

Pollination Efficiency of *Apis mellifera* L. (Hymenoptera: Apidae) on Flowers of *Sesamum indicum* L. (Pedaliaceae) at Bilone (Obala, Cameroon)

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Abstract: To evaluate *Apis mellifera* impact on pod and seed yields of *Sesamum indicum*, its foraging and pollinating activities were studied in Bilone during the rainy season of 2016 and 2017. Treatments included unlimited floral access by all visitors, bagged flowers to avoid all insects' pollinators and limited of single visit of *A. mellifera* worker. For each year of study, observations were made on 40 ± 7 flowers per treatment. *Apis mellifera* foraging behavior and its pollination efficiency were evaluated. Results show that this bee foraged nectar and pollen on *S. indicum* flowers during the whole blooming period. The number of seeds per pod and the percentage of normal seeds of unprotected flowers were significantly higher than those of flowers protected from insects ($P < 0.001$). Through its pollination efficiency, *A. mellifera* provoked a significant increment of the number of seeds per pod by 17.54% in 2016 and 8.77% in 2017, and the percentage of normal seeds by 9.53% in 2016 and 7.70% in 2017. The installation of *A. mellifera* colonies close to *S. indicum* fields is recommended to increase pod and seed yield in the region.

Keywords: *Apis mellifera*, *Sesamum indicum*, flower, pollination.

1. INTRODUCTION

Several plant species depend on insect pollinators for their reproduction [1, 2]. In agro ecosystems, these pollinators have a great ecological and economic importance because they influence positively plant productions [2, 3]. *Sesamum indicum* is annual plant originated from Africa [4]. Sesame plants grow upright to 3 m and carry many flowers per branch; these flowers are white, but can vary from pink to red and produce nectar and pollen that attract insects [5, 6]. China and Indian are the largest producers of sesame in the world [7]. The fruit is a pod containing four row of twelve seeds [8]. Seeds contain oil (63%), proteins (31%), glucose (3%) and minerals that are important for human's diets [9]. The research conducted in India by Viraktmath et al. [10], in Egypt by Kamel et al. [11] and in Northwest Region of Cameroon by Atibita et al. [12] have revealed that *Apis mellifera* visits *S. indicum* flowers and collect nectar and pollen. No previous research has been reported on the relationships between *S. indicum* flowers and *A. mellifera* in Bilone (Center Region, Cameroon), although, the activities of insects on the flowers can vary with regions [3, 13]. The main objective of this research was to gather more data on the relationships between *S. indicum* and *A. mellifera*. Specific objectives were to study the activities of this bee on *S. indicum* flowers and to evaluate its pollination efficiency on pod and seed yields.

2. MATERIALS AND METHODS

2.1. Site and biological materials

The studies were conducted twice, March to June in 2016 and 2017 in a field located at the campus of the Institute of Agronomy at Bilone (Obala) (Latitude: N 04.20514°, Longitude: E 011.51694°, Altitude: 525 m) in the Center Region of Cameroon. This Region belongs to the tropical rainforest

agro-ecological zone [14]. The climate is equatorial guinean-type with four seasons: the peak rainy season (August to November), the peak dry season (November-March), the mild rainy season (March-July) and the mild dry season (July-August), the mean annual temperature is 25°C, while the mean annual relative humidity is 79% [15]. The experimental plot was an area of 800 m². The animal material was represented by insects naturally present in the environment and a colony of *A. mellifera* (Hymenoptera: Apidae) located 22 m from the experimental field. Vegetation was represented by wild species and cultivated plants. The plant material was represented by the seeds of *S. indicum* provided by the Institute of Agricultural Research for Development in Nkolbisson (Yaoundé).

2.2. Planting and maintenance of culture

On March 15, 2016 and 2017, the experimental plot was divided into six subplots (6 * 5.5 m²). Seeds were sown on five lines per subplot; each line had five holes and each hole received 10 to 14 seeds. The spacing was 1 m between rows and 1 m on rows. Each hole was 4 cm depth. Two weeks after germination, the plants were thinned and only two were left per hole. Weeding was performed manually as necessary to maintain plot weeds-free.

