

Research Article

Impact of Plant Extracts on the Pollination Activity of *Apis mellifera* Linnaeus, 1758 (Hymenoptera: Apidae) on Flowers of Cowpea Variety Feekem, in Dang (Adamaoua, Cameroon)

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Abstract

Synthetic pesticides present worldwide risks of contamination of humans, livestock and the environment due to the strong persistence and the toxic residues in fruits and vegetables. Natural biopesticides of local plant origin present low persistence and are the best alternative for the control of crop pests. In the Adamaoua region (Northern Cameroon), few studies exist concerning effects of botanical pesticides on the behavior of beneficial insects. Studies aimed to draw up a list of pollinating insects on flowers of *Vigna unguiculata* (L.) Walp., 1843 (Fabales: Fabaceae), in situations of treatment with botanical pesticides compared to the situation of the use of synthetic insecticide and to determine the effect of the biopesticides on the behavior of the main floricultural insects. Field investigations were carried out during two cowpea cultivation campaigns (June to September 2021 and June to October 2022) in Dang (suburb of Ngaoundere) on the effect of leaves extracts of local plant origin on the foraging behavior of *Apis mellifera* Linnaeus, 1758 (Hymenoptera: Apidae) and the main sap-sucking insect *Aphis craccivora* Koch, 1854 (Hemiptera: Aphididae). Forty-four cowpea plots of 4x3.5 m each distributed according to the randomized complete block model (four untreated plots as negative control, four plots treated with the synthetic insecticide Parastar (40EC 535/ 10/IN, 20 g/l of imidacloprid and 20 g/l of lambda-cyhalothrin) as positive control, and 36 experimental plots treated with three concentrations (10%, 20% and 30%) of aqueous leaves extract of *Calotropis procera* (Gentianales: Apocynaceae), *Eucalyptus camaldulensis* (Myrtales: Myrtaceae), and *Tithonia diversifolia* (Asterales: Asteraceae) respectively, made it possible to conduct four treatments: (1) flowers left to freely pollination, (2) flowers protected against pollinators, (3) flowers visited exclusively by *Ap. mellifera* and (4) flowers protected against insects. Among eight species (four orders, four families and seven genera) recorded on the flowers of *V. unguiculata*, the domestic bee *Ap. mellifera* was the most common and collected nectar and pollen. The control plots and those treated with 10% or 20% aqueous leaves extracts allowed the bee to carry out its activity. Plots treated with 30% extract of each plant and those treated with the synthetic insecticide Parastar, drastically altered the rhythm and speed of activity in *Ap. mellifera* foragers. This behavior became less coordinated and slow

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on treated plants. It would be wise to use 10% or 20% aqueous extracts as botanical insecticides and an alternative to the synthetic insecticide Parastar.

Keywords

Floricultural Insects, *Vigna unguiculata*, Botanical insecticides, Chemical Pest Control

1. Introduction

Cowpea *Vigna unguiculata* (L.) Walp., 1843 (Fabales: Fabaceae) is an annual seed legume that can be creeping, semi-erect or erect depending on the variety [1]. Its leaves are opposite, alternate and trifoliate [2] and its stem, which can reach four meters long, is angular or almost cylindrical, slightly striated and sometimes hollow [3]. Bisexual flowers with 10 stamens are made up of five sepals fused into a tube of five petals which have changing colors depending on the variety [4]. This Fabaceae produces indehiscent pods with eight to 20 ovoid, kidney-shaped, smooth or wrinkled seeds of variable color and size [4]. Cowpea plays a crucial role in feeding humans and livestock and the creation of income for farmers as well as sellers of food products in sub-Saharan Africa [1, 2, 5, 6]. Cowpea seeds are highly rich in proteins ($\geq 25\%$) (lysine: 427 mg.g⁻¹ of azotes, and tryptophan: 68 mg.g⁻¹ of azotes although poorly rich in sulfur amino-acids; [7]). The consumption of cowpea helps fight against malnutrition and much more, the folic acid content is of importance in pregnant women (protection against malformation of the newborn) [8]. It is also an excellent source of antioxidants for the body [8]. Global cowpea production is estimated as 6.4 million tones per year, of which Sub-Saharan Africa accounts for approximately 95.0% [9]. Nigeria is the main producer and consumer with production estimated as 3.2 million tones per year [10]. In Cameroon, the cowpea annual production is low and occupies the eighth position among the main African cowpea producing countries, with an annual production estimated as 156.2 tones per year [10]. In African countries, the cowpea production is limited by several factors [5], among which the shortage of agricultural land, the low soil fertility, the poor management of pollinating insects, the pressure from insect pests in the fields and the post-harvest losses in warehouses, are frequently reported [5, 11]. Fields and warehouses pests control is mainly based on the spraying of approved synthetic insecticides [12, 13], which have proven their harmful effect to humans, livestock, the environment in general and the beneficial insects in particular [14]. Many plants species depend on the pollination by insect [15, 16]. Efficient pollination by insects increases fruit yield [17-21]. In natural environments as well as in agro-ecosystems, floricultural insects in general and Apo ïdae (Hymenoptera) including *Apis mellifera* Linnaeus, 1758 (Hymenoptera: Apidae) in particular have great ecological

and economic importance as pollinators, because they positively influence agro-food production [18, 22, 23]. Hence the preservation of pollinating insects, particularly *Ap. mellifera*, in cultivated plots, is nowadays recommended [14]. In Cameroon, the use of synthetic pesticides improves farmers' potential yield, but handling them with inexperienced hands increases the risk of human contamination and environmental pollution, and also reduces any prospect of sustainable agricultural development [24]. These products exterminate not only the target insect pests in treated fields, but also negatively affect the behavior of non-target organisms such as useful insects [14, 25, 26], destroy pollinating insects [27], directly contaminate the environment, plant production, farmers and livestock [24, 28], and indirectly contaminate the consumer due to the presence of toxic pesticide residues in fruits and vegetables [29, 30]. Due to the confirmed toxicity of synthetic pesticides which results in the high toxicity of synthetic chemicals, of the degradation products, and their strong persistence in the environment [31, 32], it is nowadays imperative to consider the use of control methods that respect the environment, beneficial insects and consumers. A new alternative is the use of botanical pesticides from local plants extracts that are weakly persistent and naturally degraded [33]. They are less expensive and accessible to farmers since the concerned plant species naturally grow in the nearby fallows. It is for example the case of aqueous leave extracts of the fake Kinkeliba *Cassia occidentalis* L. (= *Senna occidentalis* (L.) Link, 1829) (Fabales: Fabaceae), the apple tree of Sodom *Calotropis procera* (Aiton) W. T. Aiton, 1811 (Gentianales: Apocynaceae), the red gum tree *Eucalyptus camaldulensis* Dehnh., 1832 (Myrtales: Myrtaceae), the Chan grass *Hyptis suaveolens* (L.) Poit., 1806 (Lamiales: Lamiaceae) and the Mexican sunflower *Tithonia diversifolia* (Hemsl.) A. Gray, 1883 (Asterales: Asteraceae) [11, 14]. Floricultural entomofauna of *V. unguiculata* is well documented in cowpea producing countries in general and more particularly in Benin [34], in Ghana [35], in Nigeria [36], and in Cameroon [19, 23, 37]. These authors have reported the pollinating abilities of several insects including *Ap. mellifera*, *Halictus* sp. Latreille, 1804 (Hymenoptera: Halictidae), the several other Apidae (Hymenoptera) such as *Xylocopa olivacea* (Fabricius 1778) *X. caffra* (L. 1767), *X. erythrina* Gribodo 1894, *X. imitator* Smith, 1854, *X. inconstans* Smith

F. 1874 and *X. nigrita* (Fabricius 1775) on cowpea flowers. In Cameroon, it is reported that *Megachile eurymera* Smith 1864 (Hymenoptera: Megachilidae) and *Ap. mellifera* are frequently recorded on the cowpea flowers. In Cameroon, although many natural additives based on plant extracts have proven effective against harmful insects [11, 14, 38], their effects on pollinating insects in particular *Ap. mellifera* remains less known and it is necessary to carry out studies in the Adamaoua Region (Cameroon) on the effect of local plants on the foraging behavior of *Ap. mellifera*, with a view to completing the available information. Specifically the study aimed to draw up a list of cowpea pollinating insects in situation of treatment with botanical pesticides and to determine the effect of these natural products on the behavior of the bee.

2. Materials and Methods

2.1. Study Site

The study was conducted from June to September 2021 and from June to October 2022 in Dang (7°25'26.42"N, 13°32'24.46"E; 1107.40 m a.s.l.) in Adamaoua Region (North-Cameroon) (Figures 1A and 1B). The site of the experimental plots was located not far from the Ngaoundere University campus and the municipal lake of Dang (Ngaoundere III) (Figure 1B). The Adamaoua region is located in the agro-ecological zone of the high Guinean savannahs (which is a transition zone between the rain forest zone in South-Cameroon and the wooded savannah in North-Cameroon, covering the Adamaoua Plateau and a part of the Eastern Region) [39, 40]. Dang is located in the 3rd district of Ngaoundere, in the suburbs, approximately 12.9 km from the urban center. Resulting from the emergence of the old crystalline base, the Adamaoua Region is covered in places with basalt rocks and there are also humid valleys covered with rocky outcrops and basaltic cones [41]. The surface area of the agro-ecological zone of the high Guinean savannahs is 123,077 km² [39, 42]. The surface area of the high savannahs of Adamaoua is 123,077 km² [39] and the locality of Ngaoundere (Figure 1A) covers an area estimated at 62,000 km² [42]. The soils are permeable, with an average water retention capacity, brown or red ferralitic and hydromorphic [39]. The prevailing climate is Sudano-Guinean type with two seasons: a rainy season (from mid-April to mid-October of the same year) and a dry season (from mid-October to mid-April of the following year). The average temperature is 22.9 °C and precipitation is approximately 1,500 mm to 2,248 mm per year with around 150 rainy days [39, 43]. It is a tropical savannah climate of type "Aw" according to the Köppen-Geiger classification [44]. The lowest relative humidity is in February (21.7%) and the average annual humidity is 70% [43, 45]. The average annual temperature varies from 22.1 °C to 22.9 °C; the average annual

humidity varies from 64.1% to 67.6%; annual rainfall varies from 1227.9 mm to 1675.8 mm; the annual insolation duration varies from 2321.1 hours to 2557.9 hours [43]. According to the same source of information, the average monthly temperature varies from 22.34 °C to 24.70 °C; the average monthly humidity varies from 37.7% to 81%; monthly rainfall varies from 0 mm to 274 mm; the duration of monthly insolation varies from 133 hours to 293.3 hours. According to Djoufack-Manetsa [42] and Tounsi [39], the vegetation of the Adamaoua plateau is a shrub or tree savannah and the frequently plant species found in fallow are *Calotropis procera* (Gentianales: Apocynaceae), *Daniellia oliveri* (Rolfe) Hutch. & Dalziel, 1928 (Fabales: Fabaceae) and *Lophira lanceolata* Tiegh. ex Keay, 1954 (Malpighiales: Ochnaceae), *Cosmos sulphureus* Cav., 1791 (Asterales: Asteraceae), the sunflower *Helianthus annuus* L., 1753 (Asterales: Asteraceae), the Mexican sunflower *Tithonia diversifolia* (Hemsl.) A. Gray, 1883 (Asterales: Asteraceae), the pigeon pea *Cajanus cajan* L., 1753 (Fabales: Fabaceae), the common bean *Phaseolus vulgaris* L., 1753 (Fabales: Fabaceae) and the sesame *Sesamum indicum* L. (1753) (Scrophulariales: Pedaliaceae) [41], and *Ti. diversifolia*. Plant species frequently found along watercourses and lakes are the false kapok tree *Bombax costatum* Pellegr. & Vuillet, 1914 (Malvales: Malvaceae), Ethiopian *Borassus aethiopicum* Mart., 1838 (Areciales: Arecaceae), *Boswellia dalzielii* Hutch., 1910 (Sapindales: Burseraceae), *Commiphora africana* (A. Rich.) Engl., 1883 (Sapindales: Burseraceae), *Eucalyptus camaldulensis* (Myrtales: Myrtaceae), *Hyparrhemia rufa* (Nees) Stapf, 1919 (Poales: Poaceae) and *Lannea microcarpa* (Sapindales: Anacardiaceae) (pers. com.). The most common woody species are *Annona senegalensis* (Annonaceae), *Croton macrostachyus* (Euphorbiaceae), *Entada africana* (Mimosaceae), *Ficus* spp. (Moraceae), *Hymenocardia acida* (Euphorbiaceae), *Strychnos spinosa* (Loganiaceae), *Syzygium guineense* var. *macrocarpum* (Myrtaceae), *Terminalia macroptera* (Combretaceae), *Vitex madiensis* (Verbenaceae), *Vitellaria paradoxa* (Sapotaceae). In certain areas there are meadows and river banks with gallery forests [41]. The development of natural resources is mainly done through agriculture, cattle breeding, beekeeping and fishing [46]. The cultivated areas are small plots of mixed food crops sometimes neighboring wooded grassy fallows [61]. The main crops are corn *Zea mays* L., 1753 (Cyperales: Poaceae), cotton *Gossypium hirsutum* L., 1763 (Malvales: Malvaceae), the millet-sorghum *Sorghum bicolor* (L.) Moench, 1794 (Poales: Poaceae), the white yam *Dioscorea alata* L., 1753 (Liliales: Dioscoreaceae), the yellow yam *Dioscorea dumetorum* (Kunth) Pax, 1887 (Liliales: Dioscoreaceae), the potato *Solanum tuberosum* L., 1753 (Solanales: Solanaceae) [39]. We also find *Prosopis africana* (Guill. & Perr.) Taub., 1893 (Fabales: Fabaceae), the shea tree *Vitellaria paradoxa* C. F. Gaertn., 1807 (Ebenales: Sapotaceae) and the neem plantations *Azadirachta indica* A. Juss., 1830 (Sapindales: Meliaceae), and *Hyptis suaveolens* (L.) Poit., 1806 (Lamiales:

Lamiaceae).

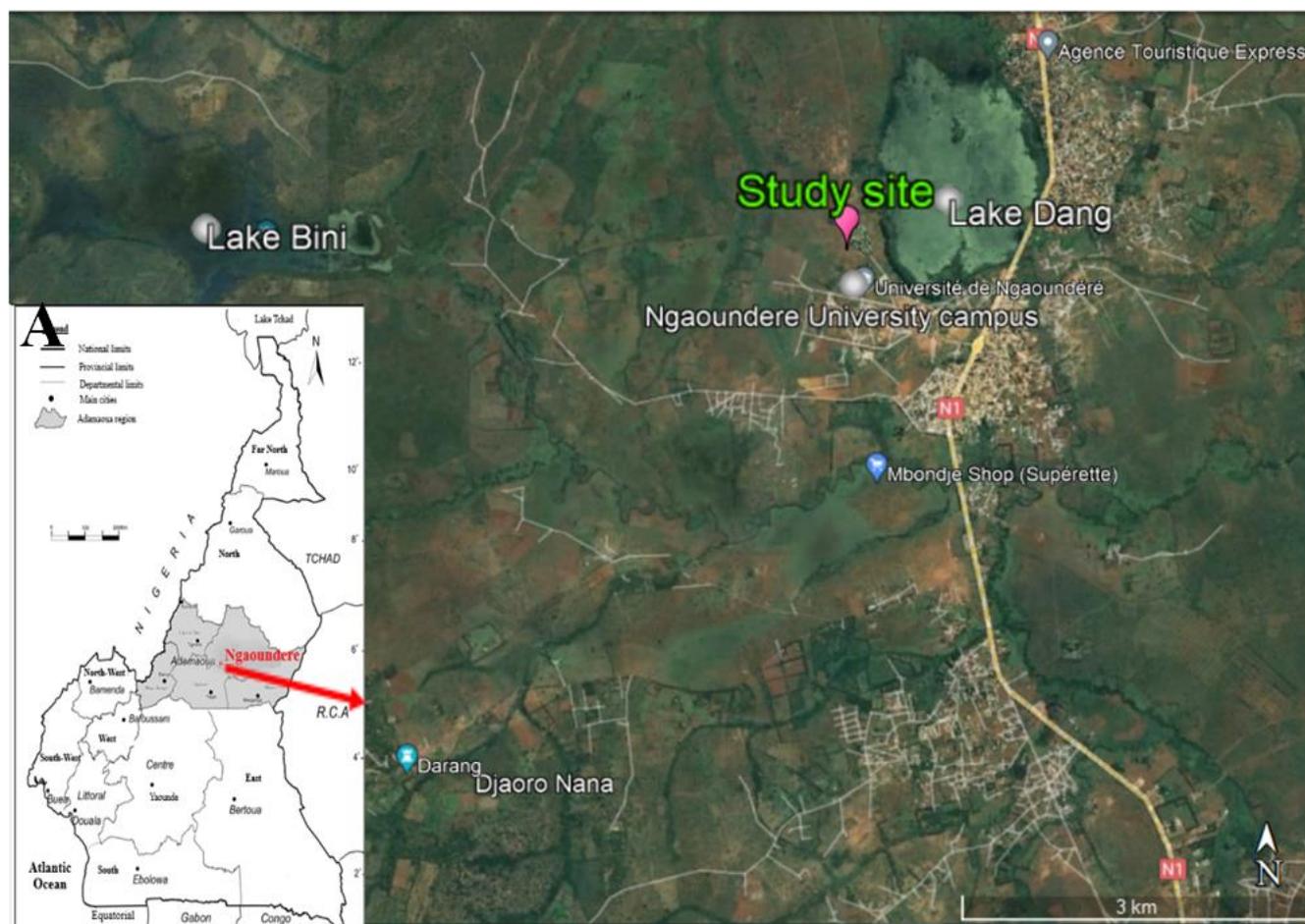


Figure 1. Localization map of the study site. A. Adamaoua Region in Cameroon (adapted from Sehou [47]); B. study site at Dang (Ngaoundere III suburb area) (Google Earth Pro for windows version 7.3.4.8642).

