

Pollination efficiency of *Apis mellifera* (Hymenoptera: Apidae) on *Cosmos sulphureus* (Asteraceae) capitula at Dang (Ngaoundéré, Cameroon)

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Abstract

To study the foraging activity of *Apis mellifera* (Hymenoptera: Apidae) on *Cosmos sulphureus* (Asteraceae) and to evaluate its impact on fruits yields, *C. sulphureus* capitula were observed on Dang (Ngaoundéré, Cameroon) from September to November 2016 and from August to October 2017. Each year, observations were made every day, from 06.00 am to 6.00 pm on 540 capitula divided in four treatments: 120 capitula left in free pollination, 120 capitula protected from insects ; 200 capitula protected, then uncovered, exclusively visited by *A. mellifera* and rebagged ; 100 capitula protected, then uncovered and rebagged without insect or any other organism visit. The foraging behaviour on *A. mellifera* capitula, its pollination efficiency, the fruiting rate, the percentage of mature achenes and the percentage of normal (well developed) achenes were evaluated. The results show that among 10 insect species recorded on *C. sulphureus* capitula after two seasons of observation, *A. mellifera* was the most represented with 95.57 % of 1128 visits. This bee harvested intensely nectar and pollen on *C. sulphureus* florets. The fruiting rate, the percentage of mature achenes and the percentage of normal achenes of treatments with unprotected capitula were significantly higher than those of treatments with capitula protected from insects. Through the pollination efficiency of a single capitule visit, *A. mellifera* increased the fruiting rate, the percentage of mature achenes and the percentage of normal achenes by 50.58 %, 14.37 % and 40.52 % respectively. The installation of *A. mellifera* colonies close to *C. sulphureus* fields is recommended to increase achenes and honey yields, as well as pollen production as a hive product.

Keywords: *Apis mellifera*; *Cosmos sulphureus*; Capitula; Pollination efficiency; yields.

1. Introduction

In ecosystems, the reproduction of many flowering plants needs pollination; this action can be done through agents such as wind, water and animals among which insect are found [1]. The pollination of a great number of plants species mainly depends on insects of the Order Hymenoptera [2]. In many regions of the world, the activity of pollinating insects is judiciously exploited, because it is well known [3]. In Tropical Africa, the impact of pollination on the fruits yields of plants and the contribution of plants to the feeding of pollinating insects is slightly known [4].

Apis mellifera is a social insect, native from Europe, Near and Middle East and Africa [5]. It is the most important bee especially for its honey, wax and propolis production [6]. During their foraging trips, the workers of this bee can harvest nectar, pollen or the two products used as food and achieve at the same time an excellent work of pollination [1].

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Cosmos sulphureus is an herbaceous of the family Asteraceae, native from America [Bindurani, 2013]. It is used in the textile industry for making dyes; in pharmaceutic, it presents anti-malaria, anti-inflammatory and anti-bacterial properties; it is also used in gardening [7].

The productivity of *A. mellifera* colonies depends mainly on the attractiveness and the abundance of polleniferous and nectariferous plants in the environment of the apiaries [8]. The apicultural value of a plant species represents its potential in ressources exploitable by bees like pollen, nectar and honeydew [3].

The number of investigations carried out in Cameroun on the relations between plants and insects is increasing [e.g. 3, 9]. In spite of the preliminary studies of Tchuenguem *et al.* [9] in Cameroon and Wróblewska *et al.* in Poland [10] showing that *A. mellifera* visits *C. sulphureus*, there are no published data to our knowledge on the pollination efficiency of *A. mellifera* on *C. sulphureus*. In this same country, the demand for seeds of *C. sulphureus*, honey and pollen is high, while their production is low [11].

This work carried out in Ngaoundéré, had as main objective to contribute to the mastery of the relations that exist between *C. sulphureus* and *A. mellifera*, for their optimum management. The specific objectives were: determination of the place of *A. mellifera* into the floral entomofauna of *C. sulphureus*; study of the activity of *A. mellifera* on the capitula of this plant species; estimation of the apicultural value of the Asteraceae; evaluation of the impact of the flowering insects on the pollination and akenes yields of *C. sulphureus*; estimation of the pollination efficiency of *A. mellifera* on this plant species.

2. Material and methods

2.1. Material

2.1.1. Study site and biological material

Experiments were carried out from May to November 2016 and from May to October 2017 at Dang (Latitude 7°25.365 N, Longitude 13°32.572 E and Altitude 1083.), a village in the town of Ngaoundéré, in the Adamawa Region of Cameroon [12]. This Region covers 62000 km² and belongs to the high-altitude Guinean-savanna agro-ecological zone. This climate is characterized by two seasons: a rainy season (April to October) and a dry season (November to March) [3]. The annual rainfall is about 1500 mm [13]. The mean annual temperature varies from 22.08°C to 22.93°C and the mean annual relative humidity varies from 64.1 % to 67.6 % [12]. The vegetation is a savannah and gallery forests in some places [3].

The plant material was made up of achenes of *C. sulphureus* grown in the study site. The animal material included: a) 52 to 72 *A. mellifera* colonies numbered from September 2016 to August 2017; b) unidentified colonies of this bee, since they can attain a radius of 12 km around the hive in search of food [14]; c) many other insects naturally present in the environment of the study site.

