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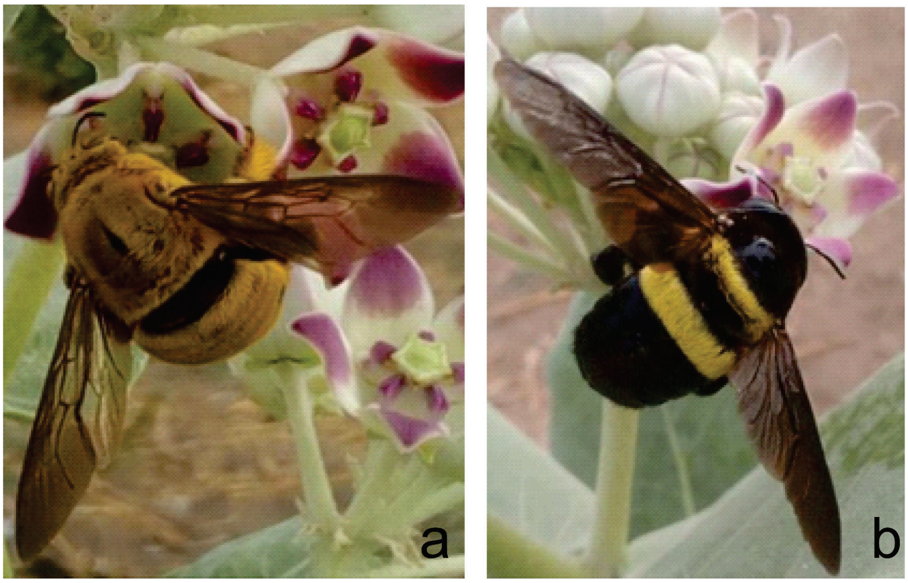

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Front cover: *Xylocopa inconstans* Smith, 1874 on the flowers of *Calotropis procera*. a, ♂. b, ♀. © Michelson Azo'o Ela.

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Abstract

Calotropis procera (Asclepiadaceae) flowers were observed in order to evaluate the impact of foraging activity of bee visits on seed yield and germinability in Maroua (Sudano-Sahelian region of Cameroon) in 2019 and 2020. Comparisons were made between bagged and unbagged inflorescences. *Xylocopa inconstans* Smith, 1874 (male and female), *X. pubescens* Spinola, 1838 (male and female), *X. ustulata* Smith, 1854 (female), and *Amegilla* sp. were recorded as major flower visiting insects of this plant species. The activities of these wild bee species were predominant at dawn (6:00 – 7:00 a.m.) and declined sharply after 11:00 a.m. Honeybee *Apis mellifera* L., 1758 was sporadic. All observed floral visits were devoted to nectar collection during which pollination behaviour occurred. The 12.01% (in 2019) and 19.59% (in 2020) of flowers visited mainly by carpenter bees *Xylocopa* spp. were able to produce fruits and seeds; while, the bagged flowers all aborted. No variation was observed between both years about the bee fauna, the floral phenology and preference for floral products harvested. The proportion of seed germinability was 93.25% in 2019 and 92% in 2020 from unbagged treatment. The regeneration of *C. procera*, which is possible through seeds, is preconditioned by the anthophilous activity of insects.

Keywords: carpenter bee, pollination, production, regeneration, seed, Sodome apple

Introduction

Calotropis procera (Ait.) Ait. (Asclepiadaceae) or 'Sodom apple' is a Sudanese and East Saharo-Arabian evergreen shrub (EISIKOWITCH, 1985). This plant species is widely distributed in the tropics and subtropics of Africa and Asia where it grows under extremely hot conditions (MILLAR & MORRIS, 1987). Owing to its thermophilic nature, *C. procera* is among the plants whose distribution is drastically restricted by thermal conditions (ZOHARY, 1962). In Cameroon, this plant species is widely grown in the North and Far-North regions where the average air temperature value varies between 30 and 36°C with a peak of about 42°C in April–May (MORIN, 2000).

Calotropis procera has several therapeutic properties which are widely exploited in traditional medicine and pharmacology (BATELLO *et al.*, 2006). This plant species is used to treat rheumatism, asthma, syphilis, and skin diseases such as lupus erythematosus, eczema and leprosy (BURKILL, 1985; PARROTTA, 2001). The latex of this plant species has several properties: anti-inflammatory (KUMAR & BASU, 1994), analgesic (DEWAN *et al.*, 2000a), antipyretic (DEWAN *et al.*, 2000b), anticoagulant (ATAL & SETHI, 1962), antidiarrheal (KUMAR *et al.*, 2001), antimicrobial (SRINIVASAN *et al.*, 2001), anthelmintic (DERASARI & SHAH, 1965; SHIVKAR & KUMAR, 2003).

Calotropis procera has the ability to multiply through vegetative and generative methods. Vegetative propagation which is an asexual method of plant reproduction that occurs in its leaves, roots, and stem, is very useful in large-scale multiplication of the superior genotypes (AHMED *et al.*, 2005). Generative multiplication of *C. procera* is made naturally from seeds which are disseminated by the wind, water and animals including humans (MOSTAFA *et al.*, 2013). The plant has spread widely from its original range as a weed in many areas, further, *C. procera* has been domesticated and is cultivated in India for instance, for fiber for rugs and sheets, bioplastics, rubber, bio-fuels, and for pharmaceutical purposes (AHMED *et al.*, 2005).

A diverse assemblage of pollinators will often visit a single plant species (OLLERTON *et al.*, 2011). Traditionally, pollination biology has focused on the visitors of flowering plants in general i.e., those visitors that play large roles in the evolution of floral traits (MICHENEAU *et al.*, 2006). Thus, most research to date has focused on evaluating the relationship between the visits and effects of major visitors on seed production (HERRERA, 2000).

In many cultivated and wild plant species, the honey bee (*Apis mellifera*) is known to be the main pollinator (KLEIN *et al.*, 2007). However, there are many other insect species that also play a key role in this biological process (LARSON *et al.*, 2001). It seems that the genus *Calotropis* is coevolved with several species of carpenter bees (HAGERUP, 1932; PIJL, 1954; JAEGER, 1971; WANTROP, 1974; RAMAKRISHNA & AREKAL, 1979; GERLING *et al.*, 1983; EISIKOWITCH, 1985) which are therefore the best suited to extract the floral resources of *C. procera* (WILLMER, 1988). According to ALI & ALI (1989), the foraging behaviour of these bee species make the pollination of *C. procera* easier. Indeed, after landing on an open flower, they insert their strong proboscis into the narrow entrance of the cuculli. The lips of the narrow entrance to the nectar are pressed together very strongly so that it only can be opened by the hard proboscis of a carpenter bee (SCHREMMER, 1972). However, while gripping the flowers, they insert their mesothoracic or prothoracic legs into the stigmatic chamber. Later, during the withdrawal of their legs, carpenter bees frequently and incidentally extract pollinia from the flower (JAEGER, 1971). The extracted pollinia adhere firmly to the numerous bristles of the foraging bee's legs. When the bees loaded with pollinia on the legs repeat the same behavioral pattern on a new flower visited, the paired pollinia, are inserted into the stigmatic chamber of the new flower and released into the nectar secreted there. Thus, *C. procera* nectar serves both as a substrate for pollen germination and as a reward for pollinators (ALI & ALI, 1989).

Despite the high involvement of Carpenter bees in the pollination of *C. procera*, it is well known from the literature that the flower visiting entomofauna of a given plant species can vary from one biogeographic region to another (KLEIN *et al.*, 2007; OLLERTON *et al.*, 2011). In Cameroon, no study has yet been done on the relationship between *C. procera* and its flower-visiting insects. Though, the general policy for improving agricultural development consists in diversifying production systems to make the best possible use of available resources (DE SILGUY, 1997).

