Diversity of insect's pollinators of Cowpea, Vigna unguiculata L. Walp small white variety seed (Fabaceae) and assessment of its impact on yields at Malang (Cameroon)

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ABSTRACT

Vigna unguiculata (cowpea) is an important food plant whose production and valorization are of high priority for improving the food security of the population. The objective of this study is to study the activity of insects and its impact on the yield of *V. unguiculata* in a farming environment. From August to October 2022, the experiments were carried out mainly on 360 flowers divided in three treatments two treatments differentiated according to the presence or absence of protection of the flowers from other insects; the third treatment with flowers protected then open to foraging by insects. Insect foraging behavior, fruiting rate and percentage of normal seeds were assessed. The insects collected nectar intensively and regularly. The fruiting rate from treatments 1, 2 and 3 is 92.50%, 62.50% and 85% respectively. For the average number of seeds per pod, the figures corresponding to T1, T2 and T3 are 7.51, 4.46 and 6.61. The percentage of normal seeds corresponding to these batches is 88.58%, 63.80 and 84.13% respectively. Flowering insects significantly increased the fruiting rate, the average number of seeds per pod and the percentage of normal seeds.

Keywords: *Vigna unguiculata*, flowers, pollination, insects, Malang.

RESUME

Vigna unguiculata (niébé) est une importante plante alimentaire dont la production et la valorisation sont d'une grande priorité pour l'amélioration de la sécurité alimentaire de la population. L'objectif de cette étude est d'étudier en milieu paysan l'activité des insectes et son impact sur le rendement de *V. unguiculata*. D'août à octobre 2022, les expériences ont porté sur 360 fleurs divisées en trois traitements deux traitements différenciés selon la présence ou l'absence de protection des fleurs vis-à-vis d'autres insectes ; le troisième traitement aux fleurs protégées puis ouverts au butinage par les insectes. Le comportement de butinage des insectes, le taux de fructification et le pourcentage de graines normales ont été évalués. Les insectes récoltaient intensément et régulièrement le nectar. Le taux de fructification, issues des traitements 1, 2 et 3 est de 92,50 %, 62,50% et 85% respectivement. Pour le nombre moyen de graines par gousse, les chiffres correspondants au T1, T2 et T3 est de 7,51, 4,46 et 6,61. Le pourcentage de graines normales correspondant à ces lots est de 88,58%, 63,80 et 84,13% respectivement. Les insectes floricoles ont augmenté significativement le taux de fructification, le nombre moyen de graines par gousse et le pourcentage de graines normales.

Mots clés : *Vigna unguiculata*, fleurs, pollinisation, insectes, Malang.

INTRODUCTION

Agriculture is one of the key and priority sectors of the Cameroonian economy. It is the main provider of employment, since it employs nearly 70% of the active population, contributes 42% to the GDP and represents 51% of exports (Cameroon-Report, 2014). Among the cultivated plants we have among others the cowpea.

Cowpea, *Vigna unguiculata is* a plant traditionally cultivated in Africa, most often in association with other food crops such as corn, millet, sorghum (Mako *et al*., 2013). In most growing regions, the young shoots and leaves are eaten as leafy vegetables. Cowpea plays a very important role in the diet in Africa. Its seeds, rich in protein, are made up of most of the essential amino acids, with the exception of sulfur amino acids (Bressani, 1985). It is therefore an interesting nutritional supplement in the case of protein-deficient diets (Mako *et al*.,2013). The stems and leaves, rolled into bundles after the pods are harvested and dried on sheds, provide excellent fodder for animals during the dry season (Mako *et al*., 2013). Cowpea has interesting agronomic characteristics. Indeed, it can be grown on poor soils, as its cultivation requires little fertilizer due to its ability to achieve symbiotic fixation of atmospheric nitrogen (Rachie, 1985). This legume is therefore recommended in crop rotations and associations.

Despite the importance of cowpea in human nutrition, the climatic and ecological diversity enjoyed by the country not only favors the development of various plants but also promotes the proliferation of various parasites that harm the growth and production of plants (Tchatat, 1996).

Crop damage is caused by infestations of these pests, which often lead to serious consequences, thus justifying the need for the use of pesticides. According to the FAO definition, a pesticide is a substance used to neutralize or destroy a pest, a vector of human or animal disease, a harmful or troublesome plant or animal species during the production or storage of agricultural products to ensure sustainable food production with higher yields and greater availability of food all year round.