2.2. Methods

2.2.1. Determination of the reproduction mode of *Cosmos sulphureus*

In September 2016 and August 2017, from the emergence of the first capitule with florets at bud stage, 240 of them have been labelled each year, and divided into two treatments:

Treatment 1 (2016) or 3 (2017): 120 capitula labelled and left unprotected to allow insects visits and on which no insect capture was made;

Treatment 2 (2016) or 4 (2017): 120 capitula labelled and bagged to avoid insects' visits using gauze bags [15].

For each study year, at the harvest, the number of formed achenes was assessed in each treatment. The fruiting index was then calculated as described by Tchuenguem *et al.* [15]: $P_i = F_2 / F_1$, where F_2 the number of formed achenes and F_1 the number of viable florets initially set.

The allogamy rate (TC) from which is deduced the autogamy rate (TA) was calculated as the difference of fruiting indexes between treatment X (unprotected florets) and treatment Y (protected florets) [3].

$TC = [(PiX - PiY) / PiX] * 100$, where PiX and PiY are respectively the mean fruiting indices in treatment X and treatment Y .

$$TA = 100 - TC$$

2.2.2. Determination of the place of *Apis mellifera* in the floral entomofauna of *Cosmos sulphureus*

To determine the place of *A. mellifera* in the floral entomofauna of *C. sulphureus*, observations were made on treatments 1 and 3 every day, from the opening of the first capitule bud (12th of September 2016 and of 21st August 2017). Data were taken from 06.00 am to 6.00 pm according to six daily time frames: 6 - 7 am, 8 - 9 am, 10 - 11 am, 12 am - 1 pm, 2 - 3 pm and 4 - 5 pm. For each of these time slots, the different insects encountered on blooming florets of *C. sulphureus* were counted [3]. Cumulative results were expressed as the number of visits [15]. Data on the frequency of visits of the various identified flowering insects have made it possible to determine the place of *A. mellifera* in the anthophilous entomofauna of *C. sulphureus*. The frequency of visits of the insect i on *C. sulphureus* florets (Fi) was calculated using the following formula: $Fi = [(Vi) / VI] * 100$, with Vi the number of insect i visits on unprotected capitula, and VI the number of visits of all recorded insect on the same capitula [3].

Specimens of all insect taxa, excluding *A. mellifera* were captured on unlabelled capitula using an insect net and kept in 70 % ethanol, excluding butterflies that were kept dry [16], for later taxonomic identification.

2.2.3. Study of *Apis mellifera* activity on *Cosmos sulphureus* florets

Observations of the foraging activity of *A. mellifera* on the florets of *C. sulphureus* were made according to six daily time frames: 7 - 8 am, 9 - 10 am, 11 - 12 am, 1 - 2 pm, 3 - 4 pm and 5 - 6 pm. On each sampling day, the number of opened florets was counted. The floral product harvested (nectar or pollen) by worker bees during each floral visit was registered; nectar gatherers were seen extending their proboscis into the base of the corolla, while pollen harvesters directly scratched anthers with their mandibles and legs [13]. We also determined the abundance of foragers (highest number of individuals foraging simultaneously on a floret, a capitule or 1000 florets) and the foraging speed (number of florets visited by *A. mellifera* per minute). Abundances per floret and per capitule were recorded following the direct counting, on the same dates and daily periods as for the registration of the duration of visits. For the abundance per 1000 florets (A_{1000}), some foragers were counted on a known number of florets; A_{1000} was then calculated using the formula $A_{1000} = ((Ax/Fx)*1000)$, with Fx and Ax are the number of opened florets and the number of foragers effectively counted on these florets at time x [15]. The foraging speed was recorded. It corresponds to the number of flowers visited per minute [3]. The zeroed stopwatch was started as soon as a worker landed on a floret and stopped as soon as this bee was lost to follow-up or if it left the visited capitulum for another plant species. Concomitantly, the number of florets visited was counted. The foraging speed (Fs) was calculated using the formula $Fs = (Fl/du)*60$, where du is the duration (sec) given by a stopwatch, and Fl , the number of visited florets during du [3]. This parameter was recorded on the same dates and daily periods as for the registration of the duration of visits, at the rate of at least five values per time frame when the activity of the foragers allowed it [3]. Moreover, the number of pollinating visits (a bee comes into contact with the stigma) [3] were assessed on the same dates and time frames as for the registration of the duration of visits.

The interruptions of the activity of foragers by competitors or predators and the attractiveness of other plant species flowers with respect to *A. mellifera* were evaluated. During each observation day, ambient temperature and relative humidity were registered after every 30 minutes from 6 am to 6 pm, using a mobile thermo-hygrometer installed in the shade [3].

2.2.4. Evaluation of the apicultural value of *Cosmos sulphureus*

The apicultural value of *C. sulphureus* was assessed as in other plant species [11], using data on flowering intensity and the attractiveness of *A. mellifera* workers with respect to nectar and pollen. The concentration in total sugars of *C. sulphureus* florets nectar was recorded using a portable refractometer (0 - 90 % Brix) and a thermometer giving the ambient temperature [17]. *Apis mellifera* workers in full activity of nectar harvest were captured on the capitula and anesthetized by their introduction into a small bottle containing cotton moistened with chloroform, since the nectar of *C. sulphureus* is not directly accessible to the investigator [11]. Then, by small pressures on the bee abdomen placed between the thumb and the forefinger of the experimenter, the nectar was expelled from the crop and its concentration in total sugars (in dry matter g/100g) measured [17].

The recorded values were corrected according to the ambient temperature. Data were recorded in 2016 and 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 [17].