Considering the medicinal and pharmacological importance of *C. procera*, we undertook this study with a view to optimize its productivity in Cameroon. In order to achieve the above goal, the following specific objectives were; 1) to study the diversity of the flower visiting entomofauna associated with *C. procera*; 2) to study the foraging activity of pollinators on *C. procera* flowers; 3) to assess the impact of pollinating insects on the seed yields of this plant species and 4) to assess the germinability of seeds resulting from *C. procera* open-pollinated flowers.

Material and methods

STUDY SITE

Field experiments took place in Domayo behind the TOTAL filling station and in the vicinity of the Grammar School of Domayo in the neighbourhood of Maroua (Far-North region of Cameroon; Fig. 1). Maroua is the capital of the Diamaré division and the capital of the Far North region of Cameroon. The Far North region is located between latitude 10°35'North and longitude 14°20' East (DUMORT & PERONNE, 1966); its average altitude is about 500 meters (MORIN, 2000).

This region belongs to the Sudano-Sahelian zone (MULLER & GAVAUD, 1979). The prevailing climate here is of the Sudano-Sahelian type with two seasons: the dry (November to May) and rainy (June to October) seasons. The annual rainfall varies from 400 to 1200 mm and the mean annual temperature varies from 29°C to 36°C (MORIN, 2000).

The vegetation is dominated by plant species such as *Azadirachta indica* (Meliaceae), *Kaya senegalensis* (Meliaceae) and *Cassia* (*Senna*) *siamea* (Caesalpiniaceae) which occupy large areas in urban agglomerations and reforestation areas (RAUNET, 2003). In this environment, *C. procera* grew naturally as a woody perennial plant species. In the study site, this flowering shrub grows up to 0.7–2 m tall (Fig. 2). Each plant bears 3 to 8 inflorescences with about 7 to 43 bisexual flowers each. Previous description of the flowers of *C. procera* has been already done in the literature. According to EISIKOWITCH (1985), the flower of *C. procera* comprises 5 sepals and 5 grey, violet-tipped petals. The two free styles are conjoined to form the stout gynostegium. There are 5 coronal extensions from the base of each stamen, forming 5 cuculli or nectar containers. The nectar is produced in the stigmatic chamber which connects to the cuculli through spongy, diffused channels (GALIL & ZERONI, 1965). Flower morphology of *C. procera* is adapted towards the concealment of nectar, in order to protect it against robbery by ants and from evaporation. Pollinia while being inserted within the stigmatic chamber, are soaked in liquid nectar which, by its concentration controls pollen germination (EISIKOWITCH, 1985).

STUDY ON THE FLOWER VISITING INSECTS OF *Calotropis procera*

Field investigations were made during the blooming period of *C. procera*. Preliminary trials started from January 15 to June 15, 2019 which were duplicated from January 12 to June 15, 2020 inside a public space of about 1.2 ha, where several *C. procera* grew naturally. For each year, 40 inflorescences from the uppermost part of the 40 tagged plants were randomly grouped into two treatments of 20 inflorescences each. In treatment 1, flowers were freely exposed to the activity of visiting insects (Fig. 3a) while flowers of treatment 2 were prevented from visiting insects using gauze bags (Fig. 3b).

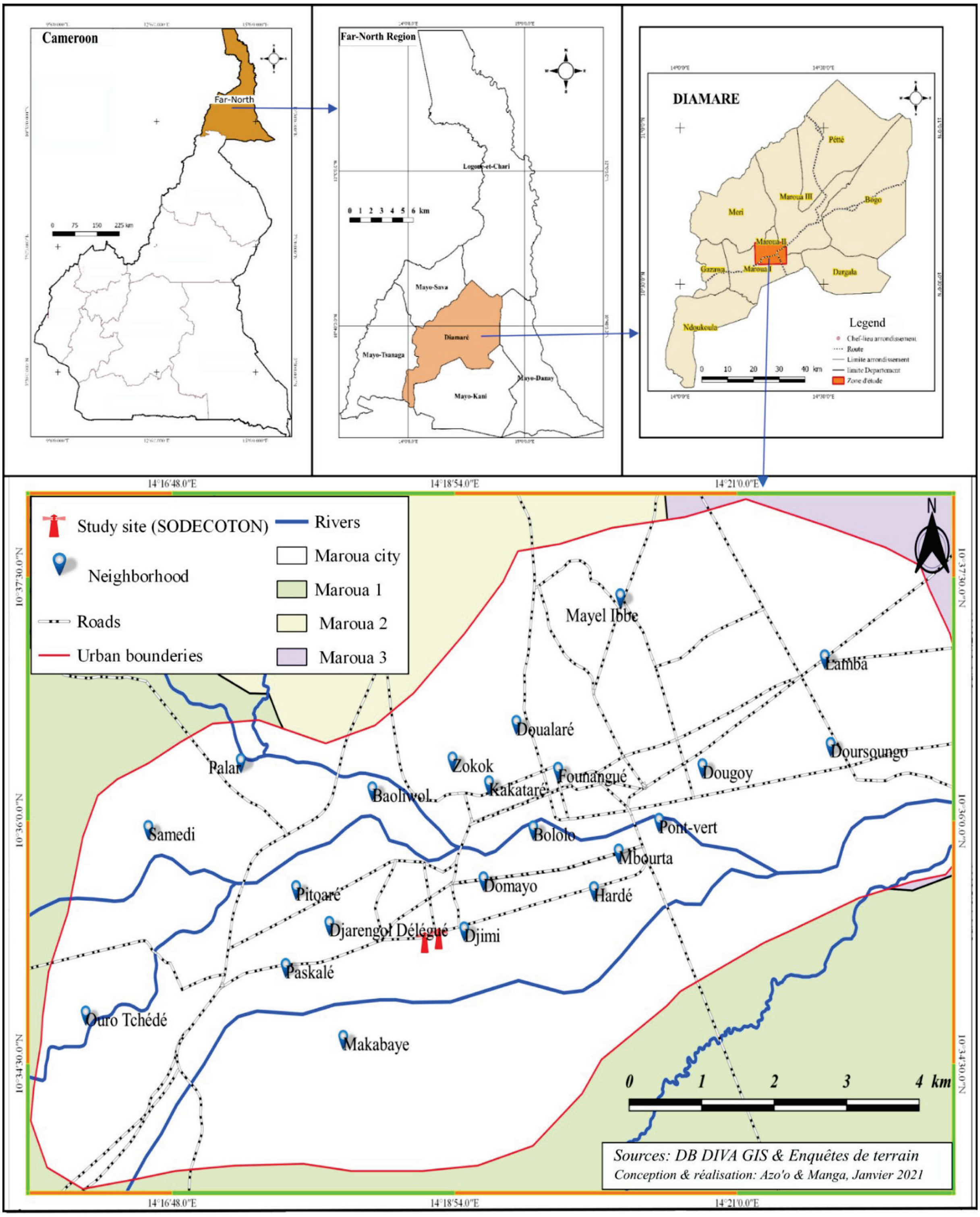

Fig. 1. Location map of the study area. © Michelson Azo'o Ela & Pierre Manga.

Observations on *C. procera* flowers were made daily between 06:00 a.m. and 07:00 p.m. (local time). The number of insect morphospecies visiting *C. procera* flowers were counted each day on tagged plants for 5 to 10 minutes at the following time intervals: 06:00–07:00 a.m., 08:00–09:00 a.m., 10:00–11:00 a.m., 12:00–1:00 p.m., 02:00–03:00 p.m., 04:00–05:00 p.m. and 06:00–07:00 p.m. We assigned a recognition code to each insect morphospecies recorded. Since some specimens could have been observed more than once, counts were expressed as a number of visits.

Fig. 2. *Calotropis procera* in field experiment. © Michelson Azo'o Ela.

