

*Full Length Research Paper*

# **Pollination by *Xylocopa olivacea* Fabricius 1871 (Hymenoptera: Apidae) and potential benefits on *Vigna unguiculata* (L.) Walp. 1843 (Fabaceae) production in Djoumassi (North Region, Cameroon)**

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The investigation aimed to assess the impact of *Xylocopa olivacea* on *Vigna unguiculata* yields, and its foraging and pollinating activities were studied at Djoumassi from October 30th to November 04th in 2018 and from 04 to 10th October in 2019. A total of 540 flowers were observed, divided into four treatments. Two treatments were differentiated based on the presence or absence of flower protection against insect visits. The third treatment consisted of protected and opened flowers, allowing *X. olivacea* visits, while the fourth treatment involved opened flowers that were later closed without any visit. The daily rhythm of the bee's activity, as well as its foraging and pollination activities, was evaluated. Results indicated that *X. olivacea* intensely and exclusively collected nectar from *V. unguiculata* flowers. Its pollination efficiency increased the podding rate by 19.44 and 27.01%, the mean number of seeds per pod by 33.42 and 29.38%, and the percentage of normal seeds by 30.12 and 25.43% in 2018 and 2019, respectively. Therefore, it is recommended to establish and increase nests of *X. olivacea* near *V. unguiculata* fields to enhance pod and seed yields.

**Key words:** *Xylocopa olivacea*, *Vigna unguiculata*, nectar, pollination, yield, Garoua.

## **INTRODUCTION**

The sustainability of ecosystems, environmental quality, and biodiversity conservation relies on the propagation of

wild species facilitated by insect pollination (Rollin et al., 2015; Abajue, 2023). More than 85% of described

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flowering plant species are, to some extent, dependent on animal pollination, with mobile foraging insects constituting the majority of this activity (IPBES, 2016). Insect pollination offers benefits such as enhanced fruit quality, increased seed production, and improved fertility (Robinson et al., 2023). Social bees are frequent and tongued wild pollinators can effectively pollinate plants that may not be accessible for short-tongued pollinators effective pollinators of wildflowers and agricultural crops, garnering attention in insect pollinator studies (Feketéné et al., 2023). While research on honeybees has been more prominent compared to the numerous solitary bees; wild insect pollinators exhibit greater efficiency in pollinating certain plant species than honeybees. Long-like honeybees (Eeraerts, 2023). Many cultivated plant species, including *Vigna unguiculata*, have benefited from pollination by wild bees (Mohammadou et al., 2023).

This plant is utilized as food, animal feed, and a cash crop, especially in Central and West Africa. Cowpea leaves are promoted for consumption, providing valuable nutrients such as calcium, zinc, fiber, and phytonutrients (Enyiukwu et al., 2018). Additionally, the seeds are a rich source of protein, along with vitamins A and C, and potassium (Klein et al., 2020). Cowpea flowers produce nectar, which is attractive to insects, especially bees. However, these flowers have a higher level of self-pollination (Blackhurst and Miller, 1980). According to the Organization for Economic Co-operation and Development (2016), the pollination process in cultivated cowpeas is complete before the flower opens. Consequently, some opportunities for cross-pollination occur, providing pollinators are present (Hordzi, 2011). Before the present study, the flowering insects of *V. unguiculata* were studied in the Central Region of Ghana, where frequent visitors included *Apis mellifera*, *Ceratina* species, *Megachile* species, *Xylocopa calens*, *Xylocopa imitator*, *Brausepsis* species, *Lipotriches* species, *Melecta* species, *Amegilla* species, and Lepidopterans (Hordzi, 2014). In Cameroon, Tchuenguem et al. (2009) and Pharaon et al. (2019) observed *A. mellifera* as the abundant insect pollinator of cowpea, while Pando et al. (2013) and Djonwangwé et al. (2017) highlighted that the main insect visitors of cowpea flowers were *Chalicodoma cincta cincta* and *Megachile eurymera*, respectively. According to Ndayikeza (2014), cowpea flowers were visited mainly by *A. mellifera*, *Xylocopa caffra*, *Xylocopa flavorufa*, *Xylocopa hottentota*, *Xylocopa inconstans*, *Xylocopa nigrita*, *Xylocopa olivacea*, and *Xylocopa scioensis* in the agro-ecosystem of Burundi. Thus, the floral entomofauna of a plant varies in time and space (Tchuenguem et al., 2005). The main objective of this work was to contribute to the understanding of the relationships between *V. unguiculata* and *X. olivacea* for their optimal management in Cameroon. Specific objectives were to: (a) determine the place of *X. olivacea* in *V. unguiculata* flowering insects; (b) study the activity of this Apidae on cowpea flowers; (c) evaluate the impact

seeds yields; and (d) estimate the pollination efficiency of *X. olivacea* on this plant species.

## MATERIALS AND METHODS

### Study site, experimental plot, and biological material

The studies were conducted from June to October, in 2018 and 2019, in the locality of Djoumassi (latitude of 9°23'16.512' N, a longitude of 13°23'20.627' E, an altitude 169 m.a.s.l.), Benoue Division, North Region (Cameroon).