2.2.5. Evaluation of the impact of flowering insects including *Apis mellifera* on *Cosmos sulphureus* yields

In parallel to the implementation of treatments 1 to 4, 600 capitula with florets at bud stage were labelled to set four other treatments:

Treatments 5 (2016) or 7 (2017): 200 capitula protected, destined to be uncovered, visited once by *A. mellifera* then rebagged. As soon as the florets on each capitula of treatment 5 or 7 bloomed, the gauze bag was gently removed and the florets left in free pollination, observed for 1 to 10 minutes to note their possible visit by *A. mellifera*. After this manipulation, the capitula were reprotected and no longer handled. The unvisited capitula were included in treatments 6 or 8 [9].

Treatments 6 or 8 (2017): 100 capitula protected, then uncovered and reprotected without insects or any other organism visits. As soon as the florets on each capitula of treatments 6 or 8 bloomed, the gauze bag was delicately removed and the blooming florets observed for 1 to 10 minutes, while preventing insects or any other organism visits. After this manipulation, the capitula were rebagged and no longer handled [9].

At the maturity of achenes in treatments 5, 6, 7 and 8, they were harvested, their quality appreciated and their number determined.

The evaluation of the impact of anthophilous insects including *A. mellifera* on achenes production of *C. sulphureus* was based on the impact of anthophilous insects on pollination, the impact of pollination on the fruiting of *C. sulphureus* and the comparison of achenes production (fruiting rate, percentage of mature achenes and percentage of normal or well-developed achenes) of treatments 1, 2, 3, 4, 6 and 8 [3]. For each year of investigation, the fruiting rate due to flowering insects (*Fri*) is calculated using the following formula:

$Fri = \{[(FX - FZ) / (FX + FY - FZ)] * 100\}$, where *FX*, *FY* and *FZ* are the fruiting rates in treatments *X* (capitula left in free pollination), *Y* (capitula protected from insects) and *Z* (capitula protected, uncovered then rebagged, without insects or any other organism visits), respectively [13].

For each treatment, the fruiting rate (*F*) is:

$F = (\text{number of formed achenes} / \text{number of viable florets initially borne}) * 100$ [13].

The percentage of mature achenes and the percentage of normal achenes due to flowering insects were calculated according to the same method as for the fruiting rate.

2.2.6. Assessment of the pollination efficiency of *Apis mellifera* on *Cosmos sulphureus*

The contributions of *A. mellifera* in the fruiting rate, the percentage of mature achenes and the percentage of normal achenes were calculated using data from treatments 5, 6, 7 and 8.

For each observation period, the fruiting rate due to *A. mellifera* (*Fam*) was calculated using the following formula:

$Fam = \{[(FA - FZ) / FA] * 100$ [13], Where *FA* is the fruiting rate in treatment 5 or 7 (capitula protected, then uncovered, visited exclusively by *A. mellifera* and reprotected) and *FZ* the fruiting rate in treatment 6 or 8 (bagged capitula, opened and closed without insects or any other organism visit).

The contributions of *A. mellifera* in the percentage of mature achenes and the percentage of normal achenes were calculated in the same way as for the fruiting rate.

2.2.7. Data analysis

Data were subjected to descriptive statistics (means, standard deviations and percentages), ANOVA (*F*) for the global comparison of more than two means, Student's *t*-test for the comparison of two means, Pearson correlation coefficient (*r*) for the study of the linear relationships between two variables and Chi-square (χ^2) for the comparison of percentages. Microsoft Excel 2010 software was used for this purpose.

3. Results and discussion

3.1. Reproduction mode of *Cosmos sulphureus*

The fruiting indices were 0.53, 0.19, 0.42 and 0.17 in treatments 1, 2, 3 and 4 respectively. Thus, in 2016, *TC* was 64.15 % and *TA* was 35.85 %; in 2017 *TC* was 59.53 % and *TA* was 40.47 %. For the two cumulated years; *TC* was 61.84 % and *TA* was 38.16 %. Consequently, *C. sulphureus* has a mixed reproduction mode (allogamy and autogamy) with the predominance of allogamy over autogamy.

3.2. Concentration in total sugars of *Cosmos sulphureus* nectar

The mean concentration in total sugars of *C. sulphureus* nectar was 32.90 % ($n = 35$; $s = 2.14$; minimum = 25.25 %; maximum = 33.81 %) in 2016 and 31.81 % ($n = 37$; $s = 3.35$; minimum = 25.39 %; maximum = 33.91 %) in 2017. The difference between these two means is significant ($t = 1.99$; $df = 68$; $P < 0.05$). For the two years, the mean concentration in total sugars of *C. sulphureus* was 32.36 % ($n = 72$; $s = 2.75$).

3.3. Place of *Apis mellifera* in the floral entomofauna of *Cosmos sulphureus*

Among the 601 and 527 visits of 9 and 5 insect species recorded on *C. sulphureus* flowers in 2016 and 2017 respectively, *A. mellifera* was the most represented insect species in each study year, with 563 visits (93.68 %) and 515 visits (97.72 %), in 2016 and 2017 respectively (Table 1). The difference between these two percentages is highly significant ($\chi^2 = 10.85$; $df = 1$; $P < 0.001$). Similar results were obtained by Wróblewska *et al.* [9] in Poland, presenting *A. mellifera* as the most frequent floral insect of *C. sulphureus* with 51.7 % of visit. Honeybees are known to be among the most frequent floral insects present on other plant species such as *Bidens bipinnata* and *Bidens pilosa* [18]; *Achillea millefolium*, *Brachyscome multifida*, *Senecio icoglossus*, *Tithonia diversifolia*, *Trichocline catharinensis* and *Zinnia elegans* [6]; *Ceratotheca sesamoides* [9]; *Helianthus annuus* [11]. The significant difference between the frequencies of visits of this bee and those of other insects can be explained by the strategies adopted by this bee, which consists in recruiting a great number of workers for the exploitation of an interesting food source [19]. This invasion may then cause a limitation of the number of visits of other insect species, that find the open florets almost completely occupied by *A. mellifera*.