2.3. Determining the reproduction mode

In May 18 and May 23 respectively in 2016 and 2017, 90 flowers of *S. indicum* at the bud stage were labeled. Forty five of the total number flowers were allowed for treatment 1 (open pollination) and 45 others flowers belong to treatment 2 (bagged with gauze bags to prevent visitors or external pollinating agents) (figure 1). For each year, fifty days after the wilting of the last flower, the number of pod formed in each treatment was counted. For each treatment, the fruiting index (*Ifr*) was then calculated using the following formula: $Ifr = (F1/F2)$, where *F1* is the number of pod formed and *F2* the number of flowers initially labeled [16]. The out crossing rate (*TC*) was calculated using the formula: $TC = \{[(Ifr_x - Ifr_y/Ifr_x)*100]\}$, where *Ifr_x* and *Ifr_y* are mean fruiting indexes in treatments 1 and 2 respectively [17]. The rate of self-pollination in the broad sense (*TA*) was calculated using the formula: $TA = (100 - TC)$.



Figure1. Plants of *Sesamum indicum* showing flowers isolated from insects

2.4. Study of the activity of *Apis mellifera* on *Sesamum indicum* flowers

Observations were conducted on individually opened flowers of treatment 1 each day, from May 26 to June 12 in 2016 and from May 21 to June 18 in 2017 at one hour interval from 9 to 16 h (9-10 h, 11-12 h, 13-14 h, 15-16 h). In a slow walk along all labeled flowers of treatment 1, the identity of all insects that visited *S. indicum* flowers was recorded. All insects encountered on flowers were recorded and the cumulated results expressed in number of visits to determine the relative frequency of *A. mellifera* in the anthophilous entomofauna of *S. indicum*. Direct observations of the foraging activity of worker bees on flowers were made. The floral products (nectar and/or pollen) collected by the foragers were recorded for the same dates and time slots as that of the insect counts. The duration of visits and the foraging speed (number of flowers visited per minute) [16] were timed to the same dates and in four time slots (10-11 h, 12-13 h, 14-15 h, 16-17 h). Abundances (larger numbers of individuals simultaneously active) per flower and per 1000 flowers (*A₁₀₀₀*) were recorded on the same dates and time slots as that of the registration of the duration of visits. The first parameter was recorded as a result of direct counts. For the abundance per 1000 flowers, honeybees were counted on

a known number of open flowers; $A_{1000} = [(Ax/Fx)*1000]$, where F_x and A_x are respectively the number of flowers and the number of individual bees actually counted on F_x [16]. During the days of investigation, the temperature and humidity of the study site were recorded every 30 min, from 9 am to 17 pm, using a thermo hygrometer installed in the shade.

2.5. Evaluation of the impact of pollinating insects on *Sesamum indicum* yield

This evaluation was based on the impact of flowering insect on pollination, the impact of pollination on fruiting and the comparison of yields (fruiting rate, mean number of seeds per pod and percentage of normal i.e. well developed seeds) of treatment 1 (unprotected flowers) and treatment 2 (bagged flowers). The fruiting rate due to the influence of foraging insects (Fr_i) was calculated using the formula: $Fr_i = \{[(Fr_x - Fr_y) / Fr_x] * 100\}$, where Fr_x and Fr_y are the fruiting rate in treatments 1 and 2 respectively. The fruiting rate of a treatment (Fr) is $Fr = [(F_2 / F_1) * 100]$, where, F_2 is the number of pods formed and F_1 the number of viable flowers initially set [16]. At maturity, pods were harvested from all treatments. The mean number of seeds per pod and the percentage of normal seeds were then calculated for each treatment.

2.6. Assessment of the pollination efficiency of *Apis mellifera* on *Sesamum indicum*

In 2016, along with the development of treatment 1 and 2, 30 flowers were protected using gauze bag (treatment 3). In 2017 the same experience was repeated with the same number of flowers. Between 6 and 9 am, the gauze bag is gently removed from each newly bloomed flower which was then observed during for one to 20 min. Flowers visited by *A. mellifera* were marked. After this manipulation, the flowers were protected once more. The impact of *A. mellifera* on fruiting rate (Pfx) was calculated using the following formula: $Pfx = \{[(f3-f2)/f3]*100\}$, where $f3$ and $f2$ are the fruiting rates in treatment 3 (protected flowers visited exclusively by *A. mellifera*) and treatment 2 (protected flowers) [16]. The number of seeds per pod, the percentage of normal seeds was then calculated for each treatment 3.

3. Data analysis

SPSS software and Microsoft Excel were used for three tests: Student's (t) for the comparison of means, correlation coefficient (r) for the study of linear relationship between two variables, Chi-square (χ^2) for the comparison of percentages.

