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RESEARCH ARTICLE

POLLINATORS' DIVERSITY 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 which produc tion and valorizationare of high priority in sustaining food security. The objective of this study was to investigate insects' activity and its impact on V. unguiculata yield in a farming environment. From august to october2022, experiments were carried out on 360 flowers divided into three treatments, two of them differentiated according to the presence or absence of protection from other insects; the third treatment with flowers protected then uncovered to allow insects' visit. The foraging behavior, the fruiting rate and the percentage of normal seeds due to insects visit were assessed. Insects were intensively and regularly collecting nectar. The fruiting rate from treatments 1, 2 and 3 was 92.50%, 62.50% and 85% respectively. F or the mean number of seeds per pod, figures corresponding to T1, T2 and T3 were 7.51, 4.46 and 6.61 respectively. The percentage of normal seeds corresponding to these treatments was 88.58%, 63.80 and 84.13% respectively. Flower visiting insects significantly increa sed the fruiting rate, the mean number of seeds per pod and the percentage of normal seeds.

Introduction:-

Agriculture is one of the key priority sectors of Cameroon's economy. It is the main source of employment, since it employs nearly 70% of the active population, contributes 42% to the GDP and represents 51% of exports (Cameroon-Report, 2014).Vigna unguiculata also known as Cowpea, is a plant traditionally cultivated in Africa, most often in association with other crops such as corn, millet, sorghum (Mako et al., 2013). In most emerging regions, its young shoots and leaves are eaten as leafy vegetables (Mako et al., 2013). Cowpea plays an important role in the diet in Africa (Mako et al., 2013).

The 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 for protein-deficient diets (Mako et al.,2013).

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The stems and leaves (which are rolled into bundles after the pods are harvested and dried in sheds), provide excellent fodder for animals during the dry season (Mako et al., 2013). Cowpea has interesting agronomic characteristics (Rachie, 1985). Actually, 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 in Cameroon, the country climatic and ecological diversity do not only favor the growth of diverse plants, but also promotes the proliferation of various parasites that harm the growth and production of plants (Tchatat, 1996).

Crop damage is caused by these pests' infestations, which often lead to serious negative consequences, thus justifying the need for the use of pesticides. According to the FAO, 2013, a pesticide is a substance used to neutralize or kill 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 (Tchatat, 1996).

At the same time, in spite of their advantages, the use of pesticides generates a certain number of risks about the chemical composition of air, water and soil, which result in pollution which toxicological (for human beings) and ecotoxicological (for living organisms) consequences can be detrimental to the quality of the environment. These pesticides can also cause the destruction of several insects (Diptera, Heterocera Lepidoptera, Coleoptera, Hymenoptera or even Lepidoptera) which possess anthophilous activity and which generally contribute to the pollination of these plants (Calvet et al., 2005; Fenster et al., 2004).

The main objective of this work was to evaluate the impact of flower visiting insects on the yield of V. unguiculata for sustainable agriculture in Dang. Specific objectives included: a) inventory of the insects' diversity of the studied plant; b) study of insects' activity on the flowers of this Fabaceae; c) evaluation of the impact of floral insects on pod and seed yield of this species.

Material and Methods:-

Location of the study site

Investigations were carried out from august to october 2022 at Malang in Ngaoundere, Adamaoua Region of Cameroon (Figure 1) (latitude 07°42.26'N, longitude 13°53.94'E and altitude 1106 m above sea level). 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 high Guinea Savanna (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).