This region belongs to the Sahel-Sudanian ecological zone (MINEPAT, 2014). It has Sudanian climate type characterized by two annual seasons: a rainy season (April to October) and a dry season (November to March). August is the wettest month of the year. Annual rainfall varies from 750 to 1250 mm and the mean annual temperature is 27°C (Donfack, 1998; Hieng, 2009; MINEPAT, 2014).

The experimental plot was an area of 437 m<sup>2</sup>. Insects naturally present in the environment and three and five nests of *X. olivacea* in 2018 and 2019, respectively mainly represented the animal material, located near the experimental field.

The plant material was *V. unguiculata* variety BR<sub>1</sub> seeds obtained from the Institute of Research and Agricultural Development (IRAD) of Maroua in the Far North Region of Cameroon.

### Preparation of experimental plot, sowing, and weeding

From 24 to 29th June 2018 and from 3rd to 6th July 2019, the experimental plot was cleaned and divided into eight subplots, each measuring 8 × 4.5 m<sup>2</sup>. Four seeds were sown per hole and six lines per subplots were made, each line having six holes. Holes were separated by 50 cm from each other; while lines were 75 cm. Weeding was performed manually before the flowering periods.

### Determination of the reproduction mode of *V. unguiculata*

On September 25th, 2018, 240 flowers from untreated subplots at the bud stage were labeled among which 120 flowers were left unprotected (treatment 1), while 120 others were bagged using gauze bags nets (treatment 2) to prevent insects' visits. In similar subplots, on October 4th, 2019, 240 flowers at the bud stage were labeled of which 120 were unprotected (treatment 5), while 120 were bagged (treatment 6). For each cropping year, two weeks after shedding of the last labeled flower, the number of pods was assessed in each treatment. The fruiting index was then calculated as (Tchuenguem et al., 2001):

$$P_i = \frac{F_b}{F_a} \quad (1)$$

where  $F_b$  is the number of pods formed and  $F_a$  the number of viable flowers initially set. The allogamy rate ( $TC$ ) from which derives the autogamy rate ( $TA$ ) was expressed as the difference in fruiting indexes between treatment X (unprotected flowers) and treatment Y (bagged flowers) (Tchuenguem et al., 2001):

$$TC = \frac{(P_iX - P_iY) \times 100}{P_iX} \quad (2)$$

where  $P_iX$  and  $P_iY$  are respectively the mean fruiting indexes of

treatment X and treatment Y;  $TA = 100 - TC$ .

### Study of the foraging activity of *X. olivacea* on *V. unguiculata* flowers

From the opening of the first flower (occurred October 30th in 2018 and 4th October in 2019) to the fading of the last flower (November 4th in 2018 and 10th October in 2019), observations were done on flowers of treatments 1 and 5, according to six daily time frames: 6 - 7, 8 - 9, 10 - 11, 12 - 13, 14 - 15, and 16 - 17 h. Flowering insects that visited *V. unguiculata* flowers were recorded at each daily time frame during the blooming period. All insects encountered on flowers were recorded and the cumulated results expressed as number of visits have been used to determine the relative frequency of *X. olivacea* ( $F_x$ ) among flowering insects of *V. unguiculata* was calculated using the following formula for each year of study (Tchuenguem et al., 2001):

$$F_x = \frac{V_x}{V_i} \times 100 \quad (3)$$

where  $V_x$  is the number of visits of *X. olivacea* on flowers of free treatment and  $V_i$  the total number of insect visits on flowers of the same treatment.

During our investigations, before starting with the record of visits, the number of opened flowers was counted. The same days as for the registration of the frequency of visits, the floral products (nectar and/or pollen) collected by each worker bee were recorded for the same date and daily time frame. The study of this parameter indicates whether *X. olivacea* is strictly polliniferous and/or nectariferous on *V. unguiculata* flowers. This can give an idea of its involvement in the pollination of this plant and honey production. The duration of the individual flower visits was recorded (using a stopwatch) according to six daily time frames: 7 - 8, 9 - 10, 11 - 12, 13 - 14, 15 - 16, and 17 - 18 h. The foraging speed, expressed as the number of flowers visited by a worker bee per minute according to Jacob-Remacle (1989), was calculated using the following formula:

$$Vb = \frac{Fi}{di} \times 60 \quad (4)$$

where  $di$  is the time given by the stopwatch and  $Fi$  is the number of flowers visited during  $di$ .

The abundance of foragers (highest number of individuals foraging simultaneously) per flower or per 1000 flowers ( $A1000$ ) were recorded on the same dates and time slots as the duration of visits. Abundance per flower was recorded as a result of direct counting. To determining the abundance per 1000 flowers, some foragers were counted on a known number of opened flowers and  $A1000$  was calculated using the following formula (Tchuenguem et al., 2004):

$$A1000 = \frac{Ax}{Fx} \times 1000 \quad (5)$$

where  $F_x$  and  $A_x$  are respectively the number of flowers and the number of foragers effectively counted on these flowers at time  $x$ . The disruption of the activity of foragers by competitors and/or predators and the attractiveness exerted by other plant species on this insect was assessed by direct observations. For the second parameter, the number of times the worker bee went from *V. unguiculata* flowers to another plant species' flowers and vice versa was noted throughout the periods of investigations. During each day, temperature and relative humidity of the station were recorded after every 30 min using a mobile thermo-hygrometer (HT-9227) installed in the shade.