4. RESULTS

4.1. Reproduction mode

In 2016, the podding index was 0.77 for treatment 1 and 0.25 for treatment 2, while in 2017, it was 0.73 and 0.62 for the two treatments respectively. Consequently, in 2016 the allogamy rate was 25.97 % and the autogamy rate was 74.03 %. In 2017, the corresponding figures were 15.06 % and 84.94 %. It appears that the variety of *S. indicum* used in our experiments has a mixed reproduction mode, autogamous -allogamous, with the predominance of autogamy over allogamy.

4.2. Activity of *Apis mellifera* on *Sesamum indicum* flowers

4.2.1. Seasonal frequency of visits

Amongst the 467 and 715 visits of 13 and 15 insects species recorded on *S. indicum* flowers respectively in 2016 and 2017, *A. mellifera* was the most represented insect with 194 visits (41.54%) in 2016 and 327 visits (45.73%) in 2017 (Table 1). The difference between these two percentages is not significant ($\chi^2 = 2.02$; $df = 1$; $P > 0.05$).

Table 1: Diversity of insects on *Sesamum indicum* flowers in 2016 and 2017, number and percentage of visits of different insects at Bilone

Insects			2016		2017		Total	
Order	Family	Genus, species, Sub -	n_1	$p_1\%$	n_2	$p_2\%$	$n_{1,2}$	$p_{1,2}\%$
Diptera	Calliphoridae	(1 sp.) ^{nt}	8	1.71	0	0	8	0.68
	Muscidae	<i>Musca domestica</i> ^{nt}	29	6.21	39	5.45	68	5.75
Hymenoptera	Apidae	<i>Apis mellifera</i> ^{po, nt}	194	41.54	327	45.73	521	44.07
		<i>Xylocopa olivacea</i> ^{po, nt}	37	7.92	58	8.11	95	8.04

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		<i>Amegilla</i> sp. ^{po, nt}	59	12.63	46	6.43	105	8.89
	Eumenidae	<i>Delta</i> sp. ^{nt}	0	0	12	1.67	12	1.02
	Formicidae	(1 sp.) ^{nt}	6	1.28	53	7.41	59	4.99
	Halictidae	<i>Lasioglossum</i> sp. ^{po, nt}	0	0	37	5.17	37	3.13
	Megachilidae	<i>Chalicodoma</i> sp. ^{nt}	81	17.34	38	5.31	119	10.07
		<i>Megachile</i> sp. ^{po, nt}	16	3.43	63	8.81	79	6.68
	Vespidae	<i>Synagris cornuta</i> ^{po, nt}	4	0.86	7	0.97	11	0.93
Lepidoptera	Acraeidae	<i>Acraea acerata</i> ^{nt, rt}	9	1.93	6	0.83	15	1.27
	Pieridae	<i>Catopsilia flerella</i> ^{nt, rt}	5	1.07	3	0.41	8	0.68
	Nymphalidae	(1 sp.) ^{nt, rt}	0	0	7	0.97	7	0.59
Orthoptera		(1 sp.) ^{df}	7	1.50	15	2.09	22	1.86
Neuroptera		(1 sp.) ^{pr}	12	2.58	4	0.55	16	1.35
Total	16 species		467	100	715	100	1182	100

n_1 : number of visits on 45 flowers in 16 days. n_2 : number of visits on 45 flowers in 23 days. p_1 et p_2 : percentages of visits. $p_1 = (n_1 / 467) * 100$. $p_2 = (n_2 / 715) * 100$. $n_{1,2} = (n_1 + n_2)$. $p_{1,2} = [(n_1 + n_2) / 1182] * 100$. nt: visitor collected nectar. po: visitor collected pollen. df: defoliator. rt: rest. pr: predator. sp.: undetermined species.

4.2.2. Floral substances harvested

From our field observations, *A. mellifera* workers were found to collect pollen and to harvest nectar on *S. indicum* flowers (Figure 2). In 44 and 56 visits counted on flowers respectively in 2016 and 2017, 26 (59.09%) and 30 (53.57%) were for nectar collection, 11 (25.00%) and 17 (30.36%) for pollen collection, respectively in 2016 and 2017 (Table 2). For the total of 100 visits recorded during the two seasons, the number of visits allocated to nectar harvest was 56 (56.00%) and that for pollen collection was 28 (28.00%).