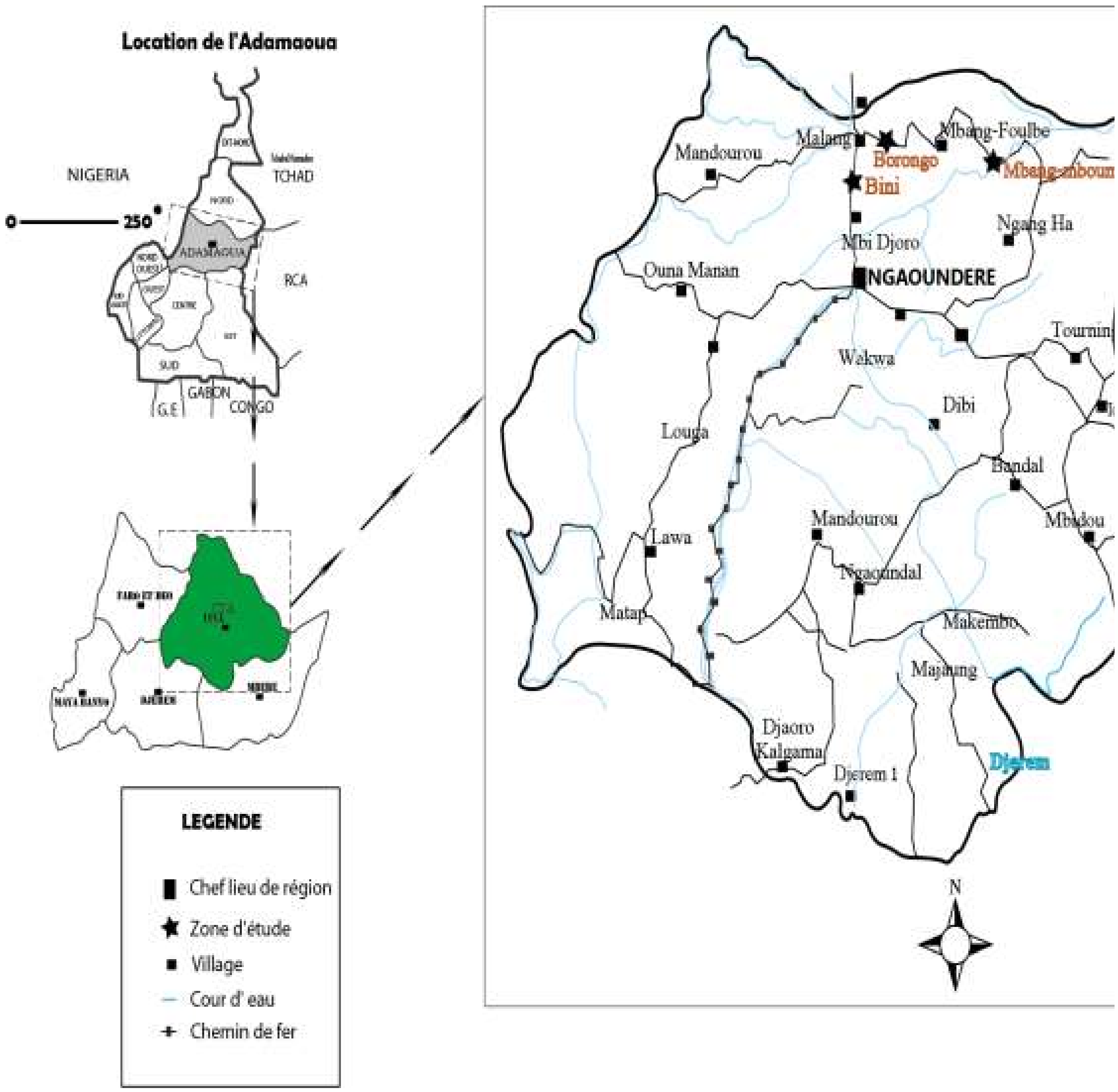

Figure 1:Location of the study site

Plant and animal material

The plant material was made up of the seeds of V. unguiculata Var. small white seed (figure 2) obtained from the ARID Touboro and the animal material consisted of flower visiting insects naturally present in the study site.