Table 1 List of flowering insects on *Cosmos sulphureus* in September 2016 and August 2017, number and percentage of visits of different insects.

Insects			2016		2017		Total	
Order	Family	Genus and species	n_1	p_1 (%)	n_2	p_2 (%)	n_T	p_T (%)
Diptera	Bombyliidae	(sp. 1) (ne)	5	0.83	0	0	5	0.44
		(sp. 2) (ne)	1	0.17	0	0	1	0.09
Hymenoptera	Apidae	<i>Apis mellifera</i> (ne, po)	563	93.68	515	97.72	1078	95.57
		<i>Braunsapis</i> sp. (po)	2	0.33	3	0.57	5	0.44
		<i>Xylocopa olivacea</i> (ne)	1	0.17	0	0.00	1	0.09
	Halictidae	<i>Halictus</i> sp (ne)	0	0.00	2	0.38	2	0.17
Lepidoptera	Nymphalidae	<i>Acraea</i> sp. (ne)	10	1.66	1	0.19	11	0.97
		<i>Hypolimnas misippus</i> (ne)	14	2.33	6	1.14	20	1.77
		<i>Precis</i> sp. 1 (ne)	2	0.33	0	0.00	2	0.17
	Geometridae	(sp. 1) (ne)	3	0.50	0	0.00	3	0.27
Total		10 species	601	100	527	100	1128	100

n_1 : number of visits on 120 capitula in six days ; n_2 : number of visits on 120 capitula in seven days ; n_T = total number of visits on 240 capitula in 13 days ; p_1 and p_2 : percentages of visits ; $p_1 = (n_1 / 601) * 100$; $p_2 = (n_2 / 527) * 100$; p_T = total percentage of visits ; sp. : unidentified species ; ne : nectar harvest ; po : pollen harvest

Comparison of percentages of *Apis mellifera* visits for the two study years: $\chi^2 = 10.85$ ($df = 1$; $P < 0.001$).

3.4. Activity of *Apis mellifera* on *Cosmos sulphureus* capitula

3.4.1. Products harvested

During each flowering season, *A. mellifera* regularly and intensively gathered nectar. It regularly and less intensively harvested pollen. While harvesting nectar (Figure 1), this bee collected pollen in a passive way, in addition to the active collection of pollen (Figure 2).



Figure 1 *Apis mellifera* harvesting nectar in a floret of *Cosmos sulphureus* at Dang in 2017.



Figure 2 *Apis mellifera* harvesting pollen on florets of *Cosmos sulphureus* at Dang in 2017

3.4.2. Rhythm of visits of according to the blooming of florets

Visits increased with the opening increase of capitula and florets of *C. sulphureus* (Figure 3). Moreover, we found a positive and significant correlation between the number of opened florets and the number of visits in 2016 ($r = 0.94$; $df = 4$; $P < 0.05$) and a positive and a highly significant correlation between the number of opened capitula and the number of visits in 2017 ($r = 0.92$; $df = 5$; $P < 0.001$). These positive and significant correlations between the number of *C. sulphureus* opened florets and the number of visit of *A. mellifera* underscores the attractiveness of *C. sulphureus* nectar and/or pollen for *A. mellifera*. A positive and significant correlation between this bee and the blooming progression of florets was also obtained by Egono *et al.* [11] on *H. annuus*.

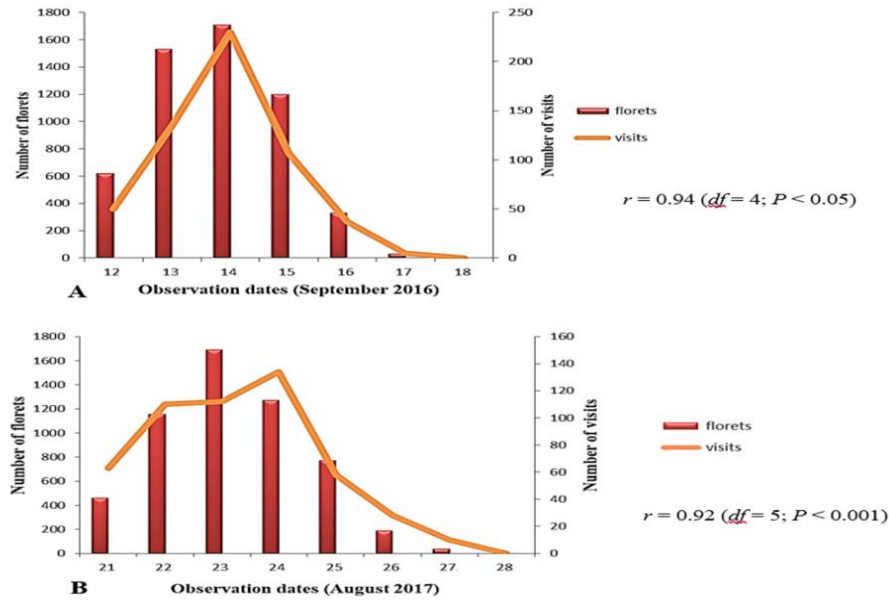


Figure 3 Variations of the number of *Cosmos sulphureus* opened capitula and the number of *Apis mellifera* visits on these organs according to the observation dates in 2016 (A) and 2017 (B) at Dang.

