and 94.75% in 2016, respectively.

For the two cumulate years, the numeric contributions of flowering insects were 91.14%, 48.01% and 94.27% for the fruiting rate, the percentage of fruits with seed and the percentage of normal seeds, respectively.

Pollination efficiency of Braunsapis sp. on Helianthus annuus

During pollen and/or nectar harvest in sunflower florets, individuals of *Braunsapis* sp. regularly came into contact with anthers and stigma, increasing the possibility of *H. annuus* pollination.

The comparison of the fruiting rate (Table 8) showed that the differences observed were highly significant between treatments 5 and 6 (χ2 = 2195.43; *df* = 1; *P* <

0.0001) and treatments 7 and 8 (χ2 = 4375.83; *df* = 1; *P* < 0.0001). Hence, in 2016 and 2017, the fruiting rate of capitula protected and visited exclusively by *Braunsapis* sp. was higher than that of capitula protected, opened and closed without a single visit.

The comparison of the percentage of fruits with seed (Table 8) showed that the difference observed were highly significant between treatments 5 and 6 (χ^2 = 178.29; *df* = 1; *P* < 0.0001) and treatments 7 and 8 (χ^2 = 171.38; *df* = 1; *P* < 0.0001). For the two years, the difference was highly significant between the yields of flowers protected and visited exclusively by *Braunsapis* sp. and those of flowers protected, then opened and closed without any visit.