### Evaluation of the effect of insects including *Xylocopa olivacea* on *V. unguiculata* yields

The evaluation of the effect of insects including *X. olivacea* on *V. unguiculata* yields was based on the impact of flowering insects on pollination, the impact of pollination on *V. unguiculata* fruiting, and the comparison of fruiting rate, the mean number of seed per pod and the percentage of normal seeds of treatments 1 and 2 (2018) and 5 and 6 (2019).

For each year, the fruiting rate due to the foraging insects including *X. olivacea* ( $Fri$ ) was calculated using the following formula (Diguir et al., 2020):

$$Fri = \frac{(FX+Eg)-FY}{(FX+Eg)} \times 100 \quad (6)$$

where  $FX$  and  $FY$  are the fruiting rates in treatment X (flower left in free pollination) and treatment Y (flower protected from all insect visits), and  $Eg$  the effect of the gauze bag net who can be calculated using the formula  $Eg = FY - FZ$ , where  $FZ$  is the fruiting rate in treatment Z (flowers protected then opened and closed without insect or any other organism visit). Finally (Diguir et al., 2020),

$$Fri = \frac{FX-FZ}{FX+FY-FZ} \times 100 \quad (7)$$

The fruiting rate of a treatment ( $F$ ) is (Diguir et al., 2020):

$$F = \frac{b}{a} \times 100 \quad (8)$$

where  $b$  is the number of seeds formed and the number of viable flowers initially set. The impact of flower visiting insects including *X. olivacea* on the number of seeds per pod and the normal seeds were evaluated using the same method as mentioned earlier for the fruiting rate.

### Evaluation of the pollination efficiency of *X. olivacea* on *V. unguiculata*

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

- (1) Treatment 3 (2018) or 7 (2019): 400 flowers protected using gauze bag nets to prevent insect or any other organism visits and destined to be visited exclusively by *X. olivacea*. As soon as the first flowers of treatments 3 and 7, the gauze bag was gently removed and the flower observed for up to 10 min; the flower visited once by *X. olivacea* was marked and then protected again;
- (2) Treatment 4 (2018) and 8 (2019): 200 flowers protected using gauze bag nets and destined to be opened then closed without the visit of insects or any other organism. As soon as the first flowers of treatments 4 and 8 were opened, the gauze bag was gently removed and the flower was observed for up to 10 min, while avoid insect or any other organism to visit it. At the maturity, pods from each treatment were harvested and counted from.

To evaluate the pollination efficiency of *X. olivacea*, the fruiting rate, the number of seeds per pod, and the percentage of normal seeds were then calculated in these different treatments (treatments 3, 4, 7 and 8).

### Statistical analysis

Data were analyzed using descriptive statistics, student's  $t$ -test for

the comparison of means of the two samples, Pearson correlation coefficient ( $r$ ) for the study of the association between two variables, and Chi-square ( $\chi^2$ ) for the comparison of percentages, Microsoft Excel 2013 software was also used.

## RESULTS

### Reproduction mode of *V. unguiculata*

The podding indexes of *V. unguiculata* were 0.91, 0.51, 0.85 and 0.67 for treatments 1, 2, 5 and 6, respectively. Thus, in 2018, the allogamy rate was 43.95% and the autogamy rate was 56.05%. In 2019, the corresponding figures were 21.18 and 78.82%. It appears that, *V. unguiculata* variety BR<sub>1</sub> has a mixed mating system with the predominance of autogamy.

### Activity of *X. olivacea* on *V. unguiculata* flowers

#### Frequency of visits

Amongst the 485 and 433 visits of 25 and 23 insect species recorded on its flowers in 2018 and 2019, respectively, *X. olivacea* ranked second insect with 74 visits (15.26%) and 56 visits (12.93%) in 2018 and 2019, respectively (Table 1). The difference between these two percentages is not significant ( $\chi^2 = 1.02$ ;  $df = 1$ ;  $P > 0.05$ ).

#### Floral products harvested

During each flowering period, *X. olivacea* was seen harvesting intensively and exclusively nectar on *V. unguiculata* flowers (Figure 1).

#### Relationship between visits and flowering stages

The visits of *X. olivacea* were more numerous on treatments 1 and 3 when the number of opened flowers was high (Figure 2A and B). The correlation was highly significant between the number of *V. unguiculata* opened flowers and the number of *X. olivacea* visits in 2018 ( $r = 0.92$ ;  $df = 4$ ;  $P < 0.01$ ) as well as in 2019 ( $r = 0.95$ ;  $df = 5$ ;  $P < 0.01$ ).

#### Daily visits

The carpenter bee visits were registered on *V. unguiculata* flowers between 8 and 13 am. The bee activity period coincided with the opening flowers of this Fabaceae. The correlation was not significant between the number of *X. olivacea* visits and relative humidity in 2018 ( $r = 0.01$ ;  $df = 1$ ;  $P > 0.05$ ) and in 2019 ( $r = 0.85$ ;  $df$

$= 1$ ;  $P > 0.05$ ) (Figure 3A and B). The correlation was not significant between the number of *X. olivacea* visits and the temperature in 2018 ( $r = 0.48$ ;  $df = 1$ ;  $P > 0.05$ ) and in 2019 ( $r = 0.11$ ;  $df = 1$ ;  $P > 0.05$ ).