2.2. Biological Material

Three biological materials were used: (1) the cowpea plants (Figure 2A), (2) leaves aqueous extract of plants species, and (3) floricultural insects. Cowpea plants were obtained from seeds of the Feekem variety (Figure 2B), sown in the experimental plots. Leaves of three plant species were: (1) *Calotropis procera* (Gentianales: Apocynaceae) (Figure 2C), *Eucalyptus camaldulensis* (Myrtales: Myrtaceae) (Figures 2D and 2E), and *Tithonia diversifolia* (Asterales: Asteraceae) (Figure 2F). *E. camaldulensis* and *Ti. diversifolia* leaves were collected in neighboring fallows in Dang. *C. procera* leaves were collected in Bockle (North, Cameroon). Floricultural insects came naturally from neighboring fallows and a bee hive was positioned not far from the experimental plots.

2.3. Experimental Device and Procedure

The study was carried out on an area of 1,064 m² and the experimental design was that of blocks (3.5×4) m² completely randomized to 4 treatments repeated 4 times. Negative control plots were four plots having no spraying, while positive control plots were four plots treated with the synthetic insecticide Parastar (20 g/l of imidacloprid and 20 g/l of lambda-cyhalothrin, one l p.c./ha). Experimental plots were 36 plots treated using the aqueous leaves extract of *Ca. procera* (Apocynaceae), *E. camaldulensis* (Myrtaceae), and *Ti. diversifolia* (Asteraceae), at concentrations 10%, 20%, and 30%, in accordance of the procedure clearly described by Mohammadou *et al.* [14]. A total of 44 plots were controlled from 3rd to 7th October (five consecutive days).

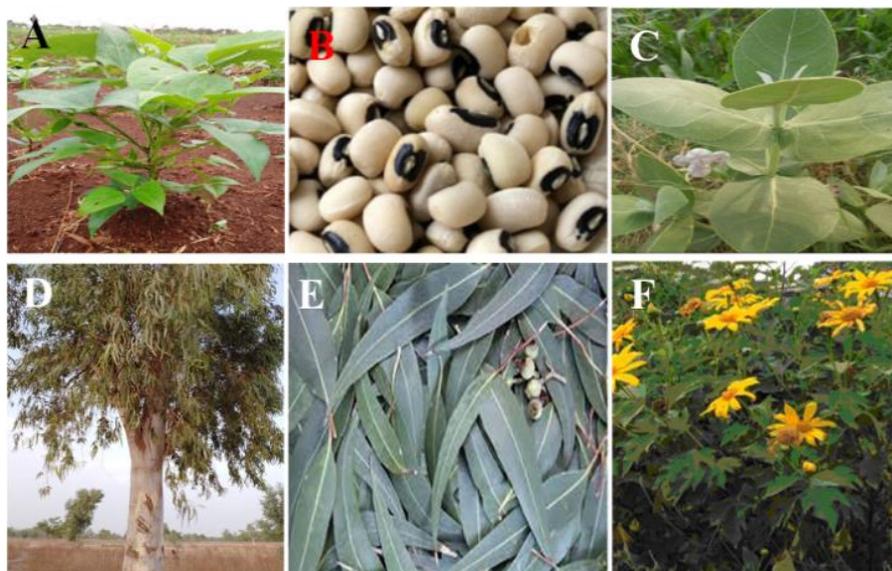


Figure 2. A: a cowpea plant 42 days after sowing; B: cowpea seeds or black-eyed bean “Feekem” variety from IRAD Garoua (North-Cameroon); C: a branch of *Calotropis procera* (Aiton) W. T. Aiton, 1811 (Gentianales: Apocynaceae) showing a blooming flower and green fleshy leaves; D: *Eucalyptus camaldulensis* Dehnh., 1832 (Myrtales: Myrtaceae); E: Some leaves and fruits of *E. camaldulensis*; F: a clump of *Tithonia diversifolia* (Hemsl.) A. Gray, 1883 (Asterales: Asteraceae) plants showing yellow blooming flowers.

2.4. Formulation of Botanical Extracts

The aqueous extracts were formulated based on the method described by Sreekanth [48]: one kilogram of the vegetal powder was diluted using one liter distilled water and the solution homogenized and sprayed in the plots. Field applications started when the first cowpea flowers appeared, were executed in the evening from 5 p.m. each day, and were repeated during four days with seven days intervals.

2.5. Field Observations

Data collection was set up in the field by direct observation on the foraging activity of *Ap. mellifera* foragers on blooming cowpea flowers. Observations were made every day from the start of flowering of cowpea plants, during six daily time slots each day: 6-7 a.m., 8-9 a.m., 10-11 a.m., 12 a.m.-1 p.m., 2-3 p.m. and 4-5 p.m. Field controls were repeated during five consecutive days in each plot. Insects on the blooming flowers were counted and captured when possible. As the insects were not marked, the recorded parameter was the number of visits of *Ap. mellifera* foragers as well as that of other floricultural insects, as proposed by Tchuen-guem [49]. The frequency of visits of the various floricultural insects made it possible to determine the place of *Ap. mellifera* in the anthophilic entomofauna of *V. unguiculata* using the following formula: $F_i = (V_i/V_1) * 100$, where V_i represented the number of visits of i^{th} insect on the flowers of each category (untreated or treated flowers) and V_1 represented the number of visits of all insects on flowers of the same category [21]. The products (pollen or nectar) collected by the insects were noted [49]. Bee that buried the head or probos-

cis in a flower were nectar harvesters; while those who scraped the flower anthers using mandibles and legs, were pollen harvesters [50]. The abundances of foragers per 1000 flowers (A_{1000}) were determined as $A_{1000} = (A_x/F_x) * 1000$, where F_x represented the number of controlled flowers at x time period and A_x represented the number of the target pollinator insect recorded at x period of time on 1000 blooming flowers [49]. The duration of visits per flower was the time taken by an individual of *Ap. mellifera* to collect the floral product [49]. The parameter was recorded in each plot according to the procedure described by Jacob-Remacle [51], during six daily time slots: 7-8 a.m., 9-10 a.m., 11 a.m.-12 p.m., 1-2 p.m., 3-4 p.m. and 5-6 p.m.. The foraging speed $V_b = (F_i/d_i) * 60$ was determined where F_i corresponded to the number of flowers visited during d_i time period [49].

2.6. Data Analysis

Collected data were stored in an Excel spreadsheet and then analyzed using SigmaStat software and StatXact software. Results are given in terms of absolute and relative abundances (qualitative variables) or mean \pm standard error (quantitative series). Comparison of two percentages was made using the Fisher's exact test and the simultaneous comparison of several percentages was made using the asymptotic chi-square test or the Fisher-Freeman-Halton test from StaXact software. Pairwise comparisons were carried out when necessary, the risk probabilities being corrected according to the number of comparisons using the sequential Bonferroni procedure [52]: for k pairwise comparisons of several independent proportions (our situation), at the significance level α , the Fisher's exact test probabilities were ordered from the smallest p_1 to the largest p_k and the test probability p_i was significant if $p_i < \alpha' = (1-\alpha)^{(1/(1+k-i))}$.

Comparison of two mean values was made using the Student's t test when conditions of normality and equality of variances passed and otherwise we used the Mann-Whitney rank sum test. The simultaneous comparison of several means was made using the ANOVA test when the conditions of normality and equality of variances passed, followed by the post-hoc Student-Newman-Keuls test. Otherwise, we used the Kruskal-Wallis test followed by the Dunn's post-hoc test.

3. Results

3.1. Floricultural Insects

During the period of the field checks, the number of blooming flowers of *V. unguiculata* visited by insects varied from one to eight flowers on the same plant (360 flowers, mean \pm se: 3 ± 0 flowers, 132 plants in 2021; 362 flowers, 3 ± 0 flowers, 132 plants in 2022; and 722 flowers, 3 ± 0 flowers, 264 plants in the pooled campaigns). Visits began early in the morning at 6 a.m. and ended at 13 p.m. The two-way ANOVA test with "Years" and "Treatment" as factors, applied to the number of flowers showed a not significant effect of each factor and even the interaction of the two factors ($p=0.982$ for "Years", $p=0.963$ for "Treatment", and $p=0.982$ for the interaction). The same test with "Treatment" and "Time" as factors, showed a significant effect of "Time" ($p<0.001$) while "Treatment" and the interaction were not significant ($p=0.951$ and $p=0.461$ respectively). Pairwise comparisons showed a not significant difference between 6-7 a.m. and 10-11 a.m. while other differences were significant: 6-7 a.m. (176 flowers, 3 ± 0 flowers, 66 plants) vs. 8-9 a.m. (294 flowers, 4 ± 0 flowers, 66 plants): $p=2.0 \times 10^{-5}$; 6-7 a.m. vs. 10-11 a.m. (162 flowers, 2 ± 0 flowers, 66 plants): $p=0.632$; 6-7 a.m. vs. 12-13 p.m. (90 flowers, 1 ± 0 flower, 66 plants): $p=0.023$; 8-9 a.m. vs. 10-11 a.m.: $p<0.001$; 8-9 a.m. vs. 12-13 p.m.: $p<0.001$; 10-11 a.m. vs. 12-13 p.m.: $p=0.017$. The two-way ANOVA test with "Years" and "Treatment" as factors, applied to the number of the pollinator insect *Ap. mellifera* showed a significant effect of "Years" ($p=0.024$), the insect being more numerous in 2021 (2 ± 0 individuals, 132 flowers) than in 2022 (1 ± 0 individual, 132 flowers) (Student-Newman-Keuls test: $p=0.023$). The global occurrence

of the pollinator insect was 1 ± 0 individual (264 flowers). Effects of "Treatment" and the interaction of the two factors were not significant ($p=0.456$ and $p=0.476$ respectively). During the two-year study, 220 collection sessions (five sessions in each of the 44 plots) permitted the capture of 8,987 specimens of the floricultural insects on flowers of *V. unguiculata*.

Percentage of captures was low during the 2021 campaign (48.9%) compared to that recorded during the 2022 campaign (51.1%) (Fisher's exact test: $p=2.610^{-3}$) (Table 1). Captured insects belonged to four orders (Hemiptera Linnaeus, 1758, Hymenoptera Linnaeus, 1758, Lepidoptera Linnaeus, 1758, and Orthoptera Latreille, 1793), four families (Acridae Macleay, 1821, Aphididae Latreille, 1802, Apidae Latreille, 1802, and Nymphalidae Rafinesque, 1815), seven genera and eight species (Table 1). The most species-rich order was Hymenoptera with four species (50.0%) followed by Lepidoptera with two species (25.0%) while the two other orders were each represented by one species (12.5%) (Table 1). Overall, *Ap. mellifera* was the most collected pollinator insect (25.7% of the total collection) with a low entomophilic rate ($F=25.7\%$). It was highly represented compared to the cumulus of the other pollinator insects (22.9%) (Fisher's exact test: $p=1.0 \times 10^{-5}$) but it was lowly represented compared to the cumulus of all other floricultural insects (74.3%) (Fisher's exact test: $p<0.001$). It was the most collected pollinator in the 2021 campaign (14.9% of the total collection) with a low entomophilic rate ($F=30.5\%$) than the 2022 campaign (10.9%; $F=21.2\%$) (Fisher exact test: $p=2.1 \times 10^{-26}$) (Table 1). Other floricultural insects were *Amegilla* sp. Friese, 1897 (Hymenoptera: Apidae) (3.8% of the total collection exclusively in 2021, entomophilic rate: $F=3.8\%$), *Amegilla calens* (Le Peletier, 1841) (Hymenoptera: Apidae) (2.2% and $F=4.5\%$ in 2021, 0.7% and $F=1.3\%$ in 2022, 2.9% and $F=2.9\%$ in the pooled data), *Aphis craccivora* Koch, 1854 (Hemiptera: Aphididae) (17.9% and $F=36.7\%$ in 2021, 33.4% and $F=65.4\%$ in 2022, 51.3% and $F=51.3\%$ in the pooled data), *Danaus plexippus* (Linnaeus, 1758) (Lepidoptera: Nymphalidae) (1.0% and $F=2.1\%$ exclusively in 2021), *Hypolimnas misippus* (Linnaeus, 1764) (Lepidoptera: Nymphalidae) (0.8% and $F=1.6\%$ in 2021, 0.1% and $F=0.3\%$ in 2022, 0.9% and $F=0.9\%$ in the pooled data).

Table 1. Absolute and relative abundances of the floricultural insects on 360 flowers of cowpea in Dang.

Orders / Families / Species	Products	Collection periods		
		2021: n (%)	2022: n (%)	Total (%)
Hemiptera Linnaeus, 1758 / Aphididae Latreille, 1802				
<i>Aphis craccivora</i> Koch, 1854	Sap-sucking	1,611 (17.9)	3,003 (33.4)	4,614 (51.3)
Hymenoptera Linnaeus, 1758 / Apidae Latreille, 1802				

Orders / Families / Species	Products	Collection periods		
		2021: n (%)	2022: n (%)	Total (%)
<i>Amegilla calens</i> (Le Peletier, 1841)	Nectar	199 (2.2)	61 (0.7)	260 (2.9)
<i>Amegilla</i> sp. Friese, 1897	Nectar	338 (3.8)	-	338 (3.8)
<i>Apis mellifera</i> Linnaeus, 1758	Nectar, and pollen	1,338 (14.9)	976 (10.9)	2,314 (25.7)
<i>Xylocopa olivacea</i> (Fabricius 1778)	Nectar	501 (5.6)	236 (2.6)	737 (8.2)
Lepidoptera Linnaeus, 1758 / Nymphalidae Rafinesque, 1815				
<i>Danaus plexippus</i> (Linnaeus, 1758)	Nectar	94 (1.0)	-	94 (1.0)
<i>Hypolimnas misippus</i> (Linnaeus, 1764)	Nectar	69 (0.8)	12 (0.1)	81 (0.9)
Orthoptera Latreille, 1793 / Acridae Macleay, 1821				
<i>Tettigonia viridissima</i> (Linnaeus, 1758)	Phytophagous	242 (2.7)	307 (3.4)	549 (6.1)
Total		4,392 (48.9)	4,595 (51.1)	8,987 (100.0)

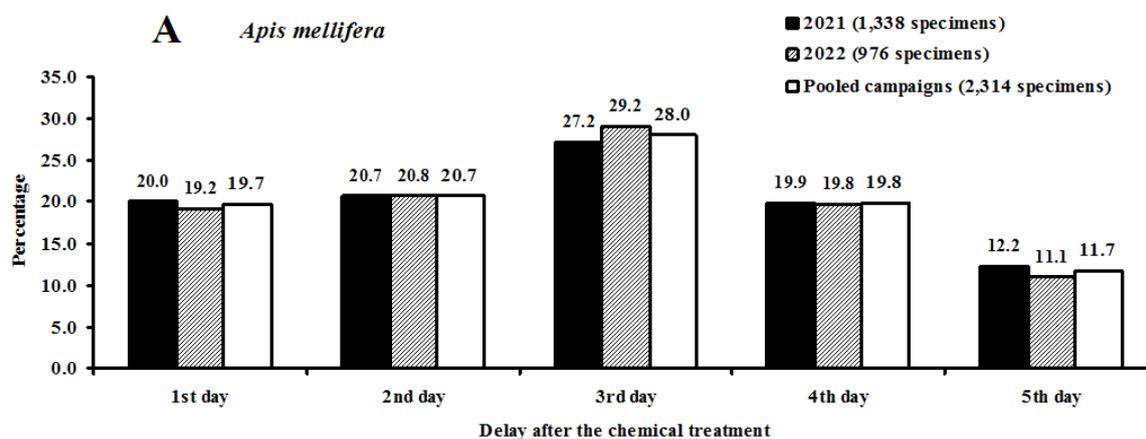
n: sample size or the number of the insects captured on 360 flowers

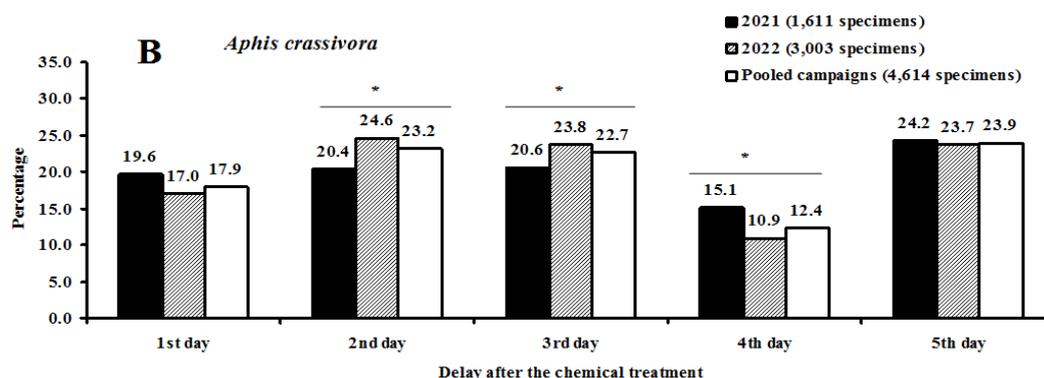
These species were followed by *Tettigonia viridissima* (Linnaeus, 1758) (Orthoptera: Acrididae) (2.7% and F=5.5% in 2021, 3.4% and F=6.7% in 2022, 6.1% and F=6.1% in the pooled data), and *Xylocopa olivacea* (Fabricius 1778) (5.6% and F=11.4% in 2021, 2.6% and F=5.1% in 2022, 8.2% and F=8.2% in the pooled data). Amongst these insects, *Ah. craccivora* was mostly represented and the butterfly *H. misippus* was less recorded (Table 1). Apart from sap-sucking *Ah. craccivora* which collected sap from twigs, bugs, leaves and flowers, pollinator insects collected nectar and/or pollen. *Ap. mellifera* raised its abdomen on the style and pressed it. By this action, the bee slightly deviated petals, and then landed on the hull, the head facing toward the stamens and floral anthers and it rubbed stamens using legs. Pollen grains were thus collected and accumulated in the ventral brush. Sometimes, the individual stopped harvesting pollen and settled on the corolla and completed the pollen collection. After completing pollen harvest, foragers flew

directly to another flower on the neighboring pigeon pea *Cajanus cajan* (Fabales: Fabaceae) or another plant species. After pollen collection, it usually harvested nectar by introducing its proboscis in the bottom of the flower and by that action sucked nectar. Studies of the effect of aqueous botanical extracts were subsequently focused on the occurrence of the sap-sucking insect *Ah. craccivora* and the activity of the pollinator bee *Ap. mellifera* which were both mostly frequent on the flowers (cumulus occurrences: 77.0% of the total collection).