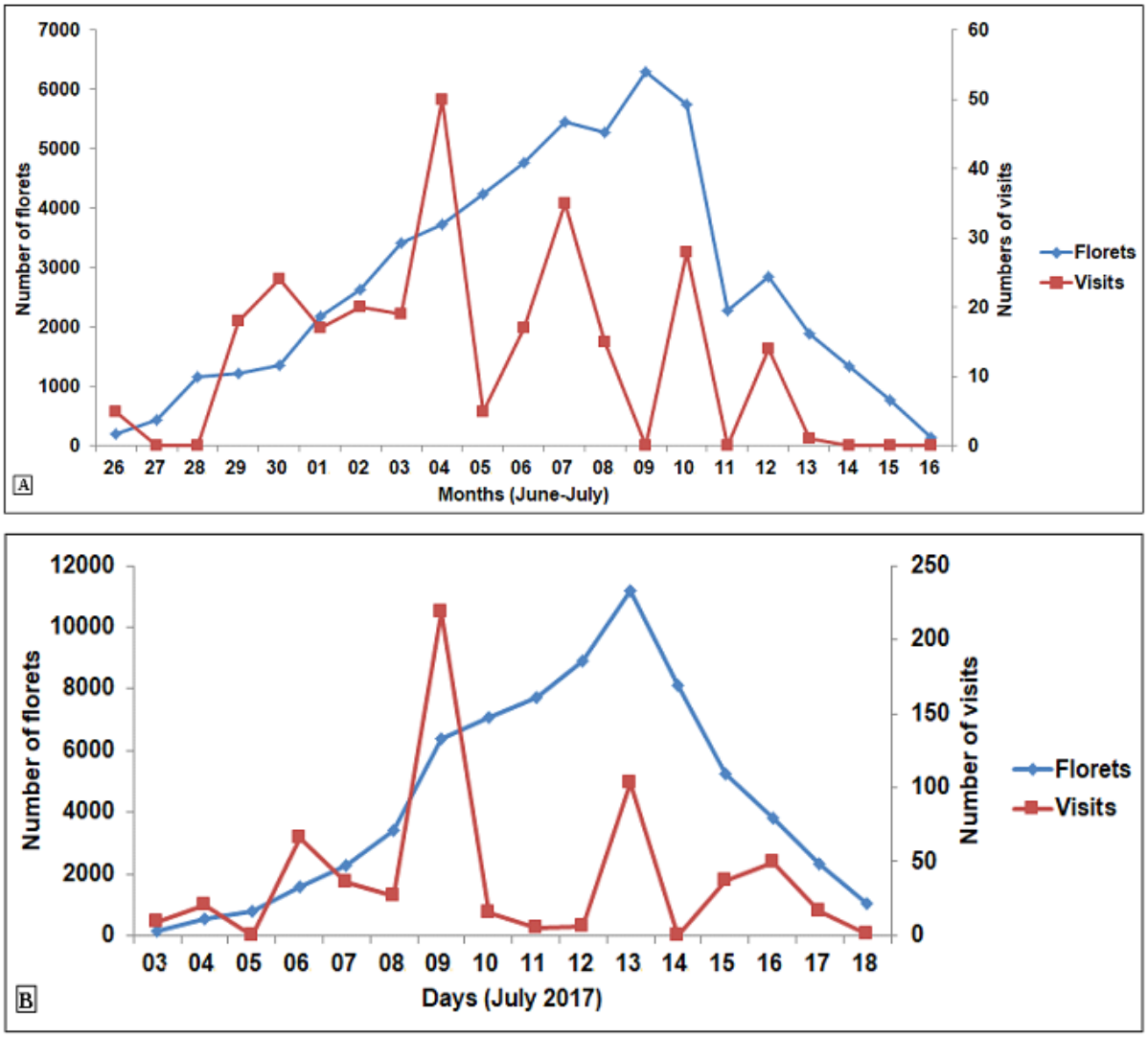

Fig. 2. *Braunsapis* sp. collecting nectar on a floret of *Helianthus annuus* at Dang in 2017.

The comparison of the percentage of normal seeds (Table 8) showed that the difference observed were highly significant between treatments 5 and 6 (χ^2 = 430.03; *df* = 1; *P* < 0.0001) and treatments 7 and 8 (χ^2 = 149.21; *df* = 1; *P* < 0.0001). Our observations pointed out that capitula visited by *Braunsapis* sp. 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 *Braunsapis* sp. on the fruiting rate, the percentage of fruits with seed and the percentage of normal seeds via a single capitula visit were 49.66%, 32.32% and 84.00%% respectively. In 2017, the corresponding figures were 55.69%, 46.70% and 63.04%% respectively.