r: correlation coefficient between the number of florets and the number of visits ; df : degree of freedom ; P : probability

3.4.3. Rythm of *Apis mellifera* visits according to the daily time frames

Apis mellifera started its foraging activity on *C. sulphureus* flowers at around 6 am and ended at about 6 pm, with a peak of visits situated between 8 am and 9 am in 2016 as well as in 2017 (Figure 4). The peak of activity of *A. mellifera* observed on *C. sulphureus* capitula could be linked to the fact that these time frame may be the period of highest secretion of nectar, greatest availability of pollen on anthers, and secretion of a large quantity of volatile substances attracting bees [1]. This result is not in accordance with those of Wróblewska *et al.*, [1] that found a peak of foraging insects including *A. mellifera* between 12 pm and 4 pm. This difference may be due to the fact that insect activity vary over time and space [3].

3.4.4. Abundance of *Apis mellifera* foragers

In 2016, the highest mean number of *A. mellifera* simultaneously in activity was 2 per floret ($n = 1626$; $s = 0.06$), 53 per 1000 florets ($n = 415$; $s = 28.28$) and 151 per 1000 capitula ($n = 776$; $s = 97.78$). In 2017, the corresponding values were 2 per floret ($n = 1274$; $s = 0.07$), 35 per 1000 florets ($n = 537$; $s = 19.27$) and 222 per 1000 capitula ($n = 598$; $s = 119.81$). For the two study periods, the greatest mean number of *A. mellifera* workers simultaneously in activity was 1 per floret, 44 per 1000 florets and 187 per 1000 capitula. The difference between the highest mean numbers of *A. mellifera* simultaneously in activity per 1000 florets ($t = 11.65$; $df = 949$; $P < 0.001$) in 2016 and 2017 was highly significant. The difference between the highest mean numbers of *A. mellifera* simultaneously in activity per 1000 capitula ($t = 12.08$; $df = 1372$; $P < 0.001$) in 2016 and 2017 was highly significant. The observed high abundance of workers on *C. sulphureus* capitula was due to the ability of honeybees to recruit a great number of foragers for the exploitation of profitable food sources [1].

3.4.5. Duration of visits

In 2016, the mean duration of one visit of *A. mellifera* was 12.64 sec per capitulum ($n = 623$; $s = 7.95$) for nectar harvest and 9.47 sec per capitulum ($n = 169$; $s = 4.29$) for pollen collection; the mean duration of visit was 1.79 sec per floret ($n = 977$; $s = 0.88$) for nectar harvest and 1.52 sec per floret ($n = 195$; $s = 0.75$) for pollen collection. In 2017, the mean duration of a visit was 12.81 sec per capitulum ($n = 554$; $s = 8.67$) for nectar gathering and 10.09 sec per capitulum ($n = 110$; $s = 5.58$) for pollen collection; the mean duration of one visit of *A. mellifera* was 1.80 sec per floret ($n = 821$; $s = 0.80$) for nectar harvest and 1.39 sec per floret ($n = 180$; $s = 0.60$) for pollen harvest.

For the two study periods, the difference between the mean durations of a visit of *A. mellifera* per capitulum for nectar collection is not significant ($t = 0.35$; $df = 1175$; $P > 0.05$); the difference between the mean durations of a visit per capitule for pollen collection is not significant ($t = 1.04$; $df = 277$; $P > 0.05$); the difference between the duration of visit

of *A. mellifera* per floret for nectar collection is not significant ($t = 0.25$; $df = 1796$; $P > 0.05$) ; the difference between the duration of one visit of *A. mellifera* per floret for pollen collection is not significant ($t = 1.84$; $df = 373$; $P > 0.05$).

For the two cumulative years, the mean duration of a visit for nectar harvest was 12.73 sec and 1.78 sec per capitule and per floret respectively. For pollen collection, it was 9.78 sec and 1.46 sec per capitule and per floret respectively. The variation between the mean durations of visit for nectar and pollen harvest could be linked to the accessibility and availability of these floral products. The *C. sulphureus* pollen which is produced by anthers is easily accessible to *A. mellifera* whereas nectar is produced between the base of the style and stamens and is thus less accessible. Under these conditions, a forager was obliged to spend more time on a floret to harvest nectar, compared to the needed time for pollen collection; similar observations were made on *H. annuus* in Ngaoundéré [20].

3.4.6. Foraging speed

On *C. sulphureus* plots, *A. mellifera* visited between 7 and 200 florets per min in 2016 and between 9 and 150 florets per min in 2017. The mean foraging speed was 51.39 florets per min ($n = 657$; $s = 32.01$) in 2016, 56.87 florets per min ($n = 702$; $s = 23.81$) in 2017 and 54.13 florets per min for the two cumulated years. The difference between the first two means per floret is highly significant ($t = 3.59$; $df = 1357$; $P < 0.001$). The observed variations in the foraging speed may be due to the distance between the capitula during the various foraging trips and the frequency of interruption of *A. mellifera* visits. In Ngaoundéré, on *Helianthus annuus* with bigger florets, *A. mellifera* visited an average of 24.37 florets per minute [21], a more than twice lower foraging speed compared to what we obtained. This difference may be due to the variation of the activity of this bee over time and with plant species as mentioned by Tchuenguem *et al.* [22].