Figure2. *Apis mellifera* collecting nectar in a flower of *Sesamum indicum*

Table2. Products harvested by *Apis mellifera* on flowers of *Sesamum indicum* in 2016 and 2017 at Bilone

year	Number of visits studied	Visits for nectar harvest		Visits for pollen harvest		Visits for nectar and pollen harvest	
		Number	%	Number	%	Number	%
2016	44	26	59.09	11	25.00	7	15.91
2017	56	30	53.57	17	30.36	9	16.07

4.2.3. Abundance of *Apis mellifera* workers

In 2016, the highest mean number of *A. mellifera* workers simultaneously in activity active was one per flower ($n=35$; $s=0$) and 207 per 1000 flowers ($n=27$; $s=102$). In 2017, the corresponding figures were 1 per flower ($n=35$; $s=0$) and 258 per 1000 flowers ($n=30$; $s=98$). The difference between the mean number of bees per 1000 flowers in 2016 and 2017 was significant ($t=-1.89$; $df=55$; $P<0.05$).

4.2.4. Duration of visits per flower

The mean duration of *A. mellifera* visit on flower of *S. indicum* depended on the substance collected (table 3). In 2016, the mean duration of a visit was 2.37 sec ($n=11$; $s=0.12$) for pollen collection against 3.97 sec ($n=26$; $s=1.88$) for nectar harvest. In 2017, the corresponding results were 2.17 sec ($n=17$; $s=0.47$) for pollen harvest and 4.26 sec ($n=30$; $s=0.58$) for nectar collection. The differences between the duration of a visit to harvest nectar ($t=-0.78$; $df=54$, $P>0.05$) and that for pollen ($t=-1.33$; $df=26$; $P>0.05$) collection are not significant in 2016 and 2017. The difference between the

mean duration of the visit for pollen harvest and that for nectar collection was significant ($t=3.95$; $df=80$; $P<0.001$).

Table3. Duration of *Apis mellifera* visits on flowers of *Sesamum indicum* in 2016 and 2017 at Bilone

year	Visit for nectar harvest			Visit for pollen harvest		
	<i>n</i>	<i>m</i>	<i>s</i>	<i>n</i>	<i>m</i>	<i>s</i>
2016	26	3.97	1.88	11	2.37	0.12
2017	30	4.26	0.58	17	2.17	0.47

4.3. Foraging speed of *Apis mellifera* on the flowers of *Sesamum indicum*

On the flowers of *S. indicum*, *A. mellifera* visited 5 to 12 flowers/min in 2016 and 5 to 15 flowers/min in 2017. The mean foraging speed was 9 flowers/min ($n=30$; $s=3.12$) in 2016 and 11 flowers/min ($n=30$; $s=2.83$) in 2017. The difference between these two means is highly significant ($t=-10.49$; $df=58$; $P<0.001$).

4.3.1. Daily rhythm of visits

Apis mellifera workers were active on the flowers of *S. indicum* from 9 am to 16 pm, with a peak of visits between 9 am and 10 am in 2016 and in 2017 (Figure 3). Climatic conditions have influenced the activity of *A. mellifera* workers on *S. indicum* flowers in field conditions. The correlation was positive and higher significant between the number of *A. mellifera* visits on *S. indicum* flowers and temperature in 2016 ($r=0.98$; $df=3$; $P<0.05$) and not significant between these two parameters in 2017 ($r=-0.60$; $df=3$; $P>0.05$). The correlation between the number of visits and the relative humidity of the air was not significant in 2016 ($r=0.10$; $df=3$; $P>0.05$) as well as in 2017 ($r =-0.40$; $df=3$; $P>0.05$) (figure 4).

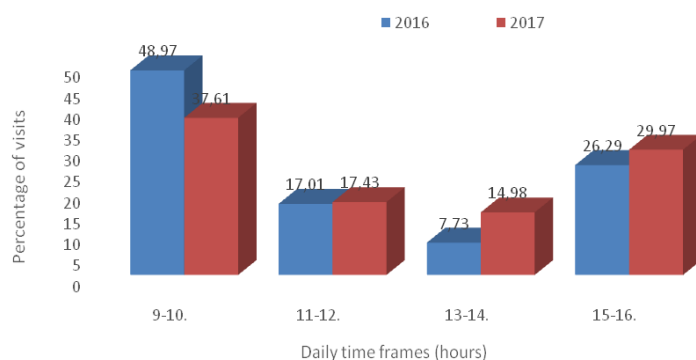


Figure3. Distribution of visits of *Apis mellifera* on the flowers of *Sesamum indicum* according to daily time in 2016 and 2017