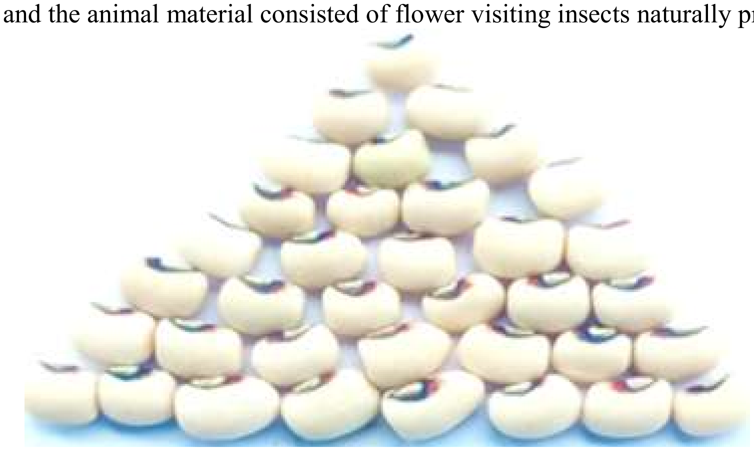

Figure 2:Seeds ofVigna unguiculataVar. small white seed

Methods:-

Preparation of the experimental plot and sowing

From August 1 to 5, 2022, an experimental field of 437 m2 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).

Treatment 1: 120 bud flowers (belonging to sub-plots 1 to 8) were labeled and left for free pollination.

Treatment 2: 120 bud flowers (belonging to sub-plots 1 to 8) were labeled and protected from insects using gauze bags. After preparing the field, the seeds of V. unguiculata, which are of small white seed variety with an intermediate cycle (85 to 95 days) were planted following the spacing of 75 cm between the rows and 50 cm on the rows corresponding to the prostrate varieties of cowpea (Kengni et al., 2015). 14 days after emergence of seedlings, thinning was carried out and two of the bests were left in pockets (Kengni et al., 2015). 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 hands.

