



Research article

Impact of aqueous extracts of *Cassia occidentalis*, *Eucalyptus camaldulensis* and *Hyptis suaveolens* on the entomofauna and the seed yield of *Gossypium hirsutum* at Boklé (Garoua, Cameroon)

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ABSTRACT

There is a frightening decline in the population pollinators around the world due to the over usage of synthetic pesticides, leading to the directly reduce of plant production. Plant extracts with insecticidal properties could be eco-friendly alternatives to synthetic pesticides in maintaining the pollinator population and the diversity of the ecosystem. The impact of aqueous extracts of *Cassia occidentalis* L., *Eucalyptus camaldulensis* Dehnh. and *Hyptis suaveolens* L. was investigated on the entomofauna and the seed yield of *Gossypium hirsutum* L. cotton. The study was carried out in RCBD, four times replicated: 3 extracts x 1 standard synthetic insecticide (TEMA) x 1 control x 4 groups of flowers (group 1: flowers free to insect visits, group 2: flowers protected from insects using gauze bags, group 3: protected flowers and opened exclusively to *Amegilla* sp. and group 4: protected flowers opened from time to time without any visit of insect). *Gossypium hirsutum* was found to be visited by the insects belonging to five orders, 10 families and 18 species. *Amegilla* sp.1 and *Apis mellifera* were the major pollinators during the rainy and dry seasons, respectively. The number and quality of seeds visited exclusively by *Amegilla* sp.1 were significantly improved by *H. suaveolens* extract. During the dry season, *E. camaldulensis* and *H. suaveolens* extracts as well as the standard insecticide improved the number of seeds and the percentage of normal seeds harvested from the flowers allowed to be visited by insects; that was probably due to their insecticidal effects which protected plants from pest damage. Therefore, aqueous extracts of *E. camaldulensis* and *H. suaveolens* are good candidates for incorporation in integrated pest management programs to minimize the risk of synthetic pesticides to pollinators, hence to increase the yield and the quality of seeds.

1. Introduction

In Cameroon, agriculture is the main activity in countryside and the main provider of jobs, since it employs around 60% of the working population (INS, 2017). One of the key objectives of agricultural research in Cameroon is the optimisation of agricultural yields to achieve a balanced diet (MINADER/DESA, 2010). The qualities of seeds and varieties have enabled farms to have yields of nearly 70% (MINADER, 2017). Cotton cultivation plays an important role in food self-sufficiency. In fact, the FAO estimates that nearly 100 million rural African families depend directly on cotton production, including more than 6 million rural African households (Abdoulaye et al., 2008). Cotton is the main lint crop in

the world, its global production declining 3% to 25.8 million tons in 2018 (OECD/FAO, 2016). Although more than 50% of its production is intended for clothing; it also produces various derivatives, including vegetable oil and 20–30% protein (Abdoulaye et al., 2008). In Cameroon, the demand for cotton seeds and fiber (350,000 tons) is greater than its production (250,000 tons) because of pests, which represent the main cause of destruction of cotton crops (MINADER/DESA, 2010). The insect pests of cotton are grouped into four main categories, namely carpophagous caterpillars, phyllophagous caterpillars, sucker and mites. Crop losses due to attacks by these pests are greater than 30% and can reach total destruction of the production potential (SODECOTON, 2019).

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Thus, in order to boost yields and meet up the ever-increasing market demand, farmers resort to the use of synthetic pesticides which has caused more damages than it has solved (Bambara and Tiemtoré, 2008). Africa uses less than 10% of global pesticide production but accounts for 75% of pesticide fatalities (Guèye et al., 2011). Indeed, they aren't just expensive, but they also acidify the soil when misuse and their residues are toxic to non-target fauna. They cause resistance in pest insects (Immaraju et al., 1992; Margni et al., 2002), and pollute surface water and groundwater (Ouédraogo, 2004). Considering the harmful effects of synthetic insecticides, it is necessary to develop and recommend alternative environmentally friendly methods to control insect pests of cotton while preserving pollinating insects. Pollinators such as bees are often involved in increasing the yield of fruits and seeds of several plants (Fluri and Frick, 2005; Djonwangwe et al., 2017).

Botanical insecticides are naturally occurring insecticides derived from plants that possess repellent, sterilant, antifeedant and toxicant properties and are environment friendly (Isman, 2000; Ayvas et al., 2009). In fact, plants through their derivatives such as powders, crude extracts, oil, essential oils and semi-purified or purified compounds provide natural insecticides for the protection of field crops and can be therefore used as a substitute for synthetic insecticides (Bambara and Tiemtoré, 2008; Cheikh et al., 2015; Barry et al., 2017, 2019). Essential oils from the leaves of *C. occidentalis*, *E. camaldulensis* and *H. suaveolens* have been reported to possess insecticidal efficacy against insect pests (Conti et al., 2012; Chibuzor and Obioma, 2018; Rezaei et al., 2019). To provide farmers with quick and cheap access to crop pest control solutions, the present study was undertaken to investigate the impact of aqueous extracts of *C. occidentalis*, *E. camaldulensis* and *H. suaveolens* on the entomofauna and the seed yield of *G. hirsutum*.

