Impact of Hymenoptera on *Abelmoschus Esculentus* (L.) Moench, 1794 (Malvaceae) Seed Yields at Bilone (Obala, Cameroon)

Pharaon Mbianda Auguste¹*, Douka Chantale², Dounia³, Fernand - Nestor Tchuenguem Fohouo³

¹Laboratory of Biological and Physiological Sciences, University of Douala, Faculty of Science, Douala, Cameroon  
²Laboratory of Zoology, University of Yaoundé I, Higher Teachers’ Training College, Yaoundé, Cameroon  
³Laboratory of Applied Zoology, University of Ngaoundéré, Faculty of Science, Ngaoundéré, Cameroon

**Abstract:** In Bilone (Obala, Cameroon), during the mild raining season (March-June) in 2019 and 2020, the flowers of *Abelmoschus esculentus* (Malvaceae) were observed for the study of the activities of Hymenoptera and the determination of the impact of these insects on the yields seedlings of this Malvaceae. Treatments included unlimited floral access by all visitors and bagged flowers to avoid all insect pollinators. The results show that on flowers of *A. esculentus*, 9 species of insects were recorded. The Hymenoptera occupied the first rank with 84.76 % of the visits. These bees prey on the flowers in the morning and evening, with a peak between 09 - 10 am. His visits correspond to a very good harvest of the nectar and a weak harvest of the pollen. Comparing the yields of free flowers to those of isolated insects, there is an increase in the number of seeds per pod of 20.59 % in 2019 and 20.87 in 2020 and the percentage of normal seeds of 02.38 % in 2019 and 5.28% in 2020 due to Hymenoptera. This improvement in yields is justified by the positive action of these arthropods on the pollination of the flowers they visit. Therefore, conservation of nests and colonies of Hymenoptera close to *A. esculentus* crop fields should be recommended to improve pod and seed production in the locality.

**Keywords:** Hymenoptera, *Abelmoschus esculentus*, flower, pollen, pollination, yield.

1. **INTRODUCTION**

Many plants depend on pollination for their production [1]. This pollination is mostly carried out by insects [2]. Effective pollination is followed by good fruiting or high seed production [3]. It produces many seeds, a suitable shape and size of fruit [4]. Insects and flowers perform mutual services, the main beneficiary of which is man. Such results are beneficial for agricultural development. In developing countries, more particularly Cameroon, whose economy is essentially based on agriculture [5], the role of pollinating insects in increasing agricultural yields is little known [6]. However, the absence of pollinating insects at the time of flowering can lead to insufficient fruit and / or seed yields in some crops [7]. In the natural environment as in agro-ecosystems, flower insects have great ecological and economic importance because they positively influence agro-food production [8, 9, 10]. Most farmers in developing countries believe that the high yields are due exclusively to various cultivation techniques, nutrients and infestation control [11]. They ignore that in the presence of an insufficient number of them during the flowering of several plants, the yields can be greatly reduced or nil [12, 13, 14].

*Abelmoschus esculentus* (L.) Moench (1794) is a Malvaceae from the tropics and subtropics widely cultivated in Africa [15]. Commonly called okra, lady’s finger or gumbo is an important vegetable in the tropics and sub-tropics [16]. This crop plant probably originated in the Ethiopian region of Africa but is now widely grown throughout Africa; especially in Sudan, Egypt and Nigeria [16]. Up to 4 m tall, erect, more or less strongly branched; cylindrical stem, with stiff hairs disseminated, glabrescent,
Vegetation was represented by wild species and cultivated plants around Yaoundé by Njoya to increase okra yields in Cameroon, it is necessary to conduct research in an agricultural environment to supplement existing data. Thus, knowledge of the diversity of okra and its anthophilous insects in Bilone is the major pollinator of this plant. In Cameroon, with the exception of the work carried out in Yaoundé by Njoya and Amada, Maroua Azo’o and Pando respectively, no other scientific production in this direction is available to our knowledge. These authors found that Apis mellifera, Megachile spp., Halictus spp. and Xylocopa spp., Tetralonia fraterna and Eucara macrognata, Lipotriches collaris, then Xylocopa olivacea, Synagris cornuta are constant pollinators of A. esculentus and significantly increase the yields of this plant. Globally, the areas devoted to the cultivation of okra are estimated at 7 million hectares. In 1969, revenues generated by the export of okra by developing countries were estimated at more than $1.1 billion. In Cameroon, okra production which is estimated at 54,776.5 tonnes/year is insufficient to meet demand estimated at 7,011,392 tonnes/year. Previous work done on the pollination of flower-growing insects of A. esculentus carried out in Yaoundé by Njoya, Amada and Maroua by Azo’o, Pando, are urbanized areas. Knowing that the pollinating insects of a plant species can vary from one region to another, according that the diversity and abundance of the pollinating entomofauna of a plant can vary in time and space and considering the need to increase okra yields in Cameroon, it is necessary to conduct research in an agricultural environment to supplement existing data. Thus, knowledge of the diversity of okra pollinating insects should allow the breeding of potential effective pollinating insects of this Malvaceae. More, no previous research has been reported on the relationships between A. esculentus and its anthophilous insects in Bilone. The main objective of this research was to gather more data on the relationships between A. esculentus and flower visiting insects for its optimal management in Cameroon. Specific objectives were: (a) to determine the place of insects in the floral entomofauna of this plant; (b) to study the activity of Hymenoptera on Abelmoschus esculentus flowers; (c) to assess the impact of flower-growing insects on the pollination of this Malvaceae; (d) to assess the impact of Hymenoptera on production of seeds yields of Abelmoschus esculentus in this locality.