#### Duration of a visit per flower

In 2018, the mean duration of *X. olivacea* was 4.20 s ( $n = 87$ ;  $s = 3$ ;  $maxi = 15$  s) in 2018 and 4.15 s ( $n = 94$ ;  $s = 2.96$ ;  $maxi = 15$  s) in 2019. The difference between these durations of the visit for the cropping season was not significant ( $t = 0.11$ ;  $df = 179$ ;  $P > 0.05$ ). For the two cumulated years, the mean duration visit was 4.18 s.

#### Abundance of *X. olivacea* foragers

In 2018, the highest mean number of *X. olivacea* simultaneously active was 1 per flower ( $n = 46$ ,  $s = 0$ ) and 121.74 per 1000 flowers ( $n = 46$ ;  $s = 51.26$ ;  $maxi = 300$ ). In 2019, the corresponding figures were 1 per flower ( $n = 58$ ;  $s = 0$ ) and 174.14 per 1000 flowers ( $n = 58$ ;  $s = 123.63$ ,  $maxi = 600$ ). The difference between the mean number of *X. olivacea* per 1000 flowers in 2018 and that in 2019 was highly significant ( $t = -2.90$ ;  $df = 102$ ;  $P < 0.01$ ).

#### Foraging speed of *X. olivacea* on *V. unguiculata* flowers

During our observations, *X. olivacea* visited between 1 and 15 flowers/min in 2018 and between 1 and 17 flowers/min in 2019. The mean foraging speed was 4 flowers/min ( $n = 101$ ,  $s = 2.71$ ) in 2018 as well as in 2019 ( $n = 91$ ,  $s = 2.77$ ). The difference between these means is not significant ( $t = -0.28$ ;  $df = 190$ ;  $P < 0.05$ ). For the two cumulative years, the mean foraging speed was 4 flowers/min.

#### Influence of neighboring floral

During each observation periods, flowers of many other plant species surrounding the study area were visited by *X. olivacea*, for nectar and/or for pollen. Among these plants were: *Solanum lycopersicum* (Ne), *Solanum nigrum* (Ne, Po), and *Vitellaria paradoxa* (Ne).

#### Influence of wildlife

The foragers of *X. olivacea* were disturbed in their foraging activity by biotic factors such as other arthropods that were either by competitors for nectar and/or pollen and abiotic factors like wind, rain, and temperature.

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

Order	Family	Genus and species	2018		2019		Total			
			$n_1$	$p_1$ (%)	$n_2$	$p_2$ (%)	$n_T$	$p_T$ (%)		
Coleoptera	Coccinellidae	<i>Cheilomenes lunata</i> (fe)	0	-	2	0.46	2	0.22		
	Meloidae	<i>Coryna</i> spp. (fe)	2	0.41	4	0.92	6	0.65		
Diptera	Calliphoridae	(1 spp.) (ne)	1	0.21	0	-	1	0.11		
	Muscidae	<i>Musca domestica</i> (ne)	7	1.44	3	0.69	10	1.09		
	Syrphidae	(1 spp.) (fe)	1	0.21	0	-	1	0.11		
Hemiptera	Pentatomidae	(1 spp.) (ne)	0	-	3	0.69	3	0.33		
		<i>Amegilla</i> spp. 1 (ne, po)	9	1.86	4	0.92	13	1.42		
		<i>Amegilla</i> spp. 2 (ne, po)	11	2.27	0	-	11	1.20		
		<i>Apis mellifera</i> (ne)	144	29.69	174	40.18	318	34.64		
		Apidae	<i>Dactylurina staudingeri</i> (po)	3	0.62	0	-	3	0.33	
			<i>Lipotriches collaris</i> (po)	8	1.65	0	-	8	0.87	
			<i>Xylocopa inconstans</i> (ne)	25	5.15	15	3.46	40	4.36	
			<i>Xylocopa olivacea</i> (ne)	74	15.26	56	12.93	130	14.16	
		Hymenoptera	Formicidae	<i>Camponotus flavomarginatus</i> (ne)	14	2.89	8	1.85	22	2.40
				<i>Myrmecaria opaciventris</i> (ne)	0	-	3	0.69	3	0.33
(1 sp.) (ne)	0			-	7	1.62	7	0.76		
Halictidae	<i>Halictus</i> spp. (ne, po)		21	4.33	19	4.39	40	4.36		
Megachilidae	<i>Chalicodoma</i> spp. (ne)		33	6.80	42	9.70	75	8.17		
	<i>Megachile</i> spp. (ne)		24	4.95	17	3.93	41	4.47		
Vespidae	<i>Belonogaster juncea</i> (ne)		11	2.27	6	1.39	17	1.85		
	(2 spp.) (ne)		9	1.86	22	5.08	31	3.38		
Acraeidae	<i>Acraea acerata</i> (ne)		7	1.44	0	-	7	0.76		
	<i>Eurema</i> spp. (ne)		19	3.92	0	-	19	2.07		
Lepidoptera	Pieridae	<i>Papilio demodocus</i> (ne)	20	4.12	18	4.16	38	4.14		
		(2 spp.) (ne)	23	4.74	10	2.31	33	3.59		
Orthoptera	Acridae	<i>Tettigonia viridissima</i> (fe)	0	-	5	1.15	5	0.54		
		(2 spp.) (fe)	19	3.92	15	3.46	34	3.70		
Total		30 species	485	100	433	100	918	100		