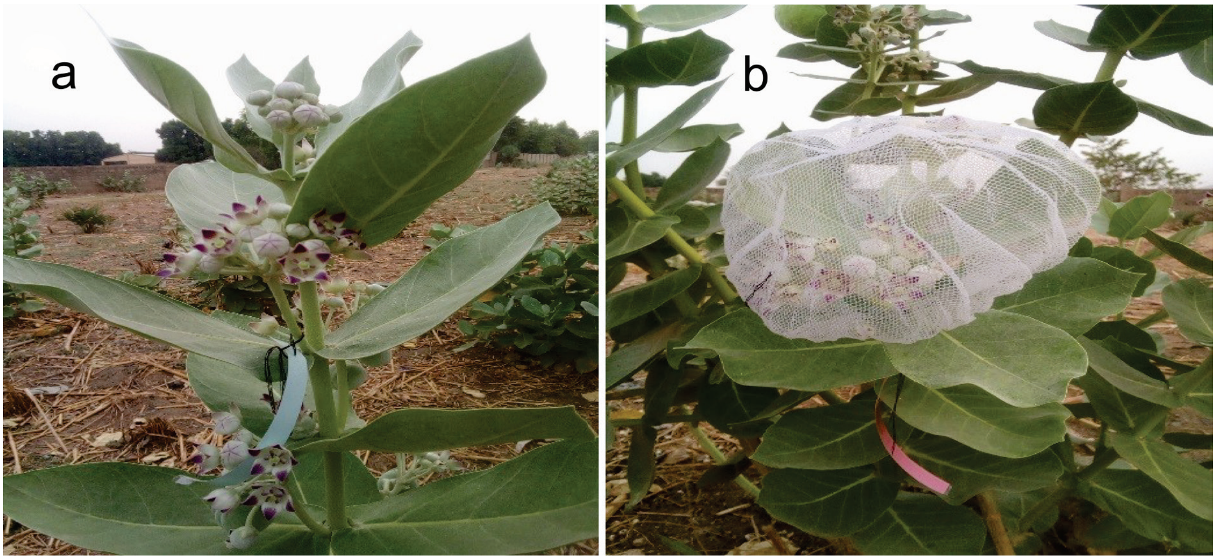

Fig. 3. Inflorescences of *Calotropis procera*. a, unbagged. b, bagged. © Michelson Azo'o Ela.

RELATIVE ABUNDANCE OF FLOWER VISITING INSECTS

The number of visits of each flower-visiting insect species was counted and species richness or the number of insect species was recorded. The centesimal frequency or relative abundance of visits, which corresponds to the percentage of total floral visits of a given insect species compared to the total of the visits of all insect species identified, was calculated as F (%) = [(ni/N) × 100], where F (%) represents the relative abundance of flower visits of species i; ni the number of visits by individuals of the species, and N the total number of visits by individuals of all species combined (ZAIME & GAUTIER, 1989).

DAILY VARIATION IN THE FORAGING ACTIVITY OF FLOWER VISITING INSECTS

From the different daily observation time intervals, the visits of insects recorded were counted and summed up. Data obtained from the observations, enabled us to assess the daily phenology of activity as a function of the daily cyclus.

OCCURRENCE OF FLOWER VISITING INSECTS ON *Calotropis procera*

The frequency of occurrence (C) or frequency of appearance which refers to the frequency of a species in the samples, i.e. in percentage it is the ratio between the number of surveys during which the species was observed (Pi) to the total number of surveys (P): C (%) = [(Pi/P) × 100] (DAJOZ, 2006). BIGOT & BODOT (1973) classify frequencies of occurrence into four classes or categories of species as a function of the variation of the C-values: very accidental species or sporadic species (C < 10 %), accidental species (10 % ≤ C ≤ 24 %); accessory species (25 % ≤ C ≤ 49 %), and constant species (C ≥ 50 %).

MEAN DURATION OF FLORAL VISIT

The duration of visit is the time taken by a given insect to collect pollen or nectar (JACOB-REMACLE, 1989). The duration of insect visit was measured using a stopwatch. It was activated at the time when an individual landed on the flower and was stopped when it left it.

INFLUENCE OF ENVIRONMENTAL PARAMETERS ON FORAGING ACTIVITY

The influence of the temperature and the relative humidity of the air on the floral activity of anthophilous insects was made by the observer using a portable hygro-thermometer of the brand HT 9227. Three values were taken at each time interval, i.e. 21 values per day. At the end of the experiments, average temperature values were calculated and correlated with the total number of floral visits corresponding to each timed pollination visit. The humidity was at saturation during experiment period.

FORAGING BEHAVIOR OF FORAGERS

For bees, besides visiting time, also the number of individual flowers from a single inflorescence was noted. The foraging speed (Vb) was calculated using the following formula: $Vb = (Fi/di) \times 60$; where (di) is the visiting time and (Fi) the number of flowers visited during the visiting time (JACOB-REMACLE, 1989).

CAPTURE, PRESERVATION AND IDENTIFICATION OF INSECTS

At least two specimens per anthophilous morphospecies were captured with an entomological hand net, preserved in vials containing 70 % ethanol in the field. In the laboratory, specimens of each morphospecies were sorted, pinned and mounted in a collection box. Field pictures of carpenter bee species were identified by Longin Ndayikeza (Burundi Environment Protection Authority, Bujumbura, Burundi) and Alain Pauly (Royal Belgian Institute of Natural Sciences, Brussels). Voucher specimens were stored at the Laboratory of Entomology (Department of Biological Sciences, Faculty of Science, University of Maroua, Cameroon). A reference collection from material collected previously in Cameroon enabled the identification of other flower visiting insect species captured.

IMPACT OF FLOWER VISITING INSECTS ON FRUIT SET RATE OF *Calotropis procera*

Mature fruits grown from each treatment were harvested. A comparison was done on the fruit set rate between both treatments. Fruit set rate or fruiting rate is defined as the ratio of fruits formed/flowers studied. The mean rate of mature fruits per inflorescence or the mean of ratio number of mature fruits/number of flowers was estimated per inflorescence.

Calotropis procera SEED GERMINABILITY TEST

This study was carried out in the laboratory of the Institute of Agricultural Research for the Development (IRAD) in Maroua. The seeds used were collected from the potential dehiscent fruits obtained from the different treatments. The experiment was made a week after fruit harvesting as seeds of *C. procera* are not dormant. The seeds were washed with 1% diluted solution of sodium hypochlorite and then rinsed with distilled water to avoid a possible attack of fungi. 400 seeds were randomly selected among those harvested from open-pollinated flowers and 4 batches of 100 seeds each were constituted in four petri dishes. Seeds were covered with filter papers and sprinkled with water at room temperature during the morning and evening of each day until seedlings emerged (LOTITO & QUAGLIOTTI, 1991). The number of seeds that had undergone germination from the four trials were checked every morning and these were withdrawn from each dish. The germination rate which is the ratio of emerged seedlings/total number of seeds (PERERA & KARUNARATNE, 2019) was evaluated; the rhythm of germination which is the number of emerged seedlings per day was also assessed.

DATA ANALYSIS

Data were subjected to descriptive statistics using SPSS 20.0 software. Linear regressions were established between parameters. Normal-theory statistical analysis was used on continuous variables by applying standard analysis of variance (ANOVA). The Honestly Significantly Difference (HSD) method was used to discriminate among the means between the two treatments. The means are given with their standard deviation (SD) and in all cases, the significant probability was computed. Means are reported as significantly different if *p* was less than or equal to 0.05.