3.2. Effect of Chemical Treatments

The two-way ANOVA on the occurrence of *Ap. mellifera* (Figure 3A) with “Years” and “Treatment” as factors showed a significant effect of each factor ($p=1.8 \times 10^{-3}$ and $p<0.001$ respectively) and a not significant interaction ($p=0.512$).





Global comparison of the occurrences in 2021, 2022 and the pooled years: Fisher-Freeman-Halton test asymptotic p-value

	1 st day	2 nd day	3 rd day	4 th day	5 th day
<i>Apis mellifera</i>	p=0.875 ns	p=0.997 ns	p=0.572 ns	p=0.998 ns	p=0.715 ns
<i>Aphis crassivora</i>	p=0.085 ns	p=0.005 *	p=0.049 *	p=2x10 ⁻⁴ *	p=0.929 ns
Pairwise comparisons for <i>Ah. crassivora</i>					
2021 vs. 2022	-	p=1.3x10 ⁻³ *	p=0.015 *	p=0 *	-
2021 vs. pooled years	-	p=0.024 *	p=0.088 ns	p=5.3x10 ⁻³ *	-
2022 vs. pooled years	-	p=0.144 ns	p=0.266 ns	p=0.050 ns	-

Pairwise comparison using the sequential Bonferroni test: α' (p-value)

Comparison	2021		2022		Pooled years	
	<i>Ap. mellifera</i>	<i>Ah. crassivora</i>	<i>Ap. mellifera</i>	<i>Ah. crassivora</i>	<i>Ap. mellifera</i>	<i>Ah. crassivora</i>
1 st vs. 2 nd	0.025 (0.701) ns	0.025 (0.597) ns	0.017 (0.396) ns	0.05 (0) *	0.017 (0.38) ns	0.005 (0) *
1 st vs. 3 rd	0.005 (0) *	0.017 (0.510) ns	0.005 (0) *	0.006 (0) *	0.005 (0) *	0.006 (0) *
1 st vs. 4 th	0.050 (0.961) ns	0.007 (0.001) *	0.050 (0.775) ns	0.006 (0) *	0.05 (0.912) ns	0.006 (0) *
1 st vs. 5 th	0.006 (0) *	0.009 (2x10 ⁻³) *	0.006 (0) *	0.007 (0) *	0.006 (0) *	0.007 (0) *
2 nd vs. 3 rd	0.013 (1x10 ⁻⁴) *	0.050 (0.931) ns	0.006 (0) *	0.025 (0.451) ns	0.006 (0) *	0.050 (0.586) ns
2 nd vs. 4 th	0.017 (0.631) ns	0.006 (1x10 ⁻⁴) *	0.025 (0.613) ns	0.009 (0) *	0.025 (0.47) ns	0.009 (0) *
2 nd vs. 5 th	0.006 (0) *	0.010 (0.011) ns	0.007 (0) *	0.017 (0.416) ns	0.007 (0) *	0.025 (0.432) ns
3 rd vs. 4 th	0.007 (0) *	0.006 (1x10 ⁻⁴) *	0.009 (0) *	0.010 (0) *	0.009 (0) *	0.010 (0) *
3 rd vs. 5 th	0.009 (0) *	0.013 (0.016) ns	0.010 (0) *	0.050 (0.976) ns	0.010 (0) *	0.017 (0.176) ns
4 th vs. 5 th	0.010 (0) *	0.005 (0) *	0.013 (0) *	0.013 (0) *	0.013 (0) *	0.013 (0) *

Figure 3. Evolution of the occurrence of *Apis mellifera* (Hymenoptera: Apidae) and *Aphis crassivora* (Hemiptera: Aphididae) on flowers of *Vigna unguiculata* (Fabales: Fabaceae) during five consecutive blooming days. ns: not significant difference ($p>0.05$); *: significant difference ($p<0.05$); α' : Bonferroni corrected significance level.

Pairwise comparison showed a significant difference between 2021 occurrences (mean \pm standard error (se): 6 ± 0 foragers, 220 flowers) and 2022 occurrences (4 ± 0 foragers, 220 flowers) (Student-Newman-Keuls test (SNK): 2021 vs. 2022: $p=1.7 \times 10^{-3}$), the global mean occurrence being 5 ± 0 foragers on 440 flowers. The difference was significant between untreated plots (13 ± 1 foragers, 20 flowers) and treated plots (5 ± 0 foragers, 420 flowers) (SNK test: $p<0.001$). The same test with the same factors on the occurrence of *Ah.*

crassivora (Figure 3B) showed a significant effect of "Years" ($p<0.001$) while effect of "Treatment" and the interaction of the two factors were non-significant ($p=0.646$ and $p=0.955$ respectively). Pairwise comparison for the factor "Years" showed a significant difference between 2021 occurrences (7 ± 1 foragers, 220 flowers) and 2022 occurrences (14 ± 0 foragers, 220 flowers) (SNK test for 2021 vs. 2022: $p<0.001$), the global mean occurrence being 10 ± 0 foragers on 440 flowers.

The two-way ANOVA test on the occurrence of *Ap. mellifera* with “Days” and “Treatment” as factors showed a significant effect of “Days” ($p=2.0 \times 10^{-5}$) and “Treatment” ($p<0.001$) and the interaction of the two factors was non-significant ($p=0.795$). Pairwise comparisons for the factor “Days” showed significant differences except between the 1st day and the 2nd day or the 4th day, between the 2nd and 4th day (Student-Newman-Keuls test: 1st day (mean \pm se: 5 ± 0 foragers, 88 essays) vs. 2nd day (5 ± 0 foragers, 88 essays): $p=0.780$, 1st vs. 3rd day (4 ± 0 foragers, 88 essays): $p=9.6 \times 10^{-3}$, 1st vs. 4th day (1 ± 0 forager, 88 essays): $p=0.875$, 1st vs. 5th day (3 ± 0 foragers, 88 essays): $p=0.033$, 2nd vs. 3rd day: $p=0.012$, 2nd vs. 4th day: $p=0.831$, 2nd vs. 5th day: $p=0.043$, 3rd vs. 4th day: $p=8.5 \times 10^{-3}$, 3rd vs. 5th day: $p=2.0 \times 10^{-5}$, 4th vs. 5th day: $p=0.044$). The same test with the same factors on the occurrence of *Ah. crassivora* showed a significant effect of “Days” ($p=0.040$) while effect of “Treatment” and the interaction between the two factors were not significant ($p=0.661$ and $p=0.619$ respectively). Pairwise comparison for the factor “Days” showed a significant difference between the 2nd and the 4th day while other differences were not significant (Student-Newman-Keuls test: 1st day (mean \pm se: 9 ± 1 foragers, 88 essays) vs. 2nd day (12 ± 1 foragers, 88 essays): $p=0.393$, 1st vs. 3rd day (2 ± 0 foragers, 88 essays): $p=0.521$, 1st vs. 4th day (1 ± 0 foragers, 88 essays): $p=0.182$, 1st vs. 5th day (13 ± 1 foragers, 88 essays): $p=0.568$, 2nd vs. 3rd day: $p=0.628$, 2nd vs. 4th day: $p=0.030$, 2nd vs. 5th day: $p=0.575$, 3rd vs. 4th day: $p=0.073$, 3rd vs. 5th day: $p=0.604$, 4th vs. 5th day: $p=0.137$). Monitoring the blooming flowers of *V. unguiculata* during five consecutive days each year, showed that the occurrence variation in insects was not significant between the two campaigns, both in *Ap. mellifera* (Figure 3A) and *Ah. crassivora* (Figure 3B) with the exception of the 2nd, 3rd and 4th days in the sap-sucking insect. Occurrences in 2022 were on average higher than in 2021 during the 2nd and 3rd days and the opposite was significant on the 4th day (Figure 3B). Pairwise comparisons of the occurrences of *Ap. mellifera* foragers showed in each year and in the cumulative data, non-significant differences between the 1st, 2nd and 4th days, the other differences being significant (Figure 3A). Similar comparisons carried out in the sap-sucking insect *Ah. crassivora* showed in 2022 and in the cumulative data, non-significant differences between the 1st, 2nd and 5th days while in 2021, non-significant differences were observed between the 1st, 2nd, 3rd and 5th days with a significant difference between the 1st and 5th days. The other differences were significant (Figure 3B).

During each campaign and in the pooled data, the harmful effect of the synthetic insecticide Parastar was noted on the occurrence of *Ap. mellifera* (Figure 4A, 4B and 4C). As for the aqueous leaves extracts, the 30% concentration of plants acted like Parastar, with no significant difference (Figure 4A and 4B). Parastar and aqueous leaves extracts showed very little effect on *Ah. crassivora* compared to the untreated

plots (Figure 4D, 4E and 4F). The concentration of 30% of aqueous extracts was therefore a toxic dose for pollinator insects. The 10% and 20% concentrations of aqueous extracts presented an average tolerable effect for floricultural insects. Overall, the variation in the occurrence of *Ap. mellifera* on the blooming flowers of *V. unguiculata* was significant during the 2021 and 2022 campaigns and even in the pooled data (Figure 4A, 4B and 4C). It was the same for the sap-sucking insect *Ah. crassivora* during both campaigns and in the pooled data (Figure 4D, 4E, 4F).

Between the two campaigns, the variation of the occurrences was globally significant (Fisher-Freeman-Halton asymptotic test: $df=10$, $p=0.015$ and $p=1.3 \times 10^{-10}$ for *Ap. mellifera* and *Ah. crassivora* respectively). Pairwise comparisons showed that *Ap. mellifera* was more active in 2021 than in 2022 in untreated plots and in Parastar treated plots. In plots treated with botanical extracts, the difference in occurrence of the bees was not significant except those treated with 30% *Ti. diversifolia* where effect was intense in 2021 than 2022 (Fisher’s exact test: untreated plots: $p=3.2 \times 10^{-219}$; Parastar: $p=0.033$; Cp10: $p=0.772$; Cp20: $p=0.544$; Cp30: $p=0.149$; Ec10: $p=0.347$; Ec20: $p=0.705$; Ec30: $p=0.050$; Td10: $p=0.896$; Td20: $p=0.660$; Td30: $p=9.8 \times 10^{-3}$). In the sap-sucking *Ah. crassivora*, the occurrence difference was not significant except in the plots treated with 10% and 30% *Ca. procera* or *E. camaldulensis*, 10% and 20% *Ti. diversifolia*, where insects were more present in 2022 than 2021 (Fisher’s exact test: untreated plots: $p=0.674$; Parastar: $p=0.872$; Cp10: $p=0.012$; Cp20: $p=0.711$; Cp30: $p=9.5 \times 10^{-6}$; Ec10: $p=0.037$; Ec20: $p=0.503$; Ec30: $p=8.4 \times 10^{-6}$; Td10: $p=2.3 \times 10^{-3}$; Td20: $p=5.8 \times 10^{-4}$; Td30: $p=0.800$) (Figure 4).

3.3. Activity of *Apis mellifera* on Flowers

3.3.1. Rhythm of Visits and Harvested Products

During periods of observation on *V. unguiculata* flowers, *Ap. mellifera* foragers collected nectar and pollen. The forager faced the anthers of the flower, scrapes the pollen and stored them through its front legs (Figure 5A) while when collecting the nectar, the head of the forager was entirely inserted into the flower to reach the sweet liquid exudates which is found deep inside the flower (Figure 5B). The activity of *Ap. mellifera* began in the morning around 6 a.m. with the blooming of the flowers and decreased sharply around 1 p.m. with their wilting. The pooled rhythm of activity was higher in 2021 than in 2022 (Student t-test: $t=2.942$, $df=64$, $p=0.004$). The daily period of optimal activity was 8-9 a.m. in both years (Figure 6A and 6B) and in the pooled data (Figure 6C). Between the two years, differences in time periods were not significant except in 6-7 a.m. where bees were more active in 2021 campaign than 2022 campaign (Figure 6). The one-way ANOVA test applied on the occurrence of the bee with “Times” as factor showed a significant between groups effect in 2021 (Figure 6). Similar results

were noted in 2022 (Figure 6) and in the pooled data (Figure 6). Differences are all significant in 2021 while in 2022 the difference was not significant between 6-7 a.m. and 10-11 a.m. and between 10-11 a.m. and 12-13 p.m. (Figure 6). In the pooled data, the difference was not significant only between 6-7 a.m. and 10-11 a.m. (Figure 6). The two-way ANOVA test on the overall occurrences of *Ap. mellifera* with “Years” and “Treatment” as factors showed a significant effect of “Years” (Fisher’s index: $F_{(1; 260)}=5.162$, $p=0.024$) while “Treatment” and the interaction of the two factors were not significant ($F_{(1; 260)}=0.569$, $p=0.456$ and $F_{(1; 260)}=0.509$, $p=0.476$ respectively). *Ap. mellifera* foragers were more active in 2021 than 2022 (1-5 foragers, 2 ± 0 foragers per flower, 132 flowers in 2021 versus 1-5 foragers, 1 ± 0 foragers per flower, 132 flowers in 2022; Student-Newman-Keuls (SNK) test: $p=0.023$) (Figure 6). ANOVA test with “Years” and “Times” as factors showed a significant effect of both factors and the interaction ($F_{(1; 256)}=15.228$, $p=1.2\times 10^{-4}$ for “Years”; $F_{(3; 256)}=65.470$, $p<0.001$ for “Times”, and $F_{(3; 256)}=2.849$, $p=0.038$ for the interaction). The nectar collection duration was significantly greater than that of pollen and the overall variation recorded was significant during the two campaigns except the case of pollen collection in 2022 (Table 2).

