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Seed Yield Improvement in *Vigna unguiculata* (L.) (Fabaceae): Efficiency of Pollinators and Impact of Aqueous Leaf Extract of Three Plant Species in North Cameroon

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MM, MA, Taïmanga and DK contributed, under the control of MK, to the establishment of the research protocol and the field experiments. The creation of crop plots and the daily monitoring of crops were done by Author MM who wrote the first draft of the manuscript. Authors MA, Taïmanga and MK managed the literature searches, performed the data analysis and corrected the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Because of the problems in agroecosystems following the anarchic use of synthetic insecticides, studies propose an alternative, the use of botanical biopesticides against pests.
Study Design: The present study was conducted to evaluate (1) the potential of leaf extract of *Calotropis procera* (Gentianales: Apocynaceae)*, Eucalyptus camaldulensis* (Myrtales: Myrtaceae) and *Tithonia diversifolia* (Asterales: Asteraceae) against insects and (2) impact of *Apis mellifera* (Hymenoptera: Apidae) on *Vigna unguiculata* (Fabales: Fabaceae) seed yield in North Cameroon.
Place and Duration of Study: A field study was set up in 2021 in North Cameroon, during the rainy season. Fourthy four plots of 4x3.5 m each were distributed according to a randomized complete block design model comprising four untreated, four treated using parastar (40EC 535/10/IN, 20 g/l imidaclopride and 20 g/l lamda-cyhalothrine), and 36 plots treated using 10%, 20% and 30% aqueous leaf extracts separately sprayed against *Aphis craccivora* (Hemiptera: Aphididae).
Methodology: Four groups of flowers were randomly selected: (1) free, (2) protected from insects, (3) free exclusively to *Ap. mellifera* and (4) protected against insects.
Results: A total of 10,984 captured flower insects belonged to three orders (Hemiptera, Hymenoptera and Lepidoptera), three families (Aphididae, Apidae and Nymphalidae) and seven species [one (14.3%) sap-sucking *Aphis craccivora* (Hemiptera: Aphididae), four (57.1%) pollinators Hymenoptera Apidae [*Amegilla calens, Amegilla* sp., *Apis mellifera* and *Xylocopa olivacea*] and two (28.6%) Lepidoptera Nymphalidae [*Danaus plexippus* and *Hypolimnas misippus*]. A total of 7,425 insects associated with *V. unguiculara* corresponded to four orders [Hemiptera (56.7%), Coleoptera (41.5%), Heteroptera and Orthoptera with 0.9% respectively], nine families [Aphididae (45.3%), Chrysomelidae (38.7%), Pyrrhocoridae (4.8%), Coreidae (3.8%), Cicadellidae (2.8%), Coccinellidae (1.9%), Alydidae, Tenebrionidae and Tettigoniidae with 0.9% respectively], 11 genera and 11 species.
Conclusion: *Apis mellifera* was the major pollinator and *Aphis crassivora* the major pest. The seed yield was improved by 30% extract of plants without impact on pollinators.

Keywords: Apis mellifera; Leaf Extract; Vigna unguiculata; Calotropis procera; Eucalyptus camaldulensis; Thitonia diversifolia; seed yield; North-Cameroon.

1. INTRODUCTION

Cowpea is an important grain legume widely grown in Sub-Saharan Africa for food and feed because grain contains high levels of protein, energy, micro- and macro-nutrients [1]. In Africa, production is considerably low due to abiotic and biotic stresses, and socio-economic constraints including the lack of improved varieties, disease and insect pests, drought, poor access to extension, poor access to credit services, low soil fertility, farmland shortage, inappropriate agronomic practices and storage pests [1, 2]. Among insects associated with the cowpea, two Hymenoptera Apidae [*Apis. mellifera* Linnaeus, 1758 and *Xylocopa olivacea* (Fabricius, 1778)] and one Halictidae (*Halictus* sp. Latreille, 1804) are frequently cited as useful pollinators [3]. In market garden crops, it is known that the beneficial activity of pollinators is counterbalanced by that of harmful phytophagous, borers and sap-sucking insects [4-8]. These insects reduce the photosynthetic potential of the plants, the quality of the seed and negatively affect yield. Many animal organisms such as bacteria and predators can protect plants against pests [9, 10] while several useful insects facilitate the pollination [11]. More than 70% of agricultural production would suffer colossal on-farm and post-harvest losses without proactive and preventive measures [12]. To improve yield and meet the ever-increasing market demand, producers generally use synthetic chemicals in abusive and inadequate manner, leading to harmful effects on humans, environment, flower insects, pest resistance and this is expected to be further amplified by the impacts of climate change [13, 14]. The negative consequences related to the inappropriate overuse of synthetic chemicals have necessitated the need of alternative methods of pest management among which is the search for genetic varieties resistant to pests [15]. Nowadays, there is a greater focus on botanical pesticides as new effective alternative of crop pest control, preserving useful pollinators. For this purpose, many natural additives from plants have been reported effective in controlling pest

insects. Leaf aqueous extract of several plant species were reported effective against pest insects [16]. The relationships between floricultural plants and their pollinators have been intensively studied in Cameroon [3, 16]. However, in the northern savannah region of the country, despite the diversified flora and a flourishing market gardening activity, there is very little information on the insecticidal potential of the local plant species extracts against pest insects [17], except few works, for example those on leaf extract of *Gnidia kaussiana* Meisner (Myrtyales: Thymeleaceae) and *Ocimum canum* Sims, 1824 (Lamiales: Lamiaceae) against *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae) [18] and that on aqueous extracts of *Cassia occidentalis* L. *(= Senna occidentalis* (L.) Link, 1829), *Eucalyptus camaldulensis* Dehnh., 1832 and *Hyptis suaveolens* (L.) Poit., 1806 on the entomofauna and the seed yield of *Gossypium hirsutum* L., 1753 [19]. In short, nothing is said about the insecticidal aptitude of common wild plant species, easily accessible and exploitable as botanical pesticides against crop pests, able to replace synthetic pesticides. The present study aimed to establish a baseline of information on the effect of aqueous extracts of three local wild plant species on the pest insects and seed yield of cowpea in Garoua and Ngaoundere (North-Cameroon).

2. MATERIALS AND METHODS

2.1 Study Site

The study was conducted from 22 June to 25 August 2021, during the rainy season, in the North Cameroon. Two sites were selected due to the collaboration of landowners and the availability of cultivable plots and georeference coordinates were taken using a Garmin GPS. The plots were therefore delimited in Bockle (9°17′29.81″N, 13°25′4.39″E, and 169 m a.s.l.) Bockle and in Dang (7°25'26.42"N, 13°32'24.46"E, 1107.40 m a.s.l.). Bockle is a third suburb district of Garoua (North region) and Dang is a third suburb district of Ngaoundere (Adamaoua Region). Both localities are situated in the high Guinean wooded tropical savannah [20] and correspond to the sudano-Sahelian agro-ecological zone, with a semi-arid and a unimodal rainfall [21, 22]. The prevaling climate both localities is globally tropical Sudano-Guinean with two seasons: a rainy season (from April to October of the same year) and a dry season (from November to March of the following year) [23].

The temperature averages 22.9°C and the precipitation is about 2,248 mm per year. The lowest relative humidity is in February (21.7%), and the average annual hygrometry is 70% [21, 22]. Frequently observed plants were *Cosmos sulphureus* Cav., 1791 (Asterales: Asteraceae), *Helianthus annuus* L., 1753 (Asterales: Asteraceae), *Tithonia diversifolia* (Hemsley) Gray, 1883 (Asterales: Asteraceae), *Cajanus cajan* (L.) Huth, 1893 (Fabales: Fabaceae), *Phaseolus vulgaris* L., 1753 (Fabales: Fabaceae) and *Sesamum indicum* L. (1753) (Scrophulariales: Pedaliaceae). The floristic species encountered along the waterways consisted of *Bombax costatum* Pellegr. and Vuillet, 1914 (Malvales: Malvaceae), *Borassus aethiopium* Mart., 1838 (Arecales: Arecaceae), *Boswellia dalzielii* Hutch., 1910 (Sapindales: Burseraceae), *Commiphora africana* (A. Rich.) Engl., 1883 (Sapindales: Burseraceae), *Hyparrhemia rufa* (Nees) Stapf, 1919 (Poales: Poaceae), *Lannea microcarpa* Engl. and K. Krause, 1911 (Sapindales: Anacardiaceae), *Prosopis africana* (Guill. and Perr.) Taub., 1893 (Fabales: Fabaceae) and *Vittellaria paradoxa* C. F. Gaertn., 1807 (Ebenales: Sapotaceae). Plantations of *Azadirachta indica* A. Juss., 1830 (Sapindales: Meliaceae). *Eucalyptus camaldulendis* (Myrtales: Myrtaceae), *Cassia occidentalis* (Fabales: Caesalpiniaceae) and *Hyptis suaveolensis* (Lamiales: Lamiaceae) are found. Cultivated areas were small plots of polycultures family farms.

2.2 Sample Design

Field experimental design was set up according to the randomized complete block procedure with four replications using 44 plots of 4x3.5 m spaced 1 m apart. Three packets of cowpea seeds (variety Fenkem) were obtained from IRAD Garoua. After the first rains, sowing was done in rows (at 36.4 cm intra-row spacing and 50 cm inter-row spacing and thinned 14 days after sowing to two plants per hill. Six rows were formed per plot and each row consisted of eight bunches. Ten seedlings were positioned per plot (total: 440 seedlings). From germination to the appearance of the first flowers, weeding was carried out with bare hands and a hoe. Ten hives of *Apis mellifera* (Hymenoptera: Apidae) were installed around the plots of each study site. Plots were subjected to the same climate. Leaf

extract of three plant species were tested against pest insects including *Aphis craccivora* Koch, 1854 (Hemiptera: Aphididae). These plants were (1) *Calotropis procera* (Aiton) Aiton, 1811 (Gentianales: Apocynaceae), (2) *Eucalyptus camaldulensis* (Myrtales: Myrtaceae) both from Bockle, and (3) *Tithonia diversifolia* (Asterales: Asteraceae) from Dang. Collected leaves were dried, powdered with a mortar and stored in labeled plastic boxes. One hundred grams of each powder was diluted in one litter distilled water from which we formed three concentrations (10%, 20% and 30%) left for maceration during 12 hours. Leaves residues were removed, the solution filtered using a 0.2 mm mesh-sized sieve and stored in labeled closed plastic containers against chemical contaminants. Each extract concentration was introduced in a manual piston sprayer for field application. Two weeks after sowing, 32 plants were labeled for insect collection set up from 6:00 a.m. to 10:30 a.m. and for foraging behavior of pollinators on 1,000 flowers of group 1 from 7 a.m. to 6 p.m. (six time periods of 1 hr each and 1 hr interval). Plants were inspected and an insect found on leaves and flowers were counted. Collected insects were stored in labelled tubes containing 70° alcohol and butterflies were kept in folded A4 size paper devices. The number of visits and the quality of harvested products were determined. Except *Apis mellifera* (Hymenoptera: Apidae), two to three active insects were captured using a sweep net and stored in labeled vials containing 70% ethanol. Adults of butterflies were conserved in A4 size paper devices folded to keep wings intact.

2.3 Chemical Treatment

Plots were treated between 7 a.m. and 9 a.m. or between 12 a.m. and 5 p.m. We used two categories of products: (1) the synthetic insecticide Parastar [10% composed of 40EC 535/10/IN (20 g/l Imidacloprid and 20 g/l lamda-cyhalothrin, one l p.c./ha)] approved in Cameroon and usually used by farmers [13, 24], and (2) three concentrations (10%, 20% and 30%) of aqueous leaf extract of three plants species. In each locality, we considered: four untreated plots, four treated plots using Parastar, four plots for each leaf extract concentrations (10%, 20% and 30%), 12 plots for each botanical pesticide and 36 plots for all three botanical plants. At flowering, we divided the inflorescences into four groups (group 1: free flowers, group 2: protected flowers from insects with plastic bags, group 3: protected flowers opened exclusively to *Apis mellifera* (Hymenoptera: Apidae) and group 4: protected flowers opened from time to time without any insect visit). Plant extracts (714 l/ha) including parastar insecticide were sprayed in the evening using hand sprayers, at sunset (5 pm), two weeks after sowing, and repeated every two weeks until harvest. Flower visitation was recorded, concerned insects were identified or captured and the duration of each visit was recorded. Flower buds were grouped as described above (360 flowers for groups 1 and 2 respectively, 600 flowers for group 3 and 300 flowers for group 4). We recorded the population evolution of *Aphis craccivora* (Hemiptera: Aphididae) and that of pollinators.