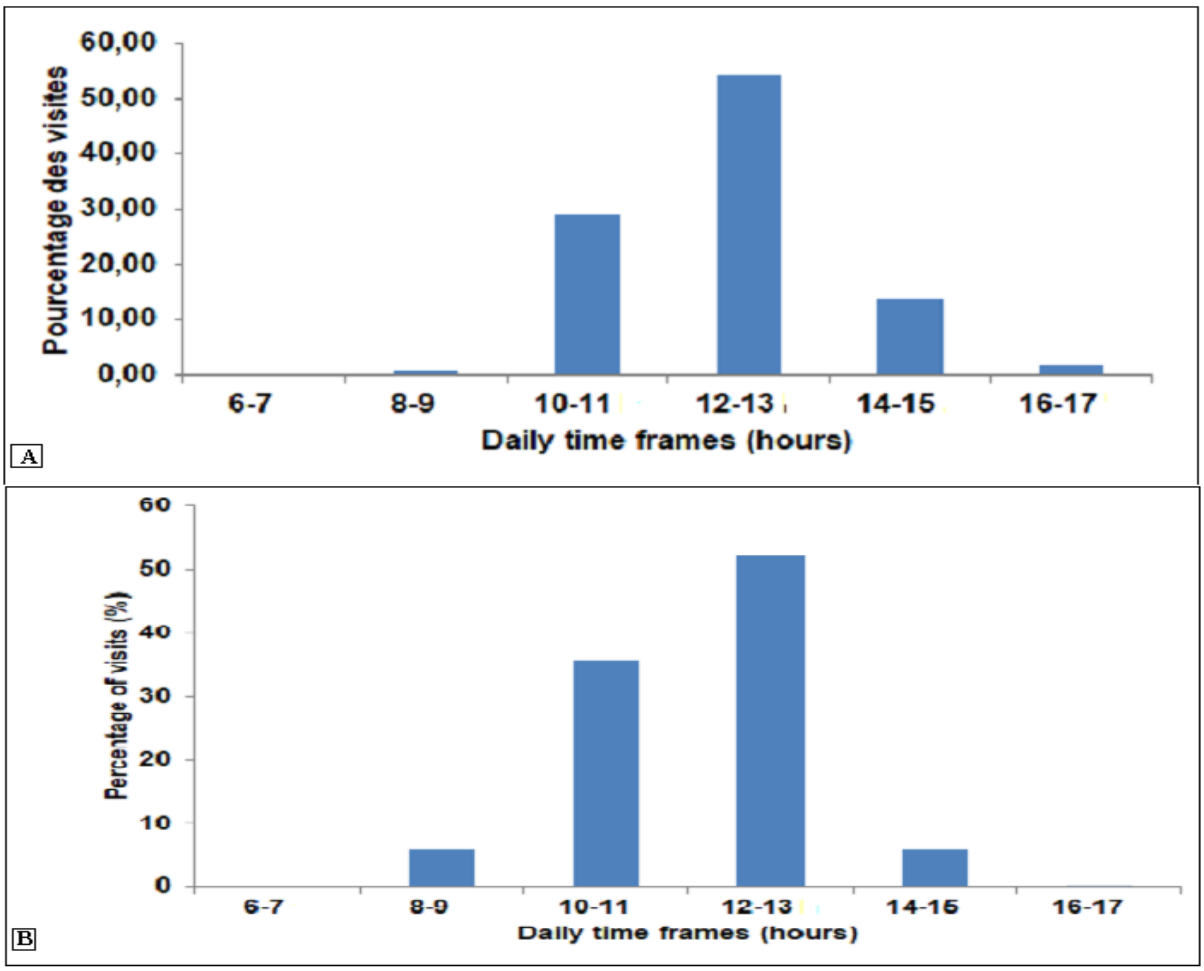

Fig. 3. Seasonal variation on of the number *Helianthus annuus* opened florets and the number of *Braunsapis* sp. visits in 2016 (A) and 2017 (B) at Dang.

For the two cumulated years, the corresponding figures were 52.68%, 39.51% and 73.52% respectively.

Discussion

Activity of Braunsapis sp. on Helianthus annuus florets

During our observation periods, we have registered 34 flower visiting insect species on *H. annuus* capitula. Among all these insects, *Braunsapis* sp. ranked third, the first and second position being occupied by *A. mellifera* and *Ceratina* sp. respectively. The weak frequency of the visit of *Braunsapis* sp. on *H. annuus* capitula compare to that of *A. mellifera* could be explained by the strategies adopted by this social bee that consist of recruiting a great number of workers for the exploitation of an interesting food source (Von Frisch, 1969; Louveaux, 1984; Kajobe, 2006).

Consequently, there may be a limitation of the number of individuals of *Braunsapis* sp. on *H. annuus* capitula due to the occupation of the majority of open florets by *A. mellfera* workers.

The existence of other plants species with flowers able to attract *Braunsapis* sp. could also explained the weak frequency of this solidary bee on *H. annuus* florets. The significant difference between the percentages of *Braunsapis* sp. visit in 2015 and 2016 (χ^2 = 119.93; P < 0.001) could be explained by the presence of a significant number of its nests (21 nests) in 2016 than in 2017 (15 nests) close to the experimental plot.

The peak of activity of *Braunsapis* sp. observed on *H. annuus* could be linked to the period of highest availability of nectar and/or pollen in florets of this Asteraceae.

The high abundance of *Braunsapis* sp. individuals per 1000 florets, and the positive and significant correlation between the number of *H. annuus* flowers and the number of *Braunsapis* sp. visits in 2016, underscores the attractiveness of *H. annuus* nectar and/or pollen for *Braunsapis* sp. The attractiveness for sunflower nectar and pollen could be partially explained by the highest availability and the accessibility of these products.