3.4.7. Influence of fauna

Apis mellifera workers were interrupted in their foraging activity on *C. sulphureus* by other individuals of the same or different species. These individuals were either predators or competitors for nectar or pollen. Disruptions occurred after collisions between visitors or by approaching a capitulum already occupied by another worker. In 2016, for 1182 visits of *A. mellifera*, 58 (4.90 %) were interrupted, whereas in 2017, for 1015 visits of this bee, 49 (4.83 %) were interrupted (Table 2). Visits' interruptions reduced the duration of certain honeybees' visits. This obliged some workers to visit more capitula during a foraging trip, in order to obtain their maximal pollen or nectar loads. Similar findings were made for *A. mellifera* workers foraging on the flowers of *Persea americana* (Lauraceae) and *Vitellaria paradoxa* (Sapotaceae) in Ngaoundere [22].

Table 2 Interrupted visits of *Apis mellifera* on *Cosmos sulphureus* capitula in 2016 and 2017 at Dang

Years	Number of studied visits	Visits interrupted by insects		Insect responsible of visits' interruption
		Number	Percentage (%)	
2016	1182	56	4.74	<i>Apis mellifera</i>
		1	0.08	<i>Acraea</i> sp.
		1	0.08	<i>Hypolimnas misippus</i>
2017	1015	47	4.63	<i>Apis mellifera</i>
		1	0.10	<i>Acraea</i> sp.
		1	0.10	<i>Halictus</i> sp.

3.4.8. Influence of neighbouring flora

During the observations periods, flowers of many other plant species situated close to *C. sulphureus* plots were visited by *A. mellifera* workers for nectar (ne) or pollen (po). Among these plants were *Bidens pilosa* (Asteraceae ; ne and po), *Bixa orellana* (Bixaceae ; ne and po), *Cajanus cajan* (Fabaceae ; ne), *Emilia coccinea* (Asteraceae ; ne and po), *Helianthus annuus* (Asteraceae ; ne and po), *Lantana camara* (Verbenaceae ; ne), *Mimosa invisa* (Mimosaceae ; po), *Sida rhombifolia* (Malvaceae ; ne and po), *Tithonia diversifolia* (Asteraceae ; ne and po) and *Vigna subterranea* (Fabaceae ; ne). During their foraging trips, *A. mellifera* foragers left *C. sulphureus* capitula for *Bidens pilosa* only twice, in 2016. This result indicates a flower constancy of *A. mellifera* on *C. sulphureus*. This floral constancy is due to the fact that, individual forager of honeybees is generally able to memorize and recognize the shape, the colour and the odour of the visited flowers during previous foraging trips [1] ; it may also be due to the good organization of honeybees, recruiting a large

number of workers for an efficient exploitation of an interesting food source [23] and to the good level of floral reward in nectar and pollen to the foragers [24] .

3.4.9. Influence of climatic factors

During the investigation periods, the temperature and the relative humidity of the air did not significantly influence the activity of *A. mellifera* on *C. sulphureus*. In 2016, there was no correlation between the number of *A. mellifera* visits and the temperature ($r = 0.13$; $df = 4$; $P > 0.05$) as well as between the number of visits and relative humidity ($r = -0.09$; $df = 4$; $P > 0.05$) (Figure 4, A). In 2017, there was no correlation between the number of *A. mellifera* visits and the temperature ($r = 0.35$; $df = 4$; $P > 0.05$) ; as well as between the number of visits and relative humidity ($r = -0.34$; $df = 4$; $P > 0.05$) (Figure 4, B).

