

The role of a non-*Apis* bee in the fructification of *Abelmoschus esculentus* (L) Moench 1794 (Malvales: Malvaceae) flowers in Bambili (Cameroon)

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Experiments were made to determine the effects of the non-*Apis* bee visits on the pollination and the productivity of *Abelmoschus esculentus* from January 9 to February 10, 2020 in Bambili. Two treatments were used on 30 randomly-selected plants for each. These included bagging flowers to avoid any other visits (treatment n°1) and bagged flowers limited on a single visit of the non-*Apis* bee (treatment n°2). The foraging behavior on flowers and the pollination efficiency (fruiting rate, number of seeds / fruit and percentage of normal seeds) of that insect species were recorded. Results showed that the fructification rate, the number of seeds per fruit and the percentage of normal seeds of treatment n°2 was significantly high than that of treatment n°1. The non-*Apis* bee foraged pollen and nectar of *A. esculentus* flowers. The insect species was effective pollinator as its visit increased the fruiting rate by 38.53%. The nests attraction of this wild bee is recommended to improve the pollination and the productivity of gumbo in Bambili.

Key words: *Abelmoschus esculentus*, non-*Apis* bee, flowers, pollination, fruiting rate, seeds.

Cette étude a été menée à Bambili du 9 janvier au 10 février 2020 afin de déterminer l'impact des visites de l'abeille non-*Apis* sur la pollinisation et la productivité de *Abelmoschus esculentus*. Deux traitements ont été réalisés sur 30 plantes sélectionnées au hasard pour chaque traitement. Le traitement n°1 comprenait des fleurs isolées de visites d'insectes à l'aide de sachets en toile gaze et le traitement n°2 était constitué de fleurs ensachées limitées à la seule visite de l'abeille sauvage. Le comportement de butinage sur les fleurs et l'efficacité pollinisatrice (taux de fructification, nombre de graines / fruits et pourcentage de graines normales) de cet insecte ont été enregistrés. Les résultats ont montré que le taux de fructification, le nombre de graines / fruits et le pourcentage de graines normales du traitement n°2 étaient significativement élevés par rapport à ceux du traitement n°1. L'abeille non-*Apis* récoltait le pollen et le nectar des fleurs de *A. esculentus*. Cette abeille sauvage est un pollinisateur efficace car sa visite a augmenté le taux de fructification de 38,53%. L'installation des nids de cette abeille sauvage près des champs de gombo est recommandée pour améliorer la pollinisation et la productivité de cette plante à Bambili.

Mots clés: *Abelmoschus esculentus*, abeille non-*Apis*, fleurs, pollinisation, taux de fructification, graines.

INTRODUCTION

Pollinators are a key component of global biodiversity, providing vital ecosystem services to crops and wild plants (Gallai *et al.*, 2009) and their role in many plants is well known throughout the world (Jacob-Remacle, 1989). Many insects visit flowers from which they obtain carbohydrate and protein food (Pesson & Louveaux, 1984). In turn, they pollinated the visited flowers (Cane, 2002). In natural environments as in agro-ecosystems, insects in general and Apoidea in particular, have a great ecological and economic value because they have a positive influence on agro-food production (Desquesne, 1996). In many crop plant species, the honey bee (*Apis mellifera*) seems to be the central pollinator insect (Klein *et al.*, 2007) but many techniques are now developed on habitat conservation to maintain the population of natural bees (Torchio, 1990) for the pollination services (Velthuis & Van Doorn, 2006), wellbeing (Gallai *et al.*, 2009) and natural world (Ricketts *et al.*, 2004).