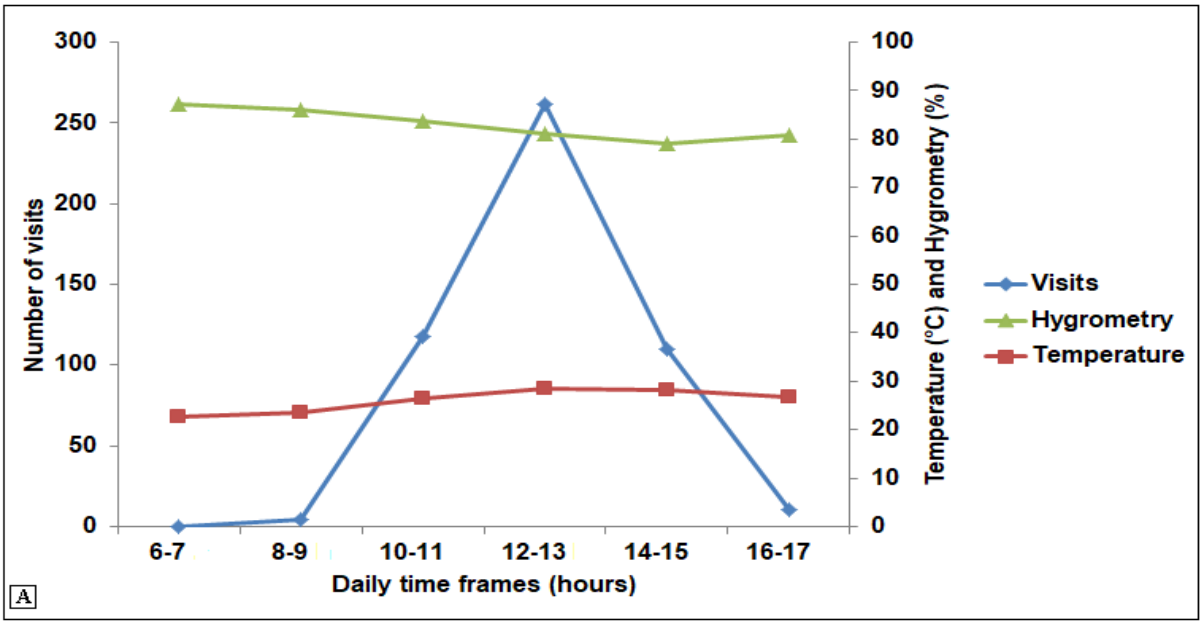

Fig. 4. Variation of number of *Braunsapis* sp. visits on *Helianthus annuus* florets according to daily time frames in 2016 (A) and 2017 (B) at Dang.

The significant difference observed between the mean duration of a pollen harvest visit and that of nectar harvest visit could be explained by the accessibility of each of these floral products. Pollen is produced by the anthers, which are on the top of the stamens, whereas nectar is between the base of the style and stamens.

Under these conditions, an individual bee must spend much more time on a floret to obtain its nectar load, compared to the time needed for the collection of pollen.

The disruption of visits by other insects reduced the duration of certain *Braunsapis* sp. visits. This obliged some individuals of *Braunsapis* sp. to visit more florets during a foraging trip to maximize their pollen or nectar loads. Similar observations have been made on *A. mellifera* workers foraging on the florets of *H. annuus* in Dang (Tchuenguem *et al.*, 2009b). *Braunsapis* sp. had a high affinity with respect to the capitula of *H. annuus* compared with flowers of the neighboring plant species, indicating their faithfulness to this Asteraceae, a phenomenon known in honey bees as floral constancy (Louveaux, 1984; Basualdo *et al.*, 2000). This flower constancy could be partially due to the high sugar content of the nectar of *H. annuus*.

Impact of Braunsapis sp. activity on the pollination and yields of Helianthus annuus

During the collection of nectar and pollen on each floret, *Braunsapis* sp. regularly come into contact with the stigma and anthers.

They could thus enhance self-pollination by applying pollen of one floret on its own stigma. *Braunsapis* sp. could provide allogamous pollination through carrying of pollen on their hairs, legs, thorax, abdomen and mouth accessories, which is consequently deposited on another flower belonging to a different plant of the same species or to a different plant of a different species (geitogamy).

The intervention of *Braunsapis* sp. on the pollination of *H. annuus* is especially probable since their density per 1000 florets and their foraging speed were high. In addition, their daily period of intense activity on *H. annuus* florets situated between 12 and 13 hours could be explained by the optimal receptivity period of the stigma of this plant species.

This result has also been observed in Ngaoundéré on this same plant by Tchuenguem *et al.* (2009b).