2.4 Identification of Insect Specimens

Plants were identified *in situ* or photographed and a sample of leaves, bark, flowers and fruits allowed identification in the laboratory. Insects were identified to the species level using a magnifying glass, keys and illustrated catalogues [25-31] in the Laboratory of Applied Zoology, Department of Biological Sciences, Faculty of Science, University of Ngaoundere, where voucher specimens were deposited. In order to consider recent developments in the taxonomy of we consulted recent reports.

2.5 Data Analysis

Data were stored in an excel spreadsheet version 2016. A data matrix of abundance counts of species for each site was constructed as well as that of fruiting rate, average number of seeds per pod, percentage of normal seeds, seed weight. Raw data were transformation using the formula $\log_{10}(x+1)$ and subjected to the ANOVA procedure when relevant from SigmaStat for Windows version 2.03. Pairwise multiple comparisons were set up using the Tukey's test. Percentages were calculated from the overall total number of the collected specimens. Abundance counts were presented in terms of mean ± standard error (se). Two means were compared using the Student t-test when relevant and when normality and equal variance tests passed. In other hand we used the non-parametric test (Wilcoxon for paired series or Mann-Whitney for independent ones). Non-parametric comparison of several abundance series was set up using the Kruskal-Wallis test from SigmaStat software 2.0® and the pairwise

comparison was set up using Dunn's procedure. Comparison of two frequencies was done using Fisher's exact-test and simultaneous comparison of several frequencies was done using Fisher-Freeman-Halton test from StatXact software 3.1 and appropriate probabilities were adjusted for the number of simultaneous tests using the sequential Bonferroni procedure [32]. Statistics of the assemblage were determined using PAST 3.05 software. These statistics were the absolute abundance of i^{th} species n_i, the sample size n (sum of n_i), the relative abundance of i^{th} species $f_i = n_i/n$, the species richness S, the Shannon-Weaver index H', the maximum Shannon-Weaver index $H'_{max} = \ln(S)$ and the Simpson's index D (D = 0 for high diversity). The Margalef's index $Mg = (S-1)/\ln(n)$ with $0 \leq Mg \leq +\infty$ (Mg = 0 for a low richness) indicated the species' richness quality. The Pielou's evenness index J and the Hill's diversity numbers $N_1 = e^{H'}$ and $N_2 = 1/D$ were determined. The richness ratio d = S/n confirmed the species richness (d = 0 for low species richness). The theoretical richness T was determined using the abundance based non-parametric estimator Chao1 and the sampling success (S/T)*100 were estimated. The degree of dominance by a few species was evaluated using Berger-Parker index $I_{BP} = n_{max}/n$ (I_{BP} = 0 for equally presence of taxa). The abundance of the main pollinator was estimated on 1,000 flowers using the formula $(n_1/n_2)*1,000$ where n_1 represented the number of foragers per flower and n_2 the number of checked flowers. The mode of reproduction was determined from group 1 (unprotected flowers) and group 2 (protected flowers). Group 3 flowers were labeled for exclusive visit of *Apis mellifera* (Hymenoptera: Apidae) and group 4 flowers were frequently opened without any insect visits. The number of pods was counted after the last fading flower. The fruiting index $FI_i = (F_2/F_1)$ was calculated, where F_1 was the number of flowers initially marked, F_2 was the number of pods formed. The out crossing rate $TC = [(FI_1-FI_2)/FI_1]*100$ was calculated, where FI_1 and FI_2 were fruiting indexes of group 1 and group 2 flowers respectively and the rate of self-pollination TA = (100-TC) was also calculated. The cumulative impact of insect pollinators and insecticide treatments on fruiting rate $FR_i = [(fr_1-fr_4)/(fr_1+fr_2-fr_4)]*100$ and the fruiting rate $fr_i = 100*FI_i$ were evaluated where fr_1, fr_2, and fr_4 were fruiting rates in groups 1, 2 and 4 respectively. The percentage of seeds per pod attributable to the cumulative impact of insect pollinators and insecticide treatments $Ps = [(s_1-s_4)/(s_1+s_2-s_4)]*100$ was calculated where s_1, s_2, and s_4 were the average numbers of seeds per pod in groups 1, 2 and 4 respectively. The percentage of normal seeds $Pn = [(Pn_1-Pn_4)/(Pn_1+Pn_2-Pn_4)]*100$ attributable to the impact of insect pollinators and insecticide treatments was calculated where Pn_1, Pn_2, and Pn_4 were percents of normal seeds in groups 1, 2 and 4 respectively. Yield was evaluated by weighting harvested pods and seeds. The average seed weight of 15 samples of 10 healthy pods, that of 10 damaged pods and the average proportion of healthy pods were calculated using the formula (number of healthy pods /total number of pods recorded)*100. The proportion of damaged pods was determined using the formula $(n_1/n)*100$ where n_1 represents the number of pods showing signs of damage and n represents the total number of examined pods. Damaged pods were recognized by the presence of black pustules representing entry points of borer insects or the presence of shrunken parts following the abortion of seeds sucked by the pests. Healthy pods have a regular shape and no aborted seeds. The yield was estimated in terms of seed weight per unit of cultivated area.

3. RESULTS

3.1 Flower Entomofauna of *Vigna unguiculata* (Fabales: Fabaceae)

A total of 10,984 insect individuals were frequently found active on flowers of 880 plants of *Vigna unguiculata* (L.) Walp., 1843 (Fabales: Fabaceae) [6,002 individuals (54.6%) on flowers of 440 plants at Bokle, suburb of Garoua and 4,982 individuals (45.4%) on flowers of 440 other plants at Dang, suburb of Ngaoundere]. These insects (collecting nectar or pollen products) belonged to three orders (Hemiptera Linnaeus, 1758, Hymenoptera Linnaeus, 1758 and Lepidoptera Linnaeus, 1758) and three families (Aphididae Latreille, 1802, Apidae Latreille, 1802 and Nymphalidae Rafinesque, 1815). Seven species were identified divided into one (i.e. 14.3%) sap-sucking species *Aphis craccivora* Koch, 1854 (Hemiptera: Aphididae), four (57.1%) pollinators Hymenoptera Apidae [*Amegilla calens* (Lepeletier, 1841), *Amegilla* sp. Friese, 1897, *Apis mellifera* Linnaeus, 1753 and *Xylocopa olivacea* (Fabricius 1778)] and two (28.6%) Lepidoptera Nymphalidae [*Danaus plexippus* (Linnaeus, 1758) and *Hypolimnas misippus* (Linnaeus, 1764)]. *Amegilla* sp. and *Danaus plexippus* were noted exclusively at Bockle while five species were recorded simultaneously at

both localities. Bockle showed a low richness (S = 7 species, maximum n_{max} = 2,676 individuals, Margalef Mg = 0.69, richness ratio d = 0.001), a median diversity (Shannon-Weaver H' = 1.57, maximum Shannon-Weaver H'_{max} = 1.95, Simpson D = 0.27), a highly even assemblage (Pielou J = 0.81), a median dominance level [Berger-Parker I_{BP} = 0.45, Hill's number N_1 = 5 (71.4%) simply abundant species, Hill's number N_2 = 4 (57.1%) codominants]. The maximum sampling effort (100%) was noted (Chao1 = 7). A similar observation was noted in Dang (S = 5 species, n_{max} = 2,412 individuals, Mg = 0.47, d = 0.001, H' = 1.06, H'_{max} = 1.61, D = 0.40, J = 0.66, I_{BP} = 0.48, N_1 = N_2 = 3 species (60.0%) codominants). The sampling effort was also maximum (100%) (Chao1 = 5). According to the rarefaction procedure for a standard sample of 4,971 individuals, the settlement in Bockle appeared most diverse [$E(S_{n = 4,971})$ = 7 ± 0 species] than in Dang $E(S_{n = 4,971})$ = 5 ± 0 species] and diversity was high in Bockle than Dang (Shannon index: t = 35.9, df = 10,960, *P* = $1.0x10^{-266}$; Simpson index: t = -23.2, df = 10,959, *P* = $7.5x10^{-116}$). In Bockle, *Ap. mellifera* (24.4%) was the most represented followed by *X. olivacea* (9.1%), *Ah. craccivora* (8.4%), *Amegilla* sp (6.2%), *A. calens* (3.6%), *D. plexippus* (1.7%) and *H. misippus* (1.3%) was the least represented (Table 1A). In Dang, *Ah. craccivora* (22.0%) was the most represented followed by *Ap. mellifera* (17.8%), *X. olivacea* (4.3%), *A. calens* (1.1%) and *H. misippus* (0.2%) was the least represented (Table 1B). In the pooled data, the ranking in descending order of percentages placed *Ap. mellifera* in the first position (42.1%) followed by *Ah. craccivora* (30.4%), *X. olivacea* (13.4%), *Amegilla* sp. (6.2%), *A. calens* (4.7%), *D. plexippus* (1.7%) and lastly *H. misippus* (1.5%) (Table 1C). Three-way ANOVA showed a significant interaction between factors "locality", "treatment" and "insect". The difference in the mean values among the different levels of each factor was greater than would be expected by chance (*P* <0.001 respectively) (Table 2). Across levels "Insect", "locality x treatment" interaction depended on what level of "insect" was present. There was not a significant interaction "locality *x* treatment" at levels *A. calens* (*P* = 0.29)*, Ap. mellifera* (*P* = 0.84)*, X. olivacea* (*P* = 0.86) and *H. misippus* (*P* = 0.11) while there was a significant interaction "locality x treatment" at level *Amegilla* sp. (*P* = 0.007), *Ah. craccivora* (*P* <0.001) and *D. plexipus* (*P* = 0.009) respectively. The mean difference between Bockle and Dang within levels *A. calens* and *Amegilla* sp. was significant (*P* <0.001).

3.2 Entomofauna Associated with *Vigna unguiculata* (Fabales: Fabaceae)

We collected 7,425 insects specimens [2,420 specimens (32.6%) in Bockle and 5,005 specimens (67.4%) in Dang] corresponding to four orders [Hemiptera Linnaeus, 1758 (56.7%), Coleoptera Linnaeus, 1758 (41.5%), rarely Heteroptera Latreille, 1810 and Orthoptera Latreille, 1793 with 0.9% respectively], nine families [Aphididae Latreille, 1802 (45.3%), Chrysomelidae Latreille, 1802 (38.7%), Pyrrhocoridae Amyot and Serville, 1843 (4.8%), Coreidae Leach, 1815 (3.8%), Cicadellidae Latreille, 1802 (2.8%), Coccinellidae Latreille, 1807 (1.9%), rarely Alydidae Amyot and Serville, 1843, Tenebrionidae Latreille, 1802 and Tettigoniidae Krauss, 1902 with 0.9% respectively], 11 genera and 11 species. *Aphis crassivora* Koch, 1854 (Hemiptera: Aphididae) was mostly represented (45.3%), followed by *Monolepta marginella* Weise, 1903 (Coleoptera: Chysomelidae) (16.0%), *Aulacophora indica* Gmelin, 1790 (Coleoptera: Chrysomelidae) (15.4%), then *Phyllotreta cruciferae* (Goeze, 1777) (Coleoptera: Chrysomelidae) (7.3%), *Dysdercus cingulata* (Fabricius, 1775) (Hemiptera: Pyrrhocoridae) (4.8%), *Anoplocnemis curvipes* (Fabricius, 1781) (Hemiptera: Coreidae) (3.8%), *Bothrogonia* sp. (Hemiptera: Cicadellidae) (2.8%), *Cheilomenes sulphurea* (Olivier, 1791) (Coleoptera: Coccinellidae) (1.9%). The rare species were *Lagria hirta* (Linnaeus, 1758) (Coleoptera: Tenebrionidae), *Riptortus dentipes* (Fabricius, 1787) (Heteroptera: Alydidae) and *Tettigonia viridissima* (Linnaeus, 1758) (Orthoptera: Tettigoniidae) each with 0.9% representation respectively (Table 2). Bockle showed a low insect species richness (S = 8 species, n_{max} = 924 individuals, Mg = 0.90, d = 0.003), a median diversity (H' = 1.67, H'_{max} = 2.08, D = 0.25), a highly even assemblage (J = 0.80), a low dominance [I_{BP} = 0.38, Hill's N_1 = 5 species (62.5%) simply abundants, Hill's N_2 = 4 (50.0%) codominants] and the maximum sampling effort (100%) (Chao1 = 8 species). A similar observation was noted in Dang [S = 11 species, n_{max} = 2,437 individuals, Mg = 1.17, d = 0.002, H' = 1.65, H'_{max} = 2.40, D = 0.29, J = 0.69, I_{BP} = 0.49, N_1 = 5 species (45.5%), N_2 = 3 species (27.3%)] and the maximum sampling effort (100%) was noted (Chao1 = 11 species). The global assemblage presented a similar information [S = 11 species, n_{max} = 3,361 individuals, Mg = 1.12, d = 0.001, H' = 1.71, H'_{max} = 2.40, D = 0.27, J = 0.71, I_{BP} = 0.45, N_1 = 6