Abelmoschus esculentus, commonly called okra, lady'sfinger or gumbo, is an important crop grown in many parts of the world, especially in tropical including Asia, Central and South America (FAOSTAT, 2008) and sub-tropical countries (Kumar *et al.*, 2010). The plant is a rich source of water, protein, carbohydrates, fiber, calcium, zinc, iron and vitamin A, B and C (Norman, 1992; Justo, 2005). Many insect species forage in the gumbo field as predators, decomposers, parasitoids and pollinators. Honey bees, bumble bees, ants, butterflies and many other pollinating insects forage in the gumbo field due to its attractive golden yellow flowers containing nectars (Bajjiya & Abrol, 2017; Nandhini *et al.*, 2018). The crop is cultivated for its edible leaves, buds, flowers and fruits and easy to farm (Angbanyere & Baidoo, 2014). Gumbo is sometimes grown purposely for the seeds because of their high amounts of edible oil (Njoya *et al.*, 2005). The plant species is of considerable economic importance (Njoya *et al.*, 2005; Sawadogo *et al.*, 2006) because the global demand for gumbo is increasing due to the growing health consciousness and its high nutritional qualities as an excellent source of food and bio medicine (reddy *et al.*, 2004) and high financial value (Sawadogo *et al.*, 2006). In Cameroon gumbo occupies a prominent place in the diet, culture and life of many ethnic groups in the Far North Region, North-West Region and West Region. *A. esculentus* is self-compatible and self-pollination can take place in its hermaphrodite flowers despite the fact that the flowers are insect-pollinated (Al-Ghzawi *et al.*, 2003).

In Cameroon one of the key objectives of agricultural research is to obtain high and sustainable yields to feed the growing population (DSCE, 2009) but the production of gumbo is low (100,025 tons) (FAO, 2018). This low production is because gumbo crop is suffering from number of biotic (like gumbo parasites) and abiotic factors (like high temperature), including lack of knowledge on the importance of flowering insect pollinators in increasing yields of vegetable crops. Therefore it is important to study the possibilities to increase the production of gumbo. Few published data do exist on the relationships between insect foragers and *A. esculentus* flowers. Njoya *et al.* (2005) in Yaounde (Cameroon) observed on preliminary trials that *Megachile* spp., *Halictus* spp., *Xylocopa* spp. and *A. mellifera* were the main okra anthophilous insects; Azo'o *et al.* (2011) in Maroua-Cameroon found that *Eucara macrognatha* Gerstaecker, 1870 and *Tetralonia fraternal* Friese, 1911 were the two main bee visitors and were effective gumbo pollinators; Amada *et al.* (2018) in Yaounde found out that *Xylocopa olivacea* Fabricius, 1787, *Synagris cornuta* Linnaeus, 1758 and the ants were the most frequently insect species pollinators of *A. esculentus*; Pando *et al.* (2020) in Maroua and Otiobo *et al.* (2020) in Bambili found that *Lipotriches collaris* Vachal, 1903 was the most pollinators of *A. esculentus*. Following the lack of complete data on the relationship between *A. esculentus* and its floral entomofauna, the emergency to know the crop-plant pollinators for their conservation and the necessity to produce higher quantity seeds to meet their various uses, it is important to carry out further researches on gumbo in order to complete the useful available data. Thus it's necessary to conduct investigations on the relationships between *A. esculentus* and their flowering insects in Bambili, North West Region of Cameroon, to increase the existing data for Cameroon. The main objective of our research was to contribute to the knowledge of the importance of a non-*Apis* bee, known to be the most prevalent for the insect visiting gumbo in Bambili. Specifically, our study was aimed at evaluating the impact of a single visit of the non-*Apis* bee on *A. esculentus* pollination and fructification.

MATERIALS AND METHODS

Study site

This experiment was carried out from January 9 to February 10, 2020. The study was carried out in Bambili (6°00'29''N 10°25'90''E and 1430 m above sea level), Tubah Sub Division, Mezam Division, in the North West Region of Cameroon during the dry season. The climate is of the tropical monsoon type with two seasons, namely the rainy season (mid-March to mid-October) and the dry season (mid-October to mid-March) (Neba & Eze, 2004). The annual average precipitation ranges from 1770 mm to 2824 mm, the mean annual temperature is 20°C and the mean annual relative humidity is above 85% (Abdoul *et al.*, 2008).