Results

DIVERSITY AND RELATIVE ABUNDANCE OF FLOWER VISITING INSECTS ON *Calotropis procera*

In the present study, 957 and 1072 visits of anthophilous insects were registered on *C. procera* in Maroua for 2019 and 2020 respectively. Table 1 shows that these insects belonged to 4 and 5 orders during both years. The order Hymenoptera was prominent with a relative abundance of 98.22% in 2019 and 95.43% in 2020. These orders of insects are grouped into four and seven families during both years with Apidae as the most important family; followed by Muscidae, Scarabeidae, Acraeidae, Formicidae, Lygaeidae and Tephritidae. 7 and 11 insect species were noted foraging on 683 and 796 *C. procera* flowers in 2019 and 2020. Among these insects, *Xylocopa inconstans* was more represented with a relative abundance of 54.44% in 2019 and 51.96% in 2020. Fig. 4 shows some of the Apidae species recorded in activity on *C. procera* flowers. Equally, some non-bee species were noted as visitors of *C. procera* flowers (Fig. 5).

Table 1. Insects recorded on *Calotropis procera* flowers in Maroua in 2019 and in 2020, number and percentage of visits; *n*: number of visits; *F* (%): relative abundance = *n*/N x 100; sp.: unidentified species.

			2019		2020	
Order	**Family**	**Species**	n_i	***F* (%)**	n_i	***F* (%)**
Hymenoptera	Apidae	*Xylocopa inconstans*	521	54.44	557	51.96
		Xylocopa pubescens	235	24.56	310	28.92
		Amegilla sp.	167	17.45	129	12.03
		Xylocopa ustulata	17	1.77	16	1.49
		Apis mellifera	-	0.00	6	0.56
Total Apidae			940	98.22	1018	94.96
	Formicidae	*Camponotus flavomarginatus*	-	0.00	5	0.47
Total Hymenoptera			940	98.22	1023	95.43
Diptera	Muscidae	(1 sp.)	-	0.00	17	1.59
	Tephritidae	*Dacus longistylus*	7	0.73	2	0.18
Total Diptera			7	0.73	19	1.77
Coleoptera	Scarabeidae	*Pelidnoda* sp.	-	0.00	15	1.40
Lepidoptera	Acraeidae	*Acraea acerata*	2	0.21	12	1.12
Hemiptera	Lygaeidae	*Spilostethus pandurus*	8	0.84	3	0.28
TOTAL (N)			**957**	**100.00**	**1072**	**100.00**

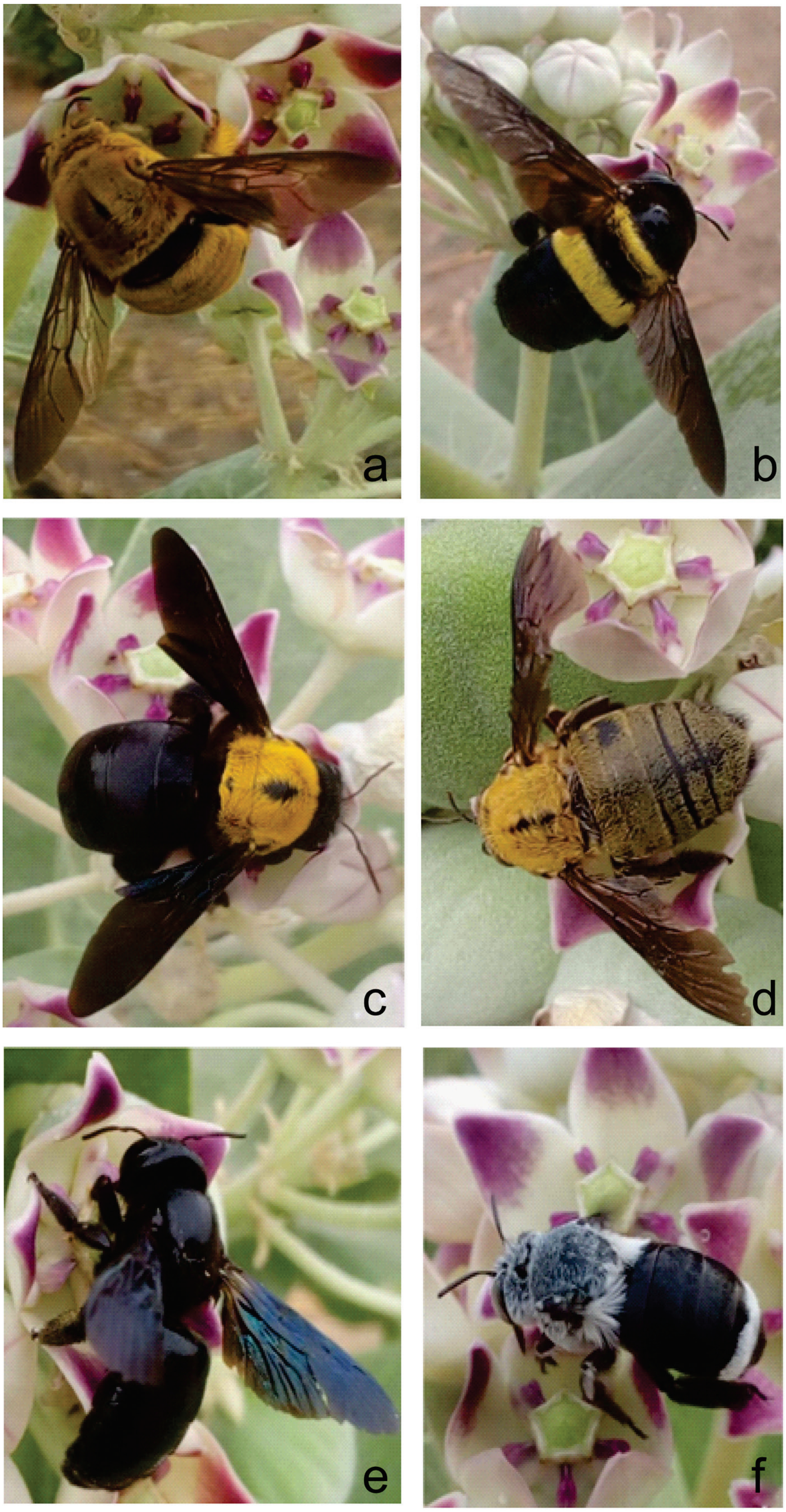

Fig. 4. Apidae recorded on the flowers of *Calotropis procera*. a, *Xylocopa inconstans* ♂. b, *Xylocopa inconstans* ♀. c, *Xylocopa pubescens* ♀. d, *Xylocopa pubescens* ♂. e, *Xylocopa ustulata* ♀. f, *Amegilla* sp. © Michelson Azo'o Ela.

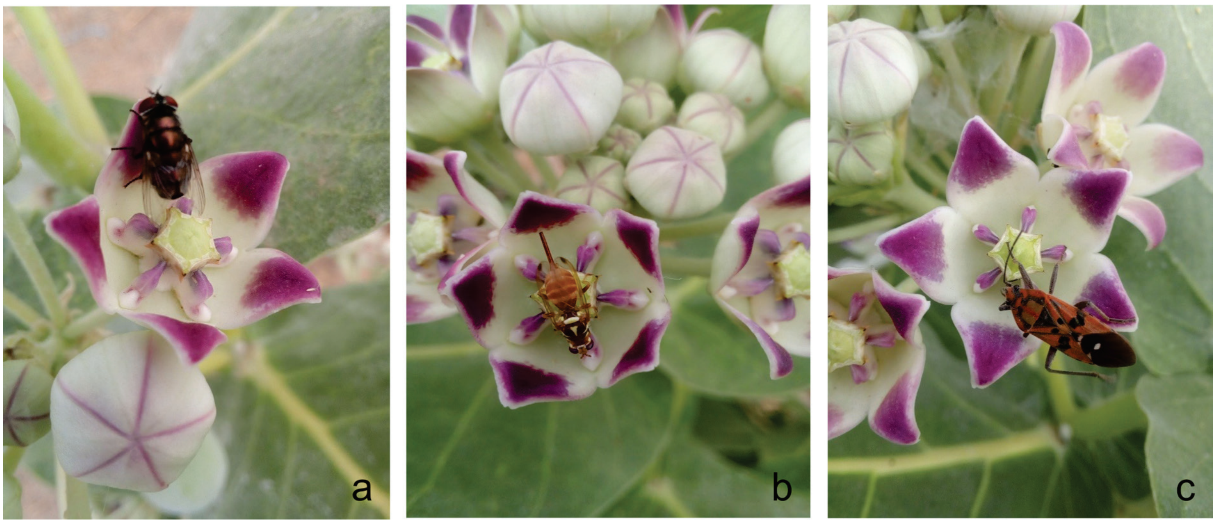

Fig. 5. Non-bees recorded on *Calotropis procera* flowers. a, fly. b, fruit fly. c, bug © Michelson Azo'o Ela.