2. MATERIALS AND METHODS

2.1. Study Site, Experimental Plot and Biological Material

The studies were conducted twice, April to July respectively in 2019 and 2020 in a field located at the campus of the Institute of Agronomy in Bilone (Obala) (Latitude: N 04.20514°, Longitude: E 011.51694°, Altitude: 525 m) in the Center Region of Cameroon. This Region belongs to the tropical rain forest agro-ecological zone. The climate is equatorial guinean-type with four seasons: the peak rainy season (August to November), the peak dry season (November-March), the mild rainy season (March-July) and the mild dry season (July-August), the mean annual temperature is 25°C, while the mean annual relative humidity is 79% [31]. The experimental plot was an area of 544 m². The animal material was represented by insects naturally present in the environment and four colonies of A. mellifera (Hymenoptera: Apidae) inhabiting six of the six beehives located at 22 meters from the experimental field (Fig. 1). Vegetation was represented by wild species and cultivated plants. The plant material was represented by the seeds of A. esculentus bought at the Mfouin market in Yaoundé (Cameroon) (Fig. 2).
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Figure 1. Six upper striped hives each housing a colony of Apis mellifera in Bilone in 2019.

Figure 2. Seeds of Abelmoschus esculentus

2.2. Sowing and Weeding

On March 26, 2019 and March 17, 2020, the experimental plot was divided into fifteen ridges of 8 meters. Seeds were sown on one line per ridges; each line had fourteen holes and each hole received four seeds. The spacing was 50 cm between rows and 2 m between ridges. Each hole was 4 cm depth. Two weeks after germination, the plants were thinned and only two were left per hole. Weeding was performed manually as necessary to maintain subplots weeds-free.

2.3. Determination of the Reproduction System of Abelmoschus Esculentus

In May 08 and 22 respectively in 2019 and 2020, 70 flowers of A. esculentus at the bud stage were labeled. 35 of the total number flowers were allowed for treatment 1 (open pollination) (Fig. 3) and 35 others flowers belong to treatment 2 (bagged with gauze bags to prevent visitors or external pollinating agents) (Fig. 4) [32]. For each year, ten days after the wilting of the last flower, the number of pod formed in each treatment was counted. For each treatment, the fruiting index (If\(r\)) was then calculated using the following formula: \(If\(r\) = (F1/F2), where \(F1\) is the number of pod formed and \(F2\) the number of viable flowers initially labeled (Tchuenguem, 2005). The out crossing rate (\(TC\)) was calculated using the formula: \(TC = \{(If\(r1\) – If\(r2\) / If\(r1\)} *100), where \(If\(r1\) and \(If\(r2\) are mean fruiting indexes in treatments 1 and 2 respectively (Tchuenguem, 2005). The rate of self-pollination in the broad sense (\(TA\)) was calculated using the formula: \(TA = (100 - TC)\).
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2.4. Foraging Activity of Flowering Insects on Abelmoschus Esculentus Flowers

Observations were conducted on 35 individually opened pollinated flowers of treatment 1 each day from May 14 to June 3 in 2019 and from May 19 to June 11 in 2020 at interval from 9am to 15pm (9-10am, 11-12, 14-15pm). In a slow walk along all labeled open flowers of treatment 1, the identity of all insects that visited A. esculentus flowers was recorded. All insects encountered on flowers were recorded and the cumulated results expressed in number of visits to determine the relative frequency and the regularity index of flowering insects in the anthophilous entomofauna of A. esculentus.