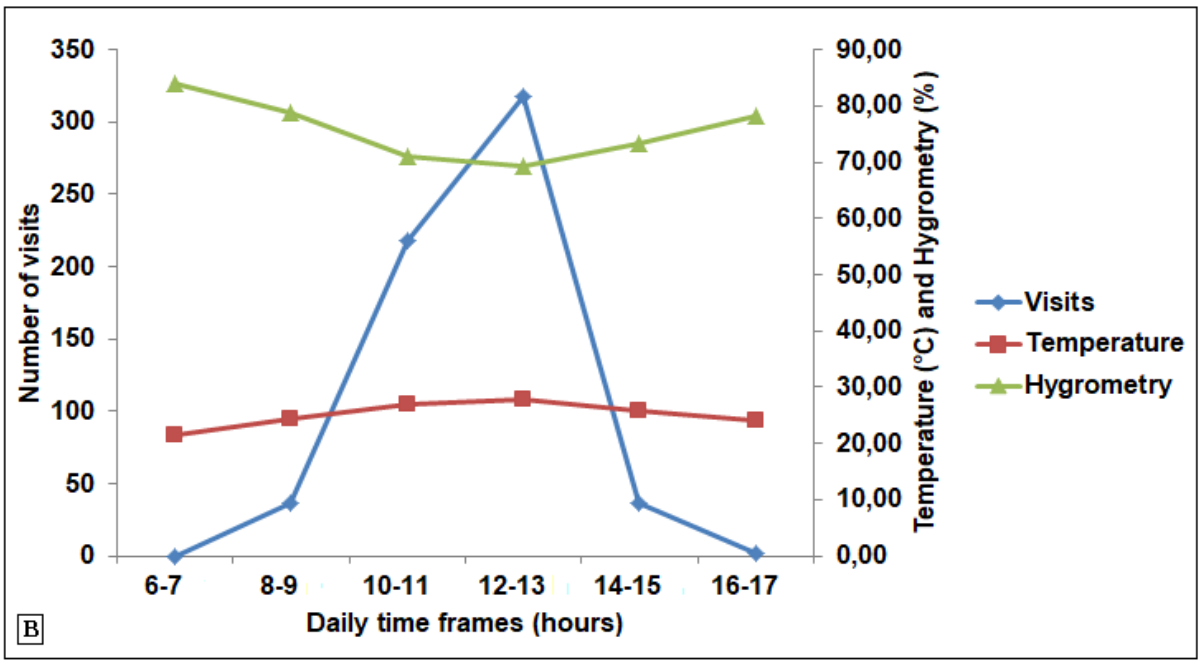

Fig. 5. Daily variation of *Braunsapis* sp. visits on *Helianthus annuus* florets in 21 and 16 days, mean temperature and mean hygrometry of the study site in 2016 (A) and in 2017 (B) at Dang.

The positive and significant contribution of *Braunsapis* sp. in the fruiting rate, the percentage of fruits 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 *Braunsapis* sp. to the yields of *H. annuus* through its pollination efficiency was significantly higher than that of all insects on the exposed capitula. On this same plant, regarding *A. mellifera*, Krishna *et al.* (2014) in India and Tchuenguem *et al.* (2009b) in Cameroon have revealed that the percentage of seed setting (86.9% and 62.21% respectively) due this Apidae through its pollination efficiency was significantly higher over the pollination without insects.Thus in Dang *H. annuus* appears to be a typical pollinating insect species plant on which *Braunsapis* sp. plays of an important role.

Conclusion

From our observations, *Helianthus annuus* is a plant species that highly benefits from pollination by insects among which *Braunsapis* sp. is the most important and harvested nectar and pollen.

The comparison of fruit and seed yields of capitula visited exclusively by *Braunsapis* sp. with the capitula protected from insects then opened and closed without the visit of insects or any other organism, underscores the value of this bee in increasing fruit and seed production as well as seed quality. Based on these results, we recommend the protection of *Braunsapis* sp. nest at the vicinity of sunflower fields to increase fruit and seed yields in the Adamaoua Region of Cameroon. Furthermore, insecticides and/or herbicide treatments should be avoided during the flowering period of *H. annuus* to protect pollinating insects such as *Braunsapis* sp.

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