INSECT ACTIVITY ON *Calotropis procera* FLOWERS

FLORAL PRODUCTS HARVESTED

According to field observation, the flower-visiting insects of *C. procera* were fond of its nectar. No direct pollen sampling was recorded for any bee species (Table 2). Most of the nectarophagous insects of *C. procera* have mouthparts adapted to this activity: stinging-sucking (*S. pandurus*), grinding-licking (*X. inconstans*, *X. pubescens*, *X. ustulata*, *Apis mellifera* and *Amegilla* sp.) and licking (*Acraea acerata*). Flies and ants which were found on flowers are considered as opportunistic nectar feeders because they generally take advantage of the droplets of nectar left on the floral surfaces by specialists such as the Apidae.

Table 2. Floral products harvested from the flowers of *Calotropis procera.*

	Floral products	
Insects	**Nectar**	**Pollen**
Xylocopa inconstans	+	-
Xylocopa pubescens	+	-
Xylocopa ustulata	+	-
Apis mellifera	+	-
Amegilla sp.	+	-
Camponotus flavomarginatus	+	-
Muscidae (1 sp.)	+	-
Dacus longistylus	+	-
Pelidnoda sp.	+	-
Acraea acerata	+	-
Spilostethus pandurus	+	-

Table 3. Daily variation of floral visits of insects on *Calotropis procera*. *= Peak of flower-visiting insect daily activity.

	Time periods (hours)												
	6–7 a.m.		8–9 a.m.		10–11 a.m.		12a.m–13 p.m		14–15 p.m.		16–17 p.m.		
Insect species	**n**	**p (%)**	**n**	**p (%)**	**n**	**p (%)**	**n**	**p (%)**	**n**	**p (%)**	**n**	**p (%)**	**Total**
Xylocopa inconstans	353	63.37*	148	26.57	14	2.51	-	-	8	1.43	34	6.10	**557**
Xylocopa pubescens	169	54.52*	104	33.55	14	4.52	4	1.29	3	0.97	16	5.16	**310**
Xylocopa ustulata	1	6.25	10	62.50*	1	6.25	1	6.25	2	12.5	1	6.25	**16**
Amegilla sp.	103	79.84*	13	10.08	5	4.58	-	-	4	3.87	4	3.10	**129**
Muscidae (1 sp.)	17	100.00	-	-	-	-	-	-	-	-	-	-	**17**
Pelidnoda sp.	6	40.00*	7	46.66	1	6.66	1	6.66	-	-	-	-	**15**
Apis acerata	-	-	7	58.33*	2	16.67	-	-	2	16.67	1	8.33	**12**
Apis mellifera	5	83.33*	1	16.67	-	-	-	-	-	-	-	-	**6**
Camponotus flavomarginatus	4	80.00*	1	20.00	-	-	-	-	-	-	-	-	**5**
Spilostethus pandurus	-	-	3	100.00	-	-	-	-	-	-	-	-	**3**
Dacus longistylus	2	100.00*	-	-	-	-	-	-	-	-	-	-	**2**
Total	**660**	**61.57***	**294**	**27.42**	**37**	**3.45**	**6**	**0.56**	**19**	**1.77**	**56**	**5.22**	**1072**

VARIATION OF INSECT VISITS ACCORDING TO THE DAILY OBSERVATION TIME INTERVALS

The distribution of floral visits by anthophilous insects according to the daily observation time frames is reported in Table 3 in 2020, the year where all insect species were recorded. Insects were found to visit *C. procera* flowers from 6:00 a.m. to 5:00 p.m. Insect visits were noted more during the morning at the first observation time interval with a dominance of the Carpenter bee species (61.57%). Apart from *X. ustulata* with a peak of activity at 8:00–9:00 a.m., *X. inconstans*, *X. pubescens* and *Amegilla* sp. visited *C. procera* flowers in the early hours between 6:00–7:00 a.m. *A. mellifera* was seen visiting *C. procera* flowers exclusively between 6:00–7:00 a.m. Insect flower visits decreased drastically after the first time interval and had practically diminished by midday. Between 2:00–5:00 p.m., there was a slight increase in the number of insect visits compared to the middle of the day, with only a few species that continued their foraging activity to the detriment of others. This was the case for *X. ustulata*, *X. inconstans*, *X. pubescens* and *Amegilla* sp. Among the bee species, only *X. ustulata* and *X. pubescens* were seen visiting *C. procera* flowers throughout the day.

FREQUENCY OF OCCURRENCE

The values of the frequency of occurrence or frequency of appearance of flower visiting insects of *C. procera* varied from one insect species to another. Bee species were classified into three categories (Table 4): 1) the constant species: *X. inconstans*, *Amegilla* sp., *X. pubescens.* 2) the accessory species: *X. ustulata* and 3) the accidental species: *A. mellifera*. The sporadic species were essentially the non-bee species. The constant species, due to their high values of relative abundance and frequency of appearance, seemed to play a major role in the pollination of *C. procera*.

Table 4. Categorization of anthophilous insects according to the variation in the frequency of occurrence on the flowers of *Calotropis procera*. $C = [(P_i/P) \times 100]$ = Frequency of occurrence P_i = Number of samples containing a given insect species; P = Total number of samples (30 in 2019 and 36 in 2020).

	2019		**2020**		
Species	***Pi***	**C(%)**	***Pi***	**C(%)**	**Categories**
Xylocopa inconstans	30	100	36	100.00	Constant species ($C \geq 50\%$)
Amegilla sp.	27	90	32	88.88	
Xylocopa pubescens	24	80	26	72.22	
Xylocopa ustulata	9	30	12	33.33	Accessory species ($25\% \leq C \leq 49$)
Pelidnoda sp.	-	-	8	22.22	Accidental species ($10\% \leq C \leq 24\%$)
Apis acerata	4	13.33	6	16.66	
Muscidae (1 sp).	-	-	6	16.66	
Apis mellifera	-	-	4	11.11	
Dacus longistylus	2	6.66	2	5.55	Sporadic species ($C < 10\%$)
Camponotus flavomarginatus	-	-	2	5.55	
Spilostethus pandurus	2	6.66	1	2.77	

Table 5. Mean duration of bee visits on *Calotropis procera* flowers; n: sample; m: mean; s: standard deviation; mean values in the same column with different letters vary significantly ($p < 0.05$).

	Duration of visits (sec)					
	2019			2020		
Insects	***n***	***m***	***s***	***n***	***m***	***s***
Amegilla sp.	50	5.78a	2.03	100	5.39a	2.60
Xylocopa inconstans	50	5.94a	3.21	100	6.43b	3.79
Xylocopa pubescens	50	7.41c	2.51	50	7.26b	4.38
Xylocopa ustulata	20	9.73d	3.21	15	10.03d	4.94

MEAN DURATION OF VISITS

The mean length of visits per flowers varied from one bee species to another and for a given bee species from year to year. Table 5 reports the mean duration of a bee floral visit. The difference in visiting time between species was significant in 2019 ($F = 10.53$ $df = 3, 166$; $p < 0.001$) and 2020 ($F = 15.36$; $df = 3, 261$; $p < 0.001$). In all, no difference of the mean value of the duration of visit of a given bee species was registered between both years.