Biological Material

The bee material was represented by the population of the non-*Apis* bee (**figure 1**) which came from the ground nests present on the study site and the plant material was made up of seeds of *A. esculentus* (**figure 2**) bought from “Farmers Pharmacy” located at “Three corners” Bambili.



Figure 1 : The non-*Apis* bee captured on *Abelmoschus esculentus* flower



Figure 2 : Dried seeds to be planted (A) and an open flower (B) of *Abelmoschus esculentus* (©Otiobo, 2020)

METHODOLOGY

The methodology used was modified from that of Azo'o *et al.* (2012). An experimental plot of 10 m long and 9 m wide was selected for the study. It was cleared and cleaned with a cutlass on 20 September, 2019. Then using a digger and a hoe, the soil was tilled and 10 ridges of 3 m long by 1 m wide and 0.3 m high each were formed. The distance between ridges was 0.5 m wide. The vegetation near *A. esculentus* field was represented by crops, ornamental plants and native plants.

Prior to planting, fowl droppings were mixed with the ground and put in holes destined for each gumbo plant. Two okra seeds were then deposited in each hole on a row of three lines while maintaining a gap of 0.5 m between two holes. Watering was done daily between 4-6 pm to prevent drying. From germination (13th October, 2019) to the opening of the first flowers (27th December, 2019), weeding was done manually every two weeks to maintain ridge weeds-free.

Direct observations on flowers were made daily from January 9 to February 10, 2020 within the blooming period and between 11:00 am and 12:00 pm (local time) since previous observations indicated that gumbo flowers were fully visited by the non-*Apis* bee species for the whole day with a peak of activity between 11:00 am and 12:00 pm (Otiobo *et al.*, 2020). At most five specimens of the wild bee species were captured globally with an insect net and were conserved in a box containing 70% of ethanol for future taxonomy. Bee identifications were done by Dr. Dounia, Laboratory of Zoology, Higher Teacher Training College, University of Yaounde I, using identification books (Delvare & Aberlenc, 1989; Borror & White, 1991; Eardley *et al.*, 2010). To assess pollination effectiveness, we used random samples of 30 experimental plants for each of the two treatments including autonomous self-pollination (ASP) and single bee visit (SBV).

In autonomous self-pollination (ASP) (treatment n°1), flower buds were isolated with gauze bags of 1mm² mesh (**figure 3**) a day before anthesis to prevent anthophilous insect visitation and airborne pollen flow the following day. The flower and the equivalent plant were labeled.



Figure 3 : *Abielmoschus esculentus* plant showing flowers protected with gauze bags
(©Otiobo, 2020)

In single bee visit (SBV) (treatment n°2) a team of four observers was positioned in the study field. The observers were placed 2 m away from a newly open flower of a given plant from 11:00 am (local time) before the arrival of bee foragers. Each flower was monitored until it received a single visit by the non-*Apis* bee.

The floral product harvested (nectar or pollen) was registered and this was done on the basis of the foraging behavior of the non-*Apis* bee. Nectar harvesters were seen going on the bottom of the flower and gathering this product at the level of the nectary while pollen gatherers directly scratched the anthers with mandibles or legs.

Moreover, the duration of bee visits was recorded by the observer using a stopwatch. After a bee visit, the flower was bagged with a gauze bag until the next day to avoid any additional insect visitation (Vaissière *et al.*, 1996; Gingras *et al.*, 1999) after which the flower and the equivalent plant were also tagged. For all treatments, only the first flower at the base of the plant was considered. Two weeks after anthesis, each fruit was harvested and tagged for future analysis according to Tchuenguem *et al.* (2004):

- The contribution (Fr) of the non-*Apis* bee in the fruiting was calculated as follows: $Fr = \{[(Fr2 - Fr1) / Fr2] \times 100\}$ where Fr2 were the fruiting rate in bagged flowers visited exclusively by the non-*Apis* bee (treatment n°2) and Fr1 were the bagged flowers (treatment n°1).

- The percentage of the number of seeds per fruit (Ps) due to the influence of the non-*Apis* bee was calculated as follows: $Ps = \{[(s2 - s1) / s2] \times 100\}$ where s2 and s1 were the mean number of seeds per fruit in treatment n°2 and n°1.