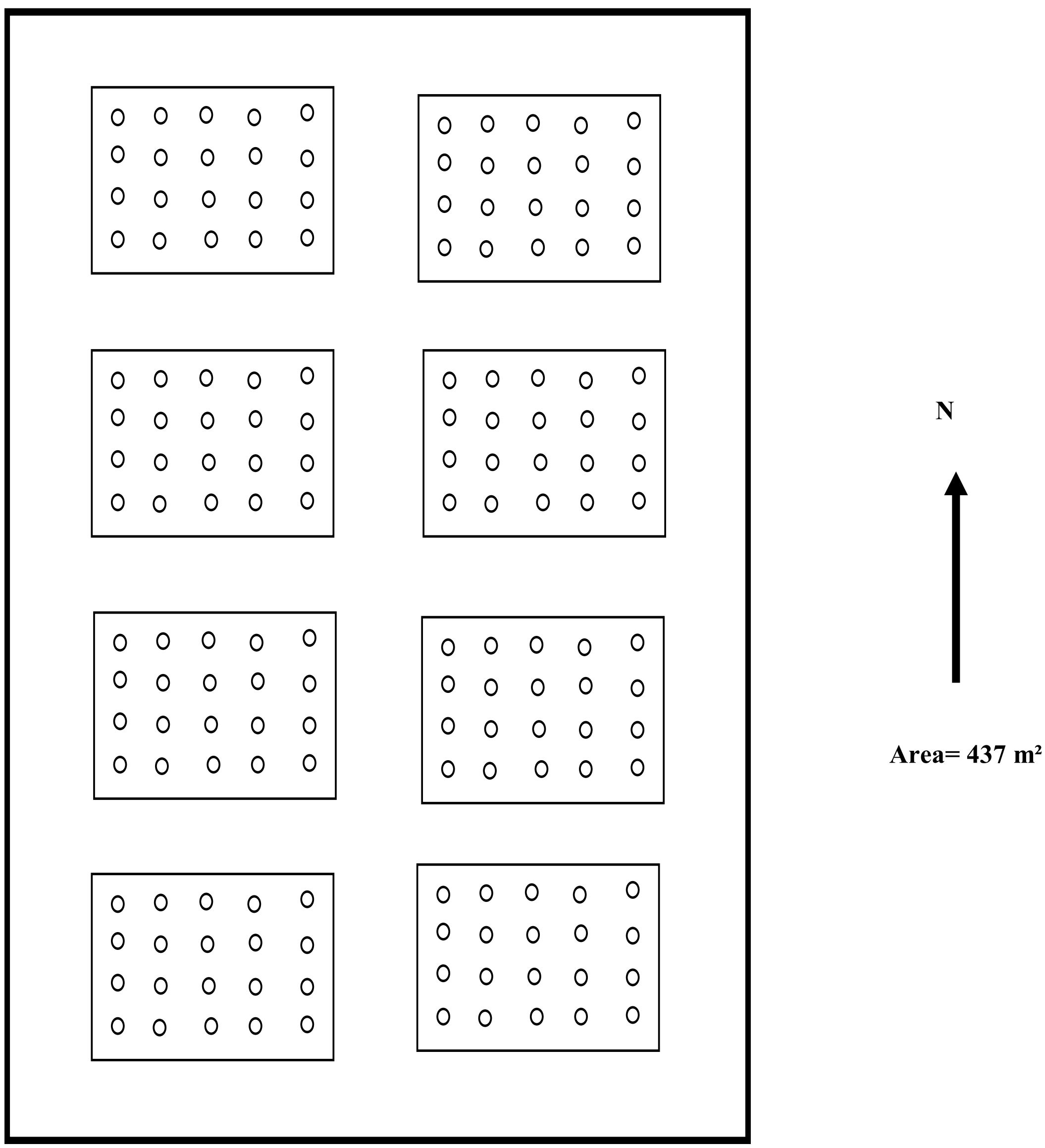

Figure 3:Experimental set-up of Vigna unguiculatain 2022 in Malang.

Hedge delimiting the experimental plot
1 to 8: Sub-plot numbers
o **:** Plant of Vigna unguiculata

Data Collection:-

The parameters taken into account were the diversity of flower visiting insects of V. unguiculate and the impact of these insects on plant yield.

Determination of the mode of reproduction ofVigna unguiculata

From august to october 2022, eight subplots carrying 240 plants were labeled among which 120 plants were left opened to insects visit (treatment 1) while 120 other plants were bagged using gauze bags (1 mm2 mesh) to prevent visiting insects (treatment 2). Thirty days after shading of the last flower, the number of pods was assessed in each treatment. The podding index (Pi) was then calculated as described by (Tchuenguem et al., 2021): Pi = Fb/Fa, where Fb is the number of pods formed and Fa the number of viable flowers initially set. The allogamy rate (TC) from which derives the autogamy rate (TA) was expressed as the difference in podding indexes between treatment X (unprotected flowers) and treatment Y (bagged flowers) (Demarly, 1977). TC = [(PiX - PiY) / PiX] * 100, where PiX and PiY are respectively the podding average indexes of treatment X and treatment Y. TA = 100 - TC.

Assessment of the foraging activity of insects on Vigna unguiculata flowers

Observations were conducted on flowers of treatment 1, each day, from august to october 2022 at 09.00 - 10.00 am, 11.00 - 12.00 am, 13.00 - 14.00 pm and 15.00 - 16.00 pm. All insect visits were recorded on the flowers each of these treatments. Specimens of all insect taxa (3 to 5 per species) were caught on flowers using an insect net, conserved in 70% ethanol, except Lepidoptera that were kept in curls for subsequent taxonomy determination (Borror, 1991). All insects encountered on flowers were registered and the cumulated results expressed in number of visits to determine the relative frequency of each insect in the anthophilous entomofauna of V. unguiculata (Tchuenguem, 2005).

In addition to the determination of the relative frequency of all insect visitors, direct observations of the foraging activity on flowers were made on insect fauna in the experimental field. The floral rewards (nectar or pollen) harvested by each insect species during each floral visit were registered based on its foraging behavior. Nectar foragers were expected to extend their proboscis or head to the base of the corolla and the stigma, while pollen gatherers were expected to scratch the anthers with their mouth ports or legs (Jean-Prost,1987). On each sampling day, the number of opened flowers was counted in each treatment. The same days as for the frequency of visits, the duration of individual flower visits was recorded (using a stopwatch) at least three times during each of the following daily time frames: 10.00 - 11.00 a.m., 12.00 a.m. - 13.00 p.m. and 14.00 a.m.-15 p.m.