species (54.5%), N_2 = 4 species (36.4%), Chao1 = 11, Sampling Effort = 100%]. The difference in species diversity was not significant between Bockle and Dang (t = -0.69, df = 6,093.7, *P* = 0.49), the two assemblages being similar (Jaccard index: 0.80). We recorded the presence of a useful predatory native species *C. sulphurea* (Coleoptera: Coccinellidae). Two harmful species were native to Africa [*M. marginella* (Coleoptera: Chrysomelidae) and *A. curvipes* (Hemiptera: Coreidae)] and eight exotic species were indomalayan phytophagous *A. indica* (Coleoptera: Chrysomelidae), palaerctic phytophagous *P. cruciferae* (Coleoptera: Chrysomelidae), holarctic sap-sucking *L. hirta* (Coleoptera: Tenebrionidae), palaerctic sap-sucking *R. dentipes* (Heteroptera: Alydidae), palaearctic sap-sucking *Ah. crassivora* (Hemiptera: Aphididae), afro-eurasian sap-sucking *Bothrogonia* sp. (Hemiptera: Cicadellidae), tropical sap-sucking *D. cingulata* (Hemiptera: Pyrrhocoridae) and eurasian phytophagous *T. viridissima* (Orthoptera: Tetigoniidae)] (Table 2).

3.3 Impact of the Aqueous Leaf Extracts

Between control plots ('Tem' and 'Para'), parastar insecticide eliminated flower insects except few *Ap. mellifera* and *X. olivacea* survivors in Bockle (Table 3A), *Ah. craccivora* in Dang (Fig. 1A and 1B; Table 3B), *Ah. craccivora, Amegilla* sp., *Ap. mellifera, X. olivacea* in the pooled data (Table 3C). Plots treated using plant extracts and those treated with parastar insecticide showed a similar negative effect against *D. plexippus* and *H. misippus* in Dang except the cases of 10% and 20% aqueous leaf extract of *Eucalyptus camaldulensis* against *H. misippus* and 30% *E. camaldulensis* against *D. plexippus* (Table 3A). A similar result was noted in Dang locality against *A. calens* and *H. misippus* (Table 3B). In the case of *Ap. mellifera* this was true only for 30% *Tithonia diversifolia* (Fig. 1G and 1H) and in the case of *X. olivacea* this was true only for 30% *Calotropis procera* (Fig. 1C and 1D) and 30% *E. camaldulensis* (Fig. 1E and 1F), the different doses of extract not having completely eliminated the flower insects, reduced the abundance (Table 3B). Whatever the plant extracts, flower insects were preserved. In short, comparisons with untreated control plots showed that chemical treatments using parastar and those of botanical origin (aqueous leaf extract of plants) have significantly reduced the abundances of insects associated with the blooming flowers of *Vigna unguiculata*. Whatever the dose, the aqueous leaf extracts of the plants

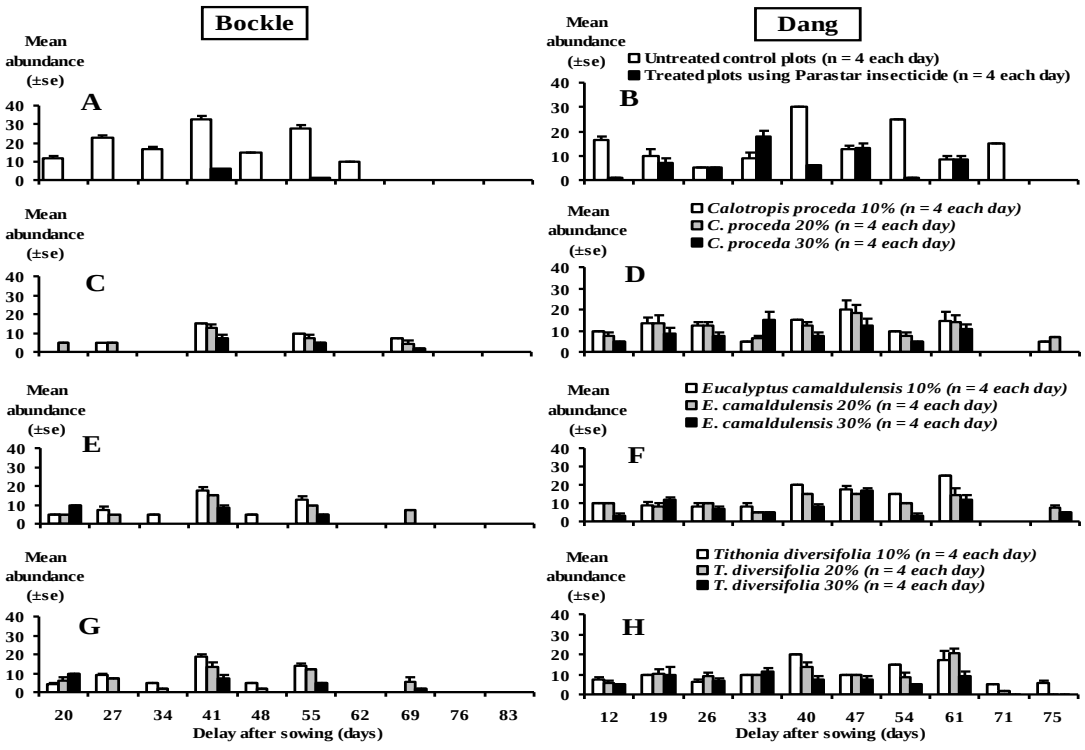

Fig. 1. Effect of Aqueous Leaf Extracts on The Population Dynamic of *Aphis craccivora* (Hemiptera: Aphidae)

Table 1. Absolute Abundance and Percentage of Flower Insects on 40 Plants of *Vigna unguiculata* in Each Category of Plot at Bockle and Dang

			Aqueous leaf extract									
	Control plots		Cp.			Ec.			Td.			Total
Insect species	Tem(%)	Para(%)	A(%)	B(%)	C(%)	A(%)	B(%)	C(%)	A(%)	B(%)	C(%)	(%)
A. Bockle suburb of Garoua (440 plants)												
Hemiptera Linnaeus, 1758 (Aphididae Latreille, 1802)												
I.	280(2.5)	7(0.1)	74(0.7)	59(0.5)	22(0.2)	95(.9)	77(0.7)	45(0.4)	154(1.4)	79(0.7)	32(0.3)	**924(8.4)**
Hymenoptera Linnaeus, 1758 (Apidae Latreille, 1802)												
II. *	68(0.6)	2(0.02)	26(0.2)	38(0.3)	36(0.3)	40(0.4)	40(0.4)	48(0.4)	46(0.4)	26(0.2)	28(0.3)	**398(3.6)**
III. *	88(0.8)	16(0.1)	56(.5)	64(0.6)	46(0.4)	76(0.7)	66(0.6)	100(0.9)	64(0.6)	64(0.6)	36(0.3)	**676(6.2)**
IV. *,#	534(4.9)	44(0.4)	252(2.3)	364(3.3)	82(0.7)	388(3.5)	334(3.0)	58(0.5)	308(2.8)	248(2.3)	64(0.6)	**2,676(24.4)**
V. *	144(1.3)	26(0.2)	106(1.0)	106(1.0)	56(0.5)	96(0.9)	114(1.0)	40(0.4)	134(1.2)	82(0.7)	98(0.9)	**1,002(9.1)**
Lepidoptera Linnaeus, 1758 (Nymphalidae Rafinesque, 1815)												
VI. *	34(0.3)	8(0.1)	14(0.1)	14(0.1)	14(0.1)	34(0.3)	16(0.1)	48(0.4)	2(0.02)	4(0.04)	-	**188(1,7)**
VII. *	32(0.3)	14(0.1)	8(0.1)	-	2(0.02)	38(0.3)	28(0.3)	14(0.1)	-	2(0.02)	-	**138(1.3)**
Total	**1,180(10.7)**	**117(1.1)**	**536(4.9)**	**645(5.9)**	**258(2.3)**	**767(7.0)**	**675(6.1)**	**353(3.2)**	**708(6.4)**	**505(4.6)**	**258(2.3)**	**6,002(54.6)**
B. Dang suburb of Ngaoundere (440 plants)												
Hemiptera Linnaeus, 1758 (Aphididae Latreille, 1802)												
I.	251(2.3)	130(1.2)	280(2.5)	263(2.4)	228(2.1)	192(1.7)	219(2.0)	203(1.8)	288(2.6)	207(1.9)	151(1.4)	**2,412(22.0)**
Hymenoptera Linnaeus, 1758 (Apidae Latreille, 1802)												
II *	48(0.4)	2(0.02)	-	8(0.1)	8(0.1)	14(0.1)	16(0.1)	12(0.1)	10(0.1)	2(0.02)	2(0.02)	**122(1.1)**
IV. *,#	438(4.0)	12(0.1)	176(1.6)	284(2.6)	40(0.4)	312(2.8)	254(2.3)	20(0.2)	228(2.1)	170(1.5)	18(0.2)	**1,952(17.8)**
V *	92(0.8)	8(0.1)	50(0.5)	50(0.5)	16(0.1)	42(0.4)	52(0.5)	10(0.1)	74(0.7)	36(0.3)	42(0.4)	**472(4.3)**
Lepidoptera Linnaeus, 1758 (Nymphalidae Rafinesque, 1815)												
VII *	8(0.1)	-	2(.02)	-	-	4(0.04)	8(0.1)	-	2(0.02)	-	-	**24(0.2)**
Total	**251(7.6)**	**130(1.4)**	**280(4.6)**	**263(5.5)**	**228(2.7)**	**192(5.1)**	**219(5.0)**	**203(2.2)**	**288(5.5)**	**207(3.8)**	**151(1.9)**	**4,982(45.4)**
C. Global (n = 880 plants)												
Hemiptera Linnaeus, 1758 (Aphididae Latreille, 1802)												
I	531(4.8)	137(1.2)	354(3.2)	322(2.9)	250(2.3)	287(2.6)	296(2.7)	248(2.3)	442(4.0)	286(2.6)	183(1.7)	**3,336(30.4)**
Hymenoptera Linnaeus, 1758 (Apidae Latreille, 1802)												
II *	116(1.1)	4(0.04)	26(0.2)	46(0.4)	44(0.4)	54(0.5)	56(0.5)	60(0.5)	56(0.5)	28(0.3)	30(0.3)	**520(4.7)**
III *	88(0.8)	16(0.1)	56(0.5)	64(0.6)	46(0.4)	76(0.7)	66(0.6)	100(0.9)	64(0.6)	64(0.6)	36(0.3)	**676(6.2)**
IV *,#	972(8.8)	56(0.5)	428(3.9)	648(5.9)	122(1.1)	700(6.4)	588(5.4)	78(0.7)	536(4.9)	418(3.8)	82(0.7)	**4,628(42.1)**
V *	236(2.1)	34(0.3)	156(1.4)	156(1.4)	72(0.7)	138(1.3)	166(1.5)	50(0.5)	208(1.9)	118(1.1)	140(1.3)	**1,474(13.4)**
Lepidoptera Linnaeus, 1758 (Nymphalidae Rafinesque, 1815)												
VI *	34(0.3)	8(0.1)	14(0.1)	14(0.1)	14(0.1)	34(0.3)	16(0.1)	48(0.4)	2(0.02)	4(0.04)	-	**188(1.7)**
VII *	40(0.4)	14(0.1)	10(0.1)	-	2(0.02)	42(0.4)	36(0.3)	14(0.1)	2(0.02)	2(0.02)	-	**162(1.5)**
Total	**2,017(18.4)**	**269(2.4)**	**1,044(9.5)**	**1,250(11.4)**	**550(5.0)**	**1,331(12.1)**	**1,224(11.1)**	**598(5.4)**	**1,310(11.9)**	**920(8.4)**	**471(4.3)**	**10,984(100.0)**