Direct observations of the foraging activity of insects on flowers were made. The floral rewards (nectar and/or pollen) collected by the foragers during each floral visit were registered based on its foraging behavior for the same dates and time slots as that of the insect counts. Nectar foragers were expected to extend their proboscis to the base of the corolla and the stigma, while pollen gatherers were expected to scratch the anthers with their mandibles or legs [33].

In the morning of each observation day, the number of opened flowers was counted. The duration of visits (Jean-prost, 1987) and the foraging speed (number of flowers visited per minute) [8, 34] were timed to the same dates and in three time slots from 8am - 14pm (8-9am, 10-11am and 13-14pm), using a stopwatch. Abundances (highest numbers of individuals simultaneously active) per flower and per 1000 flowers (A1000) were recorded on the same dates and time slots as that of the registration of the duration of visits. The first parameter was recorded as a result of direct counts. For the abundance per 1000 flowers, honey bees were counted on a known number of open flowers; A1000 = [(Ax/Fx)*1000], where \( Fx \) and \( Ax \) are respectively the number of flowers and the number of individual bees actually counted on \( Fx \) [8]. During the days of investigation, the temperature and humidity of the study site were recorded every 30min, from 9 am to 17 pm, using a thermo-hygrometer installed in the shade.

2.5. Evaluation of the Impact of Pollinating Insects on Abelmoschus Esculentus 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: 
\[ Fri = \frac{\{(Fr1 – Fr2)/Fr1\} \times 100\}, \] 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: 
\[ Fr = \frac{(F2 / F1)}{100}, \] where, F2 is the number of pods formed and F1 the number of viable flowers initially set [8]. 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.

### 2.6. Data Analysis

SPSS software and Microsoft Excel were used for three tests: Student’s (t) for the comparison of means of two samples, correlation coefficient (r) for the study of line relationship between two variables, Chi-square (\(\chi^2\)) for the comparison of percentages.

### 3. RESULTS AND DISCUSSION

#### 3.1. Pod Production of Abelmoschus Esculentus

In 2019, the podding index was 0.91 (n = 35) for treatment 1 and 0.71 (n = 35) for treatment 2, while in 2020; it was 0.86 (n = 35) and 0.77 (n = 35) for the two treatments respectively. Consequently, in 2019 the allogamy rate was 21.98% and the autogamy rate was 78.02. In 2020, the corresponding figures were 10.46% and 89.54%. It appears that the A. esculentus used in our experiments has a mixed reproduction mode with the predominance of autogamy over allogamy, which produce fruit by both autogamy (auto and self-pollination) and crossbreeding (cross-pollination), like higher plants [32]. The significantly highest (\(\chi^2 = 4.80; \ ddl = 1; \ p < 0.001\)) fruit yield as a function of the self-pollination in relation to cross-pollination agrees [35], who stated that sesame is a predominantly autogamous species. These results are in agreement with those of Free [1993], Angbanyéré & Matthew [25], Njoya [21] and Amada [26], Azo’o [18] and Pando [27] respectively in UK, Burkina Fasso, Yaounde and Maroua who indicate the best performance of self-pollination than cross-pollination. Although, these results contradict those of Andrade [36] who indicate the best performance of cross-pollination than self-pollination. This difference is due, may be to exchange of genetic material promoted by this type of pollination, increasing the heterozygosity of the embryo formed in the seeds [37].

#### 3.2. Activities of Insects

**Diversity and Frequency of Floral Entomofauna of Abelmoschus esculentus**

Table 1 shows the diversity, frequency of visits, and floral products harvested by insects observed on the flowers of Abelmoschus esculentus in Bilone.

It appears from this table that six groups of pollinators visited the okro belonging to order Hymenoptera, Diptera, Lepidoptera, Coleoptera, Orthoptera and Nevroptera of class insecta during the flowering period. The number of Hymenoptera was higher (84.76%), followed by Diptera (10.75 %), Lepidoptera (03.56 %), Nevroptera (00.39 %) and then both of Coleoptera (00.31%) and Orthoptera (00.23%). The results indicate that Hymenopterans are the major pollinators visiting Abelmoschus esculentus flowers in Bilone in 2019 and 2020. These findings are in close agreement with Azo’o [18], Pando [27] at Maroua, Amada [26] at Yaounde, in the flowers of Abelmoschus esculentus; Djonwangwé [38, 39] at Maroua, Pharaon [40] at Bilone on Vigna unguiculata and Allium cepa flowers; Pando [41, 42] in the flowers of Cajanus cajan and Zea mays at Maroua who studied the relative abundance of pollinator fauna of Abelmoschus esculentus during two successive seasons. Hymenopterans insects were higher. Also, Pharaon [14] at Obala reported nine species of Hymenopterans as predominant visitors of sesame flowers. Among the 1293 visits of 11 insect species recorded on Abelmoschus esculentus flower, Apis mellifera and Xylocopa olivacea were the most represented insects with 278 visits (44.27%) and 342 visits (51.43%), in 2019 and 2020, respectively.
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Table 1. Diversity, frequency of visits, and floral products harvested by insects in the flowers of Abelmoschus esculentus in Bilone in 2019 and 2020.