At the same time, despite their advantages, the use of pesticides generates a certain number of risks with regard to the chemical composition of the air, water and soil which result in pollution whose toxicological (for humans) and ecotoxicological (for living organisms) consequences can be detrimental to the quality of the environment and cause the destruction of several insects (Diptera, Heterocera Lepidoptera, Coleoptera, hymenoptera or even lepidoptera) which have floriculture activity and generally contribute to the pollination of these plants (Calvet *et al*., 2005, Fenster *et al*., 2004).

The main objective of this work is to evaluate the impact of flower insects on the yield of *V. unguiculata* for sustainable agriculture in Dang. Specific objectives include: a) inventory of the floricultural entomofauna of the studied plant; b) study of insect activity on the flowers of this Fabaceae; c) evaluation of the impact of floricultural insects on the pod and seed yield of this species.

I. Material and methods

I.1. Location of the study site

The investigations were carried out from August to October 2022 in Ngaoundere, in the Adamaoua Region of Cameroon (Figure 1). This Region is located between the 6th and 8th degrees of North latitude and between the 11th and 15th degrees of East longitude; it covers approximately 63,701 km²; it belongs to the agroecological zone known as the Guinean high savannahs (Djoufack-Manetsa, 2011). The climate is Sudano-Guinean, mild and cool, characterized by two seasons: a rainy season (April to October) and a dry season (November to March). The annual rainfall is 1500 mm. The average annual temperature is 22° C and the average annual humidity is 70% (Amougou *et al*., 2015).

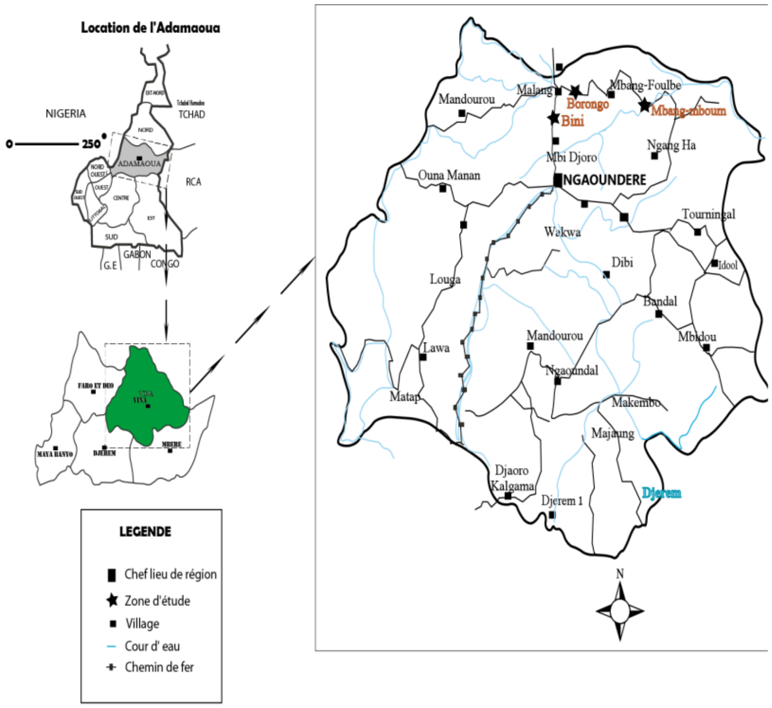

Figure 1: Location of the study site

I.2. Plant and animal material

It is made up of the seeds of *V. unguiculata* Var. small white seed (figure 2) obtained from the IRAD of Touboro and the animal material consists of flower-growing insects naturally present in the study site.

Figure 2: Seeds of *Vigna unguiculata* Var. small white seed

I.3. Methods

I.3.1. Preparation of the experimental plot and sowing

From August 1 to 5, 2022, an experimental field of 437 m^2 was cleared, plowed and divided into 8 sub-plots of 8 m length, 4.5 m width and 20 cm height. The sub-plots were separated from each other by a 1 m wide alley, to facilitate movement during observations (Figure 3).