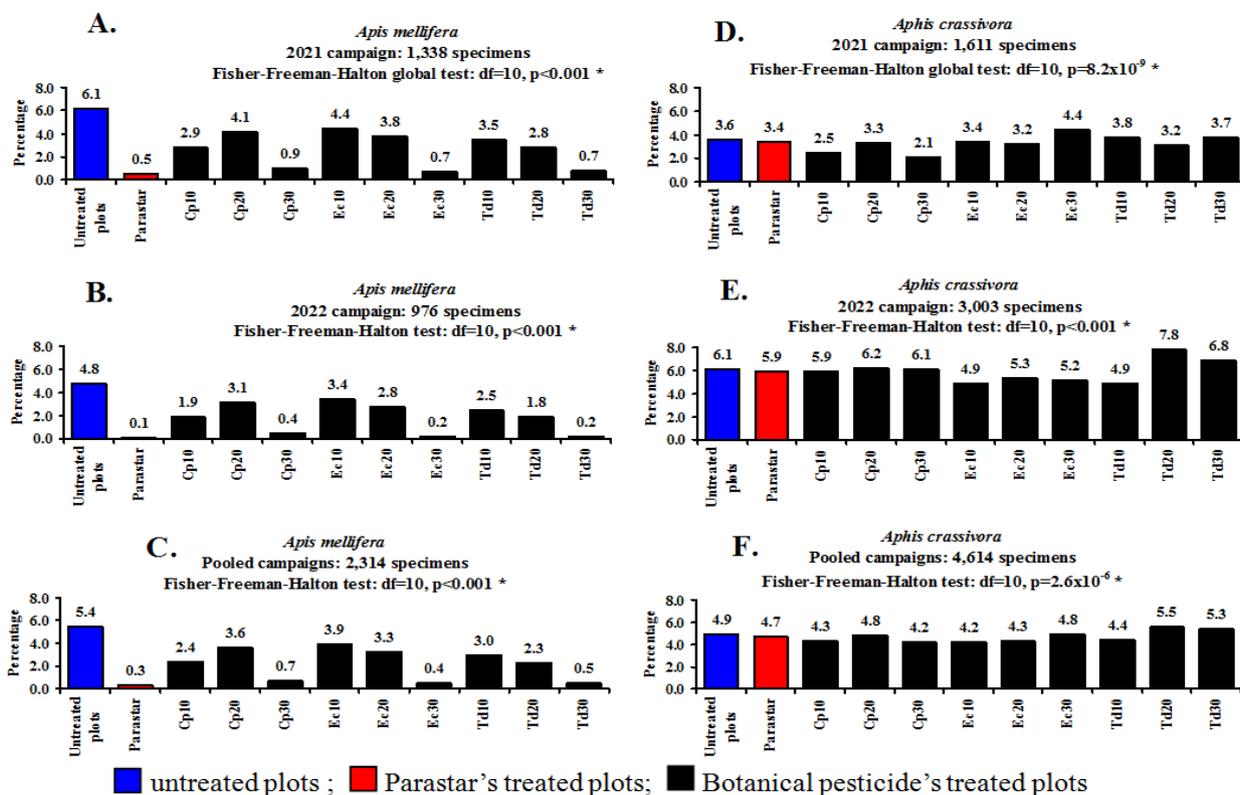
3.3.2. Product Collection

The average duration of nectar collection was in each campaign, significantly lower in Parastar treated plots than the untreated plots, which demonstrated an inhibitory effect of the synthetic insecticide on the behavior of the bee foragers.

In 2021, the average duration of nectar collection in the untreated plots was not statistically different from that recorded in the plots treated with 20% *E. camaldulensis* (Table 2). The average duration in the treated plots with Parastar was not different from that noted in treated plots with 30% *Ti. diversifolia* (Table 2). Non-significant differences were noted in combinations between 10%, 20% and 30% *Ca. procera*, 10% and 30% *E. camaldulensis*, 10%, 20% and 30% *Ti. diversifolia* (Table 2). However Cp30 and Td10 were comparable to Td30 while Td20 was comparable to Cp20 and Ec10 (Table 2).

3.3.3. Pollen Collection

The duration of pollen collection was long in treated plots, proving the disruption of the behavior of the bees not by Parastar, or aqueous extracts of the plants (Table 2). In 2021, the duration of pollen collection in untreated plots was higher than that recorded in treated plots (Table 2).



Pairwise comparisons to the control plots using the sequential Bonferroni procedure: $\alpha'(p)$

2021 campaign

	A. <i>Apis mellifera</i>	D. <i>Aphis crassivora</i>	A. <i>Apis mellifera</i>	D. <i>Aphis crassivora</i>
Untreated/Parastar	0.003 (4×10^{-60})*	0.006 (0.589) ns	Parastar/Cp10	0.005 (8×10^{-20})*
				0.003 (0.020) ns

	A. <i>Apis mellifera</i>	D. <i>Aphis crassivora</i>		A. <i>Apis mellifera</i>	D. <i>Aphis crassivora</i>
Untreated/Cp10	0.006 (1x10 ⁻¹⁴) *	0.003 (0.003) ns	Parastar/Cp20	0.004 (9x10 ⁻³⁵)*	0.050 (1.000) ns
Untreated/Cp20	0.010 (1.3x10 ⁻⁵) *	0.006 (0.547) ns	Parastar/Cp30	0.017 (0.021) ns	0.003 (2x10 ⁻⁴)*
Untreated/Cp30	0.003 (2x10 ⁻⁴⁶)*	0.003 (2x10 ⁻⁵)*	Parastar/Ec10	0.003 (4x10 ⁻³⁸)*	0.025 (0.903) ns
Untreated/Ec10	0.013 (2x10 ⁻⁴) *	0.010 (0.720) ns	Parastar/Ec20	0.004 (1x10 ⁻³⁰)*	0.009 (0.712) ns
Untreated/Ec20	0.009 (2x10 ⁻⁷) *	0.004 (0.331) ns	Parastar/Ec30	0.050 (0.397) ns	0.003 (9x10 ⁻³) *
Untreated/Ec30	0.003 (2x10 ⁻⁵⁴) *	0.004 (0.042) ns	Parastar/Td10	0.004 (4x10 ⁻²⁷)*	0.005 (0.341) ns
Untreated/Td10	0.007 (2x10 ⁻⁹) *	0.013 (0.725) ns	Parastar/Td20	0.005 (2x10 ⁻¹⁹)*	0.007 (0.621) ns
Untreated/Td20	0.006 (4x10 ⁻¹⁵) *	0.004 (0.273) ns	Parastar/Td30	0.025 (0.216) ns	0.005 (0.372) ns
Untreated/Td30	0.003 (2x10 ⁻⁵²) *	0.017 (0.769) ns			

2022 campaign

	B. <i>Apis mellifera</i>	E. <i>Aphis crassivora</i>		B. <i>Apis mellifera</i>	E. <i>Aphis crassivora</i>
Untreated vs. Parastar	0.003 (0) *	0.010 (0.655) ns	Parastar vs. Cp10	0.010 (2x10 ⁻²⁰)*	0.050 (1.000) ns
Untreated vs. Cp10	0.004 (1x10 ⁻²⁷¹)*	0.013 (0.688) ns	Parastar vs. Cp20	0.006 (5x10 ⁻³⁷)*	0.007 (0.563) ns
Untreated vs. Cp20	0.005 (3x10 ⁻²²⁶)*	0.017 (0.930) ns	Parastar vs. Cp30	0.017 (0.009) ns	0.009 (0.655) ns
Untreated vs. Cp30	0.003 (0) *	0.025 (1.000) ns	Parastar vs. Ec10	0.006 (2x10 ⁻⁴¹)*	0.004 (0.044) ns
Untreated vs. Ec10	0.005 (6x10 ⁻²¹⁶)*	0.003 (0.012) ns	Parastar vs. Ec20	0.007 (2x10 ⁻³²)*	0.006 (0.250) ns
Untreated vs. Ec20	0.004 (7x10 ⁻²³⁸)*	0.005 (0.101) ns	Parastar vs. Ec30	0.025 (0.453) ns	0.005 (0.165) ns
Untreated vs. Ec30	0.003 (0) *	0.004 (0.060) ns	Parastar vs. Td10	0.009 (3x10 ⁻²⁸)*	0.003 (0.044) ns
Untreated vs. Td10	0.004 (2x10 ⁻²⁴⁸)*	0.003 (0.012) ns	Parastar vs. Td20	0.013 (3x10 ⁻⁴)*	0.003 (4x10 ⁻²⁷)*
Untreated vs. Td20	0.003 (2x10 ⁻³)*	0.003 (6x10 ⁻²⁵)*	Parastar vs. Td30	0.050 (0.606) ns	0.004 (0.068) ns
Untreated vs. Td30	0.003 (0) *	0.006 (0.181) ns			
Pooled campaigns	C. <i>Apis mellifera</i>	F. <i>Aphis crassivora</i>	Pooled campaigns	C. <i>Apis mellifera</i>	F. <i>Aphis crassivora</i>
Untreated vs. Parastar	0.003 (4x10 ⁻¹²⁰)*	0.010 (0.474) ns	Parastar vs. Cp10	0.005 (2x10 ⁻³⁸)*	0.006 (0.196) ns
Untreated vs. Cp10	0.006 (3x10 ⁻²⁹)*	0.003 (0.041) ns	Parastar vs. Cp20	0.004 (2x10 ⁻⁶⁹)*	0.017 (0.666) ns
Untreated vs. Cp20	0.010 (4x10 ⁻¹⁰)*	0.025 (0.803) ns	Parastar vs. Cp30	0.017 (5x10 ⁻⁴)*	0.005 (0.102) ns
Untreated vs. Cp30	0.003 (8x10 ⁻⁹³)*	0.003 (0.017) ns	Parastar vs. Ec10	0.003 (3x10 ⁻⁷⁷)*	0.005 (0.138) ns
Untreated vs. Ec10	0.013 (2x10 ⁻⁷)*	0.003 (0.025) ns	Parastar vs. Ec20	0.004 (1x10 ⁻⁶⁰)*	0.007 (0.238) ns
Untreated vs. Ec20	0.009 (5x10 ⁻¹⁴)*	0.004 (0.053) ns	Parastar vs. Ec30	0.050 (0.218) ns	0.013 (0.590) ns
Untreated vs. Ec30	0.003 (7x10 ⁻¹¹⁰)*	0.050 (0.887) ns	Parastar vs. Td10	0.004 (3x10 ⁻⁵³)*	0.009 (0.339) ns
Untreated vs. Td10	0.007 (4x10 ⁻¹⁸)*	0.004 (0.088) ns	Parastar vs. Td20	0.005 (5x10 ⁻³⁷)*	0.003 (0.007) ns
Untreated vs. Td20	0.006 (2x10 ⁻³⁰)*	0.004 (0.054) ns	Parastar vs. Td30	0.025 (0.145) ns	0.003 (0.041) ns
Untreated vs. Td30	0.003 (4x10 ⁻¹⁰⁸)*	0.006 (0.198) ns			

Figure 4. Percentages of *Apis mellifera* (Hymenoptera: Apidae) and *Aphis crassivora* (Hemiptera: Aphididae) on the flowers of *Vigna unguiculata* (Fabales: Fabaceae) in untreated plots or in plots treated using the synthetic insecticide (Parastar) and three aqueous leaves extracts of three plants. Cp10, Cp20 and Cp30: 10%, 20%, and 30%: concentrations of *Calotropis procera* (Gentianales: Apocynaceae); Ec10, Ec20 and Ec30: 10%, 20%, and 30%: concentrations of *Eucalyptus camaldulensis* (Myrtales: Myrtaceae); Td10, Td20 and Td30: 10%, 20%, 30%: concentrations of *Tithonia diversifolia* (Asterales: Asteraceae). ns: not significant difference ($p > \alpha$); *: significant difference ($p < 0.05$ or $p < \alpha$).

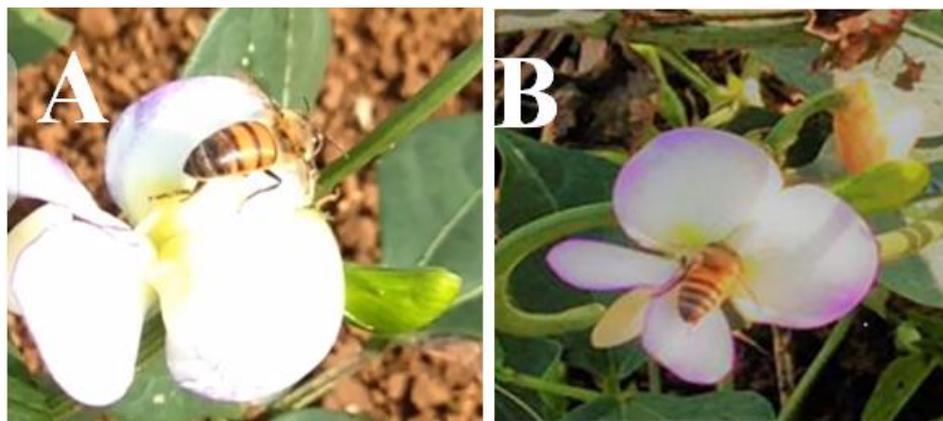


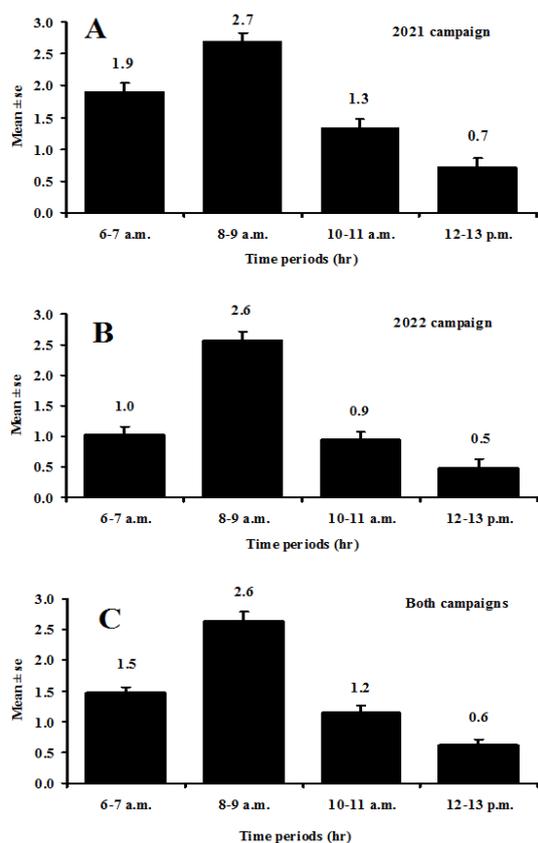
Figure 5. *Apis mellifera* (Hymenoptera: Apidae) worker harvesting pollen (A) and nectar (B) from a *Vigna unguiculata* flower in Dang.

The average duration in the Parastar treated plots was not different from that noted in plots treated with 10% and 30% *Ca. procera*, 30% *E. camaldulensis*, 10% and 30% *Ti. diversifolia* (Table 2). Records in plots treated with 20% *Ca. procera* were comparable to plots treated with 10% *E. camaldulensis*. Records in 20% *E. camaldulensis* were comparable to that noted in plots with 20% *Ti. diversifolia*. Other pairwise comparisons were significant (Table 2). In the pooled campaigns, the average duration of pollen collection in the untreated plots was high than the records in treated plots (Table 2). Non-significant differences were noted in the combinations between Parastar plots, 10% and 30% *Ca. procera*, 30% *E. camaldulensis*, 10% and 30% *Ti. diversifolia* (Table 2). However records in Cp20 were comparable to that noted in Td10, Ec10 and Ec20 plots. Records in Ec20 were comparable to that noted in Td10 plots (Table 2). Other comparisons were significant (Table 2).

3.3.4. Rhythm of Visits and Foraging Speed

Two-way ANOVA with "Years" and "Plots" as factors showed a significant effect of "Years" ($F_{(1; 242)}=8.676$, $p=0.004$) while "Plots" and the interaction were not significant ($F_{(10; 242)}=1.750$, $p=0.071$ and $F_{(10; 242)}=0.313$, $p=0.977$ respectively). The studied bees were more active in 2021 than 2022 (SNK test: $p=0.003$) (Figure 7 and Table 3). The same test with "Plots" and "Times" as factors showed a significant effect of "Times" ($F_{(3; 256)}=21.107$, $p<0.001$). "Treatment" and the interaction were not significant ($F_{(1; 256)}=0.906$, $p=0.342$ and $F_{(3; 256)}=0.312$, $p=0.817$ respectively) (Figure 7 and Table 3). In 2021, ANOVA test with "Plots" and "Time" as factors showed a significant effect of "Plots" ($p=0.002$) and "Time" ($p<0.001$) while the interaction was not significant ($p=0.636$). During the day, differences were significant between 6-7 a.m. and 10-11 a.m. (Figure 7A and

Table 3). Between plots, a significant variation was noted only during 6-7 a.m. ($F_{(10; 22)}=4.667$, $p=0.001$) and pairwise comparisons showed that the average occurrences of the bee were only significant between the plots treated with 20% aqueous leaves extract of *E. camaldulensis* and other plots including untreated and those treated with Parastar (Figure 7A and Table 3). The occurrences in Parastar treated plots were significantly different from those noted in 20% *Calotropis procera*, 20% *E. camaldulensis*, 30% *Ti. diversifolia* and the pooled data (Figure 7A and Table 3). As for the comparison between periods of daily activity during the 2021 campaign, in the Parastar plots, the variation was significant between 8-9 a.m. and 12-1 p.m. (Figure 7A and Table 3). In the plots treated with 20% *Ca. procera* the difference was significant between 8-9 a.m. and 12-13 p.m. (Figure 7A and Table 3). In the plots treated with 20% *E. camaldulensis*, the difference was significant between 6-7 a.m. and 12-1 p.m., 8-9 a.m. and 10-11 a.m., 8-9 a.m. and 12-1 p.m. (Figure 7A). In plots treated with 30% *Ti. diversifolia*, the differences were significant between 6-7 a.m. and 12-1 p.m., 8-9 a.m. and 12-1 p.m., 10-11 a.m. and 12-1 p.m. (Figure 7A). Other differences were not significant. In the pooled data, differences were significant (Figure 7A and Table 3). In 2022, the two-way ANOVA test with "Plots" and "Time" as factors showed a significant effect of "Time" ($p<0.001$) while effects of "Plots" and the interaction effect were not significant ($p=0.726$ and $p=0.966$ respectively) (Figure 7B and Table 3). Analysis of the time period showed a significant variation in plots treated with 10% and 20% *E. camaldulensis*, 10% and 30% *Ti. diversifolia* and the pooled data (Figure 7A and Table 3). In 2022, the comparison between the periods of daily activity, in the plots treated with 10% *E. camaldulensis* presented a significant difference between 8-9 a.m. and 12-1 p.m. (Figure 7B and Table 3).