| Insects | Order | Family | Genres and species | 2019 | 2020 | Total |
|---------|-------|--------|-------------------|------|------|-------|
|         |       |        | \(n_1\) | \(P_1\) (%) | \(n_2\) | \(P_2\) (%) | \(n_t\) | \(P_t\) (%) |
| Hymenoptera | Apidae | Apis mellifera (Nt, Po) | 169 | 26.91 | 203 | 30.53 | 372 | 28.77 |
|          |        | Xylocopa olivacea (Nt, Po) | 103 | 16.40 | 139 | 20.90 | 242 | 18.72 |
|          |        | Amegilla sp. (Nt, Po) | 51 | 8.12 | 45 | 6.77 | 96 | 7.43 |
| Halictidae | Lipotriches collaris (Nt, Po) | 97 | 15.45 | 66 | 9.92 | 163 | 12.61 |
| Vespidae | Lasiosglossum sp. (Nt, Po) | 23 | 3.66 | 9 | 1.35 | 32 | 2.47 |
|          | Lasiosglossum albipes (Nt, Po) | 8 | 1.27 | - | - | 8 | 0.62 |
|          | Seladonia sp. (Nt, Po) | 5 | 0.80 | 32 | 4.81 | 37 | 2.86 |
| Coleoptera | Pyrochroidae | Hyleulus affinis (Po) | 4 | 0.64 | - | - | 4 | 0.31 |
|          | Coleoptera |        | 04 | 0.64 | 00 | - | 04 | 0.31 |
| Diptera | Muscidae | Musca domestica (Nt, Po) | 65 | 10.35 | 54 | 8.12 | 119 | 9.20 |
|          | Syrphidae | Paragus barbonicus (Po) | 9 | 1.43 | 11 | 1.65 | 20 | 1.55 |
|          | Diptera |        | 02 | 11.78 | 65 | 9.77 | 139 | 18.75 |
| Lepidoptera | Acrocidae | Acroa acerata (Nt) | 23 | 3.86 | 9 | 1.35 | 32 | 2.48 |
|          | Nymphalidae | 1 sp. (Nt, Rt) | - | - | 3 | 0.45 | 3 | 0.23 |
|          | Pieridae | Eurema senegalensis (Nt) | 11 | 1.75 | - | - | 11 | 0.85 |
|          | Lepidoptera |        | 03 | 5.41 | 12 | 1.81 | 46 | 3.56 |
| Orthoptera | 1 sp. (Di) | - | - | - | - | 3 | 0.23 |
|          | Orthoptera |        | 01 | 0.48 | 00 | - | 03 | 0.23 |
| Nevroptera | Libellulidae | Corythucha erythraea (Pr) | - | - | 3 | 0.45 | 3 | 0.23 |
|          | Nevroptera | 1 sp. (Pr) | - | - | 2 | 0.3 | 2 | 0.16 |
|          | Nevroptera |        | 02 | 0.00 | 05 | 0.75 | 05 | 0.39 |
| Total | 11 | 20 | 628 | 100 | 665 | 100 | 1293 | 100 |

\(n_1\): number of visits on 35 flowers in 21 days. \(n_2\): number of visits on 35 flowers in 24 days. \(p_1\) et \(p_2\): percentages of visits. \(p_1 = (n_1 / 628)*100\). \(p_2 = (n_2 / 665) * 100\). \(n_{1,2} = (n_1 + n_2)\). \(p_{1,2} = [(n_1 + n_2)/1293]*100\). Nt: visitor collected nectar. Po: visitor collected pollen. Df: defoliator. Rt: rest. Pr: predator. sp.: undetermined species.

The total species richness of *Abelmoschus esculentus*’s flowering insects was 20 in Bilone. This specific richness is far inferior to that found by Pando et al. (2020) at Maroua, which was 32 species. The comparison between the two specific richness is highly significant \((t = 723.81 [df = 1089; P < 0.001])\). This agrees the research of Roubik [29], which revealed that the diversity and specific richness of a plant’s flowering entomofauna may vary in space and time.