$n_1$ : Number of visits on 120 flowers in six days;  $n_2$ : number of visits on 120 flowers in seven days; percentage of visits  $p_1 = (n_1/74) \times 100$ ;  $p_2 = (n_2/56) \times 100$ ; Comparison of percentages of *Xylocopa olivacea* visits (2018/2019):  $\chi^2 = 1.02$ ;  $df = 1$ ;  $P > 0.05$ ; fe: flower eater; ne: nectar collector; po: pollen collector; spp.: unidentified species.

These disturbances resulted in the interruption of some visits. In 2018, for 74 visits, 11 (14.86%) were interrupted by *A. mellifera* and 3 (4.05%) by wind. While in 2019 for 56, 2 (3.57%) were interrupted by other individuals of *X. olivacea*. For their load of floral products, some individuals of *X. olivacea* who suffered such disturbances were forced to visit more flowers during the corresponding

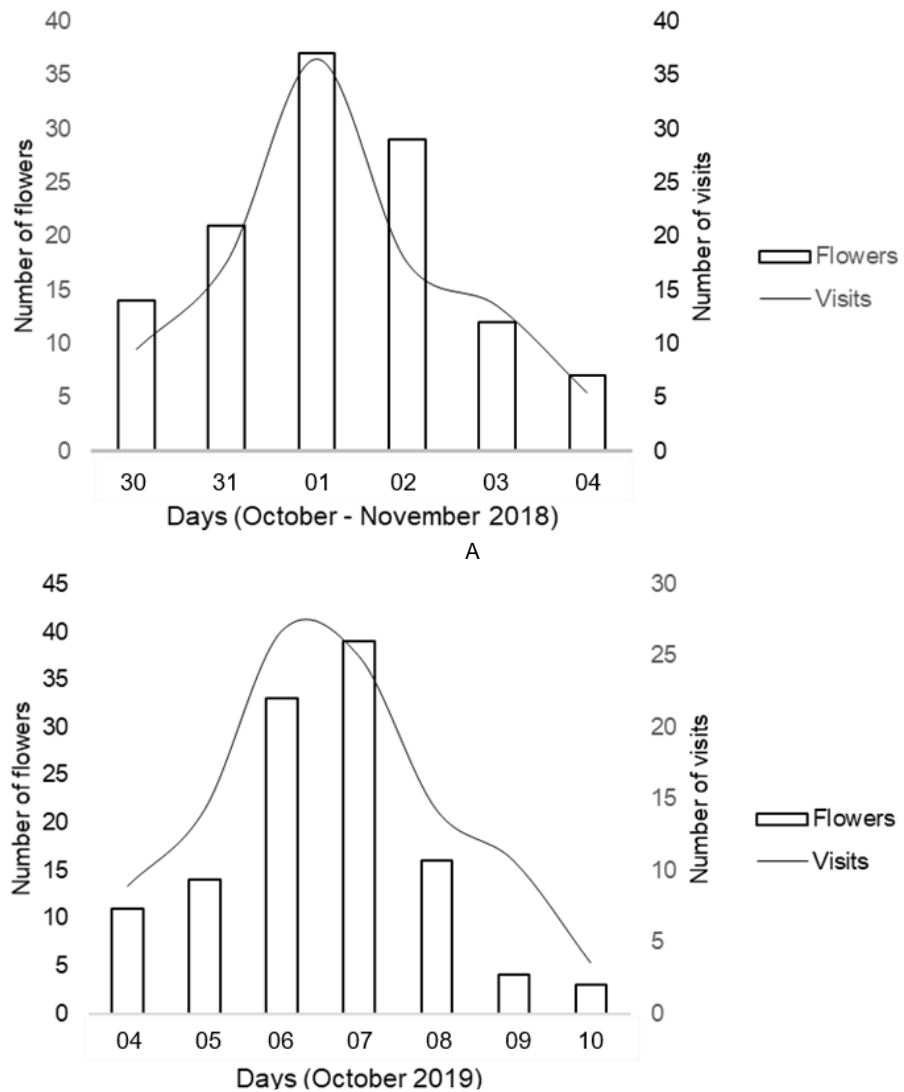
foraging trip.