FORAGING BEHAVIOUR OF BEE SPECIES

For carpenter bees (*X. inconstans*, *X. pubescens* and *X. ustulata*), the foraging behaviour was quite similar based on our observations. Individuals of carpenter bees landed on the flower, especially on a petal or the stigmatic disc in search of nectar. After landing, the bee inserted its sturdy proboscis into the entrance of the cuculli and harvested nectar. During this, an individual kept its wings opened and strongly grip on the flower with its legs. The movements of the legs were regular on the visited flower allowing the visiting bee to change posture and position for exploiting sometimes all the cuculli of the visited flower. No direct observations for pollen collection was noted, but a large amount of pollinia were clearly visible as dust on the tibial part of carpenter bee legs and the abdominal body hair. There was a 100% frequency of contact between carpenter bees and the reproductive parts of *C. procera* flowers.

Individuals belonging to the genus *Amegilla* exhibited two types of nectar-seeking behavior. The first involved bees remaining suspended in the air while vigorously beating their wings and sucking the nectar in this posture at the level of the cuculli. Secondly, they landed on the flower between the two petals, the prothoracic and mesothoracic legs grabbed the petals above and the hind legs below, whilst inserting the proboscis into the cuculli beating sometimes their wings.

Table 6. Foraging speed of bees on *Calotropis procera* flowers; n: sample; m: mean; s: standard deviation; mean values in the same column with different letters vary significantly ($p < 0.05$).

	Mean value of the foraging speed					
	2019			2020		
Insects	***n***	***m***	***s***	***n***	***m***	***s***
Amegilla sp.	25	5.03a	1.54	31	4.74a	1.93
Xylocopa inconstans	50	4.76a	1.32	52	5.38a	1.47
Xylocopa pubescens	20	4.96a	2.03	26	5.15a	1.37
Xylocopa ustulata	15	3.33b	0.49	15	3.25b	0.75

The mean values of the foraging speed of certain bee species are recorded in Table 6; the difference between these mean value was significant in 2019 ($F = 7.04$; $df = 3, 106$; $p < 0.001$) and 2020 ($F = 8.62$; $df = 3, 120$; $p < 0.001$). The important values of the foraging speed enable bee species to visit more flowers per minute and contribute highly to cross-pollination.

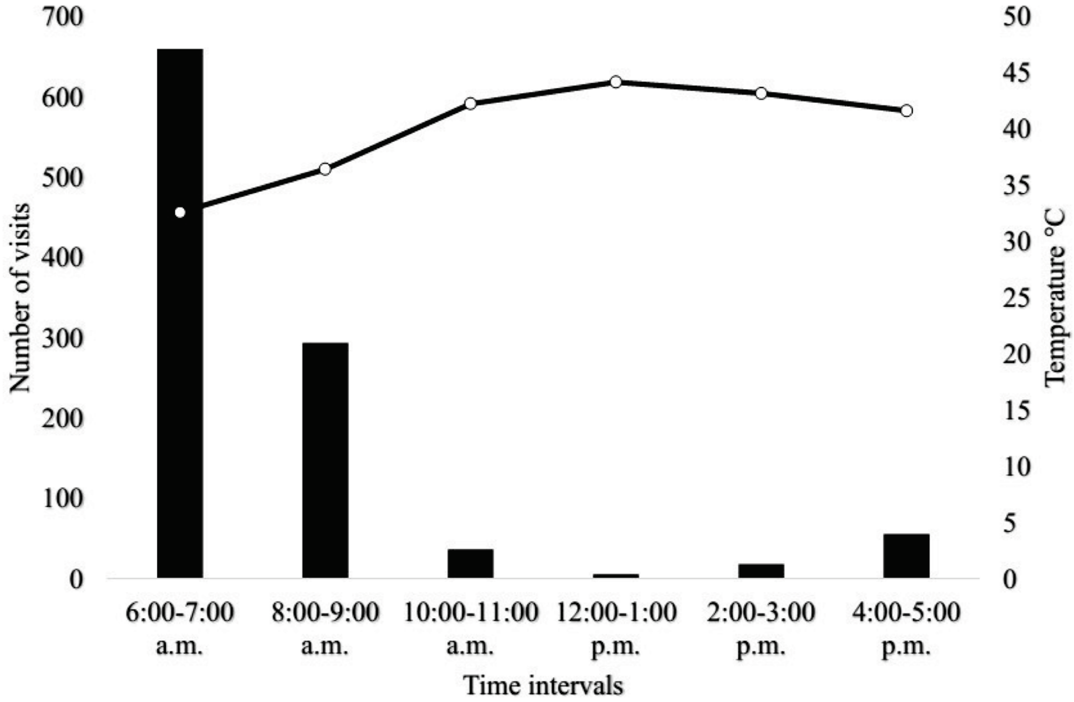

Fig. 6. Variation of flower visiting insects on *Calotropis procera* as a function of temperature and time intervals.

Table 7. Production parameters of *Calotropis procera* according to treatments 1 and 2.

	2019		2020	
Parameters	**T_1**	**T_2**	**T_1**	**T_2**
Number of inflorescences	20	20	20	20
Number of flowers studied	683	604	796	627
Number of actual fruits formed	104	0	192	0
Percentage of fruits formed (%)	15.22	0.00	24.12	0.00
Number of mature fruits	82	0	156	0
Percentage of mature fruits (%)	12.01	0.00	19.59	0.00
Total number of seeds	21778	0	48551	0
Number of mature seeds	20652	0	44604	0
Percentage of mature seeds (%)	94.83	0.00	91.87	0.00
Number of immature seeds	1126	0	3947	0
Percentage of immature seeds (%)	5.17	0.00	8.13	0.00
Mean number of mature seeds per fruit	267.58 ± 43.71	0	285.58 ± 67.02	0

INFLUENCE OF SOME CLIMATIC FACTORS

The influence of variations in mean ambient temperature on the daily flower activity of insects on *C. procera* is reported in Fig. 6. Insect activity was observed throughout the day, yet, foraging activity was high in the morning (lower temperatures), decreased at midday (high air temperature) and increased again in the evening when the temperatures tend to decrease. The linear regression between the daily variation of flower visiting insects as a function of daily fluctuation of mean temperature showed a significant negative linear slope (y = -0.091x + 34.359; $R^2 = 0.41$; $p < 0.001$) (Fig. 7).