It appears from table 1 against that there are three categories of insects: (a) species exclusively in search of nectar which was represented by *Acroa acerata* and *Eurema senegalensis*. Concerning Lepidoptera, Pando [27] reported in Maroua that these insects harvest only nectar on flowers. (b) species exclusively in search of pollen which was represented by *Hyleulus affinis*, *Paragus barbonicus* and two undetermined species bilong to Vespidae and Formicidae Family. (c) Species in search of
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nectar and pollen were represented by: Apis mellifera adansonii, Xylocopa olivacea, Amegilla sp., Lipotriches collaris, Lasioglossum sp., Lasioglossum albipes, Seladonia sp., Ammophila sabulosa, Synagris cornuta and Musca domestica. During this flowering period, these insects that collected pollen and nectar from visited the flowers of Abelmoschus esculentus, intensely collected nectar as pollen. These findings are in conformity with the observations made by Pando [27], who reported that the Hymenoptera harvested intensively the nectar than pollen. (d) One undetermined specie of Orthoptera Family consuming petals. It should be noted that some flies, butterflies, and lady beetles visit flowers for feeding on different parts of Abelmoschus esculentus and sometimes just for resting.

Frequency of flowering insects of Abelmoschus esculentus

Amongst the 628 and 665 visits of 15 and 16 insect species were recorded in two years (May 14 to June 03, 2019) and (May 19 to June 11, 2020) on A. esculentus, Apis mellifera, Xylocopa olivacea and Lipotriches collaris and Lasioglossum were the most frequently insect species observed, with 372 (28.77%), 242 (18.72%) and 163 (12.61%) visits respectively (Table 1). The difference between these three percentages of visits is high significant ($\chi^2 = 12.43$, $df = 2$, $p < 0.01$).

The distribution of flower-visiting insect species according to their daily regularity is reported in table 2.

This table presents the number and percentage of days of visits of the different flowering insects from Abelmoschus esculentus. It appears from this table that the frequency of each insect species is varied. Three groups of anthophilous insects were found on A. esculentus flowers: (a) constant visitors ($f > 50$): Apis mellifera adansonii, Xylocopa olivacea, Amegilla sp., Lipotriches collaris, and Musca domestica; (b) accessory visitors with average frequencies (25 $\leq f \leq 50%$): Lasioglossum sp., Synagris cornuta, Acraea serena and Paragus barbonicus; (c) Rare visitors ($f \leq 25%$) represented by Lasioglossum albipes, Seladonia sp., Hycleus affinis, Corcothemis erythraea, Ammophila sabulosa and Eurema lactasana. The high frequency of some species is due to their attachment to the pollen and/or nectar of A. esculentus. For Apidae and Halictidae family’s species, pollen is indispensable for their nutrition Roubik (2000). For Halictidae, this result agreed that Pando et al. (2019) were found on Glycine max flowers. In addition, the attractive nature of its flowers with insects is due to the color of the flowers, which is purple, the most attractive color, according to Faegri & Piji [43].

Table 2. Number and percentage of days of visits of the different flowering insects from Abelmoschus esculentus in Bilone in 2019 and 2020.

| Insects                  | 2019     | 2020     | Total |
|--------------------------|----------|----------|-------|
|                          | $n_1$    | $f_1$ (%)| $n_2$ | $f_2$ (%)| $n_t$ | $f_t$ (%)|
| Apis mellifera (Nt, Po)  | 21       | 100      | 24    | 100    | 45    | 100     |
| Xylocopa olivacea (Nt, Po)| 21       | 100      | 24    | 100    | 45    | 100     |
| Amegilla sp. (Nt, Po)    | 10       | 46.2     | 13    | 54.17  | 23    | 51.11   |
| Lipotriches collaris (Nt, Po) | 17      | 80.95    | 14    | 58.33  | 31    | 68.89   |
| Lasioglossum sp. (Nt, Po)| 10       | 46.2     | 5     | 20.83  | 15    | 33.33   |
| Lasioglossum albipes (Nt, Po) | 4       | 19.05    | -     | -      | 4     | 8.89    |
| Seladonia sp. (Nt, Po)   | 4        | 19.05    | 7     | 29.17  | 11    | 24.44   |
| Ammophila sabulosa (Nt, Po) | -       | -        | 3     | 12.50  | 3     | 6.67    |
| Synagris cornuta (Nt, Po) | 9        | 42.86    | 12    | 50     | 21    | 46.67   |
| Hycleus affinis (Po)     | 2        | 9.52     | -     | -      | 2     | 4.44    |
| Musca domestica (Nt, Po) | 21       | 100      | 24    | 100    | 45    | 100     |
| Paragus barbonicus (Po)  | 4        | 19.05    | 8     | 33.33  | 12    | 26.67   |
| Acraea acerata (Nt)      | 17       | 80.95    | 5     | 20.83  | 22    | 48.89   |
| Eurema senegalensis (Nt) | 6        | 28.57    | -     | -      | 6     | 13.33   |
| Corcothemis erythraea (Pr)| -       | -        | 2     | 8.33   | 2     | 4.44    |
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n1: number of days of presence of insects during N1 observation days in 2019; n2: number of days of presence of insects during N2 observation days in 2020; nt: number of days of presence of insects during N t observation days in 2015 and 2018; f1 (%): Relative frequency of insect visits (n1/N1)*100; f2 (%): Relative frequency of insect visits (n2/N2)*100; ft (%): Relative frequency of insect visits (nt/Nt)*100; N1 = 21, N2 = 24, Nt = 45