#### Impact of the flowering insects including *X. olivacea* on *V. unguiculata* yields

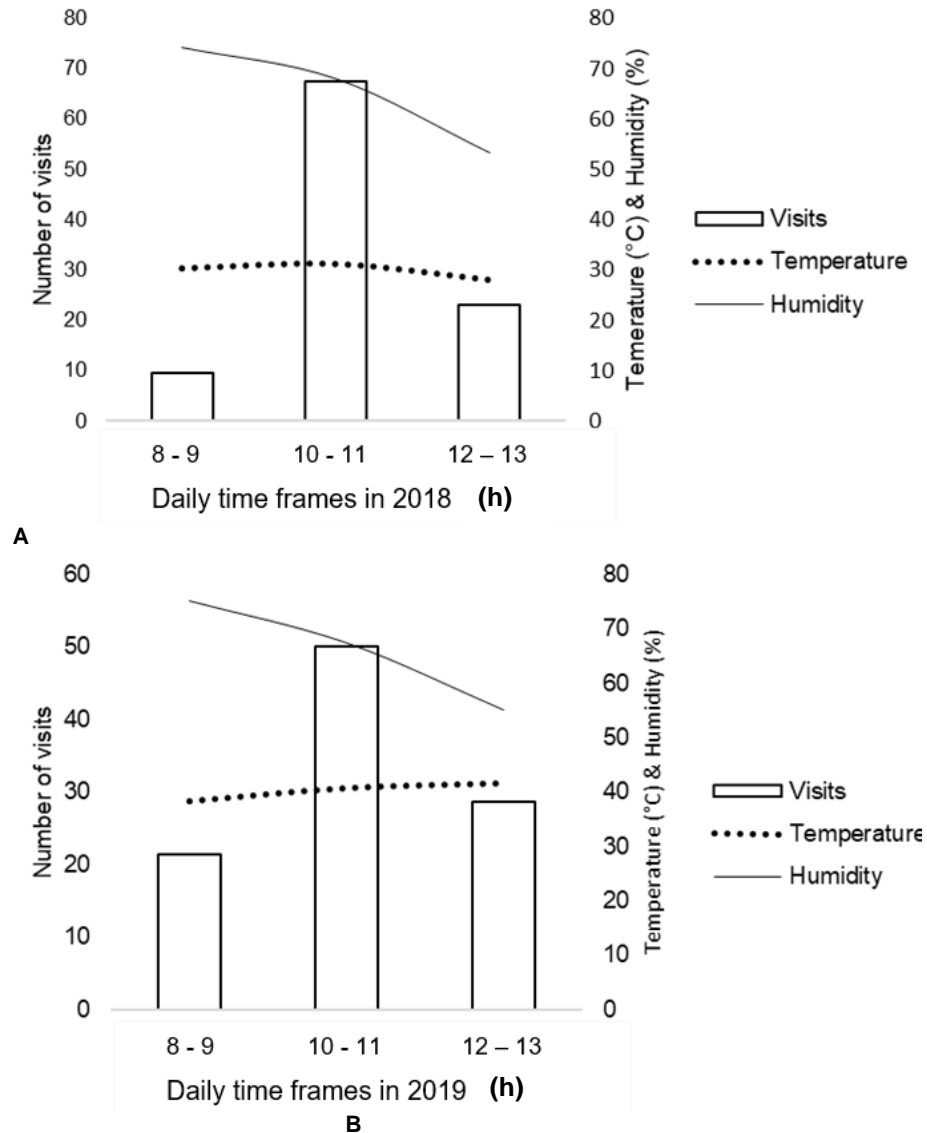
During nectar and/or pollen harvest on *V. unguiculata*,



**Figure 1.** *Xylocopa olivacea* harvesting nectar on *Vigna unguiculata* flower.



**Figure 2.** Seasonal variation of the number of *Vigna unguiculata* opened flowers and the number of *Xylocopa olivacea* visits in 2018 (A) and 2019 (B) at Djoumassi.



**Figure 3.** Daily distribution of *Xylocopa olivacea* visits on *Vigna unguiculata* flowers in 2018 (A) and 2019 (B), mean temperature and mean humidity of the study site.

flowering insects including *X. olivacea* always shook flowers and regularly contacted anthers and stigma, increasing self-pollination and/or cross-pollination possibilities of this plant species. Table 2 gives the podding rate, the mean number of seeds per pod and the percentage of normal seeds in different treatments. It appears from this table that:

a) The podding rate was 90.83, 50.83, 85, and 66.66% in treatments 1, 2, 5 and 6, respectively. The differences between these four percentages are highly significant ( $\chi^2 = 60.94$ ;  $ddl = 3$ ;  $P < 0.001$ ). Two-to-two comparisons showed that the difference observed is highly significant between treatments 1 and 2 ( $\chi^2 = 46.47$ ;  $ddl = 1$ ;  $P < 0.001$ ) and between treatments 5 and 6 ( $\chi^2 = 11$ ;  $ddl = 1$ ;

$P < 0.001$ ).

b) The mean number of seeds per pod was 14.61, 9.31, 15.02, and 9.37 in treatments 1, 2, 5 and 6, respectively. The difference between these four means are significant ( $F = 193.05$ ;  $ddl = 3$ ;  $ddl = 296$ ;  $P < 0.05$ ). Two-to-two comparisons showed that the difference observed is highly significant between treatments 1 and 2 ( $t = 9.07$ ;  $ddl = 198$ ;  $P < 0.001$ ) and between treatments 5 and 6 ( $t = 9.43$ ;  $ddl = 159$ ;  $P < 0.001$ ).

c) The percentages of normal seeds were 89.11, 37.68, 89.57 and 38.80% in treatments 1, 2, 5 and 6, respectively. The differences between these four percentages are highly significant ( $\chi^2 = 1209.88$ ;  $ddl = 3$ ;  $P < 0.001$ ). Two-to-two comparisons showed that the difference observed is highly significant between

**Table 2.** Podding rate, number of seeds per pod and percentage of normal seeds according to different treatments of *Vigna unguiculata* in 2018 and 2019 at Djoumassi.