One-way ANOVA test with “Time” as factor:
 2021 campaign: $F_{(3; 128)}=27.667, p<0.001^*$;
 2022 campaign: $F_{(3; 128)}=41.740, p<0.001^*$;
 Pooled data: $F_{(3; 128)}=37.734, p<0.001^*$.

C. 2021 vs. 2022: Student t-test (df=64)

Comparisons	p-value
6-7 a.m.	$t=5.426, p<0.001^*$
8-9 a.m.	$t=4.175, p=0.678$ ns
10-11 a.m.	$t=1.666, p=0.101$ ns
12-13 p.m.	$t=1.107, p=0.273$ ns

D. Student-Newman-Keuls test: p-value

Comparisons	2021	2022	Global
6-7 vs. 8-9 a.m.	$4.9 \times 10^{-4}^*$	$<0.001^*$	$<0.001^*$
6-7 vs. 10-11 a.m.	0.011 *	0.468 ns	0.073 ns
6-7 vs. 12-13 p.m.	$2.0 \times 10^{-5}^*$	0.036 *	$1.0 \times 10^{-4}^*$
8-9 vs. 10-11 a.m.	$2.0 \times 10^{-5}^*$	$2.0 \times 10^{-5}^*$	$2.0 \times 10^{-5}^*$
8-9 vs. 12-13 p.m.	$<0.001^*$	$<0.001^*$	$<0.001^*$
10-11 vs. 12-13 p.m.	0.007 *	0.082 ns	0.017 *

Figure 6. Daily rhythm of activity of *Apis mellifera* (Hymenoptera: Apidae) (33 trips each time period, 132 essays each campaign, 264 essays for the pooled data) in 2021 and 2022 campaigns in Dang.

Table 2. Variation in collection time (in seconds) of flower production (nectar and pollen).

	A. Duration (s) of the nectar collection					B. Duration (s) of the pollen collection					A vs. B
	n	Min.	Max.	Median	Mean ± se	n	Min.	Max.	Median	Mean ± se	Student t-test
2021 campaign											
Untreated	145	1.0	15.0	10	9.3±0.3	140	2.0	10.0	7	6.4±0.2	$t=8.46, df=283, p<0.001^*$
Parastar	156	2.0	10.0	6	5.9±0.2	141	1.0	6.0	4	3.8±0.1	$t=10.01, df=295, p<0.001^*$
Cp10	152	1.0	15.0	7	7.1±0.3	147	2.0	10.0	4	3.7±0.1	$t=11.53, df=297, p<0.001^*$
Cp20	193	1.0	20.0	9	7.5±0.3	178	1.0	10.0	4	4.8±0.2	$t=7.05, df=369, p<0.001^*$
Cp30	191	1.0	15.0	7	7.0±0.2	186	2.0	10.0	4	3.7±0.1	$t=12.87, df=375, p<0.001^*$
Ec10	211	1.0	20.0	8	7.9±0.2	203	2.0	11.0	4	4.7±0.2	$t=11.37, df=412, p<0.001^*$
Ec20	198	1.0	20.0	9	9.0±0.3	190	2.0	9.0	5	5.4±0.2	$t=10.43, df=386, p<0.001^*$
Ec30	195	1.0	15.0	7	6.9±0.2	187	2.0	10.0	3	3.7±0.1	$t=12.85, df=380, p<0.001^*$
Td10	138	1.0	15.0	7	7.0±0.3	133	2.0	10.0	4	3.7±0.1	$t=10.92, df=269, p<0.001^*$
Td20	153	2.0	15.0	10	8.3±0.3	138	1.0	9.0	6	5.8±0.2	$t=6.98, df=289, p<0.001^*$
Td30	103	1.0	15.0	7	6.6±0.3	98	2.0	10.0	4	3.7±0.2	$t=8.22, df=199, p<0.001^*$
Pooled plots	1835	1.0	20.0	8	7.5±3.5	1741	1.0	11.0	4	4.5±2.3	$t=30.7, df=3574, p<0.001^*$
ANOVA	$F_{(10; 1,824)}=15.137, p<0.001^*$					$F_{(10; 1,824)}=31.688, p<0.001^*$					
2022 campaign											

	A. Duration (s) of the nectar collection					B. Duration (s) of the pollen collection					A vs. B
	n	Min.	Max.	Median	Mean \pm se	n	Min.	Max.	Median	Mean \pm se	Student t-test
Untreated	113	1.0	15.0	7	7.2 \pm 0.3	108	2.0	8.0	4	3.9 \pm 0.2	t=10.40, df=219, p<0.001*
Parastar	128	2.0	10.0	6	5.6 \pm 0.2	113	1.0	6.0	3	3.2 \pm 0.1	t=10.37, df=239, p<0.001*
Cp10	166	1.0	15.0	7	6.9 \pm 0.2	161	2.0	10.0	4	3.7 \pm 0.1	t=11.68, df=325, p<0.001*
Cp20	78	1.0	15.0	8	7.0 \pm 0.3	73	2.0	8.0	4	3.7 \pm 0.2	t=8.25, df=149, p<0.001*
Cp30	134	2.0	10.0	6	5.9 \pm 0.2	118	1.0	6.0	4	3.8 \pm 0.1	t=9.10, df=250, p<0.001*
Ec10	186	1.0	15.0	7	6.9 \pm 0.2	178	2.0	10.0	4	3.8 \pm 0.1	t=11.90, df=362, p<0.001*
Ec20	127	1.0	15.0	7	7.1 \pm 0.3	122	2.0	10.0	4	3.9 \pm 0.2	t=10.26, df=247, p<0.001*
Ec30	170	1.0	15.0	7	6.9 \pm 0.2	162	2.0	10.0	4	3.7 \pm 0.1	t=11.62, df=330, p<0.001*
Td10	120	1.0	15.0	7	6.9 \pm 0.3	115	2.0	10.0	4	3.8 \pm 0.2	t=9.88, df=233, p<0.001*
Td20	173	1.0	15.0	7	6.9 \pm 0.2	165	2.0	10.0	4	3.7 \pm 0.1	t=11.79, df=336, p<0.001*
Td30	131	2.0	12.0	6	5.8 \pm 0.2	116	1.0	6.0	4	3.8 \pm 0.1	t=8.66, df=245, p<0.001*
Pooled plots	1526	1.0	15.0	7	6.6 \pm 0.1	1431	1.0	10.0	4	3.7 \pm 0.0	t=33.9, df=2955, p<0.001*
ANOVA	F _(10; 1,515) =5.467, p<0.001 *					F _(10; 1,420) =1.675, p=0.082 ns					
Pooled campaigns											
Untreated	258	1.0	15.0	9	8.4 \pm 0.2	248	2.0	10.0	5	5.3 \pm 0.2	t=11.90, df=504, p<0.001*
Parastar	284	2.0	10.0	6	5.8 \pm 0.1	254	1.0	6.0	3	3.5 \pm 0.1	t=14.20, df=536, p<0.001*
Cp10	318	1.0	15.0	7	7.0 \pm 0.2	308	2.0	10.0	4	3.7 \pm 0.1	t=16.40, df=624, p<0.001*
Cp20	271	1.0	20.0	8	7.4 \pm 0.2	251	1.0	10.0	4	4.5 \pm 0.2	t=9.68, df=520, p<0.001*
Cp30	325	1.0	15.0	6	6.5 \pm 0.2	304	1.0	10.0	4	3.7 \pm 0.1	t=15.47, df=627, p<0.001*
Ec10	397	1.0	20.0	8	7.4 \pm 0.2	381	2.0	11.0	4	4.3 \pm 0.1	t=16.10, df=776, p<0.001*

Table 2. Continued.

	A. Duration (s) of the nectar collection					B. Duration (s) of the pollen collection					A vs. B
	n	Min.	Max.	Median	Mean \pm se	n	Min.	Max.	Median	Mean \pm se	Student t-test
Ec20	325	1.0	20.0	8	8.2 \pm 0.2	312	2.0	10.0	4	4.8 \pm 0.1	t=13.60, df=635, p<0.001*
Ec30	365	1.0	15.0	7	6.9 \pm 0.2	349	2.0	10.0	4	3.7 \pm 0.1	t=17.34, df=712, p<0.001*
Td10	258	1.0	15.0	7	7.0 \pm 0.2	248	2.0	10.0	4	3.7 \pm 0.1	t=14.74, df=504, p<0.001*
Td20	326	1.0	15.0	7	7.6 \pm 0.2	303	1.0	10.0	4	4.7 \pm 0.1	t=12.54, df=627, p<0.001*
Td30	234	1.0	15.0	6	6.1 \pm 0.2	214	1.0	10.0	4	3.8 \pm 0.1	t=11.75, df=446, p<0.001*
Pooled plots	3361	1.0	20.0	7	7.1 \pm 0.1	3172	1.0	11.0	4	4.2 \pm 0.0	t=44.1, df=6531, p<0.001*
ANOVA	F _(10; 3,350) =18.156, p<0.001 *					F _(10; 3,350) =23.356, p<0.001 *					

C. Student-Newman-Keuls p-values for nectar collection: 2021 (upper diagonal matrix) and 2022 (diagonal lower matrix)

	Parastar	Cp10	Cp20	Cp30	Ec10	Ec20	Ec30	Td10	Td20	Td30
Untreated	1x10 ⁻⁵ *	2x10 ⁻⁵ *	4x10 ⁻⁵ *	3x10 ⁻⁵ *	9x10 ⁻³ *	0.477 ns	1x10 ⁻⁵ *	3x10 ⁻⁵ *	0.031 *	1x10 ⁻⁵ *

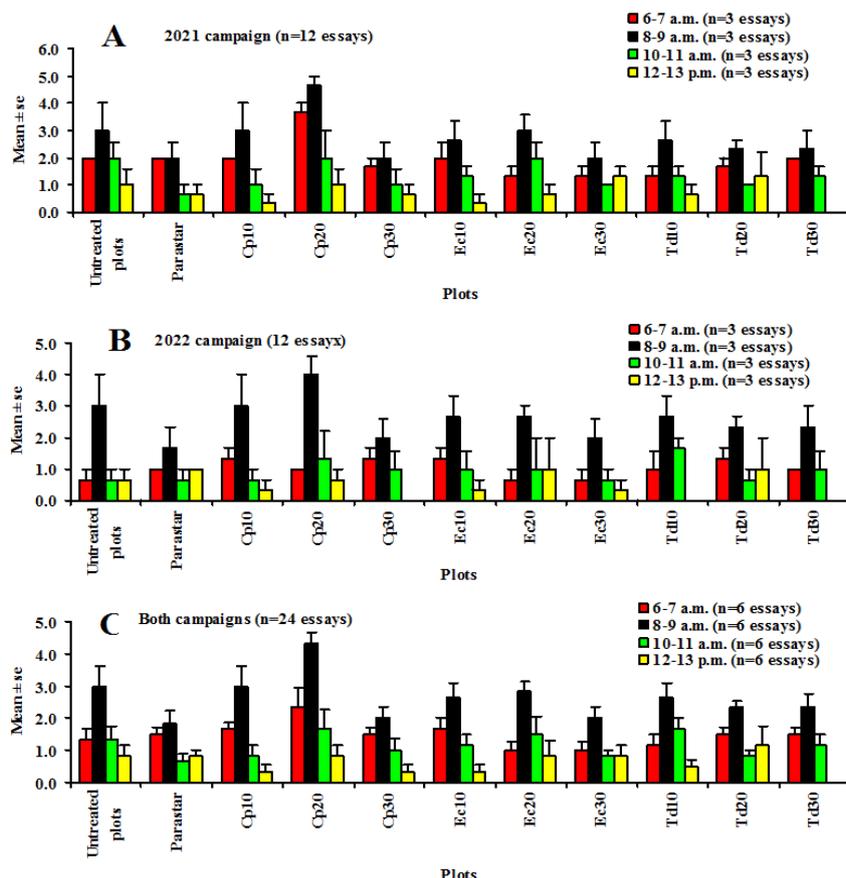
	Parastar	Cp10	Cp20	Cp30	Ec10	Ec20	Ec30	Td10	Td20	Td30
Parastar	-	0.028 *	2x10 ⁻⁴ *	0.022 *	3x10 ⁻⁵ *	1x10 ⁻⁵ *	0.013 *	0.034 *	1x10 ⁻⁵ *	0.128 ns
Cp10	7x10 ⁻⁴ *	-	0.220 ns	0.934 ns	0.054 ns	2x10 ⁻⁵ *	0.979 ns	0.914 ns	0.008 *	0.747 ns
Cp20	0,009 *	0,995 ns	-	0.335 ns	0.256 ns	7x10 ⁻⁵ *	0.415 ns	0.391 ns	0.085 ns	0.173 ns
Cp30	0,571 ns	0,009 *	0,078 ns	-	0.036 *	3x10 ⁻⁵ *	0.966 ns	0.818 ns	0.003 *	0.611 ns
Ec10	6x10 ⁻⁴ *	0,992 ns	0,948 ns	0,015 *	-	0.002 *	0.042 *	0.084 ns	0.271 ns	0.015 *
Ec20	8x10 ⁻⁴ *	0,993 ns	0,926 ns	0,020 *	0,963 ns	-	3x10 ⁻⁵ *	2x10 ⁻⁵ *	0.046 *	1x10 ⁻⁵ *
Ec30	4x10 ⁻⁴ *	0,977 ns	0,998 ns	0,003 *	0,999 ns	0,997 ns	-	0.961 ns	0.003 *	0.360 ns
Td10	0,003 *	0,996 ns	0,983 ns	0,032 *	0,918 ns	0,984 ns	0,999 ns	-	0.012 *	0.698 ns
Td20	0,001 *	0,999 ns	0,768 ns	0,024 *	0,987 ns	0,890 ns	1.00 ns	0,993 ns	-	0.001 *
Td30	0,477 ns	0,006 *	0,050 ns	0,768 ns	0,008 *	0,010 *	0,004 *	0,022 *	0,012 *	
Untreated	3x10 ⁻⁴ *	0,965 ns	0,911 ns	0,010 *	0,911 ns	0,715 ns	0,978 ns	0,953 ns	0,838 ns	0,005 *
Pooled campaigns										
Untreated	1x10 ⁻⁵ *	2x10 ⁻⁵ *	0,003 *	1x10 ⁻⁵ *	0,001 *	0,671 ns	3x10 ⁻⁵ *	3x10 ⁻⁵ *	0,006 *	1x10 ⁻⁵ *
Parastar		5x10 ⁻⁵ *	3x10 ⁻⁵ *	0,008 *	3x10 ⁻⁵ *	1x10 ⁻⁵ *	3x10 ⁻⁵ *	1x10 ⁻⁴ *	1x10 ⁻⁵ *	0,168 ns
Cp10			0,115 ns	0,273 ns	0,121 ns	2x10 ⁻⁵ *	0,957 ns	0,970 ns	0,082 ns	0,019 *
Cp20				0,008 *	0,825 ns	0,004 *	0,229 ns	0,275 ns	0,770 ns	2x10 ⁻⁴ *
Cp30					0,002 *	3x10 ⁻⁵ *	0,114 ns	0,218 ns	6x10 ⁻⁴ *	0,158 ns
Ec10						0,002 *	0,133 ns	0,234 ns	0,600 ns	4x10 ⁻⁵ *
Ec20							3x10 ⁻⁵ *	3x10 ⁻⁵ *	0,005 *	1x10 ⁻⁵ *
Ec30								0,819 ns	0,068 ns	0,011 *
Td10									0,15 ns	0,021 *
Td20										4x10 ⁻⁵ *

Table 2. Continued.