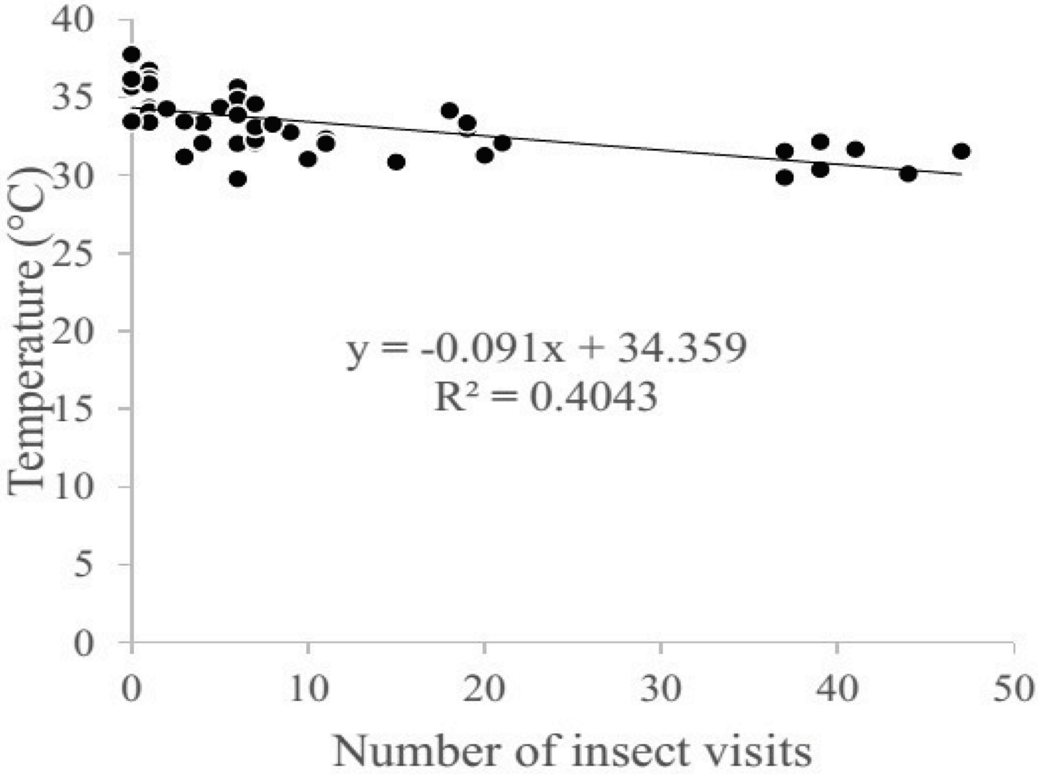

Fig. 7. Linear regression between temperature and daily variation of insect visits.

Impact of flower visiting insects on yields of *Calotropis procera*

Some parameters related to the estimates of *C. procera* yields such as the fruiting rates, the average number of mature fruits, the percentage of mature fruits, the average number of mature seeds per fruit, the average number of immature seeds per fruit, the percentage of mature seeds from both treatments are reported in Table 7. Flowers of *C. procera* which did not benefit from floral visits of insects aborted and dropped off. Only, the flowers which benefited from floral visits by carpenter bees produced fruits and seeds. The floral activity of carpenter bees recorded on *C. procera* was a precondition for fruit and seed production of this plant species. The rate of mature fruit obtained for this plant species is relatively low (12.01% in 2019 and 19.59% in 2020). The average number of mature seeds per fruit was 267.58 ± 43.71 in 2019 and 285.43 ± 37.02 2020 in treatment 1 and 0 in treatment 2 which did not produced any fruit during both years. The percentage of normal seeds from treatment 1 was very high (94.23% in 2019 and 91.87% in 2020).

SEED GERMINABILITY OF *Calotropis procera*

The parameters related to the seed germination obtained from each batch of 100 seeds studied are reported in Table 8. Of 400 seeds studied in 2019 and 2020, 374 and 368 effectively germinated between days 3 and 6 with a germination rate of 93.5% and 92% during both years. The results reported in Fig. 8 indicate the rhythm of *C. procera* seed germination. In 2019, seed germination began on the third day (3%), peaked on the fourth day (59%) dropped considerably on the fifth day (18%) and sixth day (5.75%) and then stops on the seventh day; with the same germination rhythm, the corresponding values were 1.75%, 54.75%, 20.50%, 4.75% and 0% in 2020.

Table 8. Germination parameters of *Calotropis procera* seeds.

Years	**2019**	**2020**
Number of seeds	400	400
Number of germinated seeds	374	368
Germination rate (%)	93.5	92
Germination duration (days)	3–6	3–6

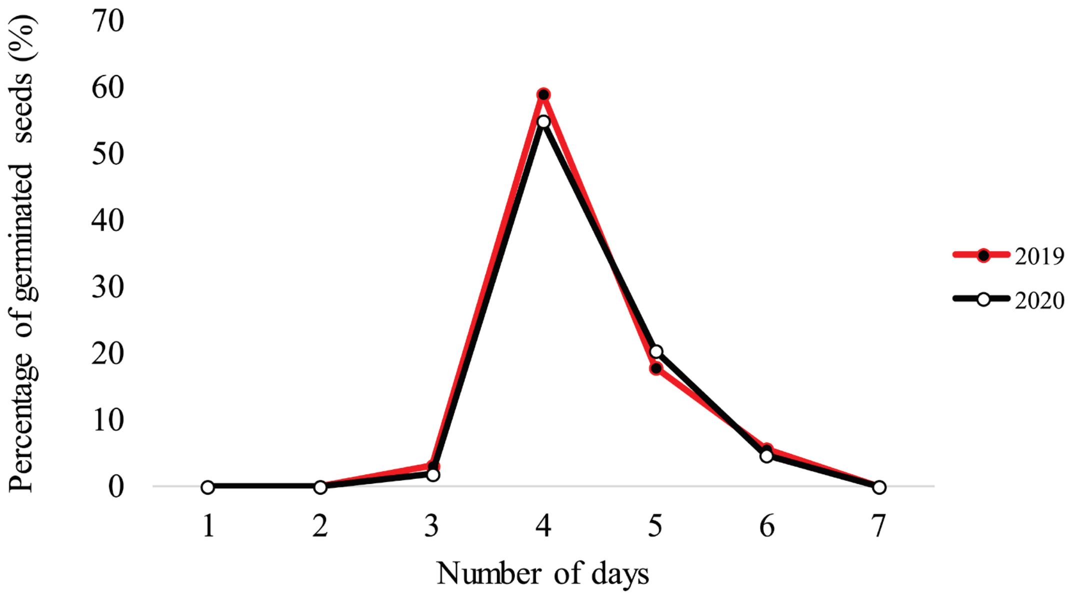

Fig. 8. Daily rhythm of seedling formation.

Discussion

This work on the foraging activity of flower visiting insects of *C. procera* revealed the presence of 11 foragers which is low in terms of the floral entomofauna of this plant species compared to work done on entomofauna of other plants. AZO'O *et al.* (2017) counted 37 insect species on watermelon flowers; BOHART *et al.* (1970) identified 334 species of insects on onion flowers; HAWTHORN *et al.* (1960) recorded 267 species of insects on carrot flowers; MESSI and TCHUENGUEM (1994) found 78 species of anthophilous insects on the flowers of *Dacryodes edulis*. In the light of these different results, it would appear that flower entomofauna varies from one plant species to another (KLEIN *et al.*, 2007).

The investigations made on the flowers of *C. procera* also revealed a prominence of Hymenoptera in general and Apidae in particular. It is known that the order Hymenoptera is the most numerous flower-visiting insects (PHILIPPE, 1991). Among these insects, the peculiarity here is the virtual absence of the honey bee, *A. mellifera* among the *C. procera* floral entomofauna. This bee species is characterized by the huge capacity of preference in its foraging area; it is able to discriminate between floral products of a less attractive plant species in favour of plant species which provide it with more energy during foraging (GUERRIAT, 1996).

The relative abundance of the carpenter bees in the floral entomofauna of *C. procera* in our study is consistent with earlier results on the dependency of *Calotropis* on the anthophilous activity of several species of Xylocopes around the world: Israel (EISIKOWITCH, 1985; RASHDA *et al*., 2018), Pakistan (ALI & ALI, 1989), India (RASHDA *et al*., 2018), Austria (MEVE & LIEDE, 1994) and United Kingdom (WILLMER, 1988). *C. procera* therefore is called a *Xylocopa*-flower (SCHREMMER, 1972). In Israel, two carpenter bee species have been identified namely *X. pubescens* (male and female) and only the male of *X. sulcatipes* (EISIKOWITCH, 1985). Carpenter bees captured in Senegal and Kenya on *C. procera* flowers have not been identified (JAEGER, 1971). In our work, 3 species were listed with an emphasis on their sex: *X. inconstans* (male and female), *X. pubescens* (male and female) and *X. ustulata* (female). In all, these results are another evidence that the floral entomofauna of a given plant species varies from one agroecological zone to another (KLEIN *et al*., 2007).