Moreover, the number of pollinating visits, the abundance of foragers (Tchuenguem et al., 2004) and the foraging speed referring to the number of flowers visited by an insect per minute (Jacob-Remacle, 1989) were evaluated. Abundance per flower was recorded by direct counting on the same dates and during the same daily periods as for the recording of the duration of visits. For determining the abundance per 1000 flowers (A1000), some foragers were counted on a known number of flowers. A1000 = (Ax/Fx)*1000), where Fx and Ax are the number of opened flowers and the number of insects effectively counted on these flowers at time x (Tchuenguem et al., 2004). The foraging speed Vb was calculated by the formula: Vb = (Fi/di)*60, where di is the time (sec) given by a stopwatch, and Fi, the number of flowers visited during di. At each observation date, every 30 minutes, ambient temperature and relative humidity were recorded using a portable thermo-hygrometer (HT-9227).

Insect activity on the plant's flowers

Harvested floral products

This involved noting whether the visiting insect collected pollen, nectar, or both on a flower. An insect that plunges its mouthparts 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). The collected pollen can be observed on transport organs, particularly, in the pollen baskets on the hind legs for the Apidae, on the collecting hair of Halictidae's legs, or on 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).

Evaluation of the Impact of Pollinating Insects on Vigna unguiculata Yields
This evaluation was based on the impact of flowering insect on pollination, the impact of pollination on fruiting and the comparison of yields (fruiting rate, mean number of seeds per pod and percentage of normal or well developed seeds) of treatment 1 (unprotected flowers) and treatment 2 (bagged flowers).

The fruiting rate due to the influence of foraging insects (Fri) was calculated using the formula: Fri = {[(Fr1–Fr2)/Fr1]*100}, where Fr1 and Fr2 are the fruiting rate in treatments 1 and 2 respectively. The fruiting rate of a treatment (Fr) was calculated as follows: Fr = [(F2 / F1)*100], where, F2 is the number of pods formed and F1 the number of viable flowers initially set (Tchuenguem et al., 2001). At maturity, pods were harvested from all treatments. The mean number of seeds per pod and the percentage of normal seeds were then calculated for each treatment.

Data processing
The software Excel 2007 was used for three tests: (a) Students' t-test at 5% threshold for comparing means of two samples; (b) Chi-square ($\chi 2$) 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.

Results and Discution :-

Reproduction mode of Vigna unguiculata
At the investigation station, 120 flowers of V. unguiculata 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.

Insect activity on flowers ofVigna unguiculata
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 appearing from this Table that among the insects who visited V. unguiculata flowers, A. mellifera was most frequently observed, followed by X. olivacea with 52.39% and 13.77% visits respectively.

Table 1:Insects recorded on the flowers of Vigna unguiculatain 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 on 120 flowers in six days; **p**: percentage of visits = (n/334) * 100; **sp**.: undetermined species;**ne**: nectar collection; **po**: pollen collection.

Floral products collected
Throughout the study period, the insects present on the flowers ofV. unguiculata in the experimental field intensively and exclusively harvested nectar.

Rhythm of visits according to the flowering stages
Overall, insect visits were more numerous on treatments 1 and 3 when the number of open flowers was highest (Figure 4). A positive and significant correlation was found between the number of opened flowers and the number of insect visits ($r = 1$; ddl = 4; $P < 0.001$).