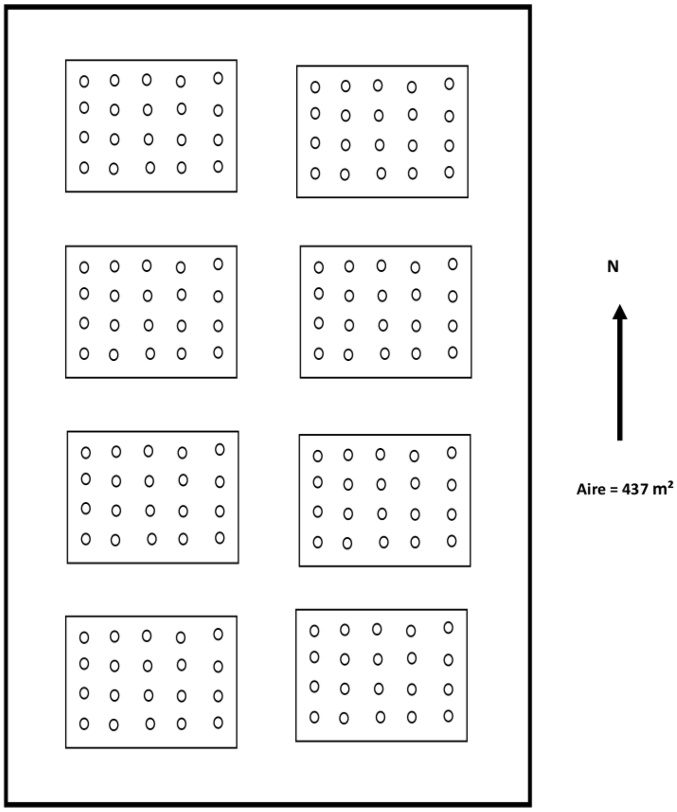

Figure 3: Experimental device of *Vigna unguiculata* in 2022 in Dang.

: Hedge delimiting the experimental plot

1 to 8: Sub-plot numbers

: Plant of *Vigna unguiculata*

Treatment 1: 120 labeled bud-stage flowers (belonging to sub-plots 1 to 8) left to pollinate freely

Treatment 2: 120 labeled bud-stage flowers (belonging to sub-plots 1 to 8) protected from insects using gauze bags

After preparing the field, the seeds of *V. unguiculata*, which is a small white seed variety with an intermediate cycle (85 to 95 days) were planted respecting the spacing of 75 cm between the rows and 50 cm on the rows corresponding to the prostrate varieties of cowpea. 14 days after emergence, thinning was carried out and two best were left in pockets. From germination to the appearance of the first flower buds, weeding was regularly carried out with a hoe, every two weeks. From flowering to fruit ripening, weeding was done by hand.

I.4. Data collection

The parameters to be taken into account were the diversity of flower-dwelling insects of *V. unguiculata*, the impact of these insects on plant yield.

I.4.1. Determination of the mode of reproduction of *Vigna unguiculata*

As soon as the first flowers appeared, 240 flower buds were labeled and two treatments were made up:

- treatment 1: 120 flower buds labeled and left to pollinate freely and on which no insects were captured;

- treatment 2: 120 flower buds labeled and protected from insects using 1mm^2 mesh gauze bags (Tchuenguem, 2005).

At harvest, the number of pods formed was counted in the subplots of treatments 1 and 2. For each treatment, the fruiting index (*Ifr*) was calculated according to the following formula: $Ifr = (Np / Nf)$ Or *Np* is the number of pods formed and *Nf* the number of viable flowers initially borne (Tchuenguem *et al*., 2001).

The difference between the fruiting indices of the two treatments (1 and 2) allowed us to calculate the allogamy rates (*TC*) and autogamy (*TA*), according to the following formulas: $TC = \{[(Ifr1 - Ifr2) / Ifr1] \times 100\}$, where *Ifr1* and *Ifr2* are respectively the fruiting indices in the treatment with flowers left to pollinate freely (T1) and in the treatment with protected flowers (T2);

$TA = [100 - TC]$ (Demarly, 1977).

I.4.2. Determination of the place of each insect in the floricultural entomofauna of *Vigna unguiculata*

Investigations were carried out on labeled flowers left to pollinate freely (T1). Observations were made every day, during four daily time slots: 8-9 am, 10-11 am, 12-1 pm, 2-3 pm. We visited each plant once for each of the above time slots. At each visit, the different insects encountered on the blooming flowers were identified by a code and counted. Since the insects were not marked, the cumulative results were expressed by the number of visits (Tchuenguem, 2005).