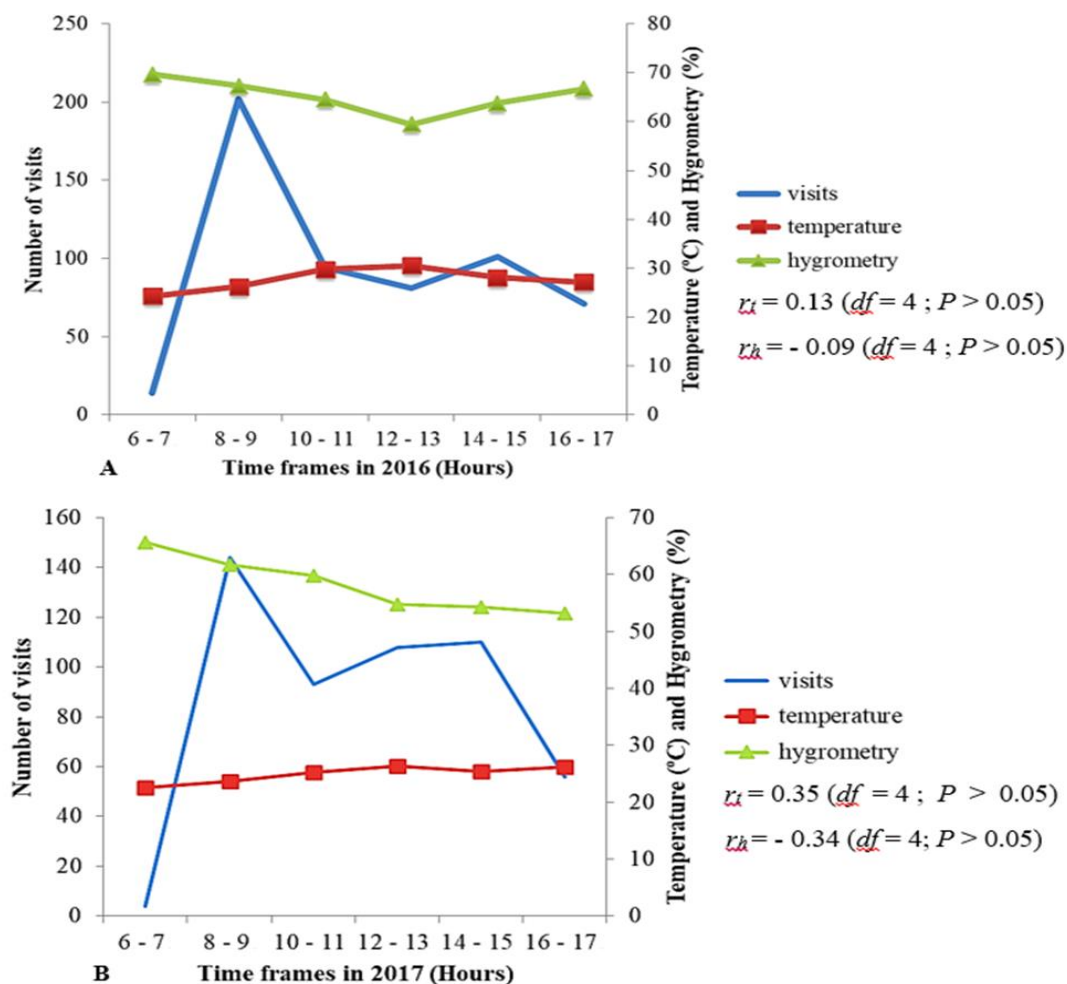


Figure 4 Daily variation of *Apis mellifera* visits on *Cosmos sulphureus* florets, mean temperature and mean relative humidity of the study site in 2016 (A) and in 2017 (B).

r_t : correlation coefficient between the number of visits and temperature ; r_h : correlation coefficient between the number of visits and hygrometry ; df : degree of freedom ; P : probability

3.5. Apicultural value of *Cosmos sulphureus*

During the flowering periods of *C. sulphureus*, a well elaborated activity of *A. mellifera* workers was observed on its capitula. There was a good daily and seasonal frequency of visits, a high density of workers per capitula, a very good nectar and pollen harvest and a fidelity of workers to the capitula. Moreover, each *C. sulphureus* plant can produce over 100 capitula ($n = 16$; $mean = 146.38$; $min = 80$; $max = 230$). These data highlight the very good attractiveness of *C. sulphureus* nectar and pollen to *A. mellifera*. Therefore, *C. sulphureus* is a highly nectariferous and polliniferous bee plant. This Asteraceae could be grown to: a) help stabilize *A. mellifera* colonies during the rainy season; b) increase honey yield; c) improve pollen production as a beehive product.

Other species of Asteraceae have been identified as honey bees polliniferous (po) and nectariferous (ne) plant species : *Vernonia amygdalina* (ne ; po) and *Vernonia cinerea* in Nigeria (ne ; po) [25] ; *Bidens pilosa* (ne ; po), *Bidens bipinnata* (ne ; po) and *Tridax procumbens* (ne ; po) in Benin [23] ; *Calendula officinalis* (po), *Centaurea macrocephala* (po), *Dahlia pinnata* (po), *Tagetes patula* (po), *Tithonia rotundifolia* (po) and *Zinnia elegans* (po) in Poland [10] ; *Helianthus annuus* (ne ; po) and *Eugenia uniflora* (ne ; po) in Cameroon [11, 26].

3.6. Impact of flowering insects including *Apis mellifera* on *Cosmos sulphureus* yields

While harvesting nectar and pollen on florets, workers got into contact with anthers and then gathered pollen over their body. *Apis mellifera* workers increased the possibilities of self-pollination and cross-pollination of *C. sulphureus* while moving from one capitulum to another. For the two study years, the frequency of contact between foragers and the stigma was 100% for nectar harvest as well as for pollen collection.

In Table 3 are presented the fruiting rate, the percentage of mature achenes and the percentage of normal achenes in the different *C. sulphureus* treatments. It appears from this table that:

- the fruiting rates were 53.29 %, 19.10 %, 42.42 %, 16.94 %, 50.43 %, 27.17 %, 35.33 % and 15.65 % in treatments 1 to 8 respectively. The differences between these eight percentages are globally highly significant ($\chi^2 = 3938.44$; $df = 7$; $P < 0.001$). Two - to - two comparisons showed that the difference observed is highly significant between treatments 1 and 2 ($\chi^2 = 1209.69$; $df = 1$; $P < 0.001$) as well as between treatments 3 and 4 ($\chi^2 = 916.31$; $df = 1$; $P < 0.001$). Consequently, in 2016 and 2017, the fruiting rate of uncovered capitula (treatments 1 and 3) was higher than that of capitula bagged during their flowering period (treatments 2 and 4);

- the percentages of mature achenes were 98.99 %, 85.72 %, 98.25 %, 73.73 %, 97.34 %, 89.01 %, 96.31 % and 73.47 % in treatments 1 to 8 respectively. The differences between these eight percentages are globally highly significant ($\chi^2 = 1688.64$; $df = 7$; $P < 0.001$). Two - to - two comparisons showed that the difference observed was highly significant between treatments 1 and 2 ($\chi^2 = 298.42$; $df = 1$; $P < 0.001$), as well as between treatments 3 and 4 ($\chi^2 = 557.10$; $df = 1$; $P < 0.001$). So, in 2016 and 2017, the percentage of normal achenes from unprotected capitula was higher than that from capitula protected from insects' visits;