- The percentage of normal seeds (Pns) due to the influence of the non-*Apis* bee was calculated as follows: $Pns = \{[(ns2 - ns1) / ns2] \times 100\}$ where ns2 and ns1 were the percentages of normal seeds in treatment n°2 and n°1.

Data analysis

The data was processed using descriptive statistics (calculation of means, standard deviations and percentages) and two tests: student's (*t*) for the comparison of means of two samples and chi-square (χ^2) for the comparison of percentages on Microsoft Excel 2010.

RESULTS

Foraging ethology

The non-*Apis* bee selectively visited the open flowers of *A. esculentus*. The wild bee comes into contact with the flower from above, either on the stamens and the stigma, or on the corolla. Subsequently, the bee scratches the anthers to collect pollen (**figure 4**). The forager temporarily stops the pollen harvest at the anthers and then lands on the corolla to complete the collection of the pollen collected. They could therefore intervene directly on self-pollination, by putting the pollen of a flower on its stigma. All the 30 (100%) single visits of the non-*Apis* bee were with stigmatic contact. The activity of this wild bee observed foraging on gumbo flowers was for pollen and nectar gathering base to its foraging behavior. The mean duration of a bee visit was 10.87s (ESM = 6.26; n = 30) for the collection of nectar and 11.33 s (ESM = 4.85; n = 30) for the collection of pollen. The difference between these two averages is significant ($t = -1.21$; $p < 0.001$)



Figure 4: The non-*Apis* bee collecting pollen in a flower of *Abelmoschus esculentus* (©Otiobo, 2020)

Impact of the non-*Apis* bee on *Abelmoschus esculentus* yields

During pollen and nectar harvest, the non-*Apis* bee species were in regular contact with the anthers and stigma. Thus this insect increased the pollination possibilities of this plant species. **Table 1** gives the fruiting rate, the number of seeds per fruit and the percentage of normal seeds in treatment n°1 (autonomous self-pollination) and treatment n°2 (single non-*Apis* bee visit). The fruiting rate was 70% in treatment n°1 and 83.33% in treatment n°2. The difference between this percentage and that obtained in treatment n°1 is not significant ($\chi^2 = 1.49$; $p > 0.05$).

The average number of seeds per fruit was 14 in treatment n°1 and 21 in treatment n°2. The difference between this average and that obtained in treatment n°1 is highly significant ($t = -3.80$; $p < 0.01$).

The percentage of normal seeds was 75.36% in treatment n°1 and 86.19% in treatment n°2. The difference between this percentage and that obtained in treatment n°2 is very highly significant ($\chi^2 = 19.86$; $p < 0.001$). Thus, the flowers protected then visited exclusively by the non-*Apis* bee gave higher fruit and grain yields than those of protected and unvisited flowers.

Table 1: Parameters associated to the production of *Abelmoschus esculentus* according to treatments

Parameter	ASP	SBV	
NFS	30	30	- Comparison of fruiting rates: SBV / ASP: $\chi^2 = 1.49$ ($df = 1, p > 0.05$, NS);
NFF	21	25	- Comparison of the average number of seeds /
FR (%)	70.00	83.33	fruit:
Seed / fruit	14 ± 7	21 ± 7	SBV / ASP: $t = -3.80$ ($df = 58, p < 0.01$, HS);
TNS	418	630	- Comparison of the percentages of normal
NNS	315	543	seeds:
NS (%)	75.36	86.19	SBV / ASP: $\chi^2 = 19.86$ ($df = 1, p < 0.001$, HS)

NFS: number of flowers studied; NFF: number of fruits formed; FR: fruiting rate; TNS: total number of seeds; NNS: number of normal seeds; % NS: percentage of normal seeds; HS: highly significant; NS: not significant; ASP: protected flowers; SBV: protected flowers and having been visited exclusively by the non-*Apis* bee.