The frequency of the insect's visits *i* (*Fi*) on the flowers of *V. unguiculata* is calculated using the following formula: $Fi = [Vi/VI \times 100]$, with *Vi* = number of visits by the insect *i* on the flowers of the treatment with flowers left in free pollination (T1) and *VI* the number of visits of all insects to these same flowers (Tchuenguem *et al.*, 2001).

Data on the relative frequency of visits of the different anthophilous insects recorded made it possible to determine the place of each of them in the floricultural entomofauna of *V. unguiculata* (Tchuenguem, 2005).

I.4.3. Insect activity on plant flowers

I.4.3.1. Harvested floral products

This involves noting whether the visiting insect collects pollen, nectar, or both on a flower. An insect that plunges its mouth parts or head deep into the corolla of a flower is a nectar forager; if it scrapes the anthers with its mandibles and legs, it is a pollen forager (Tchuenguem, 2005). Collected pollen can be observed on transport organs, particularly in the baskets of the hind legs in Apidae, the collecting hairs on the legs in Halictidae, or the ventral brush in Megachilidae (Borror & White, 1991). The floral products collected were systematically noted, by a distinctive sign, when recording the duration of visits per flower (Tchuenguem, 2005).

I.4.3.2. Insect abundance

This involves counting the greatest number of insects simultaneously active on a flower and on 1000 flowers (Tchuenguem, 2005).

Flower abundances are recorded following direct counts. For abundance per 1000 flowers (A_{1000}), individuals of each insect species are counted on a known number of blooming flowers.

The abundance per 1000 flowers (A_{1000}) is calculated using the following formula: $A_{1000} = [(A_x / F_x) \times 1000]$, where F_x and A_x are respectively the number of flowers in bloom and the number of pollinating insects actually counted on the flowers left for free pollination at the moment *x* (Tchuenguem, 2005). The data are recorded on the same dates as the frequency of visits and according to six time slots (9 - 10 a.m., 11 - 12 a.m., 1 - 2 p.m. and 3 - 4 p.m.), with at least five values per time slot and for each insect, when its activity allows it.

I.4.3.3. Evaluation of the relationships between flowering rate and visit rate

The number of blooming flowers is counted every day, from the beginning of the investigations until the last flower has faded. The data obtained are compared with the number of visits by different insects to the corresponding flowers (Tchuenguem, 2005). This parameter is recorded during the same dates and time slots as the frequency of visits.

I.4.3.4. Impact of floral entomofauna on the yield of *Vigna unguiculata*

It was based on:

- the impact of anthophilous insects on pollination;
- the impact of pollination on fruiting *V. unguiculata*;
- comparison of pod and seed yields (fruiting rate, number average number of seeds per pod and percentage of normal seeds) of treatments 1 and 2.

The percentage (*Pi*) of the fruiting rate due to the influence of pesticides and flower insects is calculated using the following formula (Tchuenguem *et al*., 2001): $Pi = \{[(F1–F2) /F1]x100\}$ where *F1* and *F2* are the fruiting rates in treatments 1 and 2 respectively. For a treatment, the fruiting rate = (number of pods / number of flowers) x100.

The percentage (*Pp*) of the number of seeds per pod attributable to the influence of flower insects is calculated using the following formula (Tchuenguem *et al*., 2001): $Pg = \{[(g1– p2) /p1] x 100\}$ where *p1* and *p2* are the average numbers of seeds per pod in treatments 1 and 2 respectively.

The percentage (*Pn*) of normal seeds attributable to the influence of pesticides and flower insects is calculated using the following formula (Tchuenguem *et al*., 2001): $Pn = \{([Pn1–Pn2) /Pn1] x 100\}$ where *Pn1* and *Pn2* are the percentages of normal seeds in treatments 1 and 2 respectively.

I.4.3.5. Data processing

Excel 2007 software was used for three tests: (a) Test –t Student's t-test at the 5% threshold for comparing the means of two samples; (b) Chi-square (χ2) for comparing percentages; (c) ANOVA (F) for the difference of means of more than two samples; (d) Pearson correlation coefficient (r) for the study of linear relationships between two variables.