Years	Treatments	NF	NFP	Pr (%)	Seed/Pod			TNS	NS	% NS
					<i>n</i>	<i>m</i>	<i>s</i>			
2018	1 (UF)	120	109	90.83	88	14.61	5.09	1607	1432	89.11
	2 (PF)	120	61	50.83	51	9.31	2.21	568	214	37.68
	3 (FvX)	150	114	76	114	15.56	5.08	1789	1556	86.98
	4 (Fbwv)	98	60	61.22	47	10.36	3.35	487	296	60.78
2019	5 (UF)	120	102	85	96	15.02	5.24	1457	1305	89.57
	6 (PF)	120	80	66.66	65	9.37	2.12	637	244	38.80
	7 (FvX)	150	131	87.33	131	15.25	5.08	2013	1750	86.93
	8 (Fbwv)	91	58	63.74	52	10.77	3.52	560	363	64.82

Uf: Unprotected flowers; Pf: Protected flowers; FvX: Flowers bagged then opened and exclusively visited by *Xylocopa olivacea*; Fbwv: Flowers bagged then closed and opened without insect or any other organism visit; NF: Number of flowers; NFP: Number of formed pods; Pr: Podding rate; TNS: Total number of seeds; NS: Normal seeds; % NS: Percentage of normal seeds; *m*: mean; *sd*: standard deviation.

treatments 1 and 2 ( $\chi^2 = 603.17$ ;  $ddl = 1$ ;  $P < 0.01$ ) and between treatments 5 and 6 ( $\chi^2 = 604.97$ ;  $ddl = 1$ ;  $P < 0.001$ ).

In 2018, the contribution of anthophilous insects including *X. olivacea* in the podding rate, the mean number of seeds per pod and the percentage of normal seeds were 36.81, 31.34, and 42.92%. In 2019, the corresponding figures were 24.18, 31.20, and 39.95%. For the cumulative years, the contributions of flowering insects were 30.50, 31.27, and 41.44% for the podding rate, the mean number of seeds per pod, and the percentage of normal seeds. The insect pollinators played a significant role in increasing pod and seed yields of *V. unguiculata*.

#### Pollination efficiency of *X. olivacea* on *V. unguiculata*

During nectar harvest on cowpea flowers, carpenter bees always contact anthers and stigma, thereby increased the pollination possibilities of *V. unguiculata* as they frequently flew from flowers to flowers on the same plants and/or on other flowers of the neighboring plants of cowpea. Table 2 reveals that:

(a) The fruiting rates were 76, 61.22, 87.33, and 63.70% in treatments 3, 4, 7 and 8, respectively. The differences between these four percentages are highly significant ( $\chi^2 = 27.62$ ;  $df = 3$ ;  $P < 0.001$ ). The difference was significant between treatments 3 and 4 ( $\chi^2 = 6.18$ ;  $df = 1$ ;  $P < 0.05$ ) and significant between treatments 7 and 8 ( $\chi^2 = 18.64$ ;  $df = 1$ ;  $P < 0.001$ ). Hence, in 2018 and 2019, the fruiting rate of flowers protected and visited exclusively by *X. olivacea* was higher than those protected, opened and closed without insect or any other organism visit.

(b) The means number of seeds per pod were 15.56, 10.36, 15.25, and 10.77 in treatments 3, 4, 7 and 8,

respectively. The differences between these four means are highly significant ( $F = 128.72$ ;  $df_1 = 3$ ;  $df_2 = 340$ ;  $P < 0.001$ ). The difference was highly significant between treatments 3 and 4 ( $t = 7.59$ ;  $df = 159$ ;  $P < 0.001$ ) as well as between treatments 7 and 8 ( $t = 6.95$ ;  $df = 187$ ;  $P < 0.001$ ). These results pointed out that flowers visited by *X. olivacea* have the highest number of normal seeds compare to those protected then opened and closed without the visit of insect or any other organism.

(c) The percentages of normal seeds were 86.98, 60.78, 86.93, and 64.82% in treatments 3, 4, 7 and 8, respectively. The differences between these four percentages are highly significant ( $\chi^2 = 320.39$ ;  $df = 3$ ;  $P < 0.001$ ). The difference was highly significant between treatments 3 and 4 ( $\chi^2 = 173.29$ ;  $df = 1$ ;  $P < 0.001$ ) as well as between treatments 7 and 8 ( $\chi^2 = 145.92$ ;  $df = 1$ ;  $P < 0.001$ ). These results pointed out that flowers visited by *X. olivacea* have the highest number of normal seeds compare to those protected then opened and closed without the visit of insect or any other organism.

In 2018, the contribution of *X. olivacea* via a single flower visit were 19.44% for the podding rate, 33.42% for the mean number of seeds per pod and 30.12% for the percentage of normal seeds. In 2019, the corresponding figures were 27.01, 29.38, and 25.43%, respectively. For the two cumulative years, the contribution of *X. olivacea* on the podding rate the mean number of seeds per pod and the percentage of normal seeds were 23.23, 31.4, and 27.78%, respectively.