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**Relationship between visits and flowering stages**

We found a positive and significant correlation between the number of opened flowers and the number of insect visits (*A. mellifera*) in 2019 (r = 0.56; ddl = 9; P < 0.05). Furthermore, a positive and highly significant correlation was found (r = 0.72, ddl = 9, P < 0.05) and a positive highly significant correlation (r = 0.80, ddl = 9, P < 0.05) between the number of opened flowers and the number of Hymenoptera insect visits in 2020.

4. IMPACT OF INSECTS ON *ABELMOSCHUS ESCULENTUS* POLLINATION

When collecting pollen and/or nectar on flowers of *A. esculentus* insects were frequently in contact with the anthers and the stigma of visited flowers. They could therefore be directly involved in self-pollination, by putting pollen of one flower on to the stigma of the same flower. Table 3 presents the percentage of contacts between insect, anther, and stigma of *A. esculentus*.

It appears on that table that all the 15 insect species that visited the flowers had contact with the anthers and/or stigmas: (a) seven of these insect species have a frequency of contact with the anthers of 100%, seven have an incidence of contact with the anthers of between 50% < f <100% and one have a frequency of contact with the anthers of between 25% ≤ f < 50%; (b) three of these insect species have a frequency of contact with the stigma of 100%, eleven have an incidence of contact with the stigma of between 50% ≤ f <100% and one have a frequency of contact with the stigma of between 25% ≤ f < 50%. Individuals of each studied bee species were seen carrying pollen of *A. esculentus* from flower to flower, using the legs, mouthparts, thorax, and abdomen. Therefore, they were likely playing a positive role in geitogamy [32] by putting the pollen of one flower to the stigma of another flower of the same plant species. The foragers passing from flower to flower on different plants were seen carrying pollen from one plant to another. They could, therefore, allowed xenogamy [32], by putting the pollen of plant species to the stigma of another plant species. Several flowering insects in general and Apoidea family, in particular, were reported as being part of the pollinating entomofauna of *A. esculentus* in Cameroon by other authors such as Njoya [21], Amada [26] at Yaounde; Azoo’o [18] and Pando [27] at Maroua.

According to table 3, the different insect species found on *A. esculentus* flowers can be classified into three categories of pollinators: (a) major pollinators (*Apis mellifera*, *Xylocopa olivacea*, *Lipotriches collaris*, and *Musca domestica*) which are characterized by a high regulatory index (R > 0.05); (b) minor pollinators (*Amegilla sp.*, *Synagris cornuta* and *Acraea serena*) which are characterized by a low regulatory index (0.01 < R < 0.05). This could be explained by the low number of the species present in the experimental field, or the species were preferentially in search of nectar. (c) Occasional pollinators (*LasioGLOSSUM sp.*, *Paragus barbonicus*, *LasioGLOSSUM albipes*, *Seladonia sp.*, *Hybleus affinis*, *Corcothemos erythraea*, *Ammophila sabulosa* and *Eurema lactasana*) which are characterized with a very weak regulatory index (R < 0.01) and absence of behavior, link to the search of pollen and/or nectar but may have a destructive attitude. All these species of insects carry out foraging

International Journal of Research Studies in Agricultural Sciences (IJRSAS)
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activities on the flowers of *A. esculentus*, thus contribute to auto pollination and/or cross-pollination. These, therefore, ensure the diversity of the species and increase the seeds yield and produce.