II. RESULT AND DISDUTION

II.1. Mode of reproduction of *V. unguiculata*

Vigna unguiculata at the investigation station, 120 flowers for each treatment 1 and 2 were studied. The fruiting index was 0.92 and 0.62 in treatments 1 and 2 respectively. Thus, *TC* = 32.60% and *TA* = 67.4%.

Consequently, *V. unguiculata* has a mixed allogamous - self-pollinated reproduction regime, with a strong predominance of self-pollination.

II.2. Insect activity on flowers of *Vigna unguiculata*

II.2.1. Frequency of visits

On 120 flowers of *V. unguiculata*, 334 visits of six insect species were counted. Table 1 presents the list of these insects with their visit frequencies. It is clear from this Table that among the flower-dwelling insects of *V. unguiculata*, *A. mellifera* was most frequently observed followed by *Xylocopa olivacea* with 52.39% and 13.77% visits respectively.

Table 1: Insects recorded on the flowers of *Vigna unguiculata* in Malang in 2022, number and percentage of visits of different insects.

Insects			2022	
Order	**Familly**	**Genus, Species**	***n***	***P*** **(%)**
Hymenoptera	Apidae	*Apis mellifera* (ne)	175	52.39
		Xylocopa olivacea (ne)	46	13.77
		Amegilla sp. 2 (ne)	26	7.78
	Total Hymenoptera		**247**	**73.94**
		Eurema eximia (ne)	29	8.68
		Graphilum angolanus (ne)	32	9.58
		Cotopsilia florella (ne)	26	7.78
	Total Pieridae		**87**	**26.04**
Total			**334**	**100**

n: number of visits to 120 flowers in six days; ***p***: percentage of visits = (*n*/334) * 100; **sp**.: species not determined; **ne**: collection of nectar; **po**: collection of pollen.

II.2.2. Floral products collected

Throughout the study period, insects present on the flowers of *V. unguiculata* in the experimental field harvested nectar intensively and exclusively.

II.2.3. Rhythm of visits according to the stages of flowering

Overall, insect visits were higher in the field of *V. unguiculata* when the number of blooming flowers was higher (Figure 4). The correlation between the number of blooming flowers and the number of insect visits was found to be positive and highly significant for the treatment ($r = 1$; $ddl = 4$; $P < 0.001$).

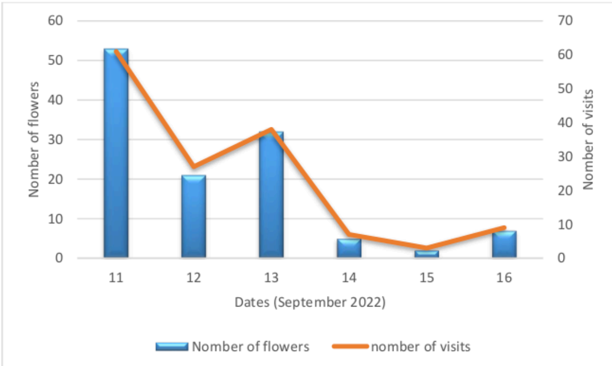

Figure 4: Variation in the number of blooming flowers and the number of insect visits to flowers *Vigna unguiculata* according to observation dates in 2022 in Malang

II.2.4. Insect abundance

The average number of insects observed active at the same time was 1.3 per flower and 74.49 per 1000 flowers.

II.2.5. Influence of some climatic factors

Figure 5 shows the daily variation of temperature, relative humidity and number of insect visits to flowers of *V. unguiculata*.

It emerges from this figure that the correlation is not significant between the number of insect visits and the temperature ($r = 0.92$; $ddl = 2$; $P > 0.05$) as well as between the number of visits and the relative humidity ($r = 0.62$; $ddl = 2$; $P > 0.05$). The peak of insect activity on the flowers of *V. unguiculata* was highlighted in the morning, between 10 a.m. and 11 a.m. This period of the day would correspond to the time of greatest availability of nectar at the level of the flowers of the Fabaceae.