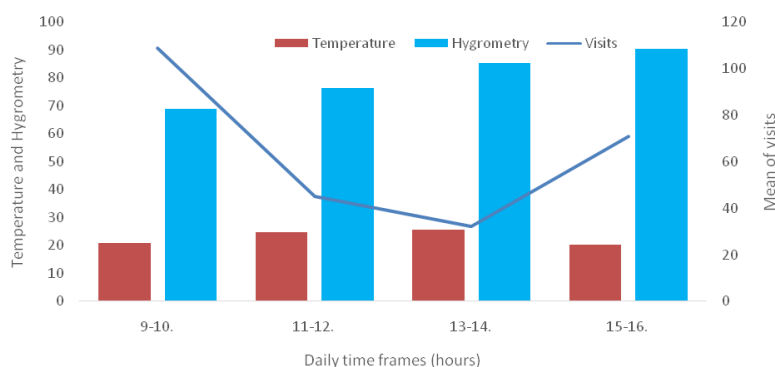


Figure4. Mean daily temperature and humidity and mean number of visits of *Apis mellifera* on *Sesamum indicum* flowers in 2016 and 2017

4.3.2. Apicultural value of *Sesamum Indicum*

During the mild rainy season in Bilone, we noted a well elaborated activity of *A. mellifera* workers on

S. indicum flowers. In particular, there was a very good nectar harvest, a good pollen collection and workers faithfulness to its flowers. These data allow to place *S. indicum* highly nectariferous and polliniferous bee plants.

4.4. Impact of flower-feeding insects in pollination and yields of *Sesamum indicum*

During pollen and nectar harvest, flowering insects of *S. indicum* were in regular contact with the anthers and stigma. Thus these insects increased the pollination possibilities of this plant species. Table 4 presents the results on the fruiting rate, the number of seeds per pod and the percentage of normal seeds in different treatments. From this table, we documented the following:

- a. The comparison of the fruiting rate showed that the difference was significant between treatment 1 (opened flowers) and treatment 2 (bagged flowers) in the first year ($\chi^2=2.71$; $df=1$; $p<0.1$) and not significant between these two treatments in the second year ($\chi^2=1.17$; $df=1$; $p>0.05$). Consequently, in 2016 and 2017, the fruiting rate of opened flowers was higher than that of bagged flowers ($\chi^2=4.99$; $df=1$; $p<0.05$).
- b. The comparison of the mean number of seeds per pod showed that the difference were highly significant between treatments 1 and 2 ($t=10.60$; $df=59$; $p<0.001$) in 2016 and in 2017 ($t=18.29$; $df=59$; $p<0.001$). Consequently, in 2016 and 2017, a mean number of seeds per pod of the unprotected flowers were higher than that of protected flowers.
- c. The comparison of the percentage of normal seeds showed that the difference were highly significant between opened flowers (treatment 1) and bagged flowers (treatment 2) in the first year ($\chi^2=6.17$; $df=1$; $p<0.001$) as well as in the second year ($\chi^2=26.62$; $df=1$; $p<0.001$). Thus, in 2016 and 2017 the percentage of normal seeds in opened flowers was higher than that of protected flowers in 2016 and in 2017.

The numeric contribution of pollinating insects to the fruiting rate, the mean number of seeds per pod and the percentage of normal seeds were respectively 26.70%, 16.07% and 3.31% in 2016. The corresponding figures were 0%, 13.33% and 6.74% in 2017. For the two cumulate years, the numeric contributions were 13.35%, 14.70% and 5.02% for the fruiting rate, the mean number of seeds per pods and the percentage of normal seeds respectively. The impact of pollinating insects on pod and seed yields was positive and significant.

4.5. Pollination efficiency of *Apis mellifera* on *Sesamum indicum*

From Table 4, it appears that:

- a. The comparison of the fruiting rate showed that the difference were not significant between treatment 3 (flowers protected and visited exclusively by *A. mellifera*) and treatment 2 (bagged flowers) in 2016 ($\chi^2=0.23$; $df=1$; $p>0.05$) as well as in 2017 ($\chi^2=0.48$; $df=1$; $p>0.05$).
- b. The comparison of the mean number of seeds per pod showed that the difference were highly significant between treatments 3 and 2 ($t=8.22$; $df=43$; $p<0.001$) in the first year and in the second year ($t=6.20$; $df=47$; $p<0.001$). Consequently, in 2016 and 2017, the pods produce by flowers bagged and visited exclusively by *A. mellifera* produced more seeds than those of protected flowers.
- c. The comparison of the percentage of normal seeds showed that the difference were highly significant between treatment 3 and treatment 2 in 2016 ($\chi^2=51.09$; $df=1$; $p<0.001$) and in 2017 ($\chi^2=28.07$; $df=1$; $p<0.001$) respectively. For the two years, the pods produce by flowers bagged and visited exclusively by *A. mellifera* have more normal seeds than those of protected flowers.