** = Nectar, # = Pollen, I. Aphis craccivora, II. Amegilla calens, III. Amegilla sp., IV. Apis mellifera, V. Xylocopa olivacea, VI. Danaus plexippus, VII. Hypolimnas misippus, Tem = untreated control plots, Para = control plots treated using Parastar, Cp = Calotropis procera (Gentianales: Apocynaceae), Ec = Eucalyptus camaldulensis (Myrtales: Myrtaceae), Td = Tithonia diversifolia (Asterales: Asteraceae), A = 10% aqueous leaf extract, B = 20% aqueous leaf extract, C = 30% aqueous leaf extract.*

Table 2. Absolute and Relative Abundance of Insects Associated with Plants of *Vigna unguiculata* at Dang and Bockle Localities

Order/Family	Species	Pest status	Reference	Dang (%)	Bockle (%)	Total (%)
Coleoptera Linnaeus, 1758						
Coccinellidae Latreille, 1807	*Cheilomenes sulphurea* (Olivier, 1791)	§, BC, WA	[33]	72 (1.0)	68 (0.9)	**140 (1.9)**
Chrysomelidae Latreille, 1802	*Aulacophora indica* Gmelin, 1790	P, pest, IM	[34]	473 (6.4)	670(9.0)	**1,143 (15.4)**
	Monolepta marginella Weise, 1903	P, pest, AF	[35]	966 (13.0)	223 (3.0)	**1,189 (16.0)**
	Phyllotreta cruciferae (Goeze, 1777)	P, pest, PA	[36]	324 (4.4)	218 (2.9)	**542 (7.3)**
Tenebrionidae Latreille, 1802	*Lagria hirta* (Linnaeus, 1758)	‡, pest, HO(WP)	[37]	67 (0.9)	-	**67 (0.9)**
Heteroptera Latreille, 1810						
Alydidae Amyot and Serville, 1843	*Riptortus dentipes* (Fabricius, 1787)	‡, ⁑, pest, PA	[38]	70 (0.9)	-	**70 (0.9)**
Hemiptera Linnaeus, 1758						
Coreidae Leach, 1815	*Anoplocnemis curvipes* (Fabricius, 1781)	‡, pest, AF	[39]	177 (2.4)	106 (1.4)	**283 (3.8)**
Aphididae Latreille, 1802	*Aphis crassivora* Koch, 1854	‡, pest, COS(PA)	[40]	2,437(32.8)	924 (12.4)	**3,361 (45.3)**
Cicadellidae Latreille, 1802	*Bothrogonia* sp.	‡, pest, OW	[41]	117 (1.6)	94 (1.3)	**211 (2.8)**
Pyrrhocoridae Amyot and Serville, 1843	*Dysdercus cingulata* (Fabricius, 1775)	‡, pest, TR, ST	[42]	236 (3.2)	117 (1.6)	**353 (4.8)**
Orthoptera Latreille, 1793						
Tettigoniidae Krauss, 1902	*Tettigonia viridissima* (Linnaeus, 1758)	P, pest, EEU	[43]	66 (0.8)	-	**66 (0.8)**
	Total			**5,005(67.4)**	**2,420(32.6)**	**7,425(100.0)**

AF: Afrotropical native species, BC: Biological control agent, EEU: native to the eastern part of Eurasia, IM: Indomalayan native species, COS: Cosmopolitan species, HO: Holarctic origin, PA: Palaearctic origin, OW: Old World origin (Afro-Eurasia region), TR; Tropical distributed species, ST: Subtropical distributed species, WA: West Africa native species, WP = western Palaearctic region, ⁑; pod-sucking insect, ‡: sap-sucking insect, §: Predator species, P: phytophagous species, pest: pest insect

Table 3. Mean Abundance (± se) of Insects on Flowers of 40 *Vigna unguiculata* Plants

	Treatment											
	Control		Cp			Ec			Td			
Insects	**Tem**	**Para**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**Global**
A. Bockle locality (n = 40 plants for each plot)												
I.	7±2	-	2±1	1±1	1±0	2±1	2±1	1±0	4±1	2±1	1±0	**2±0**
II.	2±0	-	1±0	1±0	1±0	1±0	1±0	1±0	1±0	1±0	1±0	**1±0**
III.	2±0	-	1±0	2±0	1±0	2±0	2±0	3±0	2±0	2±0	1±0	**2±0**
IV.	13±0	1±0	6±1	9±0	2±0	10±1	8±0	1±0	8±0	6±0	2±0	**6±0**
V.	4±0	1±0	3±0	3±0	1±0	2±0	3±0	1±0	3±0	2±0	2±0	**2±0**
VI.	1±0	-	-	-	-	1±0	-	1±0	-	-	-	**-**
VII.	1±0	-	-	-	-	1±0	1±0	-	-	-	-	**-**
Global	**30±2**	**3±0**	**13±1**	**16±1**	**6±1**	**19±1**	**17±1**	**9±1**	**18±1**	**13±1**	**6±1**	**14±0**
B. Dang locality (n = 40 plants for each plot)												
I.	6±1	3±1	7±1	7±1	6±1	5±1	5±1	5±1	7±1	5±1	4±1	**7±0**
II	1±0	-	-	-	-	-	-	-	-	-	-	**-**
IV	11±0	-	4±0	7±0	1±0	8±0	6±0	1±0	6±0	4±0	-	**4±0**
V	2±0	-	1±0	1±0	-	1±0	1±0	-	2±0	1±0	1±0	**2±0**
VII	-	-	-	-	-	-	-	-	-	-	-	**-**
Global	**21±1**	**4±1**	**13±1**	**15±1**	**7±1**	**14±1**	**14±1**	**6±1**	**15±1**	**10±1**	**5±1**	**9±0**
C. Global (n = 80 plants for each plot)												
I	7±1	4±0	4±1	4±1	3±1	4±1	4±1	3±1	6±1	4±1	2±0	4±0
II	1±0	-	-	1±0	1±0	1±0	1±0	1±0	1±0	-	-	1±0
III	1±0	1±0	1±0	1±0	1±0	1±0	1±0	1±0	1±0	1±0	-	1±0
IV	12±0	1±0	5±0	8±0	2±0	9±0	7±0	1±0	7±0	5±0	1±0	5±0
V	3±0	1±0	2±0	2±0	1±0	2±0	2±0	1±0	3±0	1±0	2±0	2±0
VI	-	-	-	-	-	-	-	1±0	-	-	-	-
VII	1±0	-	-	-	-	1±0	-	-	-	-	-	-
Global	**25±1**	**4±0**	**13±1**	**16±1**	**7±1**	**17±1**	**15±1**	**7±1**	**16±1**	**17±1**	**6±1**	**12±0**

c. Three-way ANOVA result				**Treatment within *Apis mellifera* (continue)**			
Source of Variation	**df**	**F**	***P***	**Comparison**	***P***	**Comparison**	***P***
Locality	1	179.61	<0.001	Tem - Td10	<0.001	Ec10 - Ec30	<0.001
Treatment	10	77.07	<0.001	Tem - Td20	<0.001	Ec10 - Ec20	<0.001
Insects	6	618.15	<0.001	Tem - Td30	<0.001	Ec10 - Td30	<0.001
Locality-Treatment	10	2.51	0.005	Para - Cp10	<0.001	Ec20 - Cp10	0.007
Locality-Insects	6	127.90	<0.001	Para - Cp20	<0.001	Ec20 - Cp30	<0.001
Treatment-Insects	60	20.40	<0.001	Para - Ec10	<0.001	Ec20 - Ec30	<0.001
Locality-Treatment-Insects	60	1.67	<0.001	Para - Ec20	<0.001	Ec20 - Td30	<0.001
Residual	6,006			Para - Td10	<0.001	Ec30 - Td20	<0.001
Total	6,159			Para - Td20	<0.001	Td10 - Ec30	<0.001

Tukey test: Treatment within *A. calens*				Para - Cp30	0.030	Td10 - Td30	<0.001
Comparison	***P***	**Comparison**	***P***	Cp10 - Cp30	<0.001	Td10 - Cp30	<0.001
Tem - Para	<0.001	Tem - Td20	<0.001	Cp10 - Ec30	<0.001	Td20 - Cp30	<0.001
Tem - Cp10	<0.001	Tem - Td30	<0.001	Cp10 - Td30	<0.001	Td20 - Td30	<0.001
Tem - Cp20	0.001	Para - Ec10	0.010	**Treatment within *Xylocopa olivacea***			
Tem - Cp30	<0.001	Para - Ec20	0.020	**Comparison**	***P***	**Comparison**	***P***
Tem - Ec10	0.021	Para - Ec30	0.004	Tem - Para	<0.001	Tem - Ec30	<0.001
Tem - Ec20	0.013	Para - Td10	0.010	Tem - Cp30	<0.001	Tem - Td20	<0.001
Tem - Td10	0.018			Tem - Ec10	0.003	Tem - Td30	0.003
Treatment within Bockle - *Amegilla* sp.				Tem - Ec20	<0.001	Td10 - Cp30	<0.001
Comparison	***P***	**Comparison**	***P***	Para - Cp10	<0.001	Ec10 - Cp30	<0.001
Tem - Para	<0.001	Para - Cp20	<0.001	Para - Cp20	<0.001	Ec10 - Ec30	0.030
Tem - Td30	<0.001	Para - Ec10	<0.001	Para - Ec10	<0.001	Ec20 - Ec30	<0.001
Ec30 - Para	<0.001	Para - Ec20	<0.001	Para - Ec20	<0.001	Ec20 - Cp30	<0.001
Ec30 - Td30	<0.001	Para - Td10	<0.001	Para - Td30	<0.001	Td10 - Td20	0.002
Para - Cp10	<0.001			Para - Td10	<0.001	Td10 - Cp30	<0.001
Treatment within *Apis mellifera*				Para - Td20	<0.001	Td10 - Ec30	<0.001
Comparison	***P***	**Comparison**	***P***	Cp10 - Cp30	<0.001	Td20 - Ec30	0.030
Tem - Para	<0.001	Cp20 - Cp10	<0.001	Cp10 - Ec30	<0.001	Td30 - Ec30	<0.001
Tem - Cp10	<0.001	Cp20 - Cp30	<0.001	Cp20 - Cp30	0.001	Td30 - Cp30	0.030
Tem - Cp20	<0.001	Cp20 - Ec30	<0.001	Cp20 - Ec30	<0.001		
Tem - Cp30	<0.001	Cp20 - Td20	<0.001	**Treatment within *Hypolimnas misippus***			
Tem - Ec10	0.002	Cp20 - Td30	<0.001	**Comparison**	***P***	**Comparison**	***P***
Tem - Ec20	<0.001	Ec10 - Cp10	<0.001	Tem - Cp20	0.020	Tem - Td10	0.040
Tem - Ec30	<0.001	Ec10 - Cp30	<0.001	Tem - Cp30	0.040	Tem - Td20	0.040 *

Table 3 (continue)

Treatment within *Hypolimnas misippus*				**Treatment within Bockle - *Aphis craccivor* (continue)**			
Comparison	***P***	**Comparison**	***P***	**Comparison**	***P***	**Comparison**	***P***
Tem - Td30	0.020 *			Tem - Td30	<0.001		
Treatment within Bockle - *Aphis craccivor*				**Treatment within Dang - *Aphis craccivor***			
Comparison	***P***	**Comparison**	***P***	**Comparison**	***P***	**Comparison**	***P***
Tem - Para	<0.001	Para - Ec10	<0.001	Tem - Para	<0.001	Para - Td10	<0.001
Tem - Cp10	<0.001	Para - Ec20	<0.001	Para - Cp10	<0.001	Cp10 - Ec10	<0.001
Tem - Cp20	<0.001	Para - Td10	<0.001	Para - Cp20	<0.001	Cp10 - Td30	<0.001
Tem - Cp30	<0.001	Para - Td20	<0.001	Para - Cp30	<0.001	Td10 - Ec10	<0.001
Tem - Ec10	<0.001	Td10 - Cp20	<0.001	Para - Ec20	<0.001	Td10 - Td20	<0.001
Tem - Ec30	<0.001	Td10 - Ec30	<0.001	Para - Ec30	<0.001	Td10 - Td30	<0.001
Tem - Td20	<0.001	Td10 - Td30	<0.001				