2. Materials and methods

2.1. Study area and biological materials

The study was carried out over two seasons from 08 July to 17 December, 2019 and from 22 December, 2019 to 17 June, 2020 in Bocklé, 3rd district of Garoua Northern Region of Cameroon (latitude: 9°17'29.814"N; longitude: 13°25'4.38636"E; altitude: 169 masl). This area belongs to the Sudano-Sahelian zone of Cameroon characterized by unimodal rainfall. The rainy season lasts 4 months from June to September with two intermediate months of unreliable rainfall in May and October. Mean total annual rainfall is approximately 1000 mm and the mean annual temperature varied between 25.5 to 32.5 °C. Mean relative humidity varied between 42 to 83% (data recorded in 2018 by the Garoua international airport: unpublished).

The floristic species of vegetation encountered are consisting of *Hyparrhemia rufa* along rivers, *Borassus aethiopicum*, *Boswellia dalzielii*, *Commiphora africana*, *Lannea microcarpa*, *Bombax costatum*, *Prosopis africana*, *Vitellaria paradoxa* and plantations of *Azadirachta indica*. *Eucalyptus camaldulensis*, *Cassia occidentalis* and *Hyptis suaveolens* used in several traditional medicines to cure various diseases can be also found. *Cassia occidentalis* and *H. suaveolens* are annual or short-lived perennial plants (Sharma et al., 2009; Yadav et al., 2010), while *E. camaldulensis* is perennial plant. There were five colonies of *Apis mellifera* and probably other non-inventoried colonies and, all other insects naturally present in the environment of the study area.

The experimental plot covered a surface area of 494 m² (26.0 m length × 19.0 m broad). It was cleared and excavated using machetes and pickaxes, and then 4 blocks of 5 experimental units each were established. Each experimental unit consisted of one row of 3.5 m broad × 4.0 m length spaced 1.0 m apart. Five cotton *Gossypium hirsutum* seeds, variety QR₁302 supplied by SODECOTON were sown at an intra-row and inter-row spacing of 36.36 cm and 50 cm respectively, and thinned to two per hill two weeks after sowing. The plot was manually weeded every two weeks.

2.2. Collection and processing of plant materials

Fresh leaves of *C. occidentalis*, *E. camaldulensis* and *H. suaveolens* were collected in the Djamboutou district at Garoua (latitude 9°31'0"N and longitude 13°41'0"E). Collection was done each two weeks on the eve of a treatment during the entire period. The aqueous plant extracts were obtained by using the method described by Sreekanth (2013): 400 g of each these leaves were weighed using a DIAMAN brand electronic balance (Capacity 3 kg, Precision 0.1 g), pounded separately using a wooden mortar and pestle before being put in a bucket containing 4L of water. Thereafter, the mixture was left to stand for 12 h. After maceration, the mixture was sifted through a 0.2mm mesh sieve into another bucket. The solutions obtained were each put into the sprayers.

2.3. Treatments

The study was carried out in a randomized complete block design (RCBD): 3 aqueous plant extracts × 1 standard synthetic insecticide (TEMA) × 1 control × 4 groups of flowers (group 1: flowers free to insect visits, group 2: flowers protected from insects using gauze bags, group 3: protected flowers and opened exclusively to *Amegilla* sp. and group 4: protected flowers opened from time to time without any visit of insect) × 4 replications. TEMA is a synthetic chemical insecticide containing 60 g of emamectin benzoate per kg and 75 g of teflubenzuron per kg. It is recommended at the rate of 200 g/ha to protect *G. hirsutum* at its different developmental stages against insect pests. In our study, 2 g was diluted in 3 L of water. Insecticide formulations were sprayed using five distinct manual gauge sprayers, each corresponding to a specific insecticidal product including the water used as control. Extracts and water at the rate of 714 L/ha and TEMA solution at the rate of 300 L/ha were applied in the evening between 5 and 6 p.m, two weeks interval, starting from the germination to the maturation of capsules. At bud stage, flowers were grouped as previously described and labelled: groups 1 and 2 = 120 flowers each, group 3 = 200 flowers and group 4 = 100 flowers.

2.4. Data collection

Flowers of group 1 were observed at the peak of flowering period and the foraging behaviour and insect species were recorded. The number of insects morphospecies visiting 120 *G. hirsutum* flowers were counted every day between 6.00 a.m. and 5.00 p.m., with six time periods per day (6:00–7:00 a.m., 8:00–9:00 a.m., 10:00–11:00 a.m., 12:00–1:00 p.m., 2:00–3:00 p.m. and 4:00–5:00 p.m.). Since the insects were not marked, the cumulative data were expressed as the number of visits. Except *A. mellifera* and *Amegilla* sp.1, other insects visiting *G. hirsutum* were captured by hand/entomology forceps (larvae) or using entomological net (adults). Specimens were stored in glass vials containing 70% ethanol, except Lepidoptera that was preserved in papillotes as recommended by Borror and White (1991). Each glass vial was labelled according to the different treatments where insects were captured. At the end of the study, insects were identified by using insect keys (Delvare and Arbelenc, 1989; Borror and White, 1991; Couilloud, 1993; Eardley et al., 2010).

The number of *A. mellifera* and *Amegilla* sp.1 simultaneously foraging one and 1000 flowers was assessed according to the method described by Tchuenguem (2005). The frequency of each insect species (Fi) was determined using the following formula: $F_i = (V_i/VI) * 100$, with V_i = number of visits of a given insect and VI the total number of visits by all insects (Tchuenguem et al., 2001).

The fruiting rate (FR), the average number of seeds (ANS) and normal seed percent (%NS) were recorded for each group as follow:

$$- FR = (F1/F2)*100: F1 \text{ is the number of boll formed and } F2 \text{ the number of flowers initially labeled.}$$

- ANS: dried capsules were opened manually and seeds were harvested and counted.
- %NS = (Number of normal seeds/Total seeds)*100.