Our observations equally revealed the dominance of carpenter bee foraging activity in the morning with a peak of activity from the early morning time interval (6:00–7:00 a.m.). This peak corresponds to the time of day when the flowers of *C. procera* are more rewarding in nectar (EISIKOWITCH, 1985). The drastic drop in foraging activity observed after 11:00 a.m. is surely related to the decrease in the amount of nectar produced which may be influenced by the daily increase in average temperature. According to PESSON & LOUVEAUX (1984), when floral products are no longer easily exploitable or decrease in quantity and/or quality, foragers also reduce their activity on flowers so that the energy spent for foraging does not exceed the one drawn from the booty. On the other hand, morning foraging behavior appears to be a specific trait in Apidae nutrition, as previously suggested by ROUBIK (1989). Moreover, the daily fluctuation of the air temperature can be responsible for the depletion of floral resources such as nectar (POLATTO *et al*., 2014).

During their flower-visiting activity, bees and non-bee species are known to be preferentially attracted to the nectar of *C. procera*. Indeed, nectar is more accessible for foragers than pollen on this plant. Observations in exclusivity of nectar harvesting by foragers from *C. procera* flowers have been documented by several other authors (ALI & ALI, 1989; EISIKOWITCH, 1985; MEVE & LIEDE, 1994).

The visiting behavior of bee species consists of the number of flowers visited per minute or the foraging speed and the length of visits per interest flowers or duration of visit (BERNADINO & GAGLIANONE, 2008). The time of visiting flowers by bee species determined the total flowers visited on *C. procera*; the shorter the duration of bee visits, the more flowers that can be visited in one foraging trip. Our results are consistent with those of HASHIFAH *et al*. (2020) on the foraging behaviour of three carpenter bee species on *Cucumis sativus* flowers in Indonesia. The highest number of flowers was visited by *X. ustulata*, while the lowest was visited by *X. inconstans* and *Amegilla* sp. (with no significant difference of time of flower visit between both bee species). In the study site it was possible to see *X. inconstans* individuals exploiting simultaneously floral products from *Borassus aethiopum* (Aracaceae) in bloom which was closer to the study site. The activity of visiting pollinating insects on flowers were also subjected to competition between insect species or different species. Certain bee species were interrupted during their foraging activity enabling them to visit more flowers during a trip. This ability to visit many flowers per time unit by bees studied allow therefore more pollen to be collected (RIANTI *et al.*, 2010) and improve pollination of *C. procera* flowers. Diversity and behavior of intensive visit of pollinating insects are positively correlated with fruit yield (DAFNI *et al.*, 2015).

Pollination of *C. procera* flowers by carpenter bees enhance fertilization and therefore the production of fruits and seeds of this plant species. Indeed, our results illustrate that in treatment 2, whose flowers are free from insect floral visits, no fruit or seeds could form under these conditions. This is an illustration that wind pollination is not efficient in the fertilization of *C. procera*. On the other hand, the flowers visited by carpenter bees in particular were able to produce a certain number of fruits and seeds for the dissemination of this plant. The bee pollination was necessary for fruit and seed production, as no fruits or seeds were produced in the treatments without visits. Indeed, carpenter bee species dominantly pollinate the flowers of *C. procera*, and therefore their absence can be severely detrimental to the reproductive success of the plant species. Similar results have been published which illustrate the important role played by insects in the reproduction, production and maintenance of certain plant species; this is particularly the case for most plant belonging to the Cucurbitaceae family (STANGHELLINI *et al.*, 1998; AZO'O *et al.*, 2017; 2020). Overall, the mutualism between bee pollinators and flowering plants provides excellent results for the ecosystem, including increased yields (HASHIFAH *et al.*, 2020).

Despite the high implication of wild bees as the main pollinators of *C. procera* flowers, the fruit set rate of 12 to 20% during both years was very low. Several factors could be combined here to explain these low values. The first factor is linked to the low pollination success from flower-visiting insects of *C. procera* among which carpenter bees were the most abundant. Unlike social bees which live in large sized colonies and can recruit large numbers of congeners to exploit an interesting source of booty and maximize pollination of the flowers of the host plants (PESSON & LOUVEAUX, 1984), carpenter bees are solitary, thus cannot have a high impact on flower pollination, perhaps at the individual level compared with social bees (MENSAH & KUDOM, 2011). Secondly, pressure from insect pests such as *D. longistylus* is detrimental to fruit set in *C. procera*. These fruit flies selectively visited more fruit than flowers of *C. procera* plants. The young fruits formed are generally preferential egg-laying sites for the females of these flies. The eggs hatch into larvae feed on the substance of the fruit which abort and drop. Finally, among the fruit plants from which *C. procera* belongs, the plant is capable of self-regulation to economize its ressources. Thus, it can limit the number of fruit formed through specific physiological phenomena such as a selective abortion strategy at the expense of certain fruits (BROWN & MC NEIL, 2006).

Several other studies on plant-pollination networks highlighted the positive influence of anthophilous insect species in the increasing fruit and seed yields of plant species. These values vary from plant to plant. A quantitative assessment of the increase in yields due to pollinating insects on cotton is known to be 53% in India (MAHADAVAN & CHANDY, 1957). Beetles of the species *Elaeidobius kamerunicus* increase oil palm yields by 45% in Malaysia and make Malaysia the world's largest producer of palm oil (FAO, 2005). The honey bee increases the yields of *Prunus dulcis* by 200 to 1000%, those of *Persea americana* by 200 to 500% in France (PESSON & LOUVEAUX, 1984); this bee also increases the grain yields of *Helianthus annuus* by 16 to 28% and those of *Glycine max* by 30% in France (PESSON & LOUVEAUX, 1984). In the UK, *Eupeodes latifasciatus* and *E. balteatus* (Diptera: Syrphidae) floral activity increased strawberry yields by over 70% and doubled the proportion of marketable fruit (HODGKISS *et* *al*., 2018). In Cameroon, insects increased the fruit yields of *Dacryodes edulis* by 55% (TCHUENGUEM *et al*., 2001), those of *Zea mays* by 30% (TCHUENGUEM *et al*., 2002), those of *Vigna unguiculata* by 32% (TCHUENGUEM *et al*., 2009), those of *Phaseolus coccineus* by 21% (PANDO *et al*., 2011a), those of *Abelmoschus esculentus* by 11% (AZO'O *et al*., 2011), those of *Cajanus cajan* by 34% (PANDO *et al*., 2011b). The results listed above are non-exhaustive.

Conclusion

We have carried out investigations on the flowers of *C. procera* in its relationship with flower-visiting insects in Domayo (Maroua, Far-North of Cameroon). This work consisted of studying the diversity of the entomofauna associated with *C. procera*; a study of the foraging activity of bees are known to be important insects in terms of pollination of the plant, and we assessed the impact of pollinating insects on the seed yield and germinability. 11 flower-visiting insects belonging to 4 orders (in 2019) and 5 orders (in 2020) and 4 families (in 2019) and 7 families (in 2020) were recorded on the flowers of *C. procera* from our site. The order Hymenoptera with a relative abundance up to 95% during both years was predominant. Within this order, the Apidae family was the most representative with over 3 species of carpenter bee which were found to play an important role in the pollination and the productivity of *C. procera*. Bee foraging activity was higher in the morning (6:00–7:00 a.m.) when the average temperature was lower. These insects were fond of nectar which was accessible for them as they have developed adaptations to enable nectar access using their mouthparts. The absence of bee pollination was detrimental to fruit set and seed production in *C. procera*, whereas flower-visiting bees promoted fruit set and seed formation. The floral activity of bees was indispensable for pollination, fruit and seed production of *C. procera*, as no fruits or seeds were produced in the treatments without visits. The overall germinability of *C. procera* due to carpenter bee pollination was estimated at 93.5% in 2019 and 92% in 2020. Protection of carpenter bees is therefore essential for the increased production of *C. procera* seeds.

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