I = Aphis craccivora Koch, 1854, II = Amegilla calens (Lepeletier, 1841), III = Amegilla sp. Friese, 1897, IV = Apis mellifera Linnaeus, 1753, V = Xylocopa olivacea (Fabricius 1778), VI = Danaus plexippus (Linnaeus, 1758), VII = Hypolimnas misippus (Linnaeus, 1764), Tem = untreated plots, Para = plots treated using Parastar, Cp = extract of Calotropis procera (Aiton) Aiton, 1811 (Gentianales: Apocynaceae), Ec = extract of Eucalyptus camaldulensis Dehnh., 1832 (Myrtales: Myrtaceae), Td = extract of Tithonia diversifolia (Asterales: Asteraceae), A = 10% aqueous leaf extract, B = 20% aqueous leaf extract, C = 30% aqueous leaf extract.

were significantly less effective than the synthetic Parastar insecticide. At Bockle, *Ah. crassivora* (Hemiptera: Aphididae) presented a significant reduction in the abundances due to the negative effect of the aqueous leaf extract of *T. diversifolia* (one-way ANOVA: $F_{(2;\ 93)} = 5.09$, $P = 0.008$; Tukey's test: Td10 *versus* Td30: $P = 0.006$, Td10 *versus* Td20: $P = 0.13$, Td20 *versus* Td30: $P = 0.45$) while the two other plant extracts did not impact significantly the pest aphid dynamic [$F_{(2;\ 90)} = 1.73$, $P = 0.18$ for *C. procera* extracts; $F_{(2;\ 84)} = 1.05$, $P = 0.35$ for *E. camaldulensis* extracts]. In Dang, similar result was recorded for *T. diversifolia* ($F_{(2;\ 114)} = 3.20$, $P = 0.04$; Td10 *versus* Td30: $P = 0.04$, Td10 *versus* Td20: $P = 0.30$, Td20 *versus* Td30: $P = 0.56$), for *C. procera* extracts [$F_{(2;\ 114)} = 0.336$, $P = 0.72$] and for *E. camaldulensis* extracts [$F_{(2;\ 117)} = 0.11$, $P = 0.89$]. Overall, 30% *T. diversifolia* reduced the aphid population size, while 10% increased it (Fig. 1G and 1H). Extracts 10% were less effective and 30% eradicated *Ah. crassivora as* did the parastar.

3.4 Visits to Blooming Flowers by Pollinating Insects

Given that the cowpea flowers bloom and attract pollinators in the morning, we conducted from 6 a.m. to 1 p.m. during three days, the study of the visit rhythm of pollinators. One to eight flowers were randomly selected per plant: 360 and 362 flowers from 115 plants from 33 plots in Bockle and Dang respectively (average: 3 ± 0 flowers per plant). A total of 700 visits to flowers was recorded: 391 visits (55.9%) in Bockle and 309 visits (44.1%) in Dang. *Amegilla* sp. and *Hypolimnas misippus* were exclusively recorded in Bockle. The difference in the visitation rate was not significant between localities (Table 4). Nectar collection was regular and intense whereas pollen collection was low. Flowers were visited from 6:00 a.m. to 1:00 p.m. with a peak of activity between 8:00 a.m. and 9:00 a.m.. Activity of *Ap. mellifera* was influenced by plant extracts. Except 20% *E. camaldulensis* and *C. procera*, other extracts were attractive to *Ap. mellifera* workers in the morning (6 to 7 a.m.). From 8 to 9 a.m. the frequency of visits was low on flowers treated with plant extracts, than on those untreated, in contrast to the results from 10 a.m. to 11 a.m.. From 12 a.m. to 1 p.m., 10% and 30% *T. diversifolia* and 30% *C. procera* prevented visits of pollinators. Parastar insecticide decreased the frequency of pollinators. During the morning (6:00-7:00, 8:00-9:00 and 10:00-11:00), the flower visitation rate was high in Bockle than Dang while during 12 p.m. to 1 p.m. the difference was not significant (Table 4).

Apis melifera was active during the four time periods with a peak of activity between 8 a.m. and 9 a.m. *Amegilla calens, Amegilla* sp. and *Danaus plexippus* were active during the three first time periods and absent during the last one. *Hypolimnas misippus* was rare during the first two time periods and absent during the two last ones. *Xylocopa olivacea* showed a peak of activity between 10 a.m. and 11 a.m. and *Ap. mellifera* was the main flower insect (384 visits, 54.9%), followed by *A. calens* (120 visits, 17.1%), *X. olivacea* (90 visits, 12.9%) and other species were rare (57 visits and 8.1% for *D. plexippus*, 25 visits and 3.6% for *H. misippus*, 24 visits and 3.4% for *Amegilla* sp.).

We focused on the behavior of *Ap. mellifera*. According to the pooled data the duration of nectar collection (3,327 cases, one to 20 seconds, mean ± se: 7.20 ± 0.05 seconds, median: 7 seconds) was in the median value greater than that of single pollen collection (3,137 cases, one to 11 seconds, 4.20 ± 0.04, median: four seconds) and even that of simultaneous nectar and pollen collection (6,494 cases, one to 20 seconds, 5.73 ± 0.04, median: 4 s) (Kruskall-Wallis test: H = 1,567.21, df = 2, P <0.001; pairwise comparisons using Dunn's method: P <0.001 for Nectar *versus* Pollen (Q = 39.34), Nectar *versus* both products (Q = 22.49) and Pollen *versus* both products (Q = 22.98) respectively). Out of 2,341 bloomed flowers visited in Bockle and Dang, *Ap. mellifera* visited several open flowers of the same plant before leaving it (676 cases, 28.9%). The visits were disturbed by the wind (399 cases, 17.0%) and the interference of another forager was by an *Ap. mellifera* congener (787 cases, 33.6%) or by an individual of *X. olivacea* (479 cases, 20.5%). A total of 3,191 recordings showed that the foraging speed varied from one to 120 flowers per minute (mean value ± es: 7 ± 0 flowers, median value: 6 flowers per minute). The time taken to forage a flower varied from one to 60 seconds (15.5 ± 0.2 seconds; median duration: 10 seconds). A significant difference in the visitation duration of *Ap. mellifera* was observed for all treatments. Tested products at their contents 10 and 30% reduced the times for collection of nectar and pollen at Bockle. Moreover, *C. procera* and *T. diversifolia*, at their content 30%, reduced the time for the collection of nectar at Dang as did parastar. Results were

significantly different between the two sites, excluding *E. camaldulensis* at 30% for nectar and pollen collection, *C. procera* and *T. diversifolia* at 10 and 30% for pollen collection. The nectar collection time on flowers treated using 10% *T diversifolia* was not different between the two sites (t = 0.44; *P* >0.05). *C. procera* and *T. diversifolia* extracts reduced the foraging speed of *Ap. mellifera* at Bockle as did parastar. The means foraging speeds varied from six flowers per minute (parastar treated group) to seven flowers per minute (untreated plots) at Dang and between five flowers per minute (10% *C. procera*) and 10 flowers per minute (untreated group) at Bockle. The number of untreated flowers visited per minute was higher in Bockle than Dang. Results at 20% and 30% *E. camaldulensis* were higher at Bockle than Dang in contrast to that recorded using 10% *C. procera*. The abundance of *Ap. mellifera* on 1,000 flowers, was high in untreated plots and low in treated plots except treatment using 10% *T. diversifolia* at Dang and 20% *C. procera*, *E. camaldulensis* and *T. diversifolia* in both localities. Compared to the parastar treated plots, 10% and 30% *C. procera, E. camaldulensis* and *T. diversifolia* did not show significant difference, unlike plots treated using 20% of each plant extract. This dose would affect the abundance of *Ap. mellifera* (Table 5).

3.5 Reproductive System

In Bockle, 360 buds marked in group 1 (free flowers) and group 2 (protected flowers) respectively, 600 buds marked in group 3 (flowers exclusively visited by *Apis mellifera*) and 300 buds marked in group 4 (flowers open from time to time without any visit of insects), produced 330 pods in group 1, 331 pods in group 2, 558 pods in group 3 and 251 pods in group 4, corresponding to a fruiting index FI_1 = 0.92 for group 1, FI_2 = 0.84 for group 2, FI_3 = 0.930 for group 3 and FI_4 = 0.84 for group 4.

The rate of out crossing was TC = 8.8% and the rate of self-pollination was TA = 91.2%. In Dang, buds marked in four groups produced 305 pods in group 1, 269 pods in group 2, 509 pods in group 3 and 204 pods in group 4, corresponding to a fruiting index FI_1 = 0.85 for group 1, FI_2 = 0.75 for group 2, FI_3 = 0.85 for group 3 and FI_4 = 0.68 for group 4. The rate of out crossing was TC = 11.8% and the rate of self-pollination was TA = 88.2%.

Overall 720 buds (group 1 and group 2 respectively), 1,200 buds of group 3 and 600 buds of group 4, produced 635 pods (group 1), 570 pods (group 2), 1,067 pods (group 3) and 455 pods (group 4), giving a fruiting index FI_1 = 0.88 for group 1, FI_2 = 0.79 for group 2, FI_3 = 0.89 for group 3 and FI_4 = 0.76 for group 4, with TC = 10.2% and TA = 89.8%. Cowpea “Feken” presented a mixed allogamous-autogamous reproductive system, with an autogamy predominance.

The cumulative impact of insect pollinators and insecticide treatments on fruiting rate was FR = 8.7% in Bockle, FR = 18.3% in Dang and FR = 13.5% for pooled data. The number of normal pods and seeds varied from two to nine (group 1: four to nine seeds, mean ± se: 7 ± 0 seeds, 321 pods and 307 seeds in Bockle, 297 pods and 286 seeds in Dang, 618 pods and 493 seeds for pooled data; group 2: two to nine seeds, 6 ± 0 seeds, 278 pods and 232 seeds in Bockle, 251 pods and 210 seeds in Dang; 529 pods and 442 seeds for pooled data; group 3: four to nine seeds, 7 ± 0 seeds, 532 pods and 496 seeds in Bockle, 486 pods and 452 seeds in Dang and 1,018 pods and 948 seeds for pooled data; group 4: two to nine seeds, 6 ± 0 seeds, 232 pods and 193 seeds in Bockle, 188 pods and 155 seeds in Dang and 420 pods and 348 seeds for pooled data). The overall variation between groups was significant at Bockle [one-way ANOVA: $F_{(3;\ 1,366)}$ = 31.25, *P* <0.001; Tukey’s tests significant except between group 1 and 3 (*P* = 1.000) and between groups 2 and 4 (*P* = 0.99)]. It was the same at Dang [$F_{(3;\ 1,226)}$ = 26.769, *P* <0.001; Tukey’s pairwise comparisons were significant except between group 1 and 3 (*P* = 1.00) and between groups 2 and 4 (*P* = 1.00)] and for the overall pooled data [$F_{(3;\ 2,596)}$ = 57.99, *P* <0.001; Tukey’s comparisons were significant except between group 1 and 3 (*P* = 1.00) and between groups 2 and 4 (*P* = 1.00)]. The percentage of normal seeds per pod attributable to the cumulative impact of insect pollinators and insecticide treatments was 11.8% in Bockle; 11.3% in Dang and 11.6% for the pooled data.

The variation in the production rate of pods was not significant between the four groups of flowers in Bockle (Fisher-Freeman-Halton test: χ^2 = 0.07, df = 3, *P* = 1.00), in Dang (χ^2 = 0.64, df = 3, *P* = 0.90) and in the pooled data (χ^2 = 0.201, df = 3, *P* = 0.98). On the other hand, the variation in the production of normal seeds was globally significant between the four groups in Bockle (χ^2= 31.33, df = 3, *P* = 6.4×10^{-7}), in Dang (χ^2= 17.90, df = 3, *P* = 4.5×10^{-4}) and in the pooled data (χ^2= 42.99, df = 3, *P* = 2.3×10^{-9}).