Table 3: Regularity index (R), numbers and percentage of insect visits in contact with the anthers and stigma of *Abelmoschus esculentus* flowers at Bilone.

| Insects                  | 2019 | 2020 | Total | R1  | R2  | Rf  | Nt  | Number of contact visits of: | Anthers | Stigma |
|--------------------------|------|------|-------|-----|-----|-----|-----|-------------------------------|---------|--------|
| *Apis mellifera*         | 0.2691 | 0.3053 | 0.2872 | 372  | 372  | 100 | 372  | 100                           |         |        |
| *Xylocopa alveoidea*     | 0.1640 | 0.2090 | 0.1865 | 242  | 233  | 96  | 238  | 98.35                         |         |        |
| *Ameigla sp.*            | 0.0379 | 0.0367 | 0.0373 | 96   | 96   | 100 | 96   | 100                           |         |        |
| *Lipotriches collaris*   | 0.1251 | 0.0579 | 0.0915 | 163  | 163  | 100 | 148  | 90.80                         |         |        |
| *LasioGLOSSUM sp.*       | 0.0171 | 0.0028 | 0.0099 | 32   | 32   | 100 | 29   | 90.62                         |         |        |
| *LasioGLOSSUM altipes*   | 0.0024 | -     | 0.0012 | 8    | 8    | 100 | 7    | 100                           |         |        |
| *Seleutonia sp.*         | 0.0015 | 0.0140 | 0.0077 | 37   | 37   | 100 | 31   | 83.78                         |         |        |
| *Amorphila sabulosa*     | -     | 0.0011 | 0.0006 | 6    | 6    | 100 | 4    | 66.67                         |         |        |
| *Synagris cornuta*       | 0.0287 | 0.0503 | 0.0395 | 109  | 102  | 93.58 | 89  | 81.65                        |         |        |
| *Hyaleus affinis*        | 0.0006 | -     | 0.0003 | 4    | 3    | 75  | 1    | 33.33                        |         |        |
| *Musca domestica*        | 0.0135 | 0.0812 | 0.0924 | 119  | 116  | 97.48 | 118 | 99.16                        |         |        |
| *Paraglossum barbaticus* | 0.0027 | 0.0055 | 0.0041 | 20   | 14   | 70  | 17   | 85                           |         |        |
| *Acrea acerata*          | 0.0296 | 0.0028 | 0.0162 | 32   | 22   | 68.75 | 25  | 78.12                        |         |        |
| *Eurema senegalensis*    | 0.0050 | -     | 0.0025 | 11   | 5    | 45.45 | 8   | 72.73                        |         |        |
| *Corcothemia erythrace*  | -     | 0.0004 | 0.0002 | 3    | 2    | 66.67 | 1   | 50                           |         |        |

R = (Pn/100)*fn/100; Pn: percentage of insect visits (Table 1); fn: Relative frequency of insect visits (Table 2); Nt: Number of total visits, n: number of contact anther visits; Pa: percentage of anther contact visits; ns: number of contact stigma visits; Ps: percentage of stigma contact visits.

The significant contribution of pollinating insects in pods and seed yield of *A. esculentus* was found in Cameroon [13] which showed that *A. esculentus* flowers produce fewer seeds per pod in the absence of pollinating insects. The weight of insect pollinators played a positive role during nectar or pollen collection, those insects shook flowers, facilitating the liberation of pollen by anthers for the optimal occupation of the stigma [17]. This Higher productivity of pods and seeds in unlimited visits when compared with bagged flowers showed that insect visits were effective in increasing cross-pollination.

**5. IMPACT OF INSECT POLLINATORS ON SEED YIELDS OF ABELMOSCHUS ESCULENTUS**

During pollen and nectar harvest, flowering insects of *A. esculentus* were in regular contact with the anthers and stigma. Thus these insects increased the pollination possibilities of this plant species. Table 4 presents the results on the fruiting rate, the number of seeds per pod and the percentage of normal seeds in different treatments.

The number of fruit set, ten days after anthesis of flowers, and harvested showed significant differences (p < 0.05) between treatments of pollination in Bilone (Table 4).

Table 4: Fruiting rate, number of seed per pod and percentage of normal seeds according to different treatments of *Abelmoschus esculentus* in 2019 and 2020 at Bilone

| Treatment     | Year | Flowers | Pods | Fruiting rate (%) | Seed / Pods Total | Normal | % normal |
|---------------|------|---------|------|-------------------|-------------------|--------|---------|
|               |      |         |      |                   | n   | s     | Seeds  | Seeds | Seeds   |         |
| Unlimited     | 2019 | 34      | 34   | 100               | 81,60 | 19,06 | 2669   | 2474  | 92.69   |         |
| Bagged flowers| 2019 | 30      | 28   | 93.33             | 61.90 | 14.96 | 2156   | 1845  | 85.57   |         |
| Unlimited     | 2020 | 34      | 32   | 94.12             | 84,02 | 3,26  | 2463   | 2374  | 96.39   |         |
| Bagged flowers| 2020 | 30      | 27   | 90                | 71,48 | 6.67  | 1561   | 1423  | 91.16   |         |