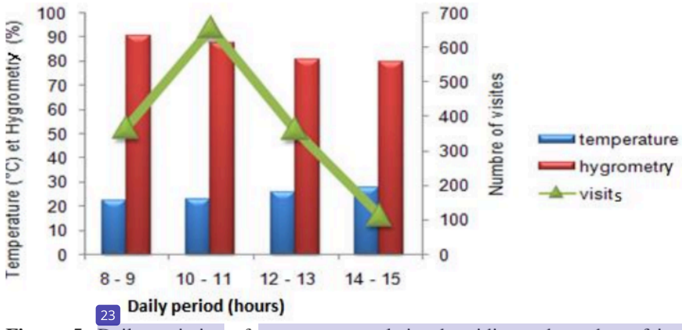

Figure 5: Daily variation of temperature, relative humidity and number of insect visits to flowers of *Vigna unguiculata* in 2022 in Malang.

II.2.6. Yields of

Table 4 summarizes the data regarding fruiting rate, mean number of seeds per fruit and percentage of normal seeds in the different treatments of *V. unguiculata*.

Table 2: Fruiting rate, average number of seeds per pod and percentage of normal seeds obtained in each of the treatments *Vigna unguiculata*

Traitements	NF	NFP	PrR (%)	NS / P		TNS	NNS	% NS
				m	***sd***			
1 (FL)	120	111	92.50	7.51	3.47	902	799	88.58
2 (Fp)	120	75	62.50	4.46	3.91	536	342	63.80
3 (FpI)	120	102	85.00	6.61	3.58	794	668	84.13

FL: flowers left to pollinate freely; **FP**: flowers protected from insects; **FpI**: flowers protected then reopened and visited once by some insect and again protected; **NF**: Number of flowers; **NFP:** Number of formed pod; **PrR:** Podding rate; **NS / P:** Number of seeds per pod; **TNS:** Total number of seeds; NNS: Number of normal seeds; **% NS:** Percentage of normal seeds; ***m*:** mean; ***sd*:** standard deviation.

It emerges from this table that:

a) the fruiting rates were 92.50%, 62.50% and 85.00% in the treatments 1, 2 and 3 respectively. The difference between these three percentages is very high significant: $\chi2_{overall}$ = 108.56 (*ddl* = 2; $P < 0.001$; THS). The two by two comparison of these percentages shows that the difference is very highly significant between treatments 1 / 2: χ2= 30.94 (*ddl* = 1; $P < 0.001$; THS); 2 / 3: χ2= 15.69 (*ddl* = 1; $P < 0.001$; THS) and not significant between treatments 1/3: χ2 = 3.38 (*ddl* = 1; $P > 0.05$; NS).

b) the average numbers of seeds per pod were 7.51, 4.46 and 6.61 in the treatments 1, 2 and 3 respectively. The difference between these three means is very high significant (F = 357; *ddl1* = 2; *ddl2* = 6.94; $P < 0.001$; THS). Comparing these means two by two shows that the difference is very highly significant between treatments 1 / 2 (t = - 6.36; *ddl* = 238; $P <$

0.001; THS); 2 / 3 (t = -4.41; *ddl* = 238; $P < 0.001$; THS) and non-significant between treatments 1/3 (t = 1.97; *ddl* = 238; $P > 0.05$; NS).

g) the percentages of normal seeds were 88.58%, 63.80% and 84.13% in the treatments 1, 2 and 3 respectively. The difference between these three percentages is very highly significant ($\chi2_{Overall}$ = 66.22; *ddl* = 2; $P < 0.001$; THS). The two-to-one comparison two of these percentages shows that the difference is very highly significant between treatments 1 / 2 ($\chi2$ = 125.93; *ddl* = 1; $P < 0.001$; THS); 2/3 ($\chi2$ = 72.35; *ddl* = 1, $P < 0.001$; THS) and highly significant 1 / 3 ($\chi2$ = 7.16; *ddl* = 1; $P < 0.01$; HS).

Discussion

In Malang in 2022, 6 species of insects were recorded on the flowers of *V. unguiculata*. Among these insects, Hymenoptera represented by the Apidae Family were in the majority followed by Diptera represented by the Pieridae Family on the flowers of this plant.