Table 4. Variation in the Number of Visits By Insect Pollinators on Bloomed Flowers of *Vigna unguiculata*

	Time period				
Pollinator Insect	**A. 6-7 hr (%)**	**B. 8-9 hr (%)**	**C. 10-11 hr (%)**	**D. 12-13 hr (%)**	**Total (%)**
Bockle (n = 12 sessions)					
I.	63 (9.0)	89 (12.7)	44 (6.3)	24 (3.4)	**220 (31.4)**
II.	21 (3.0)	31 (4.4)	16 (2.3)	-	**68 (9.7)**
III.	2 (0.3)	8 (1.1)	14 (2.0)	-	**24 (3.4)**
IV.	5 (0.7)	13 (1.9)	3 (0.4)	-	**21 (3.0)**
V.	24 (3.4)	1 (0.1)	-	-	**25 (3.6)**
VI.	1 (0.1)	9 (1.3)	20 (2.9)	3 (0.4)	**33 (4.7)**
Total	**116 (16.6)**	**151 (21.6)**	**97 (13.9)**	**27 (3.9)**	**391 (55.9)**
Dang (n = 12 sessions)					
I.	33 (4.7)	87 (12.4)	28 (4.0)	16 (2.3)	**164 (23.4)**
II.	13 (1.9)	26 (3.7)	13 (1.9)	-	**52 (7.4)**
III.	-	-	-	-	**-**
IV.	16 (2.3)	16 (2.3)	4 (0.6)	-	**36 (5.1)**
V.	-	-	-	-	**-**
VI.	5 (0.7)	14 (2.0)	31 (4.4)	7 (1.0)	**57 (8.1)**
Total	**67 (9.6)**	**143 (20.4)**	**76 (10.9)**	**23 (3.3)**	**309 (44.1)**
Pooled data (n = 24 sessions)					
I.	96 (13.7)	176 (25.1)	72 (10.3)	40 (5.7)	**384 (54.9)**
II.	34 (4.9)	57 (8.1)	29 (4.1)	-	**120 (17.1)**
III.	2 (0.3)	8 (1.1)	14 (2.0)	-	**24 (3.4)**
IV.	21 (3.0)	29 (4.1)	7 (1.0)	-	**57 (8.1)**
V.	24 (3.4)	1 (0.1)	-	-	**25 (3.6)**
VI.	6 (0.9)	23 (3.3)	51 (7.3)	10 (1.4)	**90 (12.9)**
Total	**183 (26.1)**	**294 (42.0)**	**173 (24.7)**	**50 (7.1)**	**700(100.0)**
FFH test	χ^2 **=** 156.94; df = 15; *P* <0.001 *				

FFH test for Bockle: χ^2 **=** 102.36; df = 15; *P* <0.001 * ; Dang : χ^2 **=** 60.80; df = 12; *P* = 1.6×10^{-8} *

Bockle *versus* Dang: Fisher-Freeman-Halton test (FFH)

Pollinator insect	Fisher-Freeman-Halton test	Time	Fisher-Freeman-Halton test
Apis mellifera:	χ^2 = 6.48, df = 3, *P* = 0.09 ns;	6-7 hr:	χ^2 = 0 37.99, df = 5, *P* = 6.8x10^{-8} *
Amegilla calens:	χ^2 = 0.53, df = 2, *P* = 0.79 ns;	8-9 hr:	χ^2 = 11.13, df = 5, *P* = 0.04 *
Danaus plexippus:	χ^2 = 2.50, df = 2, *P* = 0.27 ns;	10-11 hr:	χ^2 = 20.24, df = 4, *P* = 2.9x10^{-4} *
Xylocopa olivacea:	χ^2 = 1.26, df = 3, *P* = 0.77 ns;	12-13 hr:	χ^2 = 2.80, df = 1, *P* = 0.16 ns

Pairwise comparisons of the pooled data between time periods (Bonferroni procedure): α'(*P*)

	Ap. mellifera	***A. calens***	***Amegilla* sp.**	***D. plexippus***	***H. misippus***
A/B:	0.01 (2.1x10^{-9})*	0.03 (0.003)*	0.02 (0.07)ns	0.05 (0.19)ns	0.05 (9.9x10^{-12})*
A/C:	0.005 (0.05)*	0.05 (0.56)ns	0.01 (5.1x10^{-4})*	0.02 (0.004)ns	-
A/D:	0.02 (1.5x10^{-7})*	0.01 (7.6x10^{-12})*	0.05 (0.49)ns	0.01 (9.9x10^{-8})*	-
B/C:	0.009 (7.4x10^{-29})*	0.02 (2.6x10^{-4})*	0.03 (0.15)ns	0.01 (1.5x10^{-5})*	-
B/D:	0.01 (9.1x10^{-16})*	0.009 (2.0x10^{-21})*	0.01 (0.004)*	0.009 (1.7x10^{-10})*	-
C/D:	0.03 (1.4x10^{-3})*	0.01 (5.4x10^{-10})*	0.009 (8.1x10^{-6})*	0.03 (0.014)*	-

	X. olivacea
A/B:	0.02 (9.2x10^{-4})*
A/C:	0.009 (1.5x10^{-13})*
A/D:	0.05 (0.43)ns
B/C:	0.01 (3.7x10^{-5})*
B/D:	0.03 (0.02)*
C/D:	0.01 (8.3x10^{-11})*

FFH: Fisher-Freeman-Halton test; I. *Apis mellifera,* II. *Amegilla calens,* III. *Amegilla* sp., IV. *Danaus plexippus,* V. *Hypolimnas misippus,* VI. *Xylocopa olivacea,* α': Bonferroni corrected significant level; ns: not significant difference (p>α'); *: significant difference (p<α')

Table 5. True Abundance of *Apis mellifera* on 1,000 Bloomed Flowers of *Vigna unguiculata*

		Treatment										
Locality	**Statistics**	**Tem**	**Para**	**Cp10**	**Cp20**	**Cp30**	**Ec10**	**Ec20**	**Ec30**	**Td10**	**Td20**	**Td30**
A. Bockle	Sample size	242	177	225	242	205	215	229	214	185	242	204
	Minimum	2	2	1	1	1	1	2	1	1	1	1
	Maximum	800	400	800	800	400	800	800	400	800	800	400
	Mean ± se	30±4	17±3	23±4	35±4	18±2	24±4	29±4	19±2	23±5	28±4	19±2
	Median	17	10	11	18	10	12	13	10	10	15	10
	Kruskall-Wallis one way ANOVA on Ranks: H = 95.35, df = 10, *P* <.001											
B. Dang	Sample size	388	191	247	356	247	246	332	232	228	241	218
	Minimum	1	1	1	1	1	1	1	1	1	1	1
	Maximum	400	179	800	800	800	800	800	800	400	800	400
	Mean ± se	26±2	16±1	22±4	24±3	18±3	19±3	24±3	18±4	22±2	24±4	20±2
	Median	15	10	10	13	9	9	13	10	11	11	10

		Treatment										
Locality	**Statistics**	**Tem**	**Para**	**Cp10**	**Cp20**	**Cp30**	**Ec10**	**Ec20**	**Ec30**	**Td10**	**Td20**	**Td30**
		Kruskall-Wallis one way ANOVA on Ranks: H = 91.27, df = 10, *P* <0.001										
C. Global	Sample size	630	368	472	598	452	461	561	446	413	483	422
	Minimum	1	1	1	1	1	1	1	1	1	1	1
	Maximum	800	400	800	800	800	800	800	800	800	800	400
	Mean ± se	27±2	17±1	22±3	28±2	18±2	22±3	26±2	19±2	22±2	26±3	19±2
	Median	15	10	10	15	10	10	13	10	11	13	10

Pairwise comparisons to the control plots: Dunn's procedure

	Untreated plots				**Parastar treatment plots**		
Comparison	**Bockle**	**Dang**	**Global**	**Comparison**	**Bockle**	**Dang**	**Global**
Tem - Para	Q = 5.61 *	Q = 4.46*	Q = 7.11*	Para vs Cp10	Q = 2.04 ns	Q = 1.15 ns	Q = 0.60 ns
Tem - Cp10	Q = 3.71 *	Q = 6.21*	Q = 6.92*	Para vs Cp20	Q = 3.81*	Q = 2.61 ns	Q = 4.49*
Tem - Cp20	Q = 1.88 ns	Q = 2.12ns	Q = 2.84ns	Para vs Cp30	Q = 1.23 ns	Q = 1.35 ns	Q = 0.08 ns
Tem - Cp30	Q = 4.53 *	Q = 6.36 *	Q = 7.62 *	Para vs Ec10	Q = 1.73 ns	Q = 0.44 ns	Q = 1.56 ns
Tem - Ec10	Q = 4.11 *	Q = 4.35 *	Q = 5.88 *	Para vs Ec20	Q = 6.10 *	Q = 2.79 ns	Q = 6.04 *
Tem - Ec20	Q = 0.54 ns	Q = 1.98 ns	Q = 1.15 ns	Para vs Ec30	Q = 0.61 ns	Q = 1.48 ns	Q = 0.66 ns
Tem - Ec30	Q = 5.18 *	Q = 6.61 *	Q = 8.32 *	Para vs Td10	Q = 1.24 ns	Q = 1.99 ns	Q = 2.36 ns
Tem - Td10	Q = 4.34 *	Q = 2.40 ns	Q = 4.69 *	Para vs Td20	Q = 4.16 *	Q = 1.77 ns	Q = 4.22 *
Tem - Td20	Q = 1.57 ns	Q = 2.74 ns	Q = 2.88 *	Para vs Td30	Q = 0.62 ns	Q = 1.15 ns	Q = 1.26 ns
Tem - Td30	Q = 5.16 *	Q = 3.33 *	Q = 5.99 *				

Significant pairwise comparisons between botanical chemical leaf extracts: Dunn's procedure

	Bockle	**Dang**	**Global**
Cp10 - Cp20	Q = 1.84 ns	Q = 4.16 *	Q = 4.15 *
Cp10 - Ec20	Q = 4.21 *	Q = 4.38 *	Q = 5.78 *
Cp10 - Td10	Q = 0.76 ns	Q = 3.36 *	Q = 1.89 ns
Cp10 - Td20	Q = 2.18 ns	Q = 3.15 ns	Q = 3.86 *
Cp20 - Ec30	Q = 3.30 *	Q = 4.53 *	Q = 5.51 *
Cp20 - Cp30	Q = 2.64 ns	Q = 4.32 *	Q = 4.83 *
Cp20 - Td30	Q = 3.30 *	Q = 1.40 ns	Q = 3.29 *
Cp30 - Ec20	Q = 5.02 *	Q = 4.53 *	Q = 6.46 *
Cp30 - Td10	Q = 0.002 ns	Q = 3.52 *	Q = 2.55 ns
Cp30 - Td20	Q = 2.99 ns	Q = 3.31 *	Q = 4.52 *
Ec10 - Ec20	Q = 4.61 *	Q = 2.53 ns	Q = 4.74 *
Ec20 - Ec30	Q = 5.66 *	Q = 4.75 *	Q = 7.15 *
Ec20 - Td10	Q = 4.82 *	Q = 0.64 ns	Q = 3.60 *

	Bockle	Dang	Global
Ec20 - Td30	Q = 5.65 *	Q = 1.57 ns	Q = 4.87 *
Ec30 - Td10	Q = 0.65 ns	Q = 3.70 *	Q = 3.16 ns
Ec30 - Td20	Q = 3.65 *	Q = 3.49 *	Q = 5.17 *
Td20 - Td30	Q = 3.64 *	Q = 0.61 ns	Q = 3.03 ns

*Other comparisons not presented in the table were not significant. Abbreviations are presented in table 2. ns: not significant difference (P >0.05), *: significant difference (P <0.05)*

Table 6. Production Rate of Pods and Seeds of *Vigna unguiculata* at Bockle and Dang