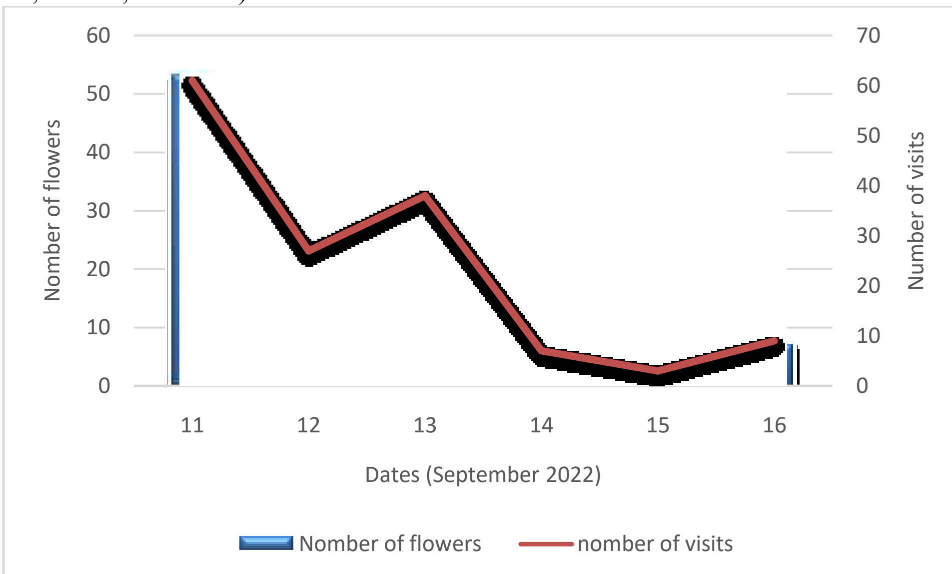

Figure 4:Variation in the number of blooming flowers and in the number of insect visits on the flowers ofVignaunguiculataaccording to observation dates in 2022 in Malang.

Insect abundance
The highest mean number of A. mellifera simultaneously in activity was 1.3 per flower and 74.49 per 1000 flowers; 1 per flower and 63.10 per 1000 flowers for X. olivacea and 1 per flower and 63.10 per 1000 flowers for E. eximia.

Influence of some climatic factors
Figure 5 shows the daily variation of temperature, relative humidity and the number of insect visits on the flowers. 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 activities on the flowers was found in the morning, between 10 a.m. and 11 a.m.

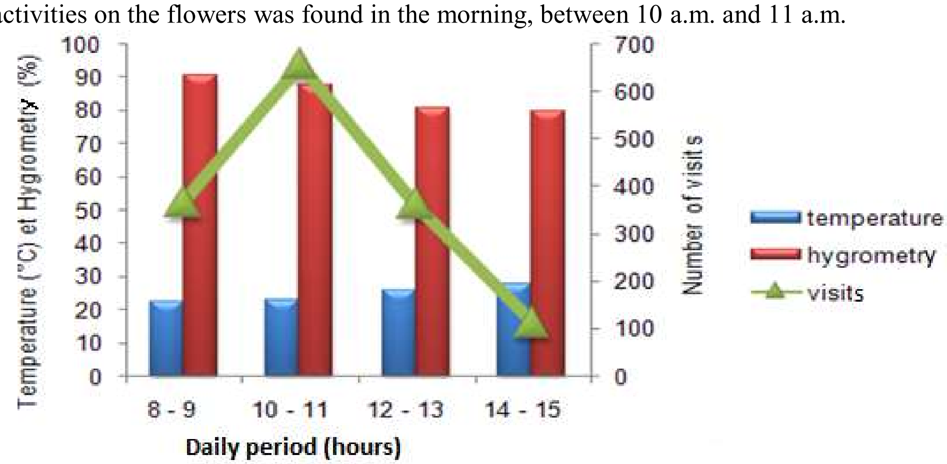

Figure 5: Daily variation of temperature, relative humidity and number of insect visits on the flowers of Vigna unguiculata in 2022 in Malang.

Yields of Vigna unguiculata
Table 2 summarizes the data regarding the fruiting rate, the mean number of seeds per fruit and the percentage of normal seeds in the different treatments ofV. unguiculata.

Table 2:Fruiting rate, mean number of seeds per pod and percentage of normal seeds obtained in each treatment ofVignaunguiculata

	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 in freepollination; **FP**: Flowers Protected from insect visits; **FpI**: Flowers protected then uncovered and visited once by some insect and rebagged; **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 highly significant: $\chi2_{overall}$= 108.56 (ddl=2;P<0.001; VHS). 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; VHS); 2 / 3: χ2= 15.69 (ddl=1;P<0.001; VHS) and not significant between treatments 1/3: χ2= 3.38 (ddl=5 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 highlysignificant (F=357;ddl1= 2;ddl2=6.94;P<0.001; VHS). 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; VHS); 2 / 3 (t= -4.41;ddl=238;P<0.001; VHS) and not significant between treatments 1/3 (t=1.97;ddl=238;P>0.05; NS).

c) 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; VHS). Comparing these means two by two percentages shows that the difference is very highly significant between treatments 1 / 2 (χ2=125.93;ddl= 1;P< 0.001; VHS); 2/3 (χ2=72.35;ddl= 1; P< 0.001; VHS) and highly significant 1 / 3 (χ2=7.16;ddl=1;P<0.01; HS).

Discussion:-

In Malang in 2022, six species of insects were recorded on the flowers ofV. unguiculata. Among these insects, Hymenoptera represented by the Family of Apidae were the mostpresent, followed by Diptera represented by the Pieridae Family.

The data in Table 1 show that the frequency of visits vary more or less with the insects. This is in agreement with the observations of Kengni etal. (2015) on the same plant species; of Kingha (2012) onPhaseolus vulgarisand those of Fardaet al. (2018) onV. subterranea white variety of a Fabaceae.Apis melliferais also known to be one of the most common flower visiting insectson the flowers of several other plant species including:Millettia laurentii(Nissoet et al., 2025);Brachiaria brizantha(Adamou & Tchuenguem, 2014);Brassica napus(Kleinet al., 2006; Jauker & Wolters, 2008; Hoyle & Cresswell, 2009);Luffa cylindrica (Farda & Tchuenguem, 2018);Cucumeropsis mannii(Azo'o & Messi, 2012); Cocos nucifera (Da Conceicaoet al., 2004);Croton macrostachyus(Népidé & Tchuenguem, 2016) andHelianthus annuus(Hoffman & Chambers, 2006; Tchuenguemet 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 ofV. unguiculatahighlight the high attractiveness of the nectar and/or pollen of this Fabaceae to insects. Peaks in insect visits onV. unguiculata could be explained by the greater availability and accessibility of nectar in the flowers concerned during the corresponding periods.

The decrease in activity observed in the flowers of this plant after the time slot 2 - 3 p.m. could 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 for 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 towards insects. Our results are in agreement with the works of Bérenger - Lévêque (1982) who mentioned 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 ofV. unguiculataduring the morning towards insects could be partly explained by the availability and quality of this foodstuff as well as by the time necessary for its harvest at the level of the corresponding flowers (Roubik, 2000). This attractiveness could also be linked to odoriferous compounds such as sterols (Pierre & Chauzat, 2005) and lipids (Sing et al., 2000).

The percentage of the fruiting rate due to the influence of anthophilous insects was 92.50%. The percentage of the number of seeds per pod due to the activity of flower visiting insects was 11.42%. The percentage of normal seeds due to the activity of flower visiting insects was 24.78% in the same plant. These data are proof that not only do flower visitng 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 Djakbéet al. (2017) showed that insect pollination increases the fruiting rate on Croton macrostachyusandPhysalis minimaof 30.29% and28.76% respectively.

The positive and significant contribution of insects in the fruiting ofV. unguiculatais thus justified by the action of insects on self-pollination and cross-pollination.
Despite the abundance of insects during the flowering ofV. unguiculata, several flowers visited by the latter did not produce pods. This result demonstrates that numerous visits by insects on the flowers ofV. unguiculataare beneficial to these arthropods but have no influence on pollination and yields of the Fabaceae.

Conclusion:-

In Malang,V. unguiculatawas studied and showed a mixed allogamous - autogamous reproduction regime, with a predominance of autogamy. Among the insects that visit the flowers of this plant, A. melliferais by far the most common.

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

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