#### DISCUSSION

*V. unguiculata* has a mixed mating system with the predominance of autogamy. The same results have been found at Ngaoundéré in the Adamawa Region of



Cameroon by Tchuenguem et al. (2009) and at Obala in the Center Region of Cameroon by Pharaon et al. (2019). Davis et al. (1991) and Asiwe (2009) reported that cowpeas are self-pollinated while Mackie and Smith (1935) and Buchmann and Nabhan (1996) observed cross-pollination in this plant species.

Among insect species recorded on cowpea flowers, *A. mellifera* was the most frequent insect. The same result was reported by Tchuenguem et al. (2009), Kengni et al. (2015), and Pharaon et al. (2019) in Cameroon and by Ndayikeza (2014) in the agro-ecosystem of Burundi. Pando et al. (2013) observed *X. olivacea* as the most frequent insect visitor of cowpea flowers. Thus, the floral entomofauna of a plant varies in time and space (Tchuenguem et al., 2005).

The peak of activity of *X. olivacea* observed on *V. unguiculata* was situated between 10 and 11 am, it is linked to the daily periods of greater availability of nectar of this plant species flowers. The pic of activity of *X. olivacea* on *V. unguiculata* flowers was situated between 8.00 and 9.00 a.m in Ngaoundéré (Kengni et al., 2015) and in Yaoundé (Pando et al., 2013).

During the flowering period of *V. unguiculata*, *X. olivacea* exclusively and regularly harvested nectar. This could be attributed to the needs of bee nests during the flowering period of this plant. Throughout the whole blooming period, this bee intensely harvested nectar and pollen on the cowpea flowers in Ngaoundéré (Kengni et al., 2015) and in Yaoundé (Pando et al., 2014). Nectar contains a wide variety of other components at lower concentrations, including inorganic ions, amino acids, lipids, and secondary plant compounds, many of which are attractant or repellent to pollinators (Roy et al., 2017; Pamminger et al., 2019).

The disruptions of visits by other insects reduced the duration of certain *X. olivacea* visits by abiotic factors such as by elevated ambient temperature, and wind velocity and biotic factors include predation and competitive interactions among flowering insects (Imam et al., 2017). Elevated temperatures are known to influence the foraging activity which affects the diversity and abundance of wild bees in agricultural ecosystems. Generally, the genus *Xylocopa* is able to move around well in a daily temperature range of 25 to 35°C (Imam et al., 2017; Elisante et al., 2020). The visits of *X. olivacea* are far more numerous on this plant species when the number of bloomed flowers is higher. The positive and significant correlation between these two parameters highlights the good attractiveness of the nectar of this Fabaceae vis-à-vis the carpenter bees. Kengni et al. (2015) in Ngaoundéré and Djonwangwé et al. (2017) in Maroua, Cameroon, have reported the same observations. Moreover, it is known that an increase in flower number and size on a plant species results in increased visitation by flowering insects (Deli et al., 2020). During their foraging activity, *X. olivacea* increased the pollination possibilities of this Fabaceae. While collecting nectar from cowpea flowers with their

proboscis, this insect came into contact with the anthers and the stigma. This facilitates the release of pollen from the anther, and therefore, optimally facilitates the occupation of the stigma by pollen grains. An optimal pollen load on the stigma would be favorable for pod and seed formation (Adamou et al., 2020). Significantly, higher yields were recorded from open-pollinated flowers compared to flowers from which pollinators had been excluded, indicating that flower visitors contribute significantly to cowpea yields. Elisante et al. (2020) reported similar observations in a smallholder bean farming system in Tanzania. Adamou et al. (2020) found significantly higher seed set and seed number in Lipstick tree flower heads that were exposed to bees compared to those that were not. During the present study, *X. olivacea* alone caused a significant increase in the podding rate, the mean number of seeds per pod, and the percentage of normal seeds. From the investigations carried out at Ngaoundéré in Cameroon by Kingha et al. (2012) and Deli et al. (2020) on *Phaseolus vulgaris*, carpenter bees were found to have a key role in increasing the fruit production and seed quality of these crops.

According to Deli et al. (2020), the seed number, dry weight of seed/pod, pod length, and the percentage of normal form seed/pod decrease with insect exclusion from flowers. Throughout the pollination efficiency of a single visit of *P. vulgaris* flower in Ngaoundéré, *X. olivacea* provoked a significant increase in the podding rate, the mean number of seeds per pod, the percentage of normal seeds, and the mean weight of a seed by 39.48, 18.19, 49.62, and 31.53%, respectively (Deli et al., 2020).

## Conclusion

*V. unguiculata* is a highly nectariferous bee plant that benefits from pollination by insects, among which *X. olivacea* was the second flower visitor after *A. mellifera*. By comparing the yields of treatments with protected flowers to those of flowers visited exclusively by *X. olivacea*, there is an increase in the podding rate, the number of seeds per pod, and the percentage of normal seeds of *V. unguiculata*. Thus, this plant could be grown or protected to stabilize *X. olivacea* nests.

This information will increase knowledge on pollination services in the North Region of Cameroon and actions for pollinator insect conservation, thus encouraging sustainable agricultural practices.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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