The numeric contribution of *A. mellifera* on the mean of number of seed per pod and the percentage of normal seeds were respectively 17.54% and 9.53% in 2016. The corresponding results were 8.77% and 7.70% in 2017. For the two years, the numeric contributions of *A. mellifera* were 13.15% and 8.61% for the mean number of seeds per pods and the percentage of normal seeds respectively. The impact of *A. mellifera* on pods and seeds yields was positive and significant.

Table4. Fruiting rate, number of seed per pod and percentage of normal seeds according to different treatments of *Sesamum indicum* in 2016 and 2017 at Bilone

Treatment	year	Flowers	pod	Fruiting rate	Seeds / pod		Total Seeds	Normal Seeds	%Normal seeds
					m	s			
Unlimited visits	2016	45	35	77.77	56	14	2205	1938	87.89
Bagged flowers		45	26	57.00	47	10	1352	1149	84.98
<i>Apis mellifera</i>		30	19	63.33	55	11	1140	1071	93.94
Unlimited visits	2017	45	33	73.33	60	9	2112	1880	89.01
Bagged flowers		45	28	62.00	52	7	1548	1285	83.01
<i>Apis mellifera</i>		30	21	70.00	57	12	1323	1190	89.94

5. DISCUSSION

Apis mellifera was the main floral visitor of *S. indicum* during the observation period. This bee has been reported as the main floral visitor of this plant in Egypt [18] and in Northwest Region of Cameroon [12]. *Apis mellifera* was also shown to be the most abundant floral visitors of other Fabaceae members such as *Glycine max* in Douala [19], *Phaseolus vulgaris* in Ngaoundere [20] and in Maroua [21]. The peak of the activity of *A. mellifera* on *S. indicum* flowers was located between 9 and 10 am, which correlated with the highest availability period of nectar on *S. indicum* flowers. The significant difference between the mean duration of the visit for pollen harvest and that for nectar collection could be attributed to the availability of floral products or the variation in the diversity of flowering insects from one year to another. During each flowering periods of *S. indicum*, *A. mellifera* harvested nectar and pollen. This could be attributed to the needs of colonies during the corresponding period. This research, indicates that *A. mellifera* can provide benefits to pollination management of *S. indicum*. During the collection of nectar and pollen on each flower, *A. mellifera* foragers regularly come into contact with the stigma. They were also able to carry pollen with their hairs, legs and mouth accessories from a flower of one plant to stigma of another flower of the same plant (geitonogamy), to the same flower (autogamy) or to that of another plant (xenogamy) [2]. The significant contribution of *A. mellifera* in pods and seed yield of *S. indicum* is in agreement with the similar findings in Egypt [18, 22] and in Northwest Region of Cameroon [12] on the same pedaliaceae. This plant species produces fewer seeds per pod in the absence of efficient pollinators [23]. The contribution of *A. mellifera* to *S. indicum* production through its pollination efficiency was significantly higher than that of all insects on the exposed flowers. The weight of *A. mellifera* played a positive role during nectar and pollen collection. *Apis mellifera* shook flowers and could facilitate the liberation of pollen by anthers for the optimal occupation of the stigma. This phenomenon was also reported by Tchuenguem and Dounia [24] on *Glycine max* in Maroua (Cameroon). The higher production of seeds per pod and that of normal seeds in the treatment with flowers visited exclusively by *A. mellifera* workers compared to that of the treatment with protected flowers showed that *A. mellifera* visit was effective in increasing pollination. Our results confirmed those of Atibita [23] who revealed that *S. indicum* flowers set little pods in the absence of insect pollinators.

6. CONCLUSION

This study reveals that the variety of *S. indicum* studied is a highly nectariferous and polliniferous bee plant that obtained benefits from the pollination by insects among which *A. mellifera* is the most important. The comparison of pod and seed sets of protected flowers to that of flowers visited exclusively by *A. mellifera* underscores the value of this bee in increasing pod and seed productions as well as seed quality. In the Center Region of Cameroon, the installation of *A. mellifera* hive close to *S. indicum* fields is recommended for the increase of the seed yields of this valuable crop.

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