	Parastar	Cp10	Cp20	Cp30	Ec10	Ec20	Ec30	Td10	Td20	Td30
D. Student-Nerwman-Keuls p-values for pollen collection: 2021 (upper part matrix) and pooled years (lower part matrix) (Continued)										
Untreated	2x10 ⁻⁵ *	3x10 ⁻⁵ *	<0,001 *	1x10 ⁻⁵ *	2x10 ⁻⁵ *	2x10 ⁻⁴ *	1x10 ⁻⁵ *	1x10 ⁻⁵ *	0,020 *	3x10 ⁻⁵ *
Parastar	-	0,879 ns	8x10 ⁻⁵ *	0,983 ns	1x10 ⁻⁴ *	<0,001 *	0,994 ns	0,992 ns	2x10 ⁻⁵ *	0,974 ns
Cp10	0,673 ns	-	5x10 ⁻⁵ *	0,995 ns	2x10 ⁻⁴ *	2x10 ⁻⁵ *	0,980 ns	0,991 ns	2x10 ⁻⁵ *	0,933 ns
Cp20	3x10 ⁻⁵ *	7x10 ⁻⁵ *	-	3x10 ⁻⁵ *	0,555 ns	0,009 *	4x10 ⁻⁵ *	1x10 ⁻⁴ *	2x10 ⁻⁴ *	5x10 ⁻⁴ *
Cp30	0,470 ns	0,986 ns	9x10 ⁻⁵ *	-	5x10 ⁻⁵ *	3x10 ⁻⁵ *	0,988 ns	0,812 ns	1x10 ⁻⁵ *	0,965 ns
Ec10	9x10 ⁻⁵ *	0,002 *	0,168 ns	0,003 *	-	0,003 *	6x10 ⁻⁵ *	5x10 ⁻⁴ *	3x10 ⁻⁵ *	0,002 *
Ec20	1x10 ⁻⁵ *	2x10 ⁻⁵ *	0,129 ns	3x10 ⁻⁵ *	0,002 *	-	1x10 ⁻⁵ *	3x10 ⁻⁵ *	0,115 ns	2x10 ⁻⁵ *
Ec30	0,279 ns	0,991 ns	5x10 ⁻⁵ *	0,895 ns	0,002 *	1x10 ⁻⁵ *	-	0,966 ns	1x10 ⁻⁵ *	0,993 ns
Td10	0,621 ns	0,955 ns	2x10 ⁻⁴ *	0,923 ns	0,006 *	3x10 ⁻⁵ *	0,973 ns	-	3x10 ⁻⁵ *	0,972 ns
Td20	1x10 ⁻⁵ *	2x10 ⁻⁵ *	0,311 ns	3x10 ⁻⁵ *	0,027 *	0,336 ns	3x10 ⁻⁵ *	2x10 ⁻⁵ *	-	3x10 ⁻⁵ *
Td30	0,750 ns	0,878 ns	3x10 ⁻⁴ *	0,991 ns	0,003 *	2x10 ⁻⁵ *	0,993 ns	0,979 ns	1x10 ⁻⁵ *	

	Parastar	Cp10	Cp20	Cp30	Ec10	Ec20	Ec30	Td10	Td20	Td30
Untreated	1x10 ⁻⁵ *	3x10 ⁻⁵ *	6x10 ⁻⁵ *	1x10 ⁻⁵ *	2x10 ⁻⁵ *	0,006 *	1x10 ⁻⁵ *	3x10 ⁻⁵ *	8x10 ⁻⁴ *	2x10 ⁻⁵ *

Cp10, Cp20 and Cp30: 10%, 20%, and 30%: concentrations of *Calotropis procera* (Gentianales: Apocynaceae); Ec10, EC20 and Ec30: 10%, 20%, and 30%: concentrations of *Eucalyptus camaldulensis* (Myrtales: Myrtaceae); Td10, Td20 and Td30: 10%, 20%, 30%: concentrations of *Tithonia diversifolia* (Asterales: Asteraceae). ns: not significant difference (p≥0.05); *: significant difference (p<0.05)



One-way ANOVA with factor “Plots”

2021 campaign:
 6-7 a.m.: F_(10; 22)=4.667, p=0.001 *;
 8-9 a.m.: F_(10; 22)=1.323, p=0.279 ns;
 10-11 a.m.: F_(10; 22)=0.880, p=0.565 ns;
 Untreated vs. Parastar: p=1.00 ns;
 12-13 p.m.: F_(10; 22)=0.880, p=0.581 ns

2022 campaign:
 6-7 a.m.: F_(10; 22)=0.800, p=0.630 ns;
 8-9 a.m.: F_(10; 22)=0.707, p=0.709 ns;
 10-11 a.m.: F_(10; 22)=0.224, p=0.991 ns;
 12-13 p.m.: F_(10; 22)=0.810, p=0.622 ns

Pooled campaigns:
 6-7 a.m.: F_(10; 55)=1.353, p=0.227 ns;
 8-9 a.m.: F_(10; 55)=2.401, p=0.019 *;
 12-13 p.m.: F_(10; 55)=1.346, p=0.230 ns
 10-11 a.m.: F_(10; 55)=0.836, p=0.597 ns;

Figure 7. Rhythm of activity of *Apis mellifera* (Hymenoptera: Apida) on flowers of *Vigna unguiculata* (Fabales: Fabaceae). Cp10, Cp20 and Cp30: 10%, 20%, and 30%: concentrations of *Calotropis procera*; Ec10, EC20 and Ec30: 10%, 20%, and 30%: concentrations of *Eucalyptus camaldulensis*; Td10, Td20 and Td30: 10%, 20%, 30%: concentrations of *Tithonia diversifolia*. ns: not significant difference (p>α'); *: significant difference (p<0.05).

In the plots treated with 20% *E. camaldulensis* the difference was significant when comparing 6-7 h to 8-9 h, 8-9 h to 10-11 h and 12-13. In plots treated with 10% *Ti. diversifolia*, the difference was significant only between 8-9 h and 12-1 p.m. (Figure 7B and Table 3). In the plots treated with 30% *Ti. diversifolia*, the differences were significant between 6-7 a.m. and 12-1 p.m., 8-9 a.m. and 12-1 p.m., 8-9 a.m. and 12-1 p.m., 10-11 a.m. and 12-1 p.m. (Figure 7B and Table 3). In the pooled campaigns, the differences were significant except between 6-7 a.m. and 10-11 a.m. where the difference was not significant (Figure 7B and Table 3)., the average variation in occurrences was significant during 8-9 a.m. (F_(10; 55)=2.401, p=0.019) and pairwise comparisons showed that the average occurrences of the bee were only significant be-

tween plots treated with 20% *E. camaldulensis* and plots treated with Parastar, plots treated with 10% or 30% *E. camaldulensis*, 30% *Ca. procera*, plots treated with 20% or 30% *Ti. diversifolia* (Figure 7C and Table 3). Occurrences in untreated plots showed significant differences between 8-9 a.m. and 6-7 a.m. or 10-11 a.m. or even 12-13 p.m. (Figure 7C and Table 3). Parastar and plots treated with 20% or 30% *Ca. procera*, 10% or 20% *E. camaldulensis*, and 10% *Ti. diversifolia* presented similar results. Differences were significant when comparing 8-9 a.m. to 10-11 a.m. or 12-1 p.m. (Figure 7C and Table 3). Occurrences in plots treated with 10% *Ca. procera* showed significant differences between 6-7 a.m. and 8-9 a.m. or 12-13 p.m. and between 8-9 a.m. and 10-11 a.m. or 12-1 p.m. (Figure 7C and Table 3). Occurrence-

es in plots treated with 30% *E. camaldulensis* presented a significant difference between 6-7 a.m. and 12-13 p.m. between 8-9 a.m. and 12-13 p.m. (Figure 7C and Table 3). Data from plots treated with 20% *Ti. diversifolia* presented a significant difference when comparing 8-9 a.m. to 10-11 a.m. (Figure 7C and Table 3). Data from plots treated with 30% *Ti. diversifolia* presented significant differences when comparing 6-7 a.m. to 12-13 p.m., 8-9 a.m. to 10-11 a.m. or 12-13 p.m. and 10-11 a.m. to 12-1 p.m. (Figure 7C and Table 3). Estimate of the rate of visitation of 1000 flowers by *Ap. mellifera* foragers compared to other recorded floricultural insects, showed a high rate in untreated plots (31±3 individuals per 1000 flowers in 2021, 26±2 individuals in 2022 and 28±2 individuals in the pooled campaigns) (Table 4A). This rate was low (<20%) in the plots treated with Parastar (17±3 in 2021, 16±1 in 2022, and 17±1 in the pooled campaigns) (Table 4A, 4B, 4C). Plots treated with 30% *Ca. procera*, *E. camaldulensis* and *Ti. diversifolia* presented rates close to that noted in plots treated with Parastar (19±2 individuals in 2021, 18±4 individuals in 2022, and 19±2 in the pooled data for *Ca. procera*; 18±2 individuals in 2021, 18±3 individuals in 2023, and 18±3 individuals in the pooled data for *E. camaldulensis*; 19±2 individuals in 2021, 20±2 in 2022, and 19±2 individuals in the pooled data for *Ti. diversifolia*) (Table 4C), proving the toxicity of 30% extracts. Other extracts presented intermediate rates between the two extremes, except 10% *Ca. procera* where it was in 2022 (19±3 individuals) close to that of Parastar (Table 5B). The number of flowers visited varied from one to three in two to 60 seconds in 2021 (Table 5A), in 2022 (Table 5B) and in the pooled campaigns (Table 5C), with a foraging speed varying from one to 120 flowers per minute in 2021, one to 60 flowers in 2022 and one to 120 flowers in the pooled campaigns (Table 5A, 5B, 5C). The overall variation in the visited flowers was not significant during the two campaigns (Table 5A, 5B, 5C).

On the other hand, the overall variation in the foraging times and speeds was insignificant in 2022 (Table 5B) but significant in 2021 (Table 5A) and in the pooled campaigns (Table 5C). Between the two campaigns, the visitation duration was long in 2021 than 2022 in 10% *Ca. procera* treated

plots and low in 2021 than 2022 in 30% *Ti. diversifolia* plots (Table 5D). The foraging speed was low in 2021 than 2022 in plots treated with 10% *Ca. procera* and the difference between the two years was significant in 30% *Ti. diversifolia* treated plots and in the pooled data (Table 5D). In 2021, the visits were very rapid (significantly low duration) in untreated plots than in plots treated with Parastar or botanical extracts, which once again demonstrated the disruption in the behavior of foragers. The longer times were noted in the Parastar plots (21±1.3 seconds), in 10% *Ca. procera* plots (23.5±1.3 seconds), in 10% and 30% *E. camaldulensis* plots (20.2±1.3 and 20.3±1.3 seconds respectively), 10% and 30% *Ti. diversifolia* (23.3±1.3 and 22.1±1.4 respectively) (Table 5D). In the 2021 campaign (Table 5A), the highest foraging speed was noted in untreated plots (9±1 flowers per minute). The low speeds were recorded in Parastar treated plots (6±0 flowers), 10% and 30% *Ca. procera* (5±0 flowers per minute, and 6±0 flowers per minute respectively), and even in 10% and 30% *Ti. diversifolia* (5±0 flowers, and 6±0 flowers respectively) (Table 5A). In the 2022 campaign (Table 5B), the foraging speed was low (5±0 flowers per minute) in untreated plots and plots treated with Parastar respectively. The speed was high (7±0 flowers per minute) in plots treated with 10% and 20% *Ca. procera* or *E. camaldulensis* or even 20% *Ti. diversifolia* respectively and in the pooled plots (Table 5B). The foraging speeds recorded in the other plots treated with botanical extracts were intermediate between the two extremes.

In the pooled campaigns, the flower visit was very rapid in untreated plots than treated plots. Between the treated plots, the duration was higher in plots treated with 10% *Ca. procera* than 20% *E. camaldulensis* or 30% *Ca. procera*. It was lower in plots treated with 30% *Ca. procera* than 10% *Ti. diversifolia*, 20% *E. camaldulensis* than 10% *Ti. diversifolia* (Table 5C). Other comparisons were not significant. Between the treated plots in the pooled campaigns, the duration was significantly lower in plots treated with 20% *E. camaldulensis* than 10% or 30% of *Ti. diversifolia* (Table 5C). The highest foraging speed (8±0 flowers per minute) was recorded in plots treated with 20% *E. camaldulensis* (Table 5C).

Table 3. Statistics on raw data presented in Figure 7.

	Parastar	Cp10	Cp20	Cp30	Ec10	Ec20	Ec30	Td10	Td20	Td30
A. Student-Newman-Keuls p-values: 6-7 a.m. in 2021 (upper diagonal matrix) and 8-9 a.m. in the pooled years (lower matrix)										
Untreated	p=0,811 ns	1.00 ns	0.705ns	0,766 ns	1.00 ns	2x10 ⁻³ *	0.933 ns	0,814 ns	0.968 ns	1.00 ns
Parastar	-	1.00 ns	0.419ns	0.535 ns	1.00 ns	0,009 *	0.443 ns	0.629 ns	0.718 ns	1.00 ns
Cp10	0.842 ns	-	0,766ns	0,814 ns	1.00 ns	9x10 ⁻⁴ *	0,968 ns	0,851ns	0,984 ns	1.00 ns
Cp20	0.878 ns	0.959 ns	-	1.00 ns	0.535 ns	6x10 ⁻⁴ *	0.718 ns	1.00 ns	0.443 ns	0.639 ns
Cp30	0.959 ns	0.878ns	0.863ns	-	0,629ns	7x10 ⁻⁴ *	0.862 ns	1.00 ns	0.718 ns	0.705 ns
Ec10	0.863 ns	0.981ns	0.959ns	0.816 ns	-	6x10 ⁻³ *	0.718 ns	0.705 ns	0.862 ns	1.00 ns

	Parastar	Cp10	Cp20	Cp30	Ec10	Ec20	Ec30	Td10	Td20	Td30
Ec20	0.019 *	0.081ns	0.074ns	0.012 *	0.032 *	-	2x10 ⁻³ *	8x10 ⁻⁴ *	3x10 ⁻³ *	4x10 ⁻³ *
Ec30	0.784 ns	0.805ns	0.811ns	1.00 ns	0.771 ns	0.010 *	-	0.933 ns	1.00 ns	0.862ns
Td10	0.921 ns	1.00 ns	0.784ns	0.925 ns	0.946 ns	0.059ns	0.878 ns	-	0.862ns	0.966 ns
Td20	0.959 ns	0.584ns	0.842ns	0.981 ns	0.878 ns	0.026 *	0.946 ns	0.846 ns	-	0.933 ns
Td30	0.784 ns	0.846ns	0.921ns	0.946 ns	0.925 ns	0.033 *	0.846 ns	0.946 ns	1.00 ns	-
Untreated	p=0,811 ns	0,946ns	0,784ns	0.771 ns	1.00 ns	0.079 ns	0.716 ns	0.846 ns	0.805 ns	0.878 ns

B. One-way ANOVA in each plot

Plots	2021 (132 essays)		2022 (132 essays)		Pooled campaigns (264 essays)	
	ANOVA	p-value	ANOVA	p-value	ANOVA	p-value
Untreated:	F _(3; 8) =1.600	0.264 ns	F _(3; 8) =4.083	0.050 ns	F _(3; 20) =4.574	0.014 *
Parastar	F _(3; 8) =4.267	0.045 *	F _(3; 8) =3.074	0.091 ns	F _(3; 20) =5.044	9.2x10 ⁻³ *
Cp10	F _(3; 8) =3.963	0.053 ns	F _(3; 8) =3.852	0.056 ns	F _(3; 20) =11.229	9.0x10 ⁻⁵ *
Cp20	F _(3; 8) =4.458	0.040 *	F _(3; 8) =2.095	0.179 ns	F _(3; 20) =8.049	7.0x10 ⁻⁴ *
Cp30	F _(3; 8) =1.267	0.349 ns	F _(3; 8) =3.278	0.080 ns	F _(3; 20) =6.162	2.9x10 ⁻³ *
Ec10	F _(3; 8) =3.769	0.059 ns	F _(3; 8) =4.222	0.046 *	F _(3; 20) =9.317	4.6x10 ⁻⁴ *
Ec20	F _(3; 8) =6.952	0.013 *	F _(3; 8) =7.606	9.9x10 ⁻³ *	F _(3; 20) =9.277	4.8x10 ⁻⁴ *
Ec30	F _(3; 8) =1.667	0.250 ns	F _(3; 8) =3.571	0.067 ns	F _(3; 20) =5.630	5.8x10 ⁻³ *
Td10	F _(3; 8) =3.619	0.065 ns	F _(3; 8) =4.400	0.042 *	F _(3; 20) =12.179	5.0x10 ⁻⁵ *
Td20	F _(3; 8) =1.296	0.341 ns	F _(3; 8) =1.556	0.274 ns	F _(3; 20) =3.429	0.037 *
Td30	F _(3; 8) =7.667	9.7x10 ⁻³ *	F _(3; 8) =4.714	0.035 *	F _(3; 20) =11.609	1.3x10 ⁻⁴ *
Global	F _(3; 128) =27.67	<0.001*	F _(3; 128) =41.74	<0.001*	F _(3; 260) =60.84	<0.001*

C. Between time periods in each category of plot: Student-Newman-Keuls test p-values

	Parastar	Cp10	Cp20	Cp30	Ec10	Ec20	Ec30	Td10	Td20	Td30
2021 campaign					2022 campaign					
	Parastar	Cp20	Ec20	Td30	Pooled	Ec10	Ec20	Td10	Td30	Pooled
6-7 vs. 8-9 a.m.	1.00 ns	0.085 ns	0.290 ns	0.545 ns	5x10 ⁻⁵ *	0.076ns	0.012 *	0.056 ns	0.065 ns	<0.001 *
6-7 vs. 10-11 a.m.	0.081 ns	0.347 ns	0.096 ns	0.242 ns	0.011*	0.438ns	0.681 ns	1.00 ns	1.00 ns	0.468 ns
6-7 vs. 12-13 p.m.	0.129 ns	0.347 ns	0.039 *	0.013 *	2x10 ⁻⁵ *	0.473ns	0.681 ns	0.413ns	0.299 ns	0.036 *
8-9 vs. 10-11 a.m.	0.035 *	0.172 ns	0.039 *	0.201 ns	2x10 ⁻⁵ *	0.050ns	9x10 ⁻³ *	0.124 ns	0.143 ns	2.0x10 ⁻⁵ *
8-9 vs. 12-13 p.m.	0.081 ns	0.033 *	0.014 *	0.010 *	<0,001*	0.046 *	0.012 *	0.030 *	0,0238 *	<0.001 *
10-11 vs. 12-13 p.m.	1.00 ns	0.174 ns	0.290 ns	0.035 *	7x10 ⁻³ *	0.694ns	0.683ns	0.217 ns	0,148 ns	0.082 ns
Pooled campaigns										
6-7 vs. 8-9 a.m.	0,015 *	0,083 ns	9x10 ⁻³ *	0,001*	0,004 *	0,023 *	9x10 ⁻³ *	0,253 ns	6x10 ⁻⁴ *	0,106 ns
6-7 vs. 10-11 a.m.	1,00 ns	0,187 ns	0,230ns	0,356ns	0,691ns	0,139ns	0,348 ns	0,253 ns	1,00 ns	0,382 ns
6-7 vs. 12-13 p.m.	0,708 ns	0,286 ns	0,024 *	0,757ns	0,915ns	0,057ns	0,102 ns	0,032 *	0,183 ns	0,506 ns
8-9 vs. 10-11 a.m.	0,038 *	0,008 *	2x10 ⁻³ *	0,005*	0,004 *	0,002 *	3x10 ⁻³ *	0,071 ns	0,002 *	0,030 *