	Group of flowers									
	I. Free		II. protected		III. *Apis mellifera*		IV. No insect		Pooled data	
	n_1	n_2 (%)	n_1	n_2 (%)	n_1	n_2 (%)	n_1	n_2 (%)	n_1	n_2 (%)
A. Bockle										
Pods	349	321(92.0)	301	278(92.4)	577	532(92.2)	251	232(92.4)	1478	1363(92.2)
Seeds	323	307(95.0)	277	232(83.8)	540	496(91.9)	230	193(83.9)	1370	1228(89.6)
B. Dang										
Pods	317	297(93.7)	269	251(93.3)	524	486(92.7)	204	188(92.2)	1314	1222(93.0)
Seeds	300	286(95.3)	250	210(84.0)	493	452(91.7)	187	155(82.9)	1230	1103(89.7)
C. Global										
Pods	666	618(92.8)	570	529(92.8)	1101	1,018(92.5)	455	420(92.3)	2792	2585(92.6)
Seeds	623	593(95.2)	527	442(83.9)	1033	948(91.8)	417	348(83.5)	2600	2331(89.7)

Global comparison between groups: Fisher-Freeman-Halton-test

Locality	Global comparison of pods	Locality	Global comparison of seeds
Bockle	χ^2 = 0.07, df = 3, *P* = 1.00 ns;	Bockle	χ^2 = 31.33, df = 3, *P* = $6.4x10^{-7}$ *
Dang	χ^2 = 0.57, df = 3, *P* = 0.90 ns;	Dang	χ^2 = 30.47, df = 3, *P* = $9.7x10^{-7}$ *
global	χ^2 = 0.17, df = 3, *P* = 0.98 ns;	global	χ^2 = 61.78, df = 3, *P* = $2.3x10^{-13}$ *

Bockle *versus* Dang: *P-value* of the Fisher's exact test

	I	II	III	IV	Global
Pods:	*P* = 0.45 ns	*P* = 0.75 ns	*P* = 0.82 ns	*P* = 1.00 ns	*P* = 0.47 ns
Seeds:	*P* = 1.00 ns	*P* = 1.00 ns	*P* = 1.00 ns	*P* = 0.79 ns	*P* = 1.00 ns

Pairwise comparisons of the seed production using Bonferroni procedure:

	I - II: α'(*P*)	I - III: α'(*P*)	I - IV: α'(*P*)	II - III: α'(*P*)
Bockle	0.009 ($5.3x10^{-6}$) *	0.03 (0.10) ns	0.010 ($1.5x10^{-5}$) *	0.01 (0.0008) *
Dang	0.02 ($8.1x10^{-3}$) *	0.05 (0.84) ns	0.013 ($5.5x10^{-3}$) *	0.01 ($2.0x10^{-3}$) *
Global	0.02 (0.08) ns	0.05 (0.47) ns	0.009 ($3.8x10^{-6}$) *	0.03 (0.21) ns

Pairwise comparisons (continue)

	II - IV: α'(*P*)	III - IV: α'(*P*)
Bockle	0.05 (1.00) ns	0.02 (0.002) *
Dang	0.03 (0.83) ns	0.009 ($2.0x10^{-3}$) *
Global	0.01 ($6.1x10^{-3}$) ns	0.01 ($1.3x10^{-5}$) *

*ns: not significant difference (p>α'), *: significant difference (p<α'), n_1 = number of production, n_2 = number of normal production.*

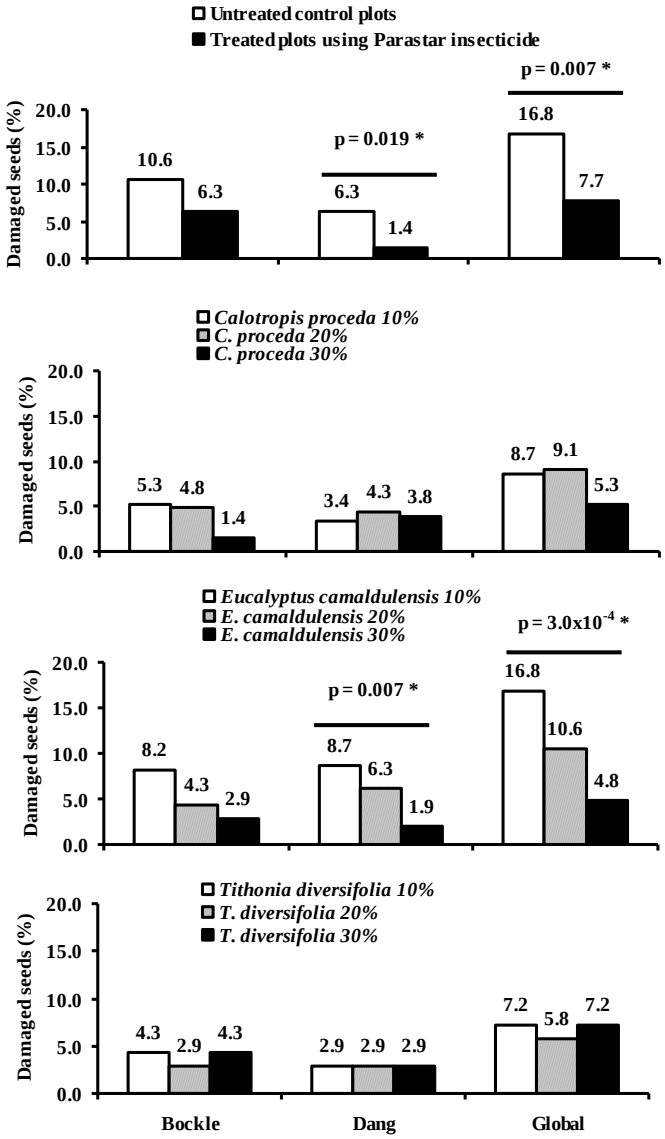

Fig. 2. Percentage Distribution of 208 Damaged Seeds Recorded in Bockle and Dang

Tukey's pairwise comparisons showed in Bockle and Dang, no significant difference between free flowers (group 1) and those visited exclusively by *Ap. mellifera* (group 3) and between protected flowers (group 2) and those not visited by floating insects (group 4). In the pooled data the difference was significant only between groups 1 and 4 and between groups 3 and 4. The other low rates recorded in groups 2 and 4 suggested a lake of positive impact of flower insects on seed production (Table 6).

Overall, fruiting rate, average number of seeds per pod and the percent of normal seeds were improved by the tested plant extracts. However, differences were not significant between treatments, except for fruiting rates recorded from free flowers. Moreover, the reproduction system did not affect the fruiting rate and the number of seeds per pod, except for fruiting rate at 20% *T. diversifolia* at Bockle. The percentage of normal seeds recorded from 10% *E. camaldulensis*, 20 and 30% *C. procera* were improved in Dang. Normal seeds improvement was recorded for control, 10% and 30% *E. camaldulensis*, *T. diversifolia* and 10% *C. procera* in Bockle. Damaged seeds were highly recorded in untreated plots (Fig. 2A) and lowly recorded in plots treated using 30% of each leaf extract except *T. diversifolia* whose reduction effect was statistically not significant (Fig. 2B, 2C and 2D). Parastar and aqueous extracts of plant

species significantly boosted seed yield, the 30% concentration being the most effective (Table 7). The tested botanical products increased significantly the seed yield of cowpea at Dang and Bockle (Table 7). Results recorded for *E. camaldulensis* 30% were similar with those recorded for parastar (Table 7). Overall, seed yield was higher at Bockle than at Dang especially for *C. procera* 10%, *E. camaldulensis* 10% and 20% and *T. diversifolia* 20% (Table 7). Between the plant species, at each of the concentrations of aqueous extracts (10%, 20% and 30%) the difference in seed yield was not significant [one-way ANOVA: $F_{(2;\ 9)} = 1.66$, $P = 0.24$ for 10% extract; $F_{(2;\ 9)} = 1.16$, $P = 0.36$ for 20% extract; $F_{(2;\ 9)} = 0.24$, $P = 0.80$ for 30% extract] at Bockle and Dang respectively.

4. DISCUSSION

4.1 Insect's Species Richness, Abundance and Dominance

The insecticidal ability of plant extract against pests of plants has been proven and validated and the relationships between floricultural plants and their pollinators are well known [5, 15, 16]. The present study is the first step to validate leaf extract of native wild plants as biopesticides against pests in cowpea fields, especially as a trial to replace synthetic pesticides whose negative impact is widely criticized [13, 14]. The cowpea plots showed a high occurrence of non-native pests as it is the case in vegetable crops [44, 45]. It is known that the anthropized areas are less diverse than that undergoing regeneration process. Our study revealed 11 species belonging to four orders and nine families associated with cowpea plants. Hemiptera represented more than 56.7% of the pests while Coleoptera represented 41.5% and Heteroptera was rare (0.9%). These insects very active on plants, suggested the recolonization from neighbouring fallows, or the cleaning of treated plants by rainwater, or an appearance of resistant individuals. Resistance would have been developed as a consequence of anarchic and uncontrolled use of parastar synthetic insecticide [13, 14, 23]. The low diversity of the pests is associated with low abundance in native species [two species (18.2%) and 19.8% of the total abundance], resulting in the weak exploitation of resources. The exploitation of food and nest sites was mostly achieved by exotic species: nine (81.8%) species and 80.2% of the total abundance. The high abundance of invasive exotic species in their introduced range is well known. The low insect diversity reflects the negative effect of the chemicals or the presence of both two native pests [*Anoplocnemis curvipes* (Hemiptera: Coreidae) and *Monolepta marginella* (Coleoptera: Chysomelidae)] and the non-native pests [*Aphis crassivora* (Hemiptera: Aphididae), *Aulacophora indica* (Coleoptera: Chrysomelidae), *Bothrogonia* sp. (Hemiptera: Cicadellidae), *Dysdercus cingulata* (Hemiptera: Pyrrhocoridae), *Lagria hirta* (Coleoptera: Tenebrionidae), *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae), *Riptortus dentipes* (Heteroptera: Alydidae) and *Tettigonia viridissima* (Orthoptera: Tettigoniidae)]. The native species *A. curvipes* and the native genera *Monolepta* are cited in West Africa as pests on soybean and cowpea [35, 46, 47]. Non-native species damage cultivated plants not only in the native range but also in areas of introduction. This is the case of polyphagous aphids in America, Europe and India where they are vectors of plant viruses [6, 7, 26, 40]. Transfer of aphids from neighbouring fallows may be the work of ants as it is the case in citrus orchards in Cameroon [48]. Exotic species would present harmful activity in cowpea fields and the low occurrence of native species could be the result of the regulation effect by natural enemies, or a negative force of introduced species. Inappropriate use of synthetic pesticides has resulted in unwanted effects including environmental pollution, non-target effect, human health hazards and the development of resistance to almost all insecticides [6, 7, 26, 40]. A similar situation would arise in North-Cameroon if the phytosanitary authorities do not take adequate measures to educate gardeners. Aphididae (45.3%), Chrysomelidae (38.7%), Pyrrhocoridae (4.8%), Coreidae (3.8%), Cicadellidae (2.8%), Tenebrionidae (0.9%), Alydidae (0.9%) and Tettigoniidae (0.8%) represented 98.0% of the total collection. The high abundance of aphids in vegetable crops is worldwide recognized. The high occurrence of Coleoptera (41.5%) and Hemiptera (56.7%) and the low presence of Heteroptera (0.9%) and Orthoptera (0.8%) may depend on the geographical area, the season, the farming and the cropping system. The insect richness was low compared to other crops. For example in Pakistan, 389 specimens, 10 orders, 33 families and 59 species were reported in olericulture spinach fields *Spinacia oleracea* L. (Amaranthaceae) while 327 specimens, nine orders, 30 families and 55 species were recorded in fenugreek fields *Trigonella foenum-graecum* (Fabaceae) [49].