The data in Table 1 show that the frequencies of visits vary more or less with the insects. This is in agreement with the observations of Kengni *et al*. (2015) on the same plant species; Kingha (2012) on *Phaseolus vulgaris* and those of Farda *et al*. (2018) on *V. subterranea* white variety of a Fabaceae. *Apis mellifera* is known to be one of the most common flower-feeding insects on the flowers of several other plant species including: *Millettia laurentii* (Nissoet *et al*., 2025); *Brachiaria brizantha* (Adamou & Tchuenguem, 2014); *Brassica napus* (Klein *et al*., 2006; Jauker & Wolters, 2008; Hoyle & Cresswell, 2009); *Luffa cylindrica* (Farda & Tchuenguem, 2018); *Cucumeropsis mannii* (Azo'o & Messi, 2012); *Cocos nucifera* (Da Conceicao *et al*., 2004); *Croton macrostachyus* (Népidé & Tchuenguem, 2016) and *Helianthus annuus* (Hoffman & Chambers, 2006; Tchuenguem *et al*., 2009a).

Overall, our results, like those of other researchers, indicate that the Specific richness of floral entomofauna varies with the plant species and the year.

The high abundance of insect foragers per 1000 flowers and the positive and significant correlation between the number of visits by these insects and the number of blooming flowers of *V. unguiculata* highlight the high attractiveness of the nectar and/or pollen of this Fabaceae to insects. Peaks in insect visits to *V. unguiculata* could be explained by the greater availability and accessibility of nectar at the flowers concerned during the periods corresponding times.

The decreases in activity observed in the flowers of this plant after the 2 - 3 p.m. time slot would be linked to the decrease in the quality and/or quantity of floral products.

According to Pesson & Louveaux (1984), when the forage is no longer easily exploitable or when it is reduced in quantity and/or quality, insects reduce their activities on the flowers, so that the energy spent on foraging is not greater than that which can be obtained from the forage. It is, in fact, known that the daily foraging activity of insects on the flowers of a plant depends on its production of pollen and/or nectar (Pouvreau, 2004).

The positive and significant correlation between the number of visits and the number of blooming flowers highlights the good attractiveness of the nectar of the corresponding flowers vis-à-vis - insects. Our results are in agreement with the research of Bérenger - Lévêque (1982) who mention that the number of flowers in bloom plays an important role in the orientation or not of insects towards the flowers.

The strong attractiveness of the nectar of *V. unguiculata* during the morning towards insects could be partly explained by the availability and quality of this food as well as by the time necessary for its harvest at the level of the corresponding flowers (Roubik, 2000). This attractiveness would also be linked to odoriferous compounds such as sterols (Pierre & Chauzat, 2005) and lipids (Sing *et al*., 2000).

The percentage of fruiting rate due to the influence of flower-feeding insects is 92.50%. The percentage of the number of seeds per pod due to the activity of flower-feeding insects is 11.42%. The percentage of normal seeds due to the activity of flower-feeding insects is 24.78% in the same plant. These data are proof that not only do flower-feeding insects effectively improve the yields of pods or fruits and seeds of these plants, but also that they play a very important role in the production of good quality seeds. According to Jean - Prost (1987), the more pollen grains a flower receives, the more potential it has to transform into a large fruit containing many seeds. Népidé & Tchuenguem (2016) as well as Djakbé *et al*. (2017) showed that insect pollination increases fruiting rate by, *Croton macrostachyus* and *Physalis minima* of 30.29% and28.76% respectively.

The positive and significant contribution of insects in the fruiting of *V. unguiculata* is thus justified by the action of insects on self-pollination and cross-pollination.

Despite the abundance of insects during the flowering of *V. unguiculata*, several flowers visited by the latter did not produce pods. This result demonstrates that numerous visits by insects to the flowers of *V. unguiculata* are beneficial to these arthropods but have no influence on pollination and yields of the Fabaceae.

Conclusion

In Dang, *V. unguiculata* studied under a mixed allogamous - autogamous reproduction regime, with a predominance of autogamy. Among the insects that visit the flowers of this plant, *A. mellifera* is by far the most common.

From our study, *V. unguiculata* White variety is a plant that highly benefits from pollination by insects. The comparison of the pod and seed sets of protected flowering plant with those of flowering plant visited by insects underscores the value of this these insects in increasing pod and seed yields, as well as improving seed quality. Our results suggest that preserving harmless anthophilous insects close *V. unguiculata* field significantly improve the pods and seed production of this important legume in the region.

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