	Parastar	Cp10	Cp20	Cp30	Ec10	Ec20	Ec30	Td10	Td20	Td30
2021 campaign					2022 campaign					
	Parastar	Cp20	Ec20	Td30	Pooled	Ec10	Ec20	Td10	Td30	Pooled
8-9 vs. 12-13 p.m.	0,012 *	0,022 *	2x10 ⁻⁴ *	0,001*	0,007 *	6x10 ⁻⁴ *	5x10 ⁻⁴ *	4x10 ⁻³ *	2x10 ⁻⁴ *	0,069 ns
10-11 vs. 12-13 p.m.	0,434 ns	0,474 ns	0,090ns	0,434ns	1.00 ns	0,366ns	0,243 ns	0,133 ns	0,081 ns	0,506 ns
	Td30	Pooled								
6-7 vs. 8-9 a.m.	0,051 ns	<0,001 *								
6-7 vs. 10-11 a.m.	0,416 ns	0,023 *								
6-7 vs. 12-13 p.m.	0,004 *	2x10 ⁻⁵ *								
8-9 vs. 10-11 a.m.	0,023 *	2x10 ⁻⁵ *								
8-9 vs. 12-13 p.m.	2x10 ⁻⁴ *	<0,001*								
10-11 vs. 12-13 p.m.	0,009 *	0,002 *								

High foraging speeds (7 ± 0 flowers per minute) were recorded in the untreated plots, in plots treated with 20% *Ca. procera*, 10% *E. camaldulensis*, 20% *Ti. diversifolia* and in the pooled plots respectively (Table 5C). The very low speed (6 ± 0 flowers per minute) was recorded in plots treated with Parastar, plots treated with 10% and 30% *Ca. procera*, 30% *E. camaldulensis*, and 10% or 30% *Ti. diversifolia* (Table 5C).

Between the two campaigns, the difference was significant between the foraging duration and speed in untreated plots, in plots treated with 10% *Ca. procera* or 30% *Ti. diversifolia* and in the pooled plots (Table 5D). In plots treated with 20% *E. camaldulensis*, the foraging durations were not different while it was the contrary in the foraging speed (Table 5D).

Furthermore, pairwise comparisons of the foraging durations recorded in plots (Table 5E) showed that during the 2021 campaign (Table 5E upper part matrix) and the 2022

campaign (Table 5E lower part matrix), the difference was significant when we compared the foraging duration recorded in untreated plots to that recorded in all treated plots. In 2021, significant differences were noted between the foraging duration recorded in plots treated with 10% *Ca. procera* and that noted in plots treated with 30% *Ca. procera* or that treated with 20% *E. camaldulensis*. It was the same between plots treated with 30% *Ca. procera* and that treated with 10% *Ti. diversifolia*, between plots treated with 20% *E. camaldulensi* and those treated with 10% *Ti. diversifolia* (Table 5E upper part matrix).

In the 2022 campaign, significant differences were noted between plots treated with 20% *E. camaldulensis* and plots treated with 10% or 30% *Ti. diversifolia* (Table 5E lower part matrix). The other comparisons were not significant (Table 5E).

Table 4. Estimation of the abundances of *Apis mellifera* per 1000 *Vigna unguiculata* flowers (A_{1000}).

	A_x		F_x		$V_{1000} = (A_x/F_x) * 1000$		
	Essays	Min.-Max.	Mean \pm se	Min.-Max.	Mean \pm se	Min.-Max.	Mean \pm se
A. 2021 campaign							
Untreated plots	362	1-9	3 \pm 0	5-689	184 \pm 10	1-800	31 \pm 3
Parastar	177	1-9	3 \pm 0	5-720	273 \pm 14	2-400	17 \pm 3
Cp10	215	1-9	3 \pm 0	5-689	260 \pm 13	1-800	24 \pm 4
Cp20	229	1-9	3 \pm 0	5-689	216 \pm 12	2-800	29 \pm 4
Cp30	214	1-9	3 \pm 0	5-689	256 \pm 13	1-400	19 \pm 2

	A_x			F_x		$V_{1000}=(A_x/F_x)*1000$	
	Essays	Min.-Max.	Mean \pm se	Min.-Max.	Mean \pm se	Min.-Max.	Mean \pm se
Ec10	225	1-9	3 \pm 0	5-689	257 \pm 12	1-800	23 \pm 4
Ec20	324	1-9	3 \pm 0	5-800	201 \pm 10	1-800	31 \pm 3
Ec30	205	1-9	3 \pm 0	5-700	260 \pm 13	1-400	18 \pm 2
Td10	185	1-9	3 \pm 0	5-689	289 \pm 14	1-800	23 \pm 5
Td20	316	1-9	3 \pm 0	5-689	199 \pm 10	1-800	28 \pm 3
Td30	204	1-9	3 \pm 0	5-689	269 \pm 13	1-400	19 \pm 2
Global	2,656	1-9	3 \pm 0	5-800	234 \pm 4	1-800	25 \pm 1
B. 2022 campaign							
Untreated plots	388	1-9	3 \pm 0	5-689	193 \pm 9	1-400	26 \pm 2
Parastar	191	1-9	3 \pm 0	24-800	269 \pm 14	1-179	16 \pm 1
Cp10	246	1-9	3 \pm 0	5-689	293 \pm 12	1-800	19 \pm 3
Cp20	332	1-9	3 \pm 0	5-689	223 \pm 10	1-800	24 \pm 3
Cp30	232	1-9	3 \pm 0	5-800	295 \pm 14	1-800	18 \pm 4
Ec10	247	1-9	3 \pm 0	5-700	274 \pm 12	1-800	22 \pm 4
Ec20	356	1-9	3 \pm 0	5-689	218 \pm 10	1-800	24 \pm 3
Ec30	247	1-9	3 \pm 0	5-800	298 \pm 13	1-800	18 \pm 3
Td10	228	1-9	3 \pm 0	5-689	239 \pm 13	1-400	22 \pm 2
Td20	241	1-9	3 \pm 0	5-689	239 \pm 12	1-800	24 \pm 4
Td30	218	1-9	3 \pm 0	5-689	249 \pm 13	1-400	20 \pm 2
Global	2,939	1-9	3 \pm 0	5-800	248 \pm 4	1-800	22 \pm 1
C. Pooled campaigns							
Untreated plots	750	1-9	3 \pm 0	5-689	189 \pm 7	1-800	28 \pm 2
Parastar	368	1-9	3 \pm 0	5-800	271 \pm 10	1-400	17 \pm 1
Cp10	461	1-9	3 \pm 0	5-689	277 \pm 9	1-800	22 \pm 3

Table 4. Continued.

	A_x			F_x		$V_{1000}=(A_x/F_x)*1000$	
	Essays	Min.-Max.	Mean \pm se	Min.-Max.	Mean \pm se	Min.-Max.	Mean \pm se
C. Pooled campaigns (Continued)							
Cp20	561	1-9	3 \pm 0	5-689	220 \pm 8	1-800	26 \pm 2
Cp30	446	1-9	3 \pm 0	5-800	276 \pm 9	1-800	19 \pm 2
Ec10	472	1-9	3 \pm 0	5-700	266 \pm 9	1-800	22 \pm 3
Ec20	680	1-9	3 \pm 0	5-800	210 \pm 7	1-800	27 \pm 2
Ec30	452	1-9	3 \pm 0	5-800	281 \pm 9	1-800	18 \pm 3
Td10	413	1-9	3 \pm 0	5-689	261 \pm 10	1-800	22 \pm 2

	A_x			F_x		$V_{1000}=(A_x/F_x)*1000$	
	Essays	Min.-Max.	Mean \pm se	Min.-Max.	Mean \pm se	Min.-Max.	Mean \pm se
Td20	557	1-9	3 \pm 0	5-689	216 \pm 8	1-800	26 \pm 2
Td30	422	1-9	3 \pm 0	5-689	259 \pm 9	1-400	19 \pm 2
Global	5,595	1-9	3 \pm 0	5-800	242 \pm 3	1-800	23 \pm 1

Cp10, Cp20 and Cp30: 10%, 20%, and 30% of *Calotropis procera*; Ec10, EC20 and Ec30: 10%, 20%, and 30% of *Eucalyptus camaldulensis*; Td10, Td20 and Td30: 10%, 20%, 30% of *Tithonia diversifolia*.

Pairwise comparisons of the foraging speeds (Table 5F) showed that during the 2021 campaign (Table 5F upper part matrix) and the 2022 campaign (Table 5F lower part matrix), the difference was significant when we compared the foraging speed recorded in untreated plots to that recorded in treated plots except comparisons in 2021 with the three concentrations of *E. camaldulensis* and 30% *Ti. diversifolia* (Table 5F upper part matrix) and it was the same in 2022 campaign (Table 5F lower part matrix). Between the treated plots, the difference was significant during each campaign, when we compared records in the plots treated with 20% *E. camaldulensis* to those noted in plots treated with Parastar or 10% *Ca. procera* (Table 5F).

4. Discussion

Floricultural entomofauna study on cowpea in Dang allowed the capture of 8,987 insects (4,392 specimens i.e. 48.9% in 2021 campaign, and 4,595 specimens i.e. 51.1% in 2022 campaign). Specimens belonged to eight species, four families and four orders. Hymenoptera was the most species-rich with four species (50.0%) followed by Lepidoptera with two species (25.0%). Hemiptera and Orthoptera were rare (one species each) (12.5%). Our records were low compared to the reports in South Cameroon on Cucurbit flowers where 66 morphospecies belonged to 37 families and five orders, Diptera (especially Tephritidae and Lonchaeidae) and Hymenoptera (Braconidae and Eulophidae) being mostly abundant and species-rich [53]. We did not find Diptera in our collec-

tion and families of Hymenoptera (Tephritidae and Lonchaeidae), certainly due to the short duration of the collections (five days from the first flowering date of the plants, on the first produced flowers). Then the existing flowers were few in number to produce enough attractive perfume for the potential floricultural insects from long distances if we look at the potential of each plant and of all plants in the cultivated plots, to produce attractive perfume for insects [54]. The extension of the control sessions until the period of maximum flowering of the plants would have made it possible to capture many more other floricultural insects. Given that the flower-dwelling insects came naturally from the surrounding savannah vegetation and that very few of them were attracted to the plants grown in our plots, it is obvious that the majority of floricultural insects were busy exploiting several other nectar-producing plants in neighboring fallows, more productive than the young flowering cowpea plants. Indeed, bees are well known for their strong ability to orient themselves in nature and memorize or even recognize the shape, color and smell of product-rich flowers visited during previous foraging trips [54-57]. For illustration, in the United States of America, surveys showed that some honey bee foragers were constant on the flowers of the same avocado tree for a minimum of 24 hours [58]. It is therefore likely that during the period of intense flowering, cowpea plants would attract many more insects compared to the present situation. But our main objective was just to identify the main pollinating insect of the locality and to evaluate the effect of botanical pesticides on their behavior and not to study the entomofauna associated with the stages of plant development.

Table 5. Foraging speed of *Apis mellifera* foragers on the blooming flowers of *Vigna unguiculata*.

	Visited flowers F_i			Foraging duration (seconds): d_i		$V_b=(F_i/d_i)*60$	
	Essays	Min.-Max.	Mean \pm se	Min.-Max.	Mean \pm se	Min.-Max.	Mean \pm se
A. 2021 campaign							
Untreated plots	221	1-3	1 \pm 0	2.0-60.0	13.5 \pm 0.6	1-60	9 \pm 1
Parastar	116	1-3	1 \pm 0	3.0-60.0	22.1 \pm 1.3	1-20	6 \pm 0

	Essays	Visited flowers F_i		Foraging duration (seconds): d_i		$V_b=(F_i/d_i)*60$	
		Min.-Max.	Mean \pm se	Min.-Max.	Mean \pm se	Min.-Max.	Mean \pm se
Cp10	140	1-3	1 \pm 0	3.0-60.0	23.5 \pm 1.3	1-20	5 \pm 0
Cp20	156	1-3	1 \pm 0	3.0-60.0	18.9 \pm 1.1	1-30	7 \pm 0
Cp30	102	1-3	1 \pm 0	3.0-56.0	17.6 \pm 1.1	1-24	6 \pm 0
Ec10	150	1-3	1 \pm 0	3.0-60.0	20.2 \pm 1.3	1-40	7 \pm 1
Ec20	201	1-3	1 \pm 0	1.0-60.0	17.8 \pm 1.0	1-40	9 \pm 1
Ec30	120	1-3	1 \pm 0	3.0-60.0	20.3 \pm 1.3	1-40	7 \pm 0
Td10	145	1-3	1 \pm 0	3.0-60.0	23.3 \pm 1.3	1-20	5 \pm 0
Td20	160	1-3	1 \pm 0	3.0-60.0	18.8 \pm 1.0	1-30	7 \pm 0
Td30	111	1-3	1 \pm 0	3.0-60.0	22.1 \pm 1.4	1-30	6 \pm 0
Global	1622	1-3	1 \pm 0	1.0-60.0	19.4 \pm 0.3	1-120	7 \pm 0
ANOVA		$F_{(10; 1611)}=0.043, p=1.00$ ns		$F_{(10; 1611)}=8.130, p<0.001$ *		$F_{(10; 681)}=4.23, p=1 \times 10^{-5}$ *	
B. 2022 campaign							
Untreated plots	230	1-3	1 \pm 0	3.0-60.0	18.4 \pm 1.0	1-24	5 \pm 0
Parastar	76	1-3	1 \pm 0	3.0-60.0	22.2 \pm 1.6	1-20	5 \pm 0
Cp10	124	1-3	1 \pm 0	3.0-60.0	19.1 \pm 1.2	1-24	7 \pm 0
Cp20	187	1-2	1 \pm 0	3.0-60.0	19.3 \pm 1.1	1-30	7 \pm 0
Cp30	103	1-2	1 \pm 0	3.0-60.0	20.9 \pm 1.4	1-30	6 \pm 0
Ec10	122	1-3	1 \pm 0	3.0-60.0	19.2 \pm 1.2	1-60	7 \pm 0
Ec20	214	1-2	1 \pm 0	3.0-60.0	19.3 \pm 1.0	1-24	7 \pm 0
Ec30	108	1-2	1 \pm 0	3.0-60.0	20.4 \pm 1.4	1-30	6 \pm 0
Td10	134	1-3	1 \pm 0	3.0-60.0	20.6 \pm 1.2	1-60	6 \pm 1
Td20	166	1-2	1 \pm 0	3.0-60.0	19.4 \pm 1.1	1-24	7 \pm 0
Td30	105	1-2	1 \pm 0	3.0-60.0	22.9 \pm 1.6	1-30	6 \pm 0
Global	1569	1-3	1 \pm 0	3.0-60.0	19.8 \pm 0.4	1-60	7 \pm 0
ANOVA		$F_{(10; 1558)}=0.100, p=1.00$ ns		$F_{(10; 1558)}=1.164, p=0.311$ ns		$F_{(10; 627)}=0.40, p=0.948$ ns	
C. Pooled campaigns							
Untreated plots	451	1-3	1 \pm 0	2.0-60.0	16.0 \pm 0.6	1-60	7 \pm 0

Table 5. Continued.