- the percentages of normal achenes were 86.55 %, 49.59 %, 97.26 %, 42.78 %, 82.37 %, 50.39 %, 94.73 % and 51.25 % in treatments 1 to 8 respectively. The differences between these eight percentages are globally highly significant ($\chi^2 = 2963.75$; $df = 7$; $P < 0.001$). Two - to - two comparisons showed that the difference observed was highly significant between treatments 1 and 2 ($\chi^2 = 477.22$; $df = 1$; $P < 0.001$), as well as between treatments 3 and 4 ($\chi^2 = 1337.94$; $df = 1$; $P < 0.001$). Thus, in 2016 and 2017, the percentage of normal achenes from unprotected capitula was higher than that from capitula protected from insects' visits.

Table 3 Fruiting rate, percentage of mature achenes and percentage of normal achenes according to the different treatments of *Cosmos sulphureus* in 2016 and 2017 at Dang.

Treatments	Years	NSF	NFA	% FR	NMA	% NMA	NNA	% NA
1 (Uc)	2016	5399	2877	53.29	2848	98.99	2465	86.55
2 (Bc)		4435	847	19.10	726	85.72	360	49.59
3 (Uc)	2017	6212	2635	42.42	2589	98.25	2518	97.26
4 (Bc)		5710	967	16.94	713	73.73	305	42.78
5 (Cpva)	2016	6562	3309	50.43	3221	97.34	2653	82.37
6 (Cpww)		3684	1001	27.17	891	89.01	449	50.39
7 (Cpva)	2017	8752	3092	35.33	2978	96.31	2821	94.73
8 (Cpww)		5228	818	15.65	601	73.47	308	51.25

Uc: capitula left unprotected to allow insect visits; Bc : capitula bagged to avoid insect visits ; Cpva : capitula protected, then uncovered, visited once by *Apis mellifera* and reprotected; Cbww : capitula protected, then uncovered and reprotected without insects or any other organism visits ; NSF : number of studied florets ; NFA : number of formed achenes ; FR : fruiting rate ; NMA : number of mature achenes ; NNA : number of normal achenes ; NA : normal achenes

In 2016, the numeric contributions of the anthophilous insects including *A. mellifera* in the fruiting rate, the percentage of mature achenes and the percentage of normal achenes of *C. sulphureus* were 59.86 %, 9.94 % and 41.06 % respectively. In 2017, the corresponding figures were 62.24 %, 22.43 % and 47.19 % respectively. For the two cumulate years, the numeric contributions of flowering insects were 61.05 %, 16.19 %, and 44.13 % for the fruiting rate, the percentage of mature achenes and the percentage of normal achenes respectively. This significant increase in achenes production in the presence of flowering insects including *A. mellifera* is the consequence of their foraging activity on the pollination of the visited flowers.

3.7. Pollination efficiency of *Apis mellifera* on *Cosmos sulphureus*

In 2016, the numeric contribution of *A. mellifera* via a single capitulum visit on *C. sulphureus* was 47.26 % for the fruiting rate, 8.89 % for the percentage of mature achenes and 36.54 % for the percentage of normal achenes. In 2017, the corresponding figures were 53.90 %, 19.85 % and 44.49 % respectively. For the two cumulated years, the numeric contribution of a single visit of *A. mellifera* on the fruiting rate, the percentage of mature achenes and the percentage of normal achenes were 50.58 %, 14.37 % and 40.52 % respectively.

The intervention of *A. mellifera* in the pollination of this plant species is clearly expressed since its density per 1000 flowers and its foraging speed were high. The positive contribution of *A. mellifera* on the fruiting rate and the percentage of normal achenes was also found in Ngaoundere, on *H. annuus* [11, 20]. *Apis mellifera* workers also provided allogamous pollination by carrying pollen with their hair, legs, mouthparts, thorax and abdomen, from floret to floret on the capitula of a same plant or to the ones of other plants of the same species.

The numeric contribution of *A. mellifera* in *C. sulphureus* fruiting rate, mature achenes and normal achenes through its pollination efficiency was significantly higher than that of other insects. This shows that *A. mellifera* is one of the major pollinator insect of *C. sulphureus*. Similar results were reported in Cameroon on *H. annuus* by Tchuenguem *et al.* [20] in 2009 and Egono *et al.* [11] in 2018.

4. Conclusion

At Dang, *Cosmos sulphureus* is a plant species that obtains benefits from pollination by insects among which *Apis mellifera* is one of the most important. This bee intensely harvests nectar and pollen of the Asteraceae. The comparison of achene yields of capitula protected, then uncovered and reprotected without insects or any other organism visit to those of capitula protected then uncovered, visited once by *A. mellifera* and reprotected, shows the value of honeybees in the increase of achenes yields and normal achenes. Data on the foraging activity of *A. mellifera* on *C. sulphureus* capitula enable the classification of this Asteraceae among the highly nectariferous and polliniferous bee plants. Through the pollination efficiency of a visit on a *C. sulphureus* capitulum, *A. mellifera* induced a significant increment of the fruiting rate by 50.58 %, the percentage of mature achenes by 14.37 % and the percentage of normal achenes by 40.52 %. Thus, *C. sulphureus* could be protected and grown in the region to : a) increase honey yield ; b) improve pollen production as a hive product ; c) stabilize *A. mellifera* colonies during the rainy season.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors report no conflict of interest in this work.

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