The mode of reproduction of *G. hirsutum* was determined from group 1 (not protected flowers) and group 2 (protected flowers). Ten days after the last flower wilted, the number of bolls formed in each group was counted. For each group, the fruiting index (Ifr) was calculated using the following formula: $Ifr = (F1/F2)$, where F1 is the number of bolls formed and F2 the number of flowers initially labelled (Tchuenguem et al., 2004). The outcrossing rate (TC) was calculated using the formula: $TC = \{[(IfrX - IfrY)/IfrX] \times 100\}$, where IfrX and IfrY are the mean fruiting indexes of free flowers (group 1) and protected flowers (group 2), respectively (Demarly, 1977). The rate of self-pollination in the broad sense (TA) was calculated using the formula: $TA = (100 - TC)$.

The impact of flower insects and insecticide treatments on the fruiting rate (Fri) was evaluated using the following formula: $Fri = \{[(F1 - F4)/F1 + F2 - F4] \times 100\}$ where F1, F2 and F4 are the fruiting rates in groups 1 (free flowers), 2 (protected flowers) and 4 (flowers opened from time to time without any visit of insect), respectively. For a given group x, the fruiting rate is: $Fr_x = [(number\ of\ capsules\ formed/number\ of\ flowers\ labelled)] \times 100$ (Diguir et al., 2020).

The percentage of the number of seeds per capsule (Pg) attributable to the impact of flower insects and insecticide treatments was calculated as follow: $Pg = \{[(g1 - g4)/g1 + g2 - g4] \times 100\}$ where g1, g2 and g4 are the average number of seeds per capsule in groups 1, 2 and 4, respectively (Diguir et al., 2020).

The percentage of normal seeds (Pn) attributable to the impact of flower insects and insecticide treatments was calculated using the following formula: $Pn = \{[(Pn1 - Pn4)/Pn1 + Pn2 - Pn4] \times 100\}$; where Pn1, Pn2 and Pn4 are the percentages of normal seeds from groups 1, 2 and 4, respectively (Diguir et al., 2020).

The yield was assessed for each treatment by weighting the harvested corresponding seeds.

2.5. Statistical analysis

Data on insect abundance, fruiting rate, average number of seeds per capsule, normal seeds percent, weight of seeds and seed yield were log-transformed (x + 1). The transformed data were subjected to the ANOVA procedure of SPSS 16.0. Tukey's (Honest Significant Difference) multiple range test (p = 0.05) was applied for mean separation.

3. Results

From the investigations of that study, 413 individuals of insect pollinators belonging to 18 species, 10 families and five orders (Table 1) were recorded. Insect population seize was higher during the dry season (290 individuals belonging to 18 species) than the rainy season (123 individuals belonging to 17 species). More than half of individuals recorded belonged to the order of Hymenoptera. *Amegilla* sp. 1 (56.10 %) and *Apis mellifera* (25.86 %) were the most frequent floral visitors of *G. hirsutum* during the rainy season and dry season, respectively. Floral products, nectar and pollen were harvested by the species belonging to the order of Hymenoptera; nectar was harvested by those belonging to Diptera and Lepidoptera orders, while pollen was harvested by Coleopteran and Orthopteran.

The impact of aqueous extracts of *C. occidentalis*, *E. camaldulensis* and *H. suaveolens* on the foraging behaviour of *A. mellifera* and *Amegilla* sp. 1 was significant (F = 12.43, 16.93; p < 0.001) (Table 2). All the tested extracts were attractive to the both insect species compare to control, and standard synthetic insecticide which was very repellent. *E. camaldulensis* and *C. occidentalis* extracts were the most attractive.

Apis mellifera and *Amegilla* sp. 1 were found to visit *G. hirsutum* flowers from 6 a.m. to 5 p.m (Figure 1). Foraging activities fluctuated from the morning to the evening. Frequency of appearance was noted more between 10 a.m. to 1 p.m. for *A. mellifera* (dry season) and between 12 to 3 p.m. for *Amegilla* sp. 1 (rainy season). The peaks of activities were 12–1 p.m. and 2–3 p.m., respectively for *A. mellifera* and *Amegilla* sp. 1. Insecticidal products were either repulsive or attractive to pollinators

Table 1. Distribution of insects depending on the species and number of visits on *Gossypium hirsutum* flowers during the rainy and dry seasons.