	Essays	Visited flowers F_i		Foraging duration (seconds): d_i		$V_b=(F_i/d_i)*60$	
		Min.-Max.	Mean \pm se	Min.-Max.	Mean \pm se	Min.-Max.	Mean \pm se
Parastar	192	1-3	1 \pm 0	3.0-60.0	22.1 \pm 1.0	1-20	6 \pm 0
Cp10	264	1-3	1 \pm 0	3.0-60.0	21.5 \pm 0.9	1-24	6 \pm 0
Cp20	343	1-3	1 \pm 0	2.0-60.0	19.1 \pm 0.8	1-30	7 \pm 0
Cp30	205	1-3	1 \pm 0	3.0-60.0	19.3 \pm 0.9	1-30	6 \pm 0

	Essays	Visited flowers F_i		Foraging duration (seconds): d_i		$V_b=(F_i/d_i)*60$	
		Min.-Max.	Mean \pm se	Min.-Max.	Mean \pm se	Min.-Max.	Mean \pm se
Ec10	272	1-3	1 \pm 0	3.0-60.0	19.8 \pm 0.9	1-40	7 \pm 0
Ec20	415	1-3	1 \pm 0	1.0-60.0	18.6 \pm 0.7	1-120	8 \pm 0
Ec30	228	1-3	1 \pm 0	2.0-60.0	20.3 \pm 0.9	1-30	6 \pm 0
Td10	279	1-3	1 \pm 0	2.0-60.0	22.0 \pm 0.9	1-60	6 \pm 0
Td20	326	1-3	1 \pm 0	3.0-60.0	19.1 \pm 0.8	1-30	7 \pm 0
Td30	216	1-3	1 \pm 0	3.0-60.0	22.5 \pm 1.1	1-30	6 \pm 0
Global	3191	1-3	1 \pm 0	1.0-60.0	19.6 \pm 0.2	1-120	7 \pm 0
ANOVA		$F_{(10; 3180)}=0.096, p=1.00$ ns		$F_{(10; 3180)}=6.23, p<0.001$ *		$F_{(10; 1319)}=3.61, p=1 \times 10^{-4}$ *	

D. 2021 campaign vs. 2022 campaign: Student t-test

	Visited flowers F_i	Duration (seconds): d_i	Foraging speed V_b
Untreated plots	t= 0.258; df=449; p=0.796 ns	t=-4.266; df=449; p=2x10 ⁻⁵ *	t=3.473; df=449; p=8x10 ⁻⁴ *
Parastar	t=-0.156; df=190; p=0.876 ns	t=-0.024; df=190; p=0.981 ns	t=0.340; df=190; p=0.734 ns
Cp10	t=0.018; df=262; p=0.986 ns	t=2.457; df=262; p=0.015 *	t=-2.200; df=262; p=0.029 *
Cp20	t=-0.189; df=341; p=0.850 ns	t=-0.305; df=341; p=0.760 ns	t=0.019; df=341; p=0.985 ns
Cp30	t=0.328; df=203; p=0.743 ns	t=-1.814; df=203; p=0.071 ns	t=-0.573; df=203; p=0.568 ns
Ec10	t=-0.225; df=270; p=0.822 ns	t=-0.590; df=270; p=0.555 ns	t=-0.461; df=270; p=0.645 ns
Ec20	t=-0.144; df=413; p=0.885 ns	t=-1.021; df=413; p=0.308 ns	t=2.333; df=413; p=0.020 *
Ec30	t=-0.373; df=226; p=0.710 ns	t=-0.043; df=226; p=0.966 ns	t=-0.514; df=226; p=0.607 ns
Td10	t=-0.154; df=277; p=0.878 ns	t=1.546; df=277; p=0.123 ns	t=1.558; df=277; p=0.120 ns
Td20	t=0.492; df=324; p=0.623 ns	t=-0.374; df=324; p=0.708 ns	t=0.034; df=324; p=0.973 ns
Td30	t=-0.476; df=665; p=0.634 ns	t=-3.219; df=665; p=1.4x10 ⁻³ *	t=3.082; df=665; p=2.1x10 ⁻³ *
Global	t=0.673; df=3189; p=0.501 ns	t=-0.825; df=3189; p=0.409 ns	t=2.008; df=3189; p=0.045 *

E. Student-Newman-Keuls p-values for foraging duration 2021 campaign (upper part matrix) and 2022 (lower matrix)

	Parastar	Cp10	Cp20	Cp30	Ec10	Ec20	Ec30	Td10	Td20	Td30
Untreated	3x10 ⁻⁵ *	1x10 ⁻⁵ *	0.002 *	0.012 *	6x10 ⁻⁵ *	0.004 *	3x10 ⁻⁴ *	1x10 ⁻⁵ *	0.001 *	1x10 ⁻⁵ *
Parastar	-	0.841ns	0.208 ns	0.188 ns	0.500 ns	0.073 ns	0.304 ns	0.757 ns	0.272 ns	0.994 ns
Cp10	0.868 ns	-	0.050 ns	0.030 *	0.305 ns	0.004 *	0.310 ns	0.896 ns	0.056 ns	0.697 ns
Cp20	0.202 ns	0.243ns	-	0.894 ns	0.381 ns	0.749 ns	0.662 ns	0.052 ns	0.976 ns	0.300 ns
Cp30	0.319 ns	0.330ns	0.905ns	-	0.574 ns	0.916 ns	0.697 ns	0.034 *	0.773 ns	0.238 ns
Ec10	0.375 ns	0.338ns	0.836ns	0.700 ns	-	0.354 ns	0.968 ns	0.290 ns	0.633 ns	0.679 ns

Table 5. Continued.

	Parastar	Cp10	Cp20	Cp30	Ec10	Ec20	Ec30	Td10	Td20	Td30
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E. Student-Newman-Keuls p-values for foraging duration 2021 campaign (upper part matrix) and 2022 (lower matrix) (Continued)

	Parastar	Cp10	Cp20	Cp30	Ec10	Ec20	Ec30	Td10	Td20	Td30
Ec20	0.081 ns	0.114ns	0.845ns	0.933 ns	0.802 ns	-	0.510 ns	0.005 *	0.486 ns	0.103 ns
Ec30	0.552 ns	0.371ns	0.741ns	0.709 ns	0.652 ns	0.638 ns	-	0.272 ns	0.806 ns	0.561 ns
Td10	0.930 ns	0.644ns	0.104 ns	0.203 ns	0.232 ns	0.030 *	0.367 ns	-	0.060 ns	0.488 ns
Td20	0.253 ns	0.324ns	0.994 ns	0.991 ns	0.941 ns	0.592 ns	0.850 ns	0.142 ns	-	0.359 ns
Td30	0.780 ns	0.843ns	0.095 ns	0.204 ns	0.256 ns	0.025 *	0.466 ns	0.917 ns	0.121 ns	-
Untreated	3x10 ⁻⁵ *	4x10 ⁻⁵ *	0.011 *	0.046 *	0.006 *	0.008 *	0.003 *	1x10 ⁻⁵ *	0.007 *	2x10 ⁻⁵ *

F. Student-Newman-Keuls p-values for foraging speed 2021 campaign (upper diagonal matrix) and 2022 (lower matrix)

Untreated	0.007*	0.002 *	0.077ns	0.040*	0.058ns	0.810ns	0.085ns	0.002 *	0.043 *	0.055 ns
Parastar	-	0.936ns	0.710ns	0.588ns	0.723ns	0.012 *	0.821ns	0.995ns	0.701ns	0.776 ns
Cp10	0.597ns	-	0.688ns	0.793ns	0.683ns	0.005 *	0.835ns	0.986ns	0.734ns	0.858 ns
Cp20	0.527ns	0.729ns	-	0.912ns	0.909ns	0.076ns	0.767ns	0.735ns	0.913ns	0.897 ns
Cp30	0.917ns	0.988ns	0.688ns	-	0.929ns	0.058ns	0.952ns	0.908ns	0.873ns	0.887 ns
Ec10	0.561ns	0.764ns	0.907ns	0.748ns	-	0.040 *	0.914ns	0.721ns	0.953ns	0.929 ns
Ec20	0.020 *	0.036 *	0.200ns	0.059ns	0.132ns	-	0.094ns	0.005 *	0.058ns	0.073 ns
Ec30	0.843ns	0.950ns	0.751ns	0.842ns	0.853ns	0.141ns	-	0.882ns	0.939ns	0.922 ns
Td10	0.807ns	0.921ns	0.703ns	0.958ns	0.748ns	0.036 *	0.919ns	-	0.806ns	0.926 ns
Td20	0.807ns	0.940ns	0.524ns	0.884ns	0.747ns	0.078ns	0.892ns	0.921ns	-	0.734 ns
Td30	0.872ns	0.961 ns	0.788ns	0.738ns	0.854 ns	0.129ns	0.830 ns	0.915ns	0.930 ns	-
Untreated	0.009*	0.015 *	0.172ns	0.031 *	0.163ns	0.734ns	0.094ns	0.016 *	0.050ns	0.080 ns

Min.: minimum value; Max.: maximum value; n: sample size; V_b : bee's foraging speed (flowers per minute); ns: not significant variation ($p \geq 0.05$); *: significant variation ($p < 0.05$)

During the observation periods, *Ap. mellifera* was the most frequent floricultural insect on *V. unguiculata* blooming flowers with 25.7% of occurrence during the two years of study. This observation is reminiscent of that reported in Cameroon by Kengni *et al.* [59], Djonwangwe *et al.* [37], Mbianda *et al.* [23], Adamou *et al.* [20, 37]. In Benin, the most frequent insect on cowpea flowers was reported as *X. olivacea* [34] while in Nigeria, *Ap. mellifera* and *X. olivacea* predominated on *V. unguiculata* flowers [36] and in Ghana, *Ap. mellifera* and *Halictus* sp. Predominated on cowpea flowers [35]. The high abundance of *Ap. mellifera* among the floricultural insects was not surprising because intense traditional beekeeping activity was practiced in the Dang locality and neighboring villages, with many hives scattered across the savannah, making Adamaoua (North Cameroon) one of the main honey producing regions in Cameroon [60, 61]. In addition, market gardening activity was carried out during dry periods along rivers and lakes, and in non-irrigated areas during the rainy season [62, 63]. *Ap. mellifera* foragers collected pollen and nectar from cowpea flowers, and their activity was noted from 6 a.m. to 13 p.m. with a peak of activity between 8 and 9 a.m.. This would be linked to the period

of greater availability of floral products at the flower level, the good attractiveness of the floral products towards the honey bee and the combination of scents of flowers and botanical products [54]. Our observations therefore show a slight shift and were a little earlier than what reported on the same bee at Obala locality (Centre region of Cameroon) where the peak activity was reported between 9 and 10 a.m. [23]. This time difference could be linked to the different ambient climatic conditions between the two localities (the central region of Cameroon with an equatorial forest climate and the Dang locality with a Sudano-Guinean climate) [41-43, 45]. During the flowering period of *V. unguiculata*, foragers of *Ap. mellifera* collected nectar and pollen from the flowers. Similar behavior of the same bee species was reported in Bɛɛabo (Centre region of Cameroon) [23]. On the other hand, in the Ngaoundere region, which is an area with high beekeeping activity and high honey production in Cameroon [60], it has been reported that the foraging workers of this domestic bee collect more nectar than pollen during the day [18, 20]. When visiting a flower for pollen collection, *Ap. mellifera* forager rubbed anthers with the metathoracic legs and harvested pollen and carried them in the metathoracic

leg baskets while for nectar harvesting, the bee spread the wings, and introduced the proboscis into the flower base and sucked the produced nectar liquid. In the Adamaoua Region, among the pesticides approved in Cameroon [13], the main insecticide usually used by farmers was Parastar (pers. com.) in which imidacloprid and lambda-cyhalothrin are known to present a strong persistence in nature, the chronic contact contaminating pollen and nectar and then indirectly affect honeybees and bumblebees [64]. A relatively long half-life in soils (32 days in sandy loam soils, 38 days in loamy sand soils, 43 days in clay loam soils) have being reported in imidacloprid (the most widely used neonicotinoid pesticide) [65-69] and in the moderately neurotoxic pyrethroid insecticide lambda-cyhalothrin (30 days in average with values ranging from 28-84 days) [70-72]. It was certain that the five-days study was insufficient, the synthetic pesticide not having acted sufficiently. However, interesting information was noted: the little effect during the two campaigns of Parastar on *Ah. crassivora*, certainly due to the cleaning of the product by rainwater, or a reinforcement of the insects from neighboring fallows. A similar phenomenon was reported in eggplant crops in Balessing (West Cameroon) [73]. The average abundance per 1000 flowers highlighted the good attractiveness of nectar and pollen towards foragers in plots treated with 20% *E. camaldulensis* or *C. procera*. Bee foragers are known to be able to recruit a large number of congeners to exploit an interesting food source [74, 75]. The low abundance of foragers in Parastar treated plots could be explained by the repellent effect of the synthetic insecticide on the floricultural insects [12]. The duration of the flower visit varied depending on the availability of nectar or pollen, and the bees stayed longer on flowers very rich in collected products than on flowers probably very poor in collected products. Consequently, the foraging visit varied according to the type of treatment, which justified the differential effectiveness of these products. The variations observed in foraging speeds could be due to availability of floral products, accessibility, the distances separating the exploited flowers during different foraging trips and also the influence of the aqueous extracts of the plants under investigation. Thus, botanical insecticides and floral scents would constitute an important factor in reinforcing foraging behavior. Although the leaves aqueous extracts of the three tested plants could have harmful effects on pollinators in general [27], tested doses (10% and 20%) were of little harm to bees. In general, the harmful effect of Parastar and 30% concentrations was noted on *Ap. mellifera*. Botanical pesticides showed very little effect on *Ah. crassivora* compared to the untreated plots, and the concentration of 30% of aqueous extracts was toxic for pollinators (10% and 20% concentrations of aqueous extracts presented an average tolerable effect for floricultural insects). In Cameroon, synthetic pesticides, although approved [13], are frequently manipulated in an anarchic and uncontrolled manner by non-expert farmers [28, 30]. The disruptive effect of synthetic pesticides on the memory and foraging behavior

of pollinators is well known, with contaminated bees not able to return to the feeding site in the same way as uncontaminated bees [25].

5. Conclusions

The present study aimed to determine the main floricultural insects on *Vigna unguiculata* (Fabales: Fabaceae), and to study the activity of *Apis mellifera* (Hymenoptera: Apidae) on the flowers under treatment using botanical insecticides compared to untreated plots and Parastar treated plots. In Dang (Ngaoundere suburb in Adamaoua Region, Cameroon), among floricultural insects on *V. unguiculata*, *Ap. mellifera* was the most recorded. Untreated plots and those treated with 10% or 20% *E. camaldulensis* or *Ca. procera* allowed normal activity of the bee. Plots treated with 30% botanical extract and even Parastar altered the rhythm and speed of nectar and pollen collection in *Ap. mellifera*. The use of 10% or 20% *E. camaldulensis* or *Ca. procera* may be recommended for insect pest control.

Abbreviations

ANOVA	Analysis of Variance
<i>Ah. crassivora</i>	<i>Aphis crassivora</i> Koch, 1854
<i>Am. calens</i>	<i>Amegilla calens</i> (Le Peletier, 1841)
<i>Ap. mellifera</i>	<i>Apis mellifera</i> Linnaeus, 1758
<i>Ca. procera</i>	<i>Calotropis procera</i> (Aiton) W. T. Aiton, 1811
<i>E. camaldulensis</i>	<i>Eucalyptus camaldulensis</i> Dehnh., 1832
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
<i>H. misippus</i>	<i>Hypolimnas misippus</i> (Linnaeus, 1764)
MINADER	Ministry of Agriculture and Rural Development (Cameroon)
MINEF	Ministry of Environment and Forestry (Cameroon)
<i>V. unguiculata</i>	<i>Vigna unguiculata</i> (L.) Walp., 1843
<i>Ti. diversifolia</i>	<i>Tithonia diversifolia</i> (Hemsl.) A. Gray, 1883
<i>X. caffra</i>	<i>Xylocopa caffra</i> (L. 1767)
<i>X. erythrina</i>	<i>Xylocopa erythrina</i> Gribodo 1894
<i>X. imitator</i>	<i>Xylocopa imitator</i> Smith, 1854
<i>X. inconstans</i>	<i>Xylocopa inconstans</i> Smith F. 1874
<i>X. nigrita</i>	<i>Xylocopa nigrita</i> (Fabricius 1775)
<i>X. olivacea</i>	<i>Xylocopa olivacea</i> (Fabricius 1778)

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Data Availability Statement

The data supporting the outcome of this research work is available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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Research Field

Ta m̄anga: Botanical insecticides, Bees, Biological agriculture, Yield agricultural products, Coton (*Gossypium hirsutum*), Pollinization

Moukhtar Mohammadou: Botanical insecticides, Bees, Biological agriculture, Yield agricultural products, Coton (*Gossypium hirsutum*), Pollinization

Mo ñe Adamou: Botanical insecticides, Bees, Biological agriculture, Yield agricultural products, Coton (*Gossypium hirsutum*), Pollinization

Ousmana Youssoufa: Botanical insecticides, Bees, Biological agriculture, Yield agricultural products, Coton (*Gossypium hirsutum*), Pollinization

Boris Fouelifack-Nintidem: Pest control, Entomology, Biology of animal populations, Insects ecology, Applied entomology, Animal ethology

Odette Massah Dabole: Botanical insecticides, Bees, Biological agriculture, Yield agricultural products, Coton (*Gossypium hirsutum*), Pollinization

Oumarou Abdoul Aziz: Biopesticides, Insect pest control, Biological agriculture, Bees, Biological agriculture, Pollinization

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Abdel Kayoum Yomon: Pest control, Entomology, Biology of animal populations, Insects ecology, Applied entomology, Animal ethology

Sedrick Junior Tsekane: Applied zoology, Quality of life and biostatistics, Wildlife Protection, Control of protected areas, Animal Ethology, Animal Ecology

Pharaon Auguste Mbianda: Applied entomology, Insects biology, biostatistics and pollinators, Apiculture, Insects-plants interactions, Animal Ethology, Animal Ecology

Martin Kenne: Biostatistics, Biology of the Animal Populations, Entomology, Myrmecology, Animal Ethology, Animal Ecology, sociobiology, Applied entomology, plant protection, Biological control, pest insects