Table 7. Estimation of the Seed Yield (± standard error) of *Vigna unguiculata* Estimated on 4 plots of Each Category

		Seed Mean Number (±se)		Seed Mean (±se) Weight (gr)	
Treatment	**Extract (%)**	**Bockle**	**Dang**	**Bockle**	**Dang**
Untreated plots		3,433 ± 438	876 ± 438	600.0 ± 54.0	450.0 ± 54.0
Parastar treated plots		11,888 ± 572	11,688 ± 572	1,330.0 ± 74.1	1,180.0 ± 74.0
Mann-Whitney Test: T(*P*)		**T = 10 (0.03) ***	**T = 10 (0.03) ***	**T = 10 (0.03)***	**T = 10 (0.03)***
Calotropis proceda	10	5,175 ± 333	4,975 ± 333	**802.5 ± 23.2**	**652.5 ± 23.2**
	20	7,375 ± 765	7,175 ± 765	**945.0 ± 67.6**	**795.0 ± 67.6**
	30	9,950 ± 479	9,750 ± 479	1,072.5 ± 43.1	922.5 ± 43.1
One-way ANOVA: $F_{(2; 9)}$		**18.52 ***	**18.52 ***	**7.85 ***	**7.85 ***
Eucalyptus camaldulensis	10	5,525 ± 320	5,325 ± 320	**810.0 ± 23.5**	**660.0 ± 23.5**
	20	7,725 ± 726	7,525 ± 726	857.5 ± 27.8	707.5 ± 27.8
	30	10,325 ± 515	10,125 ± 515	1107.0 ± 49.7	957.0 ± 49.7
One-way ANOVA: $F_{(2; 9)}$		**19.35 ***	**19.35 ***	**20.12 ***	**20.12 ***
Tithonia diversifolia	10	4,525 ± 485	4,325 ± 485	717.5 ± 60.6	567.5 ± 60.6
	20	6,775 ± 687	6,575 ± 687	**862.5 ± 29.5**	**712.5 ± 29.5**
	30	9,825 ± 375	9,625 ± 375	1067.5 ± 39.4	917.5 ± 39.4
One-way ANOVA: $F_{(2; 9)}$		**25.02 ***	**25.02 ***	**15.20 ***	**15.20 ***

	Seed yield (± se in kg/ha)			
	Extract (%)	**Bockle**	**Dang**	**Global**
Untreated plots	-	428.6 ± 38.6	321.4 ± 38.6	375.0 ± 32.4
Parastar treated plots	-	950.0 ± 52.9	842.9 ± 52.9	896.4 ± 40.1
Mann-Whitney Test: T(*P*)		**T = 10 (0.03)***	**T = 10 (0.03) ***	**T = 36 (<0.001) ***
Calotropis proceda	10	**573.2 ± 16.6**	**466.1 ± 16.6**	519.6 ± 23.0
	20	**675.0 ± 48.3**	**567.9 ± 48.3**	621.4 ± 37.6
	30	766.1 ± 30.8	658.9 ± 30.8	712.5 ± 28.6
One-way ANOVA: $F_{(2; 9)}$		**7.85 ***	**7.85 ***	**10.14 ***
Eucalyptus camaldulensis	10	**578.6 ± 16.8**	**471.4 ± 16.8**	525.0 ± 23.0
	20	612.5 ± 19.9	505.4 ± 19.9	558.9 ± 24.1
	30	790.7 ± 35.5	683.6 ± 35.5	737.1 ± 30.8
One-way ANOVA: $F_{(2; 9)}$		**20,12 ***	**20,12 ***	**15.22 ***
Tithonia diversifolia	10	512.5 ± 43.3	405.4 ± 43.3	458.9 ± 34.8
	20	**616.1 ± 21.1**	**508.9 ± 21.1**	562.5 ± 24.5
	30	762.5 ± 28.2	655.4 ± 28.2	708.9 ± 27.4
One-way ANOVA: $F_{(2; 9)}$		**15.20 ***	**15.20 ***	**18.46 ***

Comparison Bockle *versus* Dang (n = 4 plots each): Mann-Whitney rank sum test

Treatment	Extract(%)	Number of seeds	Weight of the seeds	Seed yield
Untreated plots	-	T = 20.0, *P* = 0.69 ns	T = 23.5, *P* = 0.11 ns	T = 23.5, *P* = 0.11 ns
Parastar treated plots	-	T = 20.0, *P* = 0.69 ns	T = 22.5, *P* = 0.20 ns	T = 22.5, *P* = 0.20 ns
Calotropis proceda	10	T = 21.0, *P* = 0.49 ns	**T = 26.0, *P* = 0.03 ***	**T = 26.0, *P* = 0.03 ***
	20	T = 20.0, *P* = 0.69 ns	T = 23.0, *P* = 0.20 ns	T = 23.0, *P* = 0.20 ns
	30	T = 21.0, *P* = 0.49 ns	T = 24.0, *P* = 0.11 ns	T = 24.0, *P* = 0.11 ns
Eucalyptus camaldulensis	10	T = 20.0, *P* = 0.69 ns	**T = 26.0, *P* = 0.03 ***	**T = 26.0, *P* = 0.03 ***
	20	T = 20.0, *P* = 0.69 ns	**T = 26.0, *P* = 0.03 ***	**T = 26.0, *P* = 0.03 ***
	30	T = 21.0, *P* = 0.49 ns	T = 23.5, *P* = 0.11 ns	T = 23.5, *P* = 0.11 ns
Tithonia diversifolia	10	T = 20.0, *P* = 0.69 ns	T = 23.0, *P* = 0.20 ns	T = 23.0, *P* = 0.20 ns
	20	T = 20.5, *P* = 0.69 ns	**T = 26.0, *P* = 0.03 ***	**T = 26.0, *P* = 0.03 ***
	30	T = 21.0, *P* = 0.49 ns	T = 25.0, *P* = 0.06 ns	T = 25.0, *P* = 0.06 ns

Pairwise comparisons of the seed yields (Tukey's procedure)

	Bockle			**Dang**		
	10 *vs.* 20%	10 *vs.* 30%	*20 vs.* 30%	10 *vs.* 20%	10 *vs.* 30%	*20 vs.* 30%
C. proceda	*P* =0.07ns	***P* <0.001 ***	*P* = 0.11 ns	*P* = 0.15 ns	***P* = 0.008 ***	*P* = 0.20 ns
E. camaldulensis	*P* =0.85 ns	***P* <0.001 ***	***P* <0.001 ***	*P* = 0.63 ns	***P* <0.001 ***	***P* = 0.002 ***
T. diversifolia	*P* =0.05 ns	***P* <0.001 ***	***P* = 0.005 ***	*P* = 0.11 ns	***P* = 0.001 ***	***P* = 0.03 ***

ns: not significant difference; *: significant difference. Significant diffences are in bold.

According to the same information source, 373 specimens of 11 orders, 34 families and 61 species were reported in turnip fields *Brassica rapa* var. *rapa* L. (Brassicaceae). In Balessing (Cameroon), 370 insects, four orders, 16 families and 21 species were recorded in potato fields and 155 specimens belonging to four orders, 13 families and 22 species were collected in egg-plant fields [44, 45].

4.2 Pest Insects and Impact of Aqueous Leaf Extracts

The major insect pest was *Aphis crassivora* (Hemiptera: Aphididae) on young stems, leaves, flowers and pods, as it is the case in Africa, Asia and Latin America [50, 51]. High abundance of *Ah. crassivora* was certainly due to the favorable climatic conditions (hot climate and high air relative humidity) [21-23]. The efficacy of leaf extract of *Eucalyptus camaldulensis* (Myrtales: Myrtaceae) and that of *Tithonia diversifolia* (Asterales: Asteraceae) against *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae) and that of *Calotropis procera* (Gentianales: Apocynaceae) against *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae) are known [52, 53]. Although leaf extracts may have adverse effects on pollinators [54], those tested in our study were not harmful to bees. It is also known that in Cameroon, synthetic pesticides, although approved, are frequently handled in anarchic and uncontrolled manner by non-expert, poorly educated farmers [13, 55, 56]. The disruptive effect of synthetic pesticides on the memory and foraging behavior aptitude of pollinators is well known, honey bees subjected to the synthetic pesticide being unable to return to the feeding site in the same way as untreated bees [57]. Yet we recorded that more than 50% pollinators were *Apis mellifera* (Hymenoptera: Apidae) harvesting nectar and pollen. These bees are known as the most widespread and common pollinators of crops [58]. The floral constancy phenomenon is well known in honey bees [59] and is explained by the fact that the forager is generally able to memorize and recognize the shape, color, and odor of flowers visited on previous foraging trips [60]. In the United States of America, investigations have shown that some foragers of the honey bee were constant on the flowers of the same avocado tree for at least 24 hours [61]. Honey bee's collection time, visitation frequency and pollen deposition are key factors for measuring their pollination efficiency in allogamous or allogamous-autogamous crops [62]. Then the availability of resources, the biotic and abiotic factors must be adequate with bee fitness. Bees visited flowers between 6:00 am and 1:00 pm with a peak of activity between 8:00 and 9:00 a.m.. The peak of pollination activity is known to be correlated with the flower blooming rate, the availability of floral products and the combination of scents from flowers and botanical products [63, 64]. It is also expected that foraging activity is influenced passively by elevated temperature [65]. The low activity of *Ap. mellifera* on the flowers treated using parastar synthetic insecticide would be related to the harmfulness of the product. The high abundance of *Ap. mellifera* workers per 1,000 flowers highlighted attractiveness of the floral products of the cowpea and suggested that sugar content of the nectar product (43.0%) was within the preference range for Apidae (30% to 50.0%) [66, 67]. Bees do remember position of their blooming flower plants [68]. The low abundance of foragers on parastar treated plants could be the result of the repulsion or the elimination by the toxic molecules [69]. The duration of the flower visit varied with the availability of nectar or pollen, and bees stayed long on rich flowers than on poor flowers. A forager can obtain its load by visiting a small number of rich flowers, thus saving foraging energy. The foraging visit varied according to the type of chemical treatment, which justifies the differential effectiveness of these products.

The foraging activity of *Ap. mellifera* higher in Bockle than Dang could be explained by the presence in neigbouring fallows of flowers of *T. diversifolia* (Asterales: Asteraceae), *Arachis hypogaea* L., 1753 (Fabales: Fabaceae), the cosmopolitan adventitia *Bidens pilosa* L., 1753 (Asterales: Asteraceae) and *Sida rhombifolia* L., 1753 (Malvales: Malvaceae). In Bockle, bee foragers were faithful to the exploited plant. The floral constancy phenomenon is well known in honey bees since foragers are generally able to memorize and recognize the shape, color, and odor of flowers visited on previous trips [59, 60]. Bee's collection time, visitation frequency and pollen collection are key factors for measuring their pollination efficiency on allogamous or allogamous-autogamous plants. According to our results, cowpea had a mixed allogamous-autogamous reproductive regime, with predominance of autogamy. This result is in agreement with repports from Obala (Cameroon) where the allogamy was 5.5% and the autogamy was 94.5% [62]. The contribution of *bee* to the cowpea yield improvement confirmed that bees were major cowpea pollinators. Hymenoptera in general and Apoides in particular are known to

positively influence fruit and seed yields [70]. Seed yield could be the result of the combined impact of plant extracts and bee's pollination performance as is the case with the insecticidal effect recorded in Nigeria, of aqueous leaf extracts of *Azadirachta indica, Ocimum gratisimum* and *Vernonia amygdalina* on insect field pests of *Amaranthus hybridus* [71]. According to the same authors *A. hybridus* plants sprayed with aqueous leaf extracts of the different plants had lower percentage leaf and leaf area damage compared to the control. It is also the case in India of ethyl acetate extracts of *Dillenia indica* L. (Dilleniaceae) leaves found toxic to rice weevil, *Sitophilus oryzae* (L.) (Coleoptera), lesser grain borer *Rhyzopertha dominica* (L.) (Coleoptera) and red flour beetle, *Tribolium castaneum* (Herbst.) (Coleoptera) [72]. Our observations are similar to those made in Indonesia concerning the effect of papaya leaf extract (*Carica papaya* L.) on the mortality rate of *Spodoptera litura* Fabricius larvae and the level of damage to soybean leaves [73].

5. CONCLUSION

Botanical extracts reduced the population of *Aphis crassivora* and increased the foraging ability of pollinators. The yield and quality of cowpea seeds were improved. Similar seed yield results were obtained in both study sites using the synthetic insecticide parastar and 30% extract of *Calotropis procera* (Gentianales: Apocynaceae), *Eucalyptus camaldulensis* (Myrtales: Myrtaceae) and *Tithonia diversifolia* (Asterales: Asteraceae). Parastar was harmful to honey bees unlike botanical extracts. The 30% extract of these three plants could be used as alternative to synthetic insecticides. The preservation of honey bee hives near cowpea plantations is necessary to improve the seed yields.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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