Order	Family	Genus, species, floral products	Rainy season		Dry season		Total	
			n ₁	P ₁ (%)	n ₂	P ₂ (%)	n _T	P _T (%)
Hymenoptera	Apidae	<i>Amegilla</i> sp. 1 (Ne, Po)	69	56.10	40	13.79	109	26.39
		<i>Amegilla</i> sp. 2 (Ne, Po)	4	3.25	8	2.76	12	2.91
		<i>Apis mellifera</i> (Ne, Po)	5	4.07	75	25.86	80	19.37
		<i>Xylocopa olivacea</i> (Ne, Po)	1	0.81	10	3.45	11	2.66
		<i>Xylocopa</i> sp. 1 (Ne, Po)	2	1.63	11	3.79	13	3.15
		<i>Xylocopa</i> sp. 2 (Ne, Po)	0	0.00	21	7.24	21	5.08
	Megachilidae	<i>Megachile</i> sp. 1 (Ne, Po)	5	4.07	15	5.17	20	4.84
	Vespidae	(1 sp.) (Ne, Po)	2	1.63	7	2.41	9	2.18
	3 families	8 species	88	71.54	187	64.48	275	66.59
Diptera	Syrphidae	(1 sp. 1) (Ne)	1	0.81	10	3.45	11	2.66
		(1 sp. 2) (Ne)	3	2.44	7	2.41	10	2.42
		(1 sp. 3) (Ne)	5	4.07	11	3.79	16	3.87
		1 family	3 species	9	7.31	28	9.66	37
Lepidoptera	Hesperiidae	(1 sp.) (Ne)	1	0.81	11	3.79	12	2.91
	Nymphalidae	<i>Acraea acerata</i> (Ne)	4	3.25	10	3.45	14	3.39
	Pieridae	<i>Eurema</i> sp. (Ne)	2	1.63	8	2.76	10	2.42
		3 families	3 species	7	5.69	29	10.00	36
Coleoptera	Cetoniidae	<i>Pachnoda cordata</i> (Po)	9	7.32	14	4.83	23	5.57
Orthoptera	Acrididae	(1 sp. 1) (Po)	1	0.81	13	4.48	14	3.39
		(1 sp. 2) (Po)	8	6.50	12	4.14	20	4.84
	Pyrgomorphidae	<i>Tettigonia viridissima</i> (Po)	1	0.81	7	2.41	8	1.94
		2 families	3 species	19	15.45	46	15.86	65
Total	10 families	18 species	123	100%	290	100%	413	100

n₁ and n₂: number of visits on 120 flowers; percentage of visits: p₁ = (n₁/123) * 100; p₂ (n₁/290)*100; Ne: collection of nectar; Po: collection of pollen; sp.: unidentified species.

Table 2. Abundance of *Amegilla* sp. and *Apis mellifera* foragers per 1000 flowers of *Gossypium hirsutum* treated with insecticidal products.

Insect/season	Insecticide					F
	Control	<i>C. occidentalis</i>	<i>E. camaldulensis</i>	<i>H. suaveolens</i>	TEMA	
<i>Amegilla</i> sp. 1/Rainy season	100.24 ± 0.67 ^{bc}	122.52 ± 3.54 ^a	128.59 ± 4.21 ^a	113.79 ± 2.20 ^{ab}	93.27 ± 2.87 ^c	11.47 ^{***}
<i>A. mellifera</i> /dry season	105.26 ± 1.21 ^{bc}	115.11 ± 1.52 ^b	133.86 ± 3.35 ^a	111.99 ± 1.21 ^b	101.26 ± 2.29 ^c	25.04 ^{***}

***: p < 0.001.

Means within the same line followed by the same letter do not differ significantly (p < 0.05; Tukey's test).

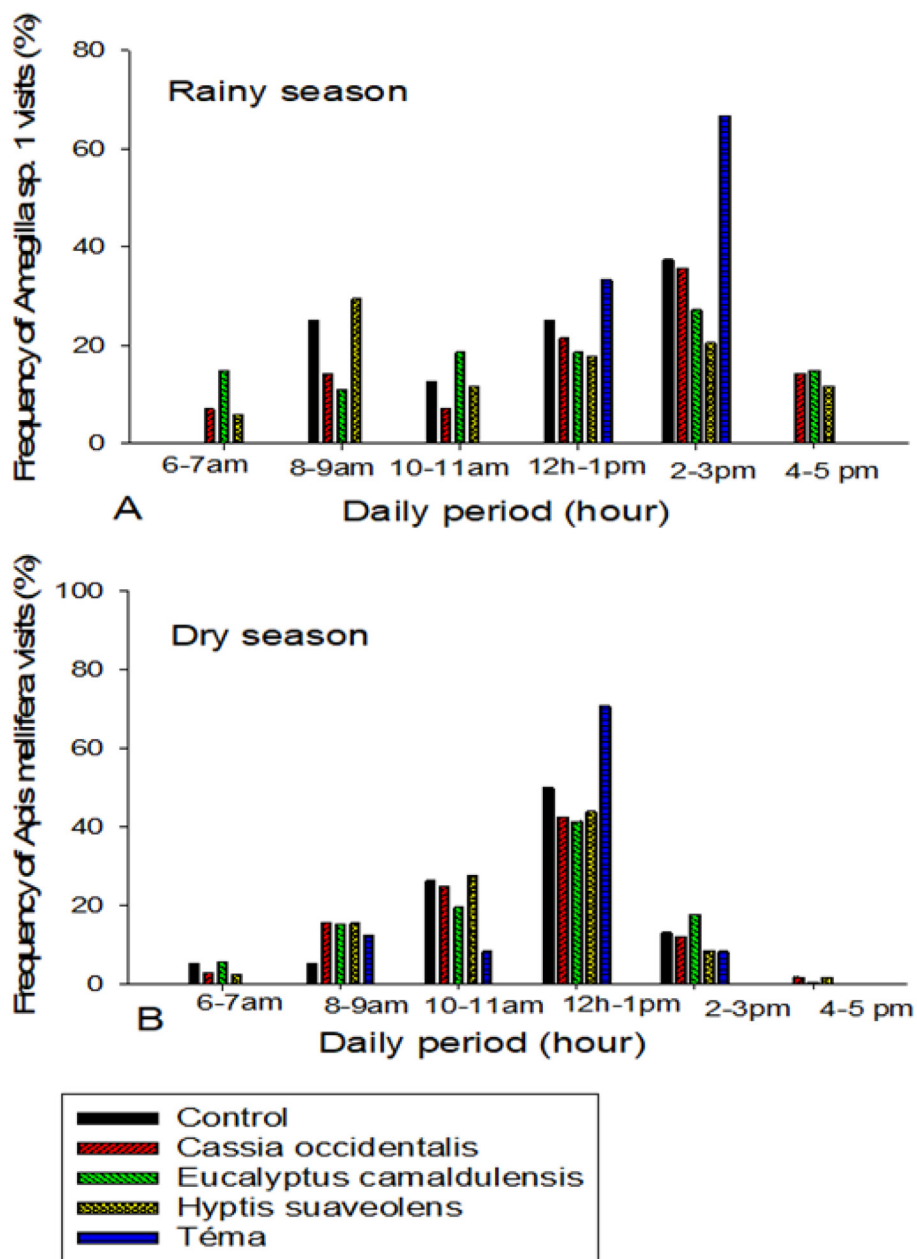


Figure 1. Frequency of *Amegilla* sp. 1 and *Apis mellifera* visits on *Gossypium hirsutum* flowers according to daily periods and insecticide treatments during the rainy (A) and dry (B) seasons.

depending on the periods of foraging activities. Overall, extracts were more moderately attractive to *Amegilla* sp. 1 than the control treatment, early in the morning (6–7 a.m.) and in the evening (4–5 p.m.). At 8–9 a.m. and 10–11 a.m., *H. suaveolens* and *E. camaldulensis* were also respectively more attractive to *Amegilla* sp. 1 compare to the control treatment. Furthermore, all the extracts were more attractive to *Amegilla*

sp. 1 than the standard synthetic insecticide TEMA, before noon and in the evening at 4–5 p.m. However, TEMA was very attractive to *Amegilla* sp. 1 compared to the extracts at 12–3 p.m. At 8–9 a.m., extracts were most attractive to *A. mellifera* than control and synthetic insecticide. In fact, the standard synthetic insecticide was repellent to *A. mellifera* compare to extracts, except at 12–1 p.m.

The number of insect pest species (Figure 2) recorded on *G. hirsutum* plants varied between treatments. Six, seven and eight species were recorded from *C. occidentalis*, *H. suaveolens* and *E. Camaldulensis* treatments, respectively. The same species were recorded on plants treated with *E. Camaldulensis* and on those without treatment. Treatment with the standard synthetic insecticide TEMA recorded the highest number of species (nine). Overall, insect abundance between treatments including the control was not significant (Table 3).

The comparison of fruiting rate (Fr), average number of seeds per capsule (ANSPC) and normal seeds percent (NSP) under diverse reproduction system (Tables 4 and 5) showed that observed differences were significant between some treatments. *Eucalyptus camaldulensis* extract significantly ($F_{4,115} = 3.13-10.83$, $p \approx 0.05-0.001$) improved Fr, ANSPC and NSP when the flowers were exclusively allowed for self-pollination during the rainy and dry seasons. During the dry season (Table 5), *E. camaldulensis* and *H. suaveolens* treatments as well as the standard insecticide improved ANSPC and NSP harvested from the flowers visited exclusively by *Amegilla* sp.1. The contributions of *Amegilla* sp. 1 to increase ANSPC and NSP during the rainy and dry seasons was significant for control ($F_{3,8} = 6.96, 7.78$, $p = 0.01, 0.05$) and treatment with aqueous extract of *H. suaveolens* ($F_{3,8} = 10.21, 18.42$, $p = 0.01, 0.001$). During the dry season, *C. occidentalis* improved the Fr and ANS of *G. hirsutum* pollinated by *Amegilla* sp.1.

The mean fruiting indexes were 0.99 and 0.87 in treatments 1 and 2, respectively. During rainy season, the allogamy rate was 12.12% and the autogamy rate was 87.88%. During the dry season, the rates of 28.72% and 71.28% were recorded in the same order. It appears that *G. hirsutum* has a mixed mating system, autogamous-allogamous, with the predominance of allogamy.

Seed yields were not significantly different ($F_{4,15} = 0.64$, $p > 0.05$) between the treatments during the rainy season and significantly different ($F_{4,15} = 3.37$, $p < 0.05$) during the dry season (Table 6). Seed

yields were higher during the dry season. The impact of insecticidal products on seed yield during the rainy season was not significant. During the dry season, aqueous extracts and the standard synthetic insecticide recorded significant higher seed yields compare to the control; *C. occidentalis* being recording the highest value.

4. Discussion

According to this study, insects pollinate *G. hirsutum* in exchange of nectar and/or pollen, thus both are mutually benefitted. Insects are important pollinators for *G. hirsutum*. However, the specific type of insect varies across the globe (Bozбек et al., 2008; Parys et al., 2020). From that investigation, *Amegilla* sp. 1 was found as constant species visiting *G. hirsutum* during the rainy season, *A. mellifera* was accessory during the dry season and all other species were sporadic either during the rainy or dry seasons according to the classification of Bigot and Bodot (1973). Relative abundance of *A. mellifera* found in the present study as the major insect pollinators of *G. hirsutum* during the dry season might be partially explained by the presence of five colonies of that Apidae identified around the study area, and optimum environmental conditions for their foraging activities. The absence during the dry season or the presence during the rainy season of others flowering plants around the site of study might be another plausible reason of that seasonal abundance of *A. mellifera*. *Amegilla* sp. 1 was found to be the major insect pollinator of *G. hirsutum* during the rainy season in contrast to the finding reported by Mazi et al. (2020), where *A. mellifera* was found to be more abundant. The contrast between the results of these studies might be due to the impact of environmental conditions on the population size of each species. According to Ghosh et al. (2020), the temperature around 20–28 °C is optimum for *A. mellifera* to forage and foragers rarely work below 13 °C and above 38 °C (Abou-Shaara, 2018). Since the temperature of



Figure 2. Insect pest species recorded on *Gossypium hirsutum* plants treated with botanical insecticides. A: *Aphis gossypii*, B: *Dysdercus delauneyi*, C: *Cheilomenes propinqua*, D: *Pachnoda cordata*, E: *Anthonomus* sp., F: *Halitrodes derogata*, G: *Diparopsis wateri*, H: *Lacusta migratoria*, I: *Zonocerus variegatus*.

Table 3. Abundance of insect pest species on *Gossypium hirsutum* plants treated with insecticidal products.

Insect pest species	Insecticide					F _{4,15}
	Control	<i>Cassia occidentalis</i>	<i>Eucalyptus camaldulensis</i>	<i>Hyptis suaveolens</i>	TEMA	
<i>Aphis gossypii</i>	4.75 ± 1.70	4.25 ± 0.25	2.50 ± 0.50	3.75 ± 0.85	4.75 ± 1.55	0.69 ^{ns}
<i>Dysdercus delauneyi</i>	2.00 ± 2.00	0.75 ± 0.47	0.25 ± 0.25	0.00 ± 0.00	0.25 ± 0.25	0.74 ^{ns}
<i>Cheilomenes propinqua</i>	2.25 ± 1.03	0.00 ± 0.00	0.75 ± 0.47	2.00 ± 0.81	2.25 ± 1.60	1.15 ^{ns}
<i>Pachnoda cordata</i>	6.00 ± 4.06	3.50 ± 1.50	3.50 ± 1.04	4.25 ± 1.43	3.00 ± 0.91	0.31 ^{ns}
<i>Anthonomus</i> sp.	0.50 ± 0.28	2.25 ± 1.03	2.75 ± 0.95	2.00 ± 1.08	1.25 ± 0.25	1.19 ^{ns}
<i>Halitarodes derogata</i>	0.00 ± 0.00	0.50 ± 0.29	0.00 ± 0.00	0.50 ± 0.29	0.25 ± 0.25	1.36 ^{ns}
<i>Diparopsis wateri</i>	0.25 ± 0.25	0.00 ± 0.00	1.25 ± 1.25	2.25 ± 2.25	1.00 ± 0.57	0.57 ^{ns}
<i>Lacusta migratoria</i>	4.25 ± 1.60	3.25 ± 1.10	0.75 ± 0.25	1.00 ± 0.71	1.75 ± 0.85	2.23 ^{ns}
<i>Zonocerus variegatus</i>	2.50 ± 1.44	0.00 ± 0.00	0.50 ± 0.50	0.00 ± 0.00	0.25 ± 0.25	0.66 ^{ns}
Total	22.50 ± 8.00	14.50 ± 2.40	25.25 ± 2.87	15.75 ± 3.77	14.75 ± 4.71	0.76 ^{ns}

ns: p > 0.05.

Table 4. Fruiting rate, average number of seeds per capsule and normal seeds percent of *Gossypium hirsutum* treated with insecticidal products under diverse reproduction system during the rainy season.

Parameter	TREATMENTS					F _{4,115}
	Control	<i>Cassia occidentalis</i>	<i>Eucalyptus camaldulensis</i>	<i>Hyptis suaveolens</i>	TEMA	
Fruiting rate (%)						
FF	100 ± 0.00	95.83 ± 4.17 ^{ab}	100 ± 0.00	100 ± 0.00	100 ± 0.00	1.00 ^{ns}
BF	83.33 ± 8.33	100 ± 0.00 ^a	100 ± 0.00	91.66 ± 8.33	100 ± 0.00	2.00 ^{ns}
FvA	88.09 ± 8.58	84.92 ± 4.76 ^{ab}	100 ± 0.00	100 ± 0.00	100 ± 0.00	1.41 ^{ns}
FOWV	74.60 ± 9.91 ^B	84.92 ± 0.79 ^{ABb}	100 ± 0.00 ^A	100 ± 0.00 ^A	95.24 ± 4.76 ^A	10.83 ^{***}
F _{3,8}	2.55 ^{ns}	4.04 [*]	-	1.00 ^{ns}	1.00 ^{ns}	
Average number of seeds						
FF	16.92 ± 2.18 ^b	18.25 ± 6.18	22.09 ± 1.17	26.71 ± 0.25 ^a	29.17 ± 0.93	3.07 ^{ns}
BF	18.33 ± 0.73 ^{BCb}	25.42 ± 1.16 ^{AB}	21.88 ± 1.96 ^{ABC}	16.67 ± 2.96 ^{Cb}	28.79 ± 1.00 ^A	8.03 ^{**}
FvA	27.53 ± 2.56 ^a	24.66 ± 0.88	28.03 ± 0.76	29.33 ± 0.35 ^a	28.61 ± 1.32	1.63 ^{ns}
FOWV	19.92 ± 0.94 ^{ABb}	10.42 ± 2.09 ^B	27.34 ± 1.81 ^A	24.96 ± 2.66 ^{Aa}	22.63 ± 3.88 ^A	6.99 ^{**}
F _{3,8}	6.96 [*]	4.35 ^{ns}	4.81 ^{ns}	7.47 [*]	2.09 ^{ns}	
Normal seeds (%)						
FF	59.03 ± 4.87	62.83 ± 19.36	81.54 ± 1.93	84.13 ± 2.47 ^{ab}	95.85 ± 0.24	2.90 ^{ns}
BF	49.01 ± 5.62 ^B	72.85 ± 1.75 ^{AB}	68.61 ± 9.73 ^{AB}	46.06 ± 9.17 ^{Bc}	82.90 ± 5.29 ^A	5.19 [*]
FvA	68.14 ± 10.48 ^{AB}	68.63 ± 6.09 ^B	84.14 ± 3.93 ^{AB}	94.40 ± 0.34 ^{Aa}	86.33 ± 8.16 ^{AB}	2.91 ^{ns}
FOWV	48.77 ± 8.49 ^C	56.38 ± 4.94 ^{BC}	84.67 ± 3.13 ^A	64.32 ± 3.10 ^{ABCbc}	75.79 ± 6.10 ^{AB}	7.17 ^{**}
F _{3,8}	1.44 ^{ns}	0.46 ^{ns}	1.84 ^{ns}	18.42 ^{***}	1.76 ^{ns}	

ns: p > 0.05; *: p < 0.05; **: p < 0.001; ***: p < 0.001.

Means within the same column and line followed respectively by the same small and capital letter do not differ significantly (p < 0.05; Tukey's test).

-: estimation of the F value is not possible due to equal variance; FF: free flowers; BF: bagged flower; FvA: flowers visited exclusively by *Amegilla* sp. 1; FOWV: flowers opened without any visit of insect.

environmental area of the study was above 30 °C during the dry season and lower during the rainy season, temperature is less important factor to explain that seasonal variation of the abundance of *A. mellifera* visiting *G. hirsutum*. The plausible reason should be the availability of diverse resources more attractive than *G. hirsutum* to honey bees during the rainy season. However, further study is required to assess the attractiveness of floristic plants surrounding cotton plantation to honey bees. The relative humidity, rainfall and wind speed might be also other factors which had significant negative effect on the foraging activity of *A. mellifera* during the rainy season. In a simple legitimate way, each bee pollinator has specific ecological threshold for foraging activity which might differ inter specifically depending upon the level of adaptation of a given species in an environment as reported by previous researchers (Burill and Dietz, 1981; Abrol and Kapil, 1986).

The peak of activity of *A. mellifera* at 12–1 p.m. and that of *Amegilla* sp. 1 at 2–3 p.m. was probably linked to the daily periods of greater availability of the floral products of *G. hirsutum*, at optimum environmental conditions which might be not the same for the both insect species. Ghosh

et al. (2020) demonstrated that the honey bees were more active in the afternoon than in the morning and found the highest foraging activity at 1 p.m. because the highest amount of pollen foraged during this period of the day. These finding including ours did not corroborate with that of Adamou et al. (2020) who reported the peak of activity of *Amegilla* sp. 1 before noon at 10–11 a.m. The peak of the pollinator activity would depend on the daily flower blooming rate which may also depend on several abiotic factors. More details concerning the assessment of available resources, flower blooming rate and abiotic factors should be considered in the further study to more elucidate these contrasts.

Eucalyptus camaldulensis extract improved Fr, ANSPC and NSP of *G. hirsutum* allowed for self-pollination and this suggest that the use of botanicals to control the pests at vegetative stage of a plant might improve some plant yield parameters. This may be attributed to insecticidal activities of that plant. Abdelkhalek et al. (2020) reported the bark extract of that plant to be effective against *Tribolium castaneum* and *Sitophilus oryzae*. According to Dwibedi et al. (2017), the grain yield is highly correlated with the vegetative parameters and this relationship

Table 5. Fruiting rate, average number of seeds per capsule and normal seeds percent of *Gossypium hirsutum* treated with insecticidal products under diverse reproduction system during the dry season.

Parameter	Treatments					
	Control	<i>Cassia occidentalis</i>	<i>Eucalyptus camaldulensis</i>	<i>Hyptis suaveolens</i>	TEMA	F _{4,115}
Fruiting rate (%)						
FF	100 ± 0.00	94.00 ± 6.00 ^a	100 ± 0.00	100 ± 0.00	100 ± 0.00	1.00 ^{ns}
BF	73.33 ± 13.64	100 ± 0.00 ^a	78.67 ± 12.72	66.67 ± 20.27	95.00 ± 5.00	1.29 ^{ns}
FvA	100 ± 0.00	100 ± 0.00 ^a	100 ± 0.00	95.00 ± 5.00	100 ± 0.00	1.00 ^{ns}
FOWV	76.00 ± 14.80 ^{AB}	49.67 ± 7.80 ^{Bb}	100 ± 0.00 ^A	77.33 ± 13.92 ^{AB}	90.04 ± 9.96 ^{AB}	3.13 [*]
F _{3,8}	2.12 ^{ns}	24.47 ^{***}	2.81 ^{ns}	1.52 ^{ns}	0.73 ^{ns}	
Average number of seeds						
FF	15.42 ± 2.20 ^{BCb}	10.75 ± 1.76 ^{Cab}	20.59 ± 1.17 ^{ABab}	22.21 ± 0.25 ^{Aab}	24.27 ± 0.93 ^A	15.29 ^{***}
BF	16.53 ± 0.73 ^{BCb}	11.28 ± 0.30 ^{Cab}	20.08 ± 1.96 ^{Bb}	14.87 ± 2.95 ^{BCb}	20.79 ± 1.00 ^A	12.49 ^{***}
FvA	26.3 ± 2.57 ^{Aa}	13.56 ± 0.88 ^{Ba}	26.93 ± 0.76 ^{Aa}	28.23 ± 0.35 ^{Aa}	25.71 ± 1.32 ^A	19.41 ^{***}
FOWV	18.02 ± 0.94 ^{Ab}	08.18 ± 0.44 ^{Bb}	25.44 ± 1.81 ^{Aab}	19.06 ± 2.66 ^{Ab}	17.73 ± 3.88 ^{AB}	7.21 ^{**}
F _{3,8}	7.78 ^{**}	4.68 [*]	5.21 [*]	7.92 ^{**}	2.75 ^{ns}	
Normal seeds (%)						
FF	39.03 ± 4.87 ^{Bab}	46.16 ± 16.13 ^{ABab}	61.54 ± 1.92 ^{AB}	57.43 ± 0.92 ^{ABab}	75.65 ± 0.77 ^A	3.47 [*]
BF	29.01 ± 5.62 ^b	52.82 ± 1.75 ^a	45.28 ± 11.99	29.28 ± 7.16 ^c	61.71 ± 8.64	3.42 ^{ns}
FvA	49.56 ± 3.88 ^{Ba}	60.26 ± 3.92 ^{ABa}	66.28 ± 5.64 ^A	71.88 ± 0.93 ^{Aa}	69.62 ± 3.00 ^A	5.55 ^{**}
FOWV	36.49 ± 1.06 ^{ABab}	14.92 ± 3.57 ^{Bb}	54.61 ± 8.5 ^A	42.80 ± 8.92 ^{ABbc}	52.92 ± 8.11 ^A	5.55 ^{**}
F _{3,8}	4.04 [*]	5.46 [*]	1.32 ^{ns}	10.21 ^{**}	2.58 ^{ns}	

ns: p > 0.05; *: p < 0.05; **: p < 0.001; ***: p < 0.001.

Means within the same column and line followed respectively by the same small and capital letter do not differ significantly (p < 0.05; Tukey's test).

-: estimation of the F value is not possible due to equal variance; FF: free flowers; BF: bagged flower; FvA: flowers visited exclusively by *Amegilla sp. 1*; FOWV: flowers opened without any visit of insect.

Table 6. Seed yield of *Gossypium hirsutum* treated with aqueous extracts of local botanicals at Bocklé during the rainy and dry seasons.

Treatment	Seed yield (kg.ha ⁻¹)
Rainy season	
Control	318.08 ± 155.02
<i>Cassia occidentalis</i> ,	192.33 ± 83.69
<i>Eucalyptus camaldulensis</i>	135.28 ± 48.35
<i>Hyptis suaveolens</i>	202.09 ± 38.27
TEMA	234.27 ± 18.44
F _(4, 15)	0.64 ^{ns}
Dry season	
Control	492.19 ± 150.47 ^b
<i>Cassia occidentalis</i>	1627.20 ± 447.50 ^a
<i>Eucalyptus camaldulensis</i>	1128.30 ± 118.20 ^{ab}
<i>Hyptis suaveolens</i>	1352.70 ± 134.93 ^{ab}
TEMA	1466.50 ± 186.98 ^{ab}
F _{4, 15}	3.37 [*]

ns: p > 0.05; *: p < 0.05; **: p < 0.001; ***: p < 0.001.

Means within the same column followed by the same letter do not differ significantly (p < 0.05; Tukey's test).

may be influenced by pests (Chastain and Young, 1998). In fact, insect pests have a direct impact on agricultural food production by chewing the leaves and stamen of crop plants, sucking out plant juices, boring within the roots, stems or leaves, and spreading plant pathogens. *Eucalyptus camaldulensis* and *H. suaveolens* aqueous extracts might be not toxic to non-target insects such as *Amegilla sp.*, but toxic to insect pests, hence the improvement of ANSPC and NSP harvested from non-protected flowers. The pesticidal activities of these botanicals were reported by the previous researchers (Appiah et al., 2018; Sabo and Knezevic, 2019). The high seed yield recorded from *C. occidentalis* treatment during the dry season may be explained by the combined impact of that plant extract and pollination done by *Amegilla sp.1*.

5. Conclusion

From the present findings, it could be concluded that the preservation of nesting sites of *A. mellifera* during the rainy season and those of *Amegilla sp. 1* all year round near cotton plantations increased fruit and seed yield. Overall, all the tested extracts were attractive to the both insect species compare to control, and standard synthetic insecticide, TEMA which was very repellent. *E. camaldulensis* and *C. occidentalis* extracts were the most attractive. *Eucalyptus camaldulensis* and *H. suaveolens* aqueous extracts improved ANSPC and NSP harvested from non-protected flowers by gauze bags and may be therefore recommended as good candidates for incorporation in the management of insect pollinators at flowering period of *G. hirsutum*. Aqueous extract of *C. occidentalis* improved Fr, ANS and seed yield, especially during the dry season. Hence, *C. occidentalis* extract may be also recommended to control field insect pests at different stages of the development of *G. hirsutum*.

Declarations

Author contribution statement

Moïse Adamou, Ph.D; Elias N. Nukenine, Ph.D; Daniel Kosini, Ph.D; A. Tchoubou-Salé, Ms: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Odette D. Massah, Ms: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

T. F.C. Tchocgnia, Ms; M. Mohammadou, Ms; O. Youssoufa, Ms: Performed the experiments.

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The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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