DISCUSSION

In this work, it is observed that self-pollination in gumbo plant allows the production of fruits and seeds without any pollen deposition by anthophilous insects. Our results agreed with other reports (Al Ghzawi *et al.*, 2003; Azo'o *et al.*, 2011, 2012). As pollen grains of gumbo are very large (Vaissière & Vinson, 1994) and are not wind borne (Mc Gregor, 1976), it is the natural contact between the uppermost anthers and the lower part of the stigma that enables self-pollination (Hamon & Koechlin, 1991). Although yields are higher with the presence of pollinators such as the non-*Apis* bee as it is showed in Table 1.

Gumbo floral products attract various insect species in natural conditions especially the non-*Apis* bee which collects both nectar and pollen on gumbo flowers in our study area. This result is in agreement with those obtained by Perera & Karunaratne (2019) in Sri Lanka with *Lithurgus atratus*. *L. collaris* was recognized as the major insect pollinator in Maroua (Cameroon) (Pando *et al.*, 2020). The genus *Apis* with the species *cerana* was identified as the main *A. esculentus* pollinator in India (Crane, 1991). *Eucara macrognatha* and *Tetralonia fraterna* were collected on the flowers of *A. esculentus* (Pauly, 1984) in some tropical country of Africa and in Cameroon particularly (Azo'o *et al.*, 2011). Amada *et al.* (2018) in Yaounde (Cameroon) found out that *Xylocopa olivacea*, *Synagris cornuta* and the ants were the most frequently insect species pollinators of *A. esculentus*. Toni *et al.*, (2020) in the Ketou community (South Benin) found that *E. macrognatha* was the most effective pollinator of the gumbo. Honeybees were found to be the main insect pollinator in the forest Region of Ghana (Angbanyere & Baidoo, 2014). This attractiveness could be due to the availability in great quantity of those floral products. Moreover, gumbo is a plant that produces nectar and pollen able enough to substantially contribute to the maintaining of the nutritional needs of the wild and domesticated bee species.

The harvesting of nectar by the non-*Apis* bee could be due to high concentration in total sugars considering the range of 15 to 75% for numerous plant species (Proctor *et al.*, 1996). According to Phillippe (1991), bees are generally constant on a plant species when the concentration in sugars of its nectar is more than 15% and Azo'o *et al.* (2012) found in their study that the mean value of the sugar content of the nectar in *A. esculentus* was 21.74%. Pollens have been collected by the non-*Apis* bee because they need them for food; moreover pollens are a rich source of protein (Pesson & Louveaux, 1984). This would be an explanation of the good attachment of this bee to the pollen and/or nectar of *A. esculentus*.

Individuals from the non-*Apis* bee spend more than 10 sec visiting a flower. During this time, they frequently contacted the anthers, got the echinate pollen grains in the body hair (Hamon & Koechlin, 1991) and could thereby do pollination with this self pollen before flying off.

The significant contribution of pollinating insects in pods and seed yield of *A. esculentus* was found. In Cameroon, Azo'o *et al.* (2012) showed that *A. esculentus* flowers produce fewer seeds per pod in the absence of pollinating insects. The weight of insect pollinators played a helpful role during nectar or pollen collection: those insects shook flowers, facilitating the liberation of pollen by anthers for the optimal occupation of the stigma (Klein *et al.*, 2007). This higher productivity of pods and seeds in single non-*Apis* bee visit when compared with bagged flowers showed that the wild bee visits were effective in increasing pollination. This result is in accordance with that of Azo'o *et al.* (2012), Amada *et al.* (2018), Perera & Karunaratne (2019) and Pando *et al.* (2020) showed the importance of wild bees as the main pollinating insects of this crop.

CONCLUSION

Abelmoschus esculentus is a plant species that benefits from the foraging activity of the non-*Apis* bee on its flowers despite its capacity of self-pollination. The significant increase in yields of *A. esculentus* in the presence of the non-*Apis* bee is the consequence of the foraging activity of these insect species on the pollination of the flowers of the plant studied. Thus the conservation of those wild-bee nests in areas surrounding gumbo crops in bloom is recommended.

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