In Table 4, we documented:
1. High fruiting rate of pod formation during unprotected visits compared with bagged flowers. The comparison of the fruiting rate showed that the differences observed were significant between treatments 1 and 2 ($\chi^2_{2020} = 2.34 \ [df = 1; P < 0.05]$) and non significant between treatments 3 and 4 ($\chi^2_{2020} = 0.38 \ [df = 1; P > 0.05]$). The results suggest that the type of pollination effect of the fruiting rate. So, the visit by biotic pollinators is important for Abelmoschus esculentus pollination, whether increasing the efficiency of pollen transfer within the same flower or even bringing pollen from other flowers, providing cross –pollination. Similar results were obtained in Maroua by Pando [27] was 9.57 %. This could be due to absence or its lower abundance of the same major pollinators.

2. High mean number of seeds per pod in unlimited visits compared with bagged flowers. The comparison of the mean number of seeds per pod has shown that the difference observed was highly significant between treatments 1 and 2 ($t_{2020} = 17.17 \ [df=60; P < 0.001]$) and treatments 3 and 4 ($t_{2020} = 25.90 \ [df = 57; P < 0.001]$). The percentage of the number seeds per pod due to the action of insects was 16.12 % for the both years. In the same idea, Azo'o [18] found the corresponding result at Maroua of 9.50 %.

3. Higher normal seed yield for unlimited visits treatment compared with bagged flowers. The comparison of the percentage of normal seeds showed that the observed differences were very highly significant between treatments 1 and 2 ($\chi^2_{2020} = 64.38 \ [df = 1; P < 0.001]$) and treatments 3 and 4 ($\chi^2_{2020} = 49.04 \ [df = 1; P < 0.001]$). The Normal seed yield in unprotected flowers for unlimited visits (1 and 3) was higher than that in the bagged flowers (2 and 4). The percentage of normal seeds attributed to the influence of insects was 2.61 % for the both years. This result is inferior to what was obtained by Pando [27] at Maroua, which was 04.34 %. The difference in this value could be explained by the presence of more pollinating species at Maroua.

The numeric contribution of Hymenoptera to the mean number of seeds per pod and the percentage of normal seeds were respectively 20.59 % and 2.38 % in 2019. The corresponding figures were 20.87 % and 5.28 % in 2020. For the two cumulate years, the numeric contributions were 20.73% and 3.83% for the mean number of seeds per pods and the percentage of normal seeds respectively. The impact of Hymenoptera on pod and seed yields was positive and significant.

6. CONCLUSION

The floral products of A. esculentus attract pollinator insects. This attractiveness is of benefit for the pollination process. The comparison of pods and seeds set of unprotected flowers with that of protected flowers indicated the value of these insect pollinators in increasing pods and seed yields. In Bilone, A. esculentus is a mixed pollination plant, able to self-pollinate and reach levels above 93% fruit set, but with potential for a significant increase in fruit production in the presence of biotic pollinators that promote cross-pollination as Hymenoptera (20.73%). Twenty species of insects distributed in eleven families and five orders visited the flowers of A. esculentus to harvest nectar and/or pollen. Hymenoptera were the most frequent order with 84.76%, followed by Diptera (10.75 %) and others (Coleoptera, Lepidoptera, Orthoptera and Nevroptera: 04.49 %). Comparing the yield of unlimited flowers with insect-bagged flowers, it is observed that Hymenoptera increase the number of seeds/fruit and the normal seeds to 20.73 % and 03.83 % respectively. The treatment of okro plants with chemical pesticides should be avoided during the flowering period in order to benefit from the ecosystem service of pollinating insects. Pollinators could be protected by rational pest management tactics, i.e., pesticide application, if needed, should be done in the late afternoon to protect the pollinators for high seed yield or spray at a time of day when crop flowers are closed. More, the installation of nests or hives of insect pollinators in general, and Hymenoptera in particular, at the proximity of A. esculentus fields should be recommended for the increase of pods and seed yields of this valuable crop.

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Citation: Pharaon Mbianda Augustine, et.al., (2021). “Impact of Hymenoptera on Abelmoschus Esculentus (L.) Moench, 1794 (Malvaceae) Seed Yields at Bilone (Obala, Cameroon)”. International Journal of Research Studies in Agricultural Sciences (IJRSAS), 7(8), pp. 1-12 DOI: http://dx.doi.org/10.20431/